Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 58 (2004) 233–243
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Conformation and d*n*/d*c* determination of cellulose in *N,N*-dimethylacetamide containing lithium chloride

Anne-Laurence Dupont[a,*], Gabrielle Harrison[b]

[a]*Centre de recherches sur la conservation des documents graphiques, Centre national de la recherche scientifique, FRE 2743, Ministère de la culture et de la communication, Muséum national d'histoire naturelle, 36 rue Geoffroy-Saint-Hilaire, 75005 Paris, France*
[b]*Preservation Research and Testing Division, Library of Congress, 101 Independence Ave, SE, Washington, DC 20540, USA*

Received 6 September 2003; revised 9 February 2004; accepted 24 March 2004
Available online 11 September 2004

Abstract

In order to characterise cellulose dissolved in lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) using size-exclusion chromatography with online multiangle laser light scattering and differential refractive index detection, a number of parameters were determined. One of them in particular, the specific refractive index increment (d*n*/d*c*) of cellulose in 0.5% LiCl/DMAc, is required in order to calculate the molar mass from the light scattering signal. Pure cellulose paper was used as sample source. The precision and reproducibility of the SEC/MALS/DRI method were evaluated. Molar mass and root mean square radii averages, molar mass distribution as well as conformation of cellulose in 0.5% LiCl/DMAc, from both unaged and artificially aged papers, were studied. The latter was determined to be random coil for cellulose and vary slightly with the polymer molar mass.

Keywords: Cellulose; d*n*/d*c*; Lithium chloride/*N,N*-dimethylacetamide; Polymer conformation; Size-exclusion chromatography; Multiangle light scattering

1. Introduction

Size-exclusion chromatography (SEC) is the technique of choice to evaluate composition and molar mass distribution (MMD) of polymers. Extremely sensitive to early changes, SEC has been widely used to characterise cellulose and monitor its degradation in the pulp and paper industry, and more recently the technique has been applied in the field of paper conservation science (Dupont, 2003a; Jerosch, 2002).

For a biopolymer such as cellulose, which has limited solubility in most common solvents used in chromatography, the polymer solubility/system compatibility (columns packing material) is not a simple issue. Derivatisation can be a solution but direct dissolution offers a better alternative being faster, easier and more reproducible. Solvent systems such as lithium chloride/*N,N*-dimethylacetamide (LiCl/DMAc) gained considerable popularity since they were first described to dissolve cellulose, by McCormick (1981) and by Turbak (1981). Moreover, the possible use of LiCl/DMAc as SEC mobile phase with column packing such as poly(styrene-divinyl benzene) (PSDVB) simplifies the procedure. A review article by Strlič and Kolar (2003) summarises the research in the field of SEC of cellulose in LiCl/DMAc since it was carried out for the first time (Ekmanis & Turbak, 1986; Ekmanis, 1987).

The work here-described constituted a preliminary step in the study of the degradation upon time of cellulose from historic and modern papers of diverse origins. Recent publications give a review of existing dissolution methods of cellulose in LiCl/DMAc (Dupont, 2003a,b). The development of the dissolution method of paper for specific applications to historic prints and archival documents, where naturally and artificially aged paper were chosen as the source for cellulose, as well as the non-aggressiveness of the LiCl/DMAc solvent towards cellulose are also reported in the aforementioned publications.

SEC coupled with multiangle light scattering (MALS) and differential refractive index (DRI) detectors was used in

* Corresponding author. Tel.: +33-14408-6992; fax: +33-14707-6295.
E-mail address: aldupont@mnhn.fr (A.-L. Dupont).

0144-8617/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2004.07.016

the characterisation of cellulose of these papers. Precision and reproducibility of the method were evaluated for validation. In order to calculate absolute values for the molar mass (M_r) averages and dimensional parameters, such as the root mean square (rms) radii ($\langle r_g^2\rangle^{1/2}$) averages, the specific refractive index increment dn/dc of cellulose in the solvent and mobile phase used, 0.5% LiCl/DMAc, needs to be known. It appeared necessary to experimentally determine this parameter since rather different values were found in the literature. The conformation of cellulose in 0.5% LiCl/DMAc was characterised, which provided information on the solvation efficiency of the solvent system. It should be stressed that, with this study being carried out at SEC concentration, the results are representative of the properties of dilute cellulose solutions in low salt concentration LiCl/DMAc, and may differ from the properties of concentrated polymer solutions in higher ionic strength solvent.

Background theory on light scattering can be found in reference work (Flory, 1953; Wyatt, 1993). Briefly, the Rayleigh-Debye-Gans model for dilute polymers (Zimm formalism), which includes both intermolecular and intramolecular effects, embodies the principles of light scattering (Eq. (1)).

$$\frac{K^* c}{R_\theta} = \frac{1}{M_w P_\theta} + 2A_2 c \tag{1}$$

where R_θ (cm^{-1}) is the Rayleigh ratio, A_2 (mol cm^3 g^{-2}) the second virial coefficient, c (g cm^{-3}) the concentration of the solute molecules, P_θ the form factor (also called particle scattering factor), a function of the mass distribution inside the molecule (defined in Eq. (2)), and K^* an optical parameter related to the polymer in the solvent (defined in Eq. (3)).

$$\frac{1}{P_\theta} = 1 + \frac{16\pi^2}{3\lambda^2}\langle r_g^2\rangle \sin^2\left(\frac{\theta}{2}\right) + \cdots \tag{2}$$

where λ (cm) is the measuring wavelength.

$$K^* = 4\pi^2 (\mathrm{d}n/\mathrm{d}c)^2 n_0^2 N^{-1} \lambda_0^{-4} \tag{3}$$

where n_0 is the refractive index of the solvent, N (mol^{-1}) is Avogadro's number, and λ_0 (cm) is the vacuum wavelength of the incident light.

In SEC/MALS/DRI, the unknown parameters are M_w, $\langle r_g^2\rangle$ and A_2. The dn/dc needs to be accurately measured or otherwise obtained from the literature. In SEC, with polydisperse polymers, each slice of a peak can be considered as having constant M_w and c. Debye plots, represented by the function

$$\frac{K^* c}{R_\theta} = f\left(\sin^2\left(\frac{\theta}{2}\right)\right)$$

(Wyatt, 1993), can be built for each data slice in the chromatogram. These plots yield $1/M_w$ (intercept) and $\langle r_g^2\rangle$ (gradient) and can be constructed using Zimm, Debye, or Berry detector fit methods, which correspond to mathematical transformations of the same function. Zimm formalism ($K^* c/R_\theta$ as a function of $\sin^2(\theta/2)$) is most widely used for mid-sized polymers (rms radius 10–100 nm) and was found the most appropriate fit in the present study.

2. Experimental

2.1. Sample preparation

The paper used as cellulose source was Whatman No.1 (pure cellulose). Papers were artificially aged at 80 °C and 50% relative humidity (rH) by suspending the sheets individually in a climate chamber Versatenn (Tenney Environmental, Parsippany, NJ, USA) for thirty-five and ninety-four days. These samples were labelled Wt_{35} and Wt_{94}, respectively. The unaged samples were designated as Wt_0.

