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Substituent distribution along the cellulose backbone in *O*-methylcelluloses using GC and FAB-MS for monomer and oligomer analysis

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Abstract

The substituent distribution along the cellulose backbone in three heterogeneously prepared methyl celluloses has been investigated by analysis of their monomer and their oligomer compositions. The oligomers were obtained by partial acid hydrolysis of the polymers. The relative hydrolysis rates of the glycosidic bonds at the C-1 positions of the monomers were determined and it was found that these hydrolysis rates are hardly dependent on any substituent combination in the monomers. Thus the partial hydrolysis of the polymers is a random process and the released oligomers are representative for the whole sample. The substituent distributions of the cellotriose units have been measured by fast atom bombardment mass spectrometry of perdeuteriomethylated partial hydrolysates. These distributions were compared with the calculated ones which are expected if the monomeric units would be arranged in a random sequence. The differences between the measured and calculated substituent distributions show that there are areas which are poorly as well as areas which are heavily derivatised in all samples, which prove that the investigated methyl celluloses have indeed an heterogeneous substituent distribution along their cellulose backbones. The degree of heterogeneity is quantified by a newly defined parameter *H*. Also the substituent distribution in the monomers has been compared with a calculated random distribution. The degree of heterogeneity derived from the monomer composition data was quantitatively in good agreement with the *H* values derived from the oligomer data, which shows that the degree of heterogeneity is also reflected in the data at the monomeric level. The *H* parameters correlate with the flocculation points of the methyl celluloses, which illustrates the correlation between the chemical structure and the physical properties of the samples.

Keywords: *O*-Methylcelluloses; Cellulose backbone; Thermogelation; Tri-*O*-methylglucose

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1. Introduction

Methyl celluloses (MCs) are from an analytical point of view the simplest cellulose ethers. They are prepared at a commercial scale by a Williamson etherification of alkali-cellulose with methyl chloride [1]. The degree of substitution (DS), i.e. the average number of methyl groups per glucosyl residue, ranges from 1.7 to 2.3 in commercial water-soluble products [2]. The properties of the product depend, among other things, on the DS and on the distribution of the substituents along the cellulose backbones. For example, aqueous solutions of MCs which are produced under heterogeneous slurry conditions form a gel at elevated temperatures, typically above 60°C. MCs which are prepared under homogeneous reaction conditions do not show this thermogelation [3].

Recently, Haque and Morris [4] postulated a model which describes the structural changes involved in the thermogelation of MCs. They proposed that MC solutions consist of dissolved bundles of partly methylated cellulose chains, which are crosslinked both by residual cellulose crystallites and by hydrophobic interactions of densely substituted regions in the cellulose backbones. These hydrophobic regions become solvated by a cage-like water structure around the polymer chain in the temperature range of 30 to 55°C, but this cage-like solvation shell melts at increased temperatures. If the solvation shell is melted, the MCs start to form a gel driven by hydrophobic interactions of densely substituted strands which radiate from different bundles. The thermogelation is a reversible process and the gel dissolves again on cooling.

Kato et al. [5] proposed that the thermogelation of MCs is specifically due to the presence of trimethylglucose regions in the cellulose backbones. However, so far no solid experimental evidence has been presented for the presence of trimethylglucose regions, or even for the presence of heterogeneously substituted regions, in the cellulose backbones. In this paper, the distribution of methyl groups along the cellulose backbones of three MCs, which are prepared by different heterogeneous processes, is determined by the analysis of the substituent distribution in their monomers and in their oligomers.

2. Methodology

Cellulose ethers are prepared on commercial scale by heterogeneous slurry reactions, which result most likely in an inhomogeneous distribution of the substituents along the cellulose backbones. In this paper, the degree of heterogeneity in the samples is determined by the deviations between experimentally measured substituent distributions in the monomers and the distributions which are calculated under the assumption that the samples are prepared under ideal homogeneous reaction conditions. It is assumed that under these conditions all the glucosyl residues in the sample are equally accessible for the reagents, and that the reactivity of the hydroxyl groups in these glucosyl units is not influenced by vicinal substituents or by any other substituents in their neighbourhood. Thus, the substituent distribution in the monomers should be random.

