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Use of Dicarboxylic Acids To Improve and Diversify the Material Properties of Porous Chitosan Membranes

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Several nontoxic dicarboxylic acid solutions (oxalic acid, succinic acid, malic acid, and adipic acid solutions) instead of an acetic acid solution were used as solvents for chitosan dissolution. The amount of free amino groups of the chitosan in the solution decreased due to the ionic cross-linking of the dicarboxylic acids with chitosan. These solutions were used to fabricate porous chitosan membranes. Replacing acetic acid with these dicarboxylic acids for membrane preparation improved the water uptake (by 35% at most), tensile strength (by 110% at most), and elongation capability (by 50% at most) of the membranes. These dicarboxylic acid solutions not only act as solvents but also improve the material properties of the chitosan membranes due to the ionic cross-linking and hydrogen bond formation. In brief, a nontoxic and straightforward cross-linking method has been developed for chitosan material; this method does not result in a brittle product, thus making it better than the use of toxic cross-linking reagents.

KEYWORDS: Chitosan; material property; porous membrane; dicarboxylic acid; oxalic acid; succinic acid; malic acid; adipic acid

INTRODUCTION

Chitosan, derived from chitin through the deacetylation process (**Figure 1**), is an abundant polysaccharide in nature. The molecular structure of chitosan is linear and is assembled with glucosamine and *N*-acetylglucosamine through β-(1−4) glycosidic linkages (**Figure 1**) (*1*−*4*), with the number of glucosamine units usually more than 60% (*1*). Chitosan is normally dissolved in acidic solutions of pH <6, making the amino groups on the polysaccharide chain positively charged (*5*); they thus become polycationic polymers.

In previous research on chitosan, the most popular acid solutions utilized for chitosan dissolution were acetic acid and formic acid (*6*−*8*). They are both monocarboxylic acids (with only one carboxyl group) and serve only as a proton donor in solution. Besides monocarboxylic acids, there are many kinds of di-, tri-, and multicarboxylic acids with more than one carboxyl group, such as succinic acid, malic acid, and citric acid. They are found naturally and are widely utilized in the food and medical-related industries. Properties of chitosan solutions prepared with these acids were investigated in previous research (*9, 10*). Furthermore, with the participation of specific reagent, the cross-linking effects of some multicarboxylic acids toward chitosan (*11*) and starch (*12*) were also reported. Those studies indicated that interactions existed between chitosan and the "solvent" (acid) in the solution, and properties of the solution were greatly altered. These interactions result from the various molecular structures of the acids utilized and the various functional groups carried on the acids. Therefore, it is expected that unlike acetic acid with only one carboxyl group, di-, tri-, and multicarboxylic acids might provide protons to "dissolve" chitosan, and they also have the potential to "interact with" chitosan through the functional groups carried on the acid molecules, thereby changing the properties of the chitosan solutions. In other studies (*13, 14*), formic acid, acetic acid, lactic acid, and propionic acid were used for chitosan dissolution and subsequent dense-film preparation. Chitosan was dissolved by all of these acids and could be fabricated into dense films. The use of different acids to prepare chitosan dense films resulted in changes in the film properties such as mechanical strength and water vapor permeability. The major weakness of dense films, however, is their instability in water (i.e., they quickly redissolve in water), because they are not treated by basic reagents, such as a NaOH solution, to neutralize the residual acids; hence, dense films prepared in that research were

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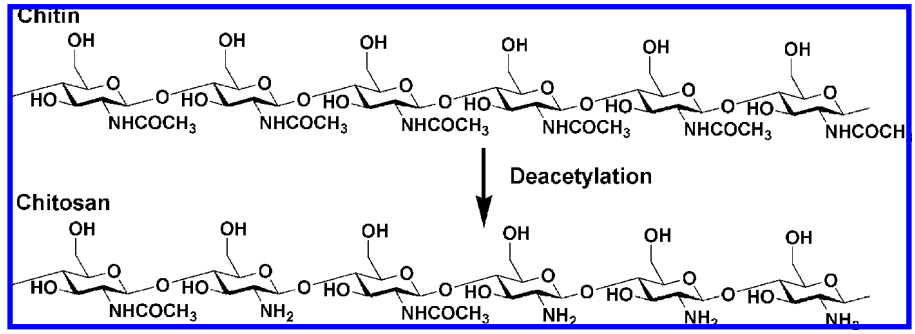

Figure 1. Structures of chitin and chitosan. Chitosan is constituted of two types of randomly distributed repeating units, glucosamine and *N*-acetylglucosamine, with the number of glucosamine units usually exceeding 60% (*1*).

of limited usefulness. In addition, the acids used in that research were limited to monocarboxylic acids. The potential advantage of using dicarboxylic acids forming ionic cross-linking has not yet been explored.

