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Separation, characterization and hydrogel-formation of hemicellulose from aspen wood

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Abstract

Hemicellulose from aspen (*Populus tremula*) was isolated by an alkali extraction method, which was followed by hydrogen peroxide treatment, ultrafiltration and recovery by spray drying. The sugar composition and lignin content were monitored with HPLC at each step of the separation procedure. Size-exclusion chromatography showed a polymeric hemicellulose of relatively high molar mass. The product was characterized by ^{1}H and ^{13}C NMR spectroscopy and was found to be composed of a linear (1 → 4)-β-linked D-xylose main chain with a 4-*O*-methyl-α-D-glucuronic acid substituting the 2-position of approximately every eighth xylose unit. Lignin and *O*-acetyl groups had largely been removed in the separation process. The xylan was soluble in hot water, and the film forming properties were examined at various mixtures of the hemicellulose and chitosan. These films formed hydrogels with a high swelling capacity at certain compositions. The morphologies of the films were examined with wide angle X-ray spectroscopy, and a pure xylan film was found to be crystalline, which was suggested to be a consequence of the lack of *O*-acetyl groups. The crystallinity of the films was found to decrease with an increasing amount of chitosan, and the film of chitosan alone showed no crystallinity. The cohesive forces of the hydrogels are suggested to be the result of the crystalline arrangement of the polymers and of electrostatic interactions between acidic groups in the hemicellulose and amine groups in the chitosan.

Keywords: Hemicellulose; Chitosan; Xylan

1. Introduction

The majority of plant materials are constructed from three major polymeric components: cellulose, hemicellulose and lignin. The term "hemicellulose" is used for polysaccharides that normally occur in plant tissues together with cellulose, and which can be isolated by extraction either with water or, more frequently, aqueous alkali (Aspinall, 1959; Timell, 1964). The main hemicellulose of hardwood is *O*-acetylated 4-*O*-methyl glucuronic acid xylan (Aspinall, 1959; Erins, Cinite, Jakobsons & Gravitis, 1976; Sjöström, 1981; Timell, 1964). The xylan is estimated to account for one third of all renewable organic carbon available on earth (Prade, 1996). The hemicelluloses are located primarily in the secondary cell walls, and together with cellulose and lignin, build up the plants in a fashion that gives the best combination of mechanical support and transport properties (Fengel, 1971; Sjöström, 1981). With a growing environmental concern, interest has evoked among material scientists in hemicelluloses as renewable raw materials for novel products. Since hemicelluloses are, in their native form, responsible for the interactions with water, hydrogel applications are evident choices (Erins et al., 1976; Fengel, 1971).

Owing to the sophisticated structure of plants and the integration of the structural polymers at a level approaching that of individual macromolecules, there are many difficulties involved with the separation of the different constituents into discrete fractions. During pulping processes, the purpose of which is to separate cellulose fibers from solid wood, hemicelluloses are completely or partially degraded together with the lignin matrix, depending on the pulping process used (Hansson & Hartler, 1969; Kleppe, 1970; Roberts & El-Karim, 1983; Vuorinen, 1995). This is the chief reason why the material properties of the polymeric hemicelluloses have not yet been exploited. Among separation methods other than those conventionally used in the pulp and paper industry, several different extraction methods have shown promising results for the recovery of hemicelluloses (Aspinall, 1959; Erins et al., 1976; Fengel, 1971; Koshijima, Yaku & Tanaka, 1976; Lindberg, Rosell &

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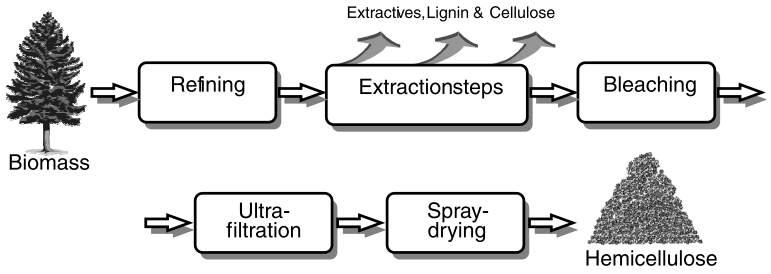

Fig. 1. The process used for hemicellulose recovery.

