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Molar mass characterization of cellulose acetates over a wide range of high DS by size exclusion chromatography with multi-angle laser light scattering detection

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ABSTRACT

A series of cellulose acetate (CA) samples with an average degree of substitution (DS) ranging from DS = 1.5 to DS = 2.3 was synthesized by partial saponification of a high DS sample (DS = 2.6). The comparison of the theoretical DS-values with ones determined by ^{1}H NMR showed that the deacetylation reaction can be well controlled by the amount of sodium hydroxide (NaOH) added. The average molar masses of the samples and their molar mass distributions were characterized by size exclusion chromatography (SEC) with multi-angle laser light scattering (MALLS) detection in N,N-dimethyl acetamide (DMAc) containing 250 mmol/L lithium chloride (LiCl) in order to effectively suppress aggregation of the samples. The dependences of molar mass versus elution volume for samples of different DS can be well described by a common calibration curve. This allows using the same calibration curve for determination of molar masses of unknown samples, irrespective of their DS. A comparison of the absolute molar masses determined by light scattering with the molar masses obtained using a poly(methylmethacrylate) (PMMA) calibration curve revealed that the PMMA equivalent molar masses overestimated the absolute molar masses by a factor of approximately 3. Correction factors were determined making it possible to convert a PMMA calibration curve into a CA calibration curve. This procedure allows determination of correct CA molar masses based on commercially available PMMA-standards without using a LS instrument for samples in the DS range investigated.

1. Introduction

Cellulose is one of the world's most abundant natural biopolymers and a renewable resource. The importance of cellulose does not arise only from the outstanding physical properties of cellulose itself, but also from the fact that cellulose can be derivatized to yield polymers having interesting properties. Cellulose acetate (CA), one of the most important cellulose derivatives, is mainly used in membranes, films, fibres, plastics, filters and as component in adhesives and pharmaceutical products (Aparecida da Silva, Maria Favaro, Pagotto Yoshida, & do Carmo Gonçalves, 2011; Baumann & Conner, 2003; Edgar, 2007; Edgar et al., 2001; Law, 2004; Mahendran, Malaisamy, & Mohan, 2002).

A fundamental understanding of the influence of the structure of cellulose derivatives on application properties requires a comprehensive characterization of the samples in terms of molar mass, degree of substitution and chemical heterogeneity. Therefore, there is a strong need to characterize these parameters.

Size exclusion chromatography (SEC) provides a convenient way for the characterization of molar masses and molar mass distributions of natural and synthetic polymers. However, SEC-separations are based on hydrodynamic volume and not on molar mass. Therefore SEC only yields equivalent molar masses with respect to the calibrant used. In order to obtain absolute molar masses from a conventional calibration curve, either standards of identical chemical nature are required or suitable correction factors have to be applied, if the calibration is based on standards of different chemical nature than the sample to be investigated. In addition, a crucial balance of the polarities of the analyte, the solvent and the stationary phase is required for a reliable SEC characterization.

A number of solvents such as acetone (Funaki, Ueda, Saka, & Soejima, 1993), dichloromethane (DCM) (Mahmud & Catterall, 1984) or tetrahydrofuran (THF) (Alexander & Muller, 1971; Tanghe, Rebel, & Brewer, 1970) and also dipolar solvents like N,N-dimethyl acetamide (DMAc), or N-methylpyrrolidone with or without salt (Fleury, Dubois, Léonard, Joseleau, & Chanzy, 1994) have been described as mobile phases for molecular characterization of cellulose diacetates (CDA) and triacetates (CTA). The resulting chromatograms showed the presence of additional peaks before the main peak of CA which were attributed to microgel fractions and ionic effects. Since the DS of cellulose derivatives alters the

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polarity, solubility and aggregation behaviour, the SEC methods found in literature are usually applicable only within narrow DS-ranges. However, due to the statistical nature of the substitution process, CAs are heterogeneous in both molar mass and chemical composition. Thus, SEC methods useful for larger DS ranges are required, in order to allow molar mass characterization of samples heterogeneous in DS. In addition such methods would allow running samples of different DS using the same chromatographic conditions instead of adjusting the phase system to the DS of the sample under investigation.

For low DS samples pyridine/water was reported to allow reliable SEC characterization of samples ranging from DS = 0.4 to DS = 2.3 (Lee, Altaner, Puls, & Saake, 2003). Samios, Dart, and Dawkins (1997) described the use of DMAc/0.5% lithium chloride (LiCl) for the determination of molar masses of the intact and enzymatically hydrolysed CAs in the range DS = 0.7 to DS = 2.5. However, they discussed the problem of decreasing solubility for the low DS samples, resulting in unreliable molar masses.

