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Determination of the degree of substitution and its distribution of carboxymethylcelluloses by capillary zone electrophoresis

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Abstract—A method based on capillary zone electrophoresis (CZE) has been developed to determine the degree of substitution (DS) of carboxymethylcellulose (CMC). Separations were performed with borate buffer (pH 9, ionic strength 20 mM) as background electrolyte in capillaries of 75 μm ID, with an applied voltage of 10 kV, and for detection UV absorption at 196 nm was measured. The use of an internal standard (phthalic acid) to correct for mobility variations resulted in a strong improvement of the precision of the DS determination. Experiments with indirect UV detection indicated that the peak widths obtained actually reflect the variation in mobility, and with that of the DS value, of CMC samples. With the proposed method not only the average DS value but also its dispersity could be established for technical CMC samples. A small but definite effect of the polymeric size on the mobilities was observed. Therefore, DS calibration curves will have to be determined for a specific MM range. Since the size effect is small, a classification of CMCs as low-, middle-, or high MM will be sufficient to obtain accurate data on the DS distribution.

Keywords: CMC; Average DS value; DS distribution; Capillary zone electrophoresis

1. Introduction

Carboxymethylcellulose (CMC) is a cellulose derivative widely used as a thickener, emulsifier, or flow controller in a broad range of products including food, textile, paper, paint, and pharmaceuticals, and in the oil industry. An important property of CMC is its water solubility, which depends on the degree of substitution (DS) of the CMCs. The DS value of a CMC sample reflects the average number of carboxylic acid groups per glucose unit. CMCs with a DS value above 0.7 are completely water soluble, giving highly viscous solutions.[1] Knowledge of the DS value of CMCs is essential to predict product properties and to qualify technical samples. Also important to know is the dispersity of the DS value. A more narrow distribution improves the quality of CMC products.

Characterization on the DS of CMC is usually carried out by ashing and titration or, alternatively, acid hydrolysis[2–5] or enzymatic fragmentation[6] and the separation of the resulting monomers using anion exchange chromatography (AEC). With these methods the average DS value of the CMC samples can be determined. Although the methods are time consuming and laborious, as depolymerization of the cellulose backbone is always needed, they are applied in every day practice. Another technique used to obtain data on the DS value of CMCs is nuclear magnetic resonance (NMR) spectroscopy.[7–9] With this technique both the average DS value and the distribution of the substituents over the positions C-2, C-3, and C-6 on the glucose units can be obtained.[9] The latter distribution indicates the reactivity

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of the hydroxyl groups of the glucose units in the cellulose backbone. Neither with NMR nor with AEC it is possible to obtain information about the DS distribution in (technical) CMC samples.

Capillary zone electrophoresis (CZE) has demonstrated its value as a highly efficient tool for the separation of a variety of analytes, including inorganic ions, small molecules, and (bio)macromolecules. Separations in CZE are based on the migration of analytes under the influence of an applied electric field according to their electrophoretic mobility as determined by their charge-to-size ratio. It has already been shown by Stefansson[10] that the CZE principle is relevant for the characterization of CMC samples. Prior to the injection Stefansson converted the CMCs to fluorescent derivatives to provide for detectability with laser-induced fluorescence detection. In the CZE system used the CMCs migrated against the electroosmotic flow (EOF) with the compounds with the lowest DS values eluting first.

In the work described in this paper a CZE system with UV detection to determine the average DS value and DS distribution of CMC samples has been developed and studied. No derivatization or any other preliminary steps were required. It is shown that this CZE system is suitable to characterize (technical) CMCs for industry in every day practice.

2. Experimental

2.1. Chemicals

CMC samples with average DS values between 0.69 and 1.64 were obtained from Akzo Nobel (Arnhem, The Netherlands). The molar masses (MMs) of the CMCs were all in the range of 200–400 kDa, as has been determined by the supplier. CMCs were dissolved in sub-boiled demi water at a concentration of 1 g L^{-1} and were stored at 4 °C for at least 16 h. Prior to the analysis phthalic acid (Acros Organics, Geel, Belgium) was added to the samples as an internal standard at a final concentration of 5 μg mL^{-1}. All other chemicals used were of analytical-grade quality and obtained from certified suppliers.