Membranes with well-distributed pores are important forms of materials for various applications (*15, 16*). For chitosan-related materials, there were several novel methods developed to fabricate porous membranes, such as CO_2-in-water (C/W) emulsions (*16*) and the freeze−gelation method (*17, 18*). The so-called freeze−gelation method is an ideal substitute for the traditional freeze-drying method because it is energy-saving. After freezing, the chitosan solutions become phase-separated into porous structures. The frozen chitosan membranes are then treated with an NaOH/ethanol solution, a nonsolvent for chitosan to fix the pore structure and exchange with the water in the pores. Chitosan membranes with a well-distributed and interconnected porous structure can be obtained by the freeze−gelation method (*17, 18*). However, in those studies, only an acetic acid solution was used for chitosan dissolution.

So far, the research mentioned above studied the "dissolution of chitosan" and "fabrication of porous membranes" separately, but unified and comprehensive research has not yet been carried out. In the present study, we combined (a) the use of other carboxylic acids for chitosan dissolution and (b) the method of freeze−gelation to fabricate porous membranes. We tried to use several kinds of carboxylic acids, especially dicarboxylic acids, to prepare solutions as solvents of chitosan and then to fabricate porous membranes, with the goal of improving the properties of the chitosan solutions and the porous membranes. The structures of carboxylic acids we used, including glycolic acid, oxalic acid, succinic acid, malic acid, adipic acid, and acetic acid (as a control), are shown in **Figure 2**. Most of these organic acids are natural and nontoxic (*19*). With the exception of acetic acid and glycolic acid, all of the other acids utilized were dicarboxylic acids with carbon backbones of different lengths and different functional groups for comparison. We successfully prepared homogeneous chitosan solutions with different kinds of carboxylic acids and fabricated porous membranes with these solutions. It was observed that the membrane properties were greatly improved by replacing acetic acid by other carboxylic acids, especially dicarboxylic acids. In addition, we demonstrated a straightforward cross-linking method for chitosan material that does not make it brittle; thus, it is better than the use of a traditional cross-linking reagent such as glutaraldehyde (*20, 21*).

MATERIALS AND METHODS

Materials. Chitosan [with a molecular weight of 3.1×10^5 and a degree of deacetylation (DDA) of about 90%] was purchased from Kiotek (Taipei, Taiwan). All of the carboxylic acids utilized, including

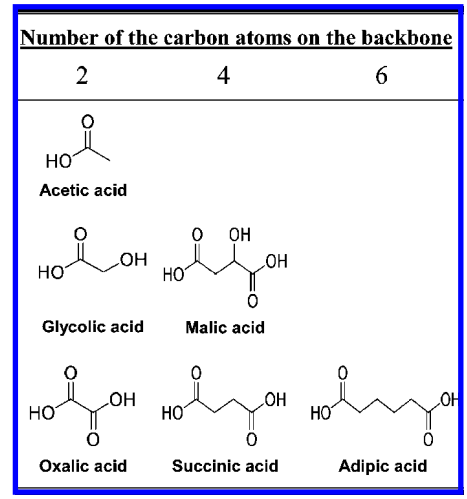

Figure 2. Structures of the carboxylic acids utilized in this study.

acetic acid, glycolic acid, oxalic acid, succinic acid, malic acid, and adipic acid, were purchased from Sigma-Aldrich (St. Louis, MO). All chemicals used in this study were of reagent grade.

Preparation and Characterization of Chitosan Solutions with Different Solvents. We tried to use the aforementioned carboxylic acid solutions as solvents for dissolving chitosan. First, chitosan powder, not a solution, was added to water with strong stirring to ensure a uniform distribution. The amounts of chitosan added to the water were 0.5, 1, 2, and 3 wt %, respectively. The various carboxylic acids (listed in **Figure 2**) were then added separately to dissolve the chitosan powder, which was uniformly distributed in the solution. The pH values of the solutions were measured with a pH-meter (model 420A, Orion Research Inc., Boston, MA).

The carboxylic acids utilized in this study, especially the dicarboxylic acids, might provide protons for chitosan dissolution as well as interact ionically with the positively charged amino groups on the chitosan. We thus tried to measure the amount of free amino groups remaining in the solution to determine the extent of the carboxylic acid−chitosan interaction. The method we utilized was described in previous research (*22*). First, 0.5 wt % chitosan solutions containing various carboxylic acids were prepared. Second, two drops of a 0.1% toluidine solution were added to the solution. After adequate mixing, each solution was titrated with an N/400 potassium poly(vinyl sulfate) solution (PVSK). Then the DDA (i.e., the percent of free amino groups remaining) was calculated from the formula (*22*)

$$\mathrm{DDA}\ (\%) = \frac{x/161}{x/161 + y/203} \times 100 \qquad (1)$$

where x is the weight of glucosamine ($= 1/400 \times 1/1000 \times f \times 161 \times v$), y is the weight of *N*-acetylglucosamine ($= 0.5 \times 1/100 - x$), v is the volume of the N/400 PVSK solution used in the titration (mL), f is the factor of the N/400 PVSK solution ($= 1.00$ at 20 °C); 161 is the

Table 1. Abbreviations and Compositions of the Various Samples

abbreviation	composition	
	chitosan	acid
C3Ace	3 wt %	acetic acid, 0.2 M
C3Gly	3 wt %	glycolic acid, 0.2 M
C3Oxa	3 wt %	oxalic acid, 0.2 M
C3Suc	3 wt %	succinic acid, 0.2 M
C3Mal	3 wt %	malic acid, 0.2 M
C3Adi	3 wt %	adipic acid, 0.2 M

molecular weight of the glucosamine unit of chitosan; and 203 is the molecular weight of the *N*-acetylglucosamine unit of chitosan.

