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Note

Determination of crystallinity in native cellulose from higher plants with diffuse reflectance Fourier transform infrared spectroscopy

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Cellulose is the main constituent of such plant fibres as flax, ramie, cotton, and wood. The structural parameters of cellulose, like lattice type, crystallinity, crystallite size, and degree of polymerization, contribute significantly to the physical properties of the fibre. The crystallinity of cellulose influences, for example, the accessibility for chemical derivatization, swelling, and water-binding properties [1,2].

Crystallinity (x_c) in semi-crystalline components like cellulose is defined as the ratio between the mass of the crystalline domains and the total mass of the material. In cellulose, these crystalline domains may consist of several lattice types (Iα, Iβ, II, $III_{I,II}$, and $IV_{I,II}$) depending upon origin and pretreatment of the cellulose. Lattice types of native cellulose are triclinic cellulose Iα and monoclinic cellulose Iβ. Differences between these two lattice types are found in the hydrogen-bonding pattern rather than in the conformation of the polysaccharide chain [3–5]. All other polymorphs of cellulose occur only after chemical treatment of the cellulose. From ^{13}C CP/MAS NMR measurements [6,7], electron diffraction [4,8], Raman spectroscopy [9], and second derivative FT-IR spectroscopy [10], it is suggested that native celluloses from different origins are composites of cellulose Iα and cellulose Iβ. Native celluloses are divided into two classes, having cellulose Iα or cellulose Iβ as the major crystalline component. Crystalline domains of algal and bacterial cellulose are composed mainly of cellulose Iα [ca. 60% (w/w)], whereas cellulose from higher plants contains cellulose Iβ as the major crystalline component [ca. 80% (w/w)] [11,12]. The IR spectra of the two classes are very similar [5,8] except for differences at 3270, 3240, 2900, 750, 710, and 650 cm^{-1}. In

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this study, cellulose from higher plants is used. This cellulose is considered as a two-phase system consisting of amorphous and crystalline cellulose. The crystalline cellulose is composed mainly of cellulose Iβ and is assumed to have a constant ratio between the amounts of cellulose Iα and cellulose Iβ.

Various methods for determining crystallinity of cellulose based on WAXS have been reported [13–17]. Polizzi et al. [17] and Fink et al. [13] developed WAXS methods for the determination of cellulose crystallinity based on the method described by Ruland [18]. In these methods, diffractograms are corrected for incoherent radiation, polarization, and absorption. Deviation of the atoms from the ideal positions (thermal vibrations, first- and second-order lattice imperfections) is corrected for by including a lattice imperfection factor. Hermans and Weidinger developed a method based on the scaling of the amorphous diffraction under the diffractogram of the semi-crystalline cellulose [15]. As a measure of crystallinity, the area of the crystalline diffraction is taken relative to the total area of the diffractogram. A prerequisite is the availability of a totally amorphous sample. Since this method depends only on the existence of a proportionality between the crystalline intensity and the crystalline fraction and between the amorphous intensity and the amorphous fraction, the lattice imperfection factor is not taken into account [16]. Although it can be concluded that the method of Hermans and Weidinger slightly underestimates ($< 10\%$) crystallinity compared to the methods of Fink et al. or Polizzi et al. by neglecting the lattice imperfection factor [13,16,17], it can nevertheless be used as a measure of crystallinity. Another measure of cellulose crystallinity can be obtained by means of ^{13}C CP/MAS NMR using the different spin–lattice relaxation times of crystalline cellulose and amorphous cellulose [6,19].

Disadvantages of the WAXS and ^{13}C CP/MAS NMR methods are their time-consuming character and the need for expensive instrumentation. WAXS crystallinity determination is also sensitive towards such noncellulosic compounds as hemicellulose and pectins which are present in plant fibres. Interference from these components will cause extra (amorphous) scattering and this will cause an underestimation of the cellulose crystallinity.

