Supplementary Material

Analysis of the substituent distribution in carboxymethyl-1,4-glucans on different structural levels – an ongoing challenge

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Table S1
Monomer distribution (Mol %) of CMC from DWC, determined by CE-UV, the substitution pattern based on the models of Spurlin and Reuben, and their deviation from the experimentally obtained data (Δ).
$H_1 = \sqrt{\Sigma \Delta s_i/(n_{S_i}-1)}$

	CMC-0.88, n=2					CMC-0.85, n=2				
	CE	**Spurlin**	**Δ**	**Reuben**	**Δ**	**CE**	**Spurlin**	**Δ**	**Reuben**	**Δ**
s_0	34.86	34.58	0.27	33.94	0.92	36.01	36.07	-0.06	35.28	0.73
s_2	21.70	21.64	0.06	22.29	-0.58	21.48	21.26	0.22	22.06	-0.57
s_3	10.12	9.55	0.57	10.20	-0.08	10.04	9.44	0.60	10.23	-0.20
s_6	12.44	13.62	-1.17	13.36	-0.92	12.76	13.79	-1.03	13.49	-0.73
s_{23}	5.07	5.98	-0.90	5.33	-0.26	4.81	5.57	-0.75	4.77	0.04
s_{26}	9.36	8.52	0.84	8.77	0.58	9.01	8.13	0.88	8.43	0.57
s_{36}	4.09	3.76	0.33	4.01	0.08	4.11	3.61	0.50	3.91	0.20
s_{236}	2.36	2.35	0.01	2.10	0.26	1.78	2.13	-0.34	1.82	-0.04
c_0	34.86	34.58	0.27	33.94	0.92	36.01	36.07	-0.06	35.28	0.73
c_1	44.27	44.81	-0.54	45.84	-1.58	44.28	44.50	-0.22	45.78	-1.50
c_2	18.52	18.26	0.26	18.12	0.40	17.93	17.31	0.62	17.12	0.81
c_3	2.36	2.35	0.01	2.10	0.26	1.78	2.13	-0.34	1.82	-0.04
H_1			0.70		0.60			0.68		0.51
x_2	0.38					0.37				
x_3	0.22					0.21				
x_6	0.28					0.28				
DS (CE)	0.88					0.85				
DS (titration)	0.86					0.84				
	CMC-0.94, n=2					**CMC-0.96, n=2**				
	CE	**Spurlin**	**Δ**	**Reuben**	**Δ**	**CE**	**Spurlin**	**Δ**	**Reuben**	**Δ**
s_0	31.28	31.85	-0.57	30.84	0.44	30.56	30.97	-0.41	30.16	0.39
s_2	23.76	22.84	0.92	23.86	-0.09	22.20	21.52	0.68	22.32	-0.12
s_3	9.91	9.20	0.71	10.21	-0.30	10.37	9.70	0.67	10.50	-0.13
s_6	12.47	13.33	-0.86	12.91	-0.44	13.21	13.96	-0.76	13.60	-0.39
s_{23}	5.55	6.60	-1.05	5.59	-0.04	5.79	6.74	-0.95	5.94	-0.14
s_{26}	10.08	9.56	0.52	9.98	0.09	10.19	9.70	0.48	10.06	0.12
s_{36}	4.58	3.85	0.73	4.28	0.30	4.86	4.37	0.49	4.73	0.13
s_{236}	2.38	2.76	-0.38	2.34	0.04	2.82	3.04	-0.22	2.68	0.14
c_0	31.28	31.85	-0.57	30.84	0.44	30.56	30.97	-0.41	30.16	0.39
c_1	46.14	45.38	0.77	46.98	-0.84	45.78	45.18	0.60	46.42	-0.65

c_2	20.20	20.01	0.19	19.85	0.36	20.84	20.82	0.03	20.74	0.11
c_3	2.38	2.76	-0.38	2.34	0.04	2.82	3.04	-0.22	2.68	0.14
H_1			0.80		0.29			0.66		0.25
x_2	0.42					0.41				
x_3	0.22					0.24				
x_6	0.30					0.31				
DS (CE)	0.94					0.96				
DS (Titration)	0.92					0.99				

Table S2

Monomer distribution (Mol %) of CMC from Sigma, determined by CE-UV, the substitution pattern based on the models of Spurlin and Reuben, and their deviation from the experimentally obtained data (Δ).

