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Molar mass characterization of sodium carboxymethyl cellulose by SEC-MALLS

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ABSTRACT

Two series of sodium carboxymethyl celluloses (NaCMCs) derived from microcrystalline cellulose (Avicel samples) and cotton linters (BWL samples) with average degrees of substitution (*DS*) ranging from *DS* = 0.45 to *DS* = 1.55 were characterized by size exclusion chromatography with multi-angle laser light scattering detection (SEC-MALLS) in 100 mmol/L aqueous ammonium acetate (NH_4OAc) as vaporizable eluent system. The application of vaporizable NH_4OAc allows future use of the eluent system in two-dimensional separations employing evaporative light scattering detection (ELSD). The losses of samples during filtration and during the chromatographic experiment were determined. The scaling exponent a_s of the relation $\langle s^2 \rangle^{1/2} \sim M^{as}$ was approx. 0.61, showing that NaCMCs exhibit an expanded coil conformation in solution. No systematic dependencies of a_s on *DS* were observed. The dependences of molar mass on SEC-elution volume for samples of different *DS* can be well described by a common calibration curve, which is of advantage, as it allows the determination of molar masses of unknown samples by using the same calibration curve, irrespective of the *DS* of the NaCMC sample. Since no commercial NaCMC standards are available, correction factors were determined allowing converting a pullulan based calibration curve into a NaCMC calibration using the broad calibration approach. The weight average molar masses derived using the so established calibration curve closely agree with the ones determined by light scattering, proving the accuracy of the correction factors determined.

1. Introduction

NaCMC is the most important cellulose derivate in terms of sales (approx. 230,000 tons per annum (Thielking & Schmidt, 2006)) up to now. NaCMCs are most often produced in a heterogeneous polymer-analogous reaction of sodium hydroxide activated cellulose with monochloracetic acid or its sodium salt (Heinze, 2005). The products obtained in this process are supposed to be heterogeneous in terms of chemical composition and molar mass (Saake et al., 2000). Thus, in order to obtain information on the influence of the molecular structure of NaCMC on application properties not only the average chemical composition and molecular mass need to be known, but also the extent of the respective heterogeneities and their correlations. The chemical composition distribution of intact NaCMC chains can be determined by gradient chromatography (Shakun et al., in preparation), while the molar mass distributions of synthetic and natural polymers can be determined using size exclusion chromatography (SEC). The correlations between chemical composition and molar mass of synthetic polymers can be investigated by two-dimensional chromatography (2D-chromatography) (Gerber & Radke, 2005; Kilz, 2004; Pasch, 2000; Raust, Brüll, Moire, Farcet, & Pasch, 2008; Schoenmakers, Vivo-Truyols, & Decrop, 2006). In the first dimension of such a 2-dimensional copolymer separation the polymer chains usually are separated according to the chemical composition. Molar masses and molar mass distributions of the eluting fractions are characterized by SEC in a second chromatographic separation. In order to perform such separations online, i.e. without manual sample treatment between the two separations, some requirements need to be met. First of all, the eluents applied in the different chromatographic dimensions have to be compatible with each other and with the column materials. In addition the detectors applied in the second dimension must be very sensitive, due to the very low sample concentrations and must also withstand the high flow rates typically applied in the second dimension. The high flow rates are required for a comprehensive separation. In the past evaporative light scattering detection

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Table 1
Summary of stationary and mobile phases applied in aqueous SEC of NaCMCs, Mark–Houwink exponents of NaCMCs in different solvents.

Stationary phase	Mobile phase	Investigated *DS* range	Mark–Houwink exponent a	Mark–Houwink exponent K [cm^3/g]	Reference
Separom HEMA mono C60 G65; Shodex OH Pak B 804 and B 805	100 mmol/L aqueous NH_4NO_3	0.80–2.90	0.84 (*DS* 1.37)	0.00138 (*DS* 1.37)	Rinaudo et al. (1993)
TSK 30/40/50/60	100 and 500 mmol/L aqueous $NaNO_3$	0.71–2.95	0.90 (*DS* 1.00, 10 mmol/L NaCl)	0.0143 (*DS* 1.00, 10 mmol/L NaCl)	Kulicke, Kull, Kull, and Thielking (1996)
G6000 PW, G5000 PW and G3000 PW	Aqueous $NaNO_3$	0.75–1.25			Hoogendam et al. (1998)
Separon HEMA 1000 (10 μm)	400 mmol/L aqueous sodium acetate buffer	0.70–0.80	0.83 (*DS* 0.76)	0.00224 (*DS* 0.76)	Eremeeva and Bykova (1998)
	500 mmol/L aqueous NaOH		0.73 (*DS* 0.76)	0.00537 (*DS* 0.76)	
TSK G (3000, 4000, 5000, 6000) PW XL	100 mmol/L aqueous $NaNO_3$, 200 ppm NaN_3	1.00–1.20	0.74 (*DS* 1.00) 0.62 (*DS* 1.20)	0.076 (*DS* 1.00) 0.344 (*DS* 1.20)	Kath et al. (1999)
Superose 12 HR 10/30	50 mmol/L aqueous NaOH	0.57			Melander and Vuorinen (2001)
TSK GMPWXL	200 mmol/L aqueous NH_4OAc buffer (pH 5)	0.7			Cohen et al. (2004)

(ELSD) was frequently and advantageously used for online 2D-chromatography. The application of ELSD however demands using vaporizable eluents in the second dimension (SEC).

A variety of different stationary and mobile phase systems has been launched in order to determine molar masses and molar mass distributions of NaCMCs by aqueous SEC. Some of these systems are summarized in Table 1. All systems contain salts to shield electrostatic interactions and to reduce aggregates. With the exception of aqueous NH_4OAc the added salts are not vaporizable by moderate temperatures and thus the applied eluents do not meet the aforementioned requirements for applying ELS-detection. Since NH_4OAc is a vaporizable salt it was used in the present work as mobile phase in SEC-MALLS of NaCMC. This was done to establish a phase system meeting the requirements for subsequent 2D-chromatography allowing investigating the heterogeneity of NaCMC with respect to molar mass and chemical composition.

As it is widely known, SEC is the relative method. Therefore the determination of molar masses and molar mass distributions requires a calibration curve. In the easiest way the calibration curve is created using narrowly distributed standards with known molar masses having the same chemical nature as the sample to be investigated. For NaCMCs this is not applicable due to a lack of suitable commercial standards.

