Starch Derivatives of a High Degree of Functionalization. VI. Multistep Carboxymethylation

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ABSTRACT: For the first time, carboxymethyl starch (CMS) samples with a very high degree of substitution (DS_{CM} = 2.1) were synthesized by multistep carboxymethylation under heterogeneous reaction conditions in methanol/water with sodium hydroxide and monochloroacetic acid as an etherifying agent. The stepwise increase in the total DS_{CM} value gradually decreased with an increasing DS_{CM} value of the starting polymer. The determination of the functionalization pattern of CMS by ^{1}H-NMR spectroscopy after chain degradation indicated a high preference for 2-O-substitution. The distribution of the carboxymethyl functions was in the order O-2 ⩾ O-6 > O-3. A detailed analysis of the depolymerized sample by means of high-performance liquid chromatography and capillary electrophoresis revealed a monomer composition that was in very good agreement with the statistical model of Spurlin. © 2002 Wiley Periodicals, Inc. J Appl Polym Sci 86: 743–752, 2002

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INTRODUCTION

Most commercially available starch derivatives have a low degree of substitution (DS) of up to 0.20. Examples include the preparation of starch acetate or succinate and the reaction with ethylene oxide or propylene oxide to form hydroxyalkyl starch, with sodium polyphosphate to form starch phosphate, and with sodium monochloroacetate to form carboxymethyl starch (CMS).[1] Many other types of starch derivatives have been prepared, including products of rather high DS values (> 0.5), but most of these compounds have not been commercialized. In this context, our interest was focused on CMS with a high DS value because carboxymethylation is a versatile transformation leading to water-soluble polymers and intermediates with various valuable features.[2–4] The properties (e.g., solution viscosity, film formation, cation interaction, and supramolecular aggregate formation) are mainly determined by the total DS, that is, the average number of carboxymethyl functions in the polymer. Moreover, the functionalization pattern may influence the properties.

CMS was first made in 1924 by the reaction of starch in an alkaline solution (40% NaOH) with sodium monochloroacetate.[5] Products with DS values of up to 1.0 have been obtained in essentially nonaqueous media.[6,7] Various studies of the carboxymethylation of starch were carried out to optimize reaction conditions, that is, to increase product yield and reaction efficiency.[8–11]

In the course of our studies on polysaccharides,[12] we found that the heterogeneous carboxymethylation of starch with methanol/water as a slurry medium, an aqueous sodium hydroxide solution for activation, and monochloroacetate as an etherifying agent led to CMS with a DS value of about 0.4 with a reaction efficiency of 60% independently of the starch type with respect to the botanical source.[13] Although products of higher DS values have been obtained with ethanol[8] and 2-propanol,[3] methanol is often used in the commercial carboxymethylation of starch.

These investigations are directly related to ongoing projects concerning the search for alternative paths of polysaccharide etherification leading to derivatives with high DS values and new functionalization patterns. One possible way in this respect is synthesis in reactive microstructures prepared via induced phase separation starting from various polysaccharide solutions in dipolar and aprotic solvents.[14–16] For starch,

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carboxymethylated derivatives with a total DS value of 1.68 were obtained in a one-step synthesis.[17]

In this article, we report on our studies of the preparation of CMS samples of high DS values by multistep carboxymethylation with the industrially important slurry medium methanol/water. The total DS values, the distribution of the functional groups within the repeating anhydroglucose units (AGUs), and the molar fractions of the differently functionalized units were determined with ^{1}H-NMR spectroscopy, high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) after complete hydrolytic chain degradation. Moreover, the intact polymers were characterized with ^{13}C-NMR spectroscopy.

TABLE I
DS of CMS Determined by HPLC, ^{1}H-NMR Spectroscopy, and CE After Depolymerization

	DS		
CMS no.	HPLC[a]	^{1}H-NMR	CE[a]
2	0.40	0.57[b]	0.37
3	0.79	0.87	0.65
4	1.08	—	1.01
5	1.31	1.40	1.23
6	1.53	1.58	1.40
7	1.72	1.59/1.74[c]	1.63
8	1.83	1.79/1.91	1.68
9	1.98	1.94/2.04	1.81
10	2.02	1.96/2.06	1.85
11	2.09	2.20/2.25	1.93

[a] DS = $\Sigma\ ic_i$ (i = 1, 2, 3, 4). See Table II.
[b] Calculated with eq. (1).
[c] Calculated with eq. (2).

EXPERIMENTAL

Materials

4-Aminobenzonitrile (ABN) was purchased from Fluka (Neu-Ulm, Germany) and used without further purification. Sodium cyanoborohydride was obtained from Sigma–Aldrich (Steinheim, Germany) and Merck (Darmstadt, Germany). Boric acid and perchloric acid (70%) were obtained from Merck.

Carboxymethylation of starch

Air-dry potato starch superior (sample 1; 425 g, 19% water content, 344.25 g and 2.125 mol of dry matter, 28% amylose content; Emsland-Stärke GmbH, Golßen, Germany) in 750 mL of methanol was stirred vigorously, and 210 mL of 45% (w/v) aqueous sodium hydroxide (4.29 mol) was added during 15 min at room temperature. Stirring was continued for another 1.5 h at 40°C, and monochloroacetic acid (MCA; 150 g, 1.59 mol) was then added during a period of 15 min. The mixture was allowed to react for 6 h at 40°C. After carboxymethylation, the mixture was filtered, suspended in 76% (w/w) aqueous methanol, and neutralized with acetic acid. The product was collected after filtration, washed three times with 76% (w/w) aqueous methanol and pure methanol (99.9%), and dried at 110°C in vacuo (sample 2). The yield was 90%, and the DS was 0.40 (determined with HPLC after complete chain degradation).

The subsequent carboxymethylation steps were run in a similar procedure (samples 3–11). DS values determined with HPLC, ^{1}H-NMR, and CE are given in Table I.

