Modification of xylan via an oxidation–reduction reaction

Citation for the original published paper (version of record):

Palasingh, C., Nakayama, K., Abik, F. et al (2022). Modification of xylan via an oxidation–reduction reaction. Carbohydrate Polymers, 292. http://dx.doi.org/10.1016/j.carbpol.2022.119660

Carbohydrate Polymers 292 (2022) 119660

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Modification of xylan via an oxidation–reduction reaction

Chonnipa Palasingh [a], Koyuru Nakayama [a,b], Felix Abik [c], Kirsi S. Mikkonen [c,d], Lars Evenäs [a,b], Anna Ström [a], Tiina Nypelö [a,b,*]

[a] Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden
[b] Wallenberg Wood Science Center, Chalmers University of Technology, 412 96 Gothenburg, Sweden
[c] Department of Food and Nutrition, 00014 University of Helsinki, Finland
[d] Helsinki Institute of Sustainability Science, 00014 University of Helsinki, Finland

ARTICLE INFO

Keywords:
Polysaccharides
Dialcohol xylan
Molecular weight
Hydrodynamic radius
Solubilization

ABSTRACT

Xylan is a biopolymer readily available from forest resources. Various modification methods, including oxidation with sodium periodate, have been shown to facilitate the engineering applications of xylan. However, modification procedures are often optimized for semicrystalline high molecular weight polysaccharide cellulose rather than for lower molecular weight and amorphous polysaccharide xylan. This paper elucidates the procedure for the periodate oxidation of xylan into dialdehyde xylan and its further reduction into a dialcohol form and is focused on the modification work up. The oxidation–reduction reaction decreased the molecular weight of xylan while increased the dispersity more than 50%. Unlike the unmodified xylan, all the modified grades could be solubilized in water, which we see essential for facilitating the future engineering applications of xylan. The selection of quenching and purification procedures and pH-adjustment of the reduction step had no significant effect on the degree of oxidation, molecular weight and only a minor effect on the hydrodynamic radius in water. Hence, it is possible to choose the simplest oxidation-reduction route without time consuming purification steps within the sequence.

1. Introduction

Polysaccharides are necessary for producing current and future materials to provide renewable polymers for structural and functional purposes. Hemicelluloses are a diverse family of polysaccharides that have up to 35% in weight availability in wood (Sixta, 2006). The most abundant hardwood hemicellulose, xylan, has been suggested for engineered films (Escalante et al., 2012; Gordobil et al., 2014; Grondahl et al., 2004; Hansen et al., 2012), as a tensioactive material (Fu et al., 2020; Nypelö et al., 2016), and in medical and hygiene applications (Fu et al., 2020; Gabrielii et al., 2000; Gabrielii & Gatenholm, 1998; Köhnke et al., 2014). Xylan is available as an extract of plant biomass (Ebringerova & Heinze, 2000) and typically exhibits a linear backbone structure comprising β-1,4-linked xylose units that may be substituted with monosaccharides, glucuronic acid groups, or be partly acetylated.

Producing engineered materials from xylan presents some interesting challenges. The current applications mainly aim for high volume and low cost. However, the processes for extracting, purifying, and concentrating xylan from wood are costly, and these costs need to be considered when selecting applications. Xylan's susceptibility to batch-to-batch variations due to its natural source necessitates robust follow-up chemistry or end uses that allow raw material diversity. Additionally, wood-based xylans can often be solubilized in water only to a limited extent (Ebringerova et al., 2005). Such limited water interactions are a challenge for the renewable polymer industry, which frequently operates in aqueous conditions.

Periodate oxidation is a modification method applied to polysaccharides to alter their chemical reactivity (Larsson & Wagberg, 2016; Vold and Christensen, 2005). Periodate oxidation of cellulose was first used for analytical purposes to characterize monosaccharide structures (Malaprade, 1928), and was later used for preparative purposes (Bobbitt, 1956; Zeronian et al., 1964). Oxidation modulates the monosaccharide ring via cleavage of the diol structure and introduces an aldehyde functionality. The aldehyde can be further oxidized to the carboxylic acid group, reduced to an alcohol, or employed for intermediate chemical modifications (Nypelö et al., 2021). The cleavage of the monosaccharide ring may also alter the polymeric properties of xylan, such as solvent interactions, while introducing the aldehyde

---

[*] Corresponding author at: Department of Chemistry and Chemical Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden.
E-mail address: tiina.nypelo@chalmers.se (T. Nypelö).

https://doi.org/10.1016/j.carbpol.2022.119660
Received 19 March 2022; Received in revised form 15 May 2022; Accepted 23 May 2022

functionality can assist reactivity or follow-up chemistry, such as thermal transitions (Börjesson et al., 2019). Periodate oxidation of xylan has been studied with a view to structural development (Painter & Larsen, 1970). Hemiacetal formation between the aldehydes and hydroxyl groups has been utilized in xylan-based hydrogels to prevent the gel structure from dispersing in water (Köhnke et al., 2014). Amination of aldehyde groups with benzylamine has been used to introduce the benzyl group into the xylan backbone to increase its use in functional biomaterials (Chemin et al., 2015).

A decrease in the molecular weight of xylan has been reported to result from the periodate oxidation (Amer et al., 2016; Palasingh et al., 2021). Depolymerization is thought to occur due to overoxidation of the polysaccharide chain reducing ends and random attacks by hydroxyl radicals generated as the periodate decomposes spontaneously in water (Vold & Christensen, 2005). Additionally, it has been found that the resulting dialdehyde is more prone to alkaline β-elimination, which can be prevented by reducing the dialdehydes to dialcohols (Kristiansen et al., 2010). The extent of depolymerization can vary depending on the reaction conditions and molar ratios between the periodate and monosaccharide units, and in certain situations, almost complete degradation can occur (Chemin et al., 2016). A low periodate-to-xylan ratio can prevent undesirable depolymerization; however, only a low degree of oxidation (DO) is reached with the low ratio (Chemin et al., 2016). An insignificant decrease in weight average molar mass but a significant decrease in number average molar mass, indicating an increase in dispersity, has been reported for periodate-oxidized arabinoxylan at up to 20% DO (Börjesson et al., 2018).

