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In Situ Chemically Crosslinked Chitosan Membrane by Adipic Acid

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ABSTRACT: Adipic acid, which is nontoxic, was used to dissolve chitosan. The chitosan/adipic acid solution was used to prepare chitosan membrane. After being heated at 80–100°C, the membrane was *in situ* chemically crosslinked by adipic acid, as verified by Fourier transform infrared and wide-angle X-ray diffractometer analysis. The crosslinked membrane did not collapse even without treatment in alkaline solution. In addition, the *in situ* crosslinking reaction was studied. The crosslinking degree (CLD) was quantitatively calculated based on the mass of water produced. The results showed that CLD was positively related to both heating temperature and time. Results of kinetic of crosslinking reaction suggested that the amidation was in agreement with the first-order rate equation and that the temperature effect could be described with the Arrhenius equation. The results of weight loss of chitosan membrane in phosphate-buffered solution (pH = 7.4) indicated that the best water resistance of chitosan membrane was obtained at 90°C. In brief, a straightforward, nontoxic, environment-friendly, and economical chemically crosslinking approach has been developed for chitosan materials. © 2012 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 000: 000–000, 2012

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INTRODUCTION

Chitosan, the deacetylated derivative of naturally abundant biopolymer chitin is a well-known filmogenic material.[1] Chitosan membranes have been explored in many uses, such as in water–ethanol pervaporation,[2] enzyme immobilization,[3,4] cationic specimen transportation,[5] protein separation,[6] controlled ingredient-release,[7] and regenerative medicine applications.[8–10] However, because of chitosan membrane's bad mechanical properties and the default that it is only dissolved in acid solution,[11–13] researchers have explored various modification methods. Modification of chitosan through cross-linking or blending with other polymers is both convenient and effective in improving its relative properties for practical applications. As the membrane prepared by blending is not stable, its application is usually limited.

Many investigations have been focused on improving the properties by crosslinking method. The crosslinking was usually about reaction of the active groups such as aldehyde groups, acid groups, and epoxy groups of crosslink reagents with amino groups in chitosan. Some metals can also be used as crosslinking reagents as their ions can form coordination linkages with chitosan's amino.

Generally, crosslinking treatment is carried out by immersing membranes in a solution bath with crosslinking reagents such as sulfuric acid,[14] aldehydes,[15,16] genipin,[17] or metals.[18] The major weakness of these crosslinked membranes, however, is their heterogeneity resulted from the fact that the crosslinking usually takes place at the membrane surface, making it difficult for crosslinking reagents to further penetrate into the interior part. To obtain homogeneous membranes, crosslinking reagents and chitosan are dissolved in a common solvent, or they are blended together after being dissolved in different solvents.[19–23] In these strategies, the solvent used for chitosan dissolution is usually acetic acid, and besides this monocarboxylic acids, there are many kinds of di-, tri-, and multicarboxylic acids, such as succinic acid, adipic acid, and citric acid. Studies indicated that interactions existed between chitosan and these acids in solution, and properties of the solution were greatly altered.[24–26] In Chen et al.'s work,[11] several dicarboxylic acid solutions, including adipic acid, glycolic acid, oxalic acid, succinic acid, and malic acid, were used as solvents for chitosan dissolution. Not only do these dicarboxylic acids solutions act as solvents, but the dicarboxylic acids perform as crosslinking reagents through ionic link formed by the carboxyl groups in these dicarboxylic acids and amino groups in chitosan. Among all the dicarboxylic

acids used, the membrane prepared with adipic acid has got the best mechanical properties because of its longer carbon bone compared to other dicarboxylic acid. But the physical crosslink formed by ionic link may not be stable when encountered with aqueous solutions.

On the other hand, in this method, because the membranes are prepared by dissolving chitosan in different acids, excessive acids are usually needed to be removed, because the membrane with residual acids is not stable in water and can even be dissolved in water. Therefore, in most cases, alkaline treatment is used, which increases the complexity of the technological process with one more step to remove the residual alkali. What is more, acids or alkalis that are usually not removed completely result in some untoward effect.

