polysaccharides

MDPI

Supplementary Material

Analysis of the Heterogeneities of First and Second Order of Cellulose Derivatives: A Complex Challenge

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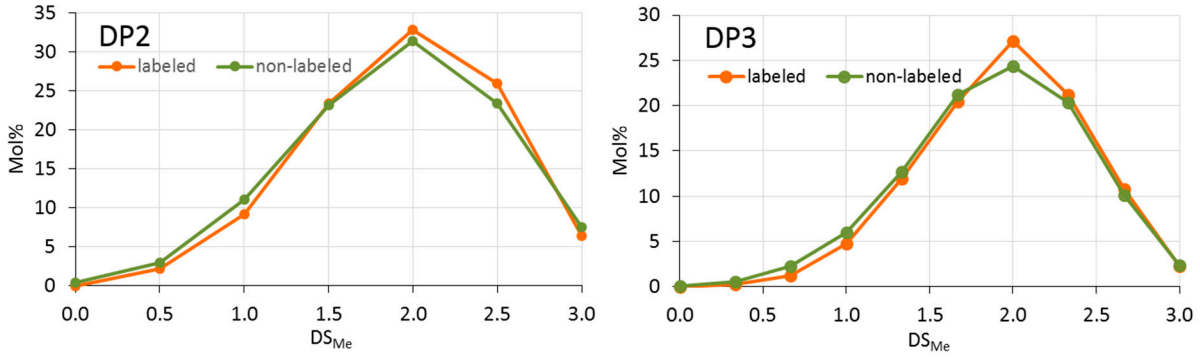

Figure S1. Methyl profiles obtained during method development to obtain about one labeled COS/MC molecule, separated from all other COS, derived from the same and different MC chains; MC, DS 1.96; Sample preparation: (1) perdeuteromethylation → MDC; (2) ultrasonic treatment; (3) labeling with *m*ABA; (4) partial hydrolysis; (5) SPE-separation of labeled and unlabeled COS; (6) LC-ESI-IT-MS, neg. mode. Labeled COS: orange; unlabeled COS: green. Because of the overall low concentration of the labeled COS fraction, the constituents being present in lowest amounts have been slightly discriminated in the LC-MS run due to insufficient number of data points due to partial separation of the consituents according to their number of CD_3 and CH_3. Therefore, the two methyl distribution profiles (labeled, originating from different cellulose chains, and unlabeled, derived from the same and different chains) are even more similar than shown here.

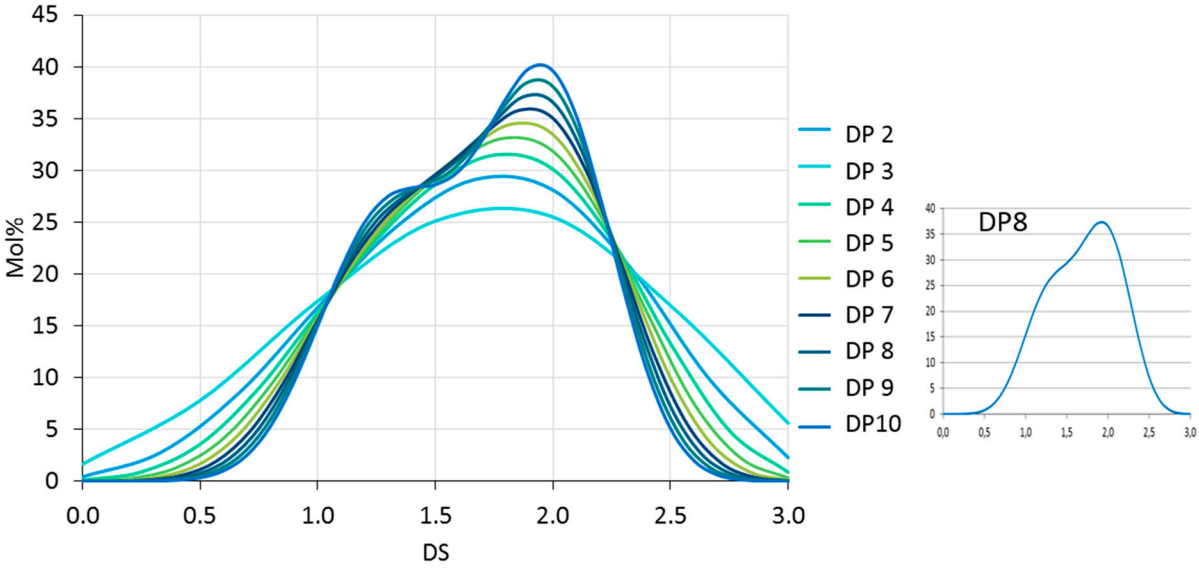

Figure S2. Methyl distribution profile calculated for a 2:3 blend of two MCs, DS 1.29 and DS 1.96, from their molar portions c_i of un-, mono-, di- and trisubstituted AGU. The saddle point between the two MCs becomes visible at DP8, shown separately.

