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Determination of the DS distribution of non-degraded sodium carboxymethyl cellulose by gradient chromatography

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ABSTRACT

Two series of sodium carboxymethyl celluloses (NaCMCs) derived from microcrystalline cellulose (Avicel samples, DP ~ 160) and cotton linters (BWL samples, DP ~ 1400) with average degrees of substitution in the range DS = 0.45–1.55 were analyzed by gradient liquid adsorption chromatography (gradient LAC) in order to determine their chemical composition distributions (DS distributions or 1st order heterogeneities). Clear separations of samples having different average DS values were achieved for both sample series, allowing determination of the DS distributions of the samples. A slight molar mass influence on the eluent composition at elution was observed. From the DS distributions the DS standard deviations were calculated and taken as a measure for the extent of chemical heterogeneity of the single samples. While no noticeable dependence of the chemical heterogeneity on average DS was observed for Avicels, the heterogeneity decreases with increasing average DS for BWLs.

1. Introduction

Carboxymethyl cellulose (CMC) is one of the most important cellulose derivatives in terms of sales (approx. 230 000 tons per annum, Thielking & Schmidt, 2006) up to now. Its applications as thickening, suspending, film-forming and binding, etc. agent reach from paper production to food and pharmaceutical industry. NaCMCs are most often produced by polymer-analogous reaction of alkali cellulose in aqueous sodium hydroxide and an organic liquid with monochloroacetic acid or its sodium salt (slurry process) (Heinze, 2005). Since the anhydroglucose units (AGU) of cellulose have three hydroxyl groups, each of which can be substituted by a carboxymethyl group, the DS of NaCMC, i.e. the average number of substituted OH-groups per AGU, can vary from 0 to 3 depending on the reaction conditions. Industrially used products synthesized by the slurry process typically exhibit average DS values ranging from 0.4 to 1.5 (Heinze, 2005). Due to the statistical nature of the substitution process the substituents are distributed within the AGUs, along single polymer chains and also among the different chains. The distributions of the substituents can potentially influence the application properties of the final products. For example, samples prepared in the slurry process with a statistic distribution of different AGUs and a substitution pattern within the AGUs with O-2 > O-6 ≫ O-3 (Heinze, 2005) become soluble in water at DS = 0.4 (Ramos, Frollini, & Heinze, 2005). Samples synthesized by dissolving of the cellulose in dimethylsulfoxide (DMSO)/tetrabutylammonium fluoride (TBAF) activated by solid NaOH exhibit a non-statistic distribution of the substituted AGUs and a substitution pattern within the AGUs with O-6 > O-2 ≥ O-3 (Ramos et al., 2005) and a more random distribution of the repeating units along the polymer chains dissolve at DS ≥ 0.85 (Heinze, 2005; Ramos et al., 2005). In contrast, CMCs exhibiting a more blocky structure within polymer chains synthesized by dissolving of the cellulose in DMAc/LiCl or via hydrolytically instable cellulose intermediates become soluble at DS ≥ 1.5 (Heinze, 2005; Ramos et al., 2005; Saake et al., 2000).

Today a variety of established methods exist to determine the substitution pattern within the AGUs: ^{1}H and ^{13}C NMR spectroscopy (Baar & Kulicke, 1994; Ho & Klosiewicz, 1980; Ramos et al., 2005), gas–liquid chromatography (Zeller, Griesgraber, & Gray, 1991), HPLC after complete chain degradation (Heinze, Erler, Nehls, & Klemm, 1994; Kragten, Kamerling, & Vliegenthart, 1992; Saake, Horner, Puls, Heinze, & Koch, 2001) or capillary electrophoresis (Lazik, Heinze, Pfeiffer, Albrecht, & Mischnick, 2002; Tüting, Albrecht, Volkert, & Mischnick, 2004). To characterize the substituent distribution along the polymer chains two different strategies were developed. The first one is based on the selective cleavage of the glycosidic bonds of cellulose derivative by the aid of enzymes (Enebro, Momcilovic, Siika-aho, & Karlsson, 2009; Gelman, 1982; Heinze et al., 2000; Horner, Puls, Saake, Klohr, &

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Thielking, 1999; Saake et al., 2000). The enzyme can cleave the glycosidic bond between two lowly substituted AGUs. The resulting oligomeric fragments therefore contain the information on the distribution between two lowly substituted AGUs along the polymer chains. The second strategy which has been used mainly for the characterization of methyl celluloses is based on a partial statistical acidic cleavage of the glycosidic bonds (Arisz, Kauw, & Boon, 1995). The obtained oligomeric fragments are analyzed with respect to the type and amount of the different AGUs and these results are compared to the expected oligomers which would result from a Bernoullian statistics. The extent of deviation of the observed and the expected oligomeric distribution allows gaining information on the homogeneity of the substituents along the polymer chains.

The abovementioned methods provide information on the chemical heterogeneity of the samples. However, due to the degradation processes, these methods cannot differentiate between heterogeneities along a given polymer chain (heterogeneity of 2nd order) and between different polymer chains (heterogeneity of 1st order). Until today there have been only a few attempts to analyze the DS distribution of intact cellulose derivatives, allowing elucidation of the extent of the heterogeneity of 1st order. Oudhoff et al. applied capillary zone electrophoresis to separate NaCMC according to DS (Oudhoff, Buijtenhuijs, Wijnen, Schoenmakers, & Kok, 2004). The detection by this method, however, is difficult and the direct coupling with spectroscopic and spectrometric methods, which would allow obtaining more detailed information of the structures, is hampered due to the very low sample quantities. Saake et al. separated NaCMCs (Saake et al., 2000), while Mischnick et al. separated methyl celluloses in fractions of different DSs using differences in solubility (Adden, Müller, & Mischnick, 2006; Mischnick & Adden, 2008). This method is, however, time consuming, needs substantial samples amounts, is not very selective and difficult to automate. Methylcellulose was analyzed by off-line coupled SEC-^{1}H-NMR (Fitzpatrick et al., 2006). This approach is suitable only for samples showing a strong dependence of DS on molar mass.