MEASUREMENTS

HPLC analysis

CMS samples were hydrolyzed with perchloric acid. CMS (0.1 g) was dispersed in 2 mL of $HClO_4$ (70%) and after 10 min at room temperature was diluted with 18 mL of distilled water. This mixture was kept at 100°C for 16 h. The solution obtained was carefully neutralized with 2*M* KOH and kept at 4°C for 1 h to guarantee maximum precipitation of $KClO_4$. The salt was filtered off and washed three times with distilled water. The volume of the solution obtained was reduced to approximately 3 mL with a rotary evaporator and was diluted with distilled water to give an exactly 5-mL sample.

An HPLC system was used that consisted of a Jasco degasser, a Knauer 64 HPLC pump, a Knauer differential refractometer, a Jasco OR 990 chiral detector, and a Jasco column heater jet stream. The sample (50 μL) was injected with a Knauer autosampler on two coupled Aminex HPX 87 H columns (300 × 7.8 mm, 9-μm particle size; BioRad Laboratories) for separation. The column temperature was 65°C. As a mobile phase, 0.01*N* sulfuric acid at a flow rate of 0.5 mL/min was used. The chromatographic data were evaluated with Jasco Borwin HPLC software.

^{1}H-NMR measurements

The ^{1}H-NMR analyses were carried out as described.[17,18] For this purpose, the CMS samples were hydrolyzed with a mixture of D_2SO_4 and D_2O (25% v/v) within 5 h at 90°C. The spectra were acquired on a Bruker AMX 250 spectrometer.

^{13}C-NMR spectroscopy

The ^{13}C-NMR spectra of CMS (without hydrolysis) were recorded on a Bruker AMX 400 spectrometer at a concentration of 5% (w/v) in D_2O at 60°C. The number of scans was 25,000–100,000.

CE

For sample preparation, 500 μL of perchloric acid (70%) was added to about 20 mg of CMS in a 5-mL V-vial, and the CMS was allowed to swell. This slurry was heated at approximately 60°C for 5–15 min. Then, 4.5 mL of water was added, and the mixture was heated under stirring for 2 h at 120°C. The solution obtained was carefully neutralized with 2*N* KOH and kept at −10°C for 30 min. The precipitated salt was filtered and washed with distilled water. For the removal of as much salt as possible, this procedure was repeated at least three times. The resulting solution was directly used for the derivatization with ABN.

A 1.5*M* methanolic solution of ABN (50 μL), a 1*M* solution of sodium cyanoborohydride in methanol/tetrahydrofuran (10/1 v/v, 50 μL), and glacial acetic acid (20 μL) were added to the CMS hydrolysate (80 μL) in a 1-mL screw-cap vial. The mixture was heated for 15 min at 60°C and subsequently evaporated to dryness under a stream of nitrogen. The residue was dissolved in methanol (250 μL) and water (750 μL), and the solution was filtered through a 0.45-μm membrane filter (Macherey & Nagel, Düren, Germany) and introduced to the capillary tube for CE.

The CE analyses were performed on a Beckman Coulter P/ACE MDQ (Fullerton, CA) CE system, which was equipped with a fused-silica capillary (eCap, Beckman) with an inside diameter of 50 μm and a total length of 60 cm (50-cm effective length). A borate buffer (150 mmol, pH 10) was used at 28 kV. Detection was carried out by on-column measurement of the UV absorption at 285 nm. Samples were loaded on the anionic site of the capillary by the application of a pressure of 34.5 mbar for 10 s. Analyses were carried out at a temperature of 25°C. Every new capillary was flushed with 1*M* NaOH for 10 min, with methanol for 10 min, with HCl for 10 min, and with 0.1*M* NaOH for 15 min. Between runs, the capillary was flushed first with water for 3 min and then with 0.1*M* NaOH for 3 min and was subsequently equilibrated with a buffer for 5 min. For quantitative evaluation, the peak areas were divided by the migration time.

RESULTS AND DISCUSSION

Carboxymethylation procedure

As the starting material, potato starch was slurried in methanol and activated by treatment with aqueous NaOH. A reactive material was obtained under these conditions.[13] In addition, sodium hydroxide initiated the reaction with MCA, which was carried out for 6 h at 40°C. The conditions used guaranteed that the reaction mixture could be mixed during the whole course of reaction and that no gelation would occur. CMS (sample 2) with a degree of substitution of carboxymethyl groups (DS_{CM}) of 0.40 was obtained at a molar ratio of 0.75 mol of MCA to 1.27 mol of NaOH/mol of AGU. The DS_{CM} values were determined by means of HPLC with completely depolymerized samples (see the next section). As found in previous studies, an increase in the MCA/AGU molar ratio to 1.5 led to a slightly increased DS_{CM}; however, the reaction efficiency decreased to less than 45%.[13] Sample 2 was activated and allowed to react with about 0.75 mol of MCA/mol of modified AGU under similar conditions, yielding CMS 3 with DS_{CM} = 0.79 (Table I). Subsequently, additional carboxymethylation reactions were carried out eight times with CMS obtained in the previous step. After 10 carboxymethylation steps, sample 11 with DS_{CM} = 2.09 was obtained. The stepwise increase of the DS_{CM} values of CMS samples 2–11 gradually decreased with increasing DS_{CM} of the starting polymer (Fig. 1).

Functionalization pattern of CMS

To analyze the functionalization pattern of the CMS samples obtained, we used an HPLC method that was developed in our laboratory.[14,17] This rapid and convenient procedure yielded DS_{CM} and the molar fractions of the differently functionalized repeating units, that is, unmodified glucose (c_0); the group of 2-, 3-, and 6-mono-*O*-carboxymethyl glucoses (CMglc; c_1); the group of 2,3-, 2,6-, and 3,6-di-O-substituted repeating units (c_2); and 2,3,6-tri-*O*-CMglc (c_3). Moreover, starting at DS_{CM} = 1.7, we detected some 2,3,4,6-tetra-*O*-CMglc units (c_4), but to a very low extent. Sample 11, for example, possessed a molar fraction of 0.02 (Table II). These repeating units resulted from the carboxymethylation of the nonreducing end groups that were present, especially in the branched amylopectin to a comparatively high extent. The potato starch contained 72% amylopectin, and so a significant number of repeating units were present with a reactive OH group at position 4.

