Association of Ethyl(hydroxyethyl)cellulose Solutions

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ABSTRACT: Ethyl(hydroxyethyl)cellulose (EHEC) forms true molecular solutions in 10 mM aqueous solution NaCl at concentrations equal or less than 0.1%. Less hydrophilic EHEC forms true molecular solution also in methanol/10 mM aqueous NaCl (1:1, v/v) mixture. An associative clustering was observed in this solvent mixture in the case of a more hydrophilic EHEC derivative. The amount and size of clusters increased with EHEC sample concentration. Repulsive forces related to some minute charge of EHEC, evidenced by ion-exclusion chromatography in the absence of salt in the methanol/water (1:1, v/v) mobile phase, became operative in clustering solution without salt; both the amount and size of clusters decreased. EHEC contains a low amount of particle impurities. The light-scattering signal related to these impurities may be misinterpreted as an aggregation when the solutions are not properly filtered.

Introduction

Ethyl(hydroxyethyl)cellulose (EHEC) belongs to a class of water-soluble cellulose derivatives with reduced solubility in water at elevated temperatures.[1] This behavior is related to the presence of two different substituents randomly distributed along the cellulose backbone; one of them (ethyl) being hydrophobic and the other one (2-hydroxyethyl) hydrophilic. The delicate balance between hydrophobic and hydrophilic interactions then determines the associative behavior of EHEC as a function of polymer concentration, temperature, and presence of additives.[2] The associative behavior as a general feature of (hydroxyalkyl)- and alky(hydroxyalkyl)cellulose derivatives in a semidilute regime is documented from their shear-flow[1,3−5] and extensional-flow characteristics.[3] It is also well evidenced in a semidilute regime from small-angle neutron scattering measurements[2] of EHEC solutions.

Any equilibrium formation of interpolymer clusters in dilute solution requires a thermodynamic equilibrium between associative forces counteracted by solvation forces and by Brownian motion of single coils. Hence, polymer concentration should be lower than the coil overlap concentration,[6] $c^* = 1.08/[\eta]$ where $[\eta]$ is intrinsic viscosity, to keep single polymer coils surrounded by a solvent and independently moving. The molecular interpenetration should then not be of great importance,[7] and well-known virial expansion regime should be valid. Contrary to this simple picture, in a semidilute regime at $c > c^*$ the system will display a lot of interchain contacts and may be described as a transient network characterized by local concentration fluctuations that relax more or less independently of the overall chain motion.[8] A discussion of the movement of single coils makes a little sense here.

The reversible temperature-dependent aggregation of dilute aqueous solutions of methylcellulose was conclusively proved by Neely.[9,10] These solutions were found to be essentially free of aggregates when refrigerated, and increasing aggregation was observed when they were stored at 24 °C for 6 days. Neely proposed that increased temperature decreases hydration of the macromolecules which are then able to form aggregates composed of several macromolecules laid down side by side. Introduction of a large number of hydroxypropyl groups into the methylcellulose removed this kind of aggregation completely as this bulky substituent prevents the ultimate contact of macromolecules and, at the same time, is more hydrated being hydrophilic.[9,11,12] Hence, formation of aggregates of a structure similar to methylcellulose is not very likely in the case of EHEC. Nevertheless, an interesting question remains, whether associative clustering due to proper balance of hydrophobic and hydrophilic interactions of EHEC substituents may be induced below c^*, e.g., by proper adjustment of solvent composition (hydrophobicity).

It will be shown in this paper that a hydrophilic EHEC derivative forms concentration-dependent clusters in a methanol/water solution. The evidence from dynamic light scattering (DLS) data will be presented. To avoid mistaking the effect of clustering as observed by DLS for the particle impurity signal, the presence of particle impurities will be analyzed using size exclusion chromatography (SEC) and filtration experiments. It will also be shown by ion exclusion chromatography that EHEC partially carries charged (carboxyl) groups originating from the oxidation of its end aldehyde and hydroxy groups similarly to other polysaccharides.

Experimental Section

Materials. Ethyl(hydroxyethyl)cellulose samples were kindly provided by Berol Kemi, Stenungsund, Sweden. The EHEC sample designated as CST-103 had a cloud point of 28 °C (in 0.5% aqueous solution) and an average degree of substitution, according to the manufacturer, of 1.5 ethyl groups and 0.7 hydroxyethyl groups per glucose unit. The sample designated as E230G had a cloud point of 65 °C and degree of substitution of 0.9 ethyl groups and 0.9 hydroxyethyl groups per glucose unit. A recent distribution analysis of these samples[13] gave $M_w = 189\,000$, $M_w/M_n = 2.1$, and $M_w = 535\,000$, $M_w/M_n = 1.8$ for CST-103 and E230G, respectively.

Standardized stock solution of the samples were prepared by dialysis in tube membranes (cutoff 12−14 000, Spectra/Por, Spectrum Medical Industries, LA) against Milli Q water (Millipore) for 1 week to remove salts and other low-molecular-weight material and filtered through 0.8 μm filters to remove large-size undissolved substances, microgels, and dust par-

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ticles. Actual concentrations were determined by drying the samples to constant weight at 105 °C.

