Macromol. Symp. **2006**, 232, 49–56 DOI: 10.1002/masy.200551406

Cationic Xylan Derivatives with High Degree of Functionalization

Katrin Schwikal,[1] *Thomas Heinze,**[1] *Anna Ebringerová,*[2] *Katrin Petzold*[1]

Summary: Xylan from birch wood was characterized regarding both the supramolecular structure (X-ray, CP/MAS ^{13}C-NMR) and the sugar composition. The reaction of the birch wood xylan with 2,3-epoxypropyltrimethylammonium chloride in 1,2-dimethoxyethane as slurry medium yields water-soluble, cationic 2-hydroxypropyltrimethylammonium xylan derivatives with high degree of substitution (DS). The DS values up to 1.6 can be controlled by adjusting the molar ratio in a one step synthesis. The structure of the cationic xylan derivatives was confirmed by means of DEPT(135) NMR spectroscopy. Film forming properties of cationic xylan derivatives were investigated with SEM measurements.

Keywords: cationic polyelectrolyte; film forming; 2-hydroxypropyl-trimethylammonium xylan; NMR spectroscopy; SEM; xylan

Introduction

In recent years, research on modified polysaccharides is focused on new functional polymers for applications in various fields. As renewable resources, they are not only unlimited available but also they have a great structural diversity. How to profit from naturally designed polymers could be shown on cellulose as a well-investigated biopolymer.[1,2]

In contrast, the chemical modification of hemicelluloses[3] is not well established up to now, even though they are 25–30% of the biomass in wood and until 50% in different annual plants. The main components in wood are xylans and glucomannans. Xylans of higher plants possess β-(1 $\rightarrow$ 4) linked xylopyranose units as the backbone, usually substituted with sugar units and *O*-acetyl groups.[4] Regarding chemically modified products, the cationic 2-hydroxypropyltrimethylammonium (HPMA) xylan is well studied.[5–7] The synthesis was carried out in alkaline medium with 3-chloro-2-hydroxypropyltrimethylammonium chloride. Dependent on the molar ratio of xylan to reagent and of reagent to NaOH, derivatives with different degree of substitution (DS) up to 0.98 were obtained.[8] Products with a DS from 0.03 to 0.25 possess microbiological activity.[9]

The present paper describes the synthesis of HPMA xylans with different DS using the 2,3-epoxypropyltrimethylammonium chloride (EPTA). To optimize the synthesis procedure, the xylan used was well characterized regarding the supramolecular structure (X-ray, CP/MAS ^{13}C-NMR) and the sugar composition. Elemental analysis (EA) and DEPT(135) NMR spectroscopy were used to characterize the HPMA xylan. To study structure property relationships, the solubility of the derivatives in water was investigated by turbidity measurements and film forming properties were studied. The films resulted were characterized by means of SEM measurements.

---

[1] Centre of Excellence for Polysaccharide Research, Friedrich Schiller University of Jena, Humboldtstraße 10, 07743 Jena, Germany

[2] Center of Excellence of the Slovak Academy of Sciences, Institute of Chemistry, Dúbravská cesta 9, 84538 Bratislava, Slovakia

Experimental

Materials

Xylan from birch wood was purchased from Roth (Karlsruhe, Germany) and was bleached with ClO_2. The solution of 70% EPTA in water (QUAB®151) was kindly supplied by Degussa. The 1,2-dimethoxyethane (DME) was purchased from Merck.

Measurements

EA was carried out by means of a Vario EL III (Elementar Analysensyteme Hanau, Germany).

The DS of HPMA xylan was calculated from the ratio of the nitrogen to the carbon content according to the equation: $DS_N = (60 \cdot \%\ N)/(14 \cdot \%\ C - 72 \cdot \%\ N)$.

CP/MAS ^{13}C NMR spectra were recorded using a Bruker AMX 400 spectrometer operating at 100.58 MHz with a 4 mm MAS double resonance probe and ZrO_2 rotors. The sample spinning frequency was 6.5 kHz, the repetition time was 2 s and the cross polarization time was 1 ms. Furthermore, two-pulse phase modulation (TPPM: $\pm 10°$, 4 μs) has been applied for proton decoupling.