Preparation and Characterization of Chitosan Porous Membranes Using Various Carboxylic Acids. The chitosan solutions prepared using various carboxylic acids were used to fabricate porous membranes by the freeze−gelation method (*17, 18*). Their symbols are shown in **Table 1**. Each solution was centrifuged for 15 min at 3500*g*. After centrifugation, the chitosan solution was poured into dishes and frozen for 12 h with the temperature maintained at −80 °C. The frozen samples were immersed in a NaOH/ethanol solution at −20 °C for 12 h, followed by rinsing with ethanol. The membranes were then washed using a phosphate-buffered saline (PBS) solution. To investigate the microstructure, the prepared porous membranes were observed by scanning electron microscopy (SEM).

Estimation of the Porosity and Water Uptake Capability of the Porous Membranes. To estimate the percentage of the volume occupied by pores in the various chitosan membranes, the volumes of wet membranes (V_{wet}, including the volume of the material and the volume occupied by the pores) were measured, and then the membranes were dried in an oven for 24 h, followed by placement in a vacuum for 24 h. The dry weights of the membranes (W_{dry}) were then measured. The dry weights were divided by the density of chitosan (1.342 g/cm^3) (*23, 24*) to obtain the estimated volumes of the dried membranes (V_{dry}, excluding the volume occupied by the pores). The porosity of the membranes was calculated using the following formula (*23*):

$$\text{porosity } (\%) = \frac{V_{wet} - V_{dry}}{V_{wet}} \times 100 = \frac{V_{wet} - W_{dry}/\rho}{V_{wet}} \times 100 \quad (2)$$

To measure the amounts of water absorbed by the various porous membranes, the samples were cut into small pieces and placed in distilled water for 6 h, and the dry (W_{dry}) and wet (W_{wet}) weights of the membranes were measured. The water uptake by the membranes was then calculated using the following formula (*25*):

$$\text{water uptake } (\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad (3)$$

Analysis of the Mechanical Properties of the Membranes. The mechanical properties of the porous membranes were determined with a tensile strength instrument (model LRX, Lloyd, Hampshire, U.K.). Before the test, the prepared porous membranes were cut into a specific dog-bone shape (6 cm long, 2 cm wide at the ends, and 1 cm wide in the middle) and allowed to absorb PBS for 2 h. The thickness of each individual membrane was measured. The mechanical analysis was performed at a stretching rate of 10 mm/min with a preload of 0.5 N to determine the maximum load and the strain at maximum load for each membrane. The measured maximum load of the membrane was then normalized by the dry weight of the membrane. The strain at maximum load was expressed as the elongated length divided by the original length of the membranes.

RESULTS AND DISCUSSION

Preparation and Characterization of the Chitosan Solutions Prepared Using Various Carboxylic Acid Solutions as Solvents. The various carboxylic acid solutions were used to replace the acetic acid solution as solvents for chitosan dissolution. As shown in **Figure 3**, all of these acid solutions could dissolve 3 wt % chitosan. We also prepared solutions containing different amounts of chitosan (0, 0.5, 1, 2, and 3 wt %). It is shown in **Figure 3** that as the concentration of chitosan increased, the pH value of the solution substantially increased, demonstrating that chitosan may serve as a base that can capture protons released by the acid. This neutralization-like behavior may be an important factor making chitosan soluble in the solution because its amino groups bind with protons and thus are positively charged. Meanwhile, from the viewpoint of the acid in the solution, neutralization of the acid by chitosan may also increase its solubility. This proposition is supported by the result shown in **Figure 3**. We found that adipic acid itself (0.2 M adipic acid solution, 0 wt % chitosan) was unable to fully dissolve in water. However, once chitosan was added to the solution (from 0.5 to 3 wt %), both the chitosan itself and all of the adipic acid added were able to fully dissolve in the solution. As mentioned above, it is traditionally pointed out that chitosan (positively charged) is dissolved by the protons released from the acid, but the effect of chitosan of increasing the solubility of the acid in the water has not previously been reported. Therefore, we can say that the chitosan does not just "dissolve" by the acid in the water but also assists the acid in "dissolving" in the water, making chitosan a "polymeric base". In other words, the relationship between chitosan and the acid in the solution is "to help each other dissolve". This proposed concept may clarify the interactions between chitosan and the various acids.

