Communications to the Editor

Degree of Acetylation of Chitin and Extent of Grafting PHB on Chitosan Determined by Solid State ^{15}N NMR

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Introduction. Chitin is a linear polysaccharide composed of β-(1−4)-linked 2-deoxy-2-acetamido-D-glucose units (Glu-NHCOCH$_3$).[1] Acetyl groups can be removed by methods such as heterogeneous alkaline hydrolysis[2] and thermomechanochemical technology.[3] The materials delivered from deacetylation of chitin with a degree of deacetylation over 75% are usually called chitosan[4] and referred to as Glu−NH$_2$. Chitin and chitosan have applications in industries ranging from cosmetics to water clarification, waste management, and pharmaceutical adjuvant, depending on the degree of deacetylation.[5] Special chemical moieties or block polymers can be grafted onto chitosan by taking advantage of the reactive amino groups on chitosan; e.g., deoxycholic acid was grafted on chitosan[6,7] and poly(3-hydroxybutyrate) (PHB) was grafted on chitosan[8] via the reaction of amino and carboxylic acid groups.

Accurate determination of the degree of deacetylation of chitin is essential when studying structure−property relations and possible industrial uses. In the case of grafting, e.g., grafting poly(3-hydroxybutyrate), PHB, on chitosan involves a small substitution degree, or the concentration of NH$_2$ undergoes a small change; hence, accuracy in their determination is even more important.

Different methods have been applied to determine the acetyl content of chitosan, including infrared spectroscopy,[9,10] solid-state NMR spectroscopy,[3] mass spectroscopy,[11] potentiometric,[11] argentmetric[11] and colloid titration,[12] ultraviolet spectrometry,[13] and gel-permeation chromatography.[13] A comparison of different methods was reported by Aiba,[13] including elemental analysis. Recently, conductometric titration[14,15] and solid state ^{13}C NMR[3,15] have also been applied to evaluation of acetyl contents of chitin or chitosan. Conductometric titration is considered accurate except for some highly crystalline hydrochitin samples[15] due to the accessibility problem. Solid state ^{13}C NMR is considered accurate for determination of acetyl contents of chitin or chitosan samples with high acetyl contents. From a solid state ^{13}C NMR spectrum, the acetyl content is calculated by comparison of the integral of methyl carbon from acetyl groups to the integrals of other carbons from the main chains. A small distortion of the spectrum baseline can greatly affect the results for the degree of acetylation of chitosan with low acetyl contents.

In this paper, we report the use of solid state ^{15}N NMR to determine the degree of acetylation of chitin and the grafting degree of chitosan. We found that solid state ^{15}N NMR is a more reliable method, especially for the determination of the extent of grafting poly(3-hydroxybutyrate), PHB, on chitosan, albeit more time-consuming due to the low natural abundance of ^{15}N.

Nitrogen NMR spectroscopy is very attractive for biophysical studies because there are relatively few nitrogen atoms in biopolymers compared to carbon or hydrogen[16] and chemical shifts of nitrogen are very sensitive to the chemical environments.[17] Solid state ^{15}N NMR has been applied to the investigation of biopolymers,[16] especially the structure and conformation of DNA or peptides.[16,18] Extensive reviews on ^{15}N NMR can be found in the literature.[17,18−20] Solid state ^{15}N NMR has also been applied to structural analysis of polymer materials, for example, analysis of nylon 66.[21] In the case of chitin and chitosan, the chemical environments for nitrogen atoms are: the nitrogen is either on amino or on amide, so that two major peaks should be expected.

Experimental Section. In this study, chitin samples from Vanson Chemical Company, Inc. (Redmond, WA), are used to establish the solid state ^{15}N NMR method. These samples were hydrolyzed for different lengths of time and were characterized by a UV technique and also characterized by conductometric titration and solid-state NMR in our previous study.[15] The chitosan used for the grafting reaction is from CarboMer, Inc. (Lot #5-0Y65). The low molecular weight PHB sample used in this study was a hydrolyzed product of a pure PHB polymer of ICI(BXG08, MBL 100/848). The conditions for the hydrolysis of PHB in a 3 N HCl solution at 104.5 °C were similar to those in ref 22. The hydrolysis took 12 h and yielded a PHB with estimated molecular weight of 1600 g mol^{-1}. The reaction scheme for grafting low molecular weight PHB on chitosan is shown in Scheme 1. The grafting reaction took place in DMSO. Acetic acid was used as a catalyst and the assistant agent for dissolution of chitosan. The detailed conditions for grafting reaction conditions were similar to the published ones described before.[8]

