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Determination of cellulose I_α and I_β in lignocellulosic materials

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Abstract

A novel method to estimate the relative amounts of cellulose I_α and I_β in lignocellulosic materials has been developed. ^{13}C-CP/MAS NMR spectra were recorded on 12 calibration samples containing cellulose of various degrees of crystallinity. Principal component analysis of the NMR data extracted qualitative information about the relative amounts of amorphous cellulose, cellulose I_α, and cellulose I_β in the calibration samples. A quantitative partial least-squares (PLS) model was established between estimates of amorphicity index, cellulose I_α-content, and cellulose I_β-content and the ^{13}C-CP/MAS NMR data of the calibration samples. The cellulose I_α and I_β content in some chemical and high-yield pulps was predicted from their NMR spectra by use of the PLS model. The existence of substantial amounts of cellulose I_α in the pulp samples was demonstrated with the PLS model.

1. Introduction

Based on results from ^{13}C-CP/MAS NMR spectroscopy on several native cellulose materials, VanderHart and Atalla discovered [1–3] that the ordered region of native cellulose is a mixture of two crystalline modifications, cellulose I_α and cellulose I_β. The existence of these two crystalline phases was recently substantiated by electron diffraction [4,5] and FTIR spectroscopy [4]. Cellulose I_α is believed to be the dominant form in bacterial and algal celluloses and cellulose I_β the dominant form in higher plants such as cotton and ramie [1–6]. The coexistence of two crystalline phases of cellulose I raises the question of their

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importance in practical life. What role do the crystalline phases play in the physicochemical properties of lignocellulosic materials, such as pulps? In which way are they affected by different manufacturing processes? In order to address these and related questions, a method for the determination of cellulose I_α and I_β in lignocellulosic materials is needed.

One method used to obtain ^{13}C NMR spectra from solid samples is cross-polarisation/magic angle spinning (CP/MAS) [7]. This method has been used extensively to study the structure and composition of cellulosic materials [8]. NMR spectra of cellulosic materials contain information regarding the degree of order in the cellulose polymer [9–11], hence the crystallinity index can be determined by integration of specific peaks in the spectra [12,13]. By utilisation of differences in ^{13}C- or ^{1}H-spin–lattice relaxation times, NMR subspectra showing either the amorphous or the ordered regions in lignocellulosic materials can be constructed [14,15].

In samples of cellulose with large, lateral, highly ordered regions (such as in *Valonia ventricosa* cellulose), the amount of cellulose I_α and I_β can be determined by integration of appropriate regions in spectra, spectral subtraction, and line-shape analysis [3,6] of ^{13}C-CP/MAS NMR spectra. NMR spectra of lignocellulosic samples with substantial amounts of amorphous cellulose, hemicellulose, and lignin, however, consist of severely overlapped signals rendering the determination of cellulose I_α and I_β by integration impossible. This spectral complexity has led to the question whether higher plant celluloses contain any cellulose I_α at all [3,4].

Statistical multivariate data analysis techniques, such as principal component analysis (PCA) [16,17], can be used to resolve spectra into several contributing subspectra. The strength of this approach is manifested when subspectra can be given a distinct physical or chemical interpretation; here properly designed experiments are a prerequisite for successful interpretation. For example, PCA has been used for classification of peracetylated oligosaccharide residues by their ^{13}C NMR spectra [18], and peat samples by their ^{13}C-CP/MAS NMR spectra [19]. Partial least-squares [20,21] (PLS) is another multivariate technique frequently used to create models for quantification, e.g., chemical constituents from FTIR spectra [22].

In this work we present a novel method for the determination of the amounts of cellulose I_α and I_β in lignocellulosic samples. A PLS model was built from the ^{13}C-CP/MAS NMR data of 12 calibration samples with known amounts of amorphous cellulose, cellulose I_α, and cellulose I_β. The amounts of cellulose I_α and I_β in some chemical and high-yield pulps were then predicted from their ^{13}C-CP/MAS NMR spectra by use of the PLS model.

