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Gelation behavior with acetylation of chitosan for membrane preparation

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ABSTRACT

The aim of this work is to investigate the gelation behavior of chitosans which has various degree of acetylation (DA) of amino group to ensure the preparation of the designed membrane structure. The DA was controlled by the amount of acetic anhydride added to the solution of chitosan/acidified water/methanol mixture. The gelation behavior was evaluated by the gelation time and the quantity of syneresis. The mechanical strength of acetylated chitosan gels was also measured. The optimum condition for the formation of the membrane, i.e. chitosan concentration, acetic acid/chitosan mass ratio, methanol/water mass ratio, and amount of acetic anhydride for acetylation, was determined. This composition gives low contractility, shorter gelation time, and high mechanical strength to the membrane and the gel. Useful information not only for a preparation of a membrane but also for an immobilized carrier or a chemical reaction system was obtained in this work.

Keywords: Acetylated chitosan; Membrane; Gelation; Degree of acetylation

1. Introduction

Chitosan is a polyaminosaccharide derived by *N*-deacetylation of chitin in aqueous alkaline medium, as shown in Scheme 1. Chitosan is suitable for a membrane material, because of its formability, chemical reactivity, biocompatibility, and antibacterial activity. Therefore, chitosan is employed for various membrane separations such as ultrafiltration [1], dialysis [2], pervaporation, evapomeation [3,4], gas separation [5,6], carrier transport [7], and fixed enzyme membrane [8]. Especially, much interest has been paid to its biomedical, ecological, and industrial applications in the past decade.

With these technical development, the chemical modification of chitosan for reforming of polymer characteristic, in particular, *N*-alkylation [9,10], *N*-acylation [11–13], *O*-acylation [14], and *N*-carboxyalkylation [15], has been studied.

Several chemically modified chitosans were reported to form gels with the increase in the amount of substituent. For example, Hirano et al. [16–19] studied the gelation mechanism of chitosans N-acylated with Scheme 2. The gelation mechanism of polysaccharides also has been studied by Moore et al. [20,21]. In spite of these superior studies, to our knowledge, there are no studies which incorporated acetylation in a membrane manufacturing process. Further, the systematic characterization of permeability and the membrane structure of gels have not been thoroughly investigated.

In our previous work, we prepared chitosan membranes with the *N*-acetylated chitosan, and the relationship between the degree of acetylation (DA) and membrane structure as well as the water permeation characteristics was investigated [22–24]. The

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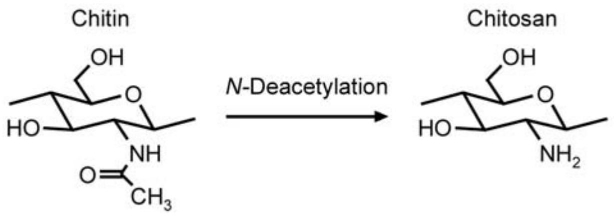


Scheme 1. Chemical structure of chitin and chitosan.

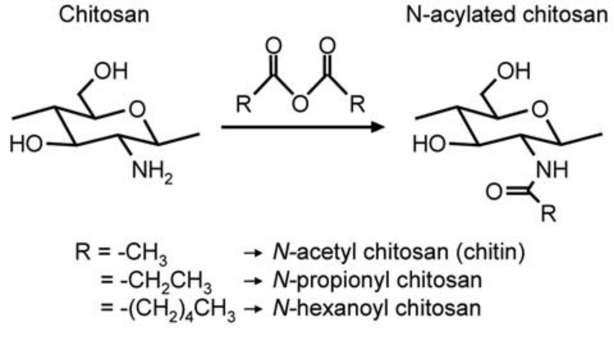
membranes had an individual so-called cellular structure, and the structure grew with the increase in DA from 0% to 50%. At the DA above 50%, hydrophobic interaction between acetyl groups induced crosslinking of the chitosan chains to form the gel. In the course of the membrane preparation, it was found that the change in the composition of reaction medium leads to the difference in the gelation behavior of acetylated chitosan.

In this study, the gelation behavior of the *N*-acetylated chitosan in various composition of reaction medium was investigated to form the membrane of designed structure. Particularly, the relationship between the composition of reaction medium and the gelation time as well as the syneresis was investigated.

