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Analysis of carboxyl content in oxidized celluloses by solid-state ^{13}C CP/MAS NMR spectroscopy

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Abstract

A noninvasive method to determine the carboxyl content in oxidized celluloses, using solid-state carbon-13 cross-polarization–magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy, has been developed. Standard samples containing 0, 4, 8, 12, 16, and 20% carboxyl content were prepared by mixing appropriate amounts of powdered cellulose, a non-oxidized cellulose standard prepared from cotton linter by ball-milling for 96 h, and a commercial oxidized cellulose sample that had a 20% carboxyl content. Standard curves were constructed by plotting the percent carboxyl content against the peak area at 171 ppm, normalized with (i) a peak area at 104 ppm and (ii) the sum of peak areas at 171, 62, and 65 ppm. The regression analysis of the curves yielded a linear relationship with correlation coefficient (R^2) values of 0.9868 and 0.9863, respectively. To validate the methods, five new samples of oxidized cellulose were prepared and analyzed. The values obtained were comparable to those determined by the calcium acetate method described in the United States Pharmacopoeia (USP), indicating that the solid-state ^{13}C CP/MAS NMR can be used to analyze carboxyl content in oxidized celluloses.

Keywords: Oxidized cellulose; Powdered cellulose; Carbon-13 cross-polarization–magic nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy

1. Introduction

Oxidized celluloses containing carboxylic groups (Fig. 1) represent a new class of biodegradable materials. They have been accepted for use in humans to stop bleeding during surgery and to prevent the formation and reformation of postsurgical adhesions (Ashton, 1968; Bowman and Cooke, 1994; diZerega, 1994). Studies also show that they possess antibacterial activities (Dineen, 1976; Abaev et al., 1986), and may be

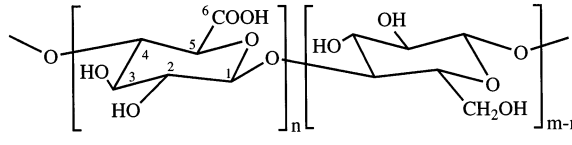

Fig. 1. Structure of 6-carboxycellulose (oxidized cellulose).

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useful in bone regeneration (Finn et al., 1992) and periodontal therapy (Pollack and Bouwsma, 1992).

Currently, oxidized cellulose containing 16–24% carboxylic groups is commercially available in powder, gauze, and fabric forms (Surgicel®, 1989). However, they are relatively expensive to be used as an excipient in the development of a pharmaceutical product. Thus, considerable efforts are being made to develop new, cost-effective methods that would also provide products with different levels of oxidation and degree of polymerization. To quantitatively determine the carboxyl content in oxidized cellulose, several methods have been developed (Samuelson, 1963; Nevell, 1985). These include: (a) an alkalimetric titration (Samuelson and Wenneerblom, 1954), (b) a calcium exchange method (Sobue and Okubo, 1956), (c) an iodometric titration method (Ludtke, 1935; Nabar and Padmanabhan, 1950), (d) CO_2 evolution method (Yackel and Kenyon, 1942; Unruh and Kenyon, 1942; Anderson, 1959; Samuelson, 1963; Franc et al., 1984); and (e) a methylene blue adsorption method (Davidson, 1948a,b). However, all these methods are destructive and have limitations. For example, the alkalimetric method is good only if no carbonyl groups are present. The calcium exchange method, on the other hand, requires steeping of the sample with an acid followed by extensive washing with water prior to analysis. During steeping, some of the carboxyl groups may convert into lactones, and consequently, may result in lower carboxylic content values. The results from iodometric titration include lactones (Nabar and Shenai, 1970) and/or ene-diols that are tautomeric with hydroxy-ketone groups at C-2 and C-3 (Singh, 1974). The CO_2 evolution method requires a small correction for the carbon dioxide given off by purified cellulose. The methylene blue adsorption method, however, is precise and can be used to quantitate materials with small amounts of carboxyl content.