Svensson, 1973; Mora, Pla & Gandini, 1989; Timell, 1960; Timell, 1964). In some cases, the hemicellulose can be extracted directly from wood but, in most cases, the wood is first delignified to holocellulose and the hemicellulose is then extracted in one or several steps. However, an advantage of direct extraction is that the chemical modification of the hemicellulose is minimized. Different hemicelluloses exhibit different solubility properties. This forces us to use specially designed separation processes depending on the particular wood we have. It also gives us the possibility to tailor-make the extraction process to yield a certain hemicellulose or to fractionate a certain mixture of hemicelluloses from wood.

In the present study, hemicellulose was separated from aspen wood using an alkali extraction process combined with ultrafiltration according to a method developed by Glasser et al. (Glasser, Jain & Sjöstedt, 1995). The sugar composition and lignin content were monitored at each step of the separation, and the hemicellulose product was characterized with regard to molar mass, sugar composition and amount of acidic groups. The film forming properties were investigated, and the films were then converted into hydrogels by dissolving them together with chitosan in acidic conditions. Both the mechanism of gel formation and the swelling properties were investigated.

2. Experimental

2.1. Recovery of hemicellulose from aspen wood

Hemicellulose was isolated from aspen wood (*Populus tremula*) using alkali extraction combined with ultrafiltration (Glasser, Kaar, Jain & Sealey, manuscript). Initially, the wood was disk refined using an 8-inch Sprout-Waldron refiner. The fiber suspension was then treated with 0.05 M hydrochloric acid at 70°C for 2 h. The fibers are then swollen, and the pectins will be soluble in ammonium hydroxide (pH 9.2), which was added after the suspension had been cooled. The suspension was stirred overnight and centrifuged to extract and remove pectins, starch and fat. (Andersson, 1935–1936) The residue was treated with 1% sodium hydroxide in 70% ethanol at 75–80°C for 2 h to solubilize lignin. After centrifugation, hemicellulose was extracted with 4% sodium hydroxide in two subsequent steps. In the first step, the suspension was kept at room temperature under constant agitation for 16 h and, in the second step, the temperature was raised to 70°C before centrifugation. The two filtrates were combined and peroxide bleached at pH 7 in the presence of Na-EDTA to minimize the residual lignin content. The suspension was finally ultrafiltered with 3000 NMWCO membrane cartridges at pH 7 and spray dried to yield the hemicellulose product. A schematic representation of the recovery process is shown in Fig. 1.

2.2. Characterization

2.2.1. Sugar, lignin and ash content during separation

The sugar composition was monitored with a High-Performance Liquid Chromatography (HPLC) method (Kaar, Cool, Merriman & Brink, 1991) at each step of the separation procedure. The method involves hydrolysis with sulfuric acid before the analysis. It follows the TAPPI Standard T249 with the exception that the acid hydrolysis was performed in sealed vessels to avoid the loss of volatile constituents that would otherwise evaporate. The lignin content was determined gravimetrically and spectrophotometrically in the usual manner (Kaar & Brink, 1991a,b), and the ash content was determined by thermogravimetric analysis, 700°C, 10°C/min.

2.2.2. Molar mass determination of the product

The molar mass of the isolated hemicellulose was examined with Size Exclusion Chromatography (SEC) after it was acetoxypropylated to yield a THF soluble derivative. The modification starts with the reaction of hemicellulose with propylene oxide in sodium hydroxide solution (pH 12.5) and is followed by precipitation in acetone. The isolated hydroxypropyl xylan was acylated with acetic anhydride in formamide solution (Jain, Sjöstedt & Glasser, manuscript). The SEC data were obtained using a high pressure liquid chromatography system utilizing a series of three Ultrastyragel columns (1000, 10,000 and 1,000,000 A), a differential refractometer and a Viscotek model 100 differential viscometer. The system was calibrated with polystyrene standards using a universal calibration-broad curve (TriSEC GPC Software Version 3, Viscotek) and run with

