

# Effect of *N*-Acetylation on the Acidic Solution Stability and Thermal and Mechanical Properties of Membranes Prepared from Different Chain Flexibility Chitosans

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## SYNOPSIS

A series of chitosan membranes and their *N*-acetylated counterparts were prepared and their differences in acidic solution stability and thermal and mechanical properties were studied. The differences were attributed to differences in crystallinity of the membrane as the result of preparation conditions and *N*-acetylation. The results show that after *N*-acetylation, the acidic solution stability of *N*-acetylated membranes increased and the tensile strengths and the enthalpies of those membranes prepared from lower DD chitosans became higher and those from higher DD ones became lower. However, the swelling index of those modified membranes prepared from lower DD chitosans decreased and that from higher DD chitosans increased. Original membranes were prepared by using chitosans with different degrees of deacetylation (DD) and solution pHs to manipulate different chain flexibilities. © 1996 John Wiley & Sons, Inc.

## INTRODUCTION

The chitosan membrane is stable in alkali and organic solvent but dissolves in acidic solution. Chemical modification to improve acidic solution stability and/or to improve permeability has been explored in ultrafiltration (UF) membranes<sup>1</sup> and reverse osmosis (RO) membranes.<sup>2</sup> Chemical modified chitosan membrane could be used to replace acetate cellulose membrane, which is widely used commercially but is restricted to solution pH values of 2 to 9. Therefore, acetate cellulose membrane cannot be used in fruit juice and biotechnology applications.<sup>3</sup> *N*-acetylation of chitosan was done by reaction chitosan membrane with carboxylic anhydrides or arylaldehydes to get *N*-acyl or *N*-arylidene chitosans. *N*-arylidene chitosans were not stable in 0.1*N* HCl.<sup>1,4</sup> However, *N*-acyl, *N,O*-acetyl chitosan membranes were sta-

ble at room temperature for more than one month in 0.5*N* NaOH or in 0.1*N* HCl solution.<sup>1</sup> However, *N*-fatty acyl (>C8) resulted in fragile membranes due to functional groups destroying the structure of the membrane.<sup>5</sup> The properties of chemical modified chitosan membranes depend on the type of modified groups used and the position of modified groups attached (e.g., thrombogenic properties were found proportional to the carbon length of acyl groups used<sup>6</sup>). However, all the aforementioned studies explored modifications on membrane prepared from single DD chitosans.

The membranes prepared from different chain flexibility chitosans showed different crystallinities and different thermal and mechanical properties.<sup>7,8</sup> The effect of *N*-acetylation on the crystallinity, physical properties, and permeability of chitosan membranes prepared from different chain flexibility chitosans (DD of chitosan and solution pH dependent) has not been explored. Therefore, the effect of *N*-acetylation on the acidic solution stability and mechanical and thermal properties of chitosan membrane prepared from different DD (60% ~ 90%) chitosans in different solution pH values (pH 2 to pH 4.5) was studied.

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## MATERIALS AND METHODS

### Chitinous Material Preparation

Chitinous material was prepared from red shrimp (*Solenocera prominentis*) waste by the method of Chen et al.<sup>7</sup> to yield about 40% DD chitinous material. The 40% DD chitinous material was alkali deacetylated at 60, 99, and 110°C for 2, 2, and 2 & 1 h to yield 60, 78, and 87% DD chitosans, respectively. The 87% DD chitosan solution was alkali deacetylated again at 140°C for 3 h to yield 90% DD chitosan.<sup>9</sup> The degree of deacetylation was determined by the titration method.<sup>10</sup>

### Membrane Preparation from Different Chain Flexibility Chitosans

Membranes were prepared by casting chitosan solutions of different chain flexibilities on clean glass plates. The chitosan solutions of different chain flexibilities were prepared from dissolving different DD chitosans in different pH solutions.<sup>7,11</sup> The cast glass plates were then oven dried at 50°C for 60 min. The dried membranes were dipped in 10% NaOH aqueous solution to neutralize the acetic acid and yield the transparent membranes.<sup>7,8</sup>

### N-Acetylation of Chitosan Membranes

The chemical modification method of Yaku and Yamashita<sup>12</sup> was followed. The membranes prepared as discussed in the previous section were dipped in methanol solution (containing 20 ~ 30 mol/GlcN of acetic anhydride in methanol) for 12 h. They were washed with distilled water to remove excess acetic anhydride to yield N-acetylated chitosan membrane.