^{1}H-NMR spectroscopy of depolymerized CMS is an efficient method for the determination of the distribution of the functional groups within the AGU.[19] Representative spectra of samples 3 and 11, including the assignments of the peaks, are shown in Figure 2. The partial DS_{CM} (x_i) values were calculated according to eq. (1), where *A* represents the peak area, O is the oxygen atom at position *i* (*i* = 2, 3, or 6), H-1 is the hydrogen atom at the anomeric C (α,β-configuration of glucose), s means substituted, and u means unsubstituted. A functionalization at position 4 was not included because the corresponding signal was not assigned. From HPLC, it is evident that even for sample 11 with a high DS_{CM} value of 2.09, the content of tetra-O-functionalized units was only 0.02. Consequently, the partial DS_{CM} value at O-4 was 0.005. This

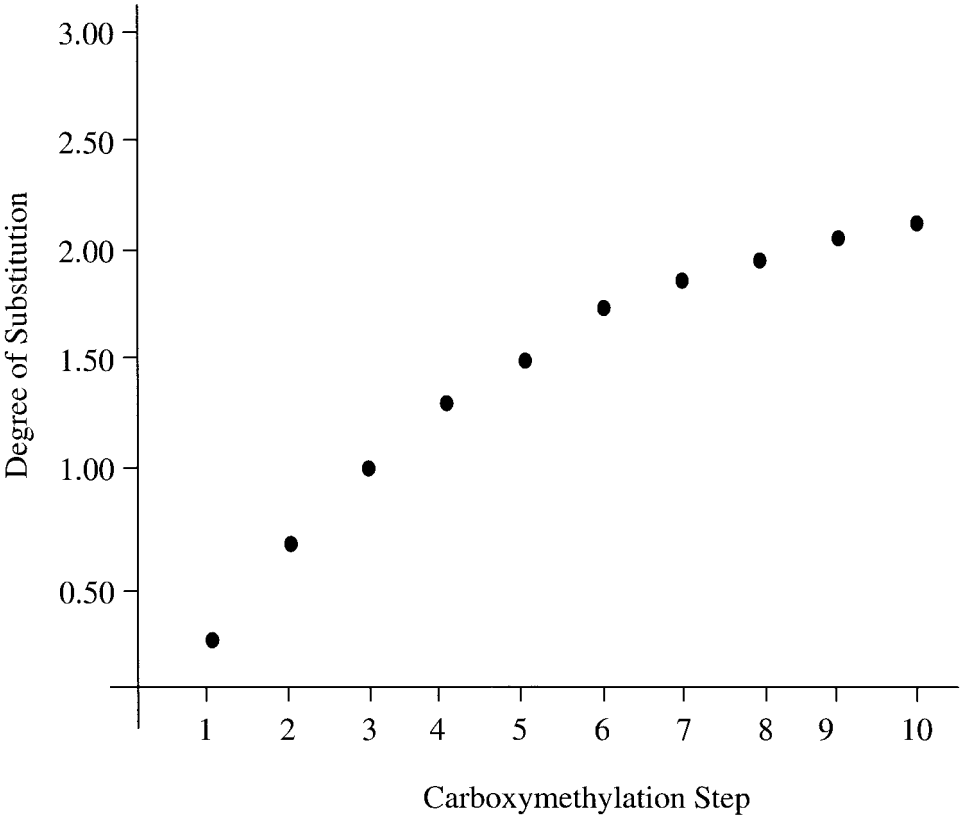

Figure 1 Dependence of DS_{CM} on the number of reaction steps.

is below the detection sensitivity limit of the NMR method:

$$x_i = \frac{\frac{1}{2}A(\text{Methylene Protons at Position O-}i)}{A(\text{H}-1\alpha,\text{O}-2\text{s}) + A(\text{H}-1\alpha,\text{O}-2\text{u}) + A(\text{H}-1\beta,\text{O}-2\text{s}) + A(\text{H}-1\beta,\text{O}-2\text{u})}$$

$$DS = \Sigma x_i \quad (1)$$

The partial DS at position 2 could also be calculated with eq. (2), which is based on the fact that 2-O-substitution usually induces a low-field shift of the vicinal H-1:[16,20]

$$x_2 = \frac{A(\text{H}-1\alpha,\text{O}-2\text{s}) + A(\text{H}-1\beta,\text{O}-2\text{s})}{A(\text{H}-1\alpha,\text{O}-2\text{s}) + A(\text{H}-1\alpha,\text{O}-2\text{u}) + A(\text{H}-1\beta,\text{O}-2\text{s}) + A(\text{H}-1\beta,\text{O}-2\text{u})} \quad (2)$$

TABLE II
Molar Fractions of Glucose (Glc) and Mono-, Di-, Tri-, and Tetra-O-carboxymethyl (CM) Glc of CMS Determined by CE and HPLC Analysis After Depolymerization

	Molar fraction (%) of									
	Glc		Mono- (c_1)		Di- (c_2)		Tri- (c_3)		Tetra-O-CMGlc (c_4)	
CMS no.	CE	HPLC	CE[a]	HPLC	CE[a]	HPLC	CE[a]	HPLC	CE	HPLC
2	0.652	0.621	0.330	0.351	0.019	0.023	0	0	0	0
3	0.417	0.312	0.520	0.592	0.061	0.092	0.002	0.004	0	0
4	0.177	0.147	0.649	0.634	0.165	0.204	0.009	0.014	0	0
5	0.093	0.080	0.605	0.568	0.276	0.315	0.026	0.037	0	0
6	0.67	0.038	0.523	0.470	0.354	0.414	0.056	0.079	0	0
7	0.039	0.023	0.400	0.373	0.452	0.469	0.108	0.128	0	0.007
8	0.022	0.022	0.405	0.311	0.447	0.483	0.126	0.170	0	0.012
9	0.013	0.011	0.347	0.252	0.459	0.493	0.181	0.234	0	0.010
10	0.040	0.015	0.291	0.232	0.449	0.477	0.220	0.262	0	0.013
11	0.016	0.018	0.298	0.213	0.438	0.453	0.253	0.296	0	0.020

[a] $c_1 = \Sigma s_i$ (i = 2, 3, 6); $c_2 = \Sigma s_{i,j}$ (i,,j = 2, 3, 6), $c_3 = s_{2,3,6}$. See Table IV for s_i and $s_{i,j}$.

