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Composition analysis of carboxymethylcellulose by high-pH anion-exchange chromatography with pulsed amperometric detection

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ABSTRACT

A rapid method for the determination of the substituent distribution in carboxymethylcellulose has been developed, involving hydrolysis of carboxymethylcellulose in 1 2 *M* perchloric acid and analysis of the mixture of carboxymethylated glucose residues and glucose by high-pH anion-exchange chromatography with pulsed amperometric detection The peaks in the chromatogram were identified by combined gas–liquid chromatography–mass spectrometry after pertrimethylsilylation Molar response factors for each of the constituent monomers were established by ^{1}H NMR spectroscopy The degrees of substitution for three carboxymethylcellulose preparations determined by the proposed method and by a standard titration method were found to be in excellent agreement

INTRODUCTION

Sodium carboxymethyl (CM)-cellulose, prepared by conversion of cellulose with sodium chloroacetate, is an industrially important polymer that has found widespread use in the food and coatings industry and in oil-well drilling The average degree of substitution (DS) of commercial, water-soluble CM-cellulose lies in the range 0 4–1 3 [1] Being only partially carboxymethylated, CM-cellulose can be regarded as a copolymer of unsubstituted (D-glucose), monosubstituted (2-, 3- and 6-O-CM-D-glucose), disubstituted (2,3-, 2,6- and 3,6-di-O-CM-D-glucose), and trisubstituted (2,3,6-tri-O-CM-D-glucose) glucose

Knowledge of the relative amounts of the constituent monomers of CM-cellulose is important in product control, and is essential for understanding structure–property relationships So far, analysis of the intact polysaccharide by ^{13}C NMR spectroscopy has only resulted in information on the molar ratio of the substituents at HO-2, HO-3 and HO-6 [2] For the determination of the monomer composition in solvolysates of CM-cellullose, different approaches have been developed Mixtures of monomers, obtained by hydrolysis or methanolysis, have been analysed by carbon column chromatography [3], gas–liquid chromatography (GLC) [4–7], ^{13}C NMR spectroscopy [1,8] and ^{1}H NMR spectroscopy [9] Recently, the substitutent distribution in CM-cellulose has been determined by GLC using a reductive cleavage procedure to obtain mixtures of the constituent monomers [10] These methods are elaborate and time consuming Recent advances in the separation of unmodified carbohydrates using high-pH anion-exchange chromatography (AEC) in combination with pulsed amperometric detection (PAD) [11] prompted us to devise a simple and rapid method for the determination of the substituent distribution in CM-cellulose

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EXPERIMENTAL

Materials

CM-Cellulose samples, A, B and C were obtained from AKZO Research (Arnhem, Netherlands)

Hydrolysis procedure

Hydrolysis was carried out according to ref 1 in modified form CM-Cellulose (5 mg) in 0 1 ml of 70% $HClO_4$ was kept for 10 min at room temperature and, after addition of 0 9 ml of doubly distilled water affording 1 2 *M* $HClO_4$, heated for 16 h at 100°C The solution was then neutralized with 2 *M* KOH, the precipitated $KClO_4$ was removed by centrifugation (2500 *g*, 5 min) and the supernatant was collected The residue was washed twice with 0 5 ml of doubly distilled water and the supernatants were pooled

High-pH anion-exchange chromatography with pulsed amperometric detection

Separation and quantification of the mixture of carboxymethylated glucose residues and glucose was carried out by high-pH AEC–PAD on a Dionex LC system consisting of a Dionex Bio-LC quaternary gradient module, a Model PAD-2 detector, a CarboPac PA-1 pellicular anion-exchange column (25 × 0 9 cm I D), (Dionex, Breda, Netherlands) and a Shimadzu C-R3A integrator An aliquot (25 μl) of the pooled supernatants was applied to the column and elution was carried out starting with 0 1 *M* NaOH (eluent A)–0 1 *M* NaOH containing 1 *M* sodium acetate (eluent B) (95 5, v/v) for 0 3 min, followed by a linear gradient to eluent B in 15 min, at a flow-rate of 4 0 ml/min and ambient temperature Detection was performed by PAD with a gold working electrode and triple-pulse amperometry with the following pulse potentials and durations E_1 = 0 05 V, 300 ms (sampling), E_2 = 0 65 V, 60 ms (cleaning), E_3 = −0 95 V, 180 ms (reduction), and a response time of 1 s For structural identification, fractions were isolated, neutralized with 4 *M* HCl, lyophilized and desalted on a column (40 × 1 5 cm I D) of Bio-Gel P-2 (200–400 mesh, Bio-Rad Labs) using doubly distilled water The eluent was monitored by UV detection at 206 nm with an LKB 2238 Uvicord S II absorbance detector Residual sodium acetate was removed by conversion into acetic acid on a column (3 × 0 5 cm I D) of Dowex 50W-X8 (H^+) resin (100–200 mesh, Bio-Rad Labs) and repeated lyophilization of the resulting solutions

