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Quantitative and Structure Analysis of Cellulose in Tobacco by ^{13}C CP / MAS NMR Spectroscopy *

by

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SUMMARY

A new method utilizing ^{13}C cross-polarization/magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectra was developed for the simultaneous quantitative determination and structure analysis of tobacco cellulose from hot water or acid detergent extraction. A reference spectrum of tobacco noncellulose components was subtracted from the spectrum of each sample to obtain a subspectrum of the cellulose components. The NMR spectra in combination with spectral fitting were analyzed in detail and some parameters, such as the content of cellulose, crystallinity, allomorph composition and lateral dimensions for cellulose elementary fibrils and microfibrils were determined. The quantitative results showed that the average recovery was 94.0% with a relative standard deviation (RSD) of 4.6–4.8%. The structure results obtained by the spectral fitting for the cellulose C1-region showed that the main allomorph composition in tobacco cellulose was I_β. The cellulose crystallinity calculated by the spectral fitting in C4 -region was about 50%. The lateral dimensions for cellulose elementary fibrils and microfibrils were in the range of 3.0–6.0 nm and 6.0–13.0 nm, respectively. Therefore, this NMR method could provide important information on both amount and structure of cellulose in tobacco. [Beitr. Tabakforsch. Int. 27 (2016) 126–135]

ZUSAMMENFASSUNG

Es wurde eine neue Methode mit ^{13}C-CP/MAS-NMR-Spektren (kernmagnetische Resonanz mit Kreuzpolarisation/Rotation um den magischen Winkel) für die gleichzeitige Mengenbestimmung und Strukturanalyse von Tabakzellulose nach der Extraktion mit Heißwasser oder saurem Lösungsmittel entwickelt. Ein Referenzspektrum von nicht-zellulosehaltigen Tabakbestandteilen wurde vom Spektrum jeder Probe subtrahiert, um ein Subspektrum der Zellulose-Bestandteile zu erhalten. Die NMR-Spektren in Kombination mit spektraler Anpassung wurden ausführlich analysiert und bestimmte Parameter wie Zellulosegehalt und Kristallinität, Allomorph-Zusammensetzung und Querabmessungen für Zellulose-Elementarfibrillen und -Mikrofibrillen wurden bestimmt. Die quantitativen Ergebnisse zeigten, dass die durchschnittliche Wiederfindungsrate 94,0% betrug, bei einer relativen Standardabweichung (RSD) von 4,6–4,8%. Die mit spektraler Anpassung erhaltenen Strukturergebnisse für die C1-Region der Zellulose zeigten, dass die Zusammensetzung des häufigsten Allomorphs in Tabakzellulose I_β entsprach. Die durch spektrale Anpassung in der C4-Region berechnete Zellulose-Kristallinität betrug etwa 50%. Die Querabmessungen für Elementarfibrillen und Mikrofibrillen lagen jeweils im Bereich 3,0–6,0 nm bzw. 6,0–13,0 nm. Daher kann diese NMR-Methode sowohl über die Menge als auch über die Struktur von Zellulose in Tabak wichtige Informationen bereitstellen. [Beitr. Tabakforsch. Int. 27 (2016) 126–135]

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RESUME

Une nouvelle méthode reposant sur les spectres RMN (résonnance magnétique nucléaire) relevés par CP/MAS (polarisation orthogonale/rotation à l'angle magique) du carbone 13 a été mise au point en guise d'analyse structurelle et quantitative simultanée de la cellulose présente dans le tabac, à partir d'une extraction au détergent acide ou à l'eau chaude. Un spectre de référence de composés non cellulosiques dans le tabac a été ôté du spectre de chaque échantillon afin d'obtenir un sous-spectre de composés cellulosiques. Les spectres RMN associés à une adaptation spectrale ont été analysés dans le détail et certains paramètres ont été définis tels que la teneur en cellulose et le taux de cristallinité, la composition des pseudomorphes et les dimensions latérales des microfibrilles et fibrilles élémentaires de la cellulose. Les données quantitatives ont indiqué que le taux moyen de récupération atteignait 94,0% avec un coefficient de variation de 4,6–4,8%. Les résultats structurels obtenus par l'adaptation spectrale pour la plage C1 de la cellulose ont révélé que la composition des principaux pseudomorphes de la cellulose de tabac correspondait à I_β. La cristallinité de la cellulose calculée par adaptation spectrale dans la plage C4 s'élevait à environ 50%. Les dimensions latérales des microfibrilles et fibrilles élémentaires de la cellulose oscillaient respectivement dans des bandeaux de 3,0–6,0 nm et 6,0–13,0 nm. Par conséquent, cette méthode RMN a permis de récolter des informations importantes concernant à la fois la teneur et la structure de la cellulose dans le tabac. [Beitr. Tabakforsch. Int. 27 (2016) 126–135]

INTRODUCTION

Cellulose is a technically important and fascinating biopolymer as well as an almost inexhaustible natural resource. The renewable material is a major component of the cell walls of higher plants, e.g., wood and cotton, and it is also produced by some bacteria, algae, fungi and sea-animals (1). Cellulose is composed of linear 1–4 linked β-D-glucose residues, and is known to exist in several polymorphic crystalline forms (2). Native cellulose, however, is always cellulose I. Cellulose II can also be formed through alkalization and regeneration. Native and regenerated cellulose fibers are both used as medical and sanitary materials (3). Chemically, cellulose can also be transformed into several derivatives, e.g., cellulose acetate, carboxymethyl cellulose and many more (4).