Two to 2.5 g of paper were sampled from different areas in separate sheets and were defibrillated by dry-milling in a two-blade cutting mill for 5 min. The defibrillated samples were placed in a controlled environment chamber at 50% relative humidity (rH), 23 °C (T 412 om-94, TAPPI test method) to equilibrate for at least 2 days in order to achieve reproducible moisture content. About 5×10^{-2} g ($\pm$0.02%) was used for activation/dissolution. These samples were subjected to an activation treatment of the cellulose substrate by solvent exchange, which consisted of two consecutive thorough swellings in 10 ml deionised water at 40 °C (milli-Q, Millipore, Bedford, MA, USA) of 1 h each, followed by two consecutive exchanges of 45 min each with 8 ml methanol (Acros Organics, Springfield, NJ, USA), and ending with two consecutive exchanges with 8 ml DMAc (Acros Organics). Anhydrous DMAc, carefully dried with aluminium sodium silicate molecular sieve, 0.4 nm effective pore size (Fisher Scientific, Springfield, NJ, USA), was used for activation, and for the preparation of the dissolution solvent and SEC mobile phase. 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 μm pore polytetrafluoroethylene (PTFE) filters Millex LCR (Millipore). After the last DMAc exchange, 5 ml of 8% LiCl/DMAc (dissolution solvent), were added to the fibres. Since it is highly hygroscopic, LiCl (Acros Organics) was oven-dried and kept in the desiccator in pre-weighted aliquots prior to use. The samples were stirred at room temperature for 24 h, and placed at 4 °C until complete dissolution, which took from 2 to 4 days. The concentration of cellulose in 8% LiCl/DMAc was about 10 mg ml^{-1}, i.e. 1% (wt/v). Details on the study that led to this activation/dissolution process, as well as referenced related work by previous researchers on LiCl/DMAc as a solvent for cellulose can be found in Dupont (2003a,b).

For SEC/MALS/DRI analysis, each solution was diluted to 0.5% LiCl/DMAc, i.e. to a concentration of about 0.625 mg ml^{-1} cellulose (0.0625% wt/v), and filtered through a 0.5 μm pore filter Millex LCR (Millipore) before injection. This dilute concentration was chosen as a good compromise between a reasonable viscosity and ionic strength of the mobile phase, and the need of staying within the optimal range of concentration of cellulose for SEC analysis, while maintaining the polymer in solution.

2.2. SEC/MALS/DRI instrumental set-up

The SEC set-up consisted of a four-channels HPLC solvent degasser Degassit™ (Metachem Technologies Int., Torrance, CA, USA), HP 1100 isocratic pump G1310A (Agilent Technologies, Palo Alto, CA, USA), and manual injector (model 7725i, Rheodyne L.P., Cotati, CA, USA) with a 100 μl loop. The precision of the flow delivery system was within less than 0.3% relative standard deviation (RSD) (typically 0.15% based on retention time at 1 ml min^{-1} flow rate). An on-line membrane 0.22 μm 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 detector (MALS) Dawn EOS (Wyatt Technologies Corp., Santa Barbara, CA, USA) equipped with heated/cooled Peltier option (−25 to +85 °C ± 0.2 °C), and interferometric differential refractometer (DRI) Optilab DSP (Wyatt Technologies Corp.) with internal temperature control (25–80 °C ± 0.005 °C). The laser source of the MALS has a nominal power of 25 mW (23.5 mW effective), and operates at 690 nm. The light source of the DRI emits also at 690 nm. The interdetector delay volume was 0.150 ml. Constants of the instruments were determined as 6.071×10^{-6} for the Dawn EOS, and 2.256×10^{-4} V^{-1} for the Optilab DSP (α constant) (Dupont, 2003a). Since the gain of the Optilab DSP was set to 10, the actual working α constant was therefore adjusted to 2.256×10^{-5} V^{-1}.

2.3. Separation

The separation was carried out on a set of three *LD* 300 × 7.5 mm MIXED-B pores poly(styrene-divinyl benzene) (PSDVB) columns packed with 10 μm particle diameter (Polymer Laboratories Inc., Amherst, MA, USA), preceded by a *LD* 50 × 7.5 mm PSDVB guard column, 10 μm particle diameter (Polymer Laboratories Inc.). The columns show a linear separation in the range 500–10^7 g mol^{-1}. The columns were placed in a thermostatted column compartment and the system was operated at 60 °C at a flow rate of 1 ml min^{-1}. This temperature was chosen as it provided a reasonable column backpressure between 65 and 69 bars and particularly, to limit the thermal degradation of the polymer as much as possible. The mobile phase, 0.5% LiCl/DMAc, was filtered and vacuum-degassed through 0.5 μm pore filters Millex LCR (Millipore) before use, and was kept at 55–56 °C under low stirring in a heating/stirring unit (Pierce, Rockford, IL, USA) during the separation. Run time was 40 min. The data acquisition was carried out in 0.5 s intervals with ASTRA software v. 4.73.04 (Wyatt Technology Corp.).

For unaged papers, three separate dissolutions were carried out (on different days, with different solvent batches). Each solution was analysed in duplicate or triplicate runs non-consecutively, for a total of 7 separate analyses. For aged paper, four samples each of Wt_{35} and Wt_{94} were dissolved and analysed also in duplicate or triplicate for a total of 8 analyses each.

2.4. dn/dc of cellulose in 0.5% LiCl/DMAc

The d*n*/d*c* of cellulose in dilute solution in 0.5% LiCl/DMAc was measured off-line with the Optilab DSP. The experiments proceeded at constant temperature (37 °C), at a flow rate of 0.2 ml min^{-1} with a manual injector (model 7725i, Rheodyne L.P.) connected directly to the DRI detector, and an injection loop of 500 μL. As the gain was set to 1, the DRI constant α used was 2.256×10^{-4} V^{-1}.

The fibres were first dried in a desiccator over drierite (Fisher Scientific Springfield, NJ, USA) for several days, and weighed in dry state in order to avoid errors on the mass due to the moisture content. A solution of 1.6% cellulose in 8% LiCl/DMAc was prepared by dissolving 49.3 mg of sample in 3.125 ml of solvent, following the same activation/dissolution sequence as reported in Section 2.1. When it cleared (optimal dissolution, no micro-gels), the solution was diluted with anhydrous DMAc to 0.5% LiCl. This was the cellulose stock solution to be used for d*n*/d*c* determination and had a concentration of 9.9×10^{-4} g ml^{-1}. Eight dilutions were made (wt/wt) from this stock solution with 0.5% LiCl/DMAc, the lowest concentration being 1.0×10^{-4} g ml^{-1}. The same 8% LiCl/DMAc and DMAc batches were used throughout the preparation of all the solutions. Pure solvent was first eluted through the detector cell to establish a stable baseline. It is important to note that the solvent must not be degassed through the degasser to avoid baseline dips, which would result from the slight difference in refractive indices between the degassed solvent and the cellulose solution. Once baseline readings were stable, each cellulose solution was passed through the DRI detector starting with the lowest concentration, and the change in the voltage (ΔV) was recorded. Time was allowed between each injection for the signal to return to baseline and solvent reinjected at the end of each series of injections in order to set the baseline. The experiment was repeated three times and the values of d*n*/d*c* obtained were averaged. The data were collected and reduced with DNDC software v. 5.20 (Wyatt Technology Corp.).

3. Results and discussion

3.1. dn/dc of cellulose in 0.5% LiCl/DMAc

From the change in the DRI detector voltage ΔV, the difference in refractive index between the pure solvent and the polymer solution (Δn) for each different concentration can be calculated from $\Delta n = \alpha \Delta V$ (where α is the DRI calibration constant). Fig. 1 represents the plot of the average Δn obtained in the three experiments as a function of c. The gradient in this plot equals $d(\Delta n)/dc$ or dn/dc, a unique variable of the studied polymer in the working solvent at the working temperature and working wavelength. The values of dn/dc obtained were 0.0805 ($\pm 1.8 \times 10^{-3}$), 0.0744 ($\pm 1.9 \times 10^{-3}$), and 0.0751 ($\pm 1.9 \times 10^{-3}$) ml g^{-1}. The mean dn/dc value for cellulose in 0.5% LiCl/DMAc thus was 0.077 ± 0.003 ml g^{-1} (measured at 37 °C, 690 nm). A plot of $\Delta n/c$ as a function of c built with the mean values is represented in Fig. 2. The gradient roughly equals zero over the larger part of the concentration spectrum. Only the first data points bear a larger RSD, which could be attributed to experimental error on the lowest concentrations. A flat line indicates invariable dn/dc at all concentrations.

