

Size-exclusion chromatography of xylan derivatives – The critical evaluation of macromolecular data

Leena Pitkänen,^{1} Vladimir Aseyev,² Christiane Laine,³ Päivi Tuomainen,¹ Maija Tenkanen¹*

¹Department of Food and Environmental Sciences, P.O. Box 66, FIN-00014, University of Helsinki, Finland

²Department of Chemistry, P.O. Box 55, 00014, University of Helsinki, Finland

³VTT Technical Research Centre of Finland, P.O. Box 1000, 02044 VTT, Finland

*Corresponding author: leena.m.pitkanen@helsinki.fi

vladimir.aseyev@helsinki.fi, christiane.laine@vtt.fi, päivi.tuomainen@helsinki.fi, maija.tenkanen@helsinki.fi

Abstract

Hydroxypropyl xylans with varying degrees of substitution were characterized by size-exclusion chromatography. Molar masses of the samples were determined using two approaches: by conventional calibration with molar mass standards and by a multi-detection method that utilizes the combination of static light scattering, viscometry, and differential refractive index detection. The molar mass results obtained by the multi-detection method were accurate, but required the determination of separate refractive index increments for each structurally different sample. The column calibration approach with standard pullulan samples gave biased results due to the differences in hydrodynamic volumes between pullulans and hydroxypropyl xylans with similar molar masses. The degree of hydroxypropylation affected the chain conformation and compactness of the polymer chains. Mark-Houwink parameters and persistence length values suggested that the hydroxypropyl substituents reduce the flexibility of the xylan chain and make the polymer chain more extended.

Keywords: xylans, hydroxypropylation, size-exclusion chromatography, chain stiffness

Introduction

Interest in the utilization of biomass has increased enormously during the past couple of decades. Two global issues have raised interest in finding ways to convert biomass to chemical products: the necessity of using renewable carbon sources instead of fossil fuels and the need to decrease greenhouse gas emissions. The use of biopolymers extracted from the biomass as an alternative to synthetic polymers is one important way of utilizing biomass. In addition to using biopolymers in their native forms, the properties of biopolymers can be altered by modification (chemical, physical, enzymatic) of the polymeric chains. This study focuses on the macromolecular characterization of chemically modified birch xylan. Xylans, the cell-wall polysaccharides in plants, are one major group of hemicelluloses and constitute the second most abundant bioresource in the earth after cellulose [1].

The composition of xylans varies depending on the plant and the part of the plant. Birch wood contains glucuronoxylans (GX) which, in their native form, consist of a (1→4)-linked β -D-xylopyranosyl (β -D-Xylp) backbone with (1→2)-linked 4-O-methyl- α -D-glucopyranosyl uronic acid residues (MeGlcA) and acetyl groups attached to the main chain [2, 3]. However, alkaline extraction that is mainly used for hemicelluloses results in modification to the xylan structure by removing the acetyl groups. Deacetylation decreases the water solubility of xylans. Production of kraft pulp further modifies the xylan structure by reducing the MeGlcA content [4, 5]. Thus, xylan from the kraft pulp process (employed in this study) is nearly linear and contains only a low charge of approximately -0.24 meq/g dry weight [6]. By chemical modification, such as hydroxypropylation, of alkali extracted xylans, the water solubility can be recovered. Previous studies on hydroxypropylation of xylans, using either propylene oxide or propylene carbonate as reagents, have shown the potential of this chemically modified, thermoplastic material [6-8]. Hydroxypropyl xylan (HPX) has been shown to be a potential material in films and coatings that

could be used in biodegradable packaging [6, 9, 10]. Also, cellulose has been modified by hydroxyalkylation (i.e., introduction of hydroxyethyl or hydroxypropyl groups at the position of C2, C3 and/or C6) in order to improve its water solubility. Hydroxypropyl cellulose is used as a thickener and emulsion stabilizer in the food industry (E number E463), and also in the pharmaceutical industry as a binding agent in tablets and a formulation agent in artificial tears [11, 12].

