

# Comparative evaluation of size-exclusion chromatography and viscometry for the characterisation of cellulose

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## Abstract

The analysis using size-exclusion chromatography (SEC) with multi-angle light scattering (MALS) and differential refractive index (DRI) detection of cellulose dissolved in lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) is evaluated and compared to two other methods currently used for cellulose analysis. These are SEC with low-angle light scattering (LALS) and ultra-violet detection of cellulose derivatised to tricarbaniates (CTC), and viscometry in cadmium triethylene diamine dihydroxide (cadoxen). The cellulose source is Whatman No. 1 paper, unaged or artificially aged with a combination of heat and humidity. The values of the molar mass ( $M_r$ ) averages of cellulose obtained with the different methods resulted quite different for both aged and unaged paper. SEC of cellulose in LiCl/DMAc provided the highest  $M_r$  averages values, followed by SEC of CTC, while viscometry yielded the lowest values. These differences were more or less pronounced depending on the initial degradation state of the paper. Several hypotheses are presented in order to explain these discrepancies and each method is discussed on the basis of its suitability to characterise the aging-induced degradation.

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## 1. Introduction

When analysing cellulose a key parameter for accuracy of the data obtained is the integrity of the polymer. A non-degradative dissolution process is paramount if the molar mass ( $M_r$ ) of dissolved cellulose is to reflect the  $M_r$  of the cellulose source.

Viscometry is fast and convenient, and is often the preferred method to estimate the average degree of polymerisation ( $DP_v$ ) of cellulose and its derivatives. However, the method has obvious limitations since it provides only the viscosity-average molar mass ( $M_v$ ), which is not an absolute average since it depends on the solvent/temperature conditions. Moreover, no information concerning the molar mass distribution (MMD) of the polymer is obtained, and the complexing organometallic solvents used often degrade the

polymer [1–3], especially when analysing oxidised cellulose [4]. Viscometry is still widely used in cellulose analysis despite the multiple advantages of size-exclusion chromatography (SEC), which are chiefly the characterisation of the MMD, and the determination of the different molar mass averages  $M_n$ ,  $M_w$ ,  $M_z$ , including  $M_v$ , under certain conditions. SEC also informs on the degraded fractions, thus leading to insights in the degradation mechanisms. In SEC, the type and quality of the data and the precision in the  $M_r$  determination depend on the sensitivity of the detection method. Light scattering (LS) detectors online with refractive index detectors or UV detectors provide an absolute determination of  $M_r$  and MMD. Insight in light scattering theory and principles of detector-coupling techniques can be found in [5,6].

In the present study, different methods for analysing cellulose were compared. The experiment was based on evaluating SEC of cellulose dissolved directly in lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc), denoted DDC, SEC of cellulose derivatised to cellulose

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tricarbanilates, denoted CTC, and viscometry in cadoxen, a cadmium triethylene diamine dihydroxide complex  $[\text{Cd}(\text{En})_3](\text{OH})_2$  (with  $\text{En} = \text{H}_2\text{N}(\text{CH}_2)_2\text{NH}_2$ ), denoted V. Detection was carried out with low-angle LS and UV for CTC, and with multi-angle LS and differential refractometer (DRI) for DDC. The solvents/analytical methods conditions were chosen because they are reported to be the least degrading ones in their respective category among those commonly used in paper and cellulose research. So far, only few studies have been dedicated to compare different methods for the characterisation of cellulose [4,7–9], and when involving viscometry, copper ethylene diamine (CED) was most often used, as it is the solvent on which most standardised methods are based. To our knowledge there is no published work on the comparative evaluation of viscometry of underivatised cellulose in cadoxen and SEC of both derivatised and underivatised cellulose. Additionally, the present study not only characterises unaged cellulose, but also cellulose artificially aged by a combination of heat and humidity. Separate aspects of the three methods were considered. Precision, accuracy and quality of the information obtained were assessed. The methods were also investigated in terms of their suitability for the analysis of undegraded as well as degraded cellulose.

## 2. Experimental

### 2.1. Paper samples description

Whatman No. 1 filter paper (pure cellulose) was used. Some papers were left unaged (samples abbreviated UA), and some were subjected to accelerated aging at 80 °C and 50% relative humidity (rH) for 94 days (samples abbreviated At<sub>94</sub>) by hanging the sheets individually in a climate chamber Versatenn (Tenney Environmental). As reported by several authors, under these aging conditions acid-catalysed hydrolysis of cellulose occurs, leading to more or less random cleavage of the polymer chain [10–14]. Oxidation probably contributes to the overall depolymerisation by facilitating the hydrolysis reactions, as recently proposed by Shahani and Harrison [15].

### 2.2. Conditions

#### 2.2.1. Chemicals

Whatman No. 1 filter paper was obtained from Fisher Scientific (Springfield, NJ, USA). Deionised water was Milli-Q from Millipore, (Guyancourt, France). Sodium borohydride, ethylenediamine and cadmium oxide were from Sigma–Aldrich Corp. (St. Louis, MO, USA). Lithium chloride, methanol and *N,N*-dimethylacetamide were purchased from Acros Organics (Springfield, NJ, USA). Dimethylsulfoxide, phenylisocyanate, acetone and ethanol were from Fluka (Saint-Quentin Fallavier, France) and tetrahydrofuran was obtained from Merck (Darmstadt, Germany).

#### 2.2.2. Preparation of cellulose solutions

**2.2.2.1. Cellulose in cadoxen.** Four samples of unaged and three samples of aged paper taken from separate sheets were dissolved on different days. The dissolution was preceded by a reduction treatment of the paper in sodium borohydride ( $\text{NaBH}_4$ ), as preventive treatment in order to decrease solvent-induced degradation during dissolution [16,17]. The papers were immersed for 16 h in  $\text{NaBH}_4$  0.5 M in ethanol, in a 1:0.1 ratio (w/v), and were subsequently thoroughly rinsed. Cadoxen was prepared following the procedure described by Donetzhuber [18]. The solvent was added to the paper cut in 2 mm × 2 mm pieces. The paper was totally dissolved within 90 min. After centrifugation, in order to eliminate eventual residues, two dilutions were made from the stock solution. This dissolution procedure follows the method developed by Kaminska [16], a modification of the method used by Doty and Spurlin [19] and Burgess [20]. Further on, cellulose analysed by viscometry in cadoxen is abbreviated V-UA and V-At<sub>94</sub>, for unaged and aged cellulose. Table 1 gives the list of the abbreviations used throughout the text.

**2.2.2.2. Cellulose tricarbanilates (CTC).** The cellulose tricarbanilate was prepared according to the procedure by Lauriol et al. [21] and Lauriol [22]. The paper from separate sheets was defibrillated by dry milling in a hammer mill (Poitemill/Forplex). Two samples of unaged and aged paper of 200 mg each were activated in 30 ml of

Table 1  
List of the abbreviations used

UA	Paper unaged
At <sub>94</sub>	Paper artificially aged at 80 °C and 50% rH during 94 days
DDC	Directly dissolved cellulose in LiCl/DMAc
DDC <sub>rec</sub>	Directly dissolved cellulose in LiCl/DMAc, $M_f$ recalculated with 39% mass increment (ASTRA software)
CTC	Cellulose tricarbanilate
CTC <sub>sno</sub>	Cellulose tricarbanilate, $M_f$ calculated using the smoothing function (CARB software)
CTC <sub>raw</sub>	Cellulose tricarbanilate, $M_f$ calculated without the smoothing function (CARB software)
SEC <sub>DDC</sub>	Size-exclusion chromatography of DDC
SEC <sub>CTC</sub>	Size-exclusion chromatography of CTC
V	Viscometry

dimethylsulfoxide (DMSO) at  $70 \pm 1^\circ\text{C}$  for 5–6 h, after which the derivatisation reaction was initiated by adding 10 ml of phenylisocyanate (PIC). The reaction took place during 48 h at  $70 \pm 1^\circ\text{C}$ , and was stopped by pouring 20 ml of acetone, which reacted with the excess PIC. The CTC of unaged and aged cellulose were then precipitated in ethanol under thorough stirring, and were cleaned by diffusion overnight in fresh ethanol. They were oven-dried at  $40^\circ\text{C}$  for 24 h, and subsequently re-dissolved in THF in order to perform SEC in THF mobile phase. The CTC solutions were filtered through a  $0.2\ \mu\text{m}$  pore polypropylene filter Anotop (Whatman) prior to the injection.

