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Targeted Functionalization of Spruce *O*-Acetyl Galactoglucomannans—2,2,6,6-Tetramethylpiperidin-1-oxyl-oxidation and Carbodiimide-Mediated Amidation

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ABSTRACT: Modified spruce *O*-acetyl galactoglucomannans (GGMs) can be used as molecular anchors to alter the surface properties or to activate the surface of cellulose. To selectively introduce functionalities, GGMs were oxidized on C-6 of hexoses by 2,2,6,6-tetramethylpiperidin-1-oxyl-mediated oxidation. Different degrees of oxidation were successfully obtained by varying the reaction parameters. Low degrees could be obtained by performing the oxidations in bromide-free conditions. The formed uronic acids were further modified by a carbodiimide-mediated amidation reaction, which opens a window for introducing various functionalities selectively on hexoses. The adsorption of the modified GGMs to various cellulose samples was investigated. Indirect bulk sorption to fibers was compared to direct adsorption to nanofibrillated cellulose ultrathin films. GGMs with low degrees of oxidation showed high affinity to cellulose surfaces and could be sorbed onto cellulose in pure water. Moderate amounts of GGMs with high degree of oxidation could be sorbed onto cellulose in the presence of salts. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 3122–3129, 2013

KEYWORDS: polysaccharides; functionalization of polymers; synthesis and processing; adsorption

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INTRODUCTION

Wood and plant hemicelluloses are potential starting materials for environmentally sustainable industrial processes and renewable materials. Polysaccharides are already widely used in, for example, the cosmetic, food, and pharmaceutical industries. A wood-derived polysaccharide that is of interest especially for the Nordic forest industry is *O*-acetyl galactoglucomannan (GGM). GGM is the main hemicellulose in industrially important softwood species and can be isolated in industrial scale from the process waters of mechanical pulping.[1] GGM has a backbone consisting of (1 → 4)-linked β-D-mannopyranosyl (Man) and β-D-glucopyranosyl (Glc) units, and single (1 → 6)-linked α-D-galactopyranosyl (Gal) units are attached to some of the mannose units. Part of the mannose units are acetylated at either two- or three-position, the degree of acetylation (DA) being ~0.3. GGM naturally has a high affinity toward cellulose, and modified GGMs can be used, for instance, for the modification of cellulosic surfaces.[2,3] The degree of sorption of GGM to bleached kraft pulp (BKP) can be increased by some chemical modifications, such as deacetylation and removal of galactose side-chains,[2] whereas introduction of anionic groups, such as carboxylic acids or carboxymethyl groups, decreases the sorption.[2,4,5] Despite that it lowers the tendency to adsorb onto cellulose, carboxylation of GGM is an interesting pathway for the modification of polysaccharides. Not only does the adsorption allow introduction of charged groups to fibers, the carboxylic acids can also be further functionalized with.[6] Other anionic polysaccharides, such as carboxymethyl cellulose (CMC), have been added to chemical pulp to improve the tensile strength of paper[7] and to be used as molecular anchors for the introduction of bioactive molecules to cellulose.[8,9]

The nitroxyl radical 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO) can catalyze the selective oxidation of primary alcohols to carbonyl compounds. De Nooy et al.[10] showed that the TEMPO-mediated system is also selective to primary alcohols in carbohydrates. TEMPO-mediated oxidation has been used for the oxidation of several different polysaccharides, such as cellulose, starch, and pullulan.[11] Recently, it has been shown that

TEMPO-mediated oxidation is an efficient method for the carboxylation of spruce GGM.[3] When using the commonly applied procedure using sodium bromide (NaBr) and sodium hypochlorite (NaClO) as cooxidants, high degrees of oxidation are obtained within minutes after addition of reagents, the maximum degree of oxidation (~80%) is reached in 50 min, and no significant degradation of the polysaccharide can be observed.

NaBr and NaClO function as regenerating oxidants: the *in situ*-formed hypobromite regenerates TEMPO to the active form, but the active species of TEMPO can also be regenerated by direct oxidation by hypochlorite.[12] Brochette-Lemoine et al.[13] showed that the primary alcohols of carbohydrates could also be oxidized without the use of NaBr, although the rate of oxidation was low. The NaBr-free oxidation has been applied on different polysaccharides.[12] Because the rate of the bromide-free reaction is lower than that of the normal bromide-containing one, this procedure is applicable when aiming at low degrees of oxidation (DO). Another benefit of the bromide-free method is a more economical and environmentally friendly oxidation procedure when using fewer chemicals. Ma et al.[14] studied controlled TEMPO-mediated oxidation of polysaccharides. By adjusting the amount of reactants, different degrees of oxidation were obtained. However, the authors concluded that the amounts of reactants needed for different polysaccharides could not be extrapolated from the results of a certain polysaccharide. Thus, optimization of the reaction parameters needs to be done separately for each polysaccharide.

The TEMPO-oxidized polysaccharides can be further derivatized, for instance, by coupling of amines to the formed carbonyl groups. A commonly used method is the carbodiimide-mediated amidation, where the coupling of amines to carboxylic acids through amide bonds is activated by *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide (EDC) and *N*-hydroxysuccidimide (NHS). One benefit of the carbodiimide-mediated reaction compared to other amidation reactions is that no organic solvents are needed, and thus water-soluble polysaccharides can be amidated with water-soluble amines in mild conditions in aqueous solutions. New biopolymers and biologically active materials have been developed by amidation of, for example, TEMPO-oxidized cellulose, CMC, chitin, and chitosan.[8,9,15–23]

In this work, we present a method for controlled TEMPO-mediated oxidation of spruce GGMs. The carboxyl groups were further amidated using arginine and the 1,6-diaminohexane. The adsorption of the modified GGMs to cellulose and nanofibrillated cellulose (NFC) surfaces was investigated.

