

STUDY ON THE CHARACTERIZATION OF DISTRIBUTION OF SUBSTITUENTS ALONG THE CHAIN OF CARBOXYMETHYL CELLULOSE

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ABSTRACT

Four new parameters, $\overline{L}_{S_n}$ and $\overline{L}_{G_n}$, $\overline{L}'_{S_n}$ and $\overline{L}'_{G_n}$ have been proposed to describe the average length of the sequence of substituted anhydroglucose units and that of unsubstituted ones respectively along the CMC chain. The methods for determining those parameters have also been presented. Six CMC samples have been characterized in terms of intrinsic viscosity, degree of substitution (DS), index of substitution (SI) and $\overline{L}_{S_n}$, $\overline{L}_{G_n}$, $\overline{L}'_{S_n}$, $\overline{L}'_{G_n}$. It has been proved experimentally that $\overline{L}_{S_n}$, $\overline{L}_{G_n}$, $\overline{L}'_{S_n}$, $\overline{L}'_{G_n}$ not only can describe the sequence distribution along the CMC chain, but also can be used for calculating SI and estimating the average degree of substitution in the substituted glucose rings.

INTRODUCTION

Carboxymethylcellulose (CMC) is an important water-soluble polyelectrolyte widely applied in textiles, detergents, cosmetics, food, paper and well drilling operations. Most properties of CMC in actual application depend to a large extent on the degree of substitution (DS) of the hydroxyl groups on the anhydroglucose unit and the distribution of the carboxymethyl substituents along the polymer chain (sequence distribution).

The DS of CMC can be determined by a number of analytical techniques, of which the most commonly used are ashing-method^[1], conductometric titration method^[2]. All these methods give only the DS value without providing any information on sequence distribution. Some papers^[3-6] had pointed out the possibility of determining the sequence distribution of CMC based on the principle that CMC will be cleaved by cellulase merely between two adjacent unsubstituted groups, but few concrete results had been given. Gelman^[7] had characterized the distribution of substituents of CMC by a procedure using enzymatic degradation techniques, monitored by viscosity and determination of reducing sugars, but, the length of the sequence of unsubstituted anhydroglucose units (UAHG) and that of substituted anhydroglucose units (SAHG) could not be obtained simultaneously. In this paper we propose four parameters $\overline{L}_{S_n}$, $\overline{L}'_{S_n}$ and $\overline{L}_{G_n}$, $\overline{L}'_{G_n}$ to describe the average length of SAHG sequence and UAHG sequence respectively, and develop the analytical

where W denotes the weight of CMC sample, M_{CMC} ($= 162.14 + 80.02\text{DS}$) denotes the mol. wt. of structural unit, -2 in the right-hand side of Eq. (1) accounts for that the two UAHG's at two ends in GSnG must be subtracted to get accurate value of Sn. As the so-called SAHG block here may consist of some sandwiched unsubstituted G ($\underline{G}$), $\overline{L}_{\text{Sn}}$ does not reflect the actual length of Sn block. Hence $\overline{L}_{\text{Sn}}$ is called the apparent length of substituted block.

According to Fig. 1, Gn and Sn (or GSnG) appear alternatively in a CMC chain, N_{Gn} should equal to N_{GSnG} or differ only by one which can be neglected when the chain is long enough. Therefore the average number of structural unit in an unsubstituted block is:

$$\overline{L}_{\text{Gn}} = \frac{N_{\text{G}}}{N_{\text{Gn}}} + 2 = \frac{N_{\text{G}}}{N_{\text{GSnG}}} + 2 \quad (2)$$

$+2$ in the right-hand side of equation (2) accounts for that the two UAHG's at two ends in GSnG must be added to unsubstituted block to get accurate value of Gn. Owing to the same reasons for $\overline{L}_{\text{Sn}}$, $\overline{L}_{\text{Gn}}$ does not reflect the actual length of Gn-block, it is called the apparent length of unsubstituted block.

2. $\overline{L}'_{\text{Sn}}$ and $\overline{L}'_{\text{Gn}}$

$\overline{L}'_{\text{Sn}}$ and $\overline{L}'_{\text{Gn}}$ are called the actual length of Sn and Gn respectively, without any $\underline{G}$ in the substituted block. It is obvious $\overline{L}'_{\text{Sn}} < \overline{L}_{\text{Sn}}$. The difference between $\overline{L}'_{\text{Sn}}$ and $\overline{L}_{\text{Sn}}$ depends on the number of $\underline{G}$. Now, let N_{G} denotes the number of $\underline{G}$ and N_{G} the total number of UAHG, then

$$N_{\text{G}} = N'_{\text{G}} - N_{\text{G}} - 2N_{\text{GSnG}} \quad (3)$$

$$\overline{L}'_{\text{Sn}} = \frac{\frac{W}{M} - N'_{\text{G}}}{N_{\text{GSnG}} + N_{\text{G}}} \quad (4)$$

$$\overline{L}'_{\text{Gn}} = \frac{N'_{\text{G}}}{N_{\text{GSnG}} + N_{\text{G}}} \quad (5)$$

where N_{G} and N_{GSnG} have the significances as before.

