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Cationised *O*-acetyl galactoglucomannans: Synthesis and characterisation

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ABSTRACT

Water-soluble *O*-acetyl-galactoglucomannans (GGMs) can be obtained from Norway spruce by hot-water-extraction of the wood or as a side product by ultrafiltration of mechanical pulping waters. Cationic and amphiphilic polysaccharides and their derivatives are of interest for a number of applications and thus quaternary nitrogen moieties with cationic charge were grafted onto GGMs in the heterogeneous reaction to render a cationic polyelectrolyte. The degree of substitution was measured by elemental analysis of nitrogen, by quantitative ^{13}C NMR and interestingly also by polyelectrolyte titration and the results were congruent. NMR, matrix-assisted laser desorption/ionisation mass spectroscopy (MALDI-TOF-MS), and FT-IR analysis were used to characterise the product. THF or DMSO with water enhanced the reaction efficiency and decreased M_w reduction in comparison to plain water as a reaction media. Cationised GGM was also successfully acetylated. The cationic derivatives of hemicelluloses can potentially be utilised as polyelectrolyte layers in packaging and pharmaceutical applications.

1. Introduction

O-acetyl-galactoglucomannans (GGMs) are the main hemicelluloses in softwoods. Norway Spruce (*Picea abies*) GGM is a heteropolysaccharide and has a structure of randomly distributed (1 → 4)-linked β-D-mannopyranosyl and (1 → 4)-linked β-D-glucopyranosyl units in the main chain, while (1 → 6)-linked α-galactopyranosyl units are attached to mannose units only as side groups. *O*-acetyl groups are attached to C2 and C3 positions of the mannose units in the main chain (Hannuksela and Hervé du Penhoat, 2004; Timell and Syracuse, 1967; Willför et al., 2003; Xu, Willför, Sundberg, Pettersson, & Holmbom, 2007). Norway spruce (*P. abies*) GGMs can be recovered from process waters in thermomechanical pulp mills by ultrafiltration (Willför et al., 2003; Xu et al., 2007). Polymeric GGMs can also optionally be isolated from wood by hot-water extraction (Leppänen et al., 2011; Song, Pranovich, Sumerskiy, & Holmbom, 2008).

There have been numerous efforts for utilising cationic polysaccharides and their derivatives. Cationised polysaccharides can be used as flocculants in kaolin suspensions (Bratskaya et al., 2005; Sableviciene, Klimaviciute, Bendoraitiene, & Zemaitaitis, 2005; Tian, Wu, Liu, & Xie, 2010; Wei, Cheng, & Zheng, 2008) or they can be used as an alternative to polyacrylamide-based flocculants in waste water treatment and in papermaking (Kuutti et al., 2011). Physical properties of paper handsheets were improved significantly by adding cationised and carboxymethylated hemicelluloses together, while using either of these alone, properties were improved less. Hemicelluloses may be used as the source for the production of wet-end additives in papermaking as a sustainable way of using the agricultural and forestry residues (Ren, Peng, Sun, & Kennedy, 2009). Small addition of cationised xylan in aqueous dispersion of thermomechanicalpulp increased retention by ~3% (Ebringerova, Hromadkova, Kacurakova, & Antal, 1994).

Adsorption of cationic polysaccharides and neutral polymers onto solid polymer surfaces has been compared to mimic the effect of hair conditioner on hair (Guzman et al., 2011). Cationic starch with adjusted charge density is used to adsorb water from rock surface pores in oil reservoirs (Leslie, Xiaoa, & Dong, 2005). Cationised konjac glucomannans showed inhibitory effects against some bacterial and fungal species (Yu, Huang, Ying, & Xiao, 2007). Chitooligosaccharide with quartenary ammonium group had antibacterial activity against the dental caries *Steptocccus*

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mutans (Kim, Lee, Lee, Lee, & Park, 2003). Anionic and cationic starches have been used as interactive polyelectrolyte multilayers on SiO_2 surfaces (Lundström-Hämälä, Johansson, & Wågberg, 2010). Cationic chitosan can also be involved in multi-component coatings for emulsion-based systems to delay the delivery of bioactive lipophilic components used for nutraceuticals or pharmaceutical (Li et al., 2010).

As an indicator of future prospective, ionic polysaccharides are involved in numerous patent applications, for example in oil recovery with cationic guar gum (Labeau et al., 2011), personal cleaning (Parker et al., 2011) detergents (Barreleiro and Ziganke, 2011), dye fixation at textiles (Bird et al., 2011), coating for solid flavour particles (Philip Morris Products, 2011) new-generation biomaterials where metal nanoparticles are dispersed and stabilised by cationic polysaccharides (Donati et al., 2011), aqueous fire preventing agents (Fraunhofer et al., 2009) and preparing a biologically active material by coating tablets (Freers and Mcpherson, 2006).

3-Chloro-2-hydroxypropyltrimethylammonium chloride (Ebringerova et al., 2004) or 2,3-epoxypropyltrimethylammonium chloride (ETA) (Bigand et al., 2011) is usually used as the reagent. Cationic groups in polysaccharides usually consist of trimethylammonium chloride or then one of the methyl groups is replaced with an aliphatic chain (Wei et al., 2008). The degree of substitution (DS) value 1.5 for starch can be achieved by carrying out the cationisation reaction two times (Heinze, Haack, & Rensing, 2004). Xylan and galactomannan type hemicelluloses were cationised, and epoxide and hemicellulose concentrations were optimised. High concentration of xylan yielded good mass balances with moderate epoxide concentrations (Bigand et al., 2011). Alkalisation in water increased reactivity also with insoluble xylans (Ebringerova et al., 1994).

Cationisation of polysaccharides can be carried out in heterogeneous manner in ethanol/water or in homogenous manner in DMSO (Heinze et al., 2004; Ren, Liu, Sun, She, & Liu, 2007). Homogenous systems yielded higher DS due to better access of reagent within the dissolved hemicellulose (Ren et al., 2007, 2008). Cationic esterification has also been carried out with trimethylglycine (Auzély-Velty and Rinaudo, 2003). Cationic esters and ethers were susceptible to hydrolysis at pH 6.1 in D_2O. Reactive extrusion has also been applied in starch cationisation (Moad, 2011).

