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# Comprehensive analysis of the substituent distribution in 3-*O*-ethyl/propyl cellulose derivatives

Julia Cuers [a], Yunhui Wang [b], Andreas Koschella [b], Thomas Heinze [b], Petra Mischnick [a,*]

[a] *Technische Universität Braunschweig, Institute for Food Chemistry, Schleinitzstr. 20, D-38106 Braunschweig, Germany*
[b] *Friedrich Schiller University of Jena, Institute for Organic Chemistry and Macromolecular Chemistry, Center of Excellence for Polysaccharide Research, Humboldtstraße 10, D-07743 Jena, Germany*

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ABSTRACT

Two 3-*O*-ethyl-3-*O*-propyl celluloses of similar ethyl and propyl content prepared under different conditions were analyzed with respect to their substituent distribution in the glucosyl units and along the polymer chain by ESI-MS analysis of labeled oligosaccharides. Both samples showed similar regioselectivity of substitution and random distribution of ethyl and propyl groups over the 3-*O*-positions. The distribution of all types of glucosyl residues identified by monomer analysis was also random over the cellulose molecules. Comparison of the substitution pattern in the glucosyl units with the pattern expected for the observed regioselectivity showed relative high 2-*O*-alkylation in spite of protecting group strategy, but no corresponding 2,3-di-*O*-substitution. This is probably the result of partial silyl migration under the alkaline alkylation conditions.



## 1. Introduction

The analysis of the substituent distribution in modified polysaccharides is of great interest for the understanding of reaction mechanisms and structure–property relationships. Differences in rheological or dissolution behavior of derivatives of same type and average DS are assumed to be caused by different substituent distributions in the polysaccharide chain and thus of sequences responsible for aggregation or other organization phenomena (Heinze & Liebert, 2012; Heinze & Petzold-Welcke, 2012). Due to the dispersity of polysaccharides with respect to their chain length (DP) and degree of substitution (DS) as well as distribution of substituents no defined sequence but only probabilities of various substitution patterns can be determined. Mass spectrometry of partially degraded cellulose ethers has been applied to determine substitution profiles of oligomeric mixtures which were compared with calculated theoretical patterns (Mischnick & Momcilovic, 2010). This approach requires quantifiability of the mass spectra, which has been achieved by permethylation with MeI-$d_3$ in case of methyl cellulose (Arisz, Kauw, & Boon, 1995) or methyl amylose (Mischnick & Kühn, 1996), and by additional labeling with ionic tags in case of hydroxyalkyl celluloses (Adden, Müller, & Mischnick, 2006; Adden, Müller, Brinkmalm, Ehrler, & Mischnick, 2006). While alkoxy groups are otherwise overestimated due to their high contribution to sodium ion complexation, ethyl or propyl derivatives of oligosaccharides show enhanced ion intensity in electrospray ionization due to their higher surface activity. Because of the interest in defined derivatives, regioselectively substituted cellulose ethers have been prepared using protecting groups (Fox, Li, Xu, & Edgar, 2011; Heinze & Liebert, 2012). Recently, 3-*O*-alkyl celluloses bearing two different alkyl moieties on one polymer chain were prepared from 2,6-di-*O*-thexyldimethylsilyl cellulose by simultaneous reaction with two alkyl iodides in the presence of sodium hydride under various conditions followed by cleavage of the TDMS groups. Two mixed 3-*O*-ethyl/propyl celluloses with similar DS and ethyl/propyl ratio prepared under different conditions, one at room temperature with TBAF as phase transfer catalyst, the other one at 50 °C without TBAF, showed different behavior in SEC analysis (Table 1) (Heinze, Wang, Koschella, Sullo, & Foster, 2012).

The lower $DP_n$ of sample **1** may be caused by polymer degradation during this multistep synthesis. However, the $DP_n$ found for sample **2** by calibration with pullulan standards is relatively higher than the $DP_n$ of the starting cellulose ($DP_n$ 117). Therefore, $DP_n$ 212 is an apparent DP that is caused by aggregation effects. Due to the fact that samples **1** and **2** possess nearly the same composition on the molecular level of the anhydroglucose unit, these differences might be due to a different functionalization pattern along the polymer chain.

Therefore, the compounds have been analyzed with respect to their substituent distribution in the chain. In this respect ethyl or propyl celluloses have not been analyzed before.

* Corresponding author. Tel.: +49 531 3917201; fax: +49 531 3917230.
*E-mail addresses:* p.mischnick@tu-braunschweig.de, p.mischnick@tu-bs.de (P. Mischnick).

