Further Study on the Distribution of Substituent Group in Cellulose Acetate by $^{13}\text{C}\{^1\text{H}\}$ NMR Analysis: Assignment of Carbonyl Carbon Peaks

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ABSTRACT: An attempt was made to determine an unsolved problem in peak assignment of carbonyl carbon region in ^{13}C NMR spectra for cellulose acetate. For this purpose, Kamide and Okajima's method for determination of the distribution of acetyl group among three hydroxyl groups in a glucopyranose unit was reexamined carefully using a 200 MHz FT-NMR spectrometer, adequate and the most reasonable assignment at present was made on the carbonyl carbon peaks in NMR spectra, applying a low-power selective decoupling method to acetyl methyl proton located at specific carbon position. All three NMR peaks in the carbonyl carbon region were assigned to be attributed to the acetyl groups located at C_6 , C_3 , and C_2 positions, from the low magnetic field side. The previous Kamide and Okajima's assignment on C_3 and C_2 peaks was found to be erroneously made by neglecting an overlapping of the three peaks due to low resolving power in the ^1H and ^{13}C NMR spectrometer used by them. The new and complete assignments were in good agreement with those by Miyamoto *et al.* The method of Miyamoto *et al.* was examined in detail in the light of the validity of the assignment of carbonyl carbon peaks for not fully substituted cellulose acetate.

KEY WORDS ^{13}C NMR / ^1H NMR / Cellulose Acetate / Distribution of
Degree of Substitution / Selective Decoupling /

Recently, attention has been focused on the study of the distribution of degree of substitution, $\langle f_k \rangle$ ($k=2, 3$, and 6), among three hydroxyl groups in a glucopyranose unit of cellulose derivatives: Wu¹, Clark, and Stephenson² evaluated $\langle f_k \rangle$ from ^{13}C NMR spectra of cellulose nitrate, Kamide, Okajima and their collaborators determined $\langle f_k \rangle$ of sodium cellulose sulfate^{3,4} and sodium carboxymethylcellulose⁵ by ^{13}C NMR, showing that the anticoagulant activity of sodium cellulose sulfate toward human blood and the absorbency of sodium carboxymethylcellulose in various aqueous solutions are closely correlated with $\langle f_k \rangle$. Goodlett and his co-workers⁶ proposed a method for esti-

imating $\langle f_k \rangle$ of cellulose acetate (CA) by analyzing the acetyl methyl proton region of their ^1H NMR spectra. They observed that the acetyl methyl proton peak at *ca.* 2 ppm of CA, whose unsubstituted hydroxyl groups were fully substituted with deuterioacetyl group by acetyl- d_3 chloride, was split into three peaks (see Figures 1, 4, and 5 of ref 6) and they assigned, by comparing the peak intensities with $\langle f_k \rangle$ chemically evaluated according to a procedure by Malm *et al.*⁷ and Gardner and Purves,⁸ three peaks from the low magnetic field, as acetyl methyl protons at C_6 , C_2 , and C_3 positions. Note that in the Goodlett *et al.* method, the experimental determination of the peak intensity is not sufficiently accurate due

