Short Communication

Size-Exclusion Chromatography and Methylation Analysis of Cellulose in *N,N*-Dimethylacetamide/LiCl*

Arne Lund Kvernheim[a] and Elizabeth Lystad[b]

[a]Department of Chemistry, University of Oslo, P.O.Box 1033, N-0315 Oslo 3, Norway and [b]Norwegian Paper Research Inst., Forskningsveien 3, N-0371 Oslo 3, Norway

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In the last ten years there has been an increasing interest in non-degradative solvents for cellulose. Such solvents will be helpful in the characterisation and derivatisation of cellulose. These solvents are rather exotic e.g. *N*-methylmorpholine *N*-oxide (MMNO),[1] trifluoroacetic acid–chlorinated alkanes,[2] liquid ammonia–NH_4SCN,[3] SO_2–diethylamine (DEA)–dimethyl sulfoxide (DMSO),[4] N_2O_4–DMSO[4] and *N,N*-dimethylacetamide (DMAc)–LiCl.[4–11] The last one seems to be the most versatile. Characterisation of native cellulose and derivatisation introducing a range of substituents has been achieved in this solvent.[4–11]

We chose the DMAc – LiCl system for our study because it is the only solvent known to be reasonably inert towards size-exclusion chromatography (SEC) column material. The BuLi/MeI methylation procedure[12] was attempted because of the rather small degree of substitution (DS 1.1) achieved by McCormick *et al.* using NaOH as the alkoxide-forming reagent in this solvent.[11] It was also interesting to find out if the lithium introduced with butyllithium (BuLi) was in any way affected by the large amount of lithium already present in the solvent. A further interesting aspect is whether SEC and methylation techniques can be used to study the degradative effect of the solvation process.

The solvation process of cellulose in DMAc/LiCl is well known. It can be achieved by preswelling cellulose in water,[10] or by activating it using refluxing DMAc.[6,7] We have used both methods. The former is probably the least degradative but the latter the more efficient. The preswelling method was not able to dissolve Whatman CF1 cellulose, but the refluxing procedure did the job. When dissolving pulp, it is important to ascertain that the pH of the pulp is neutral initially, else considerable degradation may occur before the pulp is safely dissolved in DMAc/LiCl. A buffer of pH 7 is recommended.

For the SEC analysis pullulan was chosen as the standard. It was considered likely that the hydrodynamic volume of pullulan would be closer to that of cellulose in this solvent system, than polystyrene. The standard curve gives an almost linear correlation between log $\bar{M}_w$ and retention time, and the curve chosen may be represented by $y = -0.203x + 10.96$. Calculating the $\bar{M}_w$ of several standards from this curve gave an error in the range 1.6–5.3 %. Rayon pulp from different sources, using different bleaching methods was investigated together with Whatman CF1 cellulose and purified cotton. For a typical series, see Table 1. Here the increase in viscosity (CED) is seen to follow nicely the increase in molecular weight. The solvation procedure with water activation was found not to degrade the samples significantly (less than 5 % reduction in CED viscosity). These particular pulps were all bleached using a traditional sequence (including chlorine). An investigation of oxygen-bleached rayon pulp shows no great difference in polydispersity or molecular-weight distributions. The Whatman CF1 cellulose and cotton sample was dissolved using the reflux procedure and the molecular weights indicated that some degradation had occurred.

An SEC investigation by Ekmanis of a softwood sulfite rayon pulp revealed an $\bar{M}_w$ = 790 000, and PD of 6.[13,14] These values differ somewhat from those found in this investigation, $\bar{M}_w$ being somewhat higher and PD a little lower. Ekmanis, however, used polystyrene standards for calibration. Rantanen carbanilated softwood rayon pulp, and analysed the molecular weight to be about 300 000, with a PD value as low as 2.44.[15] The system was calibrated with a broad distribution standard of carbanilated cellulose. The carbanilation may conceivably have altered the distribution of the samples.

Methylation of DMSO-insoluble polysaccharides can be a difficult task. It can be achieved in DMSO using multiple additions of base and MeI.[16] This is, however, not recommended for base-labile polysaccharides. Even if a solution of such a polysaccharide in another solvent is obtained, its methylation can be difficult because of the solubility characteristics of the base.[11,16] The technique described here, successfully used on DMSO-soluble polysaccharides in

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Table 1. Molecular weights of cellulose sulfite pulp produced from Norwegian spruce, Whatman CF1 cellulose and cotton.

Sample	$Visc_{(CED)}$[a]	$Visc_{(DMAc)}$[b]	$\bar{M}_n$	$\bar{M}_w$	PD[c]
C541	541	627	44300	383200	8.6
C560	560	654	37100	398000	10.7
C577	577	686	68100	414700	6.1
C585	585	667	47900	402500	8.4
C596	596	702	40300	420400	10.4
C614	614	699	49500	420400	8.5
C658	658	661	55640	400000	7.2
Whatman CF1[d]	152	79	27500	68800	2.5
Cotton[d]	1016	1835	212500	933800	4.4

[a] $Visc_{(CED)}$ measured according to SCAN-C15:62, in copper/ethylenediamine. [b] $Visc_{(DMAc)}$ calculated using $K = 0.000128$, $a = 1.19$.[10] [c] Polydispersity. [d] Solvated by the reflux procedure.

