Gelatine sizing of paper and its impact on the degradation of cellulose during aging: a study using size-exclusion chromatography

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Citation for published version (APA):
Chapter 5. Evaluation of different methods for the characterisation of cellulose: size-exclusion chromatography using direct dissolution, size-exclusion chromatography using derivatisation, and viscometry

Abstract

The method developed in the present study, i.e. size-exclusion chromatography (SEC) using multiangle light scattering (MALS) and differential refractive index (DRI) detection applied to what is referred as 'directly dissolved cellulose' or DDC in lithium chloride/N,N-dimethylacetamide (LiCl/DMAc), is evaluated compared to two other methods currently used for cellulose analysis. These are SEC using low-angle light scattering (LALS) and ultra-violet detection of cellulose derivatised to tricarbanilate or CTC, and viscometry (V) in cadmium tri-ethylene diamine dihydroxide or Cadoxen. The values of the molar mass ($M_r$) averages of cellulose obtained with the different methods, and the discrepancies between these values are discussed on the basis of the precision of each methodology and the action of the solvents on the polymer. As SEC of the DDC yielded the highest $M_r$ averages values and viscometry the lowest, several hypotheses are presented in order to account for these differences. Each method is also discussed on the basis of its suitability to characterise the aging-induced degradation of the paper. The complexity of carrying out such comparisons between different methods is outlined.

5.1 Introduction

When analysing cellulose a key parameter for the accuracy of the data obtained is the integrity of the polymer. A non-degradative dissolution process is paramount if the $M_r$ of dissolved cellulose is to reflect the molar mass ($M_r$) of the cellulose source. The type and quality of the data and the precision in the $M_r$ determination also greatly depend on the methods used and their sensitivity.

Viscometry (V) is fast and convenient, which often makes it the preferred method to estimate the average degree of polymerisation ($DP_v$) of cellulose and its derivatives. However, the method has obvious limitations since only one single $M_r$ average value, the
viscosity-average molar mass \((M_v)\) is determined, and no information on the molar mass distribution (MMD) of the polymer can be obtained. Additionally viscometry methods are based on the dissolving action of complexing organometallic solvents (copper ammonium hydroxide, copper ethylene diamine, cadmium ethylene diamine), which often result in degradation of the polymer [1,2,3] especially when analysing oxidised cellulose [4]. Viscometry is still widely used in cellulose analysis despite the multiple applications of size-exclusion chromatography (SEC) in polymer chemistry. The main reason is that among the solvents that are able to swell cellulose, penetrate the fibres and break the intermolecular hydrogen bonds, few are compatible with SEC column packings. But the advantages of SEC over viscometry are manifold, and these are chiefly providing information on MMD and allowing the determination of all the molar mass averages \((M_n, M_w, M_z, M_{2-1} \text{ and } M_v)\). SEC provides also information on the degraded fractions thus leading to insight in the degradation mechanisms.

In SEC, the type and quality of the data and the precision in the \(M_t\) determination depend on the detection method. The refractive index (RI) detectors require a calibration using narrowly distributed polymer standards of known \(M_t\), leading to the relative MMD and \(M_t\). The lack of structurally identical standard fractions for cellulose is responsible for the well-known problem of structural mismatch between the chosen calibration standard and the analyte, as well as the resulting uncertainty on the real \(M_t\) (section 4.1.1 of Chapter 4).

One alternative in order to avoid a calibration of the type Log \(M_t\) as a function of elution volume \((V_e)\) relative to narrowly distributed standards is the universal calibration method [5], which is based on the hydrodynamic volume of the polymers in solution. This method requires coupling SEC with RI and viscometry (V) detectors or the knowledge of accurate MHS values for both the sample polymer and the reference standards (section 4.1.2.1. of Chapter 4). It has been applied to cellulose \(M_t\) determination by Timpa et al. [6,7,8].

UV detectors can be used when cellulose has been modified to derivatives that absorb in UV like cellulose esters such as tricarbanilate (underivatised cellulose has no absorption in ultraviolet radiation) or when studying UV absorbing species such as lignins.

However, the best solution is to avoid calibration of any kind by using light scattering (LS) detectors, which online with a concentration detector - UV or RI - allow for absolute \(M_t\) determination. Low-angle LS, multiangle LS or V coupled to LS detectors can be used. Further details on detection methods are in section 4.1 of Chapter 4.

### 5.2 Aim of the study

Chapter 3 and Chapter 4 outlined the reasons based on the literature search that led us to opt for analysing cellulose in lithium chloride/\(N,N\)-dimethylacetamide (LiCl/DMAc) using SEC for the current research, and reported the development of an appropriate method to carry out the study. The present chapter is aimed at searching for experimental
evidence of the reported superiority of this method by comparing it to other commonly used methods in cellulose analysis.

The experiment was based on evaluating SEC in LiCl/DMAC of cellulose versus SEC in tetrahydrofuran (THF) of cellulose tricarbanilate (CTC), and versus viscometry of cellulose in cadmium tri-ethylene diamine dihydroxide (Cadoxen). The latter methods were chosen because they are prominently used in paper and cellulose research and are both reported to be the least degrading in their category.

So far, few studies have been dedicated to the comparative evaluation of different methods for cellulose characterisation. SEC in LiCl/DMAC and viscometry for $M_r$ determination of oxidised cellulose [4], cellulose from different sources [9], and cellulose which originated from specific pulp processes [10] have been compared in the past. In these studies, viscosity measurements were performed in copper di-ethylene diamine dihydroxide (CED). Lawther et al. [11] studied SEC of derivatised cellulose (CTC) and underivatised cellulose (dissolved in LiCl/DMAC), and compared it with viscometry of the CTC in pyridine.

To our knowledge no study including both SEC methods of derivatised and underivatised cellulose, and viscometry of underivatised cellulose in Cadoxen has been published. Moreover, the present study bases the comparisons not only on the characterisation of unaged cellulose but also on cellulose artificially aged by a combination of heat and humidity.

The task of making global experimental comparisons between different methods that involve a number of parameters ranging from sample preparation to instrumentation is far from simple. The comparison was rationalised by studying several aspects of the three methods. Precision, information obtained and its quality were assessed. The methods were also investigated in terms of their suitability for the analysis of non-degraded as well as degraded cellulose. Lastly, their relative simplicity, as for instance the ease of sample preparation was evaluated.

5.3 Experimental

5.3.1 Description of the methods

5.3.1.1 Viscometry in Cadoxen

The majority of standardised methods for viscometry measurements use CED as the solvent [12,13,14,15,16]; one method uses iron (III) sodium tartrate complex (FeTNa or EWNN) [17]. However, Cadoxen, a cadmium tri-ethylene diamine dihydroxide complex [Cd(En)$_3$(OH)$_2$ ($\text{En} = \text{H}_2\text{N(CH}_2\text{)}_2\text{NH}_2$)] is sometimes preferred over CED because the solvent induced degradation of cellulose is significantly lower [18,19,20,21]. Additionally, as opposed to CED, Cadoxen is a colourless solvent, which makes the
experiment easier to follow. In the present study, instead of the one-point viscosity measurement recommended by the standard methods, three-point measurements were carried out with solutions of different concentrations, from which the value of the intrinsic viscosity \( [\eta] \) was obtained by extrapolation to zero concentration. This provided greater accuracy in the results with a relative standard deviation (RSD) of 1.5% for repeatability of the values of \( DP_v \) [22].