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## 2. Experimental

### 2.1. Materials

The 3-*O*-ethyl/propyl celluloses were prepared from 2,6-di-*O*-TDMS cellulose in THF with sodium hydride and EtI and PrI under different conditions as has been described earlier (Heinze et al., 2012). For ESI-MS Methanol of LC-MS grade was used. The reagents for reductive amination *m*-aminobenzoic acid (mABA) and 2-picoline borane were obtained from Sigma–Aldrich (St. Louis, USA).

### 2.2. GLC-FID/MS analysis

1 mg of the samples was treated with 2 M TFA at 120 °C for 120 min. After cooling to r.t. the acid was removed in a stream of nitrogen by co-distillation with toluene. Obtained monomers were reduced with 500 μL of 0.25 M $NaBD_4$ solution in 2 M $NH_3$ at 60 °C for 120 min. After cooling to r.t., the solution was co-evaporated in a stream of nitrogen with methanolic acetic acid (15%) to remove borate as trimethyl borate. The residue was dissolved in 50 μL of pyridine and 200 μL of acetic anhydride and heated to 90 °C for 120 min.

The acetylated alditols were extracted by liquid/liquid extraction with dichloromethane and washed once with saturated $NaHCO_3$-solution, 0.1 M HCl (2 mL) and three times with 2 mL water. The organic phase is dried over $CaCl_2$ and used after dilution for gas chromatography.

*GLC-FID* analysis was carried out on a GC-2010 (Shimadzu) equipped with a ZB-5MS column (Phenomenex, length = 29 m, i.d. = 0.25 mm) and hydrogen as carrier gas (40 cm/s, linear velocity mode. Splitless injection mode (250 °C) was used for sample application. Temperature program: initial temperature was set to 60 °C and held for 1 min. Temperature was increased with 20 °C/min to 150 °C, with 2 °C/min to 190 °C and finally with 25 °C/min to 310 °C and held for 10 min. For data evaluation GCsolution (Shimadzu, Version 2.30.00 SU7) was used.

*GLC-MS* analysis was carried out on a GC 5890A (Hewlett Packard) with electron impact ionization (EI) (70 eV) used in split injection mode (1:20). The same column and temperature program as in GLC-FID was used. Data evaluation was performed with Amdis Analysis (Version 2.68).

### 2.3. Perdeuteromethylation

Perdeuteromethylation was performed with NaOH/MeI-$d_3$ in DMSO at room temperature (Ciucanu & Kerek, 1984). Ca. 15–20 mg of each sample was weighed in a Schlenck flask, dried in vacuum for several hours and then dissolved in 2 mL DMSO. To the dissolved material ca. 200 mg of freshly powdered NaOH (100 mg/mL) was added under nitrogen. After 15 min stirring MeI-$d_3$ (5 eq./OH) was added. After 12 h again 2.5 equiv./OH of MeI-$d_3$ was added and the mixture was stirred for 4 more hours. Excess alkylation reagent was removed in vacuum and the samples were isolated by liquid/liquid extraction with dichloromethane and water (yield: 69–82%).

### 2.4. Partial degradation

In a 1 mL V-vial ca. 2 mg of perdeuteromethylated 3-*O*-ethyl/propylcellulose (10 μmol AGU) were dispersed in a mixture of acetone and water (5/4.25, v/v). Then 75 μL of trifluoroacetic acid (concentrated) were added and the V-vial was kept at 120 °C for various times. After cooling, the sample was co-distilled with toluene to remove TFA and evaporated to dryness under a stream of nitrogen.

### 2.5. Labeling with m-aminobenzoic acid

The partially hydrolyzed perdeuteromethylated sample (2 mg, 10 μmol) was dissolved in 0.5 mL MeOH and 0.3 mL of mABA-solution (containing 2.7 mg; 20 μmol) was added. After addition of 0.15 mL glacial acetic acid, the mixture was heated to 40 °C for 30 min.

Reduction was performed by adding 50 μL of a 2-picoline borane solution in MeOH (containing 2.1 mg; 20 μmol) and holding the mixture at 40 °C for 45 min. Afterwards the solvent was removed in a stream of nitrogen and the residue was re-dissolved in 1 mL MeOH. For ESI-MS infusion the solution was diluted (1:100) with methanol.

### 2.6. ESI-MS

ESI-MS measurements were perfomed on a HCT Ultra ETDII (Bruker Daltonics, Bremen, Germany), equipped with an ion trap. The analyte solutions (~0.02 mg/mL in MeOH) were infused directly with a syringe at a flow rate of 200 μL/h. Each spectrum is an average of 200 scans.

Nitrogen was used as dry gas (5 L $min^{-1}$, 300 °C) and as nebulizer gas (10 psi). The following voltages were used: capillary 3500 V, end plate offset −500 V. The Trap Drive, Octopole RF amplitude, Oct 2 DC were varied. Mass spectra evaluation was performed with Data Analysis (Bruker Daltonics, Bremen, Germany).

