ELSEVIER

Journal of Chromatography A, 964 (2002) 47–54

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Enthalpic interactions in size exclusion chromatography of pullulan and cellulose in LiCl–*N*,*N*-dimethylacetamide

Matija Strlič[a,*], Jana Kolenc[a], Jana Kolar[b], Boris Pihlar[a]

[a]*Department of Chemistry and Chemical Technology, University of Ljubljana, Aškerčeva 5, SI-1000 Ljubljana, Slovenia*
[b]*National and University Library of Slovenia, Turjaška 1, SI-1000 Ljubljana, Slovenia*

Received 24 September 2001; received in revised form 12 March 2002; accepted 8 May 2002

Abstract

Enthalpic phenomena were shown to contribute to the size exclusion separation mechanisms during chromatographic analysis of solutions of pullulan and cellulose in LiCl–*N*,*N*-dimethylacetamide (LiCl–DMAc) solvent and eluent. The effect of LiCl concentration in the sample solutions and the effect of temperature were of the same order of magnitude for both pullulan and cellulose samples. This led to systematic errors in the determination of mean molecular mass in the range of tens of percent, depending on the chromatographic conditions and on the molecular mass of the analyte. The systematic error is much higher than the random errors; the typical values of the latter being up to a few percent (RSD). Low column temperature and a higher content of LiCl in the sample solution led to lower determined mean molecular mass values. This can be explained by a decrease in the interactions between dissolved macromolecules, although polymer–stationary phase interactions should also be taken into account. Furthermore, the cellulose stability in solution was determined: the zero order random degradation constant being $k = 6.9 \times 10^{-8}$ mol $\text{mol}_{\text{monomer}}^{-1}$ day^{-1}.

Keywords: Enthalpic interactions; Pullulan; Cellulose

1. Introduction

Size exclusion chromatography (SEC) of non-derivatised cellulose in the solvent and eluent *N*,*N*-dimethylacetamide (DMAc) containing LiCl has been used for characterisation of cellulose since the introductory work by Ekmanis in 1986 [1] and was already the subject of a number of reviews [2,3]. The data obtained from size exclusion chromatograms include mean molecular mass (MMM) and distribution (MMD), which are important parameters in material processing and degradation, and in biochemical and biological studies, etc. Since cellulose is a linear homopolymer (although in pulp samples, limited differences in the polysaccharide composition, i.e. ratio of monosaccharides, were recently shown to lead to fractionation in SEC [4]), it exhibits distribution in only one property—chain length. It would seem therefore that its characterization is straightforward.

However, there are still several reasons why this method of molecular characterization is not more widely used. A notable drawback is the dissolution procedure involving swelling in water, solvent exchange and finally dissolution in DMAc containing 8% LiCl (w/v), typically. The whole procedure is

*Corresponding author. Tel.: +386-61-176-0543; fax: +386-61-125-8220.
E-mail address: matija.strlic@uni-lj.si (M. Strlič).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00591-5

lengthy and may last for a few days. The early references, reviewed in Ref. [5], on the solubility of cellulose in this solvent, were encouraging and reported on solutions of up to 17% cellulose in up to 11% (w/v) LiCl in DMAc with apparently little or no degradation. That the dissolution procedure is indeed mild was shown in a chromatographic study using oxidised cellulose [6]. Certainly, due to the corrosive properties of the salt and due to the high viscosity of such solutions, low concentrations of LiCl in the eluent are desirable, and usually 0.5% [4,6–19], 0.75% [20], 0.9% [21] or 1% (w/v) [22,23] of the salt are used, rarely more, e.g. 5% (w/w) [24]. In the latter case, the increased eluent viscosity lead to a decrease in mobile phase flow-rate to 0.1 ml min^{-1}.

