UDC: 543.544.3:[544.412.3:661.728.8]
review paper

Acta Agriculturae Serbica, Vol. XII, 24 (2007) 59-68

Gas Chromatographic Determination of the Degree of Substitution of CMC

Z. Markovic
Faculty of Agronomy, Cacak

Svetlana Markovic
Faculty of Science, Kragujevac

Snezana Zlatanovic
Holding Institute of General and Physical Chemistry, Beograd

Abstract: A procedure for laboratorial determination of the degree of substitution of carboxymethylcellulose (CMC) was developed. The procedure was based on the hydrolysis of CMC and silylation by means of trifluorobis(trimethylsilyl)acetamide, enabling a chromatographic analysis of obtained sugars and determination of the degree of substitution of CMC.

Key words: CMC, degree of substitution, gas chromatography.

Introduction

Carboxymethylcellulose (CMC) is an ether of cellulose obtained by a reaction of the sodium salt of cellulose with sodium monochloroacetate, under strictly controlled conditions.

$$Cellulose-OH+NaOH \rightleftarrows Cellulose-ONa+H_2O$$

$$Cellulose-ONa+ClCH_2COONa \rightarrow Cellulose-OCH_2COONa+NaCl$$

CMC is a very important product of chemical industry. The world production of water soluble cellulose ethers is in the order of hundreds of thousands tons per year. Carboxymethylcellulose represents more than 70% of the produced derivatives. There are a lot of commercial types of CMC, with different degrees of substitution (in the range of 0.5 – 1.4), purity and rheological characteristics in water solutions. More than 300 different types of CMC are produced all over the world. CMC is usually used in the food industry in order to increase viscosity, condensation and stabilisation (Stojanovic and Stojanovic 2002). Additionally, CMC is used in the production of drugs, ceramics, detergents, paper, textile and tobacco.

Fig. 1. shows the structure of cellulose molecule. Cellulose is a polymer chain made of the cellobiose units. A cellobiose unit consists of two anhydroglucose units. Every anhydroglucose unit has 3 hydroxyl groups. Carboxymethylcellulose is obtained by the substitution of one or more hydrogen atoms in hydroxyl groups with carboxymethyl groups (Fig. 1.).

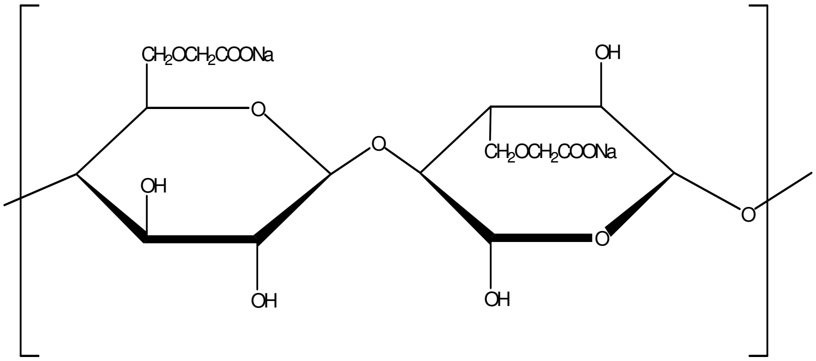

Fig. 1. The ideal structure of CMC unit with the degree of substitution of 1.0

It is evident that this substitution can produce 8 differently substituted monomers of cellulose, as follows: 1 unsubstituted (S_0), 3 monosubstituted (S_2, S_3 and S_6), 3 disubstituted (S_{23}, S_{26} and S_{36}) and 1 trisubstituted product (S_{236}). These monomers are shown in Fig. 2.

