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Conversions between ordered and disordered cellulose. Effects of mechanical treatment followed by cyclic wetting and drying

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An investigation into the effects of mechanical treatment and hydration on the order of cellulose substrates (microcrystalline cellulose and *Cladophora* cellulose) was performed by the use of ball milling followed by cyclic wetting and drying. The results, monitored by ^{13}C-CP/MAS NMR-spectroscopy, were evaluated by calculation of the crystallinity indices and principal component analysis of the NMR data acquired. The results showed that a large part of the disorder induced by the mechanical treatment of cellulose by ball milling is reversible and reordering upon hydration leads to the cellulose I form initially present. The C4 signals corresponding to the reversibly disordered cellulose chains are observed in the 'amorphous region' between 79 and 86 ppm in the ^{13}C-CP/MAS NMR-spectra together with signals from cellulose chains on the surface of ordered regions. The peak cluster which contains the C2, C3 and C5 ring carbons can be divided into two specific spectral regions; one between 74 and 77 ppm largely originates from ring carbons within disordered cellulose structures, and one between 70 and 74 ppm contains larger contributions from ordered cellulose. The behaviour of the celluloses upon milling is in accordance with a concept of ordered cellulose fibrils containing 'amorphous' cellulose mainly as surface layers and induced reversible lattice distortions.

KEYWORDS: cellulose I, cellulose II, disordered cellulose, hydration, principal component analysis, ball milling, NMR-spectroscopy

INTRODUCTION

Our knowledge of the structure of native cellulose and how it is affected during industrial processing is still limited. Any perspective on the factors influencing the physico-mechanical or chemical properties requires detailed knowledge of the structural characteristics of the processed cellulose or lignocellulose fibres.

^{13}C-CP/MAS NMR-spectroscopy is well suited for investigations of the molecular order in cellulose substrates. Several investigators have established that the cluster of fairly sharp signals with a distribution between 86 and 92 ppm corresponds to C4 carbons situated inside cellulose crystallites, while C4 carbons of disordered regions are

distributed in a broad band ranging from 79 to 86 ppm (Atalla *et al.*, 1980; Earl and VanderHart, 1980; Teeäär *et al.*, 1987). In two recent articles Newman and co-workers (Newman, 1994; Newman *et al.*, 1994) used resolution enhanced NMR-spectroscopy to investigate the molecular ordering in native celluloses. In the disordered region a pair of signals was resolved at 84.0 ppm and 84.9 ppm and assigned to crystallographic non-equivalent surfaces of cellulose crystallites.

Recently, we studied the effects on cellulose structure induced by mechanical treatment in water, i.e. beating of kraft pulps, by ^{13}C-CP/MAS NMR-spectroscopy (Wallbäcks *et al.*, 1991; Lennholm and Iversen, 1995a). To simplify the evaluation of the experiments and facilitate the interpretation of the spectra, we applied partial least squares (PLS) analysis (Geladi and Kowalski, 1986; Martens and Naes, 1989) to the acquired experimental data. Although the changes in the NMR spectra were subtle, the chemometric evaluation indicated that they correlated with changes in the physico-mechanical properties of the pulps. The spectral changes could not, however, be given any adequate explanation in terms of allomorph conversions or ordered to disordered transformations of the cellulose.

Since the molecular order is an important factor influencing the physico-mechanical properties of fibrous cellulose substrates (Krässig, 1992; Young 1994) we decided to study cellulose samples treated by methods known to induce pronounced ordered to disordered transformations. Numerous investigators have studied decrystallization of native cellulose fibres when dry milled in a vibratory ball mill and debated the reordering of the disordered cellulose to cellulose I and/or cellulose II upon wetting (Hess *et al.*, 1941; Hermans and Weidinger, 1946; Howsman and Marchessault, 1959; Leopold and Moulik, 1968; Caulfield and Steffes, 1969; Bhama Iyer *et al.*, 1984; Liang *et al.*, 1993). Since this phenomenon of disordering and the subsequent reordering of the disordered cellulose is well documented in the literature we decided to do such a series of experiments and follow the changes induced in the cellulose structure by use of ^{13}C-CP/MAS NMR-spectroscopy.

