Deacetylation of β-Chitin. I. Influence of the Deacetylation Conditions

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ABSTRACT: The influences of the deacetylation temperature, deacetylation time, and NaOH concentration on the degree of deacetylation (DD) of deacetylated products prepared from β-chitin are discussed. The DD values of deacetylated products are related to the ratio of the signal intensities of methyl on acetyl groups and the first anomeric carbon, which are obtained from ^{13}C-NMR spectra. The results show that the DD values of deacetylated product increase as the NaOH concentration, deacetylation time, or deacetylation temperature increases. The thermal properties, chemical structures, and crystalline characteristic of deacetylated products are significantly related to their DD values. Differential scanning calorimetry shows that the peak temperature is slightly increased as the DD values of deacetylated products of β-chitin increase. Thermogravimetric analysis shows that the thermal degradation onset temperature of deacetylated products decreases as the DD values increase. Fourier transform infrared spectra show that the intensity of a specific absorption peak of —NH_2 in deacetylated products significantly increases as DD increases. X-ray diffraction patterns of deacetylated products with DD values of 17.5 and 44.7% have three significant diffraction peaks. However, there are only two diffraction peaks found in products with higher DD values of 76.5 and 94.7%. © 2004 Wiley Periodicals, Inc. J Appl Polym Sci 93: 2416–2422, 2004

Key words: chitin; chitosan; NMR; Degree of Deacetylation

INTRODUCTION

Chitosan, a deacetylated product of chitin, is a high-molecular-weight heteropolysaccharide composed mainly or fully of β-(1,4)-2-deoxy-2-amino-D-glucopyranose and partially or not at all of β-(1,4)-2-deoxy-2-acetamido-D-glucopyranose units.[1] Chitosan is the fully or partially deacetylated form of chitin. Chitin and chitosan, with excellent biodegradability, biocompatibility, and bioactivity, have attracted significant interest for biomedical applications, including antithrombogenic, homeostatic, immunity-enhancing, and wound-healing applications.[2–5] Therefore, many attempts have been made to produce new biofunctional materials from chitin and chitosan.[6]

Chitin, next to cellulose, is the second most common polysaccharide on Earth. There are three crystal forms of chitin: α, β, and γ. γ-Chitin, which exists in fungi and yeasts, is a combination of α-chitin and β-chitin. α-Chitin, which is rhombic, can be prepared from the shells of crabs, shrimps, and other arthropods.[7] β-Chitin, which is monoclinic, can be produced from squid pens. Squid pens, usually treated as waste and obtained in considerable amounts, are another potential source for chitin. Normally, in the preparation of chitin from crustacean sources, the exoskeleton must be treated with acid for the removal of calcium carbonate. Because very little calcium carbonate exists in squid pens, the acid extraction step is not necessarily required for obtaining chitin from squid pens.[8] This change in the chitin extraction procedure could reduce the cost and prevent acid hydrolysis of the chitin during the process. Thus, chitin extracted from squid pens is better in quality than that extracted from other sources. In addition, chitin from squid pens is in the β form, which has a more open structure (parallel chain alignment) than the α form (antiparallel chain alignment) found in crustacean exoskeletons.[9] Therefore, β-chitin shows better solubility and swelling than α-chitin because of much weaker intermolecular hydrogen bonding ascribable to the parallel arrangement of the main chains. β-Chitin also shows better reactivity than α-chitin during deacetylation.[10]

The deacetylation of α-chitin prepared from crab and shrimp shells has been studied extensively because of their wide availability. In 1977, Sannan et al.[11] reported that the kinetics of homogeneous alkaline deacetylation of α-chitin was a pseudo-first-order reaction. Similar results were obtained for the heterogeneous deacetylation of α-chitin at the reaction temperature of 150°C.[12] Moreover, the heterogeneous deacetylation of shrimp chitin appears to be more

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complicated than a pseudo-first-order reaction under different alkali concentrations. It might be a higher order and diffusion-controlled reaction.[13] In 2003, Methacanona et al.[8] reported heterogeneous alkaline deacetylation with β-chitin extracted from squid pens under various conditions. The deacetylation reaction followed pseudo-first-order kinetics during the initial period of the process. Because the deacetylation of β-chitin has not been widely studied, here we focus on the application of the heterogeneous alkaline deacetylation process to the preparation of β-chitin or chitosan with various degrees of deacetylation (DDs). The influences of the alkaline concentration, reaction temperature, and reaction time on DD are discussed. Generally, if the DD values of deacetylated products are greater than 70%, these products can be called chitosan.

