Carbohydrate Polymers 86 (2011) 214–218

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Xylan sulphate films

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ARTICLE INFO

Article history:
Received 12 March 2011
Received in revised form 12 April 2011
Accepted 14 April 2011
Available online 21 April 2011

Keywords:
Xylan
Film
Composite
SEM
NMR
Materials

ABSTRACT

Xylan sulphate derivatives with degree of substitution (DS) up to 1.40 at 90% yield for film applications were prepared. The structure and substitution were confirmed by 2D NMR spectroscopy and elemental analysis. The highest DS was obtained by sulphation with sulphur trioxide pyridine complex in N,N-dimethylformamide (DMF) when the least degradative conditions were used. The samples at high DS did not form a compact film. The best mechanical properties of xylan sulphate film were achieved on the partially water soluble derivate with DS 0.07 when sulphated in dimethylsulfoxide (DMSO). It was observed that partially soluble films with low DS and high molar mass of soluble component exhibited better mechanical properties than films with higher DS and low molar mass. This could be explained by adhesion of insoluble components with solubilized xylan sulphate. This was supported also by the shapes of images of samples observed by scanning electron microscopy (SEM). The thermal and thermo-oxidative properties of xylan sulphate were not as good as for films from unmodified xylan, hydroxypropylsulfonated (HPSX) or hydroxypropyltrimethylammonium (HPAX) xylan studied previously. The best mechanical properties obtained on xylan sulphate were superior in comparison to those of HPSX or HPAX.

1. Introduction

Composites based on polysaccharide films could replace materials from plastics due to environmental safety, biodegradability and sustainability of used sources (Grondahl, Eriksson, & Gatenholm, 2004; Saxena, Elder, Pan, & Ragauskas, 2009; Šimkovic, 2008; Šimkovic, Gedeon, Uhliariková, Mendichi, & Kirschnerová, 2011). Xylan sulphate, as possible analog of heparin was prepared by different methods under either heterogeneous or homogeneous conditions in DMF or N_2O_4–DMF (Chaidedgumjorn et al., 2002; Philipp, Nehles, Wagenknecht, & Schnabelrauch, 1987). It was also applied for treatment of osteoporosis as hyaluronan replacement (Cullis-Hill, 2003). This polysaccharide derivate might be also helpful for inflammatory action for dermatological applications or as a component of composite. In the present paper we ascribe the film properties of xylan sulphate using NMR, SEC–MALLS, SEM, TG/DTA, and mechanical testing method.

2. Experimental

2.1. Materials

Xylan (X) was extracted from beech sawdust holocellulose with 5% NaOH analogically as ascribed for hornbeam wood (Ebringerová, Kramár, & Domanský, 1969) and contained 19.3% of carboxyls and 2.93% of methoxyls. The neutral portion formed on hydrolysis contained 98% of xylose. The water-soluble part of xylan (WX; 6% yield; M_w 9.275 kg/mol; D 3.8) was analyzed using 0.1 M acetate buffer at pH 4.5 as SEC mobile phase (35% recovered mass; for more details on SEC–MALLS see Section 2.3). Sulphur trioxide pyridine complex ($Py{\cdot}SO_3$, Aldrich) and all other chemicals used were of commercial grade and were not further purified.

2.2. Sulphation

Xylan (0.15 g; 1 mmol) was mixed with 38.72 ml of DMF (500 mmol) and stirred (500 RPM) for 20 h at room temperature (RT) and subsequently 4.7748 g of $Py{\cdot}SO_3$ (30 mmol) was added and reacted for 6 h at 60 °C. The sample was precipitated into 96% ethanol saturated with sodium acetate and the precipitate dialyzed (1 kDa MWCO) and lyophilized to yield 0.2804 g of XS (S, 13.76;

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doi:10.1016/j.carbpol.2011.04.034

Table 1
NMR data of D_2O soluble samples [ppm].