2. Experimental

NMR methods.—The ^{13}C-CP/MAS NMR spectra were recorded on a Bruker AMX-300 instrument (at ambient temperature) operating at 75.47 MHz using a

double air-bearing probe and ZrO_2 rotors. The spinning rate was 5 kHz, contact time 0.8 min, acquisition time 37 ms, sweep width 368 ppm, and a delay between pulses of 2.5 s. For each spectrum 3000 transients were accumulated with 2048 data points and zero-filled to 4096 data points. The spectra were referenced to the carbonyl in external glycine (δ 176.03 ppm). The samples were hydrated with deionised water (ca. 50%) before recording the spectra, thus resulting in significant narrowing of the signals in the spectra [15,23]. The spectra were manually phased and then normalised by setting the integrated area of each spectrum to unity. The intensities of 601 spectral points in the interval 110–56 ppm for each of the spectra were used in the data analysis. Spectra were recorded in triplets of sample 2, doublets of samples 3, 10, 12, 14, 15, 16, 18, 21, and 22, and in singlets for all other samples (Table 1). The double and triple samples were taken from the same sample batch, but were packed in rotors and measured separately.

Sample preparation.—Amorphous cellulose was prepared from dissolving pulp

Table 1
Numbers, names, and values of crystallinity index (CrI), amorphicity index (AmI), cellulose I_α-content (I_α), and cellulose I_β-content (I_β), as estimated from the NMR spectra of the studied samples

Sample no.	Lignocellulosic sample	CrI	AmI	I_α	I_β
1	Cellulose from *Valonia ventricosa* *	0.91	0.09	0.47	0.44
2	Bacterial cellulose from *Acetobacter xylinum* *	0.75	0.25	0.36	0.39
		0.76	0.24	0.37	0.38
		0.76	0.24	0.38	0.38
3	Fibrous cellulose powder *	0.71	0.29	0.25	0.46
		0.71	0.29	0.22	0.49
4	Cotton linters *	0.68	0.32	0.24	0.44
5	Cotton *	0.66	0.34	0.25	0.41
6	Cotton cambers *	0.67	0.33		
7	Microcrystalline cellulose *	0.61	0.39		
8	$NaBH_4$-reduced microcrystalline cellulose *	0.62	0.38		
9	Linen *	0.60	0.40		
10	Dissolving pulp *	0.56	0.44		
		0.57	0.43		
11	Unbleached cotton cambers *	0.62	0.38		
12	Amorphous cellulose *	0.21	0.79		
		0.22	0.78		
13	Oxygen prebleached hardwood pulp	0.44	0.56		
14	Fully bleached hardwood kraft pulp	0.43	0.57		
15	Fully bleached softwood kraft pulp	0.52	0.48		
16	Unbleached softwood kraft pulp	0.52	0.48		
17	Aspen CTMP	0.47	0.53		
18	Spruce CTMP	0.45	0.55		
19	Unbleached GWP	0.44	0.56		
20	Bleached GWP	0.45	0.55		
21	TMP	0.49	0.51		
22	Bleached spruce sulfite pulp	0.53	0.47		
		0.53	0.47		

* Denotes the calibration samples.

(Acetacraft, International Paper, USA); air-dried dissolving pulp (50 g) was mixed with 70% $ZnCl_2$ (1 kg) for 1 h at ambient temperature [24], and then washed with water until a conductivity below 10 mSi was measured for the filtrate.

Reduced microcrystalline cellulose was prepared by mixing microcrystalline cellulose (12 g) with $NaBH_4$ (5 g) in water (100 mL) and stirring for 48 h at ambient temperature. The sample was filtered, washed with AcOH and water, and then dried.

The fibrous cellulose powder, CF-11, was purchased from Whatman. The microcrystalline cellulose, Avicel, was purchased from Merck. All the pulps were mill samples manufactured from the wood species *Picea abies*, *Pinus sylvestris*, or *Populus tremula*. The *Valonia ventricosa* and *Acetobacter xylinum* cellulose samples were provided by Göran Pettersson and Jerry Ståhlberg, Uppsala University.

