Study of the stability of cellulose–holocellulose solutions in N,N-dimethylacetamide–lithium chloride by size exclusion chromatography

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Abstract

Solutions in N,N-dimethylacetamide (DMAC)–LiCl were prepared from two different pulps (sulphite pulp from softwood and cotton linters) in different ageing states. Degradation of the stirred solutions at 35–40°C was observed by determining the molecular masses by size exclusion chromatography (SEC). We showed that under these conditions cellulose and holocelluloses are degraded in DMAC–LiCl and that the rate of degradation is dependent on the temperature and the initial state of degradation of the sample. Temperature and dissolution time are recommended to be reduced, especially for aged samples. © 2001 Elsevier Science B.V. All rights reserved.

1. Introduction

For a long time cellulose studies by size exclusion chromatography (SEC) have been limited because of its poor solubility in most current solvents. For this reason, cellulose was often transformed into a soluble derivative for SEC analysis like cellulose tricarbanilate, trinitrate and others [1,2] but the derivatisation process might not be entirely uniform and may induce degradation of the polymer. Within the past 30 years, considerable effort has been directed toward finding simple, non-derivatizing solvents for cellulose [3–5]. In 1979, McCormick found cellulose to be soluble in N,N-dimethylacetamide–lithium chloride (DMAC–LiCl). A detailed literature review was published by Dawsey and McCormick [6]. Different procedures were described which involve sample activation by swelling in liquid NH_3, water, or in a NaOH solution at room temperature, or in hot DMAC, followed by solvent exchange and addition of LiCl. Cellulose from any source including cotton, wood, and regenerated viscose cellulose yarn was reported to be dissolved in DMAC–LiCl [7]. The ageing effects of cellulose solutions were monitored in McCormick's study [8] by measurement of the viscosity as a function of time. Only 2–3% loss in viscosity was observed after 30 days for a solution maintained at 30°C. McCor-

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mick explained the loss by an inter- and intramolecular hydrogen bonding rearrangement. Another viscosity study of a cellulose solution in DMAC–LiCl made by Terbojevich [9] confirmed this hypothesis of good solution stability. From these results, cellulose solutions in DMAC–LiCl seem to be stable at room temperature for a long time.

SEC is a very powerful tool and the preferred method for molecular mass determination [10]. It was applied for the first time to direct cellulose analysis in DMAC–LiCl by Ekmanis [11]. Many researchers [12–17] applied SEC analysis to cellulose with the same mobile phase and based their experiences on the description of solution stability performed by the cited studies of McCormick and Terbojevich. Because of the lack of commercial narrow distribution cellulose standards, pullulan (polymaltotriose) standards have been the preferred choice of many authors [7,9] for calibration. One can assume that this linear polysaccharide has similar interactions with the solvent system DMAC–LiCl and thus a similar hydrodynamic volume on which is based the separation by SEC [18].

In order to work out a method for molecular mass determination for pulp, our aim was to study the solution stability of cellulose in DMAC–LiCl. Because we observed shifts of peaks for both pullulan and cellulose solutions we questioned if cellulose degradation by DMAC–LiCl took place. In contrast to the earlier studies performed by McCormick and Terbojevich [8,9], our experiments using SEC showed the importance of sample preparation on the resulting molecular mass distribution (MMD). The influence of the different activation and dissolution methods on the MMD was discussed in Ref. [19]. In the present report, we investigated the solution stability of pulp in DMAC–LiCl using SEC on two types of pulp, one of pure cellulose and the other containing additional hemicelluloses. We studied solvent-caused degradation and its relation to the initial ageing state of the sample.

2. Experimental

2.1. Samples

Two different types of pulp sheets were dissolved

in DMAC–LiCl: PS-1 was made from bleached sulphite softwood, and PS-2 was made from bleached cotton linters, both containing neither dyes nor fillers. The degree of polymerisation (DP) determined by viscometry following the French norm [20] was 812 ± 7 (corresponding to the viscometric MM (M_v) = 131 500 g mol^{-1}) for PS-1 and 704 ± 7 (M_v = 114 000 g mol^{-1}) for PS-2. Hemicelluloses (glucomannans) were present in PS-1 [21] but they were not quantified. Our study concerns holocelluloses (cellulose and hemicelluloses) since a separate detection was not possible with the used characterising material. The crystallinity index (CRI) determined by X-ray diffraction of the references (R) had been described as 88 for PS-1 and as 95 for PS-2 [22]. Since in that report it was concluded that acid attack had a large effect on the DP but only a low increase in crystallinity, this type of characterisation was not used in this study. The two pulp sheets underwent different types of artificial degradation:

• Thermal aged (TA) at 90°C with 50% relative humidity for 12 days.