The aim of this study was to investigate conversions between ordered and disordered cellulose, exaggerating the conditions occurring during industrial processing of pulp fibres, with an emphasis on the fate of the cellulose during pronounced mechanical treatment followed by cyclic wetting and drying. Microcrystalline cellulose (MCC) was chosen as a model for pulp celluloses because of the chemical purity of MCC and since it has been well characterized previously by X-ray diffraction and NMR-spectroscopy (Atalla *et al.*, 1980; Earl *et al.*, 1981; Teeäär *et al.*, 1987). Experiments on a well ordered algal cellulose isolated from *Cladophora* sp. (VanderHart and Atalla, 1987) was included in the study to corroborate the observed transformations.

MATERIALS AND METHODS

Preparation of samples

Ten samples of decrystallized cellulose were prepared by dry milling 1 g of microcrystalline cellulose (Avicel PH 101), abbreviated MCC, in a Retsch vibratory mill type MM-2 for 5, 10, 15, 20, 25, 30, 35, 40, 60 and 1320 minutes with balls of zirconium oxide. Including the zero sample (unmilled) this made a total of eleven samples for investigation by NMR-spectroscopy. Spectral data were acquired for all the samples in dry, wet, redried (air dried at 23 °C/20% RH for two months) and rewetted

state. Wetting of the dry samples (5% water content) was conducted, after packing in the NMR-rotor, with de-ionized water to about 50% water content (Horii *et al.*, 1985; Willis and Herring, 1987). Algal cellulose was isolated from *Cladophora* sp. naturally grown in the Baltic sea (Gardner and Blackwell, 1974). A sample of the *Cladophora* cellulose was decrystallized by dry milling for 10 minutes. Spectral data for milled and unmilled *Cladophora* celluloses were acquired on dry and wet samples as described above.

^{13}C-CP/MAS NMR-spectroscopy

All spectra were recorded on a Bruker AMX-300 WB spectrometer operating at 7.05 Tesla, with a ^{13}C resonance frequency of 75.43 MHz, using the cross polarization and magic angle spinning (CP/MAS) technique. The spectra were acquired with a 90° proton pulse of 3.75 μs, contact time of 1 ms, delay between pulses of 2.5 s and a spectral width of 368 ppm. The 7 mm zirconium oxide rotor was spun at 5000 Hz and a total of 3000 transients for each spectra were accumulated with 2 K data points and zero filling to 4 K. The chemical shift scale was calibrated by using glycine (carbonyl peak assigned to 176.03 ppm) as an external standard.

Size exclusion chromatography (SEC)

Size exclusion chromatography was performed using a chromatographic system from Millipore Co. Three μ- Styragel^{TM} HT columns (10^6 10^5 and 10^4 Å) connected in series were used with a column temperature of 80 °C, eluent 1% LiCl (dry, P.A.) in dimethylacetamide (DMAC, HPLC quality) and flow rate, 1 ml/min. Samples of 0.1 ml containing 0.5–1 g/l of the sample were injected in each run. The system was equipped with a 410 refractive index detector. Both the columns and detector were from Millipore Co. Three pullulanes (molecular weight 1 600 000, 100 000 and 5 800) were used to calibrate the system for molecular weight determination.

Calculation of crystallinity index

The ^{13}C-CP/MAS NMR-spectra of the cellulose samples were integrated over the C4 signals at 86–92 ppm (a) and 79–86 ppm (b) and the crystallinity index (CrI) was calculated as CrI = a/(a + b) according to Teeäär (Teeäär *et al.*, 1987).

Principal component analysis

Principal component analysis (PCA) (Joliffe, 1986; Wold, 1978) was used to evaluate the NMR data of the initially dry and wet MCC sets and a combination of the two sets. The intensities of 588 data points, in the range 50 to 120 ppm, for each spectrum were used in the principal component analysis. Only PCs that were judged to result from relevant changes (both chemically and statistically) in the spectra of the samples, throughout the series, were retained.

The variations in the spectral data were visualized by projection of the objects, i.e. spectra, onto the principal components, thus giving the scoreplots of the data set. The corresponding subspectra were constructed by charting the loadings (projection of spectral variables onto the PCs) versus variable name (chemical shifts as measured in ppm relative to TMS) (Lennholm *et al.*, 1994).