There are several methods of determining the DD values of chitin and chitosan, such as infrared spectroscopy,[14] near-infrared spectroscopy,[15] UV spectrophotometry,[16] first-derivative UV spectrophotometry,[17,18] colloidal titration,[19] linear potentiometric titration,[20] enzymatic determination,[21] ninhydrin testing,[22] ^{13}C-NMR,[8] and thermal analysis.[23] Among these analysis methods, solid ^{13}C-NMR has been chosen to examine the DD values of deacetylated products prepared from β-chitin because of the following characteristics: simplicity, quickness, and accuracy. Thermogravimetric analysis (TGA), Fourier transform infrared (FTIR), and X-ray diffraction (XRD) have also been used to characterize the deacetylated products prepared from β-chitin.

EXPERIMENTAL

Materials

β-Chitin with 0.3% ash and 6% DD was supplied by Ohka Enterprises Co., Ltd. (Kaohsiung, Taiwan). It was extracted from squid pens. Its viscosity was about 155 mPa S (0.5 g of β-chitin was dissolved in a 5% LiCL solution with stirring for 6 h and then was measured with a viscometer). The material was light yellow, and the particle size was about 40-mesh. NaOH was purchased from Union Chemical Works, Ltd. (Hsinchu, Taiwan). All the reagents were used without further purification or treatment.

Deacetylation of β-chitin

A 250-mL conical Erlenmeyer flask equipped with a thermometer was used for the deacetylation experiments. A stirrer was put into the flask to ensure proper mixing. β-Chitin (5 g) and a 100-mL NaOH solution were consistently used in the deacetylation experiments. To study the influences of the deacetylation temperature, deacetylation time, and concentration of the NaOH solution on the DD values of deacetylated products prepared from β-chitin, we designed three groups of deacetylation experiments:

1. The deacetylation temperature was set at 100°C, and the deacetylation time was fixed at 60 min. The concentration of aqueous NaOH was varied from 20 to 60 wt % in 10 wt % intervals.
2. The deacetylation temperature was set at 100°C, and the concentration of aqueous NaOH was 50 wt %. The deacetylation time was set at 5, 10, 20, 40, 60, or 80 min.
3. The deacetylation time was fixed at 60 min, and the concentration of aqueous NaOH was 50 wt %. The deacetylation temperature was varied from 60 to 110°C in 10°C intervals.

After the completion of each deacetylation experiment, the flask was removed from the hot plate, and the resulting solution was quickly filtered. The filter cake was collected and washed with distilled water until the pH of the filtrate was around 7. Then, the wet cake was dried in a 60°C oven for 2 days.

Determination of DD

The cross-polarization/magic-angle-spinning (CP–MAS) ^{13}C NMR spectrum was recorded at 100.624 MHz with a Bruker (Germany) Advance DPX-400 spectrometer at a spinning rate of 6200 Hz. The NMR spectrum represents an optimized 3-s pulse delay, 10-ms contact time, and 1000 scans. The chemical shifts with respect to the intensities were measured in parts per million. The DD values of the deacetylated products were related to the ratio of the signal intensities of methyl on acetyl groups (I_{CH_3}) and the first anomeric carbon (I_{C1}) obtained from the ^{13}C-NMR spectrum.[24] The following equation was used to calculated the DD values of the deacetylated products:

$$\mathrm{DD}(\%) = \left(1 - \frac{I_{CH_3}}{I_{C1}}\right) \times 100\% \quad (1)$$

Differential scanning calorimetry (DSC) analysis

A PerkinElmer (United States) DSC 7 differential scanning calorimeter was used to examine the thermal properties of the deacetylated products prepared from β-chitin at a heating rate of 10°C/min under a nitrogen flow. Each sample was heated in two stages. First, the sample was heated from 40 to 100°C and cooled to room temperature to evaporate the moisture. Second, the sample was reheated from 40 to 250°C at a heating rate 10°C/min. The results were recorded and analyzed.