As reported by Lauriol [22], given the precision of the method, carbanilates can be considered as tri-substituted when degree of substitution (DS)  $\geq 2.8$ , which is indeed achieved under the same reaction conditions as the present ones.

#### 2.2.2.3. Cellulose directly dissolved in LiCl/DMAc (DDC).

The paper from separate sheets was defibrillated by dry-milling in a two-blade cutting mill. Three samples of unaged and two samples of aged paper of approximately 50 mg each were dissolved. The dissolution was preceded by an activation treatment of the cellulose substrate by solvent exchange. The latter consisted in two consecutive thorough swellings of 1 h each under constant stirring in a heating/stirring unit (Pierce) in 10 ml deionised water at  $40^\circ\text{C}$ , followed by two consecutive exchanges of 45 min each with 8 ml methanol, and ended by two consecutive exchanges with 8 ml anhydrous DMAc, dried with aluminium sodium silicate molecular sieve, 0.4 nm effective pore size (JT Baker). The first DMAc exchange lasted 45 min and the second was prolonged overnight. After each exchange, the activation liquids were expelled by filtering under vacuum through  $0.5\ \mu\text{m}$  pore polytetrafluoroethylene (PTFE) filters Millex LCR (Millipore). After the last DMAc exchange, 5 ml of a solution of 8% LiCl/DMAc were added to the fibres. Being highly hygroscopic, LiCl was previously oven-dried before being stored in a desiccator over drierite. When needed, aliquots were weighted swiftly and placed back in the desiccator for several hours until dryness before use. The sample was stirred at room temperature for 24 h, and placed at  $4^\circ\text{C}$  until complete dissolution, which took 2 days. The solution was then diluted to 0.5% LiCl/DMAc with anhydrous DMAc, i.e. to a concentration of roughly  $0.625\ \text{mg ml}^{-1}$  (0.0625%, w/v), and filtered through  $0.5\ \mu\text{m}$  pore filter Millex LCR before injection. Details on the experiments that led to the development of this method can be found elsewhere [14,23].

### 2.2.3. Methods and instrumentation

**2.2.3.1. Viscometry.** In practice the viscosity is not measured directly. Instead, the time of flow in a capillary viscometer of dilute solutions of the polymer, typically of the order of 1% by mass [24], and the pure solvent, the so-called

efflux time, is measured.  $M_v$  is calculated according to the Mark–Houwink–Sakurada (MHS) equation:  $[\eta] = K' M_v^a$ , where  $[\eta]$  is the intrinsic viscosity,  $K'$  and  $a$  are the MHS constants for a given polymer-solvent system, temperature and molar mass range. For cellulose in cadoxen at  $30^\circ\text{C}$ , the MHS equation is:  $[\eta] = 3.85 \times 10^{-4} M_v^{0.76}$  [18].

The efflux time of a given cellulose sample at three different concentrations was measured. This yielded a three-point plot  $\eta_{\text{sp}} = f(c)$ , where  $\eta_{\text{sp}}$  is the reduced viscosity, that allowed to extrapolate  $[\eta]$  from the value of  $\eta_{\text{sp}}$  at zero concentration. The measurements were done in a water bath at  $30 \pm 0.1^\circ\text{C}$  using a capillary glass viscometer Routine 100 (Cannon-Fenske, now Cannon Instrument Cie) with a nominal constant of 0.015 and kinematic viscosity range between  $3 \times 10^{-6}$  and  $15 \times 10^{-6}\ \text{m}^2\ \text{s}^{-1}$ . In order for the viscosity of the diluted cellulose solutions to fall within the measurable range of the viscometer, the concentrations of the initial solutions prepared were about  $1.5\ \text{g l}^{-1}$  for the unaged paper samples, and  $2.1\ \text{g l}^{-1}$  for the aged paper samples. This corresponded to about  $6.2 \times 10^{-2}\ \text{g}$  and  $8.8 \times 10^{-2}\ \text{g}$  of paper, respectively. For each solution the measurement was repeated until three consecutive efflux times agreed within 0.1 s. Every such measurement was done three times non-consecutively, and the efflux time values averaged. Acceptable values of  $[\eta]$  have a correlation coefficient  $R^2$  exceeding 0.998. Relative standard deviation (RSD) of 1.5% on the values of  $\text{DP}_v$  are thus obtained [16].

#### 2.2.3.2. Size-exclusion chromatography of CTC.

The SEC setup consisted of a P-1500 pump (Thermo Separation Products, now Thermo-Finnigan), manual injector (model 7125, Rheodyne L.P.) with a  $100\ \mu\text{l}$  loop. The exact injected volume was  $109\ \mu\text{l}$ . This value, together with the exact sample concentration, is used to calculate the  $M_r$  values. The detection was done using a low-angle light scattering (LALS) detector KMX-6 (Chromatix) operating at 633 nm with 2 mW helium–neon laser source, followed on-line by a UV detector 2000 (Spectra Physics) working at 280 nm. The scattering angles of the LALS form an annulus at  $6^\circ$ – $7^\circ$  forward (3–4 real degrees). A photomultiplier with adjustable gain measures the intensity of the scattered light  $G_\theta$  and the transmitted light  $G_0$ . A series of calibrated attenuators allow the measurement of  $G_0$  in the same sensitivity range as  $G_\theta$ . The measure of the transmittance yielded an attenuation factor ( $D$ ) of  $1.419 \times 10^{-8}$  (theoretical value for  $D$  is  $1.22 \times 10^{-8}$ ). The scattered light was reduced with the field stop  $0.2\ \text{mm}$ . The interdetector delay volume was  $0.167\ \text{ml}$ .

The separation was carried out on a set of two  $L \times D$   $300\ \text{mm} \times 7.8\ \text{mm}$  Ultrastayragel P/N 10681 poly(styrene-divinyl benzene) (PSDVB) columns (Waters), with linear separation in the range of  $2 \times 10^3$  to  $4 \times 10^6\ \text{g mol}^{-1}$ . An on-line membrane  $0.22\ \mu\text{m}$  pore PTFE filter FGLP (Millipore) was placed between the columns and the LALS detector to remedy possible particle bleeding that can severely interfere with the LALS signal. Former experience on numerous cellulose samples, among which high- $M_r$

cotton linters have ascertained that this filtration procedure is non-discriminating for the sample. The runs were done at ambient temperature at a flow rate of  $1 \text{ ml min}^{-1}$ , and lasted 30 min. The mobile phase, THF, was filtered through  $0.5 \mu\text{m}$  pore filters Fluoropore (Millipore), and was degassed under helium flow prior to use.

The samples concentration was approximately  $1 \text{ mg ml}^{-1}$ , the injected weights were  $1.461 \times 10^{-4} \text{ g}$  for the CTC prepared with aged paper (abbreviated CTC-At<sub>94</sub>), and  $1.548 \times 10^{-4} \text{ g}$  for the CTC prepared with unaged paper (abbreviated CTC-UA). The two samples were analysed in duplicate runs. Reported values are the average. The value of the specific refractive index increment,  $dn/dc$  of CTC in THF used was  $0.169 \pm 0.002 \text{ ml g}^{-1}$  [21,22]. As in SEC, the polymer concentrations are very low, the second virial coefficient ( $A_2$ ) can safely be omitted, and this had indeed been verified [22]. The data acquisition was done in 1 s intervals, and  $M_r$  calculations were performed using the software CARB adapted by Lauriol [22]. The SEC method for CTC is further abbreviated SEC<sub>CTC</sub>.