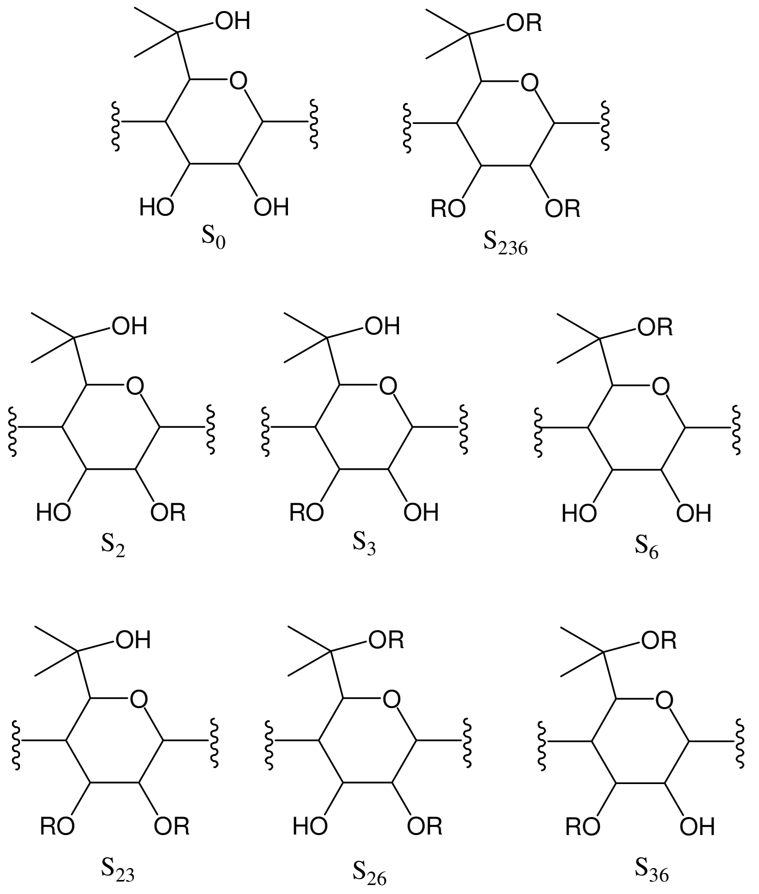

Fig. 2. The products of the substitution of hydrogen atoms of hydroxyl groups by means of carboxymethyl groups in monomeric units of cellulose

The average number of substituted hydroxyl groups in anhydroglucose unit is known as the degree of substitution (DS). In order to calculate the DS we need to define a fractional degree of substitution (f_i, where i represents C atoms where substitution is performed). According to the definition, f_2, f_3 and f_6 are related to relative quantities of corresponding glycosides, conforming to the following simple relations:

$$f_2 = s_2 + s_{23} + s_{26} + s_{236} \tag{1}$$

$$f_3 = s_3 + s_{23} + s_{36} + s_{236} \tag{2}$$

$$f_6 = s_6 + s_{26} + s_{36} + s_{236} \tag{3}$$

The average degree of substitution is equal to the sum of the fractional degrees of substitution:

$$DS = f_2 + f_3 + f_6 = (s_2 + s_3 + s_6) + 2(s_{23} + s_{26} + s_{36}) + 3(s_{236}) \qquad (4)$$

From equation (4) we can conclude that:

$$DS = S_I + 2S_{II} + 3S_{III} \qquad (5)$$

where S_I, S_{II} and S_{III} represent the respective quantities of monosubstituted, disubstituted and trisubstituted products.

Maximal theoretical value of DS is 3.0, in the case when all hydroxyl groups in the molecule of cellulose are replaced. In practice, such a result is impossible to obtain. The optimal solubility in water and other useful physical characteristics of CMC are achieved at the substitution degree far lower than 3.

The most important characteristic of the water solution of CMC is viscosity. Viscosity of CMC solution grows rapidly with the increase of the molecular mass. Since a molecular mass of the polymer is determined by the degree of polymerisation and the degree of substitution, we can conclude that the characteristics of the water solution of CMC are mainly dependent on DP and DS. (Klemn at al., 1998; Dapia at al., 2005)

For this reason the industrial practice requests simple analytical method of DS tracking in the production process of CMC. Our goal was to develop such a method, based on gas chromatography.

Hydrolysis of CMC and analysis of the products of hydrolysis

In order to determine the contents of glucose and carboxymethyl glucoses it is necessary to perform a hydrolysis of CMC, yielding the mixture of corresponding monomers. Acid catalysed hydrolysis has a very important place in the chemistry of the polymer carbohydrates (Heftmann 1967; Stojanovic and Stojanovic 2002). Acidic hydrolysis is successfully accomplished by means of $HClO_4$, H_2SO_4 and HCl. (Heftmann 1967; Stojanovic and Stojanovic 2002). Acid catalysed hydrolysis of CMC is a complex heterogenic reaction, schematically presented in Fig. 3.

