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Carbohydrate Research 271 (1995) 101–112

CARBOHYDRATE RESEARCH

# Carboxymethylation of inulin

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Received 3 May 1994; accepted 19 November 1994

**Abstract**

Inulin was carboxymethylated in aqueous alkaline medium with monochloroacetic acid as the reagent. The degree of substitution of the reaction product was determined by titration, LC analysis and $^{13}C$ NMR spectroscopy. Carboxymethylinulin with a degree of substitution between 0.2 and 1 was obtained depending on the molar ratio of inulin–monochloroacetic acid. Increasing the concentration of the reaction mixture and lowering the reaction temperature resulted in higher selectivities towards carboxymethylinulin. Determination of the molecular weight distribution was performed by GPC and by multi-angle laser light scattering. Carboxymethylation caused little or no degradation of the chain length of the starting material.

*Keywords:* Fructan; Polyelectrolyte; Monochloroacetate; $^{13}C$ NMR spectroscopy; GPC

## 1. Introduction

Inulin, a (2 → 1)-β-D-fructan with a D-glucose unit at the reducing end (Scheme 1), can be found as a reserve polysaccharide in various plants such as chicory, Jerusalem artichoke and dahlia. The average degree of polymerization (dp) varies from 5 to 30 depending on the plant origin. Recently, inulin became commercially available.

Besides the obvious use of inulin as such or as a source for D-fructose, there is a need of synthetic methods for the conversion of inulin into other useful products. A possible method, carboxymethylation, is now reported.

Carboxymethylation is a well known derivatisation process for polysaccharides, giving products in which primary and/or secondary alcohol groups are etherified with carboxymethyl groups. The derivative obtained is a polyelectrolyte and can be applied in

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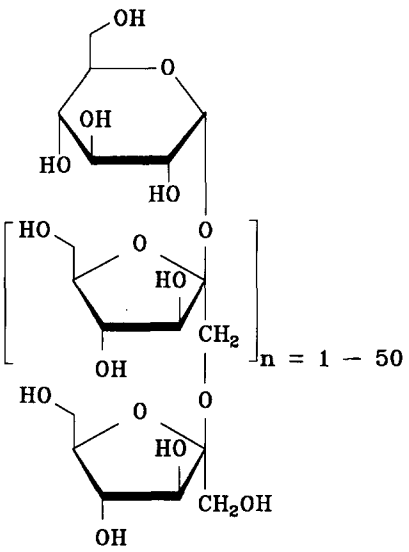
*SSDI* 0008-6215(95)00028-3




Scheme 1. Inulin.

a wide variety of fields, e.g. as dispersing agent or as metal ion carrier. Other advantages of carboxymethylation are the ease of processing, the low cost of the chemicals and the non-toxicity of the products. An example is carboxymethylcellulose (CMC), which was described for the first time by Jansen (1920) [1,2] and which is an important industrial material at present. The estimated world production in 1991 was about 300,000 tons per year. CMC is used as anti-redeposition agent in detergents (26%), in the oil (20%), paper (18%), textile (6%) and mining industry (5%), as thickener in foods and in pharmaceutical preparations (17%) [3]. Besides cellulose, other polysaccharides such as starch [4,5], guar [6] and mono- and di-saccharides such as sucrose, lactose, D-galactose and D-glucose [7–10] have been carboxymethylated.

## 2. Experimental

*Materials.*—Two types of inulin were used. The first type (type I), with an average dp = 30, was obtained from E. Merck (Darmstadt, Germany). The second type (type II), isolated from chicory root, was a gift from Suiker Unie (Roosendaal, The Netherlands) and is commercially available under the name "Fibruline". The average dp is 10.

The dp of inulin was established by LC analysis [column: Dionex Carbopac PA1; detection: Dionex PED 1 pulsed electrochemical detector; eluent: a solvent gradient starting with 70% solvent A (0.1 M NaOH) and 30% solvent B (0.1 M NaOH, 0.5 M NaOAc) and ending with 100% solvent B]. While type I inulin is essentially monosaccharide-free, type II inulin contains 5.4% of monosaccharides (D-glucose and D-fructose) and 4.5% of sucrose.