• Polluted (P . . .) by the air pollutants SO$_2$ (20 ppm) and NO$_x$ (10 ppm) at 23°C with 50% relative humidity for 14 days (P14). Pollution for 4 days (P4) and 8 days (P8) was only applied to PS-1.

In the following report, non-treated samples are called references (R).

2.2. Sample preparation

Samples were dissolved at room temperature in DMAC–LiCl by following three steps. First, pulp was blended for 30 s in a kitchen blender (SEB). Five mg of the resulting fibres was suspended in distilled water and left overnight to allow thorough swelling of the fibres (''activation''). Second, the water was removed by an adapted glass filter and DMAC was added. The solution was stirred with a glass rod and left for 1 h. This ''solvent exchange'' was repeated three more times. Finally, after DMAC had been removed, 1.25 ml of a 8% (w/v) LiCl in DMAC solution was added and stirred at 35–40°C for the ''dissolution time T_{sol}''[1] in sealed glassware to exclude moisture absorption. The initial pulp

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[1]Please note that T_{sol} does not denote the actual time a sample needs to dissolve, but the duration of the dissolution step.

concentration was 0.4% in DMAC–LiCl (8%). Before injection of the solution into the chromatographic system it was diluted to a cellulose–holocellulose concentration of 0.05% and filtered with GHP (hydroxylated polypropylene) Acrodisc filters (0.45 μm).

2.3. Conditions

The chromatographic conditions were as follows: a 515 HPLC pump (Waters), manual injector (Vici AG, Valco International), a heating system at 55°C (Interchim, model 102), four Phenogel 5-μm mixed bed columns 300×4.6 mm (Phenomenex, packing material is a crosslinked polystyrene–divinylbenzene gel, pore size 10^2–10^7 Å) with a Phenogel 5-μm guard column 30×4.6 mm and a differential refractometer detector (Kontron Instruments, 8-μl cell). The volume of total permeation was 14.6 ml, while the volume of total exclusion was calculated to be 7.3 ml. The elution range of the columns was indicated as 10^2–10^7 g mol^{-1} for polystyrene standards. The eluent, 0.5% w/v LiCl (Normapur 99%, Prolabo) in DMAC (HPLC quality 99.8%, Fluka), was pumped into the system at a flow-rate of 0.3 ml min^{-1}; the run time was 60 min. The sample loop was 20 μl. Before injection, the columns were equilibrated for 1 day in the circulating eluent. Three injections were made per sample. Data acquisition was made with the chromatographic software Gold Nouveau (Beckman) and was processed afterwards with Microsoft Excel.

2.4. Calibration

From eight narrow distributed pullulan standards (Showa Denko K.K., Shodex) we prepared three separate standard solutions, each one containing 0.05% (w/v) pullulan in 1% (w/v) LiCl in DMAC. The first solution was composed of pullulan of peak molecular mass (M_p) 788 000, 112 000 and 11 800 g mol^{-1}, the second 404 000, 47 300 and 5900 g mol^{-1}, and the third 212 000, 22 800 and 180 g mol^{-1} (glucose). Full dissolution was attempted after 1 day at 25–40°C with occasional stirring. Calibrations were repeated regularly to exclude column modification.

2.5. Calculations for polymer characterisation

To characterise a polymer it is useful to introduce averages such as the number-average molecular mass (M_n), the weight-average molecular mass (M_w), the z-average molecular mass (M_z) and the z^2-average molecular mass (M_{z^2}) or $z+1$ average molecular mass. They can be calculated as follows:

$$M_n = \frac{\sum n_i M_i}{n_i} \quad M_w = \frac{\sum n_i M_i^2}{\sum n_i M_i} \quad M_z = \frac{\sum n_i M_i^3}{\sum n_i M_i^2}$$

$$M_{z^2} = \frac{\sum n_i M_i^4}{\sum n_i M_i^3}$$

As a result, their sensitivity towards the high molecular mass is $M_{z^2} > M_z > M_w > M_n$.

