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Note

Degree of deacetylation of chitosan using conductometric titration and solid-state NMR

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Chitosan is composed primarily of 2-amino-2-deoxy-D-glucose residues and is produced by the deacetylation of chitin (2-acetamido-2-deoxy-D-glucose) by methods that include alkaline hydrolysis[1] and thermo-mechano-chemical technology[2]. This biopolymer has applications in industries ranging from cosmetics to water clarification and waste management. Accurate determination of the degree of deacetylation of chitosan is essential when studying structure–properties relations and possible industrial uses[3]. Infrared[4] and NMR spectroscopy[1], potentiometric[5], argentometric[5] and colloid titration[6], mass spectrometry[5], ultraviolet spectrometry[7], and gel-permeation chromatography[7] have been proposed as methods for the determination of the acetyl content of chitosan samples. For low acetyl contents, such methods as the IR technique, which depend on deconvoluting the amide I band (1655 cm^{-1}), show inherent resolution difficulties[4]. The need for a reliable technique to determine low acetyl contents, which are typical of commercial chitosan, is evident.

Conductometric titration is a well known analytical tool for quantifying acidic functional groups. It was popularized by the well known text "pH and Electro Titrations" by Kolthoff and Laitinen[8]. Numerous applications, such as measuring the number of carboxylic and sulfonic acid groups in wood pulps[9], quantifying strong and weak acids bound to latexes[10], and characterizing heparin[11] are known. In fact, it is probably the most accurate technique for analyzing multifunctional polysaccharides and should prove invaluable for characterizing glycosaminoglycans.

In this study, the degree of deacetylation of chitosan samples was determined by the conductometric approach and solid-state NMR. The results were compared to those obtained by ultraviolet spectrophotometry, a frequently used technique for these determinations. Also included in this study are results on a water-insoluble film of chitin crystallites (hydrochitin)[12,13], which exemplifies a high level of acetyl content.

EXPERIMENTAL

Materials.—Samples of chitosan, VNS-458, 461, and 466, were obtained from Vanson Chemical Company, Inc. (Redmond, WA). Sample MAR-1, was a sample from Stein Hall, Inc. The sample CHITO-FIB was obtained by spinning an 8.2% chitosan solution (w/w in 4% aq HOAc) into an ethylene glycol–aq KOH coagulating solution. The fibres were extruded by a continuous process and, after washing, were air dried on a drum at room temperature.[14] X-Ray diffraction examination showed that the sample was crystalline and slightly oriented. Hydrochitin was obtained by hydrolyzing a "practical grade" chitin (Sigma Chemical Company, Inc.) with 3 M HCl for 1 h. After washing by centrifugation to pH ≈ 2, the suspension was dialyzed (against distilled water) until neutral. The final product was a colloidal dispersion of rod-like microfibrillar fragments which are stabilized by NH_3^+ charges thought to reside on some free amino groups at the crystallite surfaces[12].

Procedure.—Solutions containing ~ 1 g of oven-dried chitosan in 20 mL of 1% HOAc by weight were prepared by stirring vigorously for 2 h. The viscous solutions were poured onto Teflon-coated glass plates and dried overnight. Because chitosan is only soluble in its protonated form, chitosan films prepared in this way had the amino groups protonated prior to titration. The films were transferred to a three-necked round-bottom flask containing 450 mL of 1 mM NaCl, 5 mL of 1 M HCl and a few drops of glacial HOAc to ensure swelling and dissolution. The titrations were performed under N_2 while maintaining a low stirring rate. An automated titration system, consisting of a conductivity meter (Metrohm 600, sleeveless immersion cell with built-in temperature probe) and a titration burette (Metrohm 665 Dosimat), all interfaced to an AT-286 personal computer, was utilized for the titrations. A computer program controlled the titration parameters. The 0.10 M NaOH titrant was added at a rate of 0.5 mL every 20 s (i.e., the time required to achieve a stable conductance value). A graph of conductance as a function of the volume of titrant was plotted on line and the equivalence points, corresponding to sharp changes in slope, were used to calculate the number of equivalents of amino groups.

