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The Processing of Chitosan Membranes

MIT Sea Grant
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Massachusetts
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Cambridge
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RELATED REPORTS

The following reports contain information related to the project described herein:

Ashford, Nicholas A., Dale Hattis, and Albert E. Murray. INDUSTRIAL PROSPECTS FOR CHITIN AND PROTEIN FROM SHELLFISH WASTES. MITSG 77-3. NTIS: PB-267 527. Cambridge: Massachusetts Institute of Technology, 1976. 99 pp. $12.50.

CHITIN AND CHITIN DERIVATIVES. MIT/Marine Industry Collegium Opportunity Brief. MITSG 76-5. NTIS: PB-262-557/AS. Cambridge: Massachusetts Institute of Technology, August, 1976. 21 pp. $2.50.

Muzzarelli, R.A.A. and E.R. Pariser, editors. PROCEEDINGS OF THE FIRST INTERNATIONAL CONFERENCE ON CHITIN/CHITOSAN. MITSG 78-7. Cambridge: Massachusetts Institute of Technology, 1978. 652 pp. $15.00.

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ABSTRACT

The processing steps in the production of chitin and chitosan from blue crab shells have been investigated. Crushed shells were treated with dilute hydrochloric acid to remove calcium carbonate and then with dilute sodium hydroxide to remove protein. The resultant product was chitin, N-acetyl-D-glucosamine, and this was deacetylated in concentrated sodium hydroxide to produce chitosan. Variations in the demineralization, deproteination and deacetylation reactions were studied with the objective of making chitosans which would produce membranes with good mechanical properties.

Techniques for the casting of chitosan membranes from dilute acetic acid solutions were developed, and the mechanical properties of the membranes were measured. The tensile modulus of these membranes was approximately 6300 MPa (915,000 psi). There was a distinct yield point at about 100 MPa (14,500 psi) and the tensile strengths were of the order of 150 MPa (22,000 psi) with elongations of about 40%. The membranes were clear, tough, flexible and insoluble in water. Good membranes could be produced from chitosans which exhibited molecular weights from 600,000 to 1,300,000 and viscosities from 0.15 to 2.0 $NS.m^2$ (150-2000 centipoise).

I. INTRODUCTION

Chitin is one of the principal constituents of the exoskeletons of crustaceans. It is a polysaccharide, N-acetyl-D-glucosamine, and it has a chemical structure similar to cellulose. Specific enzymes in natural environments decompose chitin, but it is resistant to the usual acid, alkali, and organic solvents. Only a few specialized organic solvent systems are able to dissolve chitin without destroying the basic chemical bonding (1).

Chitosan is obtained by deacetylating chitin in strong caustic solutions. This reaction is indicated schematically in Figure 1. The deacetylated structure has one amino group and two free hydroxyls per glucose ring. This material is hydrophillic and a cationic polyelectrolyte. Chitosan is a good chelating agent for heavy metal and various other ions in aqueous solutions, and the chemical reactivity appears to be a consequence of the free amino groups. Chitosan is soluble in weak acetic and formic acids, in contrast to the relative insolubility of chitin.

These properties of chitosan have generated considerable interest. The polycationic nature has been utilized to coagulate wastes in food processing plants and sludge in sewage treatment plants. The chelating properties of

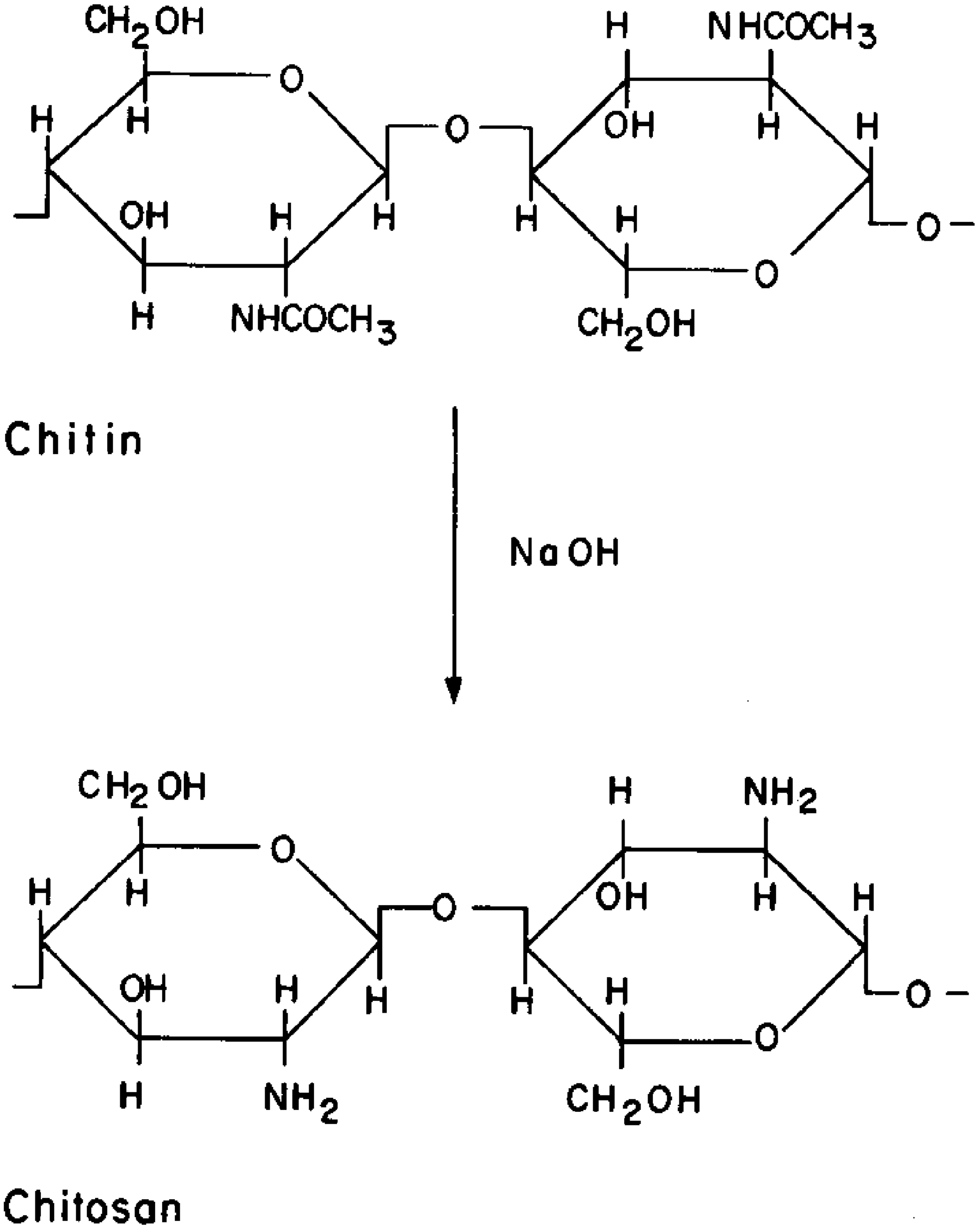

Fig. 1. The deacetylation of chitin to chitosan.

chitosan have been used for trace ion detection in sea water and for metallic ion removal from manufacturing waste waters. Films can be formed from chitosan with ease, and fibers and membranes of high tensile strength have been produced. Applications for chitosan membranes as food wraps and dialysis membranes have been proposed.