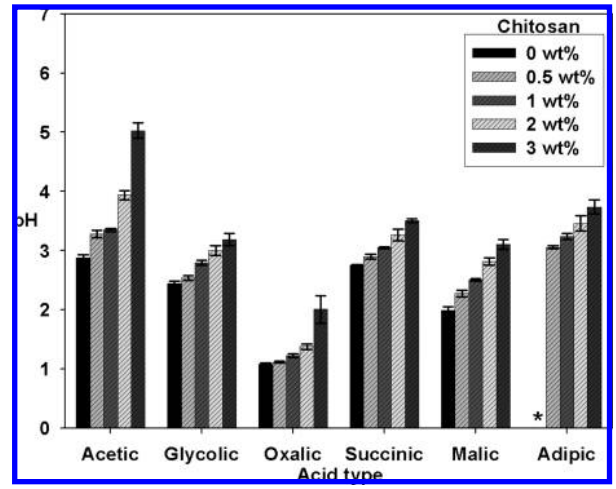

Figure 3. pH values of the various types of acid solutions (0.2 M) containing different amounts of chitosan ($n = 3$, mean ± SEM). The pH values increased with increasing amount of chitosan dissolved. *, adipic acid was unable to be fully dissolved without the addition of chitosan.

In the dissolution process of chitosan and carboxylic acid in water, it is expected that the carboxyl groups of the carboxylic acids, especially dicarboxylic acids, can ionically interact with the positively charged amino groups on the chitosan, thus masking and reducing the amount of the free amino groups (i.e., lowering the DDA of the chitosan). Therefore, the final DDA in the solution can be an index for the extent of interaction between chitosan and the carboxylic acids. From the results shown in **Table 2**, it was found that if monocarboxylic acids (acetic acid and glycolic acid) were used for chitosan dissolution, the DDA values of the solutions were both about 90%, roughly equal to the DDA of the raw chitosan powder (of about 90%). However, when dicarboxylic acids (oxalic, succinic, malic, and adipic acids) were used for chitosan dissolution, the DDA values of the solution obviously decreased, meaning that the numbers of free amino groups remaining in the solutions were fewer than

Table 2. Degree of Deacetylation (DDA) as an Index of Free Amino Groups Present on the Chitosan in Various Solutions ($n = 3$, Means ± SEM)

sample	DDA (%)
C3Ace	91.71 ± 1.55
C3Gly	89.66 ± 0.42
C3Oxa	81.15 ± 0.14
C3Suc	80.28 ± 0.52
C3Mal	78.08 ± 1.02
C3Adi	63.97 ± 0.86

Table 3. Porosity of the Various Carboxylic Acid-Prepared Chitosan Membranes ($n = 6$, Means ± SEM)

sample	porosity (%)
C3Ace	94.29 ± 0.02
C3Gly	94.92 ± 0.02
C3Oxa	94.90 ± 0.02
C3Suc	95.22 ± 0.02
C3Mal	95.38 ± 0.01
C3Adi	95.66 ± 0.01

those in the solutions prepared using monocarboxylic acids. This finding suggests that dicarboxylic acids utilized for chitosan dissolution may provide protons to dissolve the chitosan and also can serve as ionic cross-linking reagents (*9*), generating ionic interactions between amino groups and the two carboxylic groups of the acid molecules as shown in **Figure 8c**. In addition, comparing the chitosan solutions (C3Oxa, C3Suc, and C3Adi) prepared with different dicarboxylic acids, we found that the longer the carbon backbone of the dicarboxylic acid was, the lower the DDA value of the chitosan solution was (i.e., with fewer free amino groups). For the C3Adi solution, the value of DDA was 63%, the lowest among all of the chitosan solutions. On the basis of this result, we propose that a longer carbon backbone makes it easier for the two carboxylic groups of an acid molecule to contact the amino groups on separate chitosan chains, thus increasing the extent of ionic cross-linking. Therefore, with the longest carbon backbone among the acids utilized, adipic acid should have had the strongest ionic cross-linking effect toward chitosan. From these results, not only can we say that these acids serve as solvents for chitosan, but their use also has additional benefits, such as being a cross-linking reagent for chitosan.

Observations of Chitosan Porous Membranes Prepared with Various Carboxylic Acid Solutions. The freeze−gelation method was used to fabricate chitosan porous membranes. As shown in **Figure 4**, the C3Ace membrane and porous membranes produced from other kinds of carboxylic acid solutions, including C3Gly, C3Oxa, C3Suc, C3Mal, and C3Adi membranes, were successfully obtained. The pore structures of the membranes were well-distributed and interconnected, as indicated on the SEM micrographs. Among all of the membranes fabricated, the pore distribution of the C3Oxa membrane was less uniform than the other acid-prepared ones. The poor uniformity of the C3Oxa membrane may come from the unfavorable dissolubility of oxalic acid toward chitosan, because we found in our experiment that it took a longer time for the oxalic acid solution to dissolve the chitosan. This fact may result in the poor uniformity of the membrane due to the poor compatibility of chitosan with oxalic acid, even though the oxalic acid could eventually dissolve all of the chitosan added. The shapes of the pores in various membranes were slightly different. The freezing situations could be different in the various carboxylic acid-prepared solutions (due to different melting points and other properties), thus resulting in the different pore shapes. It was obvious that most of the volume of the membranes was taken up by the interconnecting pore space. The high porosities of the various membranes demonstrate that they are suitable for a variety of applications, including serving as absorption sponges and matrices for cell proliferation.