Chromatographic methods for polymers which use interactions between the macromolecules and the stationary phase are expected to have a high potential to separate cellulose derivatives according to their chemical composition as they were extensively used for the determination of chemical composition distribution of other synthetic polymers (Glöckner, 1980; Pasch & Trathnigg, 1998). Chromatographic methods have the advantage of requiring low sample amounts and being able to be coupled to spectroscopic, spectrometric and also to other chromatographic methods in order to get additional information. A separation of intact cellulose derivative chains by DS followed by the above mentioned selective or statistic cleavage approach should allow differentiation of both types of heterogeneities and therefore to achieve an extensive characterization of cellulose derivatives. Kawai and Teramachi reported on the separation of cellulose acetates according to DS using liquid adsorption chromatography on a reversed phase column (Kawai, Teramachi, Sakai, & Hibino, 1997). However, no reports on the separation of NaCMCs by DS using liquid chromatography exist to the best of our knowledge. Therefore it was the intentions of the present work to establish such a method.

2. Materials and analytical methods

2.1. Solvents and samples

Water was obtained by deionization through a Milli-Q system (Millipore). Ammonium acetate (NH_4OAc) and methanol were purchased from VWR (Haasrode, Belgium). Acetic acid and sodium acetate (NaOAc) were supplied by Merck KGaA (Darmstadt, Germany).

Table 1
Average DS, M_w, $DS_{w,rec}$ and SD of NaCMCs samples.

Sample	Average DS[a]	M_w (g/mol)	$DS_{w,rec}$[c]	SD
Avicel 1	0.45	392,000	0.46 (2.2%[d])	0.17
Avicel 2	0.75	43,000	0.72 (4.0%[d])	0.16
Avicel 3	0.98	39,000	0.98 (0.0%[d])	0.17
Avicel 4	1.23	42,000	1.23 (0.0%[d])	0.17
Avicel 5	1.54	46,000	1.48 (3.9%[d])	0.18
BWL 1	0.46 (0.49[b])	236,000	0.52 (6.1%[d])	0.26
BWL 2	0.73 (0.72[b])	341,000	0.83 (15.3%[d])	0.26
BWL 3	0.95	386,000	0.99 (4.2%[d])	0.19
BWL 4	1.25	481,000	1.29 (3.2%[d])	0.14
BWL 6	1.55	318,000	1.55 (0.0%[d])	0.10

[a] Determined by HPLC after the complete chain degradation.
[b] DS of the soluble fraction.
[c] Weight average DS recalculated from the DS distributions shown in Fig. 7.
[d] Percentage deviation from average DS listed in column 2.

NaCMCs Avicel und BWL were laboratory samples synthesized at the University of Jena. The details of the synthesis, molar mass and DS characterization were given elsewhere (Shakun, Maier, Heinze, Kilz, & Radke, 2013). The average DSs as well as the weight average molar masses M_w are given in the second and third columns of Table 1.

2.2. HPLC system

The chromatographic separations were performed using an Agilent 1100 HPLC instrument consisting of a quaternary pump (typ G1311A), degasser (typ G1322A), automated sample injector (typ G1313A) and column oven (typ G1316A). An evaporative light scattering detector ELSD 2100 (Polymer Laboratories, Church Stretton, England) set to an evaporator temperature of 120 °C, a nebulizer temperature of 40 °C and a gas flow rate of 1.5 SLM was used for the detection. The stationary phase was a Luna PFP (2) (particle size 5 μm, pore diameter 100 Å, column dimensions 250 mm × 4.6 mm) from Phenomenex (Aschaffenburg, Germany) operated at 35 °C. The flow rate was 1 mL/min. The injected sample concentration was typically 1 g/L. The injection volumes were 50 μL or 100 μL as indicated in the legends to the respective figures. All chromatographic experiments were run as duplicates. For the data acquisition and evaluation PSS WINGPC-Unity version 7.0 (PSS Polymer Standards Service GmbH, Mainz, Germany) was used.

2.3. Gradients conditions

The gradient in the buffer-free experiments was run from water/methanol = 95/5 to 0/100 within 10 min. In the buffer-containing experiments the gradient was run from water/methanol = 95/5 to either 20/80 or 65/35 in 40 min as indicated in legends to the respective figures. In these cases both solvents contained 100 mmol/L NH_4OAc as well as the appropriate amount of acetic acid to adjust the pH of water to 6.06, 4.76, 4.54, 4.10 and 3.46.

2.4. General sample preparation

Samples were prepared at a concentration of 1 g/L in the starting eluent. For sample preparation the samples were first dissolved in pure water overnight at 25 °C with agitation at a concentration of 1.25 g/L. Afterwards a mixture of water and methanol was added in order to adjust the sample concentration to 1 g/L and the water and methanol fractions to 95 and 5 vol%, respectively, for the buffer-free experiments. For the buffer-containing experiments a solution of ammonium acetate in water/methanol/acetic acid was added in order to adjust the sample concentration to 1 g/L, the salt concentration to 100 mmol/L, the water/methanol ratio to

95/5 (v/v), respectively, and the pH of water to 4.10 with acetic acid (446 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. Recovery after filtration and chromatography

Recoveries after filtration were determined as described in (Shakun et al., 2013). For all Avicels and for BWLs of DS > 0.9 (BWL 3, BWL 4, BWL 6) nearly quantitative recoveries were obtained, whereas for. BWL 1 and BWL 2 somewhat lower recoveries (85.3% and 91.0%, respectively) were observed.