For the same reason, and due to increased mass transfer, the column temperature that is usually in use, is 80 °C. In some studies the columns were kept at room temperature [6,24], 25 °C [21], or 30–45 °C [8]. In ideal SEC, the column temperature should not influence the elution volumes unless other separation mechanisms are in force. Namely, the chromatographic retention of macromolecules is conveniently described with the distribution coefficient K:

$$\ln K = -\frac{\Delta G}{RT} = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$$

for the distribution of the macromolecules between the stationary and the mobile phase, where ΔG, ΔS and ΔH are changes in Gibbs free energy, entropy and enthalpy, respectively; R is the gas constant and T is the temperature [25]. If separation of macromolecules is governed by size exclusion, enthalpic processes should be negligible ($\Delta H = 0$) and the distribution coefficient K temperature independent.

While enthalpic interactions will affect both relative and universal calibration results in SEC, they will not affect molecular mass data determined with light scattering detection as long as these effects do not result in local polydispersity. However, if an absolute chromatographic detector is not available, a further drawback is the absence of cellulose standards (reference samples) with narrow MMD. Data of comparative value may be obtained even without column calibration [24]; however, universal column calibration with polystyrene standards is usually used in such cases [10–16,18,19] and is valid if enthalpic contributions during chromatographic separation are negligible. Using a light-scattering detector, Striegel and Timpa [14] presented remarkably overlapping universal calibration curves of polystyrene and pullulan. The commercially available linear polysaccharide standards, pullulans, are an often used alternative to universal calibration [4,6,8,9,17,22,23]. The assumption that the solution behaviour of both pullulan and cellulose samples is similar, as both macromolecules are linear polysaccharides, might hold, as it has been shown that the MMM of a cotton linters sample measured by light scattering was in close agreement with the MMM determined relative to pullulan standards using LiCl–DMAc size exclusion chromatography [17]. Although this result is encouraging, the same authors also showed that aggregation of cellulose in the LiCl–DMAc solvent may occur and proposed a mechanical deaggregation treatment. This is in agreement with earlier studies [5], as complete dissolution of cellulose aggregates is expected only at LiCl concentrations >6% (w/v), even at low cellulose concentrations. Aggregation was shown to be concentration-dependent and it was observed that in freshly prepared cellulose solutions the formation of aggregates takes place in the time frame of a few days [21]. Under certain conditions and at considerably higher concentrations of cellulose than usually used for SEC, aggregates of several macromolecules, packed side-by-side in the extended conformation, were shown to be stable [26]. On the other hand, Striegel and Timpa [14] showed that pullulan aggregation is also possible. Considering the sometimes contradictory studies by different authors, it seems that the calibration in SEC of cellulose is not a clear issue and needs to be studied in more detail. It also seems that interactions between macromolecules are difficult to overcome and may be a source of systematic errors, as recently stressed [27]. These interactions may not be the only enthalpic phenomenon, as interactions between the stationary phase and the analyte are also a possibility and, as demonstrated recently [28], they may additionally depend on the way the stationary phase (typically polystyrene–divinylbenzene) is synthesised by the producer. Since size exclusion chromatography is ideally based on separation on the basis of hydrodynamic volume of the analyte (which is in some way proportional to the size of the macromolecule)

at the same time avoiding interactions between molecules, and between molecules and the column packaging, it was our intention to investigate these enthalpic interactions in order to be able to estimate the inherent errors.

2. Experimental

Purified cellulose from three different sources was used in the study: cellulose linters powder (Fluka, Buchs, CH, designated as FP), cellulose fibrous, long (Sigma, St. Louis, MO, USA, designated as SL) and cotton cellulose (Whatman filter paper No. 1, designated as WH).

The samples were dissolved in LiCl–DMAc in the following way: 10 mg of sample was weighed into a 10-ml centrifuge tube into which 5 ml Milli-Q water was added and left overnight to allow the fibres to swell thoroughly. The samples were centrifuged at 4000 rpm for 5 min after which the solvent was decanted and 5 ml of methanol was added. After 30 min of stirring, the centrifugation and decantation was repeated. In turn, methanol was exchanged with DMAc. The solvent exchange procedure was repeated five times. Finally, 1.25, 2.5, or 3.75 ml of 8% (w/v) LiCl (dry, puriss. p.a., Fluka, Buchs, Switzerland) in DMAc was added, stirred for 60 s and left at 4 °C until dissolution, with occasional stirring at low rpm. Although less laborious procedures for sample preparation exist in the literature, the solvent-exchange procedure was used as it is the least degrading for the samples. The solutions were then transferred into 10-ml volumetric flasks, diluted with DMAc to give a concentration of 0.1% (w/v) cellulose and filtered through PTFE filters (2 μm) prior to injection. Since for different series of samples different volumes of the 8% LiCl solution were added, its final concentration prior to injection was 1, 2 or 3% (w/v).