Cationised, carboxymethylated and iminated GGMs were tested for papermaking properties. These derivatives showed very different behaviour in the papermaking process. Cationic GGM increased the retention of both fibrous fine material and fillers without deterioration of the mechanical properties, indicating that cationised GGM followed an electrostatic mechanism. Iminated GGMs were suggested to act through the alkane chain attached to the reducing end, which oriented towards hydrophobic particles. Carboxymethylated GGMs increased the retention of fibrous material slightly and was thought to bind with metal ions originating from wood (Lindqvist et al., 2013). In this work we aimed to establish a convenient synthetic route options with water and water–organic solvent mixtures to cationise GGM and study the effect of reaction conditions on the reaction efficiency (RE (equiv. of ETA/DS)), the DS, and the molar mass. Furthermore, the purpose was to compare various analytical methods for DS determination and to accomplish structural characterisation by NMR and MALDI-TOF-MS.

Cationised GGM can potentially be applied also as polyelectrolyte for films and coatings (Schoeler et al., 2006), e.g. for multilayer packaging applications. This is to gain synergy of charged polymers with opposite charged or neutral polymers to improve barrier and mechanical properties. Cationised GGM was acetylated in order to more chemical alternatives for further applications. The introduced acetyl groups would possibly enhance branching of GGM backbone and possibly also of cationic side groups. Solubility in water would be affected too.

2. Experimental

2.1. Materials

Norway spruce (*P. abies*) thermomechanical pulp mill water was ultrafiltrated to isolate GGMs and the concentrate was further spray-dried. Willför et al., 2003; Xu et al., 2007). The product was washed in 90% ethanol. Technical grade (2,3-epoxypropyl)trimethylammonium chloride was purchased from Sigma–Aldrich. Sodium hydroxide titrisol for reactions was purchased from Merch, Germany. Potassium polyvinyl sulphate was purchased from Wako Pure Chemical industries. Dialysis tubing having cut-off 12–14000 Da and size 9, was purchased from Medicell International Ltd., London.

2.2. Methods

2.2.1. Cationisation of GGM

GGM (2 g, 12 mmol) was dispersed in water (15–30 mL) and 1 M NaOH (9–14 mL) added under magnetic stirring in round bottom flask. Alternately GGM was dispersed in 20–30 wt% THF or DMSO/water. The dispersion was heated to 50 °C under magnetic stirring and then (2,3-epoxypropyl)trimethylammonium chloride (0.34–14 mL, 3–48 mmol) was added. The reaction was quenched by neutralising the reaction mixture with acetic acid or 1 M HCl. The product was washed with ethanol: the resulted precipitate was centrifuged off and the purification process was repeated for three times. The final product was filtrated through a 0.16 μm glass microfiber filter, dried first in room temperature, and finally in vacuum oven at 40 °C.

2.2.2. Acetylation of Cat-GGM

Cat19-GGM (6 g, 28 mmol) was suspended in acetic anhydride (80 mL) and pyridine (5 mL) in a round bottom beaker under magnetic stirring. The reaction mixture was heated up to 70 °C for 2.5 h. The product was washed with 90% acetone three times and dialysed with a 12–14 kDa cut-off dialysis tube.

2.2.3. Nitrogen content

The nitrogen content of Cat-GGM was analysed with CHN LECO CHN-1000 elemental analyzer in Metla (Finnish Forest Research Institute). The blank sample of raw GGM was treated with 1.5% NaOH for 6 h at 50 °C and washed with ethanol. It contained 0.17 wt% of nitrogen, probably originating from protein residues in wood. This was taken into account in determination of DS with N-analysis.

The nitrogen wt% of the elemental analysis (EA) was used to deduce the DS in the following way (Bigand et al., 2011):

$$DS^{EA} = \frac{M_{man} \times N\%}{M_N \times 100 - M_{func} \times N\%},$$

where M_{man} is the molar mass of the mannose, $N\%$ equals nitrogen wt% of the sample, M_N is the molar mass of nitrogen and M_{func} is the molar mass eight of the functional group, 151.6 g/mol.

2.2.4. Polyelectrolyte titration

Particle charge detector can for instance be used to determine anionic charge in humic substances (Tan, Norde, & Koopal, 2011). It is appropriate to determine only the point of zero charge of polysaccharide by a particle charge detector to produce rational results. Adsorption of cationic polyelectrolytes onto anionic polyelectrolytes is affected by various factors like salt residues in

dispersion, molar mass and functional group properties of the polyelectrolytes (Böckenhoff, Fischer, & Frasenius, 2001; Farris, Mora, Capretti, & Piergiovanni, 2012; Lundström-Hämälä et al., 2010). The electrokinetic signal should not be interpreted quantitatively in the manner of a zeta potential, due of its high dependence on model presumptions and experimental conditions. Polyelectrolytes used for charge titration ought to have functional groups with strong acid or strong base character in order to provide almost entirely dissociated counterions (Böckenhoff et al., 2001).

Charge density of cationised GGM was measured with Mütek Particle charge detector −0.3. Cat-GGM (0.1 g) was suspended in water and 0.001 M potassium polyvinyl sulphate (KPVS) was added until reaching the zero point. The titration was repeated two times and the average value was used in calculation. The actual concentration of KPVS was determined by analysing zero charge of it by polyethene sodium sulphonate.

In our study, a particle-charge detector by polyelectrolyte expressed as equivalents/g (mol/g). Charge density of the single charged group can be converted to nitrogen wt%, from which we can deduce DS in the following way:

$$DS^{PET} = \frac{M_{man} \times N\%}{M_N \times 100 - M_{func} \times N\%},$$

where M_{man} is the molar mass of the mannose, N% equals nitrogen wt% of the sample, M_N is the molar mass of nitrogen and M_{func} is the molar mass of the functional group (without a chloride ion), 116.2 g/mol.

2.2.5. Molar mass

Average molar mass (M_w) was determined by size-exclusion chromatography (SEC) in on-line combination with a multi-angle-laser-light-scattering (MALLS) instrument (miniDAWN, Wyatt Technology, Santa Barbara, USA, (λ_0 = 690 nm) with three scattering angles of 41.5°, 90.0°, and 138.5° and a refractive index (RI) detector (Shimadzu Corporation, Japan). A two column system 2× Ultrahydrogel TM linear 7.8 mm × 300 mm + guard column was used. 0.1 M $NaNO_3$ solution, after being filtered through a 0.1 μm Anodisc 47 membrane filter, was used as the eluent at a flow rate of 0.5 mL/min. The samples were filtered through a 0.22 μm nylon syringe filter before injection. The dn/dc value 0.15 was used (Michielsen, 1999). The injection volume was 200 μL. Astra software (Wyatt Technology, Santa Barbara, USA) was used to interpret the data. Prior to HP-SEC analysis, the Cat-GGM and raw GGM was suspended to water at room temperature for overnight with shaking.