Chitosan is not a well-defined chemical, however, and the behavior of the material is influenced by the natural source of the chitin as well as by the processing steps. Crustacean shells contain not only chitin but protein and minerals, primarily calcium carbonate, and the relative amounts of the constituents vary from species to species. The processing methods used to extract chitin from the shells vary somewhat, but, in general the calcium carbonate is removed with dilute hydrochloric acid, and the protein with weak sodium hydroxide. These reactions must be carried out in such a way as to remove these constituents of the shells without affecting the chitin.

The chitin thus obtained is then deacetylated, and this step has a large effect on the properties of the resultant chitosan. Chitin is approximately 10% deacetylated in the natural form. On the other hand, chitosan, as it is usually made, is usually not completely deacetylated, and in some cases the deacetylation may be as low at 70%. This wide range in deacetylation levels, 70 to 100%, results in chitosans with a variety of properties, and the consequent variability has introduced some confusion about the material. If the deacetylation conditions are too mild, the product will be insoluble because of the residual chitin. If the conditions are too severe, the molecular weight of the chitosan polymer will be reduced and the chemical and physical properties will be affected.

In this study we report on our observations of the processing steps. The efficacy of the demineralization and deproteination procedures were judged on the basis of determinations of calcium contents, ash contents, and weight loss. In general, we selected the mildest conditions for these parts of the processing. The deacetylation was carried out with variations in the time, temperature, and concentration of caustic. The various chitosans were then dissolved in mild acetic acid solutions and the viscosities and molecular weights were determined. These data are summarized in the next section of this report.

We evaluated the applicability of these chitosans by casting membranes from dilute acetic acid solutions and then determining the mechanical properties of the membranes. The chitosan films were clear and flexible, with excellent strengths and ductility values. They were insoluble in water and impervious to air. There was sufficient latitude in the processing to make film production a practical process, and there seems to be considerable promise in the applications of these membranes. The data on chitosan membranes are reported in Section 3.

Our work suggests that chitosan processing must be carried out carefully if good reproducible material is to be made. The processing is not complicated, however, and procedures for the control of the processing are practicable.

II. BACKGROUND

Early History

Chitin was first isolated by Braconnot (2) in 1811. For many years it was thought to be a form of cellulose. By the 1890s, however, it had been established that chitin contained nitrogen, in contrast to cellulose. Tracey (3) provides a review of the early literature. In the 1920s, attempts were made to find applications for chitin. Kunike (4) dissolved it in an "acid solution" and produced chitin fibers. Also, Knecht and Hibbert (5) made chitin membranes using concentrated hydrochloric acid. These processes were abandoned by later workers, perhaps because the severe acidic treatment hydrolized the chitin.

Chitosan was produced and named by Hoppe-Seyler (6) in 1894. In 1936, Rigby (7) established the deacetylation process which is used today, and he investigated the solubility of chitosan in a wide variety of organic acids. He also produced membranes and adhesives of chitosan (8, 9), and many of the subsequent activities with chitin and chitosan are based on his pioneering work.

Membranes and Fibers

In 1926, Kunike (4) reported chitin fibers with a tensile strength of 340 MPa (50,000 psi). Strengths at this level have not been obtained by other investigators, however. In that same year, Knecht and Hibbert (5) were able to produce chitin membranes but had no success with fibers. About ten years later, Rigby (8) patented a technique for producing chitosan membranes, and, as indicated earlier, this remains the basis of the current work in this area. In 1940, Thor and Henderson (10) developed a chitin xanthate dispersion and were able to make membranes with tensile strengths of about 90 MPa (13,000 psi).

Interest in films and fibers waned for about thirty years. In 1971, Nud'ga et al. (11) made chitosan films and studied the molecular weight fractions of chitosan. Joffe and Hepburn (12) investigated the mechanical properties of the regenerated chitin films produced by Thor and Henderson over thirty years before. They observed a 45% drop in tensile strength due to aging. In 1973, Noguchi et al. (13) spun fibers from chitin viscose. Yaku and Yamashita (14) cast chitosan membranes and then reacetylated them to produce chitin. In 1974, Arai and Akiya (15) demonstrated the potential of chitosan membranes for desalinating brine. They reported that when a saline solution was poured onto a chitosan membrane and subjected to pressure, the water which permeated the membrane was almost pure.

That same year, Muzzarelli et al. (16) cast chitosan membranes from 70% formic acid solutions and examined some of the mechanical and chemical properties. In 1975, Brine and Austin (17) were able to dissolve chitin in trichloroacetic acid and produce fibers and films from the solution.

A number of papers presented at the first International Conference on Chitin/Chitosan in 1977 dealt with membranes or fibers. Averbach (18) presented work from this laboratory on the film forming ability of various chitosans. Moore and Roberts (19) reported complete reacetylation of some chitosan membranes using mixtures of methanol and acetic anhydride. Noguchi et al. (20) updated the work on chitin fibers. Hepburn and Chandler (21) presented work on the mechanical properties of films and fibers of regenerated chitosan and chitin. They found that the deformation in most of these materials was due to the basic molecular conformational changes typical of viscoelastic materials. Rutherford and Austin (1) discussed a new chitin solvent system (dimethylacetamide containing 5% lithium chloride) and reported on chitin films and filaments produced with this system. These papers indicate the growth of research interest in films and fibers of chitin and chitosan.

Chitosan Processing

Figure 2 is a schematic summary of chitosan processing from crustacean shells. First, the minerals are removed with a weak solution of hydrochloric acid. Next, a weak sodium hydroxide solution is used to remove intrinsic protein. In some instances the order of these two steps is reversed, but the resulting chitin is the same. Finally, the chitin is deacetylated in a strong caustic solution, and the resultant product is chitosan.

Some investigators have examined variations of the processing parameters to determine the effect on the product. Lusena and Rose (22) studied the effect of processing on the viscosity of chitosan solutions. They found that some polymer degradation occurred even during demineralization. They also reported that if the sodium hydroxide concentration was decreased, a longer deacetylation time was required to render the product soluble. Wu and Bough (23) reported the same effect, and noted that it was difficult to dissolve chitosans which had been deacetylated with 35% sodium hydroxide at 100°C. Madhavan and Nair (24) noted that high deacetylation temperatures caused a reduction in the viscosity.