All the DMAc solutions and the eluent were prepared with *N,N*-dimethylacetamide for chromatography (Aldrich, Gillingham, UK). The eluent was filtered through an on-line stainless-steel filter (0.45 μm).

A Hewlett-Packard series 1100 chromatographic system was used, with RI differential refractometric detection (detection cell temperature: 40 °C) and a column thermostat. The sample volume was 200 μl. The columns used were PLgel (crosslinked polystyrene–divinylbenzene gel, Church Stretton, UK) 5 μm GUARD column 7.5×50 mm, 5 μm MIXED C 7.5×300 mm and a 10-μm MIXED B 7.5×300 mm. The eluent (0, 0.5 or 1% w/v LiCl in DMAc) was pumped into the system at a flow-rate of 0.5 ml min^{-1}. The chromatographic data was processed with HP G2182AA data analysis software.

The pullulan standards (Polymer Laboratories) were prepared as mixed standards in two separate solutions containing 0.025% (w/v) of each standard in DMAc. The first standard solution contained pullulans of the following peak molecular masses (M_p): 1 600 000, 100 000, 23 700, and 5800 g mol^{-1}, the second contained 380 000, 48 000, 12 200, and 738 g mol^{-1}. The standards were weighed, transferred into 10-ml volumetric flasks and dissolved in DMAc. Finally, appropriate volumes of 8% LiCl (w/v) in DMAc were added and the flasks were made up to 10 ml with DMAc. In the same way, two separate solutions of polystyrene standards were prepared, the first containing standards with M_p: 2 000 000, 200 000, and 20 400 g mol^{-1}, the second solution containing standards with M_p: 775 000, 97 200, and 10 300 g mol^{-1}.

The M_w (weight average molecular mass) and M_n (number average molecular mass) values of the three cellulose samples, determined at column temperature 80 °C, *c*(LiCl) in the eluent and the sample solution 1% (w/v), relative to pullulan calibration curve (experimental points fitted by a third order polynomial to allow for extrapolation), obtained under the same chromatographic conditions, are the following: SL: $M_n = 27\,900$ g mol^{-1}, $M_w = 68\,700$ g mol^{-1} (RSD = 0.6%); FP: $M_n = 81\,000$ g mol^{-1}, $M_w = 264\,000$ g mol^{-1} (RSD = 5.1%); WH: $M_n = 140\,500$ g mol^{-1}, $M_w = 537\,000$ g mol^{-1} (RSD = 4.6%). The results were obtained with triplicate injections of the two separately prepared solutions.

3. Results and discussion

In contrast with cellulose, dissolution of pullulans proceeds more easily in DMAc without an addition of LiCl. If a solution of standards in DMAc without

an addition of LiCl is injected onto a column and eluted with DMAc, again without an addition of LiCl, the separation is incomplete and the four standards elute as a single broad peak. Separation is possible as soon as some LiCl is added to the eluent, indicating that the dissolution processes as described in Ref. [5] lead to a decrease in intermolecular interactions between pullulan macromolecules. Furthermore, at a constant content of LiCl in the mobile phase (1% w/v, experiments done at 80 °C), an increased content of LiCl in the solutions of pullulan standards causes molecules of a higher molecular mass to elute later and those of a lower molecular mass to elute earlier. The comparison of slopes of calibration lines (excluding the 1 600 000 g mol^{-1} pullulan standard, which elutes close to the exclusion volume) is presented in Fig. 1. The separation interval (the volume difference between elution of the highest-M_p and lowest-M_p pullulan standard) decreases with an increasing concentration of LiCl in the solution of standards, and so does the slope of the calibration line.