Calculation of crystallinity index, amorphicity index, cellulose I_β-content and cellulose I_α-content.—The ^{13}C-CP/MAS NMR spectra of the lignocellulosic samples were integrated over the peaks at 86–92 (a) and 80–86 ppm (b). The crystallinity index (CrI) was calculated for each sample; CrI = a/(a + b) according to Teeäär et al. [13]. The amorphicity index (AmI) was then calculated for each sample; AmI = 1 − CrI.

The NMR spectra of samples 1–5 were integrated over the peaks at 105.4–106.4 (c), 104.4–105.4 (d), and 103.3–104.4 ppm (e). The contents of cellulose I_α and cellulose I_β were calculated; $I_\alpha = CrI \times d/(c + d + e)$ and $I_\beta = CrI \times (c + e)/(c + d + e)$.

Data analysis.—The NMR data were mean-centred before the PCA. A number of principal components (PC) [16,17] were extracted from the ^{13}C-CP/MAS NMR data from the calibration samples, 1–12 in Table 1. The PCs that were judged to result from variations in cellulose I_α, cellulose I_β, and amorphous cellulose were retained. The two retained PCs were statistically significant according to cross validation [25], and accounted for 80.6 and 13.3% of the total variance, respectively. To visualise the variations between the different NMR spectra, the scores [t, projection of the objects (spectra) on the retained PCs] were charted. Corresponding subspectra were constructed by charting the loadings (p, projections of spectral variables onto the PCs) versus variable name (ppm scale). Spectral variable intensities that change extensively along the direction of a PC show loading-values of large magnitude.

In the next step of the analysis, a PLS model [20,21] was built using the ^{13}C-CP/MAS NMR data of the calibration samples as the X-matrix, and the estimates of amorphicity index, cellulose I_α-content, and cellulose I_β-content as the Y-matrix; here two significant [25] PLS components were retained (Table 3). The X- and Y-matrix were mean-centred before the PLS calculation. PLS correlation charts were constructed by mapping the X- and Y-scores (t and u, projections of the objects on the retained PLS components).

All computations were carried out on an IBM PS-2 microcomputer, using the SIMCA 4R software package obtained from Umetri AB, Box 1456, S 901 24, Umeå, Sweden.

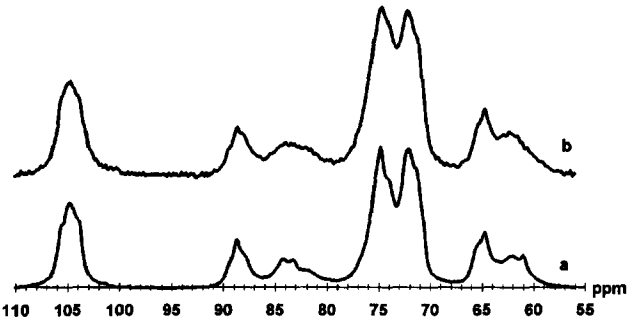

Fig. 1. a, ^{13}C-CP/MAS NMR spectrum of hydrated (50% water) bleached softwood kraft pulp; b, ^{13}C-CP/MAS NMR spectrum of dry bleached softwood kraft pulp.

3. Results and discussion

Spectra.—The addition of deionised water to lignocellulosic materials has been reported to induce signal narrowing in ^{13}C-CP/MAS NMR spectra, by increasing the mobility [15,26], or by the relaxation of distortions in the amorphous regions [23,27]. It has also been suggested that amorphous regions become more ordered in the presence of water [28]. In contrast to the ^{13}C-CP/MAS NMR spectrum of dry bleached softwood kraft pulp (Fig. 1b), the spectrum of the same hydrated sample (Fig. 1a) has much narrower resonance lines, although the chemical shifts do not appreciably differ from those of the dry sample.

