nanalysis

USP-NF: Chitosan

Degree of Deacetylation Determination in Chitosan According to USP-NF Monograph Method Using ^{1}H Benchtop NMR

Background

Chitosan is a polysaccharide composed of two units: *N*-acetyl-D-glucosamine and glucosamine (**Figure 1**).[1] These units are structurally similar, but the glucosamine fragment is deacetylated (R = H in **Figure 1**). This deacetylation occurs when chitin, a polymer composed entirely of *N*-acetyl-D-glucosamine units, undergoes an alkaline treatment, or gets degraded by chitinase.[2] The amount of deacetylation observed in the final chitosan product, also termed the degree of deacetylation, is often presented as a percentage (DDA%). This value is crucial to the physical and chemical properties of the chitosan, and while different assays exist to determine this value, NMR remains the industry standard, as demonstrated by its inclusion in the USP-NF monograph for chitosan testing.[3]

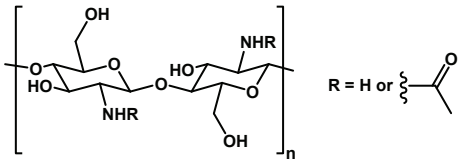

Figure 1. *General structure for chitosan. The DDA% is determined by comparing the protons of the methyl group in the remaining acetyl fragments with the seven protons with oxygen neighbors in the glucosamine ring.*

The precursor to chitosan, chitin, is typically obtained from the shells of crabs or shrimp, or the mycelia of fungi before being converted.[2] Functionally, chitosan mostly acts as a structural polysaccharide in nature, analogous to cellulose. Unlike cellulose, however, the amine group in chitosan has allowed for the formation of many substituted forms of this polymer, mainly via *N*-acylation of Schiff reactions.[4] These modified chitosan polymers have exhibited increased solubility and enhanced reactivity. Over the years, chitosan has found uses in various industries, including cosmetics, artificial skin and chitosan-based dressings, nutrition, contact lenses, and many others.[5] They have also been studied in detail for their proposed anti-microbial properties.[4]

Degree of deacetylation test

To determine the degree of deacetylation in chitosan, a sample is stirred in formic acid-d_2 for 24-48h, or until it is fully dissolved. Then, a ^{1}H NMR spectrum is collected and the integration of the methyl group in the remaining acetyl fragments (A_1) is compared to the combined integrations of the seven protons with oxygen neighbors in the glucosamine ring (A_2).[3] A representative ^{1}H NMR spectrum of chitosan in formic acid-d_2 is presented in **Figure 2**, along with labels for the regions of interest.

In total, 8 different chitosan samples from different suppliers were analyzed, and the DDA% values were obtained using qNMR, according to the USP-NF monograph method. The samples analyzed in this study were: **Low MW**, **B-95-545382**, **C-M-85-401132**, **A-HD-877**, **C-M-95-401132**, **AL-476**, **AL-120**, and **AL-801**.[6] The analyses were performed on a 60 MHz benchtop NMR instrument, in addition to a traditional 400 MHz high-field NMR instrument.

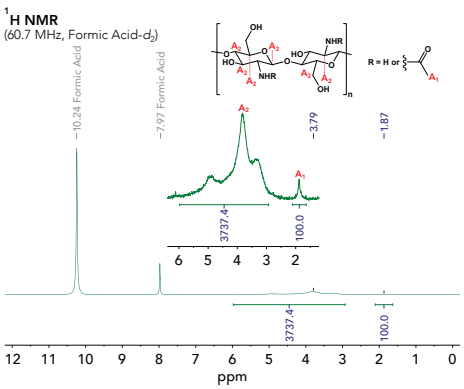

Figure 2. *^{1}H (60.7 MHz) NMR spectrum of **A-HD-877** in formic acid-d_2 with the general structure of chitosan shown. The protons of the methyl group in the remaining acetyl fragments are observed around 1.9 ppm (A_1), while the combined protons with oxygen neighbors in the glucosamine ring are observed between 2.5-6 ppm (A_2).*

Results

In total, 3 individual samples were prepared for each chitosan, and each sample was analyzed in triplicate, for a total of 9 analyses per chitosan. For all samples, very close agreement between the values obtained at 60 MHz and 400 MHz were obtained. These results are summarized in **Table 1**. Additionally, these values match the reported DDA% values[6] for these products closely, confirming that this monograph method can be performed using a benchtop NMR instrument. For certain applications, NMR analysis at lower fields can suffer from peak overlap due to decreased dispersion. However, the 60 MHz instrument can easily integrate the A_1 and A_2 regions separately, providing accurate and precise results. The analysis takes < 40 minutes, and while the chitosan requires stirring to dissolve, sample preparation is minimal.

Table 1. *Summary of the DDA% values at both 60 MHz and 400 MHz, in addition to the reported values, based on the supplier labels.*

Chitosan	Sample	DDA%		
		60 MHz[a]	400 MHz[a]	Reported[b]
Low MW	1	77 (1.2)	77 (0.7)	76[c]
	2	77 (1.1)	77 (0.4)	
	3	77 (1.3)	77 (0.5)	
B-95-545382	1	95 (1.3)	96 (2.3)	98[d]
	2	95 (0.6)	96 (1.9)	
	3	95 (2.9)	96 (1.1)	
C-M-85-401132	1	90 (0.7)	91 (0.8)	90[d]
	2	90 (0.5)	91 (1.9)	
	3	90 (4.0)	91 (0.6)	
A-HD-877	1	94 (2.0)	94 (1.8)	90[d]
	2	94 (3.3)	94 (4.6)	
	3	94 (1.0)	94 (3.5)	
C-M-95-401132	1	94 (1.6)	94 (0.8)	98[d]
	2	94 (4.6)	94 (3.4)	
	3	94 (4.1)	94 (1.4)	
AL-476	1	90 (2.8)	91 (0.6)	>90[e]
	2	90 (0.6)	91 (0.9)	
	3	90 (2.9)	91 (1.0)	
AL-120	1	84 (0.8)	86 (2.4)	>80[e]
	2	84 (0.2)	86 (0.6)	
	3	84 (0.5)	86 (0.2)	
AL-801	1	84 (0.6)	87 (0.9)	>80[e]
	2	84 (1.1)	87 (1.0)	
	3	84 (1.6)	87 (1.6)	

[a]Average of triplicate analyses, the relative standard deviation (RSD) values for which are provided in parentheses. [b]Reported DDA% values, based on the supplier labels. [c]Determined using a proprietary method. [d]Determined using a titration method. [e]Determined using high-field NMR.

If you have any questions about the incorporation of benchtop NMR into your USP-NF workflows, or about the work presented herein, please don't hesitate to contact us!

References

(1) Ravi Kumar, M. N. V. *React. Funct. Polym.* **2000**, *46*, 1–27.

(2) Kou, S.; Peters, L. M.; Mucalo, M. R. *Int. J. Biol. Macromol.* **2021**, *169*, 85–94.

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(4) Sahariah, P.; Másson, M. *Biomacromolecules* **2017**, *18*, 3846–3868.

(5) Cheung, R. C. F.; Ng, T. B.; Wong, J. H.; Chan, W. Y. *Mar. Drugs* **2015**, *13*, 5156–5186.

(6) Chitosan samples were received from various suppliers and used without further purification. MilliporeSigma: **Low MW** p/n: 448869, lot BCCF8502. ChitoLytic: B-95-401132 lot: 251716; **C-M-85-401132** lot: 251848; **A-HD-877** lot: 41001; **C-M-95-401132** lot: 251848; **AL-476** lot: 1055; **AL-120** lot: 20113; **AL-801** lot: 10447.