2. Experimental

2.1. Preparation of acetylated chitosan gels

Chitosan (low molecular weight, Sigma-Aldrich Japan) was dissolved in acetic acid (99.7%, Kanto Chemical Co., Inc.)-water solution and it was stirred for 24 h at room temperature. The chitosan/acetic acid-water solution was diluted with methanol (99.8%, Kanto Chemical Co., Inc.), and it was stirred for 24 h at room temperature. The acetylated chitosan gel was obtained in a few minutes after the addition of the acetic anhydride (97.0%, Wako Pure Chemical Industries, Ltd.) into the chitosan/acetic acid-water/methanol solution. The acetylated chitosan gels were prepared with various solution compositions of reaction medium.



Scheme 2. *N*-Acylated chitosans with different acyl chain lengths.

2.2. Observation of the gelation behavior

The gelation in this work is a phenomenon that raw material solution containing chitosan loses fluidity with the progress of the acetylation. The gelation behavior was evaluated by the gelation time and the quantity of syneresis. The gelation time was defined as the lead time from the addition of the acetic anhydride when the raw material solution loses fluidity, and is suitable to evaluate the progress of acetylation. A syneresis is the leaking out of the solvent from the inside of gel. And the amount of solvent leaked out was employed for the evaluation of the change of the three-dimensional structure of gel. The quantity of solvent leaked out was measured with a cylinder.

2.3. Determination of DA

The DA indicates molar percentages of acetylglucosamine to the sum of glucosamine and acetylglucosamine in chitosan. The DA of acetylated chitosan gels was analyzed by the colloidal titration method [22–26]. Colloidal titration is a method for measuring the quantity of free amino groups in a chitosan solution. Potassium polyvinyl sulfate solution was used as a titrant, and toluidine blue was used as an indicator. The terminal point of titration was determined by the change of color from blue to claret. It should be noted that in the previous paper, the colloidal titration method was employed for the determination of the degree of deacetylation (DD).

2.4. Mechanical properties measurements

The compressive breaking stress and strain of the acetylated chitosan gels were determined by a rheological apparatus (CR-500DX-S, SUN SCIENTIFIC Co., Ltd.). Disk shaped attachment of 10 mm in diameter was used for measurement. The gel samples of 50 mm in diameter and 10 mm in thickness were prepared, and measured 48 h after the gelation.

3. Results and discussion

3.1. Effect of the solution composition on the gelation behavior

Fig. 1 indicates the time-course of the quantity of syneresis with the acetylation of the chitosan. The quantity of syneresis almost reached to equilibrium at

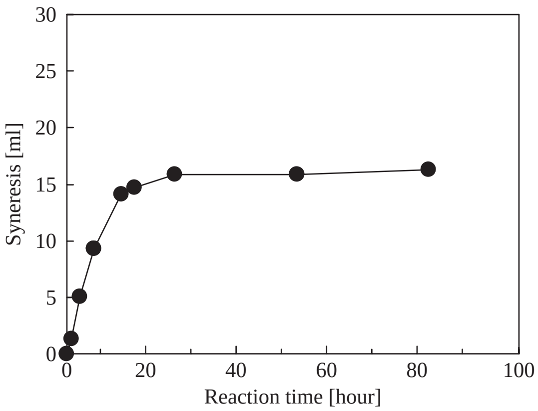


Fig. 1. The time-course of the quantities of syneresis with the acetylation of chitosan. The composition is 1 wt% in chitosan concentration, 5.0 in mass ratio of acetic acid/chitosan, 2.5 in methanol/water mass ratio, and 0.47 ml of acetic anhydride to 50 g of total mass.

24 h after the addition of the acetic anhydride. In this study, acetic anhydride was employed for the acetylation of amino groups in the chitosan. Unfortunately, it was also consumed by a hydrolysis shown in Eq. (1) [27], where Ac_2O is the abbreviated form of the acetic anhydride.

$$Ac_2O + H_2O \rightarrow 2AcOH \quad (1)$$

From these facts, the chitosan gels at 48 h after the addition of the acetic anhydride were employed in the following experiments.

Optical photograph of the acetylated chitosan gel at 48 h on Fig. 1 is shown in Fig. 2. The gel is semitransparent, and has light yellow color.

