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The Use of Titration Technique and FTIR Bands to Determine the Deacetylation Degree of Chitosan Samples

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Abstract

The use of titration technique is proposed as an alternative method to determine the degree of deacetyletion (DD) in chitin/chitosan samples, based in both volumes taken before and after titration, and the unit mass of chitosan samples. Chitosan was partially deacetylated to different levels and obtained samples were characterized using Fourier Transform Infrared Spectroscopy (FTIR) to observe the change in band formation. Moisture uptake (%) were also calculated to observe the physical changes in the polysaccharide structure. Crystalline structures were observed and analyzed to see the change in the crystal structure of the anhydrous form of chitosan after deacetylation. It is noticed that the acetyl group can contribute to the formation of hydrogen bonds that can stabilize the crystalline structure. In addition, it is found that the samples with higher acetylation degree presents a higher water absorption percentage. This study describes the role of the functional group, acetyl groups, in crystalline chitin. Such information could provide preliminary understanding of chitosan samples in different degree of deacetylation which similar functional groups are encountered.

Keywords: Chitosan; Titration; Deacetylation degree; Acetyl group; Aminopolysaccharides

Introduction

Chitosan and its parent compound chitin are naturally occurring β-(1,4)-linked linear aminopolysaccharides. Chitosan, though less prevalent in nature, is a useful and easily accessible derivative of chitin. Both two polymers are biodegradable, renewable resources with versatile chemical and physical properties. As such, they are the subject of active scientific and commercial scrutiny [1]. Chitosan is the deacetylated derivative of chitin, which is the second most abundant polysaccharide found on earth next to cellulose. Chitosan is a high molecular weight heteropolysaccharide composed mainly of β-(1,4)-2-deoxy-2-amino-D-glucopyranose units, and partially of β-(1,4)-2-deoxy-2-acetamido-D-glucopyranose [2] (Figure 1).

Chitin is an N-deacetylated product of chitin found in the main component in the shells of crustaceans, such as shrimp, crab, and lobster [3,4]. It is also found in exoskeletons of mollusks, insects and in the cell walls of some fungi [5,6]. Structurally, chitin is an insoluble linear mucopolysaccharide (Figure 2) consisting of *N*-acetyl-D-glucosamine (Glc*N*Ac) repeat units, linked by b-(1→4) glycosidic bonds [7]. Technically, the structure of chitin is highly related to that of cellulose and may be regarded as cellulose where the hydroxyl [—OH] at the C-2 position is replaced by an acetamido [—NHCOCH$_3$] group.[8] Chitin is preferred over chitin in various fields because of its solubillity in acidic, neutral and alkaline solutions, which enables further processing [8-12].

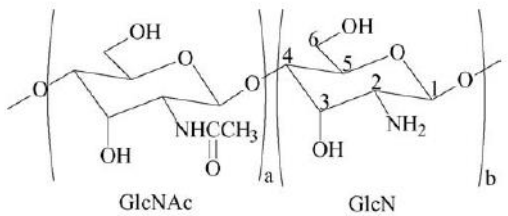

Figure 1: Chemical structure of (a) 2-acetamido-2-deoxy-D-glucopyranose (GlcNAc) and (b) 2-amino-2-deoxy-D-glucopyranose (GlcN) units joined by β (1 _4) glycosidic bond. The a and b contents distinguish chitin from chitosan.

It has many useful properties such as biocompatibility, biodegradability, antimicrobial activity, wound healing properties, antitumor effects, etc.

The only chemical difference between chitin and chitosan is the acetyl group. The presence of the acetyl group causes more high occupancy H-bonds along the inter-sheet direction of chitin model. In contrast, as there are few acetyl groups within the chitosan model, the H-bond occupancy along two directions is similar [13].

Degree of deacetylation (DD) is a percentage measurement of free amine groups along the chitosan backbone [14,15]. In this study, chitosan is deacetylated over 15.7%, then 20.9% at the next step and DD's were measured and calculated using titration technique. Then bond formations have been characterized using FTIR, moisture uptake (%) and microscopy.

