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^{13}C NMR determination of substituent distribution in carboxymethylcellulose by use of its peresterified derivatives [1]

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Abstract

The distribution of carboxymethyl groups in a series of sodium *O*-(carboxymethyl)cellulose (CMC) samples was determined by ^{13}C NMR analysis of their peresterified derivatives. Thus sodium carboxymethyl groups were first converted into methyl ester groups by treatment with dimethyl sulfate in Me_2SO at 40 °C, to produce methyl-esterified CMC (MCMC). Subsequent propanoation of unsubstituted hydroxyl groups by propanoic anhydride in *N*,*N*-dimethylacetamide–LiCl at 100 °C in the presence of DMAP produced propanoated MCMC (PMCMC), which was readily soluble in Me_2SO-d_6 regardless of the degrees of substitution. The propanoyl carbonyl carbon signal in a series of PMCMC samples was found to resolve into three peaks in Me_2SO-d_6 at 100 °C, corresponding to the position of the substitution position (2, 3, and 6) on the glucose residue, permitting subsequent determination of the distribution pattern of carboxymethyl groups in the parent CMC samples. © 1996 Elsevier Science Ltd.

Keywords: Carboxymethylcellulose; Substituent distribution in polysaccharide derivatives; Substitution mode by ^{13}C NMR

1. Introduction

Sodium *O*-(carboxymethyl)cellulose, commonly termed carboxymethylcellulose or CMC, is a water-soluble cellulose derivative of significant commercial importance.

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[1] Part 11 of a series: ^{1}H and ^{13}C NMR structural study on cellulose and polysaccharide derivatives with carbonyl groups as a sensitive probe. For the preceding papers, see ref. [20].

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Various commercial grades of CMC have applications involving human-body contact, such as in food, pharmaceuticals, and cosmetics. Another important large-scale use of CMC is in environmentally sensitive applications such as soil treatment, oil recovery, and paper sizing processes [1].

There has been an increasing demand, from product-liability considerations, for rigorous control of the final properties of these CMC products, which are governed, in principle, by their detailed structural parameters. Consequently, a reliable and convenient analytical means to provide detailed information on the distribution of carboxymethyl groups on the glucose residues in CMC is of significant importance for both quality control in the production process, and for the detailed elucidation of structure–property relationships of the product.

Although the total carboxymethyl content in CMC samples is readily determined by means of a standard titration technique, the individual DS values at the 2, 3, and 6 positions in the glucose residue are obtainable only after hydrolysis of the CMC sample. GLC [2–4] and ^{1}H [5–7], ^{13}C NMR [8–10] techniques have been applied in examining CMC hydrolyzates. This hydrolytic pretreatment of CMC samples, however, requires careful precautions to avoid incomplete reaction and incomplete recovery of the hydrolyzate. In addition, the hydrolysis is frequently accompanied by side reactions involving intra- and inter-molecular lactonization between carboxylic acid and hydroxyl groups. GLC analysis of the hydrolyzate also requires additional multistep pretreatments prior to injection, such as the reduction of carbonyl groups and the appropriate protection of hydroxyl groups. In ^{1}H and ^{13}C NMR spectroscopic analysis, on the other hand, a spectral complication arises from the presence of α and β anomers in the hydrolyzate. Therefore, an alternative analytical means to determine the substituent distribution in CMC samples while retaining its polymeric form [11–13], would be of significant practical importance.

We have proposed a new analytical technique for cellulose derivatives, in particular cellulose ethers, in which unsubstituted hydroxyl groups on the glucose residues (and those at the end of substituent groups in some cases) are peracetylated and examined by ^{13}C NMR [14–18]. We have also demonstrated recently that the distribution of substituents in partially substituted cellulose acetate, and also the distribution pattern of two ester substituents in cellulose acetate butyrate, are readily determined by means of a ^{13}C NMR technique by making use of either a propanoated derivative or directly via their carbonyl groups as a sensitive NMR probe [19,20].

As an extension of the preceding studies, we propose herein a convenient ^{13}C NMR technique for determining the substituent distribution in CMC samples by making use of peresterified derivatives.