$H_1 = \sqrt{\Sigma \Delta s_i / (n_{s_i} - 1)}$

	CMC-0.73, n=2					CMC-0.82, n=2				
	CE	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**	**CE**	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**
s_0	43.66±0.19	42.53	1.13	42.35	1.30	39.34±0.03	38.02	1.33	37.96	1.39
s_2	21.24±0.09	21.88	-0.63	22.06	-0.81	20.84±0.02	21.52	-0.68	21.58	-0.74
s_3	7.37±0.14	7.44	-0.07	7.62	-0.25	8.22±0.01	8.39	-0.17	8.46	-0.23
s_6	12.31±0.06	13.67	-1.36	13.61	-1.30	12.88±0.05	14.29	-1.41	14.27	-1.39
$s_{2,3}$	3.40±0.04	3.83	-0.43	3.65	-0.25	4.27±0.03	4.75	-0.48	4.69	-0.42
$s_{2,6}$	7.90±0.04	7.03	0.87	7.09	0.81	8.85±0.04	8.09	0.76	8.11	0.74
$s_{3,6}$	2.70±0.02	2.39	0.30	2.45	0.25	3.41±0.00	3.16	0.26	3.18	0.23
$s_{2,3,6}$	1.42±0.03	1.23	0.19	1.17	0.25	2.18±0.00	1.79	0.39	1.76	0.42
c_0	43.66	42.53		42.35		39.34	38.02		37.96	
c_1	40.92	42.99		43.29		41.95	44.20		44.30	
c_2	14.00	13.25		13.19		16.53	15.99		15.98	
c_3	1.42	1.23		1.17		2.18	1.79		1.76	
H_1			0.81		0.84			0.87		0.88
x_2	0.34	0.34		0.34		0.36	0.36		0.36	
x_3	0.15	0.15		0.15		0.18	0.18		0.18	
x_6	0.24	0.24		0.24		0.27	0.27		0.27	
DS	**0.73**					**0.82**				
	CMC-1.26, n=3					**CMC-0.75, n=2**				
	CE	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**	**CE**	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**
s_0	18.65±0.02	18.29	0.36	17.04	1.61	41.11±0.02	41.63	-0.51	41.49	-0,37
s_2	24.36±0.04	23.75	0.61	25.00	-0.64	20.39±0.14	19.78	0.61	19.92	0,47
s_3	8.27±0.01	7.08	1.18	8.34	-0.07	8.48±0.07	8.69	-0.21	8.83	-0,35
s_6	10.57±0.01	13.07	-2.51	12.18	-1.61	14.78±0.03	14.45	0.32	14.40	0,37
$s_{2,3}$	7.04±0.09	9.20	-2.15	7.94	-0.90	4.24±0.03	4.13	0.11	3.99	0,25
$s_{2,6}$	18.51±0.07	16.97	1.54	17.87	0.64	6.45±0.04	6.87	-0.42	6.92	-0,47
$s_{3,6}$	6.03±0.02	5.06	0.97	5.96	0.07	3.41±0.06	3.02	0.40	3.07	0,35
$s_{2,3,6}$	6.58±0.04	6.57	0.00	5.68	0.90	1.13±0.00	1.43	-0.30	1.39	-0,25
c_0	18.65	18.29	0.36	17.04	1.61	41.11	41.63		41.49	
c_1	43.20	43.90	-0.72	45.52	-2.32	43.65	42.92		43.16	
c_2	31.58	31.23	0.36	31.77	-0.19	14.11	14.02		13.97	
c_3	6.58	6.57	0.00	5.68	0.90	1.13	1.43		1.39	
H_1			1.52		1.04			0.40		0.42
x_2	0.56	0.56		0.56		0.32	0.32		0.32	
x_3	0.28	0.28		0.28		0.17	0.17		0.17	
x_6	0.42	0.42		0.42		0.26	0.26		0.26	
DS	**1.26**					**0.75**				

Table S3
Monomer distribution (Mol %) of CMA, determined by CE-UV, the corresponding substitution pattern based on the models of Spurlin and Reuben, and their deviation from the experimentally obtained data (Δ). $H_1 = \sqrt{\Sigma \Delta s_i}/(n_{S_i}-1)$