This dn/dc of 077 ml g^{-1} was considered the most appropriate to be used in the determination of M_r averages in the following study because it was determined using exactly the same cellulose source as the samples analysed and under optimal instrumental conditions (same working wavelength of MALS and DRI). The dn/dc of cellulose in LiCl/DMAc is indeed particularly difficult to determine, as observed by several authors (McCormick, Callais, & Hutchinson, 1985; Terbojevich, Cosani, Conio, Ciferri, & Bianchi, 1985). With 0.5% LiCl salt concentration, values reported were 0.091 ml g^{-1} (measured at 25 °C) (Sjöholm, Gustafsson, Eriksson, Brown, & Colmsjö, 2000), 0.104 ml g^{-1} (measured at 40 °C) (Schult, Hjerde, Optun, Kleppe, & Moe, 2002), 0.108 ml g^{-1} (measured at 40 °C) (Berggren, Berthold, Sjöholm, & Lindström, 2003), and 0.163 ml g^{-1} (unspecified temperature) (Striegel & Timpa, 1996). At higher salt concentration (9% LiCl), the values for dn/dc of cellulose from different sources and representing a large range of M_w ($1.25–7 \times 10^5$ gmol^{-1}) were found to vary within 0.0403–0.0698 ml g^{-1} (measured at 30 °C) (McCormick et al., 1985). This surprisingly wide range of

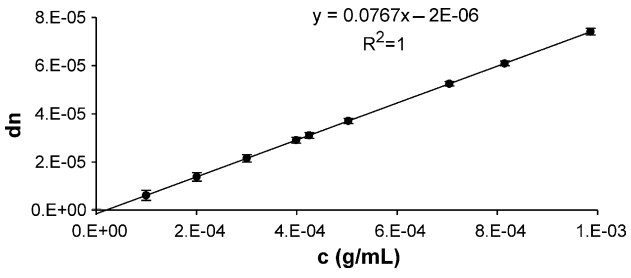

Fig. 1. dn as a function of concentration c.

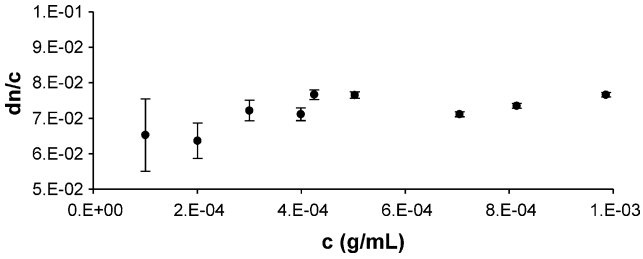

Fig. 2. dn/c as a function of c.

reported values for dn/dc can be explained by the influence of instrumental as well as sample related parameters. Furthermore, the lack of cellulose standards of known M_r impedes any verification of the measures. The instrumental variable that plays a major role is the measuring wavelength. For accurate determination and thus optimal results in the M_w determination, the DRI wavelength at which dn/dc is determined should be the same as that of the laser of the LS detector. Unfortunately, in the literature the measuring wavelengths data are sometimes lacking, and thus the values of dn/dc proposed cannot always be used. Poor reproducibility in the dn/dc measured values has also been attributed to solvent hygroscopicity (Debzi, 1992). As recently reported by Berggren et al. (2003), dn/dc can be influenced to a certain extent by small variations of the LiCl concentration in the solvent. This is probably related to the degree of dissolution and bonding achieved between solvent molecules and cellulose molecules. Sample related variability thus includes the degree of dissolution of cellulose in LiCl/DMAc, and the possible molecular associations or aggregation. Indeed several studies have reported association of molecules and formation of aggregates in solutions of cellulose in LiCl/DMAc (Sjöholm et al., 2000; Terbojevich et al., 1985). Röder, Morgenstern, Schelosky, and Glatter (2001) showed that aggregation can result from too low LiCl concentration (6%) or too high cellulose concentration (1% wt/wt). Strlič, Kolenc, Kolar and Pihlar (2002) also acknowledged the role of the salt concentration in relation with aggregation. Aggregates can arise from the presence of water in the solvent system. This effect of water was found to be more pronounced on diluted solutions (SEC concentration), where within one day, as low as 0.05 M water can lead to considerable increase in the measured hydrodynamic radius (Potthast et al., 2002). Similarly, Röder et al. (2002) found that, depending on the pulp type, 0.01–0.05 M water can disturb a thermodynamically good solution and promote aggregation. With more than two water molecules per LiCl molecule, the concentration of the salt is not sufficient for a good dissolution of cellulose (Chrapava, Touraud, Rosenau, Potthast, & Kunz, 2003). Indeed a specific number of complexed sites are required to keep the polymer in solution as proposed in several reviews of the solvation mechanism (Dawsey & McCormick, 1990; Morgenstern & Kammer, 1996; Striegel, 1997, 2003). Nevertheless the exact structure of the complex cellulose-LiCl/DMAc is still not fully elucidated.

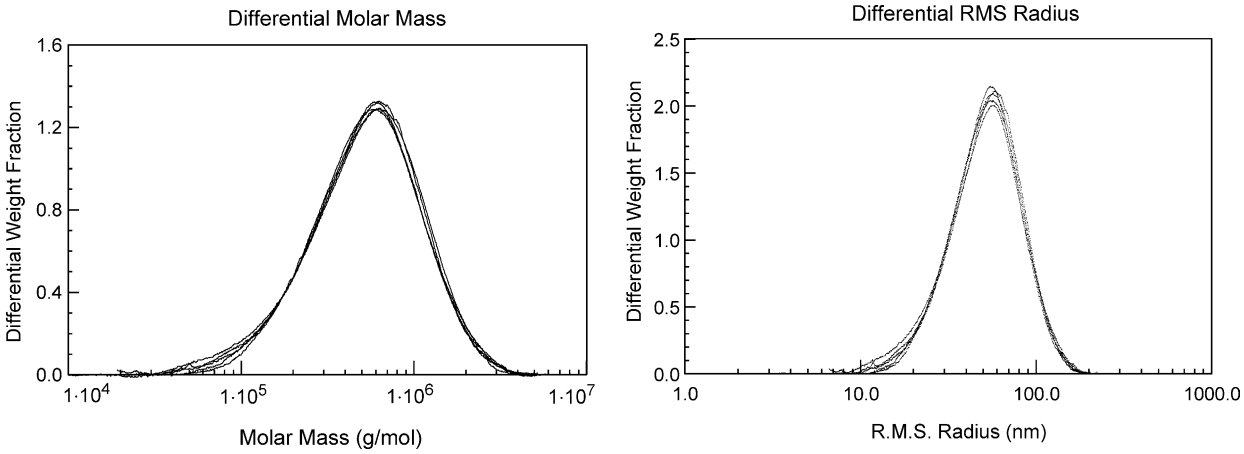

Fig. 3. Overlaid differential molar mass (left), and rms radii (right) graphs of Wt_0 (5 analyses, 0.0625% cellulose in 0.5% LiCl/DMAc) obtained using Zimm detector fit method and first order polynomial results fit.

Recent publications present experimental evidence of a strong bonding between the anionic moiety of the solvent complex (Cl^-) and the hydrogen atoms of the cellulose hydroxyl groups (Dupont & Mortha, 2004; Spange et al, 1998; Striegel; 2003).

3.2. Influence of the second virial coefficient A_2

The second virial coefficient A_2 is a thermodynamic parameter indicative of solvent–solute interactions. In other words, it is a measure of the amount of energy gained by the system upon surrounding polymer molecules by solvent molecules. Since with polydisperse polymers, multiple concentrations of the same sample are not available, and for monodisperse polymers, the concentration range represented within the peak region is usually too low and too narrow, A_2 cannot be determined with SEC. However, at very low concentrations (e.g. 0.5–1 mg ml^{-1}), A_2 can safely be omitted as long as the relationship $2A_2\,cM_w \ll 1$ is verified.