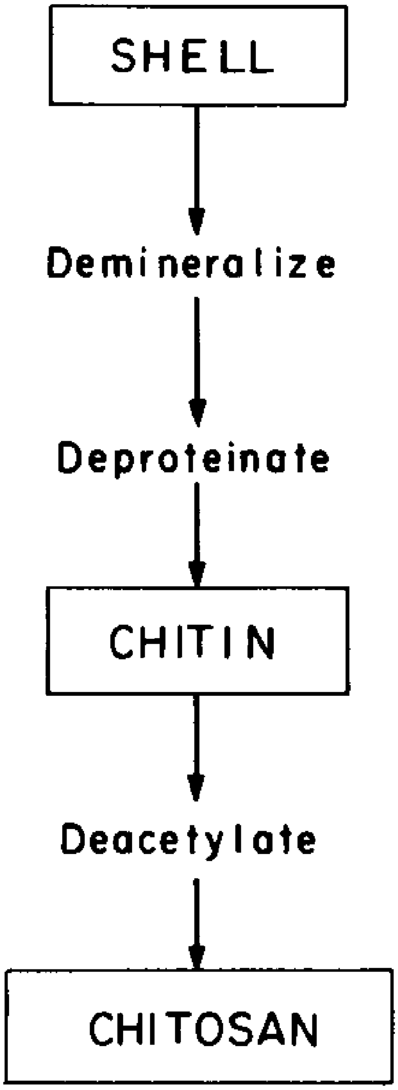

Fig. 2. Steps in the processing of chitosan.

Other Applications

Many other chitosan applications have been proposed. Wu and Bough (23) studied the effectiveness of chitosan in coagulating wastes. Bough and Landes (25) undertook a nutritional evaluation of food processing waste coagulated with chitosan. They found that rats suffered no adverse effects at chitosan levels up to 5% of their diet. Balassa and Prudden (26) reported that both chitin and chitosan can be used to accelerate wound healing in animals and humans. Allan et al. (27) proposed chitosan as a bonding system for paper.

There has also been a substantial amount of work on the chelating ability of chitosan. Considerable data have been presented by Muzzarelli and colleagues (28-34), and they have considered a series of selective chelation reactions for the separation of cobalt, nickel, and copper from iron and manganese (33). This procedure is of potential importance in the recovery of metals from manganese nodules.

Andreev et al. (35) demonstrated that "chitin derivatives" could extract uranium, both as anions and cations, from bottom sediments of water basins. Masri et al. (36) compared the chelating ability of chitosan to that of tree bark, activated sludge, and other substances. They found chitosan to be a superior chelating agent. Masri and Randall (37) investigated the potential of chitosan for the purification of manufacturing effluents. Yaku and Koshijima (38) investigated various chitosan-metal complexes. Lepri et al. (39) separated various inorganic ions using chitosan in chromatography with thin layers and columns, and also examined thin layer chromatography of organic compounds (40). They found that chitosan was superior to treated cellulose for separating phenols.

There are several excellent general summaries of the work on chitin and chitosan. Muzzarelli has written two books "Natural Chelating Polymers" (41) and "Chitin" (42) which provide a good systematic summary of the work to date. Pariser and Bock compiled a complete bibliography of the work on chitin up to 1970 (43) and along with Muzzarelli edited the Proceedings of the First International Conference on Chitin/Chitosan (44). These publications are excellent benchmarks for those undertaking work in this field.

There has been relatively little work on the atomic configurations in processed chitin and chitosan. Some preliminary X-ray diffraction studies were undertaken here in an earlier Sea Grant Program (45), but additional work is needed in light of subsequent developments in the processing of these materials.

III. The Processing of Chitin and Chitosan

Chitosan is not a well-defined material. The starting material, chitin, varies from one crustacean species to another and even from part to part in the same animal. When these variations are compounded with variations in the processing steps it is not unusual to find that chitin and chitosan from various sources may behave differently.

The raw material for this research was restricted to the shell of one species of crab in an attempt to minimize this problem. Shells of blue crabs landed in Hampton, Virginia were used, and only the bottom shells were utilized. The bottom shells are free of pigment and are of a uniform texture and composition. The chitins and chitosans produced from these bottom shells were white and clean, and data from lot to lot were reproducible. Approximately 100 batches were made in the course of this study of the processing of chitosan and chitosan membranes.

Figure 2 provides a summary of the principal steps in deriving chitosan from crustacean shells. The first chemical step is demineralization to remove the calcium carbonate, and this procedure was investigated in some detail since it was known that extreme acid treatments hydrolize chitin (22). Table 1 indicates the effects of the demineralization parameters on impurity content. The first four entries show that the mineral content dropped markedly as the strength of

Table 1

Demineralization of Blue Crab Shells

l concentration %	time hrs.	Calcium content, weight %	Ash, * weight %
2	18	10.	48.5
3	18	6.	26.1
4	18	0.05	.64
4.7	16.5	0.07	1.31
4.7	2	0.48	3.74
5	4	0.13	.64

ll reactions at room temperature.

ethods of analysis outlined in the Appendix.

hydrochloric acid was increased from 2% to 5%. A demineralization treatment in 5% hydrochloric acid for 5 hours at room temperature was selected for all the subsequent processing.

Determinations of the extent of the deproteination process were difficult since the usual protein analysis is based on a measurement of the nitrogen content, and both chitin and protein contain nitrogen. In order to avoid this difficulty the shells were deproteinated under a variety of conditions and the weight loss caused by protein removal was measured. The maximum weight loss was obtained after 2 hours at 85°C in 2% sodium hydroxide, and it was assumed that the protein extraction was near completion after this treatment. All of the material was deproteinated under these conditions. Deproteination was also carried out at 65°C and there were no apparent differences in the chitosans which were eventually produced with either deproteination treatment.

The deacetylation treatments were carried out at relatively low temperatures in an effort to retain relatively high molecular weights in the polymer. The range of temperature chosen was 75° to 100°C. The concentration of sodium hydroxide was varied from 41% to 50%, and the reaction times were 2, 3, and 4 hours. These conditions produced a wide variation in deacetylations and supplemented other work which had been carried out at higher temperatures (23). The chitosan thus produced was in the form of small white flakes. The reactions were carried out with large excesses of the reagents, and the depletion of the active chemical was thus small in each case.

Table 2 summarizes the deacetylation conditions used in this study and indicates the resultant mineral and ash contents of the chitosan. The impurity levels were low in all cases. Batches A, B and C were made from the same lot of chitin and were identical except for the time of deacetylation. Samples D, E and F were intended to explore the effects of caustic concentration and in G, H and J the temperature of deacetylation was varied. These nine batches were selected for our study of the deacetylation procedure and the investigation of the properties of chitosan membranes.