GLC and GLC–electron impact mass spectrometry (EI-MS)

Aliquots of the desalted high-pH AEC fractions were pertrimethylsilylated using pyridine–hexamethyldisilazane–trimethylchlorosilane (5 1 1, v/v/v) GLC was carried out on a Perkin-Elmer Model 8410 gas chromatograph equipped with a SE-30 bonded-phase, fused-silica capillary column (25 m × 0 32 mm I D) (Pierce) and a flame ionization detector, and connected to a Shimadzu C-R3A integrator The temperature of the column was increased from 150 to 250°C at 4°C/min, and then kept at 250°C for 5 min GLC–EI-MS was performed on a Carlo Erba GC–Kratos MS 80–Kratos DS 55 system (electron energy, 70 eV, accelerating voltage, 2 7 kV, ionizing current, 100 μA, ion-source temperature, 225°C, BP-1 capillary column) The initial temperature of the column was 200°C for 2 min, then increased to 300°C at 4°C/min

^1H NMR spectrocopy

Prior to ^1H NMR spectroscopic analyses, samples were repeatedly treated with ^2H_2O (99 9 atom% ^2H) (MSD Isotopes), finally using 99 96 atom% ^2H at $p^2H \geqslant 7$ 300-MHz ^1H NMR spectra were recorded using a Bruker AC-300 spectrometer at a probe temperature of 20°C Chemical shifts (δ) are expressed in ppm downfield from the signal for internal sodium 4,4-dimethyl-4-silapentane-1-sulphonate, but were actually measured by reference to the signal for internal acetone (δ 2 225) with an accuracy of 0 002 ppm To obtain quantitatively reliable results, no resolution enhancement was applied, the HO^2H signal was not suppressed and a repetition delay of 5 s was used

RESULTS AND DISCUSSION

For a reliable analysis of its substituent distribution, solvolysis of CM-cellulose has to be complete and the occurrence of side-reactions during solvolysis has to be avoided A brief incubation with 70% $HClO_4$ at room temperature was applied to improve the solubility of the material, thereby making it accessible for complete hydrolysis in 1 2 *M*

$HClO_4$ (16 h, 100°C) without browning of the hydrolysate Then, most of the $HClO_4$ was removed as $KClO_4$ after precipitation with KOH This procedure gave samples that could be analysed directly by high-pH AEC–PAD

A typical high-pH AEC–PAD trace for a hydrolysate separated on CarboPac PA-1 is shown in Fig 1 For identification purposes fractions 1–8 were collected, neutralized, desalted on Bio-Gel P-2, pertrimethylsilylated and identified by GLC–EI-MS Fraction 1 was found to correspond to D-glucose Fractions 2, 3 and 4 contained 6-, 2- and 3-O-CM-D-glucose, respectively Fractions 5, 6 and 7 corresponded to 2,6-, 3,6- and 2,3-di-O-CM-D-glucose, respectively, whereas fraction 8 contained 2,3,6-tri-O-CM-D-glucose Apart from some minor differences in relative peak intensities, the mass spectra were in agreement with reference mass spectra of these derivatives [4,12] It is evident that the compounds elute in groups according to the number of substituents, indicating that the interaction with the anion-exchange resin is dominated by the carboxymethyl groups Completely resolved peaks were obtained for the positional isomers within each group, with the exception of 2-O- and 3-O-CM-D-glucose However, the fractionation was sufficient to allow accurate integration (Fig 1) The order of elution of the carboxymethylated glucose residues on CarboPac PA-1 was identical with that of the corresponding sulphoethylated glucose residues, separated on the same resin [13] This suggests that the type of interactions of these compounds with the anion-exchange beads is similar, irrespective of the character of the anionic substituent However, compared with the sulphoethylated glucose residues, a lower concentration of sodium acetate is sufficient for the elution of the carboxymethylated glucose residues, probably reflecting the weaker binding of the less acidic carboxymethyl group to the anion-exchange beads