*Carboxymethylation.—Method (a).* In a 100-mL round-bottom vessel, inulin (3.4 g, 20 mmol monomeric units) was dissolved in water (25 mL). To this solution,

monochloroacetic acid (MCA) and NaOH were added in a molar ratio of 1:2.0 to 1:2.1. The solution was heated to the reaction temperature and was stirred magnetically for 5 h. After cooling, the reaction mixture was neutralised by addition of a 2 M HCl-solution. The required amount of HCl corresponded to the excess of NaOH at the beginning of the reaction (0 to 0.1 equiv.).

*Method (b).* Inulin (410 g, 2.5 moles) was mixed with water until a kneadable paste was formed. A 50% NaOH solution (1 mole) was added and the mixture was kneaded for 1 h. After that the sodium salt of MCA (117 g, 1 mole) was mixed thoroughly with the paste. The mixture was heated at 70° C during 3 h. A syrupy product was obtained.

*Isolation of the product.*—The neutralised reaction mixture was concentrated under reduced pressure to a vol of about 10 mL. The resulting mixture was poured into 100 mL well-stirred absolute MeOH. The sodium salt of carboxymethylinulin (CMI) precipitated as a white solid. Sodium glycolate, formed by hydrolysis of MCA, remained in soln. The residue was filtered off, washed with absolute MeOH and dried under reduced pressure. Finally, NaCl and traces of glycolate and MeOH were removed by membrane filtration (UTC 60, Toray Industries, Inc., Tokyo, Japan) at a pressure of 20 bar. The solution was freeze-dried to yield pure (> 95%) CMI. The product had a slightly brown color.

*Determination of the degree of substitution (ds).*—The ds of CMI was determined by titration of the carboxylic acid groups, by LC analysis and by $^{13}C$ NMR spectroscopy.

For the titration of the carboxylic acid groups, the sodium salt of CMI was converted into the acid form by treatment with an acidic cation exchange resin (Dowex 50X8–100, $H^+$) and subsequent freeze-drying. A portion of the carboxymethylated product (100 mg) was dissolved in 20 mL of distilled water. The carboxylic acid content was then determined by back-titration with 0.1 N HCl after addition of a known amount of NaOH.

In addition, LC analysis was used for the determination of the ds of CMI. To that end, CMI was hydrolysed to monosaccharides by heating (70° C) in aqs soln at pH 1.5 for 1 h. After neutralization, the solution was analysed by LC [column: Phenomenex (Bester, Amstelveen, the Netherlands), Rezex Organic Acid, 300 × 7.8 mm; eluent: 0.01 M trifluoroacetic acid; 60° C; flow rate: 0.6 mL/min; RI and $UV_{215}$ detection]. The products were identified by LC-MS analysis using the same column and conditions. In this case, the LC system was coupled to a VG 70-SE mass spectrometer. Monosubstituted, disubstituted and non-substituted monosaccharides were found. The carboxylic acid content of the product was calculated from the integrals of the peaks.

The last technique used for determination of the ds was $^{13}C$ NMR spectroscopy. The spectra were recorded on a Varian VXR-400 S spectrometer using $D_2O$ as solvent and *tert*-butanol as internal standard. $^{13}C$ NMR spectra were measured quantitatively (relaxation delay: 30 s.; pulse angle: 45° ; decoupler on during acquisition). The carboxylic acid content was calculated from the peak integrals of carboxylic acid groups ($\delta$ 179 ppm) relative to those of C-2 of inulin ($\delta$ 104 ppm).