MATERIALS AND METHODS

All chemicals were of commercial grade. TEMPO and 1,6-diaminohexane were purchased from Merck (Hohenbrunn, Germany), NaClO solution (available chlorine 10–15%), EDC (purum $\geq$ 98.0%), and NHS (98%) from Sigma-Aldrich (Steinheim, Germany). Arginine was purchased from Merck (Darmstadt, Germany).

GGM was prepared from spruce thermomechanical pulp (TMP) by a large laboratory-scale method modified from the method reported by Willför et al.[1] In short, a suspension of TMP in hot tap water was stirred for 3 h, and the pulp was removed. The extract water was purified from colloidal wood resin and aromatic residues using a cationic coagulant (Raifix 120, Raisio Chemicals Oy, Finland) and XAD-7 resin (Amberlite, Rohm and Haas, UK). The water was concentrated by rotary evaporation before GGM was isolated by precipitation in ethanol and air-dried.

Analytical Methods

The carbohydrate content was determined by gas chromatography (GC) and by gas chromatography and mass spectroscopy (GC–MS) after methanolysis and silylation.[24,25] GC analysis was done on a PerkinElmer AutoSystemXL Instrument (Norwalk, USA) equipped with an HP-1 column. The temperature program used was 100–175°C, 4°C/min, 175–290°C, 12°C/min. Injector 260°C; detector 290°C. GC–MS was done on an HP 6890-5973 GC-MSD instrument equipped with an HP-1 column. The temperature program used was 80°C (0.5 min) – 300°C at 8°C/min.

Acetyl groups released from GGM were determined in the form of acetic acid by high-pressure liquid chromatography (HPLC) with a refractive index (RI) detector (Shimadzu Corporation, Japan). The original GGM was first deacetylated by alkali treatment at pH 11 at 60°C for 2 h. The deacetylated GGM solution was titrated to pH 2.7–2.9 by 3% H_3PO_4 and then sampled for HPLC analysis. A Synergi 4u hydro-RP 80A 250 × 4.60 mm column (Phenomenex, CA) equipped with a guard column was used. About 0.02*M* KH_2PO_4 was used as the elution solvent at a flow rate of 0.5 mL/min. The samples were filtered through a 0.22-μm nylon syringe filter before injection. The injection volume was 100 μL.

Weight–average molar mass, M_w, and number–average molar mass, M_n, were determined by size-exclusion chromatography (SEC) in online combination with a multiangle laser-light-scattering instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA) and with a RI detector (Shimadzu Corporation, Japan). A two-column system, 2× UltrahydrogelTM linear 7.8 × 300 mm column (Waters, Milford, USA), in series was used. About 0.1*M* $NaNO_3$ was used as the elution solvent at a flow rate of 0.5 mL/min. The injection volume was 100 μL. Astra software (Wyatt Technology, Santa Barbara, USA) was applied to analyze data.

FTIR spectra were recorded on an FTIR spectrophotometer (Bruker ALPHA series) using a KBr disc containing 100–120 mg of dried KBr and about 1–5 mg sample. The spectra were obtained in the frequency range of 4000–400 cm^{-1} at a resolution of 2 cm^{-1} in the transmittance mode.

NMR spectra of the modified GGMs were recorded on a Bruker AV 600 instrument. D_2O was used as solvent.

TEMPO-Mediated Oxidation of GGM

GGM (200 mg) was dissolved in distilled water (40 mL). The oxidation was performed either at room temperature or at 2–4°C. The influence of NaBr was evaluated by performing the oxidation either with or without NaBr. TEMPO (2 mg), NaClO (10–15% solution, pH adjusted to 10, 4 mL), and, in some

cases, NaBr (300 mg) were added. The pH of the solution was kept at 10 by adding 0.5*M* NaOH. After 120 min, the reaction solution was neutralized by the addition of 1*M* HCl. To remove salts and oxidation reagents, the TEMPO-oxidized GGM (GGM_{PolyU}) was dialyzed against distilled water for 3 days (membrane cut-off 12,000–14,000 g mol^{-1}). The purified products were freeze-dried.

The DO was determined by GC-analysis after methanolysis and silylation. During oxidation, samples (0.1 mL) were taken every 15 min, and they were directly precipitated in ethanol, isolated by centrifugation, washed a few times, and dried in a vacuum desiccator. The DO was calculated by comparing the amount of uronic acids to the total amount of sugars in the sample. For the determination of the DA, samples were taken every 15 min. To inhibit any further oxidation or deacetylation, the pH of the samples was directly adjusted to ~3, and the samples were kept frozen until analysis. Samples for the determination of the molar mass (0.5 mL) were directly precipitated in ethanol, washed, and finally dried in a vacuum desiccator. The samples were redissolved, reduced by $NaBH_4$, and filtered through a 0.22-μm nylon syringe filter before injection to the HPLC.