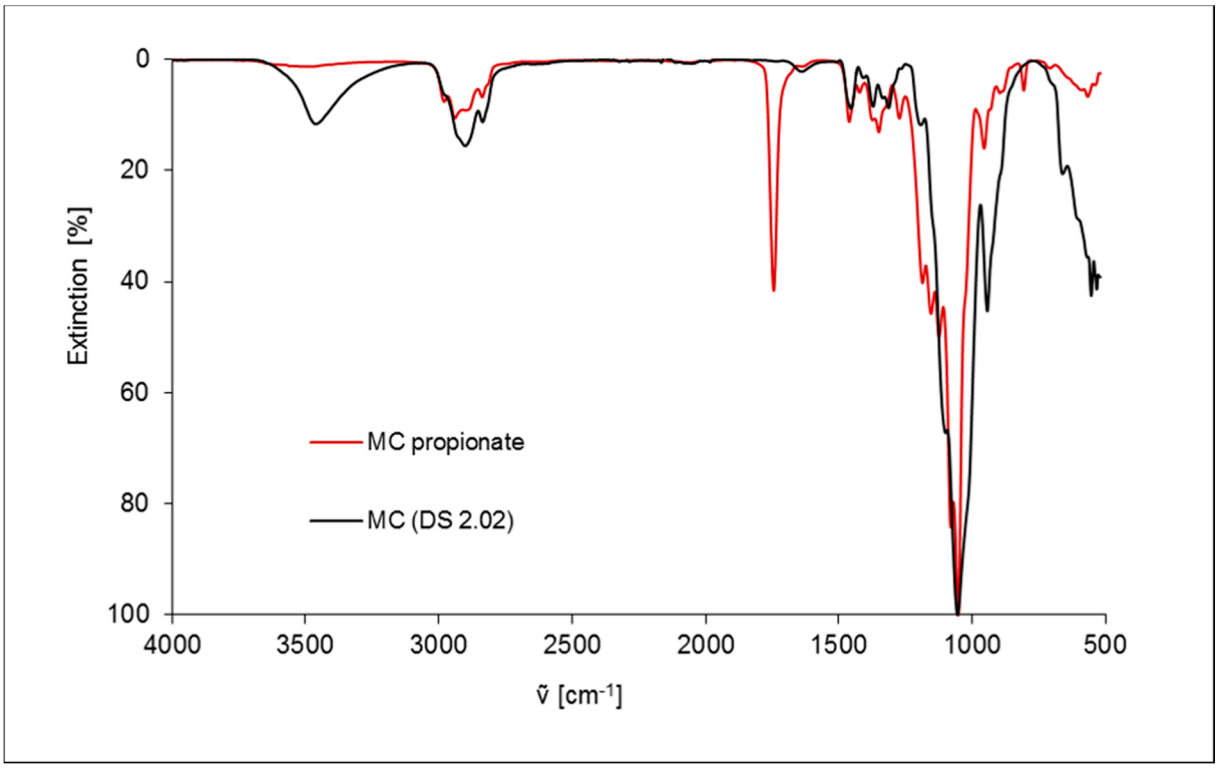

Figure S3. ATR-IR spectrum of MC (DS_{Me} 2.02) and the precipitated MC propionate; for comparison, extinction is normalized to the C-O vibration at about 1050 cm^{-1}.