*Gel-permeation chromatography (GPC).*—The molecular weight distribution of inulin and CMI was determined by GPC on a Bio-Gel P-6 (Bio-Rad) column, using the following conditions: column diameter: 0.8 cm; column length: 65 cm; eluent: 0.02 M $NH_4HCO_3$; flow rate: 6 mL/h; sample: 100 mg/0.5 mL; detection: RI. Fractions of 3

mL were collected with an automatic fraction collector. The fractions containing organic material were combined, hydrolysed and analysed by LC as described above. The column was calibrated by fractionation of D-fructose, nystose ($GF_3$) and inulin with an average dp = 30 ($GF_n$).

*GPC coupled with multi-angle laser light scattering.*—A high-performance GPC system (GPC 150 C, Waters) was coupled with a multi-angle laser light scattering (m.a.l.l.s.) detector (Dawn-DSP-F, Wyatt Technology). A refractometer was used as a concentration-sensitive detector. The samples were chromatographed on two GPC-columns coupled in series (TSKgel G3000$PW_{XL}$ and TSKgel G5000$PW_{XL}$) using 0.1 M $NaNO_3$ (1 mL/min) as eluent. The specific refractive increment ($dn/dc$) was determined on an interferometric refractometer (Optilab, Wyatt Technology) equipped with a 633 nm filter to isolate the wavelength produced by the laser used in the light scattering detector. For inulin and its carboxymethyl derivatives, $dn/dc = 0.131$ mL/g.

## 3. Results and discussion

*Carboxymethylation of inulin.*—Inulin (type I) was carboxymethylated by heating an aqueous solution at 95° C for 5 h with monochloroacetic acid (MCA) and sodium hydroxide. The molar ratio MCA-inulin was varied between 0.5:1 and 4:1. The degree of substitution of the isolated reaction products was determined by titration, LC-analysis and $^{13}C$ NMR. The results are compiled in Table 1.

Since the results obtained by the three analytical techniques agree well, it can be concluded that all are suitable methods for determining the ds of CMI. Under the conditions employed, reaction products with a ds of 0.21 to 1.05 were obtained.

The distribution of the substituents was determined by LC-analysis, separating the group of monosubstituted monosaccharides (3 regioisomers) from the disubstituted (3 isomers) and the non-substituted monosaccharides. An example of a LC-chromatogram is given in Fig. 1. Table 2 shows the distribution of the substituents for the various products.

The product with the lowest ds (ds 0.20) still contains disubstituted monomers, while the product with ds 1.05 still contains 25% of non-substituted monosaccharide residues. When products with increasing ds were prepared, the increase in proportion of disubsti-

Table 1
The ds of CMI as determined by titration, LC-analysis and $^{13}C$ NMR spectroscopy. Starting materials: inulin (Type I, 20 mmol), MCA and NaOH (molar ratio 1:2) dissolved in 25 mL water; Reaction temperature: 95° C; Reaction time: 5 h

| Molar ratio | ds as determined by | | | MCA |
|---|---|---|---|---|
| MCA-inulin | Titration | LC | $^{13}C$ NMR | efficiency [a] |
| 0.5 | 0.22 | 0.21 | 0.21 | 0.42 |
| 1 | 0.40 | 0.36 | 0.33 | 0.36 |
| 2 | 0.66 | 0.70 | 0.67 | 0.34 |
| 4 | 1.05 | 0.99 | 1.10 | 0.25 |

[a] MCA efficiency is calculated as (average ds / molar ratio MCA-inulin)