Additionally, van der Waals interaction within chitin crystals is significantly enlarged due to the larger molecular mass of acetyl group. The effect of acetyl group on these non-bonded interactions results in not only distinct mechanical properties between chitin and chitosan but also moisture uptake and change in crystal orientation which has also shown in this study. The physical properties of chitosan arise from its crystalline polymorph and biological activities. Crystal structure of the anhydrous form of chitosan provides knowledge of the molecular and packing structure of the chitosan chains in the crystal.

Chitosan is known to have high affinity for dyes belonging to the acid, direct and fiber reactive dye classes. Chitosan or modified chitosan is dyeable with vat sulfur, and disperse dyes. Basic dyes are the only

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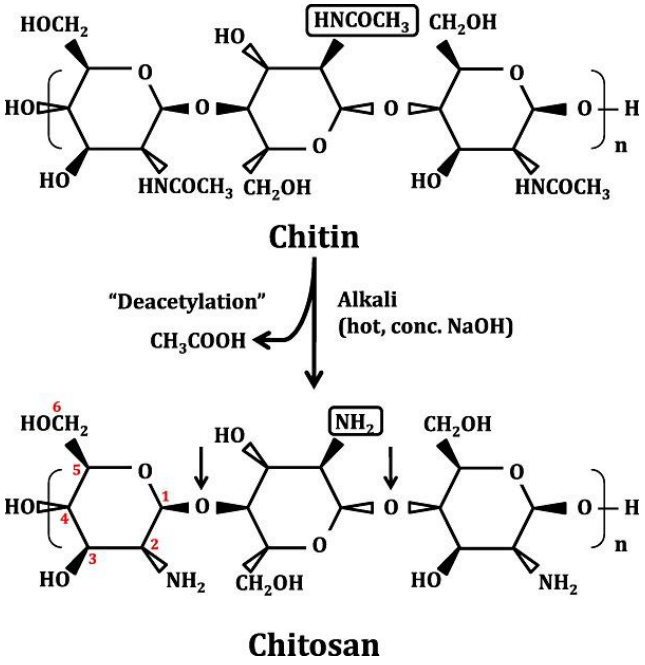


Figure 2: Chemical structure of chitosan, its production from chitin and the specificity of chitonases.

dye classes that have inherent low affinity for chitosan due to charge repulsion. The C (2) basic amine group in each glucosamine group of chitosan is a potential site for ionic interaction with acidic functional groups. Protonation of the basic amine group makes chitosan soluble in dilute aqueous organic and mineral acid solutions. In some cases the ionic interaction is strong enough to render the salt (especially, chitosan sulfate and sulfite salts insoluble in aqueous solution, though the effect can sometimes be overcome by heat or treatment with an excess of other acids [16]. Chitosan ionic interactions also have been characterized in terms of change in crystal orientation with the change of DD.

Experimental

Chitosan purification

Commercial samples of chitosan obtained from Sigma- Aldrich Company with a viscosity average molecular weight of 1.0×10^6. Chitosan particles were first rinsed with methanol/DI water (50:50) and the excess water was filtered using a nylon fabric in order to prevent contamination and excess bond formation with chitosan. Then the particles dried at 80°C for 30 min. The solvents used were hydrochloric acid to solve chitosan.

Measuring the degree of decaetylation (DD)

The degree of deacetylation of chitosan was measured by acid-base titration dissolving chitosan in an acidic solution by protonation of its amine groups, which was calculated by Equation 1

$$DD\% = \{[(V_2 - V_1)\ (L) * M_{NaOH}\ (mol/L) * \text{Unit mass of chitosan } (g/mol)]/(\text{Mass of chitosan sample } (g/mol)\} * 100\backslash$$

In this method, 0.0976 g chitosan completely dissolved in 10 mL of 0.1 N HCl solution and was titrated with a 0.1 N NaOH solution. The initial volume was 16.7 mL. After titrating 0,1 N HCl with 0,1 N NaOH, the final volume was measured as 20.9 mLl and the degree of deacetylation (DD%) was measured and calculated as 70% using Equation 1.