IR spectroscopy proved to be a simple and fast method for determining crystallinity in several compounds [20]. Furthermore, the IR crystallinity determination can be developed to be independent of the noncellulosic components in plant fibres. In the past, several IR ratios for predicting cellulose crystallinity semi-quantitatively have been reported [21–24]. A limiting factor was the highly overlapping, poorly resolved bands in the spectrum of cellulose. With the development of Fourier transform instruments and the possibility to enhance resolution mathematically (deconvolution), there is renewed interest in IR spectroscopy [25].

Our research programme is focused on the relationship between the structural parameters and the physical properties of such agrofibers as flax (*Linum usitatissimum* L.) and fibre hemp (*Cannabis sativa vulgaris* L.); one such parameter is cellulose crystallinity. The aim of this study is to develop a quantitative method for the determination of cellulose I crystallinity in samples which may contain significant amounts of noncellulosic components like hemicellulose and pectins. IR

spectroscopy was used to develop a method which will not be disturbed by these noncellulosic components.

1. Results and discussion

Fig. 1 shows the WAXS diffractograms of the cellulose samples which were ball-milled for 0, 2, 10, 25, 62, 120, 173, and 500 min. Since the diffractogram of 500-min, ball-milled microcrystalline cellulose shows no discrete diffractions and further milling did not change the diffractogram, this cellulose sample can be assumed to be totally amorphous. In Fig. 2, the original and deconvoluted DRIFT spectra in the range 850–1500 cm^{-1} for all cellulose samples are given. The absence of derivative-like bands in the spectra indicates that specular reflectance contributions can be neglected. When moving from highly crystalline to amorphous cellulose, the spectra of these samples show decreasing bands at 1429 (CH_2 bending), 1372 (CH bending), 1336 (OH rocking), 1313 (CH_2 wagging), 1280 (CH bending), 1160 (COC antisymmetric stretching), 1105 (antisymmetric in-phase ring stretching), and 1080 cm^{-1} (CO stretching); the published peak assignments [22,26–28] are given in parentheses. However, the assignments are not always unambigious due to the fact that most bands are combination bands. When changing from highly crystalline to amorphous cellulose, the atoms or atom groups

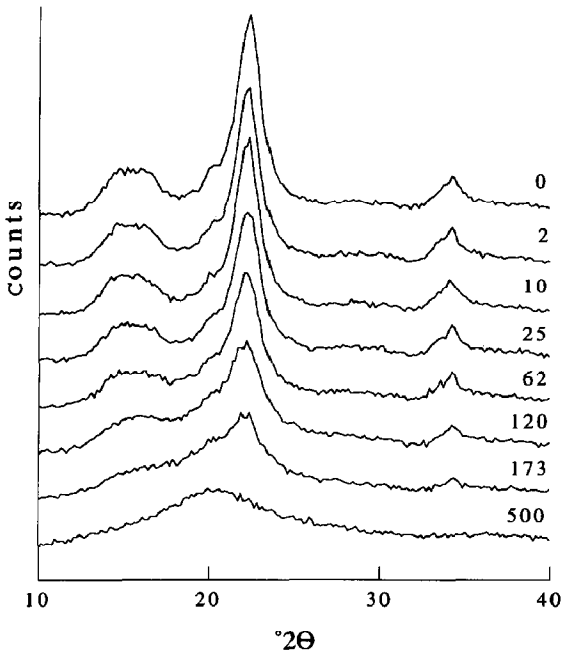

Fig. 1. WAXS diffractograms of ball-milled (0–500 min) cellulose samples.

in the polysaccharide chains lose the specific molecular environment as present in the cellulose Iα and Iβ lattices. Due to this loss in short-range order, the spectrum of amorphous cellulose shows no discrete bands at the wavenumbers mentioned. In contrast to the above, bands at 1200 (OH bending) and 898 cm^{-1} (anomeric

