Carbohydrate Polymers 85 (2011) 738–746

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Molecular characterization of hydrolyzed cationized nanocrystalline cellulose, cotton cellulose and softwood kraft pulp using high resolution 1D and 2D NMR

Hanna de la Motte[a,*], Merima Hasani[a], Harald Brelid[b], Gunnar Westman[a]

[a] Organic Chemistry, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Göteborg, Sweden
[b] Forest Products and Chemical Engineering, Department of Chemical and Biological Engineering, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

ARTICLE INFO

Article history:
Received 3 December 2010
Received in revised form 11 March 2011
Accepted 16 March 2011
Available online 6 April 2011

Keywords:
Softwood kraft pulp
Cotton linters
Nanocrystalline cellulose
2D NMR
Cationization
Characterization

ABSTRACT

Nanocrystalline cellulose, cotton linters and softwood kraft pulp were reacted with the cationic epoxide 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) according to two different procedures, whereof one was a solvent and reagent minimizing spray technique. The samples were subjected to acid hydrolysis and characterized by solution-state nuclear magnetic resonance (NMR). The spectra were obtained using a 600 MHz cryoprobe NMR instrument. The use of ion exchange SPE-column chromatography enhanced the intensity of the peaks derived from the modified monosaccharide residues and opened for detailed NMR characterization at low degrees of substitution. In addition to a consistent 9 ppm change in the initial shifts of carbons adjacent to substituted hydroxyl groups, the NMR spectra proved that other neighboring atoms were affected by the substitution. Unlike nanocrystalline cellulose and cotton cellulose, the softwood kraft pulp cationized with the spray technique demonstrated a higher preference for O-6 (83%), on the expense of O-2 substitution (16%).

1. Introduction

Polysaccharides are the most abundant biopolymers produced in nature and chemical modifications of these biopolymers may be applied in the development of new materials. Such materials, originating from a renewable source are, in principle, in agreement with an eco-effective material development and are of interest in many different industrial application areas (Rahman & Brazel, 2004; Shahidi, Arachchi, & Jeon, 1999).

Polysaccharides are polyhydroxylic polymers, which can be functionalized by a wide selection of chemical reactions. The physical and chemical properties obtained are highly dependent on how and where substituents are located along the polymer chains (Mischnick & Hennig, 2001; Richardson & Gorton, 2003). Thus, characterization methods for modified polysaccharides are of high interest. Nuclear magnetic resonance (NMR) is a powerful characterization technique indispensable for analyses in the field of organic chemistry. In the early 1980s, new NMR techniques with improved magnets and pulse sequences were introduced and the characterization of polysaccharides in the pulp and paper industry advanced in several function areas (Ek, Henriksson, Nystrom, & Odberg, 1994; Gabrielii, Gatenholm, Glasser, Jain, & Kenne, 2000; Haggkvist, Li, & Odberg, 1998; Jansson, Kenne, & Widmalm, 1989; Lennholm, Larsson, & Iversen, 1994; Olson, Chang, & Wang, 1990). ^{13}C NMR has become a great tool for the characterization of both native and modified polysaccharides on a molecular level (Atalla & VanderHart, 1999; Nehls, Wagenknecht, Philipp, & Stscherbina, 1994) and recently the two-dimensional ^{13}C–^{1}H correlated solution state NMR has improved the characterization further (Kim & Ralph, 2010).

Cationic modified polysaccharides are used extensively in the industry. One important example is cationic starch which is used as an additive to improve the dry strength of paper, the filler and fines retention and drainage rate of the pulp in papermaking. Quaternary ammonium starches are used in laundry detergents for improved stiffness and smoothness of fabrics and as flocculants for suspensions of inorganic and organic matter having a negative charge (Solarek, 1986). Other examples of cationic modified polysaccharides are hydroxyethyl cellulose derivatives, which are used as conditioners in personal hygiene products (Ungewiss et al., 2005).

In this paper, a convenient method for molecular characterization of cationic polysaccharides is described. The method is suitable for cellulosic substrates with a low degree of substitution (DS). These materials are of interest when the initial properties of the fibers, such as the fiber structure, the nanocrystalline structure or water insolubility, should be retained after modification.

Two-dimensional ^{1}H–^{1}H and ^{13}C–^{1}H correlated solution state NMR were used to study the reaction of the epoxide 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) with three

---

[*] Corresponding author. Tel.: +46 31 772 30 65; fax: +46 31 772 36 57.
E-mail address: hanna.delamotte@chalmers.se (H. de la Motte).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.03.038

Table 1
Reaction conditions and results of the different reaction procedures of 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) with three different types of cellulosic substrates.

Cellulosic substrate	Temperature (°C)	Time (h)	Sodium hydroxide (M)	Molar ratio (EPTMAC/AGU)	Solvent	Nitrogen content (%)	Degree of substitution (DS_n)
Cotton linters (P1)	50	5	1.15	1.5	Water	0.44	0.053
Nanocrystalline cellulose (P1)	65	5	2	3.0	Water	0.37	0.044
Softwood kraft pulp (P2)	140	1	–[a]	0.2	Dry	0.13	0.015[b]

[a] The softwood kraft pulp was activated in a 0.1 M sodium hydroxide solution.
[b] The degree of substitution is calculated based on hexoses exclusionary.

types of cellulosic substrates: nanocrystalline cellulose, cotton cellulose and softwood kraft pulp. The approach was to perform the chemical modification of the cellulosic substrates and then hydrolyze the product in order to liberate the monosaccharides. To facilitate the NMR analyses of the sugar residues that had been cationized by reaction with EPTMAC, the cationized sugar residues were concentrated by using preparative ion exchange chromatography.

2. Material and methods

2.1. Materials

A never-dried, industrially produced Scandinavian softwood kraft pulp bleached in a totally chlorine free peroxide-based sequence was used in the experiments. Nanocrystalline cellulose was prepared by sulphuric acid hydrolysis of the softwood kraft pulp according to the previously reported procedure (Beck-Candanedo, Roman, & Gray, 2005). The cotton linters were obtained from Munktell Filter AB, Sweden. 2,3-Epoxypropyltrimethyl ammonium chloride (EPTMAC) was obtained from Shandong Guofeng Fine Chemical Co., Ltd., China, and used without further purification. The cationic exchange sorbent ISOLUTE® CBA was obtained from Sorbent AB, Sweden.

2.2. Methods

2.2.1. Cationization of softwood kraft pulp with 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC)

The fibers (1 g) were activated by washing the pulp with a sodium hydroxide solution (0.1 M) followed by solvent exchange with acetone. The fibers were oven-dried at 105 °C for 1 h. After drying, the fibers were sprayed with EPTMAC (molar ratio EPTMAC/AGU = 0.2) dissolved in water (6 wt% EPTMAC) followed by kneading to distribute the reagent. The EPTMAC impregnated pulp was placed in an oven at 140 °C for 1 h. Following heat treatment, the sample was stored over night at room temperature (weight gain 12%). The sample was then thoroughly washed with water and

