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Preparation of cellulose samples for size-exclusion chromatography analyses in studies of paper degradation

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Abstract

Hydrolytic degradation of cellulose was shown to take place during the activation procedure in distilled water during the dissolution procedure of cellulose samples from papers for size-exclusion chromatography analyses in the lithium chloride–*N*,*N*-dimethylacetamide (DMAc) solution system. The use of dilute aqueous sodium hydroxide solution in the activation procedure prevents hydrolytic degradation of cellulose during the dissolution procedure, especially in the case of samples of aged papers with low pH. The use of the freeze-drying technique provides samples of cellulose ready-made for dissolution in lithium chloride–*N*,*N*-dimethylacetamide solution.

Keywords: Activation procedure; Dissolution procedure; Cellulose

1. Introduction

Studies of cellulose depolymerisation are very important for determination of paper ultimate lifetimes in archives and libraries as well as in electric power transformers and cables. The cellulose depolymerisation can be evaluated by size-exclusion chromatography (SEC) analyses of cellulose samples dissolved in LiCl–*N*,*N*-dimethylacetamide (DMAc). Although the standard viscometric technique is fast and convenient [1] the SEC method for determination of the molar masses and the molar mass distribution (MWD) of cellulose from samples of paper has some advantages. From results of these analyses, MWD and molar masses of cellulose, i.e. the number average molar mass ($\overline{M}_n$) and the weight average molar mass ($\overline{M}_w$) can be obtained. We studied paper conservation by treatment with aqueous solutions of calcium hydroxide and calcium hydroxide–methyl cellulose, respectively [2]. The papers were subjected to an accelerated ageing procedure in order to compare the permanence of the untreated paper, the calcium hydroxide treated, and the calcium hydroxide–methyl cellulose treated paper, respectively. The changes in MWD of the cellulose and in polydispersity index (PDI) during accelerated ageing provide evidence of the mechanism of cellulose chain scission and help in the choice of kinetic model of cellulose degradation [3,4]. The paper samples consisted of cellulose, papermaking additives, conservation chemicals and products of paper degradation. The presence of other substances in the sample besides the main polymer

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influences the viscometric average molar mass [5]. Furthermore, the cupri-ethylenediamine (CED) system used in standard viscometric techniques has a detrimental effect on oxidised cellulose, which may form during accelerated ageing. In addition, a reduction step prior to the dissolution of cellulose samples cannot completely prevent the cellulose degradation in CED [6].

The dissolution procedures of cellulose samples in LiCl–DMAc have been described in several reports [7–13]. The dissolution of cellulose requires the heating of the cellulose in the LiCl–DMAc mixture [7,8], or an activation procedure by swelling in water followed by solvent exchange [9–11]. Some researchers also have used liquid ammonia [12,13], or sodium hydroxide [14] as the activation procedure. In studies of paper permanence it is very important to minimise cellulose degradation during the sample preparation. Furthermore, any heating of paper samples during the dissolution procedure is undesirable since it has recently been shown that reactive structures are formed in cellulose when refluxed in LiCl–DMAc [15]. Old papers produced in the second half of the 19th century as a rule have a pH of the cold extract below 5.0 [16]. The activation procedure of such samples in water may result in acid hydrolysis of the cellulose. Furthermore, Zou et al. have shown that significant cellulose degradation takes place, although the hydrogen ion concentration is small (pH$>$7) [17]. Therefore, we used aqueous NaOH solution in the activation procedure of paper samples with pH of the cold aqueous extract below 8.0. Then we investigated the effect of this modification by measuring the cellulose molar masses and PDI of the samples. Another modification of the dissolution procedure was using freeze-drying of samples swelled in DMAc. The use of freeze-drying in the dissolution procedure of cellulose in LiCl–DMAc was published quite recently [18]. The freeze-drying of cellulose swelled in DMAc simplifies the preparation of samples with exact concentration for SEC measurements and makes possible the storage of activated, readily soluble cellulose in dry state in a desiccator. It is known that cellulose is a polymer with broad and often bimodal MWD [19]. For such polymers the effect of sample concentration on the elution volume can be significant [20,21]. We examined the effect of the cellulose concentration in LiCl–DMAc solution and revealed that it is quite small.