GC analysis of the alditol trifluoracetates of neutral sugars, obtained after total hydrolysis of the polysaccharide using 2M trifluoracetic acid[10], was carried out by means of a Hawlett-Packard 5890 Series II chromatograph equipped with a PAS-1701 column (0.32 mm × 25 m), 110–125 °C ($2\,°C \cdot min^{-1}$) to 165 °C ($20\,°C \cdot min^{-1}$), flow rate of hydrogen: 20 ml min^{-1}.

The DEPT(135) spectra were recorded with a Bruker AVANCE 400 NMR spectrometer at 60 °C in D_2O.

Turbidity measurements were carried out with a Turbiquant®3000 IR from Merck, which was calibrated with an AMCO Clear®calibration standard kit.

The X-ray powder diffraction was recorded on a Bruker D8 Advance diffractometer using CuK_α radiation. The generator system operated at 40 kV.

SEM-investigation of Films from HPMA Xylan

The wet membranes (see below) were dried by using a critical point dryer CPD030 (BAL-TEC, Balzers, Liechtenstein), mounted on stubs for SEM and were subsequently coated with gold using a sputter coating device BAL-TEC-SCD005. The measurements were carried out with a LEO-1450 VP (LEO, Oberkochen, Germany) with a Cryo-transfer BAL-TEC-VCT 100. An EHT potential of 15.00 kV was applied. The micrographs were taken at a magnitude of 5000 X and working distance of 9–10 mm.

Preparation of HPMA Xylan

In a typical synthesis, 5 g of xylan (0.038 mole anhydroxylose units (AXU)) suspended in 17.5 ml H_2O was heated under reflux for 15 min. The mixture was cooled to room temperature and a solution of 0.9 g (0.023 mole) NaOH in 5 ml water was added. After 30 min at room temperature, 35 ml DME are added to the dissolved xylan to yield a heterogeneous reaction mixture. To the reaction mixture 24.6 g of a 70% solution of QUAB®151, representing 0.114 mole EPTA was added dropwise. The reaction mixture was stirred for 24 h at room temperature. After neutralization with 1 N HCl, the product was precipitated with 200 ml ethanol, dialyzed (M_W cut-off $= 3500\ g \cdot mol^{-1}$) in water and lyophilized.

Yield: 5.38 g (51.4%), DS = 0.96 (**2**) (determined by means of EA: C = 38.53%; H = 8.31%; N = 4.01%; Cl = 10.33%).

Film Preparation

A solution of 0.35 g HPMA xylan in 0.93 ml H_2O was cast with a doctor on a glass plate with constant velocity. A film of 100 μm was obtained. This film was immediately treated with ethanol for 2 min and stored in ethanol.

Results and Discussion

Sugar Composition of the Birch Xylan

Xylans may possess different sugar side chains.[4] The xylan used in our studies was isolated from birch wood. Sugar analysis revealed xylose as the main neutral sugar component (93.9%) and the presence of low amounts of arabinose (3.6%) and rhamnose (2.5%), originating very probably from co-extracted, contaminating polysaccharides. The xylan contains also 9.7% uronic acid and 1.48% ash based on the air dried substance. These data indicate the xylan to be a very pure 4-*O*-methylglucuronoxylan, what was supported by the FT-IR spectrum (not shown) with a spectral pattern typical of this xylan type.[11]

Morphology of the Birch Xylan used

If the xylan was treated in hot water before used, different authors described a improved solubility of xylan (4-O-methylglucuronoxylan from aspen) in aqueous NaOH[12] and showed also a higher reactivity[8] for different xylan types during the reaction with 3-chloro-2-hydroxypropyltrimethylammonium chloride. Horio and Imamura[13] show a decreasing crystallinity, if the xylan membrane from beech was treated at 100 °C, immersed the xylan in hot glycerol for 2 h. The X-ray diffraction pattern of the birch wood xylan before (D) and after different treatments (A–C) is shown in Figure 1.