Recoveries after the chromatographic separation were determined only for the buffer containing system using isocratic elution at the eluent composition water/methanol = 65/35 (v/v) (both solvents contained 100 mmol/L ammonium acetate and 446 mmol/L acetic acid at pH of water 4.1). For this purpose well-known amounts of samples dissolved in the buffer containing solvent were injected onto the column. Well defined volumes of the effluent covering the elution region of the polymeric material were collected. The theoretical sample concentration in the collected volume was calculated based on the injected sample mass and the collected volume. The true sample concentrations were determined by re-injecting the collected solutions into the chromatographic system without column using a detector calibration curve, which has been previously established by injecting well known sample amounts of the respective sample without the column. The ratio of the experimentally determined over the theoretical sample concentration was taken as the sample recovery based on the filtrated sample. The determined recoveries were nearly quantitative for all samples within the inaccuracy of the method.

2.6. Determination of dependencies of ELSD response on DS, molar mass and eluent composition

The dependencies of the ELSD response on DS and molar mass were determined by isocratic runs using an eluent composition of water/methanol = 95/5 (v/v) (both solvents contained 100 mmol/L ammonium acetate and 446 mmol/L acetic acid at pH of water 4.1) without column. 10 μL of the sample at a concentration of 1 g/L in the buffer-containing solvent but without filtration (in order to exclude sample loss by filtration) were injected into the chromatographic system. The ELSD responses resulting for samples of different DS and molar masses were compared to each other.

The dependence of the ELSD response on the eluent composition was investigated isocratic without column at eluent compositions of water/methanol = 95/5, 88.5/11.5, 87/13, 85.5/14.5, 65/35 for Avicel 3 and water/methanol = 95/5, 87/13, 86.2/13.8, 83.9/16.1, 65/35 for BWL 3. Both water and methanol contained 100 mmol/L ammonium acetate and 446 mmol/L acetic acid at pH of water 4.1. For these experiments the samples were prepared at 0.5 g/L in the corresponding eluent. 50 μL and 70 μL of the sample solutions were injected into the chromatographic system without column. The ELSD responses resulting in different eluents were compared to each other.

2.7. LC-FTIR-coupling

For the FTIR-characterization of the eluting fractions a LC-transform interface model 600xy (LabConnections, Carrboro, USA) was applied. The main element of the interface is a heated nozzle positioned above a moving germanium plate. The eluent is heated upon flowing through the nozzle. Since the backpressure decreases toward the end of the nozzle, the solvent vaporizes at the exit of the nozzle. Non-volatile analytes, e.g. polymers, will not evaporate, but will deposit on the germanium plate. Due to the plate movement fractions corresponding to different chromatographic elution times will be placed at different positions on the plate. After the end of the chromatographic experiment the plate is placed on a motor driven optical device within a conventional FTIR-spectrometer. The optical device consists of a number of mirrors, which redirect the FTIR-beam onto the germanium plate and further to the detector of the FTIR-instrument. The motor allows moving the plate, such that the fractions at the different positions can be positioned in the FTIR-beam. By this approach a solvent free FTIR-spectrum is obtained for every position of the deposited track. Each spectrum corresponds to a certain elution time of the chromatographic run.

In the gradient experiment the nozzle temperature has to be adjusted during the run in order to maintain a good vaporization despite the changing eluent composition. The use of water in the eluent required reducing the flow rate in order to provide a good evaporation of the solvent. Therefore the buffer-containing gradient was run from water/methanol = 95/5 to 65/35 (v/v) at 0.2 mL/min. Therefore the gradient time was set to 200 min in order to maintain the gradient steepness. The nozzle temperature was changed from 185 °C at the beginning to 174 °C at the end of the gradient. The gas flow rate was set to a meter reading of 30 psi. The same HPLC system as described above was used. However, the ELS detector was replaced by the LC-transform interface. Spectra were acquired after every 1 mm using a Nicolet Protégé 460 FT-IR spectrometer (Thermo Electron, Waltham, USA) in the spectral region of 700–4000 cm^{-1} using 32 scans. For the data acquisition and evaluation Omnic Software version 7.3 (Thermo Electron Corporation, Waltham, USA) was used.

3. Results and discussion

In order to separate NaCMC according to the chemical composition (DS) different stationary and mobile phases were tested. The water content required to achieve solubility prevents adsorption of NaCMC on normal phase columns. C18- and C8-reversed phase columns did not shown efficiently adsorption due to the deficit of hydrophobic organic groups in NaCMC. Highly polar compounds containing hydrogen bound to heteroatoms can be efficient separated on stationary phases which allow for formation of hydrogen bonds with the analyte molecules. Such stationary phases are pentafluorophenylpropyl (PFP) modified silica columns. Electron poor hydrogen atoms covalently bound for example to oxygen will interact electrostatically with the electronegative fluorine atoms of the PFP ligands. Additionally, low pH mobile phases, which cause protonation of bases, increase the possibility for hydrogen bonding interactions with the fluorine atoms. The suitability of such a PFP stationary phase was checked in the given investigation.

Methanol was used as organic modifier because it provided the best solubility of the samples among the different tested modifiers. While nearly 70% (v/v) of methanol could be added to the aqueous NaCMC solutions without any visible precipitation, the addition of more than 50% (v/v) of ethanol, isopropanol, THF or acetone led to precipitation of the samples.

