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Effect of molecular weight of chitosan with the same degree of deacetylation on the thermal, mechanical, and permeability properties of the prepared membrane

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Different molecular weight, 90% deacetylated chitosans were obtained by ultrasonic degradation on 90% deacetylated chitosan at 80°C for various times.

Ninety percent deacetylated chitosan was prepared from alkali treatment of chitin that was obtained from red shrimp waste. Number average-, viscosity average- molecular weights were measured by gel permeation chromatography and the viscometric method, respectively. Degree of deacetylation was measured by the titration method. Enthalpy, maximum melting temperature, tensile strength and elongation of the membranes, flow rate of permeates and water are properties measured to elucidate the effect of molecular weight of chitosan on the above thermal, mechanical, and permeation properties, respectively of the prepared membranes. Results show tensile strength, tensile elongation, and enthalpy of the membrane prepared from high molecular weight chitosans were higher than those from low molecular weight. However, the permeability show membranes prepared from high molecular weight chitosans are lower than that from those of low molecular weight. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Chitosan is a biodegradable biopolymer. Its membrane has been proposed for use in food processing (Bai *et al.*, 1988; Muzzarelli, 1977), membrane separation (Aiba *et al.*, 1986), chemical engineering, medicine and biotechnology areas (Senstad & Mattiasson, 1988). Mechanical properties, permeability, thermal decomposition points, solvent stability, etc. are parameters considered in the selection of the right film used in each application (Collins *et al.*, 1973).

The selectivity of the membrane is a critical parameter in membrane separation. Factors affecting the selectivity of a membrane are numerous, membrane pore size (Craig *et al.*, 1970), swelling index (Mochizuki *et al.*, 1989), thickness (Nakatsuku & Andrady, 1992), charge effect (Uragami *et al.*, 1983, Sawayanagi *et al.*, 1982), membrane making condition (Kienzle-Sterzer *et al.*, 1982; Aiba *et al.*, 1986; Hwang *et al.*, 1986), annealing treatment (Aiba *et al.*, 1985), casting method (Ogawa *et al.*, 1992, Samuels, 1981), solute characteristics such as molecular weight (Blair *et al.*, 1987; Samuels, 1981), chain flexibility (Chen *et al.*, 1995) and its derivative (Hirano *et al.*, 1981), etc. Biocompatability is another parameter considered in choosing a membrane applied in biomedical field. Chitosan membrane is not antigenic in a mammalian test system and is non-thrombogenic (Muzzarelli, 1977). It is very suitable to use as an artificial kidney (Hirano & Noishiki, 1985), drug delivery system (Sawayanagi, 1982).

Numerous studied are aimed at improving the selectivity of chitosan membrane. Blending with hydrophilic compounds such as chitosan-poly(vinyl pyrolidine) membrane (Qurashi *et al.*, 1992), chitosan-poly (vinyl alcohol) membrane (Blair *et al.*, 1987; Nakatsuka and Andrady, 1992), chitosan-cellulose membrane (Hosokawa *et al.*, 1990) allows manipulation of the chain flexibility of chitosan used (Chen *et al.*, 1995).

Effect of molecular weight on the physical properties of chitosan membrane has been explored. Blair *et al.*, (1987) reported that tensile strength and elongation increased proportionally with M_v of chitosan used. Samuels, (1981) reported that molecular weight of chitosan affects the crystal size and morphological character of its cast film. Ogawa *et al.*, (1992) reported crystallinity of membrane increased with decreasing chitosan molecular weight. However, the molecular weight of chitosan used in their reports were not controlled to a certain distribution due to laborious tasks required. The chitosans employed in those reports varied in the degree of deacetylation. This may due to the lack of easy, cheap preparation method to prepare a large amount of different molecular weights. However, the degree of deacetylation affects the physical properties of the chitosan membrane (Samuels *et al.*, 1981; Mochizuki *et al.*, 1989; Chen *et al.*, 1994, 1995). Wang & Qin (1989) reported that the degree of deacetylation did not change during ultrasonic degradation on the chitosan. Molecular weight distribution of treated chitosan approached a finite value after some time at constant sonification conditions.