2.2. Capillary zone electrophoresis

CZE experiments were performed on a HP^{3D} instrument (Agilent, Waldbronn, Germany). Fused silica capillaries of 75 μm ID obtained from Composite Metal Services (The Chase, UK) were used with a detection window at 45 cm and a total length of 53.3 cm. The phosphate buffer (pH 7.5) consisted of 2.2 mM sodium dihydrogenphosphate and 7.7 mM disodium hydrogenphosphate. Borate buffer (pH 9) was made by dissolving 10 mM disodium tetraborate-decahydrate in sub-boiled demi water. Prior to all injections the capillary was flushed with 0.1 M NaOH and buffer solution, both for 2 min. Following this, a high voltage of 10 kV was applied for 1 min and finally the capillary was filled with fresh buffer solution. Samples were injected by a pressure plug of 20 mbar for 5 s. Separations were carried out at 35 °C. The applied voltage was 10 kV and UV detection was performed at wavelength of 196 nm with a bandwidth of 8 nm.

Indirect UV detection was carried out with benzenesulfonic acid and picric acid as the monitoring ions, both at concentrations of 4.5 mM, in a background electrolyte (BGE) of 10 mM KOH (1 mM excess). Detection of the displacement of benzenesulfonate and picrate was performed using diode array detector (DAD) at wavelengths of 210 and 357 nm, respectively. Other experimental parameters were the same as in the direct detection mode.

2.3. Fractionation by size-exclusion chromatography

Size-exclusion chromatography (SEC) experiments were done using a Waters 410 Alliance 2690 separations module (Milford, MA, USA) combined with a differential refractive index detector. The stainless steel column was a 300 × 7.5 mm aquagel-OH mixed 8 μm obtained from Polymer Laboratories (Shropshire, UK), which was thermostated at 30 °C. In all experiments the injection volume was 40 μL and the flow rate 1 mL min^{-1}.

SEC separations were carried out using an eluent of 5 mM sodium nitrate ($NaNO_3$) in sub-boiled demi water. The CMC 1.22 sample was prepared by dissolving the sample in sub-boiled demi-water at a concentration of 5 g L^{-1}. Before use, the sample was stored at 4 °C for at least 16 h. Finally, $NaNO_3$ was added until the same concentration as in the eluent was obtained. This addition was carried out after 16 h because, according to a previous publication, dissolving of CMC in the presence of salt may result in scaly particles in the solution.[11] Fractionating was carried out with a Waters fractionater that was connected to the SEC equipment and was controlled by Millenium Software. The interval time was 30 s and the total number of runs was 10, yielding six fractions of 5 mL. After fractionating, a volume of 1 mL of each fraction was placed in an oven at a temperature of 95 °C and was pre-concentrated by evaporation to a final volume of 0.1 mL. The pre-concentrated samples were analyzed using the CZE system.

3. Results and discussion

3.1. CZE system

A method for the analysis of CMCs by CZE has previously been described by Stefansson.[10] In this work, prior to the injection the CMC samples were derivatized

to be able to measure them by fluorescence detection. A disadvantage of the derivatization is that it is laborious and an extra source of imprecision. Moreover, it cannot be excluded a priori that the derivatization yield may depend on the molecular size or DS of the CMC samples. This will influence the CZE separation and thereby the determination of the DS value. We preferred to operate a CZE method without any derivatization or other preliminary step.

Preliminary experiments showed that CMCs can be detected in the direct UV mode at a low wavelength of 196 nm. However, the sensitivity was only moderate. Applying relatively wide capillaries of 75 μm ID we succeeded in decreasing the detection limit. Still, the injection of CMC samples with high concentrations ($1\,g\,L^{-1}$) was essential. To prevent sample overloading we tried the use of relatively high concentrations of the BGE salt in the buffer solutions. First, a phosphate buffer of pH 7.5 with a final ionic strength of 25 mM was investigated. Electropherograms obtained for a specific CMC sample in various concentrations ($0.5–5\,g\,L^{-1}$) showed triangular peaks for all the injected samples with a peak top shifting with the sample concentration (Fig. 1). It is clear that the overloading of the system could not be avoided using this buffer composition. A further increase of the ionic strength of the solution was not possible since the current observed was already quite high (~29 μA at 10 kV).

