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Fractionation of Polysaccharide Derivatives and Subsequent Analysis to Differentiate Heterogeneities on Various Hierarchical Levels

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Summary: A set of methyl celluloses of DS 1.8 has been fractionated by stepwise solvent extraction. All fractions have been investigated with respect to their monomer composition and methyl distribution in the polymer chains. Deviation from the random model and differences between the four samples could be determined with higher sensitivity and more differentiated than for the unfractionated material. Structure complexity of cellulose ethers on different hierarchical levels is outlined and discussed.

Keywords: cellulose; fractionation of polymers; modification; substituent distribution

Introduction

The analysis of polysaccharide derivatives is a very complex task. Obtained by post-modification of homoglycans as cellulose they can be considered as co-polymers of up to eight constituents and even more if more than one type of substituent has been introduced (Figure 1). But also those polysaccharides and derivatives occurring in nature like sulfated carrageenans, partially methyl esterified pectins or alginates, which are products of enzymatic post-modification of biopolymers, in principle raise the same questions:

- How are substituents distributed on the positions available in the monomeric unit?
- How are the resulting monomer units located in the polymer chain?
- In which respects do the polymer molecules vary? – Molecular weight (dispersity), degree of substitution (DS), substituent distribution? And: are these variations independent from each other or is there a correlation of M_w and DS?
- Is the distribution uni- or bimodal?
- Are in the case of cellulose and starch: Are there heterogeneities on the morphological level?

During our longtime studies on the substituent distribution of starch and cellulose derivatives we have stepwise advanced from the monomer level, over the polymer chain (intramolecular heterogeneities) to the intermolecular level, the distribution over distinct polymer molecules. Without fractionation according to size or chemical properties, only average values over the mixture under consideration are obtained.

Before presenting the results of our fractionation studies, I will amplify complexity of structures and show that the pattern on one level influences the next one.

The number of various monomer units is given by a^3 with a = number of different residues R (Figure 1). For methyl cellulose (R = H or Me) it is 8, for methyl ethyl cellulose (R = H, Me or Et) it is 27, and for hydroxypropylmethyl cellulose (R = H, Me, HP, HPme) it is 64 without considering

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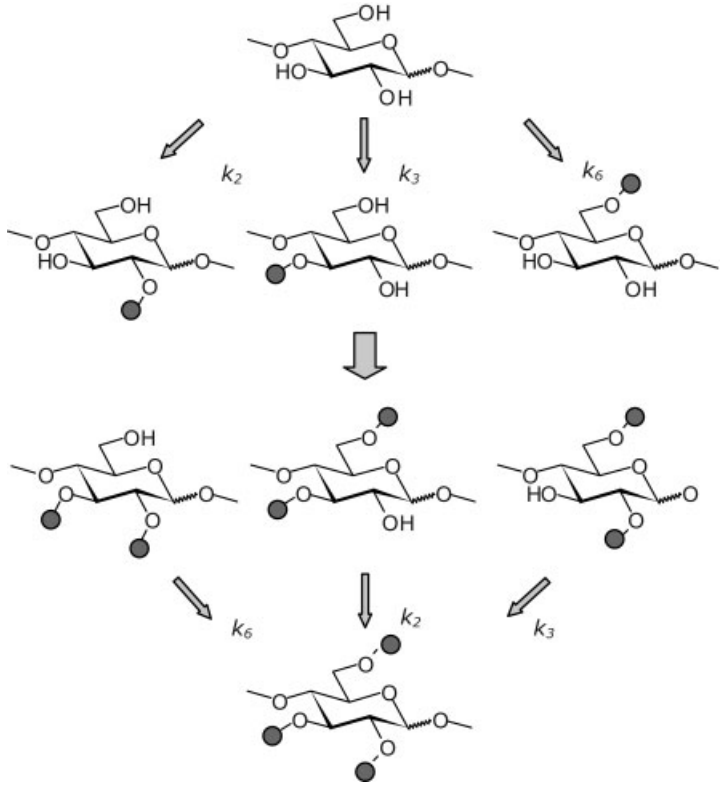

Figure 1.
Polymer analogous modification of glucans.

tandem reaction ($(HP)_n$). The kinetics of the post-modification process of cellulose was described by Spurlin and Reuben.[1,2]