Is there a method by which without removing excessive acid or without alkaline treatment the membrane, which is relatively stable in water, can be prepared? To achieve the aim, adipic acid, a kind of dicarboxylic acid, which is nontoxic and biocompatible, was chosen to perform as an acid to dissolve chitosan and as a crosslinking reagent at the same time. Besides, a more stable crosslinking structure was formed by chemical linking other than ionic linking as expected. As carboxyl and amino groups react easily under certain conditions resulting in amide bonds[27,28] and some attempts forming amide bonds have been achieved with carboxyl to react with the amino from chitosan with heating,[12,29,30] anticipation was reasonable that carboxyl in adipic acid would react with amino groups in chitosan to form the chemical linkage during heating. Here, the formation of the amido link was verified by Fourier transform infrared (FTIR) and wide-angle X-ray diffractometer (WAXD) analysis. The result showed that the membrane with this chemical linkage would not be dissolved in water compared to the membrane just with the ionic linkage that would collapse in water. The crosslinking degree (CLD) was calculated based on the mass of water produced. The factors affecting the crosslinking reaction, including temperature and time, were studied. The activation energy of the crosslinking reaction was also calculated according to the kinetic plot. Furthermore, weight loss in phosphate-buffered solution (PBS, pH = 7.4) as well as the mechanical properties of the crosslinked chitosan membrane was characterized and compared to chitosan membrane just with the ionic crosslink.

EXPERIMENTAL

Materials

Chitosan was purchased from Jinan Haidebei Marine Bioengineering Co. (Jinan, China) and purified by the method described in previous literature.[31] The viscosity average molecular weight and deacylation degree of the chitosan were 465,600 Da and 82.7% ± 0.5%, respectively. Adipic acid was of analytical grade and used without further purification. Water used in this study was double-distilled water.

Preparation of Chitosan Membrane

Chitosan solution of 2 wt % was prepared by dissolving chitosan in 0.75 wt % adipic acid solution under stirring overnight. Ten-gram solution was cast into a petri dish (6 cm in diameter) after air bubbles in the solution were removed. Then the petri dish was placed on an aclinic table at room temperature for 3–5 days. Chitosan membranes were peeled off from the dish and were further dried in vacuum.

In Situ Chemical Crosslinking of Chitosan Membrane

The chemical crosslinking of chitosan membrane was carried out by heating the membranes at given temperatures (that is 80, 90, and 100°C) in vacuum for 40 or 60 min. The membrane cooled down to room temperature in a desiccator without further treatment to remove residual adipic acid.

Calculation of CLD and Kinetics of Crosslinking Reaction

The crosslink reaction was the amide between amnio group in chitosan and carboxyl in adipic acid, which resulted in the production of water. Here, the crosslinking degree (CLD) was defined as a half of the ratio of the actually produced H_2O (m_r) to theoretically produced value (m_{th}).

$$\mathrm{CLD}\% = \frac{1}{2} \times \frac{m_r}{m_{\mathrm{th}}} \times 100\% \tag{1}$$

The m_r was obtained based on the loss of the membrane weight during the reaction. To get accurate m_r, the chitosan membrane was dried in vacuum at 45°C for 38 h and cooled down to room temperature in a desiccator before the crosslinking reaction. The weight of the dried membrane was measured on an analytical balance (m_0). After the crosslinking reaction, the membrane cooled in a desiccator, and its weight was measured again (m_t). The m_r was ($m_0 - m_t$). The theoretical value m_{th} was calculated based on the constitute of the membrane. The equation could be finally deducted to

$$\mathrm{CLD}\% = K \times \frac{m_0 - m_t}{m_0} \times 100\% \tag{2}$$

where K is a constant (=7.7698) only correlated to the raw materials including chitosan and adipic acid.

To better investigate the effect of temperature and time on the crosslinking reaction of chitosan membrane, we selected CLD as a parameter to evaluate the extent of crosslinking reaction, and we assumed that the crosslinking reaction was in agreement with the following equation, the first-order rate equation.

$$Ln\frac{1}{1 - 2\mathrm{CLD}} = k_{(T)}t \tag{3}$$

where t, k, and T represent time, rate constant, and temperature, respectively.