	CMA-1.06, n=4				
	CE	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**
s_0	16.89±0.14	17.22	-0.33	15.85	1.03
s_2	54.60±0.28	54.35	0.25	55.72	-1.12
s_3	3.29±0.06	1.77	1.52	3.14	0.15
s_6	3.90±0.14	4.59	-1.40	4.23	-1.03
$s_{2,3}$	4.17±0.03	5.60	-1.43	4.23	-0.06
$s_{2,6}$	15.98±0.56	14.49	1.49	14.86	1.12
$s_{3,6}$	0.69±0.01	0.47	0.22	0.84	-0.15
$s_{2,3,6}$	1.19±0.06	1.49	-0.30	1.13	0.06
c_0	16.89	17.22		15.85	
c_1	61.08	60.72		63.09	
c_2	20.84	20.57		19.93	
c_3	1.19	1.49		1.13	
H_1			1.12		0.82
x_2	0.76	0.76		0.76	
x_3	0.09	0.09		0.09	
x_6	0.21	0.21		0.21	
DS	**1.06**				

	CMA-0.65, n= 4				
	CE	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**
s_0	43.38±0.52	43.35	0.03	42.33	1.05
s_2	41.29±0.62	41.25	0.04	42.27	-0.98
s_3	4.39±0.04	3.37	1.01	4.39	0.00
s_6	3.04±0.03	4.19	-1.15	4.09	-1.05
$s_{2,3}$	2.12±0.03	3.21	-1.09	2.19	-0.07
$s_{2,6}$	5.06±0.10	3.99	1.07	4.09	0.98
$s_{3,6}$	0.43±0.02	0.33	0.10	0.42	0.00
$s_{2,3,6}$	0.29±0.02	0.31	-0.02	0.21	0.07
c_0	43.38	43.35		42.33	
c_1	48.72	48.81		50.75	
c_2	7.61	7.52		6.70	
c_3	0.29	0.31		0.21	
H_1			0.82		0.77

x_2	0.49	0.49		0.49	
x_3	0.07	0.07		0.07	
x_6	0.09	0.09		0.09	
DS	**0.65**				
	CMA-0.64, n= 3				
	CE	**Spurlin**	**Δs_i**	**Reuben**	**Δs_i**
s_0	51.00±0.17	45.83	5.17	46.44	4.56
s_2	27.44±0.12	32.15	-4.70	31.53	-4.09
s_3	1.68±0.10	2.31	-0.64	1.70	-0.02
s_6	5.70±0.03	10.12	-4.42	10.26	-4.56
$s_{2,3}$	1.79±0.01	1.62	0.17	2.23	-0.45
$s_{2,6}$	11.06±0.02	7.10	3.95	6.97	4.09
$s_{3,6}$	0.40±0.00	0.51	-0.11	0.37	0.02
$s_{2,3,6}$	0.94±0.00	0.36	0.58	0.49	0.45
c_0	51.00	45.83		46.44	
c_1	34.82	44.58		43.49	
c_2	13.24	9.23		9.58	
c_3	0.94	0.36		0.49	
H_1			3.48		3.28
x_2	0.41	0.41		0.41	
x_3	0.05	0.05		0.05	
x_6	0.18	0.18		0.18	
DS	**0.64**				

Carboxymethyl distribution in the glucosyl unit of CM-glucans: deviation of s_i and c_i from the Spurlin and Reuben model