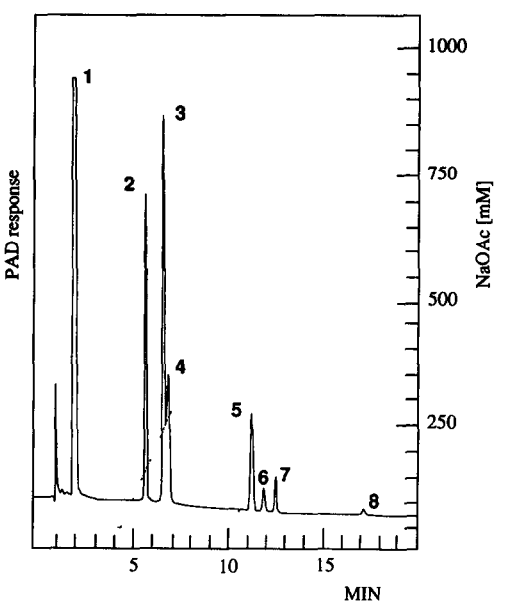

Fig 1 Typical high-pH AEC trace of a CM-cellulose hydrolysate separated on a CarboPac PA-1 anion-exchange column (250 × 9 mm I D) using a gradient of sodium acetate (NaOAc) (dotted line) in 0 1 *M* NaOH at a flow-rate of 4 ml/min and PAD Peaks 1 = D-glucose, 2 = 6-O-CM-D-glucose, 3 = 2-O-CM-D-glucose, 4 = 3-O-CM-D-glucose, 5 = 2,6-di-O-CM-D-glucose, 6 = 3,6-di-O-CM-D-glucose, 7 = 2,3-di-O-CM-D-glucose, 8 = 2,3,6-tri-O-CM-D-glucose

The high-pH AEC–PAD trace shows no additional peaks, suggesting that the formation of O-CM-glucoselactones (*ca* 3 mol%) [5], which occurred during hydrolysis (6 *M* HCl, 2 h, room temperature, and subsequently 2 *M* HCl, 30 min, 120°C) or sample concentration, does not take place in the present procedure In the method presented here the hydrolysis conditions are milder However, when a higher concentration of $HClO_4$ (2 *M*) or a longer treatment (30 min) with 70% $HClO_4$ was supplied, minor additional peaks were observed on the high-pH AEC–PAD trace, which probably originate from O-CM-glucoselactones This suggests that lactonization takes place only when carboxymethylated glucoses are exposed to strongly acidic conditions for a prolonged period of time

For the determination of the substituent distribution in CM-cellulose from the peaks on the high-pH AEC–PAD trace, the molar response of each monomer to the pulsed amperometric detector was obtained from 300-MHz ^1H NMR spectra, recorded without resolution enhancement, of solutions containing one of the monomers and a defined amount of methyl β-cellobioside as an internal standard In each instance the molar ratio of monomer to methyl β-cellobioside was determined by integration of the signals for the respective anomeric protons in the ^1H NMR spectra [14] Then, each of the mixtures of monomer and methyl β-cellobioside was lyophilized, dissolved in doubly distilled water

TABLE I

CALCULATED PAD RESPONSE FACTORS OF THE MONOMERS OF CARBOXYMETHYL- AND SULPHOETHYLCELLULOSES

Monomer	PAD response[a]	
	R = CM[b]	R = SE[b]
D-Glucose	1 00	1 00
2-O-R-D-glucose	0 71	0 71
3-O-R-D-glucose	0 37	–
6-O-R-D-glucose	0 77	0 77
2,3-Di-O-R-D-glucose	0 26	0 16
2,6-Di-O-R-D-glucose	0 38	0 36
3,6-Di-O-R-D-glucose	0 24	0 17
2,3,6-Tri-O-R-D-glucose	0 20	–

