Determination of the Distribution of Substituent Groups in Sodium Cellulose Sulfate: Assignment of ^1H and ^{13}C NMR Peaks by Two-Dimensional COSY and CH-COSY Methods

Keisuke KOWSAKA, Kunihiko OKAJIMA, and Kenji KAMIDE*

*Fundamental Research Laboratory of Fibers and Fiber-Forming Polymers,
Asahi Chemical Industry Co., Ltd., 11-7 Hacchonawate,
Takatsuki, Osaka 569, Japan*

(Received September 25, 1990)

ABSTRACT: An attempt was made to give full assignment to the ^1H and ^{13}C NMR spectra of sodium cellulose sulfate (NaCS) dissolved in D_2O and to determine the molar fractions of all possible 8 kinds of anhydroglucopyranose (AHG) units constituting NaCS molecule. For this purpose five NaCS samples with the total degree of substitution $\langle F \rangle$ ranging from 0.61 to 2.42 were synthesized by three different methods. Using a NaCS sample ($\langle F \rangle = 2.42$) ^1H and ^{13}C peaks for 2,3,6-trisubstituted AHG were reasonably assigned by two-dimensional (2D) homonuclear ^1H correlation spectroscopy (COSY) and heteronuclear ^1H – ^{13}C correlation spectroscopy (CH-COSY) techniques. In the same manner, ^1H and ^{13}C NMR peaks originating from unsubstituted AHG were assigned by analysis on NaCS with $\langle F \rangle = 0.61$. Chemical shifts (δ) of protons and carbons in monosubstituted and disubstituted AHG units were calculated using shift factors empirically estimated from the observed δ values of trisubstituted and unsubstituted AHG units. The assignments on ^1H NMR peaks for NaCS previously made by Kamide and Okajima [*Polym. J.*, **13**, 163 (1981)] on the basis of the peak assignment of cellulose acetate were found to be partly incorrect. The average degree of substitution for each three hydroxyl groups attached to C_2 , C_3 , and C_6 carbon positions $\langle f_k \rangle$ ($k=2, 3$, and 6) was estimated based on the assignment made here and a relation $\langle f_6 \rangle > \langle f_2 \rangle > \langle f_3 \rangle$ was obtained for the NaCS samples synthesized here.

KEY WORDS Cellulose / Sodium Cellulose Sulfate / Degree of Substitution / Distribution of Sulfate Group / Nuclear Magnetic Resonance / Two-Dimensional NMR /

Cellulose sulfate (CS), one of the oldest cellulose derivatives, resembles mucopolysaccharides such as heparin and chondroitin sulfate in its chemical structure and possesses blood anticoagulant activity.¹ Kamide and his coworkers systematically studied dilute solution properties,² physiological activities,³ and pharmacodynamic properties⁴ of CS.

Experimental determination of the average degree of substitution for three hydroxyl groups attached to C_2 , C_3 , and C_6 carbon positions in anhydro-glucopyranose (AHG) units constituting cellulose derivative mole-

cules $\langle f_k \rangle$ ($k=2, 3$, and 6) has attracted keen attention because this kind of molecular characterization unquestionably affords the strong possibility of establishing the structure–property relations of cellulose derivatives. For example, Kamide and his coworkers proposed the ^1H and ^{13}C NMR spectroscopic methods for estimating $\langle f_k \rangle$ of cellulose acetate (CA),^{5–8} carboxymethyl cellulose (CMC),⁹ cellulose xanthate (CX),¹⁰ carboxyethyl carbamoyl ethyl cellulose,¹¹ and CS.^{3,12} They successfully demonstrated that the properties of these cellulose derivatives are

* To whom all correspondence should be addressed.

controlled not only by the total degree of substitution $\langle F \rangle$, which was the sole parameter representing molecular structure employed for long years, but also by $\langle f_k \rangle$.

For cellulose derivatives 8 different AHG units are theoretically expected to exist: 2-, 3-, 6-monosubstituted, 2,3-, 2,6-, 3,6-disubstituted, 2,3,6-trisubstituted, and unsubstituted ones (see Figure 1). Then, one can imagine easily that even $\langle f_k \rangle$ is not always sufficient to explain completely the properties of cellulose derivatives and the distribution of these AHG units should be determined.

Wu,¹³ Clark and Stephenson¹⁴ proposed the ^{13}C NMR spectroscopic method for estimating the molar fraction of 4 AHG units (2,3,6-tri-, 2,6-di-, 3,6-di-, 6-monosubstituted ones) of cellulose nitrate (CN) when the hydroxyl group at C_6 position was fully substituted. Very recently, Kowsaka *et al.*⁸ defined the molar fractions of these 8 AHG units as $\langle f_{lmn} \rangle$ ($l=1$ or 0 means that a hydroxyl group attached to C_2 position is substituted or not, m and n indicate corresponding values in C_3 and C_6 positions) and succeeded in determining $\langle f_{lmn} \rangle$ of CA samples by analyzing the carbonyl carbon region of their $^{13}\text{C}\{^1\text{H}\}$ NMR spectra.

Studies on $\langle f_k \rangle$ of CS were first (in 1981) made by Kamide and Okajima,¹² who measured the ^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of sodium salt of CS (NaCS) with $\langle F \rangle = 1.96$ dissolved in deuterium oxide (D_2O) and assigned 6 ^1H peaks originating from AHG units from lower magnetic field as H_3^* , H_2^* , H_1 , H_6^* , (H_3 , H_4 , H_6) and H_5 protons, with reference to the assignment made for CA.¹⁵ Here, H_k ($k=1-6$) denotes each proton attached to the corresponding C_k carbon position in AHG unit and $*$ denotes the corresponding proton when the hydroxyl group at the C_k position is substituted. They demonstrated that blood anticoagulant activity and acute toxicity (lethal dose 50 for rat, administrated intravenously) of NaCS were mainly governed by $\langle f_2 \rangle + \langle f_3 \rangle$ determined by NMR spectroscopy. Note that in some cases

they unavoidably estimated $\langle f_6 \rangle$ as the difference between $\langle F \rangle$ determined chemically and $\langle f_2 \rangle + \langle f_3 \rangle$ determined above.³ However, recent advance in NMR techniques brings about the following questions regarding the previous ^1H NMR peak assignment for NaCS carried out by Kamide and Okajima¹²: (1) Resolution of their 100 MHz ^1H NMR spectra was not enough to separate peaks accurately and this situation might be enhanced when D_2O is used, (2) oversimplified assumption, by them, of a one-to-one correspondence of the peak assignment between CA and NaCS cannot always be accepted and the possible difference in magnetic influence on other protons caused by substitution at C_k position between CA and NaCS should have carefully been considered, (3) the assumption by them that only nine non-equivalent protons (H_1 — H_6 , H_2^* , H_3^* , H_6^*) exist is crude and rather wrong at present. These questions lead us to the conclusion that the past determination of $\langle f_k \rangle$ of CS by ^1H NMR analysis is intrinsically accompanied with low accuracy.