Molar mass (and especially molar mass distribution) is one of the most important factors influencing the polymer properties [13]. In order to get information on the molar mass distribution, the polymeric sample needs to be separated using methods such as size-exclusion chromatography (SEC) or field-flow fractionation (FFF). The simplest way to get information on molar mass distribution is to couple the separation technique with a concentration-sensitive detection method, commonly differential refractive index (DRI) or ultraviolet (UV). This approach requires the analysis of low dispersity molar mass standards using analytical conditions identical to those for the samples. The detector signal intensity thus gives the concentration for each eluted fraction, and the elution volume defines the molar mass (when the retention volume of the sample is compared with the elution volumes of the standards). This so-called conventional calibration method is, however, prone to errors caused by the different molar mass-to-hydrodynamic volume ratio of the sample and the standards used for calibration. Also, molar masses determined by conventional calibration are sensitive to errors caused by enthalpic interactions between column stationary phase and analytes. Adding other detectors to the detector train, especially light scattering detectors (multi-angle light scattering detectors [MALS] and dynamic light scattering detectors [DLS]) and viscometers, allows for the determination of “absolute” molar masses as well as size and conformation parameters for each separated fraction [14, 15].

As already discussed, interest in modification of biopolymers is rapidly increasing. Thus, there will also be growing demand for the development of analytical techniques for these novel biomacromolecules. Even though the analytical toolbox for polymer separation and characterization already exists, critical evaluation of the use of these techniques for biomacromolecules with complex and heterogeneous structures is important. In this study, hydroxypropyl xylans with varying degrees of hydroxypropylation were characterized by SEC using multi-detection approach (light scattering, viscometer, and differential refractometer). SEC with conventional molar mass calibration (employing narrow dispersity pullulan standards) was used as a reference method [16]. The accuracy of both techniques was evaluated for molar mass analysis. Also, the difference in the chemical composition of the HPX samples was taken into account when the accuracy of the multi-detection data was evaluated. This was accomplished by investigating the effect of hydroxypropylation degree on the refractive index increment ($\partial n/\partial c$); the constant that is needed both for concentration determination by differential refractometer and for the determination of molar mass by light scattering.

Materials and methods

Materials

The hydroxypropyl xylans used here were produced previously for film formation studies [10]. The alkaline-extracted birch xylan was hydroxypropylated under alkaline conditions [6, 10]. The chemically modified xylans formed dispersions, with the dry matter content ranging from 8% to 12%. Part of the dispersion was freeze-dried for SEC analysis. HPLC grade dimethyl sulfoxide (DMSO) for the SEC was obtained from Fisher Scientific (Loughborough, UK) and lithium bromide (LiBr) was obtained from Sigma-Aldrich (Steinheim, Germany). Narrow dispersity

pullulan standards came from Agilent/Polymer Laboratories (Amherst, USA) and Postnova Analytics (Landsberg/Lech, Germany).

SEC analysis

The SEC instrument consisted of an integrated autosampler and pump module (Viscotek GPCmax, Malvern Instruments Ltd, Worcestershire, UK), a column oven (CROCO-CIL, Cluzeau Info Labo, Sainte-Foy-la-Grande, France), a combined static light scattering and viscometric detector (Viscotek 270 Dual Detector, Malvern Instruments Ltd), and a differential refractive index (DRI) detector (Viscotek VE 3580, Malvern Instruments Ltd). The light scattering detector ($\lambda_0 = 670$ nm) included two scattering angles: 7° and 90° . The separation of samples was conducted using two Jordi (Jordi Labs, Mansfield, USA) columns: X-stream mixed bed (covers molar mass range from 100 g/mol to 10000000 g/mol) and X-stream 500 Å (dimensions of both columns 250×10 mm) at 60°C . The flow rate was 0.8 ml/min and the injection volume was 100 μl . HPX samples were dissolved in mobile phase DMSO containing 0.01 M LiBr at the concentration of 5 mg/ml, and the samples were filtered using 0.45 μm syringe filters (GHP Acrodisc 13, Pall Corp., Ann Arbor, MI) before analysis. The molar mass averages were calculated based on the light scattering/viscometry method [14, 17] or by constructing a relative calibration curve using narrow dispersity pullulan standards (pullulans with nominal molar masses of 1320, 5900, 11800, 22800, 47300, 112000, and 212000 g/mol). Calibration curve is shown as Supplementary material (Fig. 1S). Column recovery values were determined based on the DRI signals.