In addition, we also assumed that the reaction rate complied with the well-known Arrhenius equation.

$$\ln k_{(T)} = \ln k_0 - \frac{E}{RT} \tag{4}$$

where E represents the activation energy and k_0 represents the frequency factor.

Fourier Transform Infrared Spectrometer

Fourier transform infrared (FTIR) analysis was performed on a Nicolet 560 FTIR 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 were mixed with KBr and pressed into disks (0.5 mm in thickness) for test.

Wide-Angle X-ray Diffractometry

To investigate the crystalline characteristics of chitosan membranes, a wide-angle X-ray diffractometer (WAXD, Rigaku Denki) was used. WAXD patterns were recorded by the reflection method with nickel-filtered Cu Kα radiation operated at 30 kV and 20 mA in the 2θ scanning mode between 5° and 55°. The relative crystallization degree (CD) of chitosan membrane was calculated according to the following equation.[32]

$$\mathrm{CD}\% = [A_c/(A_c + A_a)] \times 100\% \quad (5)$$

where A_c and A_a are the areas of the crystalline and amorphous peaks in WAXD spectrum, respectively.

Weight Loss in Water

Chitosan membranes (size: 2 cm × 2 cm, weight: W_0) were immersed in PBS (pH = 7.4) at 37°C for 1 week. The membranes were washed with double-distilled water and then dried at 45°C under vacuum until constant weight (W_t). The weight loss (W_L) was calculated according to the equation below.

$$W_L\% = (W_0 - W_t)/W_0 \times 100\% \quad (6)$$

Mechanic Properties Test

The mechanical properties of the chitosan membranes were determined with a tensile strength instrument (AGS-J, Shimadzu, JP). 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 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.

Scanning Electron Microscope Morphology

The fracture surface morphologies of these samples were studied using an SEM at an accelerating voltage of 20 kV. All samples were coated with gold, before SEM photographs were taken at different magnifications (SEM, JSM-5900 LV, JEOL, Japan).

RESULT AND DISCUSSION

Changes in Molecular Structure of Chitosan Membranes After In Situ Crosslinking

FTIR was used to analyze the changes in the molecular structure of chitosan membranes caused by the heating treatment. The spectra were summarized in Figure 1. Curve CS and curve S0-0 represent pure chitosan membrane and the chitosan/adipic acid membranes before heating, respectively. Because of the high-deacylation degree (82.7%), the flexural vibration of amide I at around 1632.9 cm^{-1} was relatively strong, the amide III band at 1310 cm^{-1} was very weak, and the amide II band at about 1570 cm^{-1} almost disappears. It also showed that the peak assigned to amide I in the curve of chitosan/adipic acid became weaker (narrower) compared to that of pure chitosan, which might

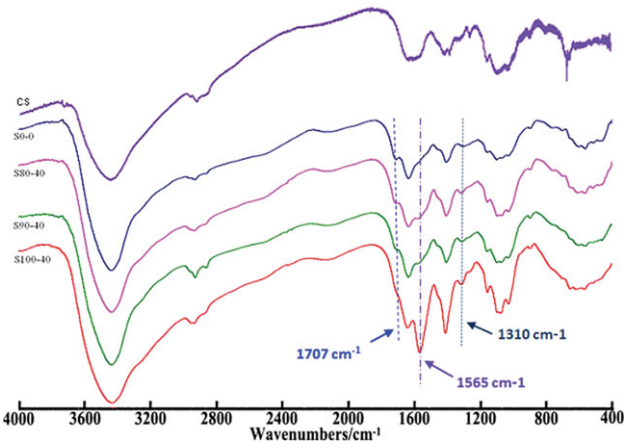

Figure 1. FTIR spectra of chitosan membranes prepared by casting/evaporation of its AA solution: CS for pure chitosan; S0-0 for uncrosslinked membranes; S80-40, S90-40, and S100-40 for the membranes heated for 40 min at 80, 90, and 100°C, separately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

result from the ionic bonds in the complex membrane.[33] Besides, the obvious peak at around 1707 cm^{-1}, which did not occur in the curve of pure chitosan, was assigned to the —COOH in adipic acid.

