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Evaluation of size-exclusion chromatography and viscometry for the determination of molecular masses of oxidised cellulose

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Abstract

The molecular masses of oxidised cellulose samples from two different sources were determined by size-exclusion chromatography (SEC) and viscometry. SEC was performed at room temperature using a cross-linked polystyrene–divinylbenzene column and 1% LiCl (w/v) in *N,N*-dimethylacetamide (DMAc) as the eluent. Cellulose samples were oxidised using aqueous solutions of H₂O₂, NaClO or KIO₄, and dissolved in the LiCl–DMAc solvent system using activation with water and a solvent exchange procedure. Viscometry in the cupriethylenediamine (CED) solvent system was performed following the standard technique. Oxidised cellulose samples are prone to degradation by alkalis. While the dissolution in LiCl–DMAc was shown not to have a degrading effect, the oxidised cellulose samples are unstable in the highly alkaline CED solvent, thus introducing a systematic error to the viscometric measurements. A stabilising reduction procedure usually recommended for such samples was tested, and shown to be advantageous, although degradation cannot be completely avoided. © 1998 Elsevier Science B.V.

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1. Introduction

The application of size-exclusion chromatography (SEC) for the analysis of cellulose is not as widespread as it is for synthetic polymers. The main reason for this is that the highly ordered crystalline structure of cellulose does not allow solvents to easily penetrate the cellulose fibres and break the intermolecular hydrogen bonds. The need, therefore, to use either cellulose derivatives or complex solvent

systems has made a rapid and easy determination of relative molecular masses (M_r) or relative molecular mass distributions (MWD) impossible. Cellulose derivatives are generally more soluble in organic solvents, such as tetrahydrofuran, methylethylketone or dimethylsulfoxide, but the derivatisation process may not be entirely uniform and may induce degradation of the polymer [1]. After the description of the capacity of the LiCl–*N,N*-dimethylacetamide (DMAc) system for the dissolution of cellulose [2] different procedures were described which involve sample activation by swelling in hot DMAc, liquid NH₃, water, or NaOH solution followed by solvent exchange and addition of LiCl [3]. The exact struc-

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ture of the cellulose–LiCl–DMAc complex has been the subject of many studies [4–6] and reviewed recently [7]. It is clear that no derivatisation occurs and that a true solution of cellulose is formed. It has also been reported that these solutions are stable for months at room temperature [8], and a 2–3% decrease in viscosity of a cellulose solution has been observed after being stored for 30 days at 30°C [3]. This indicates that the solvent itself does not have a pronounced degrading effect on pure cellulose.

Size-exclusion chromatography of cellulose in the LiCl–DMAc solvent system was first reported by Ekmanis in 1987 [9]. In this study, M_r were calculated using polystyrene calibration ($\log M_r$ vs. V_R). Since the hydrodynamic volume is the most important parameter in SEC, the application of polymer standards, chemically different to the sample, leads to relative values having only a comparative significance. Similarly, the same calibration method was used in a more recent study [10].

However, when the universal calibration method is used [11], the calibration curve $\log([\eta] \times M_r)$ vs. V_R may be applied to any polymer as long as the size-exclusion mechanism holds. The latter requirement is somewhat limiting and is not fulfilled in the case of SEC of cellulose in LiCl–DMAc, since the strong interactions between the solvent and cellulose influence its hydrodynamic volume. On the other hand, when the solute is a polystyrene standard, such interactions are minimal. The universal calibration concept thus also has limitations which have to be respected. It was used for M_r determination of cotton fibre cellulose [1]. Even without calibration, SEC is useful for a limited description of MWD. In this manner, chromatograms of four cellulose and two chitin samples were published [12].