Specific refractive index increment ($\partial n/\partial c$) determination

Specific refractive index increment ($\partial n/\partial c$) values for the HPX samples (degrees of substitution [DS] of 0.3, 0.6, and 1.1) were determined offline using a T-rEX differential refractometer (vacuum wavelength of light $\lambda_0 = 658$ nm, Wyatt Technology Co., Santa Barbara, USA). HPX samples were dissolved in DMSO containing 0.01 M of LiBr at six concentrations of approximately 1, 2, 3, 4, 5 and 6 mg/ml, and each solution was injected directly into the refractometer cell using disposable 1 ml syringes. The temperature of the refractometer was set to 40 °C. ASTRA software (Wyatt Technology Co.) was used for data collection and processing. The $\partial n/\partial c$ values were obtained from the slopes of a plot of concentration versus differential refractive index for each HPX sample.

Results and discussion

The series of HPX samples with varying DS were produced from the alkaline-extracted birch xylan using propylene oxide as a reagent for derivatization. The DS for each HPX was determined using quantitative ^{13}C NMR spectroscopy and average DS values of 0.3, 0.6, and 1.1 were obtained [10]. The HPX samples were separated and characterized using two SEC approaches: with conventional pullulan calibration (single concentration-sensitive detector) and by coupling the SEC with multiple detectors (light scattering [LS], viscometry [VISC], and differential refractometry [DRI]). An organic solvent was used as the eluent (DMSO + 0.01 M LiBr) in all the SEC analyses. Initially, aqueous eluent was also tested for HPX samples, but SEC-LS/VISC analyses in 0.1 M NaNO_3 (aq.) revealed that HPX chains were not dissolved at the level of individual polymer chains and that the samples contained high-molar mass aggregates (previous SEC studies in DMSO/water eluent have shown that the molar masses of nitren-extracted birch pulp xylans are relatively low, in the range of

~10,000 g/mol [18]). Aqueous eluent was an obvious first choice for the HPX samples, because the purpose of hydroxypropylation is to increase the water solubility of polysaccharides, such as cellulose and various hemicelluloses. The alkaline-extracted birch xylan used as the starting material for hydroxypropylation was not soluble in either of the eluent systems tested in this study for SEC analyses (organic or aqueous).

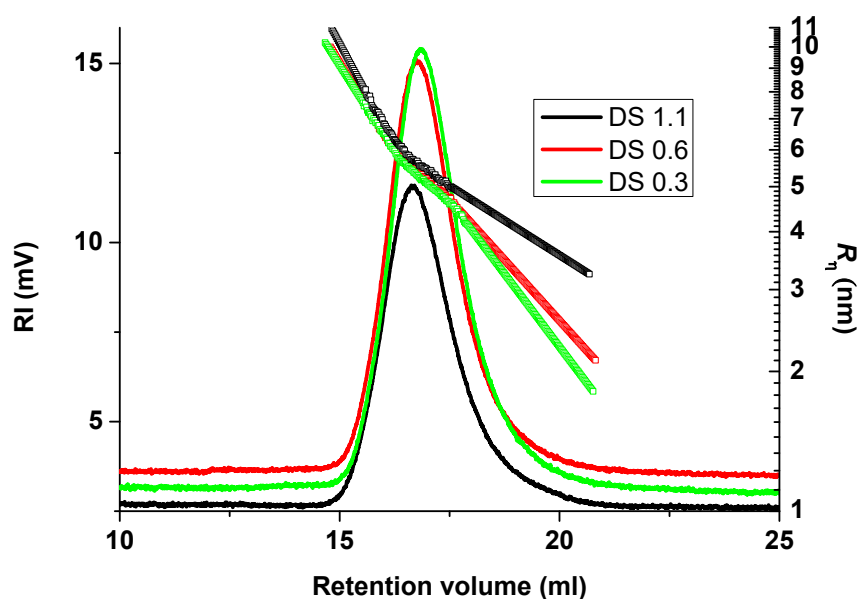


Fig. 1 SEC chromatograms for hydroxypropyl xylans with varying degrees of substitution (DS). The viscometric radii (R_{η}) for each xylan is plotted across the peak detected with a refractive index detector.