All measured solutions were prepared by weight. Water from a Millipore MilliQ$_{\mathrm{PLUS}}$$^{\mathrm{UF}}$ ultrapure water purification unit was used. Millex-AA, HA, and GS filters (0.8, 0.45, and 0.22 μm, respectively, mixed cellulose ester membrane) (Millipore, Bedford, MA) and Millex-FG and SR (0.2 and 0.5 μm, respectively, polytetrafluoroethylene membrane) from the same supplier served for the sample filtrations. All filters were 25 mm in diameter. Analytical reagent grade NaCl and methanol were obtained from Merck, Darmstadt, Germany and used without further purification.

DLS Experiments. An Inova-series 4 W argon ion laser (Coherent Laser Division, Palo Alto, CA) tuned to 514.5 nm was focused onto a precision-scattering 10 × 10 mm cell (Hellma, Mullheim Baden, Germany). Time fluctuations in light scattering were detected at $\Theta = 90°$ using a commercially available Brookhaven photomultiplier tube and a 128-channel BI-8000 digital correlator (Brookhaven Instruments, Holtsville, NY). The Brookhaven particle size distribution software package contains five of the most common analytical procedures;[14] the Brookhaven CONTIN version 3.0 (including Mie correction) was used here. Due to approximative character of this correction, dimensions of large clusters should be, in this sense, taken as apparent values. All solutions were filtered through selected filters directly into the scattering cells. In clustering experiments where polymer concentration was varied, the solutions were prepared by the dilution of the maximum polymer concentration (0.1%) as well as directly from the stock solution. Identical DLS behavior within the experimental error was observed. All solutions were tested in nonlinear (wide-window) mode for the presence of slower modes, and their absence was proved. An optimum linear mode setting (64 channels) selected to minimize the effect[15] of base-line error on the CONTIN procedure was then used in all experiments.

SEC Experiments. Two modular systems were used; one of them with refractometric (RI) detection and the other one with dual RI and low-angle laser light-scattering (LALLS) detection. System I consisted of a VCR 40 HPLC pump (Academy Development Works, Prague, Czech Republic), an injection valve Rheodyne 7125 (Rheodyne Inc., Cotati, CA) with 100-μL loop, and a RIDK 101 differential refractometer (ECOM Ltd., Prague, Czech Republic). Two stainless steel columns (250 × 8 mm i.d. supplied by Tessek Ltd., Prague, Czech Republic) packed with diol-modified LiChrospher 1000 and 4000 (Merck, Darmstadt, Germany) packings prepared according to a recently described procedure[16] were connected in series.

System II consisted of an identical pump and injection valve with a 200-μL loop, an R 401 differential refractometer (Waters Assoc., Milford, MA), and a Chromatix KMX-6 LALLS detector (LDC/Milton Roy, Sunnyvale, CA) that operated at a 6−7° measuring angle. One more column as above packed with diol-modified LiChrospher 300 was mounted in series to two columns as in system I. Both SEC systems were connected through a Black Star 2308 A/D converter to an IBM-compatible computer.

Results and Discussion

The CST-103 and E230G samples used have[13] $c^* = 0.22\%$ and 0.24% in water and $c^* = 0.31\%$ and 0.37% in a methanol/10 mM NaCl mixture (1:1, v/v), respectively. Recent model calculations[7] have shown that both chain entanglement and overlaps can take place even at $c = 0.3c^*$. Hence, all experiments in this paper were performed at $c \leq 0.1\%$ to keep overlapping at a reasonably low level.

The possible presence of impurities in the samples and their relation to DLS data obtained deserves attention before conclusions concerning clustering are drawn. In particular, compact dense particle impurities may give a DLS signal comparable to the polymer under study even when they are present at a very low level. Strazielle[17] has shown that such spherical particles of the same density like solid poly(ethylene oxide) having the same radius of gyration as dissolved poly(ethylene oxide) coils would give an increase in the intensity of the scattered light 2 × 10^3 times as compared to that of dissolved coils if they were present in the solution at the same concentration. Accordingly, it was believed for a long time that dextrans and poly(ethylene oxide) aggregate in aqueous solutions;[18] recent studies[19,20] have shown that the apparent aggregation behavior of these polymers in water as observed by light scattering is the result of the presence of a very low amount of dense particle impurities. Jullander[21] pointed out that fragments of fibers and gel particles are always present to some extent in solutions of cellulose ethers. In a detailed study, Manley[22] described great difficulties in different attempts to obtain optically clean solutions of EHEC for light-scattering experiments and proved the presence of a very low amount of insoluble particulate matter (fiber fragments, of different sizes and different degrees of swelling, and other particles). Hence, the presence of such impurities must be assumed also in our samples. As there is no reason to believe that all the particles are larger than 0.8 μm, their complete removal in the preparation of our stock solutions cannot be assumed. Filtrations with hydrophobic and hydrophilic filters of different pore size were therefore applied to investigate the effect of the removal of filterable particles/aggregates on the DLS data obtained after the filtrations. The changes in the scattered intensity obtained after filtration with different filters are displayed as a function of concentration of the sample E230G dissolved in 10 mM aqueous NaCl in Figure 1 (dashed lines). It is seen that a significant decrease in scattered intensity was obtained when the solutions were filtered with 0.2-μm FG (hydrophobic) filter as compared to 0.22-μm GS hydrophilic one. This result indicates the presence of similar impurity observed recently in poly(ethylene oxide)/water solutions, i.e., microdroplets or microparticles formed from a hydrophobic organic substance insoluble in water and sterically stabilized by surface-active[23] EHEC molecules. Using a hydrophobic filter with a larger pore size (0.5 SR), the scattered intensity increased, indicating measurable presence of particles removed by mechanical (size) filtration. On the other hand, the scattered intensity observed after the filtration with a 0.22-μm GS filter is even slightly higher than that obtained after filtration with a 0.5-μm SR filter. Hence, it is possible