In this paper, we report the use of solid-state carbon-13 cross polarization–magic angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy as a non-invasive analytical tool to determine the carboxylic content in oxidized celluloses. Solid-state ^{13}C CP/MAS NMR spectroscopy has been extensively investigated to study the bulk properties of solids (e.g. presence or absence of water of hydration, different conformers in crystalline forms, polymorphism, different tautomers, drug–polymer interactions, solid-state reactions, and structure elucidation) (Aboul-Enein, 1990). In the field of cellulose chemistry, it has been used for the characterization of various polymorphs of cellulose (VanderHart and Atalla, 1984; Stephenson, 1985; Horii et al., 1987a; VanderHart and Atalla, 1987), determination of crystalline and amorphous contents in cellulose and purified celluloses (Teeaar et al., 1987; Ek et al., 1995), and identification of the cellulose chain conformations within crystalline and non-crystalline regions (Horii et al., 1987b). The results presented in this paper show that the ^{13}C CP/MAS NMR can also be used to quantitate carboxyl content in oxidized celluloses.

2. Experimental

2.1. Materials

Oxidized cellulose containing 20% carboxyl content was received from Eastman Chemicals Co. (Knoxville, TN) and used as a standard. Powdered cellulose, used to prepare standard and test oxidized cellulose samples, was prepared from cotton linter by ball milling for 96 h. To avoid any effect of the particle size on the NMR spectra, both of these materials were sieved and the fraction containing particles ranging in size between 74 and 105 μm was collected and used in the study.

2.2. Preparation of standard samples

The commercial oxidized cellulose and powdered cellulose served as 20 and 0% carboxyl content standards, respectively. Standard samples containing 4, 8, 12, and 16% carboxylic contents were prepared by mixing appropriate amounts of the commercial oxidized cellulose standard and powdered cellulose.

2.3. Preparation of test samples

Test samples of oxidized cellulose were prepared from cotton linter or powdered cellulose by treatment with a mixture of mineral acids and $NaNO_2$ at room temperature for 12, 24 and 48 h (unpublished results). The solid remained after the reaction was filtered, washed first with water (until the pH of the supernatant showed a pH of 3–4) and then with acetone, and finally air-dried.

2.4. COOH content analysis

The same procedure as described in the United States Pharmacopoeia (USP, 1990) was used. Briefly, about 0.5 g of the sample was accurately weighed and dispersed in 50 ml of a 2% weight-by-weight solution of calcium acetate for 30 min. The suspension was then titrated with standardized 0.1 N NaOH solution using phenolphthalein as an indicator. The volume of NaOH solution consumed was corrected for the blank. The carboxylic content in the sample was calculated from the following relationship:

$$\text{COOH content} = \frac{N \cdot V \cdot M_{\text{W COOH}}}{\text{Weight of the sample (mg)}} \times 100\%$$

where N is the normality of NaOH, and V is the volume of NaOH in mls consumed in titration, after correcting for the blank.

2.5. ^{13}C CP/MAS NMR spectroscopy

The solid state ^{13}C CP/MAS NMR spectra of the samples were obtained on a Bruker MSL-300 spectrometer at room temperature using the true 90° pulse calibration time of 6 μs, the proton transmitter dead time of 2 μs, and the contact time for polarization transfer with Hartmann–Hahn match of 3 ms. The data acquisition time was 29 μs and a line broadening of 100 Hz was applied to the spectra. A spectrum width of about 510 Hz was acquired, but only the region between 0 and 200 Hz was plotted. The number of scans used to obtain the spectra was 1200. To discern spinning side bands, each sample was run at two different spinning rates, 4500 cycles/s and 5000 cycles/s. The areas under the peaks were calculated by the line fitting technique using a NUTS data processing software (Acron NMR Inc., Fremont, CA). This method adjusts frequency, height, width at half height, and ratios of Lorentzian–Gaussian line shape of a set of lines to fit peaks in the spectrum.

3. Results and discussion

3.1. ^{13}C CP/MAS NMR spectra

The ^{13}C CP/MAS NMR spectra of powdered cellulose (PC), standard oxidized cellulose (OC-S), and test oxidized cellulose samples (OC-1–OC-5) are shown in Fig. 2. The solution ^{13}C NMR spectrum of 6-carboxycellulose in $NaOH/D_2O$ (Andersson et al., 1990; Nehls et al., 1991; Bertocchi et al., 1995; Heinze et al., 1996) and the solid-sate ^{13}C CP/MAS spectrum of cellulose (VanderHart and Atalla, 1984; Stephenson, 1985; Horii et al., 1987a,b; Teeaar et al., 1987; VanderHart and Atalla, 1987; Ek et al., 1995) have been previously reported. Thus, by analogy, in the spectrum of powdered cellulose (Fig. 2, PC), the peak at 104 ppm is assigned to C-1, the strong signal at 74 ppm to C-2, C-3, and C-5, and the two doublets appearing in the regions 81–90 ppm and 60–65 ppm to C-4 and C-6 resonances. The splitting of C-4 has been previously interpreted as being due to magnetic non-equivalent arising from chain polarity in the unit cell or from an alternating glycosidic linkage along a chain (Stephenson, 1985). Horii et al. (1987a) and VanderHart and Atalla (1984), however, have noted that the splitting of C-4 and C-6 signals occurs due to carbons on chains located in the crystalline and amorphous interiors. They suggested that the peaks at 85–90 ppm and 63–65 ppm are due to noncrystalline carbon resonances, whereas those in the regions 81–85 and 60–63 ppm originate from carbons located in the amorphous regions.