In the present experiment, dissolution was preceded by a reduction of the paper in sodium borohydride (NaBH\(_4\)) [4,22]. The measurements were done with a capillary glass viscometer Routine 100 (Cannon-Fenske). The method and the calculation of the \( DP_v \) are described in Appendix 5-1. From this point onward, cellulose analysed by viscometry in Cadoxen will be abbreviated V.

### 5.3.1.2 Size-exclusion chromatography (SEC) of cellulose tricarbanilate in THF

Both SEC methods used allow for absolute \( M_r \) determination by using LS detectors. The cellulose tricarbanilate was produced by reaction of cellulose with phenylisocyanate (PIC) in dimethylsulfoxide (DMSO). Cellulose was activated in DMSO prior to the derivatisation. After the reaction, CTC was precipitated out of the derivatising mixture and re-dissolved in THF in order to allow SEC in THF mobile phase. The concentration detector was a UV detector UV 2000 (Spectra Physics) working at 270 nm, and was connected online with the \( M_r \) detector, a low-angle laser-light scattering detector (LALS) KMX-6 (Chromatix). Sample preparation, activation and dissolution, instrumentation and chromatographic conditions for SEC of CTC (further abbreviated SEC\(_{CTC}\)) are described in Appendix 5-2. The data reduction and \( M_r \) calculations were performed using SEC software CARB made by Lauriol [23] at EFPG\(^1\) especially for the analysis of CTC.

### 5.3.1.3 SEC of underivatised cellulose in LiCl/DMAC

As seen in previous chapters, LiCl/DMAc is a direct solvent of cellulose, the solvation mechanism was described in Chapter 3. One of the advantages is that LiCl/DMAc can be used as mobile phase in SEC. Detection was done with an interferometric differential refractometer (DRI) Optilab DSP (Wyatt Technologies) as concentration detector and online multiangle light scattering detector (MALS) Dawn EOS (Wyatt Technologies) as \( M_r \) detector. In this method, dissolution was preceded by activation of the cellulose substrate followed by water/solvent exchange (water/methanol/DMAc). The activation and dissolution methods are described in section 3.2.4 of Chapter 3, and the SEC procedure is described in section 4.2.3 of Chapter 4. The data reduction and \( M_r \) calculations were performed with ASTRA software v. 4.73.04 (Wyatt Technologies).

\(^1\) Ecole Française de Papeterie et des Industries Graphiques (St-Martin d’Hères).
Cellulose dissolved in LiCl/DMAc is further referred to as DDC (Directly Dissolved Cellulose) and the SEC method as SEC_DDCC.

5.3.2 Sample preparation

5.3.2.1 Preparation of the paper samples

Whatman No.1 pure cellulose filter paper was used as cellulose source. A pool of papers were left unaged (abbreviated UA) and a pool was subjected to artificial aging at 80°C and 50% relative humidity (rH) for 94 days (abbreviated At94) by hanging the sheets individually in a climate chamber Versatenn (Tenney Environmental). As shown in section 6.3.2.1 of Chapter 6, and supported by literature evidence [24,25,26] these aging conditions favoured the degradation of the cellulose by acid-catalysed hydrolysis.

For the viscosity measurements, the paper was cut in small pieces of 2 mm × 2 mm. For SEC, the paper was defibrillated by dry milling. This was done in a hammer mill (Poitemill/Forplex) for SEC_CTC, and in a two-blade cutting mill for SEC_DDCC. This type of preparation allows to further facilitate the access of the swelling liquids to the fibres during the activation phase. Activation is necessary in order to make cellulose more readily accessible to solvent molecules, as the solvents compatible with SEC have a lower swelling power than the complexing solvents used for viscometry (see section 1.3 of Chapter 1).

5.3.2.2 Sampling for viscometry and SEC

In order to fall within the viscosity range of the capillary glass viscometer, the required concentration of cellulose was about 1.5 g L\(^{-1}\) for the unaged paper, and 2.1 g L\(^{-1}\) for the aged paper, which corresponded to sample weights of 6.2×10\(^{-2}\) g and 8.8×10\(^{-2}\) g respectively. Three different solutions of each paper were prepared and three measurement repeats per solution were carried out.

CTC of aged and unaged paper were prepared. Injected weights were 1.461×10\(^{-4}\) g and 1.548×10\(^{-4}\) g respectively. The two samples were analysed in duplicate runs. The values reported are the average. The value of \(dn/dc\) of CTC in THF 0.169±0.002 mL g\(^{-1}\) was used [23,28]. As in SEC the working concentrations are very low, the second virial coefficient \(A_2\) can safely be omitted (see section A-5.2.2.2.2 of Appendix 5-2).

For DDC, three unaged and two aged paper samples were dissolved, each one was run two to three times. The runs were non-consecutive. The values reported are the average of the multiple runs. The sample preparation was performed according to the method described in section 3.2.4 of Chapter 3. In the case of DDC, the knowledge of the exact injected weights was not required in order to calculate \(M_w\) with SEC/MALS. The injected
mass is calculated by ASTRA software by inputting the values of the calibration constant of the DRI ($\alpha$) and the refractive index increment ($dn/dc$) of cellulose in LiCl/DMAc. These two parameters were determined experimentally as $\alpha = 2.25655 \times 10^{-4}$ V$^{-1}$ and $dn/dc = 0.077$ mL g$^{-1}$ (section 4.2.2 of Chapter 4). Here also, the second virial coefficient $A_2$ can be neglected (see section 4.2.2.4 of Chapter 4).

The RSD% on the mass of cellulose injected calculated by the ASTRA software for all the DDC unaged papers was $4.2\%$. RSD% refers here to the uncertainty related to the statistical fluctuation in each detector's signal (including all photodiodes and the DRI) and does not include any of the many possible systematic errors that may be present. The precision and repeatability of the SEC/MALS method was studied in section 4.2.3.4 of Chapter 4.

5.4 Results and discussion

5.4.1 Unaged cellulose (UA)

5.4.1.1 SEC of UA

The overlaid UV and LS signals obtained in one of the SEC$_{\text{CTC}}$ runs of unaged paper (UA) are represented in Figure 5.4-1 and the DRI and LS (90° angle photodiode) signals obtained in one of the SEC$_{\text{DDC}}$ runs are in Figure 5.4-2. The small peak present on the UV signal at high elution volume ($V_e$) for the CTC was due to residual diphenylurea that was still present despite the thorough washing in ethanol. The compound was trapped in the CTC network during the precipitation phase.