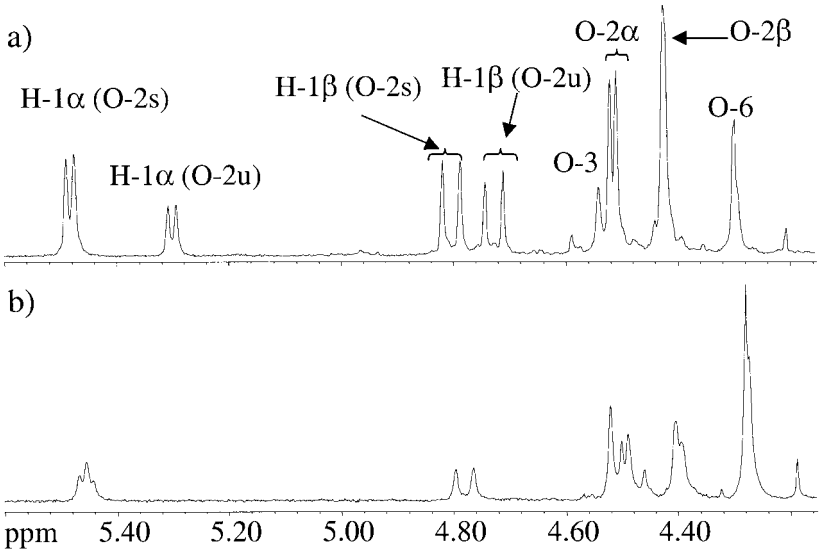

Figure 2 ^{1}H-NMR spectra of CMS samples after hydrolytic chain degradation: (a) sample 3 [DS_{CM} (determined by HPLC) = 0.79] and (b) sample 11 (DS_{CM} = 2.09).

The results are given in Table I for the total DS_{CM} values [calculations with eqs. (1) and (2)] and in Table III for a comparison of the partial DS values. The partial DS_{CM} value of position 2 was comparatively high in any case. With respect to the different methods of calculation that could be applied, it became obvious that samples 6–11, with rather high total DS_{CM} values, gave different results for the x_2 values. In the spectra of samples 6–11, no hydrogen signals of the unmodified position could be detected; this indicates that complete carboxymethylation of this position occurred. Consequently, x_2 was 1.0. However, the calculation of x_2 with eq. (1) gave slightly lower values for samples 6–11, reaching x_2 = 0.93 for sample 11. Noncarboxymethylated glucose was also detected for the higher substituted CMS by HPLC and CE (Table II), as later reported in more detail. This means that even at a total DS_{CM} value of 2.09 (determined with HPLC), some OH groups at C-2 were not carboxymethylated. This discrepancy was not found in the analysis of carboxymethyl cellulose of high DS_{CM} at all.[21] The values of x_3 were always the lowest ones independent of the total DS_{CM} value. However, with increasing DS_{CM}, the values for O-6 functionalization increased and reached a value of 0.91 (x_6) for sample 11, as expected. The high regioselectivity of 2-O-etherification was favored by a ratio of NaOH to AGU of only 1.27:1, where strongly preferred deprotonation of the most acidic hydroxyl group at position 2 took place. The latter was caused by the vicinity to the glucosidic

TABLE III
Values of the Partial Degree of Substitution (PDS) at Positions 2, 3, and 6 of the Repeating Unit of CMS Determined by ^{1}H-NMR Spectroscopy and CE after Depolymerization

	PDS at position x_i[a]					
	x_2		x_3		x_6	
CMS no.	^{1}H-NMR	CE	^{1}H-NMR	CE	^{1}H-NMR	CE
2	0.387[b]	0.300	0.048	0.041	0.134	0.027
3	0.602	0.531	0.093	0.057	0.172	0.061
4	—	0.782	—	0.078	—	0.147
5	0.862	0.866	0.139	0.115	0.404	0.253
6	0.887	0.893	0.204	0.159	0.490	0.347
7	0.851/1.00[c]	0.929	0.216	0.210	0.521	0.490
8	0.879/1.00	0.943	0.253	0.239	0.659	0.495
9	0.896/1.00	0.971	0.291	0.276	0.748	0.560
10	0.901/1.00	0.955	0.314	0.307	0.742	0.587
11	0.933/1.00	0.970	0.340	0.347	0.912	0.614

[a] $x_i = s_i + s_{i,j} + s_{i,j,k}$ (i,j,k = 2, 3, 6). See Table IV.
[b] Calculated with eq. (1).
[c] Calculated with eq. (2).

