

Highly Deacetylated Chitosan and Its Properties

SEIICHI MIMA, MASARU MIYA, REIKICHI IWAMOTO, and SUSUMU YOSHIKAWA, *Government Industrial Research Institute, Osaka, Midorigaoka 1-8-31, Ikeda, Osaka, Japan*

Synopsis

A preparative method has been established for obtaining chitosan products which have a desired degree of deacetylation of up to virtually 100%. Effective deacetylation was attained by intermittently washing the intermediate product in water two or more times during the alkali treatment. The weight average molecular weight (\bar{M}_w) of the product, which was measured by gel permeation liquid chromatography, was about 5×10^5 at the highest deacetylation of nearly 100%, and the degradation of the molecular chain was not so significant. Tensile strength of the wet film increased markedly with increasing degree of deacetylation, while the dry film did not show a corresponding significant increase of the tensile strength. In the infrared spectra of chitosan films new sharp bands appeared especially at the high degree of deacetylation. This was attributed to increased "crystallization" brought about by high deacetylation.

INTRODUCTION

Chitosan is the deacetylated product of chitin. While chitin is insoluble in most solvents, chitosan is readily soluble in acidic solutions and more available for industrial applications. However, little work has been done on chitosan, especially on the properties of the solid, and it is desirable to carry out intensive studies on the preparation of chitosan with a desired deacetylation, molecular weight of the product, structure and properties of the solid film, etc.

According to Wu and Bough,¹ deacetylation proceeds to about 70% within the first 1 h of alkali treatment in 50% NaOH solution at 100°C, but it progresses only gradually after this, reaching 80% in 5 h. Further alkali treatment does not deacetylate significantly and only degrades the molecular chain. Thus, it is difficult to prepare by the usual method, chitosan that has a degree of deacetylation >90% and that has not suffered chain degradation. Some attempts have been made to prepare chitosan whose deacetylation is >90%, but the methods used have disadvantages in sample processing resulting in degradation of the molecular chain.²

We have developed a preparative method for chitosan having a desired degree of deacetylation of up to 100%, without serious degradation of the molecular chain. In the present paper we report on the characterization of the obtained chitosan products and show evidence that the highly deacetylated chitosan has excellent mechanical properties, so that in the wet state the film maintains half the tensile strength as in the dry state.

EXPERIMENTAL

Preparation of Chitin

Shells of the crab, *Chionoecetes opilio*, were used as the raw material of chitin. The shells were cut into pieces with the longest side of <2–5 cm only to facilitate treatment in a reaction beaker. The shell pieces were cleansed from proteins and lipids by treatment for 3 h in 1N NaOH solution at about 80°C and washing in water. They were then digested for 12 h in 1N HCl solution at room temperature. The alkali and acid treatments were repeated twice. The chitin was decolorized by refluxing in acetone. The obtained product was snow-white and the ash content was <0.17%.

Preparation of Chitosan

The chitin was treated for 1 (or 2) h in 47% NaOH solution in a Ni crucible at 110°C (or 60°C) under nitrogen atmosphere, without making the sample pieces smaller. The chitosan product obtained by the alkali treatment was washed in water at about 80°C to neutrality, the deacetylation being about 80% or less by the first treatment. The chitosan after being washed in water was treated again in the alkaline solution for further deacetylation. The alkali treatment and washing in water were repeated two or more times to obtain chitosan products which are 90–95% deacetylated. For even further deacetylation, threadlike chitosan was prepared by pouring a 1.5–2% chitosan aqueous solution containing 2% acetic acid in a small stream into a large amount of 1N NaOH solution. The threadlike chitosan was then subjected to alkali treatment.

Determination of Degree of Deacetylation

The colloid titration method was used for determining the free amino group content in chitosan.³ Alternatively, the acetyl content was determined by infrared spectroscopy.⁴

Infrared Spectral Measurements

Infrared spectra were measured by the use of a Nicolet 7199 Fourier Transform Infrared Spectrometer with a resolution of 2 cm⁻¹, 400 scans, and a MCT detector.

