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Problems of the MMD analysis of cellulose by SEC using DMA/LiCl: A review

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Abstract

The state of the art in the determination of cellulose molecular mass (MM) by the size-exclusion chromatography (SEC) method during the last 10 years was examined. The separation of cellulose/pulp during SEC under conditions usually applied, i.e. 0.5–1% LiCl in N,N-dimethylacetamide (DMA) at 80°C on styrene-divinylbenzene packing, was recognized as pseudo-exclusion. The salt sensitivity, over-salting and polyelectrolyte interactions have been proposed as the secondary phenomena accompanying the separation of polysaccharides in DMA with 0.5–1% LiCl added. Chemical heterogeneity as well as the conformation and rigidity of the polymer backbone are the governing factors of the distorting effect. It has been concluded that the SEC technique requires further improvement aiming at optimising the ionic strength and pH of the mobile phase and determining the Mark-Houwink constants.

Keywords: Cellulose; N,N-dimethylacetamide; Size-exclusion chromatography; Non-exclusion effects; Mark-Houwink constants

1. Introduction

Cellulose is a commercially important material, and the determination of its molecular mass (MM) parameters (average M_w, M_n, and polydispersity) as well as the chemical uniformity is necessary. During the last 10 years, the size-exclusion chromatography (SEC) method using DMA/LiCl as a cellulose solvent and eluent has become the general technique for characterisation of cellulose by MM. This technique was applied to study the effect of pulping, bleaching, enzymatic treatment and viscose process to the MM of cellulose (Sjöholm, Gustafsson, Kolar & Pettersson, 1994; Sjöholm, Gustafsson & Lindström, 2000c; Hortling, Färm & Sundquist, 1994; Silva & Laver, 1997; Karlsson & Westermark, 1997; Strlič, Kolar, Žigon & Pihlar, 1998; Rahkamo, Viikari, Buchert, Paakkari & Suorti, 1998).

For the SEC method, the column calibration in terms of MM is an important task. Surprisingly, however, we did not find any indications in the literature to a direct calibration with cellulose samples. To estimate the cellulose MM, calibration with commercial polystyrene (Timpa, 1991; Striegel & Timpa, 1995; Silva & Laver, 1997; Sjöholm, Gustafsson & Colmsjö, 1999) or pullulan standards (Kennedy, Rivera, White, Lloyd & Warner, 1990; Hortling et al., 1994; Karlsson & Westermark, 1997; Strlič et al., 1998; Sjöholm, Gustafsson, Berthold & Colmsjö, 2000a; Sjöholm, Gustafsson, Eriksson, Brown & Colmsjö, 2000b) were used. Although the data obtained are relative, they can be applied to monitor the MM and MMD changes during the cellulose/pulp processing and the comparison of the samples (Silva & Laver, 1997; Strlič et al., 1998; Rahkamo et al., 1998). However, relative data do not characterise real MM properties and are practically unsuitable for describing and predicting the physico-chemical properties of cellulose.

To avoid this problem, Striegel and Timpa (1995) proposed a very convenient approach for MM determination using a viscometric detector (VD). Based on the principle of universal calibration, they evaluated MM for different polysaccharides utilising polystyrene standards for calibration. Additionally, they calculated Mark-Houwink constants for cellulose, pullulan, dextran, amylose and others in DMA/0.5% LiCl (80°C).

Some cellulose researchers (Westermark & Gustafsson, 1994; Hortling et al., 1994; Karlsson & Westermark, 1997; Rahkamo et al., 1998) have groundlessly concluded that pullulan and cellulose have the same hydrodynamic properties in DMA/LiCl and considered the pullulan MM as the cellulose MM. However, a drastic discrepancy between the MM from SEC and the standard viscometry method in cupri-diethylenediamine (CED) or cadoxen was noted. Recently, Sjöholm et al. (2000a) have well noted that

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Table 1
Mark-Houwink constants for cellulose and related polymers in DMA/LiCl solvent

Solvent	K 10^{-3}, ml/g	α	Reference
	Cellulose		
DMA/9% LiCl	1.278	1.19	McCormick et al., 1985
DMA/0.5% LiCl	0.794	1.00	Striegel & Timpa, 1995
	10.0	0.70	
	2.78	0.957	The present work
	Pullulan		
DMA/0.5% LiCl	0.25	1.10	Striegel & Timpa, 1995
	5.01	0.80	
	Polystyrene		
DMA	28	0.63	Welch, 1974
DMA/0.03 M LiBr/0.03 M H_3PO_4	12.1	0.69	Walker, 1988
DMA/0.5% LiCl	17.35	0.642	Timpa, 1991

cellulose and pullulan have a different relationship between the MM and elution volume, in other words, they are different in terms of hydrodynamic properties.