(1) Molecular weight of solute affects its chain flexibility in solution which in turn affects its physical properties of the membrane prepared. (2) Contrasting results of Blair *et al.* (1983) and Ogawa *et al.* (1992) regarding the effect of molecular weight on the tensile strength of the prepared membrane. Furthermore, different molecular weight, same DD chitosans are possible to prepare. The effect of molecular weight of chitosan (same DD) on the thermal, mechanical, and permeability properties of the chitosan membrane can be more specifically studied. The combined effect of molecular weight and chain flexibility (solution pH dependent) can also be explored.

MATERIALS AND METHODS

Chitinous materials preparation

Red shrimp (*Solenocera prominentis*) waste was used to prepare Chitinous material with a degree of deacetylation of about 40% by Chen *et al.*, (1994) method.

Preparation of 90% DD, different molecular weight chitosans

Ninety per cent DD, different molecular weight chitosans were prepared by alkali (50%) deacetylation on 40% DD Chitinous materials prepared from; (1). Chitosan alkali solution heated at 110°C for 2h, washed and heated again at 110°C for another hour to get 87% DD chitosan solution. It was continuously deacetylated at 140°C for 3 h to get 90% DD chitosan (Hwa, 1994). The 90% DD chitosan was dissolved in 2% acetic acid and then ultrasonically degradaded (Crest 950 D, USA) for 4, 12, 24 h at an energy levels of 180 watts at 80°C. The degraded solutions were neutralized with 0.1 N NaOH to precipitate the degraded chitosans. They were collected and washed with water until neutral then dried to get different molecular weight, 90% DD chitosans (Wang & Qin, 1989).

Degree of deacetylation determination

Titration method reported by Toei & Kohara (1976) was followed. 0.5 g of chitosan was dissolved in 99.50 g 5% (v/v) formic acid. 1.00 g of chitosan/formic acid solution was mixed with 30 ml distilled water. After adding 2 to 3 drops of 0.1% toluidine blue (indicator), the solution was titrated with n/400 PVSK (potassium polyvinylsulfate) which had been calibrated with cetylpyridinium chloride monohydrate. The degree of deacetylation was calculated as follow:

$$DD(\%) = [X/161/(x/161 + Y/203)] \times 100$$

$$X = 1/400 \times 1/1000 \times f \times 161 \times V$$

$$Y = 0.5 \times 1/100 - X$$

V: Titrated volume (mL) of n/400 PVSK; *f*: Factor of n/400 PVSK solution.

Molecular weight determination

Viscosity molecular weight determination

Five concentrations of 0.01, 0.025, 0.05, 0.10, and 0.20% chitosan solutions were prepared. Relative viscosity was measured with Cannon Fenske, No. 100 capillary viscometer at 30±0.05°C in bath water (Tamson, TMV 40, Sweden) and an extra temperature controller (Firstek, B403, Taipei). Intrinsic viscosity is defined as

$$[\eta] = (\eta_{\text{red}})c \rightarrow 0$$

It was obtained by extrapolating the reduced viscosity vs concentration data to zero concentration. The intercept on the abscissa is the intrinsic viscosity. Whereas, viscosity molecular weight was calculated based on Mark Houwink equation ($[\eta] = KM^a$) with $K = 1.64 \times 10^{-30} \times DD^{14}$ and $a = -1.02 \times 10^{-2} \times DD + 1.82$ (Wang *et al.*, 1991) here, DD is the degree of deacetylation of chitosan.