![UV and LS signals of CTC UA (unaged)](image-url)

Figure 5.4-1. UV (270 nm) and LS (LALS) signals of CTC UA (unaged).
SEC<sub>CTC</sub> and SEC<sub>DDC</sub> differential molar mass graphs are overlaid in Figure 5.4-3. The MMD profile of CTC was considerably smaller than that of DDC. In order to better compare the shape and position of the peaks, the MMD of CTC normalised to that of DDC is added. The correction factor was 7.24, which is the ratio of the weight fraction (wt frt) at peak molar mass ($M_p$) of DDC (wt frt = 1.325%, $M_p$=6×10<sup>5</sup> g mol<sup>-1</sup>) to the weight fraction at $M_p$ of CTC (wt frt = 0.183%, $M_p$=2.9×10<sup>5</sup> g mol<sup>-1</sup>). Figure 5.4-4 shows the cumulative molar mass graphs of SEC<sub>CTC</sub> and SEC<sub>DDC</sub>. Table 5.4-1 reports the values of the $M_t$ averages obtained with the three methods for UA.

![Figure 5.4-2. DRI and LS (MALs) signals of DDC UA (unaged).](image)

![Figure 5.4-3. Overlaid differential molar mass graphs of DDC UA, CTC UA and CTC UA normalised to DDC UA.](image)

![Figure 5.4-4. Overlaid cumulative molar mass graphs of DDC UA and CTC UA.](image)
Both the MMD and the cumulative molar mass graphs of CTC were shifted towards lower $M_t$ compared to DDC. The values of $M_n$, $M_w$, $M_z$ and $M_p$ obtained with SEC_{CTC} were all lower than with SEC_{DDC}, respectively by 57%, 40%, 28% and 53% (Table 5.4-1). Among the literature cited earlier, Lawther et al. [11] also found significant differences, of about 43% between CTC and DDC in the $M_w$ of cotton cellulose. However, Kennedy et al. [27] reported only a small difference between $M_t$ of CTC determined by viscometry and $M_w$ of DDC (in 10% LiCl/DMAc) for cotton linters determined by SEC, which they attributed to incomplete substitution of the CTC.

In the present study, in order to explain this significant difference, possible reasons can be ventured, including on the one hand the accuracy and limits of the two SEC methods and on the other, the dissolution procedures that can have a more or less degrading or modifying effect towards cellulose. The comparison between the two SEC methods in the following section is based on $M_w$, as it is the average $M_t$ directly calculated by the LS measurements.

Table 5.4-1. $M_t$ averages, corresponding DP and polydispersity (PD) obtained for CTC, DDC and V for unaged cellulose (UA).

<table>
<thead>
<tr>
<th>Method</th>
<th>$M_n$ ($\times 10^3$ g mol$^{-1}$)</th>
<th>$M_w$ ($\times 10^3$ g mol$^{-1}$)</th>
<th>$M_z$ ($\times 10^3$ g mol$^{-1}$)</th>
<th>$M_p$ ($\times 10^3$ g mol$^{-1}$)</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEC_{CTC} UA</td>
<td>1.72 1062</td>
<td>4.03 2485</td>
<td>7.23 4462</td>
<td>2.92 2344</td>
<td>2.38</td>
</tr>
<tr>
<td>SEC_{DDC} UA</td>
<td>3.96 +/- 0.31</td>
<td>6.68 +/- 0.14</td>
<td>10.09 +/- 0.46</td>
<td>6.24 +/- 0.35</td>
<td></td>
</tr>
<tr>
<td>V UA</td>
<td>2.88 +/- 0.18</td>
<td>3.80 2344</td>
<td>6.44 3973</td>
<td>1.70 +/- 0.12</td>
<td></td>
</tr>
</tbody>
</table>

(*): The values of the $M_t$ averages for CTC are adjusted to the molar mass of an anhydroglucose monomer, i.e. 162 g mol$^{-1}$.

5.4.1.1.1 Precision of the SEC methodologies

In this section, the precision and possible sources of uncertainties in both methods were investigated in order to explain the difference in $M_w$ between CTC and DDC.

5.4.1.1.1.1 Precision of the methodology of SEC_{CTC}

In the SEC_{CTC}, the precision was inherent to both the chemistry parameters (related to the derivatisation reaction) and the instrumental parameters.

The factor related to the instrumental parameters that most probably bore the greatest uncertainty in LALS was the attenuation factor (D) of the incident beam (Appendix 5-2). The attenuation factor was assumed to be correct from previous calibration measurements. Experimental records showed that the attenuation factor varied within ±10%. Errors associated with the value of the $dn/dc$ could not be evaluated as this parameter was a literature value, but underestimating slightly $dn/dc$ would result in overestimating slightly $M_w$ (in the same proportion), and vice-versa.
The injected mass and the degree of substitution (DS) of the cellulose tricarbanilate were the two chemistry factors that in all likelihood had the highest uncertainty. The error on the injected mass can arise from the presence of residual diphenylurea. This error was estimated to ±3% by Laurio [23]. Incomplete substitution reaction can lead to errors on the $M_t$ of the CTC monomer. As the lowest DS expected using the present derivatisation procedure was 2.8 [23], and the highest DS being 3, the uncertainty on the $M_t$ of the CTC monomer was estimated at 5%.

An experimental variability plan was designed where the three above mentioned parameters were cross-varied within their maximal and minimal values. The variability equation obtained with one of the SEC_{CTC} runs was:

$$DP_w = 2525 + 253 x_1 - 75 x_2 - 60 x_3 - 6 x_1 x_2 - 6 x_1 x_3 + 2 x_2 x_3$$

Equation 5-1

Where,

- $x_1 = \text{attenuation factor (D)}$ (variation limits: $1.277 \times 10^{-8}$ to $1.561 \times 10^{-8}$)
- $x_2 = \text{injected mass}$ (variation limits: $1.502 \times 10^{-4}$ to $1.594 \times 10^{-4}$ g)
- $x_3 = \text{molar mass of the CTC monomer}$ (variation limits: 495 to 519 g mol$^{-1}$)

The parameter that most influenced the value of $M_w$ was found to be the attenuation factor, highest coefficient in Equation 5-1.

Across the variability plan, $M_w$ varied within $3.49 \times 10^5$ g mol$^{-1}$ to $4.74 \times 10^5$ g mol$^{-1}$, which corresponds to ±13%.

### 5.4.1.1.1.2 Precision of the methodology of SEC_{DDC}

In the SEC_{DDC} methodology, the uncertainty can only arise from instrumental parameters. In MALS, $M_w$ is calculated by the software. Therefore the uncertainty on the value of $dn/dc$ and on the values of the MALS and DRI calibration constants can lead to errors.

The uncertainty on the $dn/dc$ taken within the repeatability of the three values obtained experimentally (section 4.2.2.3.2 of Chapter 4) was ±2%. The errors on the instruments calibration constants taking into account the experimental measures and the value provided by the manufacturer were ±2% for the LS constant, and ±0.6% for the $\alpha$ constant (DRI) (sections 4.2.2.2.1 and 4.2.2.1.2 of Chapter 4).

An experimental variability plan was designed where the three parameters were cross-varied within their maximal and minimal values. The variability equation obtained was:

$$DP_w = 4199 + 97 x_1 - 28 x_2 - 165 x_3 - x_1 x_2 - 4 x_1 x_3 + x_2 x_3$$

Equation 5-2

Where,

- $x_1 = \text{LS constant}$ (variation limits: $6.069 \times 10^{-6}$ to $6.356 \times 10^{-6}$)
\[ x_2 = \text{DRI constant } \alpha \text{ (variation limits: } 2.231 \times 10^{-5} \text{ to } 2.261 \times 10^{-5} \text{ V}^{-1}) \]

\[ x_3 = \text{dn} / \text{dc} \text{ cellulose/0.5% LiCl/DMAc (variation limits: } 0.0744 \text{ to } 0.0805 \text{ mL g}^{-1}) \]

The \( \text{dn} / \text{dc} \) showed the highest coefficient in Equation 5-2, followed by that of the LS constant. These were therefore the two parameters that most influenced the value of \( M_w \) in the present experimental conditions.

