

Studies on Chitin. 2. Preparation and Properties of Chitin Membranes

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SUMMARY

A chitin membrane was prepared by a new procedure involving coagulation of the chitin solution in N,N-dimethyl acetamide, N-methyl 2-pyrrolidone and lithium chloride (DMA-NMP-LiCl) with 2-propanol. The solute permeability, water sorption and mechanical properties were compared with membranes prepared by two previously reported methods (coagulation of a formic acid and dichloroacetic acid (FA-DCA) solution of chitin with 2-propanol; and coagulation of a trichloroacetic acid and dichloroethane (TCA-DCE) solution of chitin with acetone). The permeability coefficients of the three chitin membranes were higher than a regenerated cellulose membrane (Cuprophane®). The membrane prepared from DMA-NMP-LiCl solution had a higher tensile strength (3.3 Mpa) in the wet state than the others. The membrane obtained from TCA-DCE solution absorbed more water (360%) and the membrane prepared from FA-DCA solution was relatively weak (1.8 MPa) in the wet state. It was suggested that 2-propanol was a favourable coagulant for membrane production. In addition, the effect of the origin of chitin on molecular weight and tensile properties of the membranes was studied.

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1. INTRODUCTION

Chitin, (1,4)-2-acetamide-2-deoxy- β -D-glucan, is one of the most important renewable resources (Muzzarelli, 1977; Hirano & Tokura, 1982). Chitin is almost insoluble in many solvents and so is difficult to process. Muzzarelli (1983) has reviewed recent trends in applied research on chitin and summarized a number of new ways of dissolving chitin and preparing membranes and fibres.

Chitin is a hydrophilic polymer and has structural similarities to cellulose. It has potential as a blood dialysis membrane. A number of reports have appeared which have implied the feasibility of chitin for a dialysis membrane. Sannan *et al.* (1975) and Tokura *et al.* (1979) prepared chitin films from alkali and formic acid/dichloroacetic acid (FA-DCA) solutions, respectively; film properties, however, were not studied. Kataoka & Ando (1979) prepared semipermeable chitin membranes from a trichloroacetic acid/dichloroethane (TCA-DCE) solution and evaluated these using 0.1% dextran (Agency of Industrial Science and Technology, 1979). Hirano *et al.* (1980) studied the ultrafiltration properties of a regenerated chitin membrane and Uragami *et al.* (1981) reported the water flux and poly(ethylene glycol) rejection of chitin membranes cast from *N,N*-dimethyl acetamide/*N*-methyl 2-pyrrolidone/lithium chloride (DMA-NMP-LiCl) solution. Rutherford & Dunson (1984) found that chitin films cast from the DMA-5%LiCl solvent system could potentially be used as reverse osmosis membranes.

To the best of our knowledge, the permeation characteristics of chitin and its derivatives when used as a dialysis membrane have been described in only three papers. Miya *et al.* (1982) studied the feasibility of chitosan membranes for dialysis and their permeability to vitamin B₁₂ was about twice that of a regenerated cellulose membrane (Cuprophane®, ENKA Granzstoff AG). In two patents it was claimed that the dialysis characteristics of chitin and acetyl chitin membranes were superior to that of Cuprophane (Mitsubishi Rayon Co., 1979, 1981).

In these publications and patents, the origin and the method of preparation of chitin and the molecular weight varied, so it is difficult to compare the data.

In this paper we report on a new method of preparing a chitin membrane and describe its permeation and mechanical properties. The same starting material (crab shells) was used throughout the work.

2. EXPERIMENTAL

2.1 Materials

A chitin sample (C-1) processed according to the method of Hackman (1954) from the shells of Benizuwaigani (*Chionoecetes japonicus*) was obtained from the Katakura Chikkarin Co. Ltd, Wakkanai, Japan. In addition, three chitin samples, C-2, C-3 and C-4, were prepared in our laboratory from the shells of Benizuwaigani, Zuwaigani (*Chionoecetes opilio*) and Tarabagani (*Paralithodes camtschaticus*), respectively. In Table 1 the ash content, water content, elemental analysis data, degree of *N*-acetylation of the chitins, and intrinsic viscosity and viscosity-average molecular weight (M_v) of chitosans obtained from the chitins are reported. M_v was estimated by the method of Roberts & Domszy (1982).