First a 10 min linear gradient from water/methanol = 95/5 to 0/100 was run. The corresponding chromatograms for the samples of highest and lowest DS are shown in Fig. 1. Each chromatogram consists of two peaks, one at approx. 1.8 mL and another one between 3 and 8 mL. The void volume for the given column amounted to approx. 3 mL as determined by injection of sodium acetate. Thus it is clear that the peak at 1.8 mL is caused by non-retained but excluded polymeric material and the sample is only partially adsorbed at the beginning and desorbed within the gradient, resulting in the peak between 3 and 8 mL. Despite of that it is apparent that the sample of higher DS elutes later than the sample of lower one both for Avicel and BWL. This is a first

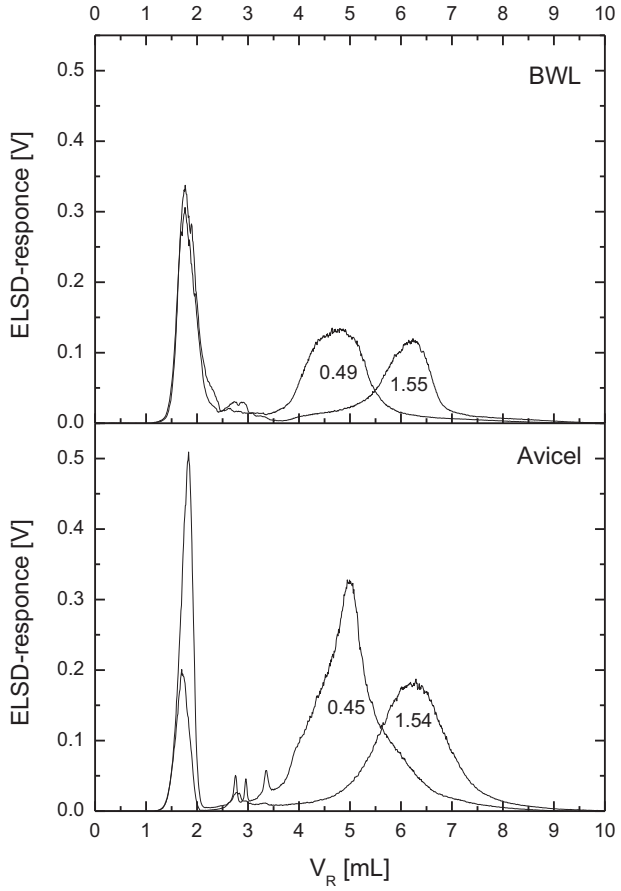

Fig. 1. Overlays of chromatograms of BWL (above) and Avicel (below) in a linear 10 min gradient from water/methanol = 95/5 to 0/100. Samples dissolved in water/methanol = 95/5 at 1 g/L. Injection volume: 100 μL. Values besides the curves represent average DSs.

indication that a DS-dependent adsorption might be realized on the selected stationary phase. It is not possible to explain the observed chromatographic behavior purely based on polarity, because the carboxymethyl groups are highly polar substituents. Thus, samples having high DS should actually elute earlier at higher water contents in the eluent than samples of lower DS. This is, however, not the case. The following effects might explain these results. First the conformation of the polymer chains can influence the separation. Without shielding the electrostatic interaction the substituted chains are expected to be stretched because of the repulsion of the like charged carboxymethyl substituents. This effect should be more pronounced for chains of higher DS than for less substituted chains. Due to the stretched conformation, the adsorbing groups might provide a better accessibility for interactions with the column material. Consequently, the higher substituted chains should adsorb stronger, resulting in later elution. Secondly, in aqueous mobile phases protolysis of sodium carboxymethyl substituents will result in acidic groups which can interact with the PFP ligand of the stationary phase via hydrogen bonding. The amount of these hydrogen bonds increases with DS resulting in stronger retention.

Fig. 1 shows nearly identical elution volumes of the lower molar mass Avicel and the higher molar mass BWL samples at comparable DSs. Thus, we can state that the molar mass does not strongly influence the retention at the selected chromatographic conditions. This means that the widths of the resulting peaks are an indication of a substantial chemical heterogeneity of the samples, i.e. the existence of chains of different DS within a single sample.

In order to realize a full adsorption of the samples, the pH of the mobile phase was lowered. pHs in the range pH = 3.46–6.06 were established using acetic acid, in 100 mmol/L vaporizable ammonium acetate buffer. The use of a volatile buffer was obligatory due to the application of the ELSD detection, which in turn was required because of the gradient elution. The lack of suitable chromophores in NaCMC prevents the use of UV-detection, while application of RI-detection in gradient elution is not feasible. The buffer concentration 100 mmol/L was selected in order to suppress the electrostatic repulsion of the charged polyanions and to eliminate the possible influence of the conformation on the retention. pH-values lower than 3.46 were not realized due to the limited solubility of the acidic form of CMC. Moreover ammonium acetate buffer allows only pH adjustment in the given range. The same concentrations of ammonium acetate and acetic acid were added to methanol in order to realize constant salt and acid concentrations throughout the gradient. First linear 40 min gradients from water/methanol = 95/5 to 20/80 were run at pH-values of water 6.06, 4.76, 4.54, 4.10 and 3.46. The resulting chromatograms are shown for the Avicel samples in Fig. 2. Chromatograms of BWLs show the same tendencies but are slightly shifted in elution volume (see discussion below).

In each chromatogram a peak at approx. 3 mL can be observed which is caused by sodium acetate as identify by the FTIR spectroscopy (see below). For the highest and lowest pHs of 6.06 and 3.46 a peak close to the exclusion volume of the column (approx. 1.8 mL) can be observed resulting from non-retained and excluded polymeric material. It is remarkable that the elution order for these both pH values is reversed. While at pH 6.06 samples of low DS adsorb stronger, the opposite is true for pH 3.46. This inversion of elution order with pH might be explained by the presence of acidic carboxymethyl substituents as well as non-substituted hydroxyl groups in CMCs. Both are able to interact with the stationary phase via hydrogen bonding, however, the interaction strength will vary with pH. The acidic carboxymethyl substituents control the retention at low pH whereas they are partially dissociated at higher pH resulting in weaker interaction with the stationary phase. As a consequence the retention is governed by the non-substituted hydroxyl groups.