TGA

A PerkinElmer TGA 7 thermogravimetric analyzer was used to measure the thermal weight losses of the deacetylated products prepared from β-chitin from 50 to 700°C at a heating rate of 10°C/min under a nitrogen stream. The weight losses at different stages were analyzed.

FTIR analysis

The chemical structures of β-chitin and the deacetylated products were identified with a PerkinElmer Spectron One FTIR spectrometer with attenuated total reflectance in the wave-number range of 400–4000 cm^{-1} at a rate of 32 scans per second.

XRD analysis

XRD patterns of β-chitin and the deacetylated products were obtained with an X-ray diffractometer (DMAX-2200, Rigaku Co., Japan). Cu Kα radiation with a wave number of 0.154 nm was used. The scanning rate was 2°/min from 5 to 35° (2θ).

RESULTS AND DISCUSSION

Solid-state ^{13}C-NMR spectroscopy has been identified as the most useful tool for structurally analyzing insoluble complex biopolymers without their conformational destruction.[25] Figure 1 presents ^{13}C-NMR spectra of deacetylated products prepared from β-chitin with various concentrations (wt %) of NaOH. The deacetylation temperature and deacetylation time were consistently set at 100°C and 60 min, respectively. Each spectrum contains seven well-defined resonances of C1–C6 and acetyl. They are δ = 174 ppm for C═O, δ = 104 ppm for C1, δ = 83–84 ppm for C4, δ = 75 ppm for C5, δ = 75 ppm for C3, δ = 61 ppm for C6, δ = 56 ppm for C2, and δ = 23 ppm for CH_3. The same chemical shift, δ = 75 ppm, appearing in C3 and C5 indicates the high structural homogeneity in the corresponding spectrum of the deacetylated product prepared from β-chitin. However, the ^{13}C-NMR spectrum of α-chitin consists of eight well-defined resonances, and the chemical shifts of C3 and C5 are separated into two close signals.[26] Figure 1 also shows that the intensities of CH_3 and C═O are reduced significantly as the concentration of NaOH is increased. This is because the DD value of the deacetylated product is increased as the concentration of NaOH increases.

The DD values of deacetylated products prepared from β-chitin at various NaOH concentrations (wt %) can be calculated with eq. (1) when the deacetylation temperature is 100°C and the deacetylation time is 60 min. The effect of the NaOH concentration on the DD values of deacetylated products prepared from β-chitin is shown in Figure 2. Figure 2 shows that the DD values of deacetylated products prepared from β-chitin increase gradually as the NaOH concentration increases up to 40 wt % and eventually approach a steady level when the NaOH concentration is greater than 50 wt %. The DD values of deacetylated products prepared from β-chitin are no greater than 45% when the concentration of NaOH is lower than 40 wt %. However, the DD values of deacetylated products prepared from β-chitin increase dramatically up to 90% and are almost proportional to the second order of the NaOH concentration when the NaOH concentration is within 40–50 wt %. When the concentration of NaOH is 60 wt % or higher, the DD values of deacetylated products prepared from β-chitin are almost constant.

