

# THE UNIFORMITY OF DISTRIBUTION OF SUBSTITUENTS ALONG THE CHAIN OF CARBOXYMETHYL CELLULOSE SYNTHESIZED IN TWO-PHASE MEDIUM $C_6H_6$ — $C_2H_5OH$

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**Abstract** Based on the property that carboxymethylcellulose (CMC) can be degraded to anhydroglucose residues by cellulase, the rate constant ( $K$ ) of enzymatic degradation of CMC synthesized in the benzene-ethanol medium has been determined. Furthermore, experimental equation  $K = 2.71 \times 10^{-2} (DS)^{-1.2}$  reflecting the relationship between  $K$  and the degree of substitution (DS) is correlated and used to describe chemical microstructure uniformity of the distribution of substituents along chains effectively. Chain structure parameters of enzymatic degradation products of CMC—the number of chain breaks and the percentage of glucose released have been also measured. Average length of substituted and unsubstituted chain segments are calculated simultaneously. Through the study of static and dynamic procedures of enzymatic degradation, the method to characterize the distribution of substituents of CMC along the chain has been improved.

**Keywords** Carboxymethylcellulose; Distribution of substituents; Anhydroglucose unit; Enzymatic degradation

## INTRODUCTION

The distribution of substituents is a highly important index for CMC, an important form of expression of which is the distribution of substitution along the molecular chain. Because the carboxymethylation of cellulose is multiphase system and not homogeneous, the possibility or the degree of substitution of different glucose units along the same chain is not consistent. The uniformity of substituent distribution along CMC chains has great influence on the solubility, shearing stability and rheological behaviour of CMC solution and its stability against temperature and low molecular additives<sup>[1~4]</sup>.

By the methods of  $^1\text{H-NMR}$  and X-ray diffraction analyses, the distribution of substituents at single glucose unit of CMC in the benzene-ethanol-water medium has been reported<sup>[5]</sup>. In this paper, characteristics of the distribution of substituents along the chain have been studied continuously on the basis of property that CMC can be degraded to glucose by cellulase.

## EXPERIMENTAL

### *Synthesis of CMC and determination of DS*

CMC sample was prepared in a mixed solution of benzene and ethanol (95%). The reaction condition was identical with that described in the paper<sup>[5]</sup>.

DS was determined by the method of ashing.

### *Determination of number average molecular weight*

Weighted exactly 0.5g CMC and dissolved it in aqueous solution of potassium ferricyanide and then boiled for 15min. After cooling, neutralized it suitably and titrated the  $\text{Fe}^{2+}$  with 0.01mol/L solution of cerium sulfate. The end point was determined by SPM-10A type potentiometer. In order to improve analytical accuracy,  $\text{Ce}^{4+}$  was standardized by quantitative standard solution of maltose. Number average molecular weight of CMC was calculated by the following equation:

$$\overline{M}_v = \frac{M_0 W}{C \cdot V}$$

where  $W$  is the weight of CMC samples.  $M_0$  is the molecular weight of maltose.  $C$  and  $V$  are the concentration and volume of standard solution of maltose, respectively.

### *Enzymatic hydrolysis of CMC*

Cellulase, the degradation agent, was supplied by SIGMA (5.1u/mg). Concentrations of CMC and enzyme were 0.0125g/mL and 0.2% ( $v/v$ ), respectively. Enzymatic degradation proceeded in a buffer solution of  $\text{CH}_3\text{COOH-CH}_3\text{COONa}$  ( $\text{pH}=4.5$ ) at  $40^\circ\text{C}$  for a certain time, and then the reducing value was determined.

### *The action of the glucose oxidase*

Glucose oxidase was added into the enzymatic and acidic hydrolytic solutions of CMC and reacted at  $35^\circ\text{C}$  for 30h in buffer solution  $\text{KH}_2\text{PO}_4\text{-NaOH}$  ( $\text{pH}=7.2$ ), then the reducing value was determined.

