Possibilities of Mass Spectrometry and Tandem-Mass Spectrometry in the Analysis of Cellulose Ethers

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Summary: The investigation of the substituent pattern of cellulose and starch ethers and esters on various structural levels as in the glucosyl unit, along the polymer chain, and over the polymer molecules, still is a very challenging task. By means of mass spectrometric methods as ESI-MS or MALDI-TOF-MS the composition of oligosaccharide mixtures which represent the substituent distribution in the original polymer can principally be determined. However, to obtain reliable quantitative data mass spectra must be recorded under appropriate instrumental conditions and after special sample preparation While *O*-methyl/*O*-methyl-d_3-oligosaccharides give representative data with the mentioned MS techniques and FAB-MS as well, hydroxyalkyl ethers require labelling with a quaternary ammonium tag and MALDI-TOF-MS to prevent discrimination of lower substituted oligosaccharides. In this way, information on the neighbourhood of glucosyl units in the polymer chain is available which - together with the monomer composition - is a valuable parameter to describe a random or a heterogeneous pattern with regions of local higher densities of substituents compared to the random model, a regular distribution, or a block-like substituent pattern, i.e. a more clustered localisation of substiuents in the polymer chain. In addition, tandem-mass spectrometry (ESI-MS^n) allows to gain further insight in the composition of isomeric oligosaccharide derivatives of the same *m/z* ratio and to calculate the complete monomer composition of methyl celluloses.

Keywords: cellulose ethers; collision induced dissociation (CID); electrospray ionisation mass spectrometry (ESI-MS); MALDI-TOF-MS; substitution pattern

Introduction

The investigation of the substituent pattern of polysaccharide derivatives on various structural levels is still a challenge. Structural levels are related to the monomer unit, along the polymer chain and over the polymeric molecules. Due to the complex structure of cellulose including amorphous and crystalline domains the functionalisation pattern obtained by a polymer analogous reaction might more or less deviate from an ideal random distribution. To gain insight in the distribution of substituents along the polymer chain,

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enzymic degradation followed by SEC and further analysis of fractions obtained, NMR-spectroscopy and mass spectrometry have been applied.[1-4] Mass spectrometric methods as FAB-MS, ESI-MS, or MALDI-TOF-MS allow to determine the composition of oligosaccharide mixtures from random degradation which represent the substituent distribution in the original polymer. However, until now, reliable quantitative data are only available for such derivatives that can be transformed to mixed *O*-methyl/*O*-methyl-d_3 ethers. For these chemically uniform derivatives, information on the neighbourhood of certain glucosyl units in the polymer chain is available which. This - together with the monomer composition - is a valuable parameter to describe a random, a comparably more heterogeneous, a more regular, or a block-like substituent pattern with regions of clustered substituents. This strategy has been successfully applied to methyl ethers, trialkyl silyl ethers, acetates, and sulfates of cellulose and starch.[5-10] Tandem mass spectrometry has become a powerful tool for sequence analysis of oligosaccharides.[11-16] Fragmentation patterns obtained by collision induced dissociation (CID) depend on the linkage positions of connected sugars. We have investigated the influence of certain *O*-methyl patterns on the daughter ion spectra.[17] ESI-MS/CID of enzymatically degraded cationic starches allowed us to differentiate between products from slurry, paste, dry and extruder preparation.[18] So, our efforts are directed to extend the mass spectrometric approach to other polysaccharide derivatives such as hydroxyalkyl ethers and to elucidate the potential of tandem mass spectrometry for further improvement of these methodologies.