Amidation of Oxidized GGM

The amidation of GGM_{PolyU} was performed using a method reported earlier by Araki et al.[15] GGM_{PolyU} (150 mg, corresponding to ~0.8 mmol of uronic acids) was dissolved in distilled water (10 mL). NHS (140 mg, 1.2 mmol) was added, and the polysaccharide solution was stirred until the NHS was completely dissolved. The amine was added (1.5 equiv. relative to the amount of uronic acid groups), and the pH was adjusted to 7.5. EDC (220 mg, 1.2 mmol) was dissolved in distilled water (2 mL) and added dropwise to the reaction solution. The pH was kept at ~7.5 by the addition of NaOH (0.5 M), and the reaction was stirred at room temperature over night. Afterward, the pH was lowered to 2, and the solution was dialyzed for 2 days (membrane cut-off 12,000–14,000 g mol^{-1}). The product was finally freeze-dried.

Sorption of Modified GGMs onto Pulp Fibers

Fully bleached BKP (in dry lap form) was obtained from a Finnish pulp mill. The pulp was suspended in distilled water and homogenized by a household mixer. The pulp suspension, with a fiber concentration of 13%, was stored at −18°C. Aliquots of this suspension were used for the bulk-sorption experiments. Polysaccharide solution (~40 mg/g fiber) in distilled water was added to a suspension of BKP (100 mg o.d.), giving a final fiber concentration of 1%. The suspension was stirred overnight, after which the fibers were removed by centrifugation. The carbohydrate content of the supernatant was analyzed by GC after methanolysis and silylation. The amount of sorbed polysaccharides was calculated by subtracting the amount of polysaccharides left in the solution from the amount added. The effect of the ionic strength of the sorption medium was investigated by performing the sorption in water and in 0.01*M* and 0.1*M* NaCl. The samples were analyzed in duplicate or triplicate.

Adsorption of GGM onto NFC Ultrathin Films Monitored by Quartz Crystal Microbalance with Dissipation

The adsorption onto NFC ultrathin films was monitored using QCM-D (Q-Sense E4, Västra Frölunda, Sweden). NFC was prepared from fully bleached hardwood pulp, from which excess of salt had been removed[26] by disintegration in a Microfluidizer (M110P fluidizer, Microfluidics Corp, Newton, USA). Possible aggregated fibril bundles were removed by ultrasonification and centrifugation.[27] The nanofibrils were collected from the supernatant. An anchoring layer of poly(ethylene imine) was adsorbed onto silica-coated quartz crystals, and the adsorption of NFC (~0.17 g/L) onto these sensors was monitored in the QCM-D. Excess of NFC was removed by rinsing before adsorption of GGM samples. Solutions of GGM samples were prepared fresh before experiments and stirred for at least 2 h. All adsorption measurements were done in a clean room at ambient temperature of 24°C. The GGM samples were adsorbed from 0.5 g/L aqueous solutions at a flow rate of 100 μL/min. Fully oxidized GGM_{PolyU} was also adsorbed from 0.1*M* NaCl solution. Adsorption was monitored until no significant changes in frequency were observed, but for at least 1 h. Afterward, the loosely attached polysaccharides were removed by rinsing with either water or electrolyte solution. Measurements were repeated at least twice.

The frequency shift Δf of the quartz crystal resonator can be related to the adsorption of material on top of the quartz crystal. The basic relationship between frequency shift and the adsorbed mass Δm is given by the Sauerbrey equation

$$\Delta m_n = -\frac{C\Delta f}{n}, \tag{1}$$

where C is a device sensitivity constant (0.177 mg/m^2) for a quartz resonator at 5 MHz, and n is the number the used frequency overtone. The Sauerbrey equation holds only for rigid, sufficiently thin-adsorbed layers, and underestimates the adsorbed mass in the case of viscoelastic films.

In the case, the Sauerbrey conditions are not met, and the sensed mass was calculated from a linear extrapolation to zero frequency from a (f^2, Δm_n) dataset, where f is the overtone frequency, using overtones 3, 5, and 7. The method is justified by the equation originally given by Johansmann et al.[28]

$$\hat{m} = m_0\left(1+\hat{J}(f)\frac{d^2\rho f^2}{3}\right), \tag{2}$$

where $\hat{m}$ is the equivalent mass, ρ is the density of the fluid, d is the thickness of the film, $\hat{J}(f)$ the complex shear, and m_0 is the sensed mass. The equivalent mass Δm can be interpreted as the Sauerbrey mass Δm_n at overtone frequency f.

RESULTS AND DISCUSSION

TEMPO-Mediated Oxidation of GGM

GGM was oxidized to its polyuronic acid derivatives (GGM_{PolyU}) by TEMPO-mediated oxidation. During the oxidation, free hydroxyls at C-6 are converted into carboxylic acids. As previously has been reported, the highest DO obtained for GGM was ~80%.[3] In GGM, ~10% of the mannose units are substituted with galactose side groups and cannot therefore be oxidized. Part from the mannose (Man) units and also some of the glucose (Glc) and galactose (Gal) units remain unoxidized. During the conventional method, where NaClO and NaBr were used as co-oxidants at 2–4°C, the reaction proceeded fast, and high DO

was reached within minutes; after 15 min, the DO was already 65% (Figure 1). Performing the reaction at room temperature increased the rate, and the maximum DO of ~70% was achieved within 15 min. In bromide-free conditions, a notably slower conversion of GGM was observed. At room temperature, a DO of only 40% was achieved, and at 2–4°C, only 16% was reached within the first 15 min. The highest degrees of oxidation were reached when the oxidations were performed at 2–4°C. The lower reaction rate caused by the bromide-free conditions could be compensated by a longer reaction time.

