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Carbohydrate Polymers 49 (2002) 457–469

Carbohydrate Polymers

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Molar mass distribution of hydroxypropyl cellulose by size exclusion chromatography with dual light scattering and refractometric detection

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Accepted 22 October 2001

Abstract

Physico-chemical properties of cellulose derivatives are of considerable interest in many technical applications, for example, in the food and drug industry. Efficient and careful characterisation of these properties is thus highly desirable. In this study, two different size exclusion chromatography (SEC) systems, connected on-line either to a low-angle laser light scattering detector (LALLS) or to a multi-angle laser light scattering detector (MALLS), are employed for size characterisation of three batches of hydroxypropyl cellulose (HPC) from different manufacturers. All three samples turned out to have a weight average molar mass around 100,000 g/mol, but considerable differences concerning conformational properties were found. Two of the samples contained compact components, presumably aggregates of HPC, which were clearly detected by both SEC-systems. The third sample, obtained from another manufacturer, did not show any indication of aggregation. Both SEC-MALLS and SEC-LALLS are proven to be efficient techniques for characterisation of complex polysaccharides like HPC containing mixtures of solvated polymer chains, as well as micelle-like aggregates.

Keywords: Hydroxypropyl cellulose; Size exclusion chromatography; Light scattering detection; Molar mass distribution

1. Introduction

Hydroxypropyl cellulose (HPC) belongs in a group of water-soluble cellulose derivatives and finds many industrial applications like in suspension polymerisation of vinyl chloride, as a protective colloid, binder for ceramics, paper, textile, food coating and others. Commercially produced HPC is usually characterised by a single point viscosity value as a relative measure of molar mass; this is probably adequate in most of industrial applications. A significant part of HPC production relates to cosmetics and pharmaceutical industry applications (Dönges, 1990) like tablet binder, film coating, etc. Correct performance of Hi-tech procedures used here does not depend only on HPC average molar mass, but frequently also on the width and shape of its molar mass distribution (MMD), as well as on the presence of aggregates/particles in a particular HPC product. An improved characterisation procedure pertaining to a proper HPC MMD determination including a presence of particles/aggregates among products of various suppliers and/or batches is highly desirable.

A presence of a small amount of particles/aggregates in ethyl(hydroxyethyl)cellulose was evidenced already in 1956 including a warning that this should be a general feature of all water-soluble cellulose derivatives (Manley, 1956). Harding, Vårum, Stokke, and Smidsröd (1991) reviewed techniques available for molar mass and MMD determination of polysaccharides, analysed general obstacles encountered in and stated that these polymers appear to be very difficult to analyse in terms of molar mass and MMD. Using dynamic light scattering, it was shown recently that ethyl(hydroxyethyl)cellulose contains a low mass amount of two filterable (i.e. stable) particle/aggregate populations differing in hydrophobicity (Porsch, Nilsson, & Sundelöf, 1997). More or less general presence of stable particles/aggregates in water-soluble cellulose derivatives was conclusively proved by Burchard and co-workers (Burchard & Schulz, 1989; Burchard & Vogel, 2000; Schulz & Burchard, 1993). A most probable structure of these aggregates, termed fringed micelle, consisting of microcrystalline core and a hairy corona of water-soluble (i.e. properly derivatised to obtain water solubility) chains, emerged from these studies. A general appearance of such structures in water-soluble cellulose polymers is not so surprising; the first step in derivatisation chemistry (sodium hydroxide dissolution) is more or less a general one and very

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0144-8617/02/$ - see front matter
PII: S0144-8617(01)00352-6

small amount of highly micro-crystalline regions of native cellulose may remain intact during subsequent modification. Hence, MMD having long high-molecular tail (a minor one on a mass scale) must be frequently expected in MMD analysis of water-soluble cellulose derivatives.

Light scattering is well known to be very sensitive to the presence of aggregates in polymer solutions. A multi-angle laser light scattering (MALLS) or a low-angle laser light scattering (LALLS) in combination with a refractometric (i.e. mass concentration) detection (RI) should be an optimum detector combination in size exclusion chromatography (SEC) of HPC as no well characterised standards are available here. The advantage of MALLS detection consists in an additional possibility to determine dimensions of dissolved polymer in terms of gyration radius (R_g) from the angular dependence of scattered light. On the other hand, this extrapolation may be difficult to perform properly in the case of large particles. LALLS detection is free of this difficulty, but does not give polymer size. Difficulties may arise here from very large sensitivity of low-angle detection (6–7°) to foreign large particles (dust) as these particles scatter light most intensively in the forward direction of the laser beam.

