Properties of Chitosan-Based Biopolymer Films with Various Degrees of Deacetylation and Molecular Weights

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ABSTRACT: An increase in the depolymerization of chitosan was found with an increased concentration of sodium perborate. Acetic anhydride was added to reacetylated chitosan in a molar ratio per gulcosamine unit, and the amide I band of IR spectra changed with the addition of acetic anhydride. Sixteen chitosans with various molecular weights (MWs) and degrees of deacetylation (DODs) were prepared. X-ray diffraction patterns indicated their amorphous and partially crystalline states. Increases in the chitosan MW and DOD increased the tensile strength (TS). TS of the chitosan films ranged from 22 to 61 MPa. However, the elongation (*E*) of chitosan films did not show any difference with MW. TS of chitosan films decreased with the reacetylation process. However, *E* of chitosan films was not dependent on DOD. The water vapor permeabilities (WVPs) of the chitosan films without a plasticizer were between 0.155 and 0.214 ng m/m^2 s Pa. As the chitosan MW increased, the chitosan film WVP increased, but the values were not significantly different. Moreover, the WVP values were not different from low DOD to high DOD. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 89: 3476–3484, 2003

Key words: depolymerization; modification; mechanical properties

INTRODUCTION

Chitosan, a copolymer derived from the abundant natural polymer chitin, is composed of 2-amino-2-deoxy-D-glucose and 2-acetoamido-2-deoxy-D-glucose units. In recent years, both chitin and chitosan have received great attention as biologically active substances. Chitosan and its derivatives have been used in a wide variety of applications. The effectiveness of these materials is dependent on their molecular weight (MW) and degree of deacetylation (DOD).[1] Ikeda et al.[2] reported that chitosan hydrolysates with low MWs of 10 and 20 kDa effectively lowered plasma and liver cholesterol concentrations. Tokura et al.[3] reported that 9.3-kDa chitosan inhibited the growth of *E. coli*, but 2.2-kDa chitosan promoted its growth. Hirano et al.[4] reported that an enhanced chitinase activity was found in the seedling growth of black pine seeds coated with a depolymerized chitosan (MW = 3–5 kDa) and that the activity was 1.3–1.5 times higher than that of uncoated seeds. Tomihata and Ikada[5] revealed a reduction in the biodegradation of chitosan films *in vivo* and *in vitro* with increased DOD.

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Chitin can be converted into chitosan or chitooligosaccharide by a chemical process[6,7] or an enzymatic preparation.[8,9] Commercially, chemical processes, ultrasonic waves, and enzymatic preparation are used for chitosan production concomitantly. Chemical methods have several defects, such as low product yields and chemical modifications of the glucose ring, but they are used extensively for commercial chitosan preparation because of their low cost and mass production. In an ultrasonic treatment, the efficiency of the chemical and enzymatic treatments is maximized, so that the chemical and enzyme treatment times can be shortened. Enzymatic methods can minimize alterations in the chemical nature of the reaction products, but few commercially acceptable enzymatic methods have been described. Chitosan has been depolymerized with $NaBO_3$ without the deacetylation of chitosan molecules.[10,11] MW varies according to the concentration and the reaction time. According to Kawamura et al.,[10] hydrogen peroxide is directly isolated from the reaction of water and $NaBO_3$ and dissociated *meta*-sodium borate. The pH of the solution in this reaction ranges from 10.0 to10.4 with a 1–5% sodium perborate concentration, and in this range of pHs, *meta*-sodium borate shows a highly stable buffering effect. Therefore, sodium perborate does not react with the amino group of chitosan, and it is known to react selectively with the glucosyl linkage of the chitosan main chain.[10]

Kubota and Eguchi[11] reported that chitosan samples could be selectively depolymerized to different MWs with similar DODs.

Chitosan is known to have good film properties. Among the various properties of chitosan, the physicochemical properties of its films are interesting for pharmaceutical applications, the food industry, and membrane separation. In some cases, chitosan films have been used as edible films and coatings in the food industry. In particular, edible films and coatings can extend the shelf life and improve the quality of food by providing barriers to mass transfer, carrying food ingredients, and improving the mechanical integrity or handling characteristics.[12] For many food applications, the most important functional characteristic of an edible film or coating is its resistance to the migration of moisture. This is because critical levels of water activity must be maintained in many foods if the product, or a certain component of a multiphase food, is to exhibit optimum quality and acceptable safety. In the field of pharmaceuticals, many studies on the permeation of drugs through chitosan membranes have been performed. Drug-loaded chitosan films have also been prepared, and most investigations have focused on drug-release studies. Many studies have reported that the physicochemical properties of polymer films, such as the mechanical properties, water uptake, and additive content of the films, the MW of the polymer, the drug concentration in the films, the type of polymer, the swelling properties, and the drug–polymer interactions, affect the drug release from the matrix film systems as well as the drug permeation through the films.[13]

In this study, chitosan was depolymerized with an $NaBO_3$ treatment and reacetylated with an acetic anhydride molar ratio treatment. Then, chitosan samples with various MWs were prepared by depolymerization and reacetylation. For an understanding of the effect of MW and DOD on films, the mechanical properties [tensile strength (TS) and elongation (E)] and water vapor permeability (WVP) of chitosan films were measured.

EXPERIMENTAL

Materials

Chitosan (MW = 880,000 and DOD = 89.3%) was provided by Biotech Co., Ltd. (Mokpo, Korea). $NaBO_3$ and acetic anhydride were purchased from Sigma–Aldrich Co. (St. Louis, MO).