2.2.6. Thermal characterisation

Glass transition temperature (T_g) was measured by differential scanning calorimetry using Mettler Toledo Differential Scanning Calorimeter model DSC820 system, STARe SW 9.20, Mettler Toledo GmbH, Switzerland, in a range from −60 °C to 150 °C. T_g was taken from mid-point of the change in slope in a second scan.

Thermal decomposition was measured with a Thermo Gravimetry/Differential Thermal Analyzer TG/DTA SDT Q600 in. About 3 mg of sample was heated from 25 °C to 600 °C at 10 °C/min heating rate under argon flow (100 mL/min).

2.2.7. NMR

NMR spectra were recorded using Bruker Avance 600 or 500 spectrometers (Bruker Biospin, Fällanden, Switzerland) operating at frequencies 600.13 MHz (^{1}H) and 150.90 MHz (^{13}C) or at 500.13 MHz (^{1}H) and 125.77 MHz (^{13}C), respectively, and equipped with BBO-5 mm-Zgrad probes. Results are expressed in ppm scale using TSP (0.00 ppm for ^{1}H and ^{13}C) or DMSO solvent signal (39.50 ppm for ^{13}C) as internal standard. The temperature during the experiments was kept at 50 °C. D_2O for Cat-GGMs and DMSO-d_6 for Cat-GGM-Ac were used as solvents. The following techniques were utilised: quantitative ^{13}C, DEPT-135, CH_2 edited HSQC and ^{1}H NMR. A 15 s pulse delay (d1) and an inverse-gated decoupling pulse sequence were used for quantitative ^{13}C measurements, and 2 s pulse delay (d1) for (non-quantitative) ^{13}C measurements. A 1 s pulse delay (d1) was used for ^{1}H, as well as, DEPT-135 and CH_2 edited HSQC measurements. About 15 000 scans were collected for quantitative ^{13}C NMR and 24 000 for ^{13}C NMR and 16 for ^{1}H NMR and 128 for CH_2 edited HSQC. HSQC spectrum was optimised for 145 Hz one bond and for 10 Hz long range ^{1}H–^{13}C coupling constants.

2.2.8. FT-IR

FT-IR spectra were obtained on a Fourier transform infrared spectrophotometer (Bruker Vector 22) using a KBr pellets containing 1 wt% of a sample. 16 scans were accumulated with a resolution of 4 cm^{-1}, at 400–4000 cm^{-1}.

2.2.9. MALDI-TOF-MS

MALDI-TOF-MS was performed with a SAI LT3 Plus MALDI-TOF mass spectrometer (SAI Ltd., Manchester, United Kingdom) equipped with a nitrogen laser of 337 nm and operated in positive ion mode (Xu, Spadiut, Araújo, Nakhai, & Brumer, 2012) MALDI-TOF-MS samples were prepared by mixing 0.5 μL of sample solution with 0.5 μL of matrix solution (10 mg/mL 2,5-dihydroxybenzoic acid in acetone) on the MALDI-TOF-plate followed by drying under a stream of air. The Cat-GGM8 was enzymatically digested prior to the analysis. Cat-GGM was dispersed in acetone (1–10 mg/mL), and the endo-1,4β-mannanase was added. The solutions were stirred at room temperature for 48 h, and were then heated in boiling water for 10 min to inactivate the enzymes.

2.2.10. Solubility

Solubility of Cat-GGM or Cat-GGM-Ac in water is measured gravimetrically after centrifugation in 1% (w/w) concentration.