In the simplest model, the relative ratios of the rate constants k_2, k_3 and k_6, assigning the etherification in positions 2, 3 and 6 of the glucosyl unit, respectively, remain constant during the course of the reaction. In this ideal case the molar monomer composition (s_i with i = substituted position) can be calculated from the partial DS values x_2, x_3 and x_6. Considering border cases, two extremes shall be differentiated: very high regioselectivity, e.g. $k_2 \gg k_3$ and k_6, i.e. $k_2 = 1$, $k_3 = k_6, = 0$, and no regioselectivity, i.e. $k_2 = k_3 = k_6 = 1$. In the first case O-2 will be occupied by and by in a random process, and at DS 1 all glucosyl units are monosubstituted. Thus $c_1 = 1$ and c_0, c_2, $c_3 = 0$ with c_i = mol fraction with i substitutents, and $x_2 = 1$, $x_3 = x_6 = 0$. In the other border case, at a DS of 1 the partial DS values $x_i = 0.33$ for all three positions, and the mol fractions c_i will be as follows: $c_0 = 0.30$, $c_1 = 0.44$, $c_2 = 0.22$, and $c_3 = 0.04$. What does this implicate for the substituent density along the chain? Taken a random distribution (''Independence model[3]) the density is completely different for these both cases as can be seen from Figure 2, only due to different regioselectivities, and with consequences for the properties. The patterns related to all other cases of relative reactivities lie in between and are shifted with increasing DS.

As mentioned above, analysis of the unfractionated sample will only give average values. However, the molecules of a material cannot be expected all to have the same DS. There should be a certain DS distribution as is commonly known from the molecular weight. Figure 3 presents the

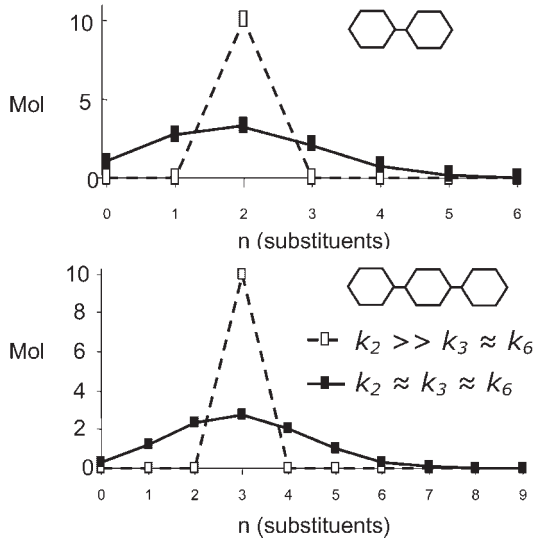

Figure 2.
Substituent distribution in diades and triades, calculated for very high and no regioselectivity (for details see text).

simulation of such a DS gradient and compares the weighted average substituent distribution in diades, which is expected from analysis, with the pattern calculated on basis of the monomer composition. Mol fractions C_i (with i = number of substituents in the dimers) are given below the diagram. Absolute deviations are small, but relative deviations are between 4 and 8% for the lowest and highest substituted dimers, while being only 0.4–1% for C_1–C_4, indicating that the shape of the distribution curve changes in the same way as for a more heterogeneous distribution on all chains.

In contrast or in addition to such a more or less narrow or broad symmetrical DS-distribution, the sample can contain very low substituted sequences as a result of

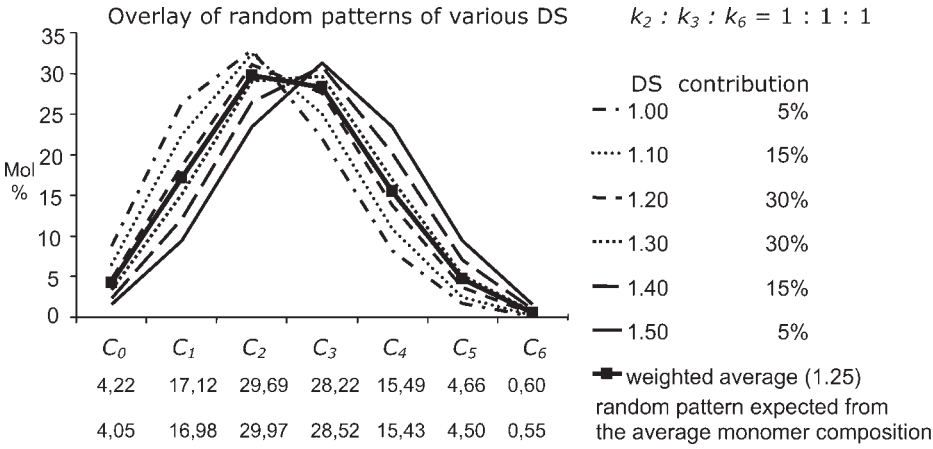

Figure 3.
Simulation of a DS gradient (1.00–1.50) in a cellulose ether of average DS 1.25, weighted average and random pattern of diades expected from the monomer composition.