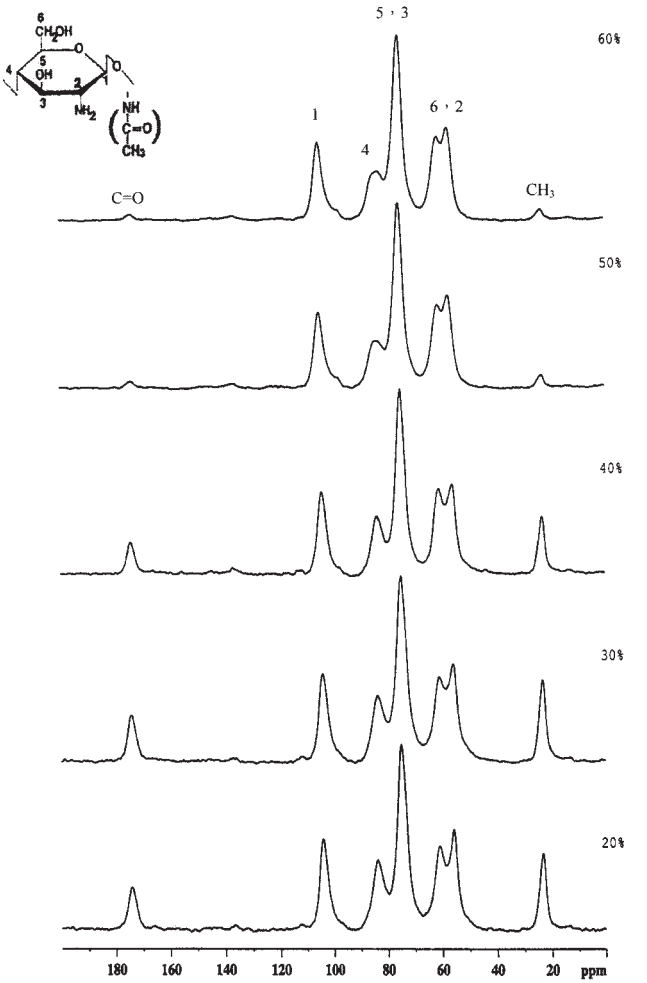

Figure 1 ^{13}C-NMR spectra of deacetylated products prepared from β-chitin at NaOH concentrations of 20, 30, 40, 50, and 60 wt % (deacetylation temperature = 100°C; deacetylation time = 60 min).

Figure 3 presents the relationship between the DD values of deacetylated products prepared from β-chitin and the deacetylation time. The deacetylation temperature is 100°C, and the NaOH concentration is

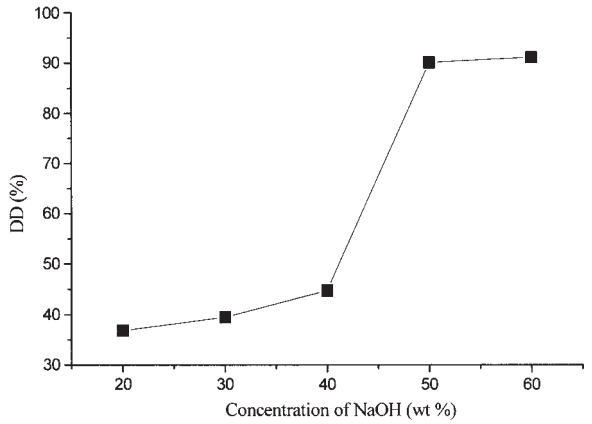

Figure 2 Effect of the NaOH concentration on the DD values of deacetylated products prepared from β-chitin (deacetylation temperature = 100°C; deacetylation time = 60 min).

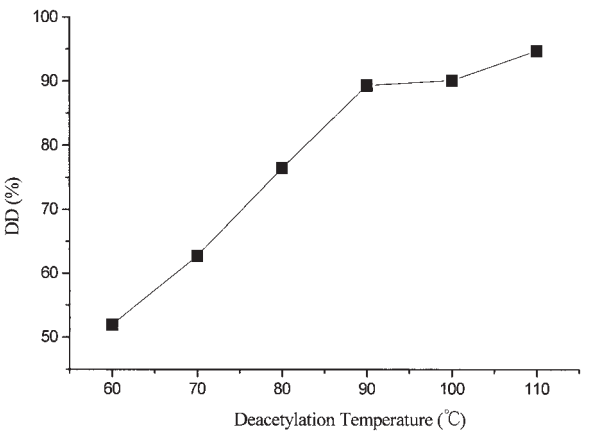

Figure 4 Effect of the deacetylation temperature on the DD values of deacetylated products prepared from β-chitin (NaOH concentration = 60 wt %; deacetylation time = 60 min).

50 wt %. The DD values of deacetylated products prepared from β-chitin increase quickly with increasing deacetylation time during the first 20 min of deacetylation. Figure 3 also shows that the DD values of deacetylated product prepared from β-chitin are about 50%, even after only 5 min of deacetylation. If the deacetylation time is longer than 20 min, the DD values of deacetylated products prepared from β-chitin slowly increase and eventually reach approximately 90% when the deacetylation time is 60 min.

Figure 4 shows the relationship between the DD values of deacetylated products prepared from β-chitin and the deacetylation temperature. The deacetylation conditions are a 60-min deacetylation time and a 50 wt % NaOH concentration. The DD values of deacetylated products prepared from β-chitin increase slowly if the deacetylation temperature is less than 50°C. However, if the deacetylation temperature is higher than 60°C, the DD values of the deacetylated products prepared from β-chitin apparently increase with the deacetylation temperature. When the deacetylation temperature is 90°C, the DD values of β-chitin begin to reach a constant level. The DD values of deacetylated products prepared from β-chitin increase dramatically (from 52 to 90%) and are almost proportional to the first order of the deacetylation temperature within 60–90°C.