2. Experimental

Two paper samples were tested: (1) paper which consisted of 100% of bleached sulfite softwood pulp, without filler or sizing, prepared in 1991; and (2) an old paper from a book printed in 1903, the composition of which is 94.3% cotton fiber, 5.7% china clay and aluminium resinate sizer. The paper consisting of bleached sulfite pulp was designated SP and the old paper as OP.

The pH of cold water extracts of the samples was determined according to the ISO 6588-1981(E) standard testing procedure with the modification that the pH of the paper extract was measured in a 0.1*M* sodium chloride solution [22,23].

In order to estimate the paper permanence an accelerated ageing procedure was carried out for both papers. The conditions of accelerated ageing were the following: temperature 90 °C, relative humidity (RH) 50% and time of accelerated ageing 12 days.

The dissolution procedure of the sample in LiCl–DMAc was the following: 0.2 g of sample was disintegrated by stirring in 100 ml of distilled water or in 100 ml of 0.1 *M* NaOH for 1 h. The water or NaOH solution was removed by suction filtration through a glass filter (grade 3, the diameter of pores is 17–40 μm), and vacuum filtration. The samples treated in NaOH solution were washed with distilled water until the pH of the rinsing water reached a value of approximately 8. Fifty millilitres of methanol was added to the sample and after 5 min of ultrasonic treatment the methanol was filtered off. The methanol treatment was repeated three times. After this the samples were washed three times with 30 ml of DMAc. The filtered sample was transferred into a glass jar, frozen in a refrigerator (−22 °C, 24 h) and freeze-dried overnight at 1 mbar in a Heto Dry Winner drier (Denmark). The jar with the sample was closed and stored in a desiccator. In a recent study it was shown that concentrations of LiCl and cellulose in stock solutions as well as in dilute solutions for SEC measurements can influence the aggregation of cellulose molecules. For the stock

solution, 0.01 g of the dry sample was weighed into 10-ml volumetric flasks and 1.25 ml of 8 wt.% LiCl–DMAc was added. After 30 min the sample was left to dissolve at 4 °C for 5 days. The solution was then diluted with DMAc to give a concentration of cellulose of 0.1 wt.% and LiCl 1 wt.%. The sample was filtered through a 0.45-μm filter prior to injection. Before filtration the old paper samples were centrifuged at 5000 rpm for 30 min in order to remove paper fillers.

The SEC was performed using 1 wt.% LiCl–DMAc as eluent, three columns connected in series (Waters Styragel HR6, HR4, HR2), guard column Waters Styragel WAT 054415 and differential refractometer (Waters 410) thermostatted at 30 °C. The temperature of the columns was 25±2 °C. The pressure of the 515 HPLC pump (Waters) was 9 859 507 Pa (1430 p.s.i.). The injection volume was 20 μl and the flow-rate of the eluent was 0.8 ml/min. The chromatographic data were processed with Waters Millennium 32 software.

The DMAc solutions and the eluent were prepared with *N*,*N*-dimethylacetamide (99+%; Sigma). LiCl (99+%) was supplied by Aldrich. LiCl was dried in a vacuum at 100 °C overnight. DMAc was used without further purification.

Because of the absence of Mark–Houvink–Sakurada parameters for polystyrene and cellulose in 1 wt.% LiCl- DMAc we used relative calibration by pullulan standards to obtain apparent molar masses and PDI. Furthermore, Strlič et al. [24] demonstrated that the validity of universal calibration should be confirmed for each particular chromatographic system. The pullulan Shodex P82 standards (Shadeko) with narrow MWD were used for the calibration curve. The concentrations of pullulan and LiCl in solutions of standards were the same as cellulose and LiCl in samples for SEC in order to minimise the effect of LiCl concentration on the systematic error in SEC measurement [24]. Three solutions of pullulan standards were prepared: (1) standards with $\overline{M}_w$ 788 000, 212 000 and 5800 g/mol; (2) standards with $\overline{M}_w$ 380 000, 100 000 and 22 800 g/mol; and (3) standards with $\overline{M}_w$ 47 300, 11 800 and 180 g/mol; 0.01 g of each standard was transferred into a 10-ml volumetric flak and dissolved in 1 wt.% LiCl–DMAc solution to give a concentration of each standard of 0.1 wt.%. A third-order calibration curve was fitted to allow for the calculation of apparent molar masses.