Another possibility to reduce sample overloading is to change the type of BGE salt. As has been described in the literature the system capacity against overloading is a complex function of the mobilities and pK_a values of analytes and BGE compounds.[12] A considered choice is to select a buffer with a better ratio of the buffer capacity in relation to the conductivity than phosphate. The choice of buffers that can be tested was limited since most organic BGE salts will show a high background absorbance at the wavelength to be used for detection (196 nm). We selected a borate buffer (pH 9) with an ionic strength of 20 mM. Symmetrical peak shapes with the CMC samples were detected for concentrations of up to $1\,g\,L^{-1}$ (Fig. 2). All further experiments were performed with this selected borate buffer, capillaries of 75 μm ID, sample concentrations of $1\,g\,L^{-1}$ and an applied voltage of 10 kV (~33 μA).

The repeatability of the migration times of the analytes is an important issue, since the differences in mobility between CMC samples with different DS values are small. During the development of the CZE conditions it was found that the run-to-run repeatability of the CMC migration times was better when a preconditioning procedure was applied before each injection. The procedure consisted of flushing with a NaOH solution followed by the borate buffer, both for 2 min. Next, a voltage of 10 kV was applied for 1 min. Finally, new in- and outlet vials were placed and the capillary was filled with fresh buffer solution. The run-to-run repeatability was investigated with the individual measurements of two selected CMC samples, which were injected seven times. From the electropherograms obtained experimentally the apparent mobilities and the effective (EOF-corrected) mobilities of the CMCs were calculated. As can be seen in Table 1 the relative standard deviations (RSDs) for the EOF-corrected mobilities were lower than for the apparent mobilities. No systematic relation between the apparent or EOF-corrected mobilities and the order of injections was found. This indicates that, despite the precondition step, there is a random difference in velocity of the EOF between two measurements. These results suggest that the velocity of the EOF could also be unstable during a separation run.

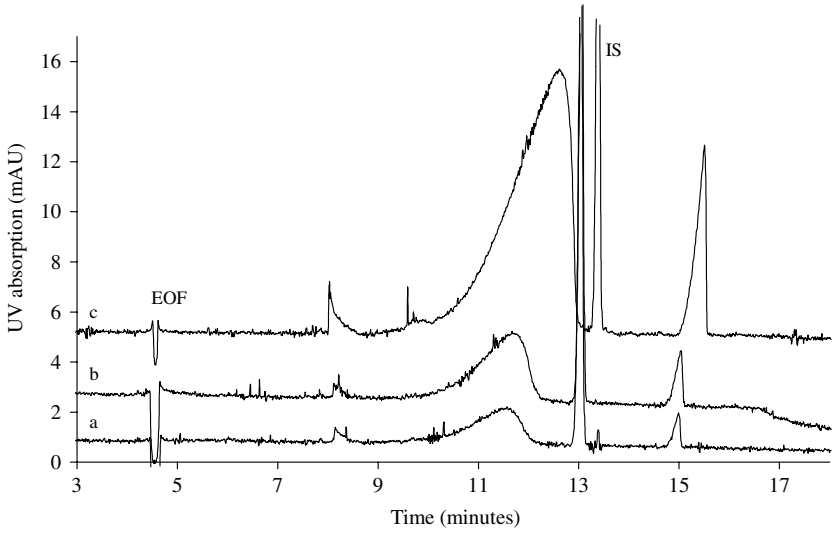

Figure 1. Electropherograms of CMC 1.22 of (a) 0.5, (b) 1, and (c) $5\,g\,L^{-1}$ using phosphate buffer (pH 7.5) as the BGE. Voltage 10 kV.