Molecular Weight Determination

Molecular weight of chitosan was determined by GPC using a HLC-803B Chromatograph of Toyosoda Manufacturing Co., Ltd. with a RI detector (RI-8) and a low angle light scattering detector (LS-8). The column used was packed with Toyopal HW75 connected to a TSK-G5000PW column. An aqueous solution containing 0.2M acetic acid and 0.1M sodium acetate was used as the eluent in the solvent delivery system.

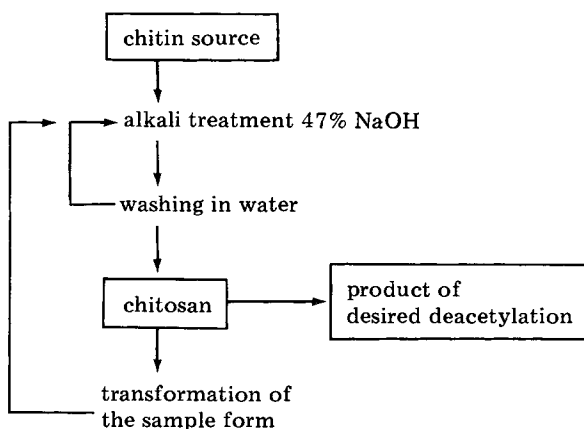
Measurements of Mechanical Properties

Films of chitosan were prepared by casting a 2% acetic acid solution of chitosan on a glass plate and drying thereon at room temperature. The film on the glass plate was neutralized in 1N NaOH solution and then washed thoroughly with distilled water. Tensile strength was measured for the sample pieces (3×50 mm) of about $20 \mu\text{m}$ thickness with a tensile tester (Toyo Measuring Instrument Co. Ltd., Tensilon Model OTM-4). Tensile strength in the wet state was measured immediately after taking out the samples that had been soaked to the equilibrium absorption in water.

RESULTS AND DISCUSSION

Preparation of Highly Deacetylated Chitosan

Effective deacetylation was attained by alkali treatment intermitted by washing in water of the intermediate product instead of the continuous processing. The sample was treated in the form of cut shells in the reaction process, and deacetylation did not proceed homogeneously through the whole bulk of the sample piece. Some portion in the bulk of the sample was less deacetylated as proved by the fact that the product especially of low deacetylation contained some insoluble parts in dilute acetic acid solution. This inhomogeneous reaction prevented further deacetylation especially when the degree of deacetylation was 90% or more. In order to prepare further deacetylated chitosan, the intermediate product obtained by the foregoing treatment was transformed into threadlike pieces which were then subjected to alkali treatment. By a series of alkali treatments, the chitosan product was easily obtained with a degree of deacetylation of up to 99%. The whole process of the alkali/washing treatment is schematically shown below. The results are summarized in Table I.



The progress of the deacetylation reaction is shown in Figure 1, where the H' or L' indicates as denoted in Table I the case in which the treatment was carried out continuously during the whole treatment at 110°C or 60°C , respectively. The accelerating effect of the intermittent washing on the deacetylation rate is clear from comparison of the H and H' or L and L' series. When the sample was

TABLE I
Deacetylation of the Obtained Chitosan Products and Their Molecular Weights

Sample ^a name	Reaction time (h)	Deacetyl- ation (%)	Isoluble parts (%)	\bar{M}_w ($\times 10^{-5}$)
L-1	2	57	40	—
L-2	4	67	11	7.9
L-3	6	84	Trace	8.5
L-4	8	90	Trace	7.0
L-3F	8	95	0	5.9
L'-1	8	70	14	—
H-1	1	78	Trace	6.0
H-2	2	91	0	5.7
H-3	3	96	0	5.7
H-3F	4	99	0	5.0
H'-1	4	82	Trace	6.3

^a L and H denote reaction temperatures of 60°C and 110°C, respectively, and the number after these means times of separate alkali treatment. The letter F indicates additional alkali treatment after the transformation of the sample form into threadlike pieces. The prime means continuous treatment.

continuously treated at 110°C without intermission, the deacetylation was 78% after 1 h, and it increased by only 4% even if the treatment time was extended to 4 h. The saturated deacetylation after the first 1 h was similarly reported by Wu and Bough¹ as shown in Figure 1. On the other hand, if the intermediate product was intermittently washed in water every hour during the treatment, the deacetylation reached nearly 100% in the same total reaction time of 4 h.