2. Experimental

Samples.—A series of sodium *O*-(carboxymethyl)cellulose (CMC) samples having different DS values were supplied from Dai-ichi Kogyo Seiyaku Co. Ltd. Methyl esterification of sodium carboxymethyl groups of a series of CMC samples was performed using dimethyl sulfate in Me_2SO solution. Thus in a 300 mL round-bottom

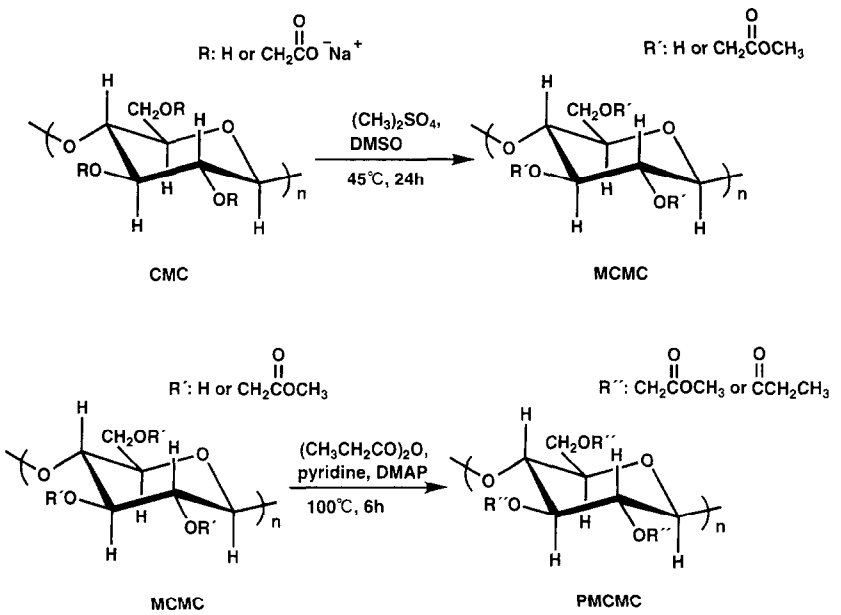
flask, 5.0 g of CMC sample, 100 mL of Me_2SO and 20.0 mL (206 mmol) of dimethyl sulfate were introduced and the mixture was heated for 24 h at 45 °C. The mixture became homogeneous during the course of the reaction. The reaction product was isolated by pouring the mixture into 2 L of methanol containing 0.1 vol% of HCl, and the product was finally dried in vacuo. Subsequent propanoation of a series of methyl-esterified CMC (MCMC) samples was carried out by propanoic anhydride–pyridine–4-(dimethylamino)pyridine in DMAc–LiCl solution. Thus in a 100-mL round-bottom flask, 1.0 g of the MCMC sample, 5.0 mL (39 mmol) of propanoic anhydride, 50 mL of pyridine, and 0.20 g (1.64 mmol) of 4-(dimethylamino)pyridine were dissolved in 50 mL of *N,N*-dimethylacetamide containing 6 wt% of LiCl. The mixture was then heated for 6 h at 100 °C, during which time the mixture became homogeneous and dark-brown. The product was isolated by pouring the mixture into 2 L of water (for the CMC of DS = 0.76) or 2-propanol (for others), and finally dried in vacuo. The isolated yield of propanoated MCMC (PMCMC) was not less than 83%.

Measurements.—^{13}C NMR measurements were performed with a Jeol EX-400 spectrometer at 100 MHz with a 5-mm wide C–H dual probe, for solutions in D_2O at 80 °C, in $CDCl_3$ at 40 °C, or in Me_2SO-d_6 at 100 °C. Chemical-shift values were referenced from the solvent signal of either $CDCl_3$ (77.0 ppm) or Me_2SO-d_6 (43.5 ppm), which was added also in the D_2O measurements as a chemical-shift standard. Quantitative-mode ^{13}C NMR measurements were performed by a non-NOE gated decoupling technique with a pulse repetition time of 30 s, and with up to 2000 transients. IR spectra were recorded on a Shimadzu Model FT-IR 8100 spectrophotometer.

3. Results and discussion

Sodium *O*-(carboxymethyl)cellulose (CMC), possessing ionic substituents, is soluble in water but insoluble in most organic solvents. Because of this particular solubility property, attempts at direct esterification (acetylation or even succinylation) in various solvents, including water-containing mixtures, have failed to produce a CMC derivative upon which quantitative conversion of hydroxyl groups could be achieved. The acid treatment of CMC, to convert carboxylate salt groups into carboxylic acid groups, led to crosslinking because of spontaneous lactonization between carboxylic acid and unsubstituted hydroxyl groups. Consequently, in the present study, we first converted the sodium carboxymethyl groups directly into methyl carboxylate ester groups by reaction with dimethyl sulfate. The residual hydroxyl groups in methyl-esterified CMC (MCMC) samples were subsequently propanoated (or acetylated) to introduce carbonyl groups as an NMR structural probe for determining the substituent distribution of the ester groups introduced, and consequently that for the carboxymethyl groups in the starting CMC samples (Scheme 1).