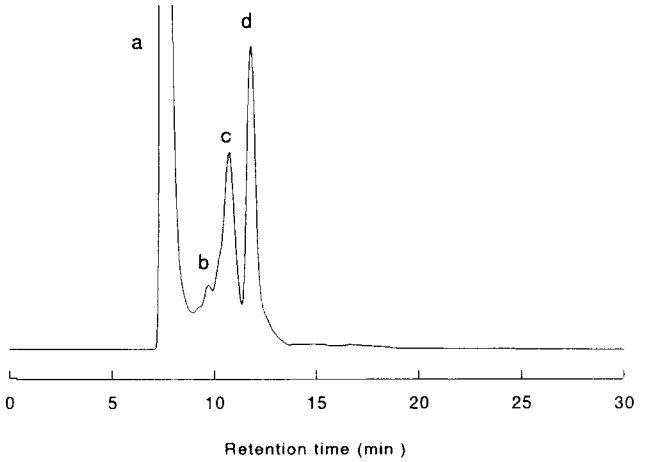



Fig. 1. LC analysis (RI detection) of products formed upon hydrolysis of CMI obtained by carboxymethylation of type II inulin: (a) exclusion peak (inorganic acids and solvent); (b) disubstituted monosaccharides; (c) monosubstituted monosaccharides; (d) non-substituted monosaccharides.

tuted units was larger than that of monosubstituted units (Fig. 2). The conclusion is that carboxymethylation is not selective for a specific position in the monosaccharide unit. In contrast, it has been shown that in cellulose the C-2 position of the glucose units is more reactive towards MCA than the C-6 and the C-3 position [11,12].

An example of a $^{13}C$ NMR spectrum of CMI (type I inulin as starting material, ds 0.68) is given in Fig. 3. In contrast to the $^{13}C$ NMR spectrum of inulin, which shows six well-defined peaks due to the six carbon atoms of the fructose units, the spectrum of CMI is more complex because of the substituents at various positions on the fructose units.

The carboxymethylation reactions were monitored by taking samples of the reaction mixture at regular time intervals and analysing them by LC. In Fig. 4, the conversion as a function of time of type II inulin in reaction with MCA and NaOH (1:2:4.2) is given.

Under the conditions used, inulin was rapidly substituted with carboxymethyl groups. A reaction time of less than 2 h appeared to be sufficient to attain the final ds. After this period, only hydrolysis of MCA occurred.

Table 2
Distribution of the substituents in mono-, di- and non-substituted monosaccharide units (in mol %) as determined by LC analysis

| | Number of substituents/monosaccharide unit: | | |
|---|---|---|---|
| ds of CMI | 0 | 1 | 2 |
| 0.21 | 83.2 | 13.0 | 3.8 |
| 0.36 | 67.6 | 28.4 | 4.0 |
| 0.68 | 40.9 | 48.1 | 11.0 |
| 1.05 | 24.1 | 52.5 | 24.1 |

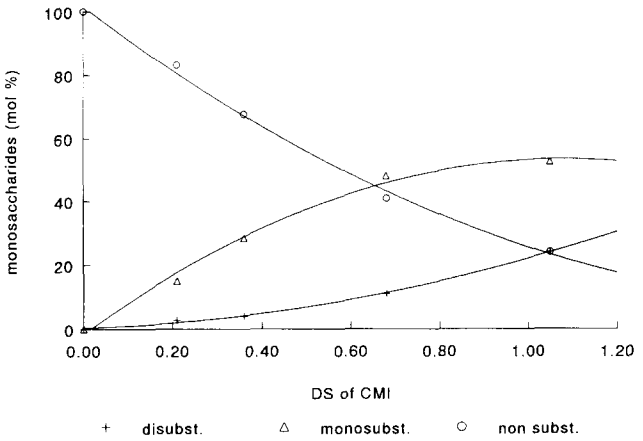



Fig. 2. Monosaccharide composition of carboxymethylated inulin with varying ds.

*Efficiency of the carboxymethylation reaction.*—During carboxymethylation reactions of polysaccharides in aqueous medium, there is a competition between the carboxymethylation and the hydrolysis of MCA into glycolate. The extent to which the carboxymethylation is favored can be expressed by MCA efficiency (selectivity of the reaction towards carboxymethylinulin).

With standard reaction conditions, the MCA efficiency (calculated as [ds obtained/ratio MCA-inulin]) was higher at lower molar ratio of MCA-inulin (see Table 1).