### Degree of Substitution Measurement

The degree of deacetylation was used to calculate the degree of substitution. Degree of deacetylation (DD) on the chitosan membrane was determined with infrared spectroscopy.<sup>13</sup> The absorbance of 1655 cm<sup>-1</sup> (amide I) and 3450 cm<sup>-1</sup> (OH peak, as an internal standard) was measured. The degree of deacetylation was calculated as

$$DD = 100 - (A_{1655}/A_{3450}) \times 115$$

### Tensile Strength Measurement

Tensile strength and elongation of membranes were measured by a rheometer (Rheometer CR-200D, Sun

Scientific, Japan) at a strain rate of 20 mm/min. The membrane was cut into strips (10 × 40 mm) by a punch press tester (Hung Ta HT-8024, Taiwan).

### Swelling Index Measurement

The swelling index is calculated as the ratio of weight difference to the original weight of the membrane strip. The weight difference was obtained by subtracting the weight of the membrane strip that had been immersed in water for 1 h from the weight of the original membrane strip. It was calculated as follows:

$$\text{Swelling index (\%)} = \frac{W_2 - W_1}{W_2} \times 100$$

Here  $W_1$  and  $W_2$  are the weight of the membrane strip before and after immersion in water, respectively.

### Enthalpy and Maximum Melting Point Temperature Measurement

The enthalpy and maximum melting point temperature were measured by a differential scanning calorimeter (DSC, DuPont DSC 10). A piece of membrane was punched and sealed in an aluminum pan and then heated at 10°C/min to 200°C. Enthalpy was calculated from the energy consumed, whereas the maximum melting point temperature is the temperature at which the membrane melted.

## RESULTS

### Degree of Substitution

Table I shows that the degree of substitution of the majority of membranes ranged from 80 to 90%.

**Table I** The Degree of N-Substitution after N-Acetylation of the Membranes Prepared from Chitosans of Various Degrees of Deacetylation and Solution pH Values

Chitosan Membranes	pH		
	2.0	3.0	4.5
60 DD	70.8	76.1	82.6
78 DD	88.7	92.6	87.0
87 DD	79.0	87.9	91.5
90 DD	88.8	80.2	68.9

DD: Degree of deacetylation.

Membrane preparation conditions did not affect the degree of substitution of the resulting membranes.

### Maximum Melting Point Temperature

Table II shows that the maximum melting point temperature of chitosan membranes before and after *N*-acetylation were between 137 and 110°C. Neither the *N*-acetylation nor the membrane preparation conditions before *N*-acetylation affected the melting point significantly.

### Tensile Strength

Table III shows that before *N*-acetylation, the tensile strength of the membranes prepared from lower DD chitosans was lower than those prepared from higher DD chitosans. The tensile strength was 171.6 kg/cm<sup>2</sup> and 234.2 kg/cm<sup>2</sup> for the membranes prepared from solution pH 4.5, 60% DD and 90% DD chitosan, respectively. Membranes prepared from pH 2 or pH 3 solutions have similar trends. However, after *N*-acetylation, tensile strengths of membranes that were prepared from lower DD chitosans became higher, and those from higher DD chitosans became lower. The tensile strength was 205.5 kg/cm<sup>2</sup> and 89.1 kg/cm<sup>2</sup> for the same membranes mentioned. After *N*-acetylation, tensile strengths of the membranes prepared from different solution pH values became different. The membranes prepared from solution pH 3 have the highest tensile strength. However, the tensile strengths of membranes pre-

pared from different solution pH values were not significantly different before *N*-acetylation.

### Enthalpy of Membranes

Table IV shows that before *N*-acetylation, the enthalpies of the membranes prepared from lower DD chitosans were lower than those of higher DD chitosans. Before *N*-acetylation, the membranes prepared from 60 DD, 78 DD, 87 DD, and 90 DD chitosan pH 2 solution had an enthalpy of 25.49, 27.20, 36.18, and 36.83 cal/g/s, respectively. After *N*-acetylation, the enthalpies of the membranes that were prepared from lower DD chitosans became higher and those of higher DD ones became lower. The enthalpies were 37.29, 32.84, 28.77, and 32.60 cal/g/s for pH 2, *N*-acetyl-60, *N*-acetyl-77, *N*-acetyl-87, and *N*-acetyl-90 membranes, respectively. The enthalpies of the membranes prepared from the same DD chitosan but different pH solution values were not different significantly before or after the *N*-acetylation.