Changing the degree of deacetylation (DD)

Two gram of chitosan was solved in 150 mL of 50% w/w NaOH. Experiment was set up as seen in Figure 3. The solution temperature was kept between 40°C~120°C using a water bath in order to control the heating rates. The nitrogen pressure was adjusted to 6 kPa (6 psi). Three neck round bottom glass flask was used dring the experiment. One of the neck of the glass flask was used for the excess of nitrogen into the solution, the other neck was closed to prevent oxygen excess into the flask and the third neck was used for the thermometer to measure and control the temperature of the solution during the experiments. After reaching 120°C solution temperature, it cooled down to 50°C. Then the samples washed with DI water, then with methanol, till the pH checked as 7. Finally all samples were left for air dry for 2 days [16-19].

Measuring the degree of decaetylation (DD)

A total of 0.0940 g of chitosan sample was dissolved in 10 mL of 0.1 N HCl solution. Then 10 mL DI water was added. The temperature was kept constant at around 20.4°C-20.5°C room temperature using a water bath. A few drops of phenolphatelein was poured into HCl chitosan solution. Then the solution was titrated with 4 mL of 0.1 N NaOH solution using a buret. After titration, the final volume was measured as 8.7 mL. Using Equation 1, DD (%) was calculated as 81%. After changing and measuring the DD (%) as 81, the same procedure was repeated using DD (%) of 81 of chitosan. And the final DD (%) of chitosan was measured as 98%.

FTIR spectroscopy

Infrared spectroscopy was performed on the samples using the Nicolet Nexus 470 Spectrophotometer FTIR infrared analyzer with AVATAR Omni Sampler in the Attenuated Total Reflectance (ATR) mode. The specimen was mounted onto the surface of the Germanium (Ge) crystal in the ATR assembly. A total of 64 scans were aggregated between 1000 cm^{-1} and 4000 cm^{-1} with each spectrum having a 4 cm^{-1} resolution. The aggregated scans showing the absorbance across the infrared spectrum was acquired using OMNIC™ software.

Moisture uptake measurements, WPU (%)

The moisture uptake measurements were carried out in order to

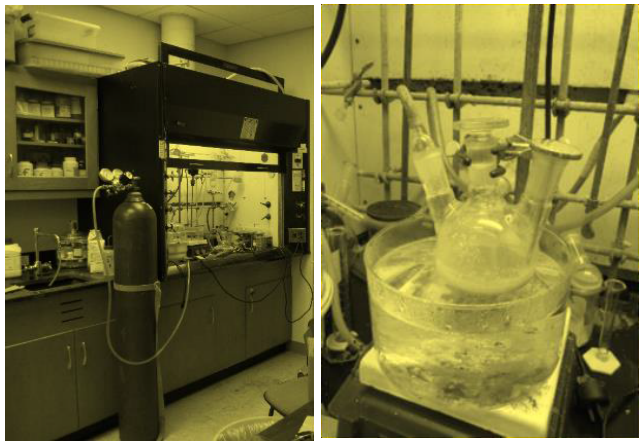


Figure 3: Experiment set up for changing the Degree of deeacetylation (DD) % of chvitosan samples.

see the effects of DD (%) on water absorption of chitosan samples. The water absorption of the chitosan samples with DD (%) of 70-81-98 were measured and calculated as given in Equation 2.

WPU (%)={[(Weight of wet samples–Weight of dry samples) (g)]/(Weight of dry samples) (g)} * 100

Acid dye interactions on chitosan fibrids for microscopic images

Acid salts are forming strong salts on chitosan. Chitosan fibrids were dyed separately for 30 min to equilibrium with Acid Red 360 under isothermal conditions (ΔT=0). Isothermal exhaustion curves of dye on chitosan were obtained to establish the time required to reach equilibrium in the Acid Red 360 experiment. After dyeing, samples were removed from the dye bath, rinsed briefly in DI water to remove surface dye, and air dried separately with Telon Red AFG (Acid Red 360) Dystar with a concentration of 5 g/L using exhaustion method. Shear precipitated dyed chitosan fibrids were examined by polarized light microscopy using a Nikon Labophot-Pol microscope (Nikon, Japan, Serial #951848). Photograph was taken with ISO400 mm film using a Nikon N6006 AF camera (Nikon, Japan).