Quantitative Analysis by Mass Spectrometry

Data obtained by mass spectrometry are usually interpreted in a qualitative way: Structures of compounds are deduced from the fragmentation pattern in EI-MS, molecular masses are obtained by chemical ionisation or by soft ionisation techniques as FAB, MALDI or ESI, which are also applicable to larger molecules. To characterise the substitution pattern along the chain of a polysaccharide derivative information about neighbourhoods, i.e. the structure of diades or triades is required. To gain a representative picture of the average substituent distribution, quantitative evaluation of MS data is necessary. The abundance of a *m/z* signal, for example in electrospray MS, depends on the efficiency of pseudo-molecular ion formation (a), the distribution of the analyte between surface and inner part of the droplets of the spray (b), and on the ion transmission of the mass analysator (c). While (a) is influenced by the basicity of the analyte (positive mode), its size and

coordinating/ chelating properties and the types and concentration of cations available, (b) can be influenced by the polarity of the analyte and solvent, and (c) by instrumental dimensions, type of mass analysator, trap drive of the ion trap, skimmer voltages, dynode and multiplier potentials etc. From this short list of parameters it is obvious that quantitative evaluation can only be obtained within a narrow *m/z* range from molecules of similar size and polarity. We had fit this requirements by isotopic labelling. In our special case this means transformation of a methyl cellulose to a mixed *O*-Me/*O*-Me-d_3 permethylated derivative. From this chemically uniform polymer disaccharides in the mass range of *m/z* 449 - 467 (sodium adducts), trisaccharides (*m/z* 653 - 680), and higher homologous are obtained by partial hydrolysis. Whether the real composition of derivatives of a certain DP is represented by relative intensities of *m/z* signals can be controlled by calculation of the average DS, which must be in agreement with the average DS or MS (in case of hydroxyalkyl) of the polymer. If this requirement is fulfilled the experimental data can be compared with the random distribution calculated from monomer composition, and deviations can be interpreted. This approach is applicable to methyl ethers (permethylation with MeI-d_3),[5,6] silyl ethers (permethylation, desilylation, permethylation with MeI-d_3), acetates (non-alkaline permethylation, acetyl-Me-d_3-exchange),[7] and sulfates (permethylation, desulfation under acetylating conditions, permethylation with MeI-d_3).[8] Problems arise with derivatives which cannot be transformed to these chemically uniform products. When we measured a total hydrolysate of a hydroxypropylmethylcellulose (HPMC) with a DS_{Me} of about 2.0 and a MS_{HP} of 0.2, we found a strong increase of the relative signal abundance with increasing number of HP (hydroxypropyl) groups (Figure 1). Glucose molecules with up to four HP residues could be detected at this low MS_{HP} value, while in GC-FID analysis of the corresponding alditol acetates only mono-HP derivatives can be detected, while the tri-*O*-methyl and di-*O*-methyl-glucitols are the main products (Figure 2). After permethylation, 1 - 2 Mol% of di-*O*-HP-glucoses can be determined. This marked discrimination of DP1 can be explained by the strongly reduced capabilities for sodium coordination for a simple glucose. Obviously, each additional HP residue with an additional OH or OMe group significantly improves this pseudomolecular ion formation. This effect is much less pronounced for oligomers obtained by partial hydrolysis, since the di- and higher saccharides offer more coordination sites and conformational flexibility.

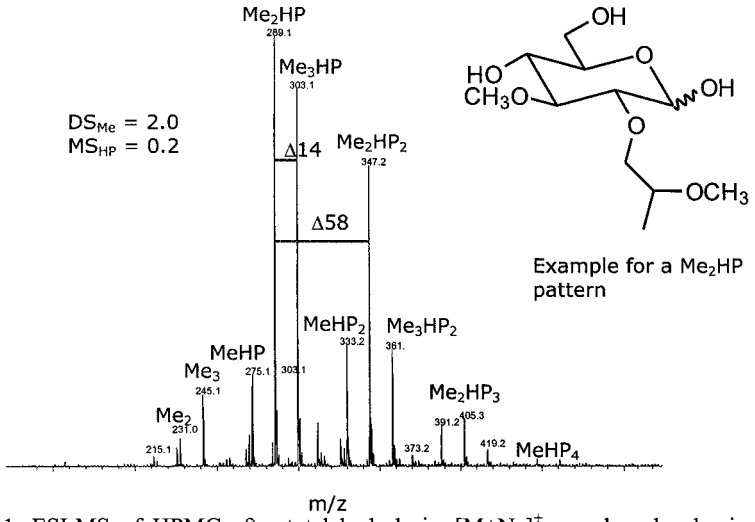

Figure 1. ESI-MS of HPMC after total hydrolysis. $[M+Na]^+$ pseudomolecular ions are observed. Δ 14 = mass shift caused by an additional methyl group, Δ 58 = mass shift caused by an additional HP group.