## 3. Results and discussion

Two samples of 3-*O*-ethyl/propyl cellulose (**1** and **2**, see Table 1) prepared from 2,6-di-*O*-TDMS-celluose by alkylation with a mixture of EtI and PrI as described (Heinze et al., 2012) were analyzed with respect to the substituent distribution in the glucosyl residues and in the cellulose chain. First the monomer composition of the two cellulose derivatives was determined by GLC-FID and GLC-MS of the corresponding alditol acetates to obtain detailed information about the substitution pattern in the glucosyl residues (Voiges, Adden, Rinken, & Mischnick, 2012). A typical chromatogram is shown in Fig. 1.

The gas chromatograms showed that not only mono-substituted glucoses were present but also di-substituted units with either only

**Table 1**
Properties of 3-*O*-ethyl/propyl cellulose obtained by simultaneous conversion of 2,6-di-*O*-thexyldimethylsilyl cellulose with ethyl- and propyl iodide in the presence of sodium hydride under different reaction conditions and subsequent cleavage of the protecting groups (Heinze et al., 2012).

| Alkylation condition | Sample | $DS_{Et}$ [a] | $DS_{Pr}$ [b] | $DS_{Total}$ [c] | LCST [d] (°C) | $DP_n$ [e] |
|---|---|---|---|---|---|---|
| 4 d, room temperature, tetra-*n*-butylammonium iodide | **1** | 0.59 | 0.43 | 1.02 | 38 | 20 |
| 4 d, 50 °C, no phase transfer catalyst | **2** | 0.54 | 0.42 | 0.96 | 38 | 212 |

[a] Degree of substitution of ethyl groups determined by $^1$H NMR spectroscopy after peracetylation.
[b] Degree of substitution of propyl groups determined by $^1$H NMR spectroscopy after peracetylation.
[c] Total degree of substitution.
[d] Lower critical solution temperature determined by turbidimetric photometry of 1% (w/v) aqueous solution.
[e] Number average degree of polymerization determined by size exclusion chromatography in *N*,*N*-dimethyl acetamide/LiCl.

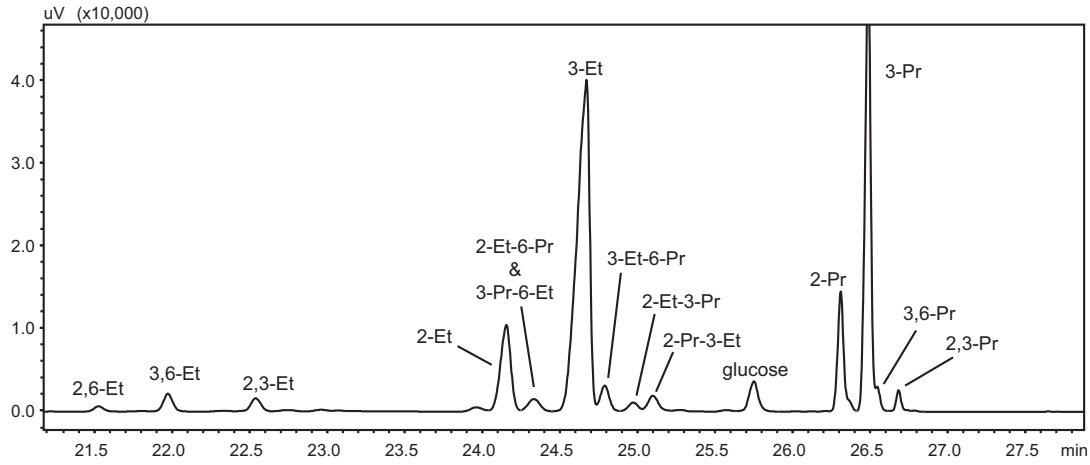


**Fig. 1.** Gas chromatogram of 3-*O*-ethyl/propyl cellulose (**1**) after hydrolysis, reduction and acetylation.

one type of substituent or Et and Pr substitution. The total monomer composition ($s_i$) of both samples the partial DS values ($x_i$) as well as DS values obtained by NMR spectroscopy (Heinze et al., 2012) are shown in Tables 2 and 3.