Sample	C_1/H_1	C_2/H_2	C_3/H_3	C_4/H_4	C_5/H_5	C_6/H_6	OCH_3
XS/MeGlcAs	–	–	–	–	–	–	61.28/3.49[a]
XS/Xyls	101.16/5.16	73.70/4.47	73.21/4.79	75.10/3.97	59.14/3.85[b]	–	–
XS*/MeGlcAs	98.97/5.32	72.55/3.61	73.54/3.79	83.82/3.26	73.31/4.36	178.37/–	61.19/3.50
XS*/Xyls	100.39/5.19	74.33/4.50	73.89/4.82	75.15/4.00	60.15/4.44[c]	–	–
XS″/MeGlcA	102.66/4.66	77.65/3.47	74.33/3.66	77.58/3.85	64.12/4.18	–	–
XS″/Xyl	102.97/4.51	73.98/3.32	74.93/3.59	77.58/3.82	64.27/4.14[d]		

Key: XS – xylan sulphated in N,N-dimethylformamide; XS* – xylan sulphated in dimethyl sulphoxide; XS″ – xylan sulphated in pyridine; MeGlcAs – sulfated 4-O-methyl-D-glucuronic unit; Xyls – sulfated xylose unit; MeGlcA – non-substituted 4-O-methyl-D-glucuronic unit.

[a] Methoxyls; Xyl – non-substituted xylose unit.

[b] CH_2 with H_{5eq} at 4.43 and CH_2 with H_{5ax} at 3.85 ppm; Xyl′ – xylose substituted with MeGlcA.

[c] CH_2 with H_{5eq} at 4.44 and CH_2 with H_{5ax} at 3.88 ppm.

[d] CH_2 with H_{5eq} at 4.14 and CH_2 with H_{5ax} at 3.42 ppm.

C, 15.68; H, 2.20). The DS {[sampes sulphur content (%)/sulphur content of fully substituted anhydroxylose (%)] × 2} was 1.40. The yield (90%) was calculated from the amount of sample obtained in relation to theoretical yield at determined DS. When treated as above at X/DMF/Py·SO_3 = 10/500/30 ratio at 40 °C for 8 h and 16 h at RT and after precipitation and dialysis the sample was preconcentrated with vacuum evaporator and after filtration poured on Petri dish and dried to constant weight (XS′, 0.3140 g; S, 1.96; C, 31.87; H, 5.46; DS, 0.2; 16% yield).

When reaction was run in pyridine (Py) at X/Py/Py·SO_3 = 25/567/50 for 7 days/RT, it was stopped by dilution with water and the pH attenuated from 5.75 to 8.00 with NaOH, dialyzed, filtered, and lyophilized. In this way 0.8892 g of water soluble (S, 1.13; DS, 0.12; 20% yield; XS″) and 3.2404 g of insoluble (S, 0.34; DS, 0.04; 76% yield; IXS) material was obtained.

By the treatment in DMSO when X was mixed with DMSO and stored at 5 °C overnight and subsequently sulphating agent was added at X/DMSO/Py·SO_3 = 2.5/500/30 and after 6 h/60 °C stirring, dialysis, and lyophylization XS* (0.4180 g; S, 10.57; DS, 1.11; 60% yield) was obtained. At X/DMSO/Py·SO_3 = 1/1000/30 and stirred for 14 days/RT, dialysis, and lyophylization XS‴ (0.1090 g; S, 3.83; C, 34.17; H, 5.69; DS, 0.50; 56% yield) was prepared. When X/DMSO/Py·SO_3 = 10/1000/30 was stirred at RT/24 h and treated as above, than water-insoluble product (XSI; 1.4346 g; S, 0.78%; DS, 0.07) was obtained.

2.3. Analytical methods

The elemental analyses of all the samples were performed in duplicate using a Fisons EA-1108 instrument. The water solubility of X was determined as described before (Šimkovic, Synytsya, Uhliariková, & Čopíková, 2009).