Molar mass determination of hydroxypropyl xylans by SEC with conventional pullulan calibration

The SEC chromatograms of HPX samples detected by DRI are presented in Figure 1. As expected, only minor differences in the retention volumes could be observed because the birch xylan used as a starting material for hydroxypropylation was the same in all three samples, and the only difference in the molar masses should have resulted from the differences in the hydroxypropyl group content. The percentage increase in molar mass can be calculated for HPX samples when the average DS is known (here, the DS is obtained from NMR spectroscopy). The molar mass of xylan is expected to

increase 14% when the DS is 0.3, 26% when the DS is 0.6, and 49% when the DS is 1.1 (Table 2). The “traditional” way of determining the molar mass for SEC separated samples is to construct a calibration curve using narrow dispersity molar mass standards. Here, the pullulan calibration curve was used for molar mass determination, and the molar mass averages (M_w , weight-average molar mass, and M_n , number-average molar mass) are presented in Table 1. As can be seen from the values in Table 1, only very small differences could be observed between the samples. This is in accordance with the chromatograms (Figure 1), which show very minor differences between the samples in their elution volumes (in conventional calibration, the peak position directly determines the molar mass). However, the differences in molar masses between the samples with varying DS should be larger, as indicated by the percentages presented in Table 2. HPX samples with varying DS have been previously analyzed by SEC using pullulan calibration and 0.1 M NaOH as eluent [10]. These previously published data show the same trend as that observed in this study: only very small differences in molar masses between the samples were observed. Also, the molar mass results from the calibration curve obtained in this study seemed somewhat high because, as already discussed, the molar mass of birch pulp xylans has been reported to be in the range of ~10,000 g/mol [18]. Due to these facts, the molar mass results obtained using pullulan calibration seemed biased, and another approach was applied, in which SEC was coupled with LS/VISC/DRI detectors.

Table 1 Molar mass averages and dispersities for hydroxypropyl xylans obtained by light scattering/viscometry (LS/VISC/DRI) method and by conventional calibration.

SAMPLE	LS/VISC/DRI			Conventional calibration		
	M_w (g/mol)	M_n (g/mol)	D	M_w (g/mol)	M_n (g/mol)	D
HPX DS 0.3	16,500	14,300	1.15	32,000	25,000	1.28
HPX DS 0.6	18,700	16,300	1.15	34,000	27,000	1.26
HPX DS 1.1	23,500	22,200	1.06	36,000	27,000	1.33

M_w , weight-average molar mass, M_n , number-average molar mass, D , dispersity

Table 2 Relative residue molar mass (m) and persistence length (L_p) for hydroxypropyl xylans.

	Theoretical increase of molar mass due to hydroxypropylation (%)	Relative residue molar mass m (g/mol)	L_p (nm) ^a
HPX DS 0.3	14	150	1.3
HPX DS 0.6	26	167	1.3
HPX DS 1.1	49	197	4.1

^aDetermined from the slopes of the Bohdanecký plots

Macromolecular characterization of hydroxypropyl xylans by multi-detection SEC

The coupling of SEC with multiple detectors (LS/VISC/DRI) offers many benefits over the method in which the SEC is employed with a single concentration-sensitive detector and the molar mass results are obtained from the calibration curve constructed using the series of standard samples.

When the LS/VISC/DRI method is employed, the molar mass obtained for every eluted fraction is not dependent on the peak position. The signal from the LS detector is directly proportional to the molar mass, and thus gives “absolute” molar masses that are independent on the calibrants. Also, additional information on the size and conformation of the analyte can be obtained when LS and VISC detectors are included in the detector train. The static light scattering detector allows the determination of a size parameter, the radius of gyration (R_G) for each eluted fraction; the viscosity detector gives another radius, namely the viscometric radius (R_η). The molar mass dependence of these radii provides information about the conformation of the macromolecules. Additionally, the viscosity detector together with DRI gives intrinsic viscosity ($[\eta]$) values across the separated sample. Intrinsic viscosity can be considered as “inverse density,” and it describes the compactness of the sample in solution.

As can be seen in Table 1, the molar masses obtained with the triple-detection method are much lower than those obtained using the pullulan calibration curve (the M_w of HPX DS 0.3 and HPX DS

0.6 are almost half of the values obtained via the calibration method). Also, the dispersities obtained with the triple-detection method are lower than those obtained using conventional calibration. Molar mass values obtained by the LS/VISC/DRI method are in the “expected range,” based on the earlier results on the average molar masses of birch pulp xylans. Also, when the M_w values of HPX DS 0.3 and HPX DS 0.6 samples are compared with each other, the difference in their masses seems reasonable in light of the theoretical mass increase caused by the higher number of hydroxypropyl substituents in the latter compared to the lowest DS sample. Theoretically, the molar mass should increase 11% when the average DS increases from 0.3 to 0.6. The experimental M_w for HPX DS 0.3 was 16,500 g/mol, and for HPX DS 0.6, it was 18,700 g/mol (Table 1); thus, the increase in the M_w value is 13%, which is very close to the value predicted by the calculations. When DS increases from 0.6 to 1.1, the mass should increase 18%. Experimentally, using SEC/LS/VISC, the increase is somewhat higher at 26%. Overall, the experimental molar masses obtained with SEC/LS/VISC are reasonable, and can be considered more reliable than the ones obtained by employing the pullulan calibration curve.