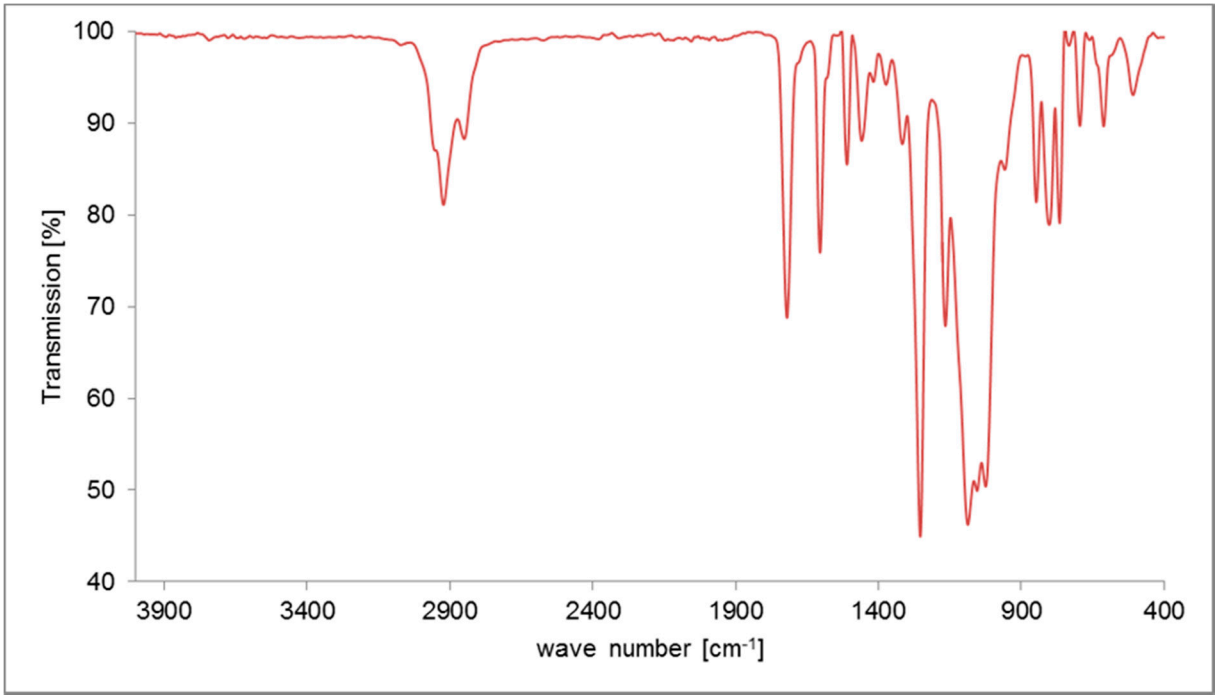

Figure S4. ATR-IR spectrum of MC 4-methoxybenzoate, prepared with 4-methoxybenzoyl chloride in pyridine. DS_{Me} 1.92.

Table S1. Yields and molar portions of monosaccharide constituents of fractions obtained by stepwise Soxhlet extraction of MC, DS 1.98, %Me = 66.0%, with THF (F1) and MeOH (2) and of the residual MC as determined by GLC of the corresponding alditol acetates. % Me = % of OH of a constiuent which are methylated. .

Scale: 2 g	**F1 (THF)**		**F2 (MeOH)**		**Residue**		**weighted average**
Constituent	**Mol%**	**% Me**	**Mol%**	**% Me**	**Mol%**	**% Me**	**% Me**
Glucose	90.4	76.1	80.1	71.9	99.1	65.1	
Mannose	3.9	86.7	3.4	74.2	0.2	88.3	
Xylose	5.7	81.6	16.5	74.7	0.7	96.9	
weighted average		**76.8**		**72.4**		**65.4**	**65.9**
Scale: 0.2 g	F1 (THF)		F2 (MeOH)		Residue		
Constituent	Mol%	% Me	Mol%	% Me	Mol%	% Me	
Glucose	89.8	75.0	88.6	72.1	99.7	65.1	
Mannose	1.8	77.8	2.6	80.2	0.1		
Xylose	8.4	94.7	8.9	84.9	0.2		
weighted average		**76.7**		**73.4**		**65.0**	**65.7**

Table S2. Fractionation of MC propionate and MC methoxybenzoate (DS_{Me} 2.02) by SPE on silica (Me propionate) and RP_{18} cartridge (MC-MeOBz), respectively. Eluents, volume ratio, fractions yields and total recovery.