Values of A_2 for cellulose in LiCl/DMAc found in the literature vary widely, probably due to solubilisation difficulties: 3×10^{-4} and 1.5×10^{-3} mol ml g^{-2} for microcrystalline cellulose and softwood Kraft pulp, respectively, in 2.6% LiCl/DMAc (Röder et al., 2001), from 3.5×10^{-3} mol ml g^{-2} (cotton cellulose) up to 5.3×10^{-3} mol ml g^{-2}, depending on the cellulose origin, in 9% LiCl/DMAc (McCormick et al., 1985), and 1.33×10^{-3} mol ml g^{-2} for cotton linters in 8% LiCl/DMAc (Matsumoto, Tatsumi, Tamai, & Takaki, 2001).

As they were done close to SEC concentrations, the experimental conditions of Röder et al. (2001) are those that most closely resemble the conditions of the present study. Simulations with ASTRA software were made by entering the value 3×10^{-4} mol ml g^{-2} as A_2 coefficient instead of the default value 0. The sample of Wt_0 chosen for the simulation had one of the highest M_w of all cellulose samples analysed (6.65×10^5 g mol^{-1}). The recalculated M_w was then only 1.5% higher than when A_2 was set to 0. Inputting A_2 value of 1.5×10^{-3} mol ml g^{-2}, the increase in M_w was 7.2%. A similar simulation was carried out with a Whatman No.1 paper (initial M_w as Wt_0) that had been immersed in a solution of aluminium potassium sulphate (0.83 g ml^{-1}), and then artificially aged (same conditions as Wt_{35}), which accelerated the acid-catalysed hydrolysis of cellulose, thus leading to significant depolymerisation (Dupont, 2003a). The cellulose of this sample had a very low M_w (1.71×10^5 g mol^{-1}) when calculated with A_2 set to 0. In this case, A_2 set to 3×10^{-4} mol ml g^{-2} resulted in a marginal 0.4% increase of M_w, while A_2 set to 1.5×10^{-3} mol ml g^{-2} resulted in 2.1% increase in M_w. These simulations showed that indeed the second virial coefficient can be omitted in the calculations of M_w in

Table 1
M_r and rms averages of cellulose of Wt_0 (2 samples, 5 analyses) calculated using Zimm detector fit method, and first order polynomial results fitting

Wt_0	$M_n\times10^{-5}$ (g mol^{-1})	$M_w\times10^{-5}$ (g mol^{-1})	$M_z\times10^{-5}$ (g mol^{-1})	r_n (nm)	r_w (nm)	r_z (nm)
1A	4.18	6.96	10.62	45.6	62.1	80.1
1B	3.69	6.65	10.39	42.9	61.0	79.8
1C	3.68	6.52	9.99	43.6	61.8	80.2
2A	4.01	6.73	10.24	45.9	63.1	81.5
2B	3.52	6.63	10.39	41.9	61.8	81.6
Average	3.81	6.70	10.33	44.0	62.0	80.6
RSD	0.27	0.17	0.23	1.73	0.76	0.84
RSD (%)	7.0	2.5	2.3	3.9	1.2	1.0

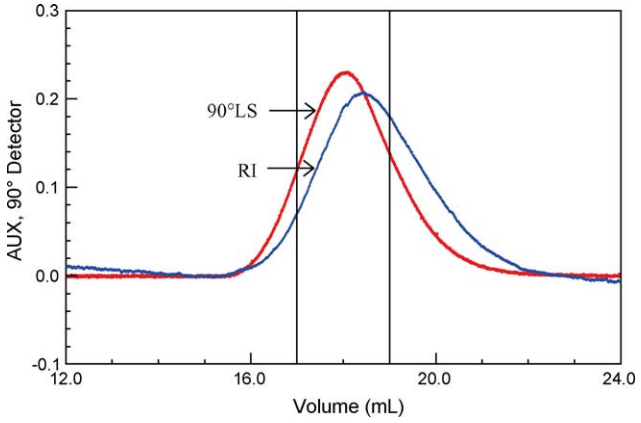

Fig. 4. 90° photodiode LS and DRI signals as a function of V_e for sample Wt_0 3A. Vertical bars enclose the fraction of V_e used in the calculations of q shown in Table 5.

the SEC/MALS/DRI experiments, as the resulting error in M_w would not exceed 2% in the majority of cases.

3.3. Precision, reproducibility and accuracy of the method

The precision of the method related to the instrumentation and to sample preparation was determined over five non-consecutive analyses: three (1A, 1B and 1C) and two (2A and 2B) replicates of two samples Wt_0 (1 and 2) dissolved on different days, respectively. Fig. 3 shows the overlaid differential molar mass and rms radii graphs of the five analyses.

Table 1 lists the values of the M_r averages and rms radii averages obtained. The small RSD on M_w and M_z shows a good reproducibility of the method. The RSD on M_n indicates a slightly larger error is made on the low-M_r molecules than on the high-M_r molecules. This could be attributed to the precision of the MALS detector, which is lower in the low-M_r range.

3.4. Conformation of cellulose in 0.5% LiCl/DMAc and quality of the solvation

Direct relationship between M_r and polymer size is usually represented by the scaling law (De Gennes, 1979; Laguna, Medrano, Plana, & Tarazona, 2001; Pincus, 1976):

$$\langle r_g^2 \rangle^{1/2} = QM_r^q \quad (4)$$

The scaling factor q is the gradient in the log–log plot of rms radius as a function of M_r. As polymer dimensions depend on polymer–solvent interactions, q yields information on the properties of the polymer in solution and relates to the shape of the chains, i.e. macromolecular conformation. Values of q comprised between 0.5 and 0.6 are expected for random coil polymer chains; q is closer to 0.5 for a polymer in a theta solvent ($A_2=0$), and to 0.6 for a polymer in a good solvent ($A_2>0$). For rigid rods, $q=1$ (or very close to 1), and for spherical polymers $q\approx0.33$. Most real random coils have $q\approx0.55$–0.60.

The parameter a in the Mark-Houwink-Sakurada (MHS) equation $[\eta]=K'M_v^a$, where $[\eta]$ is the intrinsic viscosity, and K' and a are constants for a given polymer–solvent system, temperature and molar mass range, is related to q, with: $q=(a+1)/3$(ASTRA manual).

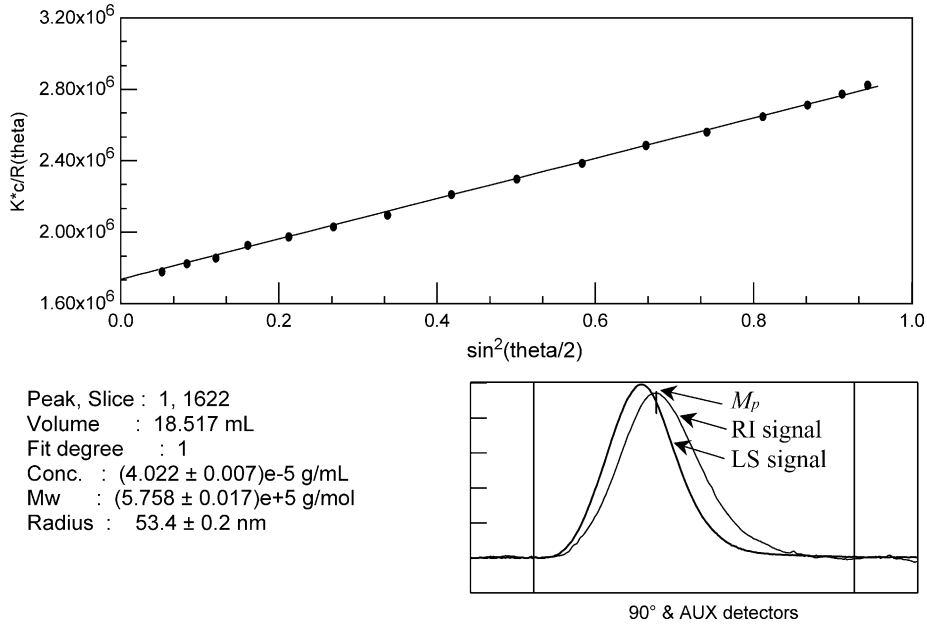

Fig. 5. Debye plot ($K^*c/R_\theta = f(\sin^2(\theta/2))$ (Zimm formalism, first order polynomial fit) of cellulose (Wt_0) in 0.5% LiCl/DMAc, from the slice of the chromatogram at $V_e=18.517$ ml. The error bars represent the baseline noise for each detector's photodiode. The data in the lower left part are calculated from the slice selected by moving the vertical ticker over the LS chromatogram. Thus, values of M_w can be obtained for each V_e. On the chromatograms (bottom right) the ticker is placed on the LS signal at the location corresponding to the apex of the DRI signal, which yields peak molar mass $M_p=5.76\times10^5$ g mol^{-1}.