The procedure for the periodate oxidation and reduction of xylan is well established. Pandeirada et al. (2022) elucidated on structural development from polysaccharides to oligosaccharides. However, the procedures aiming for oxidation and preservation of polymerix xylan rely mostly on procedures optimized for cellulose. Although xylan and cellulose are chemically similar, the cellulose reaction is almost always heterogeneous. This is not obviously the case for xylan since, unlike cellulose, the starting material is not in a fiber, fibril, or crystal form. Furthermore, analysis of the DO of xylan products has been approached using analytics optimized for cellulose. However, a few significant differences affect the applicability of the same procedures, one of which is that oxidized xylan products may be progressively solubilized in water during oxidation, complicating the typical analysis of DO, which is performed by observing the consumption of the oxidant during oxidation.

We examined the modification procedure for oxidation and reduction of xylan. We aimed to establish a process with minimal number of modular unit operations by evaluating the necessity of quenching and purification steps. The modification was sought to enable solubilization in water that extends engineering applications of xylan. Moderate water-solubility would, for example, enable the xylan derivates to be used as additives to functionalize wood fiber products. We provide insight into the challenge of analysis of degree of modification using UV–Vis and NMR spectroscopy, and molecular weight, and water-solubilization of the product from selected preparation routes to facilitate the choice of modification process unit operations.

2. Experimental

2.1. Materials

Beechwood xylan was purchased from Megazyme (Co. Wicklow, Ireland) and, according to the manufacturer, contained 13 wt% of glucuronic acid *O*-methyl substitution. Dimethyl sulfoxide (DMSO) and pullulan standards were purchased from Fisher Scientific (MA, United States) and Postnova Analytics (Landsberg am Lech, Germany), respectively. Other chemicals were purchased from Sigma-Aldrich (MO, United States) and all chemicals were used without further purification. Xylan was oxidized with sodium periodate according to Amer et al. (2016). In brief, 4 g of xylan was dispersed in 115 ml of water. A sodium periodate solution was prepared by dissolving 5.9 g (1:1 mol equivalent to anhydroxylose units [AXU]) of sodium periodate in 75 ml of water, which was added to the xylan solution. The oxidation was performed in darkness at room temperature. After 24 h, the oxidized xylan solution was divided into four batches and reduced using $NaBH_4$. Fig. 1 shows the oxidation, reduction, and purification sequences used to prepare the selected grades. The oxidation reaction was optionally quenched with ethylene glycol. Two reduction conditions were investigated: for the first, DalX1, DalX2, and DalX3 were reduced with 0.46 g $NaBH_4$ dissolved in 10 ml of water for 2 days (Leguy et al., 2018), and in the second, DalX4 was reduced with 0.4 g of $NaBH_4$ and 0.06 g of NaH_2PO_4 (buffer) dissolved in 10 ml of water for 4 h (Börjesson et al., 2019). A buffer was added to maintain a constant pH throughout the reaction. Dialysis was used to purify the products, and they were freeze dried for further use. A yield of products was approximately 40–45% after the oxidation and reduction step.

3. Methods

3.1. Compositional analysis

The composition of xylan was determined by hydrolyzing xylans with 72% sulfuric acid at 125 °C for 1 h (Theander & Westerlund, 1986). The hydrolyzed xylan was filtrated using a 0.2 μm PDVF filter. A fucose standard (200 mg/l) was added to 1 ml of filtrate, which was then diluted 50 times with water. The composition was analyzed with high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (Dionex™ ICS-3000 equipped with a CarboPac™ PA1 analysis column; Dionex Corporation, USA). NaOH/NaAc and NaOH were used as eluents.

3.2. Size exclusion chromatography (SEC)

The molecular weight of the xylans was analyzed with SEC using 0.01 M LiBr in a DMSO-based eluent. Approximately 4 mg of the xylan was dissolved in 2 ml of the eluent for several days at room temperature and then filtered through a 0.45 μm PTFE syringe filter. All xylan grades were dissolved directly in the solvent, except for the unmodified xylans, which were first swollen in 30 μl of water overnight, followed by dissolution in 2 ml of eluent. An amount of 100 μl of the filtered xylan solution was injected into the SEC system equipped with a Jordi xStream GPC column (Jordi Labs, MA, USA) and analyzed using refractive index (RI) and right-angle light scattering (RALS, 670 nm, 90°) detectors. The column temperature was 60 °C, the detector temperature was 40 °C, and the flow rate was 0.8 ml/min.

3.3. Ultraviolet–visible light (UV–Vis) spectroscopy

The oxidation progress was monitored by observing periodate consumption during oxidation based on the absorbance intensity of periodate at 290 nm using UV–Vis spectroscopy (Cary 60 UV–Vis spectrophotometer); Agilent, USA; (Maekawa et al., 1986). The intensity was used to quantify how much reactant was consumed according to a calibration curve for the sodium periodate solution, which was translated into a DO using Eq. (1) (Amer et al., 2016; Malaprade, 1928):

$$\%\mathrm{DO} = \frac{\text{mole of periodate consumed}}{\text{mole of AXU}} \times 100 \qquad (1)$$

3.4. Nuclear magnetic resonance (NMR) spectroscopy

Solid-state NMR spectroscopy was performed on a Bruker 400 MHz dynamic nuclear polarization (DNP) operating at 100.6 MHz for ^{13}C with a 3.2-mm solid-state magic-angle-spinning (MAS) probe head (Fällanden, Switzerland). Measurements were conducted at 298 K with a