The probability that position X_i ($i = 2$, 3, or 6) is substituted can be derived [6,7] from the mole fractions of the individual monomers, S_i (the subscript i indicates the position of substitution), by

$$X_2 = S_2 + S_{23} + S_{26} + S_{236}$$

$$X_3 = S_3 + S_{23} + S_{36} + S_{236}$$

$$X_6 = S_6 + S_{26} + S_{36} + S_{236}$$

The sum of X_2, X_3, and X_6 gives the DS of the sample, i.e. a value between 0 and 3. The probability that a position i is not substituted (P_i) is given by

$$P_i = 1 - X_i.$$

A statistical DS-distribution, i.e. the ratio of non-, mono-, di-, and tri-substituted glucose units, can be calculated by

$$DS(0) = P_2 \cdot P_3 \cdot P_6;$$

$$DS(1) = X_2 \cdot P_3 \cdot P_6 + P_2 \cdot X_3 \cdot P_6 + P_2 \cdot P_3 \cdot X_6;$$

$$DS(2) = X_2 \cdot X_3 \cdot P_6 + X_2 \cdot P_3 \cdot X_6 + P_2 \cdot X_3 \cdot X_6;$$

$$DS(3) = X_2 \cdot X_3 \cdot X_6.$$

where DS(0), DS(1), DS(2), and DS(3) stand for the mole fractions of non-, mono-, di-, and tri-substituted glucose units, respectively. It was proposed that the difference (Δ) between the experimentally measured DS-distribution and the calculated DS-distribution of the monomers is a measure for the degree of homogeneity of a sample [8,9].

It should be mentioned that neglecting the vicinal substituent effects in the model is not correct, since it has been shown [7] that the reactivity of the C-3 position increases threefold if the vicinal C-2 position is methylated. This vicinal substituent effect causes some deviations in the substituent distribution in the glucosyl residues, which makes the validity of the ΔDS-distribution a point of discussion.

The DS-distribution of the oligomers in a sample provides directly information about the substituent distribution along the cellulose backbone. In products produced under homogeneous reaction conditions, the monomers will be arranged in a random sequence in the cellulose backbone. Thus, also the monomer sequence in the oligomers will be at random, and can be calculated from the experimentally measured monomer compositional data by binomial distribution statistics of Newton. A statistically expected DS-distribution of the oligomers can be derived from these calculated oligomer compositions. This approach also accounts for the occurrence of vicinal substituent effects in the glucosyl units. Therefore, the degree of heterogeneity can be established unambiguously by the difference between the experimentally measured oligomer DS-distribution and the calculated one.

3. Results

Flocculation points. — The flocculation points of the samples are 60, 66, and 61°C

Table 1
Monomer composition of the samples

Monomer substitution	ECN [a]	Mole fractions		
		MC-1	MC-2	MC-3
236	725	0.222	0.208	0.257
26	760	0.271	0.257	0.288
36	760	0.032	0.036	0.034
23	780	0.096	0.107	0.095
6	795	0.114	0.125	0.100
2	815	0.167	0.166	0.158
3	815	0.014	0.017	0.012
0	850	0.084	0.085	0.055

[a] The effective carbon numbers (ECN) are calculated according to the rules formulated by Ackman (ref. [11]). The ECN of a methylene unit is taken to be 100.

for MC-1, MC-2, and MC-3, respectively (see Experimental section).

Monomer analysis. — The monomer compositions of three MC samples were determined by gas chromatography-flame ionisation detection (GC-FID) of their reduced and acetylated derivatives. The GC-peaks corresponding to the eight monomers were identified using literature data [10]. The measured peak areas were corrected for the effective carbon number of the compounds, which were derived using the rules formulated by Ackman [11]. These effective carbon numbers (ECN), which are listed in Table 1, deviate only slightly from the ones used by Sweet et al. [12]. The derived mole fractions of the monomers listed in Table 1 are average values of duplicate analyses.

The DS values calculated from the monomer compositional data are 1.76, 1.73, and 1.88 for MC-1, MC-2 and MC-3, respectively. These values are in good agreement with those determined by Hercules, i.e. 1.75, 1.74, and 1.92, respectively.

The difference between the measured and the calculated DS-distributions of the

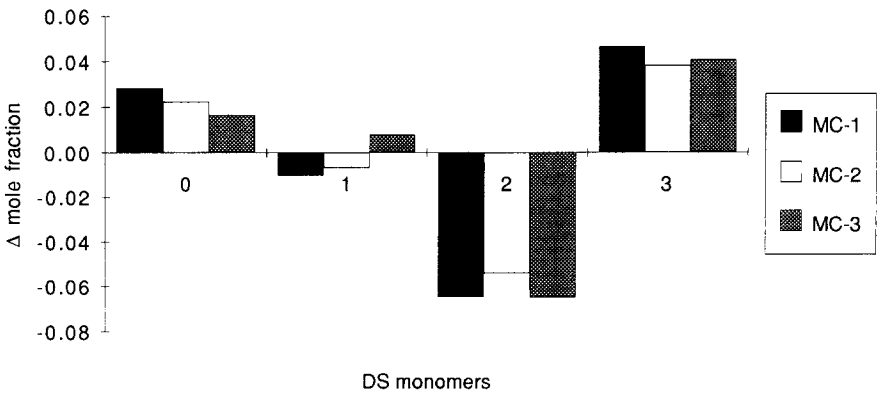

Fig. 1. The differences between the experimental and the theoretical DS-distributions in the monomers of the MC samples.

samples is shown in Fig. 1. This figure shows that in all samples the amount of non-substituted as well as trimethylglucosyl units is underestimated by their calculated DS-distributions. This implies that there are regions in the MC which are poorly derivatised as well as regions which are densely derivatised. The degree of heterogeneity of the samples can be quantified by the parameter H_1, which is defined as

$$H_1 = \sqrt{\Delta DS(0)^2 + \Delta DS(1)^2 + \Delta DS(2)^2 + \Delta DS(3)^2} \quad (1)$$

where the index 1 indicates that H is derived from the monomer compositional data. The H values are 0.085, 0.070, and 0.077 for MC-1, MC-2 and MC-3, respectively. This indicates that MC-1 is the least and MC-2 is the most homogeneous in this series.