To allow an inter-laboratory comparison and to assure maximum reliability of results especially in the case of aggregate containing samples, independent MALLS/RI and LALLS/RI SEC systems using different SEC columns are used in this paper to investigate MMD of HPC. The use of various SEC packings differing in both surface and bulk chemistry is believed to assure that a bias of results due to possible non-inertness of the stationary phase (Harding et al., 1991) is absent. The agreement of SEC-LALLS and SEC-MALLS results, if obtained, should significantly substantiate the reliability of angular extrapolations of MALLS or, if reversed, the reliability of LALLS detection. A flow rate and mobile phase ionic strength effects on SEC performance of HPC are also investigated.

2. Experimental

2.1. Materials

Three HPC samples from two different manufacturers were examined: Nisso 'HPC L' (Nippon Soda Company, Japan), Nisso 'HPC LM' (Nippon Soda Company, Japan) and Klucel 'HPC LF' (Hercules, Wilmington). All samples are relatively highly substituted having an $MS_{hydroxypropyl}$ in the interval of 3–4. According to manufacturers, the viscosity grades were similar, corresponding to 75–150 cps for a 5% solution. The solid material (water content 3%) was dispersed in the mobile phase and was stored at room temperature in darkness under gently stirring for 10–12 days.

2.2. Chromatography

2.2.1. SEC-MALLS/RI system (Mölndal)

The separation column was a TSK-GEL $GMPW_{XL}$ 7.8 × 300 mm, particle size 13 μm, linear mixed bed size exclusion column. The pump was a Shimadzu LC10AD liquid chromatography pump (Shimadzu Corporation, Tokyo, Japan). The degasser used was a Gastorr 154 (Gastorr, Japan). The flow rate of the mobile phase was held at 0.11 or 0.45 ml/min, respectively. The polymer sample was injected on the column by a Perkin–Elmer 200 LC autosampler (Perkin–Elmer Corporation, Norwalk, CT) equipped with a 100 μl sample loop. The injected sample amount was thus 100 μg. The polymer concentration in solution was held at 1 mg/ml. The mobile phase was a 0.10 M sodium chloride (p.a., Merck, Darmstadt, Germany) solution filtered with a 0.22 μm mixed cellulose ester filter GSWP (Millipore Corporation, Bedford, MA). The temperature in the carrier was approximately 297 K.

A stainless steel High Pressure Filter Holder, 25 mm, (Millipore Corporation, Bedford, MA), with a 25 mm 0.025 μm VSWP Filter (Millipore Corporation, Bedford, MA) was connected directly to the pump on-line. A small volume Precolumn Filter A315 (Upchurch Scientific, Oakharbor, WA) with a replaceable stainless steel frit A101 × (pore size 2 μm) was employed in-line between the column and the detector. Its frit served as support for a post-column cellulose acetate filter (Sartorius Cellulose Acetate 111 11106-047 N, Sartorius AG, Goettingen, Germany) the pore size of which was 0.45 μm. It cannot be excluded that such a filter would influence the results by removing sample material. Therefore, it is important that results, with and without this filter are compared for every different experimental condition. This was done in this study and it was found that the post-column filter greatly decreased the noise of the baseline without causing any detectable losses of the sample.

The light scattering photometer was a DAWN-EOS multi-angle light scattering instrument (Wyatt Technology, Santa Barbara, CA). Simultaneous concentration detection was performed using an Optilab DSP interferometric refractometer (Wyatt Technology, Santa Barbara, CA). Both detectors used a wavelength of 690 nm. Filtered toluene (Merck, Darmstadt, Germany) was used for calibration of the MALLS-detector and sodium chloride (Suprapur, Merck, Darmstadt, Germany) for calibration of the refractive index detector. The detectors at different angles in the MALLS instrument were normalised to the 90° detector using low polydisperse pullulan P-50 (Shodex STANDARD P-82, Showa Denko, Tokyo, Japan). Bovine serum albumin (BSA) (Sigma Chemical Company, St Louis, MO) was used to determine the interconnection volume between the detectors to 0.129 ml. The signals from the two detectors were analysed by ASTRA software (ASTRA for Windows 4.73) (Wyatt Technology, Santa Barbara, CA). The angular dependence of the scattered light was extrapolated to zero