Solid state ^{15}N NMR spectra were recorded at 30.35 MHZ on a Chemagnetics CMX-300 spectrometer. Approximately 200 mg of sample was inserted into a 7.5-mm rotor. To minimize errors in the quantisation of the spectra due to differing cross polarization rates involving NH$_2$ vs NH groups or incorrect setting of the Hartmann−Hahn match, cross polarization was achieved using a variable amplitude contact time.[23] The contact time consisted of 10 steps in amplitude each of 300 ms duration for a total contact time of 3ms. Samples were spun at the magic angle at 4000 Hz. A recycle delay of

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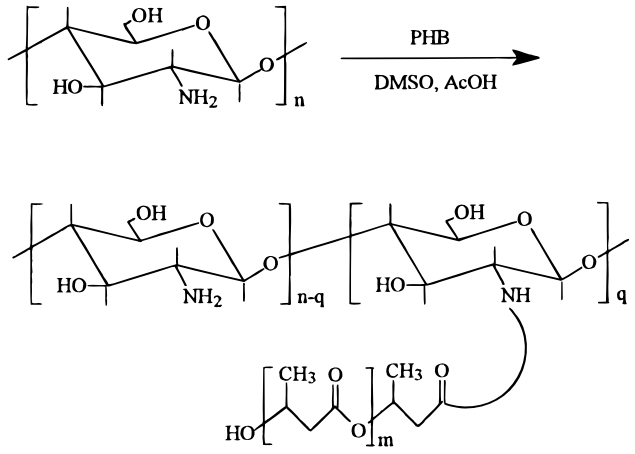
Scheme 1



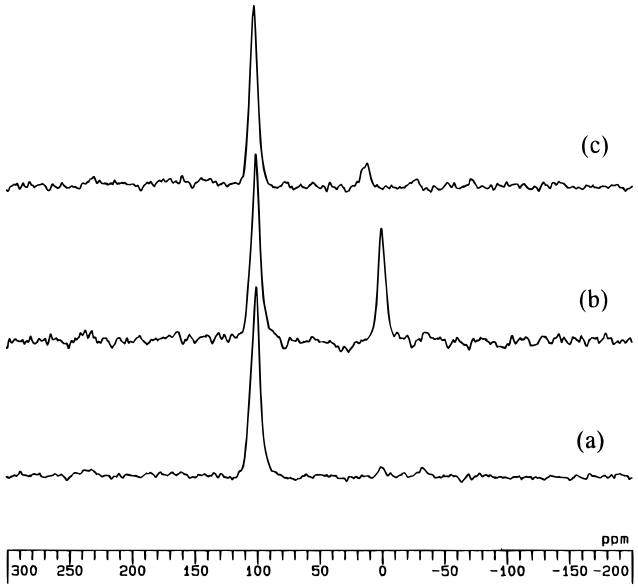


Figure 1. Solid state ^{15}N NMR spectra of chitins: (a) the original chitin; (b) the sample after NaOH hydrolysis of (a) for 6 h; (c) the chitin sample obtained from a further treatment using 3 N HCl at boil for 1.5 h after NaOH hydrolysis for 7 h.

1 s was used. Usually 50000−80000 scans were taken for one spectrum. The chemical shifts were externally referred to NH_4^+ of enriched ammonium nitrate.

Results and Discussions. Figure 1 shows solid state ^{15}N NMR spectra for an original chitin sample and the chitin sample which was alkali-treated for 6 h to deacetylate. The spectrum from the original purified chitin (Figure 1a) shows mainly one peak for the nitrogen of the acetamides at chemical shift $\delta = \sim 101$ ppm. The spectrum from the alkali-deacetylated chitin sample (Figure 1b) shows a second peak at $\delta = \sim 0$ ppm, which should be from amino groups. Actually, a small peak assigned to amino could be seen at ~0 ppm in Figure 1a. The integral ratio from the peak at 101 ppm to the total integrals from both peaks at 101 and 0 ppm in Figure 1b should give the mole fraction of acetamide. The spectra show the simplicity of the ^{15}N NMR technique for evaluation of the deacetylation of chitin. The most striking characteristic of the spectra is the pronounced separation of the two peaks from amide and amino groups. ^{15}N NMR spectra are easier to analyze compared to ^{13}C CPMAS spectra, in which there is overlap of all the oxygen bearing carbons of both chitin and chitosan and this must be taken into account in the calculation of percentage of deacetylation.