After the membrane was heated in vacuum, the peak intensity of —COOH in adipic acid decreased steadily as the heating temperature increased. At the same time, amide II band, including ν(C—N) and δ(N—H) at around 1565 cm^{-1}, appeared, suggesting the formation of amide bond. This was further confirmed by the increased amide III band at about 1310 cm^{-1}.[34] Namely, it is easy to form amide bonds by carboxyl in adipic acid and amino in chitosan just by heating. This has been confirmed by other studies.[12,34] The redshift of amide I peak also implied the weakened intermolecular hydrogen bond resulted from the decrease in —NH_2 number. When the temperature rose to 100°C, the —COOH peak was very weak, and the peak intensity of amide II band and amide III band increased to the largest. These results suggested that the increase of temperature benefited the amide formation.

Moreover, the spectra also gave an information that —COOH in adipic acid did not react with —OH in chitosan, because the stretching vibration of C=O from ester bond (at around 1730 cm^{-1}) was not found.[35–37] On the basis of these results, it can be proposed that the fundamental reaction of the chemical crosslinking is virtually the amidation between the carboxyl in adipic acid and the amino groups in chitosan chains (Figure 2).

CLD of Chitosan Membranes

The crosslinked chitosan membranes were obtained simply by heating adipic acid-containing chitosan membranes. The influences of temperature and time on the CLD were investigated. CLD was calculated based on eq. (2). When the temperature was raised to 120°C, the color of membranes turned from light brown to dark brown probably due to oxidation. Therefore, the crosslinking treatment was carried out just below 100°C in this study. As can be seen from Table I, CLD were 12.1% ± 2.0%,

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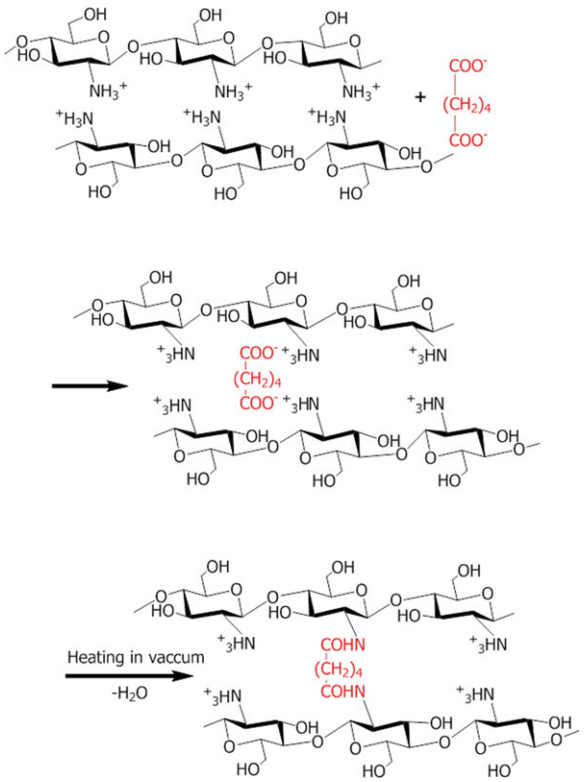

Figure 2. Proposed reactions between adipic acid (in red) and chitosan. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

18.5% ± 0.8%, and 28.4% ± 0.9% when the membranes were heated for 40 min at 80, 90, and 100°C, separately. The higher the temperature was, the higher the CLD was. A similar phenomenon was observed when the membranes were heated for 60 min. On the other hand, the influence of heating time on CLD was more complicated. At 80°C, the CLD of membranes heated for 60 min was equivalent to that of membranes heated for 40 min. In contrast, CLD was clearly elevated when the temperature was at 90°C, especially at 100°C.

Kinetic of Crosslinking Reaction

To further investigate the relationship between CLD and temperature, or time, the first-order rate equation and Arrhenius equation were used.

According to eqs. (3) and (4), the Arrhenius plots were shown in Figure 3. A good linear relationship between ln $k(T)$ and $1000/T$ was found when the heating treatment was kept for 40 and 60 min, implying that the crosslinking reaction was authentically in agreement with the first-order rate equation and that the temperature effect could be described with the Arrhenius equation.