Fig. 1 shows IR spectra of the starting CMC sample, together with those of the methyl-esterified (MCMC) and the propanoated (PMCMC) samples. The absorption due to carboxylate salt groups at 1600 cm^{-1} visible in the CMC diminished after the methyl esterification treatment, and that due to ester groups were onserved instead at 1740



Scheme 1.

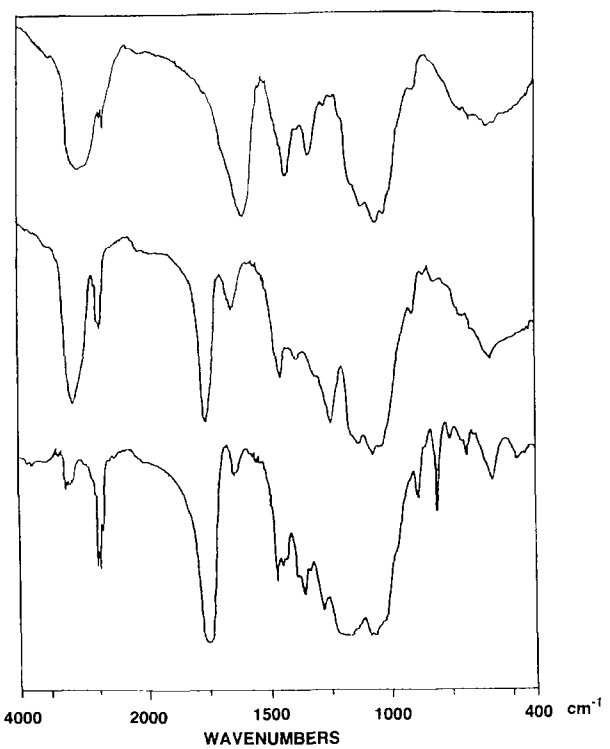


Fig. 1. IR spectra of CMC (top), MCMC (middle), and PMCMC (bottom). (sample; DS = 0.76)

Table 1
Solubility of CMC, MCMC and PMCMC samples [a]

Type	DS [b]	Solvent		
		H_2O	$(CH_3)_2SO$	$CHCl_3$
CMC	0.76	○	×	×
CMC	1.44	○	×	×
CMC	2.04	○	×	×
CMC	2.24	○	×	×
MCMC	0.76	△	○	△
MCMC	1.44	△	△	△
MCMC	2.04	△	○	△
MCMC	2.24	△	○	△
PMCMC	0.76	×	○	○
PMCMC	1.44	×	○	○
PMCMC	2.04	×	○	○
PMCMC	2.24	×	○	○

[a] ○: Soluble, △: swelling, ×: insoluble, 5 wt%, r.t.
[b] By titration.

cm^{-1}. The subsequent propanoation reaction eliminated the hydroxyl groups absorption at 3500 cm^{-1} and led to further increase in the absorption due to ester groups. This confirms the efficient esterification of carboxymethyl groups, and the subsequent propanoation of the MCMC sample.

The results of solubility tests for CMC, MCMC, and PMCMC samples of different DS values are collected in Table 1. PMCMC samples of a wide range of DS values became readily soluble in such common NMR solvents as $CDCl_3$ and Me_2SO-d_6. Since the signal separation for measurements in Me_2SO-d_2 at 100 °C was superior to those in $CDCl_3$ at 40 °C for a series of PMCMC samples, the following structural analysis for PMCMC samples was conducted with the former solvent system.

Full-range and carbonyl-region ^{13}C NMR spectra of CMC, MCMC, and PMCMC samples of identical DS values are compared in Figs. 2 and 3, respectively. In the spectrum for unmodified CMC in D_2O, the signal for the unsubstituted C-6 appears at 64.4 ppm and that for C-1 at 106.3 ppm, with peak broadening. Other signals for the glucose residues appear at 75–84 ppm as poorly resolved, broad peaks. The carboxymethyl methylene carbon signal is also visible at 74.2 ppm. The carboxymethyl carbonyl signal appears at 180–183 ppm, and is resolved into three peaks only when the DS is higher than ~2.0. Thus none of these signals, except for the carboxymethyl carbonyl signal for CMC of high DS values, are directly informative in providing the substituent pattern of carboxymethyl groups.