Across the variability experiment \( M_w \) varied from \( 6.34 \times 10^5 \text{ g mol}^{-1} \) to \( 7.28 \times 10^5 \text{ g mol}^{-1} \), \text{i.e.} \( \pm 6.5\% \). It has to be noted that this is a very small error, at half that of the estimated error for SEC_{CTC}. This shows further that the SEC_{DDC} method developed in the present study is quite precise.

The sum of the possible cumulated errors of SEC_{CTC} and SEC_{DDC} brought a maximal possible difference \( \Delta M_w \) of about 40\% (\( \pm 19.5\% \)). However, it is quite unlikely that all these statistical errors be cumulated as to yield such difference in the experimental \( M_w \) and other possible error sources had to be investigated.

### 5.4.1.1.2 Study of the discrepancies due to the dissolution processes

Factors, in particular related to the dissolution processes have to be investigated in order to account for a difference in \( M_w \) as large as 40\% between CTC and DDC. Several propositions are made hereafter.

The first hypothesis is that the procedure leading to CTC degraded the cellulose molecules to a certain extent. The second hypothesis is related to the complex chemistry involved during the dissolution of cellulose in LiCl/DMAc, that could lead to a possible overestimation of \( M_w \). The third hypothesis is related to non-steric elution phenomena of DDC and pseudo-exclusion behaviour of cellulose dissolved in LiCl/DMAc.

**Hypothesis 1. Degradation of cellulose during derivatisation to CTC**

The differences in the values of \( M_n, M_w \) and \( M_z \) of DDC and CTC for the unaged papers (\( M_r \text{ DDC UA } - \text{ M_r CTC UA} \)) followed the sequence \( \Delta M_n\% > \Delta M_p\% > \Delta M_w\% > \Delta M_z\% \). Such sequence indicated that the number of low-\( M_r \) fractions in the MMD of CTC is comparatively smaller than in the MMD of the DDC (knowing that the low- and high-\( M_r \) fractions in DDC and CTC are relative to each respective MMD). Another explanation would be that these low-\( M_r \) fractions are underestimated in the CTC- hence the latter appear as if containing proportionally more of the high-\( M_r \) fractions than the DDC. Concerning the polydispersity PD (PD = \( M_w / M_n \)), that of CTC was 1.4 times higher than that of DDC.

It has to be noted at this point that the software program CARB used to calculate the values of \( M_w \) of the CTC applies a smoothing equation based on a polynomial regression.
in the higher elution volume portion in order to recalculate values of $M_n$ [23, 28]. This is aimed at correcting the value of $M_n$ for axial diffusion because $M_n$ is most affected by the lower signal to noise ratio of the LALS at the end of the elution (low-$M_r$ and low concentration). Since this phenomenon does not affect the signal of the UV detector, the elution curve of the LS is smoothed according to the curve of the UV. After smoothing the data, $M_w$ remains unchanged while $M_n$ is significantly decreased.

Using the raw (not smoothed) data, the PD of CTC was 1.69, which was equal to the PD of DDC, and $M_n$ was $2.38 \times 10^5$ g mol$^{-1}$, which was 28% higher than $M_n$ using smoothing (but still about 40% lower than $M_n$ of the DDC). Smoothed CTC data is further referred to as $\text{CTC}_\text{smo}$ and raw CTC data as $\text{CTC}_\text{raw}$.

Based on hypothesis 1, and according to the results, cellulose appeared as if undergoing significant degradation during the derivatisation process to tricarbanilates, resulting in an overall decrease in the $M_r$ averages. The degradation seemed more directed towards the elimination of the lower-$M_r$ molecules of the CTC. This could result from the washing in ethanol after the precipitation phase of the CTC (see Appendix 5-2), as ethanol can dissolve the low-$M_r$ CTC [23]. The precipitation phase was unavoidable since the preparation of the CTC and the SEC runs were carried out in two different solvents.

The literature is unanimous on the fact that degradation occurs upon derivatisation of cellulose to CTC depending on reaction time and temperature as well as on the co-reactant, but no real agreement could be found among the different authors as to the best procedure in order to minimize this degradation. According to Evans et al. [29], in the preparation of CTC of high-$M_r$ cellulose from bleached cotton linters with phenylisocyanate (PIC) in DMSO at 70°C, reaction times above 32 hours resulted in a depolymerisation. The $M_w$ decreased by 9% when the reaction time increased from 32 to 56 hours, and by 24% when it increased from 32 to 96 hours. According to the authors, the lower temperature had to be compensated by a longer reaction time thereby also resulting in increased degradation. The least degrading conditions achieved in this case were using PIC in pyridine at 80°C, which resulted in a slight decrease in $M_w$ of only 5.4% and 6.4% when the reaction time increased from 32 to 56 hours, and from 32 to 96 hours respectively. Additionally, under these conditions, the initial $M_r$ after 32 hours of reaction was higher by 7.2% than when the reaction was carried out with PIC in DMSO. The authors concluded that the drawback of DMSO was that it led to partial degradation of high-$M_r$ cellulose during derivatisation but the advantage lied in faster reaction rates than with pyridine.

Contrary to the findings of the previous authors, Danheleka et al. [30] found no degradation during the carbanilation of cellulose with PIC in pyridine at 110°C for 12 hours when SEC data was compared to viscosity data (using Cadoxen). But Shroeder and Haigh [31] showed that, although no degradation occurred when carbanilation was carried out at 80°C for up to 48 hours, higher temperatures induced depolymerisation already within the first hours of the reaction.
For Laurio et al. [23,28], and Lapierre and Bouchard [32], using PIC in DMSO led to the most appropriate carbanilation procedure. The latter found that PIC in pyridine resulted in an overestimated \( M_t \) in the case of softwood Kraft pulps, and attributed the fact to aggregation possibly caused by incomplete carbanilation.

Hill et al. [33] showed that complete substitution (DS=3) was achieved by derivatising cellulose with PIC in four times the reaction stoichiometry, for 48 hours at 80°C in pyridine. Laurio et al. advocated using 10 times the reaction stoichiometry in order to obtain complete substitution [23,28].

In the present experiment the procedure of Laurio was followed, which used a derivatisation temperature of 70°C, that is even lower than most of the above-mentioned studies. Therefore, according to the authors cited above, such conditions should lead to no or little degradation in the worst case.

**Hypothesis 2. Overestimation of \( M_t \) averages of cellulose when dissolved in LiCl/DMAc**

According to the considerations described earlier, another cause for the molar mass discrepancy between the two SEC methods would help in the interpretation. This could arise from an overestimation of \( M_w \) by the SEC\textsubscript{DDC} method. This hypothesis is examined below.