Degradation of chitosan

Purified chitosan (10 g) was added to distilled water (2 L) in a round-bottom flask, and then $NaBO_3$ of a desired concentration was added. This solution was reacted at 60°C under N_2 gas. After stirring at a predetermined temperature and for a predetermined time, the reaction mixture was cooled, and a 1*M* NaOH solution was added up to pH 8–9. The obtained precipitate was centrifuged and washed well with deionized water until it was neutral. Finally, it was washed with distilled water and freeze-dried *in vacuo* at −80°C.[10,11,14]

N-acetylation of chitosan

On the basis of Kubota and Eguchi's[11] and Knaul et al.'s[15] methods, chitosan (0.3 g) was dissolved in 10% aqueous acetic acid (50 mL), and acetic anhydride was added by the ratio of the molar amount versus the glucosamine unit. After 5 h of stirring at ambient temperature, 5 mol/dL NaOH was added up to pH 8–9 to stop the reaction. The reaction mixture was then dialyzed against pure water for 2 day for the removal of ions and was freeze-dried. The acetylated chitosan was treated with 1 mol/dL methanolic KOH at room temperature for 5 h. After an alkaline treatment, N-acetylated chitosan was washed with methanol repeatedly with a centrifuge. At last, it was washed again in deionized water and freeze-dried.

X-ray diffraction

Powder X-ray diffraction patterns of chitosan powders and films were measured with an X-ray diffractometer (model MO3XHF22, MAC Science Co., Ishikawa, Japan) with Ni-filtered Cu radiation generated at 40 kV and 30 mA as the X-ray source. X-ray diffraction was measured at a scanning rate of 0.5° min^{-1} and over a scanning range of 5–50° (2θ).

Determination of DOD

Fourier transform infrared (FTIR) method

Chitosan (40 mg; particle size ≤ 125 μ), previously dried *in vacuo*, was mixed and triturated with 120 mg of potassium bromide and was then dried at 80°C in a hot air oven for at least 12 h before analysis.[16] Approximately 40 mg of this blend was placed in an IR press and subjected to compression for at least 30 min; this resulted in a transparent disk. The IR spectrum was then scanned with an FTIR instrument (model 430, Jasco, Tokyo, Japan) scanning between 400 and 4000 cm^{-1}. Three such disks were prepared for each sample of chitosan.[17] The IR spectroscopy method involved the determination of the absorbance at 1655 cm^{-1} (amide I band) as a measure of the N-acetyl group content and at 3450 cm^{-1} (hydroxyl band) as an internal standard used to correct for differences in the chitosan concentrations, as employed by Sannan et al.[18] and Sabnis and Block.[16]

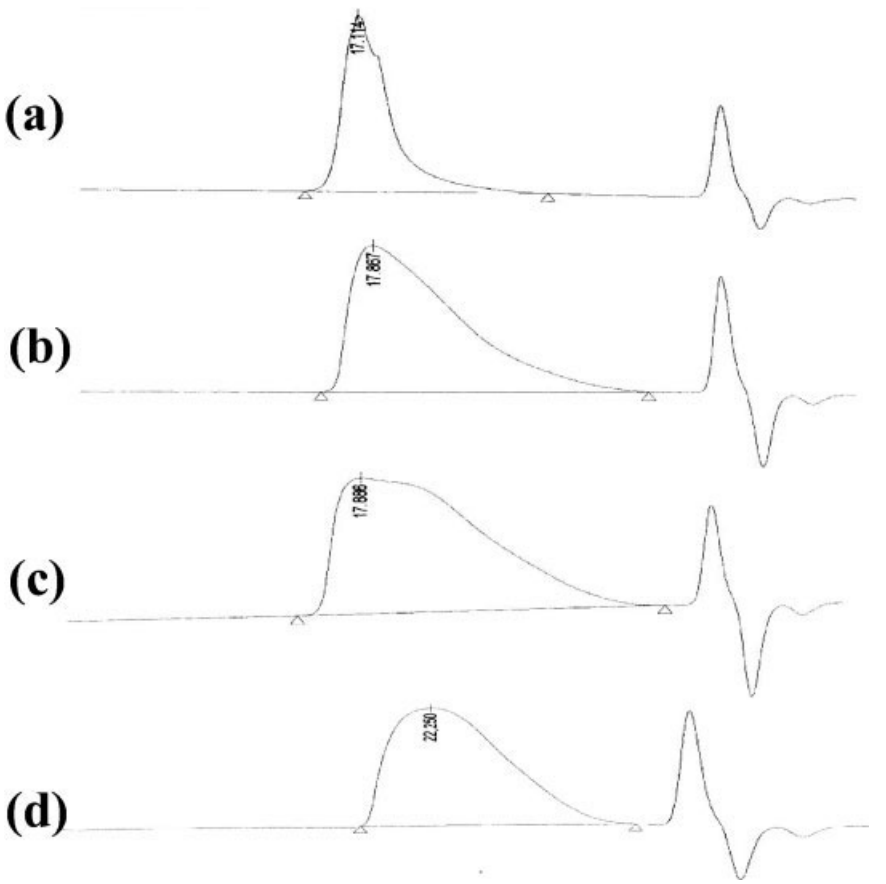

Figure 1 Chromatograms of depolymerized chitosan: (a) MW = 880,000 and DOD = 89.3%, (b) MW = 580,000 and DOD = 87.3%, (c) MW = 240,000 and DOD = 84.7%, and (d) MW = 90,000 and DOD = 86.3%.