NMR measurements were performed in D_2O at 25 °C on Varian 600 MHz UNITY INOVA spectrometer equipped with 5 mm 1H {13C, 15N} PFG Triple Res IDTG600-5 probe head. ^1H and ^{13}C chemical shifts are referred to 3-(trimethylsilyl)-propionic acid (TSP) internal standard. Multiplicity edited ^1H–^{13}C HSQC spectra were recorded in phase-sensitive pure absorption mode with optimization on one bond coupling constant $^1J_{CH}$ = 165 Hz. ^1H–^1H COSY spectra with gradient selection and ^1H–^{13}C HMBC spectra were measured in absolute intensity mode. The spectral widths employed in 2D NMR experiments were typically 6000 Hz (^1H) and 20,000 Hz (^{13}C), respectively.

SEC–MALLS characterization was performed using 0.1 M acetate buffer at pH 4.5 as SEC mobile phase (two TSK PW columns; G4000 and G3000; 35 °C; 0.8 ml/min; dn/dc = 0.146 g/ml). The rest of instrument setting was published previously (Šimkovic et al., 2009).

The SEM analysis was performed as described previously (Šimkovic et al., 2011).

The mechanical properties of films conditioned at 50 ± 2% humidity and 23 ± 1 °C (24 h) were determined on a universal tensile strength tester INSTRON 1122, Instron Ltd., High Wycombe, Buckinghamshire, England. The jaws distance used was 25 mm at film width of 10 mm. At least five replicated measurements were run for each sample. The thickness of films measured when using micrometer was within 10 μm accuracy. Thermogravimetric measurements were run on SDT 2960 (TGA-DTA module; TA Instruments) thermobalance at 90 cm^3/min nitrogen or air flow and 10 K/min heating rate from 20 to 1000 °C using 3–24 mg sample amounts in platinum pan.

2.4. Film preparation

Samples (0.1–2 g) were mixed with 5–30 ml of deionized water, heated up to boiling point, filtered and introduced on the surface of plastic Petri dish (8.5 cm in diameter) to dry out at RT. They were separated from the plastic surface during the drying process without further assistance. When less material than 0.1 g was introduced the films remained bonded on the plastic surface. When more material was used the films were thicker and mechanically more stable. In some cases as listed above the produced derivative was directly dried on Petri dish instead of lyophylization. Films were prepared also by repeating dissolving of the broken pieces of films after mechanical testing without observable effect on the results. Some samples (XS, XS*, IXS) broke into small peaces after drying even at several grams amount and could not be used for mechanical testing.

3. Results and discussion

3.1. Sulphation of (4-O-methyl-D-glucurono)-D-xylan

By sulphation of X in DMF with Py·SO_3 at 1/500/30 ratio a water soluble polysaccharide was prepared (XS) in 90% yield with DS, 1.40. According to ^{13}C-NMR spectrum there were only five signals of xylose (Xyl) units and methoxyl signal related to 4-O-methyl-D-glucuronic acid (MeGlcA). According to COSY, TOCSY, and HSQC experiments the corresponding signals were assigned as listed in Table 1. The ^1H-NMR spectrum was identical with values assignment by Chaidedgumjorn et al. (2002). The rest of signals of MeGlcA could not be assigned due to low intensity of the corresponding signals. We assume that during the sulphation the MeGlcA units were eliminated. When the sample was sulfated in DMSO at X/DMSO/Py·SO_3 = 2.5/500/30 under identical conditions as XS slightly lower yield (60%) and DS (1.11) were obtained for XS*. The NMR data were identical with XS sample. By treatment in pyridine at X/Py/Py·SO_3 = 25/567/50 for 7 days/RT lower DS (0.12) and yield (20%) were obtained for XS″. Additionally also water-insoluble part (DS, 0.04; 76% yield; IXS) material was obtained. The NMR data of

Table 2
SEC–MALLS characterization of samples.