Narrow distribution polysaccharide standards of a chemical composition very similar to cellulose have been the preferred choice of many authors for calibration [13–16]. The pullulan (polymaltotriose) standards differ from cellulose in that every third glycosidic bond is 1→6 (α) instead of 1→4 (β). Despite this structural difference, pullulans are also linear polymers and it may be assumed that interactions with the LiCl–DMAc solvent system are similar to those of cellulose which may also be true of the hydrodynamic volume. Pullulans are commercially available in a wide range of M_r . However,

until cellulose standards become available, we can only partially rely on the accuracy of SEC results.

The standard viscometric technique [17] is a fast and convenient method for determination of M_r of cellulose. It is invaluable for a rapid evaluation of material degradation during natural and artificial ageing. It requires dissolution in 0.5 mol l^{-1} $[\text{Cu}(\text{NH}_2\text{CH}_2\text{CH}_2\text{NH}_2)_2]^{2+}$ in an ethylenediamine–water mixture (the CED solvent system), which forms a stable complex with monomer units. The dissolution is fast and the whole procedure takes less than 1 h. The CED solvent system, however, has a pH of approx. 11 and the effect on the unstable oxidised cellulose molecule can be detrimental. Knowing this, some authors use a reduction step prior to the dissolution procedure [18–20] to attempt to stabilise the oxidised samples.

Our aim was to analyse oxidised cellulose samples by SEC using the LiCl–DMAc solvent system and compare the M_r values to those obtained by viscometry using the highly alkaline CED medium. The authors have shown in a previous contribution [21] that the number of oxidised groups introduced into cellulose during oxidation under conditions similar to those in this study is less than 1 per 100 glucose units. It may be estimated that this change in the chemical composition of the analyte causes no significant change in its hydrodynamic volume. Oxidised cellulose is especially sensitive to degradation due to the introduction of oxidised groups that induce chain breaking reactions in media of favourable (alkaline) pH. The mechanisms are generally well known [22]. To avoid any degradation during the sample dissolution and SEC analysis, we tried to keep all the critical parameters as optimal as possible. Therefore we chose to activate the cellulose by soaking in water prior to dissolution and SEC at room temperature.

It has been our intention to quantify the systematic error in M_r determination inherent to the viscometric technique when mildly oxidised samples are used. It should be noted, however, that most industrial procedures of cellulose production include an oxidising bleaching step so that all cellulose products are in fact already oxidised. In addition, we quantified the effect of the reducing step prior to viscometry and showed that the dissolution and SEC procedure we used is non-degrading.

2. Experimental

Cellulose from two different sources was used in the study: cellulose linters powder (Fluka, Buchs, Switzerland) and cellulose fibrous, long (Sigma, St. Louis, USA). Different samples were obtained by oxidation with $0.02 \text{ mol l}^{-1} \text{ H}_2\text{O}_2$, $0.02 \text{ mol l}^{-1} \text{ NaClO}$ and $0.01 \text{ mol l}^{-1} \text{ KIO}_4$, for 1, 2, 3 or 4 h without controlling the pH and without access of light. For each sample, 1 g of cellulose per 100 ml of the oxidising aqueous solution was stirred at room temperature (22°C). Subsequently, the samples were vacuum filtered through a filter paper with medium wide pores and rinsed with Milli-Q water and air-dried.

The samples were dissolved in LiCl–DMAc in the following way: 5 mg of sample was weighed into a 10-ml centrifuge tube into which was added 5 ml Milli-Q water, and left overnight to allow the fibres to swell thoroughly. The samples were centrifuged at 4000 rpm for 15 min, after which the supernatant was decanted and 5 ml of DMAc was added. After 15 min of heavy stirring with a PTFE bar, the centrifugation and decantation was repeated. The whole solvent exchange procedure was repeated three times. Finally 1.25 ml of 8% (w/v) LiCl in DMAc was added, stirred for 60 s and left for approximately 24 h to dissolve completely, with occasional mild stirring (100 rpm). The solutions were then transferred into 10-ml volumetric flasks and diluted with DMAc to give a concentration of 0.05% (w/v) cellulose and 1% (w/v) LiCl. All the DMAc solutions and the eluent were prepared with *N,N*-dimethylacetamide puriss. p.a. (Fluka, Buchs, Switzerland).