In the cigarette industry, cellulose content is a useful indicator for characterizing tobacco blends (5). The content of cellulose, as a kind of primary component of tobacco cell organization and skeleton, is about 11% in tobacco. Cellulose can increase the combustion and constancy of burning. With increasing cellulose content, the tobacco becomes coarse and brittle, leading to throat irritation and coughing during smoking, which would prevent the expansion of tobacco flavor and decrease the quality of a cigarette (6–7). So far, considerable attention has been paid to the improvement of chemical and physical properties of cellulose materials. Chemical or biological technology treatment may change fibril aggregates and thus reduce accessibility for solvents and reactants, which has been shown to further influence the chemical reactivity of cellulose I. The physical properties of cellulose materials, such as crystallinity, crystallite dimensions, density and tensile strength, are influenced by such ultra-structural changes as well (8). During acid or alkaline extraction of cellulose, there are changes in lateral cellulose fibril aggregate dimensions. This is attributed to an increased contact between cellulose fibril surfaces, as a result of the removal of hemicelluloses and lignin (9). Proper treatment could decompose cell wall substances effectively, change the structure of cellulose selectively, increase the contents of neutral aroma substances and improve the quality of tobacco (10). Therefore, content and structure of cellulose are very important for tobacco quality, processing and industrial applications. This information could help to understand the mechanism of degradation and improve the internal quality of tobacco.

The quantitative determination of cellulose in plants by chemical means can be especially troublesome since the nonglucan substances associated with cellulose in the plant cell walls (i.e., hemicelluloses, lignin, and minerals) are difficult to remove without the destructive degradation of the cellulose (11). The quantitative determination of cellulose in tobacco often includes several steps: extraction, hydrolyzation by acid or enzymes and weighing. At present, the acid detergent extraction of cellulose followed by desiccation and weighing is the tobacco industry standard method in China (12). But it is also very time-consuming and troublesome, and moreover, there is no information about cellulose structure during these processes.

Solid-state nuclear magnetic resonance (NMR) spectroscopy is an informative method for characterizing the composition and sequence of the polysaccharide units (13–15). It has the potential to enable quantitative determination of functional groups in a complex material because all equivalent nuclei potentially give rise to signals of equal intensity regardless of their chemical environment (16). Up to now, this NMR technique has been used extensively to study the supramolecular structure of cellulose materials (1–2, 4). High resolution solid state NMR may enable various crystalline allomorphs of cellulose to be distinguished. So far, most of the NMR work which has dealt with highly crystalline samples, only related to the cellulose structural information. A spectroscopic method for simultaneous measuring cellulose is thus very desirable. However, solid state NMR spectra are not always quantitative. The NMR signal of some ^{13}C nuclei may be diminished or be rendered completely unobservable, especially when the cross-polarisation (CP) technique is used. If the loss of NMR signal intensity is equal across all functional groups, their relative ratios will not be changed, even though the total signal is decreased. SMERNIK and OADES (16) have investigated the quantitative reliability of solid state ^{13}C NMR spectra to different materials and classified the materials into three categories based on their chemical structures: one category comprises materials that give quantitative signals with both CP and Bloch decay (BD) NMR techniques. These materials include cellulose, pectin, chitosan, lignin, and palmitic acid (14). That is to say cellulose can be quantitatively measured by CP technique.

In this work, we have developed a method for the quantitative determination and structure analysis of cellulose in tobacco utilizing ^{13}C CP/MAS NMR spectra. On the basis of solid state ^{13}C NMR spectroscopy, not only the content of cellulose in extracted tobacco was measured, but also crystallinity, allomorph composition and lateral dimensions of it were estimated by peak fitting analysis of the spectra. Through this work, we were able to monitor the changes in both content and structure of cellulose in tobacco samples during its processing.

EXPERIMENTAL

Chemicals and materials

Flue-cured tobacco samples of three grades were from Guizhou, China. Oriental and Burley tobacco samples were from Xinjiang and Hubei, China, respectively. The tobacco samples were dried at 40 °C in an oven for 4 h and ground to powder. This ground tobacco was stored in a clean brown bottle for use. Hexadecyl trimethyl ammonium bromide ($C_{19}H_{42}BrN$, ≥ 99.5%), *n*-octanol ($C_8H_{18}O$, ≥ 99%) and sulfuric acid (98%) were purchased from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China). Standard sample cellulose from J&K Chem-Tech Ltd. (Shanghai, China) was used as product without further purification. All other reagents employed were of analytical grade quality unless otherwise indicated and distilled water was used throughout the work.

Preparation of acid detergent solution

20g Hexadecyl trimethyl ammonium bromide was dissolved in 800 mL of 1.0 mol/L sulfuric acid solution and completed to 1000 mL. The solution could be stored at 4 °C for a week.

Cellulose and lignin extracted from tobacco sample

5 g of weighed, oven-dried tobacco sample was extracted with 500 mL acid detergent solution in an extractor. 1.0 mL *n*-octanol was added. The extractor was heated to boiling quickly and kept at slightly boiling status for 1.0 h. Then the extract was filtered under low pressure and rinsed with 90 °C hot deionized water three times. The residue was washed and settled with acetone three times until the filtrate was colorless. The residue was dried at 40 °C for 24 h and weighed to calculate the yield of cellulose. Lignin was simultaneously extracted in the same manner but with 500 mL 72% sulfuric acid solution instead of acid detergent solution. The cellulose and lignin products were ground to power about 150 μm and stored under phosphorus pentaoxide for the NMR analysis.

