CHARACTERIZATION OF HARDWOOD-DERIVED CARBOXYMETHYLCELLULOSE BY HIGH pH ANION CHROMATOGRAPHY USING PULSED AMPEROMETRIC DETECTION

P. DAMLIN, J.-P. MOKKOLA* and T. SALMI

Åbo Akademi University, Process Chemistry Centre, Laboratory of Industrial Chemistry and Reaction Engineering, FIN-20500 Turku/Åbo, Finland
[*]Umeå University, Department of Chemistry, Chemical-Biological Centre, Laboratory of Technical Chemistry, SE-90187 Umeå, Sweden

Received November 16, 2009

An approach for the quantitative analysis of substituent distribution in carboxymethylcellulose (CMC) is presented. In short, the high-pH anion-exchange chromatography method, coupled to pulsed amperometric detection (PAD), is introduced. Each of the seven derivatives in CMC is presented by a single peak on the PAD trace, thus enabling an easy quantification. New inside information on monomer composition is obtained by this novel method, which is essential for understanding the structure *versus* property relationships in the CMC samples.

Keywords: carboxymethyl cellulose, high-pH anion-exchange chromatography, substituent distribution

INTRODUCTION

Cellulose is a fibrous, insoluble and crystalline polysaccharide polymer composed of linear chains of 1,4-linked β-D-glucose units. Since each glucose unit can be modified on the C2, C3 or C6 hydroxyl groups, up to three different modifications per glucose unit are possible (Fig. 1). Carboxymethylcellulose (CMC), one of the most important water soluble derivatives of cellulose, is traditionally prepared *via* a reaction with sodium hydroxide and monochloroacetic acid. The degree of substitution (DS) of commercial CMCs, typically in the 0.5-1.4 range, is a key parameter controlling their final applications. Among all polysaccharides, CMC is widely used in many applications, such as detergents, textiles, paper, food, drugs and oil-drilling operations. Investigation on the distribution of the carboxymethyl substituents along the polymer chain has been the focus of considerable effort, since the complex structure–property relationships involved in the synthesis of CMCs is essential for a deeper understanding of the substitution process. Successful approaches have been presented, such as hydrolysis of CMC, followed by various chromatographic or spectroscopic analysis techniques.[1-5] These analytical methods are time-consuming and result only in information on the molar ratio of the substituents at OH-2, OH-3 and OH-6, without considering the individual yields of the monomers. The aim of this study is to investigate unmodified carbohydrates using a highly sensitive and efficient separation technique for carbohydrate analysis, namely high-pH anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD).[6-8] According to this technique, separation is based on the weakly acidic properties of sugar molecules, whereas detection is performed by taking advantage of their electrocatalytic oxidation mechanism at the gold working electrode, in basic media.

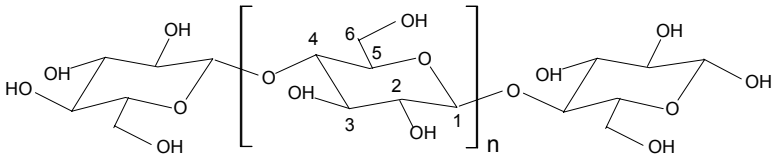

Figure 1: Molecular structure of cellulose

EXPERIMENTAL

Materials

The cellulose used in carboxymethylation, obtained from Metsä-Serla chemicals, was screened to a size of 0.35 mm. Heterogeneous carboxymethylation experiments were performed in a glass laboratory scale autoclave with fixed molar ratios of cellulose-to-monochloroacetic acid-to-NaOH of 1:4:8, according to a previously published experimental procedure.[9] 2-propanol was used as a solvent and the reaction temperature during carboxymethylation experiments ranged between 30 and 60 °C, respectively. The reaction time was of 120 min, the samples being withdrawn from the reaction mixture every 20 min.

High-pH anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

Hydrolysis

Prior to D analysis, the CMC samples were subjected to acid hydrolysis. In the hydrolysis with trifluoroacetic acid (TFA), 50 mg of CMC was weighed in pear-shaped flasks and mixed with 2 mL of 2 M TFA. The mixture was kept in an oven at 100 °C for 2 h. After cooling, TFA was evaporated with nitrogen gas, on keeping the sample in a water bath at 40-50 °C.