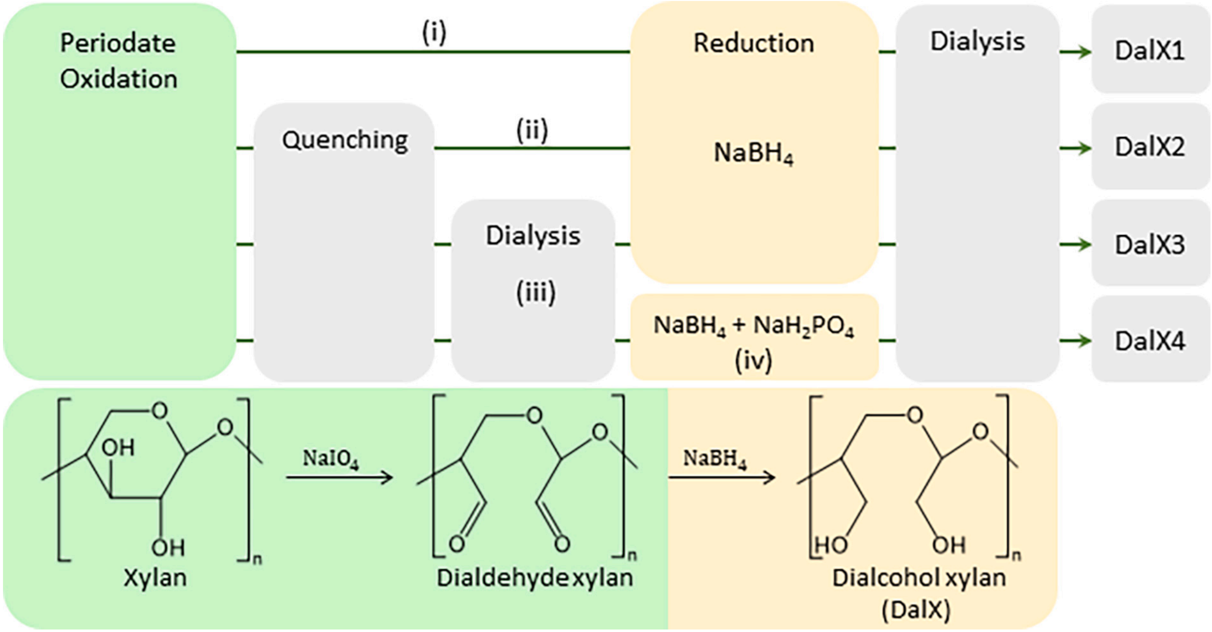

Fig. 1. Oxidation, reduction, and purification sequences for preparing dialcohol xylans via periodate oxidation and sodium borohydride reduction.

MAS spinning rate of 8 kHz. One-dimensional ^{13}C cross-polarization magic-angle spinning (CP/MAS) spectra were acquired with a 3.0 ms ^{1}H 90° pulse, 1500 ms CP contact time, 33 ms acquisition time with proton decoupling, a 5-s recycle delay, and 2048 acquisitions. The number of acquisitions of CP/MAS spectra was 2048 times with ^{13}C in natural abundance. Chemical shifts were referenced to tetramethylsilane, using the carbonyl resonance of α-glycine at 176.5 ppm as a secondary external reference.

Solution-state NMR experiments were performed at 298 K on an Oxford 800 MHz ^{1}H frequency Bruker AvanceIII HD spectrometer equipped with a TXO cryoprobe (Karlsruhe, Germany) using solutions of 20 mg/ml xylans in DMSO-d_6. The ^{1}H and ^{13}C sequential assignments were obtained using standard pulse sequences of HSQC, COSY, and HMBC with deuterium decoupling. The chemical shifts were referenced to the methyl groups of DMSO on the tetramethylsilane scale (39.52/2.50 ppm δ^{13}C/δ^1H). The spectra were processed and analyzed using MestReNova (version 14.2, Mestrelab Research, Santiago de Compostela, Spain). All spectra were normalized to unity with respect to the full integral intensity at 50–110 ppm, a frequency range encompassing all five ring carbons.

The degree of glucuronic acid side groups per xylan monomer, DGA$_{\text{NMR}}$, was determined and calculated using the signal intensity integral of the C4 of glucuronic acid side units and the total integral of all signals corresponding to the C1 carbon, according to the following equation (Eq. (2)):

$$\text{DGA}_{\text{NMR}} = \frac{\int \text{C4s}_{\text{xylan}}}{\int \text{C1}_{\text{xylan}}} \tag{2}$$

The DO was determined and calculated using the total area assigned to the intact C3 peak of the backbone repeating unit, according to Eq. (3):

$$\text{DO}_{\text{NMR}} = \frac{\int \text{C3}_{\text{dialcohol xylan}}}{\int \text{C3}_{\text{xylan}}} \tag{3}$$

3.5. *Dynamic light scattering (DLS)*

The hydrodynamic diameters (D_h) of oxidized xylans in solution were determined with a particle size analyzer (Zetasizer® Nano ZS90; Malvern Panalytical, UK). Xylan and dialcohol xylans were dissolved in Milli-Q® water (Merck KGaA, Darmstadt, Germany) at concentrations of 0.5, 1, and 2 wt%. The solutions were filtered using a 0.45 μm PTFE syringe filter before analysis. The refractive indexes of xylan and dialcohol xylans were determined using a refractometer (Abbemat 550; Anton Paar, Austria). The measurements were performed at 22 °C with a 120 s calibration time. The Stokes–Einstein equation was used to calculate D_h (Eq. (4)):

$$D_\text{h} = \frac{\text{k}T}{3\pi\eta D} \tag{4}$$

where k is Boltzmann's constant, T is the absolute temperature, η is viscosity, and D is a translational self-diffusion coefficient. D and the number distribution of D_h were estimated using the autocorrelation function. The viscosity was that of water at 22 °C (0.954 cPa), as given by the Zetasizer software.

4. Results and discussion

4.1. *Characteristics of xylan*

The xylan contained 96% xylose, <2% glucose, <2% galactose, and <1% arabinose as determined by HPAEC-PAD. It also contained approximately 12% glucuronic acid side groups, as determined by NMR analysis using Eq. (2), close to the value provided by the manufacturer. Xylan was found to be completely amorphous, based on the solid-state NMR spectrum, which was compared to previously published assignments of amorphous and crystalline xylan (Meng et al., 2021). This observation was further supported by a comparison of solid- and solution-state NMR spectra. The chemical shift positions (particularly those of the well-resolved C1 and C5 carbons) were similar in both solid and solution states, reflecting the distribution of conformations in the former, unlike for crystalline structures.

