

Improvement in the Properties of Chitosan Membranes Using Natural Organic Acid Solutions as Solvents for Chitosan Dissolution

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Abstract

Chitosan-based biomaterials are commonly fabricated using an acetic acid solution as the solvent for chitosan dissolution, but this acid has a strong, unpleasant smell. For this study, other types of natural organic acid solutions, including glycolic acid, citric acid, tartaric acid, malic acid, and ascorbic acid solutions, were used for chitosan dissolution. We found that glycolic acid, malic acid, and ascorbic acid solutions effectively dissolved chitosan similar to acetic acid solution, and the pH value of the solution went up as the concentration of chitosan increased. The viscosity of the chitosan solution was the greatest if glycolic acid solution was used for chitosan dissolution. Further, porous chitosan membranes were fabricated using the freeze-gelation method. The use of ascorbic acid solution as the solvent for chitosan enhanced the tensile strength (by about 100%) of the membranes, as compared with those prepared in the acetic acid solution. The use of glycolic acid solution as the solvent for chitosan greatly increased the water uptake (by about 100%) of the membranes. We concluded that the use of these organic acid solutions for chitosan dissolution produces chitosan membranes with improved mechanical properties and enhanced hydrophilicity for various biomedical applications.

Keywords: Chitosan, Glycolic acid, Malic acid, Ascorbic acid, Solvent

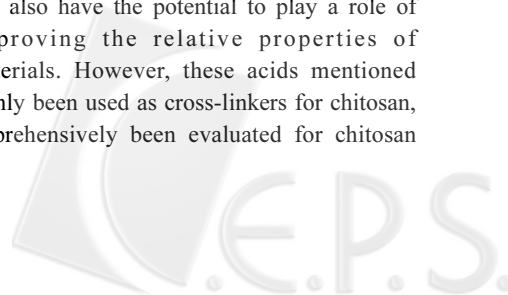
Introduction

Chitosan is a functional linear polysaccharide which can be produced from N-deacetylation of chitin (Figure 1). Chitosan is a biopolymer composed of glucosamine and N-acetyl-glucosamine units connected by β -1,4-glycosidic linkages (Figure 1) [1]. Because of the amino groups on the polysaccharide chain, chitosan can be positively charged and solubilized when the solution pH is below 6, thus becoming a polycationic polymer. Chitosan is degraded into nontoxic products *in vivo* [2], and therefore it has been widely used for various biomedical applications [3-10].

In previous research on chitosan, the most popular solvents for chitosan dissolution have been acetic acid and formic acid solutions. They are both monoacids (with only one carboxylic group), and only serve as proton donors in solution. In addition to acetic acids, there are many kinds of natural carboxylic acid, such as glycolic acid, tartaric acid, malic acid,

and citric acid (Figure 2). Among these organic acids, glycolic acid is of almost the same molecular structure; the only difference is the hydroxyl group, and other acids are binary or tertiary acid. All these acids are widely used in the food and medical industries. Some binary and tertiary acids can react with two or more amino groups on separate chitosan chains, causing cross-linking of the chitosan into a large structure [11]. In addition to the carboxylic acids mentioned above, ascorbic acid (Figure 2) is also an essential acid for human health, and it is found in many foods, especially fruits. The original structure of ascorbic acid has no carboxylic acid, but by changing the position of its long pair of electrons, a proton is released to make the solution acidic (Figure 3) [12]. Ascorbic acid is an antioxidant (i.e., it is easily oxidized) (Figure 3) [12-13]. So we thought that, unlike acetic acid solution, these organic acid solutions might not only provide protons to dissolve the chitosan, but would also have the potential to play a role of cross-linker, improving the relative properties of chitosan-based materials. However, these acids mentioned above have so far only been used as cross-linkers for chitosan, and have not comprehensively been evaluated for chitosan