This present paper reports on the molar mass characterization of high DS CAs within the range DS = 1.5 to DS = 2.9 using SEC coupled to a multi angle laser light scattering (MALLS) photometer.

2. Materials and analytical methods

2.1. Solvents and samples

1,4-Dioxane, dimethyl sulphoxide (DMSO) (VWR, Darmstadt, Germany) and DMAc (MERCK, Hohenbrunn, Germany) of HPLC grade were used as received. The water content of DMAc was specified by the manufacturer to be below 0.3%. No additional analysis was carried out by us, as a freshly opened bottle was used. Water was obtained by deionization through a Milli-Q system (Millipore water). LiCl and sodium hydroxide (NaOH) pellets were purchased from MERCK (Darmstadt, Germany).

Five of the CA samples (sample 4, 13, 14, 15 and 17 having DS = 1.72, 2.42, 2.45, 2.45 and 2.92, respectively) were obtained from industrial sources. The others were prepared by partial saponification of a commercial high DS sample (sample 16, DS = 2.60, Acetati, Italy). A typical protocol for the saponification was as follows: 9.18 g of sample 16 were dissolved in 255 mL of 1,4-dioxane (36 g/L). The required amount of NaOH was added as a aqueous solution (0.2–0.5 M) under stirring. The mixture was allowed to stand for one hour at room temperature. Afterwards the polymer was precipitated by dropwise addition into deionized water. The precipitates were washed twice with deionized water and dried in vacuum oven at 80 °C.

2.2. ^{1}H NMR-spectroscopy

Around 5–10 mg of each polymer was dissolved homogeneously in 1 mL of DMSO-d_6 at 60 °C. DS of the samples was determined by ^{1}H NMR-spectroscopy using the signals at 2.2–1.6 ppm (acetyl protons) and 5.5–2.8 ppm (cellulosic protons), upon subtracting the integral intensity of the sharp peak arising from residual water protons (δ = 3.14 ppm). The ^{1}H NMR spectra were acquired on a 400 MHz (9.4 Tesla) Mercury-VX (Varian Inc., Sao Palo, USA) NMR-spectrometer equipped with a 5 mm inverse-probe. ^{1}H measurements were executed using a 90° pulse, 2.6 s acquisition time (32 k data points, 16 ppm spectral width), 10 s relaxation delay, 64–256 accumulated scans depending on the analyte concentration, simple zero addition and exponential multiplication with lb = 0.3 Hz, respectively. The ^{1}H NMR spectra were recorded at 60 °C and processed and evaluated using ACD 11 software (Advanced Chemistry Development Inc., Toronto, Canada).

2.3. SEC-MALLS

SEC-MALLS experiments were performed in DMSO and DMAc as solvents at different concentrations of LiCl using a TOSOH Bioscience (Tokyo, Japan) EcoSEC Micro-SEC-System with build-in RI-detector. A MALLS detector (DAWN DSP, Wyatt Technology, Santa Barbara, USA) was attached between the column and the RI-detector.

The LS-detector was calibrated using pure toluene assuming a Rayleigh ratio of 9.78×10^{-6} cm^{-1} at 633 nm. The refractive index increments (dn/dc) of the samples were determined from the responses of the RI-detector presuming complete sample recovery. The RI-detector was calibrated using PMMA in THF assuming a dn/dc-value of 0.089 cm^3/g (Berkowitz, 1983). No attempts were undertaken to correct for the differences in the wavelengths between the RI-detector (white light) and the one of the light scattering instrument (633 nm).

A single PSS-GRAM linear XL column (10 μm particle size, 30 cm × 0.8 cm, PSS Polymer Standards Service GmbH, Mainz, Germany) was applied at a flow rate of 1.0 mL/min. The samples were prepared by adding the DMAc/LiCl to the polymer and allowing to stand overnight at room temperature. Sample concentrations of 3.0 g/L and an injection volume of 100 μL were used, if not stated otherwise. The SEC calibration was performed with poly(methylmethacrylate) (PMMA) standards (PSS Polymer Standards Service GmbH, Mainz, Germany) having molar masses ranging from 3600 to 1.2×10^6 g/mol. For data acquisition and evaluation PSS WINGPC-Unity version 7.0 (PSS Polymer Standards Service GmbH, Mainz, Germany) and ASTRA version 4.90.08 (Wyatt Technology, USA) software were used.