Table 1
Composition of material obtained during separation process

Sample	Sugar composition (%)						Lignin (%)			Ash content (%)
	Glc	Xyl	Gal	Ara	Man	Total sugar	Klason	Acid soluble	Total lignin	
Aspen chips	41.1	16.7	0	0	3.9	61.7	19.3	2.9	22.2	0
Refined aspen chips	40.4	16.1	0	0	3.0	59.5	19.7	2.8	22.5	0.2
Ammonium hydroxide extracted aspen	41.3	16.4	0	0	2.9	60.6	18.7	2.8	21.5	0
Alcoholic-alkali extracted aspen	45.5	16.8	0	0	2.3	64.6	16.0	2.6	18.6	11.0
Cold alkali extracted aspen	52.3	8.3	0	0	1.9	62.5	16.8	2.7	19.5	26.4
Hot alkali extracted aspen	57.9	7.3	0	0	1.7	66.9	19.3	2.5	21.8	0.5
Bleached and recovered hemicellulose	0	65.2	0	0	0	65.2	4.3	2.1	6.4	2.6

non-stabilized tetrahydofuran as the mobile phase. A flow rate of 1.0 ml/min was used.

2.2.3. NMR spectroscopy of the product

^{1}H and ^{13}C NMR experiments were performed on samples in D_2O solutions (3 and 10 mg/ml, respectively) at 85°C, using a Bruker DRX 400 instrument. ^{1}H and ^{13}C chemical shifts are reported in ppm, using acetone (δ_H 2.225 and δ_C 31.0) as the internal reference. Two-dimensional (COSY, HMQC and HMBC) experiments were performed according to standard pulse sequences available in the Bruker software.

2.3. Material properties

2.3.1. Solubility, film formation and swelling

The aspen hemicellulose was examined with regard to water solubility, film forming properties and hydrogel forming properties. When the films and hydrogels were prepared, the hemicellulose was mixed with various amounts of chitosan (Fluka, Product No: 22743). The hydrogels were obtained by immersing the films in water and were studied with regard to degree of swelling. In the first step, hemicellulose was mixed in dry form with chitosan in amounts ranging from 0 to 100% in steps of 5%. The mixtures were then stirred into water that was acidified with hydrochloric acid to about pH 1.7. The amount of dry matter was 1.5% by weight. The solutions were heated to 95°C and kept at this temperature for 20 min for the complete dissolution of the polymers. They were then cooled to room temperature and cast onto polystyrene dishes, and the film forming properties were monitored as the solvent evaporated. Swelling measurements were performed by placing small pieces (ca. 1.5×1.5 cm^2) of films in glass dishes of known weight. Deionized water (25 ml) was added, left for 30 min and then carefully removed, leaving the sample on the dish. The dish was wiped dry with a tissue and weighed. The swelling ratio (S) was defined as: (Weight of hydrated gel − Weight of dry gel)/(Weight of dry gel). The results were plotted as mean averages of at least five samples with 95% confidence intervals.

2.3.2. Morphology

Wide-angle X-ray spectroscopy (WAXS) was used to characterize the morphology of the films. The films were milled into a fine powder under liquid nitrogen and kept at ambient conditions for two weeks. A Siemens D5000 diffractometer was used in the reflection geometry, and diffractograms were taken between $2\theta = 5°$ and $2\theta = 30°$ with a step size of 0.1°. The relative crystallinity was calculated by dividing the area of the diffraction peak at $2\theta = 17 - 21°$ by the total area under this peak (Hermans & Weidinger, 1948). In the same manner, samples of the hemicellulose together with chitosan were examined. Mixtures of hemicellulose and chitosan at the ratios 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1 were examined.

3. Results

3.1. Characterization

3.1.1. Sugar, lignin and ash content during separation

The sugar composition was monitored with HPLC during the separation of the hemicellulose, and the results are presented in Table 1. This table also gives the lignin content determined gravimetrically and spectrophotometrically and the ash content determined by thermogravimetric analysis (700°C, 10°/min). No significant amounts of sugars are liberated by the refining step, the purpose of which is only to make the wood susceptible to the subsequent treatments. The only exception is the low content of mannose, which declines further at every step in the procedure. The amount of mannose in the starting material corresponds well with the amounts of glucomannan that are usually found in hardwood (Sjöström, 1981). The refined wood was further extracted with mild hydrochloric acid and subsequently with ammonium hydroxide (pH 9.2) to remove any pectinous matter (Andersson, 1935–1936). The compositional analysis (Table 1) indicates that about 3% weight loss occurs in lignin and almost no loss of hemicellulose during this step. The amount of lignin declines from 18.7 to 16.0% upon alcoholic-alkali extraction, a somewhat unexpected