Equipment used

In a further step, the products were analyzed by HPAEC with PAD, on a Hewlett Packard 1100 Series LC system with a Dionex ED50 electrochemical detector, a Dionex CarboPac PA1 anion-exchange column (semi-preparative 9×250 mm and analytical 4×250 mm combined with guard 4×50 mm) and a Chemstation chromatographic software. The column was maintained under a controlled temperature of 30 °C. Separation, using the analytical column, was performed with a linear gradient of 95:5 (v/v) of eluent A (0.1 M NaOH) / eluent B (0.1 M NaOH containing 1 M NaOAc) to 100% eluent B for 12 min, followed by 100% B for 12-15 min. Prior to the following analysis, the column was reconditioned for 7 min with a 95:5% mixture of A/B eluent. The flow rate was set at 1 mL/min. Detection was performed with a PAD unit with a gold working electrode and triple pulse amperometry, by applying the following pulse potentials and durations: E_{DET} = 0.05 V (200 ms), E_{OX} = 0.75 V (190 ms) and E_{RED} = -0.15 V (380 ms), with a total response time of 1 s for every pulse. Such a repeating step of surface oxidation and reduction is necessary to maintain a highly reproducible (state of) activity of the gold electrode surface. The counter electrode was a titanium cell body, an Ag/AgCl combination electrode being used as a reference electrode.

Standard preparation

In the preparation of standards, one CMC sample with DS 1.1 was hydrolyzed in 0.5 g scale in TFA, for obtaining a sufficient amount of mono-, di-, and tri-substituted CMG. After separation, using the semi-preparative anion-exchange column, fractions were collected and concentrated by freeze-drying, neutralized with 2 M acetic acid and desalted on a column (430×15 mm) filled with Sephadex G-10 (Sigma-Aldrich). The sugar-containing fractions were detected on a CDM210 Conductivity Meter (MeterLab^{TM}), reconcentrated by freeze-drying and silylated before analysis by GC-MS.

RESULTS AND DISCUSSION

Carboxymethylation of cellulose

The experimental results describing the effect of the reaction temperature on the kinetics of carboxymethylation are shown in Figure 2. The degree of substitution (DS) was determined[9] by titration of the pyrolyzed sample with 0.05 M H_2SO_4, *via* the HPEAC-PAD method.[2] According to titration results, the DS value was of about 0.55, at a temperature of 30 °C and, after a reaction time of 120 min, the degree of substitution reached a value around 1.5, at 60 °C. The DS values of 0.7 and 1.4, obtained at 30 and 60 °C, respectively, and determined by the HPEAC-PAD method, were in good agreement with the titrimetric values. Consequently, the values obtained for the degree of substitution of CMC agreed with the experimental results published,[9] obtained with the same raw material and by the same method of carboxymethylation.

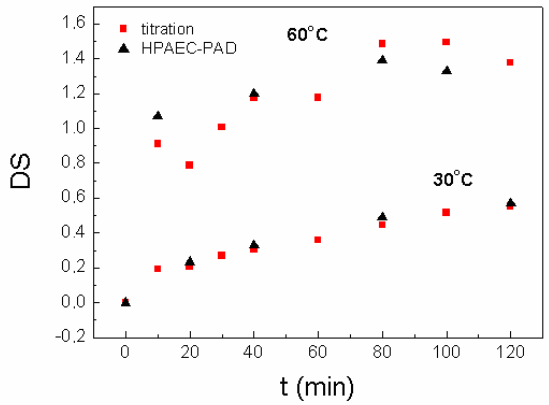

Figure 2: Kinetics of carboxymethylation of cellulose at 30 °C and 60 °C determined by titration and by HPEAC-PAD

Composition analysis

Figure 3 introduces a typical HPAEC-PAD trace for a hydrolyzed CMC sample, when using a CarboPac PA1 analytical anion-exchange column and a sodium acetate gradient in an eluent containing 0.1 M NaOH. The NaOH is present to ionize the saccharide hydroxyl groups to oxy-anions, so that it tends to bind to the anion-exchange column. Sodium acetate in NaOH is added to the mobile phase, as these anions interact much more strongly than the hydroxide with the anion-exchange sites in the column, hereby drastically decreasing the retention time values. It can be seen that elution of all fractions has taken place after a total analysis time of 17 min (Fig. 3). This was possible when using a linear gradient elution of NaOAc from 5-100%, at a total time of 12 min. One should point out that, if the gradient elution is changed so that a final [NaOAc] of 0.5 M was used, it is not possible to detect the last fraction before a total analysis time of 35 min.