Upon the methyl-esterification treatment, the methyl ester signal appears at 54.5 ppm. The unsubstituted C-6 signal is also visible at 63.9 ppm. Interestingly, the C-1 signal was found to be resolved into two peaks according to the substitution mode on the C-2 position, namely that with substitution at 104.3 ppm and another without substitution at 106.0 ppm. The carbonyl-carbon signal appears at 173–174 ppm, and is resolved into three peaks (173.2, 173.6, and 173.8 ppm), as in the case of unmodified CMC of high

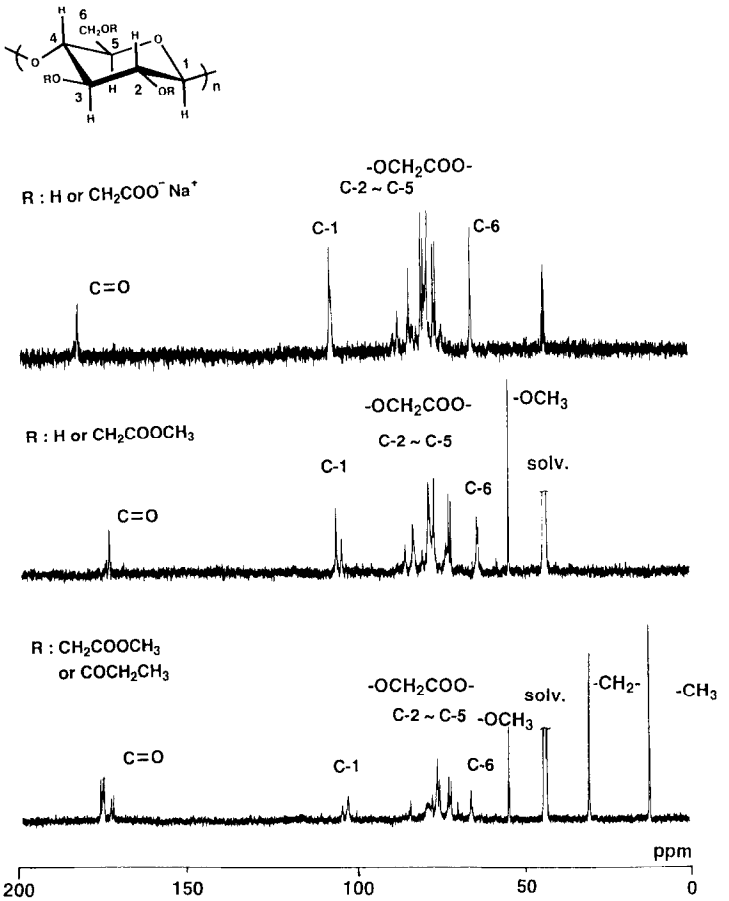


Fig. 2. 100-MHz ^{13}C NMR full-range spectra of CMC (top), MCMC (middle), and PMCMC (bottom). (Sample: DS = 0.76, in D_2O at 80 °C for CMC and in Me_2SO-d_6 at 100 °C for MCMC and for PMCMC).

DS ($> \sim 2.0$). The signal separation in MCMC was found to be superior as compared with that in CMC samples, and the distribution of substituents at C-2 and at C-6 positions may be obtained either directly or indirectly through integration and comparison of the relevant signal areas, in particular for samples of high DS values. In practice, however, a quantitative-mode ^{13}C NMR measurement requires a relatively long repetition-time, and this mode of operation gives rise to a serious line-broadening for the ring carbon signals on the glucose residues because of their relatively short transverse relaxation times (T_2). This adverse effect is often a limitation in determining an accurate area ratio of signals in the glucose residue.

By subsequent propanoation of the residual hydroxyl groups of the MCMC samples, additional signals are introduced, such as propanoyl methyl and methylene signals at 12.5 and 30.6 ppm, respectively. Importantly, the propanoyl carbonyl signal appears as a resolved triplet at 175.7, 176.2, and 176.7 ppm, which is separated from the car-