Table 1. Degree of Acetylation of Chitin or Chitosan Determined by Solid State ^{15}N and ^{13}C NMR

		degree of acetylation	
sample	NaOH hydrolysis/h	by ^{15}N NMR	by ^{13}C NMR
1	0	97	
2	1	92	94
3	2		83
4	3	75	78
5	4		70
7	6	66	63
8	7	65	63
J1[a]	7*	89	91
chitosan[b]	~30	8	
chitosan[b]	~30	9	

[a] Sample was further treated after alkali-deacetylation using 3 N HCl at boil for 1.5 h.[15b] [b] The chitosan sample from CarboMer. The sample was run twice to compare the reproducibility of the method.

Table 1 compares the results from ^{15}N NMR and those from ^{13}C NMR in our previous publication.[15] These results demonstrate the accuracy of ^{15}N NMR compared to that of ^{13}C NMR.

As indicated by other researchers, the ^{15}N resonances are very sensitive to its chemical environment.[17] One of the samples studied corresponds to a hydrochitin which was prepared by 3 N HCl acid hydrolysis (at the boil for 1.5 h, cf. Table 1) leaving the amino groups in the form of positively charged NH_3 leading to a clearly observable chemical shift of NH_3^+ ($\delta = \sim 13$ ppm) relative to NH_2 ($\delta = \sim 0$ ppm). Thus, ^{15}N spectra provide direct evidence of amino group protonation; see Figure 1c.

It is well-known that it is difficult to determine the extent of reaction after grafting PHB[8] or other materials[6,7] on chitosan. Solution ^1H and ^{13}C NMR were not very practical due to the solubility problem.[8] Solid state ^{13}C NMR spectra of grafted chitosan with low molecular weight PHB showed overlapping of the methyl groups from the acetyl moiety and those from the PHB backbone, which prevents accurate analysis. In this study, we have successfully applied the solid state ^{15}N NMR method to evaluate the extent of grafting PHB block to chitosan (see reaction Scheme 1). The satisfactory results demonstrate the significance of the development of the method. Figure 2 shows the spectra of before and after grafting PHB on chitosan. Figure 2b shows clearly the increase of the acetamide groups after the grafting reaction (ca.. 12% increase in the ratio of amide to amino), though the ratio of signal-to-noise is rather low.

A sample (chitosan sample from CarboMer) has been run twice to check the accuracy and reliability of the method. The results are listed in Table 1, which indicates that the method gives reproducible results even at a low degree of acetylation. The accuracy of the method can be estimated to be ±10%.

Conclusions. In this study, solid state ^{15}N NMR has been successfully applied to characterization of chitin or chitosan. This technique gives simple and unique spectra for evaluation of the degree of acetylation of chitin or chitosan. The present study also shows the utility of the solid state ^{15}N NMR method for evaluation of grafting on chitosan.

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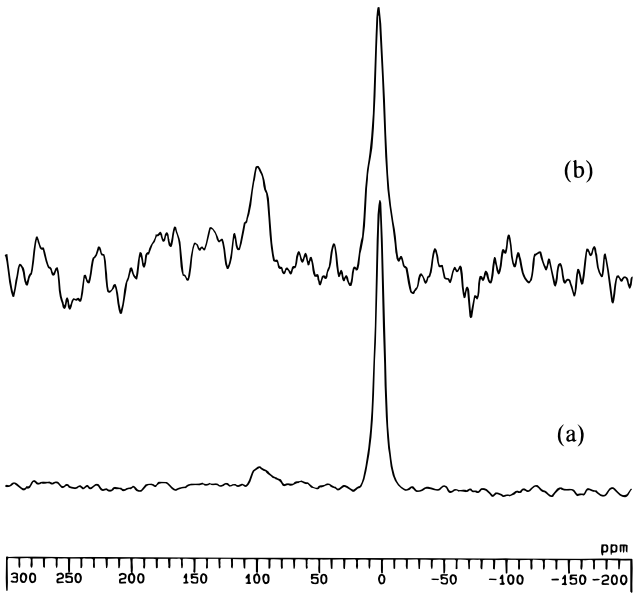

Figure 2. Solid state ^{15}N NMR spectra of (a) the chitosan sample from CarboMer Inc. (Lot #5-0Y65) and (b) the chitosan grafted with hydrolyzed PHB.

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