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Structural characterization by ^{13}C nuclear magnetic resonance of hydrolysed carboxymethylcellulose

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SUMMARY

In order to characterize heavyweight carboxymethylcellulose (C.M.C) by ^{13}C NMR it is necessary to hydrolysis them. This paper proposes and compares three different ways of degradation: acidic hydrolysis, sonication and enzymatic degradation. Acidic hydrolysis leads to poor results due to a strong alteration of the sample. Sonication gives quite good results but the enzymatic degradation is the most efficient method for degrading C.M.C under good conditions.

After hydrolysis, the sample was analysed by ^{13}C NMR and HPLC to obtain structural information such as substitution degree, preferential substitution sites and regularity of the substitution along the polymeric chain.

INTRODUCTION

As for the numerous industrial applications of carboxymethylcelluloses, the use of these types of polymer for oil-well drilling requires a good understanding of their structural characteristics. Indeed a better understanding of the mechanisms of the action of additives in drilling muds is necessary to improve their performances and thus the economics of drilling. It has been shown that drilling-fluid properties are governed to a large extent by interactions between polymeric additives and clays and that such interactions are strongly dependent on polymer microstructure. As such, great differences among properties of drilling fluids containing CMC from different origins have been observed during drilling operations. This study provides a methodology for determining CMC structural characteristics. ^{13}C NMR is one of the most powerful tools for characterizing the structure of molecules (1,2). Because of the low mobility of their functional groups, NMR spectra of heavyweight polymers are not clearly resolved (this slow motion induces very efficient dipolar relaxation in T_2 and then very poor resolution). To improve the resolution of NMR spectra, and to get some structural information, it is necessary to degrade polymers. This paper proposes and compares three different methods of degradation: acidic hydrolysis, sonication and enzymatic degradation.

EXPERIMENTAL

- ^{13}C NMR spectra were recorded with a Brüker CXP 200 spectrometer operating in the pulse mode. To obtain quantitative informations, a "gated inverse" pulse sequence with broadband proton decoupling was used. The flip angle was about 60° and the delay time was 12 s. Water was used as the solvent, and 400 µl of methanol was added as an internal chemical shift reference. Operating temperature was 80°C for sonicated samples and room temperature for enzymatically degradated samples.

- HPLC was performed on a Waters chromatograph equipped with a R410

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refractive index detector. A 30 cm sugar pak column maintained at 90°C was used for the separation. Water was the mobile phase and the flow rate was 0.4 mL/min. The first glucose oligomers were used as retention time calibration.

- Sonication was performed using a Branson B15 apparatus. It generates an ultrasonic frequency of 20 kHz.

Degradation of polymers

Three types of degradation were tested:
- acidic hydrolysis
- sonication
- enzymatic hydrolysis

Acidic hydrolysis

Several operating conditions were tested. They are shown in Table 1.

For the first five tests, the solutions became black and solid particles appeared in the tube, showing a strong alteration of the samples.

	ACID	NORMALITY	POLYMER CONCENTRATION (% weight)	TEMPERATURE	ATMOSPHERE
Test 1	HCl	10 N	10%	50°C	argon
Test 2	HCl	10 N	5%	50°C	argon
Test 3	HCl	10 N	5%	AMBIENT	argon
Test 4	H_2SO_4	5N	5%	50°C	argon
Test 5	H_2SO_4	5N	5%	AMBIENT	argon
Test 6	H_2SO_4	1 N	5%	AMBIENT	argon
Test 7	H_2SO_4	1 N	5%	50°C then 80°C	argon

Table 1 : Operating Conditions for acidic hydrolysis

For the last two tests, after two weeks, the viscosity of the solution remained equal to its initial value, which means that no hydrolysis occurred. No more tests were performed to optimize the operating conditions, and this type of hydrolysis was not studied anymore.

Sonication

It has been effectively now established that the prolonged exposure of solutions of macromolecules to high-energy ultrasonic waves produces permanent reductions in the solution viscosity (3,4).

Schmid and Mark have suggested that degradation occurs as the result of the increased frictional forces developed between the faster moving solvent molecules and the larger, less-mobile, macromolecules. Although envisaging different modes of interaction between the solvent and the macromolecule, both authors concluded that the increased frictional forces are sufficient to break an atomic c-c bond.

The intensity of sound is attenuated as it progresses through a medium. As molecules of a liquid vibrate under the action of a sound wave they experience viscous interactions that degrade the acoustic intensity, and some energy is lost in the form of heat. In order to prevent this effect, a quite low concentration of the sample was chosen, about 200 mg/liter (to reduce the viscosity). Sonicated time was

10 min per experiment for 30 mL of solution. This operation was done several times to have enough degradated polymer to prepare samples with a concentration compatible with NMR sensitivity. To prevent thermal degradation of the polymer, the sample holder was maintained at 40°C by water circulation. Total preparation time was about one day per sample. After sonication, to be in a concentration range compatible with NMR sensitivity, the solution was concentrated up to 10 weight % of polymer.