Molecular weight determination by gel permeation chromatography

Arcidiacono and Kaplan (1992) method was followed. Amicon Model G22 × 100 column packed with Fractogel TSK HW65 (S) gel loaded with 0.1% (w/v) samples were eluted with 0.15 M acetic acid, 0.35 M sodium acetate, 0.08 M sodium azo. Elution times and volumes of samples and markers (dextran standards, Fluka, with molecular weight ranged $4.0 \times 10^5 \sim 6.7 \times 10^6$ Dalton) were determined and recorded with a RI detector (Gilson Model M132, USA) and a recorder (Servocorder SR 6311, Watanabe, Japan), respectively.

The number average molecular weight was determined from K_{av} vs. log MV (logarithm molecular weight) standard curve. Kav was obtained by the following equation:

$$K_{av} = (V_e - V_o)/(V_t - V_o)$$

Here, V_e is the elution volume; V_0 is the void volume; V_t is the total volume.

Chitosan membrane preparation

Casted method was used to prepared the membrane. The chitosan solution was spreaded on a clean glass plate. The glass plate was then oven dried at 50°C to get the membrane.

Enthalpy and maximum melting point determination

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

Mechanical properties determination

Tensile strength and elongation of membrane were measured by a rheometer (Rheometer CR-200D, Sun Scientific, Japan) at a strain rate of 20 mm/min. The membrane was cut into strip (10 × 40 mm) by a punch press tester (Hung Ta HT-8024, Taiwan).

Determination of the membrane permeability

Membrane disc of 4.5 cm diameter that has an effective permeation area of 10 cm^2 was sealed in the cell of a reverse osmosis apparatus resembling C-54402001A (Abcor Inc., USA) reported by Yang and Zall, (1984). The dextran 4 (molecular weight 4000–6000 Dalton), cytochrome C (Mw 12500) were eluted at an operation pressure of 20 kg/cm^2. The permeate was collected by a fraction collector (Gilson F203, USA) at the rate of one tube every 10 min. The concentrations of permeate was monitor by a RI detector. The permeability was calculated from the concentration changes of feed and permeates by using the following equations:

$$P = 100 - R$$

$$R = \frac{C_F - C_P}{C_F} \times 100$$

Here P is the permeability, R is the rejection, C_F is the concentration of the feed, and C_P is the concentration of the permeate.

RESULTS

Preparation of different molecular weight, 90% DD chitosans

Table 1 shows viscosity average molecular weight (M_v) and number average molecular weight (M_n) of 90% DD chitosan decreased with increasing sonification time. However, the degradation rate also decreased with sonification time. After 4 h sonification, the M_v decreased to 3.41 × 10^5 from 4.10 × 10^5, whereas after 8 h treatment, the M_v decreased to 2.96 × 10^5 from 3.41 × 10^5. After 24 h degradation, the M_v decreased to 2.40 × 10^5. The decrease in Dalton rate was 1.75, 0.50, and 0.25 × 10^5 Dalton/h during 4, 8, and 24 h treatment, respectively. The trends in decreasing M_n were similar to that of M_v (Table 1).

Effect of molecular weight, solution pHs on the enthalpy of the membranes prepared

Table 2 shows that the membrane prepared from M_v 4.10 × 10^5 Dalton, pH 3 solution has an enthalpy of 39.40 cal/g/s, the highest. Membranes prepared from the same solution pHs, had enthalpies which decreased to 16.71 cal/g/s as M_v decreased to 2.40 × 10^5 Dalton. Similar trends occurred in membranes prepared from pH 2 and pH 4.5 solutions. Of the same M_v or M_n, the

Table 1. The changes of viscosity average-, number average-molecular weight of 90% DD[1] chitosan along with ultrasonic time

Time[2]	M_v[3]	M_n[4]	M_v/M_n
0	4.10 × 10^5	1.61 × 10^5	2.54
4	3.41 × 10^5	1.81 × 10^5	1.88
12	2.96 × 10^5	1.32 × 10^5	2.24
24	2.40 × 10^5	1.06 × 10^5	2.26

1. DD: Degree of deacetylation.
2. Hour of ultrasonic degradation at 80°C, 180 W.
3. M_v: viscosity average molecular weight.
4. M_n: number average molecular weight.