The effect of transformation of the sample form is shown by the solid and broken line courses for the L-4 and L-3F samples, respectively, in Figure 1. For the former the progress of deacetylation was 6% by the fourth treatment, while,

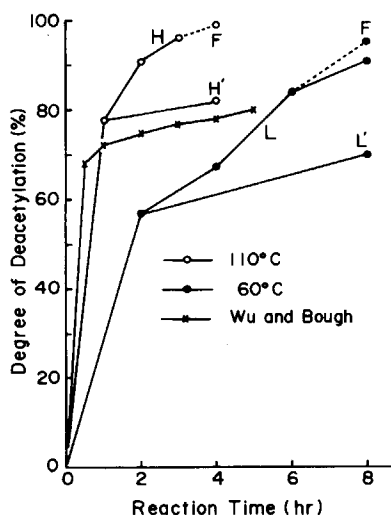


Fig. 1. Progress of deacetylation of chitosan by alkali treatment. The results of Wu and Bough are at the treatment at 100°C and taken from Ref. 1. The notations are the same as in Table I.

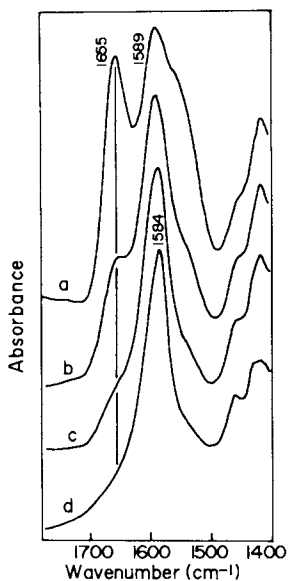


Fig. 2. Infrared absorption intensity of the Amide I band for (a) 78%, (b) 91%, (c) 96%, and (d) 99% deacetylation.

for the latter, which was subjected to the fourth treatment after the transformation of the sample form into the threadlike pieces, it was 11%. At these levels of deacetylation, this progress was very important.

Reaction temperature has a significant effect on the deacetylation as is seen from Figure 1. The rate at 110°C is more than twice as rapid as at 60°C. High reaction temperature, however, has the effect of decreasing the molecular weight to some extent, as is discussed below.

The attainment of virtually 100% deacetylation was also proved by the infrared spectra of the product. The amide I band at 1655 cm⁻¹ gives the amino content in chitosan as reported.⁴ Figure 2 gives the infrared spectra of chitosan samples with different degrees of deacetylations. The top spectrum a has a clear amide I band but the band decreases in intensity down to the bottom. The spectrum c has only a slight upward swelling at the frequency. In the spectrum d, we cannot detect any trace of the band at the frequency, proving nearly complete deacetylation.

Molecular Weight

Weight average molecular weights \bar{M}_w of the products are given in Table I. The \bar{M}_w values are those measured after 2-day storage of the solution after the preparation. The \bar{M}_w tends to decrease gradually as deacetylation progresses, but the degradation is not so serious; for example, the \bar{M}_w of the nearly 100% deacetylated sample H-3F is 5.0×10^5 in comparison with 6.0×10^5 of the H-1 having a deacetylation of 78%. Reaction temperature has a significant influence on the degrading effect of alkali treatment as noted by Numazaki.⁵ The samples H-2 and L-4 have nearly the same deacetylation, but the \bar{M}_w of the former, 5.7×10^5 , is appreciably lower than that of the latter, 7.0×10^5 . A similar difference

TABLE II
 Properties of Chitosan Films

Sample ^a name	Water sorption (%)	Tensile strength (kg/cm ²)		Elongation (%)	
		Wet	Dry	Wet	Dry
L-3	160	225	950	110	68
L-4	135	315	1020	145	86
L-3F	110	510	900	135	70
H-1	165	165	610	110	27
H-2	115	225	690	120	51
H-3	110	340	730	145	30
H-3F	105	355	620	160	30
H'-1	180	210	760	150	75

^a The notations are the same as in Table I.

is seen for the samples H'-1 and L-3, both of which are deacetylated to a similar extent. The results indicate that the alkali treatment should be carried out at as low temperature as possible to avoid degradation of the molecular chain.