^{13}C NMR spectroscopy

High resolution ^{13}C NMR spectra of samples were measured using a Bruker AVANCE AV 400 (Bruker, Karlsruhe, Germany) spectrometer operating at 400 MHz employing a double-tuned solid-state probe equipped with 4 and 7 mm (o.d.) spinners. The ^{13}C CP/MAS spectra were recorded using ^{13}C strengths of 62.5 kHz and a spin rate of 15 kHz. The spectra were obtained applying the following parameters: 2 ms contact time, acquisition times of 25.4 ms, recycle delay of 2 s and sweep width of 30 kHz. The typical number of scans was 1024 for the CP/MAS spectra. All spectra were referenced to the carbonyl peak of glycine at 176.03 ppm. All the spectra were weighted with a Lorentzian function using a line broadening of 10 Hz prior to Fourier transformation. Fitting of line widths using a Lorentzian line shape was performed by the built-in procedure in the MestReNova 6.1.1 software.

FTIR and XRD experiments

The FTIR spectra were obtained using MAGNA-8700 FTIR (Nicolet, Madison, WI, USA). The spectra were recorded in transmission mode from 4,000 to 400 cm^{-1} (mid-infrared region) at a resolution of 0.1 cm^{-1}. The sample was diluted with KBr (1:100, w/w) before acquisition and the background value from pure KBr (potassium bromide) was acquired before the sample was scanned. Monochromatic Cu Ka radiation (wavelength = 1.54056 Å) was produced by a MXPAHF X-ray diffractometer (MOX Corporation, Rigaku, Tokyo, Japan). The samples were exposed to the X-ray beam from an X-ray generator running at 40 kV and 200 mA. The scanning regions of the diffraction angle, 2θ, were 3–50°, which covered most of the significant diffraction peaks of the cellulose crystallites. Other operation conditions included: scan rate 2°/min, receiving slit 1°, and scattering slit 0.16°. Duplicate measurements were made at ambient temperature.

Sample preparation and analysis

A dried portion of the cellulose standard or tobacco residue from acid detergent extraction was loaded into the NMR rotor cell and weighed to within ± 0.1 mg on an analytical balance. Typically, the signal-to-noise ratio for the C1 resonance at 105 ppm in our tobacco spectra was in the range of (100–200):1. Approximately 200 mg of the cellulose material was encapsulated in a cylindrical cell produced from an aluminum nitride ceramic rod to precisely fit inside the 7 mm zirconia NMR rotor. The same rotor end caps were used for all measurements, and the zirconia rotor itself was always oriented in the same direction inside the NMR probe stator.

Chemical shift calculation and peak fitting

The ^{13}C carbon chemical shifts were calculated by MestReNova 6.1.1 software for model structures. Decomposition of ^{13}C CP/MAS NMR spectra in the regions of 95–110 ppm, 80–92 ppm was pursued by peak fitting module of MestReNova 6.1.1 software. The results of peak separation were applied to obtain the crystallinity, allomorph composition and lateral dimensions on the basis of the relative areas of separated peaks.

RESULTS AND DISCUSSION

^{13}C CP/MAS NMR spectra of cellulose

The ^{13}C CP/MAS NMR spectra of cellulose samples were presented in Figure 1. Obviously, a very good spectral resolution was obtained that facilitated detailed assignment of different functional groups present in cellulose. Typical CP/MAS ^{13}C-NMR spectra from cellulose I are made up of six signals from the anhydroglucose unit split into fine structure clusters due to the supra-molecular structure of the cellulose I fibril (17–18). The chemical shifts of cellulose carbon resonances were summed up in Table 1. The resonances at 95–110 ppm were assigned to C1 carbons of cellulose, which increased with the content of cellulose and were of particular interest for the allomorphism evaluation. The resonances at 80–90 ppm originated from C4 glycosidic bond carbons, which could be decomposed into disordered and crystallized regions and thus used for the estimation of the crystallinity and lateral dimensions. The peaks at 68–76 ppm came from the other carbons (C2, C3, C5) of pyranoid ring. Some weak peaks at ~63 ppm (Table 1) represent C6 carbons of cellulose molecule.

Elimination of interference of cellulose in tobacco

Because the nonglucan substances associated with cellulose in the plant cell wall (i.e., hemicelluloses, lignin, pectin, and minerals) were difficult to remove from the tobacco sample, there were interfering resonances in spectra to eliminate, especially in quantitative determination of cellulose (11). As shown in Figure 1, water extraction could remove hydrosoluble amino acid, sugar, minerals and part of pectin from tobacco sample. In addition to cellulose, the spectrum of the other substances in the plant cell walls showed a broad peak (165–180 ppm) which was probably due to signals from C6 of the galacturonic acid residues in homogalacturonans (171–175 ppm) (19) and from protein (165–180 ppm) (18). Acid detergent extraction could remove the rest of the pectin as well as most of the hemicelluloses but there still remained some impurity, such as lignin. A sub-spectrum of only the cellulose component of the tobacco residue was generated with this spectrum by the method shown in Figure 2. A spectrum of the cellulose reference material with a very high signal-to-noise ratio was subtracted from the tobacco residue spectrum to yield a subspectrum of the noncellulose components (mostly pectine and hemicelluloses).