From NMR measurements of cellulosic materials with various degrees of hydration (0–50%), we concluded that the maximum effect of signal narrowing was reached when the water content was above 20%. Variations in the degree of hydration above the 20% level did not alter the shape or position of the signals, hence NMR spectra in this work were recorded on hydrated samples containing ca. 50% water.

Since there are no known samples containing pure cellulose I_α, a difference spectrum representing cellulose I_α (Fig. 3a) was constructed from the ^{13}C-CP/MAS NMR spectra of samples containing substantial amounts of cellulose I_α (*Acetobacter xylinum* cellulose, Fig. 2a) and cellulose I_β (fibrous cellulose powder,

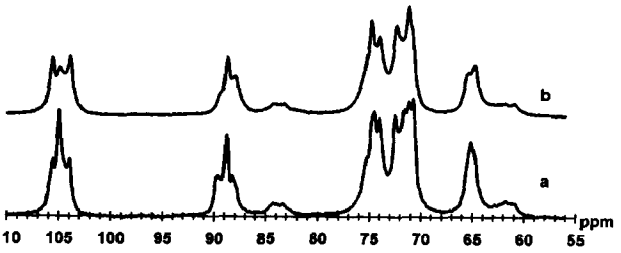

Fig. 2. ^{13}C-CP/MAS NMR spectra of a, *Acetobacter xylinum* cellulose; b, fibrous cellulose powder.

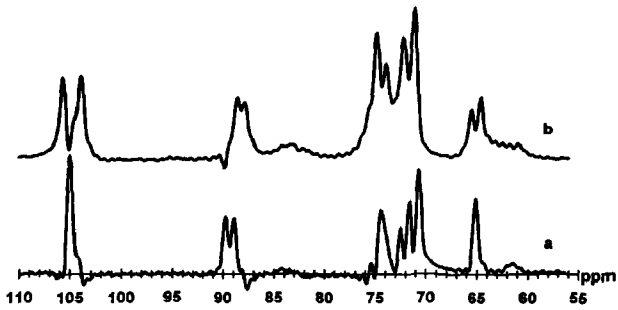

Fig. 3. Difference NMR spectra representing a, cellulose I_α; b, cellulose I_β.

Table 2
Peak assignments in the NMR difference spectra (Fig. 3) and PCA subspectra (Figs. 5 and 6): + denotes positive peaks and − denotes negative peaks

	C-1	C-4	C-2, C-3, C-5	C-6
Cellulose I_α	105.1	89.8, 89.0	74.5, 72.6 71.7, 70.8	65.2
Cellulose I_β	105.7, 104.0	88.7, 88.0	75.0, 74.1 72.3, 71.3	65.7 64.8
PC1+	105.5, 105, 104	90, 89, 88	71	65
PC1−	103	84–86	76, 73	60–63
PC2+	105	90, 89	74, 72.5, 70.5	65
PC2−	105.5, 104	88.5, 87.5	75, 72, 71	66, 64

Fig. 2b, cf. refs. 1–3). Tunicin cellulose consists of pure cellulose I_β [29], but was not available for this work; therefore the difference spectrum representing pure cellulose I_β (Fig. 3b) was constructed from the same NMR spectra as above, and corresponded well to the NMR spectrum of tunicin [29]. The peak assignments of the difference spectra in Fig. 3 are given in Table 2 and refer to the different carbon atoms in the "anhydroglucose" units [2].

PC calculations of ^{13}C-CP/MAS NMR data of the calibration samples.—The calibration samples were chosen to exhibit large variations in cellulose I_α and cellulose I_β; e.g., cotton, microcrystalline cellulose, dissolving pulp, bacterial cellulose, *Valonia* cellulose, and a sample of amorphous cellulose (samples 1–12 in Table 1). A PCA model was built from the NMR data of the calibration samples. The scores of the NMR spectra are mapped on the two retained principal components (PC) in Fig. 4, t2 vs. t1. NMR spectra of samples known to contain high amounts of cellulose I_α (*Valonia* cellulose and bacterial cellulose, samples 1 and 2) have positive t1 values, spectra of samples containing high amounts of cellulose I_β (the fibrous cellulose powder and the cotton samples, samples 3–6) have t1 values around zero, whereas spectra of the sample expected to contain high amounts of amorphous cellulose (sample 12) have negative t1 values. The