DMSO,[12] was found to be excellent in the DMAc/LiCl system. Methylation data for three pulp samples are presented in Table 2. C457 was a rayon pulp with a $visc_{(CED)}$ value of 457. It is evident from these results that the BuLi methylation is superior to the NaOH and KOH methylations[17] of cellulose in this solvent system. When cellulose is 100 % methylated, the ratios of 2,3,6-tri-*O*-Me-/2,3,4,6-tetra-*O*-Me-Glc constitutes an estimate of the DP. In the BuLi methylated samples these values were found to be about a half of the $\bar{M}_n$ values found by SEC, when using a 30 min reaction time with BuLi. This difference is partially accounted for by undermethylation, partially by degradation during methylation, and partially because of demethylation during work-up and derivatisation. The apparent degradation can be seen as a decreasing 2,3,6-tri-*O*-Me-/2,3,4,6-tetra-*O*-Me-Glc mol ratio of the C658 sample from 270 for 5 min and 118 for a 160 min reaction time with BuLi before MeI was added.

Whatman CF1 cellulose was methylated by the same method as above with reaction times with BuLi varied from 5 to 160 min. After 10 min a DS of 2.8 was obtained, recoveries 60–100 %. These DS values are far better than the DS obtained by McCormick *et al.*,[11] and are comparable to the DS obtained by Joseleau *et al.*[1] for the methylation of low molecular-weight cellulose in a MMNO–DMSO solvent system using Hakomori methylation. Prolonged reaction times gave no further methylation. The 3 position was the most difficult to methylate probably because of the intramolecular OH-3′–O-5 hydrogen bond. Cotton gave a DS of 2.6 on being methylated.

Experimental

Cellulose. Bleached sulfite pulps were from Scandinavian Spruce (for rayon production) containing about 3 % hemicellulose, $visc_{(CED)}$ 457 (C457) to $visc_{(CED)}$ 658 (C658); also used were Whatman CF1 cellulose powder, $\bar{M}_w$ 178000;[10] and purified cotton. Cellulose samples were dried for 24 h under vacuum over phosphorus pentaoxide at 80 °C.

Table 2. Molar percentages of methylated sugars, degree of substitution (DS) and recovery of methylated cellulose samples, using NaOH, KOH or BuLi as alkoxide-forming reagents.

Sample:	C457			C541			C658		
Methylated sugar	NaOH	KOH	BuLi	NaOH	KOH	BuLi	NaOH	KOH	BuLi
2,3,4,6-Glc	0.5	0.3	0.7	–	0.6	0.6	–	1.1	0.6
2,3-Xyl	0.9	0.7	0.9	1.0	0.6	1.4	1.0	0.8	1.4
2,3,6-Man	2.1	1.8	1.8	2.0	0.6	2.1	1.8	1.0	1.9
2,3,6-Glc	91.1	62.3	92.8	73.5	13.2	93.4	76.5	38.4	94.3
2,6-Glc	1.1	13.3	1.4	2.0	5.5	0.9	1.7	4.2	0.9
3,6-Glc	0.7	2.8	0.6	1.5	1.7	0.3	1.2	1.3	0.3
2,3-Glc	1.7	5.5	1.5	2.9	3.1	0.8	2.3	2.6	0.5
2-Glc	0.4	5.1	0.1	3.0	10.2	–	2.4	6.8	–
3-Glc	0.3	1.2	–	1.4	4.0	0.1	1.1	2.6	–
0-Glc	1.3	7.0	0.2	12.8	60.4	0.4	11.9	41.3	0.1
DS	2.9	2.5	3.0	2.5	0.8	3.0	2.5	1.5	3.0
Recovery[a]	82.1	98.3	91.6	47.0	39.6	114.0	50.0	44.5	132.0

[a] Recovery in % calculated relative to myo-inositol hexaacetate added as myo-inositol in the TFA hydrolysis step.

Solvation of cellulose in DMAc/LiCl. Solvation of pulp samples was carried out using the method of McCormick *et al.*,[10] except for heating to 100 °C for 1 h in the preswelling with water. Solvation of Whatman CF1 and cotton cellulose was performed using a modification of the procedure by Turbak *et al.*.[6,7] Cellulose (2 g) was refluxed (165 °C) in DMAc (50 ml) for 20–60 min under an N_2 atmosphere. The reaction mixture was cooled to 100 °C and LiCl (9 % w/v) was added. The suspension was stirred for 30 min at 100 °C, 2 h at 80 °C and 24 h at room temperature. The cotton sample was adjusted to 1 % in cellulose and 9 % in LiCl by adding DMAc and LiCl, heated to 100 °C for 2 h and stirred for another 48 h period at room temperature before being centrifuged. The concentration of cellulose was found to be 0.7 % after the sample had undergone a seven-times repeated shaking, centrifugation and removal of water, followed by freeze drying of the precipitated material.

SEC analysis. Apparatus: Waters model 510 pumps, U6K injector, Styragel columns 10^3 to 10^6 Å, Waters RI 410 detector, and Shimadzu CR3A data unit. Chromatographic parameters: eluent DMAc/LiCl 0.5 % (v/w) eluting at 1.0 ml min^{-1}, pressure 1500 psi (approx), temperatures 30–45 °C, sample volume 40 µl. Standards: Pullulan Shodex P-82 standard kit (Showa Denko KK). Molecular-weight determination was done by size-exclusion chromatography (SEC) after the method of Ekmanis,[13] except for the standard used to calibrate, and the column temperature.

Methylation procedure. Of the dissolved samples in DMAc/LiCl, a volume containing 2 mg of cellulose was withdrawn and diluted to 400 µl with DMAc in a 5 ml Reacti-vial equipped with a magnetic stirrer. The vial was purged with argon and BuLi (100–160 µl, 1.6 M in hexane; Merck) was added with stirring. After 5–30 min at room temperature the vials were cooled in ice/water for 5 min. MeI (200 µl, ice cold) was added and the samples were stirred for 30 min at room temperature. Reagents were added via syringe. Further work-up and derivatisation was by the method of Harris *et al.*[16] Analyses of methylated alditol acetates were carried out as described previously[12] or by using an SP 2380 fused capillary column instead of the SP 2340.

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