Hydrolysis constants. — Due to differences in polarity as well as steric hindrance, the hydrolysis rates of the glycosidic bonds might be different between the various monomers in a MC. The relative hydrolysis rates at the C-1 position of the monomers have been checked by the methodology described by Valent et al. [13] which encompasses first a partial acid hydrolysis of the sample. Then the reducing endgroups in the released monomers and oligomers are reduced using $NaBH_4$. Subsequently, the remaining oligomeric and polymeric material in the sample is hydrolysed completely in a second hydrolysis step, and the monomers which were not accessible for the $NaBH_4$ after the first hydrolysis step are labelled with a deuterium atom at their C-1 position by a reduction using $NaBD_4$. The reaction mixture is finally acetylated, and the monomer mixture is separated and analysed by GC-ammonia chemical-ionisation mass spectrometry. The D/H ratio of the monomers is determined by the peak ratios of their pseudomolecular ions, which were detected as ammonium adducts ($M + NH_4^+$).

The signals corresponding to the D-reduced monomers were corrected for the overlapping isobaric, i.e. having the same nominal mass, ^{13}C-isotope peak of their H-reduced analogues. The intensities of the ^{13}C-isotope peaks are 17.56, 18.72, 19.88, and 21.05% of the intensities of the mass peaks of the H-reduced tri-, di-, mono-, and non-methylated glucitols, respectively. The corrected D/H ratios for the monomers in the hydrolysates obtained after 30, 60, 90 and 180 min allowed for the first hydrolysis step are given in Fig. 2. A large D/H ratio of a monomer points to a large resistance against acid hydrolysis of it's glycosidic linkage at the C-1 position. The signal to noise ratio for the 3-*O*-methyl and 3,6-di-*O*-methyl glucitols was found to be too low for giving a reliable D/H ratio.

$$\%\ \text{hydrolysed} = \left(1 - \frac{D/H\ \text{ratio} + 1}{D/H\ \text{ratio}}\right) \times 100\% \quad (2)$$

Fig. 3 shows the percentages of monomers which were accessible for the $NaBH_4$ after 30, 60, 90, and 180 min of hydrolysis time, respectively. These percentages were calculated from the D/H ratios via the formula in Eq. (2). On average, 48% of the glycosidic bonds have been hydrolysed after 30 min, and only 85% have been hydrolysed after 180 min of hydrolysis time. Fig. 3 shows that there is no evidence that the presence of methyl groups in any of the measured monomers influences the relative rate of hydrolysis to a large extent. Also the effect of 3-*O*-substituents in S_{23} and S_{236} appear to be small to absent compared to S_2 and S_{26}, and analogous, no large deviation

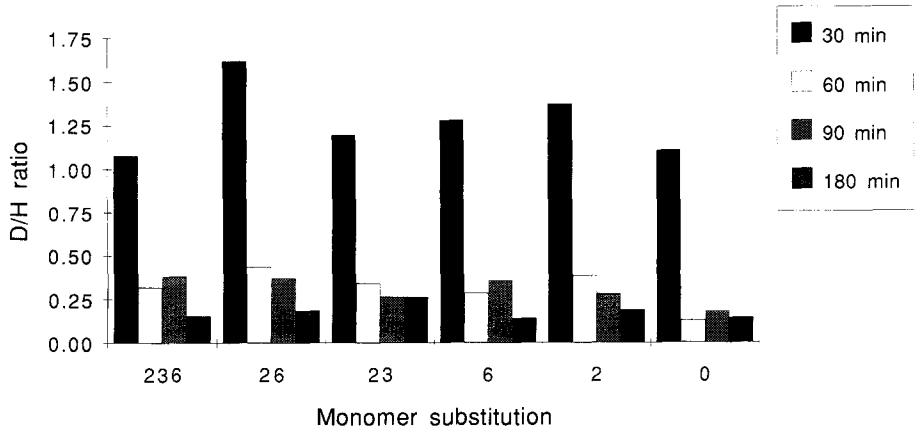

Fig. 2. The D/H ratios of the major methyl cellulose monomers after 30, 60, 90, and 180 min time allowed for the first hydrolysis step.

in the hydrolysis rates of the lacking S_3 and S_{36} will be expected. It can be concluded that the hydrolysis of the glycosidic bonds will occur at random in the polymer chain, and thus, the oligomers in partial hydrolysates of MCs will be representative for the whole sample. An hydrolysis time of 30 min is found to be appropriate for the preparation of partial acid hydrolysates of MCs.