3. Results and discussion

3.1. Alkaline partial saponification of CA-DS 2.6

Two major synthetic pathways exist for the preparation of CA of a given DS, namely esterification of cellulose and hydrolysis of highly acetylated cellulose. Esterification of cellulose uses acetylating agents like acid anhydrides or acid chlorides in heterogeneous media or in homogeneous medium applying special solvent-systems for cellulose dissolution (Kosan, Dorn, Meister, & Heinze, 2010; Li et al., 2009; Peres de Paula, Lacerda, & Frollini, 2008; Wu et al., 2004). The latter approach has the advantage of a high accessibility of the cellulose hydroxyl functions to the reactants, allowing a better control of the functionalization reaction and uniformly distributed substituents (Nagel & Heinze, 2010; Sealey, Samaranayake, Todd, & Glasser, 1996). The hydrolysis of CA with high DS can be performed by acidic or alkaline catalysis. The main advantages of alkaline over acidic hydrolysis are the short reaction times and the simplicity (Hiller, 1953; Howlett & Martin, 1947; Malm, Glegg, Salzer, Ingerick, & Tanghe, 1966; Usmanov, Tashpulatov, Abdullaev, & Sultanov, 1997). Therefore partial alkaline saponification of a highly substituted CA (sample 16, DS = 2.60) in NaOH-1,4-dioxane solution was chosen in our laboratory. The degree of saponification was adjusted by changing the amount of NaOH added.

The average DS of the CAs samples were determined by ^{1}H NMR spectroscopy (Goodlett, Dougherty, & Patton, 1971). In contrast to the given literature, where the NMR experiments were performed in CD_2Cl_2, we used DMSO, which prevents the exchange of OH-protons of the AGU with those of residual water in the solvent. Therefore in our study the region from 5.5 ppm to 2.8 ppm of the ^{1}H NMR spectrum includes both the protons of the AGU units and the protons of the OH-groups. This fact was considered in the calculation of the DS values.

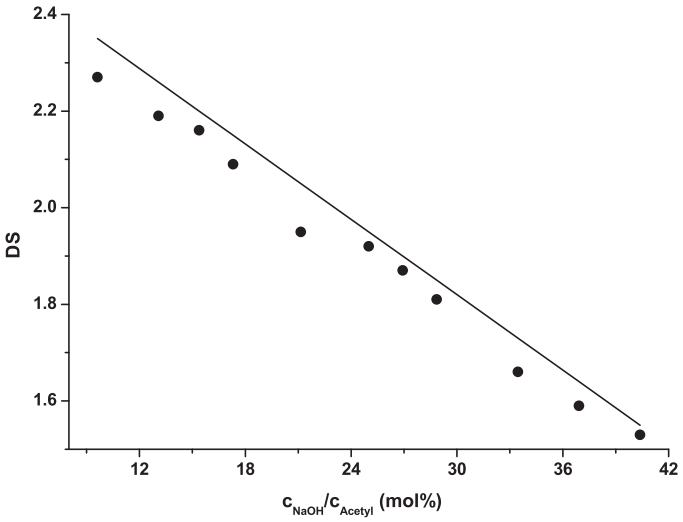

Fig. 1. Dependence of DS on the amount of NaOH for partial deacetylation of CA in dioxane. The line represents the theoretical DS.

In Fig. 1 the percentage of NaOH added relative to the acetyl groups is plotted against the average DS determined by ^{1}H NMR. As expected, a decrease of DS with increasing amount of NaOH is observed. Furthermore there is a good agreement between the theoretical DS-values and the ones obtained from ^{1}H NMR. The systematic deviation to slightly lower values might be the result of the additional reaction of water with acetyl groups since only the NaOH concentration was considered for the calculation of the theoretical DS. Nevertheless the results show that alkaline partial saponification is a suitable method for the preparation of CA of various DS.