If a pure SEC mechanism without any interaction between the polymer and the stationary phase is operating, the elution volumes of both, the calibrant and the analyte, are solely determined by their hydrodynamic sizes. Under these conditions, the universal calibration curves of the calibrant and the analyte, i.e. plots of the product of intrinsic viscosity and molar mass ($[\eta]\cdot M$) versus elution volume, result in a common calibration curve. In such cases the calibration curve of the analyte can be constructed from the calibrants calibration curve, if the Mark–Houwink parameters for the calibrant and the analyte are known in the applied eluent and at the given temperature. This approach is referred to as universal calibration (Benoît, Grubisic, Rempp, Decker, & Zilliox, 1966; Grubisic, Rempp, & Benoît, 1967). The universal calibration principle has been revealed for NaCMCs with *DS* = 0.7–0.8 by Eremeeva et al. (Eremeeva & Bykova, 1998) in 500 mmol/L NaOH and 400 mmol/L acetate buffer as eluents using dextrans as calibrants. Rinaudo et al. (Rinaudo, Danhelka, & Milas, 1993) demonstrated that in 100 mmol/L NH_4NO_3 the universal calibration curves merge for poly(maltotrioses) as calibrants and for NaCMCs in the range *DS* = 1.0–2.9. However, no information is given in literature on the Mark–Houwink parameters of NaCMCs in 100 mmol/L aqueous NH_4OAc, the solvent used in the present work, to the best of our knowledge.

For copolymers, and NaCMC has to be regarded as a complex copolymer composed of eight different monomer units, which differ by the number and positions of the substituents within the anhydroglucose unit (AGU), the situation is even more complicated, as the Mark–Houwink parameters might vary with chemical composition. This transforms into a variation of the Mark–Houwink parameters with *DS*. Therefore, for accurate application of the universal calibration approach, the dependence of the Mark–Houwink parameters on *DS* in the given *DS*-range needs to be thoroughly investigated. Rinaudo et al. (Rinaudo et al., 1993) observed only a minor influence of *DS* on the Mark–Houwink parameters for NaCMCs in range *DS* = 1.0–2.9 in 100 mmol/L NH_4NO_3. However, the situation can change in other solvents or for NaCMCs with other *DS* ranges.

SEC with light scattering or viscosity detection is suitable to determine true molar masses and molar mass distributions. However the higher complexity of the instrumentation often prevents the application in routine analysis. The problems are even more pronounced when such methods should be added to a 2-dimensional chromatographic setup, the final goal in our analytical approach. Therefore the aim of the present work was developing a reliable routine method for molar mass determination of NaCMCs in the range *DS* = 0.45–1.55, meeting the requirements of online 2D-chromatography using ELS-detection. The method should allow using commercially available standards in conjunction with suitable correction factors to determine the molar masses.

For this purpose the true molar masses and molar mass distributions of a set of NaCMCs were first determined by SEC-MALLS in aqueous NaCl, a solvent formerly applied in light scattering experiments of NaCMCs (Schneider & Doty, 1954; Sitaramaiah & Goring, 1962; Trap & Hermans, 1954). These molar masses and molar mass distributions served as reference values for the subsequent method development in aqueous NH_4OAc.

2. Materials and analytical methods

2.1. Solvents and samples

Water was obtained by deionization through a Milli-Q system (Millipore water). Sodium chloride (NaCl) and ammonium

Table 2
Amounts of sodium monochloroacetate used for the carboxymethylation reaction.

Sample (*DS*)	Amount of sodium monochloroacetate [g]
Avicel 1/BWL 1 (0.45/0.46)	1.85
Avicel 2/BWL 2 (0.75/0.73)	2.78
Avicel 3/BWL 3 (0.98/0.95)	3.80
Avicel 4/BWL 4 (1.23/1.25)	5.50
Avicel 5/BWL 6 (1.54/1.55)	1.85 + 3.40/1.80 + 3.80

acetate (NH_4OAc) were purchased from Sigma–Aldrich (Steinheim, Germany) and VWR (Haasrode, Belgium), respectively.

NaCMCs Avicel and BWL were laboratory samples synthesized at the University of Jena. Typical protocols for the synthesis are given below.

2.1.1. Synthesis of Avicel 1–4 and BWL 1–4

5 g of Avicel or BWL were suspended in 150 mL of 2-propanol for 20 min and 13.3 mL of a 15% (w/v) of aqueous sodium hydroxide was added within 1 h at room temperature under stirring. Sodium monochloroacetate (amounts see Table 2) was added and the reaction mixture was allowed to react for 5 h at 55 °C under stirring. After cooling to room temperature, the solid material was separated by filtration, suspended in 300 mL of 80% (v/v) aqueous methanol and neutralized with diluted acetic acid. The solid material was separated again and washed three times with 80% (v/v) aqueous ethanol and finally with pure ethanol. The solid material was dried in vacuum at 40 °C yielding products Avicel 1–4 or BWL 1–4 with average degrees of substitution determined by HPLC after chain degradation given in Table 2. This instruction, however, is valid only for *DS* less than 1.35.

2.1.2. Synthesis of Avicel 5

In order to get *DS* higher than 1.35 the product Avicel 1 was carboxymethylated again under comparable conditions. That means that NaCMC was suspended in 150 mL of 2-propanol, treated with 13.3 mL of a 15% (w/v) of aqueous sodium hydroxide and allowed to react with 3.40 g of sodium monochloroacetate. After the reaction time of 5 h at 55 °C and usual work-up, the product Avicel 5 with *DS* 1.54 was obtained.

2.1.3. Synthesis of BWL 6

Analogously, for synthesis of BWL 6 the carboxymethylation reaction was carried out twice. First the same procedure as described above for synthesis of BWL 1–4 was carried out by using 1.80 g of sodium monochloroacetate. The obtained product with *DS* 0.46 was carboxymethylated again under comparable conditions. That means that NaCMC was suspended in 150 mL of 2-propanol, treated with 13.3 mL of a 15% (w/v) of aqueous sodium hydroxide and allowed to react with 3.80 g of sodium monochloroacetate. After the reaction time of 5 h at 55 °C and usual work-up, the product BWL 6 with *DS* 1.55 was obtained.

2.2. DS determination

The average degrees of substitution *DS* were determined by HPLC after chain degradation as described in (Heinze, Erler, Nehls, & Klemm, 1994).

The characterization results of the samples are given in the second column of Table 3.

2.3. SEC/SEC-MALLS

SEC and SEC-MALLS experiments were performed using a TOSOH Bioscience (Tokyo, Japan) EcoSEC Micro-SEC-System with built in RI-detector to which a DAWN DSP MALLS detector (Wyatt

Table 3
DS_{HPLC}, recovery after filtration, dn/dc, $M_{w,ref}$, M_w, $M_{w,pull}$, $M_{w,recalc}$ of NaCMCs.