### Swelling Index

Figure 1(a) shows that before *N*-acetylation, the swelling index decreased proportionally with increasing DD of chitosans used. The swelling index was 227.6 and 50% for membranes prepared from solution pH 2, 60% DD, and 90% DD chitosan, respectively. The swelling index of the membranes prepared from 90% DD chitosan was near 50% for all three membranes regardless the solution pH differences. This indicated that solution pH did not affect the swelling index of membranes prepared from 90% DD chitosan before *N*-acetylation. However, solution pH does affect those membranes prepared from 60% DD chitosan. The swelling indexes of those membranes were 228, 213, and 171%, respectively. After *N*-acetylation, the swelling index increased proportionally with increasing DD of chitosan used. The swelling index of the membrane prepared from 90% DD chitosan, pH 4.5, pH 3, and pH 2 was 108%, 100% and 106%, respectively. It was 55.0%, 57.5%, and 60.4% for the membranes prepared from 60% DD chitosan, pH 4.5, pH 3, pH 2, respectively [Fig. 1(b)]. The swelling index of the membranes that were prepared from the same DD chitosan but different solution pH values was different for three solution pH values studied.

**Table II The Maximum Melting Point Temperatures\* (°C) of the Original Membranes Prepared from Chitosans with Different Degrees of Deacetylation and Solutions pH Values and Their *N*-Acetylated Counterparts**

Chitosan Membranes	pH		
	2.0	3.0	4.5
60 DD	137.13 <sup>b</sup>	125.24	130.86
78 DD	132.83	132.81	136.62
87 DD	125.46	113.51	118.64
90 DD	130.53	127.26	119.96
<i>N</i> -acetyl-60 <sup>c</sup>	131.08	120.81	117.01
<i>N</i> -acetyl-78	126.55	117.77	122.56
<i>N</i> -acetyl-87	132.49	121.97	120.44
<i>N</i> -acetyl-90	115.26	108.76	109.63

\* Heating rate: 10°C/min.

<sup>b</sup> The data are the mean of three samples.

<sup>c</sup> *N*-acetyl-xx: *N*-acetylation of the membranes prepared from chitosan of xx degree of deacetylation.

**Table III The Tensile Strength (kg/cm<sup>2</sup>) of the Original Membranes Prepared from Chitosans with Various Degrees of Deacetylation and Solution pH Values and Their Modified Counterparts**

Chitosan Membranes	pH		
	2.0	3.0	4.5
60 DD	188.0 ± 19.9 <sup>a</sup>	187.5 ± 22.3	171.6 ± 18.9
78 DD	211.4 ± 23.7	220.2 ± 18.7	206.0 ± 16.8
87 DD	243.6 ± 25.9	228.0 ± 12.7	223.2 ± 21.9
90 DD	247.5 ± 23.3	239.6 ± 25.7	234.2 ± 30.8
<i>N</i> -acetyl-60 <sup>b</sup>	176.4 ± 21.5	261.6 ± 25.0	205.5 ± 18.9
<i>N</i> -acetyl-78	143.1 ± 17.6	171.6 ± 15.6	148.9 ± 20.4
<i>N</i> -acetyl-87	132.9 ± 15.0	168.1 ± 17.1	129.3 ± 17.5
<i>N</i> -acetyl-90	90.7 ± 10.0	108.1 ± 11.6	89.1 ± 7.4

<sup>a</sup> The data are the mean of seven samples.

<sup>b</sup> *N*-acetyl-*xx*: *N*-acetylation of the membranes prepared from chitosan of *xx* degree of deacetylation.

## DISCUSSION

### Degree of Substitution

The degree of substitution of the majority of membranes ranged from 80 to 90%. The results were similar to those of Hirano et al.<sup>1</sup> The degree of substitution of the membranes prepared from 60% DD chitosan, pH 2 solution and from 90% DD chitosan, pH 4.5 solution was around 70%. However, the two membranes were stable in acidic solution.

### Maximum Melting Point Temperature

Table II shows that neither the *N*-acetylation nor the membrane-making conditions before chemical modification affected the maximum melting point temperature of membranes. *N*-Acetylation with acetic anhydride caused structure changes and crystallinity changes that were too small to result in any significant difference in maximum melting point temperature. However, Hirano et al.<sup>1</sup> reported that *N*-acetylation resulted in elevation of the maximum melting point temperature. This discrepancy may be because the membranes used by Hirano et al.<sup>1</sup> were thicker than those used in this study. The thicker membrane may result in better crystallinity due to higher chances of intermolecular interaction.