The PCA was performed on an IBM (486) computer using the SIMCA 4R software package obtained from Umetri AB, Box 1456, S-901 24, Umeå, Sweden.

RESULTS

^{13}C-CP/MAS NMR-spectra

Figure 1 shows the spectra recorded on dry and wet samples of MCC not milled, milled for 60 minutes and a wet sample milled for 1320 minutes. A comparison of the spectra demonstrates the spectral changes induced by the mechanical treatment during the milling operation. The apparent change upon milling is the degeneration of spectral features, producing spectra with broad and apparently less well-resolved signals.

In the spectra of the milled samples recorded either wet or dry, the signals at 87 to 91 ppm (C4 carbons) and 64 to 67 ppm (C6 carbons) assigned to ordered regions in the cellulose matrix have decreased. In the C1 region the most pronounced change is an intensity increase in the tail of the C1 cluster around 102 ppm, probably due to signals from small disordered cellulose fragments, and the appearance of a diminutive signal at 97 ppm. The 97 ppm signal corresponding to reducing ends appeared after 15 minutes of milling (Maciel *et al.*, 1982). The integration of the signal at 97 ppm in the C1 region of this spectra gave a value of about 0.1% for the reducing end content.

In the peak cluster between 70 and 77 ppm containing the C2, C3 and C5 ring carbons there are slight changes observed in the distribution of the signal intensities as a consequence of the milling. The cluster of signals between 74 and 77 ppm exhibits a relative intensity increase as compared to the cluster of signals between 74 and 70 ppm.

Compared with the dry samples of equal milling time, the corresponding spectra of wetted samples shows sharper signals (Horii *et al.*, 1985; Willis and Herring, 1987).

Figure 2 shows the spectra recorded on *Cladophora* cellulose not milled, milled for

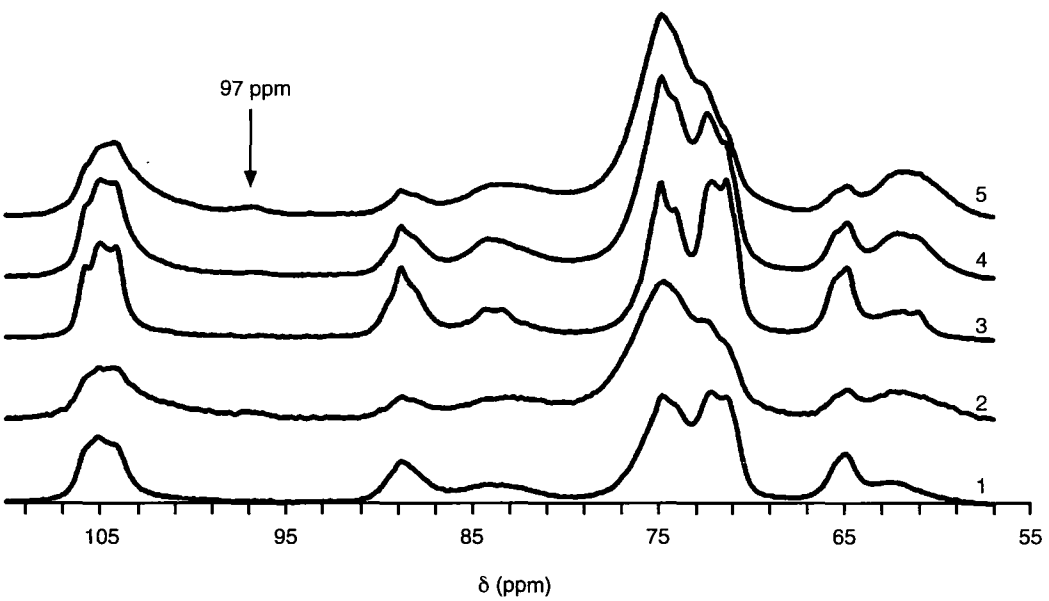

FIGURE 1. The NMR spectra of: 1, MCC recorded dry (not milled); 2, MCC recorded dry (milled 60 minutes); 3, MCC recorded wet (not milled); 4, MCC recorded wet (milled 60 minutes); 5, MCC recorded wet (milled 1320 minutes).

