Characterization of Carboxymethylcellulose: Distribution of Substituent Groups along the Chain*

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Synopsis

A procedure has been developed for characterizing cellulose ethers on the basis of the distribution of substituent groups along the polymer backbone. This method has been applied to carboxymethylcellulose. The technique uses cellulase, a cellulose-degrading enzyme. Degradation as a function of time is followed by viscosity and reducing sugar measurements. The combination of these data, along with other analytical results, provides information on the number of unsubstituted anhydroglucose residues and the number and average length of blocks of two or more contiguous unsubstituted residues. The results obtained on a series of carboxymethylcellulose samples indicate that the rate of degradation and the number and length of blocks of unsubstituted residues depends not only on the number of substituent groups, but also on other molecular parameters.

INTRODUCTION

Cellulose ethers are a series of high molecular weight polymers prepared by derivatizing cellulose. The hydrogen atom of the hydroxyl groups of the monomeric glucose residue of cellulose is, in these reactions, replaced with alkyl or substituted alkyl groups such as methyl, ethyl, sodium carboxymethyl, hydroxyethyl, hydroxypropyl, or hydroxybutyl. There are, for each anhydroglucose residue, three hydroxyl groups available for reaction with a substituent group. Thus, the polymer can have a maximum degree of substitution (DS), the average number of substituent groups per glucose residue, of 3.0; the range of typical commercial material is 0.4–1.2. When alkylene oxide is used as a reactant, the new hydroxyl group of the substituent can be further substituted, forming short side chains.

Production of cellulose ethers in the United States was 175 million lb in 1979. European and Japanese production totaled 375 million lb.[1] Cellulose ethers are used in the adhesives, cosmetics, building, detergent, food, paint, paper, pharmaceutical, petroleum, superabsorbent, and textile industries. The important properties of these materials include solubility, viscosity in solution, stability against heat and oxidation, and biostability. The commercially important properties of cellulose ethers are determined by the molecular weight of the cellulose, the chemical structure and distribution of the substituent group, and the DS.

The work reported here considers the structure of carboxymethylcellulose (CMC) and the distribution of the substituent groups along the chain. Carboxymethylcellulose is prepared by reaction of monochloracetic acid with alkali cellulose; alkali cellulose is formed by reacting cellulose with sodium hydroxide. The presence of crystalline and amorphous regions in the cellulose fiber requires

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precise control of the alkali cellulose formation, for nonuniformity can cause major problems in solution quality and performance properties. It is known that the uniformity of the distribution of the substituent groups is vital to the performance of the material, and differences in uniformity can result in materials of the same molecular weight and DS having different performance properties.

EXPERIMENTAL

The carboxymethylcellulose samples were production lots manufactured by Hercules Incorporated. The cellulase used was Cellase 1000 (GB Fermentation, Des Plaines, Ill.). Poly(vinyl pyrrolidone) (PVP), type NP-K30, was obtained from GAF Corp. All other chemicals used were reagent grade, and distilled deionized water was used throughout this study.

The samples were selected to represent a wide range of DS and weight average degree of polymerization ($\overline{DP}_w$). The weight average degree of polymerization is determined from the intrinsic viscosity measured in 0.02*M* KH_2PO_4, pH 5.5, at 25°C using the following relationship:

$$[\eta] = 1.95 \times 10^{-2} [\overline{DP}_w]^{0.83} \quad (1)$$

This was determined from standards whose weight average degree of polymerization was determined by intrinsic viscosity in 0.1*M* sodium chloride using the previously reported Mark–Houwink equation[2]

$$[\eta] = 2.0 \times 10^{-2} [\overline{DP}_w]^{0.78} \quad (2)$$

Equations (1) and (2) were found to hold over the entire molecular weight range. The DS was determined by the ASTM method D1439-72[3] and the percentage of unsubstituted anhydroglucose residues by a procedure using glucose oxidase.[4,5]