The analytical data show that regioselective protection of O-6 and O-2 using thexyldimethylsilyl chloride (TDMS-Cl) enables high regioselectivity in favor of O-3 with 75–76% of all ethyl and 67% of all propyl groups located in this position in both samples. In total, 80% (**1**) or 81% (**2**) of hydroxyl groups at position 3 are alkylated. Regioselectivity is higher for ethyl than for propyl, where 10–12% of the substituents are found at O-6 (Et: 6 or 7%) and even 21% (**1**, Et: 17%) or 23% (**2**, Et: 19%) at O-2. The relative high substitution at O-2 probably is at least partially an effect of silyl rearrangement during alkaline alkylation (Mischnick, Lange, Gohdes, Stein, & Petzold, 1995). This assumption is supported by the observation that the content of 2,3-di-*O*-substituted monomers is lower than expected from the amount of corresponding mono-substituted glucosyl residues. The determined amount only shows on average less than 30% of the expected 2,3-disubstitution ($x_2 \cdot x_3$) while the determined extent of 2,6- or 3,6-substitution matches the estimations ($x_2 \cdot x_6$ and $x_3 \cdot x_6$). If rearrangement of 2-*O*-TDMS

**Table 2**
Monomer composition (mol%) of ethyl-propyl celluloses **1** and **2** as obtained by GLC-FID analysis of corresponding alditol acetates ($n = 4$ (**1**) and $n = 5$ (**2**) independent analyses). For evaluation peak areas were corrected according to the ECR (effective carbon response) concept.

| Substitution pattern | **1** (mol%) | **2** (mol%) |
|---|---|---|
| 2,6-Et | 0.74 ± 0.20 | 0.66 ± 0.07 |
| 3,6-Et | 2.20 ± 0.51 | 2.33 ± 0.23 |
| 2,3-Et | 1.68 ± 0.39 | 1.19 ± 0.12 |
| 2-Et | 9.08 ± 0.65 | 7.58 ± 0.23 |
| 2-Et-6-Pr, 3-Pr-6-Et[a] | 1.86 ± 0.43 | 2.19 ± 0.22 |
| 3-Et | 42.82 ± 1.02 | 41.12 ± 0.80 |
| 3-Et-6-Pr | 2.78 ± 0.61 | 3.42 ± 0.27 |
| 2-Et-3-Pr | 0.84 ± 0.04 | 0.75 ± 0.06 |
| 2-Pr-3-Et | 1.67 ± 0.17 | 1.48 ± 0.18 |
| Non-subst. | 2.78 ± 0.78 | 2.94 ± 0.32 |
| 2-Pr | 7.13 ± 0.12 | 7.41 ± 0.18 |
| 3-Pr | 24.39 ± 0.50 | 26.19 ± 0.21 |
| 3,6-Pr | 1.00 ± 0.49 | 1.66 ± 0.12 |
| 2,3-Pr | 1.02 ± 0.09 | 1.06 ± 0.13 |

[a] Peaks overlap, for further evaluation a ratio of 3:7 was estimated.

**Table 3**
Partial and average DS values and monomer composition (mol%) of ethyl-propyl celluloses **1** and **2** as obtained by GLC-FID analysis of corresponding alditol acetates, compared to average DS values obtained by NMR[a] and ESI-MS. $s_i$: glucosyl residues substituted in position $i$; $c_i$: glucosyl residues $i$-fold substituted; $x_i$: partial DS-value in position $i$.

| | **1** | | | | **2** | | | |
|---|---|---|---|---|---|---|---|---|
| Et and Pr | GLC | (mol%) | NMR | ESI | GLC | (mol%) | NMR | ESI |
| $s_0$ | 2.78 | | | | 2.94 | | | |
| $s_2$ | 16.21 | | | | 14.99 | | | |
| $s_3$ | 67.22 | | | | 67.31 | | | |
| $s_6$ | – | | | | – | | | |
| $s_{23}$ | 5.21 | | | | 4.48 | | | |
| $s_{26}$ | 1.30 | | | | 1.32 | | | |
| $s_{36}$ | 7.29 | | | | 8.95 | | | |
| $s_{236}$ | – | | | | – | | | |
| $c_0$ | 2.78 | | | | 2.94 | | | |
| $c_1$ | 83.43 | | | | 82.30 | | | |
| $c_2$ | 13.80 | | | | 14.75 | | | |
| $x_2$ *Et* | 0.13 | 18.9% | | | 0.11 | 16.70 | | |
| $x_3$ *Et* | 0.51 | 74.9% | | | 0.50 | 76.32 | | |
| $x_6$ *Et* | 0.04 | 6.2% | | | 0.05 | 6.98 | | |
| $x_2$ *Pr* | 0.10 | 23.0% | | | 0.10 | 21.23 | | |
| $x_3$ *Pr* | 0.29 | 66.8% | | | 0.31 | 66.53 | | |
| $x_6$ *Pr* | 0.04 | 10.2% | | | 0.06 | 12.23 | | |
| DS Et | 0.68 | 61.5% | 0.56[a] | 0.68 | 0.65 | 58.1% | 0.59[a] | 0.65 |
| DS Pr | 0.43 | 38.5% | 0.42[a] | 0.44 | 0.47 | 41.9% | 0.43[a] | 0.50 |
| $DS_{total}$ | 1.11 | | 0.96[a] | 1.13 | 1.12 | | 1.02[a] | 1.15 |

[a] DS of ethyl- (Et) and propyl (Pr) groups determined by $^1$H NMR spectroscopy after peracetylation of the samples (Heinze et al., 2012).