Acidic hydrolysis goes in three steps. The reaction starts with an attack of an acid proton on the oxygen joining two sugar units, which is followed with a cleavage of the C-O bond. After an addition of water, sugars and the proton are released.

The products of hydrolysis of CMC are different substituted and unsubstituted sugars, where glucopyranoses are dominant components (Fig. 2.). Xylopyranoses and mannopyranoses, as well as the sugars of furanose type, produced in competitive reactions of degradation, are also present in the reaction mixture. It is well known that solutions of carbohydrates are susceptible to mutarotation. Consequently, an originally pure sugar can result into equilibrium mixture consisting of a linear form, and α- and β-anomers of piranose and furanose type.

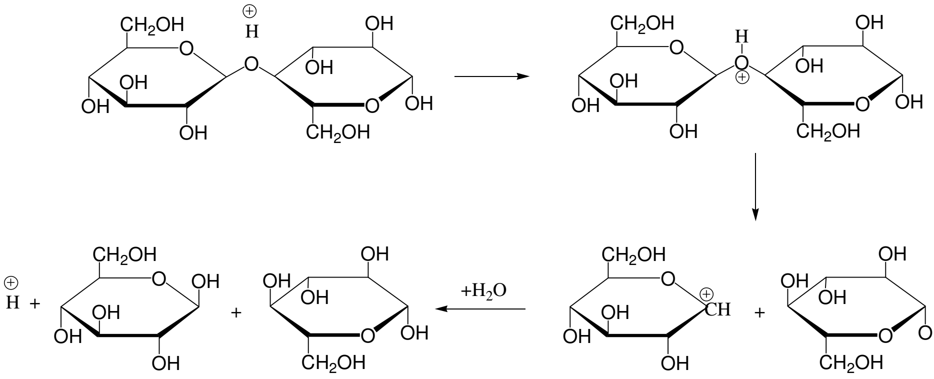

Fig. 3. The mechanisam of acid catalysed hydrolysis of CMC.

The determination of individual carbohydrates is very important. For this purpose many methods have been used, such as paper chromatography, thin layer chromatography, column chromatography, and gas chromatography (Heftmann 1967; Dickes and Nicholas 1976; Laker 1980; Honda, 1984; Patoraux-Prome and Prome 1984; Zeller et al., 1991; Rinaudo et al., 1993).

The application of gas chromatography to carbohydrates has caused more difficulties, as compared to other classes of organic compounds. The main problem is the poor evaporability of the polar compounds, and the fact that they cannot be easily transformed to evaporable compounds in quantitative amounts. A development of the reagents for derivation of carbohydrates started in early sixties of the last century. In gas chromatography, the trimethyilsilyl derivatives (TMS) are typically used as evaporable compounds of sugars (Sweeley 1963; McInnes et al., 1958; Robards and Whitelaw 1986; Sweeley 1963; Anderle 1970) was the first to apply this method to pentoses and the hexoses. Pyridine was used as a main solvent in the reaction of silylation. Some other solvents were also used, such as DMSO and dimethylformamide (Reid et al., 1970; Ramos 2005). Their advantage over pyridine is better chromatographic performance, the loss of bad odor, and lower speed of anomerization (Pierce 1968; Kim at al., 1967; Mourits et al., 1976). The appearance of more than one peak by sugar due to mutarotation, represents a difficulty in the analysis of carbohydrates.

Experimental

Materials. In this work the following samples of CMC were used: CMC-IPA, CMC-IPAR, CMC-8058, CMC-8058OP and CMC-M59.

Hydrolysis and silylation of carboxymethylcellulose. The solution of 0.0030 g of carboxymethylcellulose in 0.03 ml of 6M hydrochloric acid was stirred for two hours at room temperature, then diluted with 0.6 ml of water (to achieve 2M solution), and heated at 120°C for 30 min, while constantly stirring.

The solution was evaporated under the reduced pressure, at the temperature of 60°C. 0.3 ml of dry pyridine and 0.3 ml of trifluorobis(trimethylsilyl) acetamide with 5% of chlorotrimethylsilane were added to the dry residue. This mixture was stirred for 1 hour at room temperature, then analysed by means of gas and gas-mass chromatography.