The signal from the LS detector is directly proportional to molar mass (M) and the square of the refractive index increment ($\partial n/\partial c$) [19]:

$$LS_{Signal} \propto M \times \left(\frac{\partial n}{\partial c} \right)^2 \quad (1)$$

The $\partial n/\partial c$ value is a constant that can be determined for each polymer/solvent combination at certain wavelengths using a refractometer. Also, $\partial n/\partial c$ values can be found from the literature (please see references [20, 21]). The $\partial n/\partial c$ values taken from the literature should, however, be used only after careful consideration. The chemical structure affects the $\partial n/\partial c$ value and thus, for copolymers and biopolymers with structural variation, the constant needs to be determined for each polymer/solvent pair. Unfortunately, many researchers must rely on the literature values due to the lack of available instrumentation for the $\partial n/\partial c$ measurements. In this study, we measured the $\partial n/\partial c$

values for all three HPX samples with varying DS, and the $\partial n/\partial c$ plots (and associated slopes) are presented in Figure 2. As can be seen from the results, the DS has a significant effect on the $\partial n/\partial c$ values, and the $\partial n/\partial c$ value decreases when the DS increases. Because $\partial n/\partial c$ is a squared term in the light scattering equation, its accurate determination is highly important for accurate molar mass determination by the light scattering technique. The results obtained in this study highlight this fact: the error in M_w of HPX DS 1.1 is 34% if the $\partial n/\partial c$ value of HPX DS 0.3 (0.053 ml/g) is used for molar mass determination, and the error is even larger, at 68%, for the opposite case in which the $\partial n/\partial c$ value of HPX DS 1.1 (0.035 ml/g) is used for M_w determination of HPX DS 0.3. To our knowledge, this is the first study in which $\partial n/\partial c$ has been determined for series of structurally modified polysaccharides with varying DS. SEC with a light scattering detector has been used before for analysis of HPX samples, but in those studies [22], a constant $\partial n/\partial c$ value was used for all samples with different DS. The column recovery values obtained using the determined $\partial n/\partial c$ values for each HPX sample were between 95% and 103%, which confirms that the analyses were quantitative.

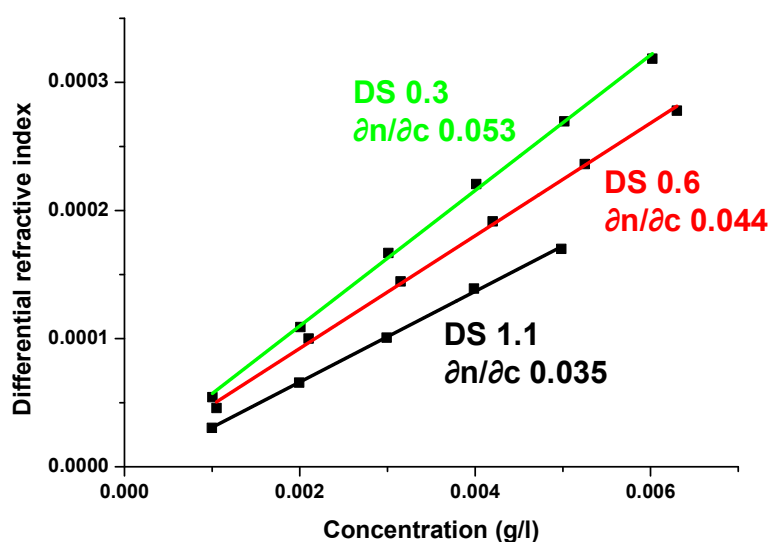


Fig. 2 $\partial n/\partial c$ plots for hydroxypropyl xylans. As indicated by the slopes, the degree of substitution (DS) affects the $\partial n/\partial c$ values.