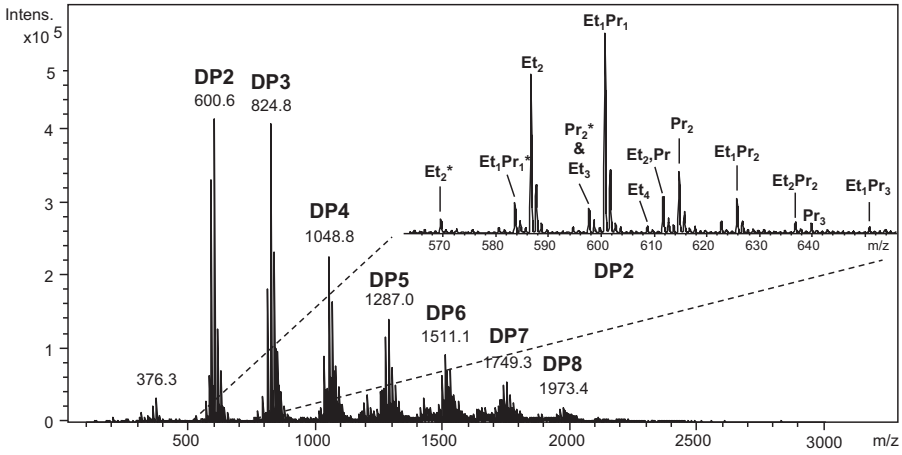


**Fig. 2.** Mass spectrum of perdeuteromethylated sample **1** after partial hydrolysis and reductive amination with mABA (negative ion mode). The annotation * denotes not completely deuteromethylated signals which have been taken into consideration in the data evaluation.