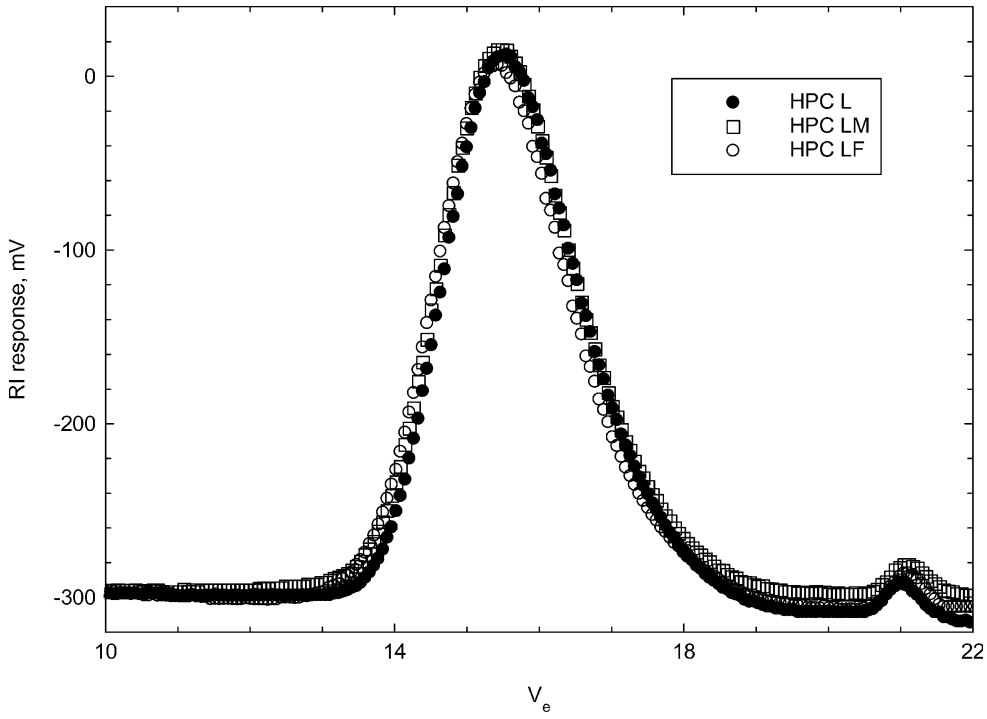

Fig. 1. SEC-LALLS: Comparison of RI signals (TSK GMPW 600 × 7.5 mm column) of various HPC samples at 0.11 ml/min in 0.1 M NaCl.

angle using the linear Berry fit method. The recovery was obtained from the ratio of the mass eluted from the channel (determined by integration of the refractometer signal) to the mass injected.

The refractive index increment (dn/dc) was determined by the injection of six different concentrations of each of the HPC-samples into the refractometer. During these measurements, the injector, equipped with a 1.0-ml loop, was connected to the refractometer directly. The flow rate employed then was 0.5 ml/min, which was found to be sufficient for obtaining good and consistent data. The data were analysed using the DNDC5 software (Wyatt Technology, Santa Barbara, CA). The (dn/dc)-value obtained for all three HPC samples was 0.141.

2.2.2. SEC-LALLS/RI system (Prague)

Modular chromatograph consisted of a Constametric® 3200 MS pump (Thermo Separation Products, Riviera Beach, FL), a Pharmacia injection valve V-7 with 200-μl loop (Pharmacia & Upjohn, Uppsala, Sweden), a Chromatix KMX-6 LALLS detector (LDC/Milton Roy, Sunnyvale, CA) and an Waters 2410 differential refractometer (Waters Association, Milford, MA) connected through a Black Star (Huntingdon, UK) 2308 A/D converter to an IBM-compatible computer. On-line RI-LALLS arrangement allows the simultaneous determination of M and c at any elution volume ('slice'). The following relation is valid for Rayleigh scattering from polydisperse polymer/solvent system at low-angle (6–7°):