The chromatographic conditions were as follows: LDC/Milton Roy constaMetric IIIIG metering pump, Rheodyne injector system, PLgel 5 μm MIXED C 7.5×300 mm column (crosslinked polystyrene–divinylbenzene gel) with a PLgel 5 μm GUARD column 7.5×50 mm (Polymer Laboratories, Church Stretton, UK) and a Waters R401 differential refractometer detector. The eluent (1% w/v LiCl in DMAc) was pumped into the system at a flow-rate of 0.7 ml min^{-1} . The pressure was 1500 p.s.i. and the sample loop volume was 100 μl . Data from the detector was sampled using a PE Nelson 900 series interface and analysed with the PE Nelson 2600

size-exclusion chromatography software. The whole apparatus was operated at room temperature.

The pullulan standards (Polymer Laboratories) were prepared as mixed standards in three separate solutions, each containing 0.05% (w/v) of each standard in 1% (w/v) LiCl in DMAc. The first standard solution contained pullulan of the following peak molecular mass 1 600 000, 48 000 and 180 g mol^{-1} , the second contained 380 000, 12 200 and 738 g mol^{-1} , and the third contained 212 000, 5800 and 180 g mol^{-1} . Standards were weighed, transferred into 10-ml volumetric flasks into which 8 ml DMAc were added. It is imperative that the solutions are left overnight to clear without stirring to prevent degradation. Finally 1.25 ml of 8% LiCl (w/v) in DMAc were added and the flasks were made up to 10 ml with pure DMAc.

Intrinsic viscosities were determined using fresh CED solvent (Carlo Erba, Milano, Italy) according to the standard method [17]. A Schott CT150 thermostatted water bath was maintained at $25 \pm 0.015^\circ\text{C}$ and controlled by using a Beckmann thermometer.

To stabilise the cellulose, the weighed samples were reduced with $0.01 \text{ mol l}^{-1} \text{ NaBH}_4$ aqueous solution for 24 h at room temperature [23], filtered through a 5- μm PTFE filter to ensure a minimal loss of mass, rinsed with diluted acetic acid (pH 5), washed thoroughly with distilled water, dried and transferred into CED solvent together with the filter.

3. Results and discussion

The concentration of LiCl in the eluent varies greatly in all the mentioned texts, from 0.5 [1,10,13,14] to 5% (w/v) [12]. The lower concentration limit is determined by the stability of the cellulose solution itself. At higher LiCl concentration, the viscosity of the eluent increases. This introduces technical as well as methodical problems, for example an increased pressure and a decreased mass transfer in the columns. These were avoided by increasing the column temperature, usually to 80°C . After the preliminary SEC analysis we decided to use 1% LiCl (w/v) as the eluent so that the viscosity of the eluent and the column pressure remained low at room temperature. The composition of the three

standard solutions was chosen so that no neighbouring peaks overlapped. For construction of the calibration graph, each standard mixture was injected three times. The calibration graph is linear if the highest M_r calibration point is omitted, as it is near to the exclusion limit of the column. However, a third-order calibration curve was fitted to allow for the M_r calculation of samples having the highest M_r beyond the linear calibration region. The equation was $\log(M_r) = 38.62 - 8.49t_R + 0.736t_R^2 - 0.0228t_R^3$, where t_R is retention time in min ($r^2 = 0.9991$). The detector response of equimolar solutions of a pullulan standard and of a cellulose sample was proven to be the same and linear throughout the whole M_r region.

The concentrations of each single standard and of the samples were equal to minimise the error due to the concentration effects [24] as a result of reduction in hydrodynamic volume from molecular crowding, as shown by the following experiment. Five and two times more concentrated solutions of a pullulan standard, not used for calibration graph construction, were injected into the chromatographic system to test the accuracy of the peak molecular mass (M_p) determinations. The results were higher by 17 and 8%, respectively, while the reproducibility in the M_p determination of an equimolar standard solution was $\pm 1.5\%$. A similar reproducibility of M_r determination of samples was found. Three injections were made per sample regularly.