The peak separation of $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum for water-soluble cellulose derivative is generally better than that for ^1H NMR spectrum when D_2O is used as solvent. The peak assignment for carbons constituting glucopyranose backbone might not be so easy but the recent remarkable advance in pulse techniques in Fourier transform NMR may overcome this situation. In fact, Kowsaka *et al.*¹⁶ succeeded in assigning the carbon peaks of CA employing two-dimensional (2D) NMR technique.

In this study, an attempt was made to give complete assignments for ^{13}C and ^1H NMR spectra of NaCS samples with the aid of 2D NMR and check the validity of the ^1H peak assignment previously given by Kamide and Okajima.¹²

EXPERIMENTAL

Sample Preparation

The sodium salt of cellulose sulfate (NaCS) was prepared according to the procedures described in the previous paper,³ using an acid-hydrolyzed soft wood pulp (the viscosity-average molecular weight $M_v = 9.7 \times 10^4$, as measured by solution viscosity in cadoxen at 25°C using the Mark-Houwink-Sakurada equation established by Brown and Wikström¹⁷). The detailed procedures are as follows:

(1) Two grams (g) of acid-hydrolyzed pulp were dispersed in 18 g of dimethyl formamide (DMF) at 25°C. The DMF-wetted cellulose was mixed with 9 g of DMF-sulfur trioxide (SO_3) complex (2:1, $\text{mol} \cdot \text{mol}^{-1}$; solid) precooled at 0°C. The mixture was stirred vigorously at 25°C for 1 h to make a transparent solution, which was poured into 2 l of NaOH-methanol-water (1:90:9, w/w/w) to precipitate the crude NaCS. The crude NaCS separated on a filter was washed with 1.5 l of methanol-water (9:1, v/v at 25°C) and then dissolved in distilled water. The polymer was reprecipitated with methanol, washed several times with a mixture of methanol-water (7:3, v/v at 25°C), and finally subjected to drying *in vacuo*. The sample thus prepared was denoted as DS-1. Two samples (denoted as DS-2 and DS-3) were synthesized by the same procedure as that described above by using 6 or 4 g of DMF/ SO_3 complex against 2 g of cellulose, respectively. In these cases the unreacted and not-dissolved cellulose in the system was first removed out by centrifuging ($1.5 \times 10^4 g$ for 1 h) the reaction mixture. The sample DSH-1 was prepared by the same procedure as for DS-1 except that the reaction temperature was 70°C.

(2) Ten grams of acid-hydrolyzed pulp were mixed with 40 g of a mixture of 98% sulfuric acid-butanol (110:29, v/v) at 20°C, stood for 10 min and this reaction mixture was poured into a system (1 l) of diethylether containing dry ice. On standing, the system

separated into a supernatant solution and brownish viscous liquid. The former phase was subjected to the same procedure as that described in (1) and the recovered polymer was coded as HBH'-1. Note that HBH series samples in ref 3 were recovered from the brownish viscous liquid.

NMR Measurements

NMR measurements on NaCS samples dissolved in D_2O were carried out using a JNM-FX200 type FT-NMR spectrometer (JEOL, Japan) with C/H 10 mm ϕ probe. ^1H and ^{13}C resonance frequencies were 199.5 and 50.18 MHz, respectively. Sodium trimethylsilylpropionate (TSP) was used as the internal standard.

^1H and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra were measured according to the method described in the previous paper.¹⁶ The water elimination Fourier-transform (WEFT) method was applied for ^1H NMR measurements to diminish peak intensity originating from HDO. Homonuclear ^1H shift correlation spectroscopy (^1H COSY) and heteronuclear ^{13}C - ^1H shift correlation spectroscopy (CH-COSY) were recorded as follows:

(1) ^1H COSY experiments were carried out at 70°C using a 90° - t_1 - 90° pulse sequence under the following operating conditions: spectral width, 500 Hz; data matrix, 512×256 ; pulse interval, 1.5 s; accumulation, 128. The polymer concentration was *ca.* 5 wt%. Homogate decoupling was employed simultaneously in order to reduce signals originating from remaining protons in the solvent. Other operating conditions are indicated in the previous paper.¹⁶

(2) The CH COSY spectrum was recorded using the pulse sequence method described in the previous study.¹⁶ The mixing time Δ was set up to be 2.0 ms ($= 1/(4J_{C-H})$), where J_{C-H} is the average coupling constant between C and H nuclei. Operating conditions are follows: ^{13}C spectral width, 2500 Hz; ^1H spectral width, 500 Hz; pulse interval, 1 s; accumulation, 2048;

data matrix; 1024×40 (1000×128 after zero-filling on t_1 axis). The polymer concentration was *ca.* 15 wt%. Other operating conditions are described in ref 16.

For ^1H and COSY measurements, DS-1 was once dissolved in D_2O , freeze dried and again dissolved in D_2O in order to diminish HDO peak.

RESULTS AND DISCUSSION

Figure 1 illustrates the possible 8 AHG units constituting NaCS chains, predicting the existence of magnetically non-equivalent 48 ^{13}C and 56 ^1H nuclei. Here, the number of ^1H nuclei does not include three ^1H nuclei for the unreacted OH protons at C_2 , C_3 , and C_6 positions in AHG unit because they are almost instantaneously exchanged by D nuclei of the solvent. Note that none of these expected peaks (*i.e.*, 48 ^{13}C and 56 ^1H peaks) will be observed actually as separate peaks because of the overlapping of the peaks.

Figure 2 shows ^1H WEFT NMR spectra of the samples DS-1(a), DSH-1(b), and HBH'-1(c). The negative resonance (arrowed in the figure) observed around 4.3 ppm might be due to a little shorter pulse interval applied for WEFT here, than the most adequate pulse interval ("null-point") and the resonance is assigned to HDO proton. The peak appearing at 3.36 ppm in Figure 2c is responsible for methanol which is introduced as a contaminant during the preparation of NaCS and this peak was ignored in further analysis. For DS-1, ^1H peaks are observed in the chemical shift (δ) range of 3.6–5.0 ppm. On the other hand, for DSH-1 and HBH'-1 ^1H peaks are observed in the chemical shift range of 3.3–4.7 ppm. Taking the estimated reading error of δ (0.02 ppm) into account, 10 ^1H peaks were detected besides HDO peak. The δ values of all the peaks detected for each NaCS sample are listed in Table I. The peaks appearing around 4.4 and 4.6 ppm in Figures 2b and 2c are triplet peaks split by vicinal coupling.