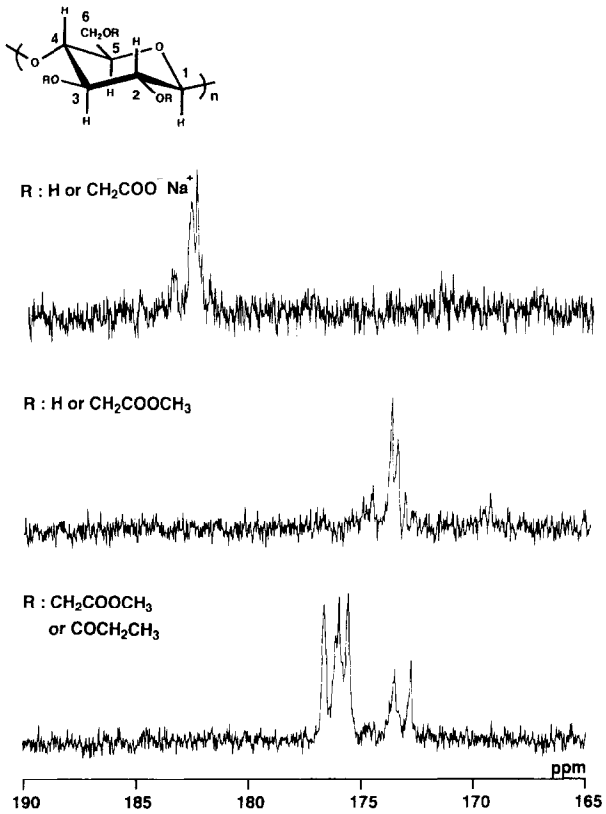


Fig. 3. 100-MHz ^{13}C NMR carbonyl region spectra of CMC (top), MCMC (middle) and PMCMC (bottom). (Sample: DS = 0.76, in D_2O at 80 °C for CMC and in Me_2SO-d_6 at 100 °C for MCMC and for PMCMC).

boxymethyl carbonyl signal at 172.8–173.9 ppm. With reference to the carbonyl signal for cellulose tripropanoate as a model compound [20], the three peaks are assigned directly as that at the C-2 (175.7 ppm), C-3 (176.2 ppm), and C-6 (176.7 ppm) positions, respectively.

Although the related acetylated derivative of the MCMC samples could be produced by a similar acylation treatment, the acetyl carbonyl signals and carboxymethyl carbonyl signals were observed as overlapped peaks in Me_2SO-d_6 at 100 °C. This problem could be solved, as shown already, by the introduction of propanoyl groups.

Since the distribution pattern of propanoyl groups in the PMCMC samples corresponds directly to that of the hydroxyl groups in the starting CMC samples, the absolute distribution pattern of the carboxymethyl groups may be obtained directly by comparison of the individual signal intensities. Thus the three peaks in the carboxymethyl carbonyl signal can be also assigned accordingly, namely, C-6 (as a doublet at 172.9 and 173.2 ppm), C-2 (173.6 ppm), and C-3 (173.8 ppm), respectively. In the carboxymethyl carbonyl signal, the difference in chemical-shift values of C-2 and C-3 is too small to determine individual DS values in the samples of low total DS. The signal for the

Table 2
Distribution of substituents in CMC samples

Sample	Total DS		Individual position [a,b]		
	Titration	^{13}C NMR [a]	2	3	6
1	0.76	0.80 (0.66)	0.31 (–) [c]	0.17 (–) [c]	0.32 (0.27)
			0.46		0.42
2	1.44	1.54 (1.48)	0.57 (0.57)	0.48 (0.34)	0.49 (0.57)
			0.58		0.48
3	2.04	2.00 (2.12)	0.73 (0.73)	0.62 (0.68)	0.66 (0.70)
			0.73		0.67
4	2.24	2.21 (2.10)	0.76 (0.71)	0.68 (0.63)	0.77 (0.76)
			0.75		0.81

[a] Top column: propanoyl and carboxymethyl (in parentheses) carbonyl analysis.
[b] Bottom column: C-1 and C-6 analysis.
[c] The sum of DS (C-2 + C-3): 0.38.

propanoated C-6 is visible at 66.1 ppm, and the C-1 signal appears as resolved peaks corresponding to the substitution mode on the C-2 position, that is, carboxymethyl-substitution at 104.5 ppm and propanoyl-substitution at 103.0 ppm, respectively. Accordingly, the individual DS values on C-2, C-3, and C-6 positions were estimated separately and compared internally to evaluate the precision of the present analytical technique (Table 2).

The unsubstituted C-1 and C-6 signals observed in MCHC samples were totally removed after the propanoation treatment, and the relative intensity of the sum of the carbonyl signals from carboxymethyl and propanoyl groups is observed to be close to three times that of the C-1 signal, indicating complete propanoation of MCMC.