Such an overestimation can happen through the formation of aggregates and/or the association of molecules in LiCl/DMAc. According to Terbojevitch et al. [34] values of \( M_w \) seven-fold those of \( M_t \) for cellulose can be found. The authors showed the formation of stable aggregates and inter-aggregate associations of cellulose molecules (especially in the case of acid hydrolysed cellulose) when dissolution took place at low LiCl concentration (5\% LiCl/DMAc). They demonstrated that the aggregates were formed by seven molecules in fully extended conformation, and proposed they arose from the native structure of the fibrils. Sjöholm et al. [9] later reported the formation of high-\( M_t \), aggregates of cellulose molecules in LiCl/DMAc solutions for softwood pulp, but these did not form with cotton linter.

A study by Röder et al. [35] showed that the solution state of cellulose molecules in LiCl/DMAc was influenced by both the cellulose and LiCl concentrations. Dissolution at high LiCl concentration (9\%) and high cellulose concentration (1\% wt/wt) resulted in a bimodal SEC molar mass distribution profile, indicating a high level of aggregation. The distribution became monomodal after 10 times dilution (to 0.9\% LiCl and 0.1\% cellulose (wt/wt)), indicating that the large aggregates had dissociated. The same initial cellulose concentration but with a lower salt concentration (6\%) resulted in large particles not totally dissolved that did not dissociate upon 10 times dilution.

Aggregates are easily detectable in MALS in the high-\( M_t \) end (small \( V_e \)) of the photodiodes signals, especially in the high degree angles. The chromatograms obtained in this study showed monomodal LS signals at all measuring angles, same as displayed in
Figure 5.4-2. Thus no evidence of aggregation was found. Additionally, our experimental
conditions were the most favourable for a total dissolution with no aggregation or
association as advocated by Röder et al. [35]. The detailed study of the conformation of
cellulose in LiCl/DMAc reported in section 4.2.4 of Chapter 4 showed a polymer in
random coil conformation and proved LiCl/DMAc to be a good solvent, and a theta
solvent in the worst case.

Excluding the formation of aggregates, a second explanation for an overestimate of the $M_r$
of cellulose using the method SEC_DDC can be one related to the chemistry of the
dissolution of cellulose in LiCl/DMAc. The dissolution involves the formation of
complexes between cellulose and LiCl/DMAc with a major role played by the chloride
anion breaking up the inter- and intra-molecular hydrogen bonds (section 2.2.2.3.1 of
Chapter 2). Previous studies showed that Cl\(^-\) formed complexes with the three hydroxyl
groups of an anhydroglucose unit (AGU) by hydrogen bonding and that the counterpart of
the solvent complex, i.e. the macrocation $[\text{Li DMAc}]^+$, was more loosely bound [36]. If
Cl\(^-\) ions were linked by hydrogen bonds to the hydroxy groups, as represented in Figure
5.4-5, the resulting apparent $M_r$ of the AGU could be considerably increased. If a bare
AGU has a $M_r$ of 162 g mol\(^{-1}\), adding three chloride atoms would increase the $M_r$ by 39%,
to 266 g mol\(^{-1}\). This could explain the difference in $M_w$ between the two SEC methods. If
this hypothesis were to be verified, it would also show that the procedure leading to CTC
is not as degrading as suggested earlier.

![Diagram of cellulose in LiCl/DMAc, CI linked to hydroxy groups of AGU through hydrogen bonds as proposed in Hypothesis 2.](image)

It has to be noted that this hypothesis holds only if there is indeed formation of a
hydrogen bond between the cellulose and the chloride ions, since the MALS detector does
not see a simple solvation layer, which has a refractive index very close to that of the
solvent.

The values of the masses injected for the seven samples of DDC UA were re-calculated
using 266 g mol\(^{-1}\) as $M_r$ for the AGU. This was achieved by decreasing the mass
calculated by the ASTRA software by 39%. $M_w$ was then re-calculated by entering the $\alpha$ constant and the new (decreased) mass as known variables (instead of $\alpha$ and $dn/dc$ as in the typical $M_w$ software calculation throughout this study). The resulting average molar masses were: $M_n = (2.42\pm0.15)\times10^5$ g mol$^{-1}$, $M_w = (4.10\pm0.15)\times10^5$ g mol$^{-1}$, and $M_Z = (6.19\pm0.40)\times10^5$ g mol$^{-1}$, with the same polydispersity of 1.69 (±0.12) as before. The value of $dn/dc$ calculated by ASTRA was then 0.126 mL g$^{-1}$.

These re-calculated values of $M_i$ averages for DDC UA resulted in a difference between DDC UA and CTC UA of: $\Delta M_n = 1.7\%$ with CTC$_{raw}$ ($\Delta M_n = 29\%$ with CTC$_{smo}$), $\Delta M_w = 1.7\%$, and $\Delta M_Z = -14\%$. DDC with the re-calculated $M_i$ are later referred as DDC$_{rec}$, and DDC with the uncorrected $M_i$ as DDC.

**Hypothesis 3. Non-steric exclusion phenomena in SEC**

The third hypothesis proposed for the difference in $M_w$ between CTC and DDC is related to the column packing. Poly (styrene-divinyl benzene) (PSDV B) is the preferred packing material used in SEC analysis of cellulose [4,6,7,8,9,10,23,27,28,29,32,33,37,38,39,40, 41,42,43,44,45]. However, little is known about the elution behaviour of solutions of cellulose in LiCl/DMAc on this column packing. A recent study by Bikova & Treimanis [46] pointed to a possible contribution of pseudo-exclusion effects in SEC of cellulose with 0.5% LiCl/DMAc as mobile phase, at 60°C to 80°C using PSDV B columns. The authors mentioned several possible causes for non-steric exclusion effects. One was the presence of electronegative groups on the cellulose. Despite the fact that cellulose is always considered as a neutral polymer, some ionisable carboxyl groups can be present from other polysaccharidic components such as hemicelluloses or from pulping and bleaching. It is noted at this point that in the present study, the accelerated aging some of the samples were subjected to could cause carbonyl and carboxyl groups to form along the cellulose chain. The second cause the authors pointed to was pH and salt concentration of the mobile phase, usually 0.5 to 1% LiCl in DMAc, i.e. 0.11 to 0.22 M, as being quite high and potentially able to lead to a modification of the viscosity of the polymer in solution due to a change in the hydrodynamic volume.

In the present case, the peaks profiles of Figure 5.4-1 and Figure 5.4-2 indicated suitable columns sets in both SEC$_{CTC}$ and SEC$_{DDC}$. Figure 5.4-6 shows the elution curves and the plot of log $M_i$ as a function of $V_e$ for DDC UA. The MMD is quasi-Gaussian and the distribution of mass across the elution is constant, which corroborates a good separation, with no retention phenomena under the current conditions. This confirmed the suitability of PSDV B as packing material for SEC columns as acknowledged in the literature [45]. Therefore, the hypothesis of a non-adequate separation range of the columns was excluded and was not investigated further in the framework of the present study.
Molar Mass vs. Volume

Figure 5.4-6. DRI signal and $M_r$ obtained from light scattering of DDC UA.