Figure 1. Substituted and unsubstituted glucopyranose units in NaCS molecules: $\langle f_{lmn} \rangle$ represents molar fraction of the unit.

Figure 2. ^1H NMR spectra of NaCS samples in D_2O solution using WEFT technique: a, sample DS-1; b, sample DSH-1; c, sample HBH'-1.

Therefore, δ values at the central peaks are employed in the Table. Note that, judging from the number in peaks observed and their δ

Table I. Chemical shifts δ of proton peaks of NaCS samples in D₂O

Peak No.	δ /ppm		
	DS-1	DSH-1	HBH'-1
1	4.93		
2	4.70		
3		4.58	4.57
4	4.41	4.43	4.42
5	4.28	4.32	4.30
6	4.18		
7		4.02	4.02
8		3.96	3.96
9	3.84	3.85	3.84
10	3.66	3.68	3.67
11		3.39	3.38

Table II. Chemical shifts δ of carbon peaks of NaCS samples in D₂O

Peak No.	δ /ppm		
	DS-1	DSH-1	HBH'-1
1	104.8	104.8	104.8
2		104.6	104.6
3	103.4	?	?
4	102.9	?	?
5	84.5	84.3	—
6	82.4	?	?
7	—	81.7	81.7
8	81.2	81.3	81.3
9	—	80.6	80.6
10	80.1	—	—
11	77.5	77.5	77.5
12	(76.8*)	76.8	76.8
13	75.8	75.8	75.8
14	75.5	75.5	75.5
	(74.3?)	(74.3?)	(74.3?)
15	69.2	69.2	69.2
16	—	63.0	63.0

? not clearly detected; —, not detected; *, included in No. 11 peak; (74.3?), detected but not be clear to arise from AHG units of polymer.

Table III. Proton and carbon chemical shifts δ of cellulose in aq. NaOD collected from Figures 4 and 6 in ref 16

Observing nucleus	δ /ppm	Assignment
¹ H	4.49	H ₁
	3.95	H _{6'}
	3.80	H ₆
	3.57	H ₃ , H ₄ , H ₅
	3.29	H ₂
¹³ C	106.7	C ₁
	81.8	C ₄
	78.2	C ₃ , C ₅
	76.8	C ₂
	63.5	C ₆

Figure 3. ¹³C{¹H} NMR spectra of NaCS samples in D₂O solution: a, sample DS-1; b, sample DSH-1; c, sample HBH'-1.

values, the sample DSH-1 is similar to that reported for NaCS with $\langle F \rangle = 1.96$, synthesized by Kamide and Okajima.¹²

Figure 3 shows the ¹³C{¹H} NMR spectra of DS-1(a), DSH-1(b), and HBH'-1(c). The spectra of DSH-1 and HBH'-1 are similar with each other, except for 9 small sharp peaks, marked by arrows in Figure 3c, which are probably originated from contaminants such

as cellulosic oligomers produced as by-product during synthesis of HBH'-1. 16 Peaks in all were observed and their δ values are indicated in Table II.

Table III gives the chemical shifts and peak

assignments of ^1H and ^{13}C NMR peaks for cellulose/ NaOD at 20°C , which was cited from Figures 4 and 6 in ref 16. Here, the assignments for the ^{13}C peaks at 81.8 and 78.2 ppm are made with reference to Nardin and Vincendon's study,¹⁸ which was carried out for the cellulose/dimethylacetamide/lithium chloride system. A comparison of Tables I and II with Table III shows that DSH-1 and HBH'-1 have NMR peaks at almost the same positions as those observed for cellulose but DS-1 has numerous peaks besides those observed for cellulose. This suggests that DS-1 is highly substituted.

Figure 4 shows the contour plot of ^1H COSY (power spectrum) of DS-1 with a projection on the horizontal axis. The projection spectrum shown in Figure 4 has apparently a higher resolution compared with the spectrum shown in Figure 2a, but a small peaks (peak 10) at 3.66 ppm is suppressed. This might be due to application of a trapezoidal window function to the observed free induction decay signal before Fourier transformation on the COSY measurement. The peaks, observed at 4.92 (doublet), 4.68 (triplet), 4.42 (triplet?), 4.14 (triplet), and 3.88 ppm (doublet) on the projection correspond to Nos. 1, 2, 4, 6, and 9 peaks in Table I, respectively. The peak at 4.92 ppm shows only one cross-peak in the contour plot in Figure 4 and is attributed to H_1 proton. The appearance of the cross-peak at 4.42 ppm, shown as " $\text{H}_1\text{-H}_2$ " in Figure 4, enables us to attribute this peak to H_2 proton. In a similar manner, H_3 , H_4 , H_5 proton peaks can be assigned and the final assignments attained, excluding HDO proton peak region, are given on the projection spectrum, that is, H_1 , H_3 , H_2 , H_4 , and H_5 proton peaks from lower magnetic field.

Figure 5 shows the contour plot of CH-COSY (power spectrum) for DS-1 with projection spectra on the horizontal (^{13}C) and longitudinal (^1H) axes. Six peaks, observed at 103.5, 81.2, 80.1, 77.5, 76.0 and 69.6 ppm on the ^{13}C projection spectrum, correspond to No.

Figure 4. Homogate decoupled proton COSY spectrum of NaCS (sample DS-1).