For the hydrochitin sample, the colloidal dispersion was dried onto a glass plate which yielded a brittle translucent film of crystalline chitin. The film was treated as with the chitosan samples although the titration proceeded on swollen fragments of the brittle film rather than dissolved macromolecules.

Solid-state ^{13}C NMR spectra were recorded at 75 MHz on a Chemagnetics CMX-300 spectrometer. Approximately 200 mg of sample was inserted into a 7.5-mm rotor. The cross-polarization pulse sequence was utilized for all the samples which were spun at the magic angle at 5000 Hz. A contact time of ~ 1 ms and a recycle delay of 3 s were used.

RESULTS AND DISCUSSION

Conductance is a function of the sum of the conductance of each type of ion present in solution. Since hydrogen and hydroxyl ions are the most conducting of all the ions, conductometric titration monitors the change in conductance due to these ions as a function of the volume of titrant added[9]. In Fig. 1, the first rapid descending branch corresponds to the neutralization of the H^+ ions of the 1 M HCl added. Less than 5 mL of the acid is neutralized by the titrant because of the consumption of acid by the chitosan to protonate remaining unprotonated amino groups. The curvature at the lower end of the branch is attributed to the initial dissociation of the protonated amino acid groups of the chitosan. The first ascending branch is due to the neutralization of the protonated amino groups. A small deviation from linearity in the plot occurs during the final phase of neutralization coinciding with the onset of the precipitation of chitosan (pH ≈ 6.5). The final upward branch corresponds to the increase in conductance due to an excess of added base. The equivalence was calculated by determining the positions of intersection of the three branches of the titration curve. The difference between the two intersection points corresponds to the volume of base required to neutralize the amino acid groups. The number of equivalents of acid groups was calculated using the following formula:

$$[NH_3^+](\text{mequiv/kg}) = \frac{1000 \times \text{molarity(moles/L)} \times \text{volume of titrant (mL)}}{\text{mass of sample (g)}} \quad (1)$$

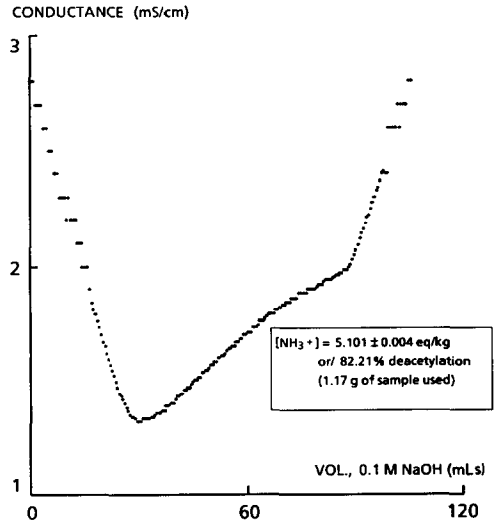

Fig. 1. Conductometric titration curve for chitosan sample VNS-458.

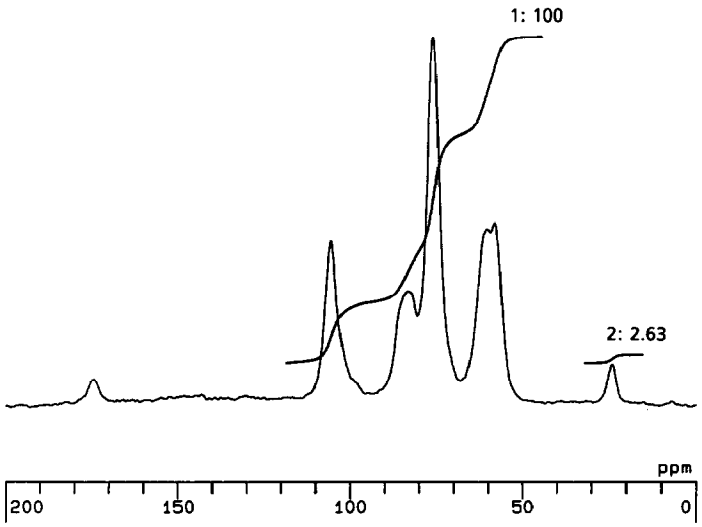

Fig. 2. CP/MAS spectrum for chitosan sample VNS-458. Integration 1 corresponds to the glucose carbon atoms, whereas integration 2 corresponds to the methyl group, which is proportional to the acetyl content.