Sample	M_p [kg/mol]	M_w [kg/mol]	M_w/M_n	Recovered mass [%]
WX	390	9.3	3.8	35.0
XS	26.6	22.3	1.2	35.1
XS′	91.6	593.8	20.0	86.1
XS″	82.0	65.4	1.6	39.8
XS*	8.1	7.1	1.2	38.8
XSI	14.9	11.1	2.1	20.8

Key: WX – water soluble part of xylan; XS – xylan sulphated in N,N-dimethylformamide with DS 1.40; XS′ – xylan sulphated in N,N-dimethylformamide with DS 0.20; XS″ – xylan sulphated in pyridine; XS* – xylan sulphated in dimethyl sulphoxide; XSI – water insoluble part of xylan sulphated in pyridine.

XS″ are listed in Table 1 and according to the integrated anomeric signals in the HSQC spectrum the ratio of substituted MeGlcA/Xyl was 1/4.37. Additionally also unsubstituted MeGlcA and Xyl units were present at 1/7 integrated ratio.

3.2. SEC–MALLS analysis

For the water-soluble part of X (WX) the biggest molar mass value at the peak was observed (Table 2). On the other hand M_w was the second smallest from all analyzed samples with polydispersity $D = 3.8$. The amounts of recovered masses could be related to the solubility of individual samples in the mobile phase as well as the retention of the sample on the column immobile phase. The XS sample prepared by treatment in DMF had more than one order of magnitude smaller M_p value, but its M_w was bigger than for WX and with the lowest polydispersity. The XS′ sample also prepared in DMF but with lower DS had the highest M_p and M_w values from all sulfated samples, but $D = 20.0$ and the amount of recovered sample was the biggest from all samples. The sample sulfated in pyridine (XS″) with similar DS like XS′ had lower M_w than XS′. The sample XS* sulfated in DMSO with DS close to XS sample had the lowest M_p and M_w values from all sulphated samples, but the polydispersity was the same as for XS. The amount of sample recovered for XS* was on the level of other samples. From the XSI sample which was also treated in DMSO but at room temperature only 21% was recovered, but M_p, M_w, and D were the second lowest from all studied samples. It means the least degradative condition for sulfation was in the presence of DMF as obtained for XS.

3.3. SEM analysis

The starting material (X), which was only partially soluble in water and the obtained film (123.2 ± 7 μm thick) looked on the surface of the fracture very inhomogeneous (Fig. 1a), but similar to WX (Šimkovic et al., 2011). The water-insoluble sample sulphated in DMSO (XSI; 60 μm thick) when analyzed with SEM on the surface (Fig. 1b) or on the fracture (Fig. 1c) looked different than X. On the other hand it was similar like positively and negatively charged xylan films ascribed before (Šimkovic et al., 2011) although its surface was more homogeneous than on X. It seems that the water soluble component covers the insoluble part and makes the smooth surface. The sample sulphated in DMF to a DS 1.18 (XS; 23 ± 3 μm thick; Fig. 2a) looks very smooth on the fracture. The sample sulphated in DMF with DS 0.2 which is just partially soluble in water is less smooth on the fracture (XS′; 23 ± 2 μm thick; Fig. 2b). The sample sulphated in pyridine formed sharper clusters on the fracture (XS″; Fig. 2c) than XS and XS′. The image of XS″ film from the surface (Fig. 2d; 51 ± 3 μm thick) even at lower magnification looked different than the surface of XSI. On the basis of these results it could be stated that the soluble part covers the insoluble and holds the film together in its form which has been developed after drying. The shape of films prepared from differently soluble derivatives is also varying probably due to different intensities of intermolecular interactions of hydrogen bonds and charged groups.