Besides, the activation energy of the crosslinking reaction was further calculated to be 60.6 and 76.17 kJ/mol when the reaction time was 40 and 60 min, respectively. These results were in agreement with what Toffey et al.[27] reported. The higher activation energy for 60 min suggested that, in the later 20 min, the rate of amidation reaction slowed down compared to the former 40 min. This might be attributed to the fact that the amount of amino groups and carboxyl groups decreased off as the amidation reaction went on.

Table I. CLD of Chitosan Membranes Prepared from Casting/Evaporation of Its AA Solution by Heating at 80, 90, and 100°C for 40 and 60 min

Sample	Time (min)	Temperature (°C)	CLD% mean ± SD
S80-40	40	80	12.1 ± 2.0
S90-40	40	90	18.5 ± 0.8
S100-40	40	100	28.4 ± 0.9
S80-60	60	80	12.9 ± 1.9
S90-60	60	90	22.1 ± 0.2
S100-60	60	100	34.9 ± 0.2

XRD Spectroscopy

Crystalline behaviors of chitosan membranes were characterized by WAXD. The quantitative CD [Figure 4(b)] was calculated based on the WAXD spectra [Figure 4(a)]. The diffractogram of CS consisted of two major peaks at 2θ around 10° and 20°, which were in agreement with the literature reports.[18,22] For those diffractograms for crosslinked CS/AA membranes, the peak at 2θ around 10° became weaker and even vanished when the temperature rose to 100°C, which indicated that hydrogen bonds in those membranes became weaker as the temperature increased, as confirmed by the FTIR spectra. A new peak at 2θ around 8.5° occurred in the uncrosslinked membrane, and the membranes crosslinked below 100°C, which was caused by the addition of adipic acid. This peak also became weaker as the temperature increased and even vanished when the temperature reached 100°C, as a result of the formation of amide bonds. Furthermore, the peak at around 20° was deformed to be wider, because adipic acid molecular attaching to the two parallel-aligned chitosan chains affects its original arrangement. But when the temperature rise to 100°C, that peak became slightly more intensive and much more symmetrical, which indicated high-degree crystallization, which could be confirmed by CD values. It is evident that CD values decreased upon heating treatment, indicating restrained motility of chitosan chains by the chemical crosslinking. However, CD value at 100°C was

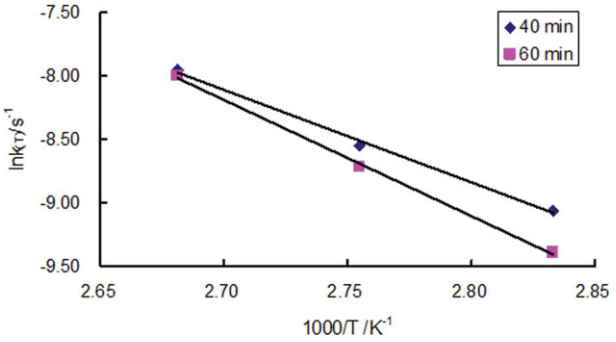

Figure 3. Arrhenius plots of rate constants versus temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