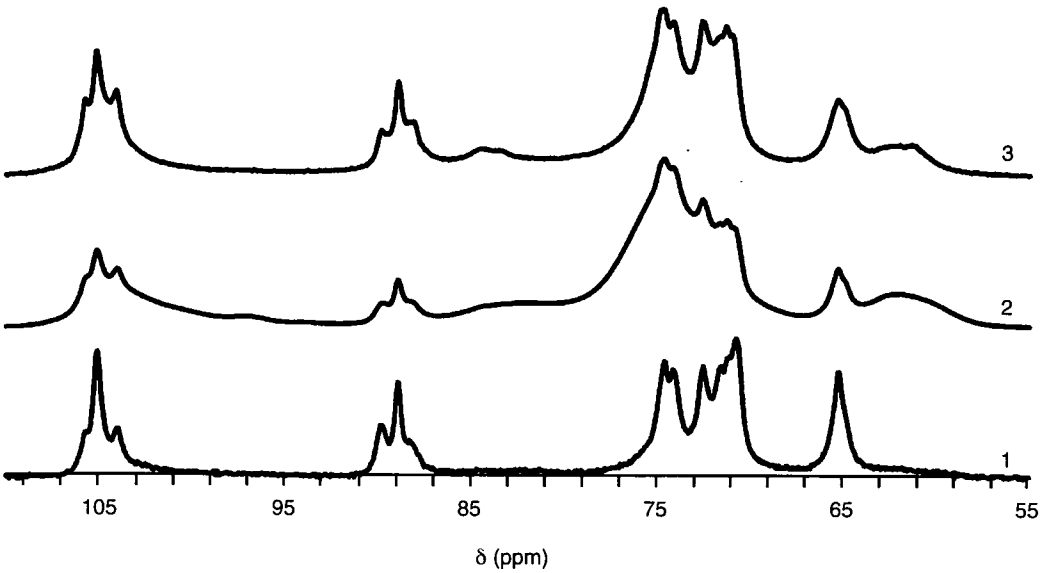

FIGURE 2. The NMR spectra of: 1, *Cladophora* cellulose recorded dry (not milled); 2, *Cladophora* cellulose recorded dry (milled 10 minutes); 3, *Cladophora* cellulose recorded wet (milled 10 minutes).

10 minutes and a wet sample milled for 10 minutes. Similar to the MCC the apparent change upon milling is a broadening of the peaks and a decrease in signal intensity in the regions assigned to ordered cellulose, likewise wetting of the milled sample results in sharper signals.

The signals in the *Cladophora* spectra are more distinct as compared with those in the MCC and allow distinction of cellulose Iα and Iβ allomorphs. Integration of the signals at 90 ppm (cellulose Iα) and 89 ppm (cellulose Iα and Iβ) in the ordered C4 region shows a relative decrease of about 25% in the cellulose Iα content due to milling. No change in allomorph composition could be observed upon wetting (or redrying) of the milled sample.

Crystallinity index

From the calculations of the crystallinity indices obtained by the integration of the C4 carbon signals (Table 1 and 2) it was evident that all the spectral sets (dry, wet, redried and rewetted) showed a decrease in crystallinity as a result of milling. Upon wetting of the initially dry set an increase in the order is observed for all the samples in the set, even for the non-milled celluloses. The subsequent redrying and rewetting seems to affect only slightly the order of the MCC samples.

Size exclusion chromatography (SEC)

The results from size exclusion chromatography of three MCC samples, not milled, milled for 30 minutes and milled for 60 minutes, showed that there was a decrease of about 40% in the degree of polymerisation as an effect of milling (Table 3).

TABLE 1. Crystallinity indices for dry, wet, redried and rewetted MCC samples as determined by NMR

Milling time (minutes)	*Dry*	*Wet*	*Redried*	*Rewetted*
0	0.55	0.60	0.58	0.60
5	0.49	0.57	0.58	0.58
10	0.48	0.55	0.55	0.58
15	0.43	0.54	0.55	0.56
20	0.45	0.53	0.53	0.55
25	0.41	0.50	0.52	0.53
30	0.40	0.49	0.52	0.54
35	0.37	0.47	0.52	0.53
40	0.33	0.46	0.48	0.52
60	0.32	0.40	0.45	0.44
1320	0.22	0.37	0.39	0.41