Differences in the oxidation rates of the different sugar species were observed. As can be seen from Figure 1, the rate of conversion of Man and Glc units to their corresponding uronic acids was similar, but the oxidation of Gal proceeded noticeably slower, and the DO obtained was much lower. In all procedures, the maximum DO of both Man and Glc was ~80%, whereas Gal only reached a DO of ~60%. This is in accordance with the results of Brenton et al.[29] They reported that during oxidation mediated by $TEMPO^+$ BF_4^-, the rate and conversion of Gal to the corresponding uronic acid were considerably lower than those of Man and Glc. They speculated that the reason for this is the β-configuration of the C-4 in Gal that might sterically hinder the formation of the oxidation intermediates.

The aldehyde form is an intermediate in the TEMPO-mediated oxidation of alcohols to carboxylic acid. During the oxidation of GGM, a few percent of the sugar units are in aldehyde form. To ensure that no aggregation through inter- and intramolecular

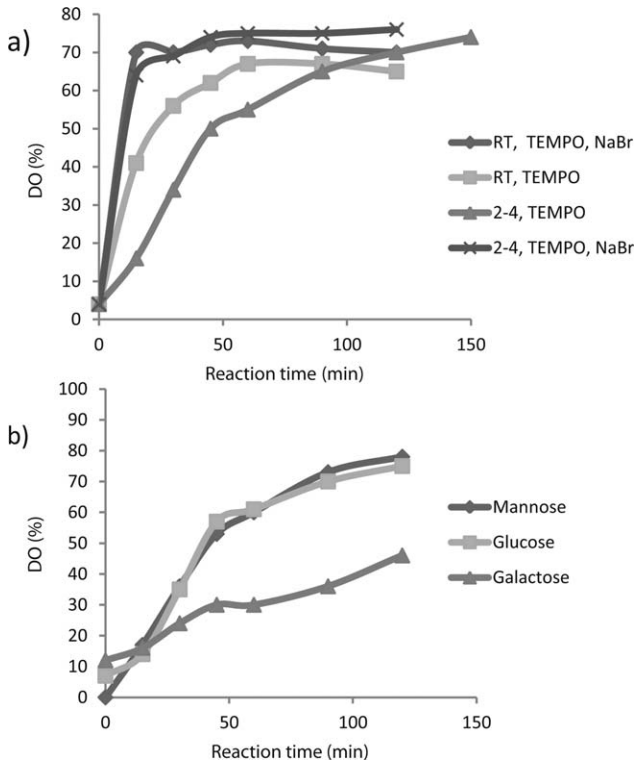

Figure 1. (a) DO of TEMPO-oxidations of GGM done at room temperature (R.T.) or at 2–4°C with or without (w/o) NaBr; (b) DO of mannose, glucose, and galactose. The TEMPO-oxidation was performed at 2–4°C without NaBr.

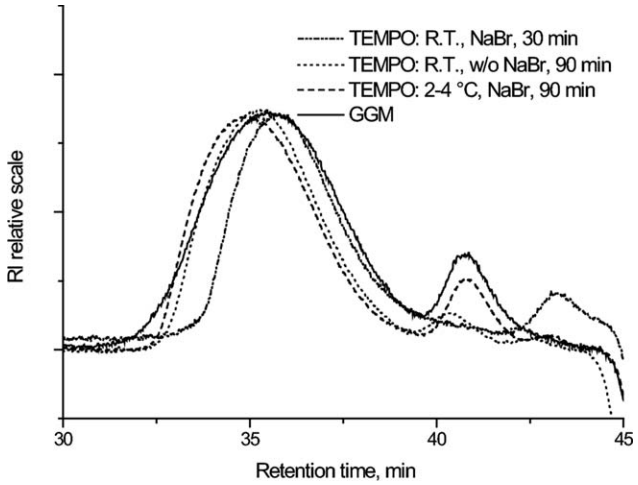

Figure 2. SEC chromatograms of TEMPO-oxidized GGMs. Oxidations were performed either at room temperature (R.T.) or at 2–4°C, with or without NaBr.

hemiacetal bonds would occur, the samples were reduced by $NaBH_4$ before SEC analysis. Polysaccharide chains degrade during TEMPO-mediated oxidation. The reaction temperature, time, and amount of reagents are factors that influence the extent of the degradation.[12,30] Furthermore, also the bond types affect the degradation: (1 → 6)-bonded side-chains of starch are cleaved of to a high degree.[31] Even if GGM contains more accessible (1 → 6)-bonded Gal side-groups, and even if some of the oxidations were performed at room temperature, no significant degradation could be observed during the oxidations (Figure 2). Analysis of samples taken when the maximum DO of each method was reached showed that GGM oxidized at room temperature in the presence of NaBr degraded the most. When oxidation was carried out at 2–4°C, a slight increase in M_w could be observed, corresponding to the increase of M_w caused by the formation of the carbonyl groups.