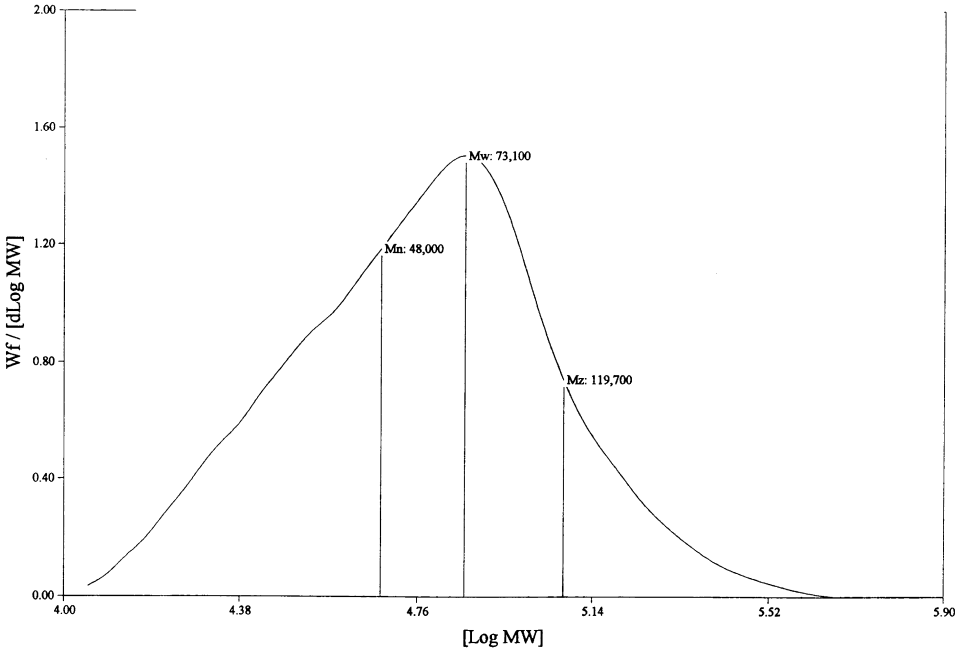

Fig. 2. The size exclusion chromatogram of acetoxypropyl modified aspen hemicellulose.

low reduction. The remaining lignin is, however, removed to a satisfactory low level at the bleaching step. Eventually, the lignin was degraded by the alcoholic-alkali treatment but only to near the limit of what is required for being solubilized in this step. During the alkali treatment of delignified wood, approximately 55% of the hemicellulose is extracted from the wood with the cold sodium hydroxide process and about 8% with the hot sodium hydroxide process. The value was calculated after normalization of sugar composition with the residue yield obtained in steps (data not shown in Table 1). The recovered and bleached hemicellulose was shown to contain xylose and glucuronic acids (6.7%) and is hence a xylan. The isolated aspen hemicellulose contained minor amounts of lignin and ash, and almost no arabinan and galactan. There was no detection of arabinan as would be expected in hardwood 4-*O*-methyl xylans. Furthermore, it is difficult to isolate lignin-free xylan from hardwood unless harsh treatment is applied in the delignification of biomass during the isolation of hemicellulose. The harsh treatment would result in a low yield of hemicellulose due to the solubilization of hemicellulose in these conditions.

3.1.2. Molar mass of the product

The molar mass of the hemicellulose was examined with SEC after conversion to acetoxypropyl xylan, which is soluble in THF. The chromatogram is shown in Fig. 2. The analysis shows that a polymeric hemicellulose product of relatively high molar mass and quite narrow molar mass distribution had been achieved with the separation procedure. The weight average molar mass was 73,100 g/mol, and the number average molar mass 48,000 g/mol. This corresponds to a polydispersity index of 1.5. The alkali treatment was consequently efficient enough to extract the hemicellulose but lenient enough not to degrade the hemicellulose significantly. The use of ultrafiltration probably contributed to the rather narrow molar mass distribution.