TABLE 2. Crystallinity indices for dry, wet and redried *Cladophora* cellulose samples as determined by NMR

Milling time (minutes)	*Dry*	*Wet*	*Redried*
0	0.80	0.88	–
10	0.39	0.60	0.55

TABLE 3. Results from SEC of three milled MCC samples

Milling time (minutes)	*Crystallinity index*[a]	*Average molecular weight* (kDA)	*Average degree of polymerization*
0	0.60	48.5	299
30	0.49	39.0	241
60	0.40	28.4	175

[a]Crystallinity index from wet samples

Principal component analysis (PCA)

PCA was performed on the spectral data of the dry and wet MCC samples and a combination of the two sets. The scoreplot of the respective PC1s and PC2s are shown in Figure 3. The first principal component, PC1, explaining 96.7, 95.5 and 90.7% respectively of the total variance from each calculation, distributed the samples according to the milling time. The PC1s were thus judged to contain information due to the effect of milling and were used for construction of subspectra.

The corresponding PC2s described only 1.98, 2.38 and 5.80% of the total variance and showed a non-linear distribution of the samples, no explanation of these PC2s was attempted.

The subspectrum, created from the loading values of PC1 from dry samples shows the variations in spectral variables between samples of different milling times (Figure 4a). The location of the positive peaks agrees well with that expected for the signals