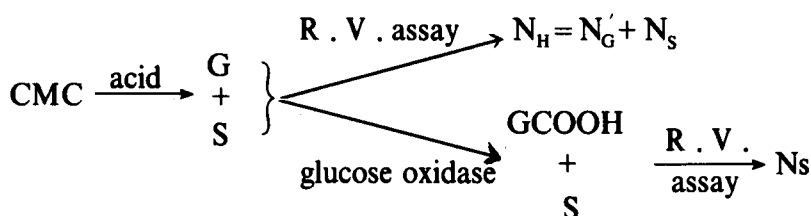
From Eq. (4) and Eq. (5), we get

$$\frac{\overline{L}'_{\text{Sn}}}{\overline{L}'_{\text{Sn}} + \overline{L}'_{\text{Gn}}} = 1 - N'_{\text{G}} = \text{SI} \quad (6)$$

N'_G being the fraction of unsubstituted glucose units in the sample weighed W . SI is called substitution index proposed by Wirick^[4] to express the percentage of substituted anhydroglucose units on cellulose derivatives.

3. Determination of N'_G

In order to calculate $\bar{L}'_{s_n}$ and $\bar{L}'_{G_n}$ by Eq. (4) and (5), it is necessary to get the value of N'_G . The determination of N'_G can be carried out by the complete scission of CMC into UAHG-units and SAHG-units by acid hydrolysis, followed by glucose analysis. The glucose analysis can be accomplished either by glucose oxidase-peroxidase method^[8] or D-glucose oxidase method^[9]. The latter one is adopted in this paper. The overall procedure is shown briefly in the following schematic diagram:



$$\therefore N'_G = N_H - N_S$$

4. Relationships between $\bar{L}_{s_n}$ and $\bar{L}'_{s_n}$, $\bar{L}_{G_n}$ and $\bar{L}'_{G_n}$

Substituting Eq. (3) into Eq. (1), (2), (4) and (5), and rearranging, we can get the following relationships:

$$\bar{L}_{s_n} = \frac{(1 - N'_G) + N'_G}{N'_{GSnG}} \quad (1')$$

$$\bar{L}_{G_n} = \frac{N'_G - N'_G}{N'_{GSnG}} \quad (2')$$

$$\bar{L}'_{s_n} = \frac{1 - N'_G}{N'_{GSnG} + N'_G} \quad (4')$$

$$\bar{L}'_{G_n} = \frac{N'_G}{N'_{GSnG} + N'_G} \quad (5')$$

It is obvious from Eq. (1') and Eq. (4')

$$\bar{L}_{s_n} > \bar{L}'_{s_n} \quad \text{when } N'_G \neq 0$$

$$\bar{L}_{s_n} = \bar{L}'_{s_n} \quad \text{when } N'_G = 0$$

and since $N'_G \gg N'_{GSnG}$ in most cases, it is evident from Eq. (2') and Eq. (5')

$$\bar{L}_{G_n} > \bar{L}'_{G_n} \quad \text{when } N'_G \neq 0$$

$$\bar{L}_{G_n} = \bar{L}'_{G_n} \quad \text{when } N'_G = 0$$

All these results are easily reasoned by intuition .

EXPERIMENTAL

1 . Preparation and characterization of CMC

Sodium carboxymethylcellulose was prepared by the reaction of monochloroacetic acid with alkali cellulose , and alkali cellulose was formed by reacting cellulose with sodium hydroxide . Both reactions were carried out in presence of isopropanol . Intrinsic viscosity of NaCMC solution was determined with Ubbelodhe viscosimeter at 25 °C with 0.1N NaCl as solvent^[4] . DS were determined by conductometric titration^[10] .

2 . Degradation of CMC by cellulase

The cellulase used was " Onozuku R-10 " (Serva Feinbiochemica , Heidelberg) . The enzymatic degradation was carried out in 0.02M HAc- NaAc (pH5.5) at 40 °C for 30h .