**2.2.3.3. SEC of DDC.** The SEC set-up consisted of a four-channels HPLC solvent degasser Degassit<sup>TM</sup> (Metachem Technologies Int.), HP 1100 isocratic pump G1310A (Agilent Technologies), and manual injector (model 7725i, Rheodyne L.P.) with a  $100 \mu\text{l}$  loop. An on-line membrane  $0.22 \mu\text{m}$  pore PTFE filter FGLP (Millipore) was placed between the pump and the injector to filter any remaining particulates in the mobile phase. The detection was done with a multi-angle light scattering (MALS) detector Dawn EOS (Wyatt Technologies Corp., Santa Barbara, CA) equipped with heated/cooled Peltier option ( $-25$  to  $+85 \text{ }^\circ\text{C} \pm 0.2 \text{ }^\circ\text{C}$ ), and interferometric differential refractometer (DRI) Optilab DSP (Wyatt Technologies Corp.) with internal temperature control ( $25$ – $80 \text{ }^\circ\text{C} \pm 0.005 \text{ }^\circ\text{C}$ ). The laser source of the MALS has a nominal power of  $25 \text{ mW}$  ( $23.5 \text{ mW}$  effective), and operates at  $690 \text{ nm}$ . The light source of the DRI emits also at  $690 \text{ nm}$ . The interdetector delay volume was  $0.150 \text{ ml}$ . Constants of the instruments were determined as  $6.071 \times 10^{-6}$  for the MALS, and  $2.256 \times 10^{-4} \text{ V}^{-1}$  for the DRI [14].

The separation was carried out on a set of three  $L \times D$   $300 \text{ mm} \times 7.5 \text{ mm}$  MIXED-B pores PSDVB columns packed with  $10 \mu\text{m}$  particle diameter (Polymer Laboratories Inc.), preceded by a  $L \times D$   $50 \text{ mm} \times 7.5 \text{ mm}$  PSDVB guard column,  $10 \mu\text{m}$  particle diameter (Polymer Laboratories Inc.). The columns show a linear separation in the range of  $500$ – $10^7 \text{ g mol}^{-1}$ . The system was operated at  $60 \text{ }^\circ\text{C}$  at a flow rate of  $1 \text{ ml min}^{-1}$ . The mobile phase,  $0.5\%$  LiCl/DMAc, was filtered through  $0.5 \mu\text{m}$  pore filters Millex LCR (Millipore) prior to use. Run time was 40 min. The data acquisition was carried out in  $0.5 \text{ s}$  intervals with the ASTRA software version 4.73.04 (Wyatt Technologies Corp.).

As reported in Section 2.2.2.3, for aged and unaged papers, two and three separate dissolutions were carried out

(on different days, with different solvent batches), respectively. Each solution was analysed in duplicate or triplicate runs non-consecutively, which yielded a total of seven separate analyses for the unaged paper and six for the aged paper. The values reported are the average of the multiple runs. Depending on the samples, the theoretical mass injected was comprised between  $5.9 \times 10^{-5}$  and  $6.3 \times 10^{-5} \text{ g}$ . This is normally merely indicative, as the ASTRA calculates the actual injected mass by integration of the DRI signal, provided that the values of  $dn/dc$  of cellulose in  $0.5\%$  LiCl/DMAc, and the calibration constant  $\alpha$  of the DRI, are known. These two parameters were experimentally determined in separate measurements, as well as the ASTRA calculation simulations showing that the second virial coefficient  $A_2$  could be omitted [14,25]. The RSD calculated by ASTRA on the injected mass of cellulose for the unaged papers analyses was  $4.2\%$ . Other possible uncertainties that can have an impact on the calculated value of  $M_r$  are discussed later. DDC of unaged and aged paper are denoted DDC-UA and DDC-At<sub>94</sub>, respectively and the SEC method is abbreviated SEC<sub>DDC</sub>.

### 3. Results and discussion

#### 3.1. Unaged cellulose (UA)

##### 3.1.1. SEC of UA

The overlaid UV and LS signals obtained in one of the SEC<sub>CTC</sub> runs of unaged paper (CTC-UA) are represented in Fig. 1, and the DRI and LS ( $90^\circ$  angle photodiode) signals obtained in one of the SEC<sub>DDC</sub> runs of unaged paper (DDC-UA) are in Fig. 2. The small peak present on the UV signal at high elution volume ( $V_e$ ) for CTC-UA was due to residual diphenylurea trapped in the CTC network during the precipitation phase, despite the thorough washing in ethanol.

SEC<sub>CTC</sub> and SEC<sub>DDC</sub> MMD graphs are represented in Fig. 3. Table 2 reports the values of the  $M_r$  averages obtained with the different methods for cellulose of unaged paper. The MMD graph of CTC-UA showed a shift towards low- $M_r$

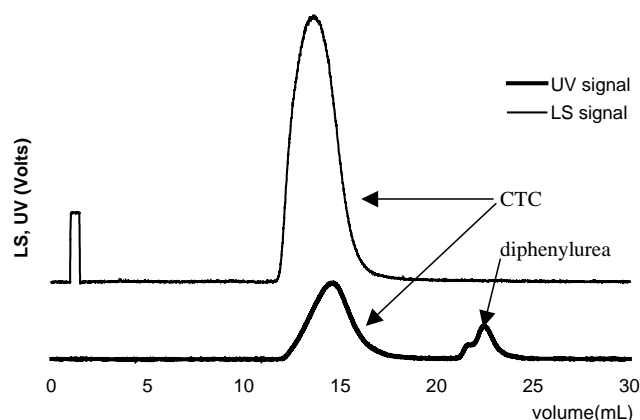


Fig. 1. UV (270 nm) and LALS signals of CTC-UA.

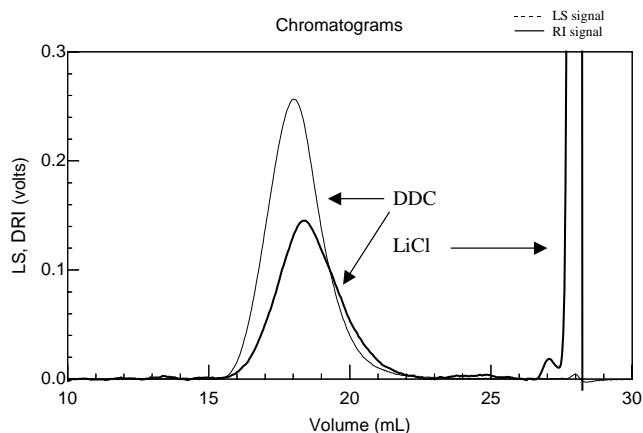


Fig. 2. DRI and MALS signals of DDC-UA.

compared to that of DDC-UA and the values of  $M_n$ ,  $M_w$ ,  $M_z$  and  $M_p$  obtained with SEC<sub>CTC</sub> were all lower than with SEC<sub>DDC</sub>, by 56, 39, 28 and 53%, respectively. In the scarce literature reporting similar comparisons, no agreement could be found regarding such disparities: if Lawther et al. [9] also reported a significant difference (43%) in the  $M_w$  of cotton between CTC and cellulose in LiCl/DMac, Kennedy et al. [26] measured a few percent difference only. In the present study, in order to explain the discrepancy on the values, possible reasons were ventured, including on the one hand the precision and possible sources of uncertainties in the two SEC methods, and on the other, the effect towards cellulose of the dissolution procedures, that can be more or less effective or degrading.

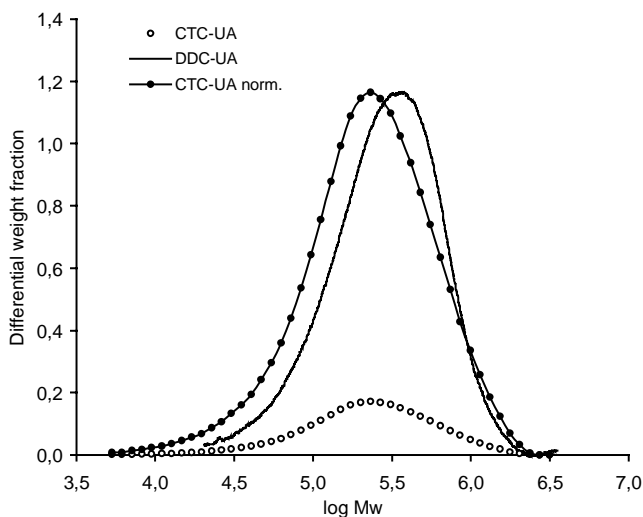


Fig. 3. Overlaid differential molar mass graphs of DDC-UA, CTC-UA and CTC-UA norm. “Norm” refers to the normalisation of CTC-UA to DDC-UA using a correction factor of 7.24 ( $=1.325/0.183$ ), corresponding to the weight fraction (wt. frt.) at peak molar mass ( $M_p$ ) of DDC-UA over the wt. frt. at  $M_p$  of CTC-UA (with  $M_p$  DDC-UA =  $6 \times 10^5$  g mol<sup>-1</sup> and  $M_p$  CTC-UA =  $2.9 \times 10^5$  g mol<sup>-1</sup>).

