# Starch Derivatives with High Degree of Functionalization. III. Influence of Reaction Conditions and Starting Materials on Molecular Structure of Carboxymethyl Starch

TH. HEINZE,[1] K. PFEIFFER,[1] W. LAZIK[2]

[1] Friedrich Schiller University of Jena, Humboldtstrasse 10, D-07743 Jena, Germany

[2] CHP Carbohydrate Pirna GmbH & Co. KG, Lohmener Strasse 12, D-01796 Pirna, Germany

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**ABSTRACT:** Carboxymethyl starch (CMS) was prepared in a completely heterogeneous procedure in a methanol/water slurry activated with aqueous sodium hydroxide (45%, w/v) using monochloroacetic acid as the etherifying agent. The influence of the reaction conditions and the type of starting starch (amylose content and preactivation) was evaluated in regard to the formation of the main repeating units (i.e., unfunctionalized and mono-, di-, tri-, and tetra-O-carboxymethylated) and the pattern of functionalization within the anhydroglucose units (AGU). The reproducible synthesis gave products with a maximal degree of substitution of CM groups ($DS_{CM}$) of 0.66, which was reached in a one-step synthesis. Repeated carboxymethylation led to products with a $DS_{CM}$ of 0.88. As revealed by means of HPLC analysis after complete acidic depolymerization, in any sample the mono-O-carboxymethylated glucose (mono-O-CMglc) was preferably present while the di-O-CMglc was formed to a very low extent only. The tri-O-CMglc was found in some samples while tetra-O-CMglc was not detected. The mole fractions determined did not follow the simple Spurlin statistic as shown for CM cellulose synthesized under comparable conditions. Within the carboxymethylated AGUs a preferred functionalization at position 2 was analyzed by means of $^1$H-NMR spectroscopy after hydrolytic chain degradation. Consequently, the CMS samples synthesized contained mainly 2-mono-O-CM-AGU. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 81: 2036–2044, 2001

**Key words:** carboxymethyl starch; degree of functionalization; reaction conditions; pattern of functionalization

## INTRODUCTION

Carboxymethylation of polyglucans such as starch and cellulose is a versatile transformation because it provides access to water-swellable or water-soluble polymers and intermediates with various valuable features.[1–5] The properties (the viscosity of solutions, interaction with multivalent cations or polycations, and formation of supramolecular aggregates) are mainly determined by the degree of substitution (DS), which is the average number of carboxymethyl (CM) functions in the polymer. Moreover, the functionalization pattern may strongly influence the properties of the biopolymer derivatives.[6]

Some of the classical methods to determine the DS values are titration of the carboxy groups with

*Correspondence to:* Th. Heinze.

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perchloric acid,[7] direct flocculation titration,[8] or precipitation of the polymer with copper salts and backtitration of the excess Cu ions,[9] which gives the total DS only. In addition to these classical methods, effective approaches for the determination of the distribution of the functional groups within the single modified anhydroglucose unit (AGU) and in the polymer chain are an important prerequisite for the establishment of structure–property relationships and for the evaluation of the influence of reaction conditions on the product structure. The most common method for the determination of the substitution pattern is $^{1}$H- and $^{13}$C-NMR spectroscopy on the intact polymer or a hydrolytically depolymerized sample. These paths provide direct access to information on the substitution at the 2, 3, and 6 position of the repeating unit. Thus, it was shown by means of NMR spectroscopy that the industrially applied carboxymethylation of starch in alcoholic slurries like ethanol or isopropanol proceeds in the order O–2 > O–6 > O–3.[10,11] Alternative approaches for the determination of functionalization patterns consist of the time-consuming complete reduction of the CM units to hydroxyethyl groups, hydrolysis of the polymer, and GLC measurements.[12] A highly efficient method was developed for CM cellulose (CMC) in which HPLC was applied on completely hydrolyzed samples.[13] This allowed the determination of the repeating units that built up the chain, which were the mole fractions of unsubstituted and mono-, di-, or trifunctionalized AGUs. A comparison of the determined values with those of statistical calculations make it possible to gain even information about the distribution of the functional groups along the polymer chain. A combination of these results with $^{1}$H-NMR analyses on depolymerized samples gives a fairly complete picture of the functionalization pattern.[14–16] These investigations were directly related to ongoing projects on the search for alternative paths of polysaccharide etherification leading to derivatives with new patterns of functionalization. One possible way is the synthesis in reactive microstructures prepared via induced phase separation.[17,18]

In this article we report on our studies about the influence of selected reaction conditions on the degree of substitution of CM groups ($DS_{CM}$) and on the functionalization pattern of CM starch (CMS) obtained by a conventional heterogeneous slurry process. Because it is very well known that starch structure and composition vary according to the botanical source, the product structure and content of amylose and amylopectin (the main macromolecular components of starch) were consequently evaluated. In order to gain reliable information on the functionalization pattern, the characterization of the products had to be carried out with advanced analytical tools. In this work a broad variety of CMS samples were prepared and investigated by means of HPLC analysis and $^{1}$H-NMR spectroscopy after complete depolymerization of the polymer chains.