Because of the high pH, the acetyl groups of GGM are partly cleaved during TEMPO-mediated oxidation. When GGM was oxidized at 2–4°C in the presence of NaBr, 18% of the acetyl groups were removed already after 5 min, and after 2.5 h, the degree of deacetylation was 27–40%.[3] The results were similar using the other methods discussed in this work. At room temperature, in the presence of NaBr, 20% of the acetyl groups were cleaved during the first 5 min (data not shown). At 15 min, when the reaction had reached its maximum DO, ~30% of the acetyl groups had been removed. Because the sodium-free reactions require longer time to be completed, also more deacetylation occur; by the time the maximum DO was reached at room temperature 50%, and at 2–4°C, up to 70% of the acetyl groups were removed.

Amidation of GGM_{PolyU}

GGM_{PolyU} was further functionalized by carbodiimide-mediated amidation. Two structurally varying water-soluble amines were chosen as model compounds; the amino acid arginine and the aliphatic 1,6-diaminehexane. Arginine has been shown to improve bioactive properties of chitosan, for example, the anticoagulant activity.[32] Derivatization with a diamine could lead to

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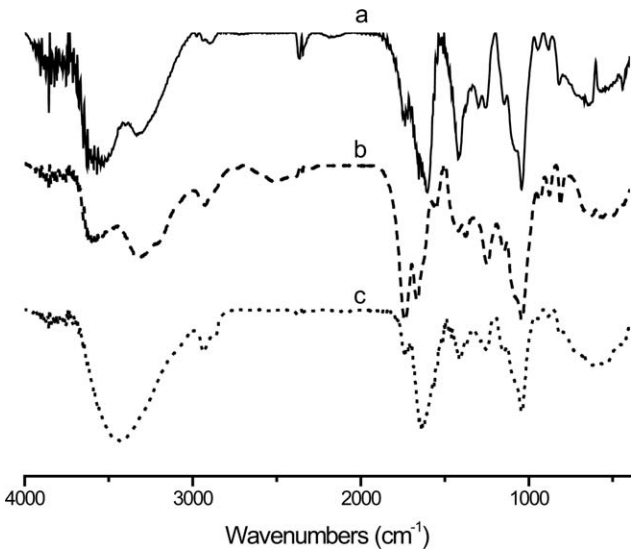

Figure 3. FTIR spectra of TEMPO-oxidized GGM (GGM_{PolyU}) (a) and its amidation products of arginine (b) and 1,6-diaminohexane (c).

cross-linking, which is known to improve the strength properties of GGM films.[33]

To remove any reagent residues and low-molar-mass by-products, the reaction solutions were purified by dialysis after the amidation reactions. Thus, it can safely be assumed that all detected compounds were bound to the polysaccharide.

In the FTIR spectrum of GGM_{PolyU}, besides the characteristic peaks of GGM, the formation of uronic acids resulted in the appearance of strong C═O stretching bands at 1603 cm^{-1} in its carboxylate form and at 1735 cm^{-1} in its acid form (Figure 3).[34] When high-DO GGM_{PolyU} was reacted with 1,6-diaminohexane in the presence of the EDC and NHS, the band of C═O stretching at 1603 cm^{-1} shifted to 1637 cm^{-1} indicating the successful formation of amide bonds between the carboxylic acid groups of GGM_{PolyU} and 1,6-diaminohexane (GGM_{PolyU}-1,6-DAH). In addition, appearance of absorption at 2865 and 2935 cm^{-1} is ascribed to symmetric and asymmetric H vibration of CH_2 groups in the alkyl chain of 1,6-diaminohexane.

NMR analysis also verified the formation of GGM_{PolyU}-1,6-DAH. The peaks between 27–40 ppm in the ^{13}C NMR spectrum are characteristic for the CH_2 groups of the amidated 1,6-diaminohexane (Figure 4).[35] The two-bond ^{1}H-^{13}C correlations in HMBC show a coupling between the carbonyl signals at 172–175 ppm and the amide CH_2 (C7) at 3.2–3.3 ppm, indicating the formation of amide bonds (Figure 5). The lack of correlations between the carbonyl signals and the signals at 2.87 ppm (N—CH_2, C12) show that not all amines have cross-linked, and there are free amino groups left.

During EDC-mediated amidation, reactive *O*-acylisourea is formed when the EDC carbocation is attacked by the carboxylate, and this *O*-acylisourea can react further with primary amines to form amides. A side-reaction is the formation of *N*-acylurea through an intramolecular acyl transfer.[36,37] The