All solvents and reagents were of guaranteed reagent grade except for NMP, which was of extra pure grade. Cytochrome C (Type III) was purchased from the Sigma Chemical Co. Ltd, and used without further purification. Cuprophane PT-150 was used after extraction of plasticizers. Elemental analysis was performed at the Ibaraki Environment Technical Center.

2.2 Preparation of chitin membrane from FA-DCA solution (FA-chitin membrane)

Chitin solution (1% w/w) in FA-DCA (5% DCA v/v) was prepared by the method of Tokura *et al.* (1979), except that they diluted the solution with diisopropyl ether whereas this was not done in our case. The solution was filtered through glass filter G-1 and degassed under reduced pressure. The solution was cast on a clean glass plate and immediately coagulated in 2-propanol. The chitin membrane thus obtained was immersed in water, 1% ammonia solution and water, in turn. The membrane was annealed in water at 80°C for 2 h, then cooled to room temperature and dried in the atmosphere and then under reduced pressure.

TABLE 1
Characterization of Chitin Samples

Sample ^a	Ash content (%)	Elemental analysis			Acetyl content ^b (%)	Chitosan	
		C	H	N		Intrinsic viscosity ^c (cm ³ g ⁻¹)	Viscosity-average molecular weight, ^d × 10 ⁶
C-1 (<i>Chionoecetes japonicus</i>)	6.3	42.01	5.90	6.06	104	1161	1.75
C-2 (<i>Chionoecetes japonicus</i>)	0.1	46.32	6.53	6.80	96	2063	3.26
C-3 (<i>Chionoecetes opilio</i>)	0.1	46.16	6.53	6.80	97	1982	3.11
C-4 (<i>Paralithodes camtschaticus</i>)	0.3	46.36	6.46	6.82	96	1954	3.06

^a C-1 chitin was supplied by Katakura Chikkarin Co. Ltd. C-2, C-3 and C-4 chitins were prepared in our laboratory.

^b Based upon C/N ratio.

^c Measured at 25°C in 0.1M acetic acid and 0.2M sodium chloride.

^d Calculated using the equation of Roberts & Domszy (1982).

2.3 Preparation of chitin membrane from TCA-DCE solution (TCA-chitin membrane)

Chitin solution (1% w/w) in TCA-DCE (weight ratio 7:13) was prepared according to the method of Kataoka & Ando (1979). The solution was cast and then immediately coagulated in acetone. The membrane obtained was then treated as described in Section 2.2.

2.4 Preparation of chitin film from DMA-NMP-LiCl solution (DMA-chitin membrane)

Chitin solution (0.75% w/w) in DMA-NMP-LiCl (100:100:5 in weight) was prepared by the method of Uragami *et al.* (1981). A chitin membrane was obtained as described in Section 2.2, except that treatment in ammonia solution was omitted.

2.5 Measurements of permeation, water sorption and mechanical properties

Permeation experiments were conducted at 37°C using the permeation cell and apparatus in a similar manner to that of Nakagawa & Fujiwara (1983). Urea (initial concentration, 0.5% in water), creatinine (0.01%), glucose (0.5%), sucrose (0.5%), vitamin B₁₂ (0.01%) and cytochrome C (0.05% in 0.05M tris-acetic acid buffer solution (pH 7.4)) were used as test solutes. A Laboratory Data Control Refracto Monitor III SF-1107 was used for the detection of urea, glucose and sucrose and a Varian VARI-CHROM UV-Vis detector for creatinine (wavelength 232 nm), vitamin B₁₂ (360 nm) and cytochrome C (410 nm). Permeability coefficients, P (cm² s⁻¹), were calculated from a plot of $\ln(C_0 - 2C')/C_0$ against time (t) according to the following equation:

$$t = \frac{-VL}{2AP} \ln \frac{C_0 - 2C'}{C_0}$$

where V , L , A and C_0 are cell volume (200 ml), membrane thickness (cm), effective membrane area (7.07 cm²) and initial concentration of the tested solute, and C' is the concentration of dialysate at time t (s). The water sorption was calculated using the following equation:

$$\text{water sorption (\%)} = ((W_2 - W_1)/W_1) \times 100$$

where W_1 and W_2 represent the weight of dry and wet membranes, respectively. The tensile properties were measured on a Shimadzu Autograph DSS-100 at a temperature of 37°C maintained by a water bath. Crosshead speed was 100 mm min⁻¹. The initial length between clamps was 3 cm and specimen width was 0.5 cm. All the samples were used after immersion in water for about 1 week.