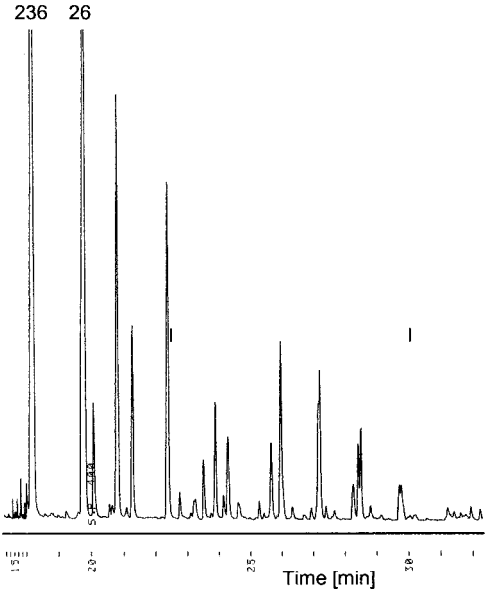

Figure 2. Gas chromatogram of a HPMC (DS_{Me} 2.0, MS_{HP} 0.2) after total acid hydrolysis (2 M trifluoroacetic acid, 120 °C, 2 h), reduction ($NaBD_4$, 60 °C, 1 h) and acetylation (Ac_2O, pyr, 90 °C, 3 h). Peaks are assigned according to positions of methyl groups.

Consequently, the apparent average MS_{HP} is much to high for DP1 and continuously decreases with increasing DP to become constant when the influence of hydroxyalkyl groups is levelled off. Figure 3 illustrates this for hydroxypropylated malto-oligosaccharides with an average MS_{HP} of 0.94.

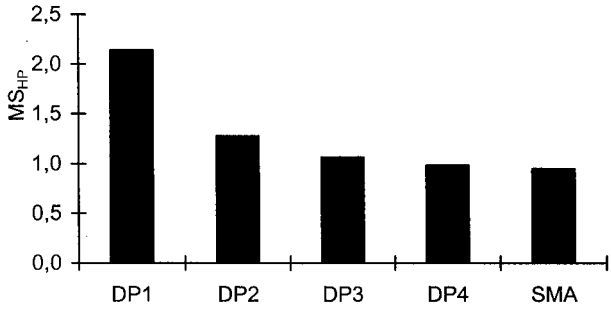

Figure 3. Average MS_{HP} in dependence of the DP for a partially degraded HP-β-cyclodextrin (MS_{HP} 0.94) as calculated from the relative ion intensities in ESI-MS. Comparison with standard methylation analysis (SMA).

An approach to diminish the influence of HP residues at lower DP is based on the introduction of a tag that strongly improves ion formation or is charged itself. Reductive amination has been widely applied for labelling of sugars for capillary electrophoresis or mass spectrometry.[19,20] Reductive amination of oligosaccharides obtained from hydroxyethylmethylcellulose (HEMC) with *n*-propylamine yielded products that preferably formed $[M + H]^+$ pseudomolecular ions and were detected with higher sensitivity, but quantitative evaluation was not significantly improved (Figure 4a). When the amino group was transformed to a dimethyl-propyl-ammonium group, coordination sites should no longer play any role since the analytes are permanently charged now (Figure 4b). With ESI-Ion trap-MS the dependence of average MS_{HE} on DP was less marked, but still observed.