Sample	*DS*	Recovery after filtr.grav. from NaCl	dn/dc in NaCl [mL/g]	dn/dc in NH_4OAc [mL/g]	$M_{w,ref}$ in NaCl [g/mol]	M_w in NH_4OAc [g/mol][b]	$M_{w,pull}$ in NaCl [g/mol]	$M_{w,pull}$ in NH_4OAc [g/mol]	$M_{w,recalc}$ in NaCl [g/mol][b]	$M_{w,recalc}$ in NH_4OAc [g/mol][b]
Avicel 1	0.45	99.4%	0.159	0.153	392,000	481,000	–	–	–	–
						23%				
Avicel 2	0.75	96.9%	0.163	0.178	43,000	42,000	–	–	–	–
						−2%				
Avicel 3	0.98	94.9%	0.157	0.157	39,000	35,000	164,000	159,000	40,000	37,000
						−10%	320%	310%	3%	−6%
Avicel 4	1.23	95.9%	0.158	0.156	42,000	39,000	178,000	171,000	44,000	40,000
						−7%	323%	308%	5%	−5%
Avicel 5	1.54	96.3%	0.144	0.140	46,000	44,000	162,000	164,000	40,000	38,000
						−4%	252%	256%	−13%	−17%
BWL 1	0.46	85.3%	n.m.[a]	n.m.[a]	236,000	219,000	967,000	936,000	244,000	228,000
						−7%	310%	299%	3%	3%
BWL 2	0.73	91.0%	n.m.[a]	n.m.[a]	341,000	260,000	1,390,000	1,160,000	351,000	280,000
						−24%	308%	240%	3%	−18%
BWL 3	0.95	95.4%	n.m.[a]	n.m.[a]	386,000	383,000	157,000	159,000	398,000	375,000
						−1%	307%	312%	3%	−3%
BWL 4	1.25	95.3%	n.m.[a]	n.m.[a]	481,000	435,000	1,770,000	1,730,000	450,000	419,000
						−10%	268%	260%	−6%	−13%
BWL 6	1.55	96.5%	n.m.[a]	n.m.[a]	318,000	339,000	1,220,000	1,320,000	309,000	311,000
						7%	284%	315%	−3%	−2%

[a] Not determined.
[b] Relative deviation to $M_{w,ref}$.

Technology, Santa Barbara, USA) was added between the column and the RI-detector. A column set consisting of a Suprema 10 μm 10^4 Å (8 mm × 300 mm), a Suprema 10 μm 30 Å (8 mm × 150 mm) and a pre-column Suprema 10 μm 30 Å (PSS Standards Service, Mainz, Germany) were used at a flow rate of 1.0 mL/min and a column temperature of 35 °C. The injection volume was 80 μL. Experiments were performed in 100 mmol/L aqueous NaCl and 100 mmol/L aqueous NH_4OAc as eluents. All chromatographic experiments were run as duplicates.

Data acquisition and evaluation was performed using PSS WINGPC-Unity version 7.0 (PSS Polymer Standards Service GmbH, Mainz, Germany) and ASTRA version 4.90.08 (Wyatt Technology, USA).

Conventional SEC calibration (base calibration) was performed using a broad pullulan membrane standard (PSS-mepulb300k, 342,000 g/mol) obtained by PSS Polymer Standards Service GmbH (Mainz, Germany) for which the cumulative mass distribution was supplied by the vendor. This sample was also used to countercheck the performance of the LS-instrument.

The MALLS-detector was calibrated using pure toluene assuming a Rayleigh ratio of 9.78×10^{-6} cm^{-1} at 690 nm. The instrument constant of the RI-detector was determined using dextran and pullulan in pure water assuming a refractive index increment (dn/dc) of 0.145 mL/g for both polymers (values provided by PSS Standards Service, Germany), ignoring the differences between the wavelengths of the RI- and MALLS-detector.

2.4. SEC sample preparation

Samples were prepared at concentrations of 3 g/L for Avicel and 0.5 g/L for BWL with benzoic acid (0.25 g/L) as internal standard. The samples were dissolved in pure water over night at 25 °C with agitation at concentrations of 4.5 g/L for Avicel and 1.25 g/L for BWL. Afterwards aqueous solutions of NaCl or NH_4OAc containing benzoic acid were added to adjust the salt concentration to the desired 100 mmol/L. The solutions were filtrated immediately through a syringe filter (PALL, Sample Acrodisc® PSF, 25 mm, GxF/0.45 μm PVDF membrane).

2.5. Agilent chromatographic system

Losses on column were determined using an Agilent 1100 instrument consisting of a quaternary pump (G1311A), degasser (G1322A), automated sample injector (G1313A) and column oven (G1316A). An ELSD 2100 (Polymer Laboratories, Church Stretton, England) operated at an evaporator temperature of 110 °C, an nebulizer temperature of 60 °C and a gas flow rate of 2.3 SLM was used for the detection. The flow rate was 1 mL/min, the injection volume 80 μL and the column temperature 35 °C. For data acquisition and evaluation PSS WINGPC-Unity version 7.0 (PSS Polymer Standards Service GmbH, Mainz, Germany) was used.

2.6. Determination of recoveries after filtration and chromatography

2.6.1. Loss by filtration

The recoveries of NaCMCs after filtration were determined by dissolving well known amounts of the samples in 100 mmol/L aqueous NaCl at a concentration 1 g/L. The sample preparation followed the same procedure as described for the chromatographic experiments. However, no benzoic acid was added. The samples were filtered through a syringe filter (PALL, Sample Acrodisc® PSF, 25 mm, GxF/0.45 μm PVDF membrane). The water was evaporated and the remaining residues were dried at 50 °C in vacuum until a constant mass was reached. The recovery of NaCMC after filtration was calculated as ratio of the solid residue over initial sample amount, taking into consideration the NaCl added. The experiments were carried out in duplicates.

2.6.2. Loss on column

The recoveries in the chromatographic experiments in 100 mmol/L aqueous NH_4OAc were determined by dissolving the samples (Avicel 1, 5 and BWL 1, 6) in the eluent at concentrations of 3 g/L and 0.5 g/L for Avicel and of 0.5 g/L for BWL using the same procedure as described above, however, without the addition of benzoic acid.

Well defined amounts of the samples were injected into the Agilent chromatographic system. However, only the Suprema 10 μm 30 Å (8 mm × 150 mm) column and the pre-column were used in order to minimize the experimental time and to maximize the sample concentration in the collected effluent. Well defined volumes of the effluent covering the elution region of the eluting polymer material were collected. The theoretical sample concentration within the collected volume was calculated based on the injected sample mass and the collected volume. The true sample concentrations were determined by re-injection of the collected solutions into the chromatographic system without column, using a calibration curve previously established by injecting well known amounts of the respective sample at the same conditions. The ratio of the experimentally determined over the calculated concentration was taken as the sample recovery.

2.7. DS determination of insoluble and soluble parts of BWL 1 and BWL 2

In order to characterize the soluble and insoluble parts of BWL 1 and 2 these samples were filtered quantitatively. Approximately 100 mg of the samples were dissolved in pure water overnight at 25 °C with agitation at a concentration 2 g/L. The solutions were filtered through a folded filter paper (Schleicher and Schuell 595½, 150 mm diameter). Subsequently, the filtrates (soluble parts) and insolubles were dried at 50 °C in vacuum to constant weight. The *DS* values of the solid materials were determined as described in Section 2.2.

3. Results and discussion

3.1. Recovery after filtration

Sitaramaiah and Goring (1962) postulated the presence of high molar mass aggregates in aqueous solutions of NaCMCs which will not fully dissolve. These aggregates might give rise to blocking of the inlet frits of the SEC columns or the SEC columns themselves and might result in incomplete recoveries in chromatographic experiments. Since in the present work the NaCMC solutions were filtrated before the SEC measurements sample loss during filtration might occur. This would change the concentrations of the analyzed material which in turn will influence the determination of dn/dc and the molar masses derived by SEC-MALLS. In order to identify possible losses during filtration NaCMCs recoveries after filtration were determined gravimetrically.