3.2. Molar mass and molar mass distribution

The solubility of the cellulose derivatives and therefore of CAs is a complex function which depends not only on the average DS but also on the distribution of acetyl substituents in the anhydroglucose units and along and among the cellulose chains (Kamide, Okajima, Kowsaka, & Matsui, 1987). In order to identify suitable solvents, 5 samples (samples 2, 4, 9, 16 and 17), having DS-values of DS = 1.59, 1.72, 2.09, 2.60 and 2.92, respectively, were selected to prepare solutions of 1 g/L, using a large variety of different solvents (Water, Acetone, Acetonitrile, Water/acetone (1:1), Chloroform, THF, THF/water (1:1), Dimethylformamide (DMF), DMF/water (1:1), 1,4-Dioxane, DMSO, DMAc/LiCl, DCM, Acetic acid, Methoxydiglycol, 2-Ethoxyethanol, 2-Butoxyethanol, 1,2,4-trichlorobenzene, Methanol, Ethyl acetate, 2-Propanol, Butanone, Tetrachloroethylene, and Xylene). The samples were kept at 90 °C overnight under constant agitation and sample dissolution was checked by visible inspection. Only DMSO and DMAc/LiCl were identified to dissolve all our samples, irrespective of DS. Accordingly, SEC of the CAs was performed in these solvents. Starting with pure DMSO, the samples showed bimodal chromatograms probably due to strong aggregations between the chains. It is known that the addition of a salt, such as LiCl can prevent the association of the polymer chains. In fact, a study on the variation of the LiCl concentration revealed that a substantially high LiCl concentration (250 mmol/L) is required before aggregation cannot be observed any longer. However, our attempts to perform SEC-LS experiments in DMSO/LiCl failed, due to the low dn/dc ranging from 0.009 cm^3/g for the sample of highest DS (sample 17, DS = 2.92) to 0.033 cm^3/g for the lowest DS sample (sample 1, DS = 1.53). Similarly to DMSO, the application of pure DMAc as eluent resulted in bimodal peaks as well. The left hand side of Fig. 2 shows the RI- and LS-traces of three CA samples of different DS in pure DMAc as the eluent. Two of the samples show a sharp peak in RI before the main one, the relative intensity of which seems to correlate with DS. The lower the DS, the more pronounced is the prehump at low elution volume. This supports the assumption that the small prepeak is due to aggregation of the CA chains, since the higher the number of hydroxyl groups, i.e. the lower DS, the higher is the probability of intermolecular interactions. The assumption of aggregates is further supported by the LS-traces. Despite the low concentration of the prehump, a very high signal intensity is observed in the LS-signal, indicating structures of very high molar mass. Even sample 17 having the highest DS (DS = 2.92), for which no evidence for aggregation is observed in the RI-trace, shows a clear bimodality in the LS-signal. Similar to the DMSO system, aggregation was suppressed by the addition of LiCl. Indeed, the addition of 250 mmol/L LiCl to DMAc was sufficient to destroy aggregation, resulting in monomodal RI- and LS-peaks as shown on the right hand side of Fig. 2.

As compared to DMSO/LiCl the LS experiments in DMAc/LiCl revealed significantly higher dn/dc-values which varied systematically from dn/dc = 0.044 cm^3/g for DS = 2.92 (sample 17) to 0.09 for the sample of lowest DS = 1.53 (sample 1). Fig. 3 depicts the determined dn/dc-values of CA in DMAc as a function of DS. The nearly linear variation of dn/dc with DS in DMAc/LiCl can be described by the equation:

$$\left(\frac{dn}{dc}\right)_{CA} = (0.129 - 0.300 \times \mathrm{DS})\,\mathrm{cm}^3/\mathrm{g} \quad (1)$$

A comparison with literature data on dn/dc for samples of different DS in pure DMAc supports our observation of decreasing dn/dc with increasing DS (Kamide, Miyazaki, & Abe, 1979; Kamide, Saito, & Abe, 1981; Saito, 1983). However, a direct comparison of the absolute values is not possible due to the different wavelengths and the addition of salt.

Although dn/dc in DMAc/LiCl was found to be significantly higher than in DMSO, rather high polymer concentrations are required to obtain a suitable S/N-ratio for the light scattering signal. However, SEC is known to be prone to column overloading, especially for high molar mass samples. Column overloading results in a variation of peak positions with concentration, which in turn will lead to concentration dependent molar masses, when evaluating the chromatograms using a calibration curve (Elsdon, Goldwasser, & Rudin, 1981; Mori, 1976; Song, Hu, Li, & Zhao, 2002). Therefore concentration dependent experiments were conducted on three representative samples of different DS. Fig. 4 shows the resulting chromatograms.

No significant and systematic variations in peak positions were observed for concentrations ranging from 0.9 to 3.0 g/L. Thus, the concentration of 3.0 g/L was chosen at best to prepare the sample solutions.

After the SEC conditions have been optimized, SEC-LS experiments were performed to measure absolute weight average molar masses and to determine the dependences of molar mass on elution volume for the CA samples.