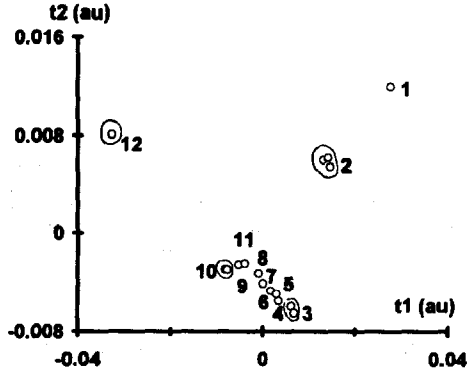

Fig. 4. PCA model built from the NMR data of the cellulose calibration samples. Map of t2 vs. t1, in arbitrary units. The numbers refer to spectra of the samples in Table 1. The double and triple samples are circled.

spectra of the samples of microcrystalline cellulose, linen, dissolving pulp, and unbleached cotton cambers (samples 7–11) were expected to have a lower degree of crystallinity than samples 1–6, and accordingly had lower t1 values.

Along PC2 in Fig. 4, the cellulose I_β-rich samples (3–11) have negative t2 values, whereas the cellulose I_α-rich samples (1 and 2) and the sample of amorphous cellulose (12) have positive t2 values.

These results have led us to interpret the PCA model as containing one principal component (PC1) describing variations in the proportion of crystalline and amorphous cellulose, and one principal component (PC2) describing variations in the proportion of cellulose I_α and cellulose I_β in the samples. Hence, the retained PCs were given plausible chemical interpretations. We therefore expected the subspectra constructed from the loading-vectors of the PCs to resemble the spectral features corresponding to cellulose I_α, cellulose I_β and amorphous cellulose.

The subspectra representing the principal components are shown in Figs. 5 and 6. The spectral variables with large magnitudes are given in Table 2. The position

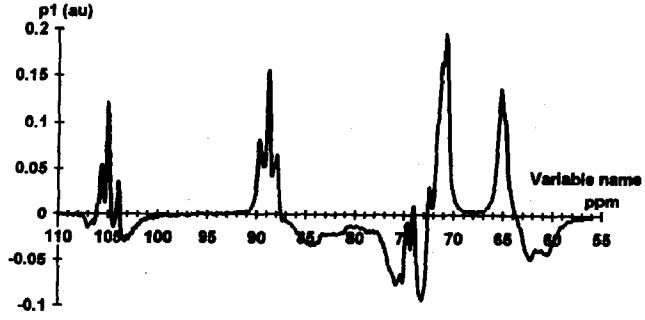

Fig. 5. Subspectrum of PC1 (p1, in arbitrary units).

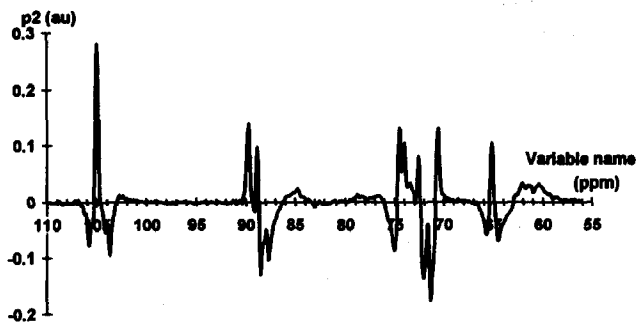

Fig. 6. Subspectrum of PC2 (p2, in arbitrary units).

of the positive peaks in the PC1 subspectrum, Fig. 5, were similar to the location of the signals in the difference spectra of cellulose I_α and I_β in Fig. 3, whereas the position of the negative peaks corresponded to the regions in NMR spectra reported for amorphous cellulose [9–11]. Furthermore, the subspectrum of PC2 in Fig. 6 resembles an NMR spectrum where the positive peaks correspond to cellulose I_α and the negative peaks correspond to cellulose I_β.

