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*Carbohydrate Polymers* **24** (1994) 41–46

0144-8617/94/$07.00

# Relationships between the chain flexibilities of chitosan molecules and the physical properties of their casted films

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(Received 8 December 1993; revised version received 14 January 1994; accepted 25 January 1994)

Relationships between the chain flexibilities of chitosan molecules in solutions and the physical properties (gel swelling index, maximum melting temperature, and tensile strength) of their casted films were studied in order to manipulate the conditions to tailor the physical properties of the films made. The chain flexibilities of chitosan molecules in solutions were manipulated by using chitosans with different degrees of deacetylation (DD), pH and ionic strength of the media, and solvent systems. The results show the gel swelling index was independent of the media pH, and decreased with increasing DD of chitosans used, whereas maximum melting temperature and tensile strength increased with the increase of DD of the chitosan molecules. The differences were attributed to different crystalline regions in the films.

## INTRODUCTION

Chitosan films have been used in many fields: membrane separation (Yang & Zall, 1984; Aiba *et al.*, 1986); preservation of fruits and vegetables (Bai *et al.*, 1988); meat casing (Muzzarelli, 1977); and biomedical applications (Olsen *et al.*, 1989). Physical properties determined the selection of the right film used in those applications. Mechanical properties, permeability, thermal decomposition points, solvent stability, etc., are physical properties considered in the selection of the right film (Collins *et al.*, 1973). However, in membrane separation applications, membrane porosity is the key factor considered besides the mechanical properties.

Film-making conditions, including solvent pH, ionic strength, type of solvent (acid) used (Kienzle-Sterzet *et al.*, 1982; Aiba *et al.*, 1986; Hwang *et al.*, 1986) and annealing treatment (Aiba *et al.*, 1985) are parameters often manipulated to alter the mechanical properties and membrane porosity. Coagulant used (Aiba *et al.*, 1985), chitosans of various degrees of deacetylation (DD) (Mima *et al.*, 1983), and chitin/chitosan derivates (Hirano *et al.*, 1980) were also the factors, but have been explored less often.

Hwang *et al.* (1986) suggested that the mechanical properties and porosity of the chitosan membrane can be controlled by the manipulation of chitosan solution conditions (pH, concentration, ionic strength, and solvent). Ionic strength or pH can be manipulated to a condition which will reduce inter- and intra-molecular electrostatic repulsions between chitosan chains. This will allow the chains to come closer together and enhance the inter- and intra-chain hydrogen bonding and cause the chains to form a special network structure that results in the increase of the average pore size in the matrix. Ogawa *et al.* (1992) reported that the functionality of the film is affected by the polymorphism and crystallinity of chitosan, which depend on different molecular weights ($\bar{W}$) and different procedure methods. There are four crystallinity polymorphs: tendon, annealed, L-2, and I-2. Samuels (1981) reported in chitosan film the presence of two polymorphic crystal forms

(forms I & II) and a morphological structure that varied from spherulites to rods. The functionality of the film also depends on the film process conditions.

We considered that chain flexibility was another factor that can be manipulated to vary the inter- and intra-chain H-bonding, resulting in different mechanical properties and membrane porosity (Lin, 1992). The chain flexibility of the chitosan molecule has been related to the DD of the chitosan (Chen *et al.*, 1994, submitted). Therefore, in this study, the effects of various DD chitosans, solution pH, ionic strength, and solvent systems on the mechanical properties, gel swelling index, and maximum melting temperature of the film were studied to relate the causes of using chitosans of different chain flexibilities and the effect upon the physical properties of the film manufactured.

## MATERIALS AND METHODS

### Preparation of chitosans with various degrees of deacetylation (DD)

Chitin was prepared from ground dried shell and head of red shrimp (*Solenocera prominentis*) (Lin *et al.*, 1992; Chen *et al.*, 1994, submitted) using 11% and 50% NaOH (v/w) at 45, 99, 110, and 140°C for 1·0–10 h. The products were washed and dried to give chitosans of various DD. The chain stiffness parameters (*B*) of chitosans of 80, 90, and 100% DD in different pH solutions, calculated from intrinsic viscosities' data using the Smidsrod and Haug method (1971), are shown in Fig. 1.