Enzymatic treatment

The enzyme used was a cellulase produced by a strain of *Trichoderma reesei*. After fermentation, the cells were removed by centrifuging or filtration. The clarified broth was concentrated by ultrafiltration (molecular weight cut off from the membrane equal to 10,000) and the retentate containing enzyme was then completely dehydrated by freeze-drying. The enzymatic activity of this freeze-dried powder, measured in filter paper units (FPU), was about 500 FPU/g of powder.

CMC was hydrolysed with a 2% polymer suspension, the pH of which was adjusted to 5.1 with 1N HCl and adding 10 FPU/g of polymer.

The reaction temperature was 50°C. After only 30 minutes, a perfectly clear solution was obtained and its viscosity was considerably lowered. These reaction conditions were maintained during one night to obtain complete hydrolysis. The enzyme was removed from the final solution by ultrafiltration through a 5000 molecular weight cut off membrane.

The free glucose concentration was determined by HPLC of the filtrate obtained. The presence of carboxymethyl oligomers was detected.

For NMR analysis, the sample was concentrated by water evaporation to obtain 25% polymer (by weight).

RESULTS AND DISCUSSION

The sample used for testing the different degradation methods was a commercial polymer supplied by Aqualon (France).

The sample was purified by washing in a Soxhlet apparatus for 7 hours. The rinsing solvent was a methanol/water (85/15) mixture.

The average molecular weight was determined by low-angle light-scattering measurements (KMX 6 Chromatix apparatus) : $Mw = 1.1\ 10^6 \pm 10^5$

Intrinsic viscosity in NaCl at 0.2 M and at 25°C was 1200 cm^3/g.

Fig. 1 shows ^{13}C NMR spectra of samples degradated by sonication and by enzymatic treatment. A comparison of these spectra shows that a better spectrum quality is obtained for the enzymatically hydrolyzed sample, especially concerning:

- The spectral resolution. This is due to the greater efficiency of the enzymatic process in degrading the polymer. The correlation time of functional groups was then shortened (because more mobile), which means that transverse relaxation time T_2 was greater and the half-width peaks were smaller.
- The signal to noise ratio. Because enzymatic hydrolysis is more efficient, the viscosity of the solution is no longer a problem, and it is then possible to use a more concentrated sample.
- The number of resolved singlets is higher. There were, at least, two "new" signals (δ=92 and 96 ppm). They were due to anomeric carbon atoms at the end of polymeric chains. The detection of the signals were detected because anhydroglucose units could be either in α or in β conformation. The detection of the signals clearly indicates shorter polymeric chain lengths (i.e. many more chain ends) in the solution after enzymatic treatment than after sonication. This result coroborates the fact that enzymatic degradation is more efficient for chain breakage than sonication.

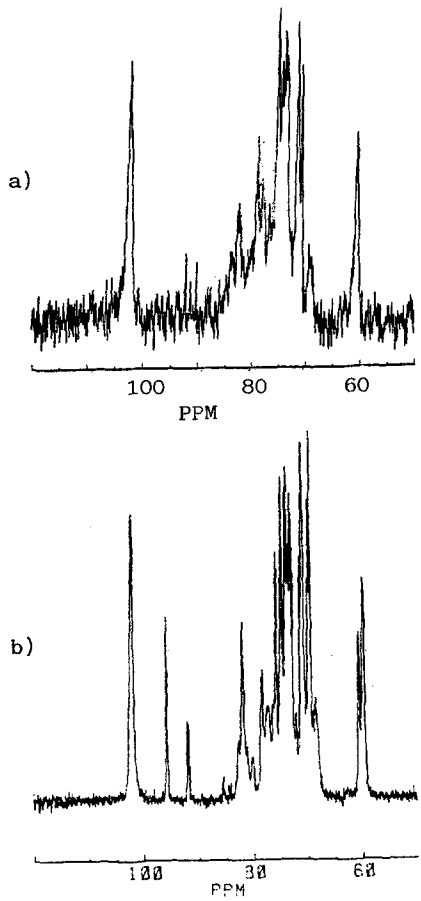
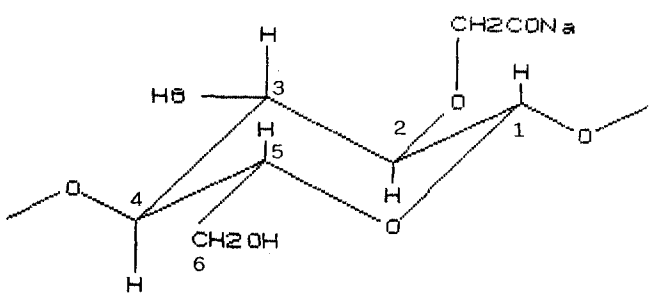