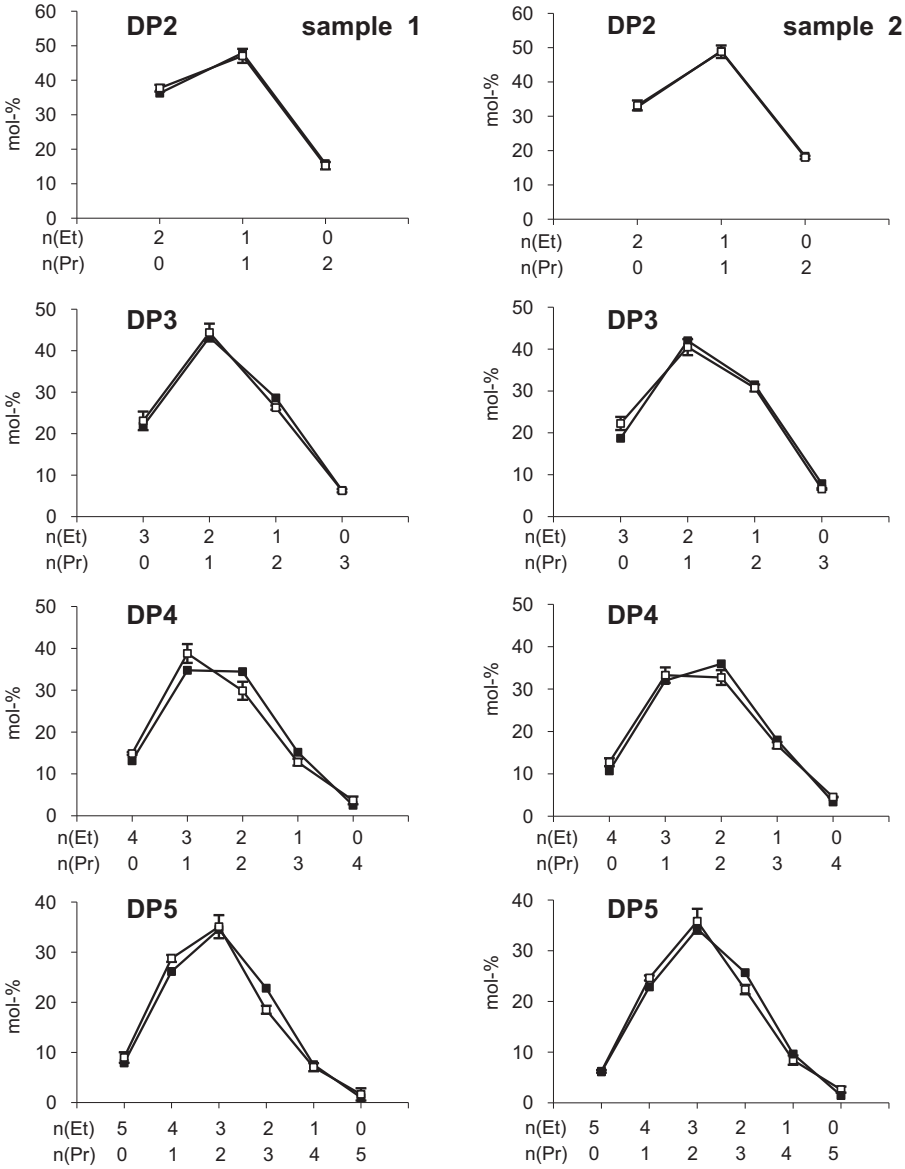


**Fig. 3.** Comparison of experimental (□) and calculated (■) distribution of ethyl and propyl substituents in monosubstituted AGUs of DP 2–5 of perdeuteromethylated samples **1** (left) and **2** (right).

is involved, O-3 becomes protected instead of O-2 and either O-2 *or* O-3 is alkylated, but not both of them. In former studies, 4–7% of rearrangement has been estimated for 2,6-TDMS celluloses (DS 1.7–1.9) during methylation with Na/MeI in THF from the ratio of mono-2-*O*-methyl-glucose to the summarized mono-2- and mono-3-*O*-methyl-glucoses. When these apparent degrees of rearrangement are calculated independently for ethyl and propyl products, 17.5 (**1**) and 15.6% (**2**) are found for ethylation, and 22.6 (**1**) and 22.0% (**2**) for propylation. This increase of rearrangement correlates with the decrease of reactivity giving more time for equilibration of the 2-*O*-/3-*O*-TDMS-glucosyl residues via the cyclic silyl ether.

Monomer data of the GLC-FID analysis are used for the calculations of a random distribution (binominal distribution) in oligomers of DP 2 to DP 5. If the experimentally determined substituent distributions show significant deviation from the calculated random pattern, for example a block-like distribution could be detected and give an explanation for unexpected behavior (Mischnick & Kühn, 1996; Mischnick & Momcilovic, 2010).

### 3.1. ESI-MS analysis of oligomers

Oligomer analysis was carried out to investigate the substituent pattern along the polymer chains. For quantitative analysis of cellooligosaccharides obtained from cellulose ethers, full alkylation of all free OH is required. To allow differentiation of originally free OH, *O*-ethyl ($\Delta M = 28$) and *O*-propyl ($\Delta M = 42$) substitution in oligomeric mixtures, the samples were perdeuteromethylated ($\Delta M = 17$) prior to partial depolymerization. In order to prevent bias due to different complexation abilities with sodium ions, oligomers were labeled with *m*-aminobenzoic acid (mABA) by reductive amination. The carboxyl group enables the measurement in the more selective negative ion mode. Analytes are detected as anions $[M-H]^-$, and ionization is independent of adduct formation with sodium ions or other cations (Cuers et al., 2012; Unterieser & Mischnick, 2011; Unterieser, Cuers, Voiges, Enebro, & Mischnick, 2011). Polarity of the analytes, which also influences the ion yield in the ESI process, is similar and leveled off by peralkylation and the effect of the introduced tag.

**Table 4**
DS values for oligomers (DP 2-DP 5) obtained from ESI-MS analysis in comparison to averages DS from GLC monomer analysis.

| | **1** | | **2** | |
|---|---|---|---|---|
| | ESI | GLC | ESI | GLC |
| DP 2 | 1.14 | | 1.17 | |
| DP 3 | 1.12 | | 1.16 | |
| DP 4 | 1.12 | | 1.15 | |
| DP 5 | 1.12 | | 1.12 | |
| Average | 1.13 | 1.11 | 1.15 | 1.12 |

The oligomer mixtures prepared from both samples were infused directly into the ESI-IT mass spectrometer by a syringe pump. A typical mass spectrum is shown in Fig. 2. To obtain reliable results each sample was measured three times with different methods using different settings for voltages of the ion trap. Measurement parameters (voltages of ion optics and ion trap) can have a strong influence on the ion yield and the relative intensities, however this was not observed for the present samples. All spectra obtained are in good agreement, proving low sensitivity of the analyte mixtures to change of instrumental parameters.