The starting xylan is partially crystalline and shows reflexes, which are characteristic for xylan dihydrate, as deduced from the X-ray data earlier reported by Marchessault and Nieduszyuski.[14] The treatments of the xylan by refluxing in H_2O (B), dissolving the xylan in 4% aqueous NaOH and precipitation (C) or by refluxing in H_2O, dissolving in 4% aqueous NaOH and precipitation (A) result in samples possessing an amorphous diffraction pattern.

Solid state NMR spectroscopy (Figure 2) was applied to verify the results from X-ray diffraction measurements. The signals of the ^{13}C NMR spectra were assigned according to Teleman et al.[15] The signals δ 102.5, 77.5 - 72.0, and 63.4 ppm were assigned to C1–C5 of the 4-linked β-xylopyranosyl residue. The spectra of xylan both refluxed in H_2O (B) and refluxed in H_2O and dissolved in 4% NaOH (A) exhibit broadened signals in contrast to the spectrum of raw xylan (D). This is a hint for a decreasing crystallinity of the samples (A) and (B).

Synthesis of the HPMA Xylan

The synthesis of HPMA xylan was carried out heterogeneously in DME. Based on previous results, method (A) was used to activate the xylan, i.e. increase the accessibility of the polymer for further chemical modification with EPTA (Figure 3).

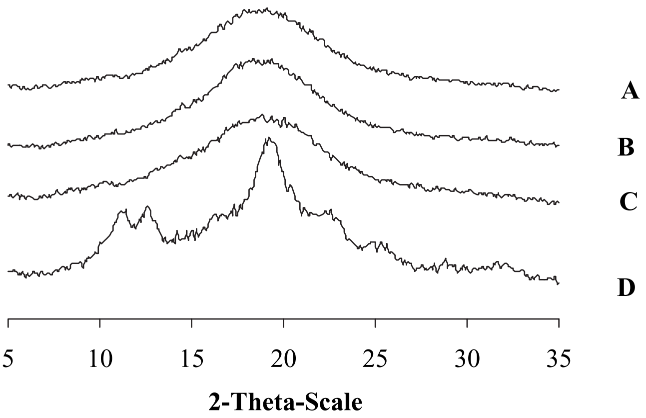


Figure 1.
X-ray diffraction pattern of xylan refluxed 15 min in H_2O, dissolved in 4% NaOH, precipitated with 1,2-dimethoxyethane (DME) and washed neutral with 80% ethanol (A); xylan refluxed 15 min in H_2O (B); xylan dissolved in 4% NaOH, precipitated with DME and washed neutral with 80% ethanol (C); xylan from birch wood (D). Sample B was lyophilized. Samples A, C and D were dried under vacuum at 60 °C.

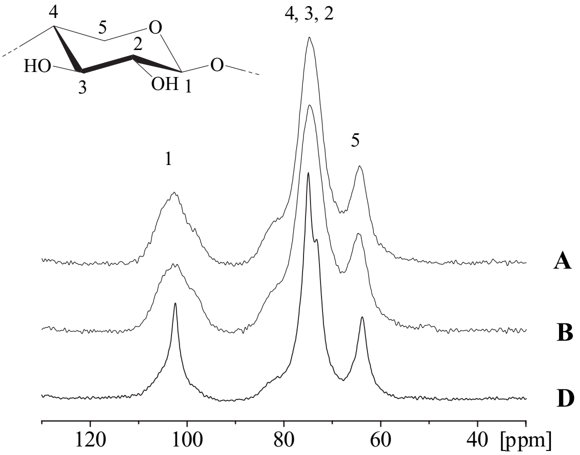
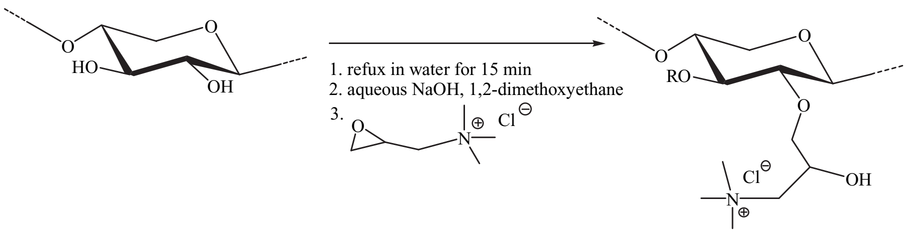


Figure 2.
CP/MAS ^{13}C NMR spectra of birch wood xylan (D), xylan reflux for 15 min in H_2O (B) and xylan refluxed for 15 min in H_2O, treated with 4% NaOH and precipitated (A).