Besides type I inulin, also type II inulin, nystose and sucrose were carboxymethylated under the conditions described above. A molar ratio of MCA-monosaccharide units of 2:1 was used. For all of the starting materials, a ds of about 0.70 was obtained. It can be concluded that the MCA efficiency does not depend on the chain length of the starting materials.

In order to improve the MCA efficiency, the influence of the reaction temperature and the amount of water in the reaction mixture was studied. In a first series of experiments inulin was carboxymethylated with 2 equiv MCA at three temperatures (95, 75 and 55° C). The volume of water used was 25 mL. The reactions were monitored by taking samples of the reaction mixture and analysing them by LC. The conversions of MCA and the selectivities towards carboxymethylinulin (calculated as [carboxymethyl groups in the product] / ($[MCA_{start}]$ – [MCA]) are given in Table 3. Decreasing the reaction temperature results in a dramatic decrease of the reaction rate. The MCA efficiency was found to be somewhat higher at lower reaction temperatures.

In a second series of experiments, the influence of the water content of the reaction mixture was studied (Table 4).

Decreasing the water content in the reaction mixture has a great influence on the MCA efficiency of the carboxymethylation. When the amount of water was reduced by a factor of five, the selectivity was twice as high. Carboxymethylinulin with the same ds was thus obtained with half the quantity of MCA and sodium hydroxide. The importance


Fig. 3. $^{13}C$ NMR spectrum of CMI (ds 0.68, prepared with type I inulin) in $D_2O$ (100.572 MHz; 25° C; pH 8.5). Peak assignment: δ 31.2 ppm: reference (*tert*-butanol); δ 62.4 – 64.4 ppm: C-1 and C-6; δ 71.0 – 71.7 ppm: $CH_2COO^-$; δ 75.8 – 88.0 ppm: C-3, C-4 and C-5; δ 104.8 – 105.3 ppm: C-2; δ 179.1 – 179.5 ppm: $CH_2COO^-$.

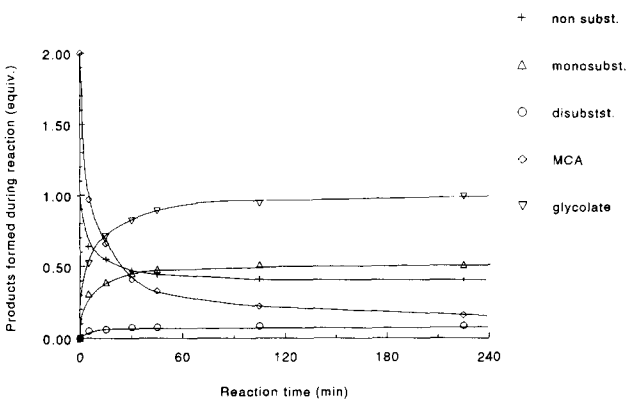



Fig. 4. Conversion of type II inulin (20 mmol) in a carboxymethylation reaction with MCA and NaOH (molar ratio 1:2:4.2) dissolved in 25 mL water at 95° C. Mono-, di- and non-substituted monosaccharides were formed upon hydrolysis of inulin and CMI.

of using low water contents has also been found for the carboxymethylation of cellulose [13,14]. In conclusion, it can be stated that the reaction temperature influences mainly the reaction rate. Decreasing the water content in the reaction mixture results in higher MCA efficiencies of the carboxymethylation process.

Neither the temperature nor the concentration of the reaction mixture influenced the distribution of the substituents in the products. The relative proportions of di-, mono- and non-substituted units in the end products corresponded to the curves presented in Fig. 2 (data not shown).

In a larger scale experiment, inulin was carboxymethylated successfully using a minimum quantity of water (experimental method b). From LC-analysis, it was concluded that the reaction product was CMI with a ds of 0.24. The conversion of MCA was 73% and the selectivity towards CMI was 81%. Sodium monochloroacetate, sodium glycolate, and sodium chloride were removed by membrane filtration as described before. With this procedure, it was possible to prepare a large amount of CMI using a small reactor volume. The MCA efficiency of the reaction was very high and, in consequence, only a small amount of by-product (glycolate) was formed.

