# Note

# Gas–liquid chromatographic analysis of carboxymethylcellulose and carboxymethylstarch

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Carboxymethylcellulose (CMC) and carboxymethylstarch (CMS) have been employed as important industrial polymers in textile processing, detergents, drilling fluids and protective coatings. The purified grades have been used extensively in the pharmaceutical, cosmetic and food industries. The suitability of CMC or CMS in actual applications is influenced by the degree of substitution (the average number of carboxymethyl groups substituted per anhydroglucose unit) and the distribution of the carboxymethyl substituents. Various methods have been reported for the determination of the DS (degree of sustitution) in CMC and CMS, such as chemical titration[1-4], spectrophotometry[1,5] and electrometric titration[1,6]. However, these methods determine only the DS without providing information on the distribution of the carboxymethyl substituents. Recently, Ho and Klosiewicz[7] reported on the distribution of the substituents on CMC by proton nuclear magnetic resonance spectrometry.

This paper deals with determination of the DS and the distribution of the carboxymethyl groups in both CMC and CMS by a gas–liquid chromatographic (GLC) method based on a modification of previous work[8].

## EXPERIMENTAL

### Reagents

All chemicals were of analytical-reagent grade. Sodium borohydride was purchased from Kishida (Osaka, Japan) and other reagents from Nacalai Tesque (Kyoto, Japan). Amberlite CG-120 was purchased from Organo (Tokyo, Japan). CMC (DS = 0.6–0.8) and CMC (DS = 1.0–1.5) were kindly provided by Daicel (Osaka, Japan) and CMS (DS = 0.3–0.5) was obtained from Nikka (Fukui, Japan).

### Evaporation

All evaporations were conducted under reduced pressure at bath temperatures not exceeding 40°C.

### Reduction of carboxymethylglucans

CMC (DS = 0.6–0.8) (100 mg) was dissolved in water (30 ml), then 1-ethyl-3-

(3-dimethylaminopropyl)carbodiimide (EDC) (1 g) was added. As the reaction proceeded, the pH of the reaction mixture was maintained at 4.75 by titration with 0.1 $M$ hydrochloric acid (HCl) with stirring for 2 h. Then 2 $M$ sodium borohydride (NaBH$_4$) (10 ml) was added slowly to the reaction mixture at room temperature. The pH of the mixture rose rapidly to 7.0 and the mixture was maintained at this pH by titration with 4 $M$ HCl. The solution was dialysed against water overnight, then the non-dialysable solution was concentrated to *ca.* 30 ml. The product was reduced twice more under the same conditions. The final non-dialysable solution was lyophilized to yield 85.3 mg as the hydroxyethyl product HCMC-1. CMC (DS = 1.0–1.5) (100 mg) was reduced under the same conditions to give 82.2 mg as the hydroxyethyl product HCMC-2. CMS (DS = 0.3–0.5) (100 mg) was treated in the same manner to yield 62.3 mg as hydroxyethyl product HCMS.

*Preparation for GLC analysis*

Reduced carboxymethylglucans (5 mg), *i.e.,* hydroxyethylglucans (HCMC-1, HCMC-2 and HCMS), were hydrolysed with 90% formic acid (1.5 ml) at 100°C for 5 h and the solution was evaporated to dryness. The residue was dissolved in 2 $M$ trifluoroacetic acid (TFA) (1 ml) and heated at 100°C for 4 h. The solution was evaporated to dryness and the dried hydrolysates were reduced with NaBH$_4$ (5 mg) in water (2 ml) for 3 h at room temperature, then passed through a column of Amberlite CG-120(H$^+$). The eluate was evaporated to dryness and the resulting boric acid in the residue was removed as trimethyl borate by repeated evaporation with methanol. The sample was dried and acetylated with acetic anhydride–pyridine (1:1) (1 ml) at 95°C for 2 h. Toluene (1 ml) was added to the reaction mixture in order to remove the residual acetic anhydride and pyridine as an azeotropic mixture[8], then evaporated to dryness. The residue was dissolved in chloroform (50 $\mu$l) and the solution was injected into the gas chromatograph.

*Gas–liquid chromatography*

The samples were analysed using a Shimadzu 4 CM chromatograph equipped with a hydrogen flame ionization detector. A glass column (1.5 m × 0.3 cm I.D.) packed with 2% EGSS-X on Chromosorb W AW DMCS (60–80 mesh) was used at 215°C and a nitrogen flow-rate of 60 ml/min. Peak areas and retention times were measured by use of a Shimadzu Chromatopac C-R5A integrator. The samples were also analysed with a CP-Sil 88 FS-WCOT fused-silica capillary column (25 m × 0.25 mm I.D.) with temperature programming (after injection at 180°C, held for 70 min, the temperature was increased at 0.5°C/min to 190°C, held for 70 min, at 0.5°C/min to 200°C held for 70 min, at 0.5°C/min to 210°C, held for 70 min, and finally at 0.5°C/min to 220°C) at a pressure of 60 kPa (1.3 kg/cm$^2$) of helium, with a splitting ratio of 1:157.