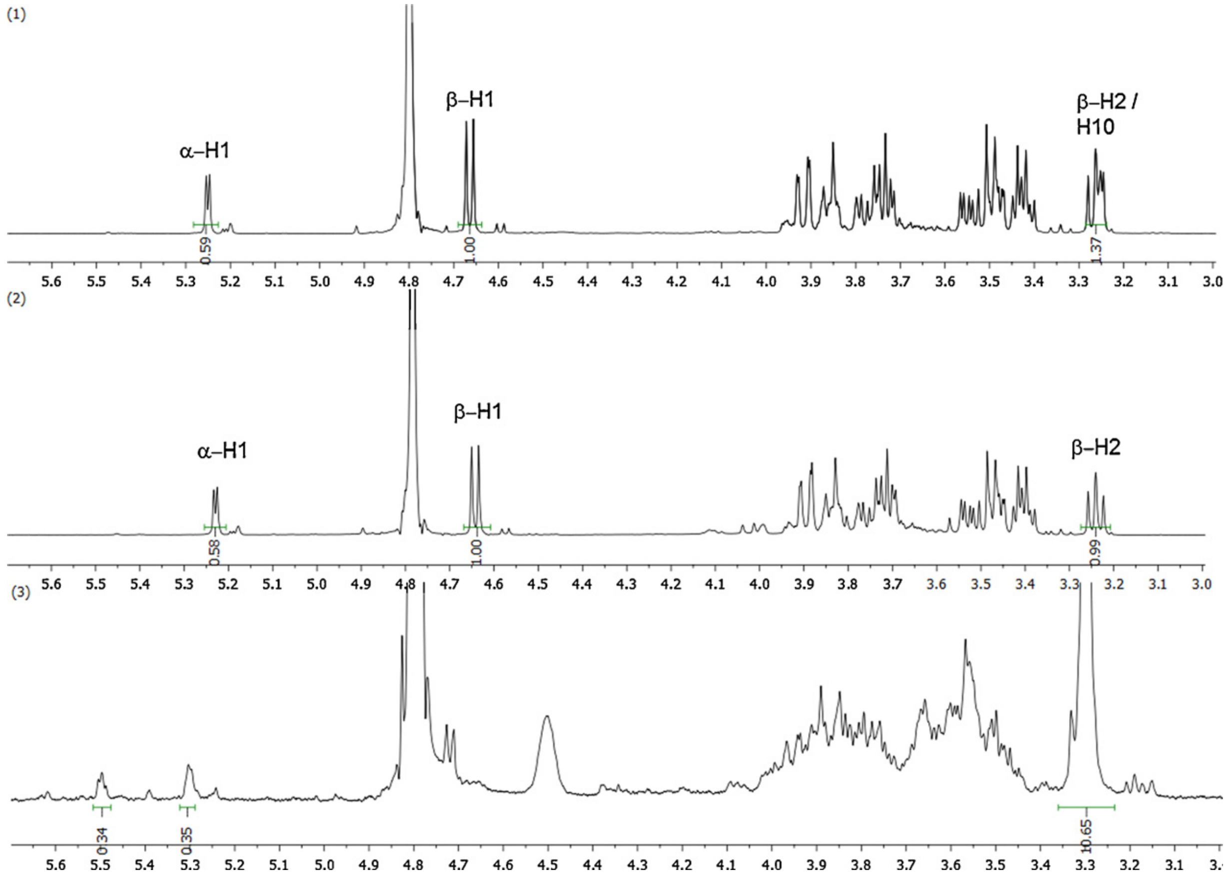

Fig. 1. ^{1}H NMR spectra displaying the integrals of the α-H1, β-H1, β-H2 and the nine methyl protons (H10) of the substituent of (1) cationic modified pulp prior to ion-exchange chromatography concentration, (2) the neutral fraction of the ion-exchange chromatography and (3) the cationic modified fraction after the ion-exchange chromatography.

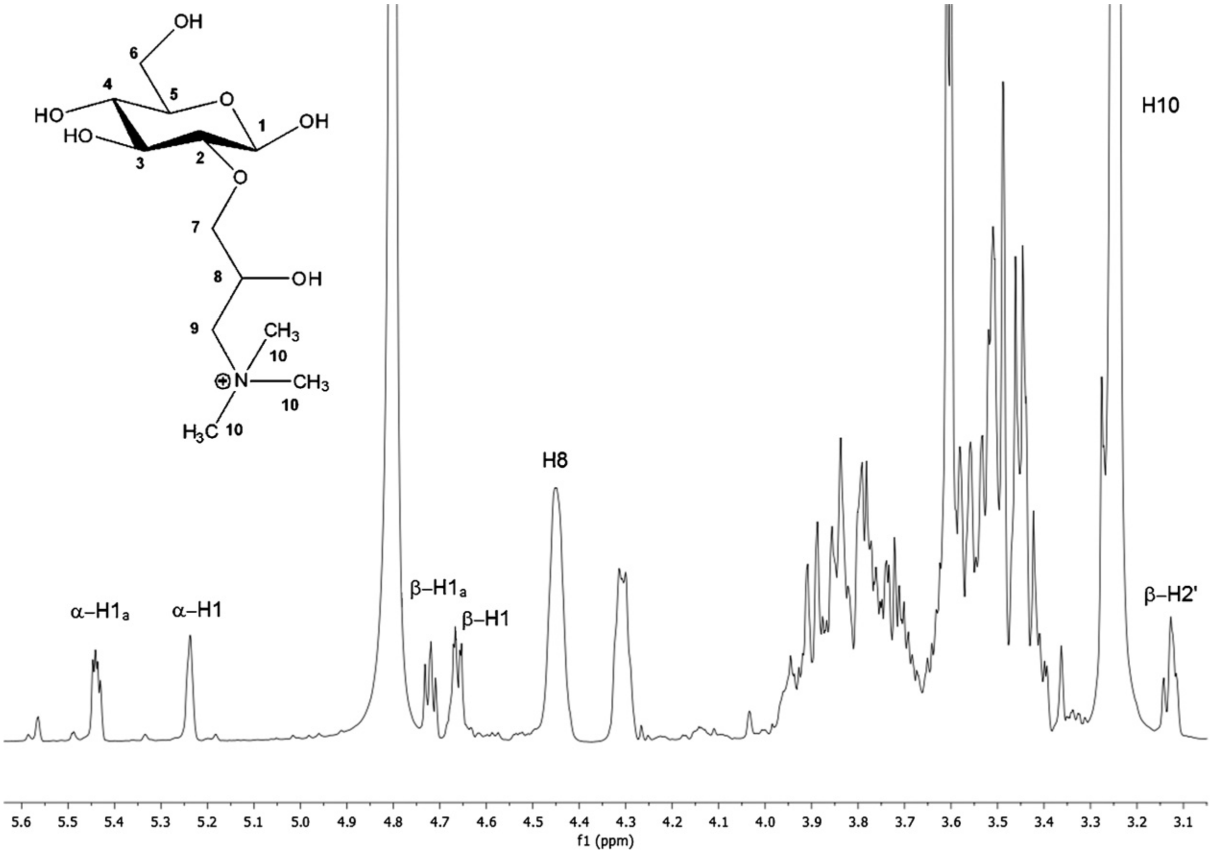

Fig. 2. ^{1}H NMR spectrum of hydrolyzed and concentrated nanocrystalline cellulose modified with the cationization reagent EPTMAC.

then subjected to solvent exchange with acetone followed by air drying (weight gain 0.4%). As a reference, softwood kraft pulp was treated according to the same procedure, but without any addition of EPTMAC to the water that was sprayed on the pulp prior to the heat treatment. The reaction efficiency (the conversion of reagent to product) was 7.3% based on nitrogen analysis.

2.2.2. Cationization of cotton linters with 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC)

The cotton linters were mixed with a sodium hydroxide solution (1.15 M) at a consistency of 11%. After 30 min at room temperature, EPTMAC (molar ratio EPTMAC/AGU = 1.5) was added and the temperature was increased to 50 °C. After 5 h, the reaction mixture was diluted with water. The product was filtered, washed thoroughly with distilled water and air-dried. The reaction efficiency was 3.2% based on nitrogen analysis.