## EXPERIMENTAL

### Materials

Sodium hydroxide, monochloroacetic acid (MCA), perchloric acid, and sulfuric acid were purchased from Fluka. Technical grade (minimum 97%) methanol was used. The starch samples were superior potato starch 1 (Emsland-Stärke GmbH, Golßen, Germany), R6 (03453) corn starch 2, C*Gel 03402 waxy corn starch 3, C*AmyloGel 03011 amylocorn starch 4 (Cerestar GmbH, Krefeld, Germany), amylose KG pea starch 5 (Stauderer & Co., Altenmarkt, Germany), Remy BKA rice starch 6 (Remy Ind., Wijgmaal-Leuven, Belgium), tapioca starch 7 (Crespel & Deiters, Ibbenbüren, Germany), Emjel E70 potato starch 8, Emdex KS 1025 hydrolyzed potato starch 9, dextrin from Emdex MTW potato starch 10 (Emsland-Stärke GmbH, Emlichheim, Germany), and Zulkowsky starch 11 (Merck). The amylose content (see Table I) was given by the producers.

### Carboxymethylation of Starch

The synthesis of CMS samples was carried out based on the procedures of Heidrich and Ullmann[19] in which 425 g (2.62 mol) of potato starch 1 in 750 mL of methanol was stirred vigorously while 210 mL of 45% (w/v) aqueous sodium hydroxide was added during 15 min at room temperature. Stirring was continued for another 1.5 h at 40°C, and 150 g (1.59 mol) of MCA 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 methanol (0.86 g/mL density), and neutralized with acetic acid. Product 12 was collected after filtration, washed 3 times with methanol (0.86 g/mL density) and pure methanol, and dried at 110°C in a vacuum. The DS was 0.36 ± 0.03, which was determined by means of HPLC after

**Table I Conditions and Results of Carboxymethylation of Various Starch Materials Using Conventional Heterogeneous Synthesis Path and Reaction Temperature of 40 ± 2°C**

| Starting Starch Sample | | | | Reaction Conditions | | CMS | | |
|---|---|---|---|---|---|---|---|---|
| No. | Type | Amylose (%) | Pretreatment | Molar Ratio[a] | Time (h) | No. | $DS_{CM}$ | Efficiency (%) |
| 1 | Potato | 28 | Without | 0.622 | 6 | 12 | 0.36 | 57.8 |
| 1 | Potato | 28 | Without | 0.622 | 6 | 13 | 0.36 | 57.8 |
| 1 | Potato | 28 | Without | 0.622 | 6 | 14 | 0.35 | 56.2 |
| 1 | Potato | 28 | Without | 0.498 | 6 | 15 | 0.28 | 56.2 |
| 1 | Potato | 28 | Without | 0.747 | 6 | 16 | 0.47 | 62.9 |
| 1 | Potato | 28 | Without | 1.494 | 6 | 17 | 0.66 | 44.2 |
| 2 | Corn | 25 | Without | 0.622 | 6 | 18 | 0.33 | 53.0 |
| 3 | Waxy corn | 1 | Without | 0.622 | 6 | 19 | 0.33 | 53.0 |
| 4 | Amylocorn | 50 | Without | 0.622 | 6 | 20 | 0.36 | 57.8 |
| 5 | Pea | 90 | Without | 0.622 | 6 | 21 | 0.37 | 59.4 |
| 6 | Rice | 24 | Without | 0.622 | 6 | 22 | 0.18 | 28.9 |
| 7 | Tapioca | 26 | Without | 0.622 | 6 | 23 | 0.37 | 59.4 |
| 8 | Potato | — | Gelatinated | 0.622 | 6 | 24 | 0.29 | 46.6 |
| 9 | Potato | — | Hydrolyzed | 0.622 | 6 | 25 | 0.41 | 65.9 |
| 10 | Dextrin | — | From potato | 0.622 | 6 | 26 | 0.30 | 48.2 |
| 11 | Zulkowsky | — | 15,000 g/mol | 0.622 | 6 | 27 | 0.33 | 53.0 |
| 9 | Potato | — | Hydrolyzed | 0.622 | 4 | 28 | 0.23 | 37.0 |
| 1 | Potato | 28 | Without | 0.622 | 3 | 29 | 0.35 | 56.2 |
| 1 | Potato | 28 | Without | 1.245 | 3 | 30 | 0.31 | 24.9 |
| 16 | CMS | — | — | 0.763 | 6 | 31 | 0.88 | 53.8 |
| 14 | CMS | — | — | 0.743 | 6 | 32 | 0.84 | 65.9 |
| 14 | CMS | — | — | 0.739 | 6 | 33 | 0.85 | 67.6 |