Chromatograms of untreated cellulose samples are shown in Figs. 1 and 2 along with the molecular mass averages. The mass distribution of the cellulose

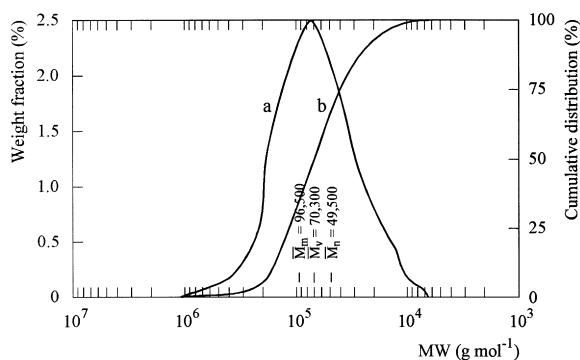


Fig. 1. Size-exclusion chromatographic mass fraction (a) and cumulative M_r distribution (b) plots for cellulose fibrous, long.

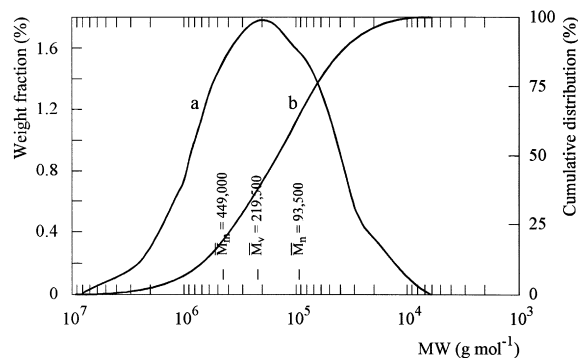


Fig. 2. Size-exclusion chromatographic mass fraction (a) and cumulative M_r distribution (b) plots for cellulose linters powder.

powder (Fig. 2) is broader and the average molecular mass is greater than fibrous cellulose (Fig. 1). In Table 1, the averages of three individual determinations for the unoxidised and oxidised cellulose samples are presented. The percent decreases in weight-average molecular mass (\bar{M}_w) and number-average molecular mass (\bar{M}_n), as determined by SEC, follow a similar pattern for both celluloses. However, the viscometric determinations of average molecular mass (\bar{M}_v) show a considerably higher sensitivity for the samples with higher M_r , i.e. the cellulose powder, while the difference in \bar{M}_v of the fibrous cellulose samples are almost within the precision of the method, that is, except for the most unstable KIO_4 -treated samples. \bar{M}_v calculated from viscosity determinations of a dissolved sample subjected to a reduction pre-treatment step, is presented as $\bar{M}_v(r)$. The significant difference between $\bar{M}_v(r)$ and \bar{M}_v for the most sensitive samples illustrate the stabilising effect of reduction prior to viscometry. Generally, viscometry appears to be a less sensitive method than SEC since the positive trend in decrease (%) in \bar{M}_n with duration of oxidation is in all cases bigger than in $\bar{M}_v(r)$.

The viscometric average molecular mass \bar{M}_v calculated from the limiting viscosity number $[\eta]$ using the Mark–Houwink–Sakurada equation is an important parameter for polymer description, although theoretically it is not an average and the mathematical relation to either \bar{M}_w or \bar{M}_n is not uniform. It is more influenced by high- M_r species. For the majority of polymers, $\bar{M}_w \geq \bar{M}_v \geq \bar{M}_n$ holds and it is true in our case. We used the parameters

Table 1
SEC and viscometric M_r averages of oxidised and unoxidised cellulose samples of two origins