The hydrolysis of CMC was also performed using perchloric, acetic and sulphuric acids. However, in these hydrolyses the degradation of CMC was a far more significant reaction, in comparison to the hydrolysis with hydrochloric acid. Instead of pyridine, DMSO and dimethylformamide were also used, but pyridine showed the best solvent characteristics in the silylation reaction of the mentioned samples of CMC.

Chromatography. Gas chromatography was used for qualitative and quantitative evaluation of silylated products of hydrolysis of CMC. The components of the mixture were identified by means of standard referent solution (Restek Corporation, Bellefonte, PA, USA), and the analysis was confirmed by means of gas chromatography-mass spectrometry.

Gas chromatography was performed by means of gas chromatograph 8610C (SRI Instruments, Torrance, CA, USA). The instrument was equipped with capillary column Zebron ZB-1 (30m x 0.53 mm I.D.) with 100% poly(dimethylsiloxane) as a stationary phase (df =1.5 µm; Phenomenex, USA). Helium at 8 psi pressure was used as a carrier gas. The analysis was performed using the following temperature programme: starting temperature was 145°C for 2 min, and then the column was heated 20°C/min up to 255°C. The column was kept at the final temperature for 2 min. The temperature of detector was 280°C. Flame-ionisation detector was used. Acquisition and data processing were performed by means of the program package PeakSimple Chromatography Data System for gas and liquid chromatography, version 1.88.

The identification of compound was performed by means of gas chromatography-mass spectrometry on the instrument Saturn 2000 GC-MS/MS (Varian, Walnut Creek, USA). The instrument was equipped with the same column and worked under the same regime and temperature program as the case of gas chromatograph. Mass spectrometer worked in the TIC (total ion current) mode, scanning from 40 to 600. Acquisition and data processing were performed by means of the program package Saturn 2000, version 5. The identification of products of hydrolysis was based on interpretation of mass spectra.

Results and discussion

Gas chromatograms of the carboxymethylcellulose hydrolysis products contained 28 peaks identified as described above, and some minor unidentified peaks. Mutarotation during the hydrolysis explains the formation of the α-pyranose compounds, and it is well known that their trimethylsilyl derivatives are eluted faster than the corresponding β-anomers. The majority of compounds were of the pyranose type, but several compounds of the furanose type were found in small amounts as well. Gas chromatogram of the hydrolytic process of CMC-8058 is shown in Fig. 4.