3, 8, 10 (and 9), 11, 13 (and 14), and 15 peaks in Table II, respectively. The peaks, assigned in Figure 4, are also observed in the ^1H projection spectrum. The contour plot revealed that the ^{13}C peak at 103.5 ppm was correlated with the ^1H peak at 4.93 ppm, assigning this ^{13}C peak to C_1 carbon. In the same manner, three ^{13}C peaks at 81.2, 77.5, and 76.0 ppm were assigned to C_3 , C_4 , and C_5 carbons, respectively. The ^1H peak at 4.42 ppm (H_2 proton) is correlated with two ^{13}C peaks at 80.1 and 69.6 ppm and therefore, the peak at 4.42 ppm is expected to be an overlapping peak of H_2 proton and an other proton. The ^{13}C peak at 69.6 ppm is assigned to C_6 carbon because the C_6 carbon peak is without exception observed in the highest magnetic field in the spectra of cellulose,¹⁶ CA,¹⁶ CN,¹⁴ and CX.⁶ This leads to the conclusion that the ^1H peak at 4.42 ppm originates from H_2 and H_6 (and H_6') protons; note that there are two magnetically non-equivalent H_6 protons, H_6 and H_6' . The difference between δ values of ^1H

Figure 5. CH-COSY spectrum of NaCS (sample DS-1).

and ^{13}C peaks for DS-1 (*cf.* Figures 4 and 5) and those for cellulose (*cf.* Table III) are summarized as follows: (1) the peaks due to H_2 , H_3 , and H_6 protons for DS-1 appear in the lower magnetic field by 0.6–1.1 ppm than those of cellulose, (2) C_2 , C_3 , and C_6 carbon peaks for DS-1 substantially shifted to a lower magnetic field (3.0–6.0 ppm), compared with the corresponding carbon peaks for cellulose, (3) hardly any of the δ values of ^1H and ^{13}C peaks for DS-1 coincide with those of cellulose. These facts suggest that almost all NMR peaks for DS-1 should appear as a result of the almost complete substitution of three hydroxyl groups at C_2 , C_3 , and C_6 positions. Therefore, almost all peaks observed in Figures 4 and 5 originate mainly from the tri-substituted AHG unit although there are some peaks (84.0, 82.4, and 75.2 ppm) without a detectable cross peak on the CH-COSY spectra.

Figure 6 shows ^1H COSY of HBH'-1. The spectrum is quite similar to that for cellulose/NaOD system,¹⁶ suggesting that $\langle F \rangle$ of this polymer is very low. The following assignments are easily made: H_1 , 4.57 ppm; H_6 , 3.99 ppm (doublet); H_6' , 3.84 ppm; (H_3 , H_4 , H_5), 3.67 ppm; H_2 , 3.38 ppm. Besides these peaks, there remain three unassigned peaks at 5.22, 4.4, and 4.3 ppm, and the full assignment is not possible by ^1H COSY alone.

Figure 6. Homogate decoupled proton COSY spectrum of NaCS (sample HBH'-1).

Figure 7 shows CH COSY of HBH'-1. On the basis of ^1H peak assignment given in Figure 6, the following ^{13}C peak assignment is made: 104.8 ppm, C_1 ; 75.8 ppm, C_2 ; 63.0 ppm, C_6 . Three ^{13}C peaks (81.3, 77.5, and 76.8 ppm) are correlated with a (H_3 , H_4 , H_5) proton peak (3.67 ppm) and then, these ^{13}C peaks should

Figure 7. CH-COSY spectrum of (sample HBH'-1).

be assigned to either C_3 , C_4 or C_5 carbons. Although these three peaks are unable to be assigned absolutely only from Figure 7, the peak at 81.3 ppm can be attributed to C_4 carbon from the assignment in Table III. The remaining two peaks at 77.5 and 76.8 ppm were assigned to C_5 and C_3 , respectively, with reference to Nardin and Vincendon's assignment¹⁸ carried out for cellulose/dimethylacetamide/lithium chloride system and to Gagnaire *et al.*'s assignment¹⁹ on low molecular weight cellulose in dimethyl sulfoxide. Since δ values of ^1H and ^{13}C peaks assigned above for HBH'-1 are confirmed to be almost the same as those for cellulose, almost all these peaks should originate from unsubstituted AHG units. Inspection of two cross-peaks marked by "*" in Figure 7 indicates that the ^{13}C peak at 69.2 ppm is due to 6-mono-substituted AHG unit because there is no cross-peaks due to substituted $C_2\text{--H}_2$ and substituted $C_3\text{--H}_3$ in the dotted circle region, as shown in Figure 7, and because the total degree of substitution of HBH'-1 is quite low. The peaks at 81.7 and 80.6 are correlated with ^1H peak around 3.67 ppm and therefore attributable to either C_3 , C_4 or C_5 carbon. The peak at 75.5 ppm is correlated with ^1H peak around 3.84 ppm.

2D NMR spectra, shown in Figures 4–7 enables us to assign the following peaks: (1) all

^{13}C and ^1H peaks for 2,3,6-trisubstituted AHG unit (C_1 ; 103.4 ppm, C_2 ; 80.1 ppm, C_3 ; 81.3 ppm, C_4 ; 77.5 ppm, C_5 ; 75.8 (75.5) ppm, C_6 ; 69.2 ppm; H_1 ; 4.93 ppm, H_2 ; 4.42, H_3 ; 4.70 ppm, H_4 ; 4.18 ppm; H_5 ; 3.84 ppm, H_6 ; 4.42 ppm), (2) all ^{13}C and ^1H peaks for unsubstituted AHG unit (C_1 ; 104.8 ppm, C_2 ; 75.8 (75.5) ppm, C_3 ; 76.8 ppm, C_4 ; 81.3 ppm, C_5 ; 77.5 ppm, C_6 ; 63.0 ppm; H_1 ; 4.58 ppm, H_2 ; 3.39, H_3 ; 3.67 ppm, H_4 ; 3.67 ppm; H_5 ; 3.67 ppm, H_6 ; 3.96 and 3.84 ppm).

Table IV gives the observed δ values of ^1H peaks for the 2,3,6-trisubstituted AHG unit and unsubstituted AHG unit. Denoting the carbon atom directly attached to the proton in question as C_α , the neighbouring carbon to C_α as C_β , the next neighbouring carbon as C_γ , ..., we here define the magnetic influence on the proton in question as shift factor ΔH_α , ΔH_β , ΔH_γ , ..., when the hydroxyl groups attached to C_α , C_β , C_γ , ... carbons are substituted, respectively. The shift factors can be estimated from the observed δ values in Table IV and using these estimated shift factors we can calculate δ values for ^1H protons belonging to other AHG units. Since δ values of H_1 and H_4 protons for the trisubstituted AHG unit are larger than those for unsubstituted one by 0.36 and 0.51 ppm, respectively, the following relations are expected to hold:

Table IV. Observed and calculated proton chemical shifts of AHG units in NaCS

AHG unit	δ/ppm						Remarks
	H ₁	H ₂	H ₃	H ₄	H ₅	H ₆	
2,3,6-Trisub.	4.93	4.41	4.70	4.18	3.84	4.41	Observed
2,3-Disub.	4.93	4.41	4.70	4.18	3.44	3.81	Calculated
2,6-Disub.	4.93	4.01	4.10	3.78	3.84	4.41	Calculated
3,6-Disub.	4.53	3.81	4.30	4.18	3.84	4.41	Calculated
2-Monosub.	4.93	4.01	4.10	3.78	3.44	3.81	Calculated
3-Monosub.	4.53	3.81	4.30	4.18	3.44	3.81	Calculated
6-Monosub.	4.53	3.41	3.70	3.78	3.84	4.41	Calculated
Unsub.	4.53	3.41	3.70	3.78	3.44	3.81	Calculated
Unsub.	4.57	3.38	3.67	3.67	3.67	3.84, 3.96	Observed

Here, $\Delta H_\alpha = 0.6$ ppm, $\Delta H_\beta = 0.4$ ppm, and $\Delta H_\gamma = 0.0$ ppm are assumed. $H_k = H_{k_0} + (\Delta H_\beta + \Delta H_\gamma)$; $k = 1-6$. H_{k_0} means standard value (tri-substituted AHG unit) of H_k .

$$0.36 = \Delta H_\beta + \Delta H_\gamma + \Delta H_\zeta \quad (\text{for } H_1) \quad (1)$$

$$0.51 = \Delta H_\beta + 2\Delta H_\gamma \quad (\text{for } H_4) \quad (2)$$

Similar analysis on H_2 and H_3 protons gives the following relations:

$$1.03 = \Delta H_\alpha + \Delta H_\beta + \Delta H_\epsilon \quad (\text{for } H_2) \quad (3)$$

$$1.03 = \Delta H_\alpha + \Delta H_\beta + \Delta H_\gamma \quad (\text{for } H_3) \quad (4)$$

Provided that the long range effects as expressed by ΔH_γ , ΔH_δ , ΔH_ϵ , and ΔH_ζ are assumed to be 0, $\Delta H_\beta = 0.36-0.51$ ppm and $\Delta H_\alpha = 0.52-0.67$ ppm are estimated from the above equations. If we take $\Delta H_\alpha = 0.6$ and $\Delta H_\beta = 0.4$, the δ values for H_1-H_6 protons belonging to any other AHG units can be calculated and the values thus calculated are listed in Table IV. Here, the observed δ values for 2,3,6-trisubstituted AHG unit were used as standards because the δ values were precisely determined with an unquestionable peak assignment. The calculated δ values for the unsubstituted AHG unit are in good agreement with those observed except for H_5 proton. This disagreement may be explained by conformational change around C_5-C_6 linkage, which is expected to depend on $\langle F \rangle$ or $\langle f_k \rangle$. The chemical shifts of the ring protons for cellulose derivatives are expected to depend on changes in the magnetic environment, conformation

and solvation state due to the introduction of substituents. Nevertheless, the relatively simple assumption introduced here proved to be adequate to predict the δ values of the protons belonging to AHG units.

Table V compiles the assignments of the 11 proton peaks observed for NaCS/D₂O systems in Table I based on the results in Table IV. In the table the symbol $H_k(lmn)$ is employed to express the carbon position k ($= 2, 3$, and 6), to which the proton in question is attached, and to denote one of 8 AHG units by (lmn) . The definition of (lmn) is the same as lmn used for $\langle f_{lmn} \rangle$. Table V indicates that the peak at the lowest magnetic field (4.93 ppm) is exclusively assigned to H_1 proton when the hydroxyl group attached to C_2 position is substituted. Thus, the peak intensity of this proton peak ($I_{4.93}$) is proportional to $\langle f_2 \rangle$. That is, $\langle f_2 \rangle$ is given by the following equation:

$$\langle f_2 \rangle = 7I_{4.93}/\sum I \quad (5)$$

Here, $\sum I$ is the integrated proton peak intensity of NaCS. Kamide and Okajima¹² erroneously assigned this peak to H_3^* proton and employed this peaks to estimate $\langle f_3 \rangle$. This means that the $\langle f_3 \rangle$ values estimated by Kamide and Okajima^{3,12} (Table I of ref 3, Table II of ref 12) should be corrected as $\langle f_2 \rangle$. The peak at 4.7 ppm, which was assigned as H_2^* by Kamide

Table V. Assignments of proton peaks of NaCS/D₂O systems

Peak No.	δ /ppm	Assignment
1	4.93	H ₁ (111), H ₁ (101), H ₁ (110), H ₁ (100)
2	4.70	H ₃ (111), H ₃ (110)
3	4.58	H ₁ (011), H ₁ (001), H ₁ (010), H ₁ (000)
4	4.42	H ₂ (111), H ₂ (110), H ₆ (111), H ₆ (101), H ₆ (011), H ₆ ' (001)
5	4.30	H ₃ (011), H ₃ (010), H ₆ (001)
6	4.18	H ₃ (101), H ₃ (100), H ₄ (111), H ₄ (011), H ₄ (010), H ₄ (110)
7	4.02	H ₂ (101), H ₂ (100)
8	3.96	H ₆ ' (110), H ₆ ' (100), H ₆ ' (010), H ₆ ' (000)
9	3.84	H ₂ (011), H ₂ (010), H ₅ (111), H ₅ (101), H ₅ (011), H ₅ (001)
		H ₆ (110), H ₆ (100), H ₆ (010), H ₆ (000)
10	3.67	H ₃ (001), H ₃ (000), H ₄ (101), H ₄ (100), H ₄ (001), H ₄ (000)
		H ₅ (110), H ₅ (100), H ₅ (010), H ₅ (000)
11	3.39	H ₂ (001), H ₂ (000)

H_k(*lmn*) means H_k (*k* = 1–6) proton attached to corresponding C_k carbon position in one of 8 AHG units denoted by *lmn*. *l* = 1 or 0 denotes that a hydroxyl group attached to C₂ position is substituted or not, *m* and *n* indicate the corresponding values at the C₃ and C₆ positions, respectively.

and Okajima,¹² now proves to correspond to two H₃ protons of 2,3,6-trisubstituted AHG unit and 2,3-disubstituted AHG unit. Therefore, the peak intensity of this peak (*I*_{4.7}) should be proportional to ($\langle f_3 \rangle - \langle f_{011} \rangle - \langle f_{010} \rangle$) but not to $\langle f_2 \rangle$. The value ($\langle f_3 \rangle - \langle f_{011} \rangle - \langle f_{010} \rangle$) (hereafter, defined to $\langle f_3 \rangle'$) can be approximated to $\langle f_3 \rangle$ only when $\langle F \rangle$ is sufficiently high. Of course, their experimentally important finding that anticoagulant activity χ , as determined by the method according to the Commentary of Japanese Pharmacopoeia,²⁰ is almost linearly governed by $\langle f_2 \rangle + \langle f_3 \rangle$ for the NaCS with $\langle F \rangle \geq 2$ keeps its validity even now. From Table V it is obvious that ¹H NMR analysis, except for above-mentioned two peaks (4.93 and 4.70 ppm), does not provide a reliable way for estimation of $\langle f_k \rangle$ owing to heavy peak overlapping.