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to the low separation of three peaks in ^1H NMR spectra and $\langle f_k \rangle$ values have a significant possibility to change during the deuteration process. Kamide and Okajima⁹ observed at 170.2, 169.7, and 169.3 ppm in carbonyl carbon region of 25 MHz ^{13}C $\{^1\text{H}\}$ NMR spectrum of CA with the total degree of substitution $\langle F \rangle = 2.92$ dissolved in trichloromethane-*d* (TCM-*d*), and their intensities were 1.00, 1.02, and 0.89, reflecting the slight deviation of $\langle F \rangle$ from 3 (see Figure 2 of ref 9). These intensities were compared with these of three acetylmethyl proton peaks in ^1H NMR spectrum of the same sample and they assigned the three carbonyl carbon peaks to C_6 , C_2 , and C_3 positions from the low magnetic field. Using the assignment made thus, Kamide and Okajima determined $\langle f_k \rangle$ of various CA samples having $\langle F \rangle = 0.49 \sim 2.92$. Note that they observed at least four peaks in the carbonyl carbon region for CA with low $\langle F \rangle$ and they assigned one of these peaks as that from acetic acid contaminated in CA sample, by assuming CA with $\langle F \rangle$ less than unity. Prepared homogeneous hydrolysis should have only three peaks corresponding to three types of mono-substituted glucopyranose residue. Thereafter, Miyamoto *et al.*¹⁰ approximately divided the carbonyl carbon region in ^{13}C NMR spectrum of CA with $\langle F \rangle = 1.91$ ($\langle f_2 \rangle = 0.63$, $\langle f_3 \rangle = 0.48$, and $\langle f_6 \rangle = 0.80$ as determined by Goodlett *et al.* method) into three major peaks, although at least four peaks were observed for CA samples with $\langle F \rangle = 0.84$ and 1.23 (Figure 3 of ref 10), assigning from low magnetic field as acetyl groups at C_6 , C_3 , and C_2 positions by comparing the peak intensities with those of ^1H NMR spectrum (Figure 4a of ref 10), which were not well resolved. In their assignment, two peaks between those responsible for carbonyl carbons of acetyl groups attached to C_6 and C_2 positions were attributed to acetyl carbon attached to C_3 position. Their assignment on the C_2 and C_3 positions is just

the reverse of that first reported by Kamide and Okajima. Miyamoto *et al.* utilized a CA ($\langle F \rangle = 1.91$) unfractionated sample for assignment of carbonyl carbon peaks. As is well known, CA with $\langle F \rangle$ ranging 0.5 $\sim$ 2.5 consists of mono-, di- and tri-substituted glucose residues and then, a total of twelve peaks (*i.e.*, four peaks due to C_2 position, four peaks due to C_3 position and four peaks due to C_6 position) are theoretically expected to appear in the carbonyl carbon region in ^{13}C NMR spectra. As far as incompletely substituted CA is concerned, any one of roughly divided three major peaks from these less resolved spectra in Miyamoto *et al.*'s paper (see Figures 3 and 4 of ref 10) is not theoretically ascertained to consist of four peaks, all belonging to a specific carbon positions, and the results on the integrated intensity estimated in this manner may not be completely reliable. This is an unavoidable methodological problem in Miyamoto *et al.*'s procedure. In contrast to this, if we employ CA with $\langle F \rangle = 3$ for the assignment as Kamide and Okajima did, tri-substituted glucose residue is a predominant component of the polymer chain and all twelve peaks theoretically expected in ^{13}C NMR spectra due to the carbonyl carbon of insufficiently substituted CA are overwhelmingly simplified, when CA with $\langle F \rangle = 3.0$ is used, so that only three peaks remain. Therefore it is recommended to assign the carbonyl carbon peaks of tri-substituted glucose residue with good accuracy. In view of this, the Kamide and Okajima method is at least in principal reasonable, but unfortunately the resolving of acetyl methyl proton was relatively poor (see Figure 2 of ref 10) because they used a 60 MHz (^1H) CW-NMR spectrometer, and also their method has a short coming that $\langle f_k \rangle$ estimation is restrictively limited to CA with almost $\langle F \rangle = 3$.

This article intends to assign the carbonyl carbon peaks on NMR spectra of CA recorded on a 50 MHz FT-NMR spectrometer by ap-

plying low-power selective spin decoupling method to acetyl methyl protons located at specific carbon positions with the help of well-resolved ^1H NMR spectra for acetyl proton region, obtained by choosing proper operating conditions. The validity of $\langle f_k \rangle$ evaluation to apply not fully substituted CA is discussed.