A break-through was achieved when a MALDI-TOF-MS instrument was used for the analysis of the quaternary ammonium compounds. Obviously ion desorption and transmission was no longer influenced by the number of hydroxyethyl groups and the DP. The average MS_{HE} was in very good agreement with the reference data from methylation analysis as well as the DS_{Me} for DP1 - 5. This is illustrated in Figure 5. Comparison of the

experimental data with the calculated substituent distribution for DP2 and DP3 indicated a slight heterogeneity (Figure 6). Calculation for hydroxyalkyl pattern is more difficult, but can be done now on basis of the quantitative MALDI-data.

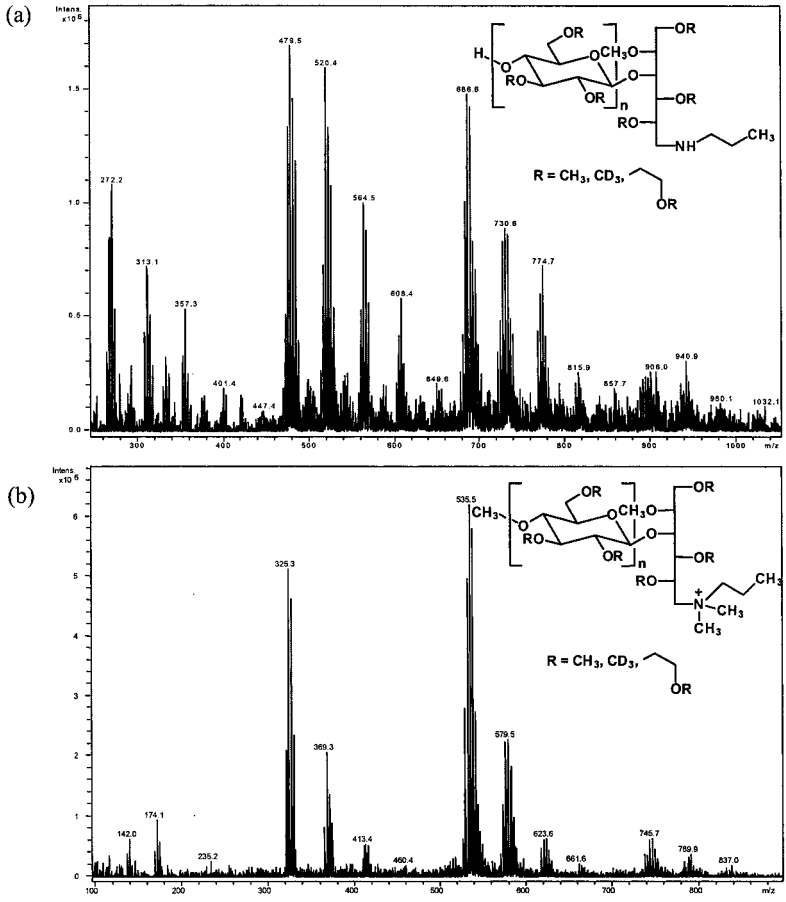

Figure 4. ESI-Ion trap-MS of HEMC (DS_{Me} 1.54, MS_{HE} 0.28) after perdeuteromethylation, partial hydrolysis, reductive amination (a) and additional methylation (b).