4.2. *Periodate oxidation of xylan and analysis of the degree of oxidation*

A DO of 84%, determined by monitoring UV-absorbance intensity due to the consumption of periodate, was reached after 24 h. Periodate consumption was fast in the beginning and then slowed down (Fig. 2a). Considering the amount of periodate added, the reaction did not consume it all within 24 h, perhaps because, as Amer et al. (2016)

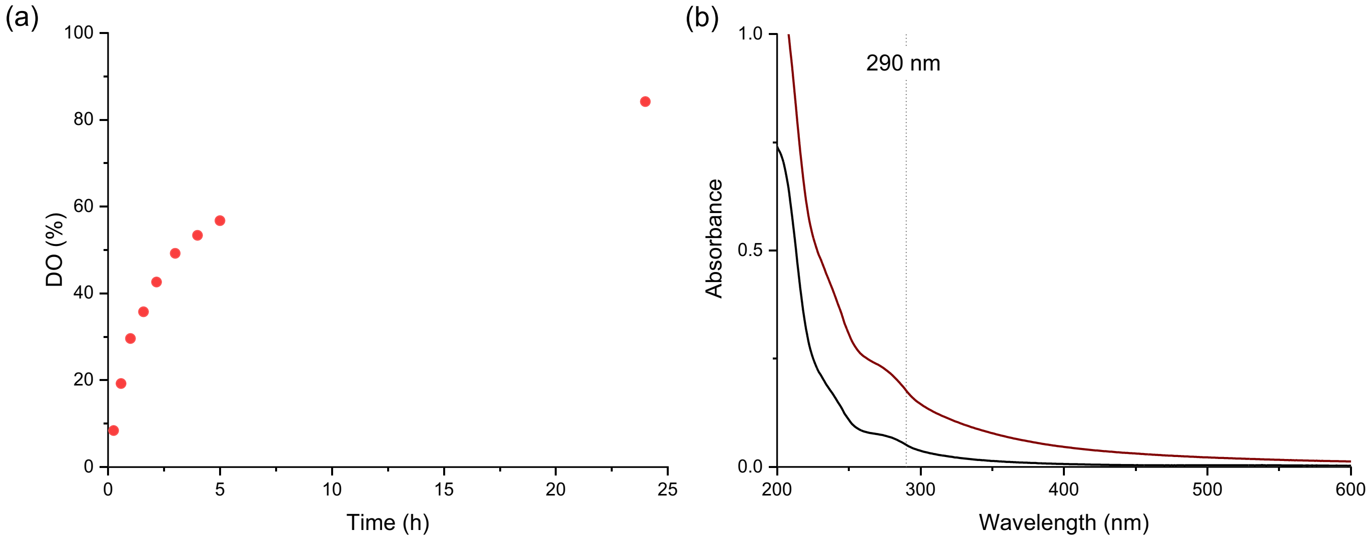

Fig. 2. (a) Degree of oxidation of xylan determined via periodate consumption monitoring using UV absorbance at 290 nm; (b) UV–Vis spectra of xylan (brown) and DalX1 product (black) solubilized in water. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

suggested, part of the newly generated dialdehyde xylan converted to its hydrated structure, which could form hemiacetal linkages and prevent periodate from cleaving C2-C3 bonds.

We highlight a challenge in the analysis of DO by monitoring periodate consumption. Xylans with a low glucuronic acid group and other monosaccharide side group content can be solubilized in water only partially or not at all. A typical approach for monitoring oxidation with UV absorbance is to filter the xylan solution to prevent the xylan from contributing to the analysis. Optimally, all xylan is filtered away, and only the unreacted periodate is analyzed; however, since some xylan is solubilized in the water, a fraction can pass through the filter, contributing to the absorbance. This contribution can be subtracted using unmodified xylan absorbance. However, as oxidation proceeds, oxidized xylan is also generated, which adds another fraction that may contribute to the absorbance. The system becomes complex when more and less solubilized xylan fractions and the oxidized fraction are present, and the value for deriving the DO becomes obscured because the baseline to be subtracted cannot be precisely determined.

Here, we demonstrate how the DO was determined without filtering the xylan fractions from the analyte (Fig. 2a). The xylan background absorbance was subtracted from the spectra at selected time intervals during oxidation on the assumption that it would not change as the DO proceeds. However, the background spectra were affected during oxidation, as can be observed based on spectra in Fig. 2b, showing that the DalX1 background had a lower absorbance intensity than xylan with the same concentration. The intensity difference corresponded to around 8.5% DO. Considering that the yield of the oxidized product was lower than that of the starting material, the actual absorbance may have been even lower. In addition, reflectance that may occur from particulate matter contributes to the absorbance intensity, hence the observed absorbance intensity is a sum of the absorbance and reflectance contributions. This further amplifies the overestimation of the consumption of the reactant, and hence, the DO. Therefore, there is an inherent overestimation of the DO of the final products. The extent of misestimation depends on the xylan's initial degree of water solubilization and its development, and the value presented here should not be generalized. An overestimation of DO was also seen in the periodate oxidation of cellulose (Kim & Kuga, 2001). Monitoring periodate consumption is useful in terms of ease of measurement, studying the kinetics of the reaction, and allowing to choose a time point to quench the oxidation to a selected degree. However, based on the observations here, a complementary analysis of DO is required. Other techniques used for determining DO are titration (Babor et al., 1973; Chemin et al., 2016; Zhao & Heindel, 1991), IR spectroscopy for quantifying the aldehydes and their derivates (Simon et al., 2022) and NMR spectroscopy (Maekawa, 1991).