An increased content of LiCl in the solutions of standards does not have a uniform effect on the higher- and lower-molecular mass pullulan standards. Hypothetically, any combination of the polymer–polymer interactions (i.e. their decrease in the case of higher molecular masses would cause such molecules to elute later) or polymer–stationary phase interactions (i.e. their decrease in the case of lower molecular masses would cause such molecules to elute earlier) or preferential solvation of lower molecular-mass material (in this case the molecules with a lower molecular mass would again elute earlier) could explain such irregular behaviour.

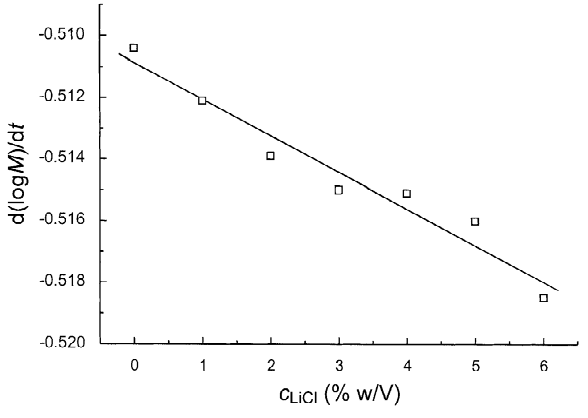

Fig. 1. The calibration line slope depending on the content of LiCl in the standard solution. Content of LiCl in the eluent: 1% (w/v); column temperature: 80 °C.

Since preparation of cellulose solutions with a higher content of LiCl might be easier and might proceed more quickly, the effect of a higher content of LiCl in the sample solution is worth to be studied. A series of solutions of a pullulan standard (M_p = 212 000 g mol^{-1}) was therefore prepared with various contents of LiCl. The M_p was determined relative to the calibration curve prepared with standard solutions containing 1% (w/v) LiCl (Table 1).

The results show that a higher content of LiCl in the pullulan sample solution results in lower determined peak molecular mass, which indicates lower intermolecular interactions or de-aggregation for which there is evidence in the literature [14]. The differences may amount up to a few tens of percent, and depend on the MMM. Since the chromatographic conditions (eluent composition and column temperature) are the same, only the concentration of LiCl in the solutions of the injected pullulan sample is varied, the differences in expansion coefficients of the solute are less probable to account for these results. The introductory experiment with no LiCl in the solution of pullulan standards, where separation was possible using the eluent containing 0.5% LiCl (w/v), but not with the one containing no LiCl, is consistent with this conclusion. That the procedure of preparation of cellulose solutions may affect the aggregation process was already demonstrated, also at low concentrations (0.3% [26]).

The comparison of polystyrene and pullulan calibration curves gives further indications of enthalpic

Table 1
Relative error in the determination of peak molecular mass of a standard pullulan sample (M_p = 212 000 g mol^{-1}) prepared in solutions with various concentrations of LiCl (w/v)

c(LiCl) (%)	Relative error (%)
0	−2
1	0
3	−13
6	−23

Column temperature: 80 °C; LiCl content in the eluent: 1% (w/v).

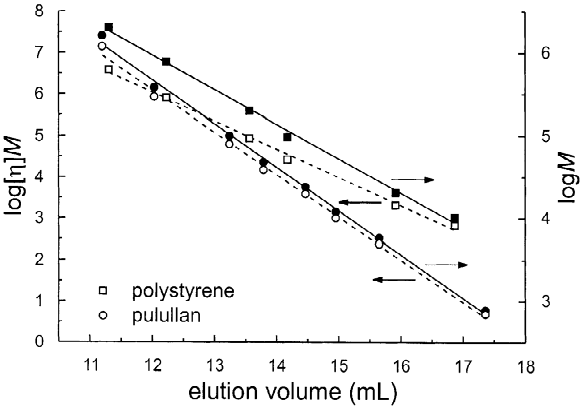

Fig. 2. Comparison of calibration and Mark–Houwink universal calibration curves for polystyrene and pullulan standard samples in 0.5% (w/v) LiCl–DMAc eluent–solvent at column temperature 80 °C.