As expected from the monomer data obtained from GLC analysis, not only oligomers containing monosubstituted AGUs were detected. Signals from higher substitution were also evaluated and used for the calculation of the DS of the particular DP. The DS values for each DP are in good agreement with GC monomer analysis (Table 4) and thus representative for the starting

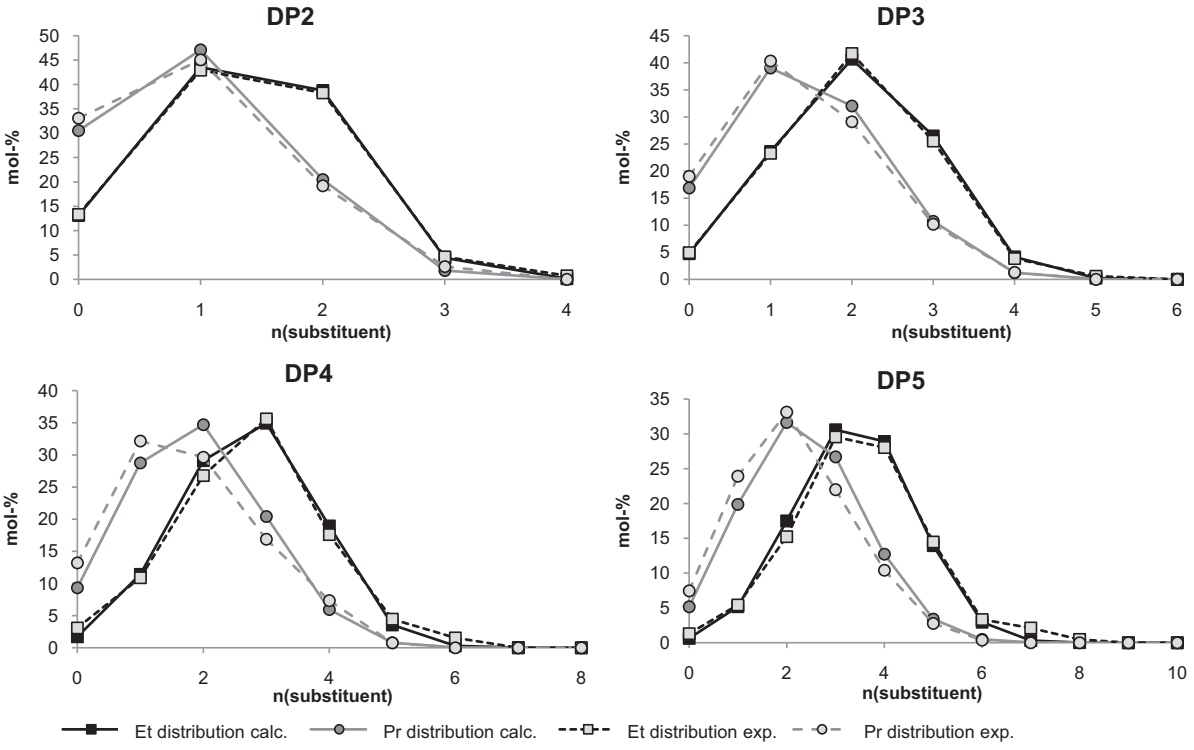


**Fig. 4.** Comparison of calculated and experimental distribution of Et- and Pr-substituents in DP 2–DP 5 of ethyl-propyl cellulose **1** obtained from ESI mass spectra of oligomers labeled with mABA (negative mode).