### Determination of the DD

Infrared spectrometry was used to determine the degree of *N*-acetylation of the chitosan (Lin *et al.*, 1992; Chen *et al.*, 1994, submitted). Chitosan powder was mixed with KBr (1:100) and pressed into a pellet. The absorbance of amide I (1655 cm$^{-1}$) and of the hydroxyl band (3450 cm$^{-1}$) were measured using an Hitachi 260-30 infrared spectrophotometer. The band of the hydroxyl group at 3450 cm$^{-1}$ was used as an internal standard to correct for disc thickness and for differences in chitosan concentration in making the KBr disc. The percentage of the amine group's acetylation in a sample was given by $(A_{1655}/A_{3450}) \times 115$. Here, $A_{1655}/A_{3450}$ are the absorbances at 1650 cm$^{-1}$ and 3450 cm$^{-1}$, respectively.

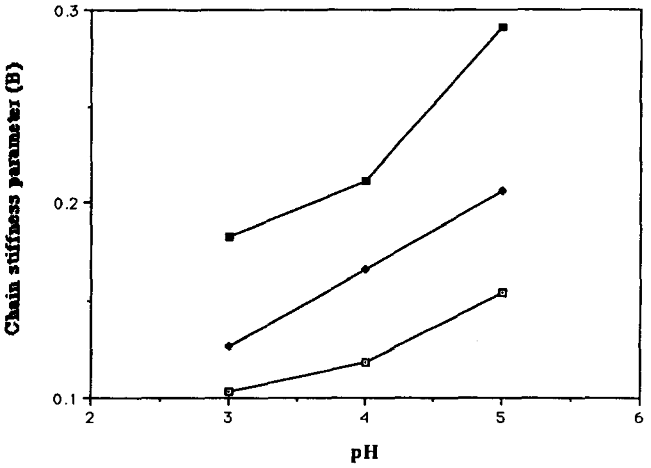

Fig. 1. The chain stiffness parameter (*B*) of chitosans with different degrees of deacetylation in different media pH values. □, 80%; ◆, 90%; ■,100%.

### Film preparation

The spread method (Aiba *et al.*, 1985) was used to prepare films for physical properties measurements. The procedures are given below.

*Films prepared from chitosans of low DD*

Chitosan concentrations of 0·5 and 1·0% with 35·2 and 43·9% DD were dissolved in a 50 ml mixture of 1:1 *N-N*, dimethyl-acetamide (DMA) and *N*-methyl-2-pyrrolidone (NMP). LiCl was added later to make the ratios of DMA:NMP:LiCl to 100:100:7·5; 100:100:10; and 100:100:12·5. The mixtures were stirred, and then diluted with four volumes of DMA + NMP (1:1). The solutions were filtered after mixing and degasing, and spread onto clean glass plates. The glass plates were dipped in 2-propanol for 2 h to coagulate the films; then the glass plates were immersed in water for 12 h to remove the 2-propanol. The glass plates were then dried at ambient temperature to obtain the films.

*Films prepared from chitosans of high DD*

One percent chitosan with 80, 90, or 100% DD was dissolved in pH 1·0, 2·0, 3·0, 4·0, and 5·0 acetic acid solutions. The mixtures were stirred to dissolve the chitosan. Solutions were then spread onto clean glass plates after degasing and filtration. The glass plates were dipped in 2% NaOH solution for 2 h to coagulate the films. The plates were immersed in water for 12 h to neutrality, then dried at ambient temperature to obtain the films.

### Measurement of physical properties

*Film thickness*

Film was fixed with paraffin, and sliced with a microton. The thickness was measured by light microscopy (Nikkon model 104) with a standard scale.

*Tensile strength*

Strips of 5 mm × 30 mm were prepared. The tensile strength was measured by a rheometer (model CR-2000, Sun Scientific Co. Ltd., Japan) at a strain rate of 20 mm/min.

*Gel swelling index*

The gel swelling index, defined as the ratio of the weight difference after immersing a film in water for 24 h to the original weight of the film, was calculated from

$$\text{Gel swelling index } (\%) = \frac{(W_2 - W_1)}{W_1} \times 100 \qquad (1)$$

where $W_1$ and $W_2$ are the weights of the film before and after immersion in water, respectively.

*Maximum melting temperature*

The energy of fusioning the film is reported to be proportional to the crystallinity of the film (Aiba *et al.*, 1985). The melting temperature was measured by differential scanning calorimetry (Du Pont TA 2000, D.S.C 10); the point at which the film melted is termed the maximum melting temperature.