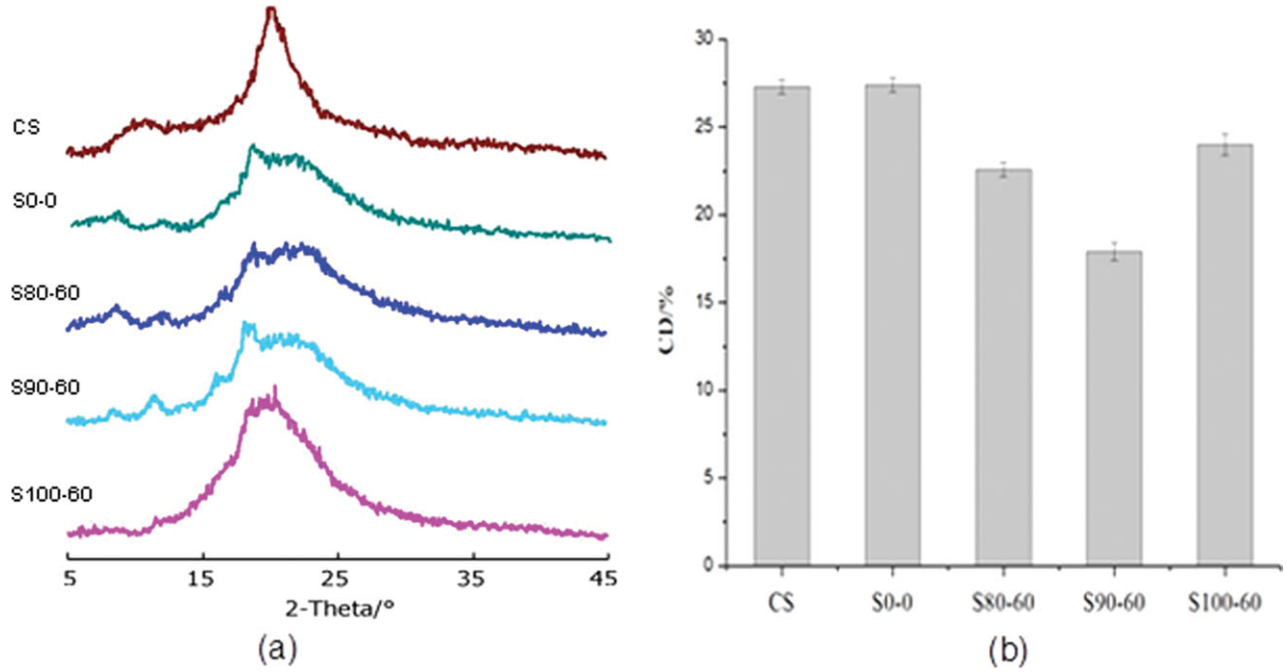

Figure 4. XRD spectra (a) and crystallization degree (b) of chitosan membranes prepared by casting/evaporation of its AA solution: CS for pure chitosan; S0-0 for uncrosslinked membranes; S80-40, S90-40, and S100-40 for the membranes heated for 60 min at 80, 90, and 100°C, separately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

higher than those at 80 and 90°C, although its CLD was much higher (Table I). We thought that higher temperature (100°C) could enhance the movement of chitosan chains and benefit rearrangement of chitosan chains, at the same time, the formation of amide bonds fixed the ordered structure. Thus, under 100°C heating treatment, the crosslinked chitosan membrane showed higher degree crystallization. When heating treatment temperature was relatively low, the crosslinking point was partial and asymmetrical, which would destruct the structure of crystal lattice, resulting in low-CD value.

Water Resistance

Generally, chitosan membranes formed with the help of acids are stable only after being dealt with strong alkaline solution, because alkaline can neutralize the acids in chitosan and makes chitosan unsoluble. In this work, all the chitosan membranes were not to be handled with alkaline. As the uncrosslinked membrane would collapse in water and other acidic solution, a kind of weak alkaline solution, namely PBS (pH = 7.4), was chosen to evaluate chitosan membrane capability of water resistance. The results were summarized in Figure 5. All the chitosan membranes showed weight loss, especially, the uncrosslinked membrane was most obvious. The weight loss included the dissolution of the unreacted adipic acid as well as small chitosan molecules in PBS. Even for crosslinked membranes, weight loss was more than 5.8%, because the residual adipic acid was not specially removed after heating treatment. The membrane by heating treatment, crosslinked by chemically amino linkage, showed better capability of water resistance, compared to the membrane without heating treatment, which was just crosslinked by ionic bonds. The best capability of water resistance was obtained at the temperature of 90°C, and the weight loss was just 5.8%. But an odd phenomenon was that when the heating temperature was raised up to 100°C, the weight loss increased abnormally. We supposed that a lot of adipic acid molecules in the crosslinked membranes formed lone amino bonds at lower heating temperature, but at high-heating temperature (100°C), ordered molecular arrangement led to adipic acid molecules reacting by their both carboxy groups. Therefore, the former used more amount of adipic acid and got lower CLD, and less adipic acid was lost after soaking in PBS. But the