5.4.1.2 Viscometry of UA

$M_r$ always falls between $M_n$ and $M_w$ (section 2.1 of Chapter 2). In the specific case where the coefficient $a$ in the Mark-Houwink-Sakurada (MHS) equation (described in Appendix 5-1) is equal to unity, $M_v$ is equal to $M_w$. For pure cellulose paper, the MHS coefficient $a$ is often found close to unity, and $M_v$ is usually reported in the literature to be closer to $M_w$ than to $M_n$. In the present study with SEC/MALS (section 4.2.4 of Chapter 4), the experimental average value of $a$ for Whatman No.1 paper in 0.5% LiCl/DMAc was found of 0.81.

Table 5.4-1 reports the average value obtained for $M_v$ of unaged cellulose in Cadoxen. This value was considerably smaller than the values obtained for $M_w$ for both SECDDC and SECCTC: 57% lower than the $M_w$ of DDC (30% lower than that of DDCrec), and 28% lower than that of CTC.

A difference in $M_r$ is often reported in the literature when comparing viscometry in CED with LS for SECCTC [23] and for SECDDC [34]. It has to be noted that CED is more aggressive to cellulose and especially to oxidised cellulose than Cadoxen [3,22]. This difference in $M_r$ was also reported when great care was taken to minimize the degradation of the cellulose during dissolution in CED by working in a N$_2$ atmosphere and in the absence of light [10].

With the data collected by the UV and the LALS detectors for SECCTC, and with the DRI and MALS detectors for SECDDC it was possible to calculate $M_v$ for CTC and DDC (see formulae in section A5-2.2.2.3 of Appendix 5-2). This was done in order to more
accurately evaluate viscometry and SEC by comparing the same $M_v$ average ($M_v$). The MHS coefficient $a$ used in the calculation of $M_v$ for DDC UA was the experimentally determined value of 0.81, and for CTC UA, the value 0.84 found in the literature was used [30,47], as the current SEC$_{CTC}$ method did not allow for the calculation of $a$.

The values thus obtained for $M_v$ were of $6.44\times10^5$ g mol$^{-1}$ for DDC UA and $3.80\times10^5$ g mol$^{-1}$ for CTC UA (Table 5.4-1). These values are indeed quite close to $M_w$. However, for V UA $M_v$ was still lower respectively by 55% and 24% than $M_v$ calculated for DDC UA and for CTC UA. Using the re-calculated average $M_w$ of DDC (DDC$_{rec}$ UA) obtained with the seven samples unaged, the average value obtained for the calculated $M_v$ for DDC$_{rec}$ UA was then $3.95\times10^5$ g mol$^{-1}$, which is still 27% higher than the value obtained for $M_v$ V UA. However, this difference approaches the 24% difference reported above obtained subtracting the $M_v$ of V UA to the calculated $M_v$ of CTC UA.

This difference can be attributed to either solvent-induced degradation or the precision in the value of the calculated $M_v$. Indeed, errors on the MHS constants $K'$ and $a$ can lead to considerable errors in the $M_v$. This is especially true for the value of $a$, as it is a power exponent in the MHS equation, and contrary to $K'$, $a$ is used in the calculation of $M_v$ based on the MMD in SEC. However, the present study (section 4.2.4 of Chapter 4) showed that the RSD on the average $a$ value of 0.81 determined for DDC UA was 4.9%, with $a$ ranging from 0.77 to 0.86. The subsequent error on the calculated $M_v$ for DDC UA was therefore $\pm 6.8\times10^5$ g mol$^{-1}$, which yields a value of $M_v$ within $6.37\times10^5$ to $6.5\times10^5$ g mol$^{-1}$. Such a very small error cannot account for the discrepancy in the values of the different $M_v$. Thus, it can be concluded that the latter is most probably due to a considerable underestimation of $M_v$ due to the method of viscometry in Cadoxen, which is then attributed mainly to solvent-induced degradation.

### 5.4.2 Aged cellulose (At$_{94}$)

#### 5.4.2.1 SEC of At$_{94}$

The overlaid SEC$_{CTC}$ and SEC$_{DDC}$ differential and cumulative molar mass graphs of cellulose from aged papers are represented in Figure 5.4-7 and Figure 5.4-8 respectively.

As already observed for unaged cellulose, the MMD profile of CTC At$_{94}$ was lower than that of DDC At$_{94}$ (Figure 5.4-7). Here also, in order to better compare the MMD profiles, the differential molar mass graph for CTC normalised to that of DDC was added. The correction factor was 6.78, which corresponds to the ratio 1.166/0.172 (ratio of the wt frt at $M_p$ of DDC ($3.5\times10^5$ g mol$^{-1}$) divided by the wt frt at $M_p$ of CTC ($2.3\times10^5$ g mol$^{-1}$)).
The values of $M_r$ averages obtained with SEC\textsubscript{CTC} (CTC\textsubscript{smo} and CTC\textsubscript{raw}) and SEC\textsubscript{DDC} for the papers aged 94 days are reported in Table 5.4-2. $M_n$ and $M_w$ resulted in lower values for CTC than for DDC, by 9% for CTC\textsubscript{raw} (40% for CTC\textsubscript{smo}), and by 15% respectively, while the two $M_z$ were similar. The PD of CTC\textsubscript{smo} was 1.4 times higher than that of DDC, while the PD of CTC\textsubscript{raw} and that of DDC were similar. Here also, it seemed more appropriate to use CTC\textsubscript{raw} rather than CTC\textsubscript{smo} for the comparison with DDC.

According to the calculations of uncertainties reported in section 5.4.1.1.1, a 15% change in $M_w$ falls within the experimental error in the precision of the methods. It has to be noted at this point that the RSD of 2.1% on $M_w$ of DDC UA, i.e. \((6.68\pm0.14)\times10^5\) g mol\(^{-1}\), and 3.4% on $M_w$ of DDC At\textsubscript{94}, i.e. \((3.81\pm0.13)\times10^5\) g mol\(^{-1}\) reported in Table 5.4-1 and Table 5.4-2 were calculated based only on the different values of $M_w$ obtained in the multiple runs repeats and do not consider the experimental uncertainties that were estimated in section 5.4.1.1.1.

The values of the $M_r$ averages for DDC\textsubscript{rec} At\textsubscript{94} were $M_n = (1.26\pm0.09)\times10^5$ g mol\(^{-1}\), $M_w = (2.33\pm0.08)\times10^5$ g mol\(^{-1}\), and $M_z = (3.70\pm0.2)\times10^5$ g mol\(^{-1}\). These values for DDC\textsubscript{rec} At\textsubscript{94} of $M_n$, $M_w$ and $M_z$ were smaller than for CTC\textsubscript{raw} At\textsubscript{94} by 33%, 28% and 39% respectively. However, the reader is reminded that these $M_r$ averages of DDC\textsubscript{rec} At\textsubscript{94} were calculated on the basis of a hypothetical 39% increment in $M_r$, as in the case of DDC\textsubscript{rec} UA. This increment was based on the assumption of the availability of three hydroxyl groups on each AGU to form hydrogen bonds with the chloride anions of the solvent complex. The number of hydroxyl groups on a chain of aged cellulose is however most probably less than three, due to oxidation as consequence of the aging process. Therefore, the average number of CI along the chain would in all likelihood be less than three per AGU. In addition, charge repulsion with the CI\textsuperscript{−} can occur if negatively charged oxidised groups are present on the chain. The average $M_r$ of the AGU of aged cellulose in LiCl/DMAc.