2.2.3. Cationization of nanocrystalline cellulose with 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC)

Nanocrystalline cellulose (cellulose nanocrystals) was mixed with a sodium hydroxide solution (2 M) at a consistency of 5.9%. After 30 min stirring at room temperature, EPTMAC (molar ratio EPTMAC/AGU = 3) was added and the mixture was stirred for 5 h at 65 °C. The reaction mixture was diluted 5-fold with water and dialyzed (cellulose dialysis membranes, molecular weight cut-off of 12 000–14 000 Da from Spectrum Labs) against de-ionized water for 15 days. The resulting suspension of hydroxypropyltrimethyl ammonium chloride cellulose nanocrystals (HPTMAC-CNC), approximately 1% (w/w), was sonicated before concentration by evaporation at reduced pressure. The reaction efficiency was 1.4% based on nitrogen analysis.

2.2.4. Hydrolysis

The cationic modified cellulose substrates were hydrolyzed according to Theander and Westerlund (1986). 3 mL of sulphuric acid (72%) was added to 200 mg of cellulosic substrate. The sample was subjected to vacuum for 15 min and then heated in a water bath (30 °C) for 1 h. Distilled water (84 g) was added and the sample was autoclaved for 1 h at 125 °C. The hydrolysate was filtered, neutralized with barium hydroxide and again filtered. The filtrate was concentrated to dryness under reduced pressure.

2.2.5. Preparative ion exchange chromatography

The hydrolyzed cationic modified cellulosic substrates were concentrated using preparative ion exchange chromatography. The sorbent was wetted with methanol and loaded on a column (Biotage 6 mL, 2.5 cm ISOLUTE® CBA sorbent). The methanol was eluted with de-ionized water and the sorbent was converted to its sodium form by the addition of a sodium hydroxide solution (0.1 M) to the column. After rinsing with de-ionized water (10 column volumes) the cellulosic hydrolysate were applied on the column (1 g/20 g sorbent). To elute uncharged molecules the column was again rinsed with de-ionized water (3 column volumes). The cationic fraction was eluted with 3 column volumes of diluted sulphuric acid (0.1 M). The cationic fraction was neutralized with barium hydroxide, filtered and concentrated to dryness under reduced pressure.

According to a control experiment starting with 750 mg of hydrolyzed cationized softwood kraft pulp, at least 77% of the carbon content is recovered from the column based on carbon analysis (presuming that the amount of carbon in the starting material is 300 mg). The amount of carbon in the neutral fraction was 213 mg, and in the cationic fraction 17 mg.

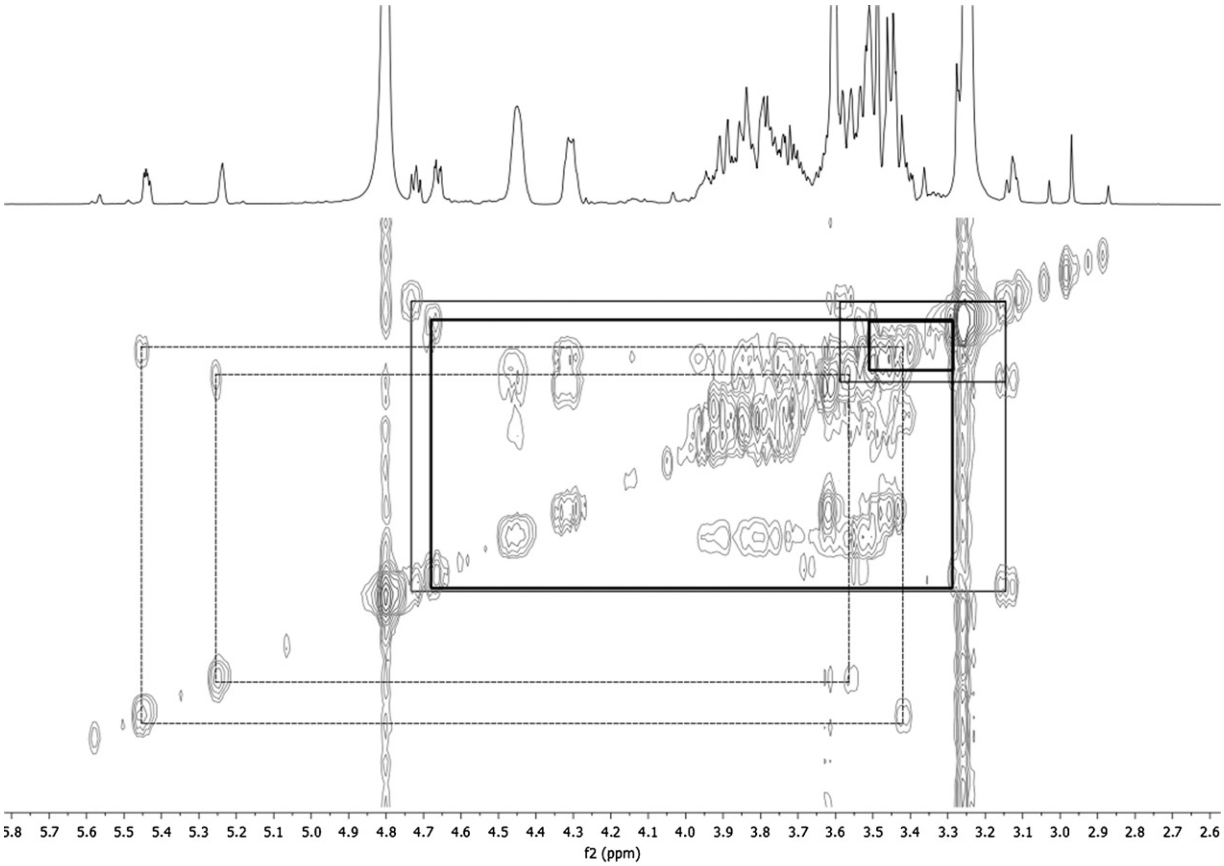

Fig. 3. COSY NMR of hydrolyzed and concentrated modified nanocrystalline cellulose. The heavy black rectangle corresponds to the couplings of the original position of the β-H2 proton, hidden in the strong peak belonging to the methyl groups of EPTMAC. The thin black rectangle shows the new couplings of the substituted position of β-H2. The dashed rectangles are the equivalent of the α-isomer. The ^{1}H NMR spectrum of hydrolyzed and concentrated nanocrystalline cellulose modified with the cationization reagent EPTMAC is inserted to make the figure more perspicuous.

2.2.6. *NMR analysis and elemental analysis*

Before NMR analysis, materials were lyophilized and dissolved in D_2O. The ^{1}H and ^{13}C–^{1}H-correlated NMR spectra were recorded at 25 °C on a Varian Inova 600 spectrometer equipped with a cold probe, operating at 599.76 MHz for proton detection and at 150.81 MHz for carbon detection. The ^{1}H–^{1}H correlated NMR spectra were recorded at 25 °C on a Varian Unity Inova 500 spectrometer operating at 499.9 MHz for proton detection. The nitrogen analysis was provided by the Mikroanalytische Laboratorium, Kolbe, Germany and at the Department of Biology, Plant Ecology and Systematics, Lund University, Sweden.