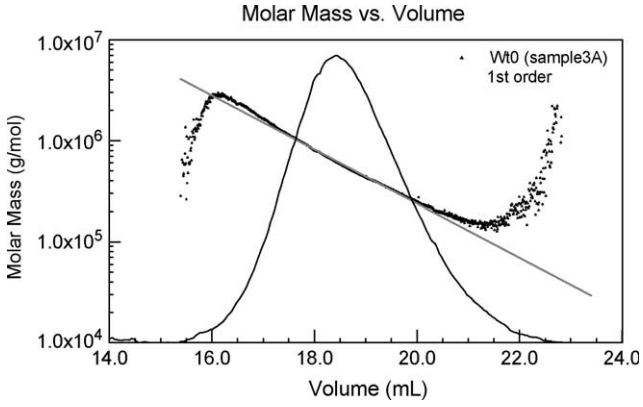

Fig. 6. Molar mass distribution across Ve for a Wt_0 sample (0.0625% cellulose in 0.5% LiCl/DMAc), DRI signal.

3.4.1. Cellulose from unaged paper (Wt_0)

The raw signals of the DRI and the 90° angle photodiode as a function of the elution volume (V_e) for a sample Wt_0 indicate that the elution profiles do correspond (Fig. 4). Fig. 5 shows a Debye plot (Zimm formalism) for a slice of the distribution near peak molar mass (M_p) for a sample Wt_0. First order polynomial results fit was chosen as second order polynomial did not yield significantly different values for both M_w and rms radii.

Figs. 6 and 7 illustrate the M_r and the rms radius versus V_e, respectively, for cellulose of Wt_0. In Fig. 6, the distribution of mass across V_e is superimposed onto the DRI chromatogram. At the very edges of the distribution curve, the signal-to-noise ratio is low (corresponding to low concentration), especially at high V_e (low concentration and low M_r), and results in an uncertainty in the values, which is visible from a dispersion of the data points. Nevertheless, across most of the elution range the cellulose showed a linear relationship of both M_r and rms radius with V_e. Thus, a good separation under the current conditions seemed achieved, which confirmed the suitability of PSDVB packing material, as acknowledged by Schult, Moe, Hjerde, and Christensen (2000) and the absence of non-steric elution phenomena, and pseudo-exclusion behaviour of cellulose in LiCl/DMAc as described by Bikova and Treimanis (2002).

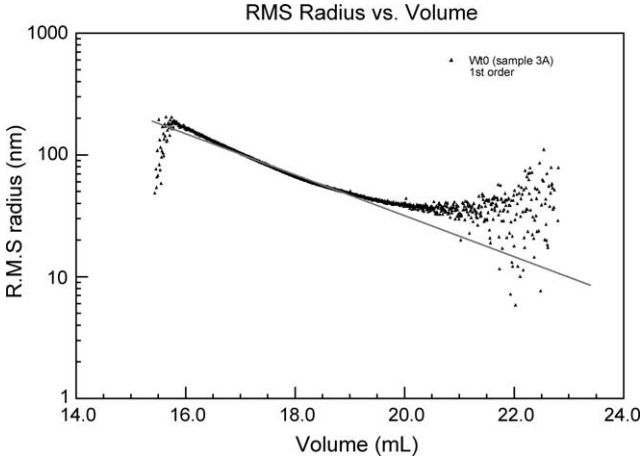

Fig. 7. rms radius distributions across Ve for a Wt_0 sample (0.0625% cellulose in 0.5% LiCl/DMAc).

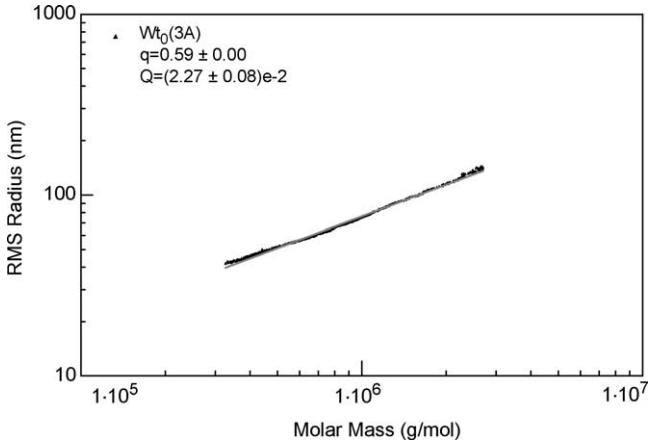

Fig. 8. rms radius *vs* M_r (log–log scale) for cellulose of Wt_0, 0.0625% in 0.5% LiCl/DMAc.

Table 2
Values of Q, q and a of Wt_0 (0.0625% cellulose in 0.5% LiCl/DMAc, 3 samples, 7 analyses)

Wt_0	Q ($\times 10^2$) nm(gmol^{-1})$^{-q}$	q	a
1A	2.38±0.14[a]	0.61	0.83
1B	2.11±0.10	0.59	0.77
1C	1.97±0.06	0.60	0.80
2A	3.62±0.10	0.57	0.71
2B	1.11±0.07	0.56	0.68
3A	2.27±0.08	0.59	0.77
3B	1.37±0.05	0.62	0.86
Average		0.59	0.77
RSD		0.02	0.06
RSD (%)		3.6	8.2

[a] The uncertainty range on the values of Q is calculated by the ASTRA software by determining the statistical fluctuation in each detector's signal (including all photodiodes). These uncertainties are statistical only, and do not include any of the many possible systematic errors that may be present from sample preparation to injection and separation.

Table 3
Values of q of cellulose in 0.5% LiCl/DMAc (3 samples Wt_0, 7 analyses) across three broad and partly overlapping peak regions, covering the entire V_e

Wt_0	q from V_e peak start to V_e peak apex	q in V_e peak 1/2 heights	q from V_e peak apex to V_e peak end
1A	0.62	0.58	0.48
1B	0.65	0.50	0.34
1C	0.63	0.57	0.41
2A	0.56	0.58	0.47
2B	0.58	0.55	0.48
3A	0.64	0.54	0.45
3B	0.68	0.58	0.37
Average	0.62	0.56	0.43
RSD	0.041	0.030	0.056

Table 4
Values of M_w and log M_w of cellulose (3 samples Wt_0, 7 analyses) over three fractions of V_e

Wt_0	V_e peak start to V_e peak apex		V_e peak 1/2 heights		V_e peak apex to V_e peak end	
	M_w (gmol^{-1})	Log M_w	M_w (gmol^{-1})	Log M_w	M_w (gmol^{-1})	Log M_w
1A	1.62×10^6	6.21	6.13×10^5	5.79	3.55×10^5	5.55
1B	1.11×10^6	6.05	6.02×10^5	5.78	3.87×10^5	5.59
1C	1.07×10^6	6.03	6.07×10^5	5.78	3.34×10^5	5.52
2A	1.07×10^6	6.03	6.14×10^5	5.79	3.66×10^5	5.56
2B	1.09×10^6	6.04	6.27×10^5	5.80	3.78×10^5	5.58
3A	1.13×10^6	6.05	6.27×10^5	5.80	3.62×10^5	5.56
3B	1.14×10^6	6.06	6.20×10^5	5.79	3.39×10^5	5.53
Average	1.18×10^6	6.07	6.16×10^5	5.79	3.60×10^5	5.56
RSD	1.98×10^5	0.064	9.4×10^4	0.007	1.9×10^4	0.023