3. RESULTS AND DISCUSSION

The chitin sample (C-1) manufactured at Katakura Chikkarin Co. Ltd is a source of chitosan which is used as a flocculant in industry. This sample had a relatively high ash content. The deviation of the acetyl content of the four samples from 100% was believed to be due to experimental error. The molecular weight of chitin can be estimated from that of the chitosan prepared from it. The viscosity-average molecular weights of the chitosans prepared from the four chitin samples ranged from 1.75 to 4.87×10^6 daltons.

Using sample C-1, a chitin solution in DMA-NMP-LiCl was prepared. The solution (1%) was very viscous because of its high molecular weight and so was diluted to 0.75%. The solution was very hygroscopic and gradually coagulated at the surface on adsorption of water vapour from the atmosphere. When the solution cast on a glass plate was immersed in water, the gel membrane formed shrunk considerably, but in 2-propanol the solution coagulated with minimal contraction. The gel membrane thus obtained in 2-propanol was then immersed in water. The membrane shrunk slightly and DMA, NMP and LiCl were washed out. On annealing in water, the membrane became transparent and the DMA, NMP and LiCl was removed. Thus, we obtained for the first time a regenerated chitin membrane from DMA-NMP-LiCl solution by coagulation with 2-propanol. Uragami *et al.* (1981) prepared chitin membranes from DMA-NMP-LiCl solution by evaporating DMA and NMP at various temperatures and then coagulating with water, whereas Rutherford & Austin (1978) prepared chitin fibers from DMA-LiCl solution by coagulating with acetone, and Rutherford & Dunson (1984) prepared chitin films from DMA-5%LiCl solution by coalescing with water vapour and then with acetone.

The DMA-chitin membrane, prepared by the procedure of coagulation with 2-propanol, was compared with other membranes. The FA-

chitin membrane and TCA-chitin membrane were somewhat translucent in the wet state, whereas the DMA-chitin membrane was transparent in both dry and wet states. In Table 2 the water sorption and mechanical properties of the membranes are summarized. All the membranes were hard, stiff and brittle in the dry state. The dry membranes absorbed about three times their weight of water. The water sorption of the membranes was larger than that of Cuprophane. The DMA-chitin membranes had higher tensile strength than the others in both the dry and the wet states and had a much higher elongation at break compared with the others in the wet state. These facts imply that FA, DCA and TCA cause the cleavage of glycoside bonds. The use of the solvent system, DMA-NMP-LiCl, and the coagulation with 2-propanol seem to be an improvement over other aspects of preparation. It was expected from structural and hydrogen bonding considerations that the chitin membrane would be stronger than Cuprophane. The DMA-chitin membrane, however, was much weaker than Cuprophane in the wet state, as is clearly shown by the data in Fig. 1. This seems to be due to the difference of crystallinity, crystal structure and orientation between chitin and cellulose. The chitin membranes absorbed more water than Cuprophane. This suggested that the chitin membranes were more amorphous than Cuprophane.

An original chitin flake prepared from a shell of Benizuwaigani by the Hackman's method was very strong and absorbed less water than the regenerated chitin membranes. The water sorption of the original

TABLE 2
Water Sorption and Mechanical Properties of Chitin^a Membranes

<i>Solvent</i>	<i>Water sorption (%)</i>	<i>Tensile strength at break (MPa)</i>		<i>Elongation (%)</i>	
		<i>25°C dry</i>	<i>37°C wet</i>	<i>25°C dry</i>	<i>37°C wet</i>
FA-DCA	260	29 ± 8	1.8 ± 0.6	3.0 ± 0.7	39 ± 14
TCA-DCE	360	5.5 ± 1.0	2.2 ± 0.4	6.1 ± 2.2	23 ± 6
DMA-NMP-LiCl	280	38 ± 4	3.3 ± 0.5	5.7 ± 2.1	71 ± 17
Cuprophane	100	—	29 ± 3	—	38 ± 7

^a Membranes prepared from Chitin sample C-1.