Different factors affect the DS, namely the NaOH concentration, the reaction temperature and the molar ratio of the cationization agent per anhydroxylose unit. Figure 4 illustrates the effect of the concentration of NaOH on the resulting DS. Under comparable reaction conditions (molar ratio EPTA:AXU = 3:1; ratio DME:H_2O = 1.5:1, v/v), the 4% concentration of NaOH (0.59 mole/mole AXU) in the reaction mixture is adequate to give a high DS of 0.96 (**2**). At a higher amount of added NaOH (0.89 mole/mole AXU), resulting in a 6% concentration of the aqueous NaOH solution, the DS of the product (**3**) was only slightly higher (1.00).

A further increase of the NaOH concentration to 10% using 1.49 mole/mole AXU leads to a decrease of the resulting DS to 0.61 (**4**). This can be explained as a consequence of increasing hydrolysis of EPTA to the corresponding diol. The hydrolysis of the reagent should be as low as possible, on one hand. On the other, a certain concentration of aqueous NaOH is necessary to activate the xylan. A very low DS (0.56, (**1**)) was obtained also at 2% NaOH concentration (0.3 mole/mole AXU). This indicates an un-sufficient activation of the hydroxyl groups. Therefore, an aqueous NaOH with a concentration of 4% is appropriate to give the highest DS.

After refluxing the biopolymer in water and dissolving in aqueous NaOH (4%), the xylan was precipitated with DME. Subsequently, EPTA was added with molar ratio of AXU to EPTA of 1:3 applying different reaction temperatures. The effect of reac-



R= H; $CH_2CHOHCH_2N(CH_3)_3{}^+Cl^-$

Figure 3.
Reaction scheme of the cationization of xylan with 2,3-epoxypropyltrimethylammonium chloride (EPTA).

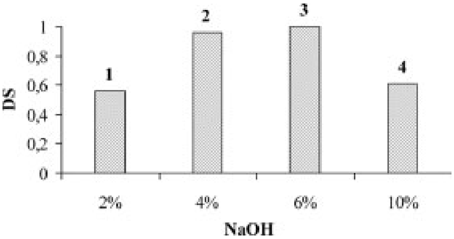


Figure 4.
The effect of the NaOH concentration on the degree of substitution (DS) of 2-hydroxypropyltrimethylammonium xylan, molar ratio 2,3-epoxypropyltrimethylammonium chloride:anhydroxylose unit = 3:1; molar ratio 1,2-dimethoxy-ethane:H_2O = 1.5:1.

tion temperature on the DS of HPMA xylan obtained depending on reaction time is shown in Table 1.

The reaction at 24 °C leads to a DS of 0.96 (**2**) after 24 hours. The prolongation of the reaction time to 48 h does not yield higher DS. At 40 °C, a product of comparable DS of 0.96 (**14**) was obtained (24 h). At higher temperature (60 °C) a lower DS value appear, which may result from the fast hydrolysis of the reagent. However, already after 1 h a DS of HPMA xylan as high as 0.72 (**16**) was reached.

Table 1.
Effect of reaction temperature on the degree of substitution (DS) of 2-hydroxypropyltrimethylammonium (HPMA) xylan depending on reaction time with molar ratio of anhydroxylose unit to 2,3-epoxypropyltrimethylammonium chloride (EPTA) 1:3.

