Determination of Intermolecular Acetyl Distribution of Cellulose Triacetate by Reversed Phase High Performance Liquid Chromatography

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Summary: The chromatographic method for the determination of the intermolecular acetyl distribution of cellulose triacetate (CTA) was established; the key point of the method is a gradient elution by gradually changing the solvent composition of the ternary mixture of chloroform, methanol, and water on a phenyl-bonded silica gel stationary phase. A positive correlation between the retention time and the degree of acetyl substitution was observed under the established chromatographic conditions. The validity of the elution peak width as the measure of intermolecular acetyl distribution was confirmed by the co-injection and fractionation experiments and the comparison of a peak width with a statistically estimated distribution.

Keywords: cellulose triacetate; intermolecular acetyl distribution; peak width; reversed phase high performance liquid chromatography

Introduction

Widely used since decades ago has been cellulose triacetate (CTA) as *e.g.* raw materials of textile filaments, and photographic films.[1] This old material is also of increasing importance in the fields of flat panel display, separation of optical isomers, and membrane separation as reviewed elsewhere.[2,3]

Although it is a common practice to call it a ''triacetate'', no peracetylated cellulose (PCTA) with the maximal degree of substitution (DS) of 3.0 is commercially available. This is partly due to the fact that a PCTA can never be obtained under normal conditions for the commercial production; the conventional catalyst for the reaction, sulfuric acid, does not allow cellulose for the peracetylation introducing itself to a certain extent to hydroxyl groups of cellulose during acetylation.[4] The DS of commercially available CTAs usually ranges from 2.8 to 2.9 depending on applications.

Highly but partially substituted CTAs may possess structural diversity in terms of distribution of acetyl groups. The acetyl distribution should be discussed at three levels: (i) the distribution of the acetyl groups at the three possible sites of anhydroglucopyranose unit, (ii) the distribution along a molecule (intramolecular distribution), and (iii) the distribution among molecules (intermolecular distribution).[5] In light of structure-performance relationships, the acetyl distributions of CTA are of industrial and scientific interests. Attempts were made to determine the acetyl distribution within anhydroglucopyranose unit by means of NMR techniques. One of the most successful studies in this field was made by Tezuka *et al.* revealing that propanoyl groups introduced to the unsubstituted hydroxyl groups of CTA serves as a sensitive probe to determine the acetyl distribution within anhydroglucopyranose unit.[6] It was also demonstrated that the determination of hydrolysis products

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of further derivatized CTA provides insight with regard to the intramolecular acetyl distribution.[7] Considering the DS dependence of solubility and other properties of cellulose acetates including CTAs, it is conceivable that the intermolecular acetyl distribution has a direct effect on the performance of material in question.[7] It is somehow astonishing that number of reports in relation to the intermolecular acetyl distribution is relatively limited. This type of distribution is what the authors focus on in this article.

Kamide *et al.* demonstrated that the intermolecular distribution in composition of cellulose acetates and cellulose nitrates could be visualized by means of thin layer chromatography (TLC).[8,9] It is unfortunate that the report lacks clear descriptions to ensure sufficient reproducibility of the TLC method. As far as cellulose diacetate (CDA) with a DS of approximately 2.5 is concerned, Floyd made a successful study on the intermolecular acetyl distribution by means of high performance liquid chromatography (HPLC).[10] Kawai *et al.* also reported a reversed phase HPLC technique for the determination of the acetyl distribution of CDA after reports on the distribution in composition of synthetic graft copolymers by means of HPLC in adsorption mode.[11–16]

Number of solvents for CTA is limited. The Solvents for CDA cannot always be that for CTA. Given the difference in nature towards solvents, the systems reported for CDA are not applicable to CTA unfortunately. A special system is required to determine the acetyl distribution of CTA. To this end, having focused on reversed phase HPLC technique and selected a suitable mixed solvent system through cloud point measurements, the authors established a method for the acetyl distribution measurement of CTA.[17–20] The validity of the method and results from it are discussed in the article.

Results and Discussion

Reversed-Phase HPLC Conditions

In the development of this HPLC method, various chromatographic conditions were examined. The choice of a suitable chromatographic system *i.e.* mobile phase(s) and stationary phase was guided by the following concepts.