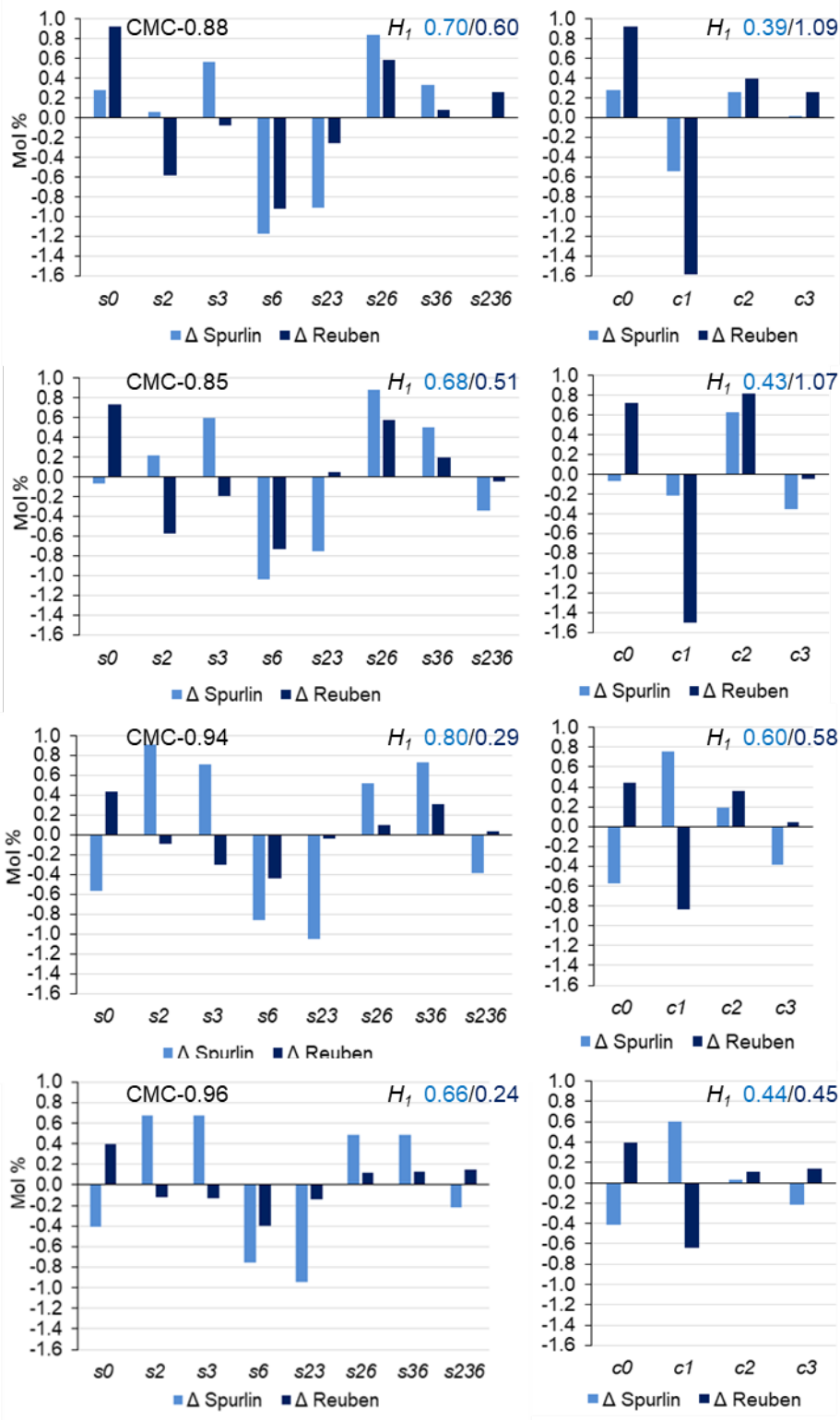


Figure S1. CMC set provided by former DWC. Deviation from the Spurlin and the Reuben model, heterogeneity $H_1(s_i)$ and $H_1(c_i)$, calculated from Δ Spurlin and Δ Reuben, respectively. $H_i = \sqrt{\Sigma(\Delta s_i)/(n-1)}$ and $H_i = \sqrt{\Sigma(\Delta c_i)/(n-1)}$, respectively. For discussion see body text, *3.1*.