As illustrated by Figure 3, quite many peaks are observed in the HPAEC trace, as it will be the case when using cellulose as starting material for carboxymethylation experiments. Figure 3A displays the whole HPEAC trace, whereas enlarged parts of this trace are shown in Figures 3B, C and D, respectively. To identify all these peaks, the column was changed to the semi-preparative CarboPac PA 1 column, capable of separating much higher sample concentrations of the hydrolyzed CMC than the analytical column. A CMC sample with DS 1.1 was hydrolyzed in a 0.5 g scale, as higher concentrations of each fraction had to be gathered for further analysis, after silylation by GC-MS. Each collected fraction was neutralized, desalted on a Sephadex G-10, freeze-dried and silylated. The identification of the silylated fractions with GC-MS showed that the hydrolyzed CMC sample contained xylose, glucose, 3-O-CM-xylose, 2-O-CM-xylose, 3-O-CM-glucose, 2-O-CM-glucose, 6-O-CM-glucose, 2,3-di-O-CM-glucose, 3,6-di-O-CM-glucose, 2,6-di-O-CM-glucose and 2,3,6-tri-O-CM-glucose. Also, some small traces of arabinose, levoglucosan and galactose were evidenced by GC-MS analysis. The numbered peaks in the HPAEC trace in Figure 3 correspond to glucose (1), 6-O-CM-glucose (2), 2-O-CM-glucose (3), 3-O-CM-glucose (4), 2,6-di-O-CM-glucose (5), 3,6-di-O-CM-glucose (6) and 2,3-di-O-CM-glucose (7), respectively. It can be mentioned that, at a reaction temperature of 60 °C, the HPAEC trace showed peaks for the di-substituted forms of CMC already after a 10 min reaction time. This should be compared with the 30 °C experiment in which di-substituted forms of CMC could be observed after a reaction time of 120 min. The tri-subtituted form of CMC could not be observed in the experiments carried out at 30 °C, yet it was visible in the HPAEC trace at 14 min, when the carboxymethylation reaction was carried out at 60 °C; at this point, the reaction continued for 10 min.