- An isocratic elution is usually inappropriate for a chromatographic separation of polymeric material; thus a design of a gradient system, changing over from a poor solvent gradually to a good solvent, is inevitable.
- In a gradient system as such, the solvent composition at the elution point should have solubility as good as possible to avoid a fractionation by precipitation (phase separation), which tends to depend more on molecular weight distribution.

To substantiate the aforementioned concepts, solvent compositions at chromatographic elution point and that of precipitation were studied over a range of good and poor solvents. The poor solvents studied were methanol-water (95/5,v/v), methanol-water (9/1,v/v), methanol-water (8/1,v/v) and the good or more elutropic solvents, chloroform-methanol (9/1,v/v), chloroform-ethanol (9/1,v/v), acetone, methanol, ethanol, tetrahydrofuran, tetrachloroethane, dichloromethane-methanol (9/1,v/v).

The type of stationary phase used in this study are CN (ZORBAX-CN 4.6 mm × 25 cm, Hewlett Packerd), ODS (TSK-gel ODS80Ts 4.6 mm × 15 cm, TOSOH, J'sphere ODS-H80 4.6 mm × 25 cm, YMC Co. Ltd.) and Ph (Novapak-Phenyl 3.9 mm × 15 cm Waters).

The following is the optimal conditions determined by these preliminary experiments.

A CTA sample is chromatographed on a Waters Novapak-phenyl (3.9 × 150 mm) under a mobile phase condition of chloroform-methanol (9/1,v/v)-methanol-water (8/1,v/v) (2:8) to 100% in 28 min at a flow rate of 0.7 ml/min. A 20 μl portion of 0.1% sample solution in chloroform-methanol (9/1,v/v) is injected at the starting. The linear relationship between injection volume and peak area was confirmed at the range from 10 μl to 30 μl and this sample amount was

chosen to avoid the polymer exclusion at high injection volume.

Detection was performed with an ELSD, Polymer Laboratories PL-ELS- 1000 evaporative light scattering detector (ELSD), the setting of which is as follows; evaporator tube temperature: 70 °C, nebulizer temperature: 65 °C, gas flow: 0.7 SLPM of nitrogen gas.

Factors Affecting Retention Time

Under the established conditions, CTAs with a variety of DSs were loaded to the chromatograph. The chromatographic retentions are shown in Figures 1 and 2 as functions of DS and viscosity-average degree of polymerization (DPv). The chromatographic retention increased with increasing DS while it did not look dependent on DPv. To quantify the observation, the partial correlation analysis was carried out (Table 1). The correlation coefficient between DS and chromatographic retention was as significant as 0.926 while that between DPv and chromatographic retention was as small as −0.195. This leads to the conclusion that, under the establishied conditions, the chromatographic retention is mainly dominated by DS.

The distribution of the acetyl groups at the three possible sites of anhydroglucopyranose unit might affect the chromatographic retention. CTAs with similar total DSs (2.808 and 2.804) but with different distribution of acetyl groups at 2, 3, and 6 positions were analyzed. The sample with a higher DS at 6 position resulted in a slightly longer elution time (Table 2).

Multi regression analysis was carried out for the results of several CTAs. The following equation was obtained by the regression.

$$\text{Retention time} = 0.834\ (\text{DS}) + 0.217\ (\text{C6-DS}) + 0.003$$

The regression was statisitically significant with a level of significance of 1%.

Looking at the equation, the slope of C6-DS corresponds to approximately 25% of that of DS. This means that the effect of acetyl distribution among glucosidic positions on chromatographic retention is not dominant. However, attention should be paid to the possibility that CTAs with an identical DS would not give an identical chromatographic retention when a significant difference in positional distribution exists.

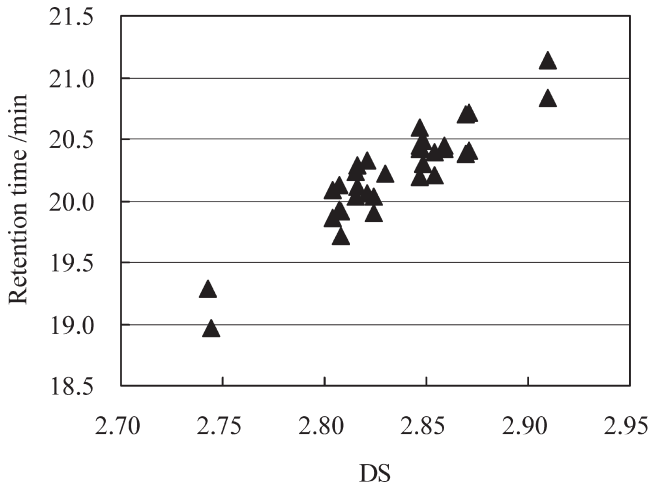

Figure 1.
Effect of DS on Chrromatographic retention.