The subspectra in Figs. 5 and 6 were similar to the spectral features in the difference spectra of cellulose I_α (Fig. 3a) and cellulose I_β (Fig. 3b), thereby verifying our chemical interpretation of the PCA model. In order to obtain quantitative information, we constructed a PLS model.

PLS modelling.—The quantities of cellulose I_α, cellulose I_β, and the amorphous cellulose were determined in the calibration samples. The crystallinity index (CrI) was measured by integrating the C-4 peaks in the NMR spectra, according to Teeäär et al. [13]. The amorphicity index (AmI) was defined as 1 − CrI. To estimate the amounts of cellulose I_α and I_β, the C-1 peaks in the NMR spectra were integrated [3,6]. The smallest error in this determination was considered to be for the NMR spectra having the best resolution; hence the amounts of cellulose I_α and I_β were determined only for samples 1–5 (*Valonia* cellulose, *Acetobacter* cellulose, fibrous cellulose powder, cotton, and cotton linters). The sample of amorphous cellulose was assumed to have no cellulose I_α or I_β.

Table 3
Characteristics of the PLS model: PLS-component no., explained sumsquares for the X-matrix in % (SSX%), and Y-matrix in % (SSY%), predicted sumsquares/sumsquares (PRESS/SS), and the correlation coefficient (R^2) for the correlation charts (Figs. 7a and b)

No.	SSX%	SSY%			PRESS/SS			R^2
		AmI	I_α	I_β	AmI	I_α	I_β	
1	81.8	98.20	93.16	79.20	0.03	0.07	0.26	0.97
2	12.9	0.98	1.83	20.31	0.45	0.78	0.03	0.87
Σ	94.7	99.18	94.99	99.51				

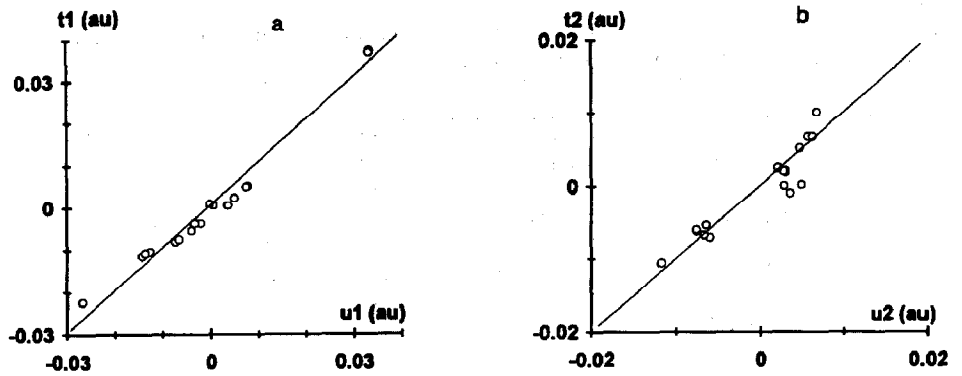

Fig. 7. Correlation charts of the PLS components calculated from the NMR data of the cellulose calibration samples: t are the scores for the X-variables (NMR data) and u are the scores for the Y-variables (AmI, I_α, and I_β), in arbitrary units. a, PLS component 1, t1 vs. u1, $R^2 = 0.97$; b, PLS component 2, t2 vs. u2, $R^2 = 0.87$.

A PLS model was established between the ^{13}C-CP/MAS NMR data and the estimates of amorphicity index (AmI), cellulose I_α-content (I_α), and cellulose I_β-content (I_β) for the calibration samples. Two PLS components were retained, by which the AmI, I_α, and I_β were sufficiently well explained, cf. Table 3. In the correlation charts of the two PLS components (Fig. 7a and b) the samples are spread along the correlation lines with good correlation coefficients. The PLS model was thus considered to be a feasible method to determine AmI, I_α, and I_β in the calibration samples.