Results and Discussion

FTIR spectroscopy for chitosan samples

The total reflection infrared spectra of chitosan samples (DD-70-81-98) are shown in Figure 4. FTIR spectra of chitosan shows characteristic bands at 3350 cm^{-1} which refers to O-H stretching and N-H stretching (1° amide). Aliphatic C-H stretching at 2929 cm^{-1} and 2874 cm^{-1}. Glycosidic linkages of C-H stretch at 1152 cm^{-1}–1156 cm^{-1} show its saccharide structure. Two peaks were identified in the resolved N1s spectra of chitosan samples. The peak at 399.9 eV was assigned to N-C=O and NH_2 chemical bindings, while the peak at 400.0 eV was assigned to amino groups in the ammonium form (NH_3^+) [17,18]. Both forms, NH_2 and NH_3^+, were likely to be present in chitosan samples, taking into account the pK_a of chitosan amine groups (ca. 6.5) [19].

Two absorption peaks at 1650 cm^{-1} and 1595 cm^{-1} which refer to C=O (acetyl group) of secondary amide and NH_2 of primary amine. Two absorption peaks at 1425 cm^{-1} and 1385 cm^{-1} can be attributed to the C-H bending. Peaks at 1261 cm^{-1} shows amide III, acetyl group. Absorption peaks at 1149-1150 cm^{-1} were assigned to amide I and II respectively. Chitosan characteristic peaks in IR spectrum confirms its saccharide structure. The peak at 1419 cm^{-1} in C3 is the joint contribution of bend vibration of OH and CH.

Moisture uptake measurements WPU (%)

The chitosan samples with DD (%) of 70 referred as 'A', DD (%) of 81 samples were indicated as 'B' samples and DD (%) of 98 were referred as 'C' samples. First all samples were weighed as 1 g before conditioning (W_1). All samples were weighed again after conditioning at 80°C for 35 min using a convection oven (W_2). The mean values of WPU (%) of chitosan samples were calculated and given in Figure 5.

The water absorption % were increased between 8-10% after increasing the DD (%) of chitosan samples. The change in DD (%) has a significant effect on moisture uptake of chitosan samples.

Acid dye ınteractions on chitosan fibrids and microscopic images

Dissolved chitosan was known to participate in ionic bonds with small water soluble parts. Chitosan-dye ionic interactions were observed to evaluate the inherent affinity of Acid Red 360 for chitosan for the observation of the crystal structure of the anhydrous form of chitosan which provides knowledge of the molecular and packing structure of the chitosan chains in the crystal. Chitosan shear precipitated fibrids were birefringent, showed positive birefringence (Figure 6), indicating that the predominant polymer chain orientation was parallel to the fibrid axis.