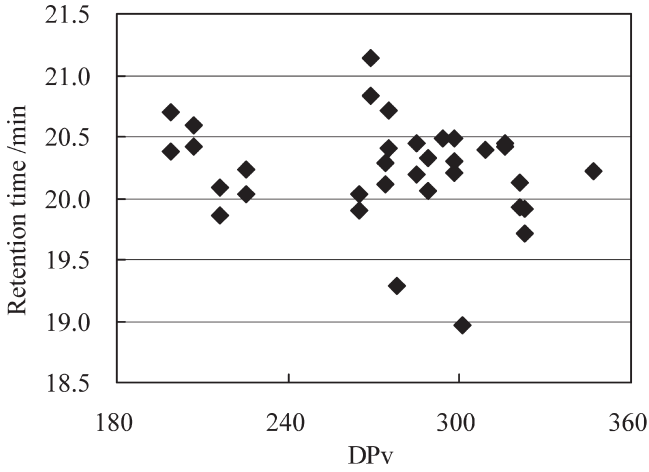

Figure 2.
Effect of DPv on Chromatographic retention.

Validity as Measure of Intermolecular DS Distribution

Co-Injection

Two solutions were prepared from CTAs with DSs of 2.83 and 2.81 (hereinafter referred to as samples C and D respectively). The third solution was prepared for the co-injection trial by a one-to-one mixing of portions of samples C and D. These three samples were loaded to the chromatograph under the established conditions. The chromatograms were converted to DS - Intensity curves according to the calibration curve obtained with CTAs with a variety of DSs. The DS – Intensity curves of samples C, D and their mixture are shown in Figure 3. The peak shape of co-injection sample agreed well with that calculated from original chromatograms of samples C and D. The result means that no interference in elution behabior occurs when CTA molecules with different DSs are mixed. It suggests that the established chromatographic method serves as a measure of intermolecular DS distribution of CTA.

Fractionation

Sometimes a chromatographic separation of polymer materials involves phase separation (precipitation and dissolution). When the phase separation is dominant for the chromatographic separation, the chromatographic diffusion (width of chromatogram) might not reflect that of chemical composition but the duration required for the phase separation. When the duration as such is dominant for the chromatographic diffusion, a narrow fraction collected by the chromatographic separation might result in, when loaded again to the chromatograph, a peak as wide as the original unfrac-

Table 1.
Results of partial correlation analysis (N = 36)

Correlation		Correlation coefficient
DPv-DS	R12 · 3[a)]	0.138
DS-retention time	R23 · 1	0.926
DPv-retention time	R13 · 1	−0.195

[a)] By assuming DPv = x1, DS = x2 and retention time = x3 for the regression analysis, the correlation coefficient between x1 and x2, that eliminating the efficient of x3, is 0.138.

Table 2.
Influence of acetyl distribution in the glucose residue on retention time

Sample	DS				DPv	Retention time (min)
	Total	C2	C3	C6		
A	2.808	0.975	0.97	0.863	613	19.47
B	2.804	0.938	0.93	0.936	345	19.85

tionated sample. A similar result might be observed when the diffusion caused by the chromatography system (*e.g.* void volume and column efficiency problems) is significant. A CTA sample was fractionated by the chromatography and re-chromatographed. The result is shown in Figure 4. The chromatogram of the fractionated sample was reasonably narrower than that of original sample. The result means the phase separation problem as well as the chromatography system problem have little contribution to the chromatographic diffusion. It also suggests that the established chromatographic method serves as a measure of intermolecular DS distribution of CTA.