2.2.7. *Carbohydrate composition analysis*

The carbohydrate composition of the hydrolyzed and concentrated cationic modified nanocrystalline cellulose, cotton cellulose and softwood kraft pulp was analyzed using ion chromatography with pulsed amperometric detection. The separation was performed isocratically in Milli-Q water on a CarboPac$^{\text{TM}}$ PA1 column (Dionex, Sunnyvale, CA, USA). The detection was enhanced by the post column addition of a sodium hydroxide solution.

2.2.8. *DS calculation*

The degree of substitution was calculated from the nitrogen analysis according to the following equation:

$$\mathrm{DS_N} = \frac{162.15 \times \%N}{1401 - 151.64 \times \%N} \tag{1}$$

The degree of substitution was estimated from NMR spectra according to the following equation:

$$\mathrm{DS_{NMR}} = \frac{\int(\beta\text{-H2} + 9\,\text{methyl protons of EPTMAC}) - \int \beta\text{-H1}}{9\left(\int \alpha\text{-H1} + \int \beta\text{-H1}\right)} \tag{2}$$

where $\int \beta\mathrm{H2} = \int \beta\mathrm{H1} = 1$ is the normalized integrated area of the β-H1 peak.

2.2.9. *Liquid chromatography–mass spectrometry (LC–MS)*

LC–MS analysis was performed using a PerkerElmer 200 series LC-pump system coupled to a PerkerElmer PE SCIEX API 150 EX instrument equipped with a TurboIon spray ion source, using positive ion mode. Separation was achieved using a Grace Genesis C8 column (4 μm particle size, 4.6 mm inner diameter, 50 mm long). The flow-rate was 1 ml/min and an aqueous buffer (18 mmol formic acid) and acetonitrile constituted the eluent. A program using an 8 min gradient of the eluent (buffer/acetonitrile, 95/5% to 5/95%) was used. The samples were dissolved in water prior to injection.

3. Results and discussion

3.1. *Cationization reactions*

The cellulosic substrates were cationized by two different procedures (Table 1): with a high amount of EPTMAC in an alkaline water solution at a relatively low temperature and with a long reaction time (P1), and with a lower amount of EPTMAC under dry conditions at high temperature and with a short reaction

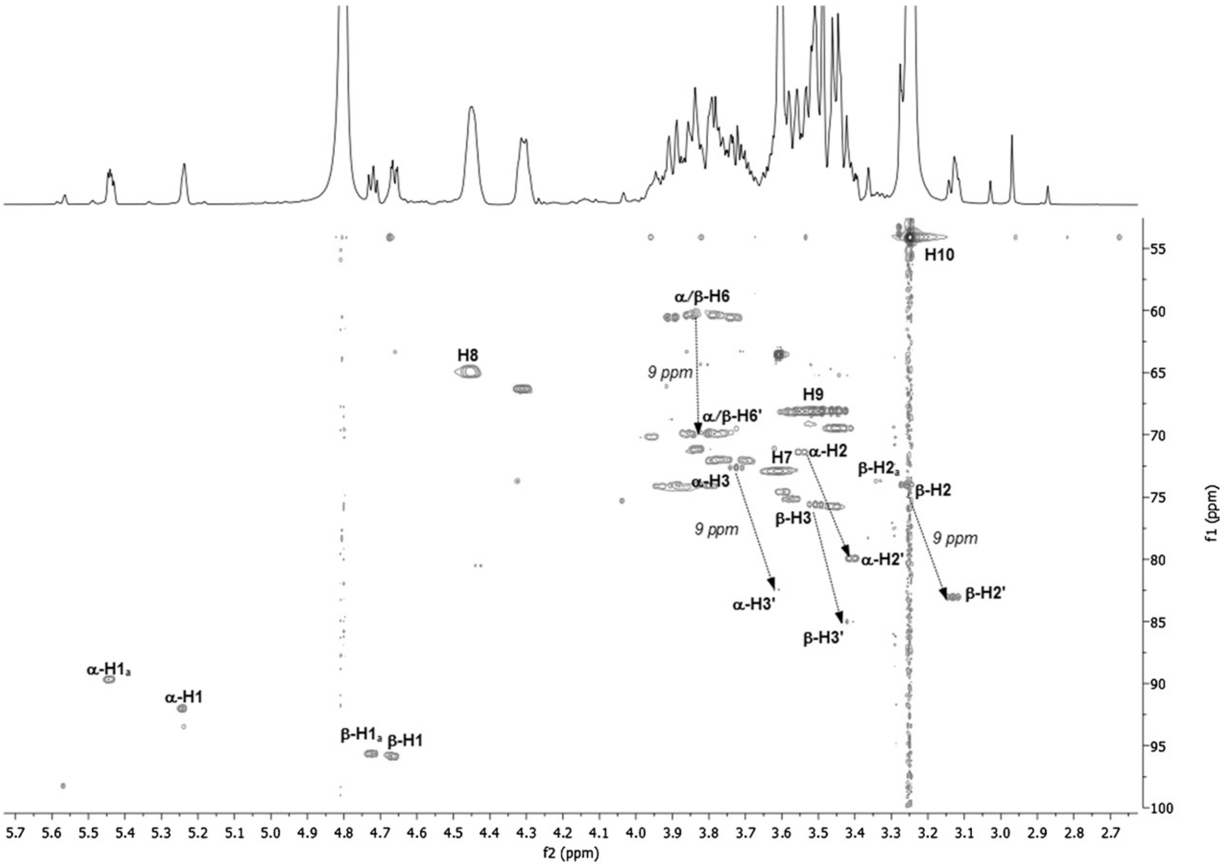

Fig. 4. HSQC NMR of hydrolyzed and concentrated nanocrystalline cellulose modified with the cationization reagent EPTMAC. The changes in shift are demonstrated using arrows. The ^{1}H NMR spectrum of hydrolyzed and concentrated nanocrystalline cellulose modified with the cationization reagent EPTMAC is inserted to make the figure more perspicuous.

time (P2). Procedure (P1) is based on previous work (Hasani, Westman, Potthast, & Rosenau, 2009). The second procedure (P2) is a dry procedure, which includes a preceding activation of the substrate with sodium hydroxide. The substrate was, consequently, washed with a sodium hydroxide solution (0.1 M) prior to solvent exchange with acetone and drying. EPTMAC, dissolved in water, was then distributed on the substrate using a spray technique. The cellulosic substrates were eventually placed in an oven where the actual reaction occurred after the evaporation of water. The results from the different procedures are presented in Table 1.

Analysis of nitrogen content in the modified cellulosic substrates indicated that the degree of substitution (DS_n) was fairly low for all reactions. However, evaluation of the outcome of the reactions promote that the solvent-minimizing spray technique (procedure P2) may have a more efficient use of the added EPTMAC. In this conclusion though, it must be taken into consideration that the three cellulosic substrates have different degrees of crystallinity and, in the case of kraft pulp, the presence of hemicelluloses should affect the reactivity.

3.2. NMR spectroscopy

To attain more information regarding the introduction of substituents on the polysaccharides, different NMR-techniques were applied. Structural characterization of reference softwood kraft pulp and the modified cellulosic substrates was accomplished using high resolution 1D and 2D NMR. For enhanced sensitivity a 600 MHz cryoprobe NMR instrument was used. In order to liberate monosaccharides, and increase solubility in deuterated solvents, the modified cellulosic materials were subjected to acid hydrolysis prior to analysis. However, when the degree of substitution is low, the signals originating from substituted monosaccharides become weak and limit the extraction of useful information from the spectra. To enhance the signals arising from the modified monosaccharide residues, the cationic monosaccharides were concentrated by the use of preparative ion exchange chromatography. The ^{1}H NMR spectra in Fig. 1 illustrate how the ion exchange chromatography opened for more detailed information. The nine methyl protons belonging to the ammonium group of the reacted EPTMAC are visible in the hydrolysate from the cationized softwood kraft pulp. The signal from the methyl protons was, as expected, strongly enhanced after ion exchange chromatography of the hydrolysate.