4.3. Reduction of dialdehyde xylan to dialcohol xylan

Xylan was modified following four pathways (Fig. 1): i) oxidation and direct reduction without quenching the remaining reactant, ii) quenching of the periodate residual prior to reduction, iii) quenching and purifying before reduction, and iv) controlling the pH (9.4) of the reduction to minimize degradation. Using the shortest pathway (producing DalX1), moving directly from oxidation to reduction without quenching the reactant, any excess IO_4^- and IO_3^- by-product remains in the solution during reduction. Besides IO_4^- potentially reacting with $NaBH_4$ in the reduction step (Lyttle et al., 1952), IO_3^- could also have consumed $NaBH_4$. Moreover, if the concentration of IO_3^- and IO_4^- was higher than that of the oxidized product, they could consume most of the reductant, and hence the dialdehyde xylan would be only partially reduced. When the excess IO_4^- was consumed by the addition of ethylene glycol via the second pathway (producing DalX2), the ethylene glycol was oxidized and was present in the solution. Since this pathway involved no dialysis step, the oxidized ethylene glycol may have consumed the reductant along with the dialdehyde xylan. Using the third pathway (producing DalX3), the reactants were removed after quenching, and no diols other than xylan were present in the reduction step. As degradation was foreseen during $NaBH_4$ reduction if pH was not controlled (Börjesson et al., 2019), using the fourth pathway (producing DalX4), NaH_2PO_4 was added to limit the pH change during a reduction step that also allowed for reduced reaction time (Börjesson et al., 2019).

NMR spectroscopy was used here to determine the DO of the xylan backbone repeating unit (Eq. (3)). The C3 ^{13}C NMR signal of the unmodified xylan backbone monosaccharide ring turned out to be spectrally well resolved when dissolved in DMSO-d_6, not only for pure xylan but also for the modified grades (Fig. 3a). As highlighted in Fig. 3b-c, the C3 chemical shift of 74.47 ppm for xylan was downfield shifted to 75.14 ppm due to the oxidation and reduction steps. The integral regions based on Eq. (3) were 74.0–75.0 and 74.75–75.6 ppm for xylan and dialcohol xylan, respectively. The error in this procedure was estimated by comparing the integral intensity of the C1 region at 96–106 ppm with the different measurements. The error estimation was found to be 1.3% of C1 intensity variability (using a 95% confidence interval), and the DO

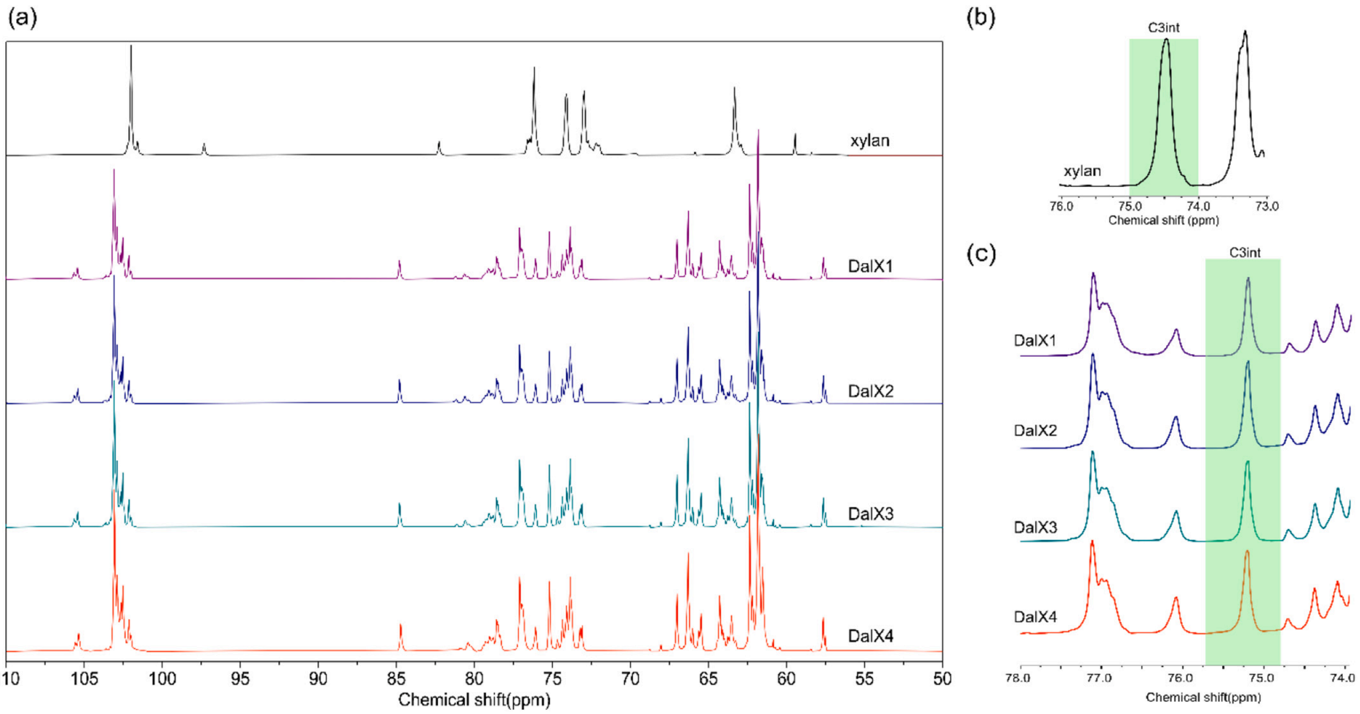

Fig. 3. a) Quantitative ^{13}C NMR spectra of xylan and DalX1–4, and the integral of total area at the intact C3 position b) between 74 and 75 ppm in xylan and c) between 74.75 and 75.6 ppm in DalX1–4. The green mask in b) and c) indicates the integral region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

values for the backbone are provided in Table 1. All the routes resulted in a DO between 77 and 80%. The values are lower than the estimation from observing reactant consumption via UV–Vis and support the hypothesis of it overestimating the DO. It should be noted that possible oxidation of glucuronic acid side groups and their connecting backbone monosaccharide were not considered here.