Comparison of Chromatographic Results with Theoretical Prediction

CTA samples were prepared from cellulose by acetylation followed by partial deacetyaltion. Assuming that the reactions takes place in a random manner, the resultant intermolecular acetyl distribution can be calculated by the binominal theorem. To perform the actual calculation, assumptions on weight-average degree of polymerization (DPw) and distribution in degree of polymerization (DP) are necessary. For the purpose of the theoretical prediction, the DPw and polydispersity factor (DPw / DPn) were assumed to be 600 and 2.0 respectively considering the analytical results of CTA samples. As the DP distribution, Schultz-Zimm type distribution was assumed.[21]

To make a better comparison between chromatographic results and the theoretical prediction, diffusion due to chromatography system was eliminated from the chromatographic results by the follwing manner.

$$(W1/2)^2 = (W1/2)^2_{obs} - (W1/2)^2_{PCTA}$$

where (W1/2): W1/2 eliminating chromatographic system diffusion $(W1/2)_{obs}$: W1/2 obsereved for a CTA, $(W1/2)_{PCTA}$: W1/2 obsereved for peracetylated CTA.

This correction is based on the fact that PCTA with a theoretical maximal DS of 3.0 can never possess the intermolecular acetyl distribution.

The result of theoretical calculation is shown in Figure 5 as half-height-widths (W1/2) as a function of DS. The chromatographic results of CTAs are also shown in Figure 5. It should be noted that, although the chromatographic results of W1/2 were systematically larger than what the binominal theorem predicts, the magnitude and

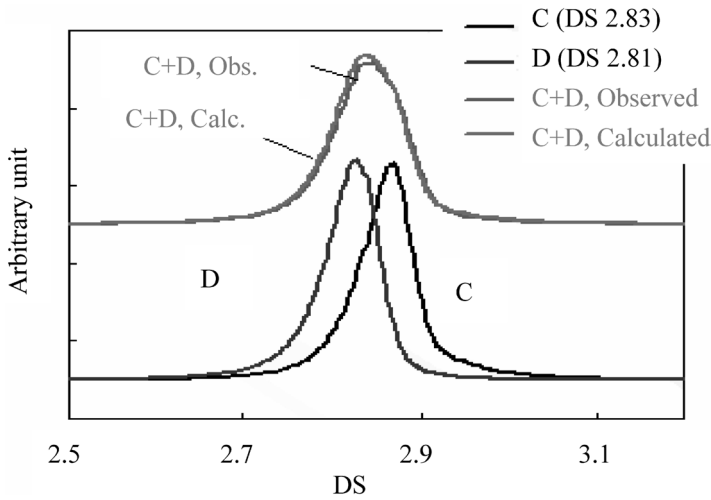

Figure 3.
Acetyl distribution curves of Co-Injection sample. DS of C; 2.83, D; 2.81.

DS dependence of the chromatographic results were basically in line with the theoretical prediction. This observation also suggests that the established chromatographic method serves as a measure of intermolecular DS distribution of CTA.

Conclusion

In search for a tool of analyzing the intermolecular acetyl distribution of CTA, the gradient elution conditions were establishied, where CTA samples were eluted in the order of increasing average DS. Under the established conditions, the chromatographic retention does virtually not depend on DP. The co-injection and fractionation experiments suggests that the observed width of chromatogram serves as a measure of the intermolecular acetyl distribution. This is supported by the fact that the observed width of chromatogram is close to what statistical theory predicts *vis-a-vis* the route for the syntesis of the polymer. Judging from the observations, the authors conclude that the set of the established conditions for HPLC is valid for the determination of the intermolecular acetyl distribution of CTA.

Experimental

CTA Samples

CTAs were prepared by the acetylation of a cotton linter or a woodpulp in a mixture of acetic acid/acetic anhydride/sulfuric acid followed by the partial deacetylation (partial hydlolysis of acetyl groups) in a mixture of acetic acid/water/surfuric acid.[4] The DS of CTA was determined by ^{13}C-NMR measurement of the propionated cellulose acetate.[6] The weight and number-average molecular weights (Mw and Mn) of CTA was determined by the size exclution chromatograph equiped with light scattering and reflactive index detectors using dichloromethane as the eluent.[22] The DPv of CTA was determined from the limiting viscocity number ([η]) of CTA in dichloromethane/ethanol mixed solvent (8 / 2, v / v). [23] The Mw and Mn were converted to DPw and DPn respectively taking into account the DS. The DS range of samples was from 2.74 to 3.00, the DPv range was from 190 to 350, and the DP_w range was from 437 to 723.