CMS, carboxymethyl starch; $DS_{CM}$, the degree of substitution of carboxymethyl groups determined by HPLC after complete depolymerization.

[a] Moles of monochloroacetic acid per mole of anhydroglucose units.

complete chain degradation. FTIR spectroscopy in KBr yielded wavelengths of 1607 and 1416 $cm^{-1}$ for $\nu$COONa.

### Measurements

A HPLC system was used that consisted of a Jasco degasser, a Knauer HPLC 64 pump, a Knauer differential refractometer, a Jasco OR 990 chiral detector, and a Jasco jet stream column heater. A Knauer autosampler was used to inject 50 $\mu$L of the sample into two coupled Aminex HPX 87 H columns (BioRad Laboratories) for separation. The column temperature was 65°C. Sulfuric acid (0.01*N*) at a flow rate of 0.5 mL/min was used as the mobile phase. The chromatographic data were evaluated by means of Borwin HPLC software (Jasco).

The $^1$H-NMR analyses were carried out as previously described.[14,16] For this purpose the CMS samples were hydrolyzed with a mixture of $D_2SO_4/D_2O$ (25% v/v) within 5 h at 90°C. The spectra were acquired on a Bruker AMX 400 spectrometer.

### Sample Hydrolysis for HPLC Measurements

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

## RESULTS AND DISCUSSION

### Analytical Tools for CMS

As mentioned in the Introduction, there are several possible ways to determine the $DS_{CM}$ and the

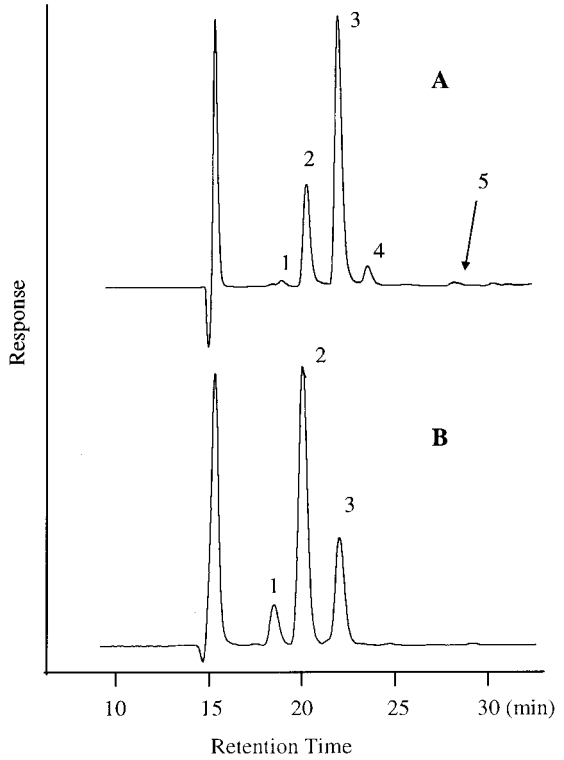



**Figure 1** The HPLC analysis of carboxymethyl starch after complete acidic depolymerization: sample 30 with a degree of substitution ($DS_{CM}$) of 0.31 (curve A) and sample 32 with a $DS_{CM}$ of 0.84 (curve B; see Table I); refraction index detection. Peak assignments: peak 1: 2,3-, 2,6-, and 3,6-di-O-carboxymethylglucose (CMglc); peak 2: 2-, 3-, and 6-mono-O-CMglc; peak 3: glucose; peak 4: diglycolic acid; peak 5: glycolic acid.