$$(K^{*}c)/R_{\Theta} = 1/M_{w} + 2A_{2}c \quad (1)$$

where c is the concentration of scattering species, R_{Θ} is the excess Rayleigh scattering factor, M_{w} is the weight average molar mass of scattering species and A_2 is the second virial coefficient. $K^{*} = (2\pi n^{2}/N\lambda^{4})\nu^{2}$, where n is the refractive index of the solvent, λ is the wavelength in vacuo (633 nm), N is the Avogadro number and ν is the refractive index increment of the scattering species in the solvent used. If correct separation takes place, the polymer seen at a slice is assumed to be monodisperse. Angular dependence of the scattered light is omitted at the low-angle used. Polydispersity and column band broadening dilutes the sample considerably; hence, the term A_2c may be neglected if the concentration of the injected solution is low enough. As the effect of second virial coefficient (thermodynamic non-ideality) may be very pronounced in the case of polysaccharides (Harding et al., 1991), the SEC sample zone dilution at the peak apex was checked by injecting a known HPC concentration directly into the system of detectors. A dilution factor was found to be 19. Hence an injection of 0.1% solution should get a maximum concentration 5×10^{-5} g/ml at the RI peak apex. A comparison with values in Table 3 compiled by Harding et al. (1991), shows that the error due to thermodynamic non-ideality would be only around 5% in the case of Alginate (laminaria) exhibiting a maximum deviation under the conditions used in this work. Thus, it seems that the influence of thermodynamic non-ideality can be safely neglected in this study.

The conventional calibration $\log M$ versus elution volume (V_e) is thus directly obtained. A home-made software (M. Netopilík, Institute of Macromolecular Chemistry) allows on-line data accumulation and all calculations of

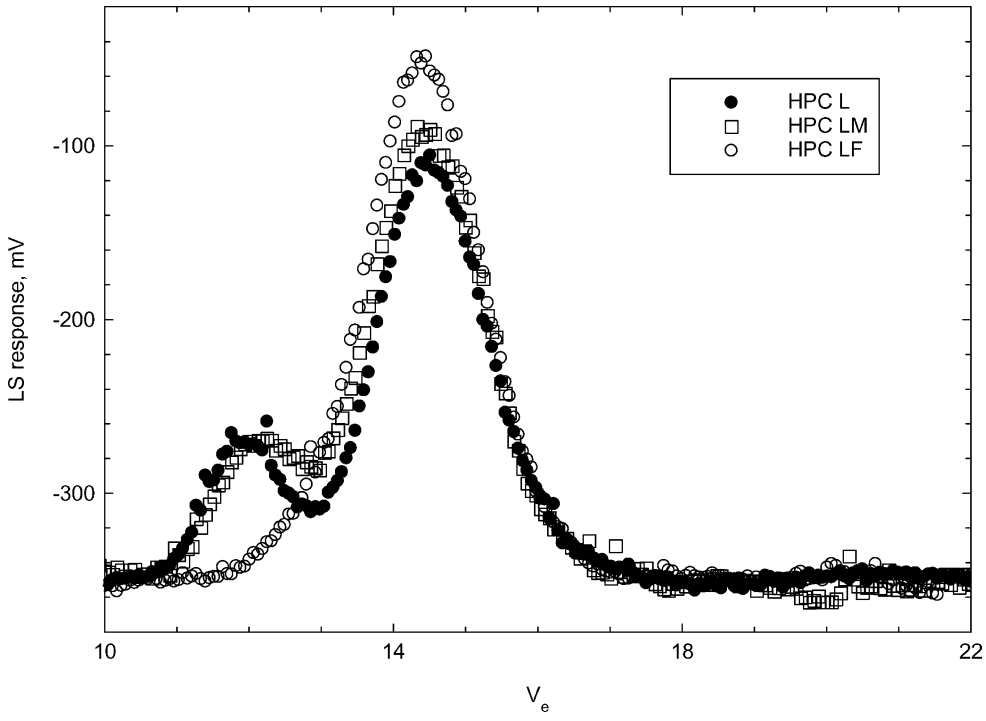

Fig. 2. SEC-LALLS: Comparison of LS signals (TSK GMPW 600 × 7.5 mm column) of various HPC samples at 0.11 ml/min in 0.1 M NaCl.