EXPERIMENTAL

Polymer Samples

Three unfractionated CA samples were used: $\langle F \rangle = 2.92$ (the weight-average molecular weight $\bar{M}_w = 2.32 \times 10^5$ and the number-average molecular weight $\bar{M}_n = 5.85 \times 10^4$), $\langle F \rangle = 2.46$ ($\bar{M}_w = 1.20 \times 10^5$) and $\langle F \rangle = 0.68$ ($\bar{M}_w = 8.0 \times 10^4$), all synthesized in the previous papers.^{11,12,13}

Solvents

Deuterated trichloromethane (TCM-*d*) and deuterated dimethyl sulfoxide (DMSO-*d*₆) were utilized as received from E. Merck Co., Inc. (Darmstadt, DBR).

NMR measurement

Seven wt% solutions were prepared. NMR spectra were recorded on a FX-200 FT-NMR spectrometer (JEOL, Japan), at 199.5 MHz for ^1H and 50.15 MHz for ^{13}C . Spectral measurement conditions utilized are as follows:

^1H NMR: flip angle 45° , spectral width 2000 Hz (10 ppm), repetition time 10 s, data points 16384, accumulation 16 times.

^{13}C NMR: ^1H noise-decoupled, ^1H low-power selective spin decoupled and non-decoupled mode, flip angle 45° , spectral width 10000 and 1000 Hz (200 and 20 ppm respectively), repetition time 2.5–5.0 s, data points 16384 and 8192 (followed by 8192 points of zero-filling), accumulation 3000–15000 times. Frequency domain accumulation was employed if necessary. Tetramethylsilane (TMS) was used as an internal standard. ^{13}C NMR spectra with 1000 Hz spectral width were

measured only over the carbonyl carbon region and signal out of this width were cut by a Butterworth type band-pass filter of 500 Hz width to avoid folding back signals. Chemical shift (0 ppm for TMS) was determined by spectra recorded with 10000 Hz spectral width. The zero-filling method was employed to save acquisition time, if necessary. The FID signal obtained for each solution was reduced by using different broadening factors ($n=4$, 0–0.3 Hz for ^1H - and $n=5$, 0–0.6 Hz for ^{13}C NMR) to 4 or 5 frequency domain spectra in order to get a reasonable average value for the peak intensity from their integral curves. Low-power selective spin decoupling technique was applied to the acetyl methyl proton located at a specific carbon position. Practically, the other nuclei having the resonance frequency in vicinity to that used for selective decoupling of the acetyl methyl proton in question are more or less decoupled and in order to make this side effect minimum, the irradiation power was carefully selected by trial-and-errors.

RESULTS AND DISCUSSION

Figure 1a and 1b show the ^1H NMR spectrum in the acetyl methyl region and ^{13}C $\{^1\text{H}\}$ NMR spectrum in carbonyl carbon region with 1000 Hz spectral width for CA ($\langle F \rangle = 2.92$)/DMSO-*d*₆ system at 100°C , respectively. In the Figure, four peaks were observed in the acetyl methyl proton region of ^1H NMR spectrum and the same number of peaks were observed in the carbonyl carbon region of $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum. In the latter spectrum, peak widths obviously differs from each other. A peak attached with an x mark in the former spectrum is attributed to an impurity (acetone) contaminated in the CA sample. The chemical shift δ , average integrated intensity I and its standard deviation σ of these peaks (A–D in ^1H and a–d in $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum) are presented in Table I. Here, the total I for A–D or a–d

Figure 1. ^1H (a) and $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of CA ($\langle F \rangle = 2.92$)/DMSO- d_6 system in the acetyl methyl proton and carbonyl carbon region, respectively.

Table I. Chemical shift δ , average integrated peak intensity I and its standard deviation σ of acetyl methyl proton and carbonyl carbon peaks

Nucleus	Peak code	δ/ppm	I^a	σ^b
Acetyl methyl proton	A	2.04	1.02	0.06
	B	1.98	0.07	0.01
	C	1.93	0.94	0.01
	D	1.89	0.91	0.02
Carbonyl carbon	a	169.8	1.01	0.02
	b	169.3	0.05	0.00
	c	169.0	0.90	0.01
	d	168.6	0.95	0.01

^a Average integrated intensity normalized as $I_{\text{total}} = 2.92$. I was determined from integral curves of frequency domain spectra reduced by Fourier transform of each FID signal using different broadening factors (0–0.3 Hz for proton and 0–0.6 Hz for ^{13}C spectra).