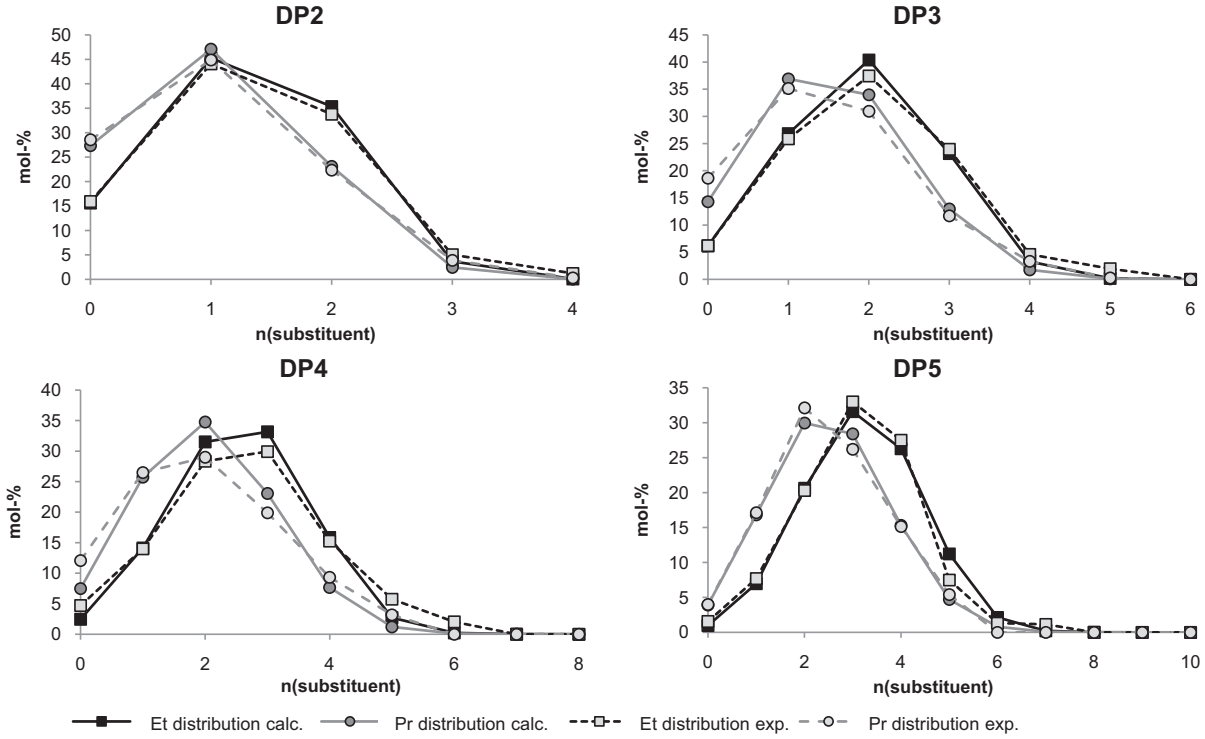


**Fig. 5.** Comparison of calculated and experimental distribution of Et- and Pr-substituents in DP 2-DP 5 of ethyl-propyl cellulose **2** obtained from ESI mass spectra of oligomers labeled with mABA (negative mode).

material which is an important indicator for a correct quantitative evaluation.

### 3.1.1. Evaluation of substituent pattern of monosubstituted glycosyl units

To get an impression of the distribution of ethyl and propyl groups in the polymer, signals of those oligomers containing only monosubstituted glucose units are evaluated first. These species account for 82–84 mol% (Table 2, $c_1$) of the material, and according to the number of Et, Pr and Me-$d_3$ substituents they can be identified and evaluated from the mass spectra. For each oligomer the distribution of substituents (Fig. 3) is compared to a random pattern calculated from the molar fractions of the monosubstituted monomers (Table 2, $s_2$, $s_3$, $s_6$).

The experimentally determined substituent distributions for the oligomers show no pronounced deviation from the random pattern. If the samples had a block-like substitution pattern, i.e. ethyl rich and propyl rich sequences, the intensities of the border cases (only Et, only Pr) should be significantly higher.

Despite the high level of regioselective substitution the amount of di-substituted AGUs cannot be neglected. Therefore the substitution pattern of Et and Pr were further investigated.

### 3.1.2. Evaluation of substituent distribution independent of DS

Since the cellulose ethers were not perfectly 3-*O*-monosubstituted, the total distribution of ethyl and propyl groups including all types of monomer patterns listed in Table 2 was also evaluated.

Taken all signals into account the substitution of both, ethyl- and propyl-substituents can be evaluated independently. The values from three measurements were averaged. The experimentally determined distributions for both samples (Figs. 4 and 5) were in very good accordance with the random distribution for ethyl, and in good agreement for the propyl pattern. The slight deviations observed for propyl, e.g. for DP 4, were not consistent over all DPs and thus not evident. This means that also the additional OH available beside O-3 were randomly distributed along the chain for both samples. While due to the protecting group strategy a high regioselectivity was achieved in the glucosyl unit, overalkylated di-*O*-alkyl residues were distributed randomly along the cellulose molecules (Kern et al., 2000).

## 4. Conclusion

An advanced method for the analysis of the substituent distribution of 3-*O*-ethyl/propyl cellulose was developed. The detailed monomer composition was determined by GLC analysis and additional information about the distribution of ethyl and propyl substituents was obtained by ESI mass spectrometry after perdeuteromethylation and labeling with mABA. Monomer analysis indicated that the amount of 2,3-di-*O*-substitution was below the level expected from the corresponding monosubstituted constituents. As possible reason rearrangement of part of the silyl protecting groups at O-2 under the alkaline conditions of alkylation is assumed. It was demonstrated that the substitution of Et and Pr proceeded randomly in both samples. Obviously, the different $DP_n$ values of samples **1** and **2** are not caused by a different functionalization pattern along the polymer chain. Thus, further studies are needed to understand the different DPn values of the two samples.

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