3.1.3. NMR spectroscopic characterization of the product

The ^{1}H and ^{13}C NMR spectra (Fig. 3) showed signals of one major and two minor components. The signals were assigned to the three components by two-dimensional COSY and HMQC experiments (Table 2). The ^{1}H and ^{13}C chemical shifts and the three-bond coupling constants showed that the major component was a 4-substituted β-D-Xyl*p* residue and the two minor components were 2,4-disubstituted β-D-Xyl*p* residues and non-reducing terminal 4-*O*-methyl-α-D-Glc*p*A groups. These identifications were also supported by similar NMR data observed previously for different xylans (Andersson, Hoffman, Nahar & Scholander, 1990; Teleman et al., 1995, 1996). The linkage positions were determined by an HMBC experiment which showed that the *O*-methyl group was substituted to the 4-position of the α-D-Glc*p*A group, that this sugar was linked to the 2-position of the disubstituted β-D-Xyl*p* residue and that the β-D-Xyl*p* residues formed a linear 1,4-linked polymer. The data support a xylan structure substituted with 4-*O*-methyl-α-D-Glc*p*A groups. The spectra were compared with those obtained for a commercial xylan also containing other acidic groups such as unsaturated hex-4-enopyranuronic acid. There were no signals for these components in the NMR spectra of the aspen hemicellulose.

The degree of branching could be determined from the relative intensities of the signals for the anomeric protons of