![Figure 5.4-7. Overlaid differential molar mass graphs of DDC At\textsubscript{94}, CTC At\textsubscript{94}, and CTC At\textsubscript{94} normalised to DDC At\textsubscript{94}.

![Figure 5.4-8. Overlaid cumulative molar mass graphs of DDC At\textsubscript{94} and CTC At\textsubscript{94}.

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would be less than 266 g mol\(^{-1}\), i.e. between 162 and 266 g mol\(^{-1}\). With such a large molar mass spread, results are rather difficult to interpret. The conformational characteristic of aged cellulose in solution studied in section 6.3.2.1.1 of Chapter 6, showed it was less well solvated than unaged cellulose, which tends to confirm the hypothesis of a lower complexation degree with the solvent.

However, it has to be noted that if aged cellulose bears fewer hydroxyl groups on its chains, this should affect the CTC prepared from aged samples as well, as PIC reacts with available hydroxyl groups to form the carbanilate. Therefore, with a DS lower than three, the average \(M_r\) of the CTC monomer would be below 519 g mol\(^{-1}\), which in turn would result in the underestimation of \(M_w\) of CTC. But this could not be investigated further here.

This shows that for aged cellulose the discrepancies in the \(M_r\) averages between the two SEC methods cannot be interpreted with certainty. It is thus extremely difficult to draw conclusions about the respective performance of the two SEC procedures for the aged samples.

Figure 5.4-9 shows on the same graph the overlaid differential molar mass graphs of aged and unaged CTC and DDC. This allows for a better visualisation of the smaller shift towards the low-\(M_r\) that exists between DDC At\(_{94}\) and CTC At\(_{94}\) compared to that between DDC UA and CTC UA (using the MMD of CTC normalised to that of DDC). Table 5.4-3 reports the percent difference in \(M_r\) averages between aged and unaged paper according to the relevant method.

![Figure 5.4-9. Overlaid differential molar mass graphs of aged and unaged DDC and CTC (CTC are normalised to DDC using same factors as in Figure 5.4-7.](image-url)
Table 5.4-2. \( M_r \) averages, corresponding DP and PD obtained for CTC, DDC and V of aged cellulose (At94).

<table>
<thead>
<tr>
<th>Method</th>
<th>( M_r ) (x 10^4 g mol(^{-1}))</th>
<th>( DP_n )</th>
<th>( M_w ) (x 10^4 g mol(^{-1}))</th>
<th>( DP_w )</th>
<th>( M_z ) (x 10^4 g mol(^{-1}))</th>
<th>( DP_z )</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>SECCTC At94</td>
<td>1.25 ± 0.16</td>
<td>1275</td>
<td>3.24 ± 0.13</td>
<td>2353</td>
<td>6.06 ± 0.34</td>
<td>3741</td>
<td>-</td>
</tr>
<tr>
<td>SECCTC At94*</td>
<td>1.88</td>
<td>1158</td>
<td>3.24</td>
<td>1997</td>
<td>-</td>
<td>-</td>
<td>1.72</td>
</tr>
<tr>
<td>SECdd At94</td>
<td>2.07 ± 0.16</td>
<td>1275</td>
<td>3.81 ± 0.13</td>
<td>2353</td>
<td>3.48 ± 0.22</td>
<td>3741</td>
<td>3.66</td>
</tr>
<tr>
<td>V At94</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.77 ± 0.19</td>
<td>1092</td>
<td>-</td>
</tr>
</tbody>
</table>

(\(*\): The values of the \( M_r \) averages for CTC are adjusted to the molar mass of an anhydroglucose monomer, i.e. 162 g mol\(^{-1}\).)

Although direct comparisons between SECCTC and SECDDC are extremely difficult, comparisons within each method between aged and unaged cellulose can be drawn. Whether using DDC or DDCrec, the difference in \( M_r \) averages between unaged and aged cellulose as characterised by SECDDC resulted in the same sequence \( \Delta M_n\% > \Delta M_w\% > \Delta M_z\% \). This indicated a cleavage process mostly governed by acid hydrolysis but with a slight preferential attack in the low-\( M_r \) fractions (kinetics of degradation are detailed in section 6.3.2.1.2 of Chapter 6). This was consistent with the very slight increase of 8% in the PD of DDC At94 versus DDC UA.

For CTC, the decrease in \( M_r \) averages between UA and At94 followed the same sequence as above, with \( \Delta M_n\% \) (CTCsm) > \( \Delta M_w\% \) > \( \Delta M_z\% \) or \( \Delta M_n\% \) (CTCraw) = \( \Delta M_w\% \) > \( \Delta M_z\% \), albeit in about halved proportions than for DDC (Table 5.4-3). Due to the step of precipitation in ethanol in the case of the CTC, which can lead to an underestimate of the low-\( M_r \) molecules, it is also possible to speculate that the difference \( \Delta M_n\% \) between CTC aged and unaged is attenuated compared to \( \Delta M_n\% \) between DDC aged and unaged, as indeed low-\( M_r \) molecules are present in higher amount in the aged cellulose.

Table 5.4-3. Percentage difference in \( M_r \) averages and in PD between aged and unaged samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>( \Delta M_n% )</th>
<th>( \Delta M_w% )</th>
<th>( \Delta M_z% )</th>
<th>( \Delta M_r% )</th>
<th>( \Delta M_z% )</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>CTCraw UA - CTCraw At94</td>
<td>27%</td>
<td>20%</td>
<td>16%</td>
<td>20%</td>
<td>20%</td>
<td>-8%</td>
</tr>
<tr>
<td>CTCsmo UA - CTCsmo At94</td>
<td>21%</td>
<td>20%</td>
<td>16%</td>
<td>20%</td>
<td>20%</td>
<td>-2%</td>
</tr>
<tr>
<td>DDC UA - DDC At94</td>
<td>48%</td>
<td>43%</td>
<td>40%</td>
<td>44%</td>
<td>43%</td>
<td>-8%</td>
</tr>
<tr>
<td>V UA- V At94</td>
<td>38.5%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

So far in the present comparison, no mention was made of the impact of the mechanical activation of the paper, as the defibrillation was carried out by different means in the case of the CTC and the DDC.

The hammering action of the hammer mill (Poitemill/Forplex) used to defibrillate the paper for the preparation of CTC leads to a slightly different shortening of the fibres than the cutting action of the two-blades mill used to prepare the paper for DDC.
As aged and unaged samples in each respective case were prepared in the same manner, the sample preparation could not account for the $\Delta M_t$ between aged and unaged cellulose within each method. However, regardless of the defibrillation method, a detrimental mechanical effect on the aged papers compared to the unaged papers, as these are less degraded to start with, cannot be ruled out. It has also to be noted that both millings induce a rise in temperature during the defibrillation. The Forplex hammering was shown to lead to the formation of carbonyl groups on the cellulose, especially ketons [48], but no information could be found in the literature concerning a similar effect by the cutting mill used for the preparation of the DDC.