3. Results and discussion

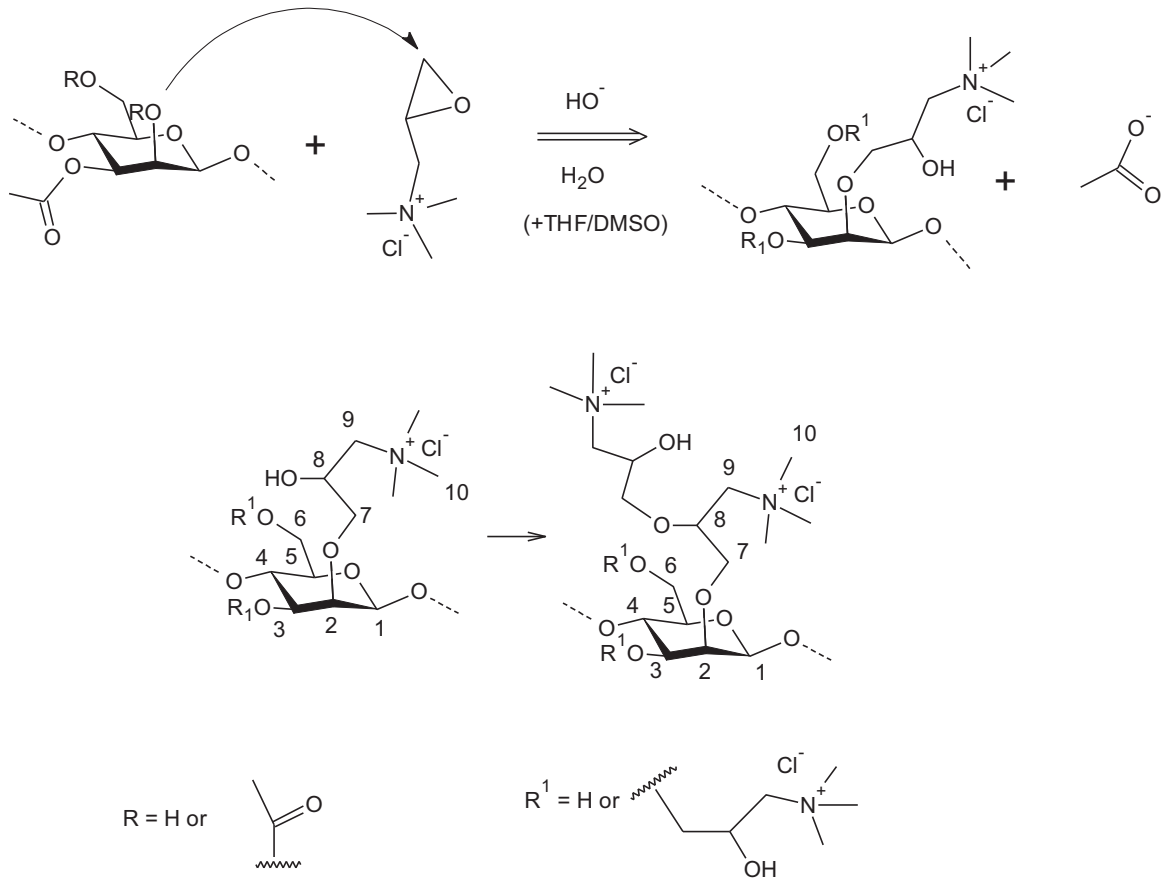
3.1. Cationisation of GGM

Cationisation of *O*-acetyl-galactoglucomannans was carried out heterogeneously in water or in water with THF or in water with DMSO in alkaline conditions with ETA as a reagent (Scheme 1). The product was neutralised and purified in ethanol. The quantity of sodium hydroxide (0.3–4 wt% of solvent), reagent (0.05–4 equiv./sugar unit) reaction temperature (30–60 °C), and reaction time (3–24 h) were altered to examine their effect on the RE, DS and molar mass (Table 1). GGM lost its water solubility when cationised upon losing its acetyl groups. GGM can lose its water solubility when de-acetylated or hydrophobised by grafting with ester groups (Kisonen et al., 2012).

3.1.1. The comparison of the results of DS determination with different methods

The reaction with the cationic moiety did not solely occur at the hydroxyls of the sugar backbone but may have also occured at the hydroxyl group of C8 of the cationised group and still further at the same manner (Scheme 1). The issue is approached by the NMR analysis at Section 3.

The DS from the charge density analysis versus DS from elemental analysis showed linear consistency (Table 1, Fig. 1). DS values under 0.25 determined with PET were lower than the ones with elemental analysis, which may arise from the acidic polymer fractions in GGM (Böckenhoff et al., 2001; Xu et al., 2008). The DS value with PET was the same or even slightly higher for Cat-GGMs with high DS. The bases for PET analysis are explained in Section 2.2.4.



Scheme 1. Cationisation of GGM with ETA at 30–60 °C. Mannosyl unit of GGM is shown. The proposed cationic reaction may also occur in cationic group.

Table 1
Reaction parameters and analysis results of cationised GGM (Cat-GGM). The GGM concentration in a dispersion with Cat15-GGM, Cat23-GGM and Cat24- was 10 wt%, with Cat16-GGM 13%, 5.5% for the Cat10-GGM, Cat11-GGM and Cat13-GGM and 6.7% for the rest.

Cat-GGM	wt% NaOH/solvent	Equiv.of ETA	wt% of THF or DMSO in H_2O	Reaction time (h)	T (°C)	DS		RE	Molar mass (kg/mol)
						EA	PET/NMR		
1	0.30	0.25		6	50	0.023	n.d.	9	23.8
2	0.30	0.5		6	50	0.034	n.d.	7	23.4
3	0.30	2		6	50	0.12	n.d.	6	n.d.
4	0.30	1.5	49^{DMSO}	6	50	0.26	n.d.	18	18.3
5	0.36	1		6	50	0.15	n.d.	15	22.7
6	0.78	1		6	30	0.14	n.d.	14	22.5
7	0.78	1		3	50	0.21	0.21^{PET} 0.19^{NMR}	21	21.3
8	0.78	1	–	6	50	0.23	n.d.	23	19.2
9	0.78	2	2^{THF}	6	50	0.27	n.d.	13	20.0
10	0.90	1.5	–	3	60	0.28	n.d.	19	16.3
11	0.90	1.5	3^{THF}	3	60	0.38	n.d.	26	19.0
13	1.2	1	2^{THF}	6	50	0.19	0.14^{PET}	19	24.2
14	1.2	4	2^{THF}	6	50	0.44	n.d.	10	16
15	1.2	6	2^{THF}	6	50	0.63	0.63^{PET}	11	n.d.
16	1.3	6	–	24	50	0.58	0.62^{PET}	10	13.0
17	1.5	0.05	–	6	50	0.022	n.d.	43	n.d.
18	1.5	0.1	–	6	50	0.036	n.d.	36	15.7
19	1.5	1	–	6	50	0.25	0.20^{PET}	25	*17.6*
20	1.5	1	–	24	50	0.24	0.25^{PET}	24	*15.4*
21	1.5	2	–	24	50	0.39	0.39^{PET}	19	14.8
22	2.0	1	–	6	50	0.25	0.19^{PET}	25	19.4
23	2.1	6	25^{THF}	24	50	0.89	0.94^{PET} 1.1^{NMR}	15	9.3
24	3.1	6	2^{THF}	24	50	0.77	0.77^{PET}	13	9.0
25	4.0	1	–	6	50	0.18	0.12^{PET}	18	17.6

DS = degree of substitution, EA = elemental analysis, PET = polyelectrolyte titration, n.d. = not determined, RE = reaction efficiency.

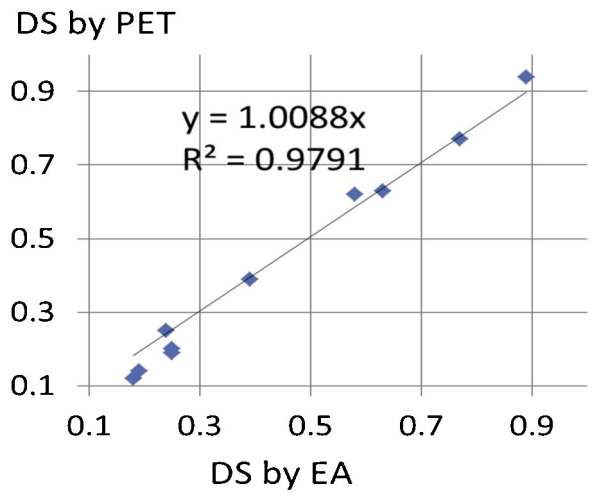


Fig. 1. Correlation of DS results determined by elemental analysis with those determined by polyelectrolyte titration.

The DS was determined by quantitative ^{13}C NMR, elemental analysis and polyelectrolyte titration gave the values 1.13, 0.89, and 0.94 values for Cat23-GGM as an example of high DS and 0.19, 0.21, and 0.21 values for Cat7-GGM as an example of medium DS (Table 1). There was some difference in the values for the DS determined by NMR for Cat23-GGM, which can be explained by trapped solvent residues in the polymer, which are not affecting the NMR results.