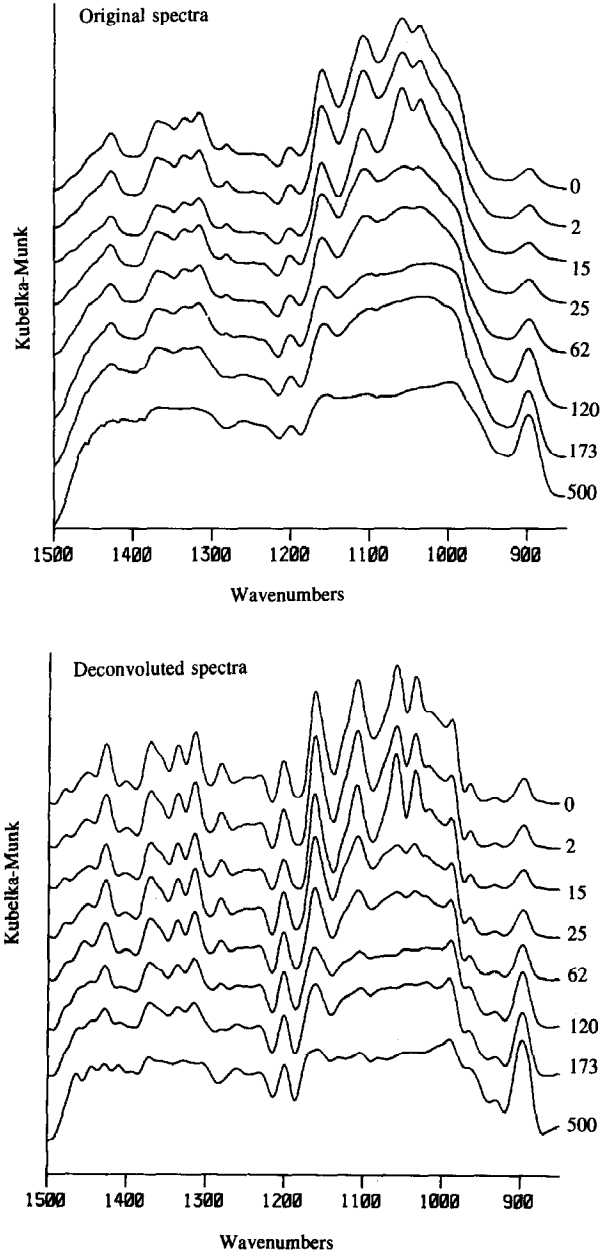

Fig. 2. Original and deconvoluted DRIFT spectra of the ball-milled cellulose samples (850–1500 cm^{-1}).

vibration, specific for β-glucosides) do not decrease with decreasing crystallinity. These vibrations seem insensitive to a change in molecular environment. The relative sensitivity of the band at 1280 cm^{-1} (CH bending) and the insensitivity of the band at 1200 cm^{-1} (OH bending) to a decrease in crystallinity are not fully understood. Changes (like a decrease in band height, band broadening, or band shifting) would be expected for bands assigned to vibrations involved in hydrogen bonds. However, this is not observed. Therefore, the assignments of the bands need further investigation.

Since this IR cellulose-crystallinity determination has been developed for plant fibres containing a significant amount of noncellulosic components [20–35% (w/w)] [29,30], a measure of cellulose I crystallinity independent of the absorbances of these components had to be selected in the DRIFT spectrum. Fig. 3 shows the deconvoluted DRIFT spectra in the range 850–1800 cm^{-1} for the major noncellulosic polysaccharides extracted nondestructively from flax fibre. Due to the overlap of bands of the noncellulosic polysaccharides with bands from cellulose, the region at wavenumbers higher than 1800 cm^{-1} is not suitable for cellulose-crystallinity determination in plant fibres. However, for pure cellulose samples, lattice type and crystallinity were determined in these regions [25]. These noncellulosic polysaccharides show minimal absorbance in the regions around 1200 and 1280 cm^{-1}.