poor activation of crystalline domains. This can result in a bimodal pattern of oligomer fractions.[4]

Without fractionation, heterogeneities of different types cannot be differentiated. Therefore, efforts have been made to fractionate cellulose ethers, especially carboxymethyl cellulose (CMC), by capillary electrophoresis,[5] or stepwise precipitation with ethanol.[6] Fractionation with respect to molecular size correlated with the determination of carbonyl groups from cellulose oxidation has also been reported.[7]

Results

A challenge of each fractionation procedure is to reassemble all pieces of the puzzle obtained to the entire picture. Methods involving solid support (e.g. chromatography) bear the risk of bias. Therefore, we decided to fractionate a series of methyl cellulose, all with DS values around 1.8, but of increasing heterogeneity, by stepwise extraction of the solid material with solvents and solvent mixtures of decreasing polarity.[8] Figure 4 shows the weight distribution of MC 1–4 on four fractions (chloroform/methanol: (F1): 10/0, (F2): 10/1, (F3): 2/1, (F4): 1/1) and the residue, which was finally obtained as the insoluble material of each MC after this stepwise procedure. Beside 1–3% in pure chloroform, the MCs for the most part are recovered in fractions 2 and 3. With increasing heterogeneity of the material, the insoluble part (''residue'') increases from about 7 to 27%.

All fractions were analyzed with respect to their substituent distribution. DS calculated by weighing the contribution of each fraction was in very good agreement with the DS of the original sample. From Figure 4 it is obvious that the DS generally decreases with increasing solvent polarity (in the range of 1.54–2.05), but that it increases within a group of fractions with increasing heterogeneities of the MC. This means that for the solubility in a certain polarity window more methyl groups are required if the material is more heterogeneously substituted. Monomer analysis showed about 40–41% of all methyl groups located at O-2, 37% at O-6, and hence about 22–23% at O-3 for MC 1–3, and a slightly higher regioselectivity for MC 4. For the fractions a trend is observed for the ratio of O-2: O-3-methylation as shown in Figure 5 for MC 1. While $x_2 : x_3$ is 1.44 for the chloroform-soluble fraction 1, it raises to 1.72 (F2), 1.91 (F3), 2.11 (F4) and finally 2.14 for the insoluble residue. However, this

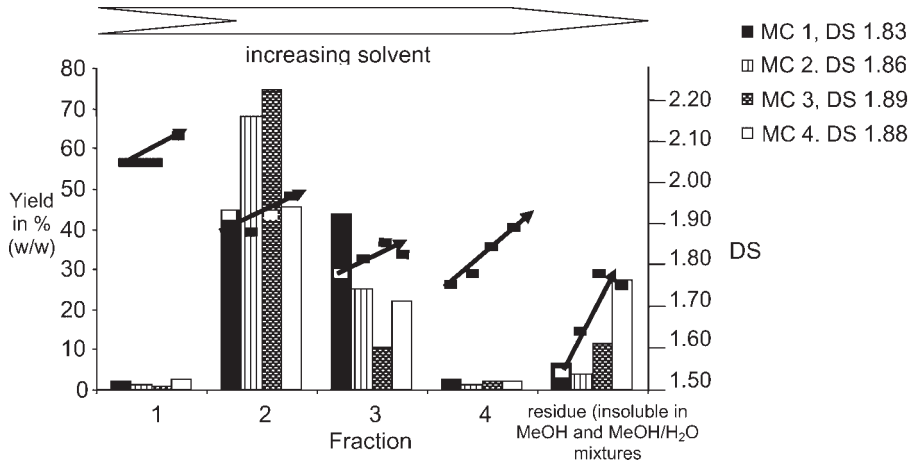

Figure 4.
Sample distribution of MC 1–4 after fractionation with chloroform-methanol mixtures. DS values for each fraction of each MC are given.