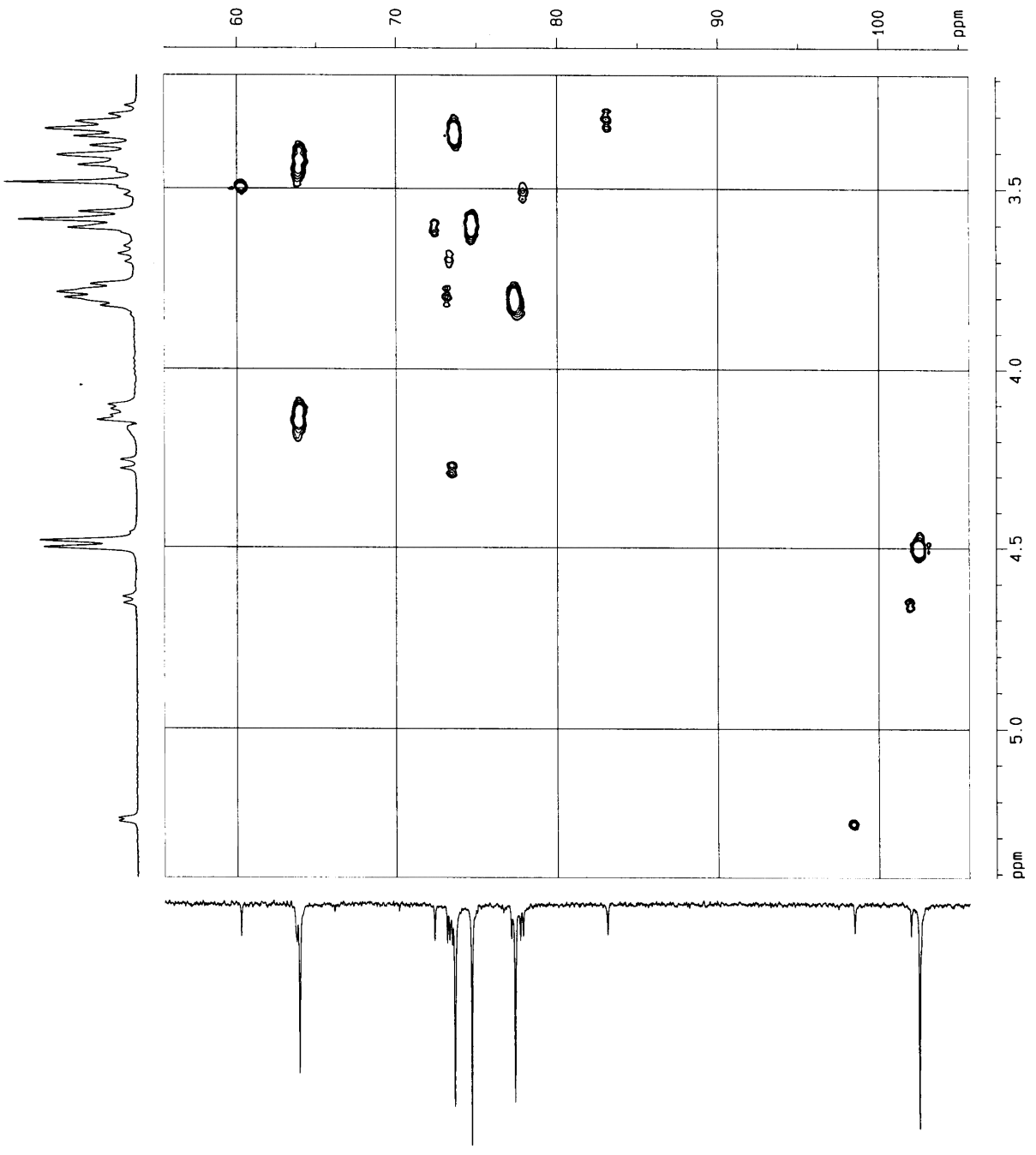

Fig. 3. The ^{1}H, ^{13}C and HMQC spectra of the aspen hemicellulose.

Table 2
^{1}H and ^{13}C NMR data obtained at 85°C for the aspen hemicellulose

Residue	Chemical shifts (δ, ppm)						
	H-1 C-1	H-2 C-2	H-3 C-3	H-4 C-4	H-5a,5b C-5	C-6	OCH_3
→ 4)-β-D-Xyl*p*-(1 →	4.50 102.5	3.34 73.6	3.60 74.7	3.81 77.4	4.13, 3.42 63.8		
→ 2,4)-β-D-Xyl*p*-(1 →	4.66 101.9	3.51 77.9	3.69 73.3	3.84 77.7	4.15, 3.44 63.6		
α-D-Glc*p*A-(1 →	5.26 98.3	3.61 72.4	3.80 73.1	3.30 83.1	4.28 73.4	176.9	3.49 60.1

the → 4)-β-D-Xyl*p* -(1 → residue (δ 4.50) and those of the 4-*O*-methyl-α-D-Glc*p*A groups (δ 5.26) or the 2,4-di-substituted β-D-Xyl*p* (δ 4.66) residues. The relative proportion was 100:14, indicating that 12% of the chain residues were branched in the following structure:

$$[\rightarrow 4)\text{-}\beta\text{-D-Xyl}p\text{-}(1]_7 \rightarrow 4)\text{-}\beta\text{-D-Xyl}p\text{-}(1\rightarrow$$
$$\begin{matrix} 2 \\ \uparrow \\ 1 \end{matrix}$$
$$\text{4-O-Me-}\alpha\text{-D-Glc}p\text{A}$$

3.2. Material properties

3.2.1. Solubility and film formation

The solubility and film forming properties of the xylan were examined. Xylan proved to be sparingly soluble in cold water but soluble in hot water. The solubility of the hemicellulose is probably affected by the separation procedure owing to deacetylation. On the one hand, acetyl groups can reduce the solubility of xylan in water, but on the other hand, prevent the alignment and formation of hydrogen bonds between xylan molecules which would make the xylan inaccessible to water (Timell, 1964). The solutions were cooled to room temperature and cast onto polystyrene dishes. The film forming properties were monitored upon drying of the solution and were found to be rather poor for the pure xylan. Instead of films, film fragments with a size of about 1×1 mm^2 were formed. The film forming properties were also tested with mixtures of the xylan with various amounts of chitosan. At as little as 5% chitosan and above, the solutions started to form films upon drying. At 10% and above, continuous, self-supporting films were achieved. The films with high xylan/chitosan ratio were quite brittle, probably because of the lower molar mass of xylan. In the following text, all samples prepared with this method are denoted as films, regardless of their size or quality.