FAB-MS of partial hydrolysates. — The response of FAB-MS is, among other things, dependent on the polarity of the sample. Since the polarity of permethylated oligomers and non-derivatised cello-oligomers differs very much, it can be expected that these two extremes will show different response factors. This problem could be solved by the methylation of the free hydroxyl groups in the oligomers using deuteriomethyl iodide (CD_3I), which reaction equation is shown in Fig. 4. The resulting oligomers are

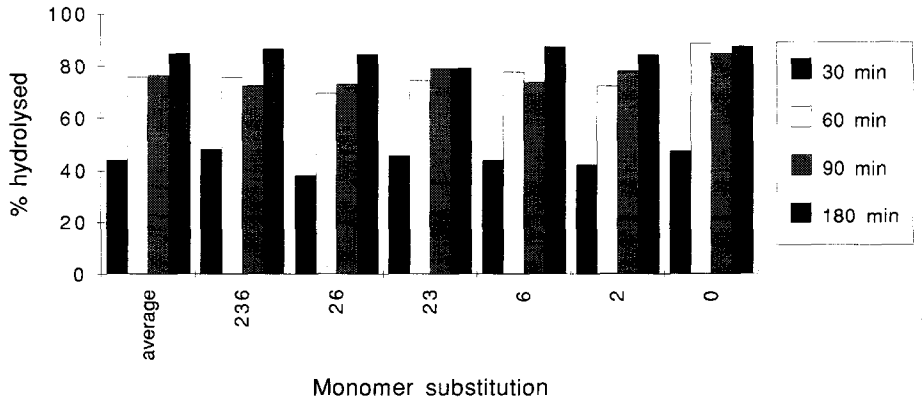

Fig. 3. The total percentage of MC-1 (average), as well as the percentages of it's major monomers which are hydrolysed after 30, 60, 90, and 180 min of hydrolysis time in 2 M trifluoroacetic acid at 100°C.

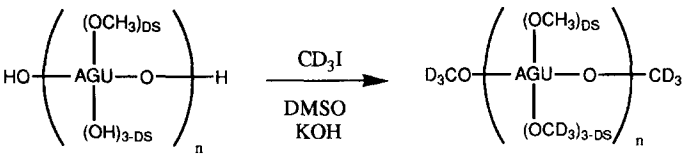

Fig. 4. The perdeuteriomethylation reaction of the oligomers in partial hydrolysates of methyl celluloses. AGU stands for an anhydroglucose unit.

chemically identical, but the originally free hydroxyl groups are substituted by the $O-CD_3$ groups, which can be distinguished from regular $O-CH_3$ groups by mass spectrometry.

The m/z values expected on FAB-MS of the deuteriomethyl-*O*-methyl cello-oligomers are given by:

$$m/z \text{ oligomer}_{(DP=n)} = n\left[159.05 + 15.02\ DS_{olig.} + 18.04(3\text{-}DS_{olig.})\right] + 75.07 \quad (3)$$

where n is the degree of polymerisation (DP) of the oligomer, $DS_{olig.}$ is the DS of the oligomer, and the addition of 75.07 amu takes account for the mass of the $-OCD_3$ and the $-CD_3$ end groups of the oligomer plus a Na^+ ion. Sodium cation adducts are usually observed on positive FAB-MS of carbohydrates, especially if some NaI is added to the matrix.