NMR spectroscopy method

^{1}H-NMR spectra were recorded on a Bruker ARX 300 spectrometer (Billerica, MA) at 25°C. The chitosan samples were freeze-dried several times from D_2O (>99.9%) solutions to minimize the H_2O by deuterium signal. All the samples were dissolved in 5-mm-diameter tubes, and the sample concentration was 20 mg/cm^{-3}. For water-insoluble samples, CF_3COOD (99.9%) was added. The chemical shifts were given on the δ scale. The measurement conditions were as follows: 8064-Hz spectral window, 32,000 data points, 3-μs pulse width, 2.03-s acquisition time, and 32 scans with delay of 1 s between the scans:[11,17,19–21]

$$\text{Degree of acetylation (\%)} = \frac{I_{\text{Ac}}/3}{I_{H2,3,1,5,6}/6} \times 100 \quad (1)$$

where I_{Ac} is the integral intensity of acetyl group and $I_{H2,3,1,5,6}$ is the summation integral intensities of H2, H3, H1, H5, H6.

Determination of MW

An Ubbelohde-type capillary viscometer was used to measure the passage of time of solutions flowing through the capillary at 25°C. There were solutions of depolymerized chitosan samples with different MWs at different concentrations (from 0.15 to 0.00125%) and solutions in 0.1*M* acetic acid/0.02*M* NaCl. The capillary viscometer was filled with 12 mL of a sample and equilibrated in a water bath (Koma Biotech Co., Seoul, Korea) at 25 ± 0.1°C for 20 min. The sample was passed through the capillary once before the running time was measured. The running time was used to calculate the relative viscosity and then the reduced viscosity. The intrinsic viscosity ([η]) was obtained by the extrapolation of η_{rel}/C to zero concentration [where η_{rel} is the relative viscosity and C is the chitosan concentration (g · mL^{-1})].[22,23] The Mark–Houwink–Sakurada equation gives the relationship between [η] and MW:

$$[\eta] = K \cdot \text{MW}^a \quad (2)$$

where K and a are empirical constants. In this study, we used $K = 1.40 \times 10^{-3}$ and $a = 0.83$ for our MW analysis, which followed the results of Knaul et al.[15]

Preparation of the chitosan films

Depolymerized and reacetylated chitosan samples (4 g) were dissolved in 200 mL of a formic acid solution

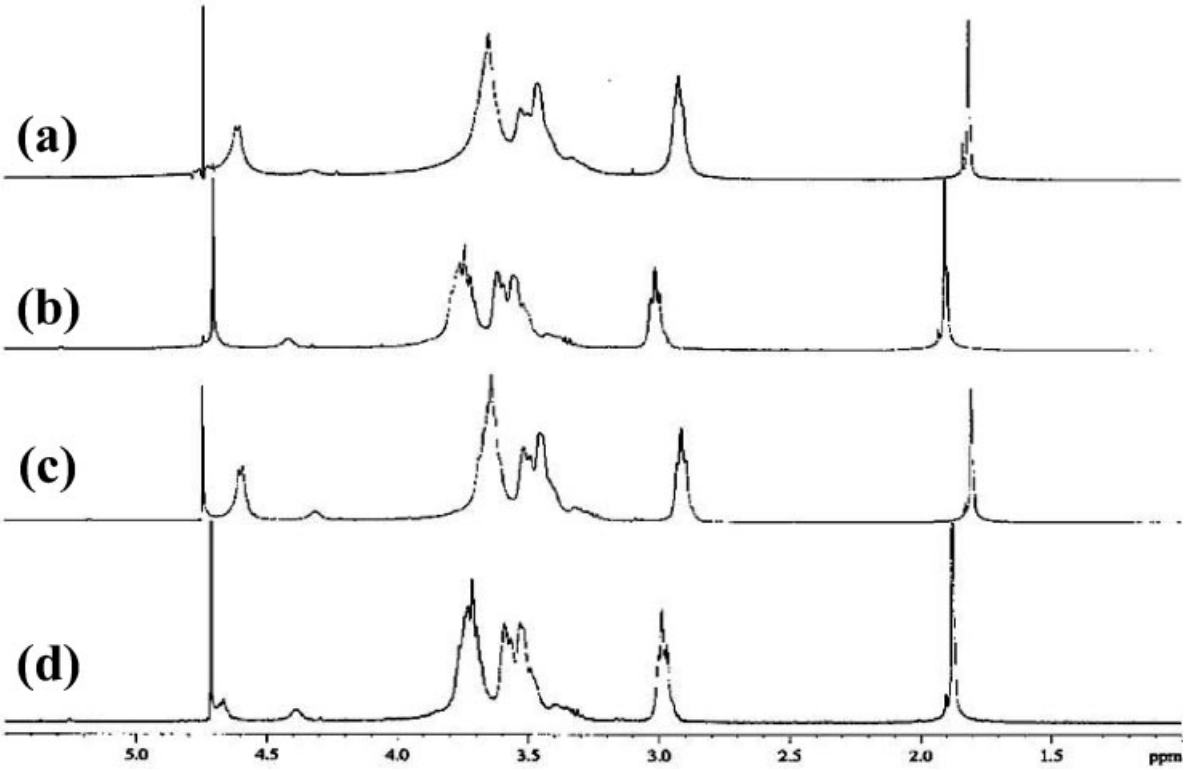

Figure 2 ^{1}H-NMR spectra of depolymerized chitosan in D_2O containing CF_3COOD: (a) MW = 880,000 and DOD = 89.3%, (b) MW = 580,000 and DOD = 87.3%, (c) MW = 240,000 and DOD = 84.7%, and (d) MW = 90,000 and DOD = 86.3%.