DSC analysis

Figure 5 shows DSC analysis curves of deacetylated products prepared from β-chitin. Five samples with different DD values (17.5, 36.8, 44.7, 76.5, and 94.7%) were tested. The results show that the peak temperature is slightly increased as the DD values of deacetylated products prepared from β-chitin increase. This

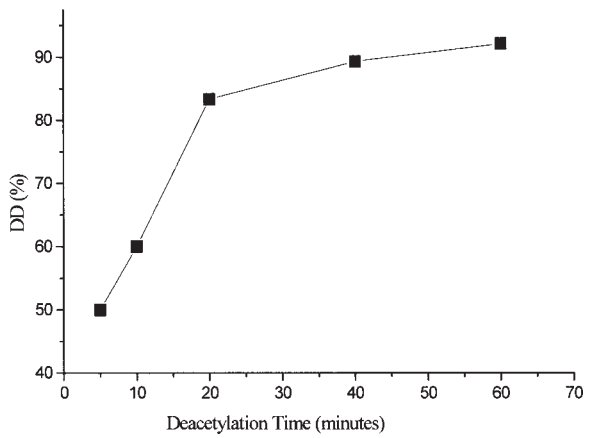

Figure 3 Effect of the deacetylation time on the DD values of deacetylated products prepared from β-chitin (NaOH concentration = 60 wt %; deacetylation temperature = 100°C).

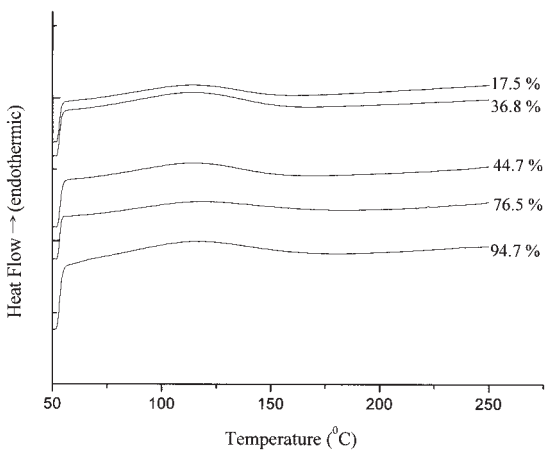

Figure 5 DSC analysis curves of deacetylated products (DD = 17.5, 36.8, 44.7, 76.5, and 94.7%) prepared from β-chitin.

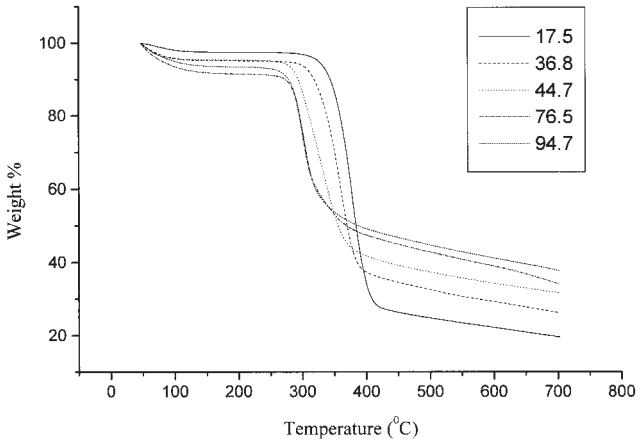

Figure 6 TGA curves of deacetylated products (DD = 17.5, 36.8, 44.7, 76.5, and 94.7%) prepared from β-chitin.

endothermic peak temperature results from the destruction of hydrogen bonding among the molecules.

TGA

Figure 6 presents TGA curves of deacetylated products prepared from β-chitin. Five samples with different DDs (17.5, 36.8, 44.7, 76.5, and 94.7%) were examined. Two weight losses can be observed in these TGA curves. One weight loss at 50–150°C is due to moisture vaporization. The other weight loss at 250–350°C is due to the thermal degradation of deacetylated products prepared from β-chitin. The thermal degradation onset temperature of the deacetylated products prepared from β-chitin decreases as DD increases. Moreover, the amount of the residue is increased as the DD values of deacetylated products prepared from β-chitin increase.