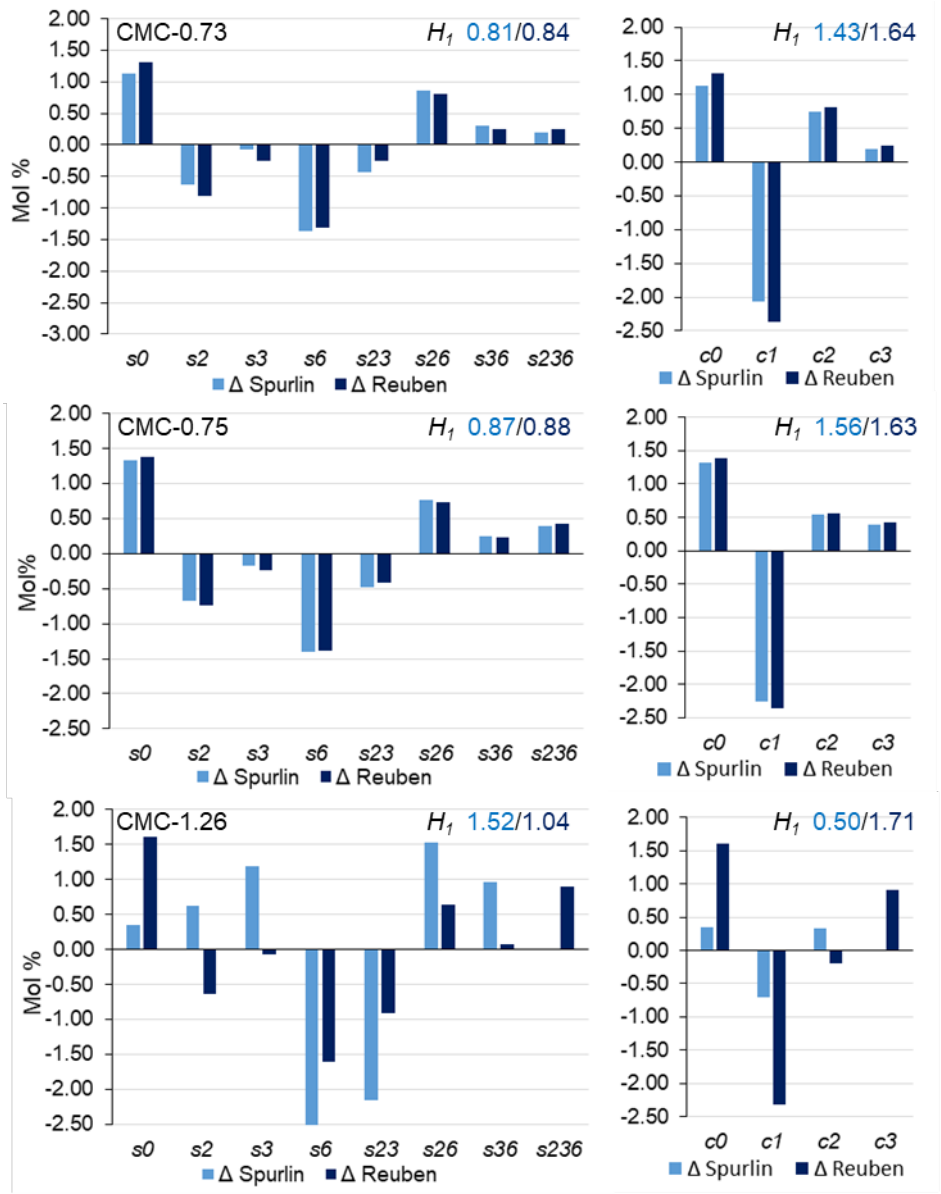


Figure S2. CMC set purchased from Sigma. Deviation from the Spurlin and the Reuben model, heterogeneity $H_1(s_i)$ and $H_1(c_i)$, calculated from Δ Spurlin and Δ Reuben, respectively. $H_i = \sqrt{\Sigma(\Delta s_i)/(n-1)}$ and $H_i = \sqrt{\Sigma(\Delta c_i)/(n-1)}$, respectively. For discussion see body text, *3.1*.