## RESULTS

### Gel swelling index of film prepared from high DD (>80%) chitosans

Figure 2 shows the effects of different DD and media pH (related to chain flexibilities) (Chen *et al.*, 1994, submitted) on the gel swelling index of chitosan films casted between pH 1 and 5. The gel swelling index decreased with increasing DD of the chitosans used. However, the media pH values did not affect significantly at the same chitosans' DD.

### Maximum melting temperature of films prepared from high DD chitosans

The maximum melting temperature of films prepared from 80% DD chitosans were lower than those from 90 or 100% DD chitosans. There were no differences between films from the 90 or 100% chitosans. Also, there were no differences observed between films from chitosans of the same DD but different media pH for the three chitosans used (Table 1).

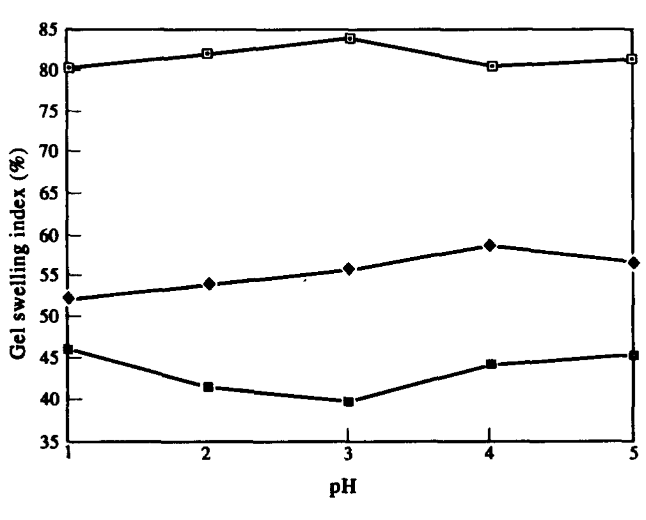

**Fig. 2.** Effect of degree of deacetylation of chitosans and media pH values on the gel swelling index of the films prepared. □, 80%; ◆, 90%; ■, 100%.

### Tensile strength

Values of tensile strength of dried or wet chitosan films prepared from chitosans of various DD (80%, 90% and 100%) are listed in Table 2. The tensile strength of dried films prepared from 80% DD chitosans, between pH 1 and 5, ranged between 921 and 952 dyne/cm$^2$. For films of 90 and 100% DD chitosans, values of tensile strength were between 1006 and 1020 and 1007 and 1018 dyne/cm$^2$, respectively, in the pH range 1–5. The tensile strength of wet films prepared from 80, 90, and 100% DD chitosans ranged from 225–278, 396–459, and 503–576 dyne/cm$^2$, respectively, for pH 1–5. The tensile strength of the wet films increased markedly with increasing DD of chitosans used. The effect of the DD of the chitosans on the tensile strength of the wet films was more obvious than on the dry films.

### Effect of LiCl concentration in casting solvent on the tensile strength of films prepared from low DD (<40%) chitosans

The effects of chitosan concentration and LiCl concentration on the tensile strength of films prepared from low DD chitosans are shown in Table 3. The concentrations of chitosan were 0·5 and 1·0% and the concentrations of LiCl were 7·5, 10·0, and 12·5%. The DD of chitosan affected the tensile strength of films prepared from 1·0% chitosan. Values of tensile strength of films prepared from 35·2% DD chitosan were lower than those from 43·9% DD chitosan when the LiCl concentrations were less than 12·5%. As the concentration of LiCl increased to 12·5%, the effect of the DD of chitosans on tensile strength of the films became insignificant in the 1·0% chitosan solution. But effect of DD did not exist in 0·5% chitosan solutions.

**Table 1. The maximum melting temperature (°C) of membranes prepared from 80, 90, and 100% DD chitosan solutions with different pH**

| Degree of deacetylation (%) | Maximum melting temperature (°C) | | | | |
|---|---|---|---|---|---|
| | pH 1 | pH 2 | pH 3 | pH 4 | pH 5 |
| 80 | 193·64 | 193·29 | 190·90 | 194·27 | 192·33 |
| 90 | 201·66 | 198·48 | 206·34 | 203·12 | 197·96 |
| 100 | 198·81 | 203·08 | 202·61 | 199·76 | 201·73 |