Irreversible adsorption or retention of possible aggregates, if any, by the guard column would be undetectable, but rather unlikely. As mentioned earlier, aggregation is promoted by the presence of water in the solutions. Great care was taken to limit water absorption as much as possible in every step of sample preparation by thoroughly drying DMAc and LiCl, and storing the solvent under nitrogen when not used immediately. Moreover, the presence of aggregates can often be detected in MALS, in the photodiodes signals in the high-*M*r end (small V_e), especially in the high degree angles. The chromatograms obtained showed monomodal LS signals at all measuring angles, similar to the 90° angle signal on Fig. 4, and consistent regular slopes in the MMD with V_e. Indeed large aggregates or associations retained on the guard column, would slowly disintegrate into smaller aggregates, which would result in their progressive elution. This co-elution with molecules of lower M_r would strongly affect the LS signal with deviating log–log plots of rms radius versus M_r, and inconsistent gradients as reported by (Olieman & Tromp, 2003). Fig. 8 shows that such plot for cellulose in 0.5% LiCl/DMAc is linear, which confirms the absence of aggregation.

Table 2 reports the values of q and Q obtained on the seven runs with three Wt_0 samples (1, 2 and 3) analysed in triplicate (1A, 1B and 1C) and duplicate (2A, 2B and 3A, 3B). The values of q of all the samples, and the mean value of 0.59 ($\pm$3.6%), indicate that cellulose chains are in random coil conformation in solution. For Wt_0 samples 1 and 3, $0.59 \leq q \leq 0.62$ (average of 0.6, RSD = 2%) pointed to optimal solvation conditions. For sample 2, a slightly lower q value (0.56–0.57) indicates that cellulose was closer to theta conditions and had a slightly more compact conformation. The corresponding values of a are in the range 0.77–0.86 (Table 2), with mean $a = 0.81$ (RSD = 4.9%). This a value corroborates those found in the literature for dilute solutions: recent studies, carried out at 0.5% LiCl/DMAc, report a of 0.957 ($q = 0.65$) (Bikova & Treimanis, 2002) and 0.65 ($q = 0.55$) (Schult et al., 2002).

By extrapolation, in order to obtain such conformational parameters with dilute solutions, good solvation of the initial concentrated cellulose solution in 8% LiCl/DMAc had to be achieved in the first place. The conformation of concentrated cellulose solutions could not be studied, but it is likely that the q values would have been larger, reflecting enhanced chain stiffness of the cellulose backbone. Indeed rigid rod conformation of cellulose in 9% LiCl/DMAc is reported by McCormick et al. (1985) and by Dawsey and McCormick (1990), with MHS coefficient a of 1.19 ($q \approx 0.73$). The suggested explanation is that, due to the complexing nature of the solvent LiCl/DMAc, the repulsive interaction of the chloride ions associated with the chain favours a fully extended conformation of the molecule. However, high cellulose and salt concentrations do not correspond to the conditions used in SEC. Schult et al. (2002) showed that at low LiCl concentration, cellulose adopts a less extended conformation probably due to a weakening of the intramolecular hydrogen bonding leading to an increased freedom of rotation of the cellulose molecule around the glycosidic bond, and consequently lower chain stiffness. The authors also proposed the hypothesis that intermolecular associations could play a role in lowering the hydrodynamic volume of the cellulose in solution. Another argument in support of the values of q obtained could be related with the polymer chains length. The cellulose used in the present study had high-M_r (Table 1), as opposed to most of the data found in the literature. Indeed long chain macromolecules are usually less stiff than short chain macromolecules (Bikova & Treimanis, 2002).

Table 5
Values of q of cellulose in 0.5% LiCl/DMAc (3 samples Wt_0, 7 analyses) over four consecutive fractions across the V_e range (17–19 ml) where the relationship between rms radii *vs* M_r (log–log) is strictly linear

Wt_0	q over V_e			
	17–17.5 ml	17.5–18 ml	18–18.5 ml	18.5–19 ml
1A	0.84	0.58	0.59	0.62
1B	0.69	0.59	0.46	0.49
1C	0.64	0.64	0.6	0.53
2A	0.6	0.61	0.6	0.57
2B	0.58	0.61	0.57	0.52
3A	0.58	0.61	0.53	0.47
3B	0.66	0.62	0.62	0.48
Average	0.66	0.61	0.57	0.53
RSD	0.091	0.020	0.055	0.054

Table 6
Values of M_w and log M_w of cellulose (3 samples Wt_0, 7 analyses) over four consecutive fractions of V_e in the range 17–19 ml

Wt_0	V_e 17–17.5 ml		V_e 17.5–18 ml		V_e 18–18.5 ml		V_e 18.5–19 ml	
	M_w (g mol^{-1})	Log M_w	M_w (g mol^{-1})	Log M_w	M_w (g mol^{-1})	Log M_w	M_w (g mol^{-1})	Log M_w
1A	1.41×10^6	6.15	1.06×10^6	6.02	7.43×10^5	5.87	5.57×10^5	5.75
1B	1.29×10^6	6.11	9.09×10^5	5.96	6.40×10^5	5.81	4.57×10^5	5.66
1C	1.36×10^6	6.13	9.59×10^5	5.98	6.90×10^5	5.84	4.96×10^5	5.70
2A	1.30×10^6	6.11	9.10×10^5	5.96	6.57×10^5	5.82	4.85×10^5	5.69
2B	1.34×10^6	6.13	9.33×10^5	5.97	6.81×10^5	5.83	5.13×10^5	5.71
3A	1.35×10^6	6.13	9.58×10^5	5.98	6.88×10^5	5.84	5.03×10^5	5.70
3B	1.25×10^6	6.10	8.73×10^5	5.94	6.11×10^5	5.79	4.28×10^5	5.63
Average	1.33×10^6	6.12	9.43×10^5	5.97	6.73×10^5	5.83	4.91×10^5	5.69
RSD	4.9×10^4	0.016	5.4×10^4	0.024	3.9×10^4	0.025	3.8×10^4	0.034

The mean value of Q using the samples that showed optimal solvation (1 and 3), was 2.0×10^{-2} nm(g mol^{-1})$^{-0.6}$ (RSD = 19.6%). Thus, it can be concluded that statistically cellulose in 0.5% LiCl/DMAc is in random coil conformation, and Eq. (4) becomes:

$$\langle r_g^2\rangle^{1/2} = 2\times10^{-2}M_r^{0.6}$$

Therefore, upon dilution from concentrated solutions in 8% LiCL/DMAc, the cellulose molecules remain in solution in good thermodynamic conditions. According to the present results, random coil conformation of cellulose in 0.5% LiCl/DMAc seems most likely. However, the possibility that this could be due to a lower complexation degree of the molecules with the solvent at low salt concentration, leading to less chain repulsion on the one hand, and to an increased chance of intramolecular association from hydrogen bonding on the other, could not be totally discarded. Nevertheless, results clearly indicate that in dilute solution at weak LiCl concentration, cellulose adopts a less extended conformation than at strong concentration, and that the critical number of complexed sites necessary to maintain the polymer solvated is undoubtedly respected, as no precipitation out of solution was observed.

3.4.1.1. Investigation into the variation with M_r of the conformation of cellulose in LiCl/DMAc. The values of q above reported were obtained from plotting and integrating across the entire peak area of the chromatograms. However, the gradient in the log–log plots may not be constant over the entire M_r range. This is most often reported in the case of linear polymers that have branched components at high-M_r. In such case the gradient becomes shallower at large M_r due to a more compact conformation. In the view of the discrepancies on the conformation of cellulose in LiCl/DMAc found in the literature, it seemed legitimate to further investigate conformation vs M_r.