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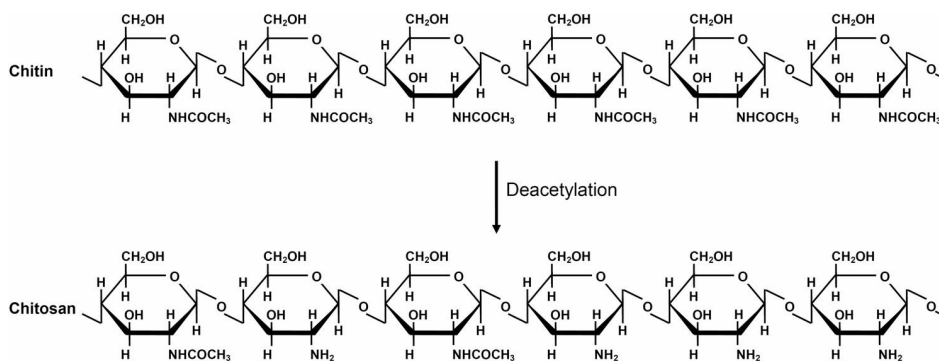


Figure 1. Structure of chitosan. Chitosan is constituted of two types of repeat units: glucosamine and N-acetyl-glucosamine.

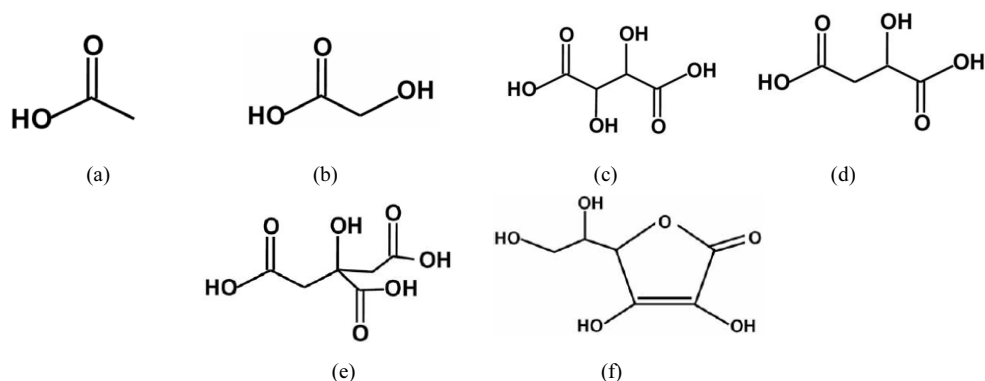


Figure 2. Structures of the organic acids used in this study. (a) Acetic acid, (b) glycolic acid, (c) tartaric acid, (d) malic acid, (e) citric acid, and (f) ascorbic acid.

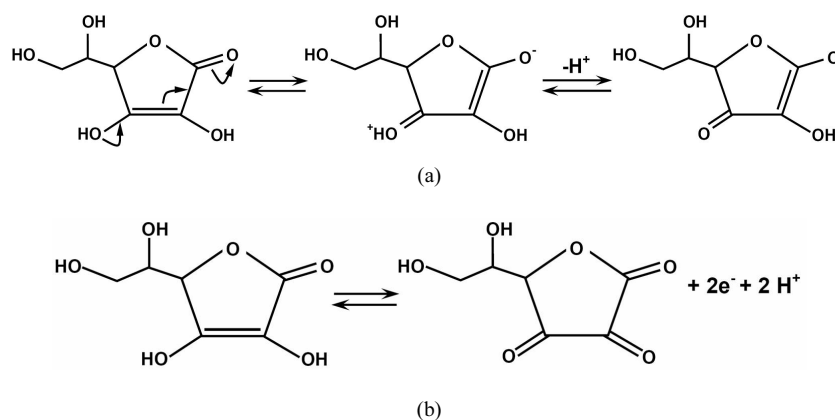


Figure 3. Acidification and oxidation of ascorbic acid. (a) Acidification: Bonding pair electrons migrate from the hydroxyl group through the ring to the carbonyl group, triggering the release of a proton from the hydroxyl group, acidifying the solution. (b) Oxidation: The two hydroxyl groups are converted to carbonyl groups, releasing two electrons.

dissolution. Therefore, additional acids like acetic acid are still added for chitosan dissolution.