The degree of substitution (DS) can be estimated from the ^{1}H NMR spectrum in Fig. 1(1). According to Eq. (2), the degree of substitution of the modified softwood kraft pulp was calculated to 0.026. The spectra in Fig. 1 also illustrate the DS-enrichment of the column concentration method, although the integrals in the weak spectrum (3) should be seen as indicative.

Carbohydrate composition analysis, using ion chromatography, of the concentrated cationic fractions of the hydrolysate showed that these fractions were virtually free from unmodified monosaccharides (less than 0.5%). The solid phase extraction-column (SPE-column) provided with propylcarboxyl acid functionalized silica could consequently be considered suitable for the concentration of quaternized materials.

To further investigate the fractions of the ion-exchange chromatography, liquid chromatography–mass spectrometry (LC–MS) was performed. In the spectrum belonging to the neutral frac-

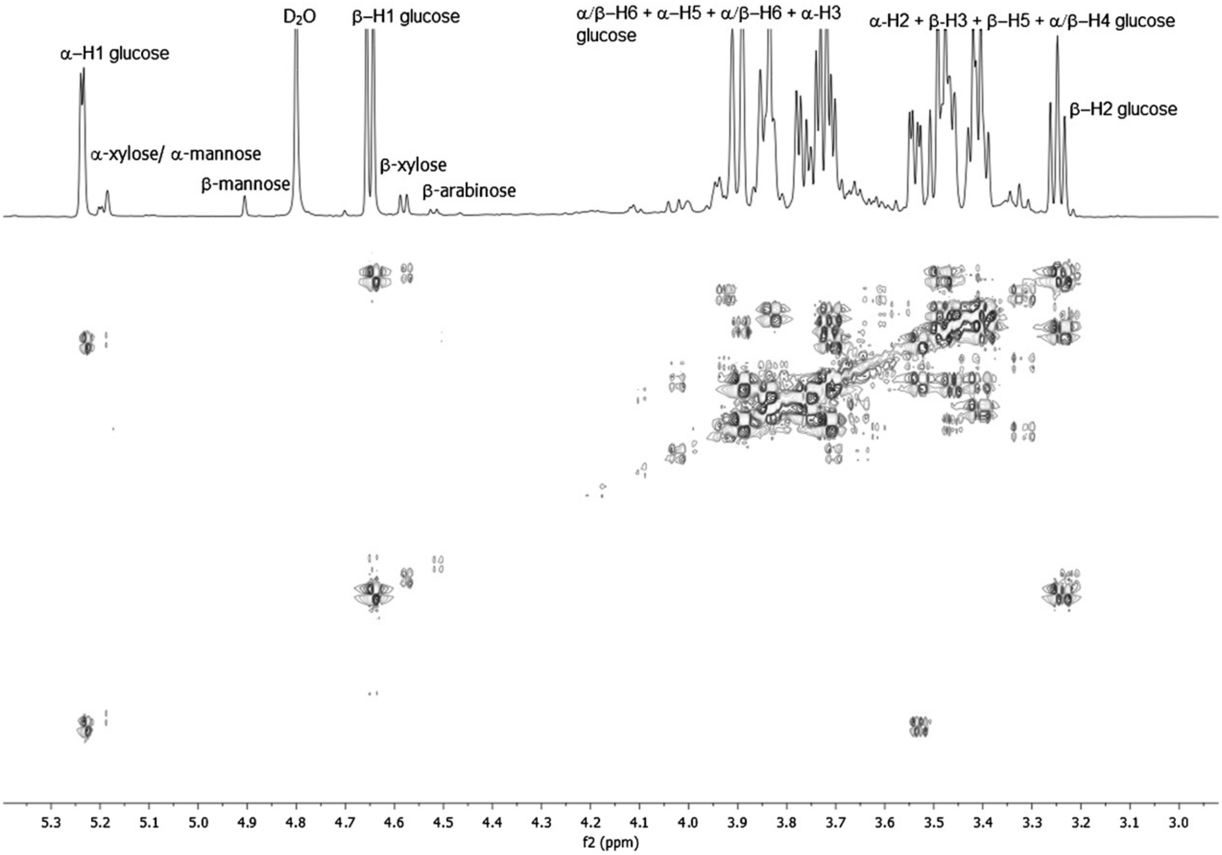

Fig. 5. DQF-COSY NMR of unmodified hydrolyzed softwood kraft pulp. In the anomeric region the peaks are assigned in order of shift values.

Table 2
The ratio of substitution at positions O-2, O-3 and O-6 for all three types of cellulosic materials according to NMR integrals.

Cellulosic substrate	O-2 (%)	O-3 (%)	O-6 (%)
Cotton linters	52	3	45
Nanocrystalline cellulose	45	7	48
Softwood kraft pulp	16	1	83

tion of the ion exchange-chromatography the molecular mass of single glucose monomers could be seen. Also small peaks of glucose dimers were observed. In the spectrum belonging to the cationic fractions, the molecular mass of cationic modified glucose monomers were observed, as well as traces of cationic modified pentoses and monosubstituted dimers. The presence of monosubstituted dimers in the samples could explain the minor variations in the NMR calculations in regard to column efficiency and DS values. The fragment pattern for single glucose monomers could not be seen in the cationic sample. The molecular mass of the by-product 2,3-dihydroxypropyl-trimethylammonium were present in the cationic fraction, although LC–MS and NMR of the washing water prior to ion-exchange chromatography confirm that this by-product, together with remaining EPTMAC, is washed out during the work-up. Di- or trisubstituted glucose could not be seen in the LC–MS sample of the cationic fraction. However, it cannot be excluded that these monomers are produced in the cationization reaction since they could be difficult to elute from the SPE-column. LC–MS and NMR spectra are accessible in the supplementary data.

Table 3
A summary of reference proton shifts and new proton shifts after substitution of different OH-protons on the nanocrystalline cellulose monomers. New shifts are seen as bold and italic.

Sample	H1	H2	H3	H4	H5	H6	H6′
β-D-Glucose							
Reference	4.67	3.26	3.51	3.45	3.47	3.73	3.90
2-OH substitution	***4.73***	***3.13***	***3.58***	3.45	3.47	3.73	3.90
3-OH substitution	4.67	***3.33***	***3.42***	3.45	3.47	3.73	3.90
6-OH substitution	4.67	3.26	3.51	3.45	3.47	***3.74***	***3.94***
α-D-Glucose							
Reference	5.24	3.55	3.72	3.45	3.83	3.78	3.85
2-OH substitution	***5.44***	***3.41***	***3.78***	3.45	3.83	3.78	3.85
3-OH substitution	5.24	***3.62***	***3.61***	3.45	3.83	3.78	3.85
6-OH substitution	5.24	3.55	3.72	3.45	3.83	***3.80***	***3.86***

3.2.1. Characterization of hydrolyzed and concentrated cationized nanocrystalline cellulose and cotton cellulose

The cationic fractions of nanocrystalline cellulose and cotton cellulose provide somewhat simpler NMR spectra compared to that of modified softwood kraft pulp, because of the absence of hemicelluloses related signals. For this reason, it seems appropriate to use the results from cotton linters and nanocrystalline cellulose as the starting point.