Table 3 gathers the values of q for Wt_0 samples across three regions of the peaks covering the entire V_e range (i.e. from 15.5 to 23 ml). The three partly overlapping regions span from the baseline to the apex (high M_r), across the peak's half height, and from the apex to the baseline (low M_r). Table 4 reports corresponding values of M_w averages over the three regions.

Table 5 lists the values of q obtained across the restricted area of the peaks over which the log–log plots of rms radius *vs* M_r were found strictly linear. This corresponded to a common V_e for all the samples between 17 and 19 ml. In this case, the calculations of q were performed over this specific peak region in four consecutive (non-overlapping) fractions of 0.5 ml each. Table 6 reports the corresponding values of M_w averages over the four volume fractions. Fig. 4 illustrates the location of the V_e fraction 17–19 ml on the chromatogram of a Wt_0 sample.

Whether calculated over the entire V_e (Table 3) or over a partial V_e (Table 5), the value of q was found to decrease from the beginning to the end of the elution. Hence, q decreased slightly with decreasing M_r. This is represented in Fig. 9, where the mean values of q (Tables 3 and 5) are plotted versus the corresponding average log M_w. Very-high-M_r chains ($M_w > 10^6$ g mol^{-1}) display slightly stiffer conformation (mean $q > 0.6$) than high and mid-M_r chains ($10^6 > M_w > 5\times10^5$ g mol^{-1}), the latter having perfect random-coil conformation with q between 0.5 and 0.6. Low-M_r chains ($M_w < 3.5\times10^5$ g mol^{-1}) have smaller q values (mean $q = 0.43$). It must be stressed that the differentiation made here for commodity between high- and low-M_r is all-relative, since the average M_r of cellulose

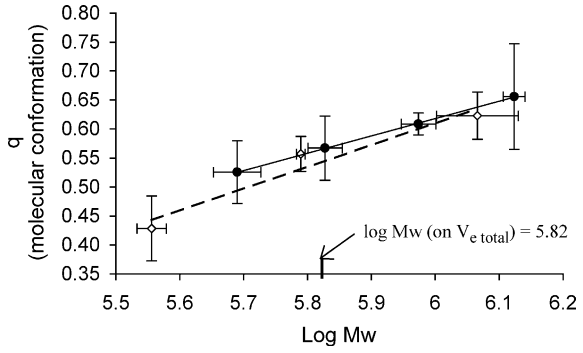

Fig. 9. (●): q as a function of log M_w over four consecutive V_e fractions of 0.5 ml between 17 and 19 ml; (◇): q as a function of log M_w over three partly overlapping V_e fractions covering the entire peak region.

Table 7
Average M_r averages, rms radii averages and values of q for Wt_{35} and Wt_{94} (four samples and 8 analyses each)

	AVG$M_n\times10^{-5}$ (g mol^{-1})	AVG$M_w\times10^{-5}$ (g mol^{-1})	AVG$M_z\times10^{-5}$ (g mol^{-1})	AVG r_n (nm)	AVG r_w (nm)	AVG r_z (nm)	AVG q
Wt_{35}	3.33	5.55	8.42	40.4	55.3	71.6	0.57
Wt_{94}	2.17	3.87	6.06	30.5	43.1	56.3	0.54
RSD (%) Wt_{35}	6.2	3.5	7.2	2.7	2.2	6.0	5.0
RSD (%) Wt_{94}	8.1	3.7	5.9	4.7	1.3	2.6	7.3

studied is rather high (Table 1). As cellulose is not a branched polymer, this change in q, and hence in conformation, as a function of M_r can be interpreted as due to a variation in the quality of the solvation depending on the length of the chains. That is, either the low-M_r chains are less well solvated than mid- and high-M_r chains, or more likely they tend to adopt a more compact conformation in solution due to less charge repulsion, as a consequence of a smaller number of negative charges. This finding can also partly explain the variations in the values of dn/dc reported in the literature for cellulose in LiCl/DMAc, depending on the polymer's origin.

3.4.2. Cellulose from aged paper (Wt_{35} and Wt_{94})

Table 7 reports the mean values of M_r, rms radii and q for Wt_{35} and Wt_{94}. Fig. 10 represents the log–log plots of rms radius versus M_r for Wt_0, Wt_{35} and Wt_{94}. It is noteworthy that all values of q were comprised between 0.5 and 0.6, which indicates here again polymers in random coil conformation in the solvent. The slight decrease in q with increasing aging time seems to point out that aged cellulose was closer to theta conditions in the solvent than unaged cellulose. As observed earlier, a somewhat more compact conformation of low-M_r than high-M_r molecules in 0.5% LiCl/DMAc could be the cause. However, it may be also that with aging, as the number of hydroxyl groups on the cellulose chains decreases due to oxidation and formation of carbonyl and carboxyl groups, a lower complexation level between solvent molecules and cellulose is reached, hence a slightly lower solvating capacity of LiCl/DMAc is possible.

From Table 7, we note that the decrease in r_z was of 11% between Wt_0 and Wt_{35}, and 30% between Wt_0 and Wt_{94} while the decrease in M_w was of 17% between Wt_0 and Wt_{35}, and 42% between Wt_0 and Wt_{94}. Indeed only a fully extended conformation (rigid rod) would yield equal changes in M_r averages and in rms radii. Thus, this tended to confirm on the one hand the random coil conformation of cellulose in dilute solution in 0.5% LiCl/DMAc, and on the other, some conformational dependency with M_r.

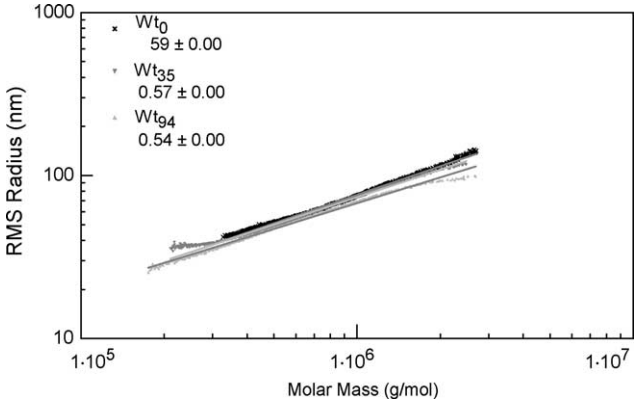

Fig. 10. Overlaid average rms radii *vs* M_r (log–log scale) for Wt_0, Wt_{35} and Wt_{94}.

4. Conclusions

SEC/MALS/DRI analyses led to a mean determined value of 0.077 ml g^{-1} for the dn/dc of cellulose in 0.5% LiCl/DMAc, in the experimental conditions of this study. The conformational study showed that with the dissolution method used, the cellulose in dilute solutions at low salt concentration adopted random coil conformation, with q values comprised between 0.5 and 0.6 for both unaged and aged cellulose. Thus, from the dilute solution behaviour, it seems that LiCl/DMAc is a thermodynamically good solvent for cellulose, or a theta solvent in the worst case. It was found that the conformation of cellulose in solution varied slightly with the polymer molar mass. The molecules with M_r above 10^6 g mol^{-1} adopted slightly stiffer conformation ($q>0.6$), while the molecules with M_r below 3.5×10^5 g mol^{-1} had a somewhat more compact conformation ($q=0.43$) in 0.5% LiCl/DMAc. This could be attributed to a different degree of complexation with the solvent between the segments of the chains, with stronger complexation yielding stiffer conformation due to charge repulsion. Similarly, the aged cellulose showed slightly more compact conformation in solution. The presence of oxidised groups on the polymer chain, decreasing the degree of complexation with the solvent, may be the cause.

Acknowledgements

This research was carried out during the Advanced Training Fellowship in Conservation Science at the National Gallery of Art, Washington DC, US, and was financially supported by the Charles E. Culpeper Foundation. Warm thanks to Prof. Dr E.R. de la Rie, Scientific Research department, National Gallery of Art, for his support and guidance, and to Dr C. Shahani, Preservation Research and Testing division, Library of Congress,

Washington DC, for providing the instrumental support. We are also indebted to Dr M. Chen, and Dr T. Scherrer, Wyatt Technology Corp., Santa Barbara, CA, US.