The viscometric detection (together with the refractometer) allows the determination of a size parameter, the viscometric radius (R_η), and a conformation parameter, intrinsic viscosity ($[\eta]$). The average R_η and $[\eta]$ values for the HPX samples are presented in Table 3, and, as can be seen, the values for each parameter are very close to each other. Both size and $[\eta]$ seem to increase slightly with increasing DS. In Figure 3, the intrinsic viscosity for each sample is presented as a function of molar mass in a so-called Mark-Houwink plot. The slope of the plot describes the conformation of a sample. As can be seen in the overlaid Mark-Houwink plots in Figure 3, the plots for the two lowest DS samples (DS 0.3 and DS 0.6) seem to have slopes that are close to each other, whereas for the sample with the highest DS (DS 1.1), the intrinsic viscosity increases more quickly as the molar mass increases, yielding a higher slope compared to the DS 0.3 and DS 0.6 samples. The constants of the double-logarithmic plots, which are also known as Mark-Houwink constants K and a in the Mark-Houwink equation:

$$[\eta] = KM^a \quad (2)$$

are presented for the HPX samples in Table 3. As already observed from the overlay graph representing the Mark-Houwink plots, the Mark-Houwink a values for the DS 0.3 and DS 0.6 samples are close to each other, at 0.63 and 0.70, respectively. The a value for the DS 1.1 sample was higher, at 0.95. Generally, a values for random coil polysaccharides range from 0.5 to 0.8. Higher values of a indicate more extended conformation [23]. Thus, it seems that when the number of hydroxypropyl side groups increases from DS 0.6 to DS 1.1, the larger number of side groups makes the polymer structure of the DS 1.1 sample more extended compared to the DS 0.6 sample. Previous results for structurally different polysaccharides support the findings of this study: e.g., the Mark-Houwink a for substituted methyl cellulose has been found to be higher than that for linear polysaccharides, such as for pullulan [24]. Similarly, the increasing Mark-Houwink K value

indicates a more extended structure, but no definite conclusions can be drawn from the data obtained in our study.

Table 3 Viscometric radius (R_η), weight-average intrinsic viscosity ($[\eta_w]$), and Mark-Houwink parameters a and K for hydroxypropyl xylans.

	R_η (nm)	$[\eta_w]$ (ml/g)	a^a	K^a
HPX DS 0.3	5.0	48	0.63	0.118
HPX DS 0.6	5.2	47	0.70	0.068
HPX DS 1.1	5.7	49	0.95	0.003

^a The Mark-Houwink parameters were obtained by fitting the straight lines to the Mark-Houwink plots presented in Figure 3.

The conformation and flexibility of polysaccharides can also be described by other parameters, such as persistence length (L_p). L_p is a quantitative measure of polymer flexibility and can be defined as the distance over which the spatial orientations of monomers are not mutually independent. Even though for a theoretical random coil the L_p approaches 0, and for a rod the L_p approaches infinity, in practice most of the polymers have L_p values that range from ~1 nm to 200 nm. The L_p can be determined using several different models [24, 25]; here the Bohdanecký model [26], which relies on the intrinsic viscosity and molar mass, was employed. In the Bohdanecký model, $(M^2/[\eta])^{1/3}$ is plotted against $M^{1/2}$, and L_p can be calculated from the slope (B_η) of the plot:

$$B_\eta = \frac{B_0}{\Phi_\infty^{1/3}} \left(\frac{2L_p}{M_L} \right)^{-1/2} \quad (3)$$

where M_L is the mass per unit length (0.54 nm), Φ_∞ is the Flory's constant, and the approximation of 1.05 is used for B_0 . As already suggested by the Mark-Houwink a values, the L_p values for HPX DS 0.3 and HPX DS 0.6 are very close to each other (29 nm and 27 nm, respectively), whereas HPX DS 1.1, with the highest number of side groups, has a higher L_p of 56 nm.