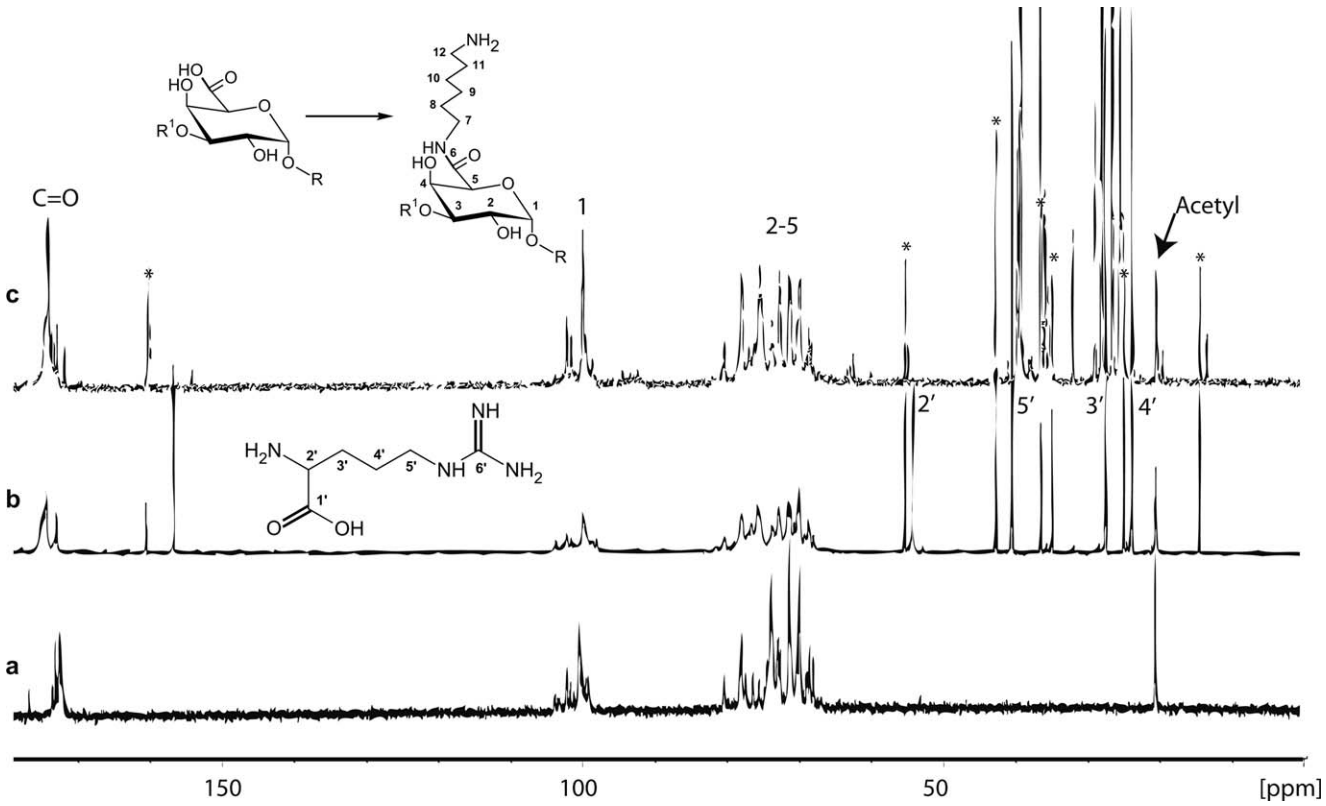

Figure 4. ^{13}C NMR spectra of TEMPO-oxidized GGM (GGM_{PolyU}) (a) and its amidation products of arginine (b) and 1,6-diaminohexane (c). * Peaks assigned to the *N*-acylurea by-product.

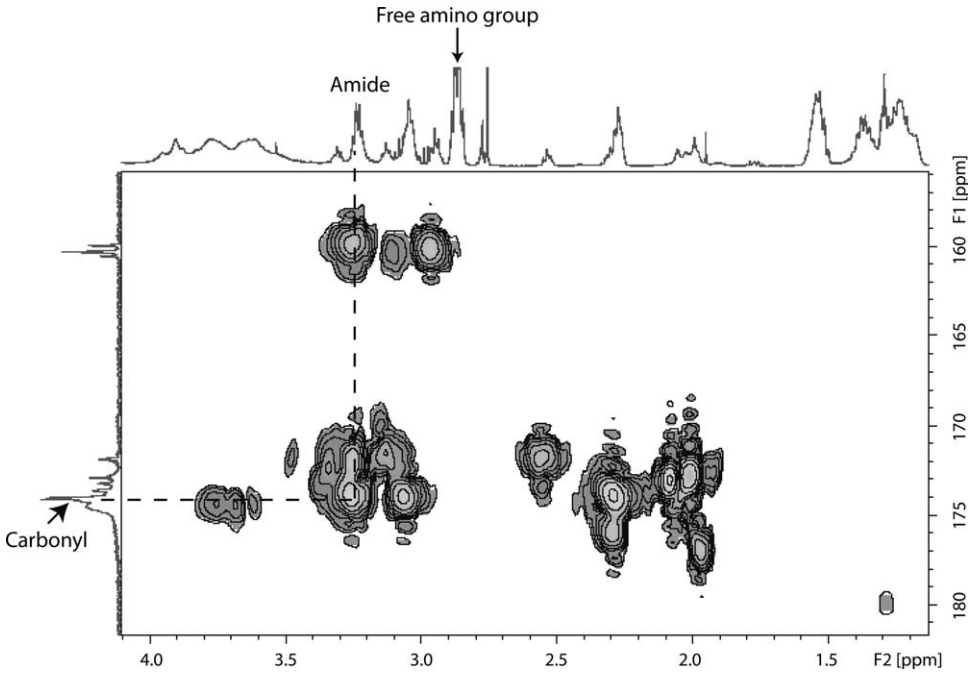

Figure 5. Part of the HMBC NMR spectra of GGM_{PolyU}-1,6-DAH. The correlation between the amide and the carbonyl signals is marked with a dashed line.

formation of *N*-acylurea can be controlled by using EDC in combination with NHS.[15,38,39] In the FTIR spectrum, *N*-acylurea has amide bands at ~1550 and ~1650 cm^{-1} and an imide band at ~1735 cm^{-1}.[37,39] These are undistinguishable from the bands of the desired amide products, but in the ^{1}H NMR spectrum, the triplet at 1.1 ppm and the multiplet at 3.0–3.2 ppm can be assigned to *N*-acylurea.[40] Thus, even if we used the reaction conditions previously optimized for the amidation of cellulose microcrystals,[15] the formation of *N*-acylurea was not completely inhibited.