3. Results

3.1. Calibration

For the calibration of the chromatographic system, a third-order curve was fitted because it describes the separation behaviour better than a linear regression [10]. The equation was $\log(MM) = -0.00067T_R^3 + 0.07002T_R^2 - 2.5962T_R + 39.041$, where MM is the molecular mass and T_R is the retention time in minutes ($R^2 = 0.9999$). We found that relatively fresh pullulan solutions have to be used for calibration because pullulan is degraded by the solvent system within about 2 weeks at 25–40°C. We observed a shift of the MMD towards the smaller MM, while a freshly prepared solution eluted at the time initially stated. Probably the relatively high storage temperature is responsible for the rapid degradation of the standard solutions. Since we did not focus on pullulan degradation, no characterisation was carried out in more detail but we were careful about using fresh standard solutions. The reproducibility of the T_R of M_p for both pullulan and pulp solutions was ±0.11 min, which can be expressed in $\log(MM) \pm 0.02$.

3.2. MMD characterisation

In order to check the stability of cellulose–

holocellulose in DMAC–LiCl, we prepared solutions of the samples mentioned in Section 2.2. In this report, we use MM averages to resume the main information and to compare samples easily. The example Fig. 1 gives the order of MM averages in a distribution. Degradation causes a shift towards the smaller masses resulting in smaller MM averages.

3.3. Shape description

The samples of PS-1 were dissolved as described in Section 2.2. with a dissolution time T_{sol} of 1, 5, 12–13 and 22 days for each one. The MMD of PS-1 reference (R) with $T_{sol} = 1$ day (see Fig. 1) has a M_p at 5.8 with a small shoulder at about log 5.5, probably resulting from degradation during the pulping and bleaching. The small peak at log 3.4 may be due to hemicelluloses but also to base line errors. According to literature, softwood hemicelluloses have a DP of about 100, but a recent study [23] showed that glucomannan can elute over the entire MM range due to association with cellulose. When T_{sol} increases from 1 to 22 days, the M_p of the initially aged samples is situated where the small shoulder was for (R) while the former M_p at 5.8 forms a shoulder and disappears completely for (P8) and (P14) after 15 and 22 days. Our characterisations concern the entire sample, i.e. cellulose and hemicelluloses (holocelluloses). Probably, shoulder formation of this relatively complex MMW shape during solvent-based degradation is caused by their different degradation reactions.

The samples of PS-2 were dissolved as described in Section 2.2. and T_{sol} was 1–2, 6–7, 15 and 22–23

days. The initial (R) and (TA) sample ($T_{sol} = 1–2$ days) show a symmetric MMD while (P14) forms an amount of low weight material around log 3.5. When T_{sol} is increased from 1–2 to 23 days, the MMD of (R) and (TA) shift towards the smaller MM without any significant modification of their initial symmetric shape. In contrast, (P14) shows a strong increase in the low mass material. The more symmetric shape of PS-2 can be explained by its composition of pure cotton cellulose.

3.4. MMD changes during the dissolution process

Table 1 shows the MM averages obtained for PS-1 whereas the relative values with respect to T_{sol} and to prior sample ageing are presented in Fig. 2. The values obtained for (R) indicate that this sample is relatively stable in DMAC–LiCl solution until a T_{sol} of 12 days. Surprisingly, the initial MMD of (TA) after a T_{sol} of 1 day is close to the initial MMD of (R). In fact, chain rupture reactions are in competition with auto-crosslinking reactions occurring in thermal ageing from 70 to 350°C [24]. However, and in contrast to (R), the solution of (TA) shows a significant solvent-based degradation after 12 days. Comparing the polluted samples, the M_z and M_{z2} values are very sensitive to the changes of the degradation rate, indicating that (P14) and (P8) are more prone to solvent-based degradation than (P4). These results are based on the global measurement of holocelluloses. Even if the degradation reactions and reaction rates of cellulose and hemicellulose are not the same, our analytical material does not allow their
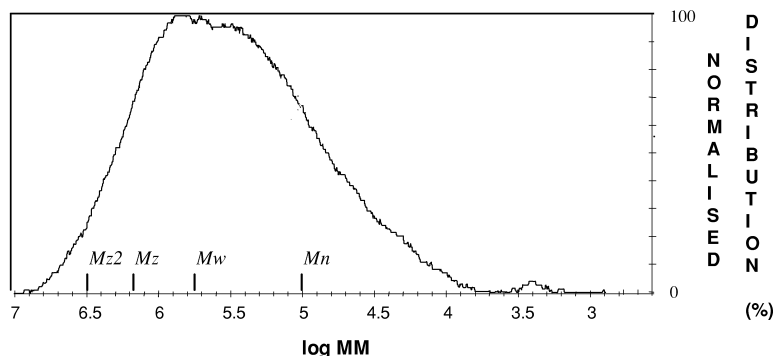

Fig. 1. Size exclusion chromatographic distribution plot for PS-1 (R) ($T_{sol} = 1$ day) with MM averages.