For the intermediate pH values no early eluting components are observed, i.e. the polymer elutes well within the gradient. At pH 4.54 obviously the contributions of both groups to the retention compensate each other resulting in a DS-independent elution for both, the low and high molecular CMCs.

At pH 4.10 the more substituted samples elute later meaning retention is controlled by the acidic substituents, whereas at pH 4.76 the inverse elution order can be observed resulting from the stronger interactions of hydroxyl groups with the stationary phase. It is interesting to note that the chromatogram of Avicel 5 at pH 4.76 exhibits a slight tailing, whereas the chromatogram of this sample at pH 4.10 shows a slight fronting and at pH 4.54 nearly symmetrical elution profile. These findings indicate that the observed curve shapes for this sample originated from the structural characteristics of Avicel 5 itself and not from experimental artefacts like band broadening. When comparing the chromatograms at pH 4.10 and 4.76 it becomes apparent that at pH 4.10 the samples adsorb stronger. Moreover, the resolution of the samples having different DS is better for pH 4.10 than for 4.76. Thus, for the further investigations the pH of water was adjusted with acetic acid to 4.10, in 100 mmol/L ammonium acetate buffer.

For further optimization of the separation the gradient steepness was decreased by running a 40 min linear gradient from water/methanol = 95/5 to 65/35. The resulting chromatograms are shown in Fig. 3.

Each chromatogram in Fig. 3 consists of two peaks: one at approx. 3 mL and another one in the elution region between

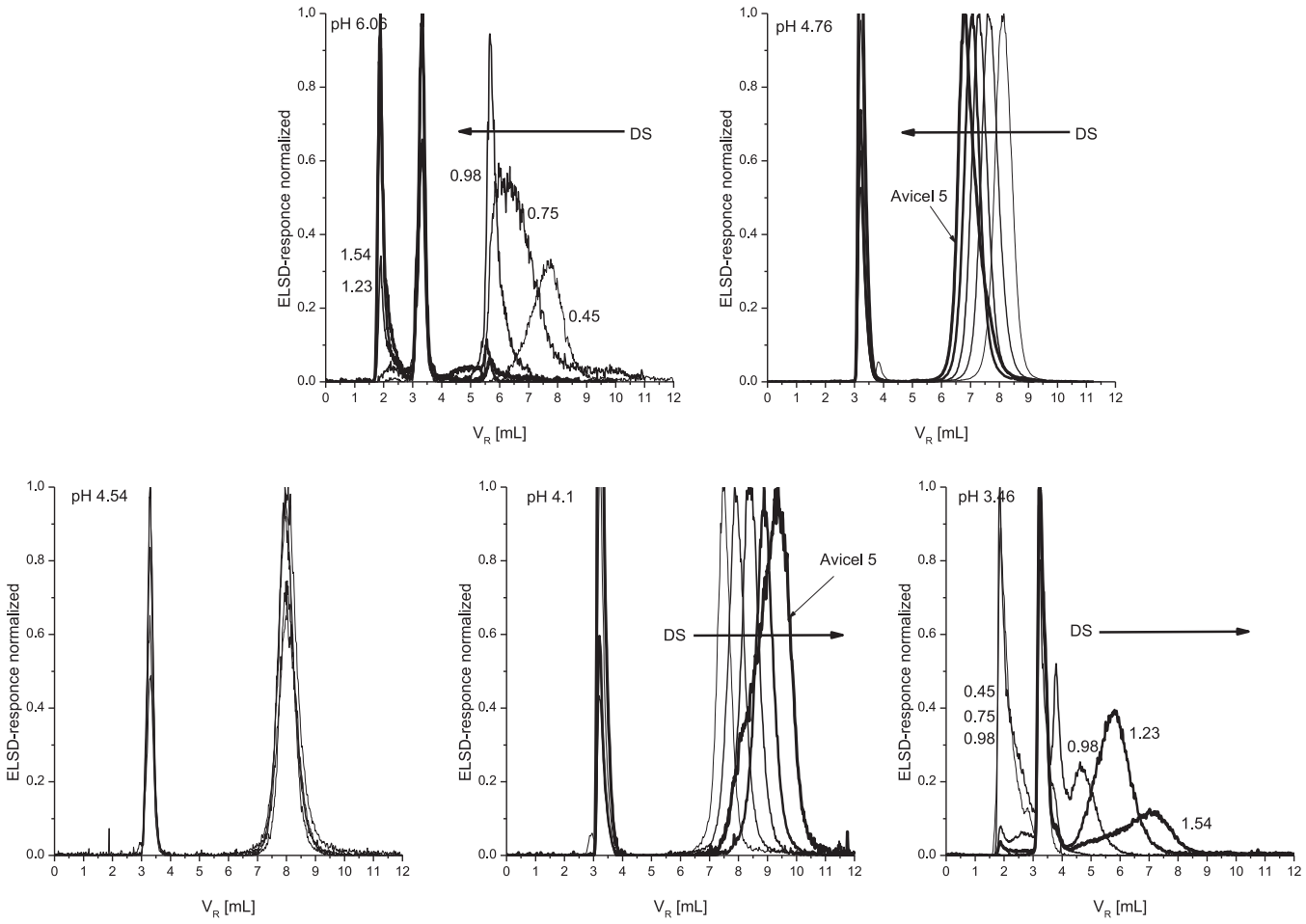

Fig. 2. Overlays of chromatograms of Avicels in linear 40 min gradients from water/methanol = 95/5 to 20/80 at different pH. Concentration: 1 g/L. Injection volume: 50 μL. The values besides the curves in the figures for pH 6.06 and 3.46 indicate the average DSs of the investigated samples.