	MC-Propionate Sample Weight 13.9 mg			MC-Methoxybenzoate Sample Weight 20.0 mg		
	Eluent	**Yield / mg**	**%**	**Elutionsmittel**	**Yield / mg**	**%**
F1	EtOAc/Toluol (5/5)	3.7	26.4	ACN/H_2O (5/5)	2.2*	10.9*
F2	EtOAc/Toluol (7.5/2.5)	1.6	11.5	ACN/H_2O (7.5/2.5)	3.4	17.1
F3	EtOAc/Toluol (9/1)	2.1	14.9	ACN/H_2O (9/1)	6.7	33.3
F4	EtOAc	1.7	11.9	ACN	4.5	22.4
F5	acetone	4.5	32.2	acetone	1.2	5.8
total		13.4	96.9		17.9	89.6

*technical losses

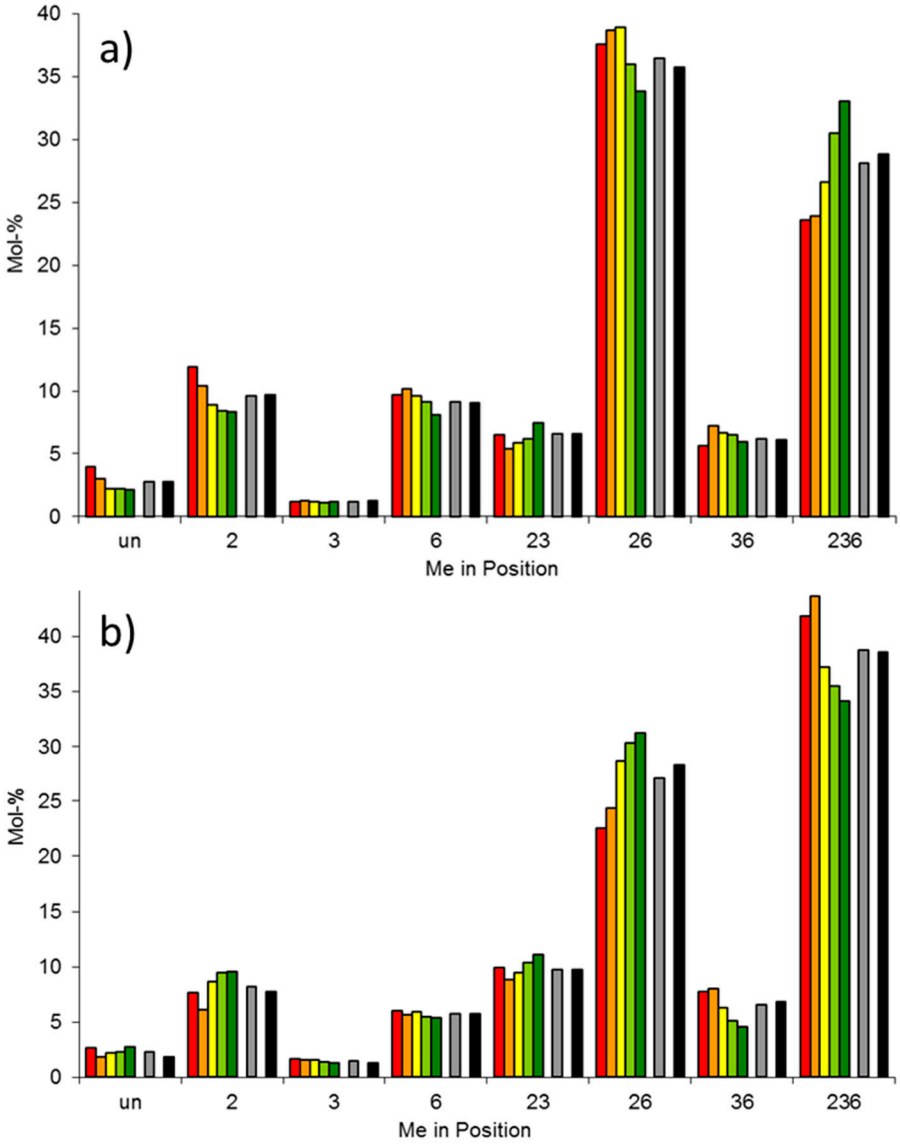

Figure S5. Methyl pattern in the glucosyl units of the MC fractions given in Table S2 (DS_{Me} 2.02). From left (red) to right (green): F1-F5 according to Table S2; gray: weighted average of all fractions; black: non fractionated MC ester. a) MC-propionate. b) MC-methoxybenzoate. In case of methoxybenzoates, ester cleavage during polysaccharide hydrolysis war not complete. Therefore, the lower O-methylated AGU (i.e. the higher esterified) were discriminated. Nonetheless, the relative change from fractions to fraction is visible.