4.4. Molecular characteristics of dialcohol xylan in solution

The modification of xylan by cleaving the monosaccharide ring is known to involve some degree of depolymerization (Amer et al., 2016; Börjesson et al., 2018; Chemin et al., 2016). Given the long reaction time of 24 h and the relatively high periodate:AXU ratio (1:1), it is unsurprising that some degradation of the xylan occurred, as indicated by the reduction of both M_w and M_n and an increase in the dispersity of the modified grades (Table 2). Interestingly, a large degree of degradation, as seen previously with similar periodate:AXU ratios after 6 h, (Chemin et al., 2016) was not observed. The quenching and dialysis steps appeared to make little difference to the extent of degradation. Meanwhile, buffering the reduction step seemed to increase the dispersity of the end product compared to the unbuffered conditions. We note that with each grade we also detect a fraction of small molecules that are below 180 Da. This fraction is present even within the starting material and hence cannot be explained to result from the modification.

The unmodified xylan solubilized in water was visibly yellowish and turbid. Turbid dispersions indicate micrometer-sized structures that scatter light. In general, it is thought that the solubilization of xylan in water is poor owing to its linear stiff backbone and ribbon-like conformation. The ribbon-like conformation promotes the close packing and interchain bonding of xylan chains, and the compact polymer structure reduces interactions with water (Guo et al., 2017). The dialcohol xylans solubilized in water were colorless and translucent independently of the modification route. The modification increased the ability to solubilize xylan in water, possibly due to the decreasing molecular weight and the opening of the monosaccharide ring. The D_h of the molecules was determined in water and as a function of xylan and dialcohol xylan concentrations. The number average D_h of the xylan and dialcohol xylans in water was in the range of 21–26 nm at a 0.5 wt% concentration (Fig. 4a), with unmodified xylan having the highest D_h. Increasing the xylan concentration to 1 wt% and 2 wt% led to an increase in D_h to 44 nm and 52 nm, respectively. Large aggregates were removed by filtering before measurement; therefore, the D_h values of xylan, as shown in Fig. 4a, represent only the fractions that were retained. The attempted D_h determination for xylan at a 2 wt% concentration without filtering resulted in a large deviation between repetitions, although large aggregates were not detected. The upper detection limit of the instrument was 10 μm.

Increasing the dialcohol xylan concentration to 1 and 2 wt% did not lead to a statistically significant increase in D_h, except for DalX1, with a DalX1 increase to 32 nm. The unchanged D_h of the dialcohol xylans (DalX2–4) as a function of concentration, compared to the increase in D_h of the xylan, indicated that the modified grades had a lower tendency to aggregate, and thus were better solubilized in water. Kishani et al. (2019) observed that the D_h of arabinoglucuronoxylan was <10 nm at a

Table 1
Summary of the degree of oxidation of dialcohol xylans.

Grade	DO_{NMR} in backbone (%)	DO_{UV} (%)
DalX1	77	84
DalX2	80	84
DalX3	77	84
DalX4	79	84

Table 2
Molecular weight and dispersity (M_w/M_n) of xylan and dialcohol xylans.

	Xylan	DalX1	DalX2	DalX3	DalX4
M_n (kDa)	18.5	9.3	9.6	9.5	8.2
M_w (kDa)	23.8	18.7	18.2	18.3	18.0
M_w/M_n	1.3	2.0	1.9	1.9	2.2

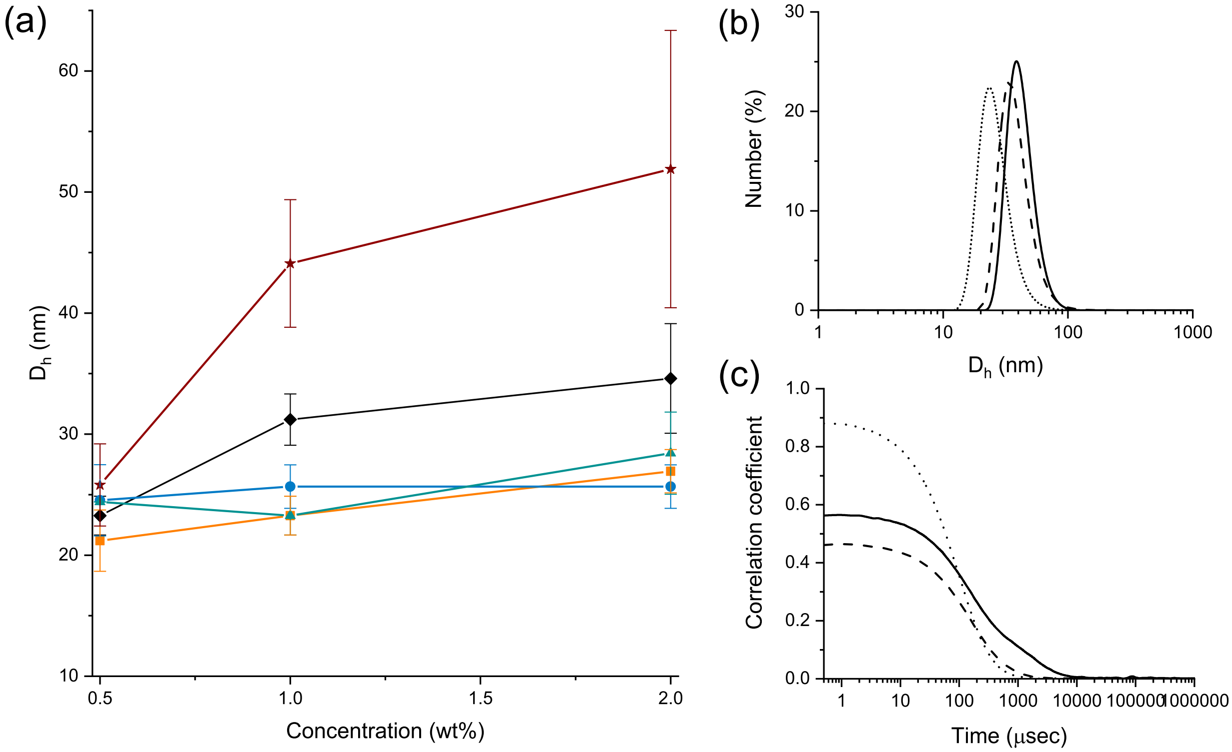

Fig. 4. a) Hydrodynamic diameters of xylan (★), DalX1 (◆), DalX2 (■), DalX3 (●), and DalX4 (▲), solubilized in water at concentrations of 0.5 wt%, 1 wt%, and 2 wt%; b) D_h by number distribution of DalX1 at concentrations of 2 wt% (solid line), 1 wt% (dashed line), and 0.5 wt% (dotted line); c) autocorrelation curves for 2 wt% (solid line), 1 wt% (dashed line), and 0.5 wt% (dotted line) DalX1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

0.1 wt% concentration, the D_h increased to >60 nm at a 1.5 wt% concentration. The dialcohol xylans obtained here exhibited a smaller increase in D_h over a similar concentration range. This implies a lower tendency of aggregation of dialcohol xylans. The solubilization of the xylan appear more complex than only a matter of molecular weight or number of side units alone, but that the cleaving modification could be used to increase solubilization at higher concentrations.