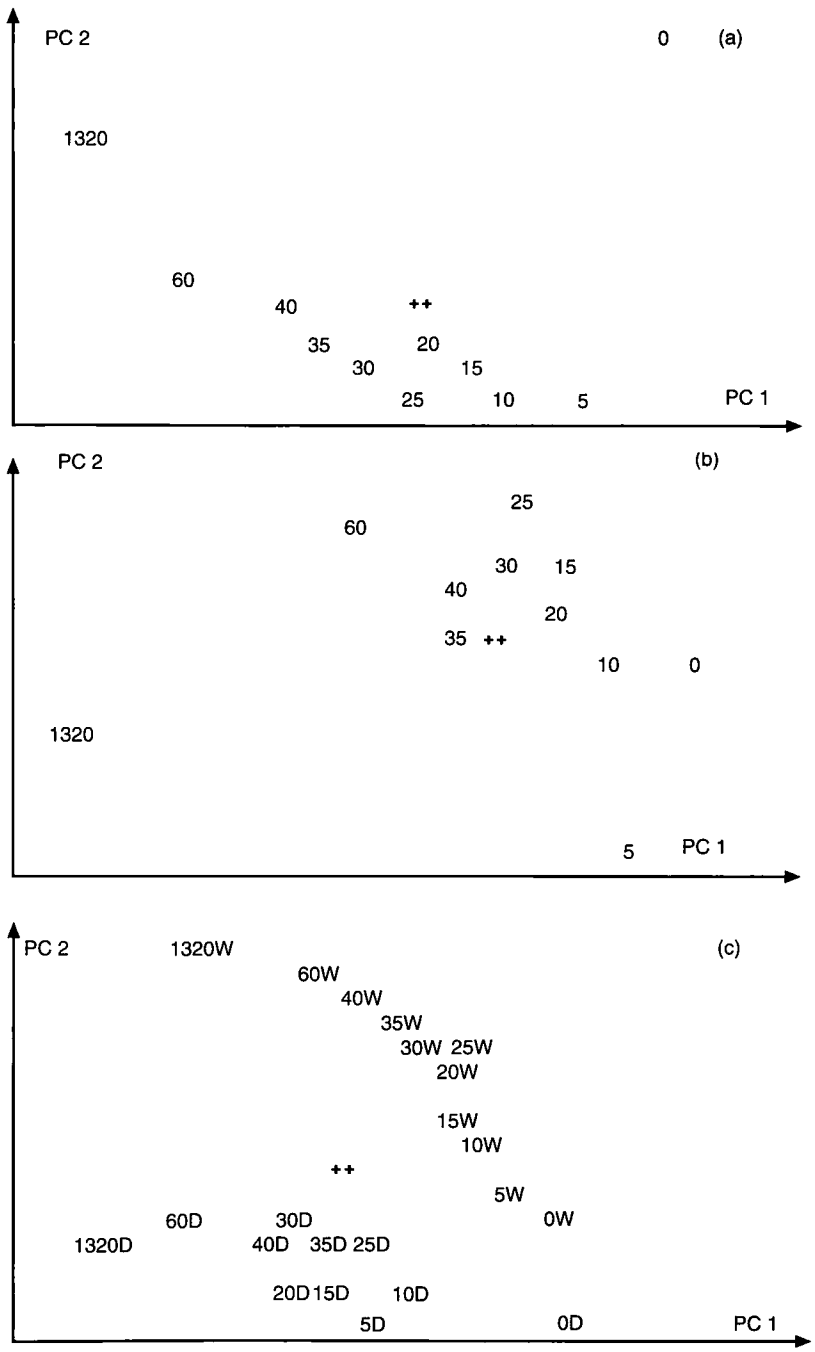

FIGURE 3. Scoreplots of the milled samples recorded dry (a), wet (b), and wet and dry (c) The direction of PC1 corresponds to an increased order within the cellulose. The notation ++ denotes the origin of coordinates and the numbers given in the figures are the milling times in minutes. In (c) milling time in minutes are followed by index W for wet samples and D for dry samples.