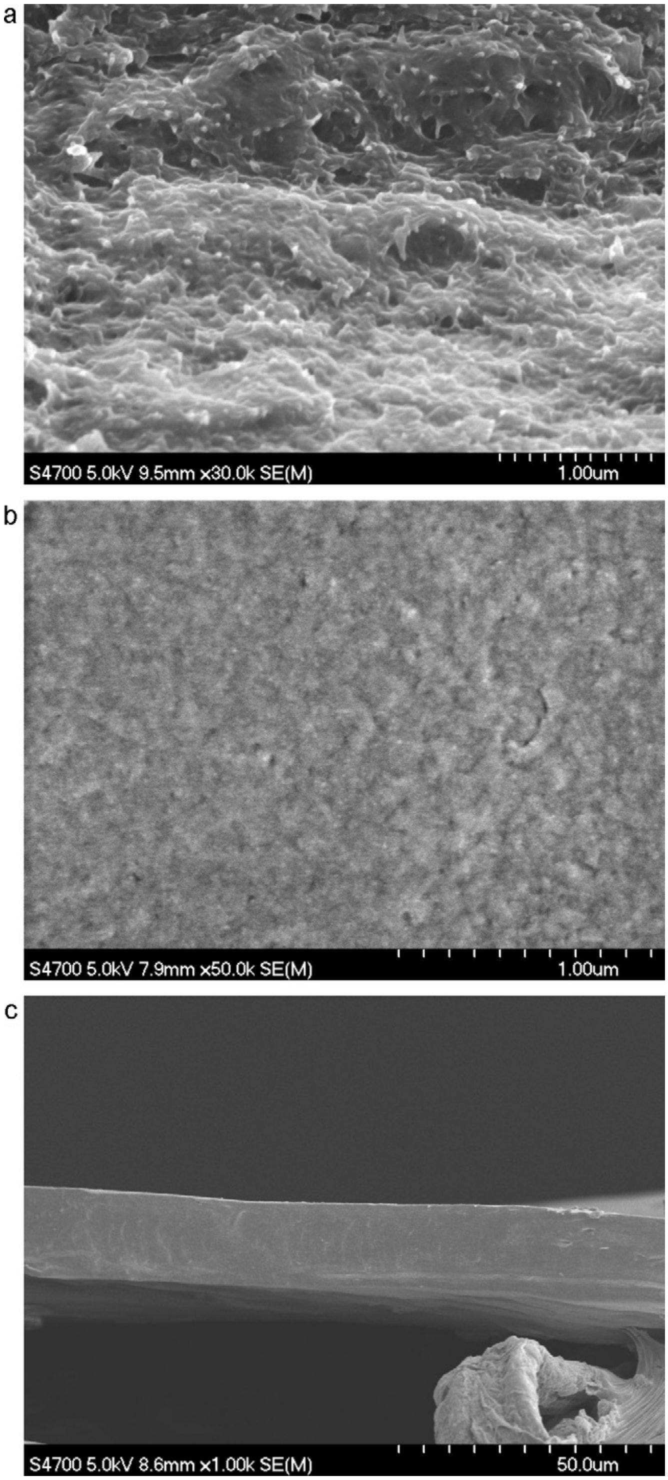

Fig. 1. SEM images of samples X on the fracture (a), XSI on the surface (b) and on the fracture at low magnification (c).

3.4. Thermal analysis features

The film prepared from sample XS was analyzed by TG/DTA methods in nitrogen and air (Table 3). In comparison to the data of the unmodified xylan X, the degradation of XS starts under nitrogen environment at lower temperature (Šimkovic et al., 2011). The first