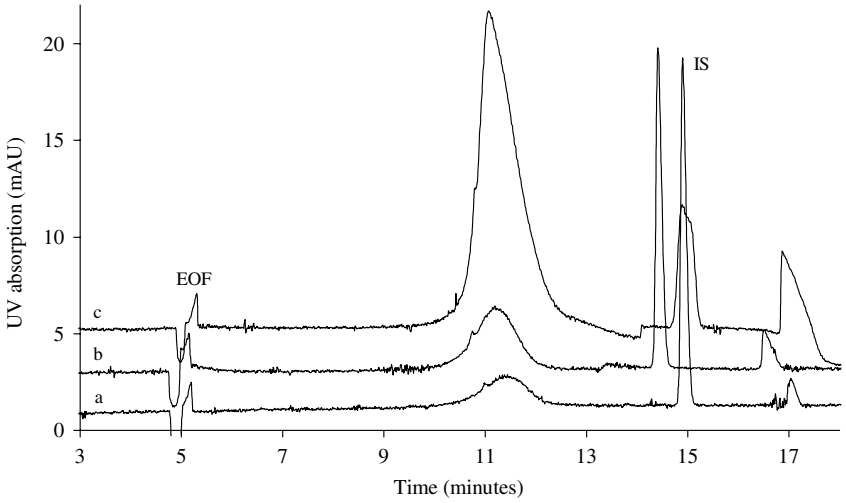

Figure 2. Separations of CMC 1.22 of (a) 0.5, (b) 1, and (c) 5 g L^{-1} with a 10 mM borate buffer at a voltage of 10 kV.

Table 1. Repeatability of mobilities for CMC 1.14 and 1.22 ($n = 7$)

	Noncorrected RSD (%)	EOF corrected RSD (%)	EOF and IS corrected RSD (%)
CMC 1.14	0.45	0.30	0.09
CMC 1.22	0.56	0.25	0.07

To make it possible to correct for the variance of the EOF velocity during a run we added phthalic acid (5 μg mL^{-1}) to the CMC solutions as an internal standard. Phthalate migrated in the borate buffer with a slightly higher mobility than the CMC polyelectrolytes. For the correction of the effective mobilities of the CMCs a normalization method was applied. The normalized mobilities of the CMC samples were calculated as the sum of their effective mobility and the difference between the effective mobility of the internal standard in the sample solution and its average value obtained with solutions without CMC ($n = 7$). The RSDs calculated for the normalized mobilities of the samples were in the order of 0.1% (Table 1).

Commercial well-characterized CMC products with an average DS value between 0.69 and 1.64 were available. The optimized CZE conditions were applied to analyze the commercial CMCs. The electropherograms obtained experimentally are shown in Figure 3. For clarity, the X-axes were translated into a mobility-scale normalized with the internal standard phthalic acid. The CMC 1.64 product contained glycolic acid as an impurity. Since the mobility of charged compounds in CZE is proportional to their charge-to-size ratio, the mobility is expected to reflect the DS of the CMCs. A plot of the

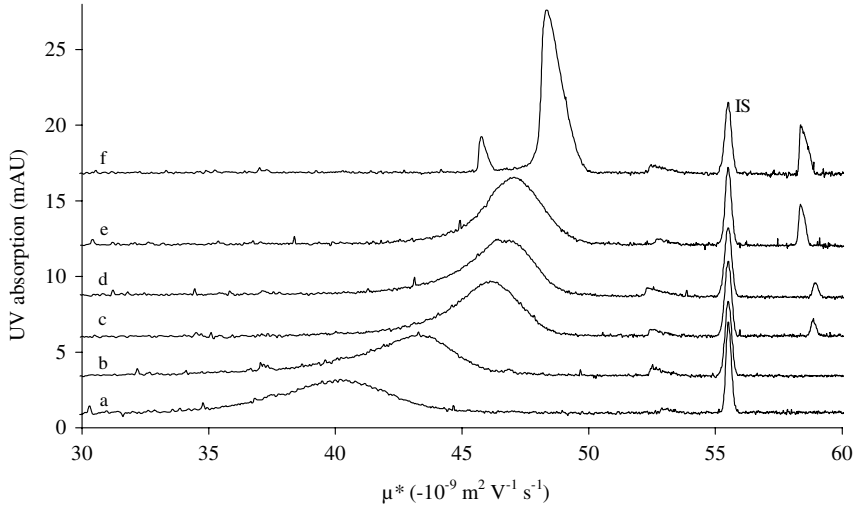

Figure 3. Electropherograms of (a) CMC 0.69, (b) 0.84, (c) 1.09, (d) 1.16, (e) 1.22, and (f) 1.64 (1 g L^{-1}). The mobility-scale of the CMCs is normalized for the effective mobility of the internal standard phthalic acid (-55.5×10^{-9} m^2 V^{-1} s^{-1}). Conditions as in Figure 2.