8 and 25 mL, i.e. well within the gradient. For identification of the chemical identity of the two peaks the FTIR spectra were acquired using the LC-transform interface. The spectra obtained are shown in Figs. 4 and 5. Clear differences can be observed, indicating that both peaks result from two chemically different species. The presence of the absorption band at 1059 cm^{-1} which is typically for the cellulose backbone, indicates that CMC elutes between 8 and 25 mL, i.e. within the gradient, as expected. In contrast to that the absence of this band in the spectrum in Fig. 4 indicates that this fraction does not contain CMC. A comparison of the spectrum obtained at 3 mL with the literature spectrum of sodium acetate identifies this peak as resulting from sodium acetate most probably formed by exchanging the sodium counter ions of the polymer chains with either hydrogen or ammonium cations from the buffer. The spectral region of the carboxyl groups in Fig. 5 shows two bands for the asymmetrical stretching vibrations at 1712 cm^{-1} and 1580 cm^{-1} indicating the presence of both acidic (at 1712 cm^{-1}) as well as carboxylate groups (at 1580 cm^{-1}) for the CMC after the chromatographic separation. This indicates that a partial protolysis of the carboxymethyl groups take place under the given chromatographic conditions. Consequently, decreasing pH of the mobile phase promotes the protonation of the carboxymethyl groups, which in turn increases the contribution of these groups to retention.

In Fig. 6 the average DS for BWLs and Avicels are plotted versus the elution volume taken at the median of the respective chromatogram, $V_{R,mc}$, in Fig. 3. Since several chromatograms show pronounced fronting or tailing, the peak maximum does not represent necessarily the weight average DS. Thereby the center of each chromatogram might better reflect the weight average DS, assuming, as a first approximation, the existence of a linear dependence of the elution volume on DS exists within single chromatograms.

The average DS increases with $V_{R,mc}$ in Fig. 6. Thus, at the selected chromatographic conditions a clear separation according to the average DS was achieved for CMCs which allows determining average DS of an unknown sample using these calibration curves. Moreover, these results allow assuming that a separation according to DS also occurs within a single sample. However, it is obvious that the high molar mass BWLs having comparable DS to the low molar mass Avicels elute at higher elution volumes (approx. 2 mL later) indicating the influence of molar mass on retention as well. For linear gradients an increase in elution volume with molar mass is usually observed at low molar masses, which vanishes, however, for sufficiently high ones. Thus it seems as if at least for the Avicels the critical molar mass has not been reached. At which molar mass the molar mass influence vanishes is presently investigated and will be published in a subsequent manuscript on 2 dimensional chromatographic separations of CMCs. It should be mentioned however that for the molar mass and DS range under investigation the variation in elution volume due to DS is stronger than the change due to molar mass. In order to properly account for the effect of molar mass on elution volume it is recommended at the present stage to establish calibration curves using CMCs of similar molar mass than the samples to be analyzed. The so obtained calibration curves can then be applied to calculate the average DS of the unknown samples. The molar masses of the calibrants need not to be absolutely identical

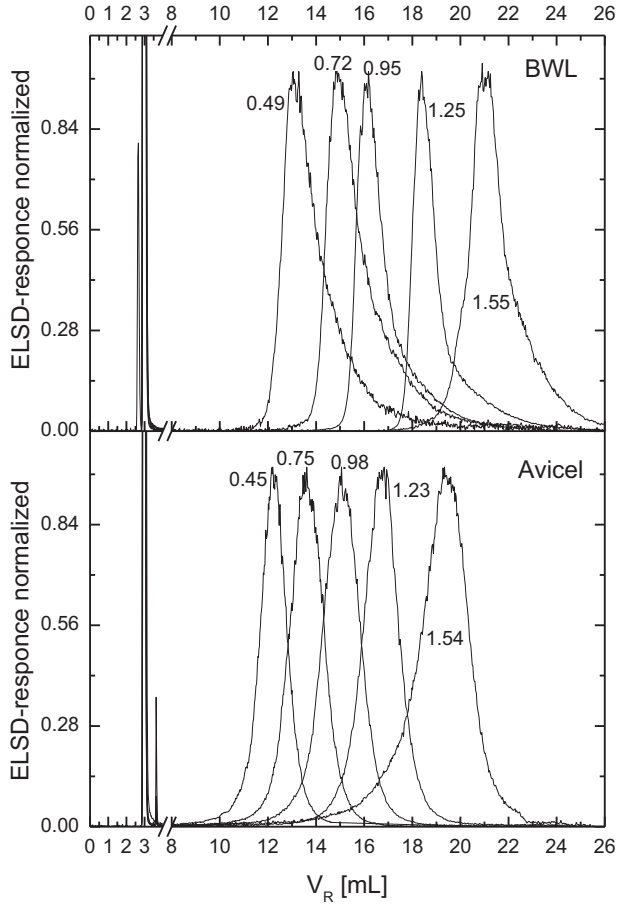

Fig. 3. Overlays of chromatograms of BWL (above) and Avicel (below) in a 40 min linear gradient from water/methanol = 95/5 to 65/35 both containing 100 mmol/L ammonium acetate buffer at pH of water 4.1. Sample concentration: 1 g/L. Injection volume: 100 μL. The values given indicate the average DS.