The demineralization and deproteination procedures varied only slightly for these batches. Samples A through F were demineralized in 5% hydrochloric acid for 16 hours at room temperature and deproteinated in 2% sodium hydroxide for 2 hours at 85°C. For samples G, H, and H, the time of demineralization was reduced to 5 hours, and the temperature of deproteination was lowered to 65°C. A comparison of the molecular weights and viscosities (Table 3) of samples F and J, deacetylated identically, indicates that these changes had no effect on the resultant chitosan.

Table 2

Deacetylation Procedures

Sample	Deacetylation Procedure NaOH %	 Temp. °C	 Time hrs.	Calcium wt %	Ash wt %
A	50	100	2	0.016	0.13
B	50	100	3	0.015	0.13
C	50	100	4	0.018	0.13
D	41	100	4	0.060	0.17
E	45	100	3	0.027	0.13
F	50	100	3	0.008	0.10
G	50	75	4	0.023	0.04
H	50	90	3	0.026	0.04
J	50	100	3	0.017	0.02

Table 3 lists the viscosity and molecular weight values. Samples A, B, and C show that the viscosity and molecular weight decreased with increasing time of deacetylation. The same phenomenon was noted by Wu and Bough (23). Samples G, H, and J indicate that an increase in temperature also lowered the viscosities and molecular weights. This agrees with the observations of Madhavan and Nair (24). These six batches show that, as expected, the polymerization of the chitosan decreases with increasing severity of the deacetylation conditions.

Samples D, E, and F appeared to deviate from this trend since the molecular weights and viscosities increased with increasing sodium hydroxide concentration. Wu and Bough (23) noted a similar anomaly, and they felt that this was related to solubility problems with the chitosan. If the deacetylation conditions are too mild, some of the larger crystallites of chitin will only be partially deacetylated and will be insoluble in acetic acid. Only the chitin of lower molecular weight will thus have been made soluble and the resultant chitosan will thus have a low viscosity. It appears that neither 41% (sample D) nor 45% (sample E) sodium hydroxide was sufficient to completely deacetylate the chitin since the viscosity and molecular weight values were unusually low for these batches. Thus, it appears that the sodium

Table 3

Viscosity and Molecular Weight Data

	Deacetylation			Viscosity,	Mw
Sample	NaOH %	Temp. °C	Time hrs.	Viscosity, $N.S/m^2$	Mw 10^6
A	50	100	2	.652	1.012
B	50	100	3	.476	.997
C	50	100	4	.297	.732
D	41	100	4	.148	.634
E	45	100	3	.142	.635
F	50	100	3	.332	.886
G	50	75	4	2.070	1.298
H	50	90	3	1.010	1.094
J	50	100	3	.354	.887

hydroxide concentration should be greater than 45% to satisfactorily deacetylate blue crab chitin at 100°C for less than 4 hours. In subsequent studies of film formation with batches D and E many insoluble translucent fibrils were observed in the membranes. These were thought to be partially deacetylated chitin which failed to dissolve in the dilute acetic acid.

These data indicate that the molecular weights and viscosities can be controlled by a selection of temperatures and times of deacetylation. The concentration of caustic is more critical and it appears that this should be maintained between 45 and 50 wt pct. The deacetylation time may be somewhat sensitive to the flake size of the initial ground shell, although the shell thickness is probably also important. The flake size in all these batches was about 1/4 in. in the longest dimension. Air must be excluded during the deacetylation and this was accomplished by keeping the chitosan below the surface of the caustic solution.

The viscosity is shown as a function of molecular weight in Figure 3. Although there is some scatter, the general trend of the data is similar to that observed by Wu and Bough (23). The high pressure liquid chromatography used to determine the molecular weights utilized a filter with a 0.3 μm pore size to filter the chitosan solution. This

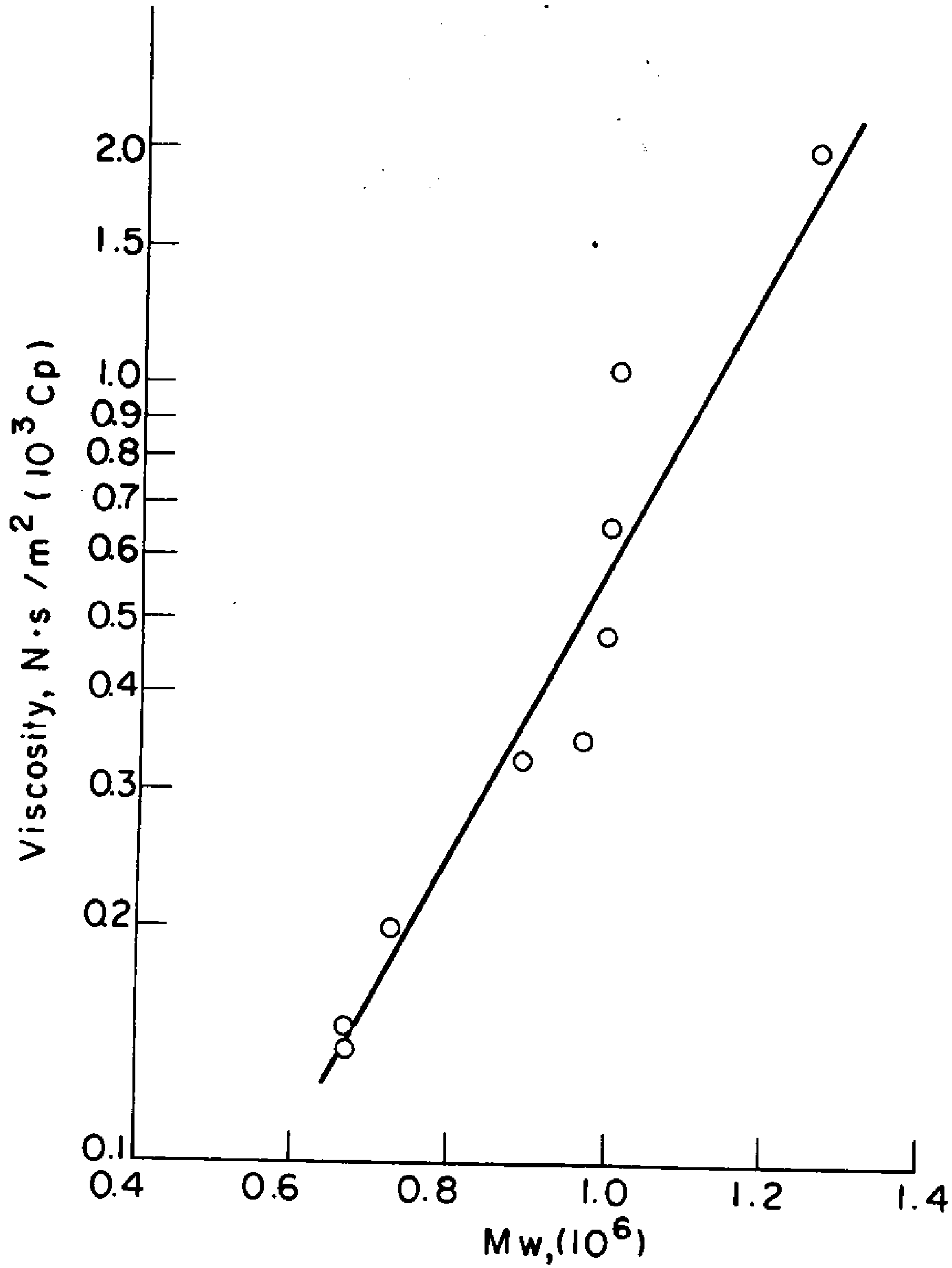

Fig. 3. Viscosity vs. molecular weight of chitosan.

probably removed most of the small fibrils of undissolved chitin, particularly in the material deacetylated under the mildest conditions. The viscosity measurements, however, were made on material which had not been similarly filtered, and this difference in material preparation may account for the scatter in the data shown in Figure 3.