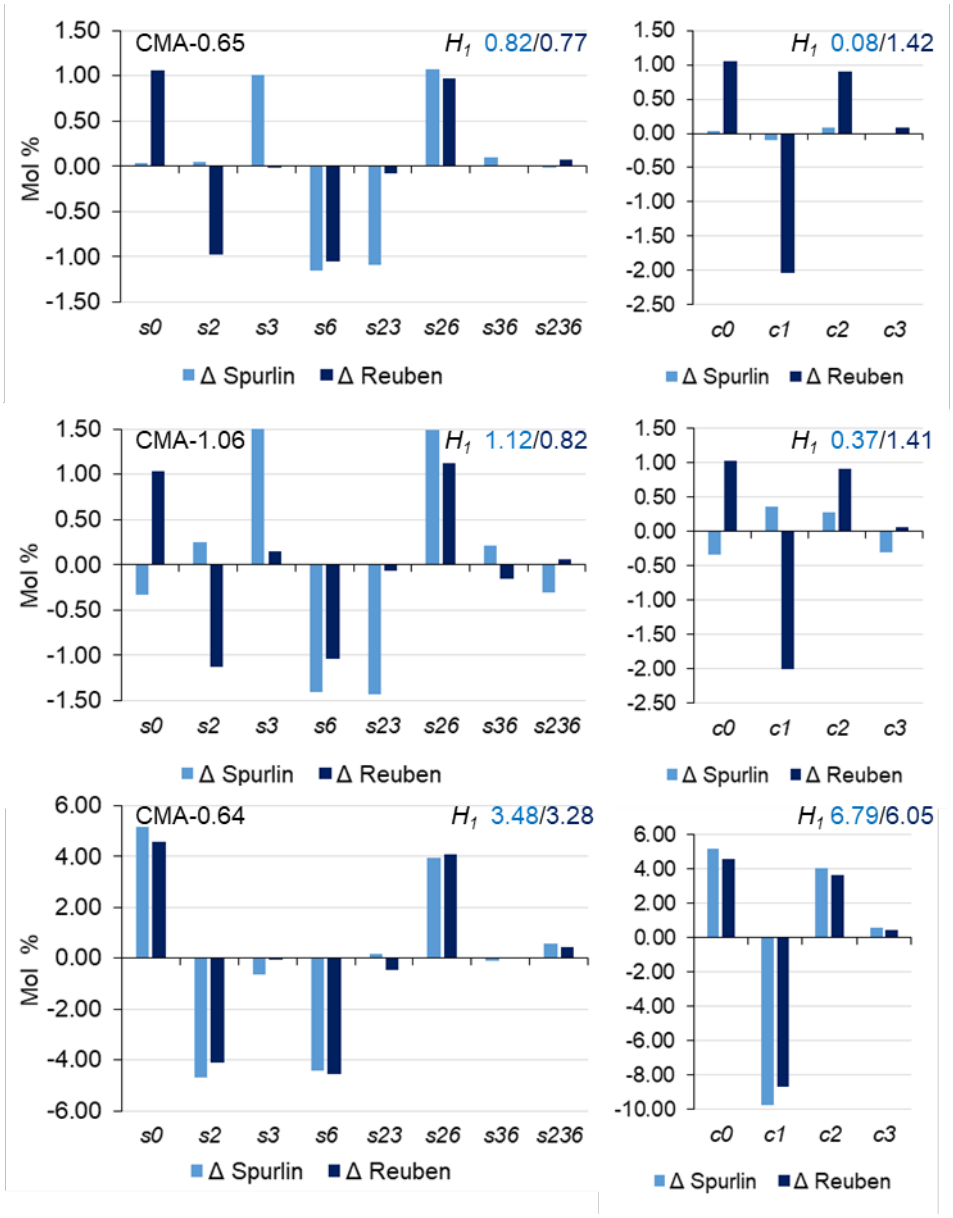


Figure S3. CMA set prepared in lab-scale. Deviation from the Spurlin and the Reuben model, heterogeneity $H_1(s_i)$ and $H_1(c_i)$, calculated from Δ Spurlin and Δ Reuben, respectively. $H_i = \sqrt{\Sigma(\Delta s_i)/(n-1)}$ and $H_i = \sqrt{\Sigma(\Delta c_i)/(n-1)}$, respectively. Consider the extended scale for CMC-0.65. For discussion see body text, *3.1*.

Evaluation of ESI-MS measurements of CM-COS

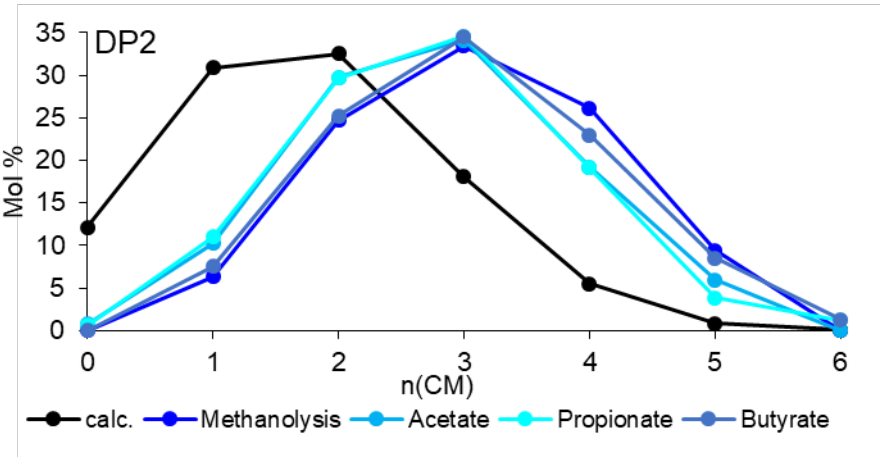


Figure S4. Distribution of the CM-groups in DP 2 of CMC-0.88 obtained from ESI-IT-masss spectra (positive mode, M+Na) of methanolysates, and in addition after acetylation, propionylation, and butyrylation of free OH, respectively. Experimentally obtained data is compared with the random distribution calculated from monomer data obtained by CE-UV-analysis (calc., DS_{CE} 0.88, see Table S1). Apparent average DS = 1.54 (methanolysate), 1.39 (Ac), 1.38 (EtCO), and 1.54 (PrCO).