**3.1.1.1. Sources of error due to the SEC methodologies.** First of all, a source of error due to a non-adequate separation range of the columns was discarded as the peaks profiles of Figs. 1 and 2 indicated suitable column sets in both SEC<sub>CTC</sub> and SEC<sub>DDC</sub>. Besides, as Fig. 4 shows, the perfectly monomodal and smooth MMD obtained for DDC-UA, and the reproducible linearity of the elution curve both corroborate a good separation, with no retention on the columns under the current conditions. This confirmed the suitability of the PSDVB packing material, as acknowledged also in [27], and the absence of non-steric elution phenomena, and pseudo-exclusion behaviour of cellulose in LiCl/DMac described in [28].

The accuracy of the final  $M_w$  is related to that of the various constants and parameters used in the calculations. Factors that can lead to significant uncertainty are the instrumental constants, such as the attenuation factor ( $D$ ) of the incident beam of the LALS for the CTC and the constants of the MALS and the DRI ( $\alpha$ ) for the DDC. Sources of error can also arise from the  $dn/dc$ : with MALS/DRI, overestimated  $dn/dc$  will result in underestimated  $M_w$  by the same order of magnitude. Finally, in the case of the CTC, errors can be made on the degree of substitution (leading to errors on the  $M_r$  of the CTC monomer), and from the injected mass (eventual presence of residual diphenylurea). In order to estimate the influence of these parameters in each respective methodology, two designs of experiments (DOE), using full factorial design, based on one of the analyses of an unaged sample for each method (CTC-UA with measured  $DP_w$  of 2463 and DDC-UA with measured  $DP_w$  of 4105), where the above-mentioned parameters were cross-varied within their maximal and minimal values, were done and yielded Eqs. (1) and (2).

$$DP_w \text{ CTC-UA} = 2525 + 253x_1 - 75x_2 - 60x_3 - 7x_1x_2 - 6x_1x_3 + 2x_2x_3 \quad (1)$$

where  $x_1$  is the attenuation factor ( $D$ ) ( $\pm 10\%$  error estimated from previous experimental records, variation limits:  $1.277 \times 10^{-8}$  to  $1.561 \times 10^{-8}$ ),  $x_2$  is the injected mass ( $\pm 3\%$  estimated error [22], variation limits:  $1.502 \times 10^{-4}$  to  $1.594 \times 10^{-4}$  g), and  $x_3$  is the molar mass of the CTC monomer (DS between 2.8 [22] and 3, variation limits: 495–519 g mol<sup>-1</sup>).

$$DP_w \text{ DDC-UA} = 4199 + 97x'_1 - 28x'_2 - 165x'_3 - x'_1x'_2 - 4x'_1x'_3 + x'_2x'_3 \quad (2)$$

where  $x'_1$  is the MALS constant ( $\pm 2\%$  error [14], variation limits:  $6.069 \times 10^{-6}$  to  $6.356 \times 10^{-6}$ ),  $x'_2$  is the DRI constant  $\alpha$  ( $\pm 0.6\%$  error [14], variation limits:  $2.231 \times 10^{-5}$  to  $2.261 \times 10^{-5}$  V<sup>-1</sup>), and  $x'_3$  is the  $dn/dc$  of cellulose in 0.5% LiCl/DMac ( $\pm 4\%$  error [14,25], variation limits: 0.0744–0.0805 ml g<sup>-1</sup>).

Eq. (1) shows that the parameter that most influenced the value  $DP_w$  CTC-UA was the attenuation factor  $D$  (highest

Table 2  
 $M_r$  averages, corresponding DP averages and polydispersity (PD) obtained for CTC (one sample, two analyses), DDC (three samples, seven analyses) and V (four samples, 12 measurements) of unaged cellulose (UA)

	$M_n$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_n$	$M_w$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_w$	$M_z$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_z$	$M_p$ ( $\times 10^{-5}$ g mol $^{-1}$ )	$M_v$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_v$	PD
SEC $_{CTC}^{smo}$ UA <sup>a</sup>	1.72	1062	4.03	2485	7.23	4462	2.92	3.80	2344	2.34
SEC $_{CTC}^{raw}$ UA <sup>a</sup>	2.38	1469	4.03	2485	—	—	—	—	—	1.69
SEC $_{DDC}$ UA	3.92 $\pm$ 0.32	2445 $\pm$ 206	6.64 $\pm$ 0.08	4127 $\pm$ 89	10.02 $\pm$ 0.44	6221 $\pm$ 305	6.24 $\pm$ 0.37	6.39 $\pm$ 0.09	3725 $\pm$ 588	1.69 $\pm$ 0.12
SEC $_{DDC}^{rec}$ UA	2.42 $\pm$ 0.15	910 $\pm$ 71	4.10 $\pm$ 0.15	1542 $\pm$ 55	6.19 $\pm$ 0.40	2327 $\pm$ 149	3.85 $\pm$ 0.20	3.95 $\pm$ 0.14	1484 $\pm$ 52	1.70 $\pm$ 0.13
V-UA	—	—	—	—	—	—	—	2.88 $\pm$ 0.18	1778 $\pm$ 111	—

<sup>a</sup> The values of the  $M_r$  averages for CTC are adjusted to the molar mass of an anhydroglucose monomer, i.e. 162 g mol $^{-1}$ .

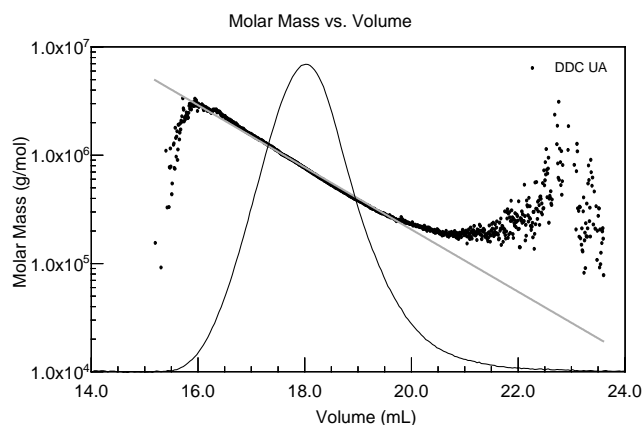


Fig. 4. Molar mass distribution across elution volume for cellulose of DDC-UA in 0.5% LiCl/DMAc.

coefficient). Eq. (2) shows that the  $dn/dc$  followed by the MALS constant were the two parameters that most influenced the value DP $_w$  DDC-UA. In the two DOEs,  $M_w$  CTC-UA varied within  $3.49 \times 10^5$  and  $4.74 \times 10^5$  g mol $^{-1}$ , that is  $\pm 13\%$ , and  $M_w$  DDC-UA within  $6.34 \times 10^5$  and  $7.28 \times 10^5$  g mol $^{-1}$ , i.e.  $\pm 6.5\%$ . These cumulated errors brought a maximal possible difference in  $M_w$ ,  $\Delta M_w$ , of about 40% ( $\pm 19.5\%$ ) between SEC $_{CTC}$  and SEC $_{DDC}$ . However, it is unlikely that all these statistical uncertainties be cumulated, and other possible error sources had to be investigated.

**3.1.1.2. Study of the discrepancies in the two SEC methods.** In order to account for  $\Delta M_w$  of 40% between DDC-UA and CTC-UA, two hypotheses, related to the derivatisation/dissolution processes, are proposed and investigated hereafter. The first is based on the procedure leading to CTC, which could degrade the cellulose molecules to a certain extent. The second is related to the complex chemistry involved during the dissolution of cellulose in LiCl/DMAc, possibly leading to an overestimation of  $M_w$ .