*Analysis of molecular weight distribution.*—Inulin is a mixture of polysaccharides with different chain lengths. The dp varies from 1 to 50, with an average dp depending on the plant it originates from.

Table 3
Effect of the reaction temperature on the conversion of MCA and the selectivity towards CMI. Starting materials: inulin (Type II, 20 mmol), MCA (40 mmol) and NaOH (80 mmol) dissolved in 25 mL water

| Temperature (° C) | Reaction time for 85% conversion (h) | Selectivity (%) | ds of CMI |
|---|---|---|---|
| 55 | 24 | 43 | 0.73 |
| 75 | 5 | 39 | 0.66 |
| 95 | 2 | 38 | 0.65 |

Table 4
Effect of the water content of the reaction mixture on the conversion of MCA and the selectivity towards CMI. Starting materials: inulin (Type II, 20 mmol), MCA and NaOH (molar ratio 1:2) dissolved in water; Reaction temperature: 75° C; Reaction time: 5 h. In all cases the conversion of MCA was 85%

| Volume water (mL) | Molar ratio MCA:inulin | Selectivity (%) | ds of CMI |
|---|---|---|---|
| 25 | 2 : 1 | 39 | 0.65 |
| 10 | 2 : 1 | 52 | 0.90 |
| 5 | 1 : 1 | 78 | 0.65 |

The average dp can be easily determined from the ratio of D-glucose:D-fructose after acidic or enzymatic hydrolysis of inulin [15]. However, this technique cannot be used for modified inulin and it is not possible to establish degradation or cleavage of the polysaccharide chains. Another disadvantage is that no information can be obtained about the molecular-weight distribution of the products. Therefore, the molecular-weight distribution of inulin and its carboxymethyl derivatives was determined by gel-permeation chromatography (GPC) on a polyacrylamide gel (Biogel P-6) [15–19]. In Fig. 5 the

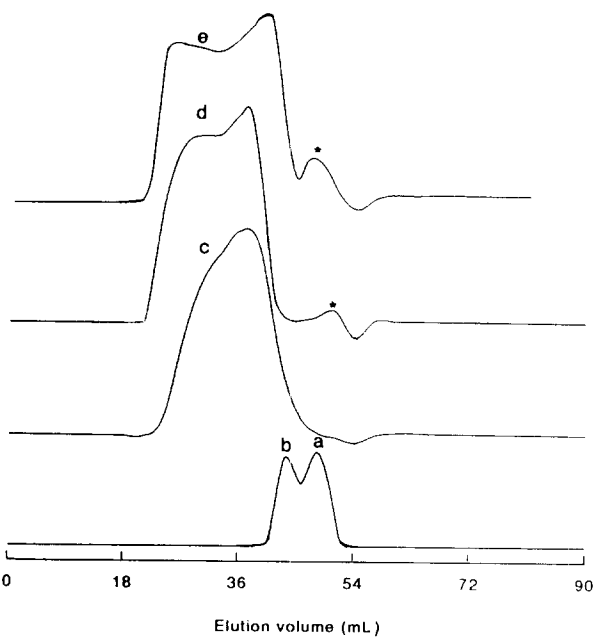



Fig. 5. GPC separation on Biogel P-6 (RI detection) of fructose (a), nystose (b), inulin (type I) (c), CMI with a ds of 0.36 (d) and CMI with a ds of 0.68 (e). Chromatographic conditions as described in the text. The small peaks marked with a " * " contained no organic material and are probably due to small amounts of inorganic salts.