The experiments were carried out only in 100 mmol/L aqueous NaCl as eluent at a polymer concentration 1 g/L. Since NH_4OAc is vaporizable and can partially be lost during drying, the same approach could not be used for the samples dissolved in aqueous NH_4OAc. Therefore the losses in NH_4OAc were assumed to be identical to those determined in NaCl solution. The recoveries are summarized in the third column of Table 3.

For all Avicel samples the recoveries after filtration from NaCl solutions were observed to be in the range 94.9–99.4% with variances of 1–2%, without any noticeable dependence on *DS*. Thus, nearly quantitative recoveries were obtained for these samples. For

the high molar mass BWL samples the situation is slightly different. While the recoveries of BWL 3–5 are nearly quantitative, BWL 1 (*DS* = 0.46) and BWL 2 (*DS* = 0.73) having the lowest *DS*-values were recovered to only 85.3% and 91.0%, respectively. Thus it appears as if a correlation of the recovery with *DS* exists for the high molar mass BWLs. The higher *DS* the higher the recovery reaching complete recovery for the high molar mass NaCMCs above $DS \approx 0.9$.

In order to obtain additional proof on the dependence of the NaCMC solubility on *DS*, the *DS*s of the filtrates (soluble parts) and insolubles of BWL 1 and 2 were determined. For BWL 1 the *DS* values for the soluble and insoluble materials were determined to be 0.49 and 0.17, respectively. For BWL 2 the soluble fraction has a *DS* of 0.72, while the *DS* of the insoluble fraction was found to amount to 0.24. These values clearly demonstrate that the insoluble fractions are of low *DS*. Consequently, unsubstituted and lightly substituted polymer chains are insoluble and remained on the filter. The results on the *DS* values of the soluble and insoluble fractions also clearly indicate that the different polymer chains vary considerably in their *DS* values, i.e. a substantial chemical heterogeneity exists.

3.2. Recovery after SEC separation

Besides sample loss during filtration, a loss of the sample due to aggregation or adsorptive interactions of the analyte with the column material might occur as well. Therefore the losses on the column during the chromatographic experiment were determined. These experiments were carried out only for the BWL and the Avicel with the lowest and the highest *DS* values. Due to the application of ELSD, the recoveries were determined in aqueous NH_4OAc only. The lack of suitable chromophores in NaCMC prevents the use of UV-detection, while the application of RI-detection without a column is not suitable due occurrence of system peaks. Two different concentrations – 3 and 0.5 g/L – were used for Avicel, while for the BWL samples only a concentration of 0.5 g/L was selected. The following recoveries were obtained which have prior been corrected for the loss during filtration: Avicel 1 (3 g/L): 75 ± 1%, Avicel 1 (0.5 g/L): 100 ± 7%, Avicel 5 (3 g/L): 76 ± 5%, Avicel 5 (0.5 g/L): 90 ± 3%, BWL 1 (0.5 g/L): 95 ± 5%, BWL 6 (0.5 g/L): 100 ± 3%.

For the low concentration of 0.5 g/L nearly quantitative recoveries (>90%) were determined for all 4 samples. Surprisingly, only recoveries of 75% and 76% were obtained for Avicel 1 and 5, respectively, if dissolved at a concentration of 3 g/L. Thus, high concentration obviously provoked lower recoveries on the column. However, concentrations of 3 g/L were required in the SEC-MALLS investigations for the lower molar mass Avicel samples in order to obtain a good signal/noise ratio. In order to account for the lower recoveries at higher concentrations, the concentrations at the detector were corrected assuming an averaged recovery of 75.5% for all Avicel samples. This average recovery determined in aqueous NH_4OAc was assumed to be valid for aqueous NaCl as eluent as well.

3.3. SEC elution curves

SEC separates polymer chains according to the hydrodynamic volume and not according to molar mass. The hydrodynamic volumes of polyelectrolytes like NaCMCs depend among other factors on the type and concentration of salt in the eluent (Brown & Henley, 1964; Contois & Trementozzi, 1955; Conway, 1955). Thus, if the hydrodynamic volumes of NaCMC chains in aqueous NaCl and NH_4OAc are different, the chromatograms obtained in both solvents will differ from each other.

Therefore the effect of the salt concentration on the chromatograms was investigated in order to identify conditions at which the electrostatic interactions of the charges along and among the polyanions are completely suppressed. Using pure water as eluent a large hydrodynamic volume, i.e. a low elution volume was observed (not shown). The addition of NH_4OAc to the eluent reduced the hydrodynamic volume, resulting in an increasing elution volume. At NH_4OAc concentrations higher than 100 mmol/L no significant changes of the chromatogram were observed. This concentration was used for aqueous NaCl as eluent as well.

Overlays of the chromatograms of NaCMCs in 100 mmol/L aqueous NaCl and NH_4OAc are shown in Fig. 1.

For all samples broad peaks are observed in the chromatograms for both, aqueous NaCl and NH_4OAc, indicating broad molar mass distributions. BWL samples elute between 9.5 and 15 mL and thereby earlier than Avicel samples, which are eluting between 11 and 16 mL, in both eluents. This indicates the higher average molar masses of BWL as compared to Avicel. One should notice the small hump between 10.5 and 12 mL in the chromatogram of Avicel 1, which at this point might be an indication for the existence of aggregates for this sample.

When comparing the chromatograms in both eluents a small shift of the chromatograms in NaCl solution toward lower elution volumes can be observed for nearly all samples, which, however, is more pronounced for BWL 2. This indicates slightly larger hydrodynamic volumes of BWL 2 in NaCl as compared to NH_4OAc. As will be shown below, this coincides with a higher M_w-value of BWL 2 determined in NaCl as compared to the M_w-value of this sample determined in NH_4OAc, which can be an indication of different degrees of aggregation in both solvents for this sample.

In addition a small shift of the chromatograms of BWL 1, 2 and 6 to higher elution volumes as compared to the chromatograms of BWL 3 and 4 can be observed in both eluents, while the Avicel-chromatograms of different *DS*s nearly coincide. A possible explanation for the observed shift of BWL 1 and 2 can be the presence of the high molecular mass insolubles in the original samples which were filtered off before chromatographic analysis. The residuals of these samples therefore have lower molar masses than original ones and their chromatograms are consequently shifted to the higher elution volumes as compared to the chromatograms of BWL 3 and 4 which do not contain insolubles. BWL 6 does not contain insolubles as indicated by the nearly quantitative recovery after filtration. The observed chromatographic shift to the smaller molar masses has therefore to be attributed to a different effect, which might be partial chain degradation during synthesis.

The dependence of the elution volume on *DS* for the BWL samples seems to indicate that standards of identical *DS* than the sample to be analyzed are required in order to establish a conventional calibration curve. However, such standards are not available commercially.