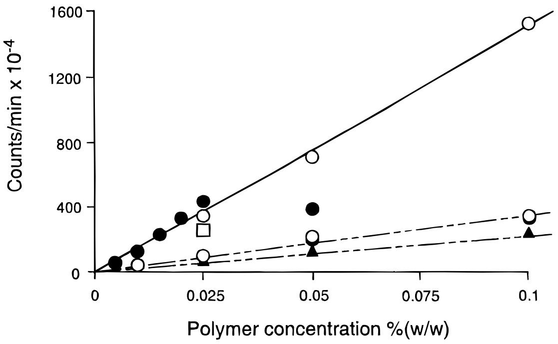

Figure 1. The concentration dependence of the scattered intensity of EHEC E230G in aqueous 10 mM NaCl (dashed lines) and in 1:1 (v/v) methanol/10 mM aqueous NaCl (full line). Solutions filtered with 0.5-μm SR (full circles), 0.22-μm GS (hollow circles), and 0.2-μm FG (full triangles) filters. The hollow square corresponds to methanol/water (1:1, v/v) filtered with 0.22-μm GS filter.

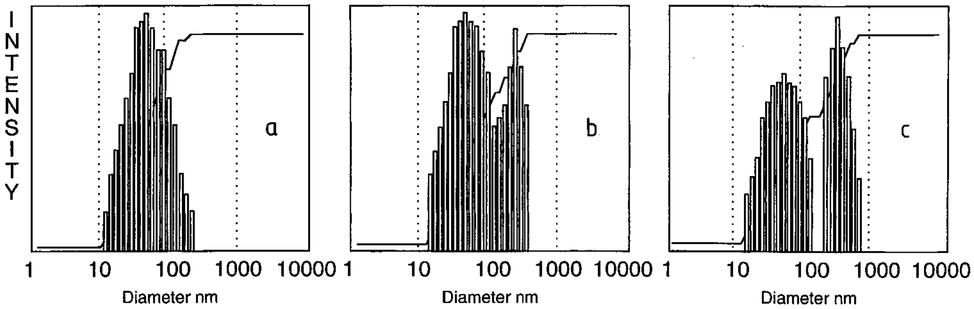

Figure 2. CONTIN plots obtained for 0.05% solutions of EHEC E230G in 10 mM aqueous NaCl after the filtration through 0.2-μm FG (a), 0.22-μm GS (b), and 0.5-μm SR (c) filters.

Table 1. Scattered Intensity of EHEC CST-103 Solutions in 10 mM Aqueous NaCl Obtained after the Filtration through Different Filters

concentration % w/w	intensity (counts × 10^{-4}/min)		
	0.5-μm SR	0.2-μm FG	0.22-μm GS
0.05	168.0	140.2	143.4
0.10	327.5	255.5	268.4

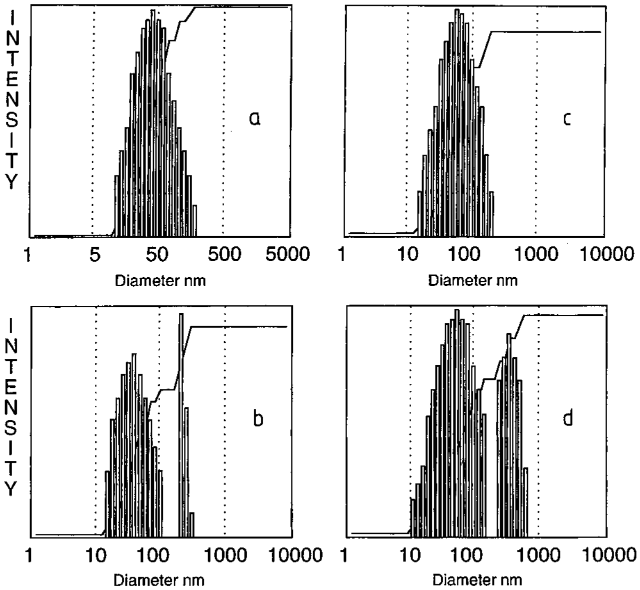

Figure 3. CONTIN plots obtained for 0.05% solutions of EHEC CST-103 in 10 mM aqueous NaCl after the filtration through 0.2-μm FG (a), 0.22-μm GS (b), 0.5-μm SR (d) filters and in 1:1 (v/v) methanol/10 mM aqueous NaCl after filtration through a 0.2-μm FG (c) filter.

to conclude that the sample contains two different impurities; one of them is removed by size filtration and the other one, being hydrophobic, is adsorbed by hydrophobic filters. The CONTIN plots obtained from DLS experiments after the filtration with these filters (E230G sample, $c = 0.05\%$) are shown in Figure 2. A different cut-off size is seen from a comparison of Figure 2b and 2c in agreement with the size of filters used. Both impurities then disappear within the experimental error after filtration with 0.2-μm FG filters (Figure 2a). Figure 2a thus unambiguously proves that sample E230G does not form aggregates/clusters having a hydrodynamic size around 250 nm (cf. Figures 4 and 5) in dilute 10 mM aqueous NaCl solution.