The degradation of the molecule by enzymatic hydrolysis was followed by viscosity, as previously reported.[2] The enzymatic degradation was carried out using 0.02*M* KH_2PO_4, pH 5.5, as the buffer. The procedure used was to prepare a stock solution of polymer by dissolving 0.2400 g of the material in exactly 100 mL of solution. For higher molecular weights, the polymer concentration was decreased. An experimental (control) sample was prepared by combining 25 mL of the stock solution with 5 mL of buffer. A one-point intrinsic viscosity procedure was used to determine the initial $\overline{DP}_w$[6] (see below). The experimental (degradation) sample was prepared by combining a 25 mL aliquot of the stock solution with 4 mL of buffer and 1 mL of enzyme solution (0.075 g Cellase 1000/L buffer). The elapsed time of degradation was taken from the time at which the enzyme was added. The solution was transferred to a Ubbelohde viscometer, and flow times were recorded.

The intrinsic viscosity was determined by a one-point intrinsic viscosity procedure using Martin's equation

$$\log(\eta_{sp/c}) = \log[\eta] + k_m[\eta]c \quad (3)$$

where $\eta_{sp/c}$ is the reduced viscosity, c is the concentration, and k_m was determined to be 0.124 for carboxymethylcellulose in 0.02*M* KH_2PO_4, pH 5.5.

The samples were analyzed for reducing sugars by a modification of the

Folin–Malmros method.[7,8] The procedure consists of placing 1.0 mL of the experimental sample into a test tube. Distilled water (3 mL), 2.0 mL of an 0.4% solution of $K_3Fe(CN)_6$, and 1.0 mL sodium carbonate/sodium cyanide solution (1.6% Na_2CO_3 in 0.3% NaCN) were added. This solution was mixed well and heated for 8 min in a boiling water bath. After cooling for 2 min in an ice bath, 5 mL of ferric iron solution (prepared by dissolving 5 g anhydrous $Fe_2(SO_4)_3$ in 75 mL syrupy phosphoric acid (85% H_3PO_4), which is then added to 100 mL of water containing 20 g PVP; the volume is adjusted to 1 L) were added. The sample was diluted to 25 mL in a volumetric flask and the absorbance read at 520 nm.

For enzymatic degradation, the sodium carbonate/sodium cyanide was used to stop the enzyme reaction. Thus, equal amounts, on a volume basis, of the digest and the sodium carbonate/sodium cyanide solution were mixed together. An aliquot, 2 mL, of this solution was mixed with 3.0 mL of water and 2.0 mL of the ferricyanide solution. The samples were then heated, and the ferric iron solution added as discussed above.

The amount of reducing sugars present relative to lactose was determined by using standards. The standard concentration versus absorbance curves were obtained as follows. A working standard was prepared from this stock solution (2 g lactose/L water) by diluting 10 mL of the stock solution to 100 mL with distilled water in a volumetric flask. This gave a standard containing 200 μg lactose/L. Serial dilutions of the working standard were used to prepare standards containing 100, 50, and 25 μg of lactose/L. The results were obtained as mg lactose/mL.

For low molecular weight materials and/or high reducing sugar content materials, such as that for long digestion times, the range of the method is often exceeded. For these cases, the amount of sample must be reduced, and the appropriate calibration correction factor used with the calibration curves.

RESULTS AND DISCUSSION

The molecular characterization procedure is based on the action of cellulase, an enzyme which degrades cellulose. Cellulase is actually a group of enzymes which contributes to the degradation of cellulose, hydrolyzing the polymer to glucose. Cellase 1000 is a fungal cellulase prepared from *Aspergillus niger*. The primary enzymatic component is a β-D-glucosidase, which cleaves the glycosidic bond of cellulose and its derivatives. It is known that cellulase will degrade carboxymethylcellulose. This cellulose ether is, however, somewhat resistant to enzymatic attack; the susceptibility decreases as the DS increases. Cleavage of the polymer chain occurs at random only between adjacent unsubstituted residues.[2,9–12] It has been suggested that the remaining segments of contiguous unsubstituted residues are cleaved until only the units adjacent to the substituted residues remain.[2] Cayle,[10] however, has suggested that, at one end, the adjacent unsubstituted residues can also be removed. McBurney[12] showed that the number of chain breaks can be related to the intrinsic fluidity, defined as the reciprocal of the intrinsic viscosity. This provides a measure of the rate of degradation.