3.1.2. The effect on reaction parameters on DS and the RE

As the highly strained three carbon ring-structure of ETA was susceptible to attack of nucleophiles, not only deprotonated hydroxyl groups of polysaccharides but also hydroxyl ions (Bendoraitiene, Kavaliauskaite, Klimaviciute, & Zemaitaitis, 2006), which may have opened up the strained epoxy ring and hence decrease the quantity of the reagent.

The highest DS values were achieved with high amount or reagent, with high NaOH concentration and having THF in solvent: 6 equiv. of EPTMAC, 2.1–3.1, wt% of NaOH and with 25% of THF (Cat23-GGM, Cat24-GGM, Table 1). A reaction time increment from 3 h to 6 h at 50 °C with 0.8 wt% of NaOH and 1 equiv. ETA increased the DS only slightly from 0.21 to 0.23 (Cat7-GGM and Cat8-GGM, Table 1), but 24 h reaction time did not enhance the DS further. Temperature increment from 30 °C to 50 °C with 0.8 wt% of NaOH and with 1 equiv. of ETA increased the DS from 0.14 to 0.23 (Cat6-GGM and Cat8-GGM).

The best RE 43% and 36% was achieved with low equivalents of ETA, i.e. 0.05 and 0.1 equiv. with 1.5 wt% of NaOH (Cat17-GGM) and (Cat18-GGM) respectively (Table 1). The reaction at 50 °C with 0.8 wt% of NaOH with 1 equiv. of ETA gave a reasonable RE (Cat7-GGM–Cat8-GGM), while around 1.5% NaOH concentration with 1 equiv. of ETA at 50 °C may have been close to optimum for obtaining high RE (Cat19-GGM–Cat20-GGM). 20–49 wt% THF or DMSO in water enhanced RE. 0.3% of NaOH gave the lowest RE at 50 °C with 0.25–2 equiv. of ETA, 6–9% (Cat1-GGM–Cat3-GGM), while the reaction in DMSO/H_2O 49:51 (w/w) at 50 °C with 0.3 wt% of NaOH and 1.5 equiv. of ETA gave a significantly higher RE value (Cat4-GGM) of 18%. Clearly the epoxide reagent is less subjected to ring opening by hydroxyl ions in presence of organic solvents.

3.1.3. Molar mass (M_w)

The GGM had an average M_w of 50 kg/mol. DMSO and THF had a beneficial effect of preventing hydrolysis. The reaction in 30% THF for 3 h at 60 °C with 0.9 wt% of NaOH and 1.5 equiv.of ETA

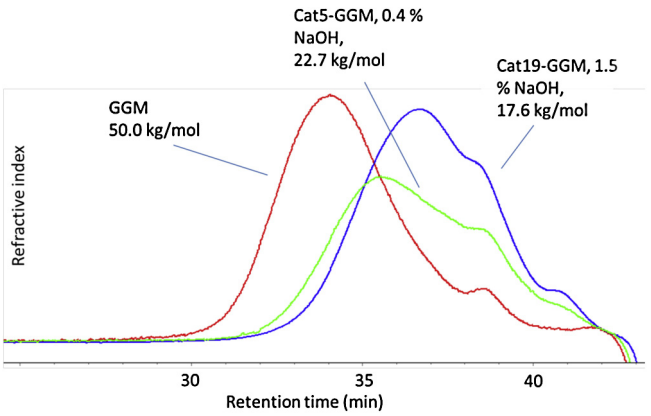


Fig. 2. The chromatograms show the reducing molar mass by increasing reaction time and sodium hydroxide concentration of raw GGM and GGM after the cationisation at different conditions. The peak for Cat24-GGM after 40 min indicates oligomer abundance.

(Cat11-GGM) gave average an M_w of 19.0 kg/mol, while the reaction in water gave 16.3 kg/mol (Cat10-GGM), clearly showing a decrease in M_w due to hydrolysis. It was observed also by Ren et al. (2007) that the molar mass preserved better in DMSO/water and EtOH/water system than in sodium hydroxide water upon cationisation of sugarcane bagasse hemicelluloses. The increase in reaction time decreased the M_w, 24 h reaction with 1.5 wt% of NaOH and 1 equiv. of ETA, (Cat20-GGM,) and 6 h reaction (Cat19-GGM) lead to 15.4 kg/mol and 17.6 kg/mol (Fig. 2) respectively. High (2.1–3.1%) concentration of NaOH at the 24 h reaction at 50 °C with 6 equiv. ETA (Cat23-GGM and Cat24-GGM) lead low molar mass of ~9 kg/mol.

3.2. Acetylation of cationised GGM

O-acetyl groups of GGM were hydrolysed in alkaline water during the cationisation. We carried out re-acetylation for Cat19-GGM (DS 0.25) with excess of acetic anhydride and pyridine (Scheme 2) according to the acetylation method by Xu et al. (2010). Acetylation was confirmed with NMR and FT-IR. Increased hydrophobicity and possible branching of cationised acetylated GGM (Cat-GGM-Ac) over GGM and Cat-GGM renders further potential for film and coating applications. In native GGM acetyl groups are essential for water solubility but they did not make Cat-GGM water soluble but rather hydrophobic. The water solubility of Cat-GGM-Ac was 20% while Cat-GGM with DS 0.39 had the value of 91%.

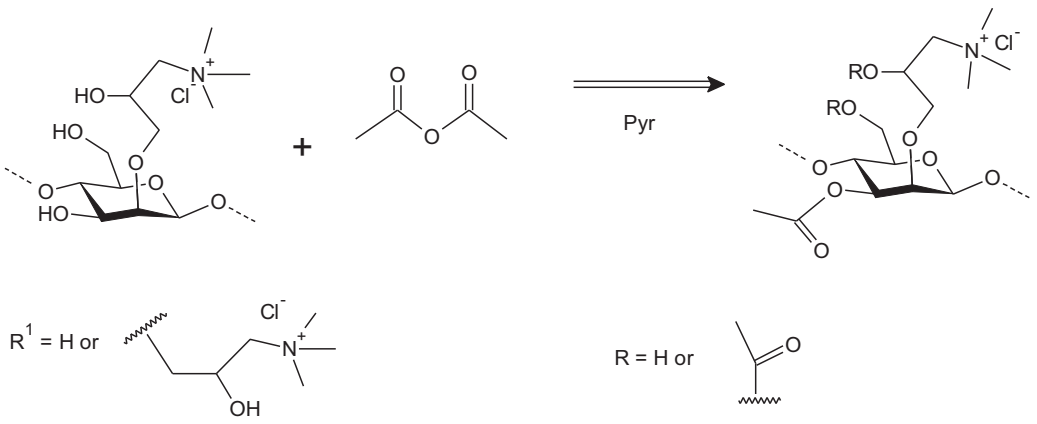
3.3. Structural characteristics of Cat-GGM and Cat-GGM-Ac by FT-IR, NMR and MALDI-TOF-MS