Similar filtration experiments were made with the sample CST-103. The scattered intensities obtained after the filtration with different filters are summarized in Table 1.

It is seen that the main part of the impurities is removed by size filtration, and only a very low (if any) amount of adsorbable impurities is present here. The related CONTIN plots at $c = 0.05\%$ are presented in Figure 3. The difference between the CST-103 and E230G samples is seen in the use of a 0.22-μm GS filter when the plots 2b and 3b are compared. The filter removes almost all impurities by size filtration from the CST-103 sample in contrast to the sample E230G where a much higher amount of hydrophobic impurities is present (cf. scattered intensities above). Again, it follows from Figure 3a that no aggregation/clustering is detected in 10 mM aqueous NaCl solutions. Figure 3c then confirms that a dilute solution of the CST-103 sample does not form clusters in the mixture methanol/10 mM aqueous NaCl (1:1, v/v) either. An entirely different behavior was obtained when the sample E230G was dissolved in this solvent mixture. A tremendous increase in scattered intensity (Figure 1, full line) observed in a methanol-containing solvent as compared to that of the aqueous solution is typical of the case of an associating system.[18] The CONTIN plots in Figure 4 clearly show the concentration-dependent formation of clusters in methanol-containing solution. It is seen that some nonaggregated coils are present in a decreasing amount with increasing concentration of solutions. At the same time, the relative scattering amplitude of clusters increases. A decrease in size of nonaggregated coils as compared to the original polymer[13] ($d_H = 42$ nm) was consistently observed. Noting that this sample has

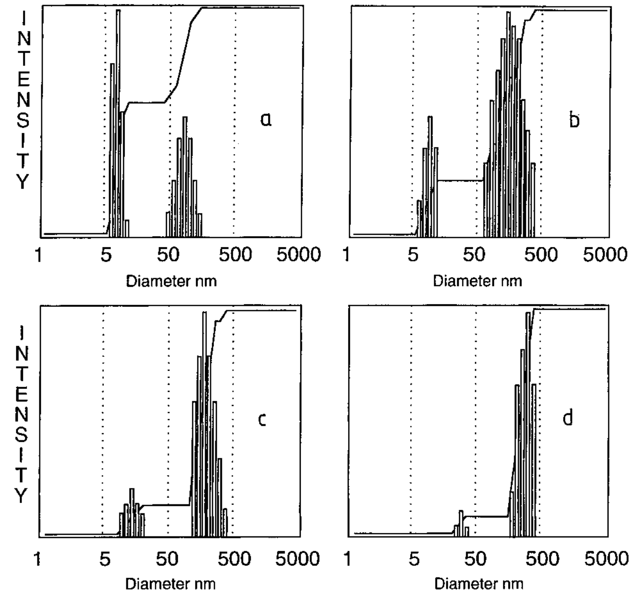

Figure 4. CONTIN plots obtained for EHEC E230G dissolved in methanol/10 mM aqueous NaCl (1:1, v/v) after fitration through a 0.5-μm SR filter at polymer concentration 0.005% (a), 0.01% (b), and 0.02% (c) and after the filtration through a 0.22-μm GS filter at concentration 0.1% (d).

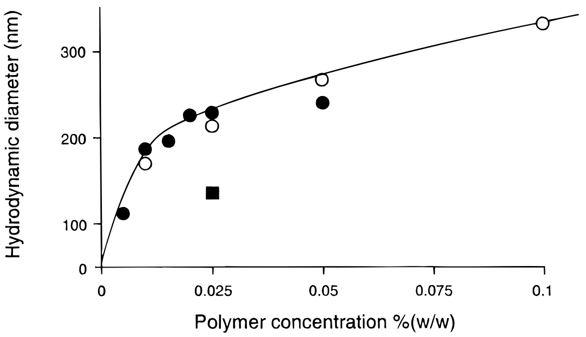

Figure 5. Hydrodynamic diameters of clusters formed in solutions of EHEC E230G in methanol/10 mM NaCl (1:1, v/v) as a function of polymer concentration. Solutions filtered through 0.5-μm SR (full circles) and 0.22-μm GS (hollow circles) filters. Full square indicates a drop in cluster size when the solvent without salt was used (0.22-μm GS filtration).