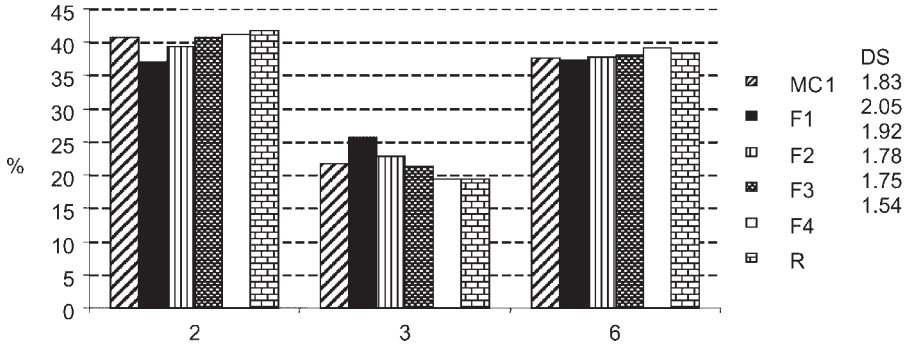

Figure 5.
Distribution of methyl groups on positions 2, 3, and 6 in the fractions obtained from MC 1.

is owed to the decreasing DS since the headstart of the most reactive position drops down during the course of the reaction (while $k_2 : k_3 : k_6$ remain constant) when the number of free OH available decreases.

The MC fractions available in sufficient amount were further investigated with respect to their substituent distribution along the monomer chain by ESI-MS after perdeuteromethylation, partial hydrolysis as has been described.[9,10] Comparison of the experimental data with a random pattern calculated from the corresponding monomer composition is shown in Figure 6. In the left column, fractions 2 of MC 1 to 4 are compared. While there is no difference between MC 1 and 2 in this fraction, slight deviation from the random model can be observed for MC 3 and much more pronounced for MC 4. From left to right, fractions 2 and 3 for MC 1 and 2 are contrasted. Now heterogeneity is already obvious for MC 2, and these two samples can be differentiated, which was not possible for the non-fractionated material. The extended contribution of unsubstituted dimers, causing even a bimodal pattern, indicates long unsubstituted sequences at a much higher probability than expected for about 5 Mol% unsubstituted residues, as was determined for F3 of MC 2 and F2 of MC 4. This has been interpreted as the result of poor activation of some of the crystalline domains of the cellulose fibre, thus remaining nearly unaffected.

Another important point should be addressed. All MCs were nearly completely soluble in water when dissolving directly, i.e. without fractionation. Thus the insoluble residue finally remaining after stepwise extraction of the solid material indicates the co-dissolving effect of cellulose molecules with different degrees of substitution and different heterogeneities for each other. This means that the portion and the composition of a sample, which can be dissolved in a solvent of certain polarity also depends on the composition of the entire sample. Similar observations were made by Kamitakahara et al.[11] when distributing amphiphilic di-block cello-oligosaccharides with different lengths of the polar part between water and chloroform.

Experimental Part

Four methyl celluloses (MC 1–4) with DS between 1.83 and 1.89 prepared under various conditions were fractionated by stepwise extraction. All MC (solid material) were subsequently treated with seven different solvent compositions at room temperature for several hours, starting with chloroform (F1), followed by mixtures of chloroform and methanol (10:1 (F2); 2:1 (F3); 1:1 (F4); v/v), pure methanol, and then mixtures of methanol and water and finally with water. After centrifugation, the dissolved material was recovered by solvent