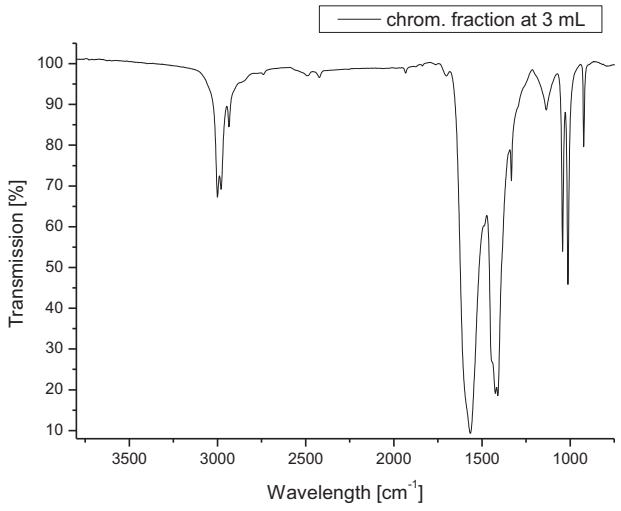

Fig. 4. FTIR-spectrum of the chromatographic fraction at 3 mL from Fig. 3.

to the molar mass of the analyte, since a factor of 10 in molar mass results in a shift in the DS calibration curve by $\Delta DS \cong 0.2$. More important than the calculation of the average DS is the potential of the chromatographic method to determine the DS distribution w(DS), i.e. the fraction of material having a specified DS within a

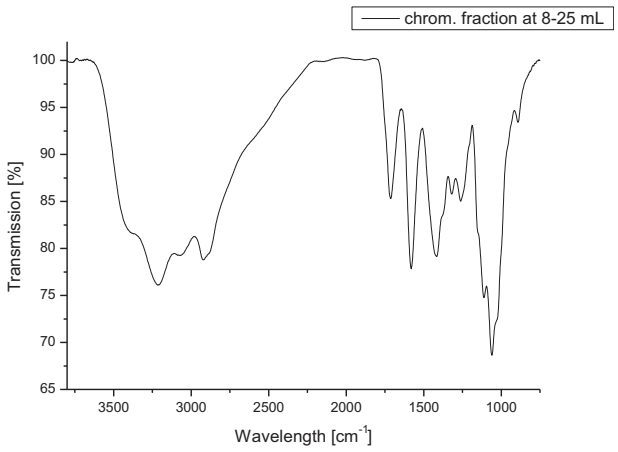

Fig. 5. FTIR-spectrum of the chromatographic fraction between 8 and 25 mL from Fig. 3.

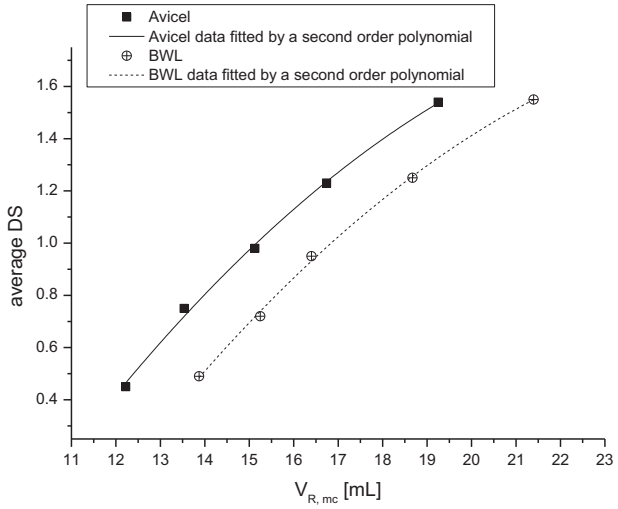

Fig. 6. Average DS as a function of $V_{R,mc}$ for Avicels and BWLs. The curves result from fitting 2nd order polynomials and just serve as a guide for the eye.

single sample. The DS distribution can be calculated from the chromatogram and the dependence of DS on elution volume via Eq. (1).

$$w(\mathrm{DS}) = \frac{S(V_R) \times \Delta V_R}{\Delta \mathrm{DS}(V_R) \times \sum S(V_R) \times \Delta V_R} \quad (1)$$

with $S(V_R)$ being the ELSD response at the elution volume V_R which to a first approximation is proportional to the mass concentration of the eluting fraction c, ΔV_R and ΔDS are the elution volume and DS intervals, respectively. The DS distributions will be affected by molar mass similar to the calibration curves. Despite that, a comparison of the DS distributions of CMCs synthesized using the same parent cellulose can be made. Such a comparison will reveal differences in the DS-distributions even if the average DS might be identical and will thus allow to obtaining valuable information on the effect of reaction conditions on heterogeneity and on final product properties.

However, from literature (Wei & Ding, 2000) it is known that the ELSD response depends on various parameters, like molar mass, chemical composition of the investigated polymer as well as on the eluent composition. Due to the applied gradient, the eluent composition changes across the chromatogram in Fig. 3, as does the chemical composition (due to the desired separation by DS) and maybe the molar mass. Therefore the influence of these parameters on the ELSD response was investigated. To study

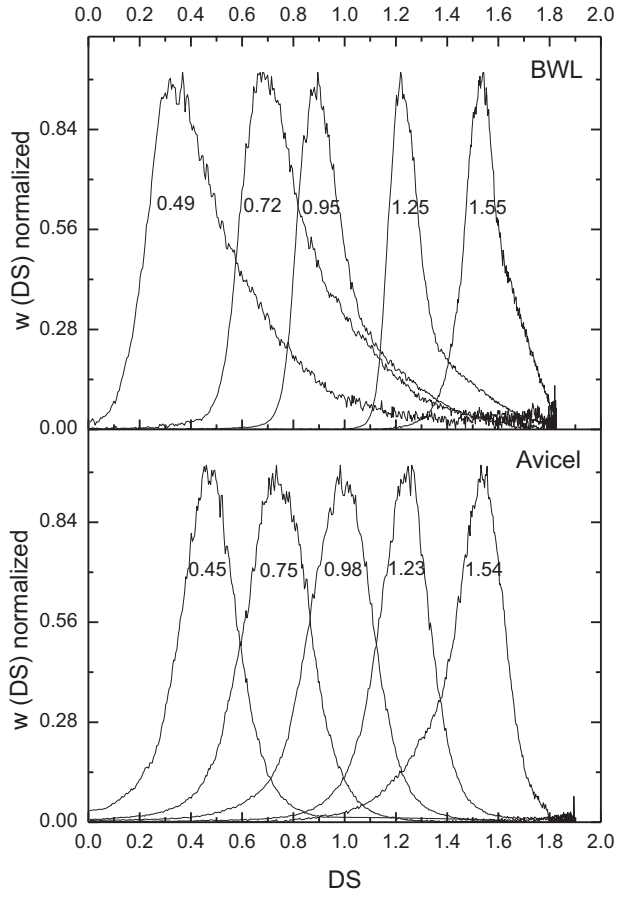

Fig. 7. Overlays of DS distributions of BWLs (above) and Avicels (below) calculated from the chromatograms in Fig. 3 and the calibration curves in Fig. 6. The values given indicate the average DS.