Another employment of the SEC method is the characterisation of wood pulp by the chemical composition. The cellulose originated from wood contains up to 20% of hemicelluloses, mainly xylan and mannan, as well as residual lignin and the lignin-carbohydrate complex (LCC). Since wood pulps chromatograms are bimodal, the second low-MM peak (10–30 kDa) was attributed to the hemicelluloses present in pulp samples (Westermark & Gustafsson, 1994; Hortling et al., 1994; Silva & Laver, 1997). Sjöholm et al. (2000b) have recently shown that hemicelluloses, i.e. xylan and mannan, both for soft and hardwood pulps eluted along the whole MM scale under SEC conditions applied. The authors have proposed the influence of the dissociation of uronic acids as well as the aggregation of macromolecules in solution on hemicellulose separation during SEC.

Residual lignin in pulps, detected by UV at 295 nm, was inadequate to this polymer MM distributed along the MM scale under the SEC conditions used for pulp analysis (Hortling et al., 1994; Westermark & Gustafsson, 1994; Karlsson & Westermark, 1997; Sjöholm et al., 1994, 1999, 2000b,c). From residual lignin elution in the high MM range (up to 10^7 Da), its sorption onto cellulose fibrils, as well as a chemical bond with cellulose have been suggested (Westermark & Gustafsson, 1994; Karlsson & Westermark, 1997).

Thus, there are many questions concerning the cellulose/pulp characterisation by SEC using DMA/LiCl as an eluent. The aim of the present paper was to discuss the state of the art in determining the MM of cellulose.

2. Mark-Houwink constants

The principle of universal calibration based on the separation of polymers according to their hydrodynamic volumes ($[\eta]M$), i.e. two polymers having equal hydrodynamic volumes elute with the same elution volume, was applied to cellulose MM calculation using the polystyrene or pullulan standards. This approach requires the application of Mark-Houwink constants estimated in a solvent used as an eluent for the polymer-calibrant and polymer under analyses. However, none of the authors mentioned above indicated the Mark-Houwink constants applied for cellulose, therefore, the relative MM for cellulose was shown.

Table 1 lists the Mark-Houwink constants cited in the literature for cellulose and polymers applied as calibrants, i.e. pullulan and polystyrene, determined in a DMA/LiCl solvent. To choose the correct constants, the well-known relationship between Mark-Houwink constants, i.e. $\lg K = C - B\alpha$ (Aharoni, 1977), was estimated using data for a wide range of solvents (Kurata, Tsunashima, Imata & Kamada, 1975; Gruber & Gruber, 1981; Kato, Okamoto, Tokuya & Takahashi, 1982; Dawkins, Gabbott, Montenegro, Lloyd & Warner, 1990). Fig. 1 shows $\lg K = f(\alpha)$ dependencies, which clearly reveal the distinctions in hydrodynamic properties for the mentioned polymers. Aharoni (1977) found the effect of the polymer backbone stiffness on the K-α dependence. Typical equations, $\lg K = 0.58 - 3\alpha$ and $\lg K = 1.45–4.63\alpha$ (ml/g), were obtained in the case of flexible and rigid polymers, respectively. In the present work, the K–α equations and K_θ values determined for the above-mentioned polymers were as follows:

cellulose: $\lg K = 2.006–4.567\alpha$; $K_\theta = 0.528$
pullulan: $\lg K = 1.48–4.648\alpha$; $K_\theta = 0.142$
polystyrene: $\lg K = 0.911–3.998\alpha$; $K_\theta = 0.081$
amylose: $\lg K = 1.225–4.437\alpha$; $K_\theta = 0.101$
dextran: $\lg K = 1.511–4.814\alpha$; $K_\theta = 0.127$.