IV. Mechanical Properties of Chitosan Membranes

It has been indicated that chitosan forms good film and there are several reports of membranes cast from various solutions. The techniques used in the production of chitosan membranes were not well established, however, and there were no data on the effects of the processing steps used in making the chitosan on the properties of the corresponding membranes. We thus undertook a program to explore the film casting technique and to then evaluate the mechanical properties of the various chitosans which had been produced in the processing study. These chitosans were all made from a single species of crab, but were processed so that the molecular weights ranged over values from 600,000 to 1,200,000. More extreme variations could have been produced by deacetylating at higher temperatures, but the processing conditions used here are convenient for practical operation, and it seemed useful to determine how closely the deacetylation must be controlled in order to obtain good membranes.

The chemical procedures involved in membrane formation are simple. Chitosan is first dissolved in an acid which will not destroy the polymer. A mineral acid, such as hydrochloric acid, hydrolyzes chitosan and should thus not be used. Acetic and formic acids are both suitable solvents. Chitosan is somewhat more soluble in formic acid, and a considerable amount of preliminary work was done with this solvent. We eventually abandoned formic acid because it appeared that acetic acid was somewhat less irritating to the laboratory personnel. All of the membranes used in this work were cast from dilute acetic acid.

Chitosan forms an acetate when dissolved in acetic acid. The acetate is then cast into a film. This film is clear and flexible and has considerable mechanical strength, but is soluble in water. The film may be treated with an alkali, such as sodium hydroxide. The acetate is removed by the alkali and the resultant film consists entirely of chitosan. The chitosan film is also clear and flexible, and it is insoluble in water. These membranes are insoluble in alkaline solutions and will resist dissolution down to a pH of about 4. X-ray diffraction work in our laboratory has shown that the structure is amorphous and that the films are impenetrable by air.

On the basis of the technology used with similar materials, we believe that an alternate procedure could be used for membrane production. A sufficiently concentrated solution of chitosan becomes viscous and forms a gel. This gel could be extruded into a sheet or tube, dried with warm air and treated with gaseous ammonia to remove the acetate. Such a procedure would be useful in high speed production.

Film Casting

The film-making procedure began with the mixing of 8 gm of chitosan with 784 gm of distilled water. While the mixture was being stirred, 16 gm of glacial acetic acid were added. The acid was added last to avoid lumps in the solution. The solution was stirred for 10 minutes and allowed to stand for 12 hours to allow complete dissolution. Usually, there were still undissolved particles at this point and the solution was filtered through a 40-mesh nickel screen to remove the coarse particles. The viscosity was then measured with a Brookfield LV Viscosimeter, using the No. 1 spindle. The solution was then centrifuged at 1500 rpm through a 30-60 μm TFE filter to remove the fine particles.

Since there was the possibility that centrifuging could degrade the polymer by a shearing process, the viscosities of some batches were measured before and after centrifuging. No changes in viscosity were detected. It should be noted that batch D, which was deacetylated in 41% NaOH at 100°C, contained so many insoluble fine particles that it clogged the filter and could thus not be centrifuged. Membranes were cast from the unfiltered solution, but the mechanical properties were adversely affected by the imperfections.

After centrifuging, the solutions were stirred thoroughly and poured into a level glass tray (29 cm x 29 cm with 3 cm sides). The tray was placed into a chamber with an even flow of forced air which was filtered with nylon mesh to remove dust.

When the film was dry, approximately 40 hours later, 1 molar sodium hydroxide was poured into the tray to convert the chitosan acetate film to a chitosan film. After 30 minutes, distilled water was poured over the film and rinsed to neutrality. At this point the film separated easily from the glass. The wet membrane was placed on a 0.01 inch thick celluloid sheet and was smoothed by hand. A square aluminum frame was placed on the edges of the film, leaving an exposed area about 24 cm by 24 cm. Hand-held blowers were used to dry the film. When it no longer felt clammy, the film was cut away along the inside edges of the aluminum frame and lifted off of the celluloid. The result was a clear, flexible membrane about 50 μm thick.

Mechanical Properties

The mechanical testing procedure followed ASTM method D882. The films were conditioned at 23° ± 3.0°C in 30% - 50% relative humidity for at least 40 hours prior to testing. Test strips 1.27 cm in width and about 16 cm in length were cut with a razor blade. We were able to obtain 223 specimens from 23 films. The minimum thickness was located on each test piece, measured to ± 1 μm with a dial micrometer, and then marked with a felt-tip pen. Because of the presence of insoluble particles, sample D was measured optically in a microscope, with somewhat less accuracy. The strips were examined for flaws and then placed in flat, rubber-faced grips with a 10 cm gage length between grips. The testing machine was an Instron model TTDL. Tensile tests were made at a strain rate of 0.5 per minute at room temperature in 30% - 50% relative humidity. The films were observed during testing to see whether yield and fracture occurred at the minima and whether flaws influenced the results.

Figure 4 shows the characteristic shape of the stress-elongation curve of a typical chitosan membrane. The two almost linear regions were also noted by Joffe and Hepburn (12) for aged regenerated chitin films. However, their material did not exhibit the sharp yield point shown by the chitosan film. The sharpness of this yield point seemed to improve with the uniformity of film thickness. Some of the thinner films exhibited a slight yield drop just past the yield point, as shown in Figure 3. Chitosan falls into the "hard and tough" class of polymers, as judged by its stress-elongation behavior. The high tensile properties and large elongation may also indicate the presence of hydrogen bonding (46).