The ^{1}H NMR spectrum of hydrolyzed and concentrated modified nanocrystalline cellulose confirms modification by comprising new peaks at 4.45 ppm (H8) and 3.25 ppm (H10), belonging to the cationic substituent (Fig. 2). The H7 (3.63 ppm) and H9 (3.52 ppm) protons of the cationic substituent are hidden in the region of the ring-protons of the glucose residues (Ebringerova, Hromadkova, Kacurakova, & Antal, 1994). The methylene protons at H7, and H9, are diastereotopic and such protons do not experience the same environment as the side chain rotates. Unfortunately the protons cannot be distinguished separately in these spectra because of overlapping signals.

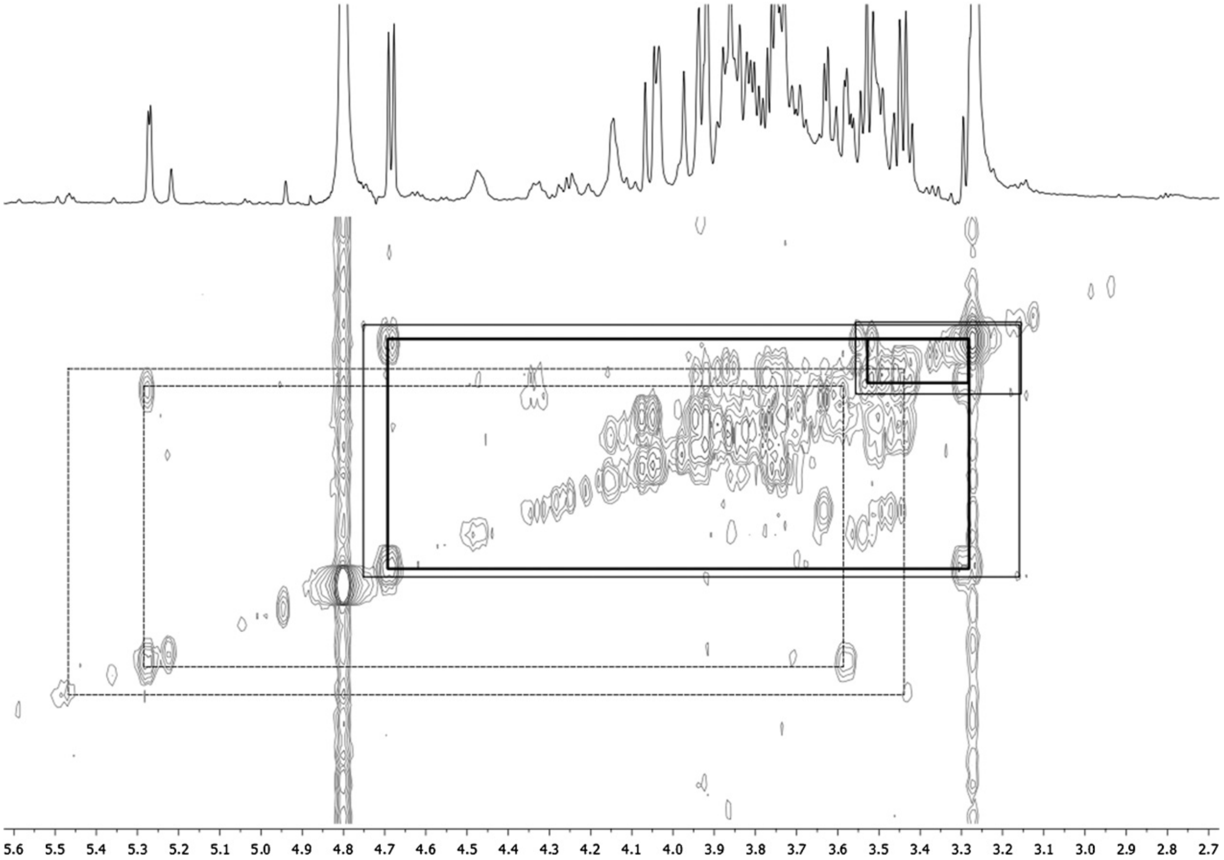

Fig. 6. COSY NMR of hydrolyzed and concentrated modified softwood kraft pulp. The heavy black rectangle corresponds to the couplings of the original position of the β-H2 proton, hidden in the strong peak belonging to the methyl groups of EPTMAC. The thin black rectangle shows the new couplings of the substituted position of β-H2. The dashed rectangles are the equivalent of the α-isomer. To make the figure more perspicuous, a ^{1}H NMR spectrum of hydrolyzed and concentrated softwood kraft pulp modified with the cationization reagent EPTMAC is inserted.

Additional peaks, not present in reference samples of cellulose, are visible at 5.45, 4.72 and 3.13 ppm in the ^{1}H NMR spectrum of hydrolyzed and concentrated modified nanocrystalline cellulose. To investigate these additional peaks, a ^{1}H–^{1}H correlated spectroscopy (COSY) NMR spectrum of the sample was recorded. The COSY spectrum proves that the additional peaks are the result of substitution of the α-2-OH and β-2-OH protons (Fig. 3) by addition of the oxirane moiety. Substitution at position 2 affect the shifts belonging to protons at position 1 as illustrated by α-H1$_a$ and β-H1$_a$ in the spectrum, where the subscribed (a) is a label for an affected proton (Schwikal, Heinze, Ebringerova, & Petzold, 2006). It can also be seen that the α-H3 and β-H3 protons are shifted downfield when substitution at the corresponding 2-OH occurs. Equally, the α-H2 and β-H2 are affected when substitution at position 3-OH occurs. It is reasonable that the α-H1 proton is more affected by the substitution of corresponding 2-OH than the β-H1 proton, because of stereoelectronic effects (de Oliveira, Tasic, Rocco, & Rittner, 2006; Juaristi, Cuevas, & Floresvela, 1992). The α-H1 proton is in the same equatorial plane as the substituted hydroxyl group and is more prone to interact with the neighboring oxygen. Substituent effects on the ^{1}H chemical shifts of monosaccharides are complex, and this observation requires further investigation.

This conclusion is further evidenced by the ^{13}C–^{1}H correlated heteronuclear single quantum coherence (HSQC) NMR, which is in line with previous work where it has been found that substitution of a hydroxy group change the shift of the adjacent carbon (Heinze, Haack, & Rensing, 2004). The HSQC NMR spectrum of nanocrystalline cellulose shows the typical ∼9 ppm change in shift for carbons next to substituted hydroxy groups (Fig. 4). It is concluded from the spectrum that substitution occurs at all three possible positions of cellulose, although positions 2 and 6 are highly favored, as determined by comparing the integrals of the peaks belonging to the original and substituted atoms (Table 2).

The peak β-H2$_a$, belonging to the β-H2 proton affected by the substitution of the β-3-OH proton, is marked in the spectrum. This proton has a somewhat higher proton shift and lower carbon shift than the original position of β-H2. Similar patterns can be found close to the peaks belonging to the α-H2, α-H3 and β-H3 protons.

A summary of the substitution patterns for nanocrystalline cellulose, including how the proton and carbon shifts are affected by different substitution positions, is presented in Tables 3 and 4.

Table 4
A summary of reference carbon shifts and new carbon shifts after substitution of different OH-protons on the concentrated nanocrystalline cellulose monomers. New shifts are seen as bold and italic.