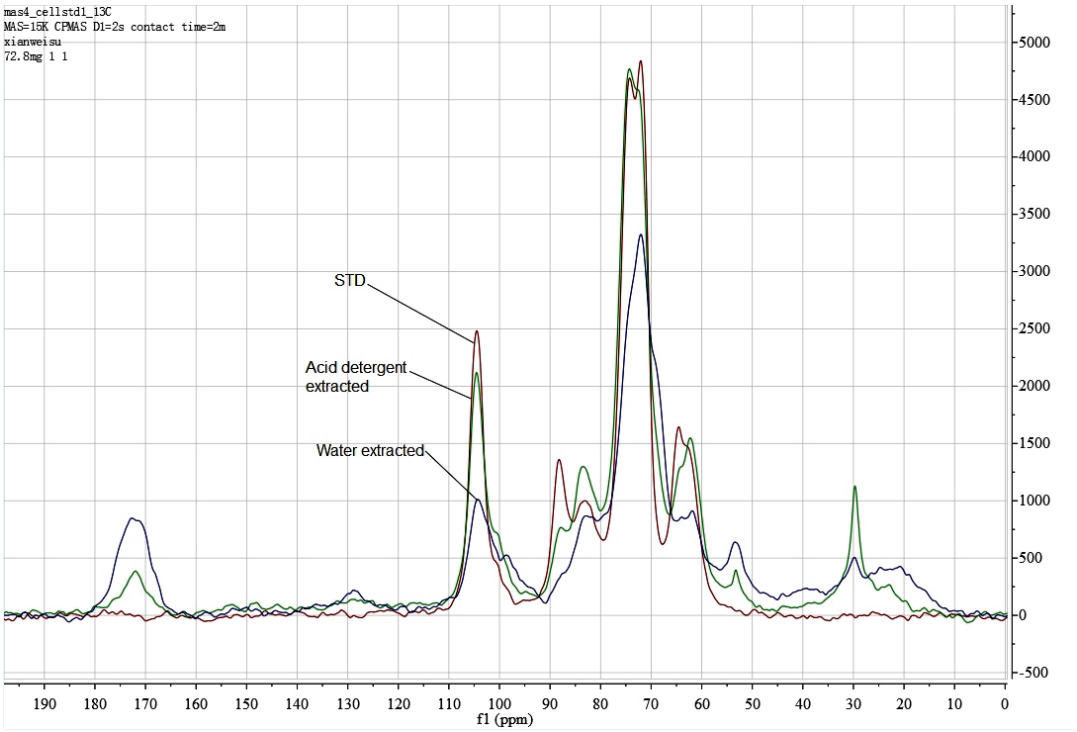

Figure 1. ^{13}C CP/MAS NMR spectra of cellulose standard and samples extracted from tobacco.

Table 1. ^{13}C CP/MAS NMR chemical shifts (in ppm) for cellulose.

Sample	Chemical shift (ppm)							
	C1		C2, C3,C5		C4		C6	
Cellulose Standard	104.49		74.25	72.07	88.21	85.04	64.54	62.51
Acid extracted cellulose	104.53		74.27	72.13	88.03	85.61	64.42	62.28
Water extracted cellulose	104.29	98.6	72.07	68.86	87.68	83.52	64.05	62.08

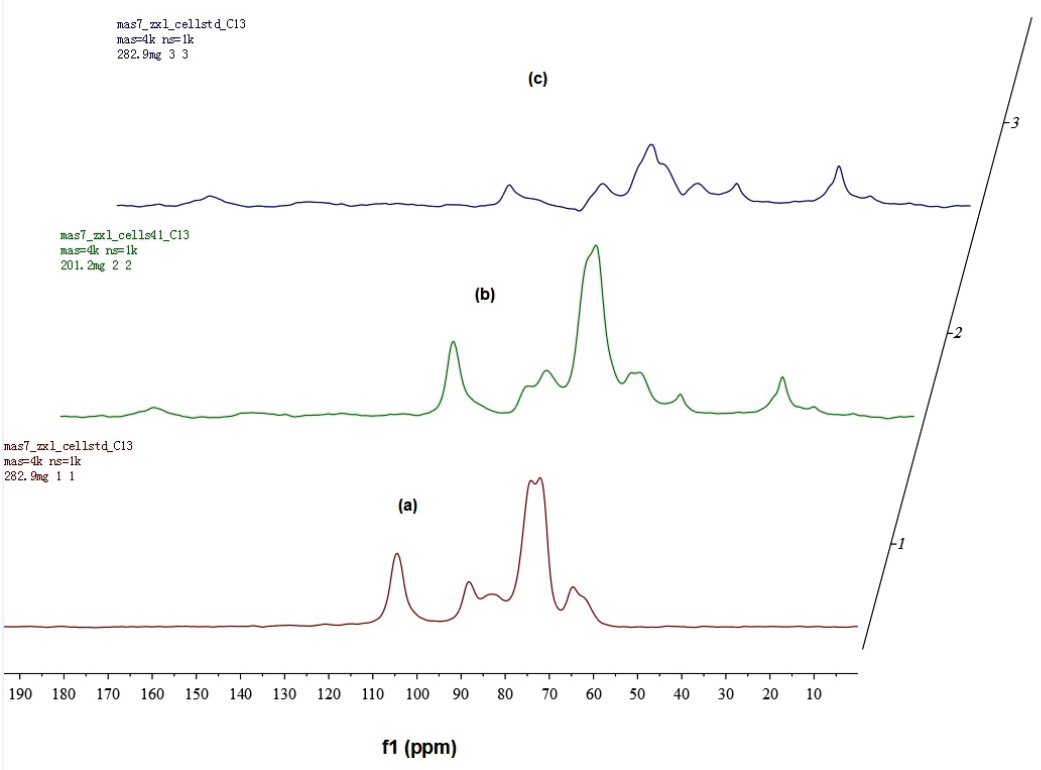

Figure 2. Deconvolution of the tobacco spectrum by spectral substraction. Carbohydrate region of the ^{13}C CP/MAS NMR spectrum of (a) cellulose standard, (b) tobacco cellulose standard samples and (c) noncellulose background spectrum obtained by subtraction of the reference cellulose spectrum.