3.4. Influence of DS and salt on refractive index increment, dn/dc

In order to determine the true molar masses of NaCMCs by SEC-MALLS and to investigate the effect of *DS* on the SEC calibration curves, knowledge of the refractive index increments, *dn*/*dc*, in the eluents is essential.

dn/*dc*-Values were experimentally determined for Avicel only, since only for these samples a complete recovery after filtration was proven, as mentioned above. However, the incomplete recoveries of these samples after the SEC separation needed to be kept in mind for *dn*/*dc* determination. No significant and systematic differences of the *dn*/*dc*-values obtained in the two eluents were observed for Avicel (fourth and fifth columns of Table 3). In addition no systematic variations of *dn*/*dc* with *DS* were observed. Therefore an average value of *dn*/*dc* = 0.156 mL/g was used for the subsequent determinations of molar masses. This value is comparable with the value *dn*/*dc* = 0.147 mL/g determined by offline light scattering experiments for NaCMCs in 100 mmol/L NaCl in the range *DS* = 0.66–0.73 (Sitaramaiah & Goring, 1962). An independence of *dn*/*dc* on *DS*

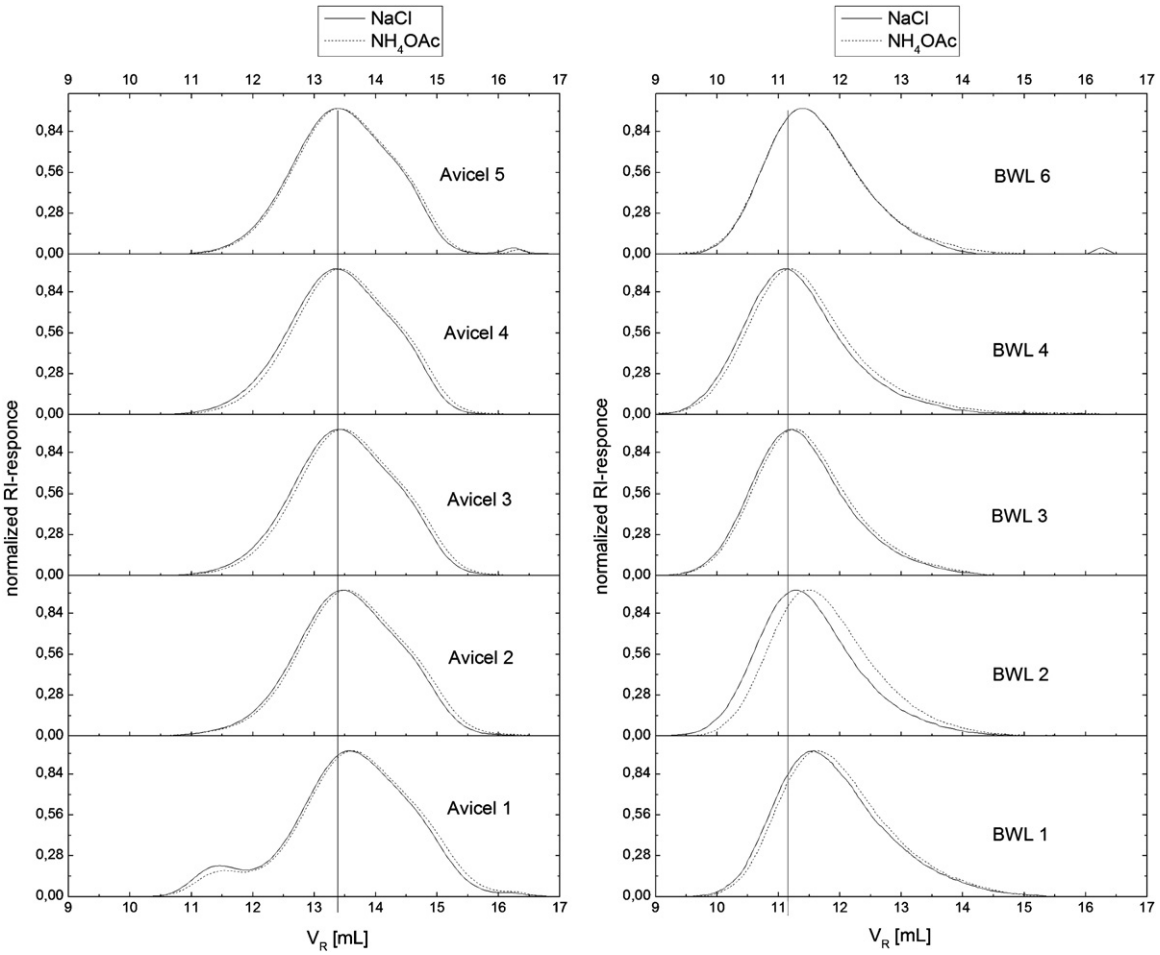

Fig. 1. Overlays of chromatograms of NaCMCs in 100 mmol/L aqueous NaCl and NH_4OAc as eluents; injection volume: 80 μL; column set: Suprema 10 μm 10,000 Å (8 × 300 mm) and Suprema 10 μm 30 Å (8 × 150 mm) at 35 °C; RI detector temperature: 35 °C; flow rate: 1 mL/min.

was also reported in 100 mmol/L NH_4NO_3 for NaCMCs in the range *DS* = 1.00–2.90 (Rinaudo et al., 1993) and for NaCMCs with *DS* ranging from *DS* = 0.75 to 1.25 in 20 mmol/L and 100 mmol/L $NaNO_3$ (Hoogendam, Keizer, Stuart, & Bijsterbosch, 1998). However Brown et al. (Brown, Henley, & Öhman, 1963) observed a slight decrease of *dn*/*dc* with increasing *DS* in the range of *DS* = 0.21–0.94 in cadoxen (triethylenediamine-cadmium hydroxide). Our results therefore coincide with the present investigations on similar systems.

For high molar mass polymers *dn*/*dc* is independent of molar mass (Wyatt, 1993), which has also been shown to be true for NaCMC (Hoogendam et al., 1998; Sitaramaiah & Goring, 1962). Therefore it is acceptable to use the average value of *dn*/*dc* determined for Avicel for the BWL samples as well.

3.5. Determination of M_w by SEC-MALLS

3.5.1. Reference $M_{w,ref}$ in 100 mmol/L aqueous NaCl

Having determined the *dn*/*dc* value, the molar masses in 100 mmol/L aqueous NaCl as eluent were determined. This solvent has been shown to be suitable for the determination of molar masses of NaCMCs by light scattering experiments (Schneider & Doty, 1954; Sitaramaiah & Goring, 1962; Trap & Hermans, 1954). Thus, the obtained molar masses were taken as reference molar masses ($M_{w,ref}$). They are listed in the sixth column of Table 3.