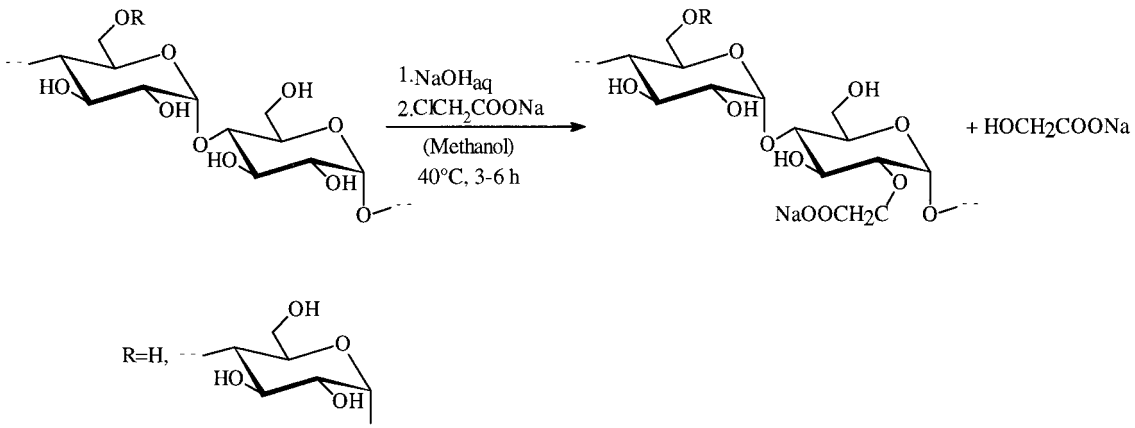
functionalization pattern of CMS.[7–14] According to our experience, it is important to apply only one method to compare different CMS samples.[20] We therefore used a HPLC method that was developed in our lab. This rapid and convenient procedure yields the $DS_{CM}$ and the mole fractions of the differently functionalized repeating units [i.e., unmodified glucose; 2-, 3-, and 6-mono-O-carboxymethylated glucoses (CMglc); the group of 2,3-, 2,6-, and 3,6-di-O-CMglc; and 2,3,6-tri-O-CMglc.[16] The 2,3,4,6-tetra-O-CMglc was not detected. Prior to the HPLC analysis a complete acidic depolymerization with perchloric acid had to be carried out. Typical chromatograms including the assignment of the peaks are shown in Figure 1 (samples 30 and 32, see Table I). Separate signals for glc, mono-O–CMglc, and di-O–CMglc were detected. No chiral components with retention times shorter than 15 min were observed, as would be expected for oligomeric fractions. That means the additional chiral detection (curve not shown) was very useful in proving the complete degradation of the polymer chains. Additional signals were determined at 24.7 and 29.5 min, which corresponded to impurities consisting of hydrolysis products of sodium monochloroacetate (see Scheme 1).

Moreover, some selected samples were investigated by means of $^1$H-NMR spectroscopy after partial chain degradation with sulfuric acid, which yielded the $DS_{CM}$ as well. In addition, the distribution of the CM groups within the single repeating unit could be evaluated.[16] The spectra of samples 19 and 32 and the assignment of the signals is shown in Figure 2.

### Influence of Reaction Conditions and Starch Material on $DS_{CM}$

Activation of the polymer is absolutely necessary in order to obtain a sufficient conversion by etherification of polysaccharides. It is common to use aqueous sodium hydroxide solutions to activate the starch samples and initiate the carboxymethylation reaction. Depending on the lye concentration, the polymers swell to various extents. According to our experience under heterogeneous reaction conditions using a slurry of starch in methanol, a treatment with 45% (w/v) aqueous NaOH under other experimental conditions (i.e., the ratio of starch to methanol and water and the temperature during activation) led to an even activation of the whole material, meaning that an even accessibility of all hydroxyl groups was guaranteed.[19] After activation the polymer was allowed to react with MCA, yielding CMS. Moreover, the reaction of sodium hydroxide and MCA produced sodium glycolate as a by-product; thereby, the reaction efficiency decreased in regard to the total $DS_{CM}$ reached (Scheme 1). The reaction conditions used guaranteed that the reaction mixture could be mixed during the whole course of the reaction without complications and that no gelatinization occurred. Compared to the carboxymethylation of other polysaccharides, the reaction temperature for starch must be low enough to prevent gelatinization during the process. Therefore, the carboxymethylation reactions were carried out at 40 ± 2°C and no gelatinization was observed under the experimental conditions used.