Two spectral peaks were chosen to aid the subtraction process (105 and 89 ppm), corresponding to the C1 and C4 (crystalline) resonances, respectively, of cellulose I. At the point where the scaling between the tobacco residue spectrum and the cellulose reference spectrum is close to correct, the exact scaling becomes somewhat subjective. The scaling of the reference cellulose spectrum was chosen such that the resulting difference spectrum had the minimum intensity possible without any dips or negative peaks at 105 or 89 ppm (11). Keeping the vertical scaling constant, the background spectrum from the deconvolution step was next subtracted from the original tobacco residue spectrum. The difference spectrum obtained from this operation consists of a subspectrum of only cellulose in which the resonance intensities are preserved from the original tobacco residue spectrum. Finally, the ratio of integral of the region carbon resonance between 100 and 110 ppm to the sample weight in the tobacco residue spectrum was compared to the standard calibration curve to determine the weight percentage of cellulose in the acid detergent residue. The percentage of cellulose in the dry tobacco was then calculated on the basis of the percentage of acid detergent residue in the original sample.

The evaluation of quantitative analyses of cellulose in tobacco

Quantitative analyses of cellulose extracted from tobacco sample were performed by ^{13}C CP/MAS NMR utilizing the above described procedures. Measurements were made on each of at least three separately weighed samples for each sample. A calibration curve for the cellulose analysis was established from the ^{13}C CP/MAS NMR spectra of a set of six cellulose standard samples with weights ranging from 50–230 mg. The integral of the region carbon resonance between 100 and 110 ppm was plotted versus the sample weights. Linear regression analysis was performed with the intercept constrained to pass through zero, yielding a straight line $A = 421.53 \times m + 2579.12$ with a correlation coefficient $r^2 = 0.9991$. Limits of detection (LOD) and limits of quantification (LOQ) were calculated at 2.9 mg/g and 8.7 mg/g with signal to-noise ratios of 3 and 10, respectively.

Table 2. Recovery and repetition data of the analytical method with cellulose or tobacco standard.

Sample	Cellulose content (%)	Spiking level (by STD/mg)	Recovery (%)	RSD (%)	Mean recovery (%)
1#	10.55	50.0	93.9	3.9	96.5
		150.0	95.2	4.4	
2#	8.62	50.0	94.1	3.1	
		150.0	102.6	2.9	
3#	10.55	98.4	92.6	4.6	94.0
4#	10.55	196.8	95.3	4.8	

To evaluate the accuracy of NMR spectra analyses of cellulose, two cellulose extraction samples (1# and 2#) added with cellulose standard samples were performed by ^{13}C CP/MAS NMR spectra. Each sample was analyzed three times separately and the results were shown in Table 2. According to the Table, the average recovery of cellulose was 96.5% (RSD: 2.9%–4.4%), which indicated that the ^{13}C CP/MAS NMR was an accurate method for determination of cellulose amount.

To verify the accuracy of the whole method, a tobacco sample added with 1 g and 2 g standard tobacco (marked by Gravimetric method, Ref. 12) was processed with acid detergent extraction, purification and NMR spectra analysis. Measurements were also made of each of three separately weighed samples and the results were shown in Table 2 (3# and 4#). The results revealed that the average recovery was 94.0% with the RSD of 4.6–4.8%. These data demonstrated that the proposed method could be used for tobacco cellulose analysis with a suitable degree of precision.

Crystalline allomorphs analysis of cellulose in tobacco sample

NMR spectral results could also be applied to determine some important cellulose characteristics in comparison with those obtained by conventional methods (20–21). It is noteworthy that each carbon signal can provide interesting information: anomeric C1 signals allow us to determine the crystalline allomorph ratio; C4 signals resonating between 80–85 ppm are used for the calculation of the amount of disordered regions. The existence of two different crystalline forms in native cellulose, triclinic (I_α) and monoclinic (I_β), was first demonstrated by CP-MAS and then further confirmed by electron diffraction and FTIR (22). The spectra are shown in Figure 3 for the C1-region of cellulose from tobacco. Three Lorentzian lines for the signals from crystalline cellulose I_α allomorph (105.1 ppm) and I_β allomorph (105.7 ppm and 104.0 ppm) are visible together with a Gaussian line for para-crystalline cellulose (104.6 ppm). Results found for the allomorphic ratios I_β/I_α in different tobacco samples are shown in Table 3.