Fig 1 : 13C NMR spectra of hydrolysed C.M.C (a) by sonication (b) by enzymatic degradation

^{13}C NMR spectrum attributions

According to CHAUDHARI and TALVITIE (5,6), identifications were made of the enzymatically degradated sample. They are summarized in Table. 2.



Carboxyméthylcellulose

ATTRIBUTIONS		δ (ppm)
glucose	$C_{1\alpha}$	92.12
	$C_{2\alpha}$	71.47
	$C_{3\alpha}$	72.78
	$C_{4\alpha}$	69.63
	$C_{5\alpha}$	71.47
	$C_{6\beta}$	59.82
	$C_{1\beta}$	95.93
	$C_{2\beta}$	74.15
	$C_{3\beta}$	75.76
	$C_{4\beta}$	69.63
	$C_{5\beta}$	75.94
	$C_{6\beta}$	59.82
C_1 : C_1 included in the chain		102.29
$C_{1\alpha}$: C_1 at the end of the chain		91.96
$C_{1\beta}$: C_1 at the end of the chain		95.76
C_{3S} : C_3 substituted		82.06
C_{2S} : C_2 substituted		78.36
CH_2 : methylene from substituent		71.11 and 69.91
C_{6S} : C_6 substituted		68.51
C_{6nS} : C_6 non substituted		60.6
C_C : Carbonyl from substituent		180

Table 2 : ^{13}C NMR attributions

From these data, it is possible to calculate several structural parameters of these polymers.

Substitution degree (SD)

Substitution degree (SD) is the average number of substitutions per anhydroglucose unit. SD varies between 0 and 3 since there are three substitution sites per anhydroglucose unit.

Several methods can be used to calculate this parameter. The NMR spectrum can be used to calculate SD in three different ways.

a) SD1: The sum of substituted carbon atoms to the number of anhydroglucose units ratio.

The number of anhydroglucose units is equal to the number of C_1 anomeric carbon atoms (one anomeric carbon atom per unit).

$$SD_1 = \frac{C_{2s} + C_{3s} + C_{6s}}{C_1}$$

with:

C_{2s} = number of substituted C_2 carbon atoms

C_{3s} = number of substituted C_3 carbon atoms

C_{6s} = number of substituted C_6 carbon atoms

Because of the poor resolution of the C_{6s} signal, a better evaluation is obtained from the difference between the number of units and the number of nonsubstituted C_6 atoms (C_{6ns}).

$$SD_1 = \frac{C_{2s} + C_{3s} + (C_1 - C_{6ns})}{C_1}$$

b) SD_2: The carbonyl number (included in the substituent) to the anhydroglucose unit number ratio.

$$SD_2 = \frac{C_c}{C_1}$$

c) SD3: The methylenic carbon number (from substituents) to the number of anhydroglucose unit ratio.

The SD values calculated from NMR data are in good agreement with those calculated with other standard analytical techniques such as atomic absorption (Na content) or nonaqueous potentiometric titration (7,8) (see Table 3)

N	SD_1	1.15
M	SD_2	1.10
R	SD_3	1.03
A.A		0.98
potentiometric titration		1.05

Table 3 : Comparison of SD values calculated from NMR, atomic absorption and potentiometric titration.

Average number of anhydroglucose units in the polymeric chain after enzymatic hydrolysis.

Because anomeric carbon atoms inside the polymeric chain and those at the end of the chain are effectively separated, (δ = 102 ppm for a carbon atom inside the chain, and δ = 92 and 96 ppm for a carbon atom at the end of the chain) the ratio plus 1 of the intensity of these peaks, gives the average number of anhydroglucose units after enzymatic degradation. For the polymer tested, it gives an average number of 4.4 units.

Average molecular weight after enzymatic hydrolysis

From the calculated values of the substitution degree and the average number of anhydroglucose units, it is possible to predict the average molecular weight after enzymatic hydrolysis. For instance, for our sample M_w = 1082. This number confirms that the enzymatic hydrolysis process is very efficient since the molecular weight has been reduced by a factor of 10^3 (M_w before hydrolysis was 1.1 10^6).