Three additional ¹³C shift factors ΔC_α , ΔC_β and ΔC_γ , which were defined in analogy with ΔH_α , ΔH_β , and ΔH_γ were also estimated by trial-and-error to minimize the difference between the calculated and observed δ values and ΔC_α , ΔC_β , and ΔC_γ were determined as 6.1 ppm, –1.4 ppm and 0.1 ppm, respectively.

Here, 12,000 combinations of ΔC_α (6.0–8.0 ppm), ΔC_β (–3.0–0.0 ppm), ΔC_γ (0.0–2.0 ppm) with 0.10 ppm interval were examined, and experimental δ values observed for ¹³C nuclei in unsubstituted AHG unit were used as standards. The values of ΔC_α and ΔC_β determined here lie well in the ranges found for CMC,⁵ CA,¹⁶ and CN⁹ (ΔC_α = 8–10 ppm and ΔC_β = –7–0 ppm). The validity of this analysis can be confirmed if we note the excellent agreement between the observed and calculated values of the carbon chemical shifts of the tri-substituted AHG unit as shown in Table VI. Comparison of Table VI with Table II also ascertains the wide applicability of our analytical procedure. However, the calculated C₄ value for tri-substituted AHG unit (C₄ (111)) proved to largely deviate from the observed value. Considering the conformational specificity around C₄–O–C₁ when the C₃ position is substituted, the calculated values for C₄ (111) (observed), C₄ (010), C₄ (011), and C₄ (110) AHG units are given in parenthesis in Table VI using the observed values for tri-substituted AHG units as standard (ΔC_α = 6.1 ppm, ΔC_β = –1.2 ppm, and ΔC_γ = 1.0 ppm).

Table VI. Observed and calculated carbon chemical shifts of AHG units in NaCS

AHG unit	δ/ppm						Remarks
	C ₁	C ₂	C ₃	C ₄	C ₅	C ₆	
2,3,6-Trisub.	103.4	80.1	81.3	77.5	75.8	69.2	Observed
2,3,6-Trisub.	103.3	80.2	81.5	80.1 (77.5)	76.2	69.1	Calculated
2,3-Disub.	103.3	80.2	81.5	80.0 (76.5)	77.6	63.0	Calculated
2,6-Disub.	103.2	81.6	75.4	81.5	76.1	69.1	Calculated
3,6-Disub.	104.7	74.1	82.9	80.0 (78.7)	76.2	69.1	Calculated
2-Monosub.	103.2	81.9	75.4	81.4	77.5	63.0	Calculated
3-Monosub.	104.7	74.1	82.9	79.9 (75.5)	77.6	63.0	Calculated
6-Monosub.	104.6	75.5	76.8	81.4	76.1	69.1	Calculated
Unsub.	104.6	75.5	76.8	81.3	77.5	63.0	Observed

Here, $\Delta C_\alpha = 6.1$ ppm, $\Delta C_\beta = -1.4$ ppm, and $\Delta C_\gamma = 0.1$ ppm are assumed. $C_k = C_{k0} + (\Delta C_\alpha + \Delta C_\beta + \Delta C_\gamma)$; $k = 1-6$. C_{k0} means standard value (unsubstituted AHG unit) of C_k . The value in parenthesis is the calculated one using the observed value for tri-substituted AHG unit as the standard.

Table VII. Assignments of carbon peaks of NaCS/D₂O systems

Peak No.	δ/ppm	Assignment
1	104.8	C ₁ (010), C ₁ (011)
2	104.6	C ₁ (000), C ₁ (001)
3	103.4	C ₁ (110), C ₁ (111)
4	102.9	C ₁ (100), C ₁ (101)
5	84.4	C ₃ (010), C ₃ (011)
6	82.4	C ₂ (101), C ₂ (100)
7	81.7	C ₄ (001), C ₄ (100)
8	81.3	C ₃ (110), C ₃ (111), C ₄ (000)
9	80.6	C ₄ (101) ?
10	80.1	C ₂ (110), C ₂ (111)
11	77.5	C ₅ (000), C ₅ (010), C ₅ (100), C ₅ (110), C ₄ (111)
12	76.8	C ₃ (000), C ₃ (001), C ₄ (011), C ₄ (110)
13	75.8	C ₅ (001), C ₅ (101), C ₅ (011), C ₅ (111)
14	75.5	C ₂ (000), C ₂ (001), C ₃ (101), C ₃ (100), C ₄ (010)
	(74.3)	C ₂ (010), C ₂ (011)
15	69.2	C ₆ (001), C ₆ (011), C ₆ (101), C ₆ (111)
16	63.0	C ₆ (000), C ₆ (010), C ₆ (100), C ₆ (110)

$C_k(lmn)$ means C_k ($k = 1-6$) carbon in one of 8 AHG units denoted by lmn . $l = 1$ or 0 denotes whether a hydroxyl group attached C₂ position is substituted or not, m and n indicate the corresponding values at the C₃ and C₆ positions respectively.

The peak assignment for the observed 16 ¹³C peaks shown in Table II was made as follows: (1) adopt the peak assignment described before (page 830) irrespective of the calculated δ values (Table VI), (2) for other ¹³C peaks, find the possible ¹H cross peaks for each ¹³C peak by referring the results in Figures 5 and 7 and Table V, (3) assign the possible AHG units

expressed as $C_k(lmn)$ to each ¹³C peak, (4) compare the observed with the calculated (Table VI) δ values and assign the AHG units having the lowest difference to the observed values considering the rough assignment made in (3) and the order of δ values. The results were summarized in Table VII. Here, for C₄ (010), C₄ (110), and C₄ (011) AHG units the

Table VIII. $\langle f_{lmn} \rangle$, estimated from ^{13}C NMR spectra using the assignments in Table VII, $\langle f_k \rangle$ calculated from $\langle f_{lmn} \rangle$ using eq 8–10, $\langle F \rangle$ calculated from $\langle f_k \rangle$ using eq 11, $\langle f_2 \rangle$ and $\langle f_3 \rangle'$ estimated from the ^1H NMR spectra of NaCS samples