Table 2. Enthalpy (cal/g/s) of chitosan membranes prepared from chitosan of different molecular weight and different solution pH

M_n ($\times 10^{-5}$)	M_v	Enthalpy (cal/g/s)		
		pH 2.0	pH 3.0	pH 4.5
1.61	4.10	36.83±2.83	39.40±6.44	32.84±9.55
1.81	3.41	33.89±7.84	28.82±3.61	25.95±3.25
1.32	2.96	22.99±1.29	17.22±1.49	21.25±10.00
1.06	2.40	22.55±9.56	16.71±0.85	21.23±4.98

M_n: Number average molecular weight.
M_v: Viscosity average molecular weight.
The data are the mean of 3 samples.
Heating rate 10°C/min.

enthalpies of membranes prepared from different solution pHs were not significantly different.

Effect of molecular weight and solution pHs on the maximum melting point of the membranes

Table 3 shows that the maximum melting point of the membranes were not affected significantly by the changes in the molecular weight of chitosan and/or solution pHs.

Effect of molecular weight and solution pHs on the tensile strength of the membrane prepared

Table 4 shows that the membrane prepared from M_v 4.10 $\times 10^5$ Dalton, pH 2 solution has a tensile strength of 247.5 kg/cm^2. It was 90.6 kg/cm^2 prepared from M_v 2.40 $\times 10^5$ Dalton of the same solution pH. The lower the molecular weight of the chitosan, the lower the tensile strength of the membrane prepared. Similar trends occurred in the membranes prepared from pH 3 and pH 4.5 solutions. However, membranes prepared from different solution pHs of the same M_v or M_n were not remarkably different.

Effect of molecular weight and solution pHs on the tensile elongation of the membrane prepared

Figure 1 shows that a membrane prepared from M_v 4.10 $\times 10^5$ Dalton, pH 4.5 solution has a tensile elongation of 110%, which is the highest. Tensile elongation was 50% for that prepared from M_v of 2.40 $\times 10^5$ Dalton with the same solution pH. Tensile elongation of membranes prepared from pH 4.5 solutions were higher than that from pH 2 or pH 3 solutions especially among membranes prepared from M_v 4.10 $\times 10^5$ Dalton samples. However, the effect was not obvious for M_v 2.40 $\times 10^5$.

Effect of molecular weight of chitosan on the permeability of the membrane prepared.

Figure 2 shows that the permeability of membrane prepared from solution pH 4.5 decreased inversely with increasing M_v of chitosan used. Membrane prepared from low M_v chitosans have higher permeability. The permeability also decreased with running time and approached nearly constant after 30 min. The highest permeability was about 50%.

Table 3. The maximum melting point temperature (°C) of chitosan membranes prepared from chitosan of different molecular weight and different solution pHs

M_n ($\times 10^{-5}$)	M_v	Maximum melting point temperature (°C)		
		pH 2.0	pH 3.0	pH 4.5
1.61	4.10	130.53	127.26	119.96
1.81	3.41	109.94	104.79	110.12
1.32	2.96	115.47	122.94	112.08
1.06	2.40	110.36	109.80	99.18

M_n: Number average molecular weight.
M_v: Viscosity average molecular weight.
The data are the mean of 3 samples.
Heating rate 10°C/min.

Table 4. The tensile strength (kg/cm^2) of chitosan membranes prepared from chitosans of different molecular weight and different solution pHs

M_n ($\times 10^{-5}$)	M_v	Tensile strength (kg/cm^2)		
		pH 2.0	pH 3.0	pH 4.5
1.61	4.10	247.5±23.3	239.6±25.7	234.2±30.8
1.81	3.41	181.9±18.7	210.0±23.5	184.0±18.2
1.32	2.96	133.0±24.9	130.6±11.9	107.2±27.4
1.06	2.40	90.6±13.1	93.8±27.8	66.9±11.5

M_n: Number average molecular weight.
M_v: Viscosity average molecular weight.
The data are the mean of 7 samples.