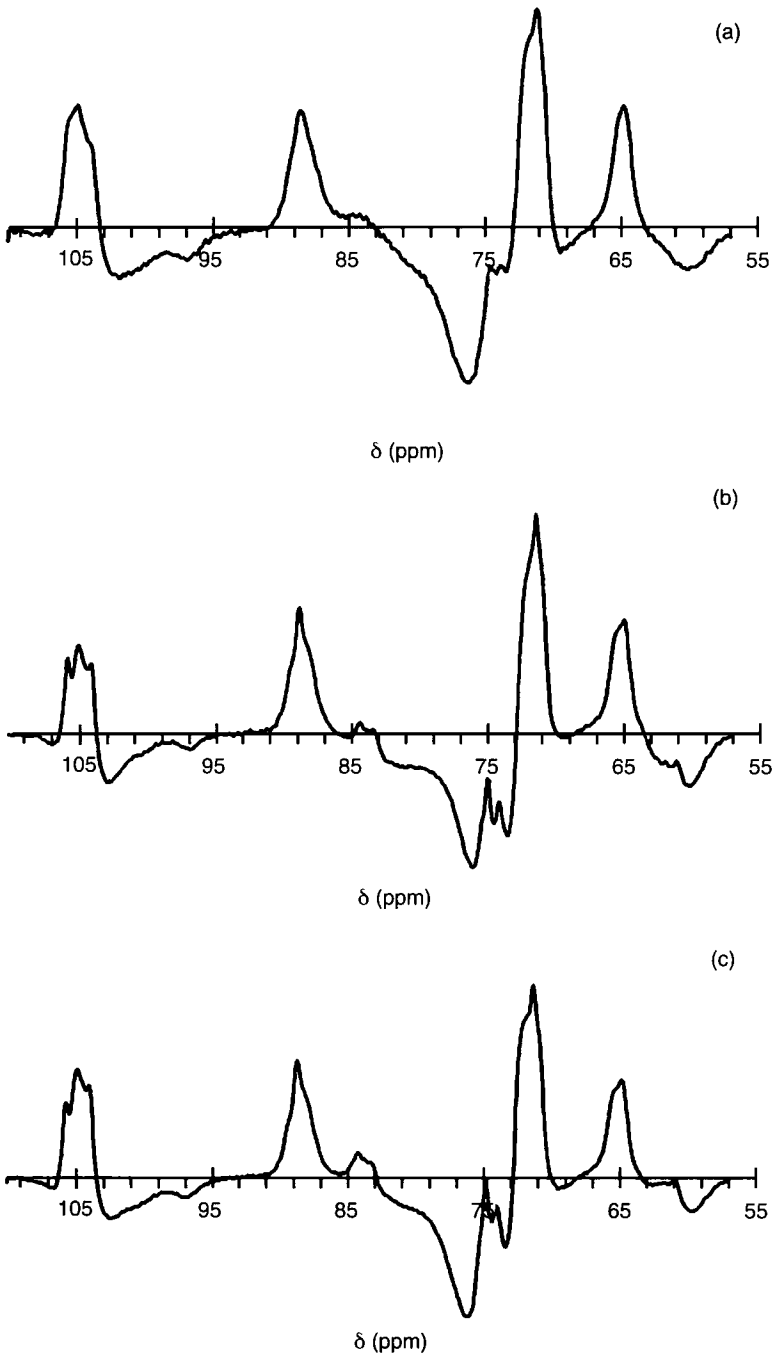

FIGURE 4. Subspectrum created from the loading values of PCA (PC1), performed on the NMR data of dry samples (a), wet samples (b), and dry and wet (c). Positive peaks correspond to signals in the NMR spectra of ordered regions and negative peaks to those of disordered signals. Intensities are in arbitrary units.

from ordered cellulose I containing both the cellulose Iα and Iβ allomorphs. The position of the negative peaks corresponds to regions in the NMR spectra reported for disordered cellulose (compare with Lennholm *et al.*, 1994).

The subspectrum created from the loading values of PC1 from wet samples shows basically the same features as the dry set, but it also indicates an increase in resolution in the spectra of the wet samples (Figure 4b). This is nicely exemplified by a well resolved cluster of small positive peaks around 84 ppm which is at a position normally assigned to disordered cellulose. The subspectrum of the combined set in Figure 4c, shows an intermediate behaviour as expected.

In all the three subspectra the region between 70 and 77 ppm corresponding to the C2, C3 and C5 ring carbons is split into two groups, a negative peak cluster between 74 and 77 ppm and a positive cluster between 70 and 74 ppm.

The scoreplot of the combined set in Figure 3c also contains information about the effect of wetting on the structure of cellulose. The two sets of samples are shifted parallel to each other along PC1. This indicates that the hydrated sample contains larger amounts of ordered cellulose I than the same samples recorded in the dry state.

DISCUSSION

The degree of crystallinity, or rather the molecular order, and the lengths and widths of ordered regions are important features of the fine structure of native cellulose fibrils. It is well known that these properties may be very susceptible to both mechanical treatments and swelling (see Krässig, 1992). When the microcrystalline cellulose (MCC) was milled, its susceptibility to mechanical treatment was observed in the ^{13}C-CP/MAS NMR-spectra as a degeneration of the spectral features due to loss of ordering in the cellulose matrix (Figure 1). Upon wetting of the initially dry samples an increase in order was recorded. As an example the crystallinity index (CrI) measured according to Teeäär *et al.* (1987) on the dry MCC samples was lowered from 0.55 in the non-milled sample to 0.33 in the sample milled for 40 minutes (Table 1). In the subsequent cycle of wetting and drying the CrI of this milled sample increased to 0.52, as compared with 0.60 for the non-milled MCC after cycling. An initial difference in CrI of 0.22 between the original dry samples was thus reduced to 0.08 after the wetting and drying cycle.

The reversibility of the disorder introduced by the mechanical treatment was further substantiated by the principal component analysis performed. The distribution of the different MCC samples along the PC1 of the combined set in Figure 3c aptly describes the disorder induced by the milling, since the samples are rated according to the milling time. The partial reordering upon wetting is observed as a shift along the PC1 towards a more ordered position of the wet samples as compared to the dry samples of the same milling time. This interpretation is confirmed by the pattern of the subspectra in Figure 4, constructed from the loading vectors of the PC1s. In these subspectra the positive peaks resemble ordered cellulose I allomorphs and the negative peaks disordered cellulose.

A notable observation was the presence of a small positive signal at about 84 ppm which was most clearly observed in the wet samples. The position of this peak or cluster of peaks agrees with the position of the C4 signals recently assigned to chains exposed on the surface of ordered regions (Earl and VanderHart, 1981; Newman, 1994;

Newman *et al.*, 1994). The fact that this cluster shows the same behaviour in the PCAs as the ordered cellulose I peaks, supports this assignment.

As seen in Figure 1 the peak cluster between 70 and 77 ppm containing the C2, C3 and C5 ring carbons showed a redistribution of the signal intensities as a consequence of the milling. The signals between 77 and 74 ppm exhibited a relative intensity increase as compared to those between 74 and 70 ppm, implicating larger contributions of ring atoms from disordered cellulose to the downfield part of the cluster. This interpretation is supported by the splitting of the same region in the subspectra of Figure 3 into a negative peak cluster between 77 and 74 ppm and a positive cluster between 74 and 70 ppm.