Sample code	^{13}C NMR							
	$\langle f_{111} \rangle$	$\langle f_{110} \rangle$	$\langle f_{101} \rangle$	$\langle f_{011} \rangle$	$\langle f_{100} \rangle$	$\langle f_{010} \rangle$	$\langle f_{001} \rangle$	$\langle f_{000} \rangle$
DS-1	0.48	0.00	0.26	0.20	0.00	0.00	0.06	0.00
DS-2	0.21	0.00	0.36	0.25	0.00	0.00	0.18	0.00
DS-3	0.02	0.00	0.16	0.11	0.02	0.01	0.61	0.07
DSH-1	0.01	0.01	0.09	0.04	0.08	0.04	0.39	0.34
HBH'-1	0.00	0.00	0.06	0.02	0.09	0.02	0.34	0.47

Sample code	^{13}C NMR				^1H NMR	
	$\langle f_2 \rangle$	$\langle f_3 \rangle$	$\langle f_6 \rangle$	$\langle F \rangle$	$\langle f_2 \rangle$	$\langle f_3 \rangle'$
DS-1	0.74	0.68	1.00	2.42	0.75	0.81
DS-2	0.57	0.46	1.00	2.03	—	—
DS-3	0.20	0.14	0.90	1.24	—	—
DSH-1	0.19	0.10	0.53	0.82	—	—
HBH'-1	0.15	0.04	0.42	0.61	—	—

calculated δ values in parenthesis were employed. From Table VII it is concluded that the peak at 63.0 ppm originates from 4 AHG units (2,3-di-, 2-mono-, 3-mono-, and unsubstituted), of which C_6 position is not substituted. The NaCS samples (DS-1 and DS-2), which do not show this peak in their ^{13}C NMR spectra, can be regarded to be constituted of only 4 other AHG units (6-mono-, 3,6-di-, 2,6-di-, and 2,3,6-tri-). The three peaks (84.4, 82.5, and 81.3 ppm) observed in DS-1 as collected in Table II are attributable to C_3 carbon in 3,6-di-substituted AHG unit, to C_2 carbon in 2,6-di-substituted AHG unit and to C_3 carbon in 2,3,6-tri-substituted AHG unit, respectively. Therefore, $\langle f_{011} \rangle$, $\langle f_{101} \rangle$, and $\langle f_{111} \rangle$ for this polymer are readily elucidated from the ratios of the corresponding peak intensities to the total peak intensity for C_2 – C_5 carbon peaks. Furthermore, for this polymer $\langle f_{001} \rangle$ can be determined through use of the following relation:

$$\langle f_{011} \rangle + \langle f_{101} \rangle + \langle f_{111} \rangle + \langle f_{001} \rangle = 1 \quad (6)$$

The results of ^{13}C NMR analysis proposed

here on DS-1 and DS-2 are summarized in Table VIII. Using the values of $\langle f_{lmn} \rangle$ obtained thus, $\langle f_k \rangle$ and $\langle F \rangle$ are also calculated from the equations as follows:

$$\sum_{l=0}^1 \sum_{m=0}^1 \sum_{n=0}^1 \langle f_{lmn} \rangle = 1 \quad (7)$$

$$\langle f_2 \rangle = \sum_{m=0}^1 \sum_{n=0}^1 \langle f_{lmn} \rangle \quad (8)$$

$$\langle f_3 \rangle = \sum_{l=0}^1 \sum_{n=0}^1 \langle f_{lmn} \rangle \quad (9)$$

$$\langle f_6 \rangle = \sum_{l=0}^1 \sum_{m=0}^1 \langle f_{lmn} \rangle \quad (10)$$

$$\langle F \rangle = \langle f_2 \rangle + \langle f_3 \rangle + \langle f_6 \rangle \quad (11)$$

Table VIII includes also the values of $\langle f_k \rangle$ and $\langle F \rangle$ evaluated for DS-1 and DS-2. The values of $\langle f_2 \rangle$ and $\langle f_3 \rangle'$ (approximately $\langle f_3 \rangle$) for the former polymer, both determined by the above mentioned ^1H NMR analysis, are included in the table for comparison. ^1H NMR and ^{13}C NMR analyses give quite similar $\langle f_2 \rangle$ values, but $\langle f_3 \rangle$ by ^{13}C NMR method is

Table IX. $\langle f_{lmn} \rangle$ of NaCS samples calculated from $\langle f_k \rangle$ in Table VIII using eq 12—19

Sample code	^{13}C NMR							
	$\langle f_{111} \rangle$	$\langle f_{110} \rangle$	$\langle f_{101} \rangle$	$\langle f_{011} \rangle$	$\langle f_{100} \rangle$	$\langle f_{010} \rangle$	$\langle f_{001} \rangle$	$\langle f_{000} \rangle$
DS-1	0.50	0.00	0.24	0.18	0.00	0.00	0.08	0.00
DS-2	0.26	0.00	0.31	0.20	0.00	0.00	0.23	0.00
DS-3	0.03	0.00	0.15	0.10	0.02	0.01	0.62	0.07
DSH-1	0.01	0.01	0.09	0.04	0.08	0.04	0.39	0.34
HBH'-1	0.00	0.00	0.06	0.01	0.08	0.02	0.34	0.47

significantly smaller than $\langle f_3 \rangle'$ by ^1H NMR method. This means that ^1H NMR analysis tends to overestimate $\langle f_3 \rangle'$ owing to considerable overlapping with neighbouring peaks, resulting in relatively low accuracy intrinsic to ^1H NMR analysis.