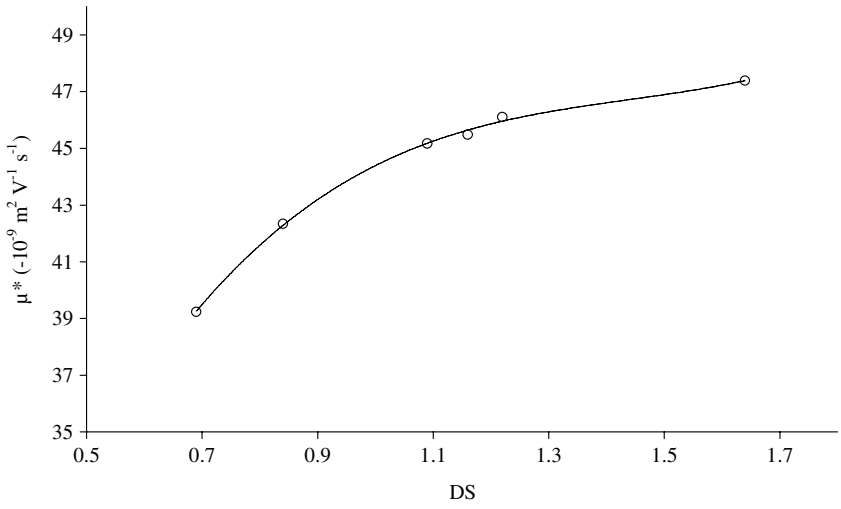

Figure 4. Plot of the normalized mobility of CMCs as a function of the average DS value.

normalized mobility (μ^*) as a function of the average DS value is depicted in Figure 4. It was found that the mobility of CMCs increased slightly with their DS. The nonlinear behavior obtained for the CMCs with a high charge density might be the result of counter ion condensation, has also been mentioned by Gao et al.[13] Polyelectrolytes with a high charge density have a net charge in an electrolyte solution that is disproportionate to their charge density due to condensation of counter ions close to the polymer backbone.[14] The significantly smaller peak width observed for CMC 1.64 also indicates the occurrence of counter ion condensation especially at high DS values. Despite the nonlinearity of the plot it is possible to apply it as a calibration curve to characterize CMC products. Since the slope of the calibration curve is low it is essential to determine the mobility of CMCs precisely. As has already been described, the internal standard phthalic acid helps to minimize the random error of the method to 0.1%, which will result in an error of the DS value of about 1%. With the calibration plot of mobility against DS the time-scale of the electropherogram of CMC 1.09 was translated into its DS-scale (Fig. 5). It was found that the CMC 1.09 product had a DS dispersity varying from 0.7 to 1.7 with a polydispersity of 1.04.

3.2. Indirect UV detection for monitoring the origin of the CZE separation

Indirect UV detection in CE is generally applied to measure ionic analytes with poor or no response with the UV detector. For indirect detection a strongly UV