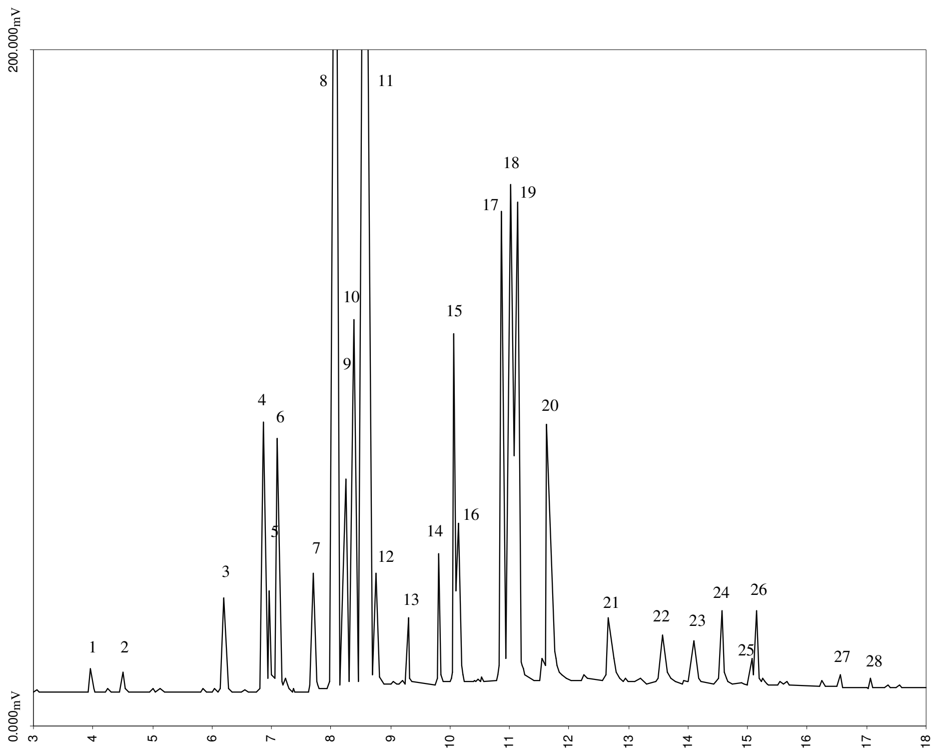

Fig. 4. Gas chromatogram of trimetylsilylated compounds obtained by the hydrolysis of the CMC-8058 sample. The names of the identified compounds are given in the text.

The products of hydrolysatis of the CMC samples examined in this work included: **1** α-D-xylofuranose, **2** β-D-xylofuranose, **3** α-D-xylopyranose, **4** β-D- xylopyranose, **5** α-D-glucofuranose, **6** α-D-mannopyranose, **7** β-D-glucofuranose, **8** α-D-glucopyranose, **9** β-D-mannopyranose, **10** 2-O-CM-α-D-xylopyranose, **11** β-D-glucopyranose, **12** 2-O-CM-β-D-xylopyranose, **13** 2-O-CM-α-D-mannopyranose, **14** 3-O-CM-α-D-glucopyranose, **15** 2-O-CM-α-D-glucopyranose, **16** 2-O-CM-β-D-mannopyranose, **17** 3-O-CM-β-D-glucopyranose, **18** 2-O-CM-β-D-glucopyranose, **19** 6-O-CM-α-D-glucopyranose, **20** 6-O-CM-β-D-glucopyranose, **21** 2,3-di-O-CM-α-D-glucopyranose, **22** 2,3-di-O-CM-β-D-glucopyranose, **23** 3,6-di-O-CM-α-D-glucopyranose, **24** 2,6-di-O-CM-α-D-glucopyranose, **25** 3,6-di-O-CM-β-D-glucopyranose, **26** 2,6-di-O-CM-β-D-glucopyranose, **27** 2,3,6-3-O-CM-α-D-glucopyranose and **28** 2,3,6-3-O-CM-β-D-glucopyranose.

One can conclude that the Zebron ZB-1 column showed very good results in the separation of sillylated sugars in examined reaction mixtures. Worse separation was observed in the case of compound pairs 15-16, 18-19, and 25-26.

Relative quantities of compound **1-28** in each sample of CMC were obtained by the integration of all peaks. The degrees of substitution of examined samples of CMC were calculated by including these values in equation (5).The results for DS are presented in Tab. 1.

Tab.1. Degree of substitution (DS) of different samples of CMC.

Sample of CMC	IPA	IPAR	8058	8058OP	M59
DS	0,58	0,53	0,77	0,66	0,69

By inspecting Table 1 one can conclude that all tested samples are poorly substituted carboxymethylcelluloses, particulary CMC-IPA and CMC-IPAR.

Conclusion

This developed procedure for the analysis of CMC by means of gas chromatography is a very simple, quick, and low cost technique for the control of the production process of CMC. The procedure is based on the hydrolysis of CMC by means of hydrochloric acid and derivation of obtained sugars using trifluorobis(trimethylsilyl)acetamide.

References

Anderle D., Kovač P., Andrelová H. (1972) Separation of some methyl O-methyl-xylofuranosides by gas chromatography; *J. chromatogr.*, 64, 368-370.

Dapia S., Tovar C. A., Santos V., Parajo J. C. (2005) Rheological behaviour of carboxymethylcellulose manifactured from TCF-bleached Milox pulps; *Food Hydrocolloides*, 19, 313-320.

Dickes G.J., Nicholas P.V. (1976) *Gas Chromatography in Food Analyses.* Butterworth, London, Ch. 15.

Ericsson A., Hansen J., Dalgaard L. (1978) A routine method for quantitative determination of soluble carbohydrates in small samples of plant material with gas-liquid chromatography. *Anal. Biochem.*, 86, 552-560.

Heftmann E. (1967) *Chromatography.* Van Nostrand-Reinhold, New York, Ch 20.