Table 1
The MM averages (in g mol^{-1}) obtained for PS-1

| PS-1	T_{sol} (days)	M_n	M_w	M_z	M_{z2}
Reference	1	114 700	583 500	1 606 100	2 914 100
	5	105 800	640 300	1 668 700	3 043 300
	12	100 200	628 800	1 641 900	3 127 600
	22	92 400	446 800	1 046 700	2 978 500
Thermal aged	1	96 400	570 100	1 622 100	3 032 500
	5	58 700	546 900	1 657 100	3 277 100
	12	113 100	456 200	1 129 100	2 089 500
Polluted 4 days	1	80 200	574 300	1 654 300	3 009 000
	5	47 600	506 800	1 415 100	2 610 800
	12	74 300	501 900	1 281 100	2 488 600
	22	104 900	346 300	889 700	2 283 600
Polluted 8 days	1	57 800	380 200	977 800	1 738 400
	5	58 500	355 400	913 100	1 630 600
	15	34 900	171 600	363 400	684 700
	22	32 800	143 700	273 000	431 600
Polluted 14 days	1	51 100	258 700	707 200	1 369 900
	5	58 500	355 400	913 100	1 630 600
	15	36 200	133 100	264 500	429 400
	22	37 000	123 500	230 600	363 500
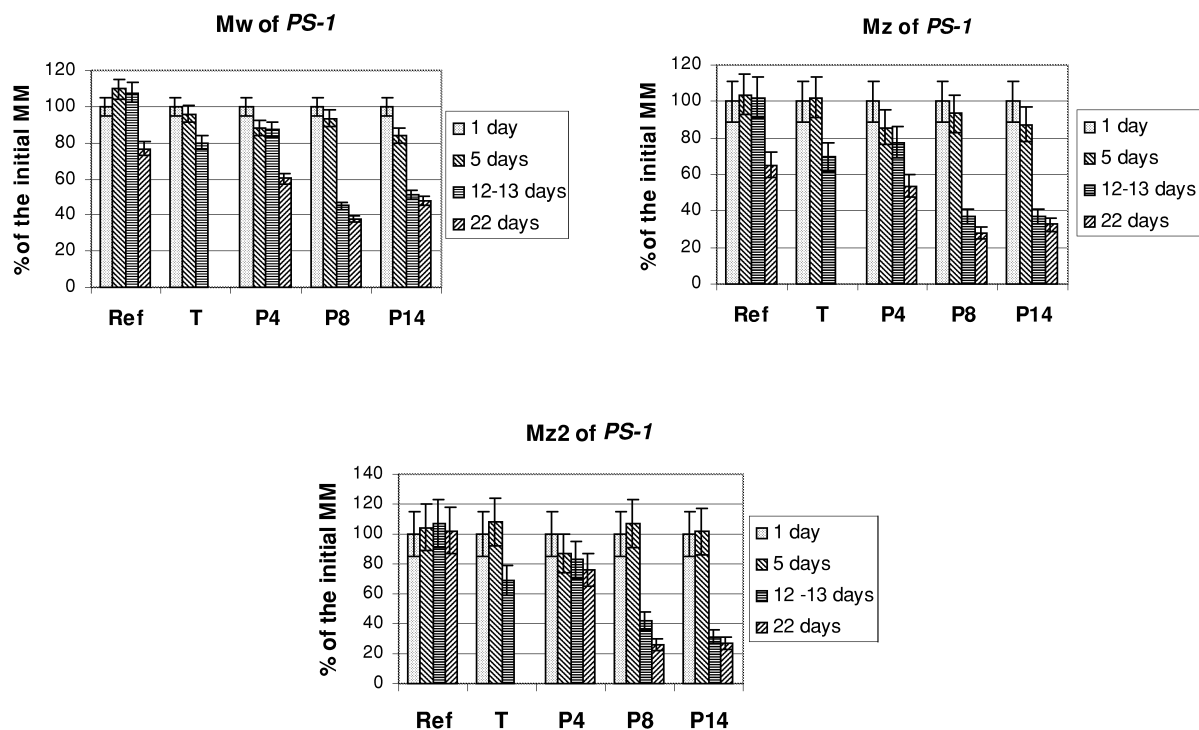
Fig. 2. The relative molecular mass averages M_w, M_z and M_{z2} in % of initial value (corresponding to 1 day) in dependence from the dissolution time for PS-1 samples. The error bars indicate the 95% confidence limit.