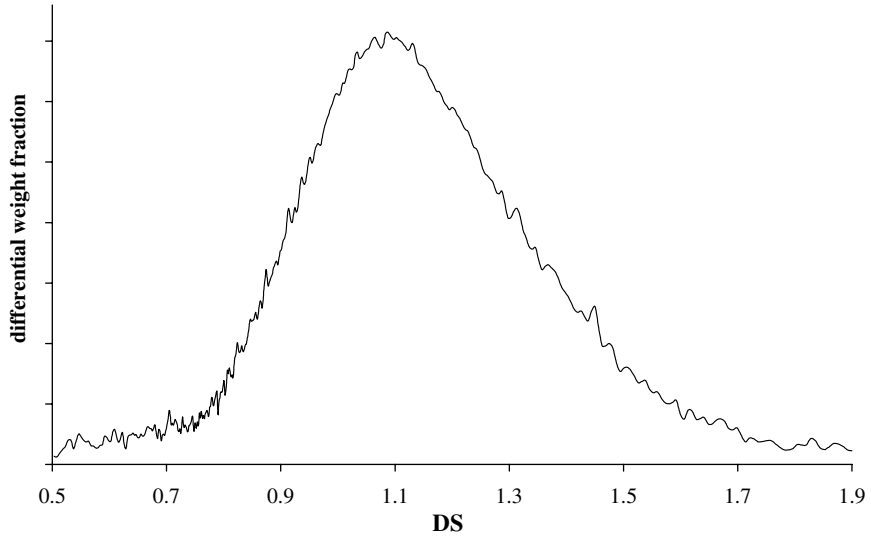

Figure 5. DS distribution of CMC 1.09 as obtained from the CZE separation.

absorbing ionic compound (the monitoring ion) is added to the BGE solution. A decrease of the UV signal, caused by the displacement of the monitoring ion by the analyte, will be detected that corresponds to the sample concentration. The degree of displacement is given by the transfer ratio. The transfer ratio can be written in equilibrium in terms of the Kohlraush functions, as has been described in detail in literature (see, e.g., Ref. 15). It was found that the transfer ratio depends on the mobilities of the analyte ion, the monitoring ion and its counter ion.

In this work we studied the origin of the CMC peak-widths using an indirect UV detection system with two monitoring ions. In a multiple ion system the principle of displacement is similar as in a single system. The UV signal detected depends on the total displaced concentration of the monitoring ions by the presence of the analyte ions. The displacement of the two monitoring ions will occur in a ratio depending on the mobilities of the ions involved. It is predicted as:

$$\frac{\Delta c_A}{\Delta c_B} = \frac{\mu_A}{\mu_B} \times \frac{(\mu_i - \mu_B)}{(\mu_i - \mu_A)} \times \frac{c^\circ{}_A}{c^\circ{}_B} \qquad (1)$$

where μ_A, μ_B, and μ_i are the mobilities of the monitoring ions A and B and the analyte ion, respectively, and $c^\circ{}_A$ and $c^\circ{}_B$ are the concentrations of the monitoring ions A and B in the buffer solution. The signs of the charge of sample ion and monitoring ions are assumed to be the same. By comparing experimentally determined transfer ratio's with the theoretical prediction, it can be verified whether peak positions and peak widths truly indicate differences and dispersities of mobilities (DS values) of CMC samples, rather than being caused by artefacts such as viscosity effects or adsorption phenomena. To obtain the experimental data on the ratio of displacement it is necessary to measure the transfer ratios of both monitoring ions at specific UV wavelengths.

We used benzenesulfonate (BS) and picrate (P) as monitoring ions. The mobility of the monitoring ions were obtained in a borate BGE with the same ionic strength (10 mM) as used in the CMC separation. Values of -39.8 and $-34.8\times10^{-9}\,m^2\,V^{-1}\,s^{-1}$ were found for benzenesulfonate and picrate, respectively. To measure the ratio of displacement of benzenesulfonate and picrate, UV detection was applied at 210 and 357 nm simultaneously. With a conventional spectrophotometer the molar absorption coefficients (ε) of benzenesulfonate and picrate were measured at these two wavelengths. At 210 nm the ε values found were 6.15×10^3 and $8.81\times10^3\,L\,mol^{-1}\,cm^{-1}$ and at 357 nm 0 and $8.78\times10^3\,L\,mol^{-1}\,cm^{-1}$, for benzenesulfonate and picrate, respectively. From the signals obtained at 210 and 357 nm the changes in benzenesulfonate and picrate concentrations and their ratio was calculated.