References

ASTRA software v. 4.73.04 manual, Wyatt Technology Corporation.

Berggren, R., Berthold, F., Sjöholm, E., & Lindström, M. (2003). Improved methods for evaluating the molar mass distributions of cellulose in kraft pulp. *Journal of Applied Polymer Science*, *88*, 1170–1179.

Bikova, T., & Treimanis, A. (2002). Problems of the MMD analysis of cellulose by SEC using DMA/LiCl: A review. *Carbohydrate Polymers*, *48*(1), 23–28.

Chrapava, S., Touraud, D., Rosenau, T., Potthast, A., & Kunz, W. (2003). The investigation of the influence of water and temperature on the LiCl/DMAc/cellulose system. *Physical Chemistry Chemical Physics*, *5*, 1842–1847.

Dawsey, T. R., & McCormick, C. L. (1990). *Journal of Macromolecular Science-Reviews in Macromolecular Chemistry and Physics*, *C30*(3,4), 405–440.

Debzi, El. M. (1992). *Celluloses issues du traitement à la vapeur: Évolution des masses moléculaires moyennes, transformations morphologiques et cristallines.* Thèse de Doctorat, Université Joseph Fournier-Grenoble I. pp. 55.

De Gennes, P. G. (1979). *Scaling concepts in polymer physics*. Ithaca, NY: Cornell University Press p. 324.

Dupont, A. -L. (2003a). *Gelatine sizing of paper and its impact on the degradation of cellulose during aging, a study using size-exclusion chromatography*. PhD thesis, University of Amsterdam, pp. 256.

Dupont, A.-L. (2003b). Cellulose in lithium chloride/*N,N*-dimethylacetamide, optimisation of a dissolution method using paper substrates and stability of the solutions. *Polymer*, *44*(15), 4117–4126.

Dupont, A.-L., & Mortha, G. (2004). Comparative evaluation of size-exclusion chromatography and viscometry for the characterisation of cellulose. *Journal of Chromatography A*, *1026*, 129–141.

Ekmanis, J. L. (1987). Gel permeation chromatographic analyses of cellulose. *American Laboratory. News*, *Jan–Feb*, 10–11.

Ekmanis, J. L., & Turbak, A. F. (1986). *GPC analysis of cellulose Lab highlights, an internal communication of applications and techniques, 251*. Millipore, Milford, MA, US: Waters Chromatography Division pp. 1.

Flory, P. J. (1953). *Determination of molecular weights. Principles of polymer chemistry*. Ithaca: Cornell University Press. pp. 266–316.

Jerosch, H. (2002). *Evaluation de l'état de dégradation de la cellulose/holocellulose dans différents types de papiers par chromatographie d'exclusion stérique.* Thèse de Doctorat, Université Versailles-St Quentin-En-Yvelines, pp. 204.

Laguna, M. T. R., Medrano, R., Plana, M. P., & Tarazona, M. P. (2001). Polymer characterization by size-exclusion chromatography with multiple detection. *Journal of Chromatography A*, *919*(1), 13–19.

Matsumoto, T., Tatsumi, D., Tamai, N., & Takaki, T. (2001). Solution properties of celluloses from different biological origins in LiCl/DMAc. *Cellulose*, *8*(4), 275–282.

McCormick, C. L. (1981). *Novel cellulose solutions*. US patent No. 4,278,790.

McCormick, C. L., Callais, P. A., & Hutchinson, B. H., Jr.. (1985). Solution studies of cellulose in lithium chloride and *N,N*-dimethylacetamide. *Macromolecules*, *18*, 2394–2401.

Morgenstern, B., & Kammer, H.-W. (1996). Solvation in cellulose-LiCl-DMAc solutions. *Trends in Polymer Science*, *4*(3), 87–91.

Olieman, C., & Tromp, R. H. (2003). *Elimination of adverse effects of aggregates (microgels) on SEC-MALLS analysis of hydrocolloids International Symposium on Separation and Characterization of Natural and Synthetic Macromolecules, Amsterdam, The Netherlands*. Oral presentation.

Pincus, P. (1976). Excluded volume effects and stretched polymer chains. *Macromolecules*, *9*, 386–388.

Potthast, A., Rosenau, T., Buchner, R., Röder, T., Ebner, G., Bruglachner, H., Sixta, H., & Kosma, P. (2002). The cellulose solvent *N,N*-dimethylacetamide/lithium chloride revisited: The effect of water on physicochemical properties and chemical stability. *Cellulose*, *9*(1), 41–53.

Röder, T., Morgenstern, B., Schelosky, N., & Glatter, O. (2001). Solutions of cellulose in *N,N*-dimethylacetamide/lithium chloride studied by light scattering methods. *Polymer*, *42*(16), 6765–6773.

Röder, T., Potthast, A., Rosenau, T., Kosma, P., Baldinger, T., Morgenstern, B., & Glatter, O. (2002). The effect of water on cellulose solutions in DMAc/LiCl. *Macromolecular Symposia*, *190*, 151–159.

Schult, T., Hjerde, T., Optun, O. I., Kleppe, P. J., & Moe, S. (2002). Characterization of cellulose by SEC-MALLS. *Cellulose*, *9*(2), 149–158.

Schult, T., Moe, S. T., Hjerde, T., & Christensen, B. E. (2000). Size exclusion chromatography of cellulose dissolved in LiCl/DMAc using macroporous monodisperse poly(styrene-co-divinylbenzene) particles. *Journal of Liquid Chromatogaphy and Related Technologies*, *23*(15), 2277–2288.

Sjöholm, E., Gustafsson, K., Eriksson, B., Brown, W., & Colmsjö, A. (2000). Aggregation of cellulose in lithium chloride/*N,N*-dimethylacetamide. *Carbohydrate Polymers*, *41*(2), 153–161.

Spange, S., Reuter, A., Vilsmeier, E., Heinze, T., Keutel, D., & Linert, W. (1998). Determination of empirical polarity parameters of the cellulose solvent *N,N*-dimethylacetamide/LiCl by means of the solvatochromic technique. *Journal of Polymer Science A: Polymer Chemistry*, *36*, 1945.

Striegel, A. M. (1997). Theory and applications of DMAc/LiCl in the analysis of polysaccharides. *Carbohydrate Polymers*, *34*(4), 267–274.

Striegel, A. M. (2003). Advances in the understanding of the dissolution mechanism of cellulose in DMAc/LiCl. *Journal of the Chilean Chemical Society*, *48*(1), 1–8.

Striegel, A. M., & Timpa, J. D. (1996). *Size exclusion chromatography of polysaccharides in dimethylacetamide-lithium chloride Strategies in size exclusion chromatography, ACS symposium series 635*. Washington, DC: American Chemical Society.

Strlič, M., & Kolar, J. (2003). Size exclusion chromatography of cellulose in LiCl/*N,N*-dimethylacetamide. *Journal of Biochemical and Biophysical Methods*, *1681*, 1–15.

Strlič, M., Kolenc, J., Kolar, J., & Pihlar, B. (2002). Enthalpic interactions in size exclusion chromatography of pullulan and cellulose in LiCl-*N,N*-dimethylacetamide. *Journal of Chromatography A*, *964*(1), 47–54.

Terbojevich, M., Cosani, A., Conio, G., Ciferri, A., & Bianchi, E. (1985). Mesophase formation and chain rigidity in cellulose and derivatives. 3. Aggregation of cellulose in *N,N*-dimethylacetamide-lithium chloride. *Macromolecules*, *18*, 640–646.

Turbak, A. F. (1981). *Solvent system for cellulose*. US patent No. 4,302,252.

T 412 om-94, TAPPI test methods (1994). *Moisture in pulp, paper and paperboard. Technical association of the pulp and paper industry*. Atlanta, US: TAPPI press.

Wyatt, P. J. (1993). Light scattering and the absolute characterization of macromolecules. *Analytica Chemica Acta*, *272*(1), 1–40.