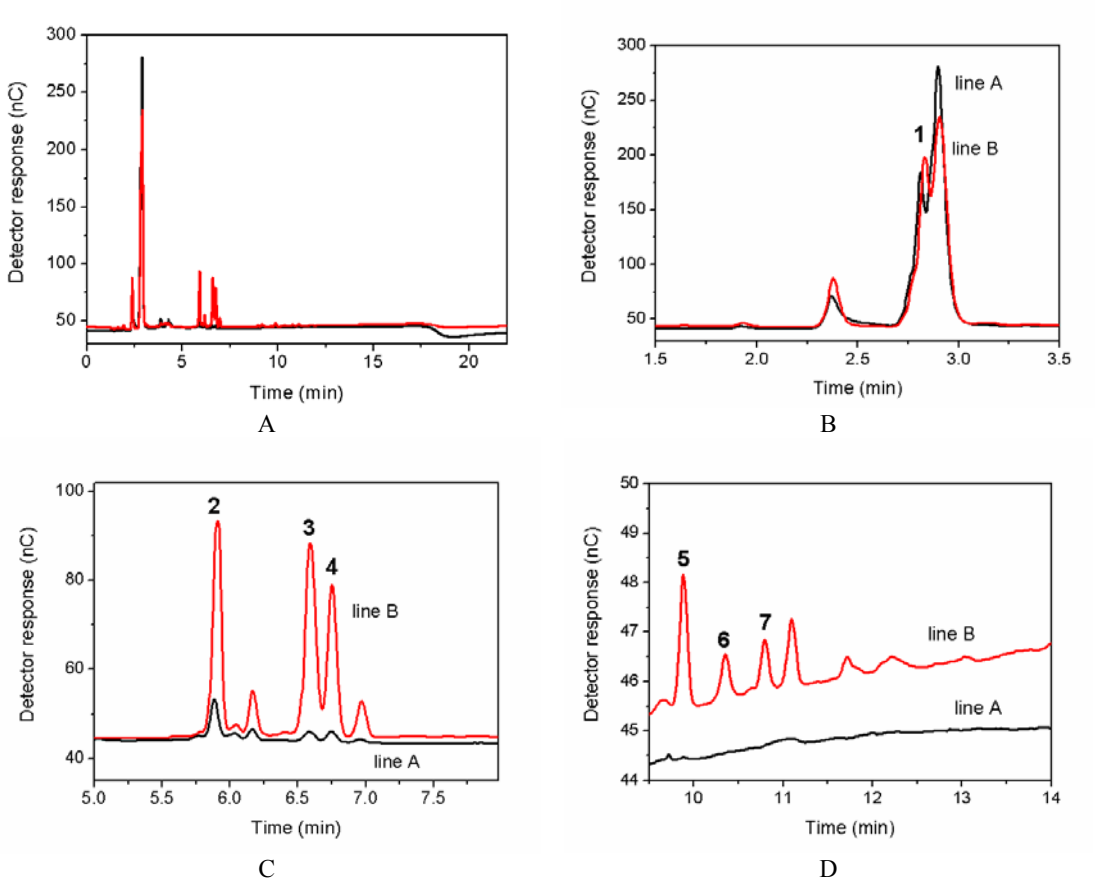

Figure 3: Separation of hydrolyzed CMC samples. Carboxymethylation at 30 °C, samples taken after 20 (line A) and 120 min (line B) reaction time

The distribution of glucose, mono-, di- and tri-substituted units is shown in Table 1 for the two CMC samples illustrated in Figure 3, with DS values 0.2 and 0.7, respectively. In the beginning of the reaction, the 6-mono-O-CM-glucose was the major component among the mono-substituted units, whereas no di-substituted units could be observed. When the carboxymethylation reaction proceeds, all forms of mono- and di-substituted units increase, with a preference for 3-mono-O-CM-glucose and 2,6-di-O-CM-glucose, respectively. Consequently, changes in the reaction temperature and synthesis time still have to be carried out, thus permitting more detailed conclusions from the substituent distribution for the CMC samples synthesized in 2-propanol.

Table 1
Product distribution in carboxymethylation determined by high-pH AEC-PAD for two different CMC samples (DS = 0.2 and DS = 0.7)

Monomer	Distribution (mol%)	
	DS = 0.2	DS = 0.7
Glucose (1)	76.6	34.6
6-O-CM-glucose (2)	12.4	16.8
2-O-CM-glucose (3)	4.2	18.8
3-O-CM-glucose (4)	6.8	24.4
2,6-di-O-CM-glucose (5)	0	2.4
3,6-di-O-CM-glucose (6)	0	1.6
2,3-di-O-CM-glucose (7)	0	1.5
2,3,6-tri-O-CM-glucose	0	0

CONCLUSIONS

It was demonstrated that, by HPAEC analysis with the semi-preparative column, standard substances of hydrolyzed CMC may be accurately isolated. Thus, quantitative determination of glucose and of its carboxymethyl derivatives from CMC samples prepared under different experimental conditions was achieved. This novel method for determining the substituent distribution of CMC offers important advantages over other existing analysis techniques, since no derivatization of the carbohydrate species is required.

ACKNOWLEDGEMENTS: This work is part of the activities developed at the Åbo Akademi Process Chemistry Centre within the Finnish Centre of Excellence Programmes (2000-2011), by the Academy of Finland. Financial support from PCC BIORAFF, the Academy of Finland, Umeå University, Kempe Foundations, Knut and Alice Wallenberg Foundation is gratefully acknowledged.

REFERENCES

[1] K. Niemelä and E. Sjöström, *Carbohydr. Res.*, **180**, 43 (1988).
[2] T. Heinze, U. Erler, I. Nehls and D. Klemm, *Angew. Macromol. Chem.*, **215**, 93 (1994).
[3] P. Käuper, W-M. Kulicke, S. Horner, B. Saake, J. Puls, J. Kunze, H-P. Fink, U. Heinze, T. Heinze, E-A. Klohr, H. Thielking and W. Koch, *Angew. Macromol. Chem.*, **260**, 53 (1998).
[4] A. Parfondry and A. S. Perlin, *Carbohydr. Res.*, **57**, 39 (1977).
[5] J. Reuben and T. Conner, *Carbohydr. Res.*, **115**, 1 (1983).
[6] T. R. I. Cataldi, C. Campa and G. E. De Benedetto, *J. Anal. Chem.*, **368**, 739 (2000).
[7] E. A. Kragten, J. P. Kamerling and J. F. G. Vliegenthart, *J. Chromatogr.*, **623**, 49 (1992).
[8] B. Saake, S. Horner, J. Puls, T. Heinze and W. Koch, *Cellulose*, **8**, 59 (2001).
[9] T. Salmi, D. Valtakari, E. Paatero, B. Holmbom and R. Sjöholm, *Ind. Eng. Chem. Res.*, **33**, 1454 (1994).