### Tensile Strength

Table III shows that the membranes with lower tensile strength became higher and higher tensile strength ones became lower after *N*-acetylation. Before *N*-acetylation, tensile strength was 171.6 kg/cm<sup>2</sup>

for membranes prepared from 60% DD chitosan, pH 4.5. For those of 90% DD chitosan of the same solution pH, the tensile strength was 234.2 kg/cm<sup>2</sup>. After *N*-acetylation, tensile strength was 205.5 kg/cm<sup>2</sup> and 89.1 kg/cm<sup>2</sup>, respectively, for the membranes mentioned. Membranes prepared from solution pH 2 or pH 3 have similar results. This may be because crystallinity in membranes can be formed in part due to the steric regularity of their constitution macromolecules and due in part to the strength of the intermolecular force.<sup>14</sup> *N*-acetylation of the membranes prepared from higher DD chitosans resulted in lower tensile strength. This may be because when the degree of substitution reaches 80 to 90%, the acetyl group will attach to amine group and ruin the crystallinity and thus decrease the tensile strength. The membranes prepared from high DD chitosan contain more crystallinity region<sup>8,15</sup> or steric regularity before *N*-acetylation. Substitution changed the steric regularity that formed before. Therefore, *N*-acetylation reduced the crystallinity structure and lowered the tensile strength of the membrane prepared from higher DD chitosan.

After *N*-acetylation, the membranes prepared from lower DD chitosan resulted in higher tensile strength. This may be because after *N*-acetylation, the acetyl group attached to the amine group in the amorphous region. The attached acetyl group in the amorphous region facilitates intermolecular interactions. This increased intermolecular force in turn increased the crystallinity and then the tensile strength of the membrane.

After *N*-acetylation, tensile strength of the membranes prepared from different solution pH values

**Table IV** The Enthalpy<sup>a</sup> (cal/g/s) of the Original Membranes Prepared from Chitosans of Various Degrees of Deacetylation and Solution pH Values and Their Modified Counterparts

Chitosan Membranes	pH		
	2.0	3.0	4.5
60 DD	25.49 ± 8.08 <sup>b</sup>	25.47 ± 1.68	25.61 ± 1.07
78 DD	27.20 ± 7.67	26.29 ± 3.66	27.28 ± 3.34
87 DD	36.18 ± 2.63	35.40 ± 9.78	31.83 ± 8.35
90 DD	36.83 ± 2.83	39.40 ± 6.44	32.84 ± 9.55
<i>N</i> -acetyl-60 <sup>c</sup>	37.29 ± 6.51	36.57 ± 6.32	35.06 ± 1.80
<i>N</i> -acetyl-78	32.84 ± 12.90	29.03 ± 1.52	31.82 ± 7.28
<i>N</i> -acetyl-87	28.77 ± 4.92	21.87 ± 1.80	29.67 ± 4.90
<i>N</i> -acetyl-90	32.60 ± 4.84	30.62 ± 6.64	24.91 ± 10.00

<sup>a</sup> Heating rate: 10°C/min.

<sup>b</sup> The data are the mean of three samples.

<sup>c</sup> *N*-acetyl-*xx*: *N*-acetylation of the membranes prepared from chitosan of *xx* degree of deacetylation.

became different. However, tensile strength of the original unmodified membranes prepared from different solution pH values did not differ significantly (Table III). This might be because the synergetic effects of intermolecular interactions in amorphous regions occurred after *N*-acetylation. The chitosan molecule in pH 3 solution showed a more random coil<sup>16</sup> than that of the other two solution pH values. The more random coil molecules might result in more amorphous membranes. After *N*-acetylation, the attached acetyl groups in the amorphous region facilitate the acetamine and hydroxyl group to interact and form hydrogen bonds, thus increasing the strength of the intermolecular force. Therefore, the tensile strength of the membrane prepared from different solution pH values differed significantly after *N*-acetylation.