A procedure has been developed for characterizing carboxymethylcellulose by using enzymatic degradation as a probe. The viscosity is monitored as a

function of time, and the change in the degree of polymerization is followed. The progressive change in chain length is recorded as the number of times the enzyme cleaves the polysaccharide backbone per 1000 residues. This treatment permits comparison of the extent of degradation, which is independent of original chain length. The number of reducing sugars present is simultaneously determined; a modified Folin–Malmros ferricyanide procedure, which gives the number of reducing sugars, is used.[7,8] There is one reducing end present for each polymer chain. In addition, each monomeric glucose residue also has a reducing end. Thus, the number of polymer plus monomer units are determined. The viscosity results are reported as the weight average degree of polymerization. The procedure, therefore, is a weight average measurement, weighted towards the high molecular weight material. The monomer material has an extremely small, essentially negligible contribution to the viscosity. We can therefore assume that the viscosity data yield the number of polymer chains. The combination of the two results gives the number of blocks of unsubstituted residues and their average length.

Seven samples of carboxymethylcellulose covering a range of molecular weight and DS were considered. Table I gives the analytical data of the samples. One sees that, as the degree of substitution increases, the percentage of unsubstituted anhydroglucose (UAHG) residues generally decreases. Over a narrow range, however, this is not necessarily the case, for samples with a very small change in DS can have a large difference in the percentage of unsubstituted residues, as seen in the data for samples A, B, and C. This indicates that material with the same level of substitution can have quite different distributions. These differences are reflected in performance in end-use applications.

It is important to note that uniformity is a relative parameter. Furthermore, while this work addresses uniformity along the chain, chemical heterogeneity among chains is also important. Molecular weight distribution also becomes a factor when uniformity within a population of macromolecules is considered. The uniformity of the sample will influence the solution properties of the molecule and, hence, its application. Aggregation is promoted by a larger number of regions of contiguous unsubstituted residues. Extreme cases of nonuniformity can lead to unique solubility behavior. It is important to note that maximum uniformity does not necessarily guarantee the best product; applications exist which might require a less uniform distribution.

TABLE I
Carboxymethylcellulose Samples

Designation	DS[a]	UAHG[b] (%)	$[\eta]$[c]	$\overline{DP}_w$[d]
A	0.78	22.2	2.8	408
B	0.81	19.6	3.3	496
C	0.77	23.0	1.8	244
D	0.75	25.0	6.9	123
E	1.11	9.0	10.9	2110
F	0.94	15.9	17.6	3820
G	0.97	12.9	24.7	5750

[a] Degree of substitution.

[b] Unsubstituted anhydroglucose residues.

[c] Intrinsic viscosity in 0.02*M* KH_2PO_4, pH 5.5, at 25°C.

[d] Weight average degree of polymerization determined from intrinsic viscosity data using the following relationship: $[\eta] = 1.95 \times 10^{-2} [\overline{DP}_w]^{0.83}$.

Cellulose derivatives are, by their very nature, polydispersed. Examination of numerous samples has indicated that the molecular weight distribution is, for a given grade of carboxymethylcellulose, constant. It has been shown that the polydispersity is rather low and the molecular weight distribution rapidly approaches 2.0 as the material is degraded.[2,12] It should be noted that any degradation occurring during the derivatization process will affect polydispersity, bringing the molecular weight distribution closer to 2.0. The polydispersity of the cellulose used as the starting material may have some effect on the distribution of substituent groups. It is, however, unlikely to affect the mechanism of enzymatic degradation and is not a factor in this study.

Figures 1 and 2 illustrate the change in intrinsic fluidity Φ, defined as the reciprocal of the intrinsic viscosity, as a function of time. This provides a measure of the rate and number of chain breaks. The initial rate is the same for the first three samples (Fig. 1). Over a longer period of time, this is not the case, indicating that some materials are degraded much more rapidly than others. The rate of degradation is consistent with the number of chain breaks per 1000 residues after 8 days, indicating that the rate of degradation is proportional to the number of times that the enzyme cleaves the chain.