3.3.1. FT-IR

The FT-IR spectra of Cat24-GGM, Cat-GGM-Ac and raw GGM are shown in Fig. 3, where the band at 2930 cm^{-1} corresponds to —CH_2 vibrations. The distinguished band of carbonyl stretching of acetyl group at ~1740 cm^{-1} was significant for the Cat-GGM-Ac, while the band is smaller for raw GGM and it is absent for Cat23-GGM. The band at 1480 cm^{-1} for Cat24-GGM indicated N—C bonding (Bigand et al., 2011; Pigorsch, 2009; Tian et al., 2010; Yu et al., 2007). The band at about 900 is assigned to the β-glucosidic linkage (Xu et al., 2010). A band at ~3400 cm^{-1} did not indicate only hydroxyl stretching but possibly also water residues.

3.3.2. NMR

The structure and DS of cationic GGM was determined also with several NMR techniques including quantitative ^{13}C and ^{1}H NMR experiments, Distortionless Enhancement by Polarisation Transfer



Scheme 2. Re-acetylation of cationised GGM at 70 °C. Pyr = pyridine.

(DEPT) with 135° pulse angle and CH_2 edited Heteronuclear Single Quantum Coherence (HSQC).

C10 at δ = 55.8 ppm and C8 at δ = 67.6 ppm had distinguished signals (Fig. 4) which were not appearing in GGM. Signals of C10 of cationic moiety can be also observed in ^{1}H NMR. The negative signals in DEPT-135 indicated the presence of CH_2 (Fig. 4). However, the negative signal of C7 is rather small. The existence of the negative signal of C7 was further tested by CH_2 edited HSQC, where —CH_2— signals (negative correlations) are distinguished from —CH— and —CH_3 signals (positive correlations). This method enables the differentiation of overlapping carbon resonances by expanding the spectrum to proton dimension. C7 signal ($\delta \sim 75.8 \leftrightarrow 4.2$ ppm, CH_2 type) indeed overlaps with another carbon signal ($\delta \sim 75.8 \leftrightarrow 3.6$ ppm, CH type) and hence restrains the C7 signal to appear rather small in DEPT-135 spectrum.

The total DS was determined by comparison of the integral value of C10 (three carbons) against the anomeric carbon signal (Hannuksela and Hervé du Penhoat, 2004) (Fig. 4). The approximate degree of substitution at C6 (Xu et al., 2011) was determined by measuring the decrease of the integral value of the non-reacted C6 signal (δ = 59–64 ppm) of the GGM ether in comparison to unreacted GGM (Fig. 4). The results showed that ~22% of C6 was reacted for Cat24-GGM with DS 1.13. Finally the DS at C2 and C3 was calculated from the difference in total DS compared to DS at C6.

The reduced size of the C8 signal in proportion to DS (0.80–1.13 respectively) was split in two tops and thus suggested a possible diastereomeric reaction of the cationic moiety on the hydroxyl group of the cationic group on C8 (Fig. 4), which has not been reported earlier.

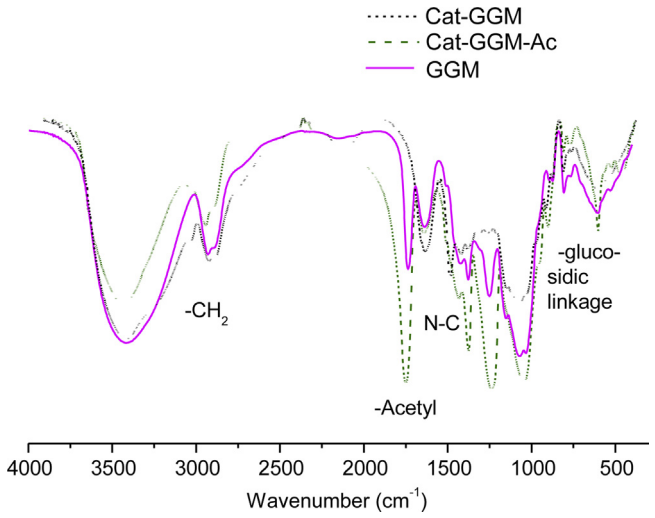


Fig. 3. FT-IR spectra of GGM, Cat23-GGM, and Cat-GGM-Ac.

Cat-GGM-Ac was possibly more branched than Cat-GGM and hence forms highly viscous gel-like solution in DMSO-d_6. Due to this, low concentration had to be used for measurements and this caused high signal to noise ratio in ^{13}C NMR, which disturbed thorough quantitative determination of the acetyl groups. However, signals of —$COCH_3$ and —$COCH_3$ can be detected, also with ^{1}H NMR indicating acetylation (Fig. 5). The appearance of CH_2 of C7 and C9 at DEPT-135 indicates the presence of a cationic moiety after acetylation. CH_3 of acetyl group was observed also in ^{1}H NMR.

3.3.3. MALDI-TOF-MS

The structure of grafted propylammonium group was also verified using oligosaccharide fingerprinting by combined mannanase treatment and MALDI-TOF-MS. The main products of enzymatic hydrolysis were assigned to the GGM oligomers with one cationised group, with a m/z increment of 116 corresponding to the [O-(2-hydroxy-3-methylammonium)propyl]$^+$ (=amm$^+$) residue. Other cationised oligomers with additional one and two O-(2-oxo)propyl (=OP) units were distinguished as well by their consequent m/z increments of 56 (Fig. 6, Table 2). These oxypropyl units (OP) are not an original functional group in the cationised GGM, but they have been formed from MALDI during laser desorption/ionisation. The presence of the OP units and the absence of multiple cations may indicate that the GGM oligomers with multiple cationic groups had undergone elimination of the trimethylammonium group during laser desorption/ionisation to their trimethylammonium residues until only one cationic trimethylammonium was left. The structures of the oligosaccharides with amm$^+$ and O.P. units identified using MALDI are illustrated in Fig. 6.