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

It is widely reported that degradation occurs upon derivatisation of cellulose to CTC depending on reaction time, temperature and co-reactant [29–31]. In the present study, the least degrading conditions as advocated by Lapierre and Bouchard [32] and by Lauriol et al. [21] and Lauriol [22] were used.

The differences between the values of  $M_n$ ,  $M_w$  and  $M_z$  of DDC-UA and CTC-UA ( $M_r$  DDC-UA– $M_r$  CTC-UA) followed the sequence  $\Delta M_n\% > \Delta M_p\% > \Delta M_w\% > \Delta M_z\%$ . Such sequence indicated that the number of lower  $M_r$  fractions is relatively smaller in the MMD of CTC-UA than in the MMD of the DDC-UA (understood that, low- and high- $M_r$  fractions are relative to each respective MMD). A possible explanation could be that these low- $M_r$  fractions are actually underestimated in the CTC.

At this point, it has to be noted that the programme CARB applies a smoothing equation based on a first order polynomial regression in the higher elution volume portion in order to readjust the values of  $M_n$ . The polynomial fit is aimed at correcting for axial diffusion because  $M_n$  is mostly affected by the lower signal to noise ratio of the LALS at the end of the elution (low- $M_r$  and low concentration) [21,22]. As a consequence while  $M_w$  remains unchanged,  $M_n$  decreases. Upon recalculations with the raw (not smoothed) data, PD of CTC-UA changed from 2.34 to 1.69, a value similar to that of DDC-UA, and  $M_n$  became  $2.38 \times 10^5 \text{ g mol}^{-1}$ , a 28% higher value than the smoothed  $M_n$  value, but still about 39% lower than the  $M_n$  value of DDC-UA. Smoothed data is further referred to as CTC<sub>smo</sub> and raw data as CTC<sub>raw</sub>. Thus, even using the raw data, compared to DDC, cellulose appears as if undergoing significant degradation during the derivatisation process to CTC, resulting in an overall decrease in the  $M_r$  averages.

**Hypothesis 2.** Overestimation of  $M_r$  averages of cellulose dissolved in LiCl/DMAc.

LiCl/DMAc has been widely applied for over three decades to dissolve cellulose and SEC is the preferred method to characterise cellulose/LiCl/DMAc systems. The solvent is reported to be non-degradative by most authors, and a recent article confirmed that the solutions prepared in the experimental conditions here reported were stable over periods of several months [23]. However, given the present findings, a possible overestimation of  $M_w$  of cellulose in LiCl/DMAc has to be considered. This could happen for instance through the formation of aggregates and/or the association of molecules. Another cause can be erroneous (underestimated) values of  $dn/dc$ . Indeed  $dn/dc$  can be influenced to a certain extent by the degree of dissolution of the sample (related with the degree of bonding achieved between solvent molecules and cellulose molecules) and by small variations of the LiCl concentration in the solvent [33]. The  $dn/dc$  of cellulose in LiCl/DMAc is difficult to determine, as shown by the wide variety of values found in the literature (0.057 [34], 0.091 [7], 0.104 [35], 0.108 [33], and 0.163  $\text{ml g}^{-1}$  [36]).

The  $dn/dc$  of cellulose in 0.5% LiCl/DMAc was experimentally determined at 37 °C with the Optilab DRI, and software DNDC (Wyatt Technologies Corp.) in a separate experiment [14,25], using a stock cellulose solution prepared from the same paper source and with the same activation/dissolution/dilution to 0.5% LiCl sequence as reported in Section 2.2.2.3. Dilutions from this stock solution were made (eight) and  $\Delta n$  for each different concentration was calculated from the change in voltage  $\Delta V$ , with  $\Delta n = \alpha \Delta V$ . The  $dn/dc$  was obtained from the gradient in the plot of  $\Delta n$  as a function of  $c$ . The average value thus obtained for the  $dn/dc$  of cellulose in 0.5% LiCl/DMAc over three separate experiments was  $0.077 \pm 0.003 \text{ ml g}^{-1}$ . This was considered the most accurate value to be used in the experiments.

Errors on  $M_w$  due to slightly varying  $dn/dc$  cannot be the cause of the large molar mass discrepancy observed, since in such case, the RSD for solutions prepared on different days with different solvent batches would in all likelihood be much larger than those found and reported in Table 2.

Several studies reported association of molecules and formation of aggregates in solutions of cellulose in LiCl/DMAc [7,37]. Röder et al. [38] showed that aggregation resulted from too low LiCl concentration (6%) or too high cellulose concentration (1%, w/w). Strlič et al. [39] also pointed the role of the salt in relation with aggregation and  $M_r$  determination, showing that upon diluting a solution of cellulose that had been dissolved in 8% LiCl/DMAc to SEC concentration, 1% LiCl in the injected sample resulted in larger  $M_r$  than 3% LiCl. Aggregates can also arise from the presence of water in the solvent system. It has been shown that as soon as more than two water molecules per LiCl molecule are present in solution, the concentration of the salt is not sufficient for a good dissolution of cellulose [40]. The effect of water was found to be more pronounced on dilute solutions (SEC concentration), where within one day, 0.05 M of water can lead to considerable increase in the measured hydrodynamic radius [41]. Also, depending on the pulp type, concentration of water between 0.01 M and 0.05 M can disturb a thermodynamically good solution and promote aggregation [42].

However, it has to be noted that aside from aggregation, degradation of cellulose is a possible consequence of the dissolution process in LiCl/DMAc. Potthast et al. [41,43,44] showed that cellulose undergoes depolymerisation upon prolonged periods in heated 9% LiCl/DMAc, via highly reactive molecules formed when heating the DMAc above 80 °C. However, such degradation was excluded in the present study, as activation of the cellulose was not carried out at high temperature. It has also been recently reported that hydrolysis of cellulose can happen during the activation in distilled water with papers that have an acidic cold extract pH (below 5.6) [45]. This should not occur with Whatman No. 1 paper unaged: its cold extract pH (measured using the TAPPI standard method T 509 om-88 [46]) was 7.01. This assumption holds also for the aged paper for which the cold extract pH was 6.47.

The presence of aggregates can be detected in MALS, in the photodiodes signals in the high- $M_r$  end (small  $V_e$ ), especially in the high degree angles, and also by inconsistent slopes in the MMD versus  $V_e$  [47]. The chromatograms obtained in the present experiment showed monomodal LS signals at all measuring angles, and consistent regular slopes similar to that on Fig. 2. Thus, no evidence of aggregation was found. Moreover, the present experimental conditions were the most favourable for a total dissolution with no aggregation or association as advocated in [38]. Besides, the solvent was carefully prepared in order to minimise the presence of water, and the detailed study of the conformation of cellulose in 0.5% LiCl/DMAc, reported in a separate work, showed that the molecules adopted a random coil conformation, proving that LiCl/DMAc was a good solvent [14,25].