The \bar{M}_w values have some unexpected variation among samples, but they are sufficiently reproducible for the same solution of each sample. The variations seem to occur partly from variations in the degrading effect of the alkali treatment or in the deacetylation rate among sample pieces.

The \bar{M}_w values obtained in the present study are generally smaller than those reported by Wu and Bough.¹ The \bar{M}_w of the sample H-1, 6.0×10^5 , is about one half of the value, 11×10^5 , given by them for the sample which was treated in alkaline solution similarly for one hour. On the other hand, Hackman and Goldberg⁶ gave a value of \bar{M}_w of 10.5×10^5 for chitin obtained from *Scylla* crab shell by light scattering. This value corresponds to the \bar{M}_w of about 8.2×10^5 for chitosan if the chitin is completely deacetylated. The maximum \bar{M}_w obtained in the present study was 8.5×10^5 for a deacetylation of 84%. Chitosan, which is prepared by alkali treatment of chitin, cannot have a larger degree of poly-

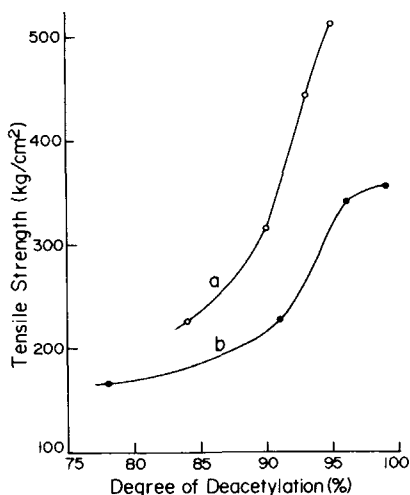


Fig. 3. Tensile strength of the wet chitosan films of (a) L and (b) H samples.

merization than the original chitin. Therefore, the \bar{M}_w values ranging from 5.0×10^5 to 8.5×10^5 in the present study seems to be generally reasonable.

Mechanical Properties

Tensile strengths of chitosan films in the dry and wet states are given in Table II. In the dry state the values vary from 610 to 760 kg/cm² for the H samples, while they are 900 to 1020 kg/cm² for the L samples. There is not a clear relation between the mechanical strength and the \bar{M}_w value and/or the degree of deacetylation in the dry state.

In the wet state, on the other hand, the tensile strengths definitely increase as the degree of deacetylation increases. The strength increases more as the deacetylation approaches 100%, and, besides, it is stronger if the \bar{M}_w is higher, as is demonstrated for the L and H samples in Figure 3. The L samples which have higher molecular weights than the H samples at the same deacetylation have appreciably higher tensile strengths. It should be noted that more than half the tensile strength in the dry state is retained even in the wet state for the highly deacetylated samples such as H-3F and L-3F. The high retainment of the strength in the wet state is comparable with the case of the α -chitin from *Locusta* tendon.⁷ This α -chitin is highly crystalline as well as oriented and has a high tensile strength of 3091 kg/cm² in the dry state and 1908 kg/cm² in the wet state. In the report⁷ by Hepburn and Chandler, chitin is the only one that retains so high a tensile strength in the wet state among the chitin and chitosan samples studied. Chitosan formate in the wet state loses its strength to about one tenth that of the dry sample.

The high degree of retainment and remarkable increase of tensile strength in the wet state of the highly deacetylated samples may be attributed to increased

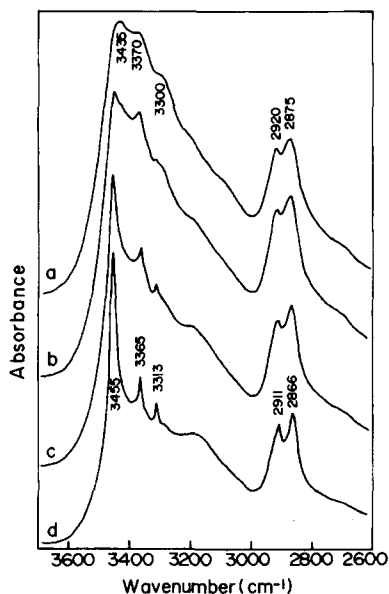


Fig. 4. Infrared spectra of chitosans of the deacetylations of (a) 78%, (b) 91%, (c) 96%, and (d) 99% in the 3600–2600 cm⁻¹ region.