The $M_{w,ref}$-values of the BWLs are higher by approx. a factor of 5 than those of Avicel (except Avicel 1), in agreement with lower SEC elution volumes (higher hydrodynamic volumes) of the BWLs. There is a remarkable high molar mass of Avicel 1 in comparison to the other Avicel samples. This supports the assumption on the presence of aggregates or other unusual structures in Avicel 1, as already indicated by the presence of the small peak between 10.5 mL and 12 mL in the SEC chromatogram of this sample.

Based on the SEC-elution volumes lower molar masses were expected for BWL 1, 2 and 6 as compared to BWL 3 and 4. This was verified experimentally, indicating the removal of the high molar mass insolubles contained in BWL 1 and 2 by filtration and the partial degradation during synthesis of BWL 6. Thus, the unexpected high elution volume of these samples observed in Fig. 1 (left) is not the result of an experimental artifact, but rather illustrates the dependence of the SEC elution volume on molar mass.

The differences in the average molar masses between BWL 3 and 4 as well as between Avicel 3, 4 and 5 can be explain by the mass increase of AGUs associated with the replacement of protons by the heavier carboxymethyl groups resulting in a growing molar mass with increasing *DS*.

3.5.2. M_w in 100 mmol/L aqueous NH_4OAc

Having determined the reference molar masses of NaCMCs in aqueous NaCl, they were compared with the molar masses obtained by SEC-MALLS in 100 mmol/L aqueous NH_4OAc (given in the seventh column of Table 3). The comparison of the molar masses obtained in both solvents is made by the relative deviation of M_w determined in NH_4OAc to the $M_{w,ref}$. With the exception of Avicel 1 and BWL 2 the relative deviations are within ±10% which is the acceptable error in M_w originating from LS based on the experience in our laboratory. Therefore the molar masses determined in

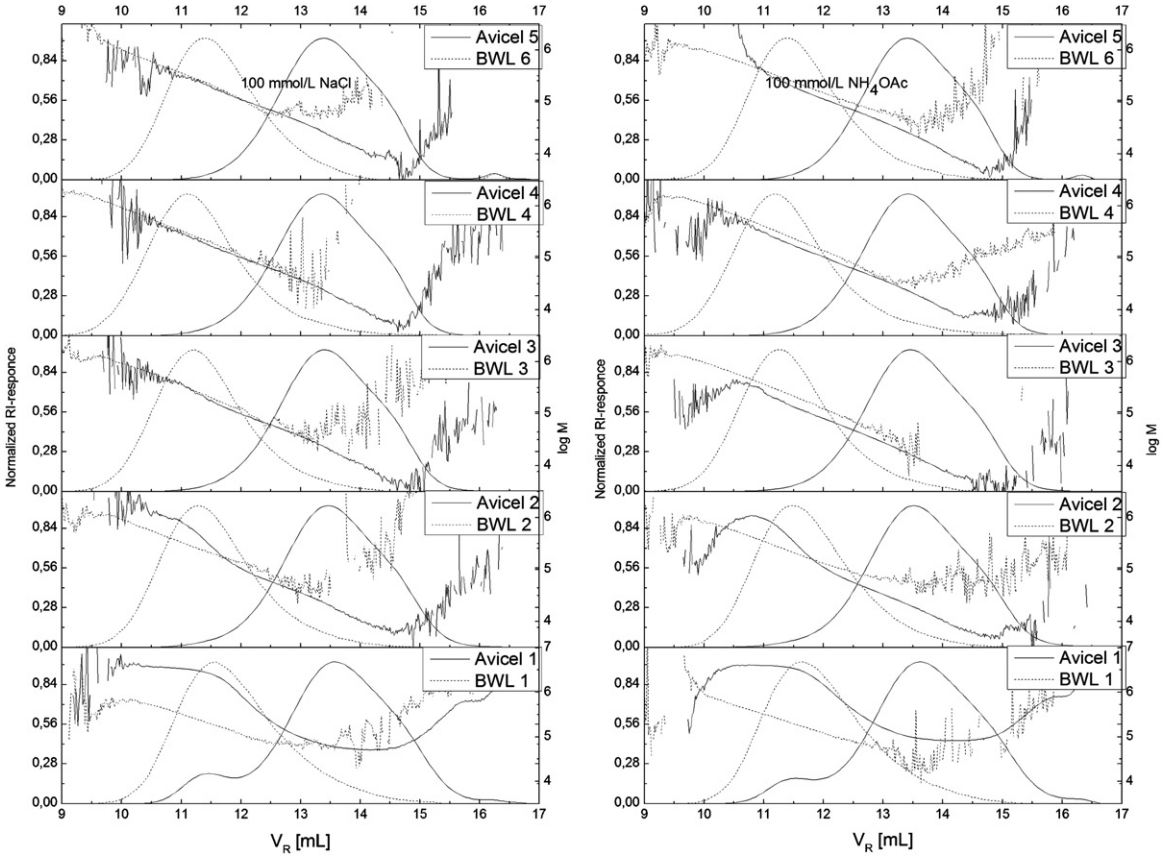

Fig. 2. Overlays of calibration curves and chromatograms of Avicels and BWLs having similar *DS*. Left: 100 mmol/L NaCl. Right: 100 mmol/L NH_4OAc. Exp. conditions see Fig. 1.

both eluents can be regarded to be identical within the accuracy of the method, especially when taking into account the experimental problems originating from sample losses during filtration and on column. Thus, aqueous NH_4OAc represents a reliable eluent for the determination of the molar masses of NaCMC by SEC-MALLS. The larger deviation of Avicel 1 might be caused by the aggregations described above. Moreover, it is obvious that the shift of the SEC chromatograms between the two solvents observed for BWL 2 (see Fig. 1) coincides with the more pronounced difference between $M_{w,\mathrm{ref}}$ and M_w for this sample. Therefore we assume that the molar mass and the hydrodynamic volume of BWL 2 dissolved in aqueous NaCl are truly different than in aqueous NH_4OAc. For Avicel 1 and BWL 2 the differences in molar mass therefore seem to be due to different degrees of aggregation in both solvents.

3.6. Calibration curves determined by SEC-MALLS

Fig. 2 shows overlays of the calibration curves and the chromatograms obtained by SEC-MALLS for BWL and Avicel samples of similar *DS* in both eluent systems. As expected for a SEC separation, the molar masses decrease with increasing V_R (except for Avicel 1 and 2 between 10.5 and 11.5 mL). The scattering of the molar masses at the high and low molar mass end of the chromatograms is typical for SEC-MALLS measurements and is attributed to the different sensitivities of the LS- and RI-detectors in the high and low molar mass regions. The calibration curves for the Avicel- and BWL-samples of similar *DS* merge closely in every plot (except sample pairs Avicel 1 – BWL 1 and Avicel 2 – BWL 2 in the region 10.5–11.5 mL) which demonstrates that the given SEC methods are useable for the separation of NaCMCs according to the molar mass.