Sample	C1	C2	C3	C4	C5	C6
β-D-Glucose						
Reference	95.9	74.1	75.6	69.4	75.8	60.5
2-OH substitution	***95.7***	***83.0***	***75.1***	69.4	75.8	60.5
3-OH substitution	95.9	***73.7***	***85.0***	69.4	75.8	60.5
6-OH substitution	95.9	74.1	75.6	69.4	75.8	***70.2***
α-D-Glucose						
Reference	91.7	71.4	72.6	69.2	71.0	60.2
2-OH substitution	***89.7***	***80.0***	***72.1***	69.2	71.0	60.2
3-OH substitution	91.7	***71.1***	***82.5***	69.2	71.0	60.2
6-OH substitution	91.7	71.4	72.6	69.2	71.0	***69.9***

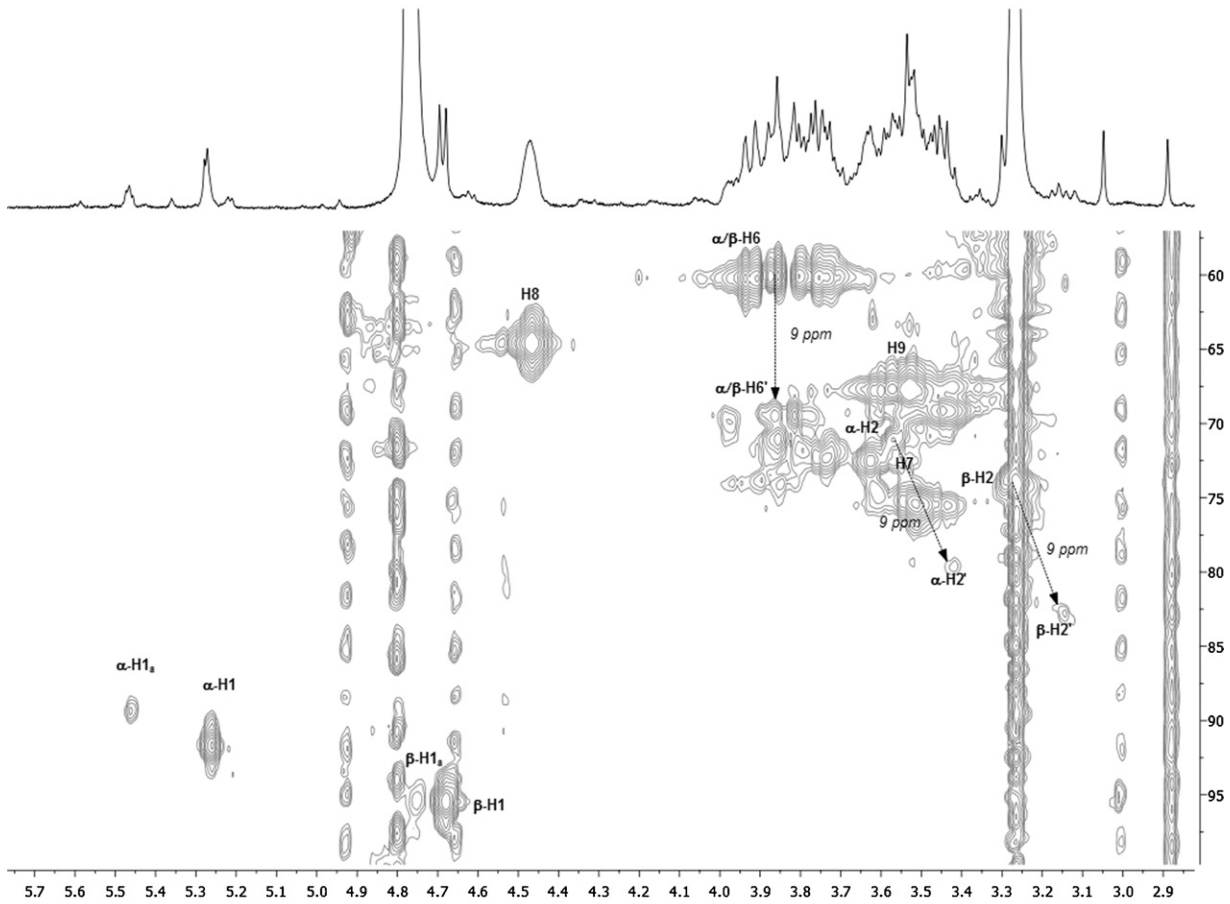

Fig. 7. HSQC NMR of hydrolyzed and concentrated softwood kraft pulp modified with the cationization reagent EPTMAC. The changes in shift are demonstrated using arrows. To make the figure more perspicuous, a ^{1}H NMR spectrum of hydrolyzed and concentrated softwood kraft pulp modified with the cationization reagent EPTMAC is inserted.

The results from the nanocrystalline cellulose are analogous to the results of the cotton cellulose (see supplementary data).

There are two strong unidentified peaks at 4.3 and 3.6 ppm found not only in the nanocrystalline cellulose NMR sample, but also in the NMR sample from cotton cellulose. The ^{1}H–^{1}H correlated spectra indicate strong couplings between these peaks. Two additional experiments were performed to investigate these peaks: the reaction of ethanol and isopropanol with EPTMAC and the reaction of EPTMAC in sodium hydroxide and water. In the second mentioned experiment the referring peaks increase with time. This study indicates that the peaks most likely belong to the by-product 2,3-dihydroxypropyl-trimethylammonium. This by-product is formed when the cationic epoxide 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) reacts with water under basic conditions.

The intramolecular side-reactions possible during hydrolysis of 2-*O*-hydroxalkyl ethers of glucans, where an intramolecular acetal can be formed, are not evidently visible in the spectra (Mischnick & Momcilovic, 2010). However, these side-reactions cannot be excluded since traces of peaks can be seen in the areas where predicted shifts are expected. The by-product *O*-(2-oxo)propyl-glucose resulting from the plausible β-elimination reaction occurring in the presence of hydroxide ions and vacuum, could not be detected using LC–MS.

3.2.2. *Characterization of hydrolyzed reference softwood kraft pulp and hydrolyzed and concentrated cationized softwood kraft pulp*

The NMR spectrum obtained from the reference softwood kraft pulp contains observable peaks belonging to hemicellulose monomers, i.e. xylose and mannose (Fig. 5). The high resolution of the double quantum filtered (DQF)-COSY NMR of the reference softwood kraft pulp enables full assignment of the anomeric protons belonging to glucose.

A coupling pattern identical to modified nanocrystalline cellulose and cotton cellulose can be seen in the ^{1}H–^{1}H correlated COSY NMR of the hydrolyzed and concentrated cationized softwood kraft pulp (Fig. 6).

The HSQC NMR of hydrolyzed and concentrated modified softwood kraft pulp also agrees with previous spectra (Fig. 7). Substitution at position 3 of glucose is very low and only visible when the spectrum is zoomed in. No clear indications of the presence of substituted xylose and mannose monomers could be seen in the spectra in Figs. 6 and 7.

The observation that substitution at position 2 (O-2) is comparatively high in crystalline cellulosic substrates is in line with previous findings (Kondo, 2005; Volkert & Wagenknecht, 2008). Because of its relatively minor involvement in the intermolecular hydrogen bonding pattern of the cellulose crystalline structure, position 2 becomes more accessible under heterogeneous conditions. It is however interesting that cationization of the softwood kraft pulp according to the spray technique shows a higher preference for O-6 (83%), on the expense of O-2 substitution (16%). The variation in crystallinity of the samples does not compensate for this difference and the result could be a consequence of the various cationization procedures.