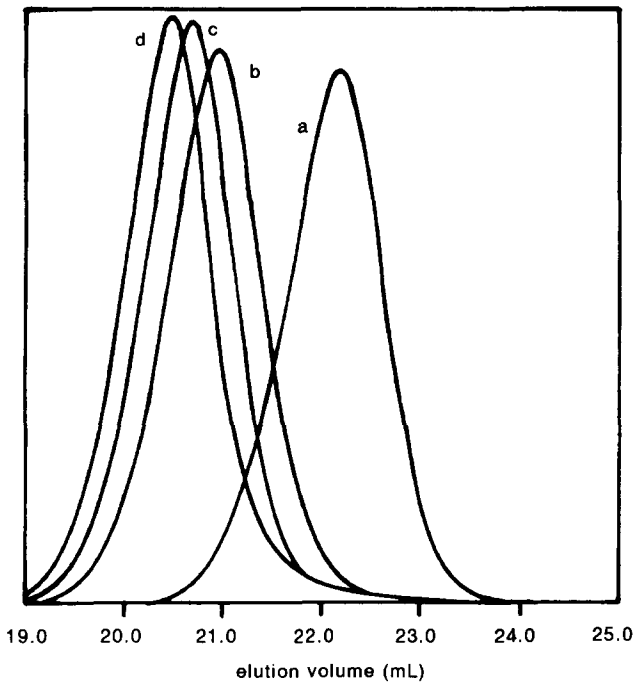



Fig. 6. Elution profiles from high performanced GPC (RI detection) of inulin (type I) (a), CMI with a ds of 0.68 (b), CMI with a ds of 0.42 (c) and CMI with a ds of 1.05 (d).

elution profiles of nystose ($GF_3$), fructose, inulin (type I) and CMI with a ds of 0.68 and 0.36 are given.

With the GPC system used, type I inulin, nystose and fructose were satisfactorily separated. Low molecular weight material can thus easily be distinguished from inulin and its derivatives. Due to chain stiffening and extension because of the electrostatic repulsion of carboxymethyl groups, CMI eluted at a slightly lower elution volume than inulin.

The fractions obtained by the GPC fractionation were hydrolysed and analysed with

Table 5
Average molecular weights of inulin and CMI from GPC-MALLS experiments

| Material | Average $M_w$ [a] (± 10 %) | Calculated [b] av. $M_w$ |
|---|---|---|
| inuline (type I) | 4300 | 4878 |
| CMI (ds 1.05) | 5300 | 7398 |
| CMI (ds 0.68) | 5700 | 6510 |
| CMI (ds 0.42) | 5300 | 5886 |

[a] The values represent averages of 2 runs. [b] Average molecular weights were calculated assuming an average dp of inulin of 30 as was established with LC (see section materials).

LC in order to determine the ds of carboxymethylated material as a function of the molecular weight. The ds was found to be the same for all the fractions containing CMI. This confirms that the selectivity of the carboxymethylation reaction does not depend on the chain length of the oligosaccharides.

Using gel-permeation chromatography no absolute value for the average molecular weight of CMI could be determined. The introduction of carboxymethyl groups affects the hydrodynamic volume of the polymer and direct comparison of a charged and and non charged material is not obvious. Therefore, some additional molecular weight determination was performed using multi-angle laser light scattering coupled with GPC (GPC-MALLS) [20]. With this technique, on-line determination of the molecular weight of the eluting material from the GPC-column was possible. The elution profiles, as detected with RI, are shown in Fig. 6. Similar to the preparative GPC profiles (Fig. 5), the carboxymethylated derivatives eluted at a lower elution volume as compared to inulin. The MALLS response of the inulin sample showed a distinct prepeak at a retention volume coinciding with the exclusion limit of the column. This prepeak is due to a small amount ($< 1\%$) of high molecular weight impurities[20] and was not taken into account for the molecular weight calculations. These impurities were not found in the samples of carboxymethylated inulin, which means that they were successfully removed during the work-up procedure of the product. The average molecular weights as obtained from the MALLS response were slightly lower (about 10%) than the calculated values for inulin as well as for CMI (Table 5). Only for CMI with the highest ds (ds 1.05) the deviation from the calculated value was somewhat higher than for the other products, which could point to some degradation due to the high sodium hydroxide concentration. However, this degradation is not detrimental for the application of the polymeric material. It can be concluded that almost no chain length degradation occurs during the carboxymethylation reaction.