Temperature [°C]	Time [h]	HPMA xylan	
		No.	DS
24	1	**5**	0.17
24	2	**6**	0.34
24	4	**7**	0.50
24	6	**8**	0.56
24	24	**2**	0.96
24	48	**9**	0.94
40	1	**10**	0.51
40	2	**11**	0.68
40	4	**12**	0.85
40	6	**13**	0.86
40	24	**14**	0.96
40	48	**15**	0.91
60	1	**16**	0.72
60	2	**17**	0.71
60	4	**18**	0.70
60	6	**19**	0.69

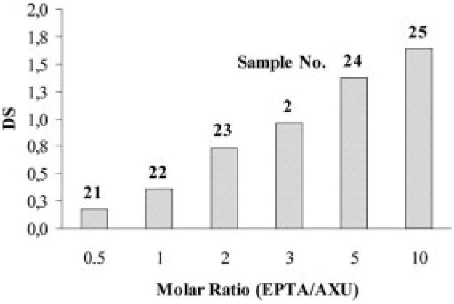


Figure 5.
Degree of substitution (DS) of 2-hydroxypropyltrimethylammonium (HPMA) xylan in dependence on the molar ratio of 2,3-epoxypropyltrimethylammonium chloride (EPTA) to anhydroxylose unit (AXU) at constant reaction conditions: 4% NaOH, 24 h, 24 °C).

The DS can be controlled by adjusting the molar ratio of cationization reagent to AXU (Figure 5).

The reactions were carried out with 4% aqueous NaOH for 24 h at 24 °C. With a molar ratio of 0.5 mole/mole AXU, a DS of 0.17 (**21**) was obtained. The DS values increase with increasing molar ratio, as expected. At a molar ratio of 10 mole/mole AXU the DS of the sample is 1.64 (**25**) yielding a product, that is highly hygroscopic.

NMR Spectroscopy

DEPT(135) spectra of HPMA xylan in D_2O were measured to characterize the substitution pattern using previously reported data.[16] The DEPT(135) spectra of samples 22 and 25 are given in Figure 6. As known, peaks of the CH_2-groups at positions 6, 8 and 5 shows negative intensities and can easily be distinguished from the CH moieties. The signals 8 and 7 of the 2-hydroxypropyltrimethylammonium chloride function were determined at δ 68.7 and 65.7 ppm. The two signals (6) observed at δ 74.9 and 71.7 ppm might result from the substitution of the hydroxyl group at position 2 and 3 of the xylan residue. The three methyl groups of the ammonium moieties show an intensive signal at δ 55.0 ppm. The C-1 signal of the 4-linked AXU appeared for an unmodified position 2 and/or 3 at δ 102.2 ppm (1) and for the

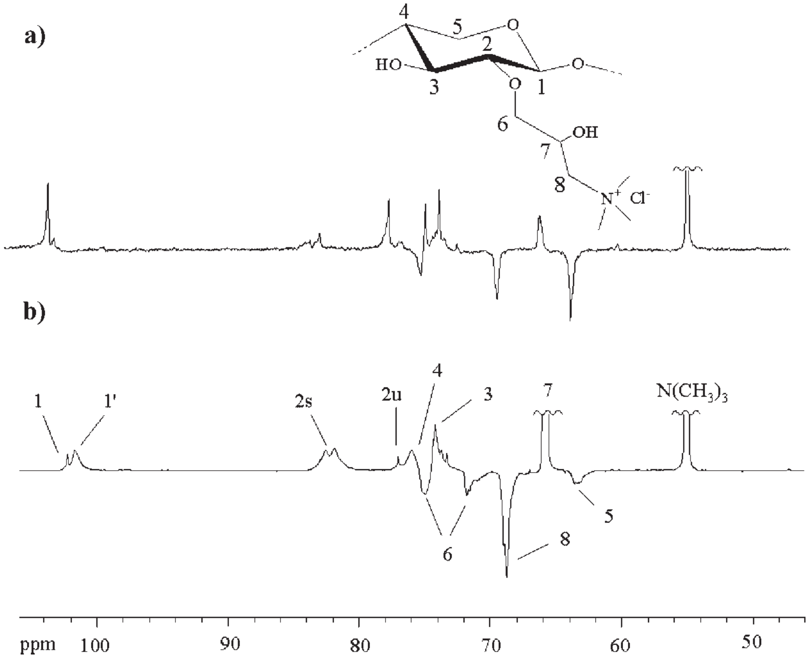


Figure 6.
DEPT(135) NMR spectra of 2-hydroxypropyltrimethylammonium (HPMA) xylans with different degree of substitution (DS) in D_2O, a) DS = 0,36 (**22**) and b) DS = 1,64 (**25**), s means substituted, u means unsubstituted in position 2, ′ means influenced by substitution at position 2.