The signal overlap in the C1 and C4 regions in the spectra of the MCC samples is too severe to enable a direct estimation of the relative amounts of cellulose Iα and Iβ. To visualise changes in the cellulose I allomorph composition a *Cladophora* cellulose rich in cellulose Iα was investigated. As seen in Figure 2 there are considerable similarities in the behaviour of the *Cladophora* cellulose and the MCC. When the *Cladophora* cellulose was milled for 10 minutes a degeneration of the spectral features was observed. The crystallinity index, measured on the dry samples was lowered from 0.80 in the non-milled sample to 0.39 in the milled sample (Table 2). In the subsequent wetting the CrI of the milled sample increased to 0.60. Integration of the C4 signals at 90 ppm (cellulose Iα) and 89 ppm (cellulose Iα and Iβ) shows a relative decrease of about 25% in the cellulose Iα proportion due to the mechanical treatment of ball milling (VanderHart and Atalla, 1987). No change in relative allomorph composition could be observed upon wetting (or redrying) of the milled *Cladophora* cellulose.

It is clear from the above results that a large part of the disorder induced by the mechanical treatment of milling is reversible and that reordering upon hydration leads to the allomorphs initially present, i.e. cellulose Iα and cellulose Iβ. The same behaviour of ball milled celluloses has been observed in earlier investigations by X-ray diffraction (Hess *et al.*, 1941; Caulfield and Steffes, 1969; Bhama Iyer *et al.*, 1984; Liang *et al.*, 1993). This is, however, a little surprising since cellulose I is regarded as being thermodynamically less stable than cellulose II.

Our interpretation is that a large part of the disorder seen in the 'amorphous' region of the NMR-spectrum between about 79 and 86 ppm, and as seen earlier by X-ray diffraction, is the result of the formation of deformed or distorted zones in the cellulose fibrils. These distorted domains are stabilized in the dry samples by mechanical interlocking or hydrogen bonding to other fibrils. Upon wetting of the otherwise largely intact cellulose fibrils with water, the distortions in the cellulose chains are released (see Hatakeyama *et al.*, 1987; Ek *et al.*, 1995). The release is not complete but this could be the result of the presence of some fully amorphous cellulose or of a lower accessibility of some regions in the fibrils to water, since the hydration in this study was conducted in a gentle way, i.e. addition of water without either agitation or addition of swelling chemicals.

It is also interesting that no conversion of disordered cellulose to cellulose II could be detected in the PCA or by visual inspection of the spectra after cycling, even in the most extensively milled sample (compare with Lennholm and Iversen, 1995b). This is probably due to the relatively 'mild' milling used in this investigation as shown by the limited decrease, about 40%, in the degree of polymerization according to the SEC analysis and the presence only of diminutive amounts of reducing end groups as

observed in the NMR-spectra. The fibril structure seems to be largely intact since, at most, only small amounts of fully disordered (i.e. amorphous) cellulose were formed (compare with Hess *et al.*, 1941; Howsman and Marchessault, 1959; Wadehra and Manley, 1965).

The above observations clearly exemplify the complex behaviour of cellulose fibres as a result of mechanical treatment and swelling. The results obtained also agree well with a model of ordered cellulose fibrils mainly containing disordered cellulose as surface layers and induced reversible lattice distortions or crystal defects (Chanzy, 1990).

CONCLUSIONS

A large part of the disorder induced by mechanical treatment of cellulose by ball milling is reversible and reordering upon hydration leads to the cellulose I allomorphs initially present.

The C4 signals corresponding to the reversible disordered cellulose chains are observed in the 'amorphous region' between 79 to 86 ppm in the ^{13}C-CP/MAS NMR-spectra together with signals from chains on the surface of ordered regions around 84 ppm.

The peak cluster between 70 and 77 ppm containing the ring carbons can be divided into a downfield part at 74 to 77 ppm and an upfield part at 70 to 74 ppm containing larger contributions from disordered and ordered cellulose I respectively.

The behaviour of the investigated celluloses upon milling is in accordance with a concept of ordered cellulose fibrils containing disordered cellulose mainly as surface layers and induced reversible lattice distortion.

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