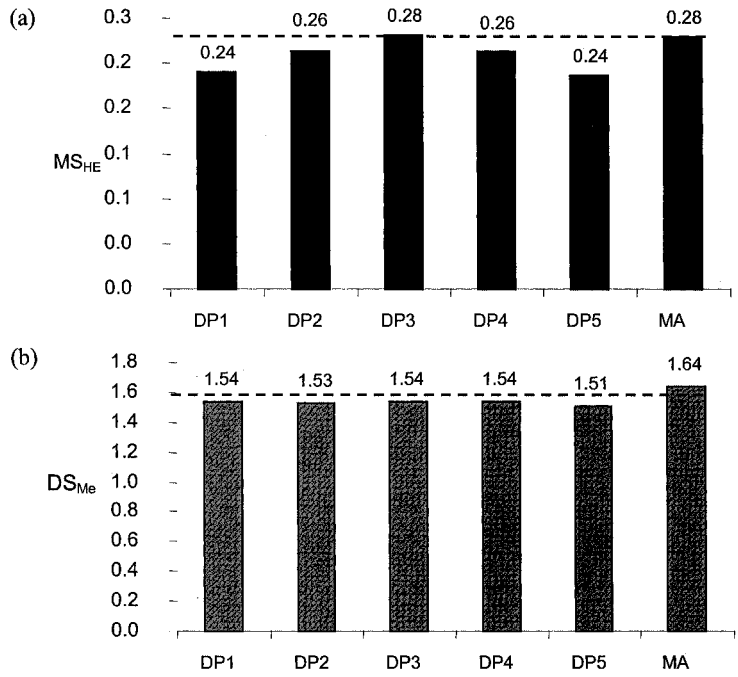

Figure 5. Average MS_{HE} and DS_{Me} of HEMC calculated from MALDI-TOF-MS after perdeuteromethylation, partial hydrolysis, reductive amination with propylamine and quaternisation with methyl iodide. MA = methylation analyis.

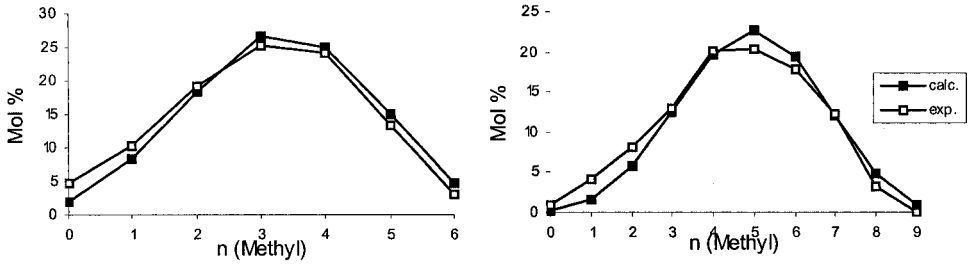

Figure 6. Comparison of experimental and calculated data of methyl distribution for the di- and trisaccharide fractions obtained from HEMC after perdeuteromethylation, partial hydrolysis, reductive amination with propylamine and quaternisation with methyl iodide (see also Figure 5). (DS_{Me} = 1.54, DS_{He} = 0.28).

Tandem Mass Spectrometry

After isolation of ions of a certain *m/z* ratio in an ion trap and subsequent collision induced dissociation (CID) or by post source decay (PSD) in MALDI-TOF-MS, sequences of amino acids, nucleic acids or sugar units can be deduced from mass intervals in the daughter spectra. We have investigated the fragmentation patterns of uniform malto- and cellooligosaccharides, which were methylated in distinct positions.[17,18] After deutero-methylation of residual OH-groups, the isomeric 2,3,6-tri-*O*-methylated disaccharides give characteristic daughter mass spectra with Y_1 as most abundant ion, followed by ring cleavage fragment $^{0,2}A_2$, and an ion corresponding to Y_1 - R^3OH. Nomenclature of Domon and Costello is used.[11] In Figure 7 the mother ESI-mass spectrum is shown.