The explanation for a possible overestimate of the  $M_r$  of cellulose using the method SEC<sub>DDC</sub> has to be found elsewhere. To this purpose, the chemistry of cellulose dissolved in LiCl/DMAc was investigated. The solvation mechanism has been thoroughly studied in the past, and several models were proposed by different authors, which are reviewed in [48–50] and more recently in [51]. All models are based on the special structure of the ion pair  $[\text{Li}(\text{DMAc})_n^+ \text{Cl}^-]$  and its capacity of forming a complex structure with cellulose. A major role is thought to be played by  $\text{Cl}^-$  breaking up the inter- and intramolecular hydrogen bonds and complexing the three hydroxyl groups of an anhydroglucose unit (AGU) by hydrogen bonding, while the counterpart of the solvent complex, the  $\text{Li}(\text{DMAc})_n^+$  macrocation, would be more loosely bound [34]. Concerning which part of the ion pair, the anionic or the cationic moiety drives the dominant force in the process, some theories attribute an equally important role to both [52–54], while other models consider that the determining action is played by the anionic moiety [34,55,56]. In a recent publication Spange et al. [57] have determined that the interaction  $\text{Cl}^-$ –cellulose contributes approximately 80% to the dipole–dipole interaction between DMAc and cellulose, whereas the specific  $\text{Li}(\text{DMAc})_n^+$ –cellulose interaction only about 10%. Based on this latest approach, Striegel recently proposed a dissolution mechanism, where  $\text{Cl}^-$  creates hydrogen bond-type interactions with the hydroxyl groups of cellulose,  $\text{Li}^+$  interacts with the carbonyl oxygens of DMAc, and the  $\text{Li}(\text{DMAc})_n^+$  macrocation creates weak interactions with both the hydroxyl oxygens and the ring oxygens of an AGU, which in a simplified form can be written  $[\text{CelloHCl}]^- [\text{DMAc}_n\text{Li}]^+$  [51]. According to this most recent model, and considering an optimally solvated cellulose, the presence of three  $\text{Cl}^-$  bound to the three hydroxyls of one AGU would considerably increase the resulting apparent  $M_r$  by 39%, from 162 to  $266 \text{ g mol}^{-1}$ . Even if the actual AGU  $M_r$  is most likely slightly below this value, as full solvation may not occur on each AGU, this hypothesis would explain most of the difference in  $M_w$  between the two SEC methods, since the consequence on LS data would be the yield of an apparent larger  $M_w$ .

In support of this hypothesis, in each analysis the theoretical values of the injected masses of the DDC (calculated as the product of the sample concentration by the injected volume) were found systematically lower than the values computed by ASTRA (calculated on the basis of  $\alpha$  constant and  $dn/dc$  pre-determined known values) by 42–45%. This mass recovery discrepancy occurred not only with Whatman No. 1 paper samples, but also in about the same proportion with other types of papers with different  $M_w$  range, for which optimal dissolution was attained (no fibres residue) and that were analysed in the same conditions. These included four linen and cotton rag papers from 18th century ( $M_w$  from  $2.6 \times 10^5$  to  $6.45 \times 10^5 \text{ g mol}^{-1}$ ) and four bleached softwood chemical pulp papers ( $M_w$  from  $4.74 \times 10^5$  to  $5.37 \times 10^5 \text{ g mol}^{-1}$ ) [14]. This large difference

can be explained by the increase in the actual mass of the AGU consequent to the solvation and the complexation with the chloride anions. It must be kept in mind that the chemical mechanism has not been fully proven yet, and it cannot be stressed enough that the hypothesis holds only if there is indeed formation of hydrogen bonding between the cellulose and the chloride ions, since a simple solvation layer, which has a refractive index very close to that of the solvent, would be invisible to the MALS detector.

The values of  $M_r$  DDC-UA were re-calculated using apparent  $M_r$  AGU =  $266 \text{ g mol}^{-1}$ . This was achieved by calculating a new injected mass: the mass computed by the ASTRA software decreased by 39%.  $M_w$  was then re-calculated by the software using  $\alpha$  constant and assuming 100% mass recovery (new—decreased—injected mass value) as known variables (instead of known  $\alpha$  and known  $dn/dc$ ). The re-calculated injected mass of the samples matched quite closely the supposed injected mass, calculated as sample concentration times injected volume. The resulting average  $M_r$  were:  $M_n = (2.42 \pm 0.15) \times 10^5 \text{ g mol}^{-1}$ ,  $M_w = (4.10 \pm 0.15) \times 10^5 \text{ g mol}^{-1}$ , and  $M_z = (6.19 \pm 0.40) \times 10^5 \text{ g mol}^{-1}$ , with the same polydispersity of 1.70 ( $\pm 0.13$ ) as before (Table 2). Under these conditions, the value of  $dn/dc$  of cellulose in 0.5% LiCl/DMAc computed by ASTRA was of  $0.126 \text{ ml g}^{-1}$ . Consequently, we can suppose that this  $dn/dc$  value would be a more accurate value of an “ideally bare” cellulose in the solvent, while the value previously experimentally determined of  $0.077 \text{ ml g}^{-1}$  would be the accurate  $dn/dc$  of cellulose complexed with LiCl/DMAc.

It is noteworthy that these re-calculated values of  $M_r$  averages for DDC-UA—which are to be considered as bearing the maximal possible correction—resulted in a significant narrowing of the difference between DDC-UA and CTC-UA with:  $\Delta M_n = 1.6\%$  (using  $\text{CTC}_{\text{raw}}$ ),  $\Delta M_w = 1.7\%$ , and  $\Delta M_z = -14\%$ . This would show that the procedure leading to CTC is not as degrading as suggested earlier. DDC with the re-calculated  $M_r$  are later on referred to as DDC<sub>rec</sub>, and DDC with the normal  $M_r$  as DDC. Fig. 5 shows the differential molar mass graphs of DDC<sub>rec</sub>-UA compared to CTC-UA and DDC-UA.

### 3.1.2. Viscometry of UA

Table 2 reports the average value obtained for  $M_v$  of unaged paper dissolved in cadoxen (V-UA). This value was considerably smaller than the values obtained for  $M_w$  with both SEC<sub>DDC</sub> and SEC<sub>CTC</sub>, by 30% compared to  $M_w$  DDC<sub>rec</sub>-UA (57% compared to  $M_w$  DDC-UA) and 28% compared to  $M_w$  CTC-UA. A difference in  $M_r$  is often reported in the literature when comparing viscometry values (in CED) with SEC values with LS detection (CTC [22] and DDC [8,37]). But it has to be noted that CED is more aggressive to cellulose than cadoxen, which is the reason why cadoxen was chosen for this study [58–61].

In order to accurately compare viscometry and SEC, it is important to compare the same  $M_r$  average, that is  $M_v$ .



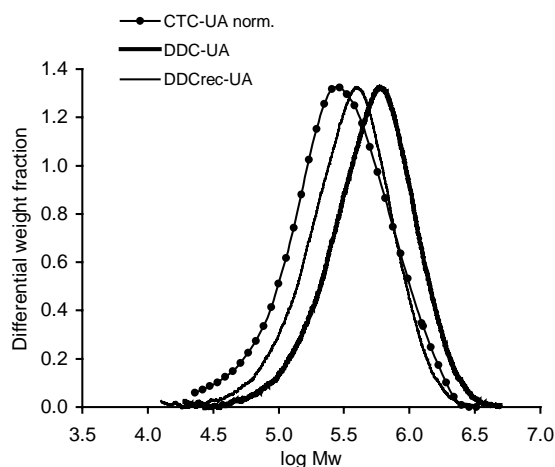


Fig. 5. Overlaid differential molar mass graphs of  $DDC_{rec}$ -UA compared to DDC-UA and CTC-UA (normalised to DDC-UA).

Provided that the MHS coefficient  $a$  is known,  $M_v$  can be obtained with SEC, with  $M_v = [\sum n_i M_i^{1+a} / \sum n_i M_i]^{1/a}$ , where  $n_i$  is the number of molecules with molar mass  $M_i$ .

The coefficient  $a$  was calculated, according to  $a = 3q - 1$  [62]. The scaling factor  $q$  in  $\langle r_g^2 \rangle^{1/2} = QM_r^q$  (where  $\langle r_g^2 \rangle^{1/2}$  is the root mean square radius) was experimentally determined at constant 60 °C using the seven separate analyses of DDC-UA [14,25]. The values of  $a$  for cellulose of Whatman No. 1 paper in 0.5% LiCl/DMAc were found comprised between 0.77 and 0.86 (average of 0.81, RSD = 5%) [14,25].

$M_v$  DDC-UA and  $M_v$   $DDC_{rec}$ -UA were thus calculated using  $a = 0.81$ , and  $M_v$  CTC-UA using the literature value  $a = 0.84$  [63,64] (the  $SEC_{CTC}$  method did not allow its calculation). The values obtained were  $M_v$  DDC-UA =  $6.39 \times 10^5$  g mol<sup>-1</sup> (with a minute error of  $\pm 6.8 \times 10^3$  considering the RSD on  $a$ ),  $M_v$   $DDC_{rec}$ -UA =  $3.95 \times 10^5$  g mol<sup>-1</sup>, and  $M_v$  CTC-UA =  $3.80 \times 10^5$  g mol<sup>-1</sup> (Table 2), which, as expected, are rather close to respective  $M_w$ . Still,  $M_v$  V-UA was considerably smaller than  $M_v$  calculated for DDC-UA (by 55%),  $DDC_{rec}$ -UA (by 27%), and CTC-UA (by 24%). As the viscometry method yields RSD of 1.5% on  $M_v$  [16], the most likely explanation retained for the considerable underestimation of  $M_v$  using viscometry in cadoxen, is solvent-induced degradation.