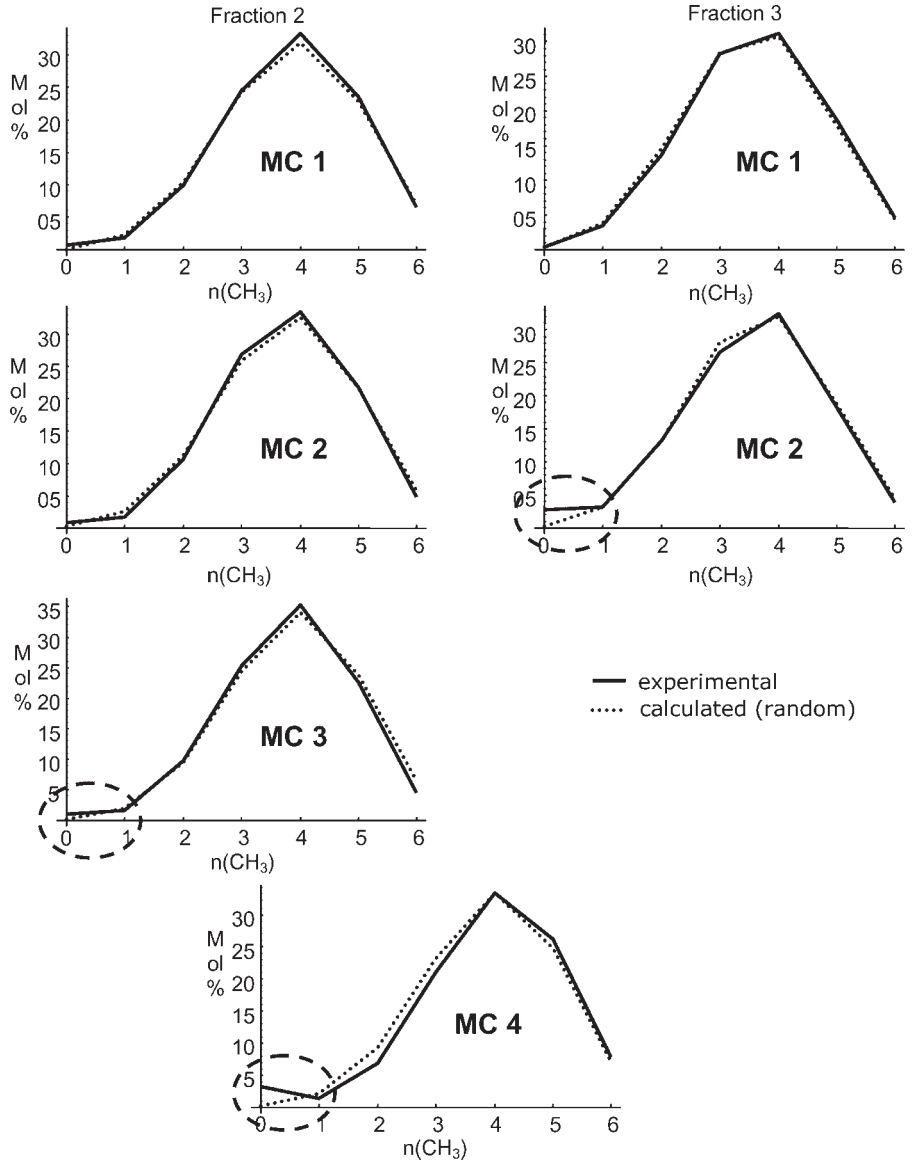

Figure 6.
Comparison of the methyl distribution in the dimers as determined for MC fractions. Left column: F2 (chloroform/methanol 10/1) of MC 1–4. MC 1 and MC 2: Comparison of F2 (left) and F3 (right) (chloroform/methanol 2/1). Increasing heterogeneity from top to down and from left to right.

evaporation of the separated solution. No material was obtained from methanol, water and mixtures thereof. From MC 1 to 4 an increasing amount remained non-dissolved (residue). Main material was obtained in fractions 2 and 3 (see Figure 4).

All fractions were further analyzed with respect to their monomer composition. Fractions 2, 3, and the residue of each MC were permethylated with CD_3I, partially hydrolyzed and analyzed by ESI-MS to determine the methyl pattern in oligomeric

sequences as has been described. For more details see Ref. [8].

Conclusion

Fractionation of polysaccharide derivatives with respect to their chemical structure and subsequent analysis gives a more detailed picture of the material. Even MC 1 with a perfect random substituent distribution yielded fractions in a DS-range from 1.54 to 2.05 at an average DS of 1.83. Slight heterogeneities or bimodal substitution patterns could be recognized with higher sensitivity and increased within the sample set from MC 1 to 4 and for a certain sample with decreasing DS. Thus differentiation of substitution patterns of MC with the same average DS is improved. However, time and effort are enormous. Due to the interrelation of cellulose chains with different DS and methyl pattern with respect to their solubility, the effect of solvents, the order of extraction (starting from the polar or unpolar side) as well as reproducibility of the fractionation procedure need to be investigated.

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