^b Standard deviation of I .

peaks was adjusted to give $\langle F \rangle = 2.92$.

Under the operating conditions of $^{13}\text{C}\{^1\text{H}\}$ NMR used in this study, the nuclear Overhauser effects (NOE) for carbonyl carbon peaks (a–d) is not so different from each other because these carbons do not directly bond to protons and their spin-lattice relaxation time T_1 (a, 2.25s; c, 2.33s, and d, 2.41s in CA/DMSO- d_6 system at 40°C)¹⁴ differs only a few percentage. Thus, I of carbon nuclei can

Figure 2. ^{13}C (a, b, c) and ^1H (d) NMR spectra of CA ($\langle F \rangle = 2.92$)/TCM- d_6 . (a), noise-decoupled; (b), non-decoupled; (c-i, c-ii, c-iii), selective-decoupled.

be regarded as quantitative, although the pulse interval employed here is not adequate to avoid the progressive saturation of the carbons. The above experimental results suggest strongly that I shown in Table I represents quantitatively the existence ratio of the nuclei concerned.

Considering I , shown in Table I, a one-to-one correspondence between peaks A–D in ^1H NMR spectrum and peaks a–d in $^{13}\text{C}\{^1\text{H}\}$ NMR spectra in the following manner: A→a, B→b, C→d and D→c.

Figure 2a shows the carbonyl carbon region of $^{13}\text{C}\{^1\text{H}\}$ NMR spectrum of CA ($\langle F \rangle = 2.92$)/TCM- d_6 system at 40°C. Here, a noise-modulated and high-powered RF wave was irradiated over all ^1H nuclei, and the spectral width was 10000 Hz (200 ppm). Three sharp peaks are observed and denoted, from the lower magnetic field, as a, c, and d based on the notation given in Figure 1b for CA ($\langle F \rangle = 2.92$)/DMSO- d_6 system.

Figure 2b shows the non-decoupled ^{13}C NMR spectrum of the same system as that

in Figure 2a. The three peaks appearing in Figure 2a becomes broad and split when the irradiation is not applied on ^1H nuclei. The above experimental result can be explained as: Each of the three peaks in Figure 2a should split theoretically into four peaks, which are not well resolved in the figure, due to the existence of the long-range coupling effect on the three protons in acetyl methyl group neighboring on the carbonyl carbon. Accordingly, when only the acetyl methyl proton located at a specific position (either C_2 , C_3 , and C_6) is preferentially decoupled, the peak of carbonyl carbon bonded directly to the decoupled methyl group can be expected to be observed very sharply. This is hereafter defined as a low-power selective spin decoupling method.

Figure 2d shows ^1H NMR spectrum in the acetyl methyl proton region and three peaks observed are denoted, from the lower magnetic field, as A, C, and D with reference to Figure 1a. We recorded the resonance frequencies as decoupling frequency of low-power selective spin decoupling method.

Figure 2c shows experimental results. In spectrum c-i, c-ii, and c-iii, A, C, and D peaks of the acetyl methyl proton are selectively decoupled. Comparison of Figure 2a and 2c shows that each a, d, and c peak becomes sharp by applying the low-power selective spin decoupling method to each A, C, and D peak. Then, we can conclude that a one-to-one correspondence exists between the acetyl methyl proton peak and the carbonyl carbon peak as follows: $\text{A} \rightarrow \text{a}$, $\text{C} \rightarrow \text{d}$, and $\text{D} \rightarrow \text{c}$.

Figure 3 shows similar results for CA ($\langle F \rangle = 2.92$)/ $\text{DMSO}-d_6$ system at 100°C . From Figure 3, we can also confirm the close correlation between $\text{A} \rightarrow \text{a}$, $\text{C} \rightarrow \text{d}$, and $\text{D} \rightarrow \text{c}$ peaks.