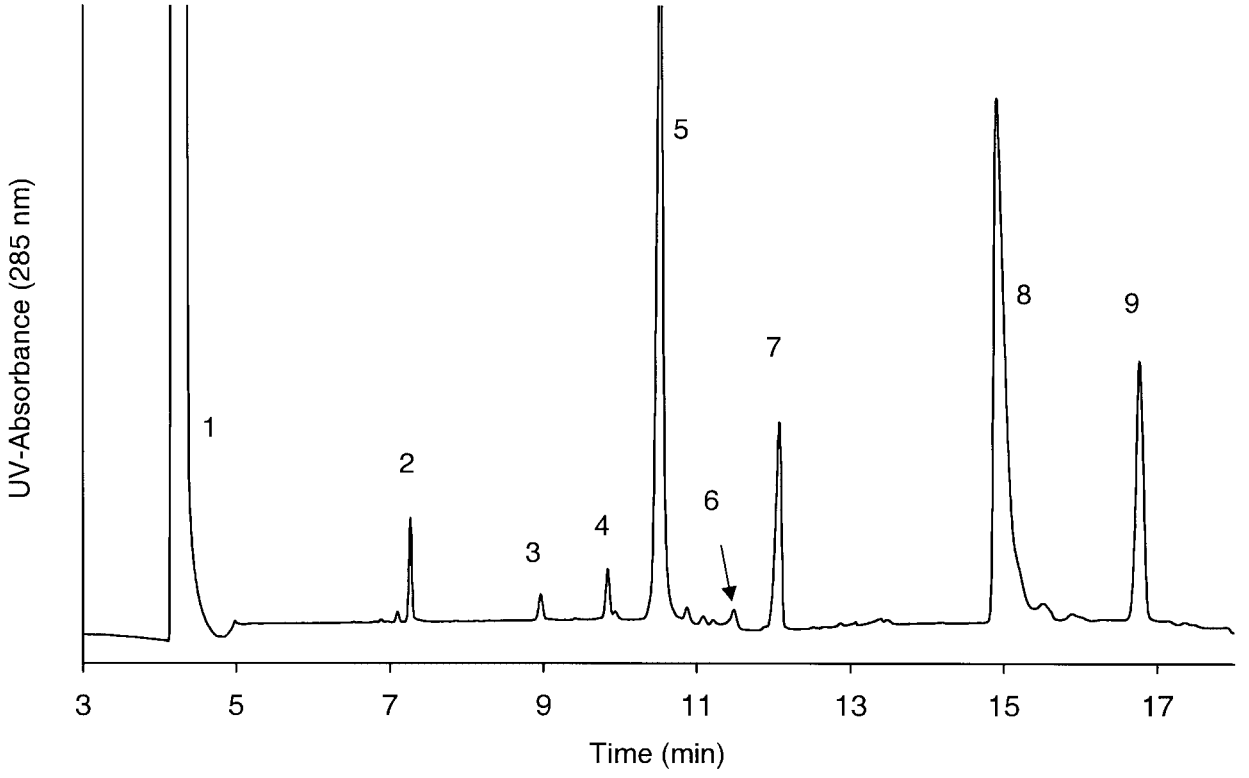

Figure 3 Electropherogram of CMS after hydrolysis and reductive amination with ABN: (1) ABN and (2–9) *N*-*p*-cyanophenyl-1-amino-1-deoxy-*O*-carboxymethyl (CM)-D-glucitols: (2) unsubstituted, (3) 3-*O*-CM, (4) 6-*O*-CM, (5) 2-*O*-CM, (6) 3,6-di-*O*-CM, (7) 2,3-di-*O*-CM, (8) 2,6-di-*O*-CM, and (9) 2,3,6-tri-*O*-CM.

carbon; it was obvious by comparison with the less regioselective reaction of cellulose that the α-configuration allowed the glucosidic O-1 to stabilize the sodium alcoholate at position 2.

Concerning the total DS_{CM}, which could be calculated from the ^{1}H-NMR data, there were differences in comparison with values obtained from HPLC (Table I). For the ^{1}H-NMR analysis of the x_6 value, it was important to use extensively purified samples because glycolate and diglycolate also gave a signal between 4.2 and 4.3 ppm, that is, in the range of the chemical shift of the CH_2 groups of O-6. In this work, samples without additional purification were used to exclude changes in sample composition. It is known that by dialysis a material of a high DS_{CM} value and a low molecular weight may be removed as well. Particularly after some carboxymethylation steps, a degradation of the polymer might be expected. Therefore, the x_6 values might be too high. By HPLC analysis, it was revealed that all the samples contained at least traces of glycolate and diglycolate.

The analysis of carbohydrates by CE is well established. Neutral and charged saccharides can be separated because carbohydrates readily react with borate to form anionic complexes.[22] For a chromophore to be introduced for UV detection, reductive amination was performed after the hydrolysis of CMS with ABN. Because the possibilities of forming borate complexes were reduced by the partial substitution of the glucose hydroxyl groups, a very effective separation of regioisomers became possible that was superior to the separation efficiency of the monosubstituted and disubstituted glucose ethers by gas chromatography. Figure 3 shows an electropherogram of the *N*-*p*-cyanophenyl-1-amino-1-deoxy-D-glucitol derivatives. Because of the counterelectroosmotic separation, the analytes with the highest charge/mass ratio were eluted last. Peaks were assigned by comparison with standards obtained from regioselectively substituted carboxymethyl celluloses and by comparison with HPAEC-PAD analysis.[23,24] The relative molar composition of the monomer mixture was calculated from the peak areas, which were corrected for their different migration times. For this quantitative evaluation, the following requirements had to be fulfilled: (1) hydrolysis had to yield a representative mixture of monomers, (2) reductive amination had to be quantitative or at least not discriminate any constituent, and (3) the molar extinction of the *N*-*p*-cyanophenyl-1-amino-1-deoxy-D-glucitols had to be determined by the chromophore only (as for all labeled analytes). To prove these requirements, we thoroughly investigated and optimized the hydrolysis step. A time course study of the reductive amination was recorded for the exclusion of kinetic discrimination, and complete transformation was proven by electrospray ionization mass spectrometry.