### *Acidic hydrolysis*

CMC sample was added to 10mol/L  $\text{H}_2\text{SO}_4$  solution and reacted at room temperature for 4h. Maintained 5h at  $100^\circ\text{C}$  after it was diluted, the solution was cooled

and neutralized. Then reducing value was evaluated.

#### *Determination of reducing ends value*

Oxidation-reduction potentiometric titration<sup>[6]</sup> was used: Beyond reducing ends of CMC oxidised by  $K_3[Fe(CN)_6]$  in basic condition, the quantity of the produced  $[Fe(CN)_6]^{4-}$  was determined by titration with  $Ce^{4+}$ . Then the reducing ends could be obtained.

## RESULTS AND DISCUSSION

### *Characterization of uniformity of substituent distribution along CMC molecular Chain*

Dry CMC is stable, but in moist condition, CMC is subjected to enzymatic hydrolysis by a variety of microorganisms. Based on the character of CMC degrading to glucose by cellulase, the distribution of substituents of CMC along chains can be investigated. To be beneficial to analysis and calculation, several basic hypotheses are made as follows:

(1) Cellulase only cleaves glycosidic bonds between adjacent unsubstituted anhydroglucose, AGU,  $(-U-U-)$ . The breakage between  $-U-S-$  and  $-S-S-$  is impossible.

(2) The cleavage of glycosidic bonds between  $-U-U-$  is random.

(3)  $U-AGU$  distributed at random.

According to statistics, (2) and (3) are approximately reasonable. The study of enzymatic degradation of CMC indicates that the group  $-CH_2COO^-$  on the  $S-AGU$  can restrain the cleavage of glycosidic bonds between it and adjacent  $U-AGU$ , so (1) is also reasonable<sup>[7,8]</sup>. It can be inferred that the ability against enzymatic degradation is due to DS and the uniformity of substitution.

### *The rate constant (K) of enzymatic degradation*

When some of the AGU are substituted, CMC are only composed of S and U if considered a single chain. We postulate the number of glycosidic bonds between  $-U-U-$  along a chain is  $n$ ,  $q$  of which are broken within time  $t$ , the rate of cleavage can be expressed by the following equation on the basis of mass action law:

$$\frac{dq}{dt} = k \cdot \left(1 - \frac{q}{n}\right) [E] \quad (1)$$

where  $[E]$  is the concentration of cellulase, which can be regarded as a constant and irrelevant to the time at initial stage. (1) can be modified as follows:

$$\frac{dq}{dt} = k_1 \left(1 - \frac{q}{n}\right) \quad (2)$$

(2) is integrated, then

$$-n \ln \left( 1 - \frac{q}{n} \right) = k_1 t \quad (3)$$

The term  $\ln \left( 1 - \frac{q}{n} \right)$  is expanded:

$-\ln \left( 1 - \frac{q}{n} \right) = -\frac{q}{n} - \frac{1}{2} \left( \frac{q}{n} \right)^2 - \frac{1}{3} \left( \frac{q}{n} \right)^3 - \dots$ , because  $n \geq q$  during the initial period of enzymatic degradation, high-order term of the expanded formula can be neglected, then (3) is simplified as follows:

$$q = k_2 t \quad (4)$$

When one  $-\text{U}-\text{U}-$  bond broken, one reducing end produces, then

$$q = \Delta H = \Delta \left( \frac{W}{M_V} \right) = \Delta \left( \frac{C \cdot V}{M_0} \right) \quad (5)$$

We known  $V_{\text{Ce}}^{4+} = k_3 V$  (maltose is the reference standard), then

$$q = \Delta \left( \frac{C \cdot V_{\text{Ce}}^{4+}}{M_0 \cdot k_3} \right) = k_4 \Delta V_{\text{Ce}}^{4+} \quad (6)$$