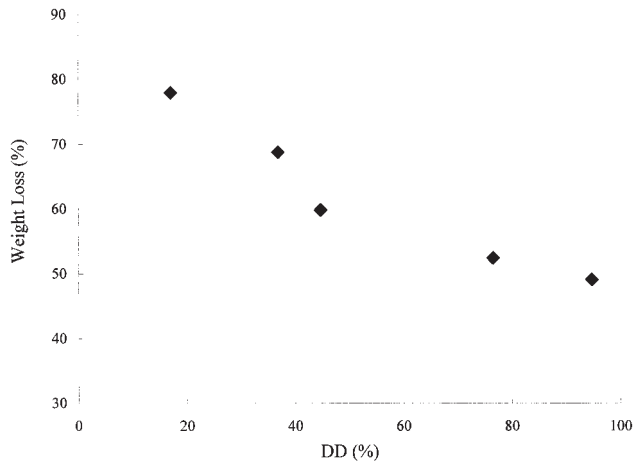

Figure 7 Relationship between the weight loss and DD of deacetylated products prepared from β-chitin.

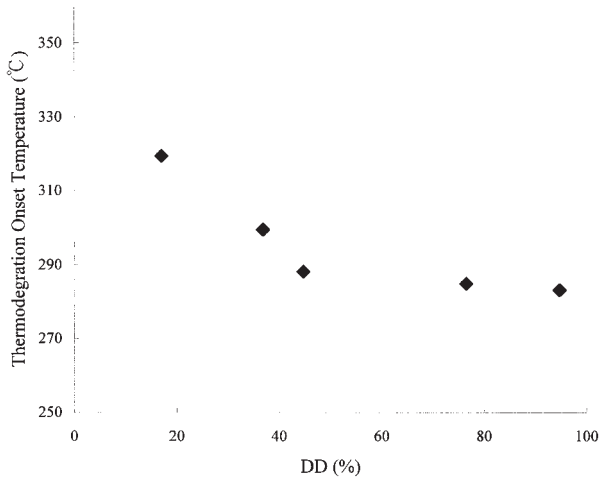

Figure 8 Relationship between the thermodegradation onset temperature and DD of deacetylated products prepared from β-chitin.

Figure 7 shows the relationship between the weight loss and the DD values of deacetylated products prepared from β-chitin. The weight loss of deacetylated products prepared from β-chitin decreases as DD increases. Figure 8 shows the relationship between the thermodegradation onset temperature and the DD values of deacetylated products prepared from β-chitin. The thermodegradation onset temperature of deacetylated products decreases as DD increases. Methacanon et al.[8] reported that the molecular weight of deacetylated products decreased as DD increased. Thus, we have to increase the concentration of NaOH, extend the deacetylation time, or raise the deacetylation temperature to obtain products with higher DD values. These treatments not only increase the DD values of deacetylated products but also reduce the molecular weights. Reducing the molecular weights of deacetylated products reduces the thermodegradation onset temperature.

FTIR analysis

Figure 9 shows the FTIR spectra of deacetylated products with DD values of 17.5, 44.7, and 94.7%. As ex-

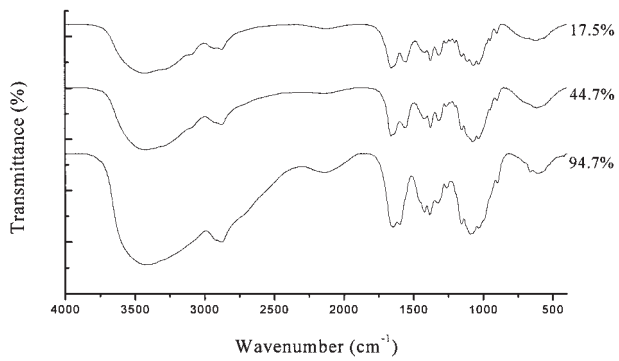

Figure 9 FTIR spectra of deacetylated products (DD = 17.5, 44.7, and 94.7%) prepared from β-chitin.