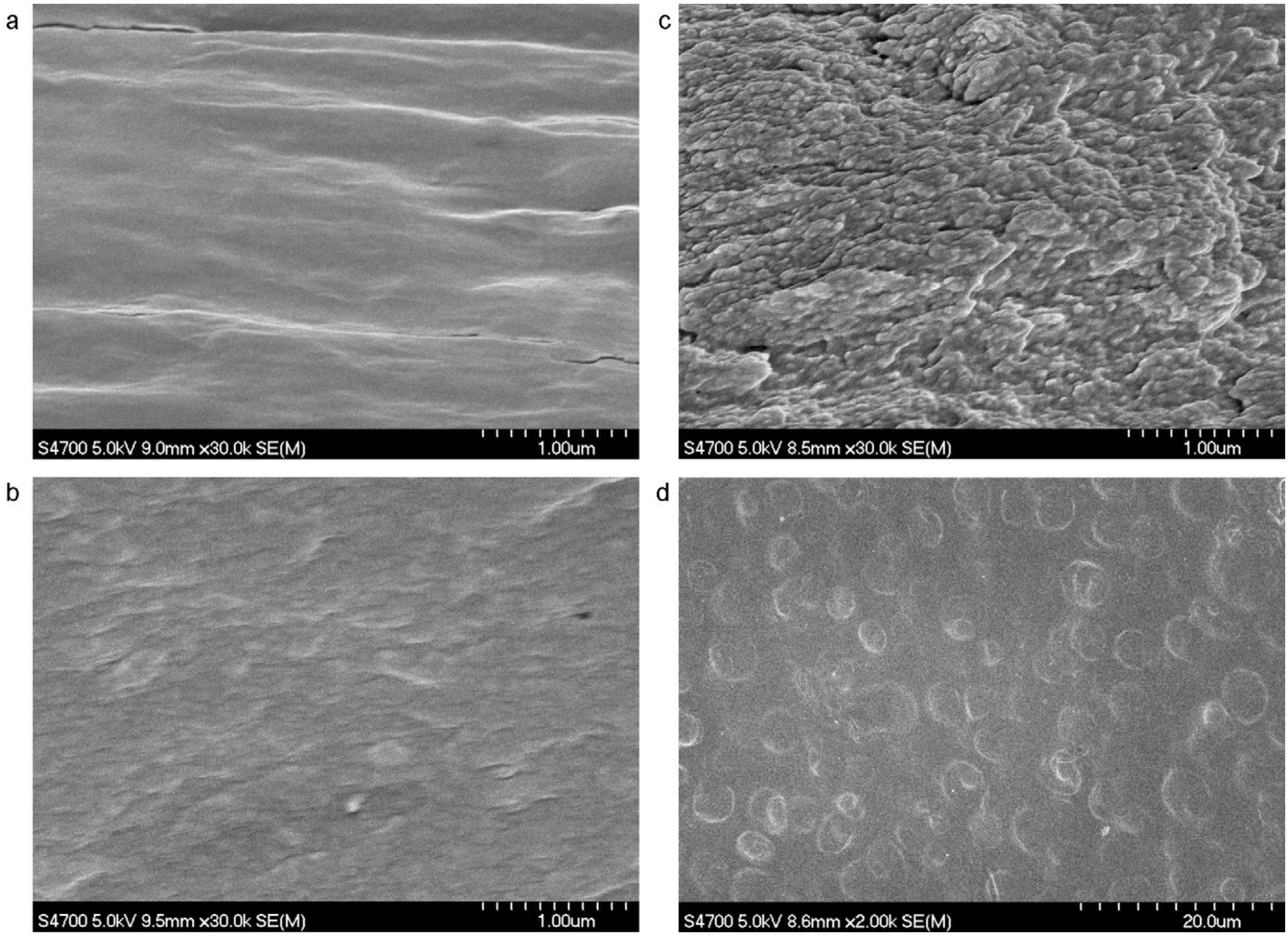

Fig. 2. SEM images of XS (a), XS′ (b) and XS″ (c) films on the fracture and XS″ (d) on the surface.

exotherm is at the same temperature as the first DTG maximum. After the second broad exotherm with maximum at 404 °C which is due to SO_2 formation (Šimkovic & Jakab, 2001) sample XS degraded further by producing an endotherm at 793 °C. The residue of 16% at 1000 °C is identical with carbon content of XS. Under the oxidative environment four separated exotherms were observed. While the first exotherm is identical with the one obtained under inert environment, the oxidation of the carbon residue at higher temperatures resulted into two sharp exotherms at 327 and 417 °C, which were not observed in inert atmosphere. We explain the last exotherm with the peak at 940 °C due to incorporation of sulphur into the residual carbon, which is supported by slight increase of the residue between 800 and 1000 °C when compared to the conditions under inert environment. In comparison to X the presence of sulphate groups in sample XS decreased the thermal as well as thermo-oxidative stability of the prepared films. At temperatures above 400 °C the residue did not drop that much as for X due to salts formed from sodium sulphate group.