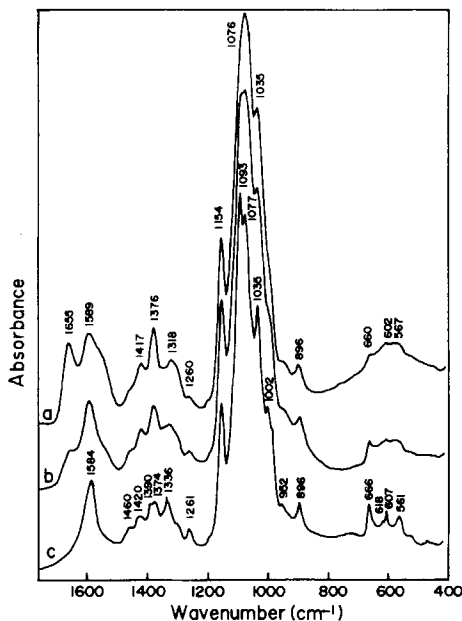


Fig. 5. Infrared spectra of chitosans of the deacetylations of (a) 78%, (b) 91%, and (c) 99% in the 1700–400 cm^{-1} region.

crystallization caused by the high deacetylation, for crystallization should prevent water from entering the grown crystalline portion and render resistance to water.⁸ Infrared spectra gave the evidence that increased deacetylation brings about higher crystallization in the chitosan film.

Figure 4 shows the infrared spectra in the 2600–3800 cm^{-1} region of a series of chitosan samples whose deacetylation varies from 78% to 99% from the top to the bottom. A broad 3435 cm^{-1} band with small shoulders at 3370 and 3300 cm^{-1} is observed when the deacetylation is 78%. Barely observable bands appear at 3455, 3365, and 3313 cm^{-1} if the deacetylation is increased to 91%. At the deacetylation of 96%, distinct and sharp peaks appear at the three frequencies. With nearly complete deacetylation, the three peaks become more enhanced, and at the same time the C—H stretching bands at 2911 and 2866 cm^{-1} become sharper. Judging from the sharpening behavior of the bands and decreasing intensity of the broad band at the base of the three peaks in the 3000–3600 cm^{-1} region against increased deacetylation, the change may be attributed to “crystallization.” It is interesting to note that the three bands appear rather abruptly at a deacetylation between 91% and 96% or when the average sequential length of the regular molecular chain consisting of the glucosamine unit increases from 10 to 20.

The crystallization should give changes in other frequency regions. In fact, similar spectral changes or appearance of sharp bands do occur in the whole frequency region as is shown in Figure 5. The sharp peak at 1376 cm^{-1} observed at the deacetylation of 78% becomes less intense with increasing deacetylation, and finally a new band appears at 1390 cm^{-1} at the highest degree of deacetylation. The 1318 cm^{-1} band for the deacetylation of 78% changes shape with increasing deacetylation, and a new band appears at 1336 cm^{-1} for the deac-

etylation of 99%. Similar and distinct changes occur for the newly appearing bands at 1093, 666, 607, and 561 cm^{-1} with decreasing intensity of the broad band at the base of the last three bands just as the change in the 3000–3600 cm^{-1} region. These spectral observations are consistent with the generally accepted concept that crystallization induces sharpening of infrared absorption bands.

CONCLUSION

A method has been developed for the preparation of chitosan having a degree of deacetylation of up to 100% by alkali treatment of chitin with intermittent washing by water. Total time of alkali treatment of chitin was less than 5 h for preparing completely deacetylated chitosan at a reaction temperature of 110°C. In order to obtain deacetylation to about 90% by the conventional continuous alkali treatment, it is necessary to treat in alkali solution for prolonged time at the reaction temperature. The shortened time in the present method avoids degradation of the molecular chains of the obtained product. This was proved by the molecular weight measurements by GPC. It was found that tensile strength of chitosan in the wet state drastically increases as the degree of deacetylation approaches 100%. Moreover, half or more of the tensile strength of the film in the dry state is retained in the wet state if the deacetylation is nearly 100%. This degree of strength retainment is comparable with the case of α -chitin of *Locusta tendon*. Chitosan is worth more intensive studies for developing applications.

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Received October 5, 1982

Accepted January 14, 1983