The relative standard deviation (RSD) of apparent $\overline{M}_n$ and $\overline{M}_w$ for SP and OP samples activated by NaOH was 1% and 0.9%, respectively.

3. Results and discussion

The results of apparent molar mass and PDI determinations are shown in Table 1. No definite conclusions on the molar masses for SP sample can be drawn as there are no reports of molar masses for the same type of pulp (bleached sulfite) under the same elution conditions. However, recent results for bleached sulfite softwood cellulose in 0.5 wt.% LiCl–DMAc solvent system [14] are in the same range as the apparent molar mass and PDI obtained for the SP sample. The molar masses of OP cellulose sample were quite small for cotton cellulose [6,19],

Table 1
Apparent molar masses and polydispersity indices ($\overline{M}_w/\overline{M}_n$) obtained by SEC of cellulose samples from two papers

Sample	Apparent $\overline{M}_n$ (g/mol)	Apparent $\overline{M}_w$ (g/mol)	$\overline{M}_w/\overline{M}_n$
SP, activation procedure in H_2O	289 800	674 300	2.33
SP aged, activation procedure in H_2O	203 800	475 700	2.33
SP, activation procedure in NaOH	289 100±3500	673 500±6700	2.33
SP aged, activation procedure in NaOH	215 900	505 500	2.34
OP, activation procedure in H_2O	94 500	206 700	2.19
OP aged, activation procedure in H_2O	36 600	80 100	2.19
OP, activation procedure in NaOH	101 800±900	221 900±2000	2.18
OP aged, activation procedure in NaOH	61 700	134 600	2.18

Chromatographic conditions: eluent 1 wt.% LiCl–DMAc; column temperature 25 °C.

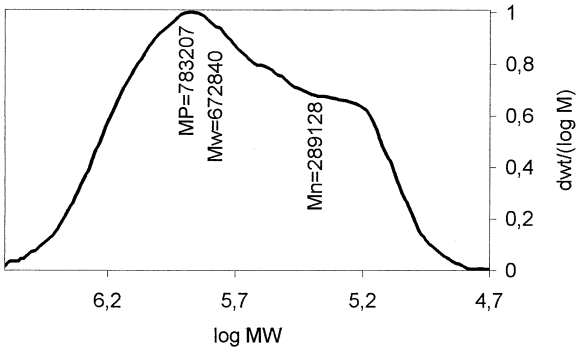

Fig. 1. Distribution of apparent molar mass of cellulose from SP paper (bleached sulfite pulp). Elution conditions: eluent 1 wt.% LiCl–DMAc; column temperature 25 °C.

but taking into account the age of this paper and the presence of acidic size makes such molar masses explainable. As can be seen from Table 1 the PDI stayed unchanged after activation in different ways, as well as after ageing. Therefore, we present only one molar mass distribution curve for each sample (see Figs. 1 and 2). It should be noted that the MWD of the SP sample possesses a shoulder that corresponds to the lower molar mass fraction, but the resolution of our SEC system is not enough to determine it as a separate peak. The low molar mass peak may be attributed to hemicelluloses [10] or products of non-random hydrolytic attack on the amorphous regions of cellulose under sulfite cooking [14]. The MWD of OP sample is monomodal, which is typical for cotton cellulose.

The activation procedure of cellulose samples

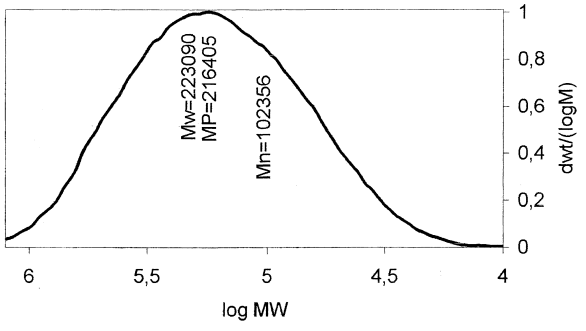

Fig. 2. Distribution of apparent molar mass of cellulose from OP paper (cotton cellulose). Elution conditions: eluent 1 wt.% LiCl–DMAc; column temperature 25 °C.