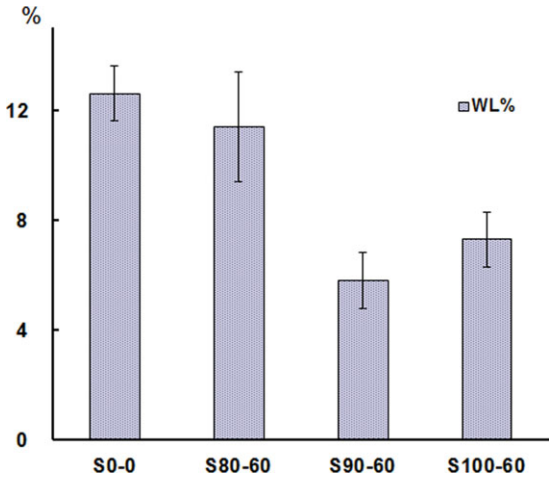

Figure 5. WL% of chitosan membranes prepared by casting/evaporation of its AA solution: S0-0 for uncrosslinked membranes; S80-40, S90-40, and S100-40 for the membranes heated for 60 min at 80, 90, and 100°C, separately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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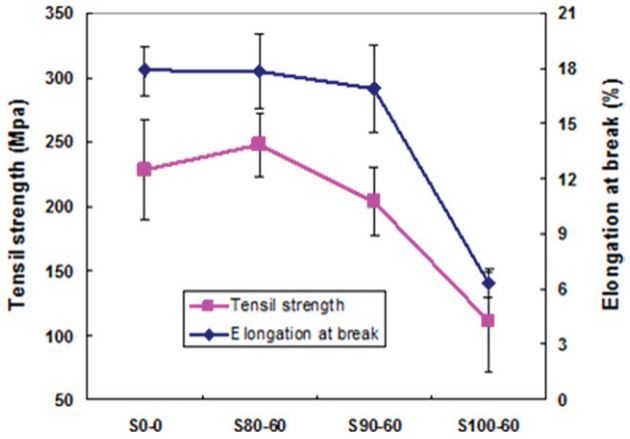

Figure 6. Tensile strength and elongation at break of chitosan membranes prepared by casting/evaporation of its AA solution: CS for pure chitosan; S0-0 for uncrosslinked membranes; S80-40, S90-40, and S100-40 for the membranes heated for 60 min at 80, 90, and 100°C, separately. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

latter got higher CLD using less amount of adipic acid by reacting their both carboxy groups; thus, more adipic acid was lost after soaking in PBS.

Mechanical Properties

The mechanical properties, including tensile strength and elongation of chitosan membranes, were summarized in Figure 6. The results showed that a slight crosslink would enhance the network and increase its tensile strength, when the heating temperature was 80°C and the CLD was 12.9% ± 1.9% (Table I). But when the heating temperature rose higher, where the CLD increased significantly (Table I), mechanical properties were influenced by excessive crosslink. When the temperature rose up to 100°C, the tensile strength and elongation degraded immensely. To investigate the reasons for tensile strength and elongation decrease at high temperature, SEM was used to examine the morphologies of the crosslinking chitosan membranes. To ensure that the morphologies would not be destroyed during the process of fracture, those samples were fractured in liquid nitrogen. The results (Figure 7) showed that the surface of the membrane heated at 100°C seemed rougher than other membranes. And some holes seemed to appear. As described earlier, the crosslinking chitosan membranes got higher CLD at 100°C, and so more porous would appear and because of that, more water was produced and evaporated in the process of heating treatment, which significantly decreased the tensile strength and elongation. On the other hand, considering the facts that the best capability of water resistance was obtained at