### Enthalpy

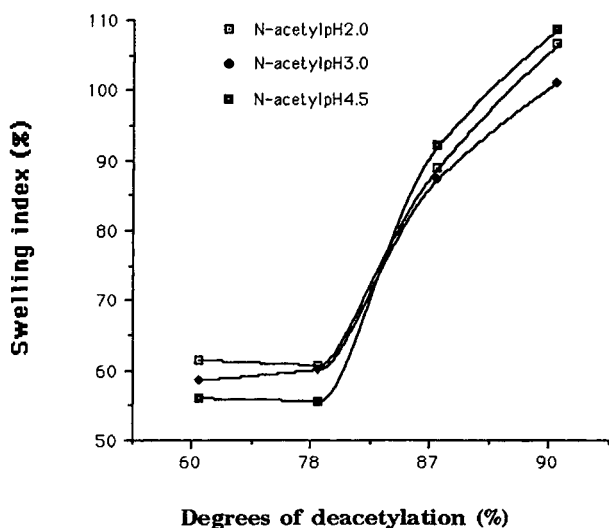
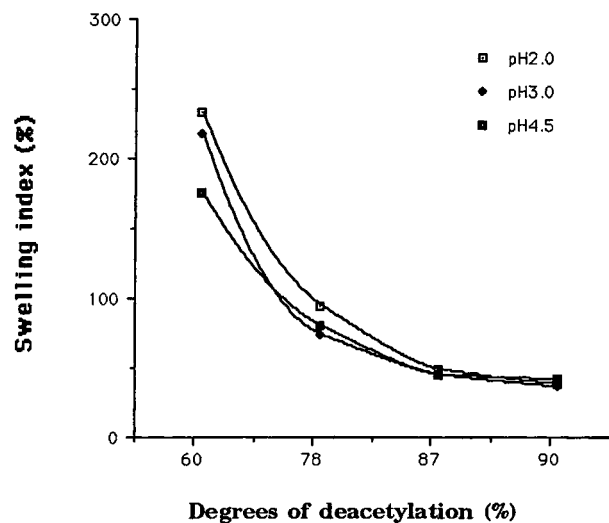
Enthalpy represents the endothermic energy needed to break the crystallinity in the membrane. The higher the enthalpy of the membrane, the higher the crystallinity in the membrane. *N*-acetylation reduced the enthalpies of the membranes prepared from higher DD chitosans, such as 87 or 90% chitosan, whereas it increased the enthalpy of the membranes prepared from lower DD chitosan, such as 60 or 78% chitosan (Table IV). These results indicated that the structure of the membranes changed after *N*-acetylation, as did the crystallinity. Therefore, *N*-acetylation caused the membranes with lower enthalpy values to become higher and those with higher values to become lower.

### Swelling Index

The swelling index is affected mainly by the hydrophilic groups in the amorphous regions of the membrane. The hydrophilic groups in the crystallinity are tightly packed; therefore, this effect is not noted. Consequently, the larger the amorphous regions, the larger their swelling index becomes. Before *N*-acetylation, the swelling index of chitosan membranes decreased proportionally with increasing DD of chitosan used. Blair et al.<sup>17</sup> and Chen et al.<sup>7</sup> have shown similar results. However, after *N*-acetylation, the swelling index increased proportionally with increasing DD of chitosan used. Figure 1 shows that the highest swelling index of *N*-acetylated membranes prepared from solution pH 4.5 was registered from 90% DD chitosan and was 110%. The tensile strength and enthalpy of its corresponding original membrane were 234.2 kg/cm<sup>2</sup> (Table III) and 32.84 cal/g/s (Table IV), respectively, the highest among the membranes prepared from pH 4.5. Table IV shows that the tensile strength and enthalpy of *N*-acetylation of the same membrane was 89.1 kg/cm<sup>2</sup> and 24.91 cal/g/s, respectively. *N*-Acetylation changed the crystallinity regions into amorphous regions and thus increased the swelling index of the membrane (Fig. 1) and reduced tensile strength (Table III) and enthalpy (Table IV). Similar trends occurred in the membranes prepared from solution pH 2 or pH 3.

### Effect of *N*-Acetylation

Hirano et al.<sup>5</sup> reported that *N*-fatty acyl (>C8) resulted in fragile membranes due to larger functional



**Figure 1** Swelling index of the original membranes (a) prepared from chitosans with various degrees of deacetylation and solution pH values and their *N*-acetylated counterparts (b). The values pH 2.0, pH 3.0, and pH 4.5 represent the original membranes prepared from 90% DD chitosan pH 2.0, pH 3.0, and pH 4.5 solutions, respectively. *N*-acetyl pH 2.0, *N*-acetyl pH 3.0, and *N*-acetyl pH 4.5 represent *N*-acetylation of the original membranes prepared from pH 2.0, pH 3.0, and pH 4.5 solutions, respectively.

groups breaking the structure of the membrane. This indicates that compounds attached to amine groups will change their steric conformation and in turn change the structure and properties of the membrane. The properties of chemical modified chitosan membranes depend on the type of modified group used and the position of modified groups attached. The thrombogenic property was found to be proportional to the carbon length of acyl groups used.<sup>6</sup>

*N*-acetylation of chitosan membranes with a tensile strength of 410 kg/cm<sup>2</sup> and maximum melting point temperature of 260°C have been reported.<sup>4</sup> Both tensile strength and maximum melting point temperature were higher than for the membranes used in this study. This may be attributed to the thickness difference. The membranes of Hirano<sup>4</sup> have a membrane thickness of 43 μm compared to around 25 μm in this study.

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