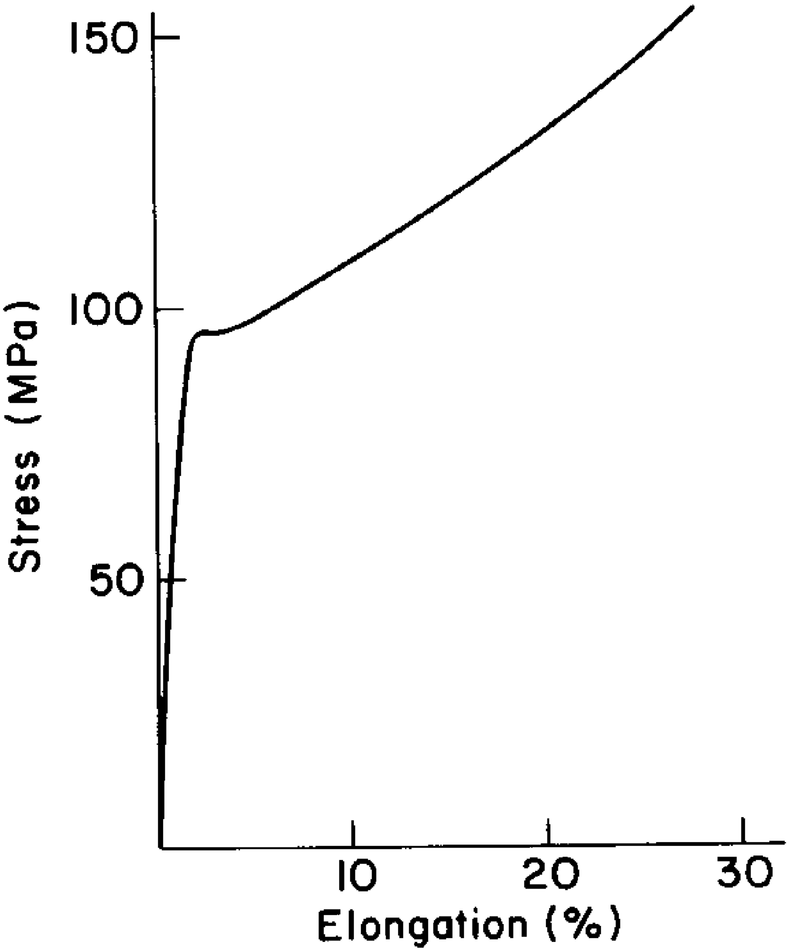

Fig. 4. A typical stress-elongation curve of a chitosan membrane, (strain rate = 0.5 per min.).

The modulus of elasticity was difficult to determine accurately because of the relatively high strain rate. The average value for 194 test pieces was 6300 MPa (914,000 psi), with a standard deviation of 710 MPa, (100,000 psi) based on the minimum rather than average areas. The strain rate was 0.5 per min. Joffe and Hepburn (12) reported a value of 1970 MPa for the regenerated chitin at a strain rate of 0.01 per minute. These values may be compatible since smaller strain rates lead to lower elastic moduli for polymers. Hepburn and Chandler (21) recently tested fibers of chitosan acetate which exhibited moduli of 10,633, 7031, and 6286 MPa for strain rates of 0.0167, 0.0017, and 0.0002 per minute, respectively. Although these moduli were also obtained at smaller strain rates than in our work with chitosan, they were larger. Thus, the elastic modulus of chitosan acetate may be larger than that of chitosan.

The tensile strength varied widely from strip to strip but showed no significant differences between batches. The average value, 135 MPa (19,600 psi), with a standard deviation of 18 MPa (2600 psi), was based only on the 166 samples that broke at or near the marked thickness minima. This result compares favorably with the value of Muzzarelli et al. (16) of 69 MPa (10,000 psi) for chitosan films.

Nud'ga et al. (11) obtained chitosan films of tensile strengths 69 - 108 MPa (10,000 - 15,600 psi). Averbach (18) reported a value of 152 MPa (22,000 psi) for chitosan films prepared earlier in this laboratory using techniques similar to those described.

Table 4 lists the yield strengths of the samples. Examination of the results for samples A - C reveals no clear trend of yield strength with respect to deacetylation time. For samples deacetylated with varying sodium hydroxide concentration (D - F), no trend could be establihsed since the apparent high yield stress for D was probably caused by the inaccurate thickness measurements. On the other hand, deacetylation temperature variations appeared to have a small effect on the yield strength (samples G - J). This is shown graphically in Figure 5. The yield strength remains constant for the lower temperatures but drops at 100°C. However, this slight trend is not comparable to the large drop in molecular weight, illustrated in Figure 6. Thus, the effect of molecular weight on the yield strength was small over this range.

For most high molecular weight polymers, the molecular weight has little effect on the mechanical properties as long as the molecular weight is above a critical minimum. It

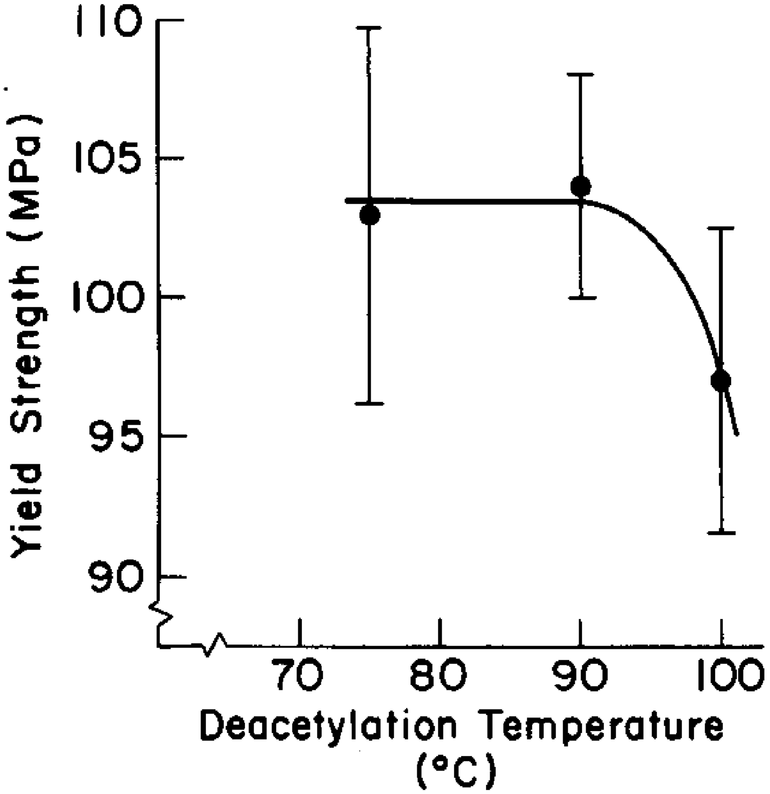

Fig. 5. The effect of deacetylation temperature on the yield strengths of chitosan membranes.