To further estimate the percentage of volume occupied by the pore space, membranes were dried to remove the water absorbed by the membrane. There are two mechanisms by which chitosan porous membranes "absorb" water: binding of water to the material itself and retaining water in the pore space (**Figure 5**). After drying in the vacuum oven, most of the water absorbed by the membranes was removed, and the dry weight of each membrane (i.e., the weight of the material itself) could be measured to determine the membrane porosity. From the results shown in **Table 3**, it was found that the porosities of the membranes prepared with various carboxylic acids were about 95%, meaning that about 95% of the volume of the membrane was pore space. This estimation again supports the proposition that membranes fabricated by the freeze−gelation method are quite porous, consistent with the SEM micrographs in **Figure 4**. Furthermore, replacing the solvent for chitosan dissolution did not affect the porosity of the membranes.

Water Uptake Capability of Porous Membranes. Biomaterials are usually used in aqueous milieus, so it is important to consider the water uptake capability in its application. From the results shown in **Figure 6**, it was found that compared with the C3Ace membranes, all other membranes, including the C3Gly, C3Oxa, C3Suc, C3Mal, and C3Adi membranes, could absorb more water. The water uptake increased from about 1500% for the C3Ace membrane to 1800−2000% for the other membranes. As mentioned above (observation of porous membranes), water is absorbed into the membranes by two processes: water binding to the material itself and water being retained in the pore space (**Figure 5**). Because the porosity was roughly the same for all of the membranes (**Table 3**), the effect of water being retained in the pore space (**Figure 5b**) might not result in a higher water uptake. Therefore, we propose that the improvement in the water uptake capability mainly resulted from the effect of the water bound to the material itself (**Figure 5a**). First, by comparison of acetic acid with glycolic acid, the latter has one more hydroxyl group that strengthens the water-binding capability of the chitosan material, so that C3Gly membranes can absorb more water than C3Ace membranes (**Figure 6**). Second, unlike monocarboxylic acid-prepared membranes, larger structures formed in the dicarboxylic acid-prepared chitosan membranes (C3Oxa, C3Suc, C3Mal, and C3Adi) by the ionic cross-linking effect mentioned above (preparation of the solutions) and shown in **Figure 8c**. The two carboxyl groups carried on one acid molecule ionically interact with the amino groups on chitosan, producing larger structures, which provide more space for water binding to the material itself, thus increasing water uptake. In addition, the carboxyl group itself is also a hydrophilic functional group, which can enhance the water-binding capability of chitosan membranes as does the hydroxyl group. As a result, the use of dicarboxylic acids instead of a monocarboxylic acid obviously improved the water uptake capability of the chitosan porous membranes.

Mechanical Properties of the Membranes. The influence of using various carboxylic acids as solvents on the mechanical properties of the membranes prepared according to the freeze−gelation method was examined using a tensile strength instrument. The tensile strength (the maximum load divided by the membrane dry weight) and elongation capability (the strain