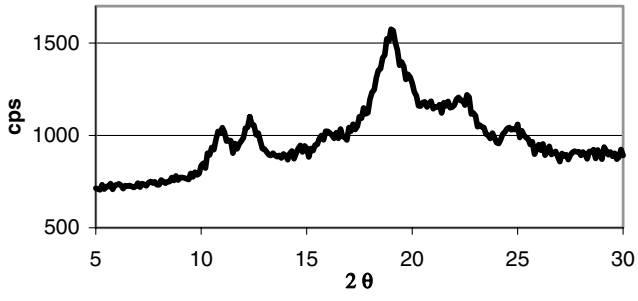

Fig. 4. The WAXS diffractogram of aspen hemicellulose film.

3.2.2. Morphology

The films composed of high amounts of xylan were found to be quite brittle. As the hemicellulose is a linear xylan it would have the potential for close packing of the polymer chains. WAXS was used for the determination of the morphology. A milled film of xylan was examined, and the diffractogram was recorded at 5–30°(2θ), as shown in Fig. 4. The relative crystallinity was calculated from the area under the peak from $2\theta = 17°$ to $2\theta = 21°$ and was found to be 0.37. The ability of the hemicellulose to crystallize is probably affected by the alkali extraction process.

Acetyl groups that are present in the native xylan prevent the xylan from crystallizing. These acetyl groups are removed in the extraction process, which leaves a xylan with a higher ability to crystallize (Marchessault, Settineri & Winter, 1967). We also recorded WAXS diffractograms of milled films of xylan with 25, 50, 75 100% chitosan. The relative crystallinities of these samples were found to decrease with an increasing amount of chitosan, and the film of pure chitosan had virtually no crystallinity (Fig. 5).

The straight line connects the relative crystallinity of a pure xylan film (0.37) with that of a pure chitosan film (0). A slight tendency can be seen that admixing of chitosan does not lower the crystallinities of the xylan films to the same extent as would be predicted from weighted averages of the pure xylan and the pure chitosan films. The same tendency has been observed earlier in films from chitosan together with xylan from birch (Gabrielii & Gatenholm, 1998b). This indicates that the chitosan may promote an increase in the degree of order of xylan in the films. It may also be possible that xylan and chitosan are able to cocrystallize. The peaks in the diffractograms are not shifted, however, and the

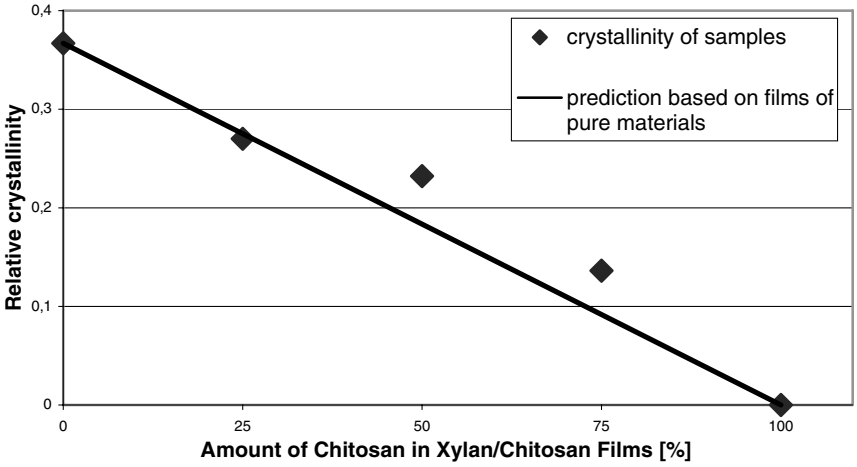

Fig. 5. The relative crystallinities of films of various mixtures of aspen xylan and chitosan.

criterion is then that the lattice distance is the same as for pure xylan.