Although the ratio of starch, methanol, and aqueous sodium hydroxide (water) was kept constant, the amount of MCA was varied to reach different $DS_{CM}$ values (see Table I).




**Scheme 1** The reaction scheme of the carboxymethylation of starch.

After the activation described, a CMS sample with a $DS_{CM}$ of 0.36 obtained by reacting potato starch with 0.622 mol MCA/mol AGU (sample 12, Table I). The reproducibility of the synthesis path was determined. The values of $DS_{CM}$ obtained under comparable conditions were equal (e.g., samples 12–14, Table I). When a higher molar ratio of 0.747 or 1.494 mol MCA/mol AGU was used, products samples 16 and 17 with higher $DS_{CM}$ values of 0.47 and 0.66 were obtained as expected. However, by the last example the efficiency of the reaction decreased to less than 50%. On the other hand, a reduction of the molar ratio to 0.498 mol MCA/mol AGU yielded a product (sample 15) with a lower $DS_{CM}$ (0.28).

In order to obtain CMS samples of high $DS_{CM}$ values it was more appropriate to use previously carboxymethylated products and repeat the reaction. In this case, $DS_{CM}$ values of up to 0.88 (samples 31–33) could be reached using the reaction conditions applied for unmodified starch.

Because one objective of our studies was to gain more insight into factors that affect the carboxymethylation reaction, different starch materials were investigated in a series of experiments. In comparing the different starch samples it became obvious that samples of the same total $DS_{CM}$ (0.33–0.37) and independent of the starch type were obtained (CMS 18–21, 23, Table I). This was also found for pretreated starch samples 10 and 11, which led to products 26 and 27 with $DS_{CM}$ values of 0.30 and 0.33, respectively. On the other hand, hydrolyzed potato starch 9 led to a CMS (sample 25) with a slightly higher $DS_{CM}$ (0.41) after a reaction time of 6 h. As expected after a 4-h reaction time, material 9 led to product 28 with a $DS_{CM}$ of only 0.23. There were only two significant exceptions: rice starch could be converted to a CMS (sample 22) with a $DS_{CM}$ of only 0.18, and gelatinated potato starch led to CMS 24 with a low $DS_{CM}$ of 0.29.

An increase of the molar ratio to 1.245 mol/mol in a one-step synthesis was not appropriate to increase the $DS_{CM}$ values. The reaction efficiency decreased drastically (sample 30). In potato starch 1 the reaction time of 3–6 h influenced the reached $DS_{CM}$ values to a minor extent only as indicated by sample 29 compared with samples 12–14.

## Functionalization Pattern Analyzed by HPLC

The mole fractions of the unmodified glucose (glc) and the three differently substituted CMglc determined by the HPLC procedure are listed in Table II. It was obvious that with increasing $DS_{CM}$ the mole fractions of glc decreased while the mole fractions of mono- and di-O-CMglc increased, dominated by monofunctionalization. In some samples traces of tri-O-CMglc were found while tetra-O-CMglc was not detected.

A very useful tool for the elucidation of the pattern of functionalization is the comparison of data acquired by HPLC with the values calculated by statistics. The statistic model applied assumed that during the functionalization no preference of any hydroxyl groups existed and the relative reactivity of the three hydroxyl groups in the AGU were constant throughout the reaction and independent of the DS of the polysaccharide chain or the state of functionalization at another position within the same AGU. This meant that all OH functions of the polysaccharide were evenly accessible during the whole synthesis step.