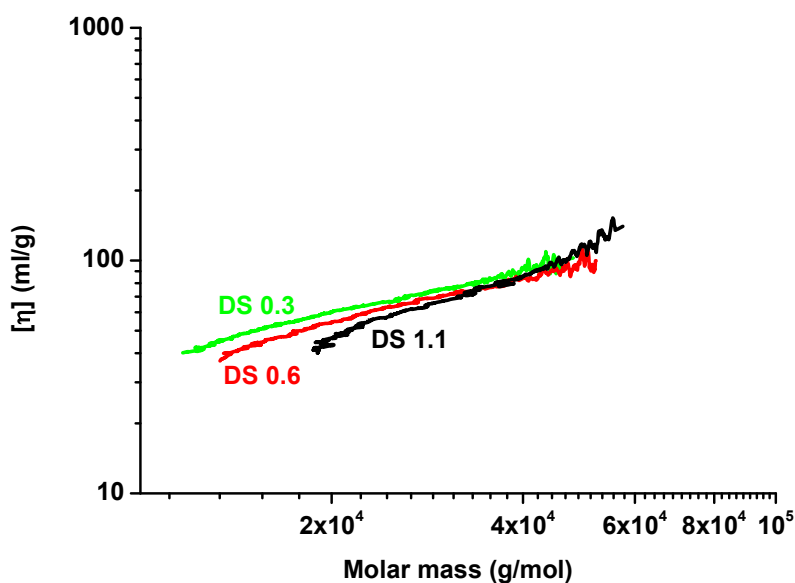


Fig. 3 Mark-Houwink plots for hydroxypropyl xylans with varying degrees of substitution (DS).

Comparison of conventional pullulan calibration and LS/VISC/DRI method

As already discussed, the LS/VISC/DRI method seemed to give more realistic results than did conventional pullulan calibration. The reason for the overly large molar masses obtained from pullulan calibration is due to the difference in the hydrodynamic sizes between pullulans and HPX samples. For comparison, the weight-average intrinsic viscosity ($[\eta_w]$) values in DMSO containing 0.01 M LiBr for pullulans with nominal molar masses of 11,800 g/mol and 22,800 g/mol were 14 ml/g and 21 ml/g, respectively. Thus, the molar masses of these pullulans are in the range of HPX samples investigated in this study, but their $[\eta_w]$ values are significantly lower, indicating more compact solution conformation of pullulans than of HPX chains. When the retention behavior in SEC separation is considered, this means that HPX that have the same molar mass as the pullulan elutes at lower retention volumes than the pullulan elutes, and thus the molar masses obtained as pullulan equivalents are overestimated. Similar to the intrinsic viscosity, the size in terms of R_η indicates the compactness of pullulans when compared to HPX samples. The R_η for pullulan 11,800

g/mol was 3.1 nm, and for pullulan 22,800 g/mol it was 4.1 nm. The difference in the conformation between pullulans and HPX samples originates from their chemical structures; pullulan consists of maltotriose units that are linked together with α -(1 \rightarrow 6)-linkages, and xylan has a β -(1 \rightarrow 4)-linked backbone. β -(1 \rightarrow 4)-linked polysaccharides are known to be more rigid than the polysaccharides that have more “nonlinear” structures.

Conclusions

HPX samples with varying DS were characterized by SEC with two different approaches: with conventional molar mass calibration and by coupling SEC with multiple detectors. Conventional calibration was shown to give biased molar mass results due to the differences in the hydrodynamic volumes between the calibrants (pullulans) and HPX chains. The molar mass results obtained by the LS/VISC/DRI method were in excellent agreement with the theoretically estimated molar masses (which were calculated based on the average DS values obtained by NMR spectroscopy). The accuracy of the molar mass results for different modified polysaccharides obtained by light scattering methods should, however, be evaluated critically because as seen in this study, the $\partial n/\partial c$ values of the HPX samples varied significantly as a function of DS (i.e., the chemical structure affected $\partial n/\partial c$). The multi-detection approach allowed the investigation of the effect of DS on conformation and chain stiffness of the HPX chains. Samples with higher degree of hydroxypropylation led to stiffer and more extended chain conformation than did the samples with lower numbers of substituents. Detailed characterizations of the macromolecular properties of modified biomacromolecules are essential, now that efforts to convert biomass into industrially applicable products have increased tremendously.