Oxidant	Duration of oxidation (h)	Cellulose fibrous, long				Cellulose linters powder			
		\bar{M}_w (g mol ⁻¹)	\bar{M}_n (g mol ⁻¹)	\bar{M}_v (g mol ⁻¹)	$\bar{M}_v(r)$ (g mol ⁻¹)	\bar{M}_w (g mol ⁻¹)	\bar{M}_n (g mol ⁻¹)	\bar{M}_v (g mol ⁻¹)	$\bar{M}_v(r)$ (g mol ⁻¹)
—	—	96 500	49 500	69 200	70 300	449 000	93 500	219 126	219 623
H ₂ O ₂	1	87 000	48 700	69 400	68 800	409 000	92 300	215 500	217 500
	2	86 200	48 200	69 900	69 000	403 000	87 100	215 000	217 000
	3	85 700	47 300	69 500	68 700	396 500	82 200	212 000	217 000
	4	85 400	45 100	69 600	69 200	390 500	78 900	208 000	217 000
NaClO	1	84 400	37 400	69 600	69 600	415 500	84 900	210 000	210 500
	2	82 200	34 100	69 300	69 500	410 500	82 600	206 500	209 000
	3	82 000	33 300	68 900	68 400	408 500	77 300	198 000	203 500
	4	81 800	32 900	68 400	68 000	401 000	73 400	183 500	190 500
KIO ₄	1	95 900	49 500	65 900	70 000	443 500	93 200	156 000	211 000
	2	95 700	49 100	62 700	69 600	444 500	92 300	113 000	197 500
	3	95 800	48 700	60 400	69 700	444 000	92 100	105 500	193 500
	4	95 800	48 300	58 100	69 900	444 500	92 500	97 100	187 000

$K=1.1 \text{ g ml}^{-1}$ and $\alpha=0.85$ as proposed by Evans and Wallis [25]. The results are the average of three measurements, with the repeatability within 1%.

To be able to compare SEC and viscometric determinations, results are presented as the percent decrease in M_r compared to the M_r of an untreated sample. The percent decrease in \bar{M}_w and \bar{M}_n are presented in Fig. 3 for cellulose linters (powder), where the differences are more prominent.

It is interesting to observe the difference in the percent decrease in \bar{M}_w and \bar{M}_n for oxidised cellulose samples, which indicates that the way in which the three different oxidants react with the cellulose molecule is profoundly different. This has been discussed thoroughly elsewhere [22]. While H₂O₂, under acidic conditions attacks the cellulose molecule randomly and has a higher mobility in the fibres, NaClO under alkali conditions mainly attacks the fibres in the freely accessible regions, and besides chain scission, mostly acidic groups are formed. Periodate ions attack the cellulose molecules in a specific way producing only aldehyde groups, without breaking the polymer chain. The SEC results are consistent with these three different reaction routes. Certainly, the increasing percent of decrease in M_r depends on the time of oxidation (Fig. 3). While the drop in \bar{M}_w is practically the same for all reaction times and for both H₂O₂ and NaClO

oxidants, the exceptionally high drop in \bar{M}_n in the case of NaClO oxidation indicates more chain scissions producing low- M_r material, which has a more pronounced effect on the \bar{M}_n average. Consistent with the literature is the almost 0% decrease in both \bar{M}_w and \bar{M}_n for samples oxidised with KIO₄. However, this result leads to a more meaningful conclusion, namely, that although these oxidised cellulose samples are by far the most sensitive, during the sample dissolution procedure no additional degradation takes place.