In the spectra of the cellulose samples, a decrease of the band at 1280 cm^{-1} is observed with decreasing crystallinity. Part of the broad bands appearing under the

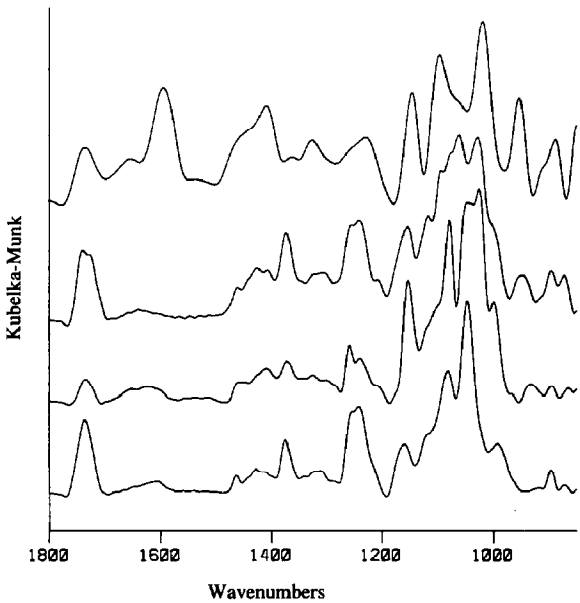

Fig. 3. Deconvoluted DRIFT spectra of the major noncellulosic polysaccharides from flax fibre (upper spectrum: pectin extract; lower three spectra: various hemicelluloses fractionated on Sephacryl S400 HR).

discrete band at 1280 cm^{-1} in the spectrum of cellulose originate from amorphous cellulose since they increase with decreasing crystallinity. The band at 1200 cm^{-1} was assumed to be constant with decreasing crystallinity. When changing from microcrystalline to amorphous cellulose, the ratio between the bands at 1280 and 1200 cm^{-1} is suitable for the determination of cellulose I crystallinity. Both the heights and the areas of the bands at 1280 and 1200 cm^{-1} are measured and their respective ratios $R_{c,h}$ and $R_{c,a}$ are calculated as shown in Fig. 4. In Fig. 5, $R_{c,h}$ and $R_{c,a}$ are plotted vs. the crystallinity (x_c) according to Hermans and Weidinger [15]. By comparison of the two lines of regression, the ratio of the heights of the bands ($R_{c,h}$) can be taken as a measure of crystallinity since it shows the best linear regression with x_c. Besides, the band at 1280 cm^{-1} showed an increasing asymmetrical shape with decreasing crystallinity due to the increase of a band at 1260 cm^{-1} which disturbs the use of $R_{c,a}$. From the line of regression depicted in Fig. 5, it was deduced that $R_{c,h}$ is related to cellulose I crystallinity x_c following the equation:

$$x_c = 1.06 \cdot R_{c,h} + 0.19 \quad (1)$$

The percentage variance accounted for is 99.7% ($n = 7$). At low crystallinities ($x_c < 0.26$), the height of the band at 1280 cm^{-1}, and therewith $R_{c,h}$, decreases to zero. This limits the DRIFT crystallinity determination to values above $x_c = 0.26$. Given the fact that x_c of the used microcrystalline cellulose is ca. 0.75, the line of regression is valid for $0.26 < x_c < 0.75$. Cellulose I crystallinity x_c could be predicted from $R_{c,h}$ with a standard error of 0.01–0.02 ($n = 3$).

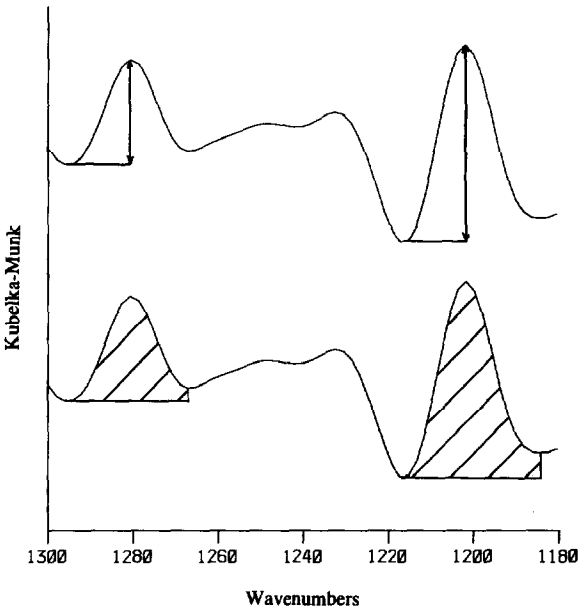

Fig. 4. Calculation of $R_{c,h}$ (upper) and $R_{c,a}$ (lower).