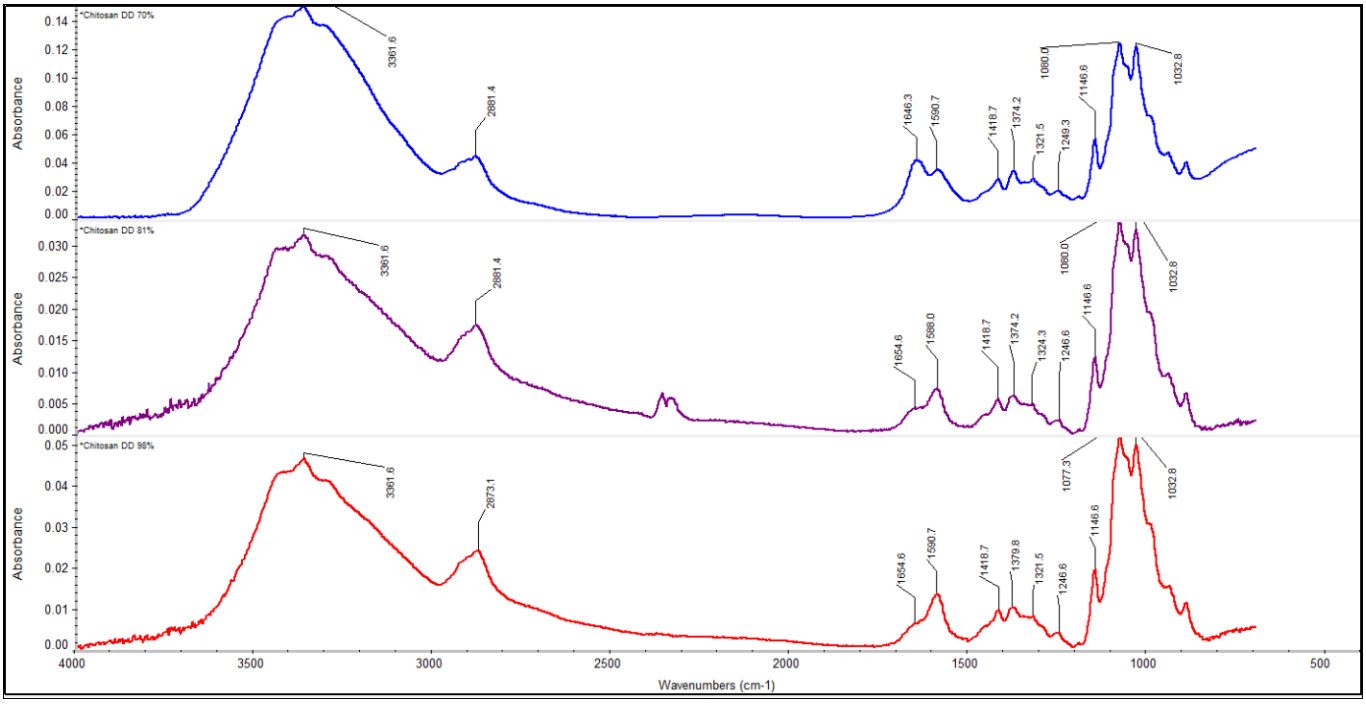


Figure 4: Infrared spectroscopy of the chitosan samples with a) DD (%) 70, b) DD (%) 81, c) DD (%) 98.

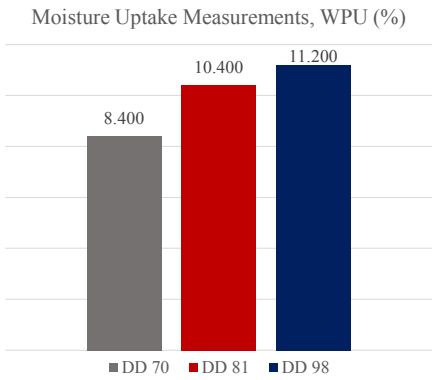
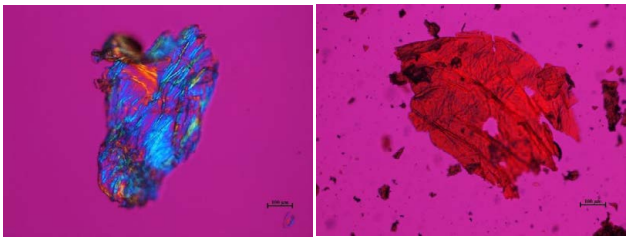


Figure 5: The mean of moisture uptake of chitosan samples (WPU (%)) in having different DD(%).



(a) (b)

Figure 6: Chitosan fibrids DD (%) of 81 undyed (a) and dyed (b) with fluorescent red.

Conclusions

The degree of deacetylation (DD) is an important property of chitosan to determine the way of application of the biopolymer. Therefore, a simple and reliable method of titration technique is proposed as an alternative method to determine the degree of deacetyletion (DD) in chitin/chitosan samples, based in both volumes taken before and after titration, and the unit mass of chitosan samples. The amide I band at 1655 cm^{-1} is used to determine the residual –CO-NH-groups. Two different i.r. bands have been proposed as internal standards depending on the range of acetyl content.[20-21] Chitosan was partially deacetylated to different levels and obtained samples were characterized using FTIR, moisture uptake (%) and microscopy. It is noticed that the acetyl group can contribute to the formation of hydrogen bonds that can stabilize the crystalline structure. In addition, it is found that the samples with higher acetylation degree presents a higher water absorption percentage. The role of the functional group, acetyl groups, in crystalline chitin were presented with this study.

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