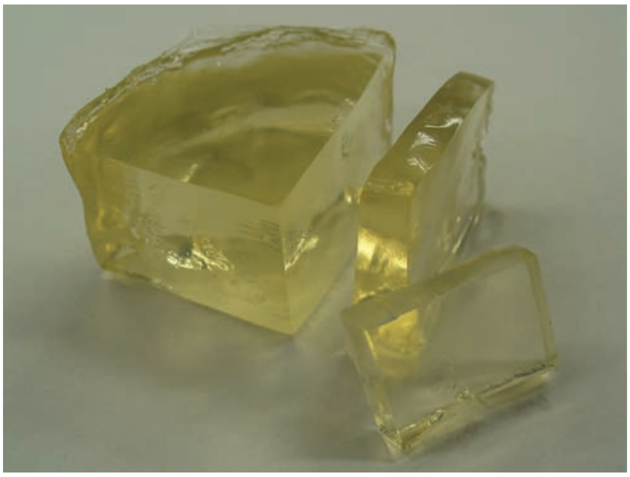


Fig. 2. The optical photograph of acetylated chitosan gel after 48 h which completed acetylation in Fig. 1.

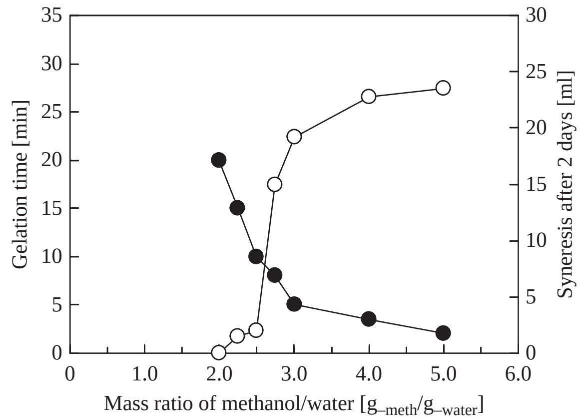


Fig. 3. Effect of methanol/water mass ratio on the gelation behavior of chitosan with acetylation. The composition is 1 wt% in chitosan concentration, 5.0 in mass ratio of acetic acid/chitosan, and 0.47 ml of acetic anhydride to 50 g of total mass. • gelation time, ○ syneresis after 2 d.

Fig. 3 shows the effect of methanol/water mass ratio on the gelation behavior of the chitosan. The gelation time became shorter with the increase in the quantity of methanol, and a significant quantity of syneresis was observed. The gelation mechanism of the chitosan on the acylation with carboxylic anhydride is the formation of bridges by the hydrophobic interaction between the acylated side-chains [16–19]. In this reaction system, the acylation of chitosan in Scheme 2 is competitive with the hydrolysis of the acetic anhydride in Eq. (1). Thus, the formation of three-dimensional crosslinked structure of chitosan is restrained by the existence of the excess water, and the amount of crosslinked structure at equilibrium is decreased. Therefore, the increase in the methanol content induced the shrinkage and a syneresis of gels. From these facts, the methanol/water mass ratio of 2.5 was decided as a suitable solution composition for the rapid gelation without the syneresis. In this work, quantity of syneresis less than 5 ml, and gelation time less than 10 min were determined as the minimum standard for the most suitable composition. The quantity of syneresis was given priority when satisfied both standard in the quantity of syneresis and the gelation time.

Fig. 4 illustrates the effect of acetic acid/chitosan mass ratio on the gelation behavior of chitosan with acetylation. The gelation time became longer and the quantity of syneresis became smaller with the increase in the acetic acid/chitosan mass ratio. These results indicate that the rate of acetylation was slowed down. As mentioned above, in the preparation of chitosan solution, chitosan was first dissolved in acetic acid-water solution, and the chitosan/acetic acid-water solution was then diluted with methanol. Thus,