4. Conclusions

Softwood kraft pulp, nanocrystalline cellulose and cotton cellulose were successfully reacted with the cationic epoxide 2,3-epoxypropyltrimethyl ammonium chloride (EPTMAC) according to two different procedures, whereof one was a solvent and reagent minimizing spray technique. After reaction, the modified cellulosic materials were subjected to acid hydrolysis and the liberated monosaccharide residues were analyzed with different NMR-techniques. The use of fast ion exchange SPE-column chromatography concentrated the cationic monomers and enhanced the intensity of the peaks belonging to the modified monosaccharides in the NMR spectra. The concentration method can beneficially be applied on a cationic cellulosic substrate with a low degree of substitution. The outcomes of the reactions were studied using high resolution 1D and 2D ^{1}H–^{1}H and ^{13}C–^{1}H solution-state NMR. The NMR results proved substitution at positions 2, 3 and 6 on the glucose units, even if substitution was favored at positions 2 and 6 for all three types of cellulosic materials. Unlike nanocrystalline cellulose and cotton cellulose, the cationic softwood kraft pulp showed a higher preference for O-6 (83%), on the expense of O-2 substitution (16%). The result could be a consequence of the various cationization procedures. The 2D correlation NMR spectra demonstrated a consistent 9 ppm change in the initial shifts of carbons adjacent to substituted hydroxyl groups. Other neighboring atoms were also affected by the substitution resulting in small changes towards higher shifts for protons and slightly lower shifts for carbons. This characterization procedure gives valuable information about the molecular composition of cationic modified cellulosic materials also with a low degree of substitution. The reactivity of the hemicelluloses, however, cannot clearly be seen in the NMR analyses. A tentative explanation may be that cationic modified hemicelluloses become water soluble and are washed out in the washing operation after reaction with EPTMAC.

Acknowledgements

The authors would like to gratefully acknowledge VINNOVA, SCA Hygiene Products AB and Södra for financial support and for giving us the opportunity to work with this interesting project. Dr. Göran Karlsson and Dr. Cecilia Persson at the Swedish NMR Centre in Göteborg, Sweden, are acknowledged for valuable support and help setting up the NMR experiments, and Tommy Olsson at the Department of Biology, Plant Ecology and Systematics, Lund University, for performing the elementary analysis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.03.038.

References

Atalla, R. H., & VanderHart, D. L. (1999). The role of solid state C-13 NMR spectroscopy in studies of the nature of native celluloses. *Solid State Nuclear Magnetic Resonance*, *15*(1), 1–19.

Beck-Candanedo, S., Roman, M., & Gray, D. G. (2005). Effect of reaction conditions on the properties and behavior of wood cellulose nanocrystal suspensions. *Biomacromolecules*, *6*(2), 1048–1054.

de Oliveira, P. R., Tasic, L., Rocco, S. A., & Rittner, R. (2006). Stereoelectronic and inductive effects on H-1 and C-13 NMR chemical shifts of some cis-1,3-disubstituted cyclohexanes. *Magnetic Resonance in Chemistry*, *44*(8), 790–796.

Ebringerova, A., Hromadkova, Z., Kacurakova, M., & Antal, M. (1994). Quaternized xylans—Synthesis and structural characterization. *Carbohydrate Polymers*, *24*(4), 301–308.

Ek, R., Henriksson, U., Nystrom, C., & Odberg, L. (1994). Pore characterization in cellulose beads from diffusion studies using the spin-echo NMR technique. *Powder Technology*, *81*(3), 279–286.

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.

Haggkvist, M., Li, T. Q., & Odberg, L. (1998). Effects of drying and pressing on the pore structure in the cellulose fibre wall studied by H-1 and H-2 NMR relaxation. *Cellulose*, *5*(1), 33–49.

Hasani, M., Westman, G., Potthast, A., & Rosenau, T. (2009). Cationization of cellulose by using N-oxiranylmethyl-N-methylmorpholinium chloride and 2-oxiranylpyridine as etherification agents. *Journal of Applied Polymer Science*, *114*(3), 1449–1456.

Heinze, T., Haack, V., & Rensing, S. (2004). Starch derivatives of high degree of functionalization. 7. Preparation of cationic 2-hydroxypropyltrimethylammonium chloride starches. *Starch-Starke*, *56*(7), 288–296.

Jansson, P. E., Kenne, L., & Widmalm, G. (1989). Computer-assisted structural-analysis of polysaccharides with an extended version of casper using ^{1}H-NMR and ^{13}C-NMR data. *Carbohydrate Research*, *188*, 169–191.

Juaristi, E., Cuevas, G., & Floresvela, A. (1992). Stereoelectronic interpretation of the unusual perlin effects and H-1-NMR chemical shifts in 1,3-oxathiane. *Tetrahedron Letters*, *33*(46), 6927–6930.

Kim, H., & Ralph, J. (2010). Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO–d(6)/pyridine–d(5). *Organic & Biomolecular Chemistry*, *8*(3), 576–591.

Kondo, T. (2005). Hydrogen bonds in cellulose and cellulose derivatives. In S. Dumitriu (Ed.), *Polysaccharides*. New York: Marcel Dekker.

Lennholm, H., Larsson, T., & Iversen, T. (1994). Determination of cellulose I_α and I_β in lignocellulosic materials. *Carbohydrate Research*, *261*(1), 119–131.

Mischnick, P., & Hennig, C. (2001). A new model for the substitution patterns in the polymer chain of polysaccharide derivatives. *Biomacromolecules*, *2*(1), 180–184.

Mischnick, P., & Momcilovic, D. (2010). Chemical structure analysis of starch and cellulose derivatives. *Advances in Carbohydrate Chemistry and Biochemistry*, *64*, 117–210.

Nehls, I., Wagenknecht, W., Philipp, B., & Stscherbina, D. (1994). Characterization of cellulose and cellulose derivatives in solution by high-resolution ^{13}C-NMR spectroscopy. *Progress in Polymer Science*, *19*(1), 29–78.

Olson, J. R., Chang, S. J., & Wang, P. C. (1990). Nuclear-magnetic-resonance imaging—A noninvasive analysis of moisture distributions in white oak lumber. *Canadian Journal of Forest Research/Revue Canadienne De Recherche Forestiere*, *20*(5), 586–591.

Rahman, M., & Brazel, C. S. (2004). The plasticizer market: An assessment of traditional plasticizers and research trends to meet new challenges. *Progress in Polymer Science*, *29*(12), 1223–1248.

Richardson, S., & Gorton, L. (2003). Characterisation of the substituent distribution in starch and cellulose derivatives. *Analytica Chimica Acta*, *497*(1–2), 27–65.

Schwikal, K., Heinze, T., Ebringerova, A., & Petzold, K. (2006). Cationic xylan derivatives with high degree of functionalization. *Macromolecular Symposia*, *232*, 49–56.

Shahidi, F., Arachchi, J. K. V., & Jeon, Y. J. (1999). Food applications of chitin and chitosans. *Trends in Food Science & Technology*, *10*(2), 37–51.

Solarek, D. B. (1986). Cationic starches. In O. B. Wurzburg (Ed.), *Modified starches: Properties and uses* (pp. 114–129). Boca Raton: CRC Press Inc.

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*, 330–336.

Ungewiss, J., Vietzke, J. P., Rapp, C., Schmidt-Lewerkuhne, H., Wittern, K. P., & Salzer, R. (2005). Quantitative determination of cationic modified polysaccharides on hair using LC–MS and LC–MS–MS. *Analytical and Bioanalytical Chemistry*, *381*(7), 1401–1407.

Volkert, B., & Wagenknecht, W. (2008). Substitution patterns of cellulose ethers—Influence of the synthetic pathway. *Macromolecular Symposia*, *262*, 97–118.