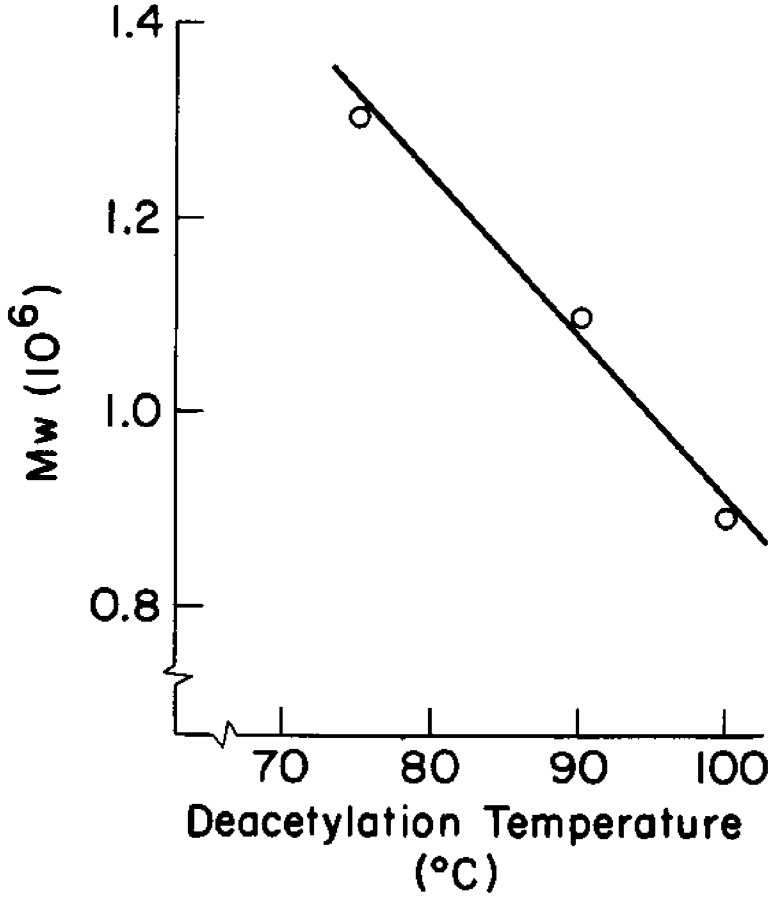

Fig. 6. The effect of deacetylation temperature on the molecular weight of blue crab chitosan.

TABLE 4

YIELD STRENGTHS OF CHITOSAN MEMBRANES

	Deacetylation					
Sample	NaOH %	Temp. °C	Time hours	Number of usable test strips*	Average yield Strength (MPa)	Standard deviation (MPa)
A	50	100	2	13	96.4	6.5
B	50	100	3	20	93.5	4.0
C	50	100	4	18	102	3.5
D	41	100	4	25	116	7.7
E	45	100	3	30	98.4	6.7
F	50	100	3	23	95.0	5.1
G	50	75	4	25	103	6.7
H	50	90	3	31	104	4.0
J	50	100	3	27	97.0	5.3

* includes only strips that yielded near measured minima

appears then that chitosan is such a polymer, and that chitin can be satisfactorily deacetylated without detriment to the mechanical properties if the resultant molecular weight is greater than 600,000. More severe deacetylation would probably affect the mechanical properties.

The total elongations of the chitosan strips are listed in in Table 5. Samples A, B, and C again show the effect of deacetylation time. More tests will be required to confirm whether the increased elongation for chitosan deacetylated for 3 hours is real. The apparent trend with sodium hydroxide concentration, samples D - F, should be viewed with caution since the insoluble particles in D probably caused premature fracture. It is felt that the 8.2% elongation of sample D is not characteristic of the material.

Samples G, H, and J indicated that increased deacetylation temperatures apparently resulted in larger elongations, shown in Figure 7. The reasons for this trend are not clear, but the increase in elongation with increasing deacetylation may be due to the more complete removal of partially deacetylated chitin from the material.

The variations of the yield and tensile strengths with molecular weight are shown in Figure 8. The yield strength is apparently invariant with molecular weight in this range. The

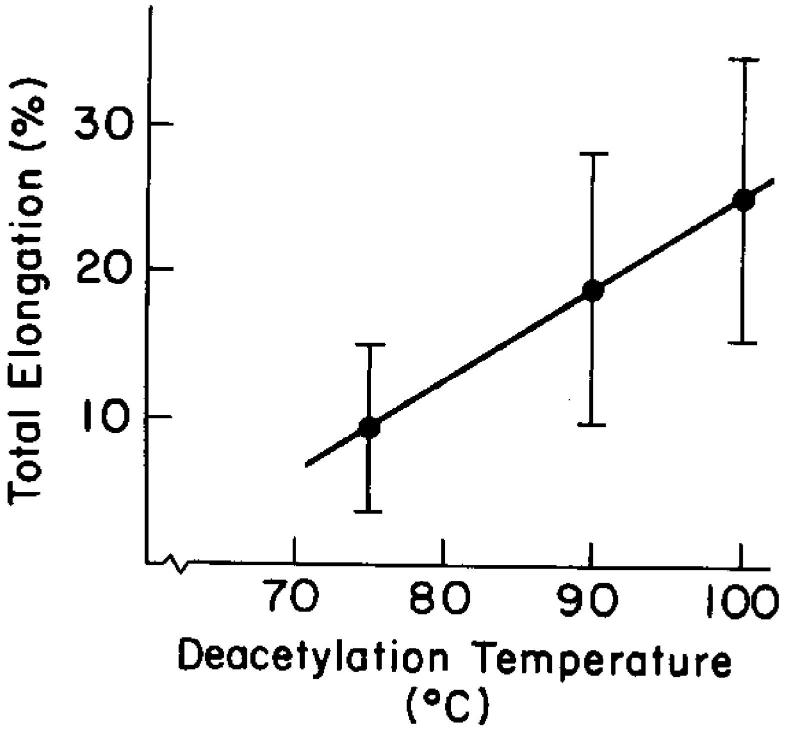

Fig. 7. The effect of deacetylation temperature on the elongations of chitosan membranes.