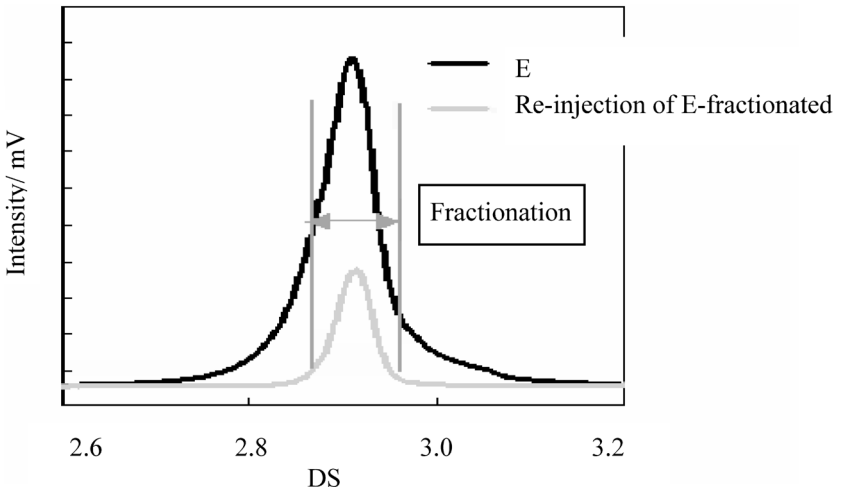

Figure 4.
Acetyl ditribution curves of sample E (DS; 2.91) before and after fractionation.

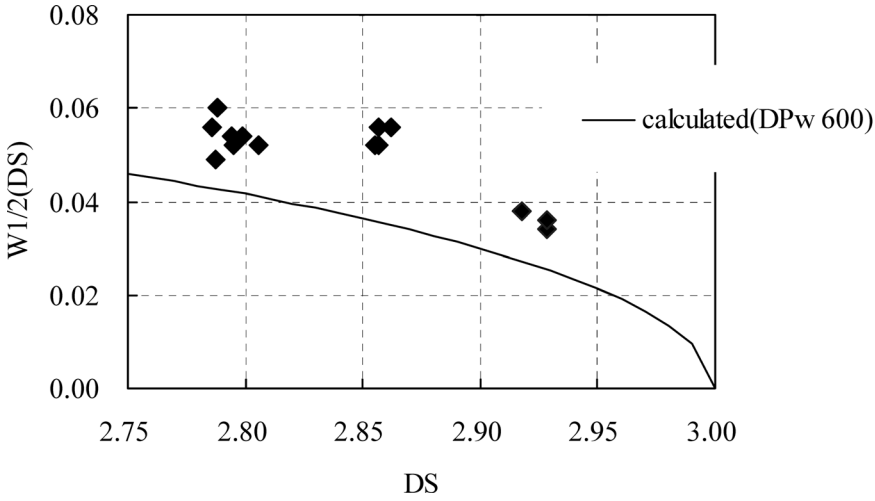

Figure 5.
Peak widths (W1/2) of CTAs and that statistically calculated by binominoal theorem. For the statistical calclation, a Schult-Zimm type DP distribution with a polydispersity factor of 2.0 is assumed. Half height widths determined by the HPLC tequnique were corrected by a deconvolution technique so as to eliminate peak broadening due to chromatographic system (See text.).

The polydispersity factors (DPw/DPn) of samples were around 2.0.

Measurement

HPLC Sysytem

Reversed-phase high-performance chro matography was performed on an Agilent LC-1100 gradient pumping system. All chromatography runs were carried out at ambient temprerature, normally 23 °C. In the study, a 20 μl portion of 0.1% CTA solution was injected and chromatographed at a flow rate of 0.7ml/min at 30 °C. Detection was performed with a Polymer Laboratories PL-ELS-1000 evaporative light scattering detector (ELSD). The several stationary phases and mobile phase conditions were examined to evaluate intermolecular acetyl distribution of CTA. They are described bellow.

The detector conditions, *i.e*, evaporator tube temperature, nebulizer temperature, gas flow, suitably set for every solvent system according to the mobile phase system.

Calibration

The calibration curve of DS vs. retention time was made with the samples of known DS (DS 2.74 ~ 3.00, NMR analysis). Chromatograms could thus be converted to acetyl distribution curve and peak width at half height ($W_{1/2}$) could also be expressed in a DS unit as the measure of intermolecular DS distribution by utilizing the calibration curve.

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