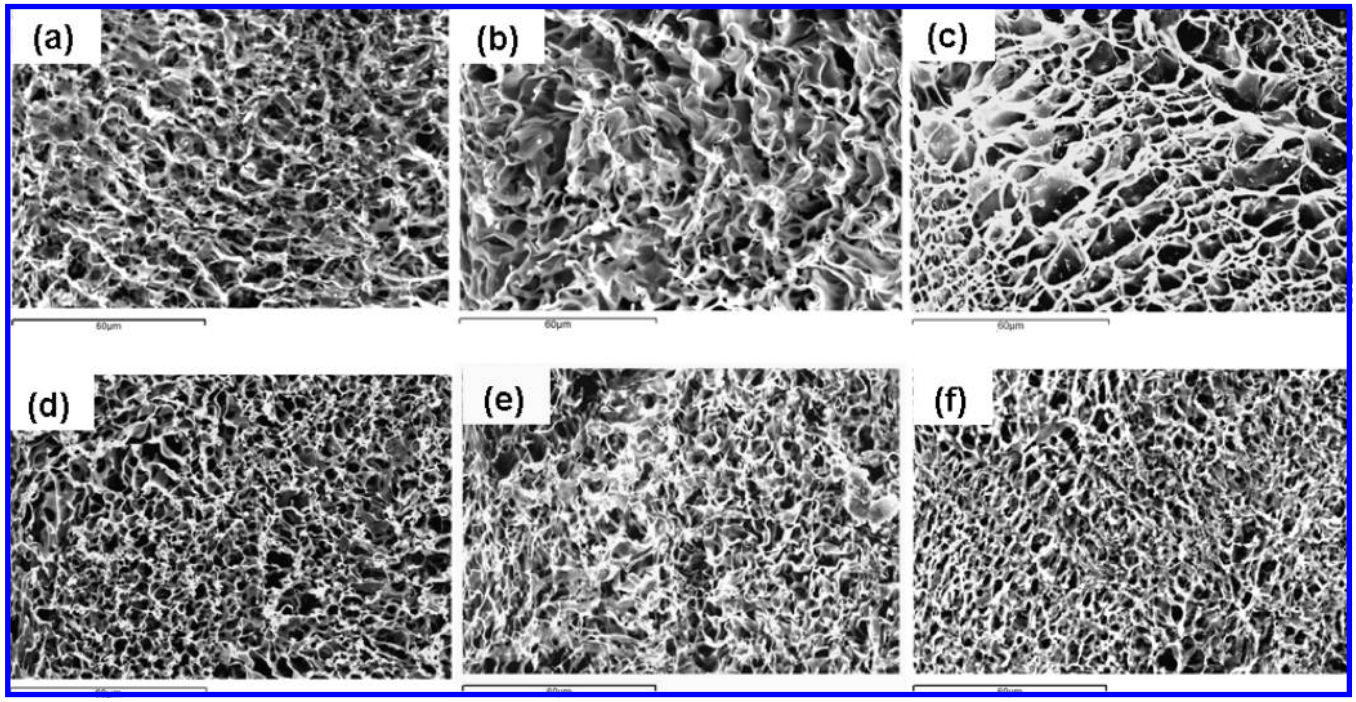

Figure 4. SEM micrographs of porous chitosan membranes prepared with different carboxylic acids: (**a**) C3Ace; (**b**) C3Gly; (**c**) C3Oxa; (**d**) C3Suc; (**e**) C3Mal; (**f**) C3Adi membranes. (Bar = 60 μm.)

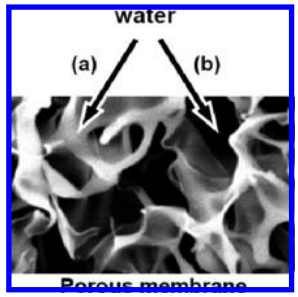

Figure 5. Schematic representation of water absorbed into the chitosan porous membrane. Water can be absorbed into the porous chitosan membrane in two ways: (**a**) water bound to the material itself and (**b**) water retained in the pore space.

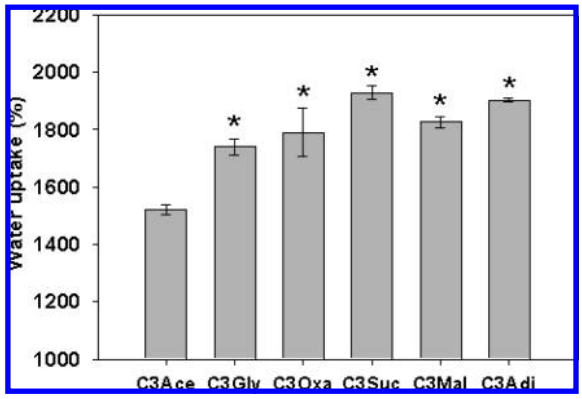

Figure 6. Water uptake of various carboxylic acid-prepared chitosan membranes. All of the membranes including C3Gly, C3Oxa, C3Suc, C3Mal, and C3Adi membranes absorbed more water than the C3Ace membranes. (n = 6, mean ± SEM; ∗, p < 0.05, vs C3Ace.)

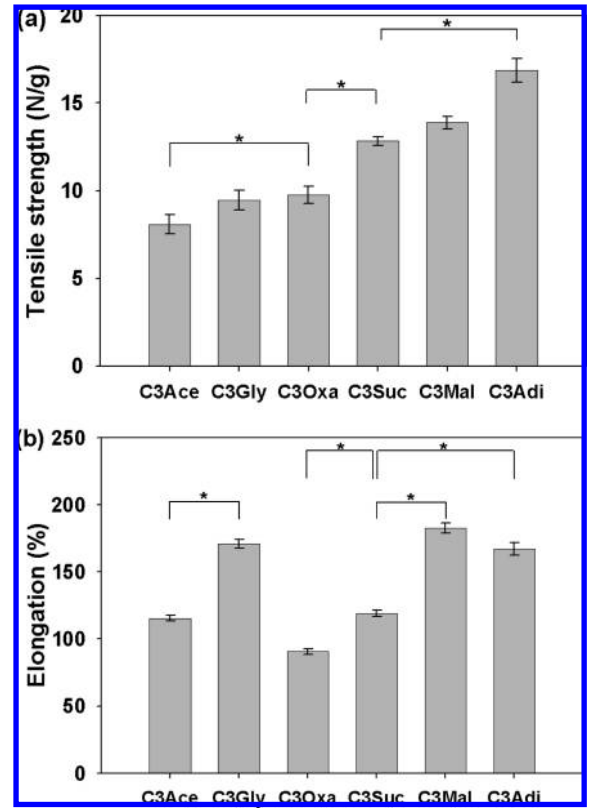

Figure 7. Mechanical properties of the various carboxylic acid-prepared chitosan membranes: (**a**) maximum tensile strength (maximum load divided by membrane dry weight); (**b**) maximum elongation (strain at maximum load). (n = 6, mean ± SEM; ∗, p, < 0.05.)