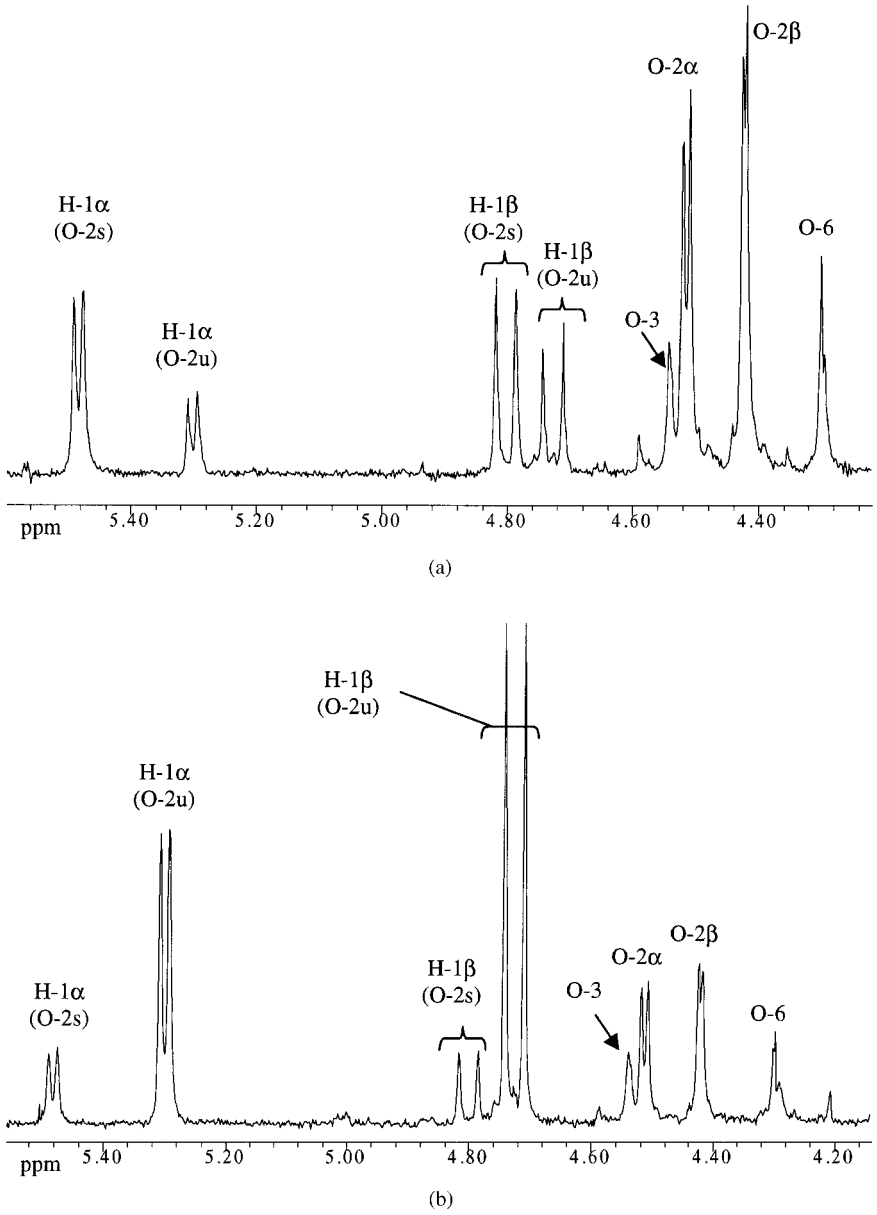



**Figure 2** $^{1}$H-NMR spectra and peak assignments of two selected carboxymethyl starch samples after hydrolytic chain degradation: (a) sample 32 with a degree of substitution ($DS_{CM}$) of 0.84 and (b) sample 19 with a $DS_{CM}$ 0.33 (see Table II).

A binomial distribution first applied to CMC by Spurlin[22] and Reuben and Conner[23] is as follows:

$$C_i = \left( \begin{array}{c} 3 \\ k \end{array} \right) (\mathrm{DS}/3)^k (1 - \mathrm{DS}/3)^{3-k} \qquad (1)$$

where $c_i$ are the mole fractions of unsubstituted, mono-, di-, and tri-O-substituted glc; $k$ is the number of substituents per AGU ($k$ = 0, 1, 2, 3); and DS is the average DS.

The data did not fit these calculations (see values in parentheses in Table II). There was always a lower content of glc, di-O-CMglc, and tri-O-CMglc while the amount of mono-O-CMglc was higher compared to the statistical calculations. This was in contrast to results obtained with heterogeneously synthesized CMC, which fit the statistic calculations exactly.[13,15,21,23] In our work dealing with CMS of different functionalization patterns[16,24] we got the impression that hetero-