from paper sources in aqueous NaOH solution can have some drawbacks. Even if the glycosidic bonds in cellulose are stable towards alkali at temperatures below 170 °C, the end-wise degradation of cellulose can occur to some extent [25,26], and so influence the molar mass of cellulose. On the other hand, the pH of cold extracts of SP paper in NaCl solution before and after ageing were 5.54 and 5.15, respectively. For OP paper the pH of cold extracts were 4.54 before ageing and 4.48 after. In this case an aqueous NaOH solution completely neutralises sulfonic and carboxylic acid groups present in cellulose due to manufacturing conditions [27] as well as acidic products of paper degradation and protects the sample cellulose against acid hydrolysis during activation. Since the apparent molar masses of non-aged SP samples stayed the same irrespective of activation medium, we conclude that the activation procedure in a dilute aqueous NaOH solution has no effect on the apparent molar mass of cellulose under the present conditions. Furthermore, the apparent molar masses of the aged SP sample activated in water is smaller than the one activated in NaOH aqueous solution. It means that during the activation procedure in distilled water observable degradation of cellulose occurs and the change of activation media to aqueous NaOH solution prevents this process. On the basis of molar mass measurements we can see that the activation procedure of OP samples in aqueous NaOH solution prevented degradation of cellulose not only for aged OP samples but also non-aged ones.

The effect of sample concentration on apparent $\overline{M}_n$ obtained by SEC is quite small as seen in Fig. 3. The concentration of cellulose in the LiCl–DMAc solvent system is an important factor influencing the aggregation of the cellulose molecules [13,20]. The activation procedure of cellulose in water and DMAc, respectively, followed by vacuum filtering provides a the content of DMAc in the sample of about 60–70%. In order to obtain an exact concentration in stock solution the dry matter content of DMAc swelled cellulose has to be determined. This procedure requires time, additional amounts of cellulose and increases errors in the measurements. Thereby the freeze-drying of activated cellulose samples is a suitable solution of the problem. In addition, the freeze-dryed activated samples are ready for dissolu-

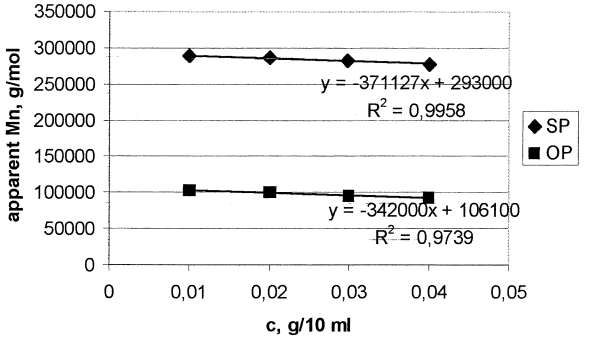

Fig. 3. The effect of sample concentration on apparent $\overline{M}_n$ of cellulose from paper source. Chromatographic conditions: eluent 1 wt.% LiCl–DMAc, columns temperature 25 °C. SP, bleached softwood sulfite pulp; OP, cotton cellulose from old paper.

tion and can be stored in a desiccator or even in liquid nitrogen to minimise cellulose degradation.

Recently, it has been proven that the water content in the LiCl–DMAc solvent system is a crucial parameter. The total amount of water in cellulose–LiCl–DMAc solution should be below 0.1 *M*. In order to avoid cellulose aggregation and obtain comparable results of SEC measurements a drying procedure of LiCl and DMAc has to be carried out [18]. Cellulose–LiCl–DMAc solutions must be injected into the SEC system immediately after dilution to final concentration [26].

4. Conclusions

In preparing the cellulose samples for SEC measurements of molar masses in LiCl–DMAc solution the activation procedure of cellulose from paper sources in distilled water results in hydrolytic degradation of cellulose especially in the case of paper with low pH of cold aqueous extract. Replacing the medium of activation procedure with dilute aqueous NaOH solution allows avoiding hydrolytic degradation.

Freeze-drying of cellulose samples swelled in DMAc allows us to minimise the concentration effect in SEC measurements and activated dry cellulose samples can be conveniently used for further studies.

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