Acknowledgements

The financial support from the Magnus Ehrnrooth Foundation for LP is gratefully acknowledged. The Finnish Bioeconomy Cluster (FIBIC) and the Finnish Funding Agency for Technology and Innovation (Tekes) are thanked for funding this work.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Aspinnall GO. Chemistry of cell wall polysaccharides. *Biochem Plants*. 1980;3:473-500.
2. Goring DAI, Timell TE. Molecular properties of six 4-O-methylglucuronoxylans. *J Phys Chem*. 1960;64:1426-1430.
3. Timell TE. Recent progress in the chemistry of wood hemicelluloses. *Wood Sci Technol*. 1967;1:45-70.
4. Teleman A, Larsson PT, Iversen T. On the accessibility and structure of xylan in birch kraft pulp. *Cellulose*. 2001;8:209-215.
5. Teleman A, Tenkanen M, Jacobs A, Dahlman O. Characterization of *O*-acetyl-(4-*O*-methylglucurono)xylan isolated from birch and beech. *Carbohydr Res*. 2002;337:373-377.
6. Laine C, Harlin A, Hartman J, Hyvarinen S, Kammiovirta K, Krogerus B, Pajari H, Rautkoski H, Setälä H, Sievänen J, Uotila J, Vähä-Nissi M. Hydroxyalkylated xylans - Their synthesis and application in coatings for packaging and paper. *Ind Crops Prod*. 2013;44:692-704.
7. Jain RK, Sjostedt M, Glasser WG. Thermoplastic xylan derivatives with propylene oxide. *Cellulose*. 2000;7:319-336.
8. Akil Y, Lorenz D, Lehnen R, Saake B. Safe and non-toxic hydroxyalkylation of xylan using propylene carbonate. *Eur Polym J*. 2016;77:88-97.
9. Bayati F, Boluk Y, Choi P. Diffusion behavior of water at infinite dilution in hydroxypropyl xylan films with sorbitol and cellulose nanocrystals. *ACS Sustainable Chem Eng*. 2014;2:1305-1311.
10. Mikkonen KS, Laine C, Kontro I, Talja RA, Serimaa R, Tenkanen M. Combination of internal and external plasticization of hydroxypropylated birch xylan tailors the properties of sustainable barrier films. *Eur Polym J*. 2015;66:307-318.
11. Guo J, Skinner GW, Harcum WW, Barnum PE. Pharmaceutical applications of naturally occurring water-soluble polymers. *Pharm Sci Technol Today*. 1998;1:254-261.
12. Chang C, Zhang L. Cellulose-based hydrogels: Present status and application prospects. *Carbohydr Polym*. 2011;84:40-53.
13. Striegel AM. Multiple-detection in size-exclusion chromatography of macromolecules. *Anal Chem*. 2005;77:104A-113A.
14. Striegel AM, Yau WW, Kirkland JJ, Bly DD. *Modern size-exclusion liquid chromatography*. 2nd ed. New Jersey: John Wiley & Sons; 2009.

15. Pitkänen L, Striegel AM. Polysaccharide characterization by hollow-fiber flow field-flow fractionation with on-line multi-angle static light scattering and differential refractometry (HF5/MALS/DRI). *J Chromatogr A*. 2015;1380:146-155.
16. Lawoko M, van Heiningen ARP. Fractionation and characterization of completely dissolved ball milled hardwood. *J Wood Chem Technol*. 2011;31:183-203.
17. Fishman ML, Doner LW, Chau HK, Hoagland PD. Characterization of hemicellulose B from corn fiber by high-performance size-exclusion chromatography with on-line molar mass and viscometric detection. *Int J Polym Anal Charact*. 2000;5:359-379.
18. Puls J, Schröder N, Stein A, Janzon R, Saake B. Xylans from oat spelts and birch kraft pulp. *Macromolecular Symposia*. 2005;232:85-92.
19. Wyatt PJ. Light scattering and the absolute characterization of macromolecules. *Anal Chim Acta*. 1993;272:1-40.
20. Brandrup J, Immergut EH, Editors. *Polymer Handbook*, 4th ed. New York: John Wiley & Sons; 1998.
21. Theisen A, Johann C, Deacon MP, Harding SE. *Refractive increment data-book*. Nottingham: Nottingham University Press; 2000.
22. Nypelö T, Laine C, Aoki M, Tammelin T, Henniges U, Etherification of wood-based hemicelluloses for interfacial activity. *Biomacromolecules*. 2016;17:1894-1901.
23. Burchard W. Solution properties of branched macromolecules. *Adv Polym Sci*. 1999;143:113-194.
24. Morris GA, Adams GG, Harding SE. On hydrodynamic methods for the analysis of the sizes and shapes of polysaccharides in dilute solution: A short review. *Food Hydrocolloids* 2014;42:318-334.
25. Mourey T, Le K, Bryan T, Zheng S, Bennett G. Determining persistence length by size-exclusion chromatography. *Polymer* 2005;46:9033-9042.
26. Bohdanecky M. New method for estimating the parameters of the wormlike chain model from the intrinsic viscosity of stiff-chain polymers. *Macromolecules* 1983;16:1483-1492.