Anhydroglucose unit conformation at the end of the polymeric chain

α and β conformations can be distinguished by the chemical shift of anomeric carbons of each form. The relative intensity of these signals gives the relative proportion of each form. For example, for our sample 37% was in α conformation and 63% was in β .

Preferential sites of substitutions

Chemical shift of C_{2s}, C_{3s}, and C_{6s} were determined and found to be quite different. The relative intensity of the corresponding signals gives the substitution's distribution at each position. For our sample we found 25% for C_2, 51% for C_3 and 24% for C_6.

HPLC analysis. Determination of the amount of glucose

It is well known that enzymatically broken positions are not random. They preferentially occur close to unsubstituted anhydroglucose units. Since enzymatic hydrolysis was maintained for at least 15 h (one night), we are convinced that the CMC was fully degradated. Accordingly no unsubstituted anhydroglucose units remained in the residual polymeric chains (i.e all unsubstituted anhydroglucose

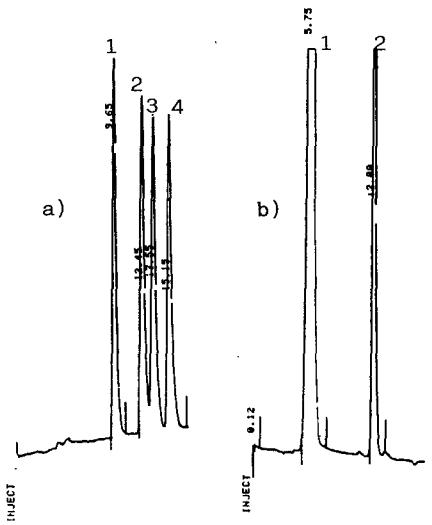


Fig n° 2 : *2a : Calibration of the column -*
1. cellobiose, 2. glucose, 3. xylose, 4. arabinose
2b : Hydrolysed C.M.C.
1. carboxymethyled oligomers, 2. glucose

units were in a glucose form, and the chromatogram (Fig 2) shows glucose but no oligomer of glucose). An HPLC glucose amount measurement gives information on the regularity of the substitution along the polymeric chain before hydrolysis. If the substitution was perfectly regular, for each SD value, there is a theoretical value for the amount of glucose (for instance, for SD ≥ 1 the amount of glucose should be zero).

Deviation between theoretical and experimental values and comparison of these deviations for polymers with identical SD values can be used to evaluate the regularity of substitutions. For our sample the amount of glucose was 5 weight %.

CONCLUSIONS

The NMR characterization of high molecular-weight CMC requires a preliminary degradation step. This paper gives the results of a comparative study of the efficiency of several methods of degradation and defines all the chemical parameters that may be deduced from ^{13}C NMR spectra. Acidic hydrolysis leads to poor results due to oxidation problems, and this method is not suitable for degrading CMC under good conditions. Although sonication gives good results for CMC degradation, enzymatic hydrolysis is more efficient. Several advantages have been pointed out.

Enzymatic hydrolysis leads to shorter polymeric chains. As such viscosity is no longer a problem, and it is possible to record spectra with a more concentrated solution (about 10 times more). Therefore, the signal-to- noise ratio is enhanced. Because of the low viscosity, the NMR resolution is better and peak indexing is easier, leading to more structural information and more accurate quantitative measurements. Moreover, spectra were recorded at room temperature, thus avoiding any thermal degradation problems.

Enzymatic hydrolysis of CMC is easier to control and faster than sonication. To prepare enough sample with the sonication method, we need about one day.

With the enzymatic method we need about half an hour to prepare the sample and, after the hydrolysis occurs without any intervention, we need about one night.

The number of unsubstituted anhydroglucose units can be measured by HPLC and provides information about the regularity of the substitution along polymeric chains.

The methodology described in this paper could be extended to different polysaccharides. Experiments with scleroglucan and xanthan are in progress to evaluate the regularity of their chemical structure.

REFERENCES

1 : Parfondry A, Perlin A.S, Carbohydrate Research, 57, 39, (1977)

2 : Reuben J, Conner H.T, Carbohydrate Research, 115, 1 (1983)

3 : Lorimer J.P, Mason T.J, Chem Soc Rev, 16, 239, (1987)

4 : Lindley J, Mason T.J, Chem Soc Rev, 16, 275, (1987)

5 : Chaudhari S.N.K, Gounden K.C, Srinivasan G, Ekkundi V, J of Polymer Science, Part A: Polymer Chemistry, 25, 337, (1987)

6 : Talvitie A, Parsivirta, J Finn Chem lett, 1, 18 (1984)

7 : MILAS M, Thesis, University of Grenoble (France) (June 1974)

8 : Gautier S, Internal report, not published, (1988)

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