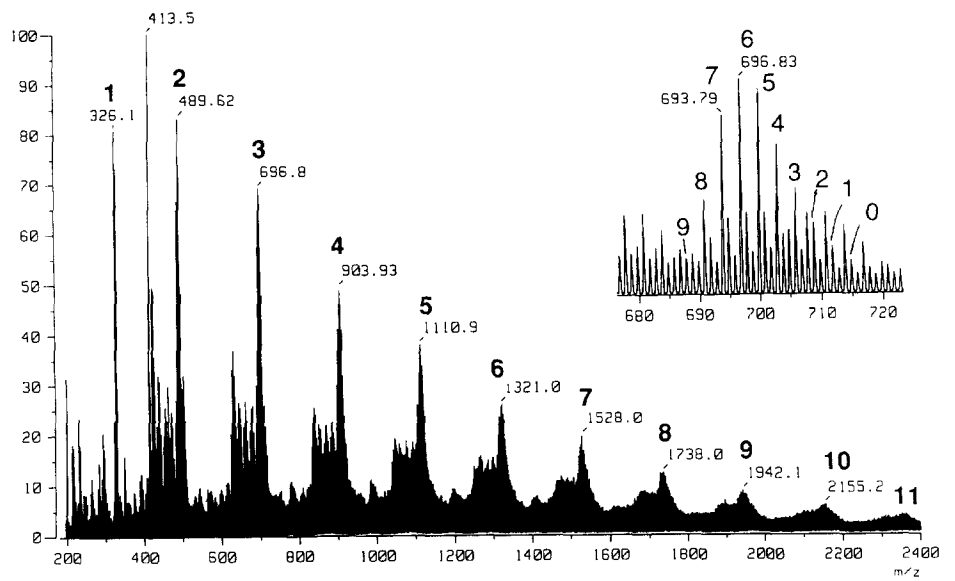

Fig. 5. A FAB-mass spectrum of a perdeuteriomethylated partial hydrolysate of MC-1. The degree of polymerisation of the oligomers is indicated by the number in bold above the peaks. The inserted section shows an enlargement of the trimer region. The numbers above the mass peaks indicate the number of CH_3 groups in the cellotrioses.

Fig. 5 shows the FAB-mass spectrum of a perdeuteriomethylated 30 min hydrolysate of MC-1. The main ion-series in the spectrum corresponds to sodium cation adducts of per-*O*-deuteriomethyl-*O*-methylcellulose oligomers, such as is described by Eq. (3). The DP of the oligomers is indicated in bold above their clusters of mass peaks. All oligomers up to the undecamers with mass peaks around m/z 2370 are observed. The clusters of mass peaks next to those masses described by Eq. (3) indicate that some fragmentation of the oligomers takes place. For example, the bunch of mass peaks around m/z 664 in the trimer region indicated that upon FAB a molecule of methanol can be released from the permethylated oligosaccharides. Also ring fragmentations occur, leading to oligomeric fragment ions which may interfere with the mass peaks corresponding to the sodium cation adducts of oligosaccharide [Eq. (3)] with a lower DP. It has been checked by FAB-MS-MS studies of various cello-oligomer derivatives [14] that there is no direct interference of any major fragment ion series with the observed per-*O*-deuteriomethyl-*O*-methylcellulose trimers.

The inserted section in Fig. 5 shows an enlargement of the trimer region of the FAB-mass spectrum. The number of OCH_3 groups in the trimers is indicated by the plain numbers above the mass peaks. Although there are no fragment ions of higher oligomers which interfere directly with the trimers, there is an ion-series of fragment ions in this trimer region at m/z 701, 704, 707, 710, 713, etc. This ion-series has been identified as being sodium cation adducts of 4′-*O*-formyl-per-*O*-deuteriomethyl-*O*-methylcellotrioses. The ^{13}C-isotope peaks of these compounds are 33.2% of the intensity of the earlier mentioned m/z values. This ^{13}C-isotope ion-series is isobaric with the $[M + Na]^+$ mass peaks of the trimers, and therefore, this fragment ion-series interferes indirectly with the trimers. In addition, there is also a significant background signal at each nominal mass in a FAB spectrum. The intensity of this background signal ($BG_{int.}$) in the trimer region can be estimated by the difference between the measured intensity of the ^{13}C isotope of the base peak in the trimer region, m/z 697.4, and it's theoretical intensity which is 33.19% of the intensity of the base peak m/z 696.4:

$$BG_{int.} = \text{int. } m/z\ 697.4 - 0.3319(\text{int. } m/z\ 696.4) \quad (4)$$

The measured peak intensities of the trimers are corrected for both the background signal and for the ^{13}C contribution of the $(m/z - 1)$ mass peaks according to Eq. (5).

$$\text{Corr. } m/z\ \text{olig.}_{\text{int.}} = (m/z\ \text{olig.}_{\text{int.}} - BG_{int.}) - 0.332[(m/z\ \text{olig.}_{\text{int.}} - 1) - BG_{int.}] \quad (5)$$

Table 2 shows the intensities of the relevant mass peaks in the trimer region measured for the three MC samples. The peak intensities of the cellotrimers were corrected according to Eq. (5), and these corrected values have been used for further data processing. The interference of ^{13}C-isotope peaks becomes more important in the higher mass regions, and therefore this study is limited to the trimer region.

The CH_3-group distributions of the cellotrioses. — The experimentally derived CH_3-group distributions of the trimers of the three MCs are given in Fig. 6. The DS values of the trimers are 1.78, 1.73, and 1.88 for the MC-1, MC-2, and MC-3 samples, respectively. These DS values are in perfect agreement with the DS values derived from

the monomer compositional data (1.76, 1.73, and 1.88). This implies that the trimers are fully representative for the whole sample composition, and that no discrimination of any fraction has occurred during the partial hydrolysis of the samples.

Fig. 6 also shows the CH_3-group distributions which are calculated from the monomer compositional data according to the binomial distribution statistics of Newton.