After amidation with arginine, the typical amide I (C═O stretching) at 1665 cm^{-1} and amide II at 1550 cm^{-1} (combination of N—H deformation and C—N stretching) were observed[41] and have revealed the successful addition of arginine to GGM_{PolyU} (Figure 4). The dramatic increase in absorption at 1735 cm^{-1} is ascribed to the introduction of carboxylate groups present in arginine as well as the formation of *N*-acylurea.

In the ^{13}C NMR spectrum, the peaks at 23.8 ppm (C4'), 27.5 ppm (C3'), 40.5 ppm (C5'), 54.2 ppm (C2'), and 156.7 ppm (C6') can be assigned to arginine, whereas the peaks at 25, 36.4, 35.7, and 55.3 ppm are from the CH group, and 14.4 and 42.7 ppm the —CH_3 groups of *N*-acylurea (Figure 4). The *N*-acylurea peaks can also be found in the ^{13}C NMR of 1,6-diaminohexane-amidated GGM.

Sorption of Modified Polysaccharides to Cellulose Fibres

It is known that unmodified GGM has a high affinity toward cellulose and that it sorbs well onto chemical pulps.[2] Introduction of carboxylic acid groups decreases the degree of sorption. When comparing the sorption in pure water of GGMs with carboxyl groups introduced selectively only on the galactose side-chains and TEMPO-oxidized GGMs containing carbonyl groups both on the back-bone and on the side-groups, the sorption of the selectively oxidized GGMs is notably higher than that of the TEMPO-oxidized ones.[3] The anionic groups located on the backbone are believed to interfere more with the interaction between the backbone of the polysaccharide and the cellulose chain than charged groups located only on the galactose side-chains, thus decreasing the sorption. Besides the location, also the amount of charged groups has been shown to affect the sorption; a higher DO leads to lower sorption.

A careful control of the TEMPO-reaction parameters is required to obtain an optimal DO for adsorption in pure water. The, for GGM novel, method of performing the oxidation in bromide-free conditions, either at room temperature or at 2–4°C, makes it possible to produce GGMs with degrees of oxidation suitable for sorption onto cellulosic fiber surfaces. Large amounts of

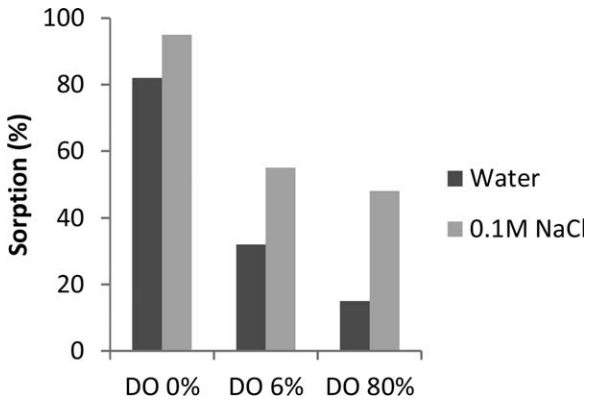

Figure 6. Sorption of GGM_{PolyU} with various degrees of oxidation to BKP. The sorption media was either water or 0.1*M* NaCl. The amount of polysaccharide added was 40 mg/g pulp. STDV < 2%.

GGM_{PolyU} with degrees of oxidation of ~6% could be sorbed, even in pure water without the addition of electrolyte (Figure 6). For fully oxidized GGM_{PolyU}(DO 80%), the sorption was noticeably lower.

The adsorption of the modified GGMs to cellulose was probed in further detail using QCM-D. In Figure 7, the sensed mass calculated using eq. (2) as a function of time is shown for the first 75 min of adsorption of the polysaccharides from pure aqueous solutions. As expected, the TEMPO-oxidation slightly decreases the affinity to cellulose, and the highest sensed mass is observed for the unmodified GGM. The rate of adsorption was also the highest for unmodifed GGM. However, the oxidized GGMs with DO 6 and 10% still adsorbed substantially to cellulose. No significant difference between the adsorption of GGMs with DO 6 or 10% was observed, and thus only the adsorption of the 6% sample is shown. Most of the adsorbed GGM remained attached after rinsing for these samples with a relative decrease in mass of 47% when compared with 31% for pure GGM. However, fully oxidized GGM_{PolyU}(DO 80%) did not adsorb irreversibly to cellulose from water solution. Only half the mass is observed when compared with the other samples, and the whole layer is removed upon rinsing with water (Figure 7).