TABLE IV
Detailed Results of the Determination of the Molar Fractions of CMS by CE After Depolymerization

CMS no.	Molar fractions (%) of glucose (Glc) and modified Glc units at position $s_{i,j,k}$							
	Glc	S_2	S_3	S_6	$S_{2,3}$	$S_{2,6}$	$S_{3,6}$	$S_{2,3,6}$
2	65.16	28.16	3.21	1.60	0.81	0.99	0.05	0.02
3	41.68	46.92	3.22	1.89	2.13	3.85	0.14	0.18
4	17.69	61.03	2.32	1.54	4.26	11.95	0.29	0.93
5	9.33	56.78	2.25	1.47	6.37	20.90	0.33	2.57
6	6.68	48.73	2.18	1.43	7.73	27.24	0.40	5.60
7	3.90	37.35	0.98	1.73	8.74	35.97	0.47	10.85
8	2.19	37.58	2.03	0.91	8.68	35.43	0.59	12.60
9	1.35	33.61	0.43	0.65	8.65	36.76	0.49	18.06
10	3.96	29.12	0	0	8.19	36.22	0.54	21.97
11	1.16	28.48	0.75	0.55	8.19	35.09	0.52	25.26

Because no conjugation was possible between the sugar and the π-system of the tag, the absorption maximum and molar extinction should not have been influenced by the functionalization pattern of carboxymethyl groups. For example, the calibration curves of the corresponding derivatives of glucose and 3-*O*-methyl glucose were in full agreement. Calibration with CMglc derivatives is presently under investigation. The detailed development of the CE method will be reported elsewhere.

In Table IV, the molar fractions of the different repeating units obtained by CE, and in Table III x_i values calculated from the CE analysis and NMR spectra are listed. The relative molar amount of the constituents is s_i, where i is equal to the positions substituted and 0 means unsubstituted ($s_0 = c_0$, $s_2 + s_3 + s_6 = c_1$, etc.; $x_2 = s_2 + s_{23} + s_{26} + s_{236}$, etc.). The CE sensitivity was very high. Small amounts of glucose could be detected even for CMS 11 with the highest DS of about 2.1, which was in agreement with the results obtained by HPLC. The average DS values calculated from the composition of the repeating units were generally lower than those calculated by HPLC and especially compared with those determined by ^{1}H-NMR spectroscopy. In a comparison of the x_i values, it became obvious that a strong deviation referred to x_6, but there was relatively good agreement for x_2 and x_3. Therefore, the assumption was supported that the interference of glycolic acid in the ^{1}H-NMR analysis caused an overestimation of x_6. Comparing the molar fractions determined by means of HPLC and CE (Table II), we found a relative discrimination of the higher substituted fractions by CE or an overestimation by HPLC to be obvious. Whether this was caused by decreasing molar extinction with increasing DS was determined by calibration with authentic standards. The average DS_{CE} was about 92% of DS_{HPLC}, whereas DS_{NMR} was about 104% of DS_{HPLC}.

To check whether substitution of the AGU in a certain position influenced the reactivity of the other hydroxyl groups, we compared the experimental data with the model of Spurlin.[25] This statistical model assumes that during the functionalization, the relative reactivities of the three hydroxyl groups in the AGU are constant throughout the course of the reaction and are independent of the DS of the polysaccharide chain or of the state of functionalization at any other position within the same AGU. Terminal groups and branching points are not considered. Tetra-O-functionalized units can be neglected because they are formed to a very small extent only.

Because of the comparably high regioselectivity of starch etherification, these different reactivities could not be neglected. Calculations were performed with the CE data with the Spurlin model, which considered the different reactivities. From Figure 4, it is obvious that the data fit these calculations very well. Starting at a DS of about 1.6, a slightly increasing deviation was observed. However, this might be the effect of possible discrimination of the higher substituted analytes by CE, as discussed in comparison with HPLC. Looking into the composition of the repeating units in more detail, we found that the amount of 2- and 3-O-monosubstituted constituents was higher, whereas the ratio of 6-*O*-mono-CMglc was lower than calculated. At higher DS values, the fractions of unsubstituted and trisubstituted glucoses showed a positive deviation at the expense of disubstituted units. This might already indicate higher and lower substituted areas and, therefore, a slight heterogeneity in the polysaccharide chain.

According to the model of Spurlin, the etherification was assumed to be a first-order process. Relative rate constants were determined by a logarithmic plot of $(1 - x_i)$ against c_0, as described by Reuben and Conner.[26] This was done in Figure 5. A linear plot was obtained. From the slopes, relative rate constants, k_2:k_3:k_6 = 11.8:1.0:2.5, could be calculated. CMS 10 and 11 were not considered because the c_0 values were too small for reliable calculations.

No better fit of the experimental data was obtained by comparison with the calculation according to the