In the present study, we tested the feasibility of using the acid solutions discussed above as solvents for chitosan dissolution and subsequent porous membrane preparation. We successfully prepared homogeneous, highly viscous chitosan solutions with some of these organic acid solutions and produced porous membranes with these solutions. To analyze and compare the effect of different acids on chitosan, we measured the pH value and viscosity of the solutions. The influence of using different acid solutions as solvents on the water uptake, porosity, and mechanical properties of the membranes was also investigated. We found that the most

commonly used solvent, acetic acid solution, could be replaced by some of these natural organic acid solutions for chitosan dissolution to prepare chitosan membranes with enhanced properties for various applications.

Materials and Methods

Materials

Chitosan (with a MW of about 3.1×10^5 and a degree of deacetylation of about 90%) was purchased from Kiotek Co. (Taipei, Taiwan). Other chemicals of reagent grade were used throughout this study.

Preparation of chitosan solutions with different solvents

Table 1. Symbols and compositions of various chitosan solutions.

Symbol	Composition		Acid conc.
	Chitosan	Acid	
C3Cit	3 wt%	Citric acid	0.2 M
C3Tar	3 wt%	Tartaric acid	0.2 M
C3Mal	3 wt%	Malic acid	0.2 M
C3Gly	3 wt%	Glycolic acid	0.2 M
C3Asc	3 wt%	Ascorbic acid	0.2 M
C3Ace	3 wt%	Acetic acid	0.2 M

Tartaric, malic, citric, glycolic, and ascorbic acid solutions were used as solvents for dissolving chitosan. First, chitosan powder was added with strong stirring to ensure that the powder was uniformly distributed in the solution. Then the various acids were individually added to the solution to dissolve the chitosan powder in the solution. The symbols and compositions of the various chitosan solutions are shown in Table 1. After preparation of these chitosan solutions, their pH values were measured with a pH meter (model 420A, Orion, USA).

Measurement of the viscosity of the solutions

In order to understand the interactions between chitosan and the different acids, a viscometer (model DV-II+, Brookfield, USA) was used to measure the viscosity of each solution at 22 °C with the spindle rotating at 0.5 rpm.

Preparation of porous membranes

The freeze-gelation method was used to prepare porous chitosan membranes [14-15]. Briefly, each chitosan 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 in phosphate buffered saline (PBS).

Measurement of the porosity of porous membranes

In order to measure the volume percentage occupied by pores in the chitosan membrane, the volume of a wet membrane (V_{wet}) was measured, and then the membrane was dried for 6 h. The weight of the dried membrane was measured, and divided by the density of chitosan (1.342 g/cm³) to obtain its volume (V_{dry}). The porosity of the membrane was calculated using the following formula:

$$\text{porosity (\%)} = [(V_{wet} - V_{dry}) / V_{wet}] \times 100\%.$$

Water uptake by the porous membranes

To measure the water uptake by the porous membranes, the weight of a wet membrane (W_{wet}) was measured. Then the membrane was dried in an oven for 24 h. The dry weight of the membrane (W_{dry}) was immediately measured. The water uptake by the membrane was calculated using the following formula:

$$\text{Water uptake (\%)} = [(W_{wet} - W_{dry}) / W_{dry}] \times 100\%.$$

Analysis of the mechanical properties

The mechanical properties of the porous chitosan membranes were determined in a tensile strength instrument (model LRX, Lloyd, Hampshire, UK). Before the test, the

Table 2. Solubility of chitosan in various acid solutions (acid conc. = 0.2 M)

	Chitosan concentration			
	0.5 wt%	1 wt%	2 wt%	3 wt%
Citric acid	0	*	*	*
Tartaric acid	0	*	*	*
Malic acid	0	0	0	0
Ascorbic acid	0	0	0	0
Glycolic acid	0	0	0	0
Acetic acid	0	0	0	0

0: fully dissolved

*: partially dissolved

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 soaked in 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 pre-load of 0.5 N to determine the maximum load (tensile strength) of each membrane.