The oligosaccharide pattern (observed after MALDI fragmentation) with repeating mono- and multiple cationised oligosaccharides with increasing sugar content (2–6 sugar units) indicates a heterogeneous distribution of the cationised groups within the GGM backbone. The main ion fragment for the Cat8-GGM sample is assigned to a cationised oligosaccharide with 3 sugar units and one ammonium residue, which corresponds well with the DS of 0.23 with the sample. At this point, from the MALDI results it is impossible to assign whether the cationised groups belong to the anhydromannose, the anhydrogalactose or the anhydroglucose, since all sugars are isobaric. Moreover, in most cases, it is impossible to infer from the current MALDI results whether the multiple cationic groups belong in the same sugar unit or different ones; that requires sequential analysis using chromatographic and

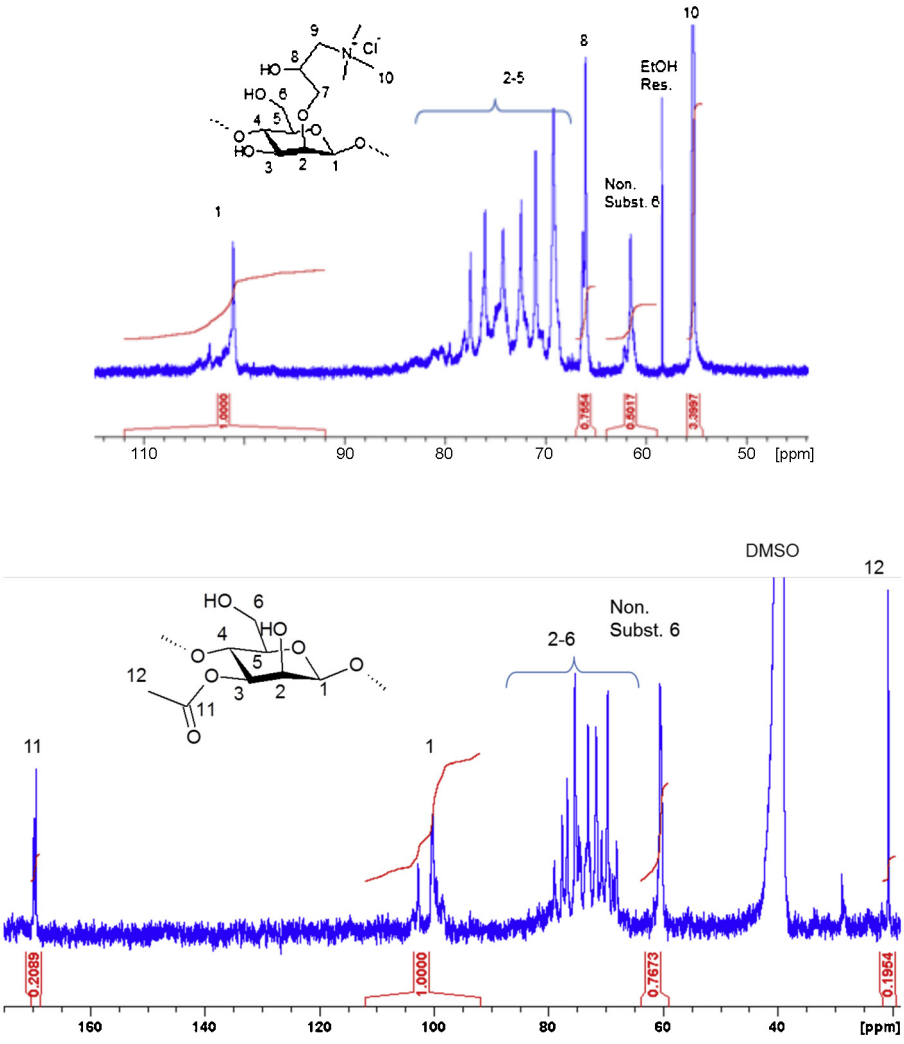


Fig. 4. The quantitative ^{13}C NMR spectrum of GGM and Cat23-GGM.

tandem mass spectrometric approaches. Nevertheless, multireaction in the same sugar unit in the GGM backbone can be observed by the signal at $m/z = 570.2$ that has two sugar units and three functional units.

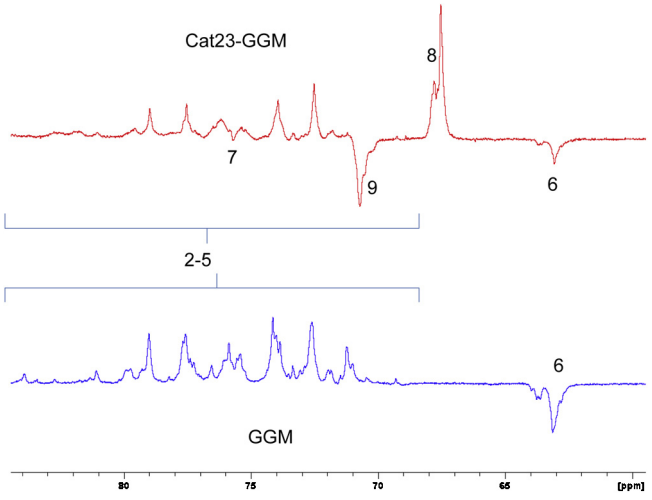


Fig. 5. DEPT-135 NMR of Cat23-GGM and GGM. CH_2 signals are negative, CH and CH_3 signals are positive.

The oligosaccharide pattern (observed after MALDI fragmentation) with repeating mono- and multiple cationised oligosaccharides with increasing sugar content (2–6 sugar units) indicates a heterogeneous distribution of the cationised groups within the GGM backbone. The main ion fragment for the Cat8-GGM sample is assigned to a cationised oligosaccharide with 3 sugar units and

Table 2
MALDI-TOF-MS spectrum of Cat8-GGM. [*O*-(2-hydroxy-3-methylammonium) propyl]$^+$ = amm$^+$, *O*-(2-oxo)-propyl residue = *O*-(2-oxo)-propyl, non-grafted GGM oligosaccharide unit with a Na^+ adduct = oligomer Na^+.

m/z	Number of sugar units	Amm$^+$	*O*-(2-oxo)-propyl	Oligomer Na^+
514.4	2	1	1	
527.1	3			×
570.2	2	1	2	
620.2	3	1		
676.3	3	1	1	
732.3	3	1	2	
782.3	4	1		
838.3	4	1	1	
894.4	4	1	2	
944.4	5	1		
1000.4	5	1	1	
1056.4	5	1	2	
1107.4	6	1		
1162.3	6	1	1	