The third column of Table 1 lists the weight average molar masses of all samples, as determined by SEC-LS. The fourth column lists the weight average degrees of polymerization (DP_w) which have been calculated from the weight average molar masses, taking into account the change in the molar mass of a monomer unit (anhydroglucose unit) due to the different DS. In contrast to the industrial materials (marked in grey in Table 1) all samples prepared in our laboratory are based on the same parent material. If the cellulose backbone was not altered by the saponification reaction, the degree of polymerization (DP) should remain constant, while the molar mass should decrease with decreasing DS, due to the lower molar mass of a repeating unit at lower DS. Indeed, despite some

Table 1
Comparison of CA molar masses obtained from LS (absolute M_w) and those determined by SEC with PMMA calibration, recalculated M_w using broad calibration and relative deviation to LS-data. The samples marked in grey are industrial samples. Samples marked by asterisks have been used to establish the calibration curve using the broad calibration approach.

Sample name	DS	LS-M_w [g/mol]	DP_w	PMMA-equivalent M_w [g/mol]	Recalculated M_w [g/mol]	%Deviation
Sample 1*	1.53	57,600	254	223,000	66,900	16
Sample 2	1.59	72,700	318	224,000	66,700	−8
Sample 3	1.66	73,500	317	224,000	66,744	−9
Sample 4*	1.72	44,600	190	131,000	44,200	−0.7
Sample 5*	1.81	68,100	286	218,000	65,500	−4
Sample 6	1.87	64,900	269	215,000	64,600	−0.4
Sample 7*	1.92	63,200	260	214,000	64,600	2
Sample 8	1.95	76,400	313	222,000	66,200	−13
Sample 9*	2.09	70,700	283	219,000	65,800	−7
Sample 10	2.16	71,900	284	217,000	65,200	−9
Sample 11*	2.19	71,000	279	220,000	65,800	−7
Sample 12	2.27	74,400	289	220,000	66,000	−11
Sample 13*	2.42	64,400	244	211,000	64,400	−0.1
Sample 14	2.45	64,700	244	212,000	64,600	−0.2
Sample 15	2.45	93,300	352	210,000	63,600	−32
Sample 16*	2.60	69,800	257	220,000	65,900	−6
Sample 17	2.92	175,000	613	387,000	103,000	−41

scattering, the weight average DP was found to be fairly constant at $DP_w = 284$ with a standard deviation (STD) of STD = ±23. Therefore, within the accuracy of the LS experiments, the calculated average DP can be assumed to be constant and it thus can be concluded that the chain length distribution of the cellulose backbone was not altered significantly by the saponification reaction. This is in contrast to the acidic saponification of CA under acidic conditions, as acidic saponification severely reduces the degree of polymerization of the cellulosic backbones during hydrolysis (Hiller, 1953; Olaru & Olaru, 2004). Therefore alkaline saponification is a better choice for deacetylation of a high DS CA.

Fig. 5 shows the dependences of molar mass on elution volume together with the corresponding RI-chromatograms. As expected for a SEC separation, the molar masses decrease with increasing elution volume. All samples are broadly distributed with molar masses covering at least two orders of magnitude (10^4–10^6 g/mol). The scattering of the data at the high and low molar mass end of the chromatograms are typical for SEC-LS measurements and is attributed to the different molar mass sensitivities of the LS- and RI-detectors.

The samples prepared in our own laboratory which are based on the same parent material, exhibit nearly identical elution profiles, indicating that the variation of DS does not change the hydrodynamic volume considerably.

With the exception of two of the industrial samples (DS = 2.45 (sample 15) and DS = 2.92 (sample 17)), the dependences of molar mass on elution volume for all samples are very close to each other, despite the differences in average DS and the origin of the samples. The reason for the deviations of the sample 15 and 17 is unclear yet. At a given elution volume the molar masses scatter by approximately ±10% relative, which is in the order of the uncertainty of LS measurements anyway. In addition, it has to be kept in mind