a rather broad molecular weight distribution, this might indicate an increased ability to form clusters with increasing molecular weight. Nevertheless, this conclusion should be accepted with caution; the general tendency of all inversion methods including CONTIN to overfitting of long-tailing unimodal broad-particle size distributions is well-known.[14] In fact, in the system composed of weak scatterers (single coils) and of strong scatterers (clusters) even a substantial number of single coils may become almost undetectable when the amount of clusters increases. On the other hand, an agreement between statistical and measured base lines always better than 0.05% was found and CONTIN was not able to find any dust term in any one of these experiments. All solutions were also completely stable, and the DLS measurements gave reproducible sizes over a week. This confirms that all solutions are in equilibrium state, and there is no indication of any phase separation within this time scale. The concentration dependence of the cluster diameter is depicted in Figure 5. Due to bimodality of the CONTIN plots, the values $(d_h)_{max}$ corresponding to the maximum frequency of the cluster size distribution were used. A considerable increase, up to 300 nm, is observed at concentration 0.1%. Some drop in cluster size (Figure 5) accompanied by a drop in scattered intensity (Figure 1), indicating some loss of sample, was observed at $c = 0.05\%$ when 0.5-μm SR and 0.22-μm GS filtered solutions were compared. At the same time, 0.2-μm FG filters could not be used with these clustering solutions as they were almost immediately blocked when tried. The explanation may again be found in different adsorption properties of the hydrophobic SR and FG filters as compared to the hydrophilic matrix of GS filters. The adsorption of EHEC on the hydrophobic filter surface is a reasonable assumption. When no clustering takes place, the adsorbed EHEC layer might only diminish the effective pore size of the filters and no blockage is seen. When the clusters are formed, an equilibrium-clustered layer is formed on the filter surface and its average layer thickness increases with increases in concentration of the solution. When the cluster size compares to filter size, considerable increase in flow resistance and blockage of the filter results. When no adsorption of EHEC on the filter membrane takes place (0.22-μm GS filter), even larger clusters than the pore size of the filter could be filtered due to their dynamic character, provided the filtration is slow enough (cf. Figure 5, $c \geq 0.05\%$).

It would be interesting to know the amount of clusters as a function of concentration in terms of their mass fractions. Unfortunately, scattering properties of clusters cannot be assumed to be identical to the unclustered polymer molecules, and the transformation of their *z*-fractions to weight fractions is not possible. Qualitatively, it follows from Figures 4 and 5 that both their size and amount increase with concentration. The same holds concerning the possibility of the determination of the mass fractions of observed filterable impurities. To get at least some crude picture of the mass content of impurities, filtrations of E230G solutions having the same concentrations as those used for DLS experiments were done in both solvents used. The RI responses of filtered solutions (the volume of the filtrate was kept constant) were determined by injecting the solutions directly into the cell of the Waters R401 differential refractometer and compared to the responses of unfiltered solutions at the same concentrations. The accuracy of such experiments cannot be expected to be very high as some variations of zero readings (both reference and measuring cell filled with solvent only) with temperature and time could not be avoided. Nevertheless, the values confident within 2-3% could be obtained. Under nonclustering conditions (10 mM aqueous NaCl), the sample recoveries after filtration with hydrophilic filters were very closely below 100% and the difference between 0.45-μm and 0.22-μm filter size was not resolved within the error of determination. Both hydrophobic filters gave on average the recovery of about 96%. These results fit the behavior observed in DLS experiments (Figures 1 and 2) and confirm a quite low level of sample contamination on the mass scale. In the case of a cluster-forming solvent, the 0.22-μm GS filter gave 95−97% and 0.45-μm HA filter 97−100% at all concentrations. When a 0.5-μm SR filter was used up to $c = 0.025\%$, the sample recovery was 95−96%, i.e., more or less the same as for the 0.22-μm GS filter. At $c = 0.05\%$, the recovery decreased to 72% and after filtration of 4−5 mL of solution the SR filter became completely blocked. The blockage of the 0.2-μm FG filter was observed during filtration of the first 2 mL of solution even at a concentration of 0.0125%. These observations thus explain the drop in size and scattered intensity in Figures 1 and 5 at $c = 0.05\%$; the use of a hydrophobic filter considerably decreases the sample concentration here.

Another interesting change of clustering behavior was observed when the same methanol/water mixture (1:1, v/v) without salt was used as a solvent for the sample E230G. Again a drop in scattered intensity (Figure 1) and cluster size (Figure 5) was observed at $c = 0.025\%$ in the case of the solution filtered with a 0.22-GS filter. As this cannot be explained by the filtration-induced change in concentration, the explanation was sought in the presence of some repulsive force present in the solution in the absence of salt. A natural assumption here is a minute charge of EHEC that is screened by the addition of salt. It was shown recently[19] by ion exclusion chromatography experiments that this is the case for dextrans, pullulans, and hydroxyethyl starch. The ion exclusion behavior of the E230G sample is illustrated as a function of NaCl concentration in the mobile phase in Figure 6. Three ion-excluded peaks are observed in the absence of salt (full curve), the first one (from the left) disappears already at a salt concentration of 0.05 mM (dashed curve). Increased salt concentration in the mobile phase moves the other two excluded peaks along the elution volume axis, illustrating increased screening of the charge effect (dash and dotted curve),

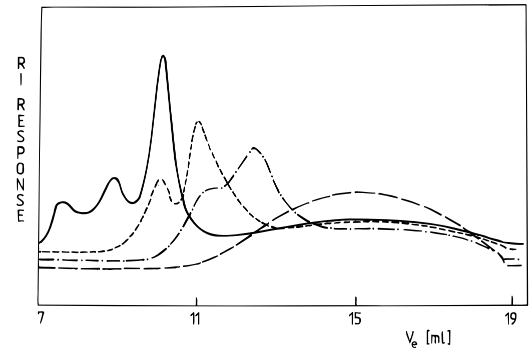

Figure 6. The effect of ion exclusion of charged components of EHEC E230G on its SEC (system I) behavior in methanol/water (1:1, v/v) containing no (full curve), 0.05 mM (dashed curve), 0.2 mM (dash and dotted curve), and 10 mM (long dashed curve) NaCl.