**Table 2. The tensile strength (dyne/cm$^2$) of membranes prepared from 80, 90, and 100% DD[a] chitosans in different solution pHs**

| pH | Tensile strength (dyne/cm$^2$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 80% DD | | | 90% DD | | | 100% DD | | |
| | Dry | Wet | Dry/wet | Dry | Wet | Dry/wet | Dry | Wet | Dry/wet |
| 1 | 948 ± 12[b] | 225 ± 11 | 4·3 | 1015 ± 17 | 459 ± 8 | 2·21 | 1007 ± 23 | 503 ± 7 | 2·01 |
| 2 | 921 ± 20 | 244 ± 9 | 3·77 | 1008 ± 13 | 438 ± 11 | 2·30 | 1018 ± 19 | 547 ± 12 | 1·86 |
| 3 | 936 ± 18 | 238 ± 8 | 3·93 | 1006 ± 23 | 414 ± 5 | 2·43 | 1016 ± 20 | 576 ± 15 | 1·76 |
| 4 | 952 ± 18 | 278 ± 11 | 3·42 | 1020 ± 20 | 396 ± 7 | 2·58 | 1011 ± 17 | 518 ± 9 | 1·95 |
| 5 | 940 ± 10 | 262 ± 7 | 3·59 | 1006 ± 18 | 408 ± 10 | 2·47 | 1013 ± 9 | 503 ± 11 | 2·01 |

[a] DD = degree of deacetylation.
[b] The data are the means of six samples.

**Table 3. The effect of chitosan and LiCl concentrations on the tensile strength (dyne/cm$^2$) of membranes prepared from 35·2 and 43·9% DD[a] chitosans**

| LiCl concentration[b] | Tensile strength (dyne/cm$^2$) | | | |
|---|---|---|---|---|
| | 0·5% chitosan | | 1·0% chitosan | |
| | 35·2% DD | 43·9% DD | 35·2% DD | 43·9% DD |
| 7·5 | 134 ± 9[c] | 149 ± 13 | 210 ± 11 | 254 ± 11 |
| 10·0 | 183 ± 12 | 166 ± 9 | 268 ± 6 | 295 ± 7 |
| 12·5 | 207 ± 15 | 212 ± 10 | 339 ± 13 | 310 ± 16 |

[a] DD = degree of deacetylation.
[b] The casting solvent varied with LiCl concentration; therefore, the ratios of DMA–NMP–LiCl are 100:100:the figures listed.
[c] The data are the means of six samples.

## DISCUSSION

### Effect of chain flexibility of chitosans on the gel swelling index of the films prepared

The results shown in Fig. 2 indicated that films prepared from 80% DD chitosans absorbed more water than those from 90% or 100% DD chitosans between pH 1 and 5. Blair *et al.* (1987) reported similar results. This may be because films prepared from high DD chitosans contain more crystallinity, which prohibits water absorption (Mima *et al.*, 1983). Using chitosans of different DD has an effect on crystalline formation in the films made; in other words, chain flexibility may effect the tendency of crystallinity formation upon film formation. This may be because higher DD chitosans were less rigid than lower DD chitosans between pH 1 and pH 5. Chain flexibility facilitated inter- and/or intra-molecular interactions, in turn increasing crystallinity formation in the film. Ogawa *et al.* (1992) reported that crystallinity formation depended on the conformation of the chitosan and the solution conditions, which is consistent with the results shown in Table 1 and Fig. 2. Crystallinity is also facilitated by interaction of polymers whose molecules are chemically or geometrically regular in structure (Billmeyer, 1966). Although chitosan molecules of the same DD in media pH between pH 1 and pH 5 had different inter- and/or intra-molecular electrostatic repulsion forces (Hwang *et al.*, 1986) should result in different network structure. In turn, it should facilitate different crystallinity formation in the film. However, crystallinity was not significantly different (Table 1), indicating that the effect of different protonated amino groups on the molecule did not result in different crystallinity formation of the film. This may be because the neutralization process employed during film preparation nullified the different crystallinity formation.

### Effect of chain flexibility of chitosans on the maximum melting temperature of the films prepared

The maximum melting temperature reflects the amount of energy needed to melt the film. It is in general, proportional to the weight fraction crystallinity of the film (Aiba *et al.*, 1985). The gel swelling index data shown in Fig. 2 indicated that the films prepared from 80% DD chitosans had less crystallinity. The maximum melting temperature of film prepared from 80% DD chitosan was lower than that from higher DD chitosan (Table 1), which supported the above finding. However, the maximum melting temperature of films prepared from 90% DD chitosan did not differ significantly from that of 100% chitosans.