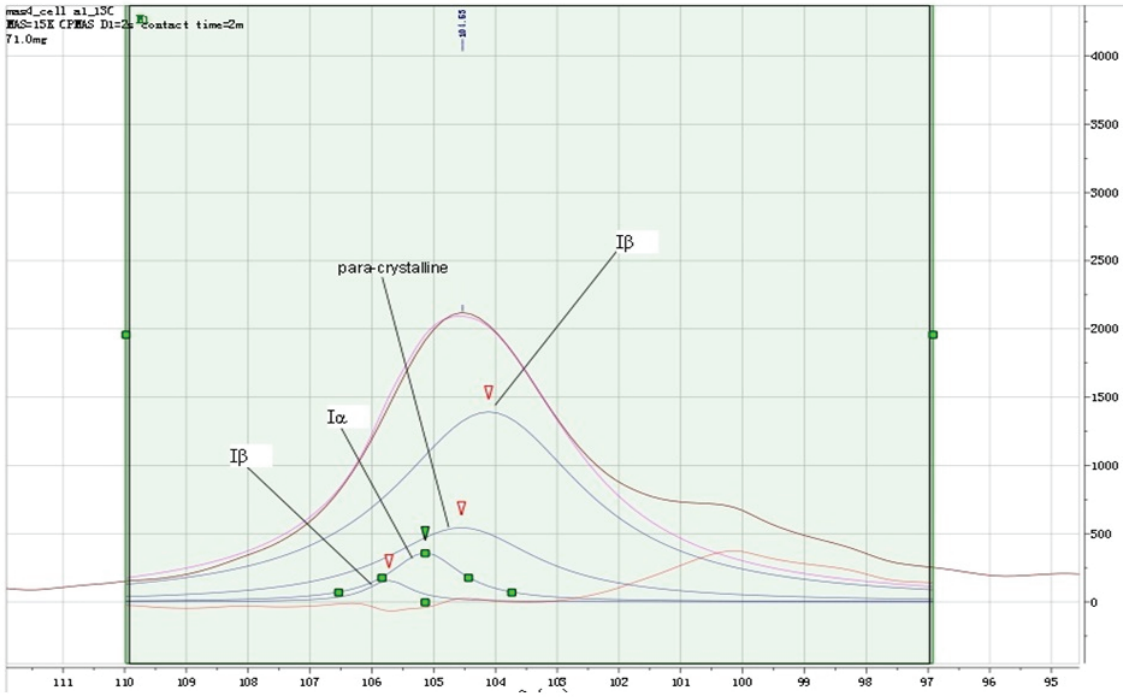

Figure 3. Fitting of C1 spectral region of ^{13}C CP/MAS NMR spectra of cellulose in tobacco.

Table 3. Contents of crystalline allomorphs obtained from ^{13}C CP/MAS NMR spectra of cellulose in tobacco samples.

Sample	I_α	$I_{\beta1}$	$I_{\beta2}$	I_α / I_β (%)	Para-crystalline
Guizhou B2F	13.77	0.82	65.99	0.21	19.43
Guizhou C3F	22.11	4.90	40.64	0.49	32.35
Guizhou X2F	8.92	4.24	51.40	0.16	35.43
Tobacco stem	9.15	1.44	62.66	0.14	26.90
Tobacco STD	20.34	13.01	34.56	0.43	32.21
Hunan Oriental	4.06	0.39	55.89	0.07	39.65
Hubei Burley	6.10	0.98	57.20	0.11	35.95
Guangzhou Bright	4.93	0.47	68.12	0.07	26.48

The results indicated that the monoclinic (I_β) were present in a larger proportion to the triclinic crystal forms (I_α) in all tobacco samples. These results were consistent with the report that the I_α phase is less stable than the I_β phase and that it can be converted into I_β during a severe treatment of the microfibrils (23). The origin of the sample, and its storage and processing conditions are thus of great importance. All our experiments have been performed using desiccated samples. Of all these samples, the ratio of I_β /I_α from Guizhou C_3F was the highest (0.49%) while Hunan Oriental and Guangzhou Bright were the lowest (0.07%). On the other hand, the ratio of para-crystalline (PC) cellulose, about 30% in most tobacco samples, is a crystalline form with more disorder and mobility than I_α and I_β. Maybe it was a good method to monitor the properties of tobacco cellulose from the changes in the ratio of para-crystalline during the process of tobacco manufacture and modification.

Crystallinity of cellulose in tobacco samples

The most informative region in a NMR spectrum of cellulose is a signal cluster with a distribution between 80 and 92 ppm (1). As shown in Figure 4, this region contains fairly sharp signals ranging from 86 to 92 ppm corresponding to C4 carbons situated in crystalline cellulose I_α and I_β domains together with paracrystalline cellulose. The C4 carbons of more disordered regions are distributed in a broad band ranging from 80 to 86 ppm. In the disordered region, a pair of signals at 83.4 and 84.3 ppm was assigned to two non-equivalent sites at the crystallite surfaces. Crystallinity of cellulose in tobacco sample was changed with the integration of the spectral signals at 86–92 ppm to 80–86 ppm and calculated by the ratio of integration at 86–92 ppm to 80–92 ppm (24).