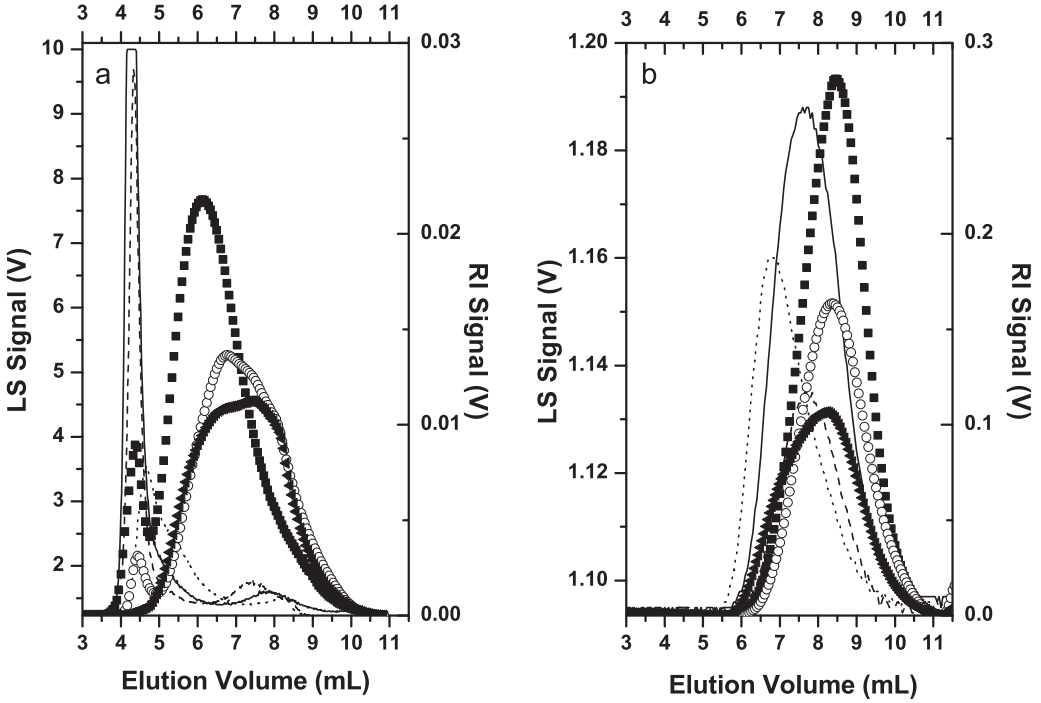

Fig. 2. RI (symbols) and corresponding LS-traces (lines) of CAs. Sample 1 (DS = 1.53, ■, solid line), Sample 14 (DS = 2.45, ◊, dashed line), Sample 17 (DS = 2.92, ◄, dotted line). (a) Eluent: DMAc; (b) Eluent DMAc/LiCl (250 mmol/L). Injection volume: 100 μL; Column: PSS GRAM Linear XL 10 μ 8 mm × 300 mm at 35 °C; Flow rate: 1 mL/min.

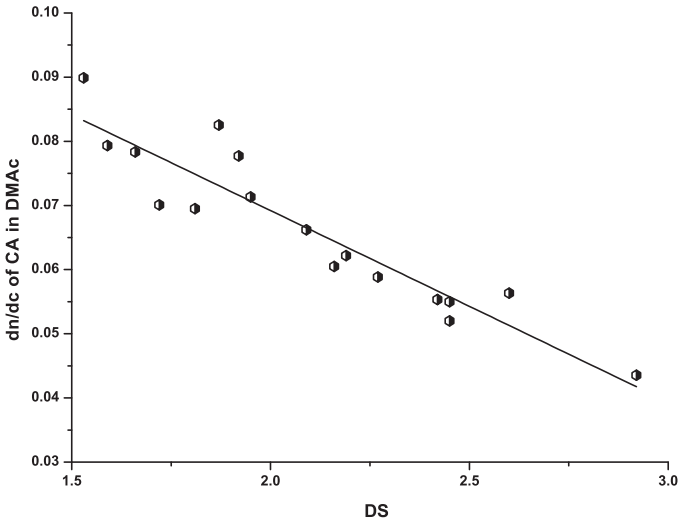

Fig. 3. *dn*/*dc* of CA in DMAc/LiCl as a function of DS.