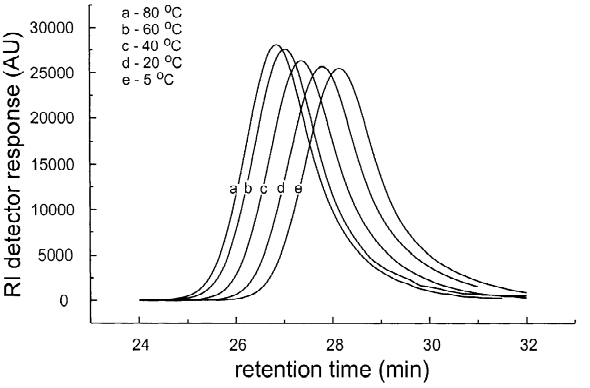

Fig. 3. Chromatograms of a pullulan standard ($M_p = 100\,000$ g mol^{-1}) dissolved in 1% (w/v) LiCl–DMAc, at column temperatures as indicated; eluent: 1% (w/v) LiCl–DMAc.

interactions (Fig. 2). Approximate hydrodynamic volumes can be calculated using the Mark–Houwink–Sakurada parameters for polystyrene in 0.5% (w/v) LiCl–DMAc: $a = 0.642$ and $K = 1.73 \times 10^{-4}$ ml g^{-1} [10] and for pullulan: $a = 0.95$ and $K = 1.12 \times 10^{-5}$ ml g^{-1}, mean values from [12]. In our case, the universal calibration curves (or, in fact, Mark–Houwink calibration curves, since they are based on constants obtained from the literature) do not overlap (Fig. 2, dashed lines), which again indicates that parallel to size exclusion, enthalpic mechanisms govern the separation. The validity of universal calibration should therefore be confirmed prior to its use, for each particular chromatographic system.

As already indicated in Section 1, in ideal SEC, the column temperature should only influence the peak width, but not its position. Due to increased viscosity of the eluent containing 1% LiCl (w/v), the pressure increases from 40 bar at 80 °C to 100 bar at 5 °C in our conditions, and at the same time, the half-height peak width of the pullulan standard sample ($M_p = 100\,000$ g mol^{-1}) increases by 8% (from 1.60 min at 80 °C to 1.73 min at 5 °C), indicating a better resolution at higher column temperatures. However, the elution volumes change significantly (Fig. 3).

The temperature dependence of elution is also reflected in the pullulan calibration curve. The elution volumes of all pullulan standards increase by approximately 0.68 ml as the column temperature is changed from 80 to 5 °C. The delay in elution is almost uniform for all standards, although it is slightly more pronounced for lower-M_p standards, thus causing a small increase in the separation interval and the slope of the calibration line (Table 2) indicating interactions between the polymer and the stationary phase.

The situation is similar with cellulose. At a lower column temperature, the retention time of the SL cellulose sample is longer and the peak broader. From 80 to 5 °C, the half-height peak width increases from 3.31 to 3.53 min, again for 7%, a similar value to the one observed with pullulan samples (Fig. 4).

A systematic error can thus be demonstrated with a series of chromatographic experiments, run at different column temperatures, whereby the M_w of a cellulose sample is always determined relative to the pullulan calibration curve obtained at the same temperature (concentration of LiCl in the sample solution and in the eluent remained the same, i.e. 1%

Table 2
Data on pullulan calibration at different column temperatures

T (°C)	Calibration line slope [d(log M)/dV]	Separation interval (ml)
5	−0.498	6.29
20	−0.500	6.29
40	−0.504	6.26
60	−0.510	6.23
80	−0.512	6.21

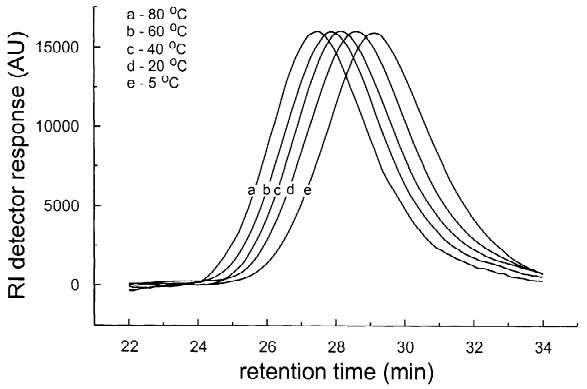

Fig. 4. Chromatograms of SL cellulose solution dissolved in 1% (w/v) LiCl–DMAc at column temperatures as indicated; eluent: 1% (w/v) LiCl–DMAc.