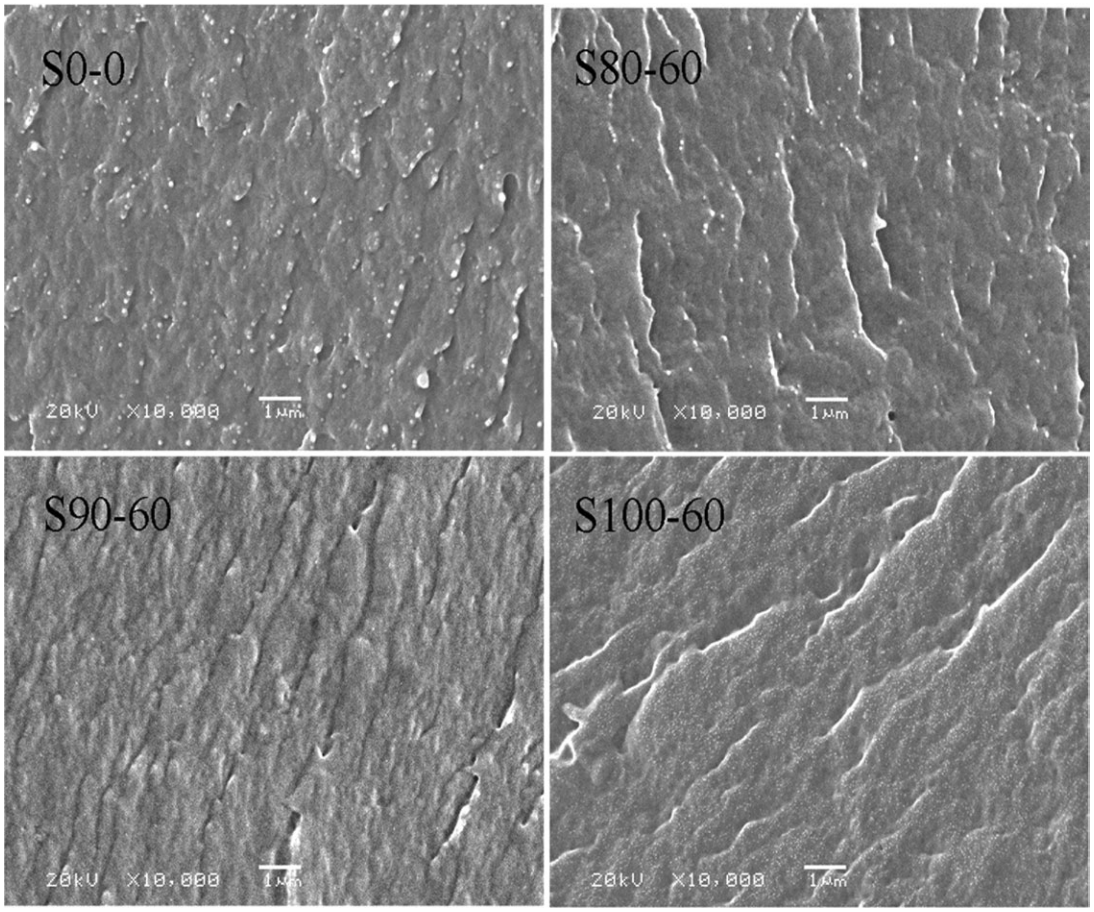

Figure 7. The SEM images of chitosan membranes prepared by casting/evaporation of its AA solution: S0-0 for uncrosslinked membranes; S80-40, S90-40, and S100-40 for the membranes heated for 60 min at 80, 90, and 100°C, separately.

the temperature at 90°C and that the mechanical properties at that temperature were similar to the membrane without heating, we could obtain the membrane with better capability of water resistance without losing its mechanical properties.

CONCLUSIONS

Chitosan membrane was prepared by casting/evaporation of chitosan/adipic acid solution and was successfully *in situ* chemically crosslinked just by heating the membrane. The CLD was calculated on the basis of weight of water produced after heating. It was positively related to both heating temperature and time. And the membrane showed good stability in PBS (pH = 7.4) as well as in water even without alkaline treatment or other processes to remove residual acids. The best capability of water resistance was obtained at the temperature of 90°C, without significant change in its mechanical properties. Thus, it could be concluded that the water resistance of chitosan membranes could be effectively improved by *in situ* chemical crosslinking by adipic acid at the temperature of 90°C without sacrificing the mechanical properties of the membranes.

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