Except for peaks at 171 ppm and 93 ppm, the ^{13}C CP/MAS spectra of oxidized cellulose standard (OC-S) and test oxidized celluloses (OC-1-OC-5), appear similar to that observed for the powdered cellulose (PC). The 171 peak is assigned

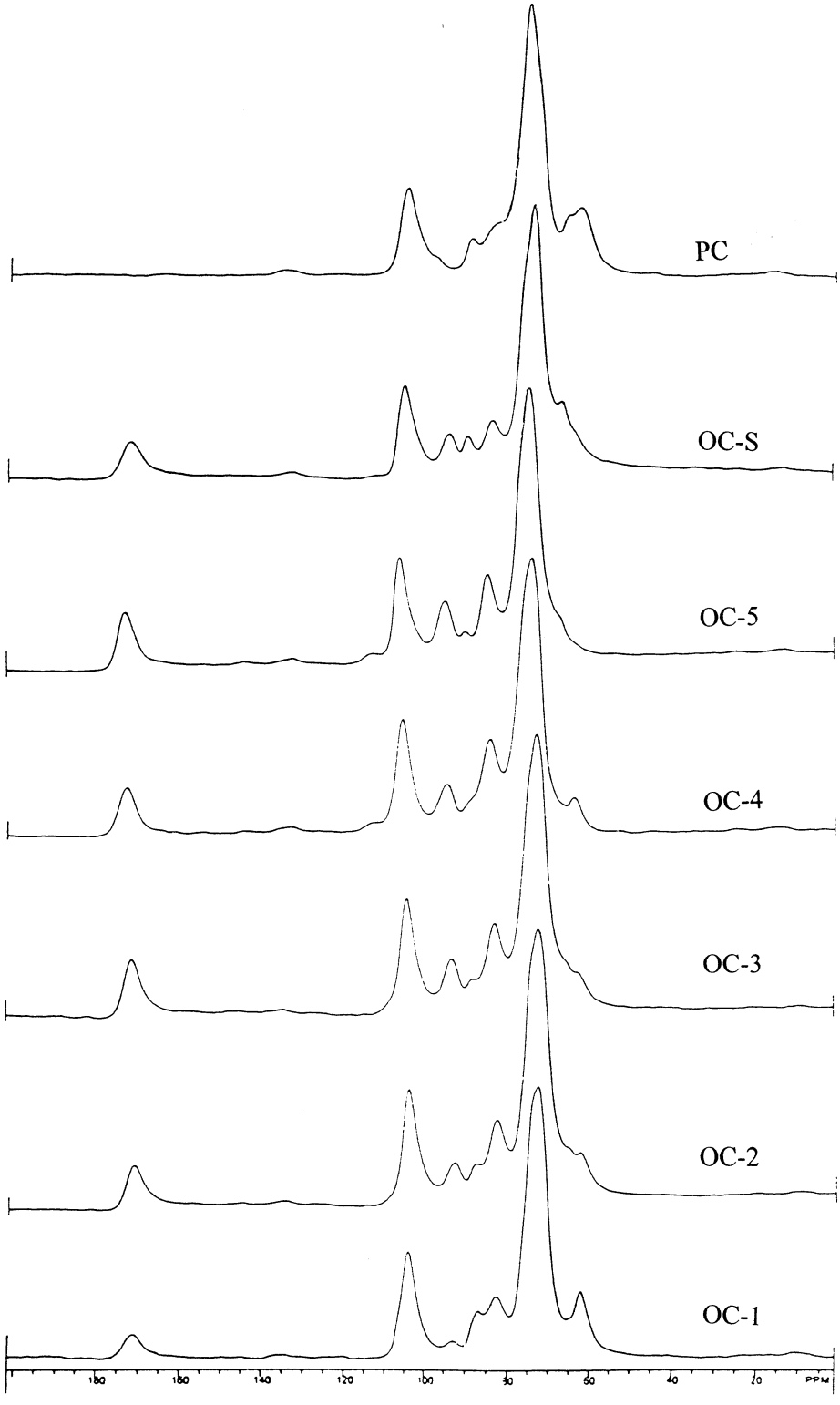

Fig. 2. Solid-state ^{13}C CP/MAS NMR spectra of powdered cellulose (PC), oxidized cellulose standard (OC-S), and test oxidized cellulose samples (OC-1–OC-5).