The data for samples E, F, and G (Fig. 2) indicate that high DS material is more resistant to degradation. The results further demonstrate that the rate is not related solely to DS; other factors are apparently important. Furthermore, the rate of degradation reaches an equilibrium value much faster, which is probably due to the much smaller concentration of cleavage sites. The crossover between F and G suggest that relative rates of degradation can also vary. This is likely

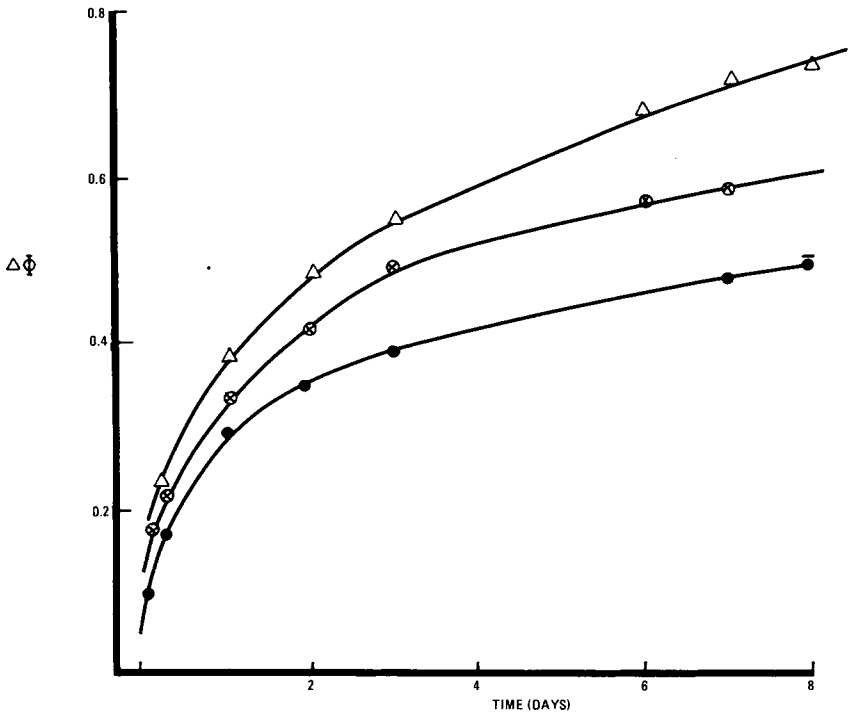

Fig. 1. Intrinsic fluidity Φ as a function of time. Sample: (△) A; (●) B; (⊗) C; and (−) D.

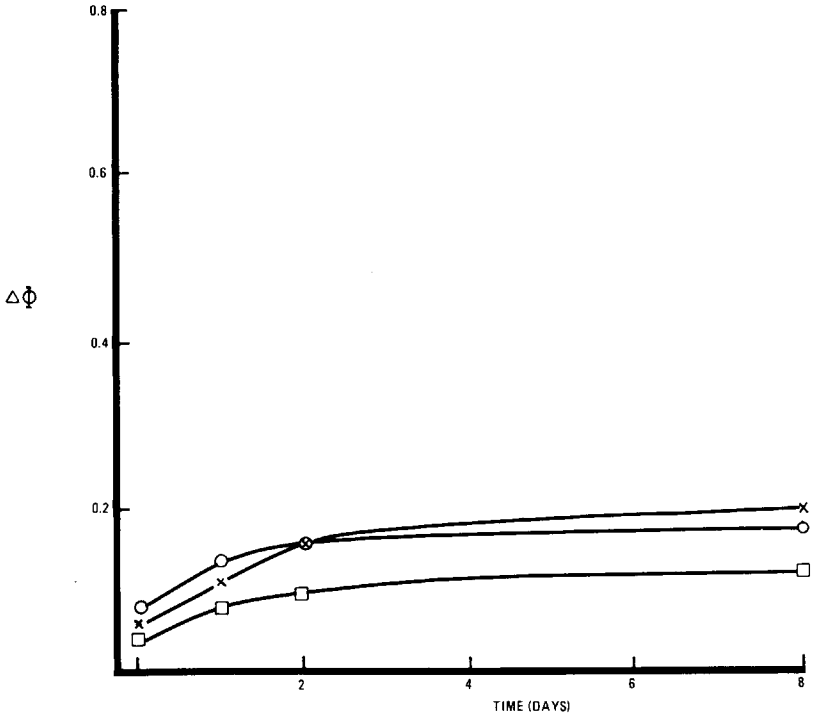

Fig. 2. Intrinsic fluidity Φ as a function of time. Sample: (□) E; (○) F; and (×) G.

to be related to the uniformity as well as the number of substituent groups along the chain.