Assuming that the reaction rate of the hydroxyl group at the C_2 , C_3 , or C_6 position with sulfating reagent is independent of whether the other two hydroxyl groups are already substituted or not, the following equations are derived:

$$\langle f_{111} \rangle = \langle f_2 \rangle \cdot \langle f_3 \rangle \cdot \langle f_6 \rangle \quad (12)$$

$$\langle f_{110} \rangle = \langle f_2 \rangle \cdot \langle f_3 \rangle \cdot (1 - \langle f_6 \rangle) \quad (13)$$

$$\langle f_{101} \rangle = \langle f_2 \rangle \cdot (1 - \langle f_3 \rangle) \cdot \langle f_6 \rangle \quad (14)$$

$$\langle f_{011} \rangle = (1 - \langle f_2 \rangle) \cdot \langle f_3 \rangle \cdot \langle f_6 \rangle \quad (15)$$

$$\langle f_{100} \rangle = \langle f_2 \rangle \cdot (1 - \langle f_3 \rangle) \cdot (1 - \langle f_6 \rangle) \quad (16)$$

$$\langle f_{010} \rangle = (1 - \langle f_2 \rangle) \cdot \langle f_3 \rangle \cdot (1 - \langle f_6 \rangle) \quad (17)$$

$$\langle f_{001} \rangle = (1 - \langle f_2 \rangle) \cdot (1 - \langle f_3 \rangle) \cdot \langle f_6 \rangle \quad (18)$$

$$\langle f_{000} \rangle = (1 - \langle f_2 \rangle) \cdot (1 - \langle f_3 \rangle) \cdot (1 - \langle f_6 \rangle) \quad (19)$$

Table IX summarizes the $\langle f_{lmn} \rangle$ values indirectly calculated from $\langle f_k \rangle$ using eq 12—19 for DS-1 and DS-2. The values for DS-1, shown in Table IX, are in excellent agreement with those directly determined from ^{13}C NMR spectrum for the same polymer, shown in Table VIII. For DS-2 it seems probable that the experimental values for disubstituted AHG units are larger than those indirectly evaluated, but the difference is not so large. This suggests

that the assumption adopted in deriving eq 12—19 is roughly acceptable to NaCS samples.

It is difficult to determine $\langle f_{lmn} \rangle$ for DS-3, DSH-1, and HBH'-1 by ^{13}C NMR method because they have AHG units whose C_6 is not substituted, as is obvious from the existence of C_6 carbon peak at 63.0 ppm. For these polymers $\langle f_6 \rangle$ can be estimated from the intensity ratio of the peak at 69 ppm to that at 63 ppm. Analysis of the peak intensities at 84.4 ppm and 80—81 ppm gives $\langle f_{011} \rangle + \langle f_{010} \rangle$ and $\langle f_{101} \rangle + \langle f_{100} \rangle$, respectively. Putting these experimental values into eq 12—19 the self-consistent $\langle f_k \rangle$ and $\langle f_{lmn} \rangle$ values are determined and are presented in Table VIII. Using $\langle f_k \rangle$ values in Table VIII, $\langle f_{lmn} \rangle$ is recalculated by using eq 12—19 and the results are shown in Table IX. For all NaCS samples, $\langle f_{lmn} \rangle$ values in Table VIII and in Table IX are consistent, supporting the validity of eq 12—19, and hence the reaction of the hydroxyl group with sulfating reagent can be regarded to occur irrespective of the substitution reaction of the hydroxyl groups at different carbon positions belonging to the same AHG unit.

From Table VIII, the relation $\langle f_6 \rangle > \langle f_2 \rangle > \langle f_3 \rangle$ holds for all NaCS samples prepared here, leading us to a conclusion that reactivity of C_6 hydroxyl is the highest in sulfation of cellulose in DMF/ SO_3 system. Similar results have been reported by us for carboxymethylation⁹ and xanthation⁶ of cellulose. The reason why $\langle f_2 \rangle$ is larger than $\langle f_3 \rangle$ is not clear at present and is open to further study.

CONCLUSIONS

Peak assignments for ^1H and ^{13}C peaks of sodium salt of cellulose sulfate (NaCS) was tentatively made with the aid of two dimensional NMR. $\langle f_{lmn} \rangle$, $\langle f_k \rangle$, and $\langle F \rangle$ of NaCS with $\langle f_6 \rangle = 1.0$, and $\langle f_k \rangle$ and $\langle F \rangle$ of NaCS with $\langle f_6 \rangle < 1.0$ can be determined on the basis of the ^{13}C peak assignment. ^1H NMR analysis is useful for estimation of $\langle f_2 \rangle$, and $\langle f_3 \rangle$ with relatively low accuracy, compared with ^{13}C NMR analysis. Sulfation reaction methods of cellulose employed in this study gave in highest $\langle f_6 \rangle$ and the reaction at a given hydroxyl group was predicted to occur irrespective of the reaction of the other hydroxyl groups at different carbon positions.

REFERENCES

1. S. Bergström, *Naturwissenschaften*, **25**, 706 (1935).
2. K. Kishino, T. Kawai, T. Nose, M. Saito, and K. Kamide, *Eur. Polym. J.*, **17**, 623 (1981).
3. K. Kamide, K. Okajima, T. Matsui, M. Ohnishi, and H. Kobayashi, *Polym. J.*, **15**, 309 (1983).
4. K. Kamide, K. Okajima, T. Matsui, and H. Kobayashi, *Polym. J.*, **16**, 259 (1983).
5. K. Kamide and K. Okajima, *Polym. J.*, **13**, 127 (1981).
6. K. Kowsaka, K. Okajima, and K. Kamide, *Polym. J.*, **18**, 843 (1986).
7. K. Kamide, K. Okajima, K. Kowsaka, and T. Matsui, *Polym. J.*, **19**, 1405 (1987).
8. K. Kowsaka, K. Okajima, and K. Kamide, *Polym. J.*, **20**, 827 (1988).
9. K. Kamide, K. Okajima, K. Kowsaka, T. Matsui, S. Nomura, and K. Hikichi, *Polym. J.*, **17**, 909 (1985).
10. K. Kamide, K. Okajima, and K. Kowsaka, *Polym. J.*, **19**, 231 (1987).
11. K. Kamide, K. Okajima, T. Matsui, and M. Ohnishi, *Polym. J.*, **19**, 347 (1987).
12. K. Kamide and K. Okajima, *Polym. J.*, **13**, 163 (1981).
13. T.-K. Wu, *Macromolecules*, **13**, 74 (1980).
14. D. T. Clark, P. J. Stephenson, and F. Heatley, *Polymer*, **22**, 1112 (1981).
15. H. Friebolin, G. Keilidh, and E. Siefert, *Angew. Chem. Int. Ed.*, **8**, 766 (1969).
16. K. Kowsaka, K. Okajima, and K. Kamide, *Polym. J.*, **20**, 1091 (1988).
17. W. Brown and R. Wikström, *Eur. Polym. J.*, **1**, 1 (1966).
18. R. Nardin and M. Vincendon, *Macromolecules*, **19**, 2452 (1986).
19. D. Gagnaire, D. Mancier, and M. Vincendon, *J. Polym. Sci., Polym. Chem. Ed.*, **18**, 13 (1980).
20. "Commentary of Japanese Pharmacopoeia, No. C1235—C1242," Nankaido, Tokyo, 1969.