DISCUSSION

Different molecular weight chitosan prepared by ultrasonic treatment

The ultrasonic degradation rate in the molecular weight of chitosan was affected by sonification energy (Watts) and solution temperature during treatment. Data in Table 1 indicate that a decrease in molecular weight slows down along with the sonification time. Wang & Qin (1989), Tsaih (1993) reported similar results. Tsaih (1993) reported molecular weight decreased to one half of its starting sample after 30 minutes of sonification at 71 watts, 98°C compared to 83% (operated at 180 watts, 80°C for 4 h) shown in Table 1. Wang & Qin (1989) reported that intrinsic viscosity decreased to 54% after sonification at 60°C for 3 h (not specified in Watts output). After 4 h treatment the M_n increased; in contrast, the M_v continued to decrease. It could be attributed to the aggregates of chitosan formed during gel permeation chromatography, as reported by Domard & Rinaudo (1985).

Crystallinity of the membrane

Enthalpies represent 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. Table 2 shows enthalpy of 39.40 cal/g/s measured from a membrane prepared from M_v 4.10 $\times 10^5$ Dalton, pH 3 solution. Of the same solution pH, enthalpy decreased with the decrease in the molecular weight of chitosans used. This indicates that the lower the molecular weight of the chitosan used, the lower the enthalpy (crystallinity) of the membrane prepared. The results were inconsistent with those reported by Ogawa *et al.*, (1992). Crystallinity increased with the increasing molecular weight of chitosan used, as shown in Table 2. This may be attributed to the higher entanglement resulting from the use of high molecular weight chitosan. It may also be due to the small intermolecular distance that facilitates the intermolecular interactions. This is because the membranes were prepared from 3% solution. From intrinsic viscosity data, 3% were over their reduced concentration ($c[\eta]$). Therefore, the solution may be considered concentrated. The inter-molecular distance was small and tended to result in an ordered structure, resulting in high crystallinity. In comparison to the results of Ogawa *et al.* (1992), crystallinity increased inversely with the molecular weight of chitosan. This may be because the membranes of Ogawa *et al.* (1992) were prepared from 2% chitosan solution. In low concentration solution, the solute has better fluidity. Furthermore, membrane formation time of their report, was longer (dried at 50°C in ambient temperature for this and their report, respectively) than the process used in this report. Longer membrane formation time, and better solute fluidity, facilitated the smaller molecule re-arrangements. Therefore, smaller molecules tended to form ordered structures. The discrepancy may also be attributed to different methods used in monitoring the crystallinity in the membrane. DSC was employed to determine the membrane enthalpy (crystallinity) for this report, whereas the X-ray diffraction method was used by Ogawa *et al.* (1992). DSC was considered more sensitive than X-ray diffraction in monitoring the small crystallinity in the membrane (Kesting, 1971).

Maximum melting temperature of the membrane

Table 3 shows no significant differences in maximum melting temperature. This may be because the molecular weight distribution was not sharp enough (Table 1) to cause any significant difference.

Tensile strength of the membrane

Table 4 indicates that the lower the molecular weight of chitosan used, the lower the tensile strength the membrane became. This may be due to the chance of entanglement differences. The use of lower molecular weight chitosan produced less entanglement. Crystallinity difference may be attributed to another factor. Table 2 demonstrates that the lower the molecular weight of chitosan used, the lower the enthalpy of the resulting membrane. This implied that the lower tensile strength of the membrane was a result of less crystallinity in the membrane prepared from low molecular weight chitosan.