(88%). The chitosan samples were stirred for about 5 h and filtered with a sintered glass filter (1G-2). A solution (200 mL) was cast on a flat and leveled glass plate to control the film thickness. The films were dried in a drying clean room with a drying machine (model AHNB16KBM, GE, Taipei, Taiwan) for 1 week and peeled from the plates. The films were then put in an environmental chamber (model 150AT/1, Jeio Tech Co., Kimpo, Korea) at 25°C and 50% relative humidity (RH) and were conditioned for further testing.

Mechanical properties

Sixteen samples, 2.54 cm wide and 10 cm long, were cut from film samples prepared on a glass plate. The samples were conditioned for 24 h at 25°C and 50% RH in an environmental chamber before TS and *E* were measured. An Instron (instrument model 4210, Instron Engineering Co., Canton, MA) was used to measure TS and *E* at break according to ASTM Standard D 882-88[24] and Park et al.[25] The film samples were cut, and the film thickness was measured. TS was calculated by the division of the maximum (peak) load by the cross-sectional area. *E* at the break point (%) was calculated by the division of *E* at the moment of rupture by the initial length of the specimen and multiplication by 100.

WVP

Standard method E 96-80,[26] the cup method, was used to determine WVP with a 50% RH gradient at 25°C. The cups were filled with distilled water, and the film samples were mounted over the cups. The weight loss was measured as a function of time for 12 h. For each WVP value, three samples were tested.

RESULTS AND DISCUSSION

Depolymerization

Chitosan was depolymerized by the treatment of sodium perborate in a molar ratio.[11] MW for chitosan samples varied according to the sodium perborate concentration, and a series of chitosan samples with MWs ranging from 880,000 to 90,000 were achieved. The decreases in MW with the amount of sodium perborate are shown in Figure 1. The high performance liquid chromatography (HPLC) chromatograms exhibit the differential MW distributions of depolymerized chitosan samples (Fig. 1). According to Figure 2, the depolymerized chitosan samples were not significantly different on the basis of NMR data for DOD. The ^{1}H-NMR spectrum of the depolymerized chitosan gave complex signals. The resonance at 1.78 ppm was easily assigned to the N-acetyl protons, but

TABLE I
Assignment of ^{1}H-NMR Chemical Shifts of Partially *N*-Acetylated Chitosan in D_2O Containing CF_3COOD

Solvent	Residue	H-1	H-2	H-3	H-4,5,6	*N*-Acetyl
CF_3COOD/D_2O	glucosamine	4.46	3.16	3.87	3.31–4.1	—
	N-acetyl glucosamine	4.62	2.98	3.64	3.32–4.1	1.78

the signal at 1.78 ppm was not different among the depolymerized processes. The peaks at 2.91, 3.64, and 4.62 ppm corresponded to H-2, H-3, and H-1 of the *N*-acetyl glucosamine residue, respectively (Table I).

Reacetylation

The chitosan samples were reacetylated for the preparation of different DODs within chitosan samples of similar MWs (Fig. 3). As the molar amount of acetic anhydride increased, DOD decreased concomitantly. The results of reacetylation are shown in Figure 4 by FTIR spectra of reacetylated chitosans. As the DOD decreased, the amide I band 1660 cm^{-1} in IR spectra increased with an increasing reacetylation time. The FTIR spectra of all the chitosan samples exhibited a broad peak at 3420 cm^{-1}. The peaks were assigned to OH stretching, indicating intermolecular hydrogen bonding of chitosan molecules, and they also overlapped in the same region of NH stretching. The peak of C═O at 1660 cm^{-1}, compared with the peak of OH at 3420 cm^{-1}, was used for the measurement of DOD. The CO stretching (amide I) peak near 1660 cm^{-1} and the NH bending (amide II) peak near 1574 cm^{-1} were not observed in the spectra of chitosan samples with 0 and 100% DODs. Takai et al.[27] also reported that a peak of NH at 1574 cm^{-1}, which predominated the peak of C═O at 1660 cm^{-1}, detected in the spectrum