**Table II Results of HPLC Analysis of Carboxymethyl Starch (CMS) Samples after Hydrolytic Chain Degradation**

| CMS | | Mole Fractions[a] | | | |
|---|---|---|---|---|---|
| No.[b] | $DS_{CM}$ | glc | Mono-CMglc | Di-CMglc | Tri-CMglc |
| 12 | 0.36 | 0.657 | 0.329 | 0.014 | — |
| | | (0.681) | (0.279) | (0.038) | (0.002) |
| 13 | 0.36 | 0.658 | 0.327 | 0.015 | — |
| | | (0.681) | (0.279) | (0.038) | (0.002) |
| 14 | 0.35 | 0.662 | 0.325 | 0.013 | — |
| | | (0.689) | (0.273) | (0.036) | (0.002) |
| 15 | 0.28 | 0.732 | 0.255 | 0.012 | — |
| | | (0.745) | (0.230) | (0.024) | (0.001) |
| 16 | 0.47 | 0.560 | 0.410 | 0.030 | — |
| | | (0.586) | (0.343) | (0.067) | (0.004) |
| 17 | 0.66 | 0.413 | 0.506 | 0.076 | 0.005 |
| | | (0.468) | (0.404) | (0.116) | (0.011) |
| 18 | 0.33 | 0.693 | 0.289 | 0.018 | — |
| | | (0.705) | (0.261) | (0.032) | (0.001) |
| 19 | 0.33 | 0.687 | 0.293 | 0.019 | — |
| | | (0.705) | (0.261) | (0.032) | (0.001) |
| 20 | 0.36 | 0.658 | 0.325 | 0.017 | — |
| | | (0.681) | (0.279) | (0.038) | (0.002) |
| 21 | 0.37 | 0.646 | 0.336 | 0.018 | — |
| | | (0.674) | (0.284) | (0.040) | (0.002) |
| 22 | 0.18 | 0.821 | 0.174 | 0.005 | — |
| | | (0.831) | (0.159) | (0.010) | (0.0002) |
| 23 | 0.37 | 0.650 | 0.332 | 0.017 | — |
| | | (0.674) | (0.284) | (0.040) | (0.002) |
| 24 | 0.29 | 0.720 | 0.271 | 0.009 | — |
| | | (0.737) | (0.237) | (0.025) | (0.001) |
| 25 | 0.41 | 0.604 | 0.380 | 0.014 | — |
| | | (0.643) | (0.306) | (0.048) | (0.003) |
| 26 | 0.30 | 0.715 | 0.270 | 0.015 | — |
| | | (0.729) | (0.243) | (0.027) | (0.001) |
| 27 | 0.33 | 0.680 | 0.307 | 0.013 | — |
| | | (0.705) | (0.261) | (0.032) | (0.001) |
| 28 | 0.23 | 0.779 | 0.211 | 0.011 | — |
| | | (0.787) | (0.196) | (0.016) | (0.0004) |
| 29 | 0.35 | 0.655 | 0.325 | 0.015 | — |
| | | (0.689) | (0.273) | (0.036) | (0.002) |
| 30 | 0.31 | 0.704 | 0.278 | 0.017 | — |
| | | (0.721) | (0.249) | (0.029) | (0.001) |
| 31 | 0.88 | 0.239 | 0.631 | 0.123 | 0.007 |
| | | (0.343) | (0.441) | (0.189) | (0.027) |
| 32 | 0.84 | 0.252 | 0.657 | 0.090 | — |
| | | (0.373) | (0.435) | (0.169) | (0.022) |
| 33 | 0.85 | 0.255 | 0.637 | 0.108 | — |
| | | (0.368) | (0.437) | (0.173) | (0.023) |

$DS_{CM}$, the degree of substitution of carboxymethyl groups. The values in parentheses were calculated by statistics (see text).
[a] The mole fraction of glucose (glc) and carboxymethyl glucose (CMglc).
[b] See Table I.

geneously synthesized CMS was also built up from statistical amounts of the different repeating units. We also studied the commercially available CMS product VIVASTAR®, which possesses a highly reproducible molecular structure and consequently identical properties. However, in

our previous study only four samples were investigated and the slight deviations of the mole fractions from the statistics were not estimated.[16] Because of the branched structure of starch (especially samples with a high content of amylopectin), there was a significant amount of repeating units with a reactive OH group at position 4 of the AGU. Consequently, a higher content of mono- and di-O-CMglc units may occur. In order to evaluate this issue a more comprehensive statistic has to be applied that considers the total structure analysis of starch; the ratio of amylose/amylopectin and the number and length of branches, including the end groups, also have to be determined. This is the subject of further studies that will include reactions with pure amylose and amylopectin.

The deviation of the mole fraction measured from the calculated values was especially high for samples that were obtained by subsequent carboxymethylation of CMS (31–33). The preferred formation of mono-O-functionalized units obviously occurred.

### Distribution of CM Groups within AGU

According to our experience and apparatus available, we chose $^1$H-NMR spectroscopy, which is also a rapid method, for the determination of the distribution of the functional groups within the AGU. Representative spectra including the assignment of the peaks are shown in Figure 2. The partial $DS_{CM}$ ($x_i$) values were determined according to eq. (2).