The polymers can be arranged according to the backbone rigidity as follows: cellulose $\gg$ dextran $\geqq$ pullulan $\geqq$ amylose $\gg$ polystyrene. This line indicates that, at the same MM value, the cellulose macromolecule has a higher hydrodynamic volume than that of other mentioned polymers under equal solution conditions (solvent, temperature) due to higher backbone rigidity. Therefore, at the same MM value, the former will elute earlier than the latter under equal SEC conditions. Thus, the statement that cellulose and pullulan have the same hydrodynamic properties is erroneous.

Pullulan has practically become a standard calibrant for cellulose MM analysis (Kennedy et al., 1990; Hortling et al., 1994; Karlsson & Westermark, 1997; Sjöholm et al., 2000a,b). However, the hydrodynamic properties of pullulan appear to be insufficiently investigated. Only several Mark-Houwink equations for it in aqueous solutions were found in the literature (Kato et al., 1982; Dawkins et al., 1990). The pair $K = 5.01 \times 10^{-3}$ (ml/g) and $\alpha = 0.8$, determined in DMA/0.5% LiCl by Striegel and Timpa (1995), that lays on the straight line, can be apparently applied for further calculations.

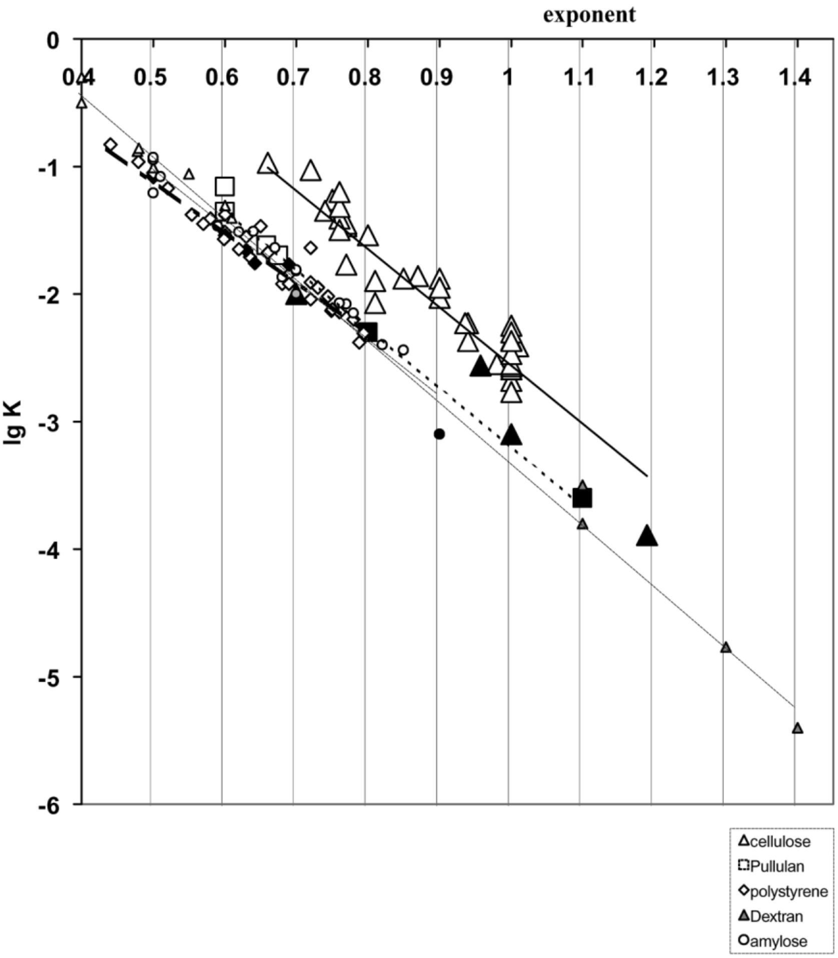

Fig. 1. Relationship between exponent α and K values in Mark-Houwink equation for cellulose (≙), pullulan (□), polystyrene (◇), amylose (○) and dextran (▽) in a wide range of organic and aqueous solutions. Bold symbols for DMA solvent.