On inspection of Table I, Figures 2 and 3 leads us to the consistent and unquestionable conclusion that peak A correlates to peak a, peak C to peak d, and peak D to peak c.

Combining the above experimental data

Figure 3. ^{13}C (a, b, c) and ^1H (d) NMR spectra of CA ($\langle F \rangle = 2.92$)/ $\text{DMSO}-d_6$. (a), noise decoupled; (b), non-decoupled; (c-i, c-ii, c-iii, c-iv), selective-decoupled.

Figure 4. Schematic representation of peak assignments of cellulose triacetate. Symbols on the carbonyl carbons and *O*-acetyl methyl protons denote the peaks shown in Table I.

with the proton assignment by Goodlett *et al.* for ^1H NMR spectra, we can assign three major peaks, except for peak b, in the carbonyl carbon region as the acetyl groups located at C_6 , C_3 , and C_2 positions, respectively from low magnetic field as illustrated in Figure 4.

These assignments are different from those by Kamide and Okajima.⁹ The former is just the reverse of the latter in the C_2 and C_3

positions. In Kamide and Okajima's earlier work, the overlapping of the acetyl methyl proton peaks and the O-acetyl carbon peaks are significantly large and in consequence, in both ^1H and ^{13}C NMR spectra, the peaks located at the middle position were unavoidably overestimated, leading to erroneous assignment. The conclusion reached here agrees well with that by Miyamoto *et al.*,¹⁰ but note that we made rigorous assignment of carbonyl carbon peaks of tri-substituted CA and peak b is still unassignable to any acetyl carbons attached to C_2 , C_3 , and C_6 positions.

Figure 5 shows $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of CA with $\langle F \rangle = 2.92$ (a), 2.46 (b), and 0.68 (c) in $\text{DMSO}-d_6$ at 100°C , with 1000 Hz spectral width. Compared with the spectrum for CA ($\langle F \rangle = 2.92$), a decrease in $\langle F \rangle$ of CA brings about peak envelopes that are broadened as a result of overlapping of peaks originating from di- and mono-substituted glucopyranose units. In peak envelopes observed for CA with $\langle F \rangle = 2.46$, in addition to three major peaks for carbonyl carbon due to tri-substituted glucopyranose units, several additional peaks (including shoulder peaks) originating probably from mainly di-substituted glucopyranose unit are observed. Inspection of the spectrum for CA with $\langle F \rangle = 0.68$ reveals that there is no peak responsible for the carbonyl carbon of the tri-substituted glucopyranose unit. Since CA samples with lower $\langle F \rangle$ were prepared by homogeneous acid-hydrolysis of CA ($\langle F \rangle = 2.92$) in acetic acid media, CA with $\langle F \rangle = 0.68$ can be regarded as constituted of mainly mono-substituted CA. Then the three peaks appearing at about 169.8, 169.4, and 168.7 ppm seem to be derived from C_6^- , C_3^- , and C_2 -mono-substituted glucopyranose units. These spectra for CA obtained here are much better resolved than those reported by Kamide and Okajima⁹ and Miyamoto *et al.*¹⁰ The lower resolution of the spectra in these papers might have given basis to roughly divide the carbonyl carbon peak region into

Figure 5. $^{13}\text{C}\{^1\text{H}\}$ NMR spectra of CA/DMSO- d_6 system in carbonyl carbon region. (a), $\langle F \rangle = 2.92$; (b), $\langle F \rangle = 2.46$; (c), $\langle F \rangle = 0.68$.

three major peaks. Unfortunately, the complete assignment for all peaks observed in Figure 5 is, at present, impossible, and a further detailed study is required on the spectra.

It is evident that a method for estimating $\langle f_k \rangle$ for CA not fully substituted, prerequisites experimental confirmation of the validity of roughly dividing the whole spectra in acetyl carbon region into three groups by detailed assignment of every peak and obtaining the relative ratio of mono-, di-, and tri-substituted groups.

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