^{13}C NMR carbonyl-region spectra of a series of PMCMC samples having different DS values are listed in Fig. 4. The propanoyl carbonyl signal was resolved into three peaks regardless of the DS of the sample, corresponding to its substitution position on the glucose residue. The carboxymethyl carbonyl signal was also resolved into three peaks, while the difference in the chemical shift for C-2 and C-3 is insufficient for estimating individual DS values for samples of low DS. Complementary information on the distribution of substituents can thus be obtained for comparison with information from propanoyl carbonyl analysis, in particular for samples of high DS, where the absolute signal intensity of propanoyl carbonyl signal becomes too weak to allow estimation of precise signal intensities.

The quantitative-mode ^{13}C NMR measurement for PMCMC samples of different DS values, thus permits determination of the distribution of substituents in the starting CMC samples. The results obtained for a series of CMC samples are collected in Table 2. The total DS value of the starting CMC, estimated by ^{13}C NMR from the signal-intensity ratio of C-1 with carboxymethyl carbonyl peaks, agrees with that obtained by the titration technique. Also the individual DS values estimated from carbonyl-region analysis and from the C-1, C-6 signals are, in general, consistent each other, although a line-broadening of C-1 and C-6 signals occurring in the quantitative-mode measurement

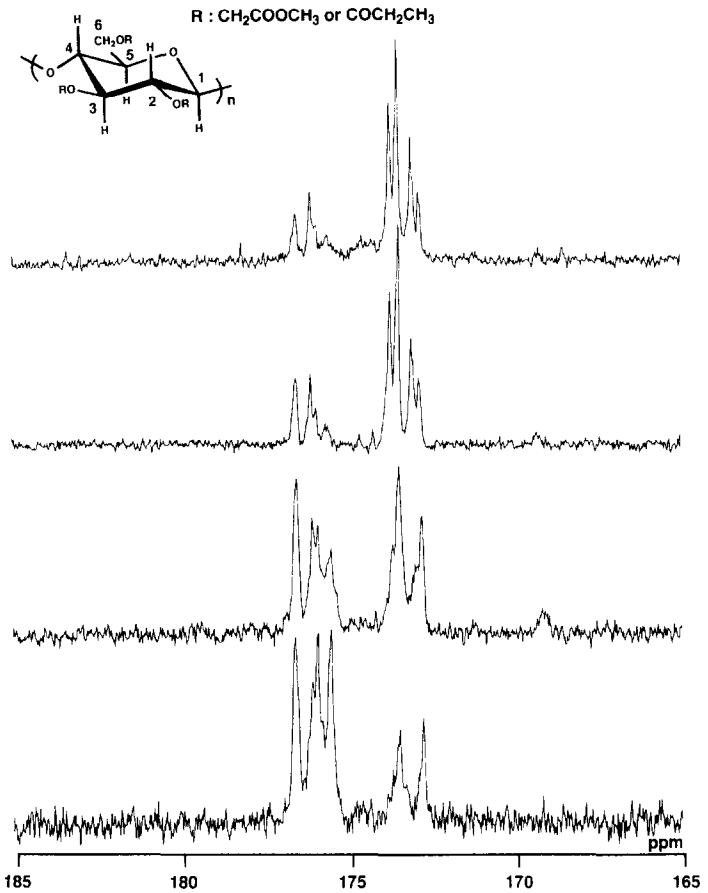


Fig. 4. 100-MHz ^{13}C NMR carbonyl-region spectra of PMCMC samples having different DS values. (Samples: 1–4 in Table 2, from bottom to top, in Me_2SO-d_6 at 100 °C).

leads to a noticeable discrepancy with those from carbonyl-region analysis for samples of lowest DS values.

It is confirmed from the present analysis that carboxymethylation takes place predominantly at the C-2 and C-6 positions in the initial stage, and reaction at the C-3 position follows at a later stage. The relative reactivities of the different hydroxyl groups in the glucose residue observed in the present study coincides with the results of previous studies on commercial CMC samples of different sources, where GLC and ^1H NMR techniques were applied to hydrolyzates of CMC [2–9]. The relative order of reactivity also agrees with that observed in other etherification reactions of cellulose by alkyl halides or epoxides [14–18].

In conclusion, the distribution pattern of carboxymethyl groups in CMC samples having a wide range of DS values is readily determined by means of a ^{13}C NMR technique that utilizes peresterified derivatives. This novel technique may offer a

significantly improved analytical means for providing detailed structural parameters in CMC samples of considerable commercial importance.

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