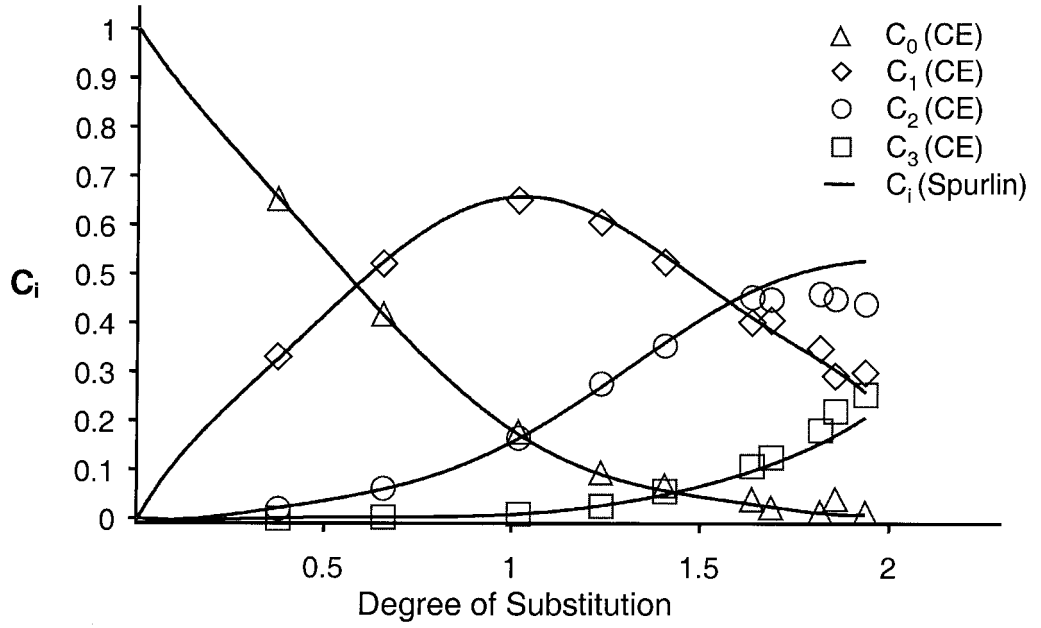

Figure 4 Comparison of experimental data for the molar fractions of unsubstituted (c_0), monosubstituted (c_1), disubstituted (c_2), and trisubstituted (c_3) AGUs from CE with those calculated by the model of Spurlin.

more refined model of Reuben,[27] which additionally considers an enhanced reactivity of O-3 if O-2 is functionalized. That means that no selective intramonomeric effect influenced the relative reactivities in an AGU during the carboxymethylation reaction of starch.

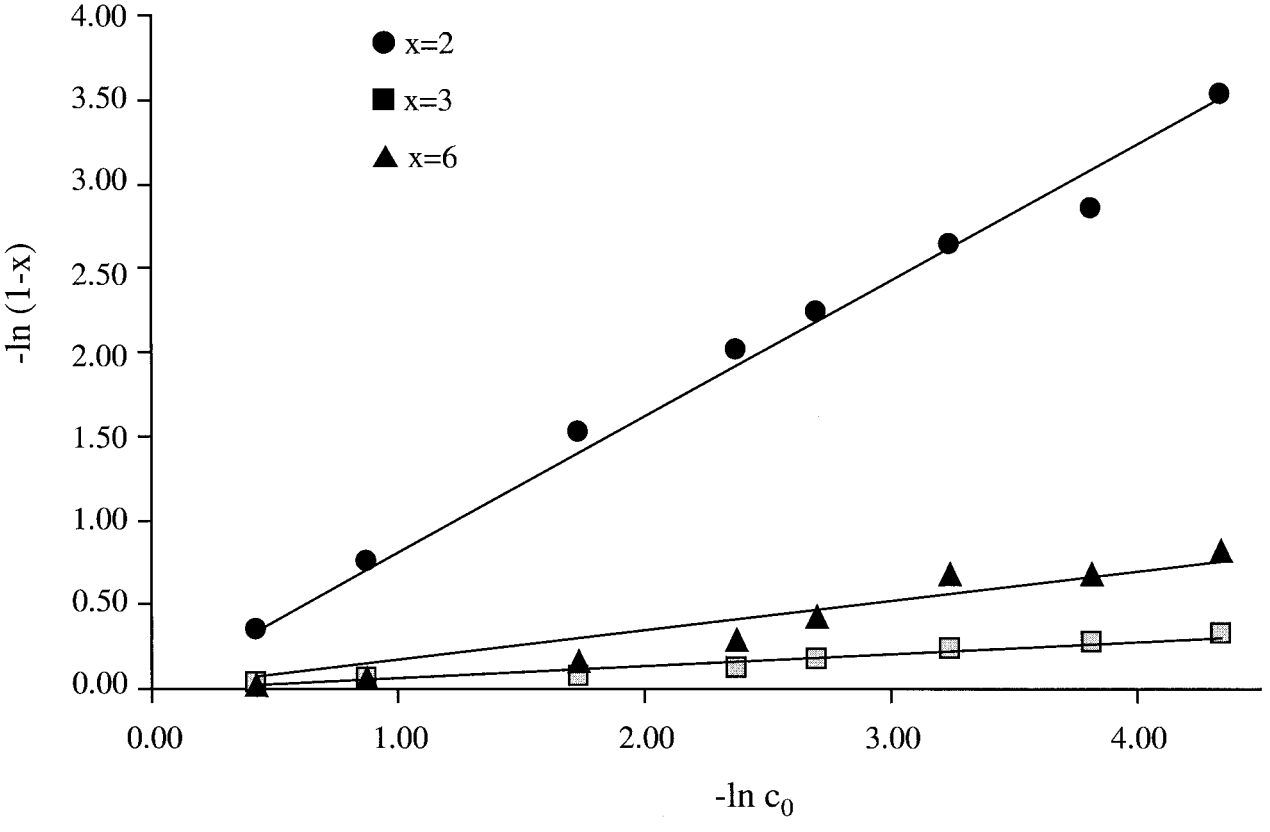

Figure 5 Logarithmic plots of the molar fractions of unsubstituted hydroxyl groups against the molar fraction of unsubstituted glucose. The slopes represent k_i, the first-order rate constants of carboxymethylation ($R^2 = 0.9901$, $i = 2$; $R^2 = 0.9025$, $i = 3$, and $R^2 = 0.9441$, $i = 6$).