at maximum load) of the membranes are shown in **Figure 7**. Because the membranes were porous, the use of the maximum load divided by the cross-sectional area (i.e., the stress at maximum load) might not be a suitable way to express the tensile strength of the membranes. Meanwhile, the thicknesses of all the membranes fabricated with different carboxylic acids were almost the same (0.2 cm), implying that the effect of the cross-sectional areas can be neglected. Therefore, a normalization method was utilized in this study: dividing the maximum load (N) of each membrane by its dry weight (g), not by the cross-sectional area, to reflect the tensile strength of each unit

amount of material, as shown in **Figure 7a**. First, comparing the C3Ace membrane (monocarboxylic acid) with C3Oxa, C3Suc, C3Mal, and C3Adi membranes (dicarboxylic acids) in **Figure 7a**, we found that all of the dicarboxylic acid-fabricated membranes had tensile strengths greater than that of the monocarboxylic acid-fabricated (C3Ace) membrane. The tensile strength of the C3Ace membrane was about 8.0 N/g. From the previous discussion, because there are two carboxyl groups carried on one dicarboxylic acid, they can interact with amino groups carried on the chitosan chains, ionically cross-linking chitosan molecules (**Figure 8c**). However, having only one carboxyl group, acetic acid lacks such a capability. This ionic cross-linking effect of dicarboxylic acids improves the tensile strength of the chitosan membrane. Therefore, in addition to being a solvent, the dicarboxylic acid also acts as a "cross-linking reagent" for chitosan, like the ionic cross-linking effect of tripolyphosphate (TPP) toward chitosan (*26*−*28*) or citric acid toward chitosan (*29*). Therefore, we concluded that from the viewpoint of fabricating stronger chitosan materials, dicarboxylic acids, especially adipic acid, are more suitable than monocarboxylic acids. Second, by comparison of the C3Oxa, C3Suc, and C3Adi membranes (all created with dicarboxylic acids), it can be seen that as the number of carbon atoms (*n*) of an acid increases, the tensile strength obviously increased from about 9.5 N/g for the C3Oxa membrane to about 12.8 N/g for the C3Suc membrane and further to about 16.8 N/g for the C3Adi membrane. According to this trend, we propose that the longer the carbon backbone of the dicarboxylic acid is, the higher is the possibility for the carboxyl group to approach and ionically interact with amino groups of chitosan (i.e., it is easier for ionic cross-linking to occur). In other words, the spatial hindrance is reduced as the backbone of the dicarboxylic acid becomes longer. In addition, the larger molecular structure in the C3Adi membrane, as a result of the longer carbon backbone of adipic acid than those of oxalic and succinic acids, may also be a factor in improving the tensile strength of the chitosan membrane. Back to the aforementioned discussion about chitosan dissolution and pH values of the solutions, adipic acid was found to be capable of dissolving chitosan, and adipic acid's solubility in water was enhanced by the addition of chitosan (i.e., in water, chitosan and adipic acid helped each other dissolve). Combined with the result of the tensile strength measurement, we propose that under the experimental conditions utilized in this study (3 wt % chitosan dissolved in a 0.2 M acid solution), chitosan and adipic acid have stronger interactions in the solution and fabricated membranes, thus generating higher mechanical strengths. Therefore, in this study, the adipic acid solution was a better choice of solvent for fabricating chitosan membranes with enhanced tensile strength (with about a 110% increase in strength).

Another important material property is the elongation capability. Values of elongation (strain at maximum load) of the various carboxylic acid-prepared chitosan membranes are shown in **Figure 7b**. A higher elongation capability means that the membrane can be elongated more than those with lower capabilities; thus, the membranes are not as easily broken. First, comparing the C3Ace and C3Gly membranes, we found that if the acid carried a hydroxyl group (glycolic acid), the fabricated membranes had greater maximum elongation, from about 115% for the C3Ace membrane to about 170% for the C3Gly membrane. What causes this improvement in the elongation capability may be the existence of the hydroxyl group carried on glycolic acid and the hydrogen bond formed between the chitosan and the hydroxyl groups (*25*) (**Figure 8b**). Previous