3 . Acid hydrolysis of CMC

CMC sample (50mg) was mixed with 0.5ml concentrated H₂SO₄ (72 %) in a testtube at room temperature for 4 h , then diluted with water to decrease the acid concentration to 5% . The testtube containing the solution was put in an autoclave at 145 °C for 1 h . The hydrolysate was neutralized with NaOH to pH 5— 6 after it was cooled to room temperature .

4 . Oxidation of the enzymatically degraded solution and acid-hydrolyzed solution of CMC in presence of glucose-oxidase

1 ml solution to be tested was mixed with 1 ml enzyme solution containing 2 mg glucose-oxidase and 1 mg hydrogen-peroxidase in 0.1 M KH₂PO₄ pH 7.2 . The oxidation reaction was carried out at 35 °C for 1 h with occasional agitation .

5 . Determination of reducing value of glucose residue

The reducing value of glucose residue was determined by DNS method^[11] , anhydrous glucose as standard and the concentration of glucose residue detected by spectrophotometer at 530 nm .

RESULTS AND DISCUSSION

1 . Experimental conditions

(1) Conditions for enzymatic degradation of CMC

In Gelman 's process^[7] , the concentration of sample CMC was 2×10^{-3} g /ml , enzyme /CMC = 1.25×10^{-3} (by wt .) in 0.02M KH₂PO₄ buffer (pH 5.5) at 25 °C and the degradation endured as long as 8 days . We tried to shorten the time by increasing the enzyme /CMC ratio and raising the degradation temperature with the results shown in Fig . 2 . Level-off of the curve reflects the completion of the

degradation . Then it can be seen the degradation time decreases to 50 h at 25 °C for enzyme/CMC = 0.01 (see curve 1) , to 30 h at 40 °C for enzyme/CMC = 0.01 (see curve 2) and to 30 h at 40 °C for enzyme/CMC = 0.02 (see curve 3) . It means that the temperature-effect is more prominent than ratio-effect . The condition finally chosen was : CMC sample concentration 5×10^{-3} g/ml , enzyme/CMC = 0.04 , at 40 °C for 30 h .

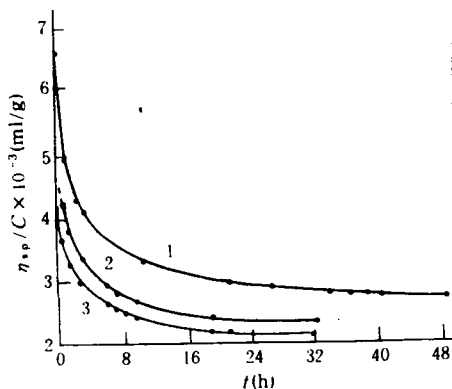


Fig. 2 Plot of η_{sp}/C vs. time for enzymatic degradation of CMC solution . CMC concn : 2×10^{-3} g/ml .

Buffer : 0.02M KH_2PO_4 , pH = 5.5 .

1 . 25 °C , enzyme/CMC = 0.01 (by wt .)

2 . 40 °C , enzyme/CMC = 0.01

3 . 40 °C , enzyme/CMC = 0.02

The concentration of glucose in enzymatic degradation solution can be detected by the absorbance at 530 nm after the solution reacting with DNS reagent^[11] . Glucose (or absorbance) should increase with the elapse of degradation time , but this was not the case when KH_2PO_4 was used as buffer as shown in Fig. 3 (a) (curve 1) , the absorbance decreased rather rapidly after 4 h . We supposed this might be attributed to the swallow of the released-glucose by microorganism , and tried to suppress this effect by adding a preservative (4-hydroxybenzoate) into the solution.

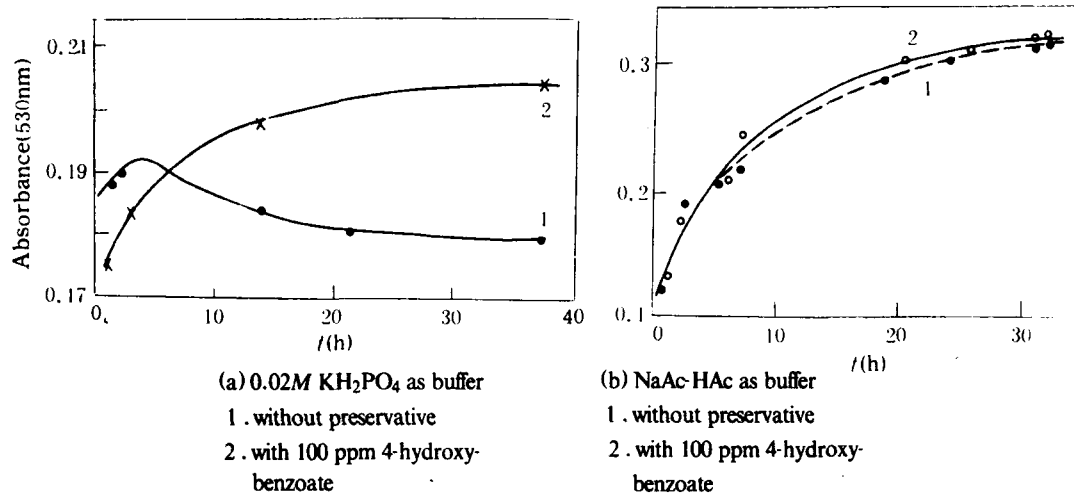
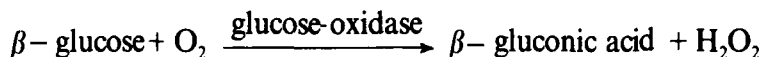