w/v). As shown in Fig. 5, the determined MMM is lower at lower temperatures, the differences amounting up to −14% at 5 °C column temperature for the FP cellulose sample with $M_w = 264\,000$ g mol^{-1} (determined at column temperature 80 °C). Considering that the elution of both the pullulan standards (and in turn the column calibration) and of the cellulose samples is strongly influenced by column temperature (Figs. 3 and 4), the 14% difference is actually surprisingly small and points to the conclusion that the behaviour of both materials may be similar. It should be noted that of the various

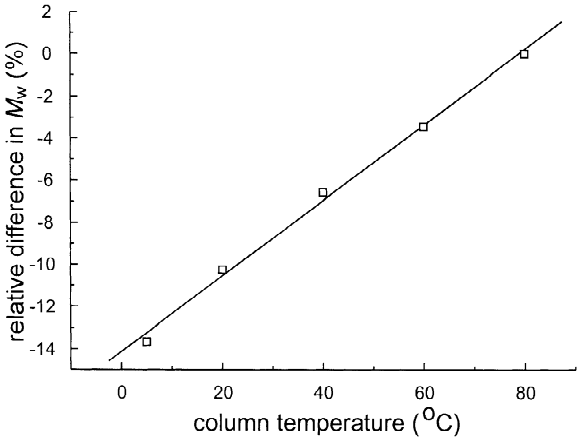

Fig. 5. Determined M_w of FP cellulose sample dissolved in 1% (w/v) LiCl–DMAc at column temperatures as indicated, eluent: 1% (w/v) LiCl–DMAc, relative to the value obtained at 80 °C.

variables that may influence the elution of polymers at different temperatures [29], hydrodynamic volumes of pullulan and cellulose may be differently dependent on temperature due to the temperature dependent nature of molecular factors such as bond lengths, freedom of rotation about bonds, torsion angles etc. However, the consequential errors are molar-mass dependent and in the absence of proper cellulose standards they are difficult to be evaluated exactly.

Similarly to pullulan samples, a higher concentration of LiCl in the cellulose sample solutions leads to lower determined M_w for all the samples studied, although the effect is more pronounced for the high-MMM cellulose samples, as shown in Table 3. The values were determined using pullulan calibration curves obtained in the same conditions as the respective cellulose sample chromatograms. A higher concentration of LiCl in the sample solution would therefore seem to decrease the intermolecular interactions and the extent of aggregation. The systematic errors associated with this phenomenon again depend on the MMM and may be as high as a few tens of percent.

It seems that aggregation during sample preparation prior to injection is the decisive process. Using 0.5% LiCl (w/v) eluent and 1% LiCl eluent, and solutions of standards in 1% LiCl (w/v) in DMAc, the calibration line slope changes within RSD (0.74%). Injection of the same cellulose solutions 1% LiCl (w/v) in DMAc, at column temperature 80 °C, using the different eluents, gives MMM data (relative to the respective pullulan calibration curves obtained at the same conditions) for SL and FP samples within the RSD values given in Section 2.

Table 3
Difference in the determination of molecular mass of cellulose samples prepared in solutions with 3% (w/v) LiCl relative to those prepared in solutions with 1% (w/v) LiCl

Sample	Relative difference (%)	
	M_n	M_w
SL	−5.1	−4.3
FP	−13.2	−5.7
WH	−21.0	−9.5

Column temperature: 80 °C; LiCl concentration in the eluent: 1% (w/v).

This result would also seem to indicate that preferential solvation does not contribute to the systematic error to a measurable extent.