3.2.3. Swelling of hydrogels

All films were investigated with regard to water solubility and water uptake. As mentioned above, the solution of pure xylan did not form films, but the fragments achieved did not appear to absorb significant amounts of water. As the films were prepared with chitosan in increasing concentrations, they absorbed water. Films with a chitosan content below 20% swelled in water and formed hydrogels. Fig. 6 shows the effect of chitosan content on the swelling ratio (S), as defined in Section 2. The swelling ratio was recorded after 30 min of exposure to water, which was found to be sufficient to reach equilibrium swelling. The degree of swelling depended strongly on the chitosan content. The presence of up to 15% chitosan does not significantly affect the degree of swelling. The addition of 15–20% chitosan results in a considerable increase in water uptake. It should be noted that, at 20% chitosan, a larger scattering of the swelling measurements was obtained. Above 20% chitosan, the samples started gradually to dissolve during the swelling measurements. Pure chitosan films were readily dissolved in the water since the chitosan in the films is in its ionized form. The larger scattering when measuring the swelling of films with 20% chitosan is probably a result of partial dissolution. A model for the morphology of the hydrogels is that the xylan forms crystallites and that the chitosan, with its higher molar mass, ties the crystallites together by cocrystallization with the xylan. This phase separation between xylan and chitosan has also been indicated by dynamic mechanical analysis and atomic force microscopy in a previous work (Gabrielii & Gatenholm, 1998a). The cohesive forces of the hydrogels may, therefore, partly be due to the crystalline arrangement of the polymers and, it should not be forgotten, to

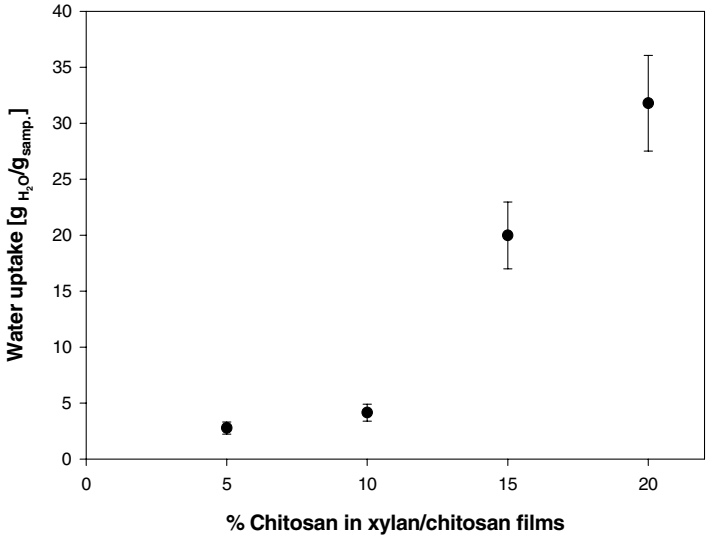

Fig. 6. The water uptake vs. xylan/chitosan composition in mixed films.

electrostatic interactions between acidic groups in the xylan and amine groups in the chitosan also discussed in the same previous work.

4. Conclusions

A polymeric hemicellulose of relatively high molar mass and narrow molar mass distribution was extracted from aspen wood with an alkali extraction method combined with ultrafiltration. The hemicellulose was constructed of a linear (1 → 4)-β-linked D-xylose main chain with a 4-*O*-methyl-α-D-glucuronic acid substituting the 2-position of approximately every eighth xylose unit. No acetyl groups and only small amounts of lignin remained in the hemicellulose product. The hemicellulose was sparingly soluble in cold water but soluble in hot water. Solutions of the hemicellulose did not exhibit good film forming properties. When mixed with chitosan, however, films were formed at compositions of 5% chitosan and above. The morphologies of these films were examined with WAXS, and a pure xylan film proved to be crystalline. The ability to crystallize was probably increased by the alkali extraction process used. The crystallinities were found to decrease with an increasing amount of chitosan, and the film of pure chitosan had virtually no crystallinity. Films of mixtures of xylan with chitosan displayed slightly higher degrees of crystallinities than would be predicted from the weighted averages of the pure xylan and the pure chitosan films. When immersed in water, films with 5–20% chitosan formed hydrogels, and the degree of swelling of the hydrogels was shown to increase as the films contained more chitosan. Films with more than 20% chitosan dissolved in water. The film and hydrogel forming properties were attributed to crystalline domains of xylan tied together with the chitosan chains, as well as to electrostatic interactions between the acidic groups in the hemicellulose and the amino groups in the chitosan.

Acknowledgements

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