Where  $C$  represents the concentration of maltose, which is a constant. To a certain CMC, the molecular weight  $M_0$  of substituted AGU is also a constant. The following equation can be inferred from (4) and (6):

$$\Delta V_{\text{Ce}}^{4+} = \frac{k_2}{k_4} t = K t \quad (7)$$

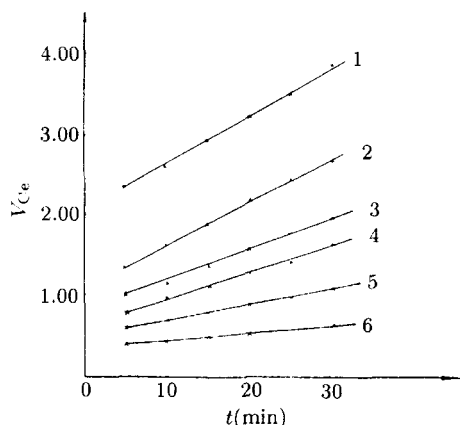


Fig.1 The rate curve of the early part of enzymatic degradation of CMC samples with different DS

Fig.1 shows that the volumes of  $C_e^{4+}$  solution vary with time as straight lines at initial period, so equation (7) is reasonable. The slope  $K$  is the rate constant of enzymatic degradation, which relate to the types of arrangement of substituted and unsubstituted AGU along the molecular backbones of CMC. Its values are summarized in Table 1.

Siü<sup>[9]</sup> and Rees<sup>[10]</sup> et al. concluded that microorganism attacks have no influence on the CMC if each AGU possesses at least one substituent group adhering.

So a certain relationship must exist between  $K$  and DS. Through  $\lg k - \lg \text{DS}$  curve, the experimental equation can be built to evaluate the sequence structure of S—AGU

of CMC samples synthesized in  $C_6H_6-C_2H_5OH$ :

$$K = 2.71 \times 10^{-2}(DS)^{-1.2} \quad (8)$$

The values of  $K$  calculated from equation (8) are summarized in Table 1.

**Table 1** The rate constant  $K$  of enzymatic degradation of CMC

| Sample no.                   |                  | 1      | 2      | 3      | 4      | 5      | 6      |
|------------------------------|------------------|--------|--------|--------|--------|--------|--------|
| $K$<br>( $\text{min}^{-1}$ ) | DS               | 0.42   | 0.59   | 0.73   | 0.83   | 1.10   | 1.28   |
|                              | Measured value   | 0.0745 | 0.0545 | 0.420  | 0.0348 | 0.0213 | 0.0144 |
|                              | Calculated value | 0.0767 | 0.0511 | 0.0395 | 0.0339 | 0.0242 | 0.0197 |

S—AGU increases with the increase of DS and has two forms of sequence structures along the macromolecules: (1) S-AUG inserts in the successive U—AGU segments at random; (2) S-AGU forms alternating copolymer chain structure with U-AGU by regular pattern (Fig.2). It can be seen from Fig.2 that six weak bonds exist in the first form while only three in the second (the number of S—AGU segments in the two forms is the same). In Table 1, measured and calculated  $K$  values are nearly identical, indicating more —S—S— and —S—U—S— sequences exist and the distribution of substituent groups are homogeneous. These results also verify that equation (8) is an excellent form to describe the chemical microstructure uniformity of the distribution of carboxymethyl groups along chain.

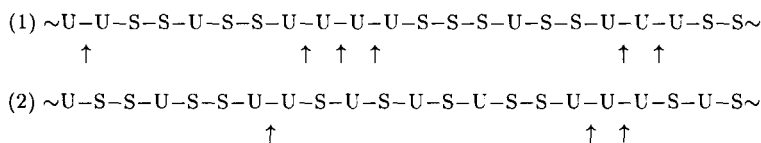


Fig.2 Schematic diagram of the distribution of S—AGU in CMC

#### *The number of chain breaks and the proportion of free sugars released*

Fig.3 is a schematic representation of enzymatic degradation of two macromolecular chains shown in Fig.2. The comparison of two chain segments (1) and (2) indicates that segment (1) has four chain segments and three free sugars obtained after enzymatic hydrolysis; while (2) has three chain segments and one free sugar. The distribution of substituents of segment (2) is more homogeneous than that of (1), which indicates that the less the quantity of cleaving points exists, the less the probability of chain cleavage and the number of chains and sugars are produced after degradation, the more homogeneous the distribution of substituents is. Chain structure parameters of products of CMC enzymatic degradation—the number

of chain breaks and sugars released——can be used to evaluate the uniformity of distribution of substituents.