The level of deacetylation of the chitosan material was calculated by comparison between the number of free amino groups (per unit weight of the sample) and the equivalent weight of the repeat unit in the polymer chitosan[6]. The results obtained by conductometric titration were compared to those values calculated using ultraviolet spectrophotometry (data from the supplier). The UV method measures the acetyl content of chitosan by relating the absorbance of the acetamide groups at 220 nm to the acetyl concentration[7].

Solid-state NMR determination of the degree of deacetylation has been reported in a study by Pelletier et al.[2] where ^{13}CNMR was used to study the deacetylation of chitin and chitosan products. The percentage deacetylation, as in the case of our analysis, was calculated by comparing the area of the CH_3 resonance to the resonances of the glucose carbons (compare Fig. 2).

The conductometric titration values, for the degree of deacetylation, are intermediate between the results obtained by UV spectrophotometry and CP/MAS (see Table I). UV determination shows lower estimates for the degree of deacetylation of the chitosan samples as compared to the other two techniques. This has also been reported in a study by Aiba[7], where the UV technique was compared to gel-permeation chromatography, to IR, and to colloid titration using elemental analysis as reference for the determination of the level of deacetylation of some chitosan materials.

By comparison, the results obtained from CP/MAS are consistently higher than results from UV and conductometric methods. The limitations of the CP/MAS technique for quantitative purposes is known and becomes important at low acetyl contents (i.e., Sample VNS-461). However, for high acetyl contents, as in the highly

TABLE I

Percentage deacetylation by conductometric titration, ultraviolet spectrophotometry, and solid-state NMR

Sample	Conductimetry	UV [a]	CP/MAS
VNS-461	97.2± < 1%	92.3	100± ~ 5%
VNS-466	91.6	83.3	92
VNS-458	82.2	76.1	83
MAR-1	83.8	–	84
CHITO-FIB	83.3	–	88
HYDROCHITIN	3.5	–	15

[a] Technique used by the supplier, Vanson Chemical Company, Inc.[7].

crystalline hydrochitin sample (see Table I), a limitation to the conductometric method is demonstrated. Thus, only the amino groups on the surface of the crystallites are accessible for titration while those within the crystalline structure are inaccessible, although CP/MAS detects all amino groups. It is noteworthy that the CHITO-FIB sample was also a crystalline material which swelled in dilute acetic acid but may have had inaccessible amino groups in the crystalline domains, thereby accounting for the large difference between the CP/MAS and conductimetry results.

A routine technique that measures a wide range of acetyl contents of chitosan is needed. Conductometric titration and solid-state NMR fulfill these requirements. A series of chitosan samples (including a sample of hydrochitin) with various degrees of deacetylation has been studied using these methods. The results obtained were comparable to values derived from UV spectrophotometry. However, conductimetry showed excellent precision (< 1%) and is recommended for characterizing low acetyl contents due to amino group accessibility factors. In the present study, the hydrochitin sample was from a liquid crystalline suspension formed by microfibrillar fragments[12,13]. The properties of such a system are probably controlled by surface-charge characteristics and a knowledge of such charges is essential for theoretical predictions. Results[15] using electrophoresis observations showed that hydrochitin has a positive charge after dialysis to neutral pH. We consider that conductometric titration is a reliable way to determine these surface charges. Solid-state NMR, however, is recommended for determinations at high acetyl content, since such samples, being intrinsically water insoluble, lack amino group accessibility.

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