the influence of the molar mass and DS on the ELSD response the ELSD peak areas resulting of samples having different DS and average molar masses were compared. This was done by injecting the same amount of sample at different isocratic eluent compositions into the chromatographic system without column. No significant (max. 10% deviation from average) or systematic dependencies of the ELSD response on DS and molar mass were observed. Besides this, no significant influence of the eluent composition on ELSD response exists in the elution region of a given sample (max. 10% variation between the initial and final eluent composition of the gradient). We therefore evaluated the DS distribution without any correction for molar mass, DS or eluent composition.

The calculated DS distributions are shown in Fig. 7. With the exception of Avicel 5 which shows a slight fronting to lower DS values, the Avicels show rather symmetric distributions. In contrast the BWL samples exhibit non-Gaussian distributions with pronounced tailing toward higher DS values. Besides that BWL 6 and Avicel 5 shows slight fronting to lower DS. It should be noted that Avicel 5 and BWL 6 both having the highest DSs, were synthesized in two-step synthesis in contrast to other samples which were synthesized in one-step. Apparently the synthesis procedure influences DS distribution within the sample.

After obtaining the DS distributions, the weight average DS values were recalculated ($DS_{w,rec}$) from these distributions using following equation:

$$DS_{w,rec} = \sum w(DS) \times DS \times \Delta DS \quad (2)$$

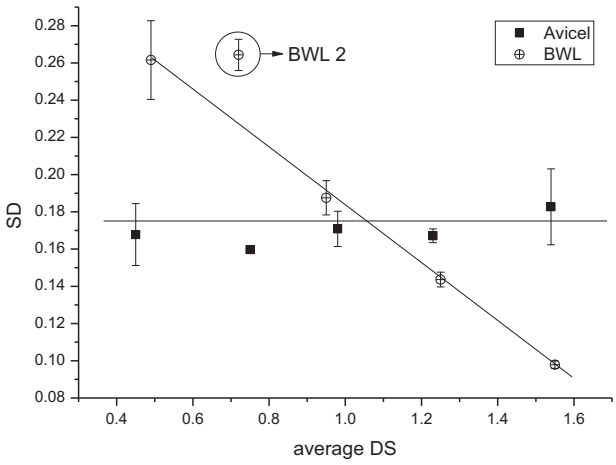

Fig. 8. DS standard deviation SD with error bars (resulted from double injections) as a function of average DS for Avicels and BWLs. The lines given serve merely as a guide for the eye.

These values and their percentage deviations to the average DS determined experimentally as described in 2.2 are tabulated in the fourth column of Table 1. Except for BWL 2 the deviations range from 0% to 6.1% indicating a good agreement between the calculated and the experimentally determined DS values and thus, a high accuracy of the calculated DS distributions. Only for BWL 2 a higher deviation of 15.3% is observed. Several factors might contribute to this deviation, e.g. a non-linear response of the ELSD, the dependence of the ELSD response on DS and/or molar mass and the fact that the assignment of the elution volumes to DS were based on the given average DS which not necessarily coincides with the peak maximum, but rather the center of the peak. In addition BWL 2 might exhibit a different distribution pattern for the AGUs which might influence the retention of this sample in a different way as compared to other BWLs.

In order to quantify the extent of heterogeneity with respect to DS, the DS standard deviations (SD) were calculated from the DS distributions via Eq. (3). These data are given in the fifth column of Table 1.

$$SD = \sqrt{\sum w(DS) \times (DS - DS_{w,rec})^2 \times \Delta DS} \quad (3)$$

These values are plotted in Fig. 8 versus average DSs for both samples sets. While the standard deviation, SD, is nearly independent of DS for the Avicel samples, a clear decrease of standard deviation with increasing average DS is observed for the BWLs. This means that the distributions become progressively narrower with increasing average DS. The sample BWL 2 exhibits a slightly higher value as would be expected if a linear decrease of SD with increasing average DS would exist for this series. This is an additional indication on the irregular behavior of this sample. Based on the results of the heterogeneity it can be concluded that high and low molecular NaCMCs behave differently during the synthesis. With respect to the above discussion on dependence of the calibration curves on molar mass it should be mentioned that the standard deviation will be less influenced by molar mass than the DS at a given elution volume or the average DS, since only the deviation to the average is of concern. Therefore application of the same calibration for both sample sets would mainly result in a shift along the *x*-axis in Fig. 8, but would not change the general dependences.

4. Conclusions

A reliable chromatographic method for the separation of intact sodium carboxymethyl celluloses in the range DS = 0.45–1.55

according to DS was developed in the present work. Despite a slight influence of the molar mass on retention volume the method allows the fast estimation of average DS of unknown samples. More importantly, the method allows determining DS distribution (1st order heterogeneity) within a single sample. Weight average DS values recalculated from the recorded distributions closely agree for the most of the samples with the ones determined by complete chain degradation. From the DS distributions the DS standard deviations were calculated and taken as a measure for the extent of the chemical heterogeneity of the single samples. No strong dependence of the width of the chemical distribution on the average DS was observed for low molecular samples synthesized from microcrystalline cellulose whereas for high molecular samples synthesized from cotton linters the heterogeneity decreases with increasing average DS.

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