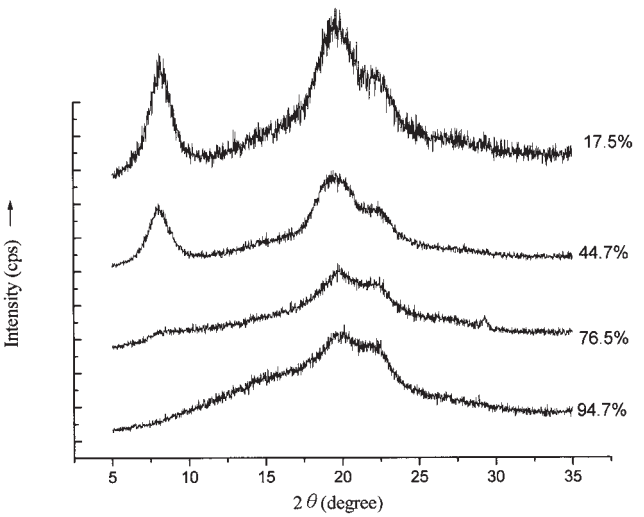

Figure 10 XRD spectra of deacetylated products (DD = 17.5, 44.7, 76.5, and 94.7%) prepared from β-chitin.

pected, N-deacetylation is associated with a progressive weakening of the band (amide I) appearing around 1661, 1662, and 1644 cm^{-1} for DD values of 17.5, 44.7, and 94.7%, respectively. Furthermore, the vibration mode of amide II is also associated with a progressive weakening around 1552, 1558, and 1598 cm^{-1} for DD values of 17.5, 44.7, and 94.7%, respectively. The absence of sharp absorptions around 3500 cm^{-1} in a deacetylated product with a DD value of 94.7% indicates that there are almost no free —OH groups. Both OH_3 and CH_2OH_6 are almost involved in intramolecular and intermolecular hydrogen bonds. The vibration mode of deacetylated products with DD values of 17.5 and 44.7% at 1377 cm^{-1} has been assigned to the —CH bending and C—CH_3 deformation mode. For the deacetylated product with a DD value of 94.7%, the —CH bending and C—CH_3 deformation mode appear at 1383 cm^{-1} .[27,28] The intensity in this region is also reduce as the DD value of the deacetylated product increases.

XRD analysis

XRD patterns of deacetylated products with different DDs of 17.5, 44.7, 76.5, and 94.7% are shown in Figure 10. For DD values of 17.5 and 44.7%, there are three significant diffraction peaks at about 7, 19, and 22°. These peaks are characteristic of the hydrated crystalline structure of β-chitin. The intensity of the diffraction peak at 7° is reduce as the DD values of the deacetylated products increase. This is due to the deacetylation process reducing the crystalline structure of β-chitin. As the DD values of deacetylated products are increased to 76.5% or even higher (94.7%), two diffraction peaks, 19 and 22°, can be observed. These are the characteristic XRD peaks for chitosan.[29]

CONCLUSIONS

The deacetylation experimental results show that the DD values of deacetylated products prepared from β-chitin increase as the concentration of NaOH, the deacetylation time, or the deacetylation temperature increase. CP–MAS ^{13}C NMR is a suitable and nondestructive method for determining the DD values of deacetylated products prepared from β-chitin. DSC shows that the peak temperature slightly increases as the DD values of deacetylated products prepared from β-chitin increase. This endothermic peak temperature results from the destruction of hydrogen bonding among the molecules. TGA shows that the thermodegradation onset temperature of deacetylated products prepared from β-chitin decrease with increasing DD. Moreover, the amount of the residue from the TGA test increases as DD increases. FTIR spectra and XRD patterns show significant differences between the samples with lower DD values (e.g.,, 17.5 and 44.7%) and higher DD values (e.g., 76.5 and 94.7%). XRD patterns for samples with DD values of 17.5 and 44.7% have three significant diffraction peaks. However, there are only two diffraction peaks for samples with DDs of 76.5 and 94.7%.

This study demonstrates the influence of three major deacetylation factors on the DD values of deacetylated products prepared from β-chitin. The influence of the concentration of NaOH, the deacetylation time, and the deacetylation temperature on the DD values of deacetylated products prepared from β-chitin has been studied systematically. In a future study, a 2^3-factorial experimental design will be applied to study the main, two-factor-interaction, and three-factor-interaction effects of these three major deacetylation factors on the DD values of deacetylated products prepared from β-chitin.

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