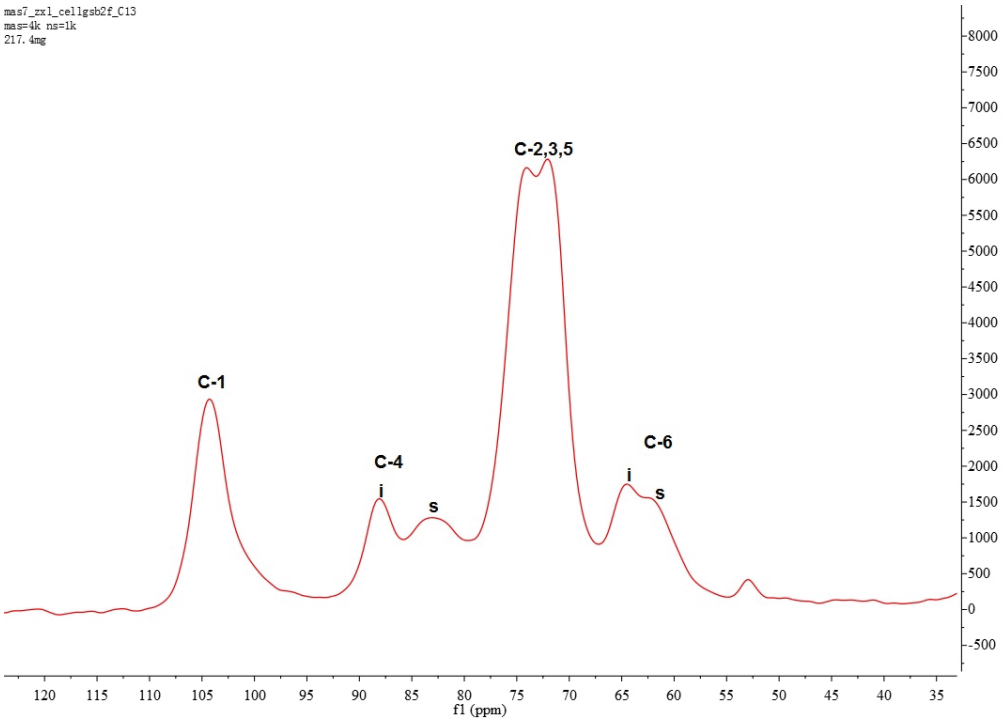

Figure 4. CP/MAS ^{13}C NMR spectra of cellulose in tobacco.

Table 4. Crystallinity of cellulose obtained from ^{13}C CP/MAS NMR spectra of cellulose in tobacco samples.

Sample	NMR method 1	NMR method 2 [a]	XRD method [b]	IR method [b]
Guizhou B2F	44.2	42.0	51.2	77.6
Guizhou C3F	47.9	43.7	51.8	90.1
Guizhou X2F	52.2	52.2	56.6	83.1
Tobacco stem	43.2	40.3	49.6	75.1
Tobacco leaf	47.9	46.0	55.5	82.5
Hunan Oriental	50.8	52.5	54.6	83.0
Hubei Burley	46.3	47.0	52.1	81.5
Guangzhou Bright	54.6	56.4	61.2	86.5
Formula	$X=\frac{S_s}{S_s+S_i}\times 100\%$	$X = I_\alpha + I_{\alpha+\beta} + I_\beta + PC$	$X=\frac{I_{002}-I_{am}}{I_{002}}\times 100\%$	$X = A_{1372\ cm^{-1}} / A_{2900\ cm^{-1}}$

[a] The data were the sum of the ratio of I_α, $I_{\alpha+\beta}$, I_β, and *PC* in Table 5.
[b] XRD method and IR method were according to Ref. (25).

Three methods were adopted to calculate the crystallinity of cellulose in different tobacco samples and the data were shown in Table 4. The results indicated that crystallinity of cellulose was a little higher when using the XRD method than those when using the NMR method and there was a strong correlation between the two methods. Compared with XRD method, ^{13}C NMR is more sensitive to the order in small regions and the intensity of the spectral signals depends on the numbers of carbon atoms within each cellulose phase region (16).

Only those small regions in the crystallite interior could be regarded as crystallite region and crystallinity of cellulose depends on the size of crystal particles and the integrity of crystallization (23). Considering Table 4, it was estimated that 50% of the cellulose molecules were in the crystallite interior of tobacco cellulose. Therefore, ^{13}C NMR was a more accurate way to analyze the crystalline structure of cellulose than other methods and could be used to detect the changes of crystallinity of cellulose in the tobacco processing.

Microfibrils size of cellulose in tobacco samples

Figure 5 shows the spectral fitting of the C4-region of a cellulose spectrum. Three Lorentzian lines for the signals from crystalline cellulose I_α allomorph (δ 89.6 ppm), I_β allomorph (δ 88.0 ppm) and $I_{\alpha+\beta}$ allomorph (δ 88.8 ppm) are visible together with a Gaussian line for para-crystalline cellulose (δ 88.5 ppm) (26, 27). The region typical of the less ordered carbohydrate forms (80–86 ppm) contained three Gaussian lines for the signals from distinct cellulose forms, accessible fibril surfaces (δ 83.4 and δ 84.3 ppm) and inaccessible fibril surfaces (δ 84.0 ppm). It has been suggested that the two signals at 83.4 and 84.3 ppm originate from cellulose at fibril (or "crystallite") surfaces, and that the wide signal at 84.0 ppm originates from amorphous cellulose.

The average lateral fibril and lateral fibril aggregate dimensions are calculated from quantitative spectra of pure cellulose isolated from tobacco. It is possible to calculate average lateral fibril dimensions from the NMR spectra if the fibrils and the fibril aggregates, as a simple approximation, are assumed to have square cross sections. The fraction of the signal intensity from accessible and inaccessible surfaces (fibril dimension) and the fraction of the signal intensity from accessible surfaces (fibril aggregate dimension) are both denoted q and are given by the equation:

$$q = (4n - 4) / n^2$$

in these models, where n is the number of cellulose polymers perpendicular to the fibril cross-section along one side of the assumed square fibril or the assumed square fibril aggregate cross-section (4, 28). A conversion factor of 0.57 nm per cellulose polymer has been used to calculate the results shown in Table 5.

As shown in Table 5, crystallinity of cellulose in different tobacco samples, calculated from the sum of I_α, $I_{\alpha+\beta}$, I_β and para-crystalline cellulose by spectral fitting of tobacco cellulose C4-region, was in agreement with the data from the integration of the spectral signals at 80–92 ppm, which confirmed the existence of para-crystalline cellulose and the reliability of spectral fitting of the C4-region.