Table 2
The MM averages (in g mol^{-1}) obtained for PS-2

PS-2	T_{sol} (days)	M_n	M_w	M_z	M_{z^2}
Reference	1	211 100	446 900	1 020 400	2 020 800
	6	196 000	451 600	1 009 000	2 166 000
	15	61 000	237 200	469 100	1 037 000
	22	78 600	189 000	349 500	651 000
Thermal aged	1	113 500	440 300	1 043 400	2 043 000
	7	170 800	372 800	717 900	1 190 800
	15	125 700	249 900	428 200	650 200
	22	99 800	184 500	300 100	448 400
Polluted 14 days	2	22 900	105 700	762 200	2 318 400
	7	25 300	123 900	714 400	1 728 700
	15	21 600	61 200	99 000	135 400
	23	20 600	57 700	94 200	129 900

differentiation. Nevertheless, it gives precious information about the pulp constituents in total.

The MM averages for PS-2 are shown in Table 2, whereas Fig. 3 presents the relative MM averages in correlation to T_{sol} and to prior sample ageing. After 1 day, this cotton pulp was not completely dissolved. Total dissolution is attained only after 2 days, probably due to cottons high crystallinity. Despite the incomplete dissolution, the MMD appears to be representative compared to a totally dissolved one of the same sample.

The MM averages of (R) decrease after 15 days in DMAC–LiCl and seem to be less stable in the solvent system than PS-1 (R). Again, the initial MMD at 2 days of (TA) is close to that of (R), and as for PS-1 the solvent-based degradation develops faster for (TA) than for (R). The most extreme decrease was observed for the M_{z^2} value of (P14).

3.5. Influence of dissolution parameters

Although mechanical treatment was reported to degrade cellulose [26], our short blending pre-treatment prior to sample dissolution was not excessive enough to affect the MMD. This conclusion was drawn from comparison with a non blended sample. Furthermore, we investigated the effect of stirring and temperature during dissolution as described in Section 2.2. with the only difference being after the addition of DMAC–LiCl (8%). The solution was divided into four parts and left for the dissolution step as shown for PS-1 (R) in Table 3.

There was no significant difference between the MMD neither of part 1 or 2 and the PS-1 (R) sample stirred for 1 day at 35–40°C, nor between part 3 and 4 while all samples were completely dissolved. This means that the temperature plays an important role
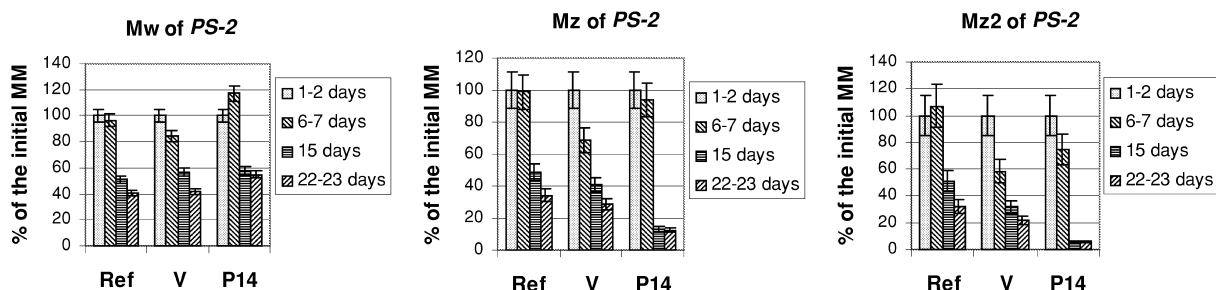

Fig. 3. The relative molecular mass averages M_w, M_z and M_{z^2} in % of initial value (corresponding to 1–2 days) in dependence from the dissolution time for PS-2 samples. The error bars indicate the 95% confidence limit.