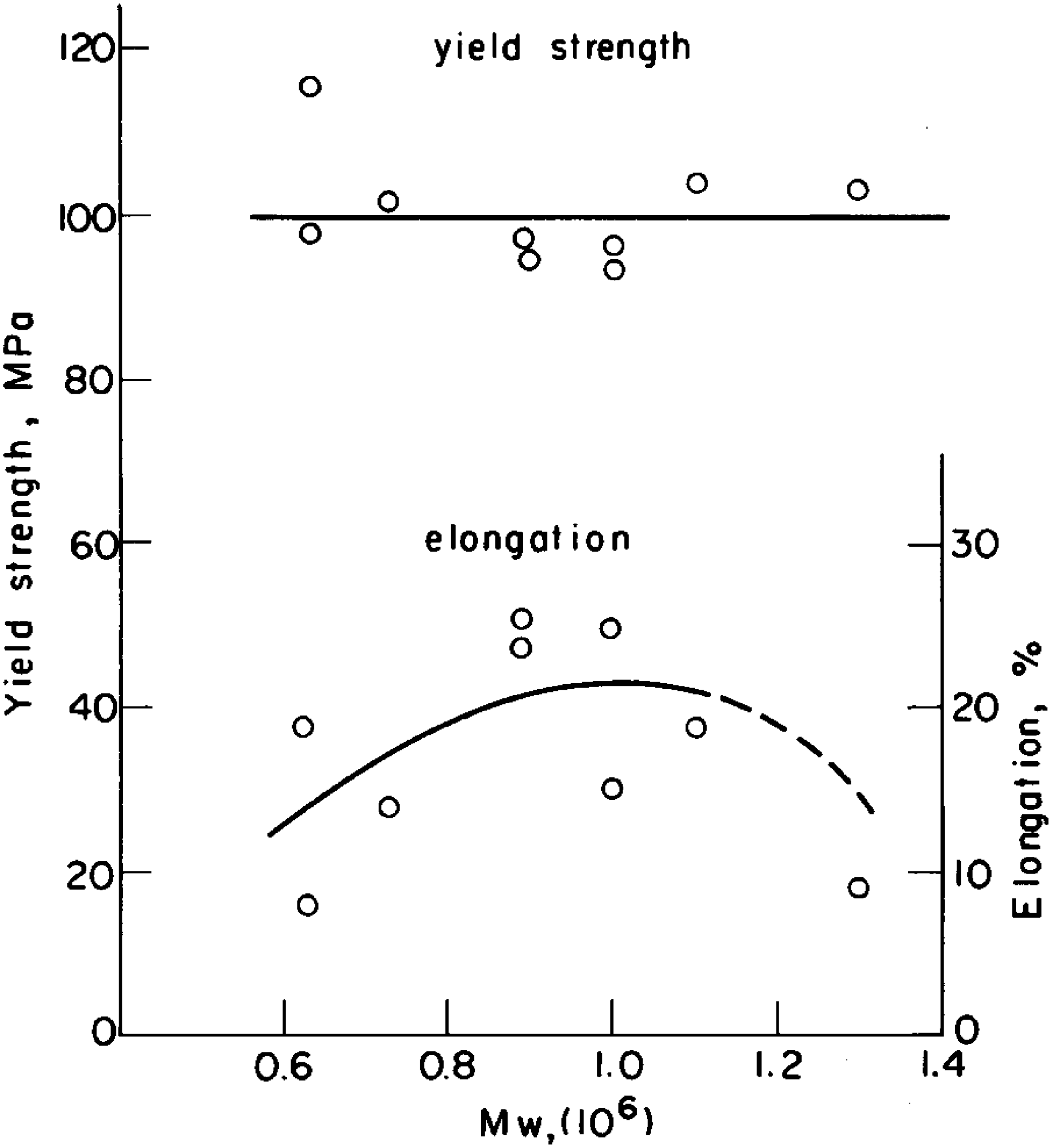

Fig. 8. Yield strengths and elongations as functions of molecular weight.

elongation exhibits a maximum at a molecular weight of about 1 million. This maximum may not be real, since the materials of high molecular weight contained undissolved fibrils which adversely affected the elongation.

TABLE 5

ELONGATION OF CHITOSAN MEMBRANES

Sample	Deacetylation NaOH %	Temp. °C	Time hours	Number of usable test strips*	Average elongation to fracture (%)	Standard deviation (%)
A	50	100	2	12	15	6.4
B	50	100	3	18	25	7.5
C	50	100	4	18	14	7.2
D	41	100	4	28	8.2	4.2
E	45	100	3	23	19	8.4
F	50	100	3	21	24	7.4
G	50	75	4	28	9.1	5.8
H	50	90	3	30	19	9.4
J	50	100	3	27	25	9.6

* excludes strips that broke at flaws

V. SUMMARY

The overall picture which emerges from this study is that chitin and chitosan must be processed with care if a useful uniform product is to be made. None of the chemical steps are severe. The deacetylation reaction is probably the most critical, but this can be carried out successfully in 50% NaOH at 90-100°C within 2-3 hours. Molecular weights in the range 600,000 - 1,200,000 can be achieved without difficulty.

Chitosan membranes can be cast from chitosan dissolved in weak acetic acid, with a concentration of about 1.5 wt pct. The resultant chitosan membranes are clear, flexible and strong. These films have high yield strengths, excellent fracture strengths, good elongations and high moduli. Chitosan membranes are insoluble in water and impervious to air. These films appear to have good potential for use as casings and wrappings in the food industry and such applications should be explored.

Although this is the subject of a subsequent report, we know that chitosan membranes chelate heavy metals and PCBs as well as the bulk flake. These properties are now being explored in our laboratory. It appears that there are several other interesting applications for chitin and its derivations, and we hope that this work on processing will help to establish a base for additional work on the application of these materials.

VI. ACKNOWLEDGEMENTS

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The contributions of a number of people at M.I.T. were greatly appreciated and are hereby acknowledged. Peter Kelleher fabricated most of the equipment used to produce the chitosan membranes. Chemical analyses of the batches of chitosan were performed by Mr. Walter Correia. Two undergraduate students, Evan Klein and Chee Wong, were of great assistance in the laboratory.

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VIII. APPENDIX

Calcium Determination

The calcium content of the chitosan was determined spectrographically. Samples were crushed to 100 mesh or smaller using a mill operated at liquid nitrogen temperatures. The samples were then mixed with high purity graphite and pure germanium, which was used as the internal standard. The constituents, all the size of 100 mesh or smaller, were 10 parts sample, 90 parts germanium, and 200 parts graphite. Calcium standards of varying concentrations were prepared in a similar manner and were used to produce a working curve for the concentrations of calcium in these samples. Standard spectrographic equipment and procedures were used to obtain the calcium contents.

Ash Determination

A weighed sample of chitosan (approximately 2 gm) was placed into a clean, previously ignited, weighed platinum crucible. The crucible and sample were put into a muffle furnace at 300°C, the temperature was gradually raised to 530°C. After approximately 24 hours at this temperature, the crucible was removed and placed in a dessicator to cool. Later, the sample was weighted, and the ash content was calculated.

Molecular Weight

Dr. Wayne Bough and Dr. Arnold Wu, both of the University of Georgia Marine Extension Serive, measured the molecular weights of the batches of chitosan using high pressure liquid chromatography. Their technique is described in reference 23. Basically, the samples were dissolved in 2% acetic acid, with 0.1 M sodium acetate. The materials for the column were Glycophase-CPG, ranging from 40 to 2500 Å pore sizes. The standard curve was calibrated with dextran standards.

Viscosity Determination

The standard solution contained 8.00 gm of chitosan, 16.00 gm of acetic acid, and 784 gm water. This was the same solution used for film casting. The solution was adjusted to 20°C and was stirred thoroughly prior to the test. Spindle #1 of a Brookfield LV viscometer was used to measure the viscosity. The motor speed was chosen to make the deflector needle stop between 45 and 90 so that the smallest possible conversion factor could be used. Four readings were averaged for each measurement.