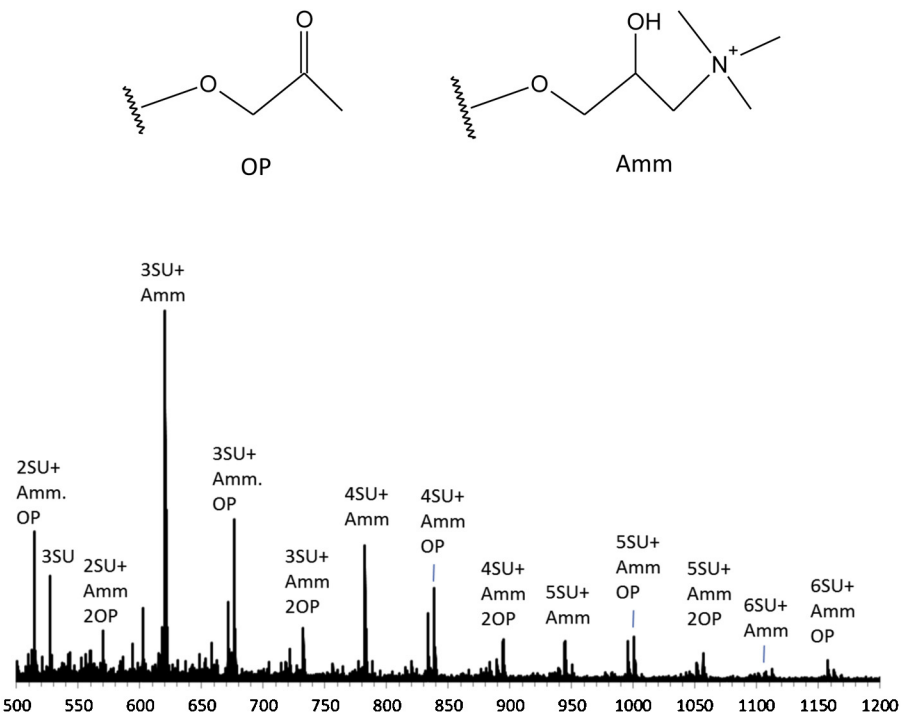


Fig. 6. MALDI-TOF-MS spectrum of Cat8-GGM (DS = 0.23) in acetone. Signals are explained in a Table 2. *O*-(2-hydroxy-3-methylammonium)propyl is marked as [Amm.] and oxypropyl units as [OP].

Table 3
$TGA^{10\%}$ and $TGA^{50\%}$ values indicating 10% and 50% weight loss, respectively, and glass transition values. DS is according to elemental analysis.

	Cat-GGM-Ac	Cat24-GGM	Cat15-GGM	Cat13-GGM	Cat6-GGM	GGM
DS^{Cat}	–	0.77	0.63	0.19	0.14	–
$TGA^{10\%}$ (°C)	262	241	n.d.	n.d.	249	241
$TGA^{50\%}$ (°C)	344	291	n.d.	n.d.	300	305
T_g (°C)	n.d.	n.d.	69	74	n.d.	86

n.d. = not determined.

one ammonium residue, which corresponds well with the DS of 0.23 of the sample. We are currently implementing further analyses involving complex chromatographic and tandem mass spectrometric approaches. Our observations of MALDI-TOF-MS spectrum of cationic resembled to the observations of Richardson et al. (2003) about MALDI study of cationised starch.

3.4. Thermal characterisation

The thermograms of differential scanning calorimetry (DSC) showed endotherm signals indicating decreasing glass transition (T_g) temperatures with increasing cationic reaction of GGM (Table 3). This was in accord with the observation of Donabedian and McCarthy (1998), for hexanoate and dilactates of pullulan, which also exhibited decreasing T_g values with increasing reaction. The thermograms of Cat-GGM, GGM and Cat-GGM-Ac did not show a crystalline melting behaviour (Duanmu, Gamstedt, Pranovich, & Rosling, 2010; Jenkins and Donald, 1998; Thomas, 2001). Low glass transition temperatures can aid material to be flexible, e.g. in packaging applications.

The decomposition temperature was analysed with TG/DTA, where $TGA^{10\%}$, and $TGA^{50\%}$ indicates 10% and 50% weight loss, respectively (Table 3). Ester groups give thermal stability to the polymer; Cat-GGM-Ac had higher TGA values in comparison to raw GGM and Cat-GGMs. This is in line with acetylated GGM, where increased degree of acetylation increased thermal stability of GGM (Xu et al., 2010). Like acetylated GGM had high TGA values in comparison to Cat6-GGM with DS 0.14 had higher TGA values than the Cat24-GGM with high DS and raw GGM.

4. Conclusion

Spruce GGM was treated with ETA in alkaline water or with THF or DMSO as co-solvent, resulting in amphiphilic polysaccharides upon introduction of cationic moieties. The DS was determined with quantitative ^{13}C NMR, elemental analysis, and polyelectrolyte titration of which elemental analysis and polyelectrolyte titration revealed linear correlation. The polyelectrolyte titration is well-functioning and rapid method to determine the DS for the cationic GGM along with the "slower" methods of elemental analysis and quantitative ^{13}C NMR. The structure of GGM with quaternary ammonium moiety bonding were characterised with ^{13}C NMR. CH_2 groups were identified separately with DEPT-135 NMR and HSQC experiments. ^{13}C NMR indicates a possible diastereomeric reaction of the cationic moiety on the hydroxyl group of the cationic group on C8. The covalent bonding of Cat-GGM was conformed with MALDI-TOF-MS. There was also evidence of tri-substitution in the two anhydrosugar units by MALDI-TOF-MS and possible A band in FT-IR was distinguished for N—C bonding. THF or DMSO with water enhanced the reaction efficiency and decreased M_w reduction in comparison to plain water as a reaction media. Reactions with low amounts of ETA and with sufficient amount of sodium hydroxide

lead to highest (36–43%) values of RE. High amounts of ETA contributed to the highest (0.77–0.89) DS values. Long reaction times (24 h) with high quantities of sodium hydroxide (>3%) resulted in low molar masses (<9 kg/mol). T_g values decreased within increasing degree of substitution for amorphous Cat-GGMs. GGM was deacetylated upon cationisation. Cat-GGM was re-acetylated with acetic anhydride; consequently the product was hydrophobic and had higher thermal stability over the Cat-GGMs and raw GGM.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2013.09.009.

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