modified position 2 and/or 3 and at δ 101.7 ppm (1′). The remaining carbons of the AXU can be assigned as follows: the signal for a substituted position 2 shifted to δ 82.1 ppm, for an unsubstituted position 2, the signal can be identified at δ 77.0 ppm. The peak area of 2s increases with increasing DS, as can be clearly seen in Figure 6. The signals of C-4 and C-3 can be detected at δ 76.0 and 73.7 ppm. At δ 64.2 ppm, the negative signal for the CH_2-group corresponds to C-5.

Solubility of HPMA Xylan

The turbidity of 1% HPMA xylan in water was determined to characterize the solubility. A decreasing Nephelometric turbidity unit (NTU) with increasing DS was observed (Table 2). The polymer solutions show no change of NTU within 10 days. The solutions are optically clear (observed by naked eye) up to a NTU of 100.

Film Forming Properties

The cationic xylan derivatives form films from aqueous solution, which can be stored in ethanol. SEM measurements show a dependence of the morphology of the films on thickness (generated by the height of the doctor) and on the concentration of the HMPA xylan solution used (Figure 7).

Table 2.
Nephelometric turbidity units (NTU) of a 1% aqueous solution of 2-hydroxypropyltrimethylammonium (HPMA) xylan dependent on the degree of substitution (DS).

HPMA xylan		NTU
No.	DS	
21	0.17	254
22	0.36	171
23	0.73	53
2	0.96	13
24	1.38	7
25	1.64	6

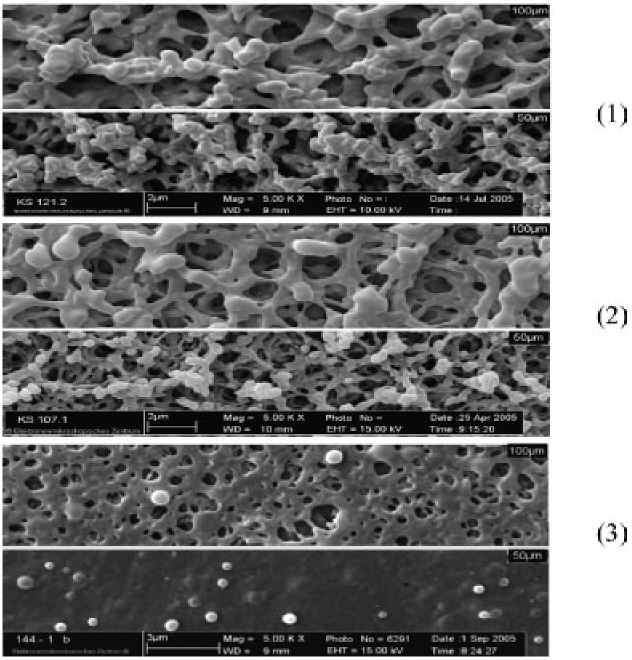


Figure 7.
SEM pictures of 2-hydroxypropyltrimethylammonium (HPMA) xylan films (degree of substitution = 0.36) with different thickness from 50 μm (lower pictures), 100 μm (upper pictures). The films were made from (1) 18.5% (w/w), (2) 27.3% (w/w) and (3) 33.3% (w/w) polymer solution.

Remarkably, the films with a thickness of 100 μm possess, independent of the concentration of the original solution, bigger pores than the films with a thickness of 50 μm. Films formed from a 18.5% (w/w) solution possess pores in the range from 250 to 500 nm (50 μm) and at 100 μm the pore size ranges between 500 and 1200 nm. The pores of the films from the 27.3% (w/w) solution exhibit a size of 300–1000 nm (50 μm) and 600–1500 nm (100 μm). No pores can be observed for the film obtained from the 33.3% (w/w) solution with a thickness of 50 μm. The film of 100 μm thickness has a pores size of 100–650 nm. Summarizing, the film morphology can be tailored by the concentration of the solution and the thickness.