The two SEC methods for cellulose characterisation lead to different estimates of the extent of the degradation upon aging as illustrated by the changes in $M_n$, $M_w$, and $M_z$. As mentioned earlier, the value that can more reliably be compared between the methods is $M_w$. This value decreased between unaged and aged papers by 20% for CTC, and by 43% for DDC. This inevitably led to the conclusion that regardless of the method used, the estimation of the extent of the degradation is significantly different. Whether this difference was due to an increased degradation of the unaged paper during derivatisation of the CTC, or to a lower complexation degree of the solvent chloride anion with the AGU along the cellulose chain of the aged paper in the case of DDC could not be elucidated here. The literature would tend to corroborate the former with the findings that the CTC method degraded preferentially the high-$M_t$ cellulose [29], i.e. that tricarbanilation would indeed be more aggressive towards unaged cellulose than towards aged cellulose.

### 5.4.2.2 Viscometry of At$_{94}$

The $M_v$ of aged cellulose in Cadoxen was 42% lower than that of CTC, and 52% lower than that of DDC (Table 5.4-2), as calculated using $a = 0.81$ for DDC, and $a = 0.84$ for CTC (see section 5.4.1.2). As was observed for the unaged cellulose, viscometry in Cadoxen of degraded (aged) cellulose resulted in underestimated values of $M_v$. The decrease in $M_v$, observed between UA and At$_{94}$ in Cadoxen was of 38.5%. This value was intermediate between that calculated between UA and At$_{94}$ for the $M_v$ of DDC (43%) and for the $M_v$ of CTC (20%) (Table 5.4-3). The value of DDC$_{rec}$ At$_{94}$ was not used here in the comparisons for the reason cited above, namely of unknown $M_v$ for the AGU of aged cellulose in LiCl/DMAC, which could be anywhere between the real value of 162 g mol$^{-1}$ and the hypothesised value of 266 g mol$^{-1}$.

It is noteworthy that the $M_v$ average closer to $M_v$ for aged paper was $M_n$ rather than $M_w$. The same sequence as with UA was obtained with At$_{94}$, i.e. $M_n$ (CTC$_{smo}$ At$_{94}$) < $M_v$ (VAt$_{94}$) < $M_n$ (DDC At$_{94}$); $M_v$ for VAt$_{94}$ was 29% higher than CTC$_{smo}$ At$_{94}$ (but only 6% lower than $M_n$ of CTC$_{raw}$ At$_{94}$), and 14.5% lower than $M_n$ of DDC At$_{94}$. 

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Compared to SEC, viscometry in Cadoxen underestimated the values of $M_r$. Moreover, the comparison between aged and unaged papers did not yield the same degradation rate as either of the two SEC methods. Although Cadoxen is reported to be relatively stable and not significantly aggressive for cellulose [18], this is contrary to the present results, as even with the reduction pre-treatment in NaBH₄ that was introduced in order to limit solvent-induced degradation, it was shown that Codoxen was quite aggressive for cellulose.

### 5.4.3 Simplicity and cost effectiveness of the methods

From the point of view of time and cost effectiveness, the time required from activation to dissolution for the CTC method was slightly lower (88 hours) than for DDC (96 hours). Both methods were found equally work-intensive and cost effective, and the solvents are both rather toxic. The sample preparation for the viscometry method in Cadoxen was faster than for both SEC methods, the dissolution taking place in 16 hours. However, the viscosity measurements themselves turned out far more time-consuming and labour-intensive than the SEC methods. A day of analysis with SEC_{CTC} or SEC_{DDC} allows about 10 to 12 samples to be run, while only about half as many can reasonably be analysed using the viscometry method. Additionally, it must be noted that pure cellulose paper is an ideal case for dissolution, and that for high lignin content papers a delignification process prior to dissolution in Cadoxen is necessary, which considerably lengthens the procedure when analysing such papers. On the other hand, delignification is necessary prior to both SEC methods too. In the case of DDC, the complete dissolution of ligneous papers in LiCl/DMAc could not be achieved even after partial delignification [49].

### 5.5 Conclusion

The present study showed the complexity of comparing methods involving a number of different parameters that cannot fully be cross-evaluated at the same level. According to our results, and considering the discrepancies in the $M_r$ averages that were obtained, it was unclear whether the derivatisation method of cellulose to tricarbanilates led to a significant degradation of the polymer, or the method of dissolution in LiCl/DMAc led to an overestimation of the $M_r$. Both those phenomena could to a certain extent be involved in order to account for the 40% difference found in the $M_w$ of unaged cellulose between the two SEC methods. This study shows also that despite the widespread use of LiCl/DMAc as a solvent for cellulose, and the numerous studies and reviews that have focus on the solvation mechanism over the past twenty years, the molecular structure of the complex formed between cellulose and LiCl/DMAc solvent is not totally understood, and more research is needed.
From the present results it appeared that the $M_t$ averages obtained with both SEC methods were more consistent for aged cellulose than for the unaged cellulose. This would support the hypothesis that if any degradation occurred, the tricarbanilation method was more aggressive towards unaged cellulose than towards aged cellulose, i.e. that, in agreement with the literature [29], the CTC method degraded preferentially high-$M_t$ rather than the low-$M_t$ cellulose.

Viscometry in Cadoxen was found to be the method that most underestimated the $M_t$ of aged as well as unaged cellulose. This was attributed to solvent-induced degradation. This method was therefore not considered the most appropriate method for $M_t$ determination. Additionally, the relative difference in $M_t$ between unaged and aged paper could not be correlated to the decrease in the $M_t$ averages between unaged and aged as observed by SEC-CTC and SEC-DDC. Viscometry was therefore also found inappropriate to estimate relative $M_t$ changes, as those occurring for instance between a sample after a certain treatment and its untreated counterpart (here: between aged and unaged samples). As such comparisons are frequent in conservation research when following the course of action of accelerated aging or other treatments on various materials, it is advised to carefully choose the analytical technique in order not to misinterpret the results.

**Chemicals and materials**

Whatman No.1 filter paper was obtained from Fisher Scientific (Springfield, NJ, USA). Sodium borohydride (NaBH₄) was obtained from Sigma-Aldrich Corp (St. Louis, MO, USA). Lithium chloride (LiCl), methanol and $N,N$-Dimethylacetamide (DMAc) were purchased from Acros Organics (Springfield, NJ, USA). Phenylisocyanate (PIC) and dimethylsulfoxide (DMSO) were from Fluka (Saint-Quentin Fallavier, France).

**Instruments**

The climate chamber Versatenn was from Tenney Environmental (Parsippany, NJ, USA).

The UV detector 2000 was from Spectra Physics (Darmstadt, Germany) and the low angle light scattering (LALS) detector KMX-6 was from Chromatix (Neckargemünd, Germany).

Multiangle light scattering detector Dawn EOS and interferometric differential refractometre Optilab DSP were from Wyatt Technologies Corp. (Santa Barbara, CA, USA).

Additional instrumentation related to the viscometry measurements and to the separation and analysis of CTC and DDC, not cited in the present chapter are reported in the sections Instruments of Appendix 5-1, Appendix 5-2 and Chapter 4 respectively.
References


49. Harrison, G. personal communication.