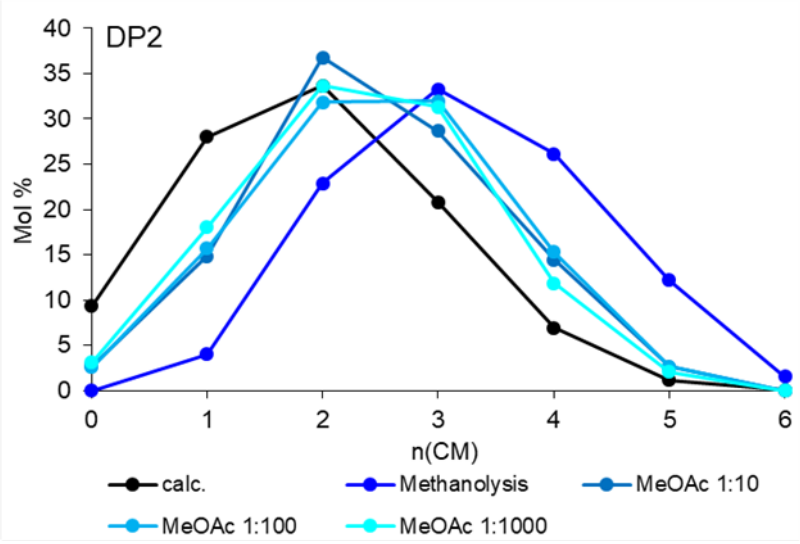


Figure S5. Distribution of the CM-groups of DP 2 from CMC-0.96 obtained after ESI-IT-MS of the d_4-methanolysate after methoxyacetylation and stepweise dilution with MeOH-d_4. Experimentally obtained data is compared with the random distribution calculated from monomer data obtained by CE-UV analysis (calc.). DS_{CE} 0.96 (see Table S1).

Light microscopic images

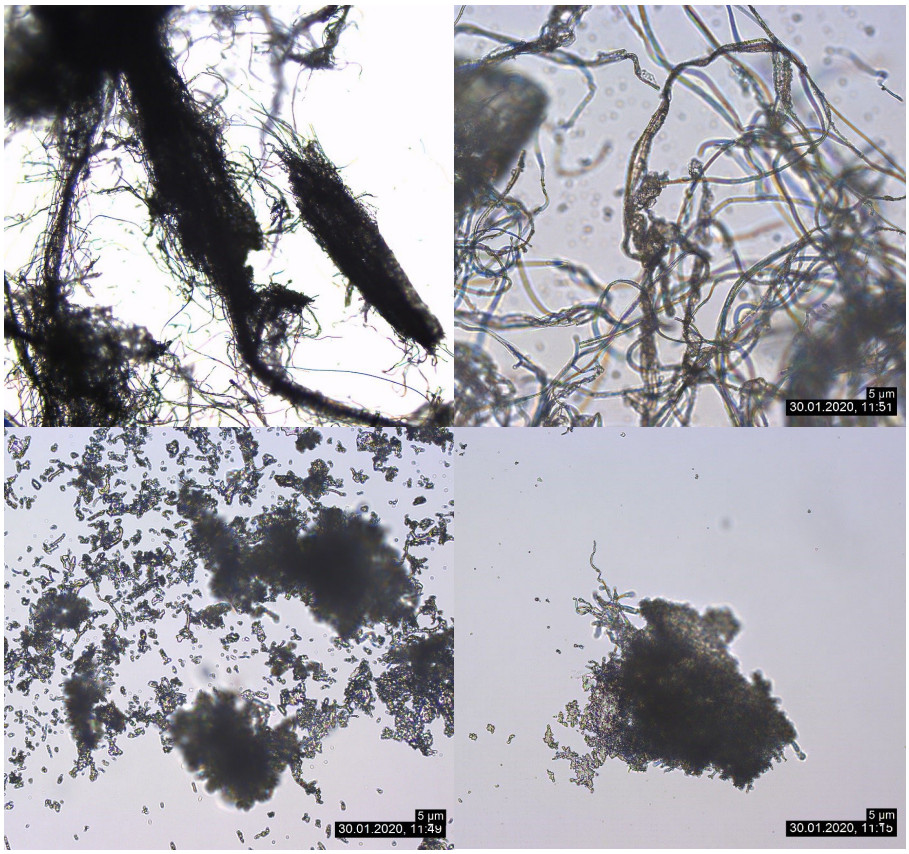


Figure S6. Light microscopic images of acetylated CMG. a) CMC-1.26, 5x (left) and 20x (right); b) CMG-0.64, 20x (left and right).