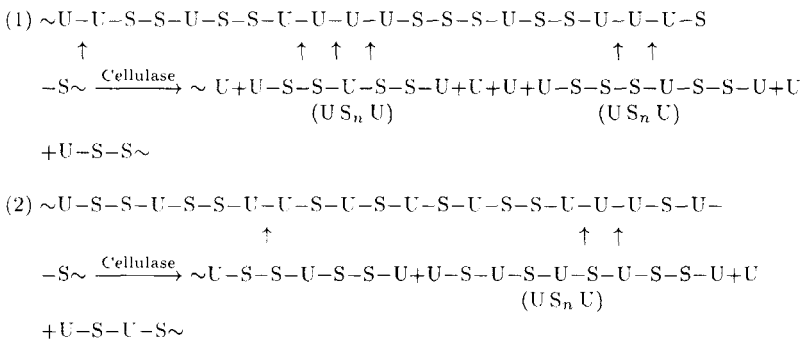


Fig.3 Schematic diagram of enzymatic degradation of CMC

The results of degradation of six CMC samples are summarized in Table 2, showing that the concentration of U—AGU, the number of chain cleavage and the amount of sugars released decrease with the increase of DS. Because the influence of distribution of substituents on the process of enzymatic degradation is concealed by DS, the distribution of substituents can not be compared among CMC with different DS through the number of chain breaks and GI. But Table 2 lists the most meaningful data: the ratios of the amount of sugars released in the enzymatic hydrolysis of CMC with low DS (sample 1, 2 and 3) to the number of chain breaks approach to 2 : 1. According to the conclusion in the literature<sup>[11]</sup>, the result indicates that the cleavage of CMC chain only occurs at the positions where several U—AGU are contiguous.

Table 2 Glucose liberated and molecular chain breaks in CMC, which is subjected to enzymatic degradation

| Sample no. | DS   | $\eta$ (2%)<br>mpn·8 | SI (%) | Measured       | DPn     |                   |                  | Number of chain ends /1000AGU |                   |                  | Number of chain breaks /1000AGU | Free sugar released /1000AGU | GI (%) |
|------------|------|----------------------|--------|----------------|---------|-------------------|------------------|-------------------------------|-------------------|------------------|---------------------------------|------------------------------|--------|
|            |      |                      |        | total          | $t = 0$ | $t = 0.5\text{h}$ | $t = 30\text{h}$ | $t = 0$                       | $t = 0.5\text{h}$ | $t = 30\text{h}$ | $t = 30\text{h}$                |                              |        |
|            |      |                      |        | U-AGU /1000AGU |         |                   |                  |                               |                   |                  |                                 |                              |        |
| (1)        | (2)  | (3)                  | (4)    | (5)            | (6)     | (7)               | (8)              | (9)                           | (10)              | (11)             | (12)                            | (13)                         | (14)   |
| 1          | 0.42 | 1450                 | 41.62  | 584            | 665     | 63.9              | 40.7             | 1.53                          | 15.6              | 24.6             | 23.1                            | 47.0                         | 8.05   |
| 2          | 0.59 | 2300                 | 58.83  | 412            | 820     | 104.2             | 82.7             | 1.22                          | 9.59              | 12.1             | 10.9                            | 22.1                         | 5.36   |
| 3          | 0.73 | 480                  | 64.47  | 355            | 272     | 169.5             | 106.9            | 3.68                          | 5.89              | 9.35             | 5.67                            | 11.8                         | 3.32   |
| 4          | 0.83 | 610                  | 77.57  | 224            | 248     | 155.9             | 104.6            | 4.03                          | 6.41              | 9.56             | 5.53                            | 7.2                          | 3.21   |
| 5          | 1.10 | 1500                 | 89.43  | 106            | 667     | 252.9             | 199.7            | 1.50                          | 3.95              | 5.06             | 3.51                            | 3.05                         | 2.87   |
| 6          | 1.28 | 620                  | 90.51  | 95             | 425     | 255.8             | 233.4            | 2.35                          | 3.91              | 4.29             | 1.93                            | 2.51                         | 2.62   |