The calibration curves of Avicel 1 and 2 (between 10.5 mL and 11.5 mL) in both eluents show strong deviations from the calibration curves of the corresponding BWL-samples. In addition, they deviate from linearity and show pronounced curvatures. The higher M_w-values determined above for these samples are logical consequences of these calibration curves. Since this unusual behavior is observed in both eluents, experimental artifacts can be ruled out. Thus, the effect originates from structural differences in the samples. Based on the calibration curve of Avicel 1 it can be concluded that the small peak between 10.5 mL and 12 mL in the chromatogram of this sample represents very high molar mass species. Sitaramaiah et al. (Sitaramaiah & Goring, 1962) suggested the presence of high molecular weight aggregates in aqueous NaCl solutions of NaCMCs, based on LS studies. However, it is rather unlikely that in both eluents the same type and amount of aggregates exists, rendering identical average molar masses. Based on the very similar molar masses in both eluents, branched or partially cross-linked structures seem to be a more plausible explanation.

Overlays of calibration curves of samples having different *DS* but similar molar masses are given in Fig. 3. Avicel 1 and 2 were excluded due to their unusual behavior. The calibration curves of samples of different *DS* closely agree to each other. The relative deviations of the molar masses at a given V_R are within app. 10%, i.e. in the order of the uncertainly of the method. Therefore the calibration curves can be regarded as being independent of *DS*. Consequently, NaCMCs within the *DS*-range under investigation can be evaluated with the same calibration curve.

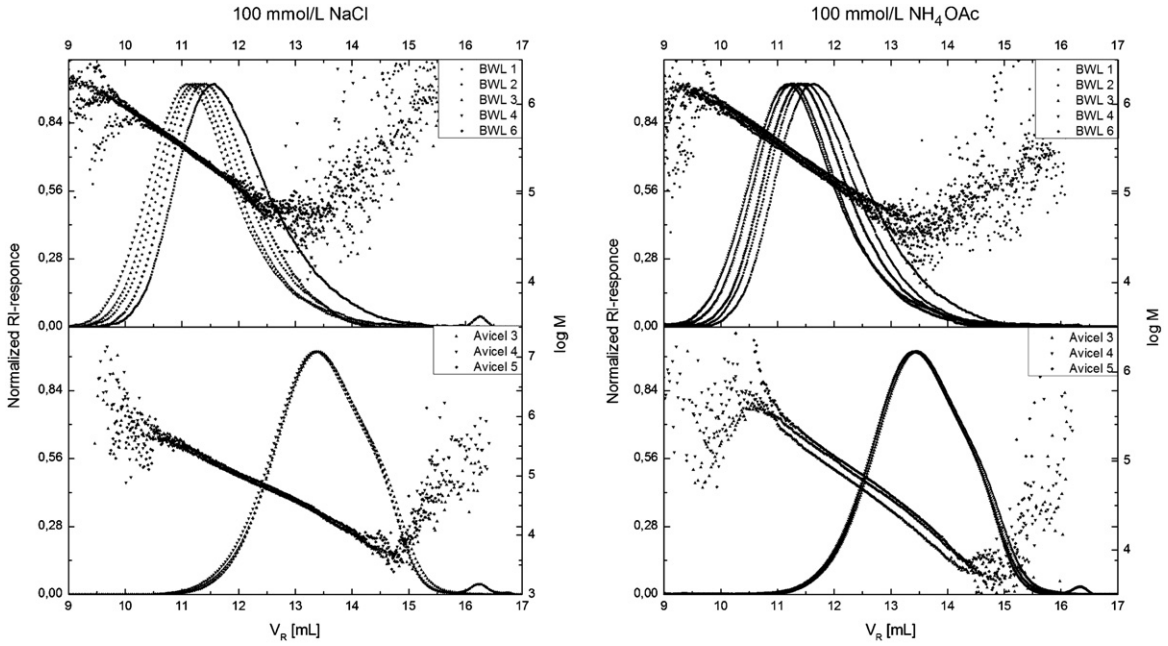

Fig. 3. Overlays of calibration curves and chromatograms of NaCMCs with different *DS* and similar molar masses in 100 mmol/L NaCl (left) and NH_4OAc (right) as eluents. Exp. conditions see Fig. 1.

3.7. Scaling behavior of NaCMCs

In order to investigate the dependence of log $\langle s^2 \rangle^{1/2}$ on log *M* relationships the exponents a_s were determined for the BWL samples (in Fig. 4 in NH_4OAc as an example). For the Avicel samples no reasonable radii could be obtained, due to the much lower molar masses. Nearly linear increases of the radii of gyration with increasing molar mass are observed in the double logarithmic plots. The scattering at the low molar mass end of the $\langle s^2 \rangle^{1/2}$ versus molar mass plot is a consequence of the lower accuracy of radii and molar masses at the low molar mass end of the chromatogram, which in turn is related to the different sensitivities of the light scattering and RI-detector. As can be seen, all double logarithmic plots fall onto a common line, thus, only minor variations of the scaling exponents with *DS* are observed. The scaling exponents a_s obtained for the individual samples are summarized in Table 4.

For both eluents and all samples the scaling exponents a_s fall into the range 0.59–0.64 indicating the structure of expanded coils in solution. These values are some higher than those reported by Kath et al. (Kath, Lange, & Kulicke, 1999) $a_s = 0.54–0.58$ in 100 mmol/L aqueous $NaNO_3$ and Hoogendam et al. (Hoogendam et al., 1998) $a_s = 0.59$ in 100 mmol/L aqueous KOH and $NaNO_3$ also by SEC coupled with light scattering. The differences might be caused by the different salts used.

However, neither in NaCl nor in NH_4OAc a significant variation of a_s with *DS* can be observed. Therefore, *DS* does not influence structure of NaCMCs in salt solution within the *DS* range investigated. This result is in accordance with the investigations of Brown et al. (Brown et al., 1963; Hoogendam et al., 1998) for the *DS* range *DS* = 0.21–0.94 in cadoxen (triethylenediamine-cadmium hydroxide) and of Rinaudo et al. (1993) who stated only a little influence of *DS* on the Mark–Houwink parameters within the *DS* range 1.00–2.90 in 100 mmol/L aqueous NH_4NO_3.

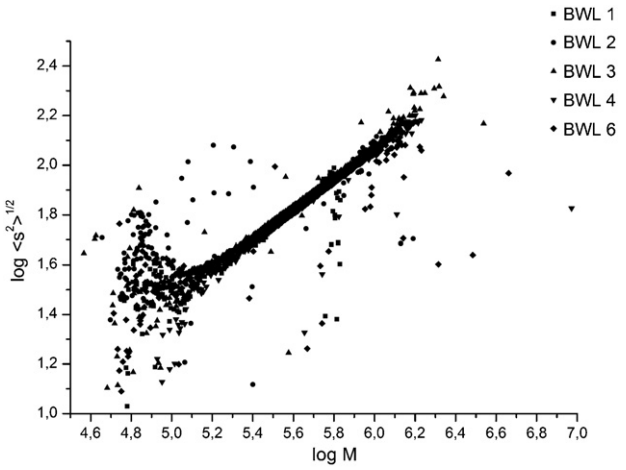

Fig. 4. Plots of log $\langle s^2 \rangle^{1/2}$ vs. log *M* for BWL in 100 mmol/L aqueous NH_4OAc determined by SEC-MALLS.