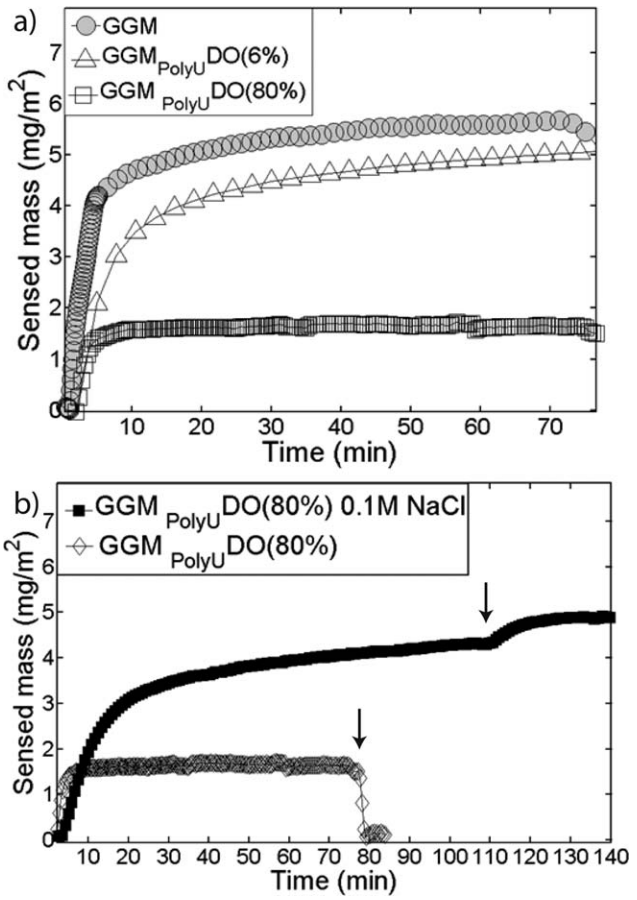

Figure 7. Sensed adsorbed mass on NFC ultrathin film substrate calculated from QCM-D responses using eq. (2). (a) Adsorption of unmodified GGM and oxidized GGMs. (b) Adsorption of GGM_{polyU}DO(80%) from 0.1*M* NaCl solution (filled squares) and pure milliQ water (unfilled diamonds). The rinsing step, when the GGM solution is exchanged to either pure water or 0.1*M* NaCl, is indicated with an arrow. For clarity, the adsorption of NFC is not shown in the figures.

The effect of the ionic strength of the sorption media was investigated by performing sorption experiments in NaCl (0.01*M* and 0.1*M*) solutions. As expected, the affinity of the GGM_{PolyU} to fibers was enhanced with increasing ionic strength (Figure 6). The sorption of low-DO GGM_{PolyU} was ~40% in 0.01*M* NaCl (data not shown) and 55% in 0.1*M* compared to ~30% in water. The sorption remained equally high even if the DO increased from 6 to 10% (data not shown). The effect of the ionic strength was more noticeable when sorbing high-DO GGM_{PolyU} to BKP; the sorption increased from 15% in water to ~50% in NaCl (0.1*M*). This was also observed in the QCM-D (Figure 7). When GGM_{PolyU} (DO 80%) was adsorbed from 0.1*M* NaCl solution, the sensed mass increased considerably. Notable is that rinsing with the same NaCl solutions did not remove any of the adsorbed GGM_{PolyU}. The higher electrolyte concentration decreases the range of double-layer repulsion between the anionic polysaccharides and slightly anionic cellulose surface, thus facilitating closer contact and van der Waals attraction. But more importantly, at high-ionic strength, anionic polysaccharides become more coiled, and more polysaccharide can fit to the surface.[42] A more commonly studied method for modifying cellulose surfaces is the sorption of CMC. The attachment of CMCs onto pulp fibers is depending on the type and pretreatment of pulp, sorption conditions used, and the degree of substitution (DS).[4,43] For high-DS CMCs (>0.5), no attachment takes place in electrolyte-free conditions, and, similarly to GGM_{PolyU}, the sorption decreases with increasing DS. When the CMC addition level is 20 mg/g, at 80°C in 0.05*M* $CaCl_2$, ~50% CMC with DS between 0.4 and 0.8 [comparable with GGM_{PolyU}(DO 80%)] sorbes onto unbeaten kraft softwood pulp.[4] These results show that sorption of GGM_{PolyU} to fibers is well comparable with the sorption of CMC when aiming at introducing carboxyl groups in pulp.

When amidated GGM_{PolyU} was sorbed to cellulose, the degree of binding decreased noticeably; 11% of the added polysaccharides attached to the fibers. The steric hindrance caused by the substituents inhibits the interaction between cellulose surfaces and the polysaccharide chains. Also, the substituents might hinder the polysaccharides from coiling, and by that decrease the amount polysaccharide that can fit on the cellulose surface. Because it is obvious that derivatization decreases the affinity to cellulose, the pathway of adsorbing the carboxylated polysaccharide before performing the amidation is to be preferred.

CONCLUSIONS

TEMPO-mediated oxidation can be used for the oxidation of spruce GGM to its polyuronic acid derivatives (GGM_{PolyU}). By careful control of the oxidation parameters and time, GGM_{PolyU} with varying degrees of oxidation can be produced. A DO of less than 10% can be obtained when the oxidation is performed in bromide-free conditions. A low-DO GGM_{polyU} shows high affinity to cellulose surfaces and can be sorbed onto cellulose in moderate amounts even without addition of salts. The TEMPO-mediated oxidations do not cause any significant degradation of

the polysaccharide chain, even though some slight decrease in M_w can be seen when the oxidation is performed at room temperature. GGM_{PolyU} can be further derivatized with amines by a carbodiimide-mediated amidation.

Even if the production cost of GGM still is high compared to commercially available polysaccharides, modified GGMs show a potential as molecular anchors for cellulose modifications. An advantage, compared to cellulose, is the ease of modification. The readily water-soluble GGM can be modified in aqueous solutions at room temperature in benign reaction conditions.

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