A set of four CMCs with the average DS values of 0.69, 0.84, 1.22, and 1.64 were separated using a buffer solution containing 4.5 mM benzenesulfonic acid and 4.5 mM picric acid dissolved in 10 mM KOH. A voltage of 10 kV was applied. As an example Figure 6 shows the two detection signals for CMC 0.69. It was found that the ratio of the displaced concentration of benzenesulfonate and picrate decreased with increasing mobility over the width of the peak. An overall overview of the ratio between the displaced concentrations and the mobilities for all CMC samples compared with the predicted trend line is depicted in Figure 7. It can be seen that the behavior obtained experimentally followed the same trend as the predicted trend line. The small systematic deviation between experimental results and the prediction may be caused by the nonlinear response of the UV

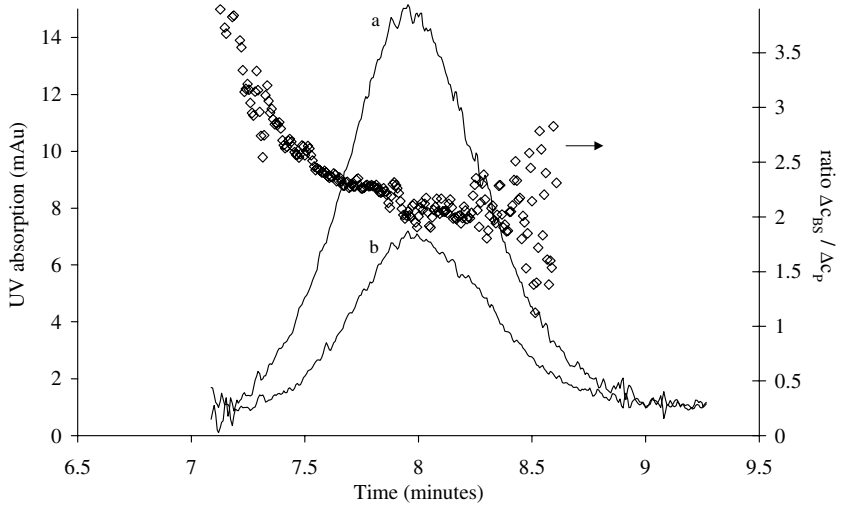

Figure 6. Indirect detection signals of CMC 0.69 with (a) benzenesulfonate (BS) and (b) picrate (P). Data on the ratio of displaced concentration of BS and P over the peak width of CMC 0.69. For conditions: see text.

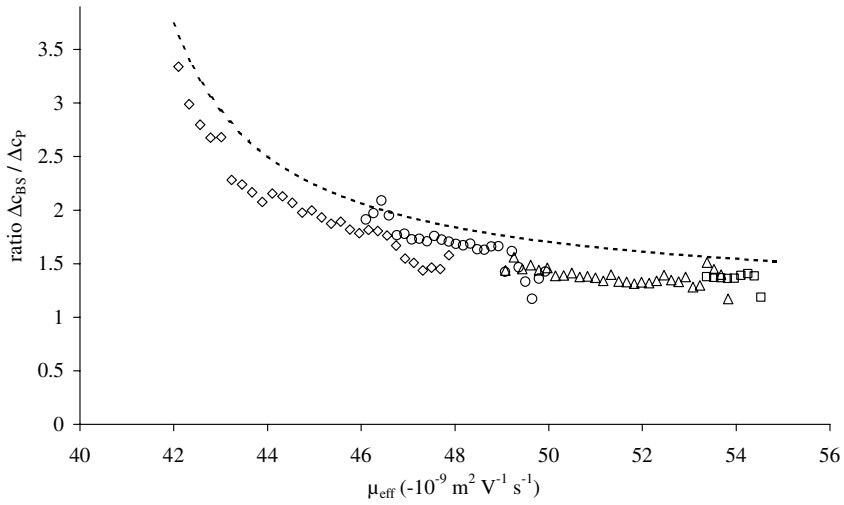

Figure 7. Ratio of displaced concentration of benzenesulfonate (BS) and picrate (P) versus the effective mobility of CMC 0.69 (◇), 0.84 (○), 1.22 (△), and 1.64 (□) compared with the predicted trend line (- - -).

detection in (cylindrical) capillaries. The sensitivities for the (displacement of the) monitoring ions in this setup may deviate to some extent from the absorbance values measured in a cuvette. The results of these experiments indicate that the CZE separations of CMCs are based on the charge density of the polyelectrolytes. It also demonstrates that it is valid to use the peak widths for the determination of the distribution of the DS of the CMCs.