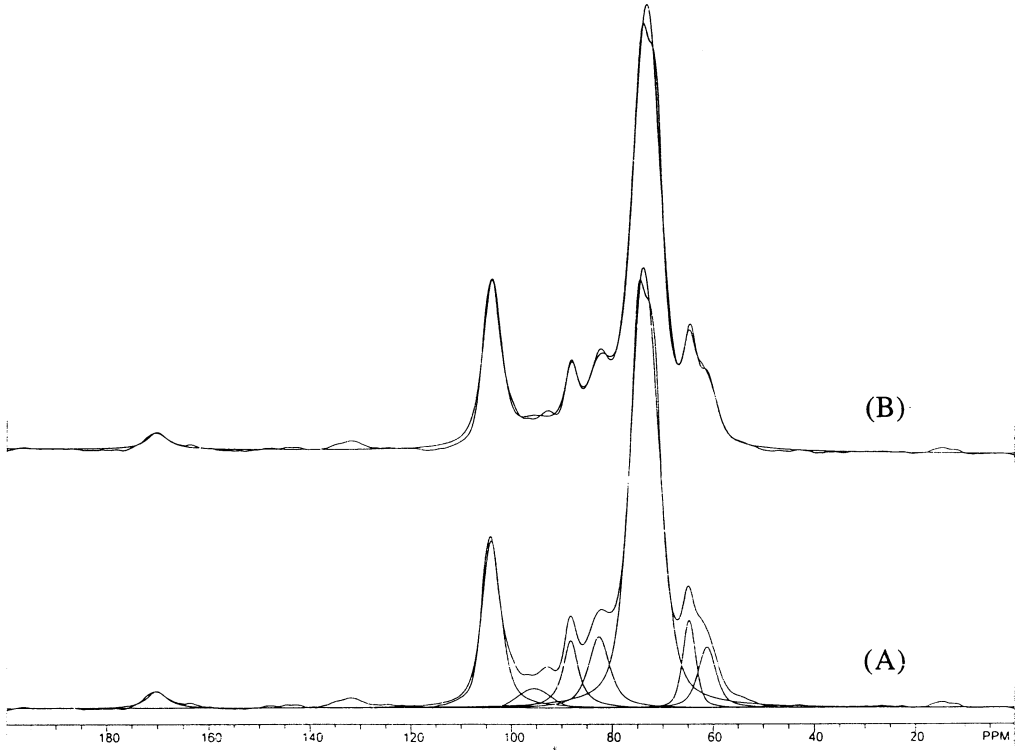

Fig. 3. A representative CP/MAS ^{13}C NMR spectrum of oxidized cellulose showing curve fitting components (A) and the fitted spectrum (B) obtained by the line fitting technique.

to the carboxyl carbon, that is, C-6 in the oxidized anhydroglucose unit of the cellulose chain. The peak at 93 ppm is attributed to C-1 of the terminal α-D-glucose unit that, according to Andersson et al. (1990), forms when cellulose is oxidized with sodium nitrite in orthophosphoric acid. Other notable features in the spectra of oxidized celluloses, compared to the spectrum of powdered cellulose, are: (a) all peaks appear relatively sharper; (b) the cluster of peaks in the region 70–76 ppm due to C-2, C-3, and C-5 resonances shows a maximum at 72 ppm versus 74 ppm seen in the spectrum of powdered cellulose; and (c) the 65 ppm peak is slightly higher in intensity than that at 62 ppm. In the spectrum of powdered cellulose, the 62 ppm component of C-6 resonance, however, is more prevalent than 65 ppm peak.

The spectra of physical mixtures of oxidized cellulose standard and powdered cellulose, used to construct standard curves, exhibited peaks that were linear combinations of those of the constituent celluloses.