[a] Relative to D-glucose
[b] CM = Carboxymethyl, SE = sulphoethyl

and subjected to high-pH AEC–PAD on CarboPac PA-1 Molar PAD responses under the conditions described under Experimental were calculated by matching the relative peak areas obtained by PAD with the relative amounts determined by ^{1}H NMR spectroscopy (see Table I) The general effect of substitution is a decrease in PAD response with an increasing number of carboxymethyl groups in the glucose residue Although little is known about the actual electrochemical reactions taking place at the gold electrode, it is likely that the response to the pulsed amperometric detector mainly results from the oxidation of unsubstituted hydroxyl groups and of the hemiacetal group in the glucose residue It is interesting that, within the group of monocarboxymethylated glucose residues, substitution of HO-3 results in the largest decrease in PAD response A similar effect is observable for the dicarboxymethylated glucose residues However, the response factors of the di- and tricarboxymethylated glucose residues cannot be calculated simply by addition of the effects of monocarboxymethylation on the PAD response For comparison, previously established molar response factors of some sulphoethylated glucose residues [13] are included in Table I Similar response factors for carboxymethylated and sulphoethylated glucose residues are observed when substituents occur at O-2 or O-6 The decrease in molar response is, however, larger with a sulphoethyl group than with a carboxymethyl group at O-3

The high-pH AEC–PAD procedure was applied twice to samples A and B and four times to sample C, and in Table II the calculated substituent distribution for each of the samples is shown The degrees of substitution (DS) of A, B and C, calculated using the data in Table II, were found to be 0 80, 0 81 and 0 68 ± 0 03, respectively The determined DS values were in excellent agreement with the degree of substitution [(A) 0 77, (B) 0 80 and (C) 0 67]

TABLE II

SUBSTITUENT DISTRIBUTIONS IN THREE CARBOXYMETHYLCELLULOSES DETERMINED BY HIGH-pH AEC–PAD

Monomer	Distribution (mol%)		
	Sample A[a]	Sample B[a]	Sample C[b]
D-Glucose	37 0	36 7	44 5 ± 1 0[c]
2-O-CM-D-glucose[d]	21 4	21 7	21 0 ± 0 3
3-O-CM-D-glucose	12 5	11 9	9 2 ± 0 2
6-O-CM-D-glucose	12 7	13 0	13 0 ± 0 3
2,3-Di-O-CM-D-glucose	4 4	4 5	3 0 ± 0 3
2,6-Di-O-CM-D-glucose	8 1	8 2	6 4 ± 0 2
3,6-Di-O-CM-D-glucose	3 1	3 3	2 4 ± 0 2
2,3,6-Tri-O-CM-D-glucose	0 8	0 7	0 5 ± 0 1

[a] Average results of two individual analyses
[b] Average results of four individual analyses with standard deviation
[c] This larger standard deviation probably results from contamination of the sample with glucose from external sources
[d] CM = Carboxymethyl

provided by the manufacturer, based on a standard acid wash procedure [15]

From the data in Table II, the order of reactivity of the hydroxyl groups in cellulose towards carboxymethylation was calculated to be HO-2 > HO-6 > HO-3, in agreement with several other reports on CM-cellulose solvolysates using ^{13}C NMR spectroscopy [1], ^{1}H NMR spectroscopy [9] or GLC [5,6,10] In two studies employing ^{13}C NMR spectroscopy [2,16] directly on the polymer it was reported that the reactivities of HO-3 and HO-6 are equal Using a statistical model for the etherification of cellulose [17], which has been confirmed for CM-cellulose [1], the relative reaction rate constants for HO-2, HO-3 and HO-6 were calculated to be $k_2\ k_3\ k_6$ = 1 8 1 1 2, 1 9 1 1 3 and 2 3 1 1 5 for samples A, B and C, respectively Comparison of these data with results from other studies is complicated by the fact that relative reaction rate constants, especially that of HO-3, depend to some extent on the reaction conditions used [8] However, the relative reaction constants presented here are similar to previously reported data [1,6,9,10], with the exception of one study presenting higher values [5]

The method for the determination of the substituent distribution of CM-cellulose reported here offers important advantages over other procedures There is no need for derivatization as required for GLC Further, contrary to the appearance in ^{13}C and ^{1}H NMR spectra and gas–liquid chromatograms, each monomer is represented by a single peak on the high-pH AEC–PAD trace, making quantification more easy

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