MMDs and their averages. Three stainless steel columns (8 × 250 mm) in series packed with hydrophilised GMB 200, 1000, and 5000 + poly(glycidyl methacrylate) packings having particle size 10 μm (Labio Ltd., Prague, CR) and TSKgel GMPW linear (7.5 × 600 mm) column, particle size 17 μm, in series with (7.5 × 75 mm) TSKgel Guard column (both Watrex, Prague, CR) were used. All measured solutions were prepared by weight. Injected concentrations were 0.2% (Labio columns) and 0.1% (TSK column). Water from a Millipore Milli-Q^{UF}_{PLUS} ultrapure water purification unit was used. Analytical reagent grade NaCl from Merck (Darmstadt, Germany) was used without further

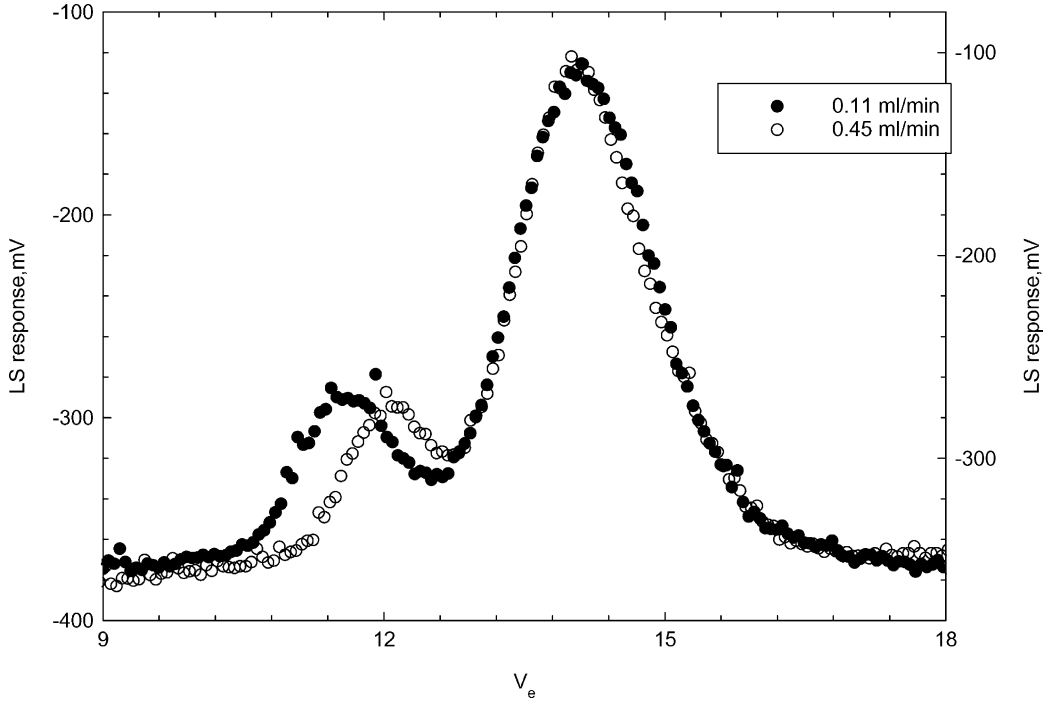

Fig. 3. SEC-LALLS: Elution of HPC L at different flow rates on TSK GMPW (600 × 7.5 mm) column in 0.1 M NaCl.