Fig. 1 illustrates that the Mark-Houwink constants for polystyrene in DMA/0.5% LiCl at 80°C, $K = 1.73 \times 10^{-2}$ (ml/g) and $\alpha = 0.642$, determined by Timpa (1991), can be used for calculations. These are in good agreement with constants for DMA and DMA in the presence of 0.03 M LiBr and 0.03 M H_3PO_4 (Table 1).

For cellulose, the equation $\lg K = 2.01\ \alpha - 4.56$ (ml/g) was evaluated from numerous data available in the literature (Kurata et al., 1975; Gruber & Gruber, 1981). From Fig. 1, it is evident that, in the case of the DMA/LiCl solvent, the Mark-Houwink pairs found by McCormick, Callais and Hutchinson (1985); Striegel and Timpa (1995) do not satisfy the $\lg K$ (α) dependence obtained. Moreover, the equation with the exponent 1.19, evaluated at a high LiCl content (9%) by McCormick et al. (1985), is not corresponding to the SEC conditions where 0.5–1% LiCl is usually applied. From the data presented by Hortling et al. (1994), we calculated the following Mark-Houwink equation: $[\eta] = 2.78 \times 10^{-3} M^{0.96}$ (ml/g). The standard $\lg[\eta]$–$\lg M$ plot was applied. Although this K–α pair satisfied the $\lg K(\alpha)$ dependence for cellulose (Fig. 1), we did not consider it as optimal. Rather a high exponent resulted from the Mark-Houwink constants applied for M_v determination in CED, cellulose samples of broad MMD used etc. In addition, the effect of MM on the conformation of cellulose backbone should be taken into account. Without going to the heart of the theory, short-chain macromolecules are stiffer and are characterised by a high exponent (about 1), whereas long-chain macromolecules are less rigid, and the exponent is obviously in a 0.7–0.8 range (Gruber & Gruber, 1981; Tscvetkov, 1986). Hence, the Mark-Houwink constants for cellulose in DMA/LiCl must be defined precisely taking into account the DP range.

We also re-examined the Mark-Houwink constants estimated by Striegel and Timpa (1995) based on the same technique for dextran and amylose. From Fig. 1, it is obvious that, in the case of amylose, only the pair $K = 10^{-3}$ and $\alpha = 0.7$ lays on the straight line plotted from numerous data (Kurata et al., 1975). Dextran is a commercially available MM standard (Pharmacia Fine Chemicals), which could be applied as a calibrant. For dextran, Fig. 1 shows that the Mark-Houwink constants in DMA/0.5% LiCl (Striegel & Timpa, 1995) prolong the $\lg K$-α line obtained byKurata et al. (1975). Formally, any K-α pairs on the straight line (e.g. with $\alpha = 10$) could be used, but the intrinsic viscosity theory restricts the value in terms of the exponent in a 0.2–1.7 range (Tscvetkov, 1986). The values of the exponent in a range of 1.1–1.8, evaluated for $MM < 250$ kDa from the SEC–VD technique (Striegel & Timpa, 1995), do not correspond to the hydrodynamic properties of dextran in dilute solutions. On the other hand, the dextran with $MM > 500$ kDa was either partly excluded from the column or eluted in the void volume. From the double log plot lg[η] – lg M, we calculated another equation, $[\eta] = 0.087\ M^{0.55}$ (ml/g), employing the Mw data provided by Pharmacia Fine Chemicals and the experimental [η] values indicated by Striegel and Timpa (1995). This K–α pair is in agreement with the one determined for dextran in DMF, 0.0165 and 0.49 (Kurata et al., 1975), calculated for DMSO (30°C) from data by Minor (1979), 0.0192 and 0.42, as well as for DMSO/0.03 M H_3PO_4 (0.063 and 0.57), estimated by us earlier.

Considering all these aspects, the violation of the pure steric separation mechanism of polysaccharides in DMA/LiCl was evident. The separation was pseudo-exclusion owing to some secondary effects, which appeared depending on the structure of the compared polysaccharides. Apparently, inappreciable polymer–polymer and/or polymer–solvent interactions for polysaccharides in a solution of DMA with LiCl added occur, which affect the hydrodynamic volume as well as the elution behaviour and separation during SEC. The general requirement of all SEC techniques elaborated for evaluation of Mark-Houwink constants is the full elimination of any secondary effects during SEC. Otherwise, the formal utilisation of these approaches leads to grave mistakes.