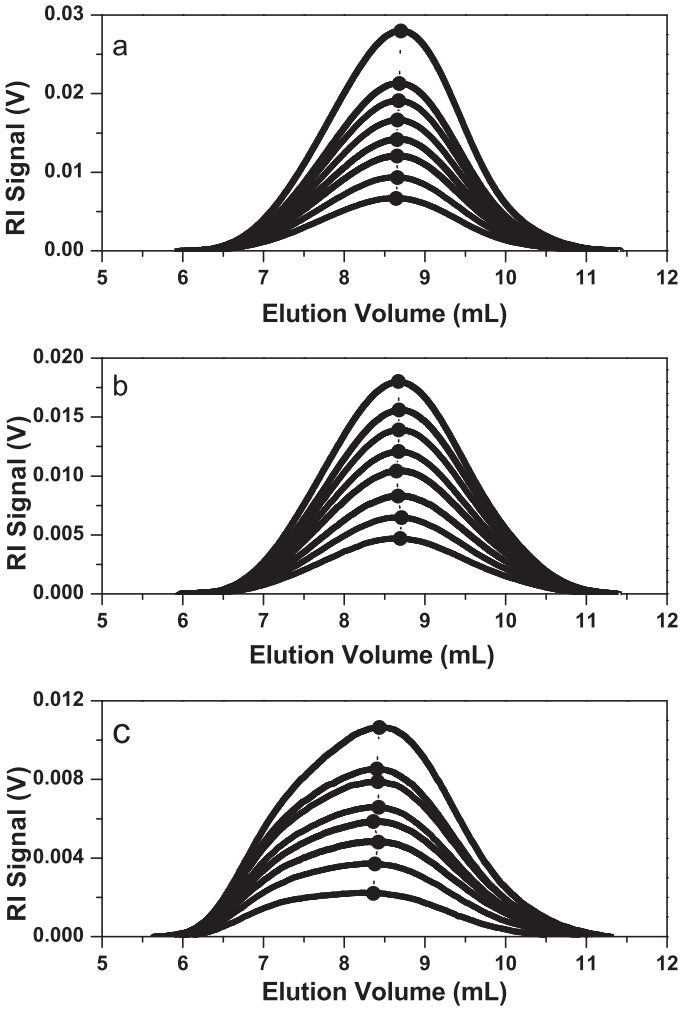

Fig. 4. Concentration dependent RI elugrams (injected concentrations 3.0, 2.7, 2.4, 2.1, 1.8, 1.5, 1.2, 0.9 g/L) for Sample 1 (DS = 1.53) (a), Sample 10 (DS = 2.16) (b) and Sample 17 (DS = 2.92) (c); Exp. conditions see Fig. 2b.

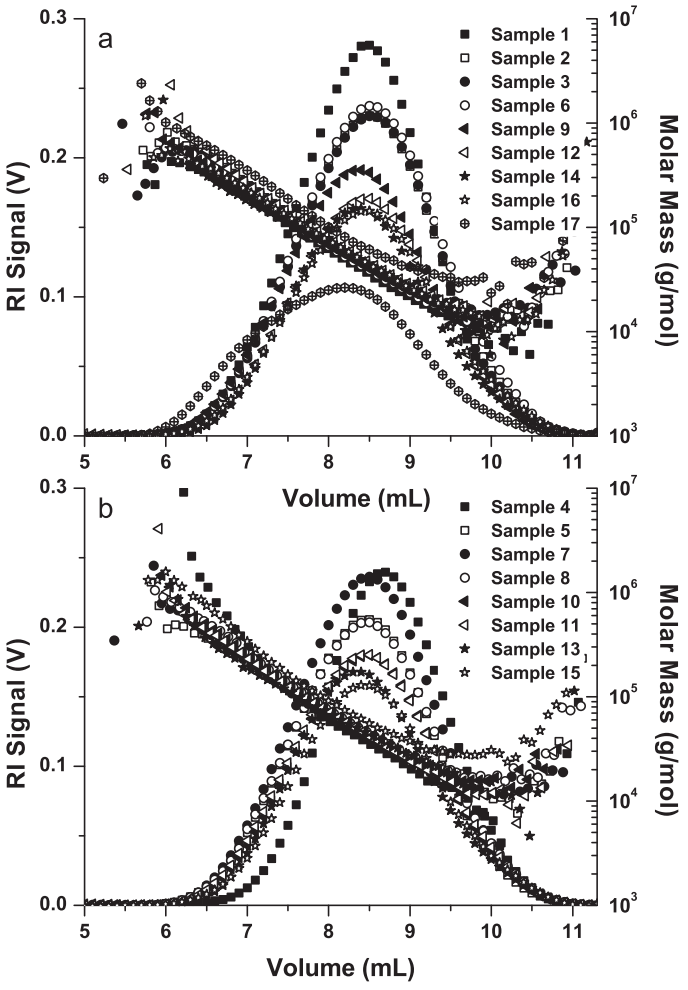

Fig. 5. RI-traces and dependences of molar mass on elution volume for CA of different DS. Exp. conditions see Fig. 2b (Sample 1 (DS = 1.53), Sample 2 (DS = 1.59), Sample 3 (DS = 1.66), Sample 4 (DS = 1.72), Sample 5 (DS = 1.81), Sample 6 (DS = 1.87), Sample 7 (DS = 1.92), Sample 8 (DS = 1.95), Sample 9 (DS = 2.09), Sample 10 (DS = 2.16), Sample 11 (DS = 2.19), Sample 12 (DS = 2.27), Sample 13 (DS = 2.42), Sample 14 (DS = 2.45), Sample 15 (DS = 2.45), Sample 16 (DS = 2.60), Sample 17 (DS = 2.92)).

that the molar mass varies with DS. However, no systematic dependence of the molar masses with DP at a given elution volume was observed. Therefore it can be concluded that the variation of DS does not alter significantly the hydrodynamic volume. As a consequence, CAs in the range investigated can be evaluated using the same calibration curve.