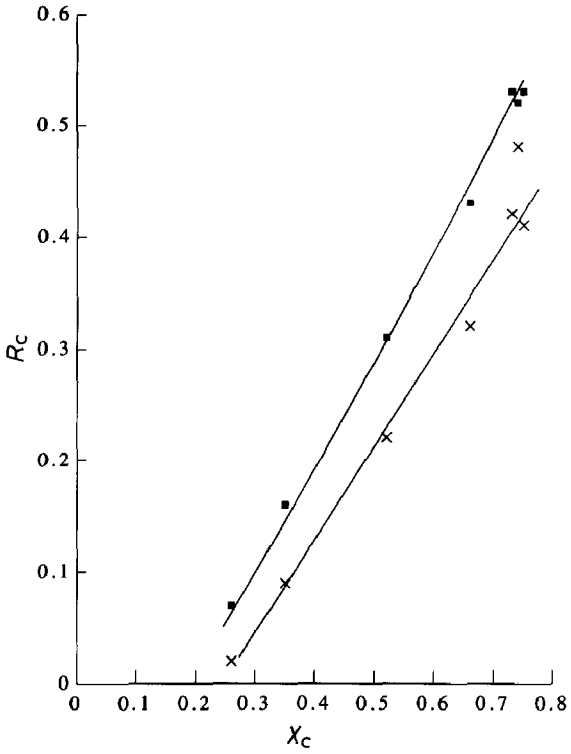

Fig. 5. $R_{c,h}$ (■) and $R_{c,a}$ (×) vs. x_c.

The method has been developed to enable determination of cellulose I crystallinity in plant fibres. It is difficult to reduce the size of these fibres to less than 50 μm without decreasing crystallinity. To make the determination of cellulose I crystallinity in these samples possible, Eq. *1* was validated with cellulose samples of different particle sizes. Native cellulose, cellulose powder, and microcrystalline cellulose with particle sizes in the ranges < 50 μm and < 150 μm were measured

Table 1
Crystallinities of commercial celluloses with different particle sizes as determined with DRIFT ($x_{c,p}$) and WAXS ($x_{c,m}$)

Sample	$x_{c,m}$ [a]		$x_{c,p}$	
	< 50 μm	Bulk	< 50 μm	< 150 μm
Native cellulose	0.40	0.41	0.41	0.41
Cellulose powder	0.48	0.55	0.47	0.58
Microcrystalline cellulose	0.74	0.74	0.73	0.73

[a] Standard errors for $x_{c,p}$ and $x_{c,m}$ were 0.01–0.02 ($n = 3$) and 0.01 ($n = 3$), respectively.

with DRIFT, and crystallinity was calculated using Eq. *1*. Crystallinity of the cellulose bulk was measured with WAXS. Values are presented in Table 1. It is concluded that crystallinity from the cellulose bulk can be predicted with DRIFT, using particles < 150 μm, while particles < 50 μm can also be used for native cellulose and microcrystalline cellulose. For cellulose powder, crystallinity was significantly lower for particles < 50 μm, as measured with both DRIFT and WAXS. For this sample, cellulose I crystallinity of the particle range < 50 μm is not a measure for the crystallinity of the cellulose bulk, probably due to severe pretreatment of the cellulose. However, cellulose I crystallinities measured with both DRIFT and WAXS are identical for both fractions.