Table 3
Dissolution conditions for PS-1 in order to evaluate the parameters stirring and temperature

	Part 1	Part 2	Part 3	Part 4
Dissolution time (days)	2	12	11	11
Dissolution temperature (°C)	4	4	40–50	40–50
Stirring	No	No	No	Yes

among the dissolution parameters while stirring is negligible and even unnecessary. Especially for initially aged samples, temperature and T_{sol} should be minimised to a few days at 4°C.

4. Discussion

4.1. Error discussion

From the calibration curve, the SEC error appears to be about 5%. This is true for values lying inside the pullulan-based calibration limits. Above 788 000 g mol^{-1}, the error is higher: first of all, because of extrapolation of the calibration curve leading to an additional systematical error, and secondly because of baseline errors raising the error of M_z and M_{z^2} up to, respectively, 11 and 15%. We included M_{z^2} and M_z in order to follow the evolution of high molecular material for comparison between the pulp samples. Baseline effects increase the error of M_n to 45% which is too high to draw meaningful conclusions. For this reason, we reported M_n values (see Tables 1 and 2) without further data exploitation.

4.2. The initial ageing state of the samples

While solvent-based degradation caused significant loss for the drastically aged samples of both PS-1 and -2, the (R) samples were almost stable until, respectively, 12 and 5 days. The growth observed in some cases in comparison to the initial value (T_{sol}=1–2 days) lies within the 95% confidence limit except for M_w of PS-2 (P14). Since its corresponding M_z and M_{z^2} are not higher than the initial value, this M_w is not considered. The more the sample is aged initially, the faster is the solvent-based cellulose–holocellulose degradation. We deduce that changes occurring during ageing as carbonyl and carboxyl group formation accelerate the solvent-based degradation. However, temperature and T_{sol} should be minimised, especially for samples with very excessive or unknown ageing treatment.

Without further and longer thermal ageing experiences of the two pulp sheets, a future perspective, it is impossible to say why the MMD of the (TA) samples are not inferior to those from the (R), but both PS-1 and -2 behave in this way. We suppose that 12 days at 90°C/50% RH is not enough to observe significant MMD changes of the two pulp types.

4.3. Kinetics

For almost each sample, the order of sensitivity to solvent-based degradation was found to be $M_{z^2} > M_z > M_w$. The example of PS-2 (Fig. 3) illustrates that M_w changes were too small to show differences in solution stability depending on the prior ageing of the samples. In contrast, M_{z^2} and M_z are able to monitor this kind of changes. The solvent-based degradation shows a tendency of a first order reaction, determined according to the Ekenstam equation for linear homopolymers depending on the total number of monomers [25], and is probably a random reaction with stirring having no significant effect on the degradation rate. However, with our results no exact kinetic characterisation was possible because of the few data that were taken.

4.4. MM determination

All samples of PS-1 were dissolved under identical conditions, and the same for samples of PS-2. The apparently faster degradation for PS-2 in comparison to PS-1 could be caused by a temperature variation between the two dissolution series. However, the initial (R) values of both samples can be compared, indicating higher averages for PS-1. That was confirmed by viscometry, even if M_v underestimates the MM which should theoretically approach M_w [17]. It should be kept in mind that the values obtained by SEC (Tables 1 and 2) are based on pullulan calibration, including a relatively high error for the high MM, but they should be closer to the absolute value and allow a more detailed study using the MM averages.

5. Conclusions

Although cellulose solutions in DMAC–LiCl had been reported to be very stable and non-degrading for the sample [8,9], our experiments made by SEC indicate an influence of the solvent system on the MMD. In this report, we investigated the solution stability of two types of pulp sheet in different ageing states. The more the cellulose–holocellulose sample was initially aged, the faster solvent-based degradation took place in DMAC–LiCl with the temperature playing an important role. In order to avoid solvent-based degradation especially of initially aged samples, it is recommended to reduce both temperature and the time of contact between sample and solvent system.

Even if the obtained MM averages are not absolute values, because of calibration curve extrapolation and errors for the high MM, they allow a comparison between different samples. The stated solvent-based degradation probably follows a random reaction kinetic, resulting in a decrease of the averages MM in the order $M_{z^2} > M_z > M_w$.

In conclusion, DMAC–LiCl is an appropriate solvent system for cellulose–holocellulose analysis by SEC and other techniques provided that the limited solution stability is taken into account.

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