Results and Discussion

Preparation of chitosan solutions with different solvents

First we tested the feasibility of using the organic acid solutions listed above as solvents for chitosan dissolution. From the results shown in Table 2, we found that citric acid and tartaric acid solutions were unable to dissolve chitosan with a concentration above 1 wt%, and thus they were unsuitable for the subsequent study. Comparing the molecular structure of citric acid with malic acid, there is only one difference between them: the branched acetic acid chain on citric acid. Although citric acid has one more carboxyl group, it cannot dissolve more chitosan than malic acid. So we proposed that there is no consequential improvement in chitosan solubility by increasing the number of carboxyl groups.

Another important factor that may affect chitosan dissolution is the pH value of the solvent (acid solution). In order to investigate the influence of the pH value, we measured the pH values of the solutions containing different amounts of chitosan (0, 0.5, 1, 2, and 3 wt%). From the results shown in Figure 4, we found for the first time that as the concentration of chitosan increases, the pH values of the solutions obviously increase, demonstrating that in solution, chitosan may play a role as a "basic" reagent and neutralize the proton released by the acid. This neutralization also causes the chitosan to dissolve in the aqueous phase because the amino groups of chitosan are positively charged by protons. However, the pH value is not the only determinant of chitosan dissolution. In Figure 4, we illustrate that citric and tartaric acid solutions had the relatively lowest pH values among the acids used, but these two solutions could not readily dissolve chitosan, indicating that the pH value is not the only dominant factor in the dissolution of chitosan. In contrast, ascorbic acid solution is a good solvent for chitosan dissolution, even though it has a relatively higher pH value. From these results, we found that a lower pH does not

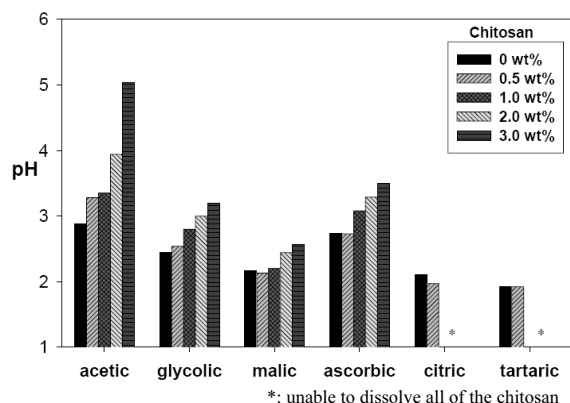


Figure 4. pH values of acid solutions containing different concentrations of chitosan. The pH value increased with an increasing concentration of chitosan. (* unable to dissolve all of the chitosan)

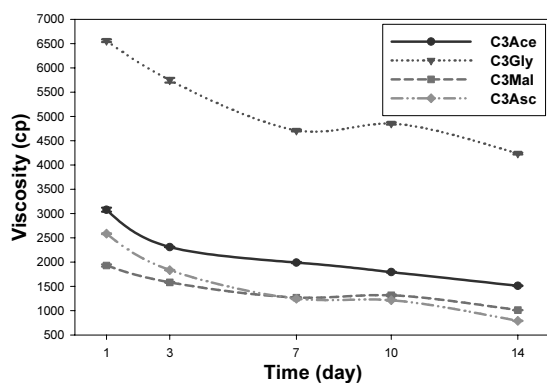


Figure 5. Decrease in viscosity with time of the various chitosan solutions. (●) C3Ace, (▼) C3Suc, (■) C3Mal, and (◆) C3Asc solutions. See Table 1 for the compositions of the solutions ($n = 6$, mean \pm SD).

necessarily lead to greater dissolution of chitosan, implying that the mechanism of chitosan dissolution is not only protonation of the amino group, but may include interactions between chitosan and the acid as well.