Similarly to the results in Ref. [21], we also observed an initial increase in the determined MMM once a cellulose solution is prepared, i.e. diluted to 1% LiCl (w/v) (Fig. 6). Afterwards, the solute slowly degrades and the data can be conveniently treated using the Ekenstam equation [30,31]. Thus the zero order random degradation constant is obtained, at room temperature and with admission of air, $k = 6.9 \times 10^{-8}$ mol $\text{mol}^{-1}_{\text{monomer}}$ day^{-1}, leading to a decrease in M_n of 47 g mol^{-1} per day, which is close to the reported approximate value [5]. Considering that aggregation takes place after dilution with DMAc to the final concentration of cellulose and LiCl prior to injection, the preparation of cellulose solutions might be further optimized.

Recently, for a cellulose linters sample, a 5% difference between the MMM value determined relative to pullulan calibration curve and the value obtained using light scattering detection off-line was demonstrated [17]. On the other hand, Bikova and Treimanis [27] demonstrated that the hydrodynamic volume of cellulose is higher than that of pullulan of the same MMM, which would lead to overestimation of cellulose MMM determined relative to pullulan standards. Even so, considering the data presented here, it seems that once the enthalpic interactions are thoroughly understood and the relationship between the hydrodynamic volumes of cellulose and pullulan is known, SEC of cellulose in LiCl–DMAc with pullulan calibration will yield reliable data.

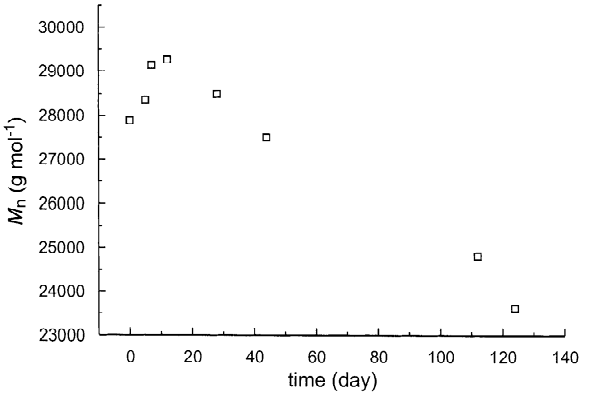

Fig. 6. M_n of SL cellulose sample dissolved in 1% (w/v) LiCl–DMAc during a prolonged period of storage. Chromatographic conditions: eluent: 1% (w/v) LiCl–DMAc, column temperature: 80 °C.

4. Conclusions

The contribution of enthalpic phenomena during size exclusion chromatographic analysis of cellulosic solutions in LiCl–DMAc gives rise to a systematic error which is difficult to evaluate exactly in the absence of proper cellulose standards. If using universal calibration, its validity should be confirmed in the particular chromatographic conditions in order to avoid unacceptable errors. If calibration relative to pullulan standards is used, it was shown that the effect of LiCl concentration in the sample solutions and the effect of temperature were of the same order of magnitude for both pullulan and cellulose samples. Nevertheless, the differences lead to systematic errors in the range of tens of percent using the specified columns. The systematic error is much higher than the random errors with typical values of up to a few percent. A lower column temperature and a higher content of LiCl in the sample solution lead to lower determined M_w values, which could be explained by a decrease in the interactions between dissolved macromolecules. Other enthalpic interactions, e.g. polymer–stationary phase interactions should also be considered. As a consequence, the systematic error depends on the molecular mass and is generally higher for high-MMM cellulose samples. Cellulose in solution degrades slowly, the first order random degradation constant, at room temperature and with admission of air, being $k = 6.9 \times 10^{-8}$ mol $\text{mol}^{-1}_{\text{monomer}}$ day^{-1}.

Acknowledgements

This work was partly supported by the Ministry of Education, Science and Sports (contract no. L2-3195) and by the Ministry of Economy of the Republic of Slovenia. The authors gratefully acknowledge the support of the European Community, 5th Framework Programme, contract no. EVK4-CT-2000-00038. The work is the sole responsibility of the authors and does not represent the opinion of the

Community. The Community is not responsible for any use that might be made of the data appearing herein.