The increasing trend in the percent of decrease of M_r is also typical for the \bar{M}_v averages, and is less pronounced in the reduced samples, indicating the importance of degradation reactions during dissolution. This is most striking in the case of samples oxidised with KIO₄, where the amount of aldehyde groups introduced during the oxidation treatment is the largest. While the SEC results prove that no scissions of the cellulose chain by KIO₄ oxidation takes place, we can observe from a comparison of $\bar{M}_v(r)$ and \bar{M}_v values (Table 1) that the reduction of aldehydes by a solution of NaBH₄ reduces the damage caused by the alkaline CED solvent, but does not totally prevent it. The reason may be that, while reduction of carbonyls is a rather slow process, the borohydride solution becomes increasingly alkaline due to the formation of sodium borate during

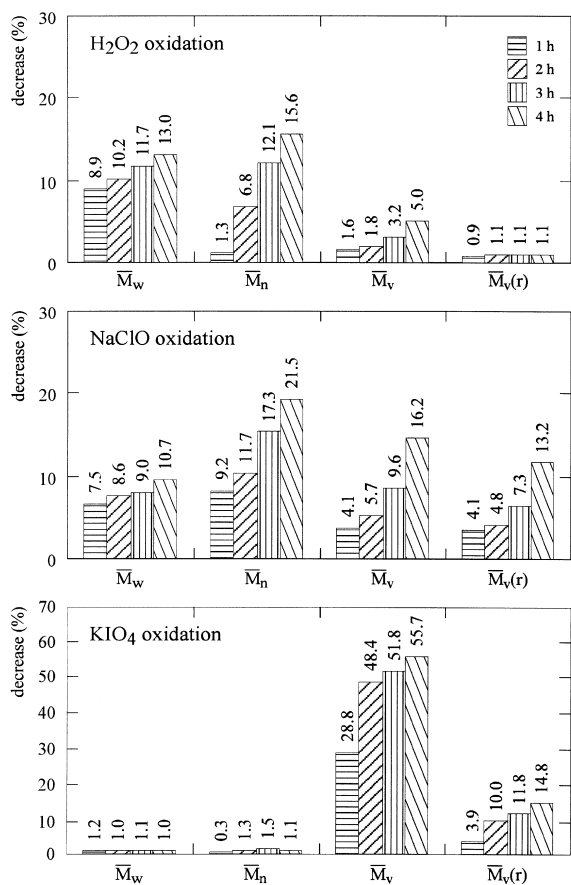


Fig. 3. Decrease of weight-average molecular mass (\bar{M}_w) and number-average molecular mass (\bar{M}_n) determined by SEC; viscometric average molecular mass (\bar{M}_v) and viscometric average molecular mass after the reduction pretreatment ($\bar{M}_{v(r)}$) for cellulose linters powder, oxidised with different oxidants; the duration of oxidation is indicated.

the treatment, thus causing the partial degradation of yet unreduced cellulose. The systematic error depends on the amount of carbonyl groups which, in turn, depends on the time of oxidation, and is between 26 and 56% for cellulose powder, but is reduced to 4–13% by the reduction pre-treatment.

4. Conclusions

The difficulties in the chemical characterisation of cellulose and its products have for many years limited its potential utility. Problems arise mainly

because of its densely packed structure and strong intermolecular hydrogen bonds which explains its solubility only in complex solvent systems, with the majority being highly degrading. Evaluation of material degradation during ageing was somewhat elusive because damaged cellulose molecules are very sensitive to degradation, caused by the carbonyl group content.

This study has revealed many new and valuable facts regarding analysis of oxidised or degraded cellulose. Most important is that the M_r and MWD data obtained by SEC indicate that the activation and dissolution procedure do not induce additional degradation even for the most sensitive KIO₄-treated cellulose samples. The effect of different oxidants showed that there was no detectable decrease of M_r of the KIO₄-treated cellulose samples but a pronounced decrease of M_r of the NaClO and H₂O₂-oxidised samples, this depending on the duration of oxidation.

The increased sensitivity of oxidised cellulose produces a systematic error in the most frequently used technique, the standard CED viscosity method, which until now had not been evaluated. With oxidised cellulose samples from two different sources we were able to compare the SEC and viscometric determinations of M_r of these sensitive samples and the systematic error, caused by degradation in the CED solvent, was shown to be up to 56%. However, when a stabilisation pre-treatment step was used, namely reduction prior to dissolution, this error, although not completely eliminated, is reduced by up to five times.

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