Using the PLS model to predict AmI, I_α, and I_β in lignocellulosic samples.—In addition to cellulose, lignocellulosic samples contain other constituents, e.g., hemicelluloses and lignin. In ^{13}C-CP/MAS NMR spectra of lignocellulosic samples the signal assigned to amorphous C-4 (80–86 ppm) contain contributions from both amorphous cellulose and other amorphous polysaccharides. For lignocellulosic samples, the definition of AmI was therefore extended to comprise amorphous polysaccharides.

NMR spectra were acquired on some chemical and high-yield pulps (samples 13–22 in Table 1). From the area of the C-4 peaks, AmI was calculated as described above and given in Table 1. By using the above PLS model AmI, I_α and I_β in the pulp samples were predicted from the spectra (Table 4). Since AmI could be measured by integration of the spectra, and predicted by use of the PLS model, the predictability of the PLS model could be probed. The chart of AmI vs. the PLS-predicted AmI is shown in Fig. 8. The open circles represent the calibration samples (defining the PLS model), and the closed circles the pulp samples. Since these samples were spread along the correlation line, the PLS model was considered to have a good predictive ability.

The amounts of cellulose I_α and I_β in the pulp samples predicted from the PLS model are charted in Fig. 9. Since carbohydrates are the main species in the pulps giving rise to NMR signals in the region 110–56 ppm, the sum of the estimates of

Table 4
Amorphicity index (AmI), cellulose I_α-content (I_α), and cellulose I_β-content (I_β) of all studied pulp samples, predicted from NMR spectra and the PLS model

Sample no.	Lignocellulosic sample	AmI	I_α	I_β	Σ
13	Oxygen prebleached hardwood pulp	0.50	0.17	0.26	0.93
14	Fully bleached hardwood kraft pulp	0.50	0.17	0.27	0.94
		0.49	0.17	0.27	0.93
15	Fully bleached softwood kraft pulp	0.43	0.21	0.32	0.96
		0.41	0.22	0.34	0.97
16	Unbleached softwood kraft pulp	0.43	0.21	0.32	0.96
		0.41	0.21	0.34	0.96
17	Aspen CTMP	0.50	0.17	0.25	0.92
18	Spruce CTMP	0.49	0.19	0.26	0.94
		0.49	0.18	0.25	0.92
19	Unbleached GWP	0.53	0.16	0.22	0.91
20	Bleached GWP	0.53	0.16	0.22	0.91
21	TMP	0.46	0.19	0.29	0.94
		0.47	0.19	0.28	0.94
22	Bleached spruce sulfite pulp	0.42	0.22	0.32	0.96
		0.42	0.22	0.31	0.95

AmI, I_α, and I_β in Fig. 9 essentially corresponds to the total amount of carbohydrates in the pulp samples. The studied pulp samples thus contained ca. 22–34% cellulose I_β and 17–22% cellulose I_α relative to the total carbohydrate content. With this PLS model it was possible to demonstrate what other methods have failed to show [3,4], i.e., the existence of substantial amounts of cellulose I_α in pulp samples.

The good stability and reproducibility of the PLS model is evident from the predicted values of the repeated measurements in Table 4. The standard deviation of the predicted I_α and I_β from repeated measurements of the same sample were

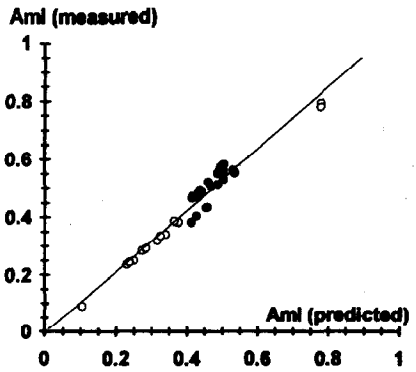

Fig. 8. Chart of measured and PLS-predicted AmI in all the samples. The open circles correspond to the calibration samples, and the closed circles to the pulp samples. The correlation coefficient (R^2) is 0.95.