SEC-LS provides a sophisticated way for determination of absolute molar masses. However, the price and the higher complexity usually prevent to use SEC-LS as routine method. Molar mass determination by SEC using a calibration curve established by standards is still the method of choice in many laboratories. However, since no commercial CA standards are available as primary calibrants, a set of well defined PMMA standards was run under the same SEC conditions to compare the PMMA-equivalent molar masses of the CAs with the absolute molar masses determined by SEC-LS.

The PMMA-equivalent molar masses are given in the fifth column of Table 1. They exceed the true molar masses derived by LS by approximately a factor of 3 revealing the differences in the hydrodynamic volumes of PMMA and CA at a given molar mass. Given the ease of setting up a conventional PMMA calibration, correction factors were aimed for, allowing calculating a CA calibration based on a PMMA calibration curve.

In order to derive suitable correction factors, the concept of broad calibration was applied (Mori, 1981). For this purpose a set of narrowly distributed standards (e.g. in this case PMMA) and at least two broadly distributed samples of the same chemical and topological structure as analyte (e.g. in this case CA) are required. Each broadly distributed sample has to be well characterized in terms of at least one average molar mass (set values). SEC chromatograms of these samples and the conventional standard calibration have to be established under the same experimental conditions.

If both CA and PMMA obey the universal calibration principle, the following equation holds true at any elution volume (Benoît, Grubisic, Rempp, Decker, & Zilliox, 1966; Grubisic, Rempp, & Benoît, 1967):

$$M_2 = \left(\frac{K_1 \times M_1^{a_1+1}}{K_2} \right)^{1/(a_2+1)} \quad (2)$$

where M_2 is molar mass of the analyte (CA), M_1 molar mass of the calibrant (PMMA) and K and a are the Mark–Houwink constants for the corresponding polymers in the respective solvent and at the respective temperature. The above equation can be written in a simplified logarithmic form:

$$\log\ M_2 = B \times \log M_1 + \log A \quad (3)$$

where $A = (K_1/K_2)^{1/(a_2+1)}$ and $B = (a_1 + 1)/(a_2 + 1)$ are the so far unknown correction factors. Variations of the parameters A and B result in a parallel shift and a change in the slope of the calibration curve relative to the PMMA calibration curve. Using suitable fitting algorithms the parameters A and B are varied automatically until the calculated average molar masses of the CAs closely agree with the given set values. In our case the resulting parameters were $A = 3.03$ and $B = 0.818$. These parameters were used to convert the PMMA calibration curve into a CA calibration curve, which was subsequently used to derive the average molar masses of all CA samples based on their chromatograms. These calculated weight average molar masses and their deviations to the absolute molar masses from LS are given in the sixth and seventh columns of Table 1, respectively. The highest deviations again arise for the two the industrial samples (DS = 2.45 (sample 15) and DS = 2.92 (sample 17)) which have also shown abnormal behaviour in Fig. 5. The good agreement between the molar masses from broad calibration and from LS for all other samples indicates the reliability of this approach for SEC calibration. It is interesting to mention once more that molar mass conversion is irrelevant to the DS of the analysed sample.

4. Conclusions

In the framework of this study it was shown that partial saponification using NaOH is a suitable method for preparing CAs of different average DS without altering the degree of polymerization. SEC-LS investigations in DMSO and DMAc revealed aggregates which can be completely suppressed by the addition of LiCl. However, the significantly lower dn/dc of CA in DMSO makes DMAc the preferred choice for SEC. Samples differing in DS but being based on the same parent material showed nearly the same elution profile and nearly identical calibration curves, indicating that variations of the DS within the range DS = 1.5–2.9 do not alter remarkably the hydrodynamic volume. As a consequence all samples can be evaluated based on the same calibration curve, irrespective of their DS. The comparison of the molar masses obtained by light scattering with PMMA-equivalent molar masses revealed that the PMMA equivalent molar masses overestimated the absolute ones significantly. Therefore correction factors were determined allowing determining of correct molar masses of a CA of any DS within the range DS = 1.5–2.9 based on a PMMA calibration curve.

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