3. Separation mechanism

We did not find in the literature any indications on the elution behaviour of cellulose during SEC in the DMA/LiCl eluent using a styrene-divinylbenzene co-polymer. The latter was the packing material of all the columns applied for cellulose analyses, i.e. μ-Styragel (Westermark & Gustafsson, 1994;Karlsson & Westermark, 1997), Ultrastyragel (Timpa, 1991; Striegel & Timpa, 1995; Silva & Laver, 1997), PLgel (Kennedy et al., 1990; Hortling et al., 1994; Strlič et al., 1998; Sjöholm et al., 1994, 1999, 2000a–c).

Cellulose is considered as an electroneutral polymer. However, it is well known that cellulose contains ionogenic carboxyl groups originated due to the oxidation destruction during pulping and bleaching. In addition, in the case of wood pulp, admixture polysaccharides (xylan, mannan) also contain carboxyl groups. In the case of unbleached and semi-bleached wood pulps, residual polyelectrolyte lignin and LCC cannot be ignored. In our opinion, ion expansion and salt sensitivity are secondary effects that accompany the cellulose and admixtures separation on the electroneutral styrene-divinylbenzene packing in the DMA/LiCl eluent. Similar phenomena were observed with cellulose nitrates, which were also considered as electroneutral, using Styragel and Zorbax PSM S columns with a THF eluent (Eremeeva, Bykova & Gromov, 1990; Bikova, 1991).

In our opinion, water traces play a significant role for the dissociation of cellulose/pulp constituents in DMA/LiCl. The negative effect of water on separation in polar solvents during SEC was noted (Coppola, Fabbri & Palesi, 1972). LiCl is a highly hygroscopic substance. In fact, water is always present in the DMA/LiCl system, has a pronounced influence on the solvent polarity (Potthast, Rosenau, Buchner, Ebner, Roder & Kosma, 2000) and induces the polyelectrolyte-type interactions for cellulose/pulp in the $\{DMA + x LiCl + y H_2O\}$ system.

Apparently, cellulose researches conclude that the LiCl present in DMA is safe against the undesirable effects during SEC. However, some aspects regarding low-MM electrolyte effects cannot be ignored.

Usually, the concentration of LiCl in the eluent is 0.5–1%, i.e. 0.11–0.22 M, which results from the cellulose dissolution procedure. However, from the standpoint of the elimination of the polyelectrolyte effect during SEC, the 0.1–0.2 M ionic strength of the eluent is rather high and can result in an over-salt effect (Barth, 1980). The low-MM electrolyte added into the polar solvent could both increase and decrease the viscosity of the polymer solution (i.e. the hydrodynamic volume of a macromolecule) depending on the stiffness of the polymer backbone (Lirova, Ljutikov & Babich, 1985). We observed a strong influence of LiCl and LiBr on the hydrodynamic properties of cellulose nitrates in THF, DMF and DMA depending on the degree of substitution (Bikova, 1991). Apparently, Striegel and Timpa (1995) observed a summated salt effect, when the polysaccharides differing in the backbone rigidity and chemical composition were compared by SEC–VD in DMA/LiCl. Therefore, the optimisation of the low-MM electrolyte concentration is an obvious but very complex problem, which must take into account different aspects of the polymer (also packing) structure.