Tensile elongation of the membrane

Figure 1 shows not only the molecular weight, but also the molecular structures that affect the tensile elongation of the membrane prepared. The larger the molecular weight of the chitosan used, the higher the tensile elongation of the membrane became. Blair *et al.*, (1989) reported similar results as shown in Fig. 1. This may be attributed to a higher molecular weight of the chitosan used, raising the chances of molecular entanglement formed during film formation. This in turn, results in better tensile elongation. With respect to the effect of molecular structure, Lin (1992) reported chain flexibility was different for chitosan molecules in different solution pH. Chain flexibility of chitosan molecules in solutions was in the order pH 4.5 > pH 2 > pH 3. The highly flexible molecules tended to form coil structure. Membranes formed by coil molecules tended to have higher tensile elongation. Therefore, molecular structures affect tensile elongation. However, the effect of solution pH on the membrane prepared from 2.40 × 10^5 was not obvious. It may be due to the combined effects of the extended structure of chitosan molecule in pH 2 solution reported by Lin (1992) and lower entanglement during film formation by using lower molecular weight chitosans.

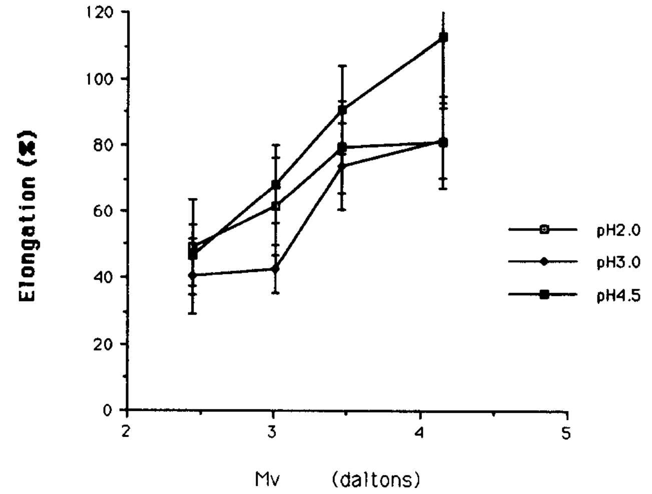

Fig. 1. Effect of molecular weight of chitosans and solution pHs on the tensile elongation of the prepared membranes. x-axis shows $M_v \times 10^5$.

Permeability

UF was usually operated at 3 kg/cm^2 and the permeability increased with running time. However, the permeability decreased with running time as shown in Fig. 2. This may be because the permeability test was operated at a pressure of 20 kg/cm^2 because of the lower porosity of the membranes prepared. At the beginning, the permeability was high, but decreased with running time due to concentration polarization (Kuo & Cheryan, 1983). However, flux of distilled water did not change with running time.

Permeability increased in the amorphous region of the membrane (Kesting, 1971). Table 2 shows enthalpies of membranes were lower for those prepared from

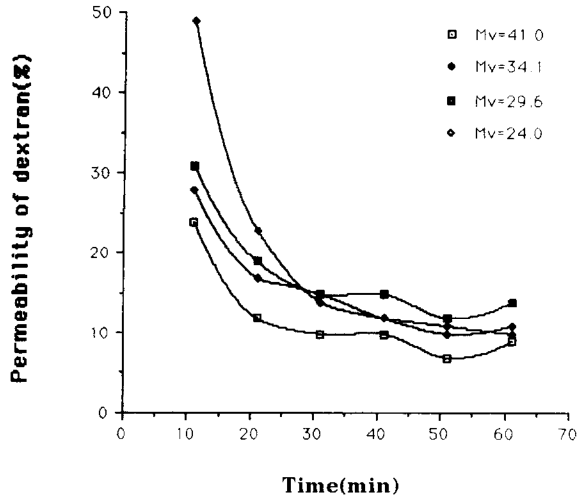

Fig. 2. Effect of molecular weight of chitosans on the permeability of the prepared membranes.

low molecular weight chitosans. The trends were consistent with the results shown in Fig. 2. Similar results were reported by Mochizuki *et al.* (1989) and Qurashi *et al.* (1992). They reported that permeability increased with the increasing swelling index of the membranes. Swelling index is proportional to the amorphous region. After 30 minutes, the permeability approached to a constant. This may due to concentration polarization.

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