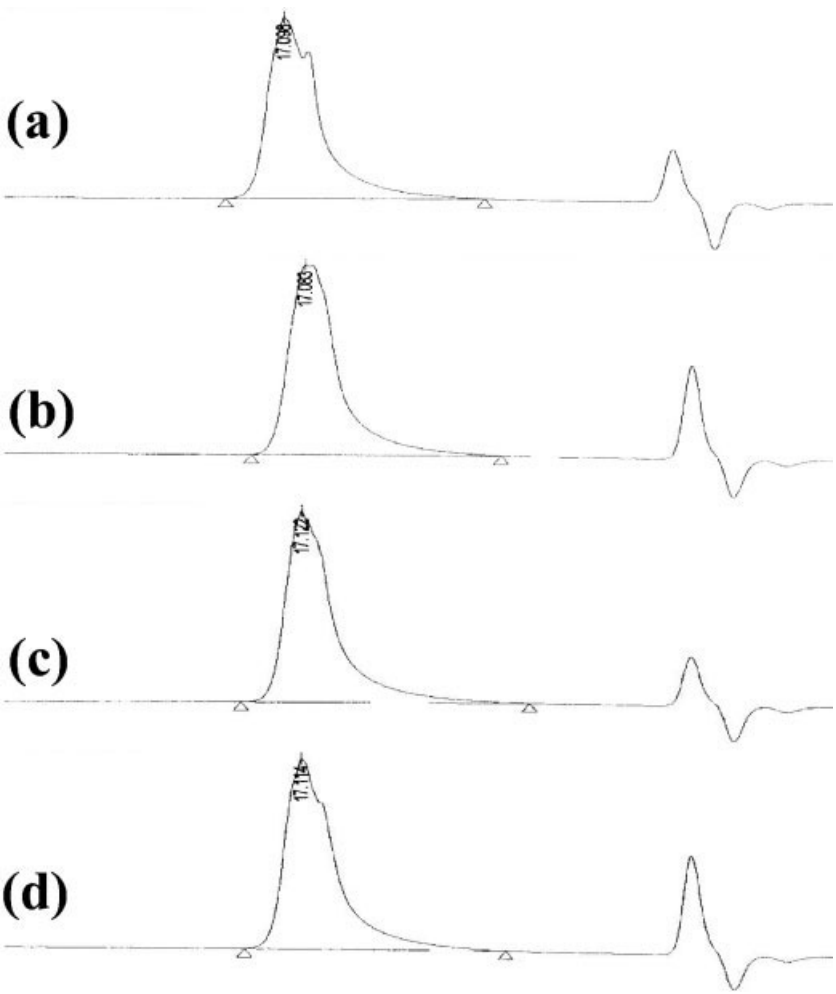

Figure 3 FTIR spectra of original chitosan and reacetylated chitosan (amide I band at 1660 cm^{-1} and OH band at 3420 cm^{-1}): (a) DOD = 89.3%, (b) DOD = 80.5%, (c) DOD = 71.5%, and (d) DOD = 65.4%.

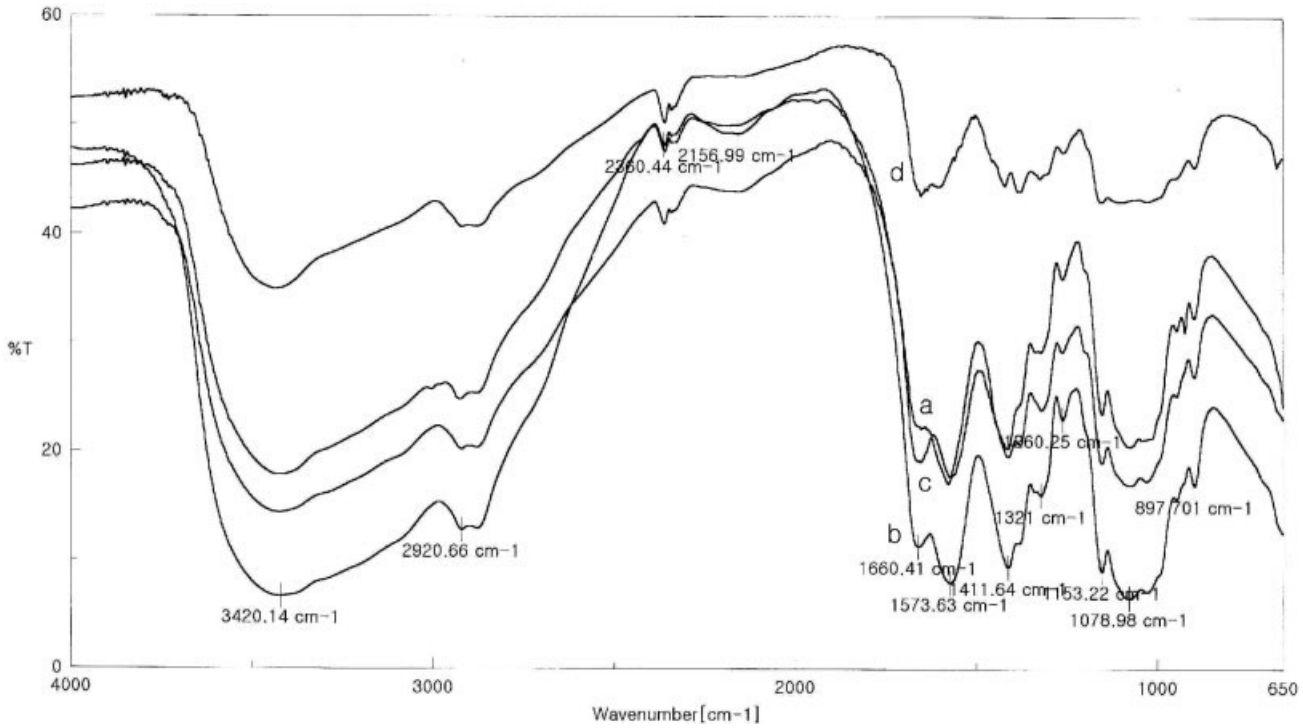

Figure 4 ^{1}H-NMR spectra of reacetylated chitosan in D_2O containing CF_3COOD: (a) DOD = 89.3%, (b) DOD = 80.5%, (c) DOD = 71.5%, and (d) DOD = 65.4%.