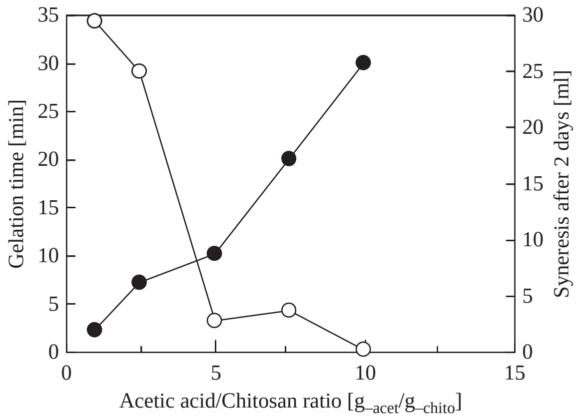


Fig. 4. Effect of acetic acid/chitosan mass ratio on the gelation behavior of chitosan with acetylation. The composition is 1 wt% in chitosan concentration, 2.5 in methanol/water mass ratio, and 0.47 ml of acetic anhydride to 50 g of total mass. • gelation time, ○ syneresis after 2 d.

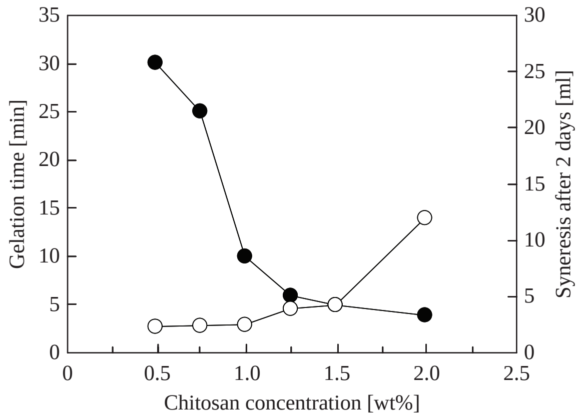


Fig. 5. Effect of chitosan concentration on the gelation behavior of chitosan with acetylation. The composition is 2.5 in methanol/water mass ratio, 0.94 ml of acetic anhydride to 1 g of chitosan, and 2.5 g of acetic acid to 50 g of total mass. • gelation time, ○ syneresis after 2 d.

changing the acetic acid/chitosan mass ratio at constant ratio of chitosan in the gelation solution means the decrease of the amount of methanol in the gelation solution. Increase in the acetic acid/chitosan mass ratio decreases the amount of methanol. The result of Fig. 4 supports the fact that methanol/(acetic acid/water) mass ratio has an influence on the gelation. Chitosan dissolves in water due to the protonation of the amino groups by the acetic acid. In our case, from considering the dissolution behavior, the degree of the protonation was sufficient. Therefore, with the existence of the sufficient amount of the acetic acid, the gelation behavior depends on the quantity of methanol. From the Fig. 4, the acetic acid/chitosan mass ratio of 5.0 was decided as a suitable solution composition for the rapid gelation without the syneresis.

Fig. 5 shows the effect of chitosan concentration on the gelation behavior. The gelation time became shorter with the increase in the chitosan concentration, and the quantity of syneresis was increased. In the low chitosan concentration region, it is hard to form gel even if acetylation completely progressed, because the quantity of the chitosan for the formation of three-dimensional structure is insufficient. On the other hand, in the high chitosan concentration region, the densification of three-dimensional crosslinking causes the syneresis. Thus, the chitosan concentration of 1.0% was decided as a suitable solution composition for the rapid gelation without the syneresis.

Fig. 6 represents the effect of the amount of the acetic anhydride on the gelation behavior of the chitosan. The gelation time decreased with the increase in the amount of the acetic anhydride. In contrast, the volume of the solution leaked out from the gel by the syneresis increased. The excessive acetylation is undesirable because it causes the shrinkage and the syneresis of the gel. Thus, the 0.95 ml of acetic anhydride per 0.5 g of the chitosan is suitable as a solution composition that ensures the rapid gelation with small syneresis (smaller than 5.0 ml).