### 3.2. Aged cellulose (At<sub>94</sub>)

#### 3.2.1. SEC of At<sub>94</sub>

$SEC_{CTC}$  and  $SEC_{DDC}$  differential molar mass graphs of cellulose from aged papers (At<sub>94</sub>) are represented in Fig. 6.

The values of  $M_r$  averages obtained with  $SEC_{CTC}$  (CTC<sub>smo</sub> and CTC<sub>raw</sub>) and  $SEC_{DDC}$  for the papers aged 94 days are reported in Table 3. Compared to DDC-At<sub>94</sub>, CTC-At<sub>94</sub> yielded lower  $M_r$  values, by 15% for  $M_w$  and by 9% for  $M_n$  CTC<sub>raw</sub>-At<sub>94</sub> (40% for  $M_n$  CTC<sub>smo</sub>-At<sub>94</sub>).  $M_z$  and PD of CTC<sub>raw</sub>-At<sub>94</sub> and DDC-At<sub>94</sub> were similar. As observed with UA, it seems also more appropriate with

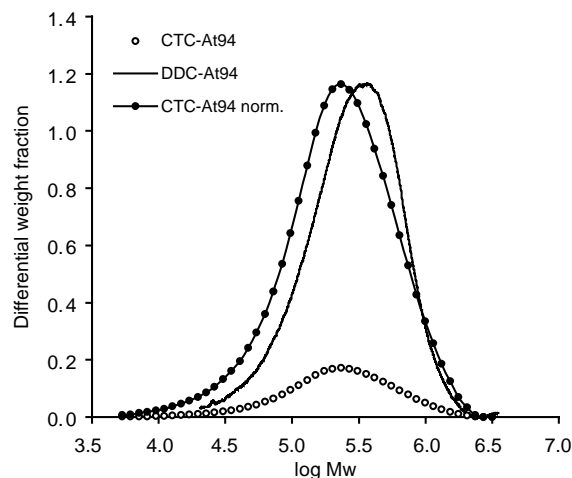


Fig. 6. Overlaid differential molar mass graphs of DDC-At<sub>94</sub>, CTC-At<sub>94</sub>, and CTC-At<sub>94</sub> norm. “Norm” refers to the normalisation of CTC-At<sub>94</sub> to DDC-At<sub>94</sub> using a correction factor of 6.78 (=1.166/0.172), corresponding to the wt. frt. at  $M_p$  of DDC-At<sub>94</sub> over the wt. frt. at  $M_p$  of CTC-At<sub>94</sub> (with  $M_p$  DDC-At<sub>94</sub> =  $3.5 \times 10^5$  g mol<sup>-1</sup> and  $M_p$  CTC-At<sub>94</sub> =  $2.3 \times 10^5$  g mol<sup>-1</sup>).

cellulose aged to use the values of CTC<sub>raw</sub> rather than those of CTC<sub>smo</sub> in the comparative evaluation with DDC.

The values of the  $M_r$  averages of DDC after recalculation considering the 39% mass increment correction,  $DDC_{rec}$ -At<sub>94</sub> were  $M_n = (1.26 \pm 0.10) \times 10^5$  g mol<sup>-1</sup>,  $M_w = (2.33 \pm 0.08) \times 10^5$  g mol<sup>-1</sup>, and  $M_z = (3.70 \pm 0.21) \times 10^5$  g mol<sup>-1</sup>. These values were smaller than those of CTC<sub>raw</sub>-At<sub>94</sub> by 33% for  $M_n$ , 28% for  $M_w$  and 39% for  $M_z$ . Here again, with  $\alpha$  constant and assuming 100% mass recovery (new—decreased—injecting mass value) as known variables, the value of  $dn/dc$  of cellulose in 0.5% LiCl/DMAc computed by ASTRA was 0.126 ml g<sup>-1</sup>. Fig. 7 shows the differential molar mass graph of  $DDC_{rec}$ -At<sub>94</sub>, DDC-At<sub>94</sub> and CTC<sub>raw</sub>-At<sub>94</sub>. It is reminded that the 39% mass increment in  $DDC_{rec}$  is based on the assumption of the availability of the three hydroxyl groups on each AGU. On a chain of cellulose aged, the number of hydroxyl groups is most probably lower, due to oxidation consecutive to the aging process. Therefore, the average number of Cl<sup>-</sup> per AGU of At<sub>94</sub> is in all likelihood less than three. Moreover, charge repulsion with the Cl<sup>-</sup> limiting the complexation, can also occur with negatively charged oxidised groups. Thus, the average apparent  $M_r$  of the AGU of aged cellulose in LiCl/DMAc is believed to be less than 266 g mol<sup>-1</sup>. The study of the conformational characteristics of cellulose in solution previously carried out by the authors indeed proved that aged cellulose was less well solvated than unaged cellulose [14,25]. Similarly, the (experimental and re-calculated)  $dn/dc$  values for DDC-At<sub>94</sub> could result slightly erroneous. All these reasons partly explain the lower values of  $DDC_{rec}$ -At<sub>94</sub> compared to CTC<sub>raw</sub>-At<sub>94</sub>.

A mention has to be made also of the impact of the defibrillation of the paper, since for practical reasons, it could

Table 3

$M_r$  averages, corresponding DP averages and polydispersity (PD) obtained for CTC (one sample, six analyses), DDC (two sample, six analyses) and V (three samples, nine measurements) of aged cellulose (At94)

	$M_n$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_n$	$M_w$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_w$	$M_z$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_z$	$M_p$ ( $\times 10^{-5}$ g mol $^{-1}$ )	$M_v$ ( $\times 10^{-5}$ g mol $^{-1}$ )	DP $_v$	PD
SEC $_{CTC_{smo}}$ At94 <sup>a</sup>	1.25	770	3.24	1997	6.07	3749	2.34	3.04	1876	2.59
SEC $_{CTC_{rw}}$ At94 <sup>a</sup>	1.88	1158	3.24	1997	—	—	—	—	—	1.72
SEC $_{DDC}$ At94	2.07 $\pm$ 0.16	1275 $\pm$ 97	3.81 $\pm$ 0.13	2354 $\pm$ 82	6.07 $\pm$ 0.34	3748 $\pm$ 209	3.52 $\pm$ 0.21	3.65 $\pm$ 0.13	2253 $\pm$ 78	1.85 $\pm$ 0.13
SEC $_{DDC_{rec}}$ At94	1.26 $\pm$ 0.10	474 $\pm$ 36	2.33 $\pm$ 0.08	875 $\pm$ 30	3.70 $\pm$ 0.21	1392 $\pm$ 78	2.14 $\pm$ 0.13	2.23 $\pm$ 0.08	837 $\pm$ 29	1.85 $\pm$ 0.13
V-At94	—	—	—	—	—	—	—	1.77 $\pm$ 0.19	1092 $\pm$ 117	—

<sup>a</sup> The values of the  $M_r$  averages for CTC are adjusted to the molar mass of an anhydroglucose monomer, i.e. 162 g mol $^{-1}$ .