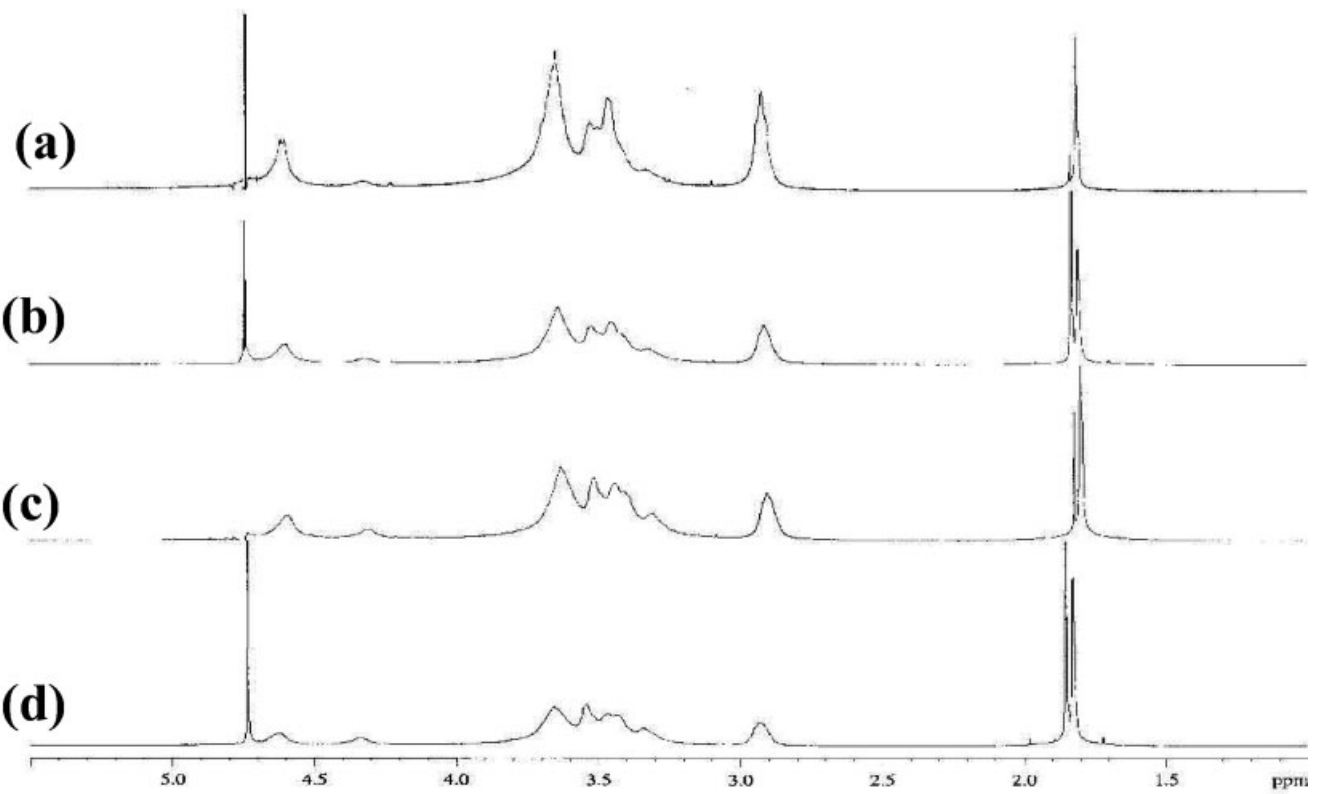

Figure 5 Chromatograms of reacetylated chitosan: (a) DOD = 89.3%, (b) DOD = 80.5%, (c) DOD = 71.5%, and (d) DOD = 65.4%.

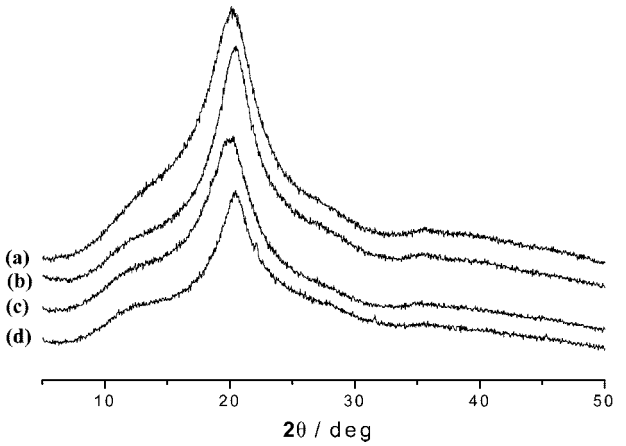

Figure 6 X-ray diffraction patterns of depolymerized chitosan: (a) MW = 880,000 and DOD = 89.3%, (b) MW = 580,000 and DOD = 87.3%, (c) MW = 240,000 and DOD = 84.7%, and (d) MW = 90,000 and DOD = 86.3%.

of chitosan, could indicate deacetylation. In the NMR spectra (Fig. 5), the peak at 1.78 ppm grew along with the increasing reacetylation time.[28,29]

Crystallinity

The depolymerized chitosan samples made at room temperature showed only one peak at $2\theta = 20°$ (Fig. 6). As the chitosan samples were depolymerized, the peak at $2\theta = 20°$ decreased more than for high-MW chitosan. The depolymerized chitosan samples gave somewhat broader patterns around $2\theta = 20°$, indicating lower crystallinity or less packing in the main chain in comparison with high-MW chitosan. This result was very similar to the work of Samuels.[28] According to Samuels, the peak at 20° was related to crystal (2) in the chitosan. The unit cell of crystal (2) was characterized by $a = 4.4$, $b = 10.0$, $c = 10.30$ Å, and $\beta = 90°$. Takai et al.[27] also reported that the peak at 20° was a typical peak for an X-ray diffraction pattern of chitosan. Ogawa[30] reported a similar result, that the crystallinity increased with decreasing chitosan MW.

X-ray diffractograms of reacetylated chitosan samples are shown in Figure 7. Compared with that of depolymerized chitosan samples, the crystallinity increased markedly for the reacetylated chitosan samples. One was at 8.5°, and the other was at 20.5°. These two peaks were related to two different types of crystal: crystal (1) and crystal (2).[28] The first peak at 5–10° was related to crystal (1), which had a unit cell characterized by $a = 7.76$, $b = 10.91$, $c = 10.30$ Å, and $\beta = 90°$. The unit cell of crystal (1) was larger than that of crystal (2). However, according to Kurita et al.,[31] although shrimp chitosan with a DOD of 80–89% was amorphous intermediately, further deacetylation caused crystallization again.