a. The number of unsubstituted AGU per 100AGU is  $(1 - \text{SI}) \times 1000$ ; b. column 12 = column 11 - column 9;  
c. SI is the substitution index —— the percentage of substituted AGU; d. GI is the percentage of free sugars

The length of chain segments

In Fig.3, chains are decomposed into several single sugars and short substituted

chain segments with U—AGU at the two ends ( $US_nU$ ). The unsubstituted glucose inserting in the substituted chain segments and not decomposing by enzyme can be regarded as substituted glucose (marked  $U^*$ ), then molecular chains of CMC are only composed of substituted and unsubstituted chain segments  $U_m$ . The degree of uniformity of distribution of different chain segments in the same molecular chain can be obtained through the determination and calculation of average length of substituted and unsubstituted chain segments of CMC molecule after enzymatic degradation. The method of determination accords with the literature<sup>[12]</sup>. The average length of substituted and unsubstituted chain segments can be calculated by the following equations:

$$\bar{L}_{S_n} = (W/M_{AGU} - E_U)/E_{US_nU} - 2 \quad (9)$$

$$\bar{L}_{U_m} = \frac{E_U}{E_{U_m}} + 2 = \frac{E_U}{E_{US_nU}} + 2 \quad (10)$$

Here,  $E_U$ ,  $E_{US_nU}$  are the number of single U and  $US_nU$ , respectively;  $W$  is the weight of samples. Equation (10) shows CMC has been substituted completely or the distribution of substituents is homogeneous if  $E_U = 0$  or  $\bar{L}_{U_m} = 2$  after enzymatic hydrolysis of CMC. Arrangement forms of chains are  $-S-U^*-S-$  or  $-S-S-$ , not  $-U-U-$ .

Based on SI,  $\bar{L}_{S_n}$  and  $\bar{L}_{U_m}$ , the number of  $U^*$  ( $N_{U^*}$ ) and the proportion of  $U^*$  to substituted chain segments ( $SU^*$ ) can be calculated:

$$N_{U^*} = \bar{L}_{S_n} - SI \times (\bar{L}_{S_n} + \bar{L}_{U_m}) \quad (11)$$

$$SU^* = N_{U^*}/\bar{L}_{S_n} \quad (12)$$

If no  $U^*$  exists in substituted chain segments, i.e.  $N_{U^*} = 0$ , the following equation is obtained:

$$SI = \frac{\bar{L}_{S_n}}{\bar{L}_{S_n} + \bar{L}_{U_m}} \quad (13)$$

To sum up, the longer the average length of substituted chain segments of CMC or the shorter that of unsubstituted chain segments is, the more homogeneous the distribution of substituents along chain will be.

Table 3 shows that  $\bar{L}_{S_n}$  increases with the enhancement of DS, the magnitude of change is very large (the maximum approaches to 10 times, such as that between sample 1 and 6). But  $\bar{L}_{U_m}$  of samples with higher DS changes within the range of only one glucose unit generally, which indicates that variation of synthesis conditions only effects the length of substituted chain segments instead of the unsubstituted. From this we conclude that there is one or two unsubstituted AGU

between the adjacent substituted segments  $-S_n-$  generally. The change of  $\overline{L}_{U_m}$  of CMC with low DS only exceed the range of one AGU, which suggests the arrangement of chain has forms  $-U-S_n-U-U-S_n-U$  or  $-U-S_n-U-U-U-S_n-U-$ . Table 3 also shows that the number of U—AGU inserting in substituted chain segments (column 8 and 9).  $U^*$ , when DS is low, have high percentage in substituted chain segments, which indicates substituents mostly adopt the arrangement form  $-U-S-U^*-S-U^*-S-\cdots-U$  to improve the uniformity of distribution of substituents and biological stability of CMC. For CMC with high DS, proportion of  $U^*$  decreases relatively.