References

[1] J.L. Ekmanis, A.F. Turbak, in: 1986 Pittsburgh Conference, Atlantic City, NJ, Published in Waters Lab Highlights No. 327, 1986.
[2] J.D. Timpa, TRIP 1 (1993) 105.
[3] S.C. Churms, J. Chromatogr. A 720 (1996) 151.
[4] E. Sjöholm, K. Gustafsson, F. Berthold, A. Colmsjö, Carbohydr. Polym. 41 (2000) 1.
[5] T.R. Dawsey, C.L. McCormick, J. Macromol. Sci. C, Rev. Macromol. Chem. Phys. 30 (1990) 405.
[6] M. Strlič, J. Kolar, M. Žigon, B. Pihlar, J. Chromatogr. A 805 (1998) 93.
[7] J.L. Ekmanis, in: The Pittsburgh Conference and Exposition, Atlantic City, NJ, 1987.
[8] A.L. Kvernheim, E. Lystad, Acta Chem. Scand. 43 (1989) 209.
[9] J.F. Kennedy, Z.S. Riviera, C.A. White, L.L. Lloyd, F.P. Warner, Cell. Chem. Technol. 24 (1990) 319.
[10] J.D. Timpa, J. Agric. Food Chem. 39 (1991) 270.
[11] J.D. Timpa, in: Chromatography of Polymers, Advances in Chemistry Series, Vol. 521, ACS, Washington, DC, 1993, p. 302.
[12] A.M. Striegel, J.D. Timpa, in: Proceedings of the 1994 International GPC Symposium, Lake Buena Vista, FL, 1994.
[13] B.A. Triplett, J.D. Timpa, In Vitro Cell. Dev. Biol. Plant 31 (1995) 171.
[14] A.M. Striegel, J.D. Timpa, in: M. Potschka, P.L. Dubin (Eds.), Strategies in Size Exclusion Chromatography, ACS Symposium Series, Vol. 635, ACS, Washington, DC, 1995, p. 366.
[15] A.M. Striegel, J.D. Timpa, Int. J. Polym. Anal. Charact. 2 (1996) 213.
[16] A.A. Silva, M.L. Laver, Trans. Am. Pulp Par. Inst. 80 (1997) 173.
[17] E. Sjöholm, K. Gustafsson, B. Eriksson, W. Brown, A. Colmsjö, Carbohydr. Polym. 41 (2000) 153.
[18] D. Ciechanska, G. Strobin, S. Boryniec, H. Struszczyk, Int. J. Polym. Anal. Charact. 4 (1997) 205.
[19] M.-A. Rousselle, P.S. Howley, Colour. Ann. 1 (1998) 153.
[20] J.M. Lawther, Z.S. Rivera, K. Jumel, C.A. White, in: J.F. Kennedy, G.O. Phillips, P.A. Williams (Eds.), Cellulose Sources and Exploitation. Industrial Utilization, Biotechnology and Physico-Chemical Properties, Ellis-Horwood, New York, 1990, p. 41.
[21] N. Schelosky, T. Röder, T. Baldinger, Papier 1 (1999) 728.
[22] U. Westermark, K. Gustafsson, Holzforschung 48 (1994) 146.
[23] O. Karlsson, U. Westermark, J. Pulp Pap. Sci. 22 (1996) J397.
[24] M. Hasegawa, A. Isogai, F. Onabe, J. Chromatogr. 635 (1993) 334.
[25] D. Berek, Prog. Polym. Sci. 25 (2000) 873.
[26] M. Terbojevich, A. Cosani, G. Conio, A. Ciferri, E. Bianchi, Macromolecules 18 (1985) 640.
[27] T. Bikova, A. Treimanis, Carbohydr. Polym. 48 (2002) 23.
[28] D. Berek, in: C. Wu (Ed.), Column Handbook for Size Exclusion Chromatography, Academic Press, San Diego, CA, 1999, p. 445.
[29] S. Mori, T. Suzuki, Anal. Chem. 52 (1980) 1625.
[30] A. af Ekenstam, Bericht 69 (1936) 553.
[31] A.M. Emsley, G.C. Stevens, Cellulose 1 (1994) 26.