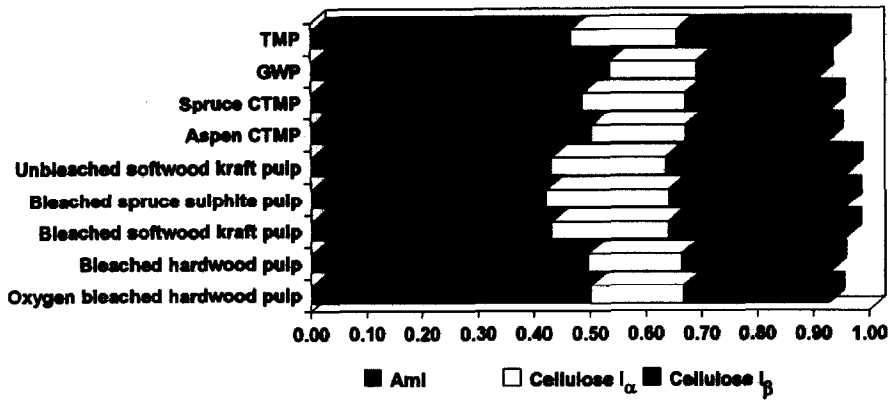

Fig. 9. The AmI, I_α, and I_β in the pulp samples, predicted from their ^{13}C-CP/MAS NMR spectra by use of the PLS model. The figure 1.00 corresponds to 100%.

within 3%. Error sources might be due to NMR signals arising from other constituents in the calibration samples, such as lignin and other cellulose polymorphs (i.e., cellulose II–IV). The elimination of these error sources should be considered in future PLS models.

Simulation of NMR spectra.—To substantiate the existence of cellulose I_α in pulp samples, we used the PLS model to simulate NMR spectra of bleached softwood kraft pulp with and without cellulose I_α. The NMR spectrum of bleached softwood kraft pulp is shown in Fig. 10a. The values AmI = 0.43, $I_\alpha = 0.21$, and $I_\beta = 0.32$ (from Table 4) were used to simulate the spectrum of the same pulp (Fig. 10b). For the simulated spectrum in Fig. 10c, identical values were used, except $I_\alpha = 0$. The simulated spectrum of Fig. 10b resembles the real spectrum in Fig. 10a, but the simulated spectrum without any cellulose I_α (Fig. 10c) exhibits a different pattern. We believe that this example substantiates and demonstrates the presence of cellulose I_α in pulp samples as well as the quality of the PLS model.

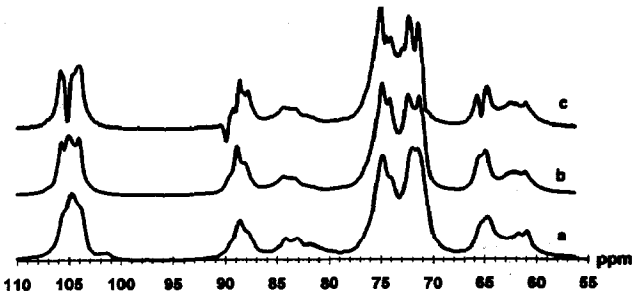

Fig. 10. a, ^{13}C-CP/MAS NMR spectrum of bleached softwood kraft pulp; b, simulated ^{13}C-CP/MAS NMR spectrum of bleached softwood kraft pulp;. c, simulated ^{13}C-CP/MAS NMR spectrum of bleached softwood kraft pulp without cellulose I_α.

4. Conclusions

A novel method to estimate cellulose I_α and I_β in lignocellulosic samples has been developed. A PLS model of ^{13}C-CP/MAS NMR data and estimates of AmI, cellulose I_α-content, and cellulose I_β-content in 12 calibration cellulose samples was established. The amounts of cellulose I_α and I_β in some chemical and high-yield pulp samples were predicted from their NMR spectra, using the PLS model. The PLS model made it possible to demonstrate the existence of substantial amounts of cellulose I_α in the pulp samples.

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