Honda S. (1984) High-performance liquid chromatography of mono- and oligosaccharides. *Anal. Biochem.*, 140, 1-47.

Kim S.M., Bentley R., Sweeley C. C. (1967) Formation of partially trimethylsilylated derivatives of carbohydrates. The trimethylsilyl tris-o-trimethylsilyl-glucopyranosides and tetrakis-o-trimethylsilyl-glucose. *Carbohydr. Res.*, 373-386.

Klemn D, Philipp B., Heinze T.,. Heinze U, Wagenknecht W. (1998) *Comprenhensive cellulose chemistry, Functionalization of cellulose.* Wiley-VCH.

Laker M.F. (1980) Estimation of neutral sugars and sugar alcohols in biological fluids by gas-liquid chromatography. *J. Chromatogr.*, 184, 457-470.

McInnes A.G., Ball D.H., Cooper F.P. Bishop C. T. (1958) Separation of carbohidrate derivatives by gas-liquid partition chromatography. *J. Chromatogr.* 1, 556-557.

Mourits J.W, Merkus H.G., Galan L.D. (1976) Gas chromatographic dete-rmination of hydroxyethyl derivatives of glucose. *Anal. Chem.*, 1557–1562.

Patoraux-Prome D., Prome J.C. (1984) *Gas Chromatography-Mass spectro-scopy: Applications in Microbiology*. Plenum New York, pp. 105-1056.

Pierce A.E. (1968) *Silylation of Organic Compounds*. Pierce Chemical Co., Rockford IL.

Ramos L.A., Frollini E. Heinze Th. (2005) Carboxymethylation of cellulose in the new solvent dimethyl sulfoxide/tetrabutylammonium fluoride. *Carbohydr. Polymers*, 1-9.

Reid P.E., Donaldson B., W. Secret D., Bradford B. (1970) A simple, rapid, isothermal gas chromatographic procedure for the analysis of monosaccharide mixtures. *J. Chromatogr.*, 47, 199-208.

Rinaudo M., Danhelka J., Milas M. (1993) A new approach to characterising carboxymethylcelluloses by size exclusion chromatography. *Carbohydr. Polymers*, 21, 1-5.

Robards K., Whitelaw M. (1986) Chromatography of monosaccharides and disaccharides. *J. Chromatogr.*, 373, 81-110.

Stojanović O., Stojanović N. (2002) *Hemija ugljenih hidrata*. Univerzitetska štampa, Beograd (in Serbian).

Sweeley C. C., Bentley R., Makita M., Wells W. W. (1963) Gas-Liquid Chromatography of Trimethylsilyl Derivatives of Sugars and Related Substances. *J. Am. Soc.*, 85, 2497-2507.

Zeller S.G., Greisgraber G. W., Gray G. R. (1991)Synthesis and mass spectra of 4-O-acetyl-1,5-anhydro-2,3,6-tri-O-(methoxycarbonylmethyl)-glucitol and the positionalisomers of 4-O-acetyl-1,5-anhydro-di-O (methoxycarbonylmethyl)-O-methyl-glucitol and 4-O-acetyl-1,5-anhydro-O-(methoxycarbonylmethyl)di-O-methyl-glucitol.*Carbohydr. Res.*, 211, 47-57.

GASNO HROMATOGRAFSKO ODREĐIVANJE STEPENA SUPSTITUCIJE CMCa

- originalni naučni rad -

Z. Markovic
Agronomski fakultet, Čačak

Svetlana Markovic
Prirodno-matematički fakultet, Kragujevac

Snezana Zlatanovic
Institut za opštu i fizičku hemiju, Beograd

Rezime

Razvijen je postupak za laboratorijsko određivanje stepena supstitucije karboksimetil celuloze (CMCa). Postupak se zasniva na hidrolizi CMCa pomoću hlorovodonične kiseline i sililovanju pomoću trifluorobis(trimetil-silil)acetamida, čime je omogućena hromatografska analiza dobijenih šećera i određivanje stepena supstitucije CMC-a. Pomoću ove metode je moguće pratiti proces karboksimetilovanja celuloze, a samim tim na indirektan način i uticati na promene u tehnološkom procesu sa ciljem dobijanja proizvoda koji će posedovati osobine koje su potrebne za njegovu dalju primenu.