ATR-IR spectra of CMG-CMOMe/Ac

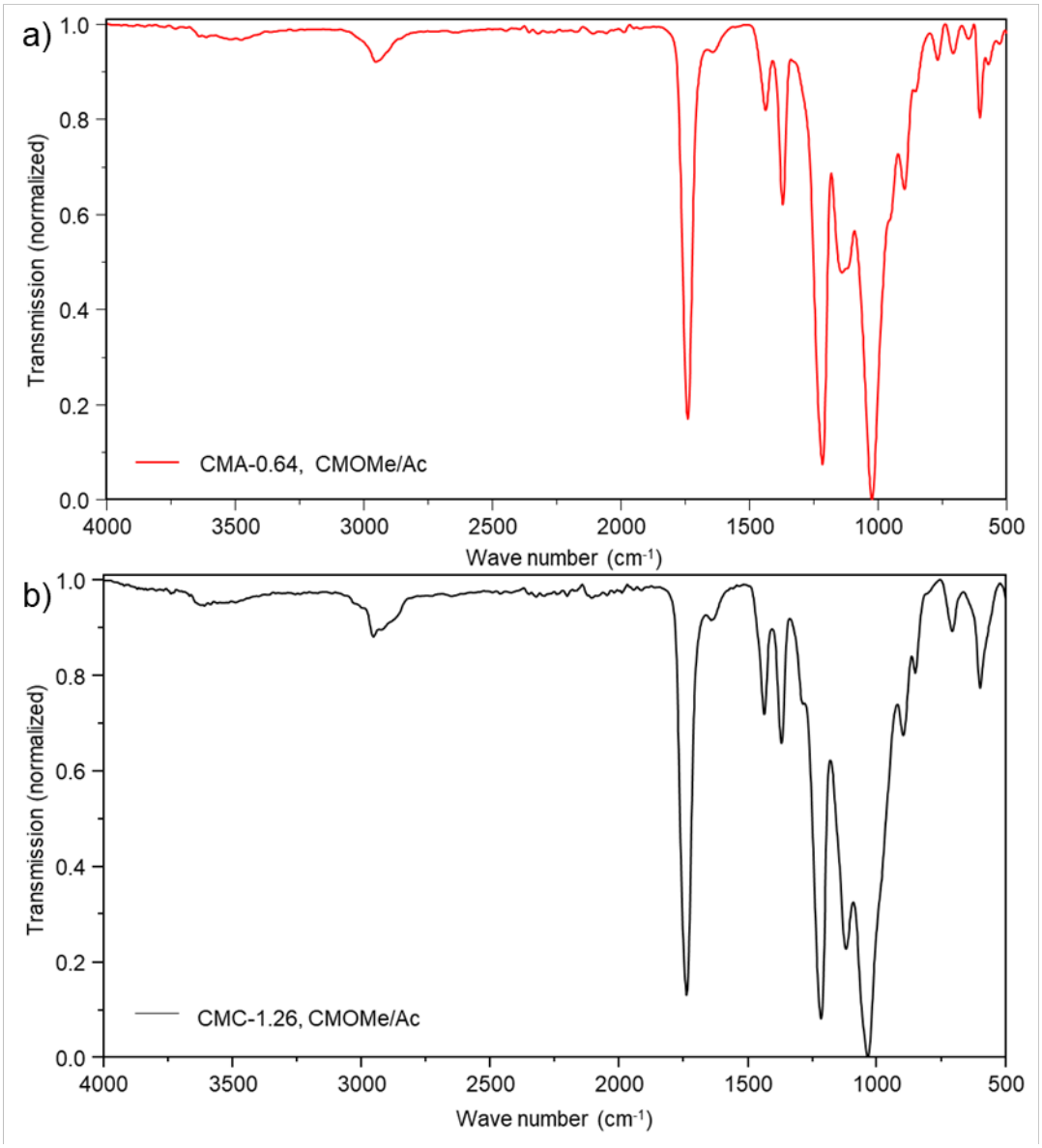


Figure S7. ATR-IR spectra of (a) CMA-0.64 and (b) CMC-1.26 after methyl esterification of CM and acetylation of OH. Sample preparation for LAH reduction.