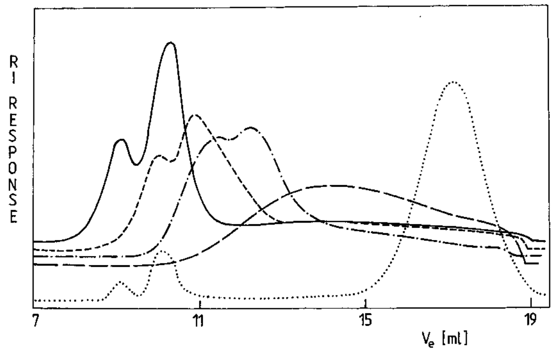

Figure 7. The effect of ion exclusion of charged components of EHEC CST-103 on its SEC (system I) behavior in methanol/water (1:1,v/v) containing no (full curve), 0.05 mM (dashed curve), 0.2 mM (dash and dotted curve), and 10 mM (long dashed curve) NaCl. Dotted curve represents ion exclusion behavior of the dextran standard sample (M_w = 99 200) in pure water as a mobile phase on the same column set.

and the ion exclusion is suppressed when the mobile phase containing 10 mM NaCl is used (long dashed curve). This behavior compares well to that of hydroxyethyl starch observed recently.[19] The ion exclusion behavior of the CST-103 sample is shown in Figure 7. The only substantial difference, as compared to E230G, is the absence of the first excluded peak from the left observed in the absence of salt in the case of the E230G sample., i.e., this sample compares to dextrans and pullulans[19] with the exception that the amount of charged molecules in the sample is higher here. The comparison of Figures 6 and 7 then indicates that the relative content of charged species is quite similar in both CST-103 and E230G samples. Figure 8 then shows that the ion exclusion is the only mechanism governing the elution of both EHEC samples as both maxima exhibit the same elution volumes and shifts along the elution volume axis, irrespective of the sample. The silica-based column system used here is incompressible; it should be therefore possible to compare ion-exclusion behavior between different mobile phases. The dotted curve in Figure 7 represents ion exclusion chromatogram of a dextran standard in pure water. It is seen that the two ion-excluded maxima of dextran have the same elution times as observed in the case of a CST-103 sample. Summarizing this full analogy of the ion exclusion behavior of EHEC to that of other polysaccharides,[19] it is possible to identify the first excluded maximum from the right in both CST-103 and E230G samples as a monocarboxy derivative (from the oxidized aldehyde group). The second one then can be identified with a dicarboxy derivative (both end groups oxidized), and the third one seen only in E230G contains higher oxidation products. In fact, it would be strange if the technology used for the production of water-soluble cellulose derivatives[1] did not partially oxidize at least the end groups of the raw material. A drop in the scattered intensity and a decrease in cluster size is thus conveniently explained by the long-range electrostatic repulsive forces that are not screened in the absence of salt in the solution.

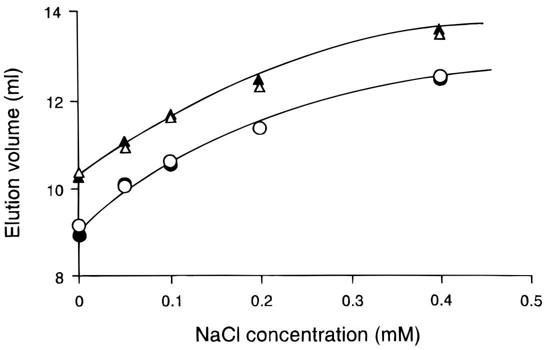

Figure 8. The dependence of the elution volumes of monocarboxy (triangles) and dicarboxy (circles) EHEC derivatives of E230G (full symbols) and CST-103 (hollow symbols) on concentration of NaCl in the mobile phase.

It can be argued that the EHEC excluded peaks might be due to association of single coils and that the addition of salt breaks down the clusters, similar to what was believed to be valid a long time ago in the case of dextrans.[24] This can be conveniently resolved when the chromatographic system is equipped with dual LALLS and RI detection. The ion exclusion behavior of EHEC CST-103, as seen by both detectors in the absence of salt in the mobile phase, is shown in Figure 9a. The correct SEC behavior of that sample when ion exclusion was suppressed by using 10 mM NaCl as a mobile phase is displayed in Figure 9b. The overlay of regression curves log M vs V_e in the absence and in the presence of salt in the mobile phase (Figure 10) confirms that there are no species of higher molecular weight present in the mobile phase in the absence of salt. Accordingly, the M_w value calculated from the data obtained in the absence of salt was identical to, within the experimental error, that obtained under correct SEC conditions.