RESULTS AND DISCUSSION

The reduction of the carboxymethylglucans (CMC and CMS) to the hydroxyethylglucans (HCMC and HCMS) was carried out by the method used by Taylor and co-workers[9,10] for the reduction of the carboxyl group of uronic acid residues in polysaccharides. The carboxymethylglucans were reacted with 1-ethyl-3-(3-dimethyl-

TABLE I

GLC AND GLC–MASS SPECTROMETRY OF SUGAR DERIVATIVES FROM HYDROLYSATES OF REDUCED CARBOXYMETHYLGLUCAN

GLC–mass spectrometry was carried out as described previously[8].

| Peak No. | Relative retention time[a,b] | Sugar derivatives[c] (as acetate) | Prominent fragments (m/z) | Percentage from peak area[a,b] | | |
|---|---|---|---|---|---|---|
| | | | | HCMC-1 | HCMC-2 | HCMS |
| 1 | 0.62(0.58) | 1,2-O-etn-α-D-Gf | 43 73 127 170 187 212 273 | 2.98 | 3.41 | 5.41 |
| 2 | 0.77(0.59) | 1,2-O-etn-α-D-Gp | 43 73 86 157 170 199 230 259 272 | 5.39 | 5.95 | 9.24 |
| 3 | 1.00(1.00) | G | 43 73 145 217 289 361 375 | 39.94 | 18.50 | 61.79 |
| 4 | 1.64(1.32) | 1,2-O-etn-β-D-Gp | 43 73 86 157 170 199 230 259 272 | 2.11 | 1.52 | 3.96 |
| 5 | 1.91(1.54) | 1,2-O-etn-6-O-he-α-D-Gf | 43 73 87 127 187 | 1.82 | 2.88 | 0.53 |
| 6 | 2.54(1.70) | 1,2-O-etn-6-O-he-α-D-Gp | 43 73 86 87 157 199 274 316 | 4.46(2.99) | 7.23(5.31) | 1.76 |
| | (1.95) | 1,2-O-etn-3-O-he-α-D-Gf | 43 73 87 127 170 212 272 | (1.47) | (1.92) | |
| 7 | 2.92(2.21) | 6-O-he-G | 43 87 115 117 375 | 14.43 | 13.52 | 1.69 |
| 8 | 3.34(2.43) | 2-O-he-G | 43 73 87 115 189 375 405 | 17.67(9.41) | 21.12(12.39) | 15.72(10.18) |
| | (2.53) | 3-O-he-G | 43 87 115 261 333 375 | (8.26) | (8.73) | (5.54) |
| 9 | 3.88(2.73) | 1,2-O-etn-3-O-he-D-Gp | 43 73 86 87 157 170 243 | 2.63 | 3.62 | 0.77 |
| 10 | 4.99(2.97) | 1,2-O-etn-6-O-he-β-D-Gp | 43 73 86 87 157 199 274 316 | 1.90 | 2.83 | 0.11 |
| 11 | 10.15(3.95) | 2,3-O-dihe-G | 43 73 87 189 305 375 449 | 6.67 | 20.22 | |

[a] Relative to hexa-O-acetyl-D-glucitol.
[b] Values in parentheses were obtained with a capillary column (CP-Sil 88).
[c] Glucitol (G) derivatives except for 1,2-ethyleneglucose (Gf and Gp) derivatives.

aminopropyl)carbodiimide in aqueous media and the resulting carbodiimide-activated carboxymethylglucans were reduced with $NaBH_4$ to yield the hydroxyethylglucans. From the results of carbon-13 nuclear magnetic resonance spectroscopy of the reduced carboxymethylglucans (hydroxyethylglucans) in 2 $M$ $NaO^2H$, the complete reduction of carboxymethyl groups were confirmed by there being no signal in the carbonyl region of the spectrum. These reduced carboxymethylglucans were hydrolysed with formic acid and then TFA. In the acidic hydrolysis, the resulting 2-O-hydroxyethylglucose derivatives were partially converted into 1,2-O-ethyleneglucose derivatives[8]. The hydrolysates thus obtained were reduced with $NaBH_4$ and acetylated with acetic anhydride in pyridine.