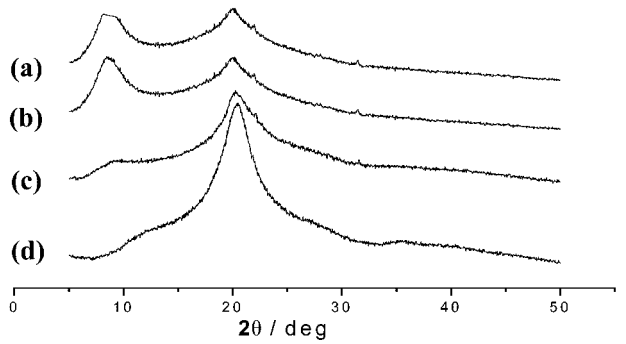

Figure 7 X-ray diffraction patterns of reacetylated chitosan: (a) DOD = 89.3%, (b) DOD = 80.5%, (c) DOD = 71.5%, and (d) DOD = 65.4%.

Film properties of chitosan

The film samples of chitosan were prepared by chitosan controlled independently with respect to MW and DOD. As shown in Figure 8, the chitosan DOD and MW influenced the TS, *E*, and WVP values of the chitosan films.

As MW of chitosan was increased, TS of the membrane also increased (Fig. 8). TS of chitosan films (MW = 90,000 and DOD = 80%) was 22 MPa and increased to 61 MPa as MW of chitosan increased to 880,000. Austin[32] reported chitosan films cast out of 5% (w/v)

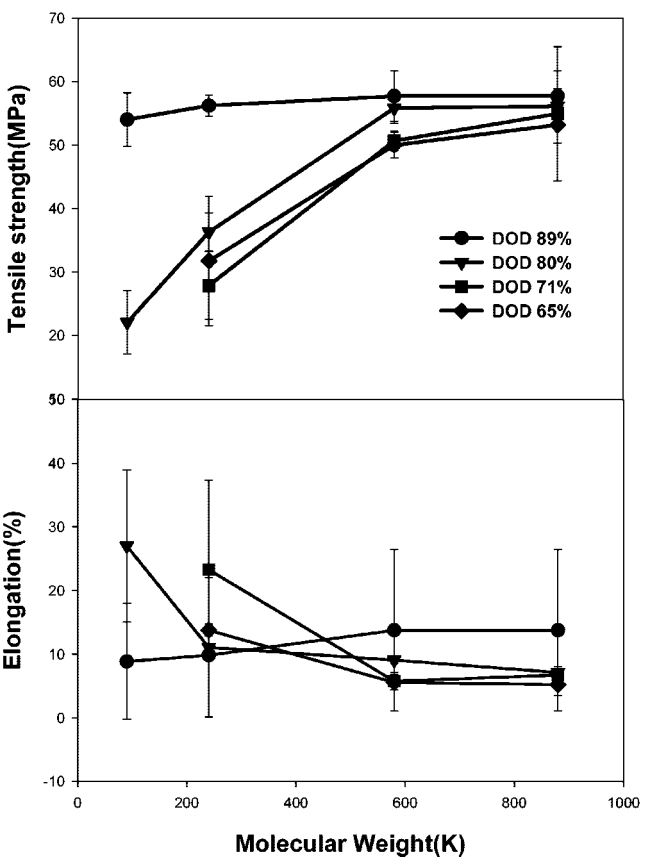

Figure 8 Effect of MW on chitosan film properties.

chitosan solutions in 4% (v/w) acetic acid and coagulated in bases. TS of the chitosan films increased from 16.9 to 23.6 MPa as MW of chitosan increased. Remuñán-López and Bodmeier[33] also reported that the mechanical properties of chitosan films increased from 7.67 to 18.01 MPa and from 19.88 to 62.73% with increasing viscosity. Chen and Hwa,[34] Chen and Lin,[35] and Blair et al.[36] also reported that TS and *E* increased with the chitosan MW. This might be attributable to an entanglement network forming during the film formation of high-MW chitosan samples.[34] The *E* values of chitosan films in this study decreased with increasing MW and ranged from 5 to 27%. According to Park et al.,[37] the *E* values of high-MW chitosan films decreased with increasing MW. As reported by Park et al., the difference may be attributed to the fact that the physicochemical properties of the films were affected by the polymer structure, plasticizer, and solvent, and other factors were considered in relation to the film properties. The lower MW chitosan films showed a large variation in *E*, and the overall trend of the chitosan films was greatly affected by increasing MWs. However, the *E* values of higher MW films ranged from 5 to 9%. According to Rhim et al.,[38] the *E* value of a chitosan film prepared with acetic acid was similar to that of a chitosan film prepared with formic acid. The *E* values of high-MW chitosan films were similar to the results (9.06%) of Remuñán-López and Bodmeier.[33]

As for the DOD effect on chitosan films (Fig. 9), TS of the chitosan films decreased with the reacetylation process. However, *E* of the reacetylated chitosan films did not show a relationship with the reacetylation process. Tomihata and Ikada[5] reported that the mechanical strength (TS) of chitosan and chitin swollen with phosphate buffer saline was lower than that of the deacetylated derivatives if the DOD was higher than 80 mol % (0.18–0.41 MPa). Chen and Lin[35] and Mima et al.[39] also reported that TS increased with an increase in the chitosan DOD. The TS values of the films tested were comparable to those of methyl cellulose, which had values of 43.7–70.2 MPa. These WVP values were comparable to the results of Kittur et al.,[40] which ranged from 67.6 to 70.3 MPa. However, they were higher than those of chitosan films reported by Butler et al.,[41] which ranged from 19 to 35 MPa. The *E* values of the chitosan films varied according to the type of acid used.[38] Except for hydrochloric acid films, which had the lowest *E* values, the *E* values of the chitosan films varied from 20 to 58%. These *E* values were higher than those in this study. The difference may be attributed to the fact that they added a plasticizer to the film. Tomihata and Ikada[5] and Blair et al.[36] reported that the strength and *E* values of films may be influenced greatly by the overall crystallinity and microcrystalline size of the films.