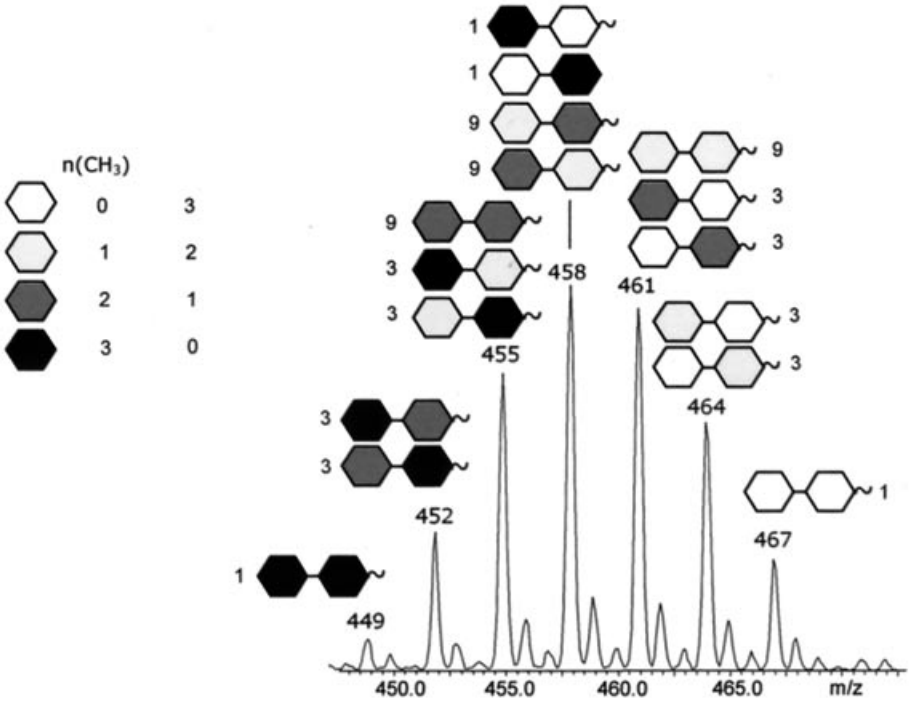

Figure 7. ESI-mass spectrum of $[M+Na]^+$ of the DP2-fraction obtained from methyl-cellulose after perdeuteromethylation and partial acid hydrolysis. Peaks are assigned with monomer composition and numbers of possible patterns.

Ions with *m/z* 452 to *m/z* 464 are isolated in the ion trap and fragmented by CID. The relative signal intensities are reproducible and therefore can be quantitatively evaluated. The contribution of each possible isomer is deduced from splitting of fragment ions due to their Me/Me-d_3-pattern. This is illustrated in Figure 8 for the isomers of *m/z* 464. In the same way, *m/z* 452 can be differentiated. Due to the claimed symmetry of dimers, it is sufficient to look on fragment ions bearing the substituents of the reducing unit of the disaccharide. Y_1 will occur at *m/z* 251 if this glucosyl unit is mono-substituted and at *m/z* 254 if it is unsubstituted. Therefore, these signals are detected with equal intensities.