Fig. 3 The relationship between absorbance (reducing value) and degradation time for CMC solution

The result shown in curve 2 (Fig. 3(a)) was quite well as expected. Considering that P and K are the essential elements for the growth of microorganism and acetic acid has some bactericidal effect, we tried again to use NaAc-HAc as buffer instead of KH_2PO_4 . The results appeared even more satisfactory. In comparison of curve 1 and curve 2 in Fig. 3(b), it is obvious no preservative is required in NaAc-HAc system.

(2) Oxidation of analytes in presence of glucose-oxidase

Glucose-oxidase selectively oxidizes glucose into gluconic acid and hydrogen peroxide



Such oxidation can be accomplished merely with frequent agitation for more than 2h. This was probably due to the accumulation of H_2O_2 in solution inhibiting the progress of reaction. The situation was much improved when hydrogen peroxidase was added to decompose H_2O_2 in time. When 1 mg glucose, 2 mg glucose-oxidase together with 1 mg hydrogen-peroxidase in 2 ml phosphate buffer were oxidized at 35°C , the reaction could be completed within 1 h with occasional agitation (see Table 1).

Table 1 The dependence of absorbance at 530 nm on the time of glucose-oxidation in presence of glucose-oxidase

Oxidation time (h)	0	0.25	0.5	0.75	1.0
Absorbance (R.V.)	0.560	0.197	0.151	0.069	0.000

2. Relationships between the characterization parameters

The characterization parameters for six CMC samples are listed in Table 2.

Table 2 Characterization parameters of CMC

Sample	$[\eta]$ (dl/g)	DS	$\overline{L}_{S_n}$	$\overline{L}_{G_n}$	(SI) _a *	$\overline{L}'_{S_n}$	$\overline{L}'_{G_n}$	SI**	DS/SI
1	6.85	0.506	2.36	3.03	0.44	2.88	3.35	0.46	1.09
2	7.46	0.655	3.66	3.01	0.55	3.24	2.83	0.53	1.23
3		0.712	5.04	3.04	0.62	4.75	2.95	0.62	1.15
4	7.53	0.839	6.56	2.93	0.69	3.74	2.21	0.63	1.33
5	6.46	1.062	10.40	3.11	0.77	7.30	2.54	0.74	1.43
6	5.63	1.553	38.62	3.38	0.92	12.03	1.78	0.87	1.78

* calculated by $\overline{L}_{S_n} / (\overline{L}_{S_n} + \overline{L}_{G_n})$. ** calculated by Eq. (6).

(1) $\overline{L}_{S_n}$ and $\overline{L}'_{S_n}$, $\overline{L}_{G_n}$ and $\overline{L}'_{G_n}$

Data in Table 2 show that for samples 2 — 6, $\overline{L}_{S_n}$ and $\overline{L}_{G_n}$ are larger than the

corresponding $\overline{L}_{S_n}'$ and $\overline{L}_{G_n}'$, and the difference $\Delta = \overline{L}_{S_n} - \overline{L}_{S_n}'$ (or $\overline{L}_{G_n} - \overline{L}_{G_n}'$) becomes larger as $\overline{L}_{S_n}$ increases. It implicates that more G exists in the S_n block of larger $\overline{L}_{S_n}$,

which is consistent with the results inferred from Eq. (1') and Eq. (4'). As regards sample 1, $\overline{L}_{S_n} < \overline{L}_{S_n}'$ (or $\overline{L}_{G_n} < \overline{L}_{G_n}'$) seems unreasonable. This may be attributed to the experimental error introduced in the complicate procedure for the determination of these parameters. Or it can be deemed that no unsubstituted glucose rings are sandwiched in such short $\overline{L}_{S_n}$ for CMC of so low DS.