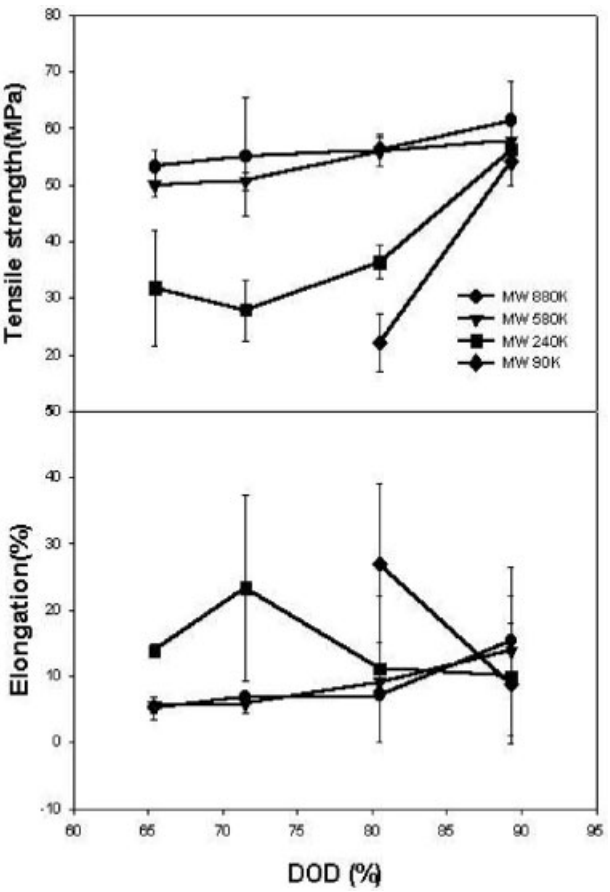

Figure 9 Effect of DOD on chitosan film properties.

The WVP values of the chitosan films without a plasticizer were between 0.155 and 0.214 ng m/m^2 s Pa (Fig. 10). As the chitosan MW increased, the WVP value of the chitosan film seemed to increase, but the values were not significantly different. However, the WVP values were not different from a low DOD to a high DOD. Park[42] found that the WVP values of chitosan films increased with increasing reacetylation. Remuñán-López and Bodmeier[33] also reported that the WVP value of a chitosan glutamate film seemed to increase with increasing polymer MW. Park et al.[37] also measured WVP values of chitosan films with various MWs and found that the WVP values did not show any trends.

CONCLUSIONS

The application of chitosan depends on its MW and DOD. Chitosan has good film-forming properties. As the chitosan MW is increased, the film TS also increases. The TS values of chitosan films range from 22 to 61 MPa. However, the *E* values of chitosan films, according to this study, do not depend on MW. As for the DOD effect on chitosan films, the TS values of chitosan films decrease with a reacetylation process. However, the *E* values of chitosan films do not depend

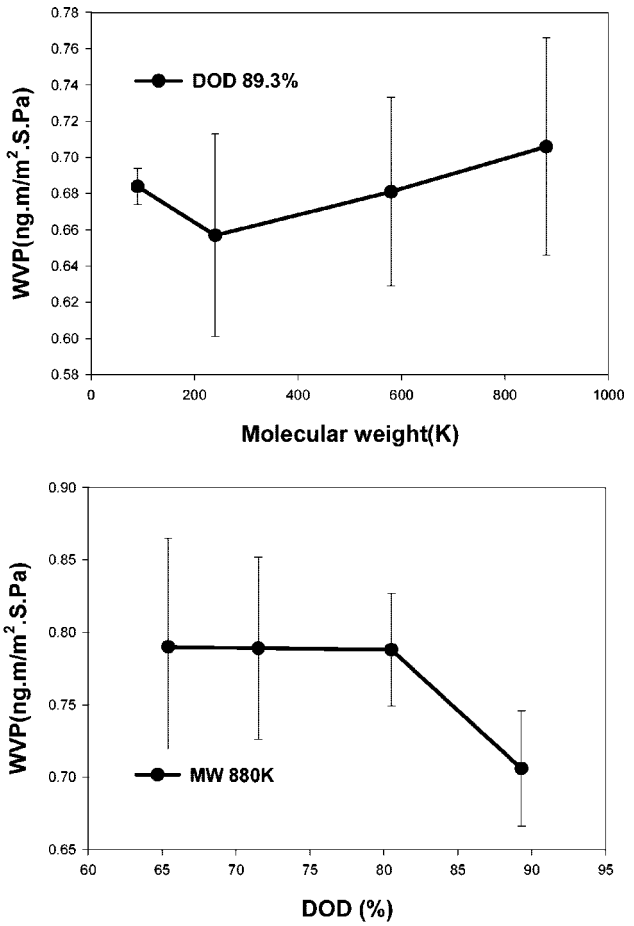

Figure 10 Effects of MW and DOD on WVP.

on DOD. The WVP values of chitosan films without a plasticizer range from 0.155 to 0.214 ng m/m^2 s Pa. As the chitosan MW increases, the WVP values of chitosan films seem to increase, but the values are not significantly different. However, WVP values are not different from a low DOD to a high DOD. On the basis of these results, chitosan films can be used in a wide range of packaging materials.

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