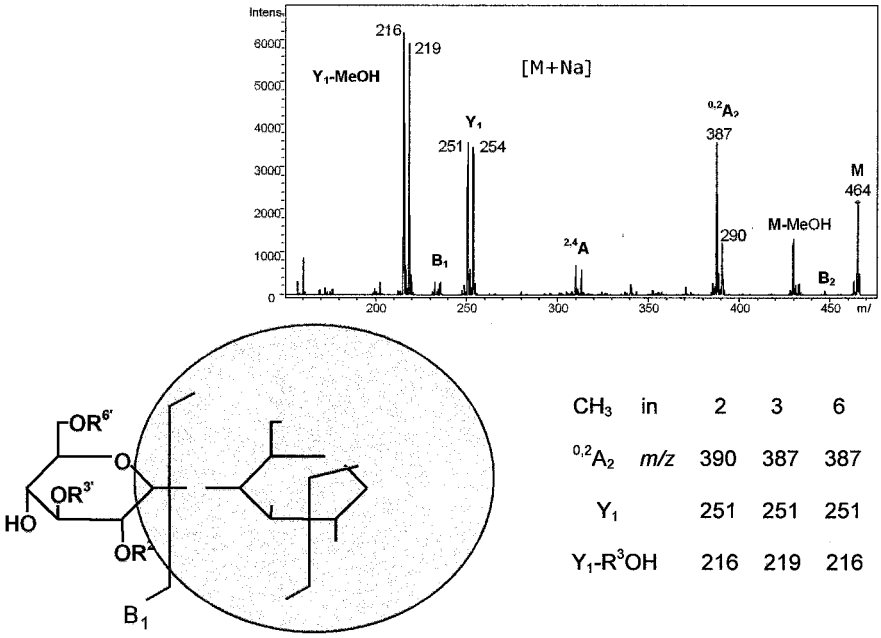

Figure 8. Fragment ions as basis for the calculation of the isomer composition in dimers of *m/z* 464 from the ESI-MS/CID spectrum. $R^{i'}$ and R^{i} = methyl or methyl-d_3 in position i or i' of the glucosyl units.

To puzzle out the composition of more complex mixtures hidden behind *m/z* 455, 458 and 461 (see Figure 7), ESI-MS^2 is no longer sufficient and additional independent information is required. This can be obtained by MS^3 of the Y_1-ions of MS^2. However, higher energy is necessary for this subsequent fragmentation. Under these conditions sodium adducts dissociate and no fragment ions can be observed. Therefore, more stable lithium adducts are used for this investigation. Seven fragment ions can be observed with reproducible

relative intensities. From these, M-R^2OH (M = Y_1 from MS^2), $^{0,2}A_1$ containing R^3 and R^6, $^{0,3}A_1$ containing R^6 only, and the complementary $^{0,3}X_1$ fragment ion, including R^2 and R^3, are used for further calculation of the contributions to distinct substitution patterns. The result of this mass spectrometric approach is compared with reference data from GC/FID analysis of partially methylated alditol acetates (PMAA) obtained after hydrolysis, reduction and acetylation of methyl cellulose. An example is given in Figure 9. The molar monomer composition is in very good agreement with the reference data. Further studies on the applicability of this new method to a wider range of methyl celluloses and starches will be reported elsewhere.[21]

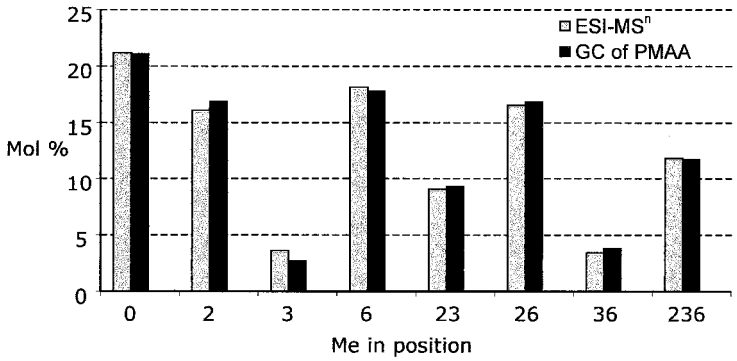

Figure 9. Substituent distribution of methyl cellulose (DS 1.32). Comparison of results from GC of partially methylated alditol acetates (PMAA) and ESI-MS^n of the perdeutero-methylated MC.

Samples: Hydroxyethylmethylcellulose (HEMC), hydroxypropylmethylcellulose (HPMC), and methyl cellulose (MC) were commercial products.

Conclusion

Mass spectrometry is a very powerful tool in structure analysis of cellulose derivatives. To elucidate the substituent distribution in the polymer chain, cellulose ethers are per-methylated with Me-d_3-I, partially degraded and submitted to MS analysis. Discrimination interfering with quantitative evaluation in case of hydroxyalkyl methyl ethers (HEMC, HPMC) can be overcome by reductive amination and subsequent quaternisation in combination with MALDI-TOF-MS. Methyl cellulose has been submitted to ESI-MS^n after

perdeuteromethylation and partial hydrolysis. From the reproducible relative ion intensities of mother, daughter and "granddaughter" spectra (n = 1-3), the complete monomer composition could be calculated. Data are in good agreement with GC analysis of partially methylated alditol acetates.

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