The LALLS detection used at the angle 6−7° should be much more sensitive to the presence of strongly scattering impurities as compared to the above described DLS experiments at the angle 90°. Neither in mobile phase without salt nor in that with salt was such a particle signal present, irrespective of the 0.22-μm GS or 0.45-μm HA hydrophilic filters used. As the particle impurities were visible by DLS in the same conditions at 90°, the only possible conclusion is that the chromatography columns remove them by adsorption. As EHEC is correctly eluted in the SEC mode under these conditions, the impurities should consist of a different species exhibiting entirely different adsorption properties. A great similarity concerning the removal of strongly scattering particle impurities from poly(ethylene oxide) in a SEC experiment should be pointed out[20] here.

The distinct difference in clustering and nonclustering behavior of E230G and CST-103 samples in a methanol/water mixture, respectively, should reflect their different cloud-point values. According to the high cloud-point value of E230G (65 °C), this polymer should be

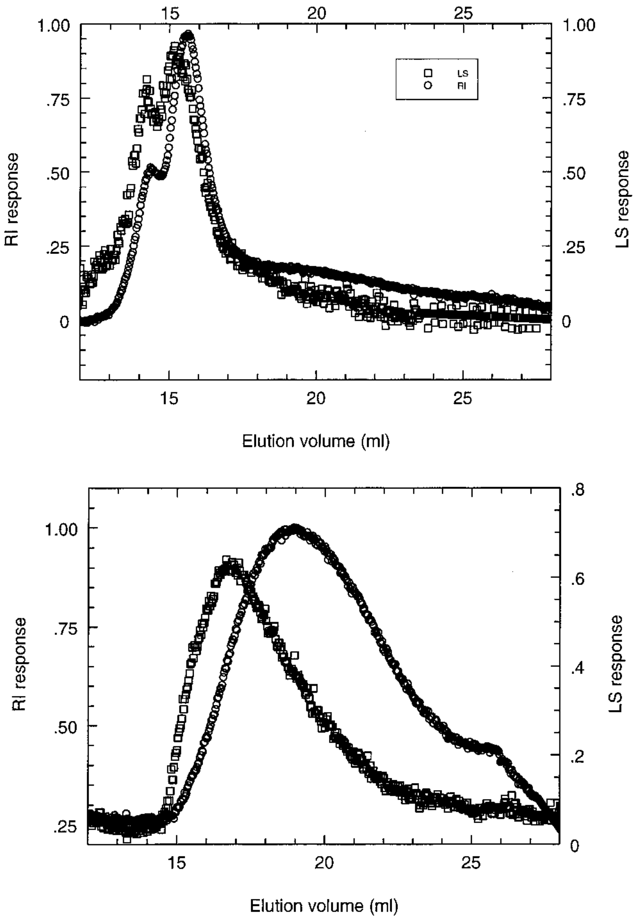

Figure 9. The LALLS/RI chromatograms (system II) of EHEC CST-103 in methanol/ water (1:1,v/v) and in methanol/10 mM aqueous NaCl (1:1,v/v) (bottom) as a mobile phase.

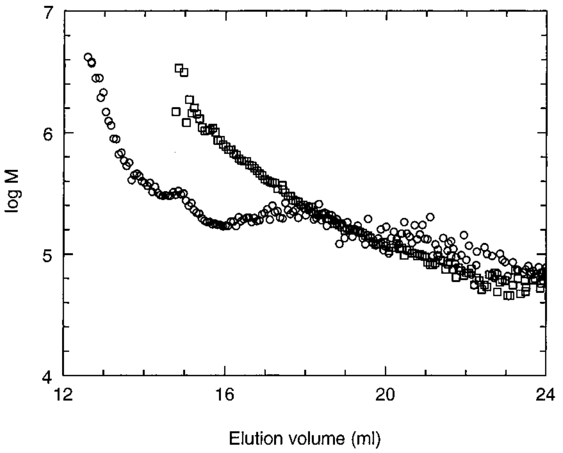

Figure 10. The comparison of log M vs V_e curves calculated from chromatograms in Figure 9 in the absence of salt (circles) and in the presence of 10 mM of NaCl (squares) in the mobile phase.

more hydrophilic than the sample CST-103 (c.p. 28 °C). The higher content of hydroxyethyl substituents, believed to be hydrophilic, and lower content of ethyl groups in E230G sample as compared to those of CST-103 fits this picture. On the other hand, methanol was found to be a nonsolvent for these polymers, and only some swelling of the samples was observed. Further, in an attempt to determine cloud point in our methanol/ 10 mM aqueous NaCl solvent, no clouding was observed up to the boiling point of methanol for both samples. Jullander[21] pointed out that water/alcohol mixtures could be the best solvents for water-soluble cellulosics as a structured mixed solvent that surrounds the polymer has a greater chance to adopt the structure reflecting the related structure of the chain with alternating hydrophobic/hydrophilic groups. Taking into account the general belief that gelling and/or precipitation of these polymers at higher temperatures relates to the loss of hydration, it is possible to assume that the addition of methanol strengthens the hydration of hydrophilic parts at higher temperatures; at the same time methanol can adsorb on the ethyl groups with hydroxy groups oriented outside of the chain and decrease the hydrophobic interaction. Hence, an increase in the observed cloud-point value does not look too surprising. In other words, the more hydrophilic E230G sample should preferentially adsorb water considerably more than the CST-103 polymer, and the observed E230G clustering might be related to an increased tendency of different coils to share a more hydrophilic milieu. This is certainly just one possible speculation, and it is believed that further investigation of the observed clustering as a function of temperature and composition of the methanol/water mixture (in progress) could clarify this point in the near future. The same holds concerning the conclusions about dynamics and/or equilibrium formation of clusters observed here. It should also be pointed out that an extrapolation of the behavior of these polymers observed in a semidilute regime could be misleading here as the entropy changes related to the cluster formation fairly below c^* and those related to the concentration inhomogeneities formed due to associative forces well above c^* could differ considerably.