The reduction pathways had little influence on the D_h for the reduction routes studied (DalX2, DalX3, and DalX4), except for DalX1. Distribution curves for DalX1 (Fig. 4b) illustrated low dispersity; however, at a 2 wt% concentration, DalX1 included a second population that was detected in the autocorrelation curves (Fig. 4c).

5. Conclusion

Quenching excess periodate, purification before reduction, and buffering the reduction pH did not significantly impact the degree of modification and molar mass of dialcohol xylan, allowing the most practical route to be selected. All selected oxidation and reduction routes, resulted in modified xylans that could be solubilized in water. There was an improvement in solubilization compared to the unmodified xylan, which was only partly solubilized, and that fraction also exhibited moieties with larger hydrodynamic radii than those of the modified grades. Furthermore, the unchanged hydrodynamic radius of the dialcohol xylans as the concentration increased indicated that the modified grades had a lower tendency to aggregate and were thus better solubilized in water compared to the unmodified xylan. The improved solubilization in water was due to the cleavage of the monosaccharide units in the backbone and a reduction in molar mass. Hence, we add a further variable to the puzzle of xylan solubilization that so far has been governed by the molecular weight, the number and kind of side units, and interchain interactions. Our focus here on the work up procedure allows to develop efficient processing for manufacturing of the dialcohol xylan grades.

CRediT authorship contribution statement

CP: Experimental work and writing the original draft. KN: NMR Spectroscopy measurements and analysis, writing, reviewing, editing. FA: SEC measurements and analysis, writing, reviewing, editing. KM: SEC analysis, writing, reviewing, editing. LE: NMR Spectroscopy analysis, writing, reviewing, editing. AS: Conceptualization, writing, reviewing, editing. TN: Conceptualization, writing, reviewing, editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We appreciate the receipt of the Swedish Research Council grant (registration number 2017-05138), SNS Nordic Forest Research grant (grant number SNS-127) and thank the Wallenberg Wood Science Center and the Materials Science Area of Advance at Chalmers for funding. We also thank the Swedish NMR Centre at the University of Gothenburg for its support. We thank Anette Larsson for discussions regarding UV–Vis spectroscopy analytics. Finally, we acknowledge the Division of Forest Products and Chemical Engineering at Chalmers for access to carbohydrate analysis.

References

Amer, H., Nypelö, T., Sulaeva, I., Bacher, M., Henniges, U., Potthast, A., & Rosenau, T. (2016). Synthesis and characterization of periodate-oxidized polysaccharides: Dialdehyde xylan (DAX). *Biomacromolecules, 17*(9), 2972–2980.

Babor, K., Kaláč, V., & Tihlárik, K. (1973). Periodate oxidation of saccharides. III. Comparison of the methods for determining the consumption of sodium periodate and the amount of formic acid formed. *Chemical Papers, 27*(5), 676–680.

Bobbitt, J. (1956). Periodate oxidation of carbohydrates*, 11. Advances in carbohydrate chemistry* (pp. 1–44). Academic Press.

Börjesson, M., Larsson, A., Westman, G., & Ström, A. (2018). Periodate oxidation of xylan-based hemicelluloses and its effect on their thermal properties. *Carbohydrate Polymers, 202*, 280–287.

Börjesson, M., Westman, G., Larsson, A., & Ström, A. (2019). Thermoplastic and flexible films from arabinoxylan. *ACS Applied Polymer Materials, 1*(6), 1443–1450.

Chemin, M., Rakotovelo, A., Ham-Pichavant, F., Chollet, G., da Silva Perez, D., Petit-Conil, M., Cramail, H., & Grelier, S. (2015). Synthesis and characterization of functionalized 4-O-methylglucuronoxylan derivatives. *Holzforschung, 69*(6), 713–720.

Chemin, M., Rakotovelo, A., Ham-Pichavant, F., Chollet, G., Da Silva Perez, D., Petit-Conil, M., Cramail, H., & Grelier, S. (2016). Periodate oxidation of 4-O-methylglucuronoxylans: Influence of the reaction conditions. *Carbohydrate Polymers, 142*, 45–50.

Ebringerova, A., & Heinze, T. (2000). Xylan and xylan derivatives– Biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromolecular Rapid Communications, 21*(9), 542–556.

Ebringerova, A., Hromadkova, Z., & Heinze, T. (2005). Hemicellulose. In T. Heinze (Ed.), *Polysaccharides 1* (pp. 1–67). Berlin: Springer.

Escalante, A., Goncalves, A., Bodin, A., Stepan, A., Sandstrom, C., Toriz, G., & Gatenholm, P. (2012). Flexible oxygen barrier films from spruce xylan. *Carbohydrate Polymers, 87*(4), 2381–2387.

Fu, G.-Q., Zhang, S.-C., Chen, G.-G., Hao, X., Bian, J., & Peng, F. (2020). Xylan-based hydrogels for potential skin care application. *International Journal of Biological Macromolecules, 158*, 244–250.

Gabrielii, I., & Gatenholm, P. (1998). Preparation and properties of hydrogels based on hemicellulose. *Journal of Applied Polymer Science, 69*(8), 1661–1667.