3.3. Influence of the size of CMC on the electrophoretic mobility

With the method of indirect UV detection described above it has been shown that the peak widths obtained experimentally represent the dispersity of mobilities of CMCs. However, it is still possible that the variety of the size of the polyelectrolytes has an effect on the electrophoretic mobility, rather than the variety of DS. This aspect was studied by CE separations of SEC fractionated samples.

A solution of CMC 1.22 (5 g L^{-1}) was fractionated using an aquagel OH-mixed column and an eluent of 5 mM $NaNO_3$ dissolved in water. This SEC system was described as very successful for the separation of CMCs when a high salt concentration was used.[11] However, in preliminary experiments it was found that such salt concentration in the fractionated samples caused severe peak distortion in the electrophoretic system. For this reason the SEC fractionating experiments were carried out using a lower salt concentration. Six fractions of the CMC 1.22 sample were collected with a fractionation

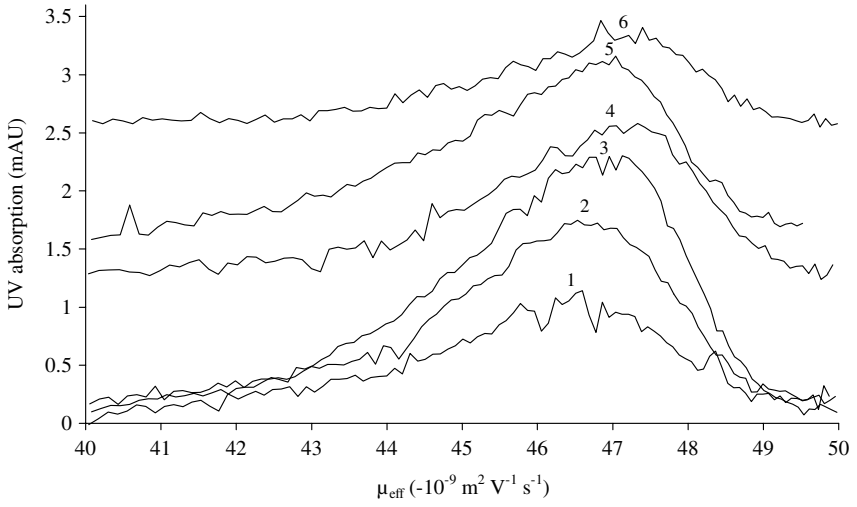

Figure 8. CZE separations of SEC fractions of CMC 1.22 (1 ~ high MM, 6 ~ low MM). Separation conditions as in Figure 2.

time of 30 s. Re-injection of the fractionated samples in the SEC system showed that the fractions contained polyelectrolytes with different average MMs.

The buffer composition used for the CZE separation of the original CMCs (borate buffer, pH 9, ionic strength 20 mM) was also used for the separation of the fractions. Figure 8 shows the electropherograms of the six fractions of the CMC 1.22 sample (fraction 1 ~ highest MM; fraction 6 ~ lowest MM). It was found that the effective mobilities of the CMC fractions increased slightly (0.6%) with increasing fraction number. This behavior suggests that there is an actual effect of the polyelectrolyte size on the CZE separation. A possible other explanation for the behavior obtained with the CMC fractions is that the charge density of polyelectrolytes with a smaller degree of polymerization is higher than that of high MM CMCs. This might be an effect of varying reaction activity for CMCs with different polymeric chain lengths. However, such dispersity in charge density is not to be expected since the chemical modification of cellulose is performed in the solid state. Another indication of the influence of the size on the CZE separation was obtained with a high MM CMC of 10^6 Da. When the normalized mobility of this sample was used in the DS calibration curve, a DS value was calculated that was approximately 10% lower than the value as provided by the supplier. This deviation is significantly higher than the imprecision obtained in the repeatability study. These results demonstrated that the size of the polyelectrolytes cannot be fully neglected, and that the calibration curves of mobility versus DS value are only valid when standards within a specific MM range are applied. However, the MM effect is relatively small. Commercial CMCs are generally classified as low, middle, or high MM products. Calibration according to this classification will be sufficient to determine data on the DS of CMCs accurately.

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