## Acknowledgements

Thanks are due to the EC (AIR program) for financial support. Dr M. Kunz and Dr A. Vogel (Südzucker AG) are acknowledged for a gift of nystose. Dr Th.L. Hoeks (Suiker Unie) is thanked for the generous samples of inulin. Dr A.C. Besemer (TNO Zeist) is recognized for the help with the desalting of samples of CMI, and Ropur International B.V. (Leusden) for providing the UTC 60 Toray membrane. Light scattering experiments were carried out at Leiden University (Laboratory of Physical and Macromolecular Chemistry) with the kind cooperation of J.A.P.P. van Dijk ing. and Drs F.A. Varkevisser.

## References

[1] E. Jansen (Deutsche Celluloid-Fabrik), Brit. Pat. 138, 116 (1920); *Chem. Abstr.* 14 (1920) 1755.
[2] E. Jansen (Deutsche Celluloid-Fabrik), Ger. Pat. 332, 203 (1921); *Chem. Abstr.* 15 (1921) 2184.
[3] CMC World Market Data (1991), provided by Metsä-Serla Chemicals B.V.

[4] I.A. Eldib, U.S. Pat. 3,629,121 (1971); *Chem. Abstr.* 76 (1972) 115231n.
[5] C. Castagna and P. Castagna, Ger. Offen. Pat. 2,731,489 (1978); *Chem. Abstr.* 88 (1978) 91367f.
[6] M. McNeil, W. Szalecki and P. Albersheim, *Carbohydr. Res.*, 131 (1984) 139–148.
[7] F. Bayerlein and P. Habereder (Diamalt A.G.), Ger. Offen. Pat. 2,149,737 (1973); *Chem. Abstr.* 79 (1973) 32255j.
[8] W.I. Lyness and J.E. Thompson (Procter and Gamble Co.), U.S. Pat. 3,634,392 (1972); *Chem. Abstr.* 76 (1972) 115222k.
[9] W.I. Lyness and J.E. Thompson (Procter and Gamble Co.), U.S. Pat. 3,686,124 (1972); *Chem. Abstr.* 77 (1972) 154303c.
[10] F. Bayerlein and P. Habereder (Diamalt A.G.), Ger. Offen. Pat. 2,161,825 (1973); *Chem. Abstr.* 79 (1973) 80662m.
[11] S.N.K. Chaudhari, K.C. Gounden, G. Srinivasan and V.S. Ekkundi, *J. Polym. Sci.*, 25 (1987) 337–342.
[12] A. Parfondry and A.S. Perlin, *Carbohydr. Res.*, 57 (1977) 39–49.
[13] A.B. Savage, A.E. Young and A.T. Maasberg, in E. Ott, H.M. Spurlin and N.W. Grafflin (Ed.), *Cellulose and Cellulose Derivatives*, Part II, Interscience Publishers INC., New York, 1954, pp. 882–945.
[14] R.R. McLaughlin and J.H.E. Herbst, *Can. J. Research*, 28B (1950) 731.
[15] W. Praznik and R.H.F. Beck, *J. Chromatogr.*, 348 (1985) 187–197.
[16] M. John, J. Schmidt, C. Wandrey and H. Sahm, *J. Chromatogr.*, 247 (1982) 281–288.
[17] K. Yamashita, T. Mizuochi and A. Kobata, *Methods Enzymol.*, 83 (1982) 105–127.
[18] M. Natowicz and J.U. Baenziger, *Anal. Chem.*, 105 (1980) 159–164.
[19] J.-F. Thibault, *J. Chromatogr.*, 194 (1980) 315–322.
[20] W.-D. Eigner, P. Abuja, R.H.F. Beck and W. Praznik, *Carbohydr. Res.*, 180 (1988) 87–95.