Variations in ionic strength (salt concentration) are not enough to eliminate some interactions, and an additional change in the pH of solvent/eluent is necessary. It has been found that, during SEC on a styrene-divinylbenzene co-polymer, DMF and DMA solvents adjusted to the pH 2–6 with inorganic acid should be used as an eluent (GPC Hitachi, 1985). Nefedov, Lazareva, Belenkii, Frenkel, Ja and Koton (1979) found that the acid–salt buffer fully suppresses the secondary effects during the SEC of polyelectrolytes in amidic solvents. In the case of hemicelluloses, lignin and LCC, 0.03 M LiBr with 0.03 M H_3PO_4 were added into DMSO and DMF to eliminate the polyelectrolyte effects during SEC (Rusakov, Lazareva, Nefedov, Shcherbakova, Ivanov & Zakharov, 1982; Zakharov, Nefedov, Lazareva, Ivanov & Ivanov, 1989; Eremeeva & Khinoverova, 1990). In addition, the problems concerning the cellulose macromolecule aggregation in DMA/LiCl (Sjöholm et al., 2000b), as well as the inadequate separation of lignin and hemicelluloses under SEC conditions employed for pulp analyses (Sjöholm et al., 1999, 2000a) could be solved by varying the pH of the mobile phase.

It should be noted that the DMA/LiCl solvent is not quite convenient as an eluent owing to its high viscosity. Moreover, Cl ions are aggressive to the chromatography equipment in the presence of water at elevated temperature (analyses are usually carried out at 80°C). It would be more useful to replace it by another eluent, e.g. DMF. This is a well-known approach in the SEC method. A good experience has been shown for polysaccharides: cadoxen is a cellulose solvent and 0.5 N NaOH is an eluent (Bao, Bose, Ladisch & Tsao, 1980; Eremeeva, Khinoverova & Treimanis, 1988); DMSO is a solvent for hemicelluloses and DMF is an eluent (Eremeeva & Khinoverova, 1990).

Finally, a mistake apparently occurred as a result of the application of viscometric and LALLS detectors. It should be emphasised that detectors do not recognise the secondary effects during SEC. It is no matter whether ion exclusion, ion expansion, sorption, etc. occurs, the viscometer and LALLS monitor the macromolecule size under the chromatographic conditions applied. Therefore, a careful investigation of the concentration dependence (Barth, 1980; Rusakov et al., 1992; Eremeeva et al., 1990) can only reveal the real mechanism of polymer separation. Additionally, the determination of the void volume of the column set is very important for the recognition of the secondary effects. From the real chromatograms presented by Westermark and Gustafsson (1994); Karlsson and Westermark (1997); Silva and Laver (1997); Strlič et al. (1998); Sjöholm et al. (2000b), it is evident that cellulose/pulp is partly eluted with a void volume of the columns applied.

4. Conclusions

An inadequate elution of wood components and the incompatible Mark-Houwink constants from SEC are indicative of a pseudo exclusion separation of cellulose/pulp in DMA/LiCl on the styrene-divinylbenzene packing. The separation according to the degree of dissociation and salt effect, as well as polymer–polymer (aggregates) and polymer–solvent interactions resulting in an increase in the hydrodynamic volume are secondary phenomena distorting the pure steric mechanism separation of polysaccharides. The chemical heterogeneity of the sample under analysis is a governing factor of the violating effects. Apparently, some previous conclusions from SEC should be re-examined after the full elimination of the secondary effects. Thus, in spite of a 15-year history of the SEC of cellulose in DMA/LiCl, there are still many problems to be solved, and much work including both chromatographic and polymeric studies must be done. We tried to be free from criticism. We aimed to analyse the mistakes and to find constructive ways for determining reliable MM data for cellulose in the commercially important DMA/LiCl cellulose solvent. Some primary chromatographic tasks to be solved connected with the separation mechanism are as follows:

1. Careful study on the elution behaviour of cellulose taking into account the degree of oxidation and admixtures such as hemicelluloses, LCC and lignin; LALLS and viscometric detectors are especially useful for this purpose.
2. Elimination of all distorting interactions (macromolecules association, polyelectrolyte) during the SEC of cellulose (hemicelluloses, lignin) by optimisation of the salt content and the pH of the mobile phase.
3. Re-examination of the mechanism of separation of calibrants, i.e. pullulan and polystyrene, under SEC conditions achieved.
4. Correct Mark-Houwink constants for cellulose (hemicelluloses, lignin, etc) in DMA/xLiCl/yH + at (t) must be determined.
5. The validity of the universal calibration between the calibrant and cellulose must be experimentally proved.
6. The DMA/LiCl eluent may be apparently replaced by the one more suitable for SEC.

As can be seen, there is a long distance to be covered from the declaration of the ability to analyse to the determination of correct MM data.

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