Gabrielii, I., Gatenholm, P., Glasser, W. G., Jain, R. K., & Kenne, L. (2000). Separation, characterization and hydrogel-formation of hemicellulose from aspen wood. *Carbohydrate Polymers, 43*(4), 367–374.

Gordobil, O., Egüés, I., Urruzola, I., & Labidi, J. (2014). Xylan–cellulose films: Improvement of hydrophobicity, thermal and mechanical properties. *Carbohydrate Polymers, 112*, 56–62.

Grondahl, M., Eriksson, L., & Gatenholm, P. (2004). Material properties of plasticized hardwood xylans for potential application as oxygen barrier films. *Biomacromolecules, 5*(4), 1528–1535.

Guo, M. Q., Hu, X., Wang, C., & Ai, L. (2017). Polysaccharides: Structure and solubility. In *Solubility of polysaccharides* (pp. 7–21). InTech.

Hansen, N. M. L., Blomfeldt, T. O. J., Hedenqvist, M. S., & Plackett, D. V. (2012). Properties of plasticized composite films prepared from nanofibrillated cellulose and birch wood xylan. *Cellulose, 19*(6), 2015–2031.

Kim, U.-J., & Kuga, S. (2001). Thermal decomposition of dialdehyde cellulose and its nitrogen-containing derivatives. *Thermochimica Acta, 369*(1–2), 79–85.

Kishani, S., Escalante, A., Toriz, G., Vilaplana, F., Gatenholm, P., Hansson, P., & Wagberg, L. (2019). Experimental and theoretical evaluation of the solubility/insolubility of spruce xylan (Arabino glucuronoxylan). *Biomacromolecules, 20*(3), 1263–1270.

Köhnke, T., Elder, T., Theliander, H., & Ragauskas, A. J. (2014). Ice templated and cross-linked xylan/nanocrystalline cellulose hydrogels. *Carbohydrate Polymers, 100*, 24–30.

Kristiansen, K. A., Potthast, A., & Christensen, B. E. (2010). Periodate oxidation of polysaccharides for modification of chemical and physical properties. *Carbohydrate Research, 345*(10), 1264–1271.

Larsson, P. A., & Wagberg, L. (2016). Towards natural-fibre-based thermoplastic films produced by conventional papermaking. *Green Chemistry, 18*(11), 3324–3333.

Leguy, J., Diallo, A., Putaux, J.-L., Nishiyama, Y., Heux, L., & Jean, B. (2018). Periodate oxidation followed by NaBH4 reduction converts microfibrillated cellulose into sterically stabilized neutral cellulose nanocrystal suspensions. *Langmuir, 34*(37), 11066–11075.

Lyttle, D., Jensen, E., & Struck, W. (1952). Simple volumetric assay for sodium borohydride. *Analytical Chemistry, 24*(11), 1843–1844.

Maekawa, E. (1991). Analysis of oxidized moiety of partially periodate-oxidized cellulose by NMR spectroscopy. *Journal of Applied Polymer Science, 43*(3), 417–422.

Maekawa, E., Kosaki, T., & Koshijima, T. (1986). Periodate oxidation of mercerized cellulose and regenerated cellulose. In *, Vol. 73. Wood research: Bulletin of the Wood Research Institute Kyoto University* (pp. 44–49). Wood Research Institute, Kyoto University.

Malaprade, L. (1928). Oxidation of some polyalcohols by periodic acid—Applications. *Comptes Rendus, 186*, 382–384.

Meng, Z., Sawada, D., Laine, C., Ogawa, Y., Virtanen, T., Nishiyama, Y., Tammelin, T., & Kontturi, E. (2021). Bottom-up construction of xylan nanocrystals in dimethyl sulfoxide. *Biomacromolecules, 22*(2), 898–906.

Nypelö, T., Berke, B., Spirk, S., & Sirviö, J. A. (2021). Review: Periodate oxidation of wood polysaccharides-modulation of hierarchies. *Carbohydrate Polymers, 252*, Article 117105.

Nypelö, T., Laine, C., Aoki, M., Tammelin, T., & Henniges, U. (2016). Etherification of wood-based hemicelluloses for interfacial activity. *Biomacromolecules, 17*(5), 1894–1901.

Painter, T., & Larsen, B. (1970). Transient hemiacetal structures formed during the periodate oxidation of xylan. *Acta Chemica Scandinavica, 24*(7), 2366–2378.

Palasingh, C., Ström, A., Amer, H., & Nypelö, T. (2021). Oxidized xylan additive for nanocellulose films – A swelling modifier. *International Journal of Biological Macromolecules, 180*, 753–759.

Pandeirada, C. O., Achterweust, M., Janssen, H.-G., Westphal, Y., & Schols, H. A. (2022). Periodate oxidation of plant polysaccharides provides polysaccharide-specific oligosaccharides. *Carbohydrate Polymers, 291*, 119540. https://doi.org/10.1016/j.carbpol.2022.119540. In this issue.

Simon, J., Tsetsgee, O., Iqbal, N. A., Sapkota, J., Ristolainen, M., Rosenau, T., & Potthast, A. (2022). A fast method to measure the degree of oxidation of dialdehyde celluloses using multivariate calibration and infrared spectroscopy. *Carbohydrate Polymers, 278*, Article 118887.

Sixta, H. (2006). *Handbook of pulp*. Wiley-vch.

Theander, O., & Westerlund, E. A. (1986). Studies on dietary fiber. 3. Improved procedures for analysis of dietary fiber. *Journal of Agricultural and Food Chemistry, 34*(2), 330–336.

Vold, I. M. N., & Christensen, B. E. (2005). Periodate oxidation of chitosans with different chemical compositions. *Carbohydrate Research, 340*(4), 679–684.

Zeronian, S., Hudson, F., & Peters, R. (1964). The mechanical properties of paper made from periodate oxycellulose pulp and from the same pulp after reduction with borohydride. *Tappi, 47*, 557–564.

Zhao, H., & Heindel, N. D. (1991). Determination of degree of substitution of formyl groups in polyaldehyde dextran by the hydroxylamine hydrochloride method. *Pharmaceutical Research, 8*(3), 400–402.