3.2. Carboxyl content analysis

Fig. 3 shows an example of the fitted components and the fitted spectrum obtained by the line fitting technique to calculate the areas under the peaks. The quantitative estimation of carboxyl content in the samples was determined using the following peak area ratios:

(a) $\dfrac{171\ \text{ppm}}{104\ \text{ppm}}$ and

(b) $\dfrac{171\ \text{ppm}}{(171\ \text{ppm} + 62\ \text{ppm} + 65\ \text{ppm})}$

The 104 peak used in method (a) showed no change with the carboxylic content and thus served as an ideal internal reference. As is evident from the data in Table 1, the area under the peak

Table 1
Relationship between peak area or peak area ratio and percent carboxyl content

	Peak area or peak area ratio			
	171 ppm	(62+65) ppm	171 ppm / 104 ppm	171 ppm / (171+62+65) ppm
PC (COOH = 0%)	0	6.9737E8	0	0
OC-S+PC[a] (COOH = 4%)	4.5601E7	4.8510E8	0.0808	0.0859
OC-S+PC[a] (COOH = 8%)	8.8250E7	3.7272E8	0.2301	0.1914
OC-S+PC[a] (COOH = 12%)	15.4640E7	2.6900E8	0.3192	0.3650
OC-S+PC[a] (COOH = 16%)	21.7789E7	2.3702E8	0.4168	0.4789
OC-S (COOH = 20%)	23.6269E7	1.9631E8	0.4777	0.5462
Intercept	−0.2263E7	5.7461	0.0051	−0.0138
Slope	1.2602	0.2115	0.0249	0.0292
R^2	0.9851	0.9419	0.9868	0.9863

[a] Physical mixture.

at 171 ppm varied directly while the sum of the areas under the peaks at 62 ppm and 65 ppm changed inversely with the carboxylic content, corresponding to correlation coefficient values of 0.9851 and 0.9419, respectively, suggesting a reasonable linear relationship. The peak area at 171 ppm, normalized with a peak area at 104 ppm (method a) and the sum of peaks at 171 ppm, 62 ppm and 65 ppm (method b), also varied linear, corresponding to correlation coefficients of 0.9868 and 0.9863, respectively (Table 1).

To validate the two approaches used, five test samples of oxidized cellulose (OC-1–OC-5), whose solid-state ^{13}C CP/MAS NMR spectra are presented in Fig. 2, were analyzed. All samples showed spectral features similar to that observed for the commercial oxidized cellulose standard (Fig. 2). The carboxylic content values determined in OC-1–OC-5 by NMR methods (a) and (b) and by the USP method are listed in Table 2. The carboxyl content values determined by the two NMR methods and the USP procedure showed a reasonable linear relationship (R^2 = 0.9597 and 0.9305, respectively). The relatively closer values obtained by the two NMR methods than those between NMR and USP methods (Table 1) further suggest that NMR offers a more precise estimation of the carboxylic content in oxidized cellulose than the USP method. The greater variability in the carboxyl content analysis by the USP method may originate from the inability of calcium ions to penetrate and interact with all the carboxylic groups present on the cellulose chains primarily due to heterogeneous reaction conditions and the complex structure of oxidized cellulose. The solid-state ^{13}C CP/MAS NMR spectroscopy, in contrast, is a direct method capable of quantitatively measuring the bulk properties of polymers (Ek et al., 1995).

Table 2
Carboxyl content values determined by solid-state ^{13}C CP/MAS NMR and the USP methods

Sample	% COOH content[a]		
	USP	NMR method (a)	NMR method (b)
OC-1	8.0	7.7	8.4
OC-2	13.4	11.3	12.0
OC-3	18.4	18.7	18.9
OC-4	21.1	21.0	19.9
OC-5	22.2	25.1	25.5

[a] Regression analysis results:

NMR method (a) versus USP: $y = -2.9218 + 0.1842x$ $R^2 = 0.9597$

NMR method (b) versus USP: $y = -1.421 + 1.104x$ $R^2 = 0.9305$.

4. Conclusions

The results presented clearly demonstrated that solid-state ^{13}C CP/MAS NMR spectroscopy offers an alternate non-invasive method to the chemical analysis of carboxyl content in oxidized cellulose. Unlike the USP and other existing methods, the NMR method is simple and requires no sample preparation. Since the NMR technique is non-destructive, it also offers potential for use in the quantitation of drug loading in prodrugs in which the drug is linked to oxidized cellulose through carboxylic groups and changes in degree of crystallinity of oxidized cellulose during processing.

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