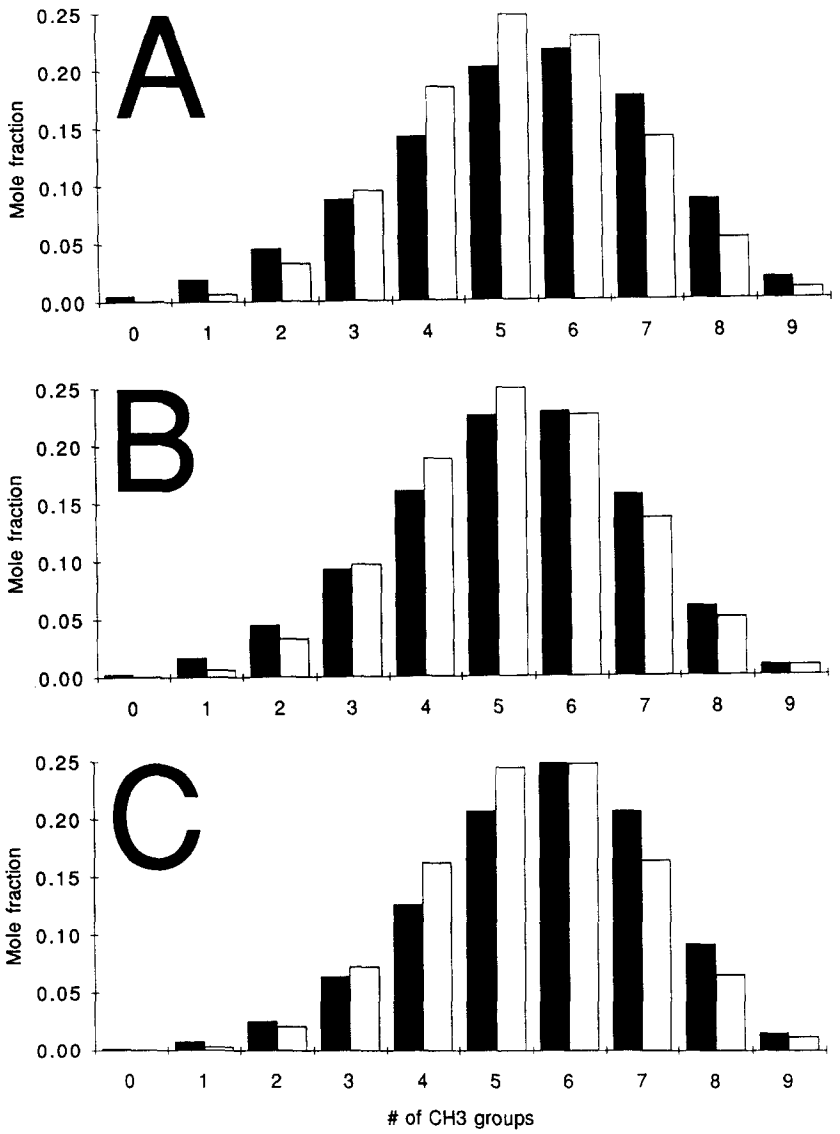

Fig. 6. The CH_3-group distributions of MC-1 (A), MC-2 (B), and MC-3 (C). The distribution measured by FAB-MS is indicated in black, and the distribution calculated from the monomer compositional data by binomial distribution statistics is indicated in white.

Table 2
Peak intensities of the relevant mass peaks in the trimer region

CH_3 groups	$DS_{olig.}$	m/z	Peak intensities		
			MC-1	MC-2	MC-3
0	0.00	714.5	6.19	5.35	3.75
		713.5	10.00	9.43	6.98
1	0.33	711.5	9.51	8.59	5.43
		710.5	11.92	11.17	8.78
2	0.67	708.5	14.31	13.80	8.40
		707.5	11.72	10.84	8.62
3	1.00	705.5	21.78	22.55	14.84
		704.5	10.62	9.66	7.54
4	1.33	702.5	31.53	35.10	25.58
		701.5	9.54	8.98	7.32
5	1.67	699.4	42.20	47.28	39.69
		698.4	8.43	8.37	7.50
		697.4	17.82	18.38	17.29
6	2.00	696.4	44.85	47.50	46.88
		695.4	8.02	7.20	6.96
7	2.33	693.4	36.85	33.32	38.93
		692.4	6.64	5.40	4.86
8	2.67	690.4	20.16	15.02	18.47
		689.4	6.52	5.50	3.75
9	3.00	687.4	8.01	5.99	5.46
		686.4	8.01	7.49	5.11

These calculated oligomer compositions would be obtained if the monomers are arranged in a completely random sequence, such as is expected for MCs which are prepared under homogeneous reaction conditions while the relative reactivities of the hydroxyl groups in the glucosyl units are dependent on vicinal substituent. There are