Fig. 7 illustrates the relationship between the amount of the acetic anhydride and the DA of the chitosan. The intersection of ordinate is the initial DA of the purchased chitosan, and it does depend on Lot number. The DA linearly increased with the increase of the amount of the acetic anhydride. Approximately

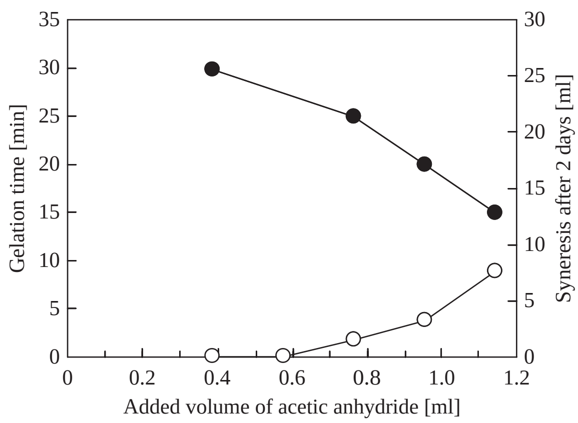


Fig. 6. Effect of the amount acetic anhydride on the gelation behavior of chitosan with acetylation. The composition is 1 wt% in chitosan concentration, 5.0 in mass ratio of acetic acid/chitosan, 2.5 in methanol/water mass ratio, and 50 g of total mass. • gelation time, ○ syneresis after 2 d.

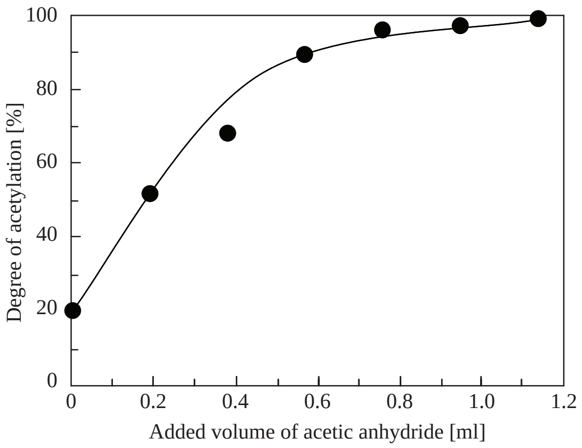


Fig. 7. Relationship between the amount of acetic anhydride and the DA of the chitosan. The composition is the same as Fig. 6.

100% of DA was achieved by the addition of about 1.2 ml of the acetic anhydride per 0.5 g of chitosan. The gel which has any DA can be prepared with using this relationship.

3.2. Mechanical properties of acetylated chitosan gels

Fig. 8 illustrates the effect of the DA on the mechanical properties of acetylated chitosan gel. The compressive breaking stress dramatically increased with the DA of above 80%. In contrast, the compressive breaking strain was decreased slightly. These results are consistent with the behavior of the gelation time and the syneresis. The mechanical strength of acetylated chitosan gels increases by the formation of the crosslinking structure that promoted by the acetylation.

4. Conclusions

In this study, the gelation behavior of the chitosan with the acetylation in various composition of reaction medium was investigated. For the low contractility, for the rapid gelation, and for the high mechanical strength, following composition was determined as the suitable composition: 1.0 wt% in chitosan concentration, 5.0 in acetic acid/chitosan mass ratio, 2.5 in methanol/water mass ratio, and 0.95 ml of acetic anhydride for 50 g of total mass. As future work, the more details of correlation between the membrane structure and composition have to be investigated to form the designed membrane structure. The information in this paper contributes for studies of material using a hydrophilic gel, e.g. an immobilized carrier or a chemical reaction system.

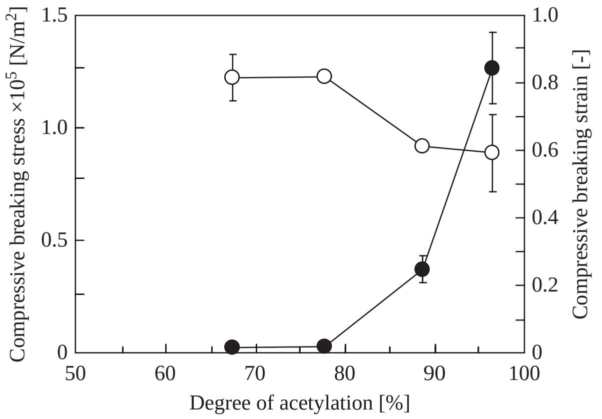


Fig. 8. Effect of the DA on compression properties of acetylated chitosan gel. The composition is the same as Fig. 6. • compressive breaking stress, ○ compressive breaking strain.

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