Conclusion

HPMA xylans were prepared easily by reaction of the polysaccharide with EPTA in water/DME slurry, which allows a low NaOH content (4 – 6%) in the reaction medium resulting in a simple purification of the products as a main advantage. Before the reaction an activation of the xylan by refluxing in water is appropriate. An explanation, why refluxing the xylan in H_2O before dissolving in NaOH results a HPMA xylan with a higher DS than without refluxing, can not be given, yet. The DS of the HPMA xylans prepared at different reaction temperatures and different reaction times shows an optimum for reaction time vs. DS at 40 °C. Under the optimized conditions, cationic xylan derivatives with high DS up to 1.64 can be synthesized. According to the uronic acid side chains of the xylan used, the resulting HPMA xylans have a polyionic structure with a permanent positive charged ammonium group and a pH sensitive charged carboxyl groups. The products starting with a DS of 0.36 are water soluble. Films can be formed with controlled pore size. Currently, the microbiological activities of HMPA xylans with high DS are investigated.

Acknowledgements: The authors thank the Bundesministerium für Wirtschaft und Arbeit (Deutsche Gesellschaft für Holzforschung, project number AiF 13698 BR) for the generous financial support. We thank Dr. J. Puls and co-workers (Federal Research Centre of Forestry and Forest Products, Hamburg, Germany) for bleaching of the xylan. Furthermore, we thank Dr. Th. Schulze (Thuringian Institute for Textile and Plastics Research, Rudolstadt, Germany) for X-ray measurements. The Slovak Academy of Sciences, COST action D29 WG No. 0008-03 is acknowledged for financial support.

[1] Th. Heinze, in: "*Structural Diversity and Functional Versatility*", 2nd edition, S. Dimitriu, Ed., Marcel Dekker, New York, Basel, Hong Kong, 2004, 551ff.
[2] Th. Heinze, T. Liebert, *Progr. Polym. Sci.* **2001**, *26*, 1689.
[3] Th. Heinze, A. Koschella, A. Ebringerová, in: "*Hemicelluloses: Science and Technologie*", ACS Symposium Series No. 864, 2004, 312ff.
[4] A. Ebringerová, T. Heinze, *Macromol. Rapid Commun.* **2000**, *21*, 542.
[5] M. Antal, A. Ebringerová, I. Simkovic, *J. Appl. Polym. Sci.* **1984a**, *29*, 637.
[6] M. Antal, A. Ebringerová, I. Simkovic, *J. Appl. Polym. Sci.* **1984b**, *29*, 643.
[7] A. Ebringerová, M. Antal, I. Simkovic, *J. Appl. Polym. Sci.* **1986**, *31*, 303.
[8] A. Ebringerová, Z. Hormádková, M. Kačurácová, M. Antal, *Carbohydr. Polym.* **1994**, *24*, 301.
[9] A. Ebringerová, A. Belicova, L. Ebringer, *World J. Microbiology & Biotechnology* **1994**, *10*, 640.
[10] A. Ebringerová, A. Kardósová, Z. Hormádková, V. Hríbalová, *Fitoterapia*, **2003**, *74*, 52.
[11] M. Kačuráková, P. Capek, V. Sasinková, N. Wellner, A. Ebringerová, *Carbohydr. Polym.* **2000**, *43*, 195.
[12] M. Gustavsson, M. Bengtsson, P. Gatenholm, W. Glasser, A. Teleman, O. Dahlman, in: "*Biorelated Polymers: Sustainable Polymer Science and Technology*", Chiellini *et al.*, Eds., Kluwer Academic/Plenum Puplishers, 2001, 41ff.
[13] M. Horio, R. Imamura, *J. Polym. Sci. (Part A)* **1964**, 2, 627.
[14] I. Nieduszyuski, R. Marchessault, *Biopolymers* **1972**, *11*, 1335.
[15] A. Teleman, P. T. Larsson, T. Iversen, *Cellulose* **2001**, *8*, 209.
[16] A. Ebringerová, Z. Hormádková, *Angew. Macromol. Chem.* **1996**, *242*, 97.