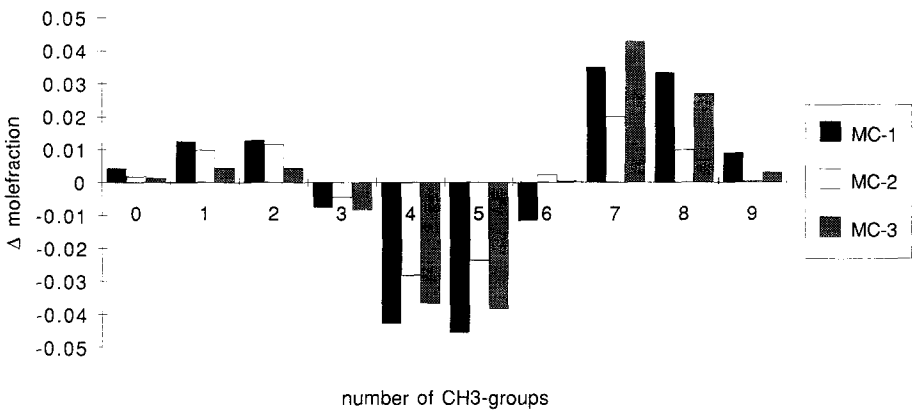

Fig. 7. The differences between the measured and the calculated CH_3-group distribution in Fig. 6.

significant differences between the experimental and the calculated CH_3-group distributions of the samples.

Fig. 7 shows the differences between the experimental minus the calculated CH_3-group distributions of the trimers. The deviations indicated in this figure are direct evidence for the heterogeneity of the substituent distributions along the cellulose backbones. In all samples, the amount of both lowly and highly substituted oligomers are underestimated by the predicted calculated oligomer composition. This result shows that there are specific areas in the MCs which are poorly derivatised, as well as areas which are heavily methylated. The degree of heterogeneity of the samples can be quantified by the parameter H, which is defined for the trimer region as

$$H_3 = \sqrt{\sum_{n=0}^{9} [\Delta DS(n)]^2} \tag{5}$$

The H_3 values for MC-1, MC-2, and MC-3 are 0.215, 0.113, and 0.167, respectively. This indicates that MC-2 has the most, and MC-1 has the least homogeneous substituent distribution along the cellulose backbones.

4. Discussion

The results in Fig. 7 are direct evidence that the substituent distribution along the cellulose backbone in the investigated MCs is indeed heterogeneous. Both the H_1 and the H_3 values indicate that MC-1 has the least homogeneous, and MC-2 has the most homogeneous substituent distribution along the cellulose backbone. This result confirms the proposal that the heterogeneity of a sample is not only reflected in the data at the oligomer level, but also in the data at the monomer level [8,9].

Fig. 6 shows that the absolute amount of nonamethyl-cellotrioses is less than 2 mol% in all MC samples. Also the deviation of the experimental and calculated mole fraction of this oligomer sequence is small, especially for MC-2, although this sample shows clearly a thermogel transition. These data cast doubt on the proposal that the thermogel transition is caused solely by pure trimethyl-glucosyl regions in the cellulose backbones. Considering the experimental evidence for crystalline order in the thermogels, which structure resembles the X-ray diffraction patterns of permethylcellulose [5], it is likely that the relatively large fractions of hepta- and octa-methyl cellotriose units play an important role in the hydrophobic interactions during the formation of firm thermogels.

The ^{1}H-NMR data published by Haque and Morris [4] showed that less than 10% of all the hydrogen atoms were visible in a thermogel of MC, which implies that at least 90% of all glucosyl units in a MC are conformationally immobile in the thermogel. The data in Fig. 6 show that 10 to 15 mol% of the trimers contains less than four methyl groups. Therefore, it is likely that besides hydrophobic interactions also hydrophilic interactions have to be taken into account during the gel formation. Khomutov et al. [15] showed by X-ray diffraction that there are indeed hydrophilic interactions in a MC thermogel as well. The question remains, however, how such a very efficient participation of almost all glucosyl units in the gel formation can occur. It might be that the

remaining crystalline cellulose regions in heterogeneous MCs are essential to align the macromolecules in such a way that a maximum interaction between both hydrophilic and hydrophobic regions is possible [4].

The flocculation points of the MC-1, MC-2, and MC-3 are at 60, 66, and 61°C. It can be expected that these flocculation points are indicative for the degree of heterogeneity in the samples, namely, more heterogeneously substituted MCs will show stronger hydrophobic as well as hydrophilic interactions, and thus the thermogelation will start at lower temperatures. The H_1 values of the samples are 0.085, 0.070, and 0.077, and their H_3 values are 0.215, 0.113, and 0.167, respectively, which show indeed that MCs with more homogeneous substituent distribution have also higher flocculation points. These results point to a direct correlation between the chemical structure, expressed in the H values, and the physical properties of the MCs.

5. Experimental

Samples and materials. — The methyl celluloses were provided by Hercules (Düsseldorf, Germany) and were produced by different heterogeneous process types. Their DS values according to Hercules are 1.75, 1.74, and 1.92, respectively. The flocculation points of the samples are also determined by Hercules. They are determined (0.5% solutions) by turbidity measurements, using a homemade device. The perdeuteriomethyl iodide was obtained from Merck, and dry dimethyl sulphoxide was obtained from Sigma.