(2) $\overline{L}_{S_n}'$, $\overline{L}_{G_n}'$ and DS

It is interesting to find from Fig. 4 that $\overline{L}_{S_n}'$ increases rapidly with increasing DS particularly for $DS > 0.8$ while $\overline{L}_{G_n}'$ decreases very slowly. That means the alternation in condition of sample preparation does not change much the length of G_n block but does change the number of G_n block. It seems the G's in some G_n blocks are converted into S's, and the S's join with the adjacent S_n block resulting in increasing $\overline{L}_{S_n}'$ and decreasing the numbers of G_n . Such case would occur when the crystalline structure of cellulose has been thoroughly destroyed so that the chemical reagents can permeate easily and react with G at random. The X-ray diffraction analysis actually reflects that all samples do not retain the specific diffraction peak of crystalline cellulose at $2\theta = 14.5^\circ$, 16.4° and 24.5° (see Fig. 5). The peak at $2\theta = 20^\circ$ is contributed by the amorphous cellulose.

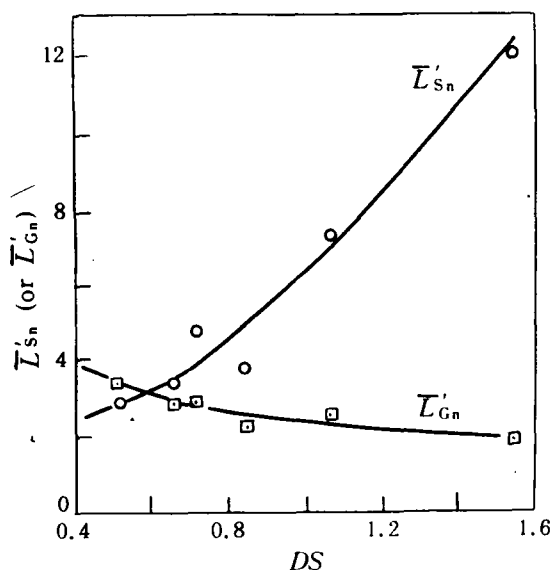


Fig. 4 Variation of $\overline{L}_{S_n}'$ and $\overline{L}_{G_n}'$ with DS

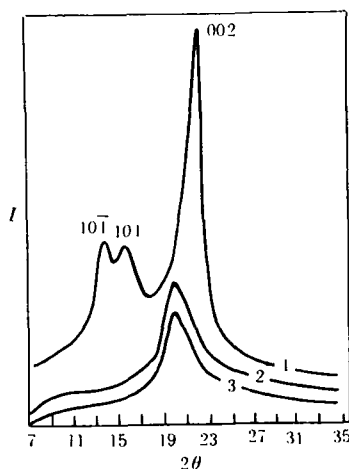


Fig. 5 X-ray diffraction of cellulose and CMC
1. cellulose; 2. CMC (DS=0.655); 3. CMC (DS=0.839)

(3) DS and SI

DS/SI represents the average number of substituent in one substituted anhydroglucose. For sample 1, monosubstitution is the principal substitution as DS/SI is only slightly larger than 1 and for sample 7, polysubstitution has already taken place as DS/SI = 1.78. For samples with DS nearly equal, the substitution in those with smaller SI inevitably occurs more concentratively in fewer glucose rings giving polysubstitution and larger DS/SI.

$(SI)_a$ is an apparent substitution index calculated by $\overline{L}_{S_n}$ and $\overline{L}_{G_n}$ as shown in Table 2. It is interesting to find the values of $(SI)_a$ close so much to those of SI that it is reasonable to take $DS/(\overline{L}_{S_n} / (\overline{L}_{S_n} + \overline{L}_{G_n}))$ as a criterion for estimating the degree of substitution in substituted rings without carrying out the complicated procedure for SI determination. Undoubtedly the estimation would be lower if some $\underline{G}$ is sandwiched in $\underline{GSnG}$ block (e.g., sample 6, DS/SI would decrease from 1.78 to 1.69), but it is still useful for rough estimation.

CONCLUSION

$\overline{L}_{S_n}$ and $\overline{L}_{G_n}$, $\overline{L}'_{S_n}$ and $\overline{L}'_{G_n}$ not only can describe the distribution of substituents along the CMC chain, but also can be used for calculating the percentage of substituted glucose ring (SI) and estimating the average degree of substitution in the substituted glucose rings. They are parameters of practical significance.

Keywords :

Carboxymethylcellulose ; Degree of substitution ; Sequence distribution

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