The insoluble XSI was thermally more stabile in inert environment than XS, but less than X. The first small exotherm at 206 °C

Table 3
Thermal analysis features of samples.

Sample	Atmosphere	DTA peaks		DTG peaks		Residue [%] at [°C]				
		[°C]	Δ [°C/mg]	[°C]	Max. rate [mg/min]	200	300	400	500	1000
XS	Nitrogen	169	–0.014	169	0.76	53	44	41	39	16
		404	–0.027	793	0.26					
		792	–0.047							
	Air	170	–0.015	170	1.29	39	32	25	21	21
		327	–0.015	406	0.19					
		417	–0.001							
		940	–0.042							
XSI	Nitrogen	206	0.011	222	0.40	86	35	27	23	11
		270	0.012	253	0.10					
	Air	481	0.503	249	0.37	87	46	35	7	7

Key: XS – xylan sulphated in N,N-dimethylformamide with DS 1.40; XSI – water insoluble part of xylan sulphated in pyridine.

was followed by the second at 270 °C in inert environment. The two DTG maxima were at lower temperature than observed on X with residue bigger at 200 °C than for XS and X. At higher temperatures the residues were similar for all three discussed samples. The surprising thermal stability of XSI was observed up to 481 °C in air environment when the only sharp exotherm occurred. The residues at 500 and 1000 °C were identical and smaller than for XS, but bigger than for X.

3.5. Mechanical properties

Table 4
Mechanical properties of studied xylan films.

Sample	Young's modulus [MPa]	Tensile strength [MPa]	Elongation [%]	Thickness [μm]
XS′	160	3.40	0.97	50
XSI	5390	140	2.78	60

Key: XS′ – xylan sulphated in N,N-dimethylformamide with DS 0.20; XSI – water insoluble part of xylan sulphated in pyridine.

According to mechanical testing results XS′ has smaller value of Young's modulus as well as tensile strength (Table 4) than X (Šimkovic et al., 2011). This might be due to the presence of sulphate group which resulted in less of intermolecular hydrogen bonds interaction and causing lower mechanical stability than X due to mixing of soluble and insoluble components. The XS′ sample has probaly in average smaller molar mass than XSI from which smaller amount of sample was recovered by SEC although the polydispesity of both samples is very high. The thickness of both films is similar, but the Young's modulus and tensile strength of XSI is superior in comparison to XS′. Also on hydroxypropylsulfonated (HPS) and trimethylammoniuhydroxypropyl (TMAHP)-xylan smaller values of Young's modulus and tensile strength at comparable thickeness were observed than on XSI (Šimkovic et al., 2011). These results were also better than those obtained on oat spelt xylan films enforced with cellulose whiskers (Saxena et al., 2009). The elongation values were similar like on other samples. As it could be predicted that the molar mass of insoluble component of sample is higher than that of the soluble part it seams to be probable that the higher molar mass of insoluble polysaccharides is responsible for the better mechanical properties. The soluble part of xylan sulphate might function as adhesive which covers the insoluble residues to form compact film.

4. Conclusions

Xylan sulphate films even at high DS are not forming mechanically stable films. On the other hand partially soluble samples were having suitable mechanical properties at lower DS. The thermal properties of XS are not as good as those observed on XSI samples. According to SEM analysis there is a difference in the shape of surface and on the fractures of individual samples. It seems likely the intermolecular hydrogen bonds are responsible for better mechanical properties of XSI in comparison to XS′. The adhesion of insoluble parts with solubilized xylan sulphate polysaccharide seams to be causing the improved mechanical properties.

Acknowledgements

The authors are pleased to acknowledge Dr. Miloš Hricovini, Dr. Maria Matulová, the State programs # 2003SP200280203 and 2003SP200280301, Slovak Granting Agency VEGA (Project No 2/7030/7 and 2/0087/11), and APVV-0650-07 for the support and helpfull discussion. The work was also supported by the research program MSM 6046137202: Preparation and research of the functional materials and material technologies using micro- and nano-scopic methods.

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