Monomer analysis. — Duplicates of ~ 10 mg MC samples were dissolved in 0.25 M H_2SO_4 as 0.5% solutions and hydrolysed at 100°C for 56 h. Even after this extensive hydrolysis time, a small amount of gel-like residue of unknown origin was present, which was removed by filtration. The samples were neutralised using Amberlite IRA-400 ion-exchange resin in the OH^- or the HCO_3^- form. One series was reduced with $NaBD_4$, the other one with $NaBH_4$. The further work-up was done according to the standard procedure for methylation analysis [9]. For the GC analysis, the samples were dissolved in 1 mL dichloromethane (DCM) and 0.3 μL was injected on GC-FID.

Capillary GC was performed on a Carlo Erba 4200 GC, which was equipped with an on-column injector and a FID. The secondary cooling of the injector was turned off after 1 min. The column was a 50 m CP Sil 5 fused silica capillary column (ID. 0.32 mm, film thickness 0.20 μm). Helium was used as the carrier gas at a flow rate of 2 mL min^{-1}. The temperature was programmed for 5 min at 50°C, subsequently to 180°C with a rate of 20°C min^{-1} and from 180 to 250°C with a rate of 2°C min^{-1}. The FID-detector was set at 350°C (air flow 340 mL min^{-1}, hydrogen flow 30 mL min^{-1}, and make-up gas 30 mL of He per min).

Relative hydrolysis rates. — 100 mg of MC-1 was hydrolysed in 10 mL 2 M trifluoroacetic acid (TFA) at 100°C. Samples of 200 μL were drawn from this hydrolysate after 30, 60, 90, and 120 min. The acid was removed under a flow of nitrogen and the samples were dried at 50°C at 200 mm Hg for 1 h. Each sample was reduced using 8 mg $NaBH_4$ which was dissolved in 100 μL 2 M ammonia. After 2 h, the excess of borohydride was destroyed by adding acetone, and the samples were dried under a flow of nitrogen. Subsequently, 250 μL of 2 M TFA was added and the

hydrolysis of the samples was continued for 18.5 h at 75°C. A similar procedure was followed for the second reduction of the samples, but now $NaBD_4$ was used instead of $NaBH_4$. The borate was removed from the residues by co-distillation with 1% acetic acid in MeOH (3 times 1 mL), followed by the co-distillation with MeOH (3 times 1 mL). The samples were acetylated using 1 mL acetic anhydride (65°C, 16 h). Water was added to the samples and the derivatised glucitols were extracted with chloroform. The chloroform was washed with water, the samples were dried under a stream of nitrogen, and the residues were co-distilled with dichloromethane in order to remove the acetic acid. The samples were dissolved in 1 mL chloroform, and 1 μL was analysed by GC-ammonia chemical ionisation mass spectrometry (CIMS).

GC-MS analysis was performed on a HRGC Mega 2 series gas chromatograph equipped with an on-column injection system connected to a JEOL DX303 double focussing mass spectrometer. A 25-m BPX-5 column (i.d. 0.32 mm; film thickness 0.25 μm) manufactured by Scientific Glass Engeering Pty. Ltd. (Sydney, Australia) was used for the gas chromatographic separation. The temperature program was 5 min isothermal at 80°C, then to 150°C at 10°C min^{-1} and from 150 to 210°C with 2°C min^{-1}. The ammonia CI-pressure was estimated to be 20 Pa, and the mass range of m/z 60–700 was scanned with a cycle time of 1 s. The GC-CIMS data was processed using a JEOL-DA 7000 data system.

Fast atom bombardment-mass spectrometry (FAB-MS) of partial hydrolysates. — 100 mg of MC was hydrolysed in 10 mL 2 M TFA at 100°C for 30 min. 0.5 mL of the hydrolysates was dried under a stream on nitrogen, and 80 mg of potassium hydroxide was added to the residue. The samples were dried over P_2O_5 at 300 mm Hg for 3 h. The samples were dissolved in 1 mL of dry Me_2SO, perdeuteriomethyl iodide (125 μL) was added in three portions and the samples were allowed to react for 15 min. Ice water was added and the permethylated oligomers were extracted with chloroform. The chloroform was washed 3 times with water (3 mL). *m*-Nitrobenzyl alcohol (1 μL), which was saturated with NaI, was applied on a FAB-probe, and 1 μL of sample was added to this matrix. The chloroform was evaporated on the air.

FAB-MS was performed on a JEOL-SX 102 double focussing mass spectrometer (B/E). A 6 kV xenon beam (10 mA emission current in the FAB-gun) was used for the ionisation. The resolution of the mass spectrometer was 3000, and the mass range m/z 180–2400 was scanned in 42 s. A JEOL-DA 7000 data system was used for the data acquisition. The data were accumulated over 7 scans.

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