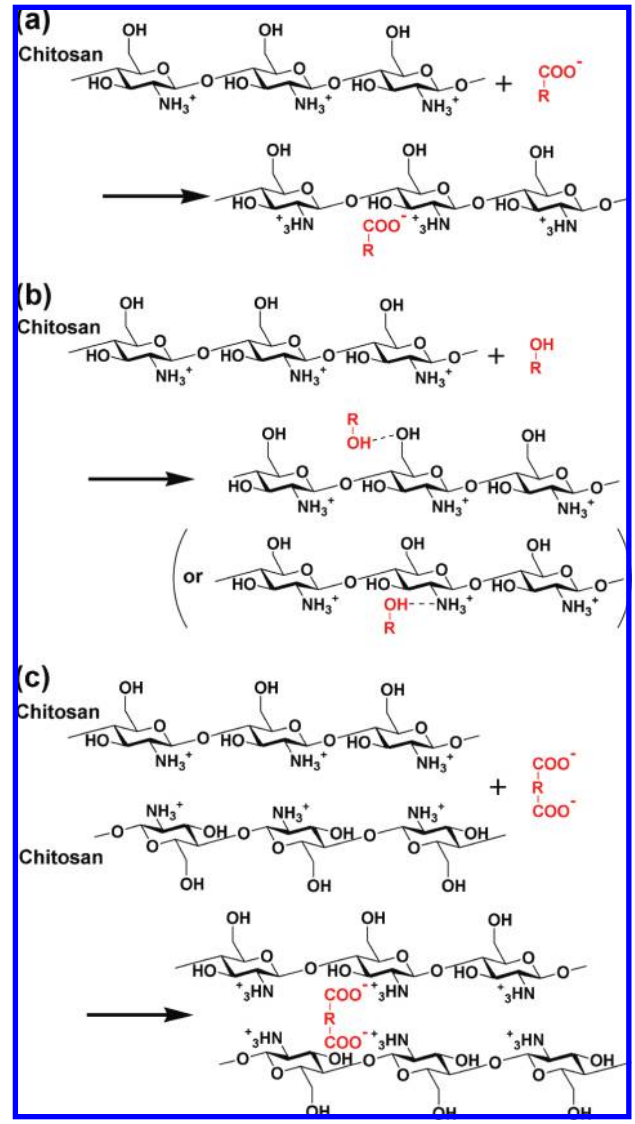

Figure 8. Proposed interactions between carboxylic acids (in red) and chitosan: (**a**) ionic interaction; (**b**) hydrogen bond formation; (**c**) ionic cross-linking.

research (*30*) demonstrated that the hydrogen bond is a more mobile bonding type. Therefore, the molecular arrangement in the C3Gly membrane is more movable than that in the C3Ace membrane, leading to the higher elongation capability. Such an improved effect also appeared in the pairwise comparison of the C3Suc and C3Mal membranes (with an additional hydroxyl group carried on malic acid), increasing the elongation capability from 120% for the C3Suc membrane to 182% for the C3Mal membrane. From the two-pair comparisons and the analogy, we concluded that carrying a hydroxyl group on the acid improves the elongation capability of chitosan membranes. This result and concept mean it is feasible to develop chitosan materials with improved elongation capability. Second, the C3Oxa, C3Suc, and C3Adi membranes were compared to identify the effect of the carbon number of the acid on the elongation capability of the chitosan membranes (**Figure 7b**). The results indicate that improvements in the elongation of the C3Oxa, C3Suc, and C3Adi membranes were about 91, 118, and 167%, respectively. Just like the trend presented in **Figure 7a**, as the carbon backbone of these acids increases in length, the elongation capabilities of the chitosan membranes are obviously enhanced. From the aforementioned proposition of dicarboxylic acid as an ionic cross-linking reagent, we inferred that dicar-

boxylic acids may react with different amino groups on chitosan via the two carboxyl groups, forming a large "cross-linked structure". As the carbon number of the dicarboxylic acid increases (i.e., as the acid molecule increases in size), the cross-linked structure forming the membranes also becomes larger. The larger structure may enhance the tensile strength of the membranes and improve the elongation capability of the membrane. It is well-known that traditional cross-linking reagents such as glutaraldehyde usually improve the mechanical strength but may not increase or may even decrease the elongation capability of a material (i.e., the materials become brittle) (*20*). However, in this study, a feasible method has been successfully developed to simultaneously improve both the tensile strength and the elongation capability of the membrane (such as the C3Adi membrane) without using any traditional cross-linking reagents. Therefore, the use of dicarboxylic acid solutions as solvents for chitosan dissolution is an effective and nontoxic choice for developing chitosan-based materials for various applications.

On the basis of our results, it can be proposed that the fundamental interaction of all carboxylic acids toward chitosan is an ionic interaction (**Figure 8a**). With the exception of acetic acid, two additional effects including hydrogen bond formation (**Figure 8b**) and ionic cross-linking (**Figure 8c**) also occur with the use of other carboxylic acids. For glycolic acid, the major effect is hydrogen bond formation with chitosan in the porous membranes, increasing the water uptake capability and the elongation capability of the membranes. For all of the dicarboxylic acids, the major effect is ionic cross-linking, which improves the mechanical strength and water uptake capability of the membranes without decreasing the elongation capability, thus making the product better than the glutaraldehyde cross-linked chitosan materials. This study clearly indicates that the use of these carboxylic acids is a straightforward and effective method to improve certain properties of chitosan materials. In addition, after treatment with these carboxylic acids, the characteristics of the chitosan solutions and porous membranes can be further diversified, thus matching the requirements needed for various applications.

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