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

$$DS = \sum_i x_i$$

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

where $A$ represents the peak area; O is the oxygen atom at position $i$ ($i$ = 2, 3, 6); H-1 is the hydrogen atom at the anomeric C; $\alpha$,$\beta$ is the configuration of glucose; $s$ stands for substituted; and $u$ stands for unsubstituted. A functionalization at position 4 was not included because the corresponding signal was not found. Moreover, as already discussed, no tetra-O-CMglc appeared. Even in case of samples with a high $DS_{CM}$ of 1.6, the content of tetrafunctionalized units was only 0.0159.[16] Consequently, the partial $DS_{CM}$ at O-4 was 0.0039. This was below the detection sensitivity limit of the NMR method.

**Table III Results of $^1$H-NMR Spectroscopy of Selected Carboxymethyl Starch (CMS) Samples**

| | $^1$H-NMR Analysis | | | |
|---|---|---|---|---|
| | | Partial DS at Position | | |
| CMS No.[a] | $DS_{CM}$ | 2 | 3 | 6 |
| 13 | 0.41 | 0.269 | 0.051 | 0.086 |
| 19 | 0.31 | 0.218 | 0.045 | 0.051 |
| 20 | 0.39 | 0.272 | 0.056 | 0.066 |
| 26 | 0.32 | 0.212 | 0.041 | 0.066 |
| 27 | 0.39 | 0.251 | 0.054 | 0.080 |
| 29 | 0.38 | 0.290 | 0.024 | 0.068 |
| 30 | 0.34 | 0.234 | 0.042 | 0.065 |
| 32 | 0.90 | 0.643 | 0.100 | 0.157 |

$DS_{CM}$, the degree of substitution of carboxymethyl groups.
[a] See Table I.

The results are summarized in Table III. In any case, the OH groups at C-3 possessed the lowest reactivity. On one hand the $x_3$ was the lowest value independent of the total DS and the reaction conditions applied. On the other hand the values for O-6 were only slightly higher. This was in contrast to CMC where an almost equal functionalization of positions 2 and 6 was found. A significantly preferred reaction at O-2 occurred in the CMS.

Concerning the total $DS_{CM}$, which could be calculated from the $^1$H-NMR data as well, there was obviously very good agreement with the values obtained from HPLC. However, for the $^1$H-NMR analysis it was important to purify the samples (e.g., by dialysis) to remove glycolate and diglycolate, which also give a signal between 4.2 and 4.3 ppm, which was in the range of the chemical shift of the $CH_2$ groups of O-6.[25]

## CONCLUSIONS

A heterogeneous carboxymethylation of starch using methanol/water as the slurry medium, aqueous sodium hydroxide solution for activation, and MCA as an etherifying agent was applied to

obtain a CMS with a reproducible $DS_{CM}$ of 0.36 and a reaction efficiency of about 60%. It was even possible to synthesize a CMS with a $DS_{CM}$ of 0.66; however, the efficiency was less than 50% in a one-step procedure. A two-step carboxymethylation was applied to produce products with a $DS_{CM}$ of up to 0.88 under comparable conditions. The reaction was applied to starch materials with different contents of amylose, leading to products of nearly equal $DS_{CM}$ values. There were only two significant exceptions: rice starch and hydrolyzed potato starch. These observations cannot be explained with the present results.

The determination of the mole fractions of the differently carboxymethylated anhydroglucose repeating units was carried out by means of HPLC after complete hydrolytic depolymerization of the CMS samples. In any product the mono-O-CMglc represented the dominant building unit. Moreover, the synthesized samples contained small amounts of di-O-CMglc; traces of tri-O-CMglc were found in only some of the samples. The tetra-O-CMglc was not present at all. The mole fractions determined did not follow the simple Spurlin statistics; however, they appeared with high reproducibility.

Some samples were included in studies of the determination of the functionalization pattern within the repeating units using $^1$H-NMR analysis after acidic chain degradation. The carboxymethylation preferably occurred at position 2 within the AGU. Consequently, the main repeating units of the CMS samples (up to a $DS_{CM}$ of 0.9) were 2-mono-O-CMglc and glucose. These results made it obvious that starch behaved differently than cellulose in carboxymethylation reactions.

The synthesis of CMS of high $DS_{CM}$ (up to a complete functionalization) was studied by using different reaction conditions including homogeneous procedures, as well as by a multistep path. A future topic of our work deals with the complete structure determination of starch and the development of an appropriate statistic model.

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