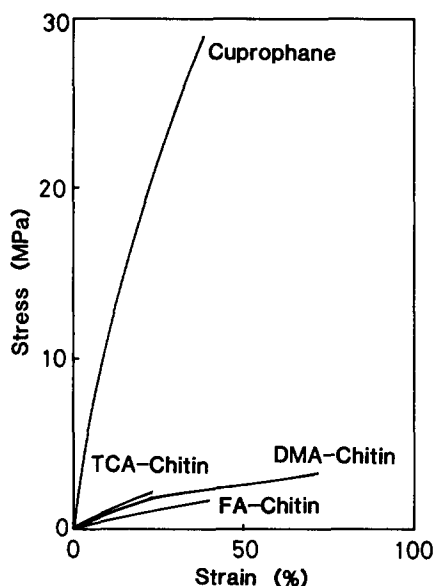


Fig. 1. Tensile stress-strain curves of the membranes at 37°C in water.

chitin flake was 77%. This suggests that the regenerated chitin membrane does not retain the original crystal structure and has a low crystallinity. It seems reasonable to assume that, in the dry state, carbohydrate chains in amorphous regions formed hydrogen bonds randomly between them but in the wet state the hydrogen bonds are broken by water. Kataoka & Ando (1979) also reported that the water content of a regenerated TCA-chitin membrane was higher than that of original chitin powder and showed that this was due to the differences in crystallinity.

Figure 2 shows the solute permeability coefficients of the membranes plotted against solute molecular weight. The three chitin membranes had greater permeability than Cuprophane, particularly for higher molecular weight solutes. This is partly due to the higher water sorption of the chitin membrane compared with Cuprophane. Two patents claimed that the dialysis characteristics of a chitin membrane were superior to that of Cuprophane (Mitsubishi Rayon Co., 1979, 1981). The permeability of their chitin membranes to vitamin B₁₂ was twice that of Cuprophane. The DMA-chitin membrane prepared by our method had four times the permeability coefficient of Cuprophane to vitamin B₁₂, and the FA-chitin membrane nine times.

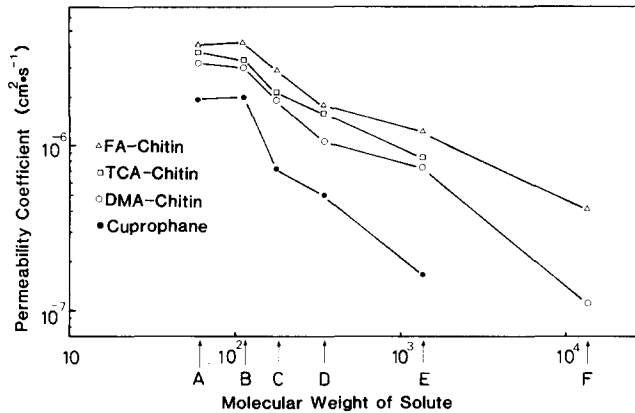


Fig. 2. Plot of permeability coefficients of the membranes as a function of test solute molecular weight: A, urea; B, creatinine; C, glucose; D, sucrose; E, vitamin B₁₂; F, cytochrome C.

TABLE 3
Properties of Chitin Membranes^a from Different Crabs

Sample	Water sorption (%)	Tensile strength ^b at break (MPa)	Elongation ^b (%)
C-2	200	2.8 ± 0.8	60 ± 9
C-3	210	3.0 ± 0.7	61 ± 10
C-4	210	3.3 ± 0.8	66 ± 12

^a Membranes prepared using DMA-NMP-LiCl.

^b Measured at 37°C in water.

The effect of the source of the chitin on the mechanical properties of the membrane are summarized in Table 3. There was no clear difference in tensile strength between membranes prepared from C-2, C-3 and C-4 because their viscosity-average molecular weights were very large and did not differ greatly (Table 1). Mima *et al.* (1983) reported that the weight-average molecular weight (M_w) of chitosan from *Chionoecetes opilio* was 0.6×10^6 . Wu & Bough (1978) obtained a value for M_w of 1.7×10^6 for chitosan produced by Kyowa Oil and Fat Co., Japan. Rutherford & Austin (1978) estimated the M_v of chitin from

Chionoecetes opilio to be 1.3×10^6 . The C-1 sample had a similar M_v to those described above, but C-2, C-3 and C-4 had higher molecular weights although the measurement procedures differed. It would be desirable to develop a standard procedure for measuring the molecular weight of chitosan.

4. CONCLUSIONS

A method for preparing a chitin membrane from DMA-NMP-LiCl solution by coagulation with 2-propanol was developed. The DMA-chitin membrane was stronger in the wet state than the other chitin membranes and had a higher permeability to solutes than Cuprophane, especially for higher molecular weight solutes. However, DMA-chitin membranes are weaker than Cuprophane membranes in the wet state. Further studies on chitin membranes are now in progress.

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