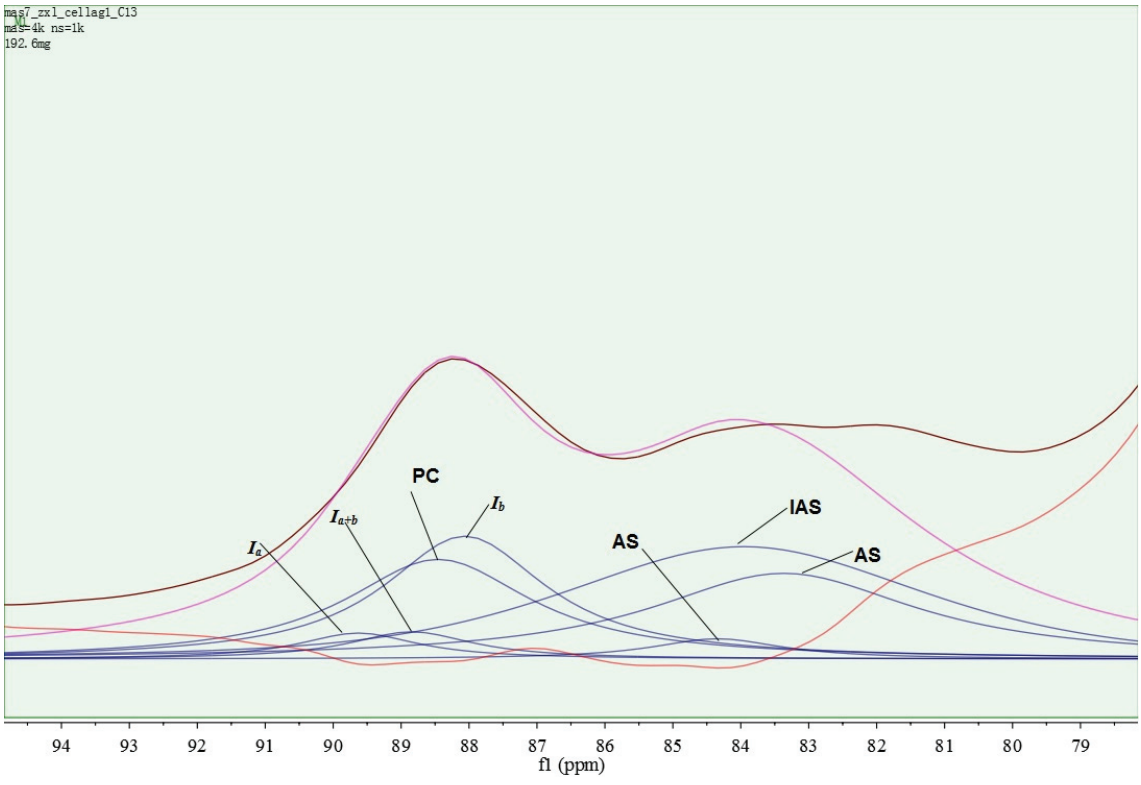

Figure 5. Results from the spectral fitting of the C4 regions in CP/MAS ^{13}C NMR spectrum.

Table 5. Quantification made by spectral fitting of tobacco cellulose C4-region.

Sample	Crystalline cellulose (%)	Para-crystalline cellulose (%)	Crystallinity [a]	Accessible fibril surfaces (%)	Inacessible fibril surfaces (%)	Elementary fibrils size (nm)	Elementary fibrils aggregate size (nm)
Guizhou B2F	27.3	14.7	42.0	31.4	26.6	3.4	6.8
Guizhou C3F	16.9	26.8	43.7	26.7	29.7	3.4	8.0
Guizhou X2F	44.6	7.6	52.2	19.9	27.8	4.0	10.8
Tobacco stem	23.3	17.0	40.3	28.5	31.1	3.4	7.4
Tobacco leaf	29.6	16.4	46.0	26.0	26.4	3.4	8.0
Tobacco cellulose	35.5	21.0	56.5	17.7	25.9	4.6	12.5
Hunan Oriental	28.0	24.5	52.5	34.0	13.4	4.0	6.3
Hubei Burley	34.7	12.3	47.0	24.5	27.5	3.4	8.6
Guangzhou Bright	27.5	28.9	56.4	22.4	21.2	4.6	9.7

[a] The data were calculated from the sum of I_{α}, $I_{\alpha+\beta}$, I_{β}, and para-crystalline cellulose by spectral fitting of tobacco cellulose C4-region, $X = I_{\alpha} + I_{\alpha+\beta} + I_{\beta} + PC$.

The results showed that the dimension of elementary fibrils and elementary fibril aggregates of tobacco cellulose were in the range of 3–5 nm and 6–13 nm, respectively. There was also a correlation between fibril dimension and crystallinity. During acid extraction of tobacco cellulose, there is an increase in lateral cellulose fibril aggregate dimensions. The change in elementary fibril aggregate dimensions is attributed to an increased contact between cellulose fibril surfaces, as a result of the removal of hemicelluloses and lignin (29).

CONCLUSION

In this work, a new ^{13}C CP/MAS NMR spectra method was proposed to analyse content and structure of cellulose following acid detergent extraction from tobacco sample. The C1 carbon region of NMR spectra was applied to study the values of cellulose and the results were in good agreement with the conventional gravimetric method. The quantitative results showed that the average recovery was 92.5% (RSD: 4.4–4.9%). Structure analysis of the spectral fittings for C1- and C4-regions showed that the main allomorph composition in tobacco cellulose was I_{β}, that cellulose crystallinity was about 50%, that lateral dimensions for cellulose elementary fibrils and elementary fibrils aggregate were in the range of 3.0–6.0 nm and 6.0–13.0 nm, respectively. Therefore, the NMR method could realize simultaneous quantitative determination and structure analysis of cellulose in tobacco.

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