Table II summarizes the enzymatic degradation data obtained on these samples. One sees a large difference in the number of times per 1000 glucose residues that the chain is cleaved; this value, after 8 days, ranges from 2.3 to 14.2. Closer examination of the data indicates that considerable differences can exist between samples which have similar values for DS and degree of polymerization. Samples A and B have the same DS and degree of polymerization, but strikingly different percentages of unsubstituted anhydroglucose residues. The enzymatic degradation results indicate that sample A, with more unsubstituted residues, is cleaved to a much larger extent. After 8 days, the number of chain breaks per 1000 residues was 8.9 for B and 14.1 for A. Furthermore, sample C has intermediate values for the number of chain breaks per 1000 residues and percentage of unsubstituted residues. These data suggest a relationship between the two parameters. The number of free sugars per chain break, which gives the average length of the unsubstituted regions, does not follow the observed order for the three samples. This indicates that CMC samples can show variations between the two parameters.

The other samples are of higher molecular weight. Samples D and E have dramatic differences in DS and, hence, in the percentage of unsubstituted residues. There is, however, only a small difference in the susceptibility to enzymatic degradation. The length of the blocks of unsubstituted residues is, as indicated in the table, much longer for sample D. This suggests that these two samples have approximately the same number of blocks of unsubstituted residues, al-

TABLE II
Enzymatic Degradation Data

Sample	$\overline{DP}_n$[b]			Chain ends/1000 residues			Chain breaks/1000 residues		Free sugars/chain break	
	t = 0	t = 0.5 h	t = 8 days	t = 0	t = 0.5 h	t = 8 days	t = 0.5 h	t = 8 days	t = 0.5 h	t = 8 days
A	205	181	53	4.9	5.5	19.0	0.9	14.1	7.3	3.2
B	248	199	77	4.0	5.0	13.0	1.0	8.9	4.0	9.9
C	122	108	48	8.2	9.3	20.1	1.0	12.4	4.8	13.9
D[a]	608	—	200	1.6	—	5.0	—	3.3	—	19.4
E	825	558	322	1.2	1.8	3.1	0.6	1.9	2.7	4.3
F	1609	777	324	0.6	1.3	3.1	0.7	2.4	2.4	1.2
G	2988	2018	669	0.3	0.5	1.5	0.2	1.1	0.9	0.5

[a] 0.5 h data not collected on this sample.
[b] Number average degree of polymerization from intrinsic viscosity data. $\overline{DP}_w/\overline{DP}_n$ assumed to be 2/1.

though their length is significantly different. It would be predicted, based on this analysis, that the solution properties would be quite different.

Samples F and G are high molecular weight materials with a DS level of ~0.95. The enzymatic degradation results indicate that the molecular structure of these materials is quite similar. The length of the unsubstituted regions is extremely small, averaging less than four residues long. It is interesting that this is shorter than that found for higher DS samples (sample E). Results of this nature have been observed for a number of samples. It is possible that the distribution does not consistently follow the DS values due to changes in the process used to make the material. The important point is that DS or percentage of unsubstituted anhydroglucose residues does not provide information on the distribution of substituent groups along the chain.

CONCLUSIONS

A procedure using enzymatic (cellulase) degradation techniques, monitored by viscosity and reducing sugar determinations, can be used to characterize the distribution of substituent groups along the chain. Based on the principle that cellulase will cleave only between two adjacent unsubstituted residues, this method allows one to determine the number and length of blocks of unsubstituted residues present in the polymer chain. The number of single unsubstituted residues can also be estimated.

The experimental results indicate that carboxymethylcellulose is made in a wide range of DS and DP_w. Differences in the distribution of substituent groups, however, can occur independent of similarities in these parameters.

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