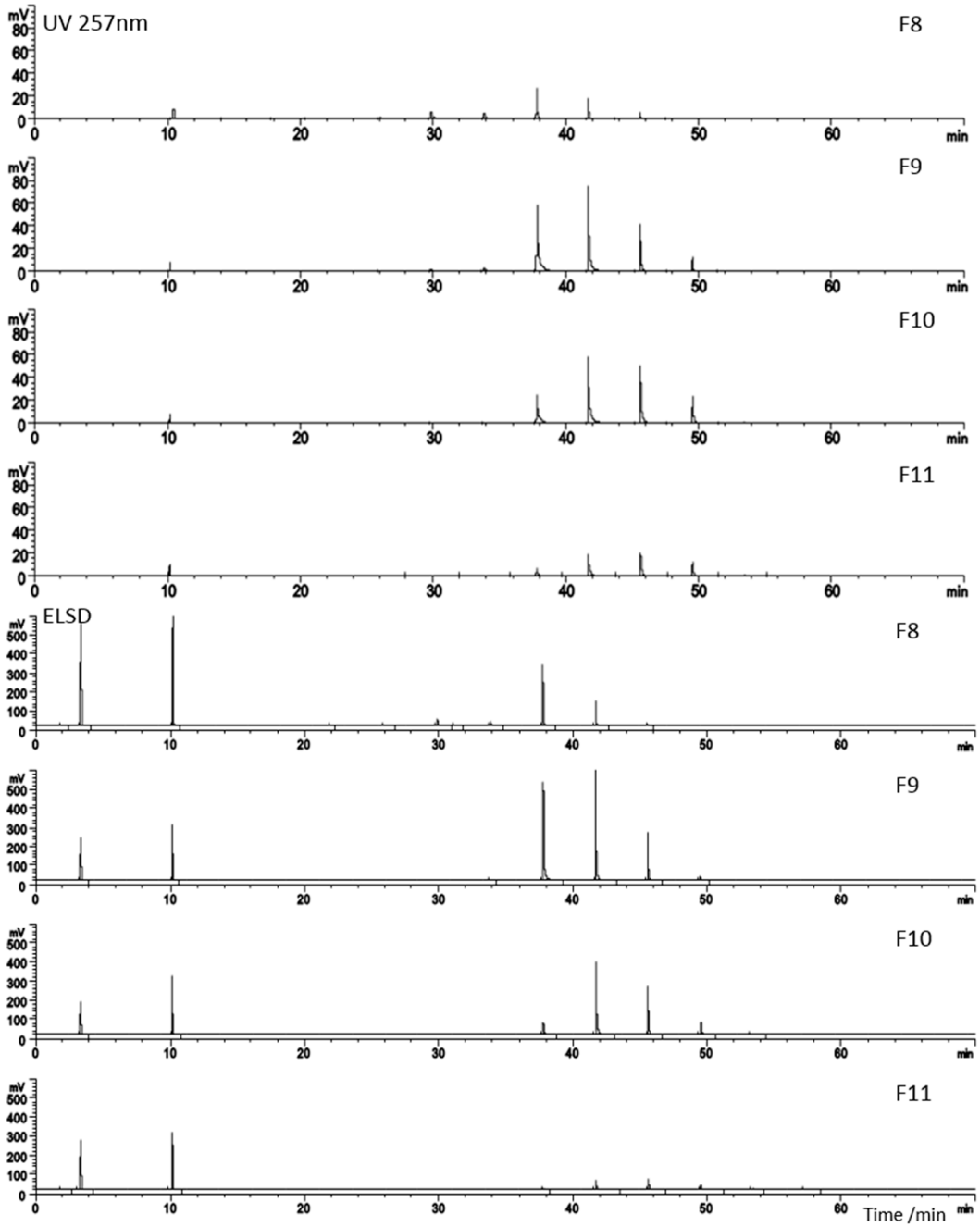

Figure S6. Analytical HPLC-runs of the semi-preparative fractions F8-F11 of Figure 9, body text (11%, 19%, 27%, 21% of the MC-MeOBz, w%) of MC-MeOBz (DS_{Me} 1.91) with stepwise adsorption/desorption; Polaris 5 Si-A 250 × 4,6 mm, DCM/2-PrOH, 1 mL/min, 2 min. hold for each, adsorption and desorption step; c=ca. 0.5 mg/mL; injection volume: 30 µL, detection: (**a**) UV 257 nm; (**b**), ELSD.