3.8. Correction factors for the determination of calibration curve of NaCMCs

While SEC-MALLS is a valuable technique for the determination of true molar masses and structural parameters, it is less applicable for a routine molar mass determination e.g. in a quality control laboratory. In order to allow for molar mass determination based on commercially available standards, calibration curves using pullulan standards were established in both eluents and the molar masses of NaCMCs ($M_{w,\mathrm{pull}}$) were derived from these calibration curves. The results are given in the eighth and the ninth columns of Table 3. The samples Avicel 1 and 2 were not evaluated due to the presence of the unusual high molar mass species. The comparison of the

Table 4
a_s Exponents of NaCMCs in 100 mmol/L aqueous NaCl and NH_4OAc.

Sample	a_s in NaCl	a_s in NH_4OAc
BWL 1	0.62 ± 0.00	0.62 ± 0.03
BWL 2	0.60 ± 0.01	0.60 ± 0.01
BWL 3	0.60 ± 0.01	0.59 ± 0.01
BWL 4	0.61 ± 0.00	0.62 ± 0.00
BWL 6	0.64 ± 0.02	0.61 ± 0.00

molar masses derived using a pullulan calibration curve with the ones determined by SEC-MALLS revealed that a pullulan calibration overestimated the true molar masses by approximately a factor of 4.0 for NaCl and 4.2 for NH_4OAc revealing the large differences in the hydrodynamic volumes of pullulan and NaCMC at a given molar mass. Obviously, pullulans cannot be regarded as suitable calibrants for NaCMCs. Therefore, it was checked, whether suitable conversion factor can be obtained using the broad calibration approach.

The broad standard approach, first described by Weiss, Mahabadi et al. and Mori (Mahabadi & O'driscoll, 1977; Mori, 1981, 1988; Weiss, 1969), utilizes a conventional calibration curve (base calibration) established using standards of arbitrary chemical structure. In the present case the base calibration was established using pullulan. Besides a base calibration one or more accurate average molar masses and the corresponding SEC-chromatograms for broadly distributed samples having the same chemical composition than the sample to be analyzed are required.

Assuming the validity of the universal calibration principle for both, pullulan and NaCMC, the following equation holds true for the molar masses, M, at any elution volume (Benoît et al., 1966; Grubisic et al., 1967):

$$M_{\mathrm{NaCMC}} = \left(\frac{K_{\mathrm{pull}} M_{\mathrm{pull}}^{a_{\mathrm{pull}}+1}}{K_{\mathrm{NaCMC}}} \right)^{1/a_{\mathrm{NaCMC}}+1} \quad (1)$$

where K and a are the Mark–Houwink parameters and exponents of pullulan (index pull) and NaCMC, respectively.

The calibration curve of pullulan can be described as:

$$\log\ M_{\mathrm{pull}} = f(V_R) \Leftrightarrow M_{\mathrm{pull}} = 10^{f(V_R)} \quad (2)$$

where $f(V_R)$ is the function of the calibration curve.

Combining (1) and (2) results in the volume dependent molar mass for NaCMC

$$M_{\mathrm{NaCMC}} = \left(\frac{K_{\mathrm{pull}} \times 10^{(a_{\mathrm{pull}}+1)f(V_R)}}{K_{\mathrm{NaCMC}}} \right)^{1/a_{\mathrm{NaCMC}}+1} = A \times 10^{Bf(V_R)} \quad (3)$$

where A and B are defined as

$$A = \left(\frac{K_{\mathrm{pull}}}{K_{\mathrm{NaCMC}}} \right)^{1/a_{\mathrm{NaCMC}}+1} ; \qquad B = \frac{a_{\mathrm{pull}}+1}{a_{\mathrm{NaCMC}}+1}$$

Knowing the yet unknown parameters A and B, it is possible to convert the pullulan calibration curve into the calibration curve for NaCMC.

The weight average molar mass M_w of NaCMCs can be calculated from the chromatogram $S(V_R)$ using

$$M_{w,\mathrm{NaCMC}} = \frac{\int S \times A \times 10^{Bf(V_R)}\, dV_R}{\int S\, dV_R} \quad (4)$$

with S being the detector response (RI) of the SEC-chromatogram of NaCMC at the elution volume V_R. It should be noted that S is assumed to be proportional to the concentration of repeating units passing the detector. The correction factors A and B can be obtained from the chromatograms by varying A and B until the best agreement of the calculated and the true M_w-values is obtained. The so obtained values can then be used to convert the pullulan calibration curve into a calibration curve for NaCMC, allowing subsequent determination of the molar masses of other NaCMC samples. (Mahabadi & O'driscoll, 1977; Mori, 1981, 1988; Radke, 2007; Weiss, 1969)

In order to determine the correction parameters A and B, the chromatograms and M_wO of Avicel 3–5 and all BWL samples determined by SEC-MALLS were used in conjunction with the pullulan base calibrations in the both eluents. Using the above described approach the following correction factors were obtained for NaCl and NH_4OAc:

NaCl : A : 0.22 B : 1.01
NH_4OAc : A : 0.21 B : 1.01

Using these correction factors and the pullulan base calibrations the weight average molar masses were recalculated from the chromatograms of the NaCMCs in the both eluents. The so obtained M_w-values ($M_{w,\mathrm{recalc}}$) and their deviations with respect to the values derived by light scattering are given in the tenth and the eleventh columns of Table 3.

The roots of the mean squared deviation of $M_{w,\mathrm{recalc}}$ to $M_{w,\mathrm{ref}}$ amount to approximately 6 and 10% for NaCl and NH_4OAc, respectively, and are indicating a good agreement of the molar masses from the broad calibration and from SEC-MALLS. A pullulan calibration curve and the above given conversion factors therefore result in reliable molar masses for NaCMCs in aqueous NaCl and NH_4OAc.

4. Conclusions

A reliable SEC method for sodium carboxymethyl celluloses in the range $DS = 0.45–1.55$ meeting the requirements of on-line 2D-chromatography using ELS-detection was developed in the present work. The characterization of the soluble and insoluble parts revealed selective removal of low DS and high molar mass material by filtration, which in turn proves significant heterogeneity with respect to DS for NaCMCs. The scaling behavior (relation $\langle s^2 \rangle^{1/2} \sim M^{as}$) exhibits expanded coil like structures in solution and is not influenced by DS in the range investigated. The dependencies of molar mass on elution volume are independent of DS. Therefore the dependence of molar mass on elution volume for sodium carboxymethyl celluloses with DS ranging from 0.45 to 1.55 can be well described by a common calibration curve. This is of advantage, as it allows the determination of molar masses of unknown samples by using the same calibration curve, irrespective of their DS. Since no commercial sodium carboxymethyl cellulose standards are available, correction factors were determined using the broad calibration approach allowing converting a pullulan based calibration curve into a sodium carboxymethyl cellulose calibration. The weight average molar masses derived using such a calibration curve closely agree with the ones determined by light scattering, proving the accuracy of the correction factors determined.

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