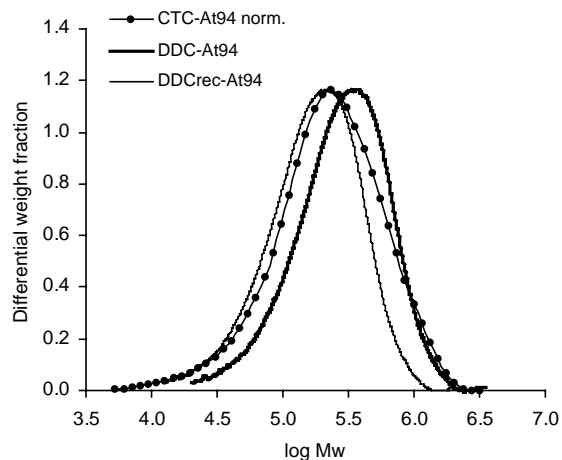


Fig. 7. Overlaid differential molar mass graphs of  $DDC_{rec}$ -At94 compared to  $DDC$ -At94 and  $CTC$ -At94 (normalised to  $DDC$ -At94).

not be carried out by the same means for the preparation of the paper for CTC and for DDC. The hammer milling used for CTC has been reported to lead to the formation of carbonyl groups on the cellulose, especially ketons [65], which is probably due to the rise in temperature during the process. Although it has never been investigated, the cutting mill used for preparing the DDC most likely has a similar effect. Moreover, while aged and unaged samples in each respective method were prepared in the same manner, regardless of the type of defibrillation, a larger detrimental effect on the aged papers compared to the unaged papers cannot be ruled out, as the former are more degraded to start with.

These results show that, in the case of aged cellulose, the discrepancies in the  $M_r$  averages obtained with the two SEC methods cannot be interpreted with certainty, and that it is extremely difficult to draw conclusions about the respective performance of the two SEC procedures. Fig. 8 shows overlaid differential molar mass graphs of aged and unaged CTC and DDC in order to better visualise the smaller shift towards

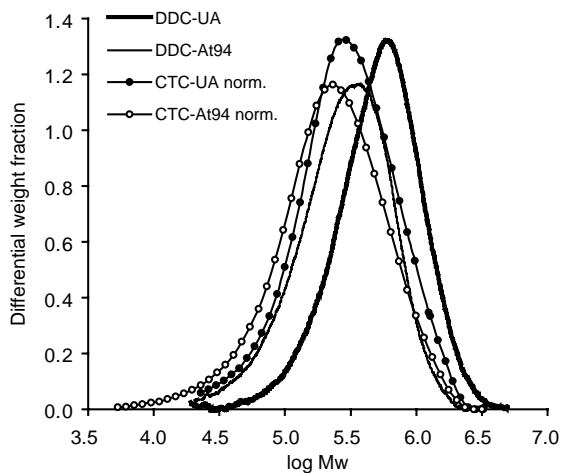


Fig. 8. Overlaid differential molar mass graphs of aged and unaged DDC and CTC (normalised to DDC using same factors as in Figs. 3 and 6).

Table 4  
Percentage difference in  $M_r$  averages and in polydispersity (PD) between unaged and aged samples

	$\Delta M_n$ (%)	$\Delta M_w$ (%)	$\Delta M_z$ (%)	$\Delta M_p$ (%)	$\Delta M_v$ (%)	$\Delta PD$ (%)
CTC <sub>raw</sub> -UA-CTC <sub>raw</sub> -At <sub>94</sub>	21	20	16	20	20	-10
CTC <sub>smo</sub> -UA-CTC <sub>smo</sub> -At <sub>94</sub>	27	20	16	20	20	-2
DDC-UA-DDC-At <sub>94</sub>	47	43	39	44	43	-9
DDC <sub>rec</sub> -UA-DDC <sub>rec</sub> -At <sub>94</sub>	48	43	40	44	43	-8
V-UA-V-At <sub>94</sub>					38.5	

low- $M_r$  between DDC-At<sub>94</sub> and CTC-At<sub>94</sub> compared to the shift between DDC-UA and CTC-UA. Table 3 reports the percent difference in the  $M_r$  averages of cellulose between aged and unaged paper according to the relevant method.

Comparisons within each method between aged and unaged cellulose can be more easily drawn. Whether using DDC or DDC<sub>rec</sub>, the difference in  $M_r$  averages between aged and unaged cellulose as characterised by SEC<sub>DDC</sub> resulted in the sequence  $\Delta M_n\% > \Delta M_w\% > \Delta M_z\%$ . This indicated a mostly random cleavage process of the molecule, but some preferential production of low- $M_r$  fractions, and was consistent with the slight increase of 8% in the PD of DDC-At<sub>94</sub> versus DDC-UA. For CTC, the decrease in  $M_r$  averages between UA and At<sub>94</sub> followed the sequence  $\Delta M_n\%$  CTC<sub>smo</sub>  $>$   $\Delta M_n\%$  CTC<sub>raw</sub>  $\approx$   $\Delta M_w\% > \Delta M_z\%$ , but in halved proportions compared to DDC (Table 4).

In summary, 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  $M_z$ .  $M_w$ , the  $M_r$  average directly measured by LS measurements, decreased between unaged and aged papers by 20% for CTC and by 43% for DDC. Thus, regardless of the method used, the estimation of the extent of the degradation upon aging is significantly different. Whether this percentage is overestimated in the case of DDC due to a different degree of complexation cellulose–solvent depending on the degradation state of the polymer, or underestimated in the case of the CTC due to an enhanced degradation of the unaged versus the aged paper during the derivatisation could not be totally elucidated here. Most likely both phenomena are involved, but previous research showing that the preparation of CTC degrades preferentially high- $M_r$  cellulose [29] tends to corroborate that tricarbanilation would indeed be more aggressive towards unaged cellulose than towards aged cellulose.

### 3.2.2. Viscometry of At<sub>94</sub>

Viscometry in cadoxen of aged cellulose resulted in underestimated values of  $M_v$ .  $M_v$  V-At<sub>94</sub> was 42% lower than  $M_v$  CTC-At<sub>94</sub>, 53% lower than  $M_v$  DDC-At<sub>94</sub> and 21% lower than  $M_v$  DDC<sub>rec</sub>-At<sub>94</sub> (Table 3), as calculated with  $a = 0.81$  for DDC, and  $a = 0.84$  for CTC (see Section 3.1.2).  $\Delta M_v$  between V-UA and V-At<sub>94</sub> was 38.5%. This value was intermediate between the  $\Delta M_v$  upon aging of DDC and CTC, 43 and 20%, respectively (Table 3).

Compared to SEC, viscometry in cadoxen underestimated the values of  $M_v$ . 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 not significantly aggressive for cellulose, this is contrary to the present findings.

## 4. Conclusion

This study shows the complexity of a comparative evaluation of methods involving a significant number of parameters. According to the results, and considering the discrepancies in the  $M_r$  averages, it was not totally clear whether the derivatisation of cellulose to tricarbonylates was responsible of a significant degradation of the polymer, or if the method of dissolution in LiCl/DMAc led to an overestimation of the  $M_r$ . It is most likely that both these phenomena are involved to a certain extent in order to account for a 40% discrepancy on  $M_w$  of cellulose from unaged paper between the two SEC methods. However, the hypothesis put forward in the present work, which is supported by past and recent literature, and by the experimental results, of a complexation between the hydroxyl groups of cellulose and the anionic moiety of DMAc/LiCl, thus leading to a significant increase in the apparent  $M_r$  of the AGU, explains most of the mass discrepancy between the two SEC analysis methods. This supposed complexation also would result in different experimental and theoretical values for  $dn/dc$  of cellulose in LiCl/DMAc. Despite the widespread use of LiCl/DMAc as solvent for cellulose, and numerous studies and reviews that have focused on the solvation mechanism over the past twenty years, the molecular structure of the complex formed between cellulose and LiCl/DMAc is still not fully elucidated, a particularly relevant point in the determination of an exact molar mass value using light scattering.

For degraded cellulose, the  $M_r$  averages obtained with both SEC methods were closer using the raw values than applying a 39% correction on the weight of the AGU unit. The difference with unaged cellulose would be a poorer solvation and less efficient complexation by chloride anions in the case of aged cellulose bearing oxidised units, yielding an undefined but significantly lower mass increment. This confirms that it is indeed difficult to conclude on the best SEC method to analyse cellulose, as both have their advantages and drawbacks, depending also on the initial degradation state of the cellulose substrate. Viscometry in cadoxen, on the other hand, was found to be the method that most

underestimated the  $M_r$  of aged as well as unaged cellulose, which was attributed to solvent-induced degradation.

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