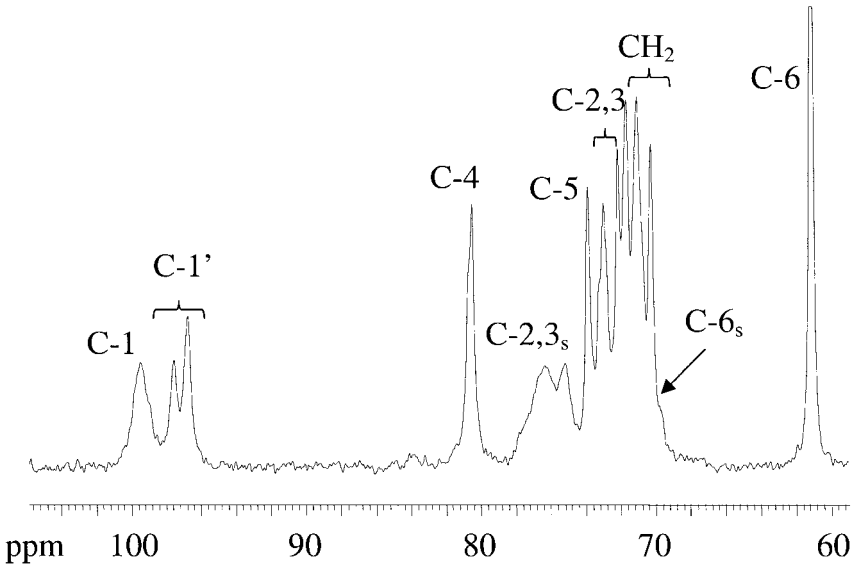

Figure 6 Standard ^{13}C-NMR spectrum of CMS sample 3 (DS_{CM} = 0.79) measured in D_2O at 60°C (100,000 scans were accumulated).

Characterization of the intact polymers by ^{13}C-NMR spectroscopy

Figure 6 shows a representative ^{13}C-NMR spectrum of CMS 3 with a DS_{CM} value of 0.79 measured in D_2O. A range from 60 to 110 ppm is shown, and 100,000 scans were accumulated. The peak for the unmodified C-6 position is visible at δ = 61.9 ppm. The high intensity of this peak agrees with the slight functionalization of O-6, as determined by means of ^{1}H-NMR spectroscopy. The signal for the carboxymethylated position 6 cannot be resolved but appears as a shoulder at $\delta \sim 70$ ppm. The signals at δ = 70.3, 71.1, and 71.7 ppm are assigned to the methylene carbon atoms of the carboxymethyl substituents. The carbon atoms of the unmodified positions 2 and 3 are found at δ = 72.2 and 73.0 ppm, and C-5 appears at δ = 73.9 ppm. The carboxymethylation of position 2 and 3 leads to a downfield shift of 2–3 ppm. The signal of C-4 is found at δ = 80.5 ppm. C-1 appears at δ = 99.5 ppm. Although the influence of O-3 carboxymethylation on C-4 cannot be seen (C-4′ does not appear), the carboxymethylation of O-2 can be clearly identified by additional signals with a high-field shift in the C-1 range (C-1′). However, it is unusual that two additional peaks at δ = 97.5 and 96.7 ppm appear. This behavior was found for all CMS samples with different relative intensities. For a homopolymer such as cellulose, just one signal appears independent of DS_{CM}. Obviously, in the CMS samples, 2-O-functionalized AGUs existed that influenced the signal of C-1 to a different extent. As is very well known, starch consists of two components, amylose and amylopectin. The chemical shift of the C-1′ of 2-O-carboxymethylated 1 $\rightarrow$ 4 and 1 $\rightarrow$ 6 linked AGUs might be different.

The signals of the carboxylate groups appear at δ = 181–185 ppm (Fig. 7). Both the intensity and number of signals increase with increasing DS_{CM}. Although the spectra of samples 2 and 3 show just one signal, the spectra of samples 4 and 5 possess an additional shoulder. At higher DS_{CM} values, three peaks clearly appear (samples 7–10). Because these signals are separated from the signals of the AGU, they can be used to gain information about the DS_{CM} as well.

CONCLUSIONS

The results clearly show that by multistep carboxymethylation of potato starch, products of a very high DS ($DS_{CM} \leq 2.1$) can be obtained that previously were not accessible. Detailed investigations of the distribution of the carboxymethyl groups by means of ^{1}H-NMR spectroscopy and CE have revealed that the reactivity of the hydroxyl groups of starch is in the order O-2 $\gg$ O-6 $>$ O-3. The relative molar compositions of the different functionalized repeating units, determined by means of CE and HPLC, well fit the Spurlin statistic. At high DS_{CM} values, even some 2,3,4,6-tetra-O-functionalized units form. From unexpected splitting of the C-1′ signal in the ^{13}C-NMR spectra, it unambiguously appears that the understanding of the functionalization pattern of CMS (and other starch derivatives) requires a more comprehensive structure analysis of the starch material with respect to the amylose/amylopectin content and the number and length of side chains and end groups.

To gain deeper insight into the molecular structure of CMS, samples will be synthesized from different starch materials, that is, with different amylose/amylopectin contents, including pure amylose and amylo-

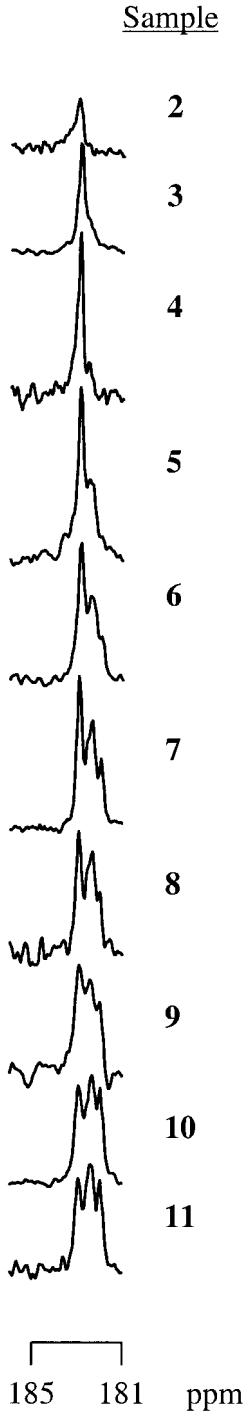

Figure 7 Carbonyl range of the ^{13}C-NMR spectra of CMS samples 2–11 for DS_{CM} = 0.41–2.09.

pectin, and will be included in this ongoing research project.

Moreover, the determination of the molecular weight and molecular weight distribution of the CMS samples with gel permeation chromatography and light scattering is under investigation. An evaluation of the effect of DS_{CM} on the properties is being made via rheology, and the results will be published elsewhere.

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