    The sugar derivatives from the acid hydrolysates of reduced CMC (DS = 0.6–0.8) (HCMC-1) were separated with a 2% EGSS-X glass column. By reference to the retention time of authentic standards and mass spectral analysis[11], the individual peaks in Table I were identified with increasing retention time as follows: 1,2-O-ethylene-α-D-glucofuranose (1,2-O-etn-α-D-Gf), 1,2-O-ethylene-α-D-glucopyranose (1,2-O-etn-α-D-Gp), glucitol (G), 1,2-O-etn-β-D-Gp, 1,2-O-ethylene-6-O-hydroxyethyl-α-D-glucofuranose (1,2-O-etn-6-O-he-α-D-Gf), 1,2-O-ethylene-6-O-hydroxyethyl-α-D-glucopyranose (1,2-O-etn-6-O-he-α-D-Gp), 1,2-O-etn-3-O-he-α-D-Gf, 6-O-hydroxyethylglucitol (6-O-he-G), 2-O-he-G, 3-O-he-G, 1,2-O-etn-3-O-he-D-Gp, 1,2-O-etn-6-O-he-β-D-Gp, 2,3-di-O-hydroxyethylglucitol (2,3-O-dihe-G). 2-O-He-G and 3-O-he-G could not be separated satisfactorily with this 2% EGSS-X glass column, so they were also analysed with a CP-Sil 88 capillary column. The assignment of 2-O-he-G for peak 8 reported previously[8] was corrected to overlap of 2-O-he-G and 3-O-he-G, and the assignment of 3-O-he-G for peak 9 was corrected to 1,2-O-etn-3-O-he-D-Gp from the present study with a capillary column. Also, the previous peak 6 was separated into 1,2-O-etn-6-O-he-α-D-Gp and 1,2-O-etn-3-O-he-α-D-Gf by the capillary column. As reported previously[8], the GLC analysis of hydroxyethylcellulose and hydroxyethylstarch synthesized by the reaction of ethylene oxide with cellulose and starch gave 2-O-(2′-hydroxyethoxy)ethylglucitol and its derivatives. However, with the hydroxyethylglucans (HCMC and HCMS) prepared by the reduction of CMC and CMS, no such compounds are afforded. CMC (DS = 1.0–1.5) and CMS (DS = 0.3–0.5) were reduced under the same conditions to give HCMC-2 and HCMS, and analysed by the same GLC method as HCMC-1. The sugar derivatives of each sample are summarized in Table I. The distribution patterns of carboxymethyl groups in each glucan are also shown in Table I. The carboxymethyl groups were located mainly at O-2 and O-2,3 of the glucosyl residue in CMC, and at O-2 of the glucosyl residue in CMS. The proportion of 6-O-carboxymethylglucosyl residue in CMC was greater than that in CMS. The DS values of these samples measured by this method are similar to those listed in commercial products (see Table II).

    In conclusion, complete reduction of carboxymethyl groups to hydroxyethyl groups was carried out by the reaction of carboxymethylglucan with carbodiimide and sodium borohydride. The hydrolysates of the resulting hydroxyethylglucans were analysed by a GLC method as the acetates of alditol and 1,2-O-ethyleneglucose derivatives. As the substituted sugars in these carboxymethylglucans can be accurately determined, the DS and the distribution of carboxymethyl substituent can be determined satisfactorily. This method simplifies the determination of the degree and pattern of carboxymethyl substitution of CMC and CMS.

TABLE II

PATTERNS OF CARBOXYMETHYL SUBSTITUTION ON GLUCOSE UNIT IN CMC AND CMS

| Compound | Percentage of total | | | | | | |
|---|---|---|---|---|---|---|---|
| | Glc | 2-cmG[a] | 3-cmG | 6-cmG | 2,3-dicmG[b] | 2,6-dicmG[c] | DS[d] |
| HCMC-1 [CMC (DS 0.6–0.8)] | 39.94 | 19.89 | 8.26 | 14.43 | 13.76 | 3.72 | 0.78 |
| HCMC-2 [CMC (DS 1.0–1.5)] | 18.50 | 23.27 | 8.73 | 13.52 | 31.07 | 5.71 | 1.19 |
| HCMS [CMS (DS 0.3–0.5)] | 61.79 | 28.79 | 5.54 | 1.69 | 0.87 | 1.30 | 0.40 |

[a] Total of 2-he-G: 1,2-O-etn-α-D-Gf, 1,2-O-etn-α-D-Gp, 1,2-etn-β-D-Gp, 2-O-he-G.
[b] Total of 2,3-dihe-G: 1,2-O-etn-3-O-he-α-D-Gf, 1,2-O-etn-3-O-he-D-Gp, 2,3-O-dihe-G.
[c] Total of 2,6-dihe-G: 1,2-O-etn-6-O-he-α-D-Gf, 1,2-O-etn-6-O-he-α-D-Gp, 1,2-etn-6-O-he-β-D-Gp.
[d] Degree of substitution.

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