The gel swelling index of films of 90% DD and 100% DD chitosan were different, although the maximum melting temperatures were the same (Fig. 2). This discrepancy may be because the crystallinities of films of 90 or 100% DD chitosans were not very different (Table 1). However, the number of free functional groups (e.g. amino groups) which tend to form hydrated water did differ between these two films. In films prepared from 90% DD chitosans, there should be more free functional groups than in films from 100% DD chitosans. Because films prepared from 90% DD chitosans absorbed more water, the gel swelling index was higher. This may be attributed to different chain flexibility which results in different probabilities of inter- and intramolecular hydrogen formation (Chen, submitted) between molecules of 90 and 100% DD chitosans. Since 100% DD chitosan is more flexible (Fig. 1) and forms more inter- and intra-molecular hydrogen bonds, less free functional groups are available to form hydrated water. Consequently, the gel swelling index between the two films is different, although the maximum melting temperatures of the films is almost the same.

### Effect of chain flexibility of chitosans on the tensile strength of the films prepared from high DD chitosans

Tensile strength is an important mechanical property to consider in many applications (Collins *et al.*, 1973). Films prepared from higher DD chitosans were stronger than those from lower chitosans (Table 2). Mima *et al.* (1983) reported similar results. This implies that chain flexibility may cause different crystallinity or junction density (Kienzle-Sterzer *et al.*, 1982) formation. Chitosans of higher DD were reported to be more flexible (Chen *et al.*, submitted). The more flexible chitosans tend to form inter- or intra-chain hydrogen bonds easier than the less flexible chitosans; therefore, the tensile strength of 90 or 100% DD chitosan films was higher than 80% DD chitosan films, both in the dry and wet state. This reaffirms that the film prepared from lower DD chitosans had lower crystallinity. Crystallinity prevents the absorption of water molecules so that the gel swelling index decreases with increasing DD of chitosans (Fig. 2). Absorbed water tended to decrease tensile strength of the film, due to a lubricating, plastic effect. Since there is less crystallinity in films prepared from 80% DD chitosans, these films absorbed more water (Fig. 2) and weakened in tensile strength (Table 2).

The ratio of tensile strength of the dry film to the wet film reflected the different crystallinity in the film (Mima *et al.*, 1983). Film of higher crystallinity was reported to have a lower ratio of tensile strength of the dry film to the wet film. Results shown in Table 2 agreed with those of Mima *et al.* (1983). Table 2 also shows that the tensile strength of films prepared from different DD chitosans in the same media pH were different. However, the effect of media pH on the tensile strength of the films prepared from the same DD chitosan was not significant. This may be attributed to the neutralization process employed during film preparation, which was explained earlier.

### Effect of LiCl concentration in the casting solvent on the tensile strength of the films prepared from low DD chitosans

The effect of LiCl concentration in the casting solvent on the tensile strength of films prepared from low DD chitosans may be due to the different chitosan solubility in DMA–NMP–LiCl systems. Rutherford (1978) reported that before the addition of LiCl, chitosan molecules swelled and dispersed in DMA or NMP solvent, but did not dissolve. Only after the addition of LiCl, did the chitosan molecules dissolve in the solvent system as a result of crystalline rupture by the LiCl. The higher the DD of the chitosan, the easier it is for the chitosan to be dissolved in the DMA–NMP–LiCl system. The positive, linear effect of the LiCl concentration in the casting solvent on the tensile strength indicated its crucial role; a minimum concentration of LiCl is required in making film that has the correct mechanical properties. Aiba *et al.* (1986) reported that DMA–NMP–LiCl had higher tensile strengths than film from formic acid and trichloroacetic acid, in both the dry and wet state. Aiba *et al.* (1985) reported a tensile strength of an unannealed chitosan membrane from DMA–NMP–LiCl of 2·2 ± 0·3 MPa, whereas a membrane annealed at 145°C for 2 h had about twice that value. Annealing made the membrane dense and strong due to the increase in crystallinity. In comparison, the tensile strength of chitosan films prepared from low DD (<44%) and from high DD (>80%), in DMA–NMP–LiCl and an acetic acid solvent system, implied the importance of hydrogen bonding in facilitating crystallinity (Tables 2 and 3).

## ACKNOWLEDGEMENTS

The authors wish to express their appreciation for the financial support of the National Science Council, Republic of China (Project No: NSC 81-0409-B-019-10). Assistance with the English was provided by Dr J.E. Lewis, Marine Biology Institute, National Taiwan Ocean University.

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