Since citric and tartaric acid solutions were not good solvents for chitosan dissolution, only malic, glycolic, and ascorbic acid solutions were used as solvents for dissolving chitosan in the subsequent study and were compared with acetic acid solution.

Measurement of the viscosity of the solutions

The viscosity of a solution is determined mainly by the molecular size and hydrogen bonds formed between molecules and water [16]. First, it can be shown from Figure 5 that among the acids we used, the C3Gly solution had a relatively higher viscosity at all time points, suggesting that because glycolic acid has the most-hydrophilic structure (i.e., the shortest backbone) and hydrophilic hydroxyl group, it can form strong hydrogen bonds with water and chitosan molecules, making the molecules less mobile; this causes the solutions to be more viscous [16]. Second, the viscosity of all solutions obviously decreased over time. This phenomenon is associated with the degradation of chitosan molecules in the acidic solution [13].

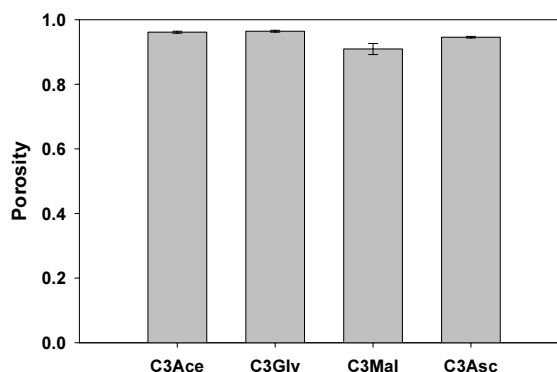


Figure 6. Porosity of various chitosan membranes. All of the membranes had similar porosities (of about 0.9). ($n = 8$, mean \pm SD)

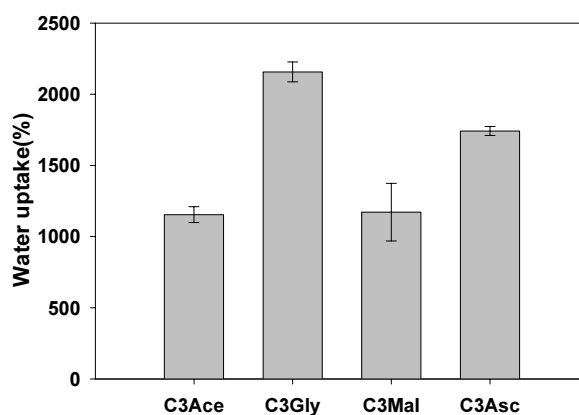


Figure 7. Water uptake by various chitosan membranes. C3Asc and C3Gly membranes absorbed about 0.5–0.9 times more water than did the C3Ace one. ($n = 8$, mean \pm SD)

With the degradation of chitosan, the size of the molecules in the solution becomes smaller, causing the viscosity of the solution to decrease.

Preparation of porous membranes

Chitosan membranes with homogeneously distributed, interconnecting pore structures were obtained by the freeze-gelation method. As shown in Figure 6, the porosity of the membranes was greater than 0.9, meaning that 90% or more of the volume of the membrane was occupied by pores, and there was no significant influence by the different acid solutions on the porosity of the membranes.