It is not known to what extent band broadening and a decrease in intensity, as mentioned by Fuller and Griffiths [31], occurred through measuring relatively large particle sizes instead of particle sizes in the range of the IR radiation used (2–15 μm). Except for cellulose powder, the DRIFT spectra from particles < 150 μm and DRIFT spectra from particles < 50 μm were identical. Particle sizes above 150 μm are not suitable for DRIFT since it is impossible to obtain an isotropic sample.

Cellulose I crystallinity of the various celluloses analyzed covers a broad range ($x_c = 0.40$–0.75) probably due to the pretreatment and origin of the cellulose. These differences may cause differences in the properties of the celluloses, for example, E-modulus, tensile strength, water absorption, or accessibility for chemical derivatization.

Compared to the traditional WAXS methods, the main advantages of the DRIFT method reported here are the small amounts of sample required and the less expensive instrumentation. Furthermore, sampling by mixing (cellulose) samples of the appropriate particle size with KBr is a fast and simple technique.

2. Conclusions

In this study, a method based on DRIFT spectroscopy has been developed for the quantitative determination of cellulose I crystallinity. The ratio ($R_{c,h}$) of the heights of the bands at 1280 and 1200 cm^{-1} showed a first-order regression with cellulose I crystallinity (x_c) as determined with WAXS according to the method of Hermans and Weidinger. The ratio $R_{c,h}$ is related to x_c following the equation $x_c = 1.06 \cdot R_{c,h} + 0.19$. This equation is valid for $0.26 < x_c < 0.75$. The percentage variance accounted for was 99.7% ($n = 7$). Cellulose crystallinity can be predicted from $R_{c,h}$ with a standard error of 0.01–0.02 ($n = 3$). Determination of $R_{c,h}$ for samples with particle sizes smaller than 150 μm accurately predicts cellulose I crystallinity of the bulk. Because cellulose crystallinity can be determined with DRIFT using small amounts of sample without pressing or grinding, information about the initial cellulose crystallinity of the sample is obtained. With this method, it will be possible to measure crystallinity of such cellulosic fibres as flax, hemp, and cotton, using particle sizes smaller than 150 μm.

3. Experimental

Materials.—Cellulose samples of different crystallinities were obtained by milling microcrystalline cellulose (Avicel®, Merck, Art. 2330) in a vibratory ball mill for 0, 2, 10, 25, 62, 120, 173 and 500 min. Samples were gently rubbed through a 50-μm sieve. Native cellulose (Merck, Art. 2351) and cellulose powder (Fluka Bio-Chemika, Art. 22183) were used to validate the method. D-Glucose content of cellulose samples was $>95\%$ (w/w). All cellulose samples originated from cotton or wood.

FT-IR.—DRIFT spectra were recorded on a BioRad FTS-60A spectrometer equipped with a water-cooled source and a DTGS detector using the Digilab diffuse reflectance accessory. Samples were prepared by shaking ca. 5% (w/w) of dried (>16 h over silica gel) cellulose in KBr (Uvasol®, Merck, <50 μm). The spectra obtained at a resolution of 4 cm^{-1} were averages of 64 scans, and were recorded as Kubelka Munk-transformed spectra against a KBr background. Spectra were baseline-corrected at 3750 and 1900 cm^{-1}, and deconvoluted (half bandwidth, 20 cm^{-1}; peak-width narrowing factor, 1.5; Bessel apodization).

WAXS.—Diffractograms were recorded on a Philips PC-APD diffractometer in the reflection geometry in the angular range 10–40(°2θ). The CuK radiation from the anode operating at 40 kV and 50 mA was monochromatized using a 15-μm Ni foil. The diffractometer parameters were: divergence slit, 1°; receiving slit, 0.2 mm; and scatter slit, 2°. The scattered radiation was detected using a proportional detector. Crystallinity was measured according to the method of Hermans and Weidinger [15]. Diffractograms were corrected by drawing a straight line between the intensities at 10 and 40(°2θ). The area of the crystalline diffraction (A_{cr}) was measured relative to the area of the total diffraction (A_t) by scaling the halo of the amorphous sample under the diffractogram of the sample ($A_{cr}/A_t = x_c$).

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