**Table 3** Parameters  $\overline{L}_{S_n}$ ,  $\overline{L}_{U_m}$ , etc. relating to the distribution of substituents of CMC, determined experiment

| Sample no | DS   | $M_{AGU}^a$ | $W(g)\times 10^2$ | $\overline{L}_{S_n}$ | $\overline{L}_{U_m}$ | $\frac{\overline{L}_{S_n}}{\overline{L}_{S_n} + \overline{L}_{U_m}}$ | SI%   | $N_{U^*}$ | SU*(%) | DS/SI | DI(%) |
|-----------|------|-------------|-------------------|----------------------|----------------------|----------------------------------------------------------------------|-------|-----------|--------|-------|-------|
| (1)       | (2)  | (3)         | (4)               | (5)                  | (6)                  | (7)                                                                  | (8)   | (9)       | (10)   | (11)  | (12)  |
| 1         | 0.42 | 195.75      | 0.81858           | 18.66                | 3.196                | 0.854                                                                | 41.62 | 9.564     | 51.25  | 1.01  | 0.38  |
| 2         | 0.59 | 209.35      | 0.83735           | 27.57                | 3.243                | 0.895                                                                | 58.83 | 9.581     | 34.75  | 1.00  | 0.17  |
| 3         | 0.73 | 220.55      | 1.00126           | 34.41                | 2.918                | 0.920                                                                | 64.47 | 10.04     | 29.92  | 1.13  | 8.53  |
| 4         | 0.83 | 228.55      | 1.20116           | 40.85                | 2.621                | 0.940                                                                | 77.57 | 7.15      | 17.48  | 1.07  | 5.43  |
| 5         | 1.10 | 250.16      | 0.99986           | 131.28               | 2.370                | 0.982                                                                | 89.43 | 11.76     | 8.95   | 1.23  | 20.57 |
| 6         | 1.28 | 264.56      | 1.00030           | 172.09               | 2.265                | 0.986                                                                | 90.51 | 15.68     | 9.11   | 1.41  | 37.49 |

a.  $M_{AGU} = 162.14 + 80.018DS$ , b. The percentage of dual-substituted AGU if no tri-substituted taking place,  $DI=DS-SI$

DS/SI (in column 11) is the average number of substituents on substituted glucose units, which indicates the substitution of AGU is single- or multi-substitution. We can see the values of DS/SI of CMC with low DS is higher than 1 slightly, which reveals the reactions are mostly single-substitution. But reactions of CMC with high DS are multi-substitution. Such as sample 6, because DS is 1.28 while the percentage of substituted AGU is 90.51%, dual-or tri-substitution must occurs.

CONCLUSIONS

The distribution of substituents along the CMC chain can be determined on the basis of the characteristic that substituted cellulose is resistant to enzyme. Attacked by cellulase, chain scission only occurs at sequences of several (two or more) adjacent unsubstituted AGU of CMC.

The parameter of dynamic process of enzymatic degradation (rate constant  $K$ ) and experimental equation  $K = 2.71 \times 10^{-2}(DS)^{-1.2}$  are excellent forms to describe

chemical microstructure uniformity of distribution of substituents along chain.

Chain structure parameter of products of CMC enzymatic hydrolysis—the number of chain cleavage and the percentage of free glucose released in unsubstituted AGU (GI) can express the uniformity of substituents on different chain segments of a chain. The lower the number of chain cleavage and GI, the more homogeneous the distribution.

Average length of substituted ( $\bar{L}_{S_n}$ ) and unsubstituted ( $\bar{L}_{U_m}$ ) chain segments can be used to gain information about the degree of uniformity of distribution of substituents. Generally, the higher  $\bar{L}_{S_n}$  is, the closer  $\bar{L}_{U_m}$  approach to 2, the more homogeneous the distribution is.

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