Water uptake by porous membranes

Biomaterials are usually used in an aqueous environment, so the water uptake capability is an important property of biomaterials. From the results shown in Figure 7, it was found that besides the malic acid solution-prepared chitosan (C3Mal) membranes, glycolic acid solution- and ascorbic acid solution-prepared (C3Gly & C3Asc) membranes could absorb more water into the membranes than acetic acid solution-prepared (C3Ace) membranes, and the water uptake values of C3Gly, C3Asc, C3Mal, and C3Ace membranes were 2150%, 1750%, 1170%, and 1150%, respectively. The results clearly indicate that the use of glycolic acid solution and

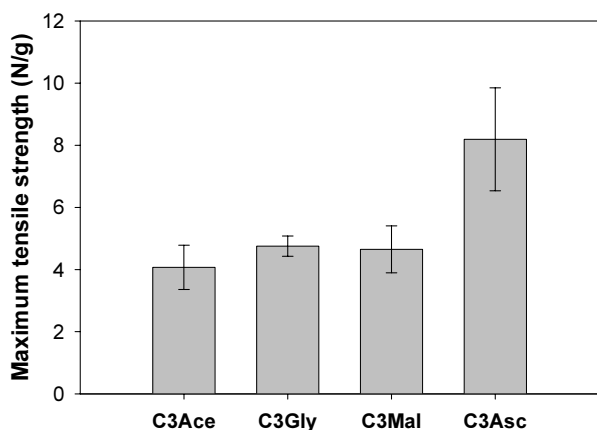


Figure 8. Maximum tensile strength of the various chitosan membranes. The maximum tensile strength is defined as the maximum load divided by the weight of the membrane. ($n = 6$, mean \pm SD)

ascorbic acid solution as solvents to prepare chitosan membranes greatly improved the water uptake capability of the membranes.

Analysis of mechanical properties

To investigate the influence of the various acid solutions on the mechanical properties of the membranes, porous membranes prepared by the freeze-gelation method were examined using a tensile strength instrument. As shown in Figure 8, the maximum tensile strength of the membranes improved from 4 to 4.6–8 N/g when glycolic, malic, and ascorbic acid solutions instead of acetic acid solution were used. For ascorbic acid solution-prepared (C3Asc) membranes, we propose that the improvement in the mechanical properties was mainly due to oxidation of ascorbic acid [12]. After the oxidation reaction, three carbonyl groups appeared on the ascorbic acid molecule (Figure 3). These carbonyl groups may have reacted with amino groups or hydroxyl groups on separate chitosan chains (reductive amination) [17], cross-linking the molecular structure of the chitosan membrane. So the mechanical strength of the C3Asc membranes was further improved and was greater than those of the C3Mal and C3Gly membranes. In addition, we propose that the presence of more ionic interactions or hydrogen bonds in the C3Gly, C3Mal, and C3Asc membranes makes the membrane become a larger molecular structure than the C3Ace membrane, thus improving its mechanical strength, like the cross-linking effect of tripolyphosphate (TPP) toward chitosan [18-19]. Therefore, C3Gly, C3Mal and especially C3Asc membranes have great potential for various biomedical applications due to their better mechanical properties than the C3Ace membrane.

Conclusions

In this work, we successfully prepared various chitosan solutions and porous membranes using glycolic, malic, and ascorbic acid solutions for chitosan dissolution. We found that the pH value of chitosan solutions for all acids went up with an

increase in the concentration of chitosan, and a more-viscous solution was obtained using glycolic acid solution as the solvent. As to the membrane properties, the tensile strength improved by about 100% using ascorbic acid solution, and water uptake capability increased by about 50%–90% when using ascorbic and glycolic acid solutions. We concluded that acetic acid solution can be replaced by these natural organic acid solutions for chitosan dissolution and subsequent preparation of porous membranes. By not only playing the role of a solvent like acetic acid solution, introduction of these organic acid solutions further improved the properties of the chitosan materials. For example, if a highly viscous solution is needed, glycolic acid solution can be used as the solvent for chitosan. If looking for greater mechanical strength, ascorbic acid solution can be utilized. Glycolic acid and ascorbic acid solutions are also suitable for preparation of chitosan membranes with a greater water-absorptive capacity. The various chitosan solutions and membranes we have prepared possess their own unique and excellent features. For further applications, the proper acid solution can be chosen for chitosan dissolution to achieve specific requirements for various biomedical applications.

Acknowledgements

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