Conclusions

The EHEC samples were shown to form true molecular solutions in 10 mM aqueous NaCl at concentrations equal or less than 0.1%. The CST-103 sample forms a true molecular solution also in a methanol/10 mM aqueous NaCl (1:1, v/v) mixture. Considerable clustering takes place in this solvent in the case of the more hydrophilic sample E230G, as evidenced by the increase in the scattering intensity. The cluster size increases with an increase in the polymer concentration as follows from DLS data. Both EHEC samples were shown to carry some minute charge (mainly mono- and dicarboxy derivatives) using the ion exclusion chromatography mode. The repulsive forces related to these charges become operative in clustering solution; both the scattered intensity and cluster size decrease in the absence of salt in the solution. Both samples studied have been shown to contain low amounts of foreign particle impurities; the light-scattering signal related to these impurities may be misinterpreted as the evidence of aggregation when the solutions are not properly filtered. SEC columns efficiently remove both impurities by adsorption to the level that LALLS detection performs properly. It follows from the results that Manley's statement[22] that "characterization of cellulose derivatives solely on the basis of light-scattering is hazardous" has been valid to some degree so far.

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References and Notes

(1) Dönges, R. *Polym. J.* **1990**, *23*, 315.
(2) Cabane, B.; Lindell, K.; Engström, S.; Lindman, B. *Macromolecules* **1996**, *29*, 3188.
(3) Meadows, J.; Williams, P. A.; Kennedy, J. C. *Macromolecules* **1995**, *28*, 2683.
(4) Greminger, G. K., Jr.; Krumel, K. L. In *Handbook of Water-Soluble Gums and Resins*; Davidson, R. L., Ed.; McGraw-Hill: New York, 1980; Chapter 5.
(5) Desmarais, A. J.; Wint, R. F. In *Industrial Gums. Polysaccharides and their Derivatives*, 3rd ed; Whistler, R. L., Bemiller, J. N., Eds.; Academic Press: 1993; Chapter 19.
(6) Weisberg, S. G.; Simha, R.; Rothman, S. *J. Res. Natl. Bur. Stand.* **1951**, *47*, 298.
(7) Brostow, W.; Drewniak, M.; Medvedev, N. N. *Macromol. Theory Simul.* **1995**, *4*, 745.
(8) deGennes, P. G. *Macromolecules* **1976**, *9*, 587, 594.
(9) Neely, W. B. *J. Polym. Sci. A* **1963**, *1*, 311.
(10) Neely, W. B. *J. Am. Chem. Soc.* **1960**, *82*, 4354.
(11) Haque, A.; Morris, E. R. *Carbohydr. Polym.* **1993**, *22*, 161.
(12) Haque, A.; Richardson, R. K.; Morris, E. R.; Gidley, M. J.; Caswell, D. C. *Carbohydr. Polym. 1993*, *22*, 175.
(13) Nilsson, S.; Sundelöf, L.-O.; Porsch, B. *Carbohydr. Polym.* **1995**, *28*, 265.
(14) Stock, R. S.; Ray, W. H. *J. Polym. Sci., Polym. Phys. Ed.* **1985**, *23*, 1393.
(15) Ruf, H.; Haase, W.; Wang, W. Q.; Grell, E.; Görtner, P.; Michel, H.; Dufour, J. P. *Prog. Colloid Polym. Sci.* **1993**, *93*, 159.
(16) Porsch, B. *J. Chromatogr., A* **1993**, *653*, 1.
(17) Strazielle, C. *Makromol. Chem.* **1968**, *119*, 50.
(18) Huglin, M. B. *Light Scattering from Polymer Solutions*; Academic Press: London, 1972.
(19) Porsch, B.; Sundelöf, L.-O. *J. Chromatogr., A* **1994**, *669*, 21.
(20) Porsch, B.; Sundelöf, L.-O. *Macromolecules* **1995**, *28*, 7165.
(21) Jullander, I. *Acta Chem. Scand.* **1955**, *9*, 1620.
(22) Manley, R. St. J. *Arkiv Kemi* **1956**, *9*, 519.
(23) Persson, B.; Nilsson, S.; Sundelöf, L.-O. *Carbohydr. Polym.* **1996**, *29*, 119.
(24) Engelhardt, H.; Mathes, D. *J. Chromatogr.* **1979**, *185*, 305.

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