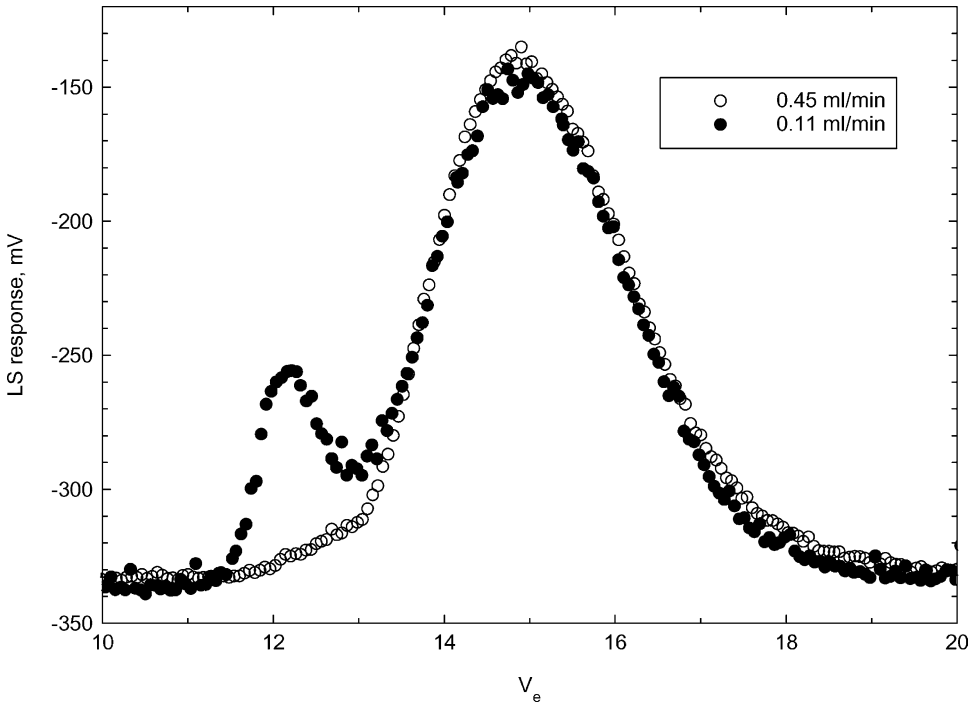

Fig. 4. SEC-LALLS: Elution of HPC L at different flow rates on Labio column set in 0.1 M NaCl.

purification. Millex GV_{13}, HV_{13} (diameter 13 mm, 0.22 and 0.45 μm, respectively, hydrophilic Durapore membrane), FG_{13}, FH_{13} (diameter 13 mm, 0.2 and 0.5 μm, respectively, Teflon membrane), filters (Millipore, Bedford, MA) were alternatively used as sample and post-column filters in combinations of the same size. Similarly like earlier, a lowering of filter pore size only reduced LS noise without affecting LS peak area. Anotop 25™ (diameter 25 mm,

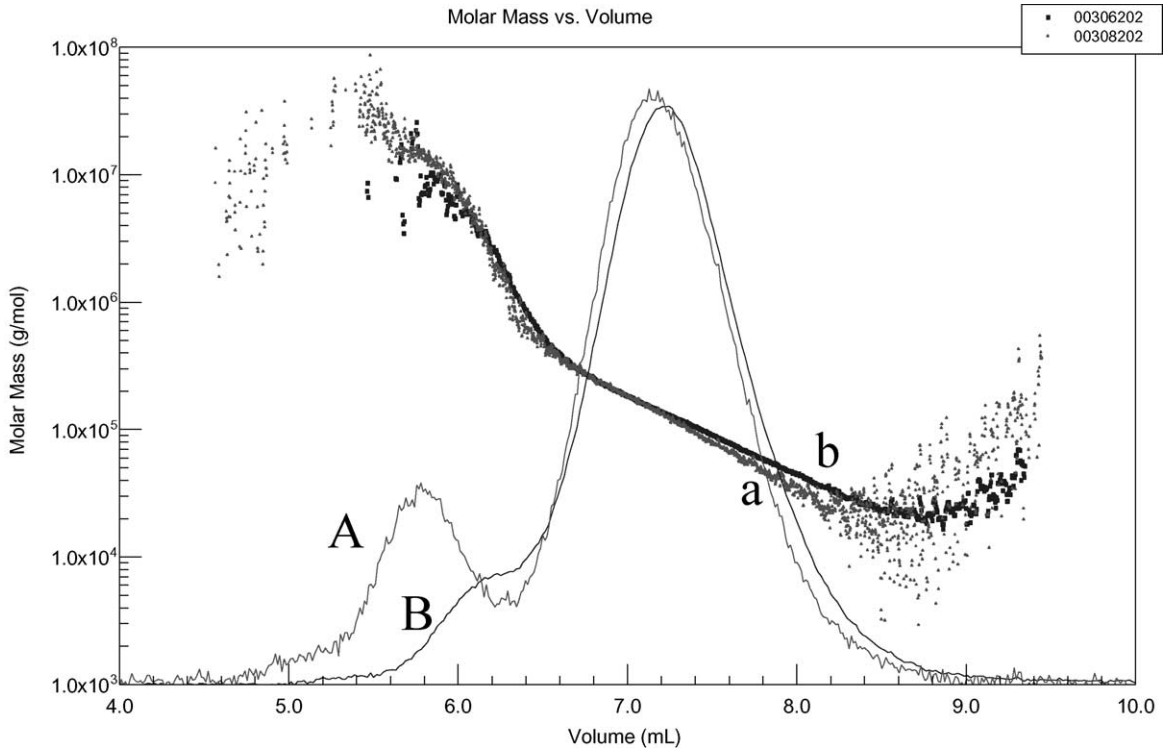

Fig. 5. SEC-MALLS: MALLS elution profiles of HPC L at different flow rates: (A) 0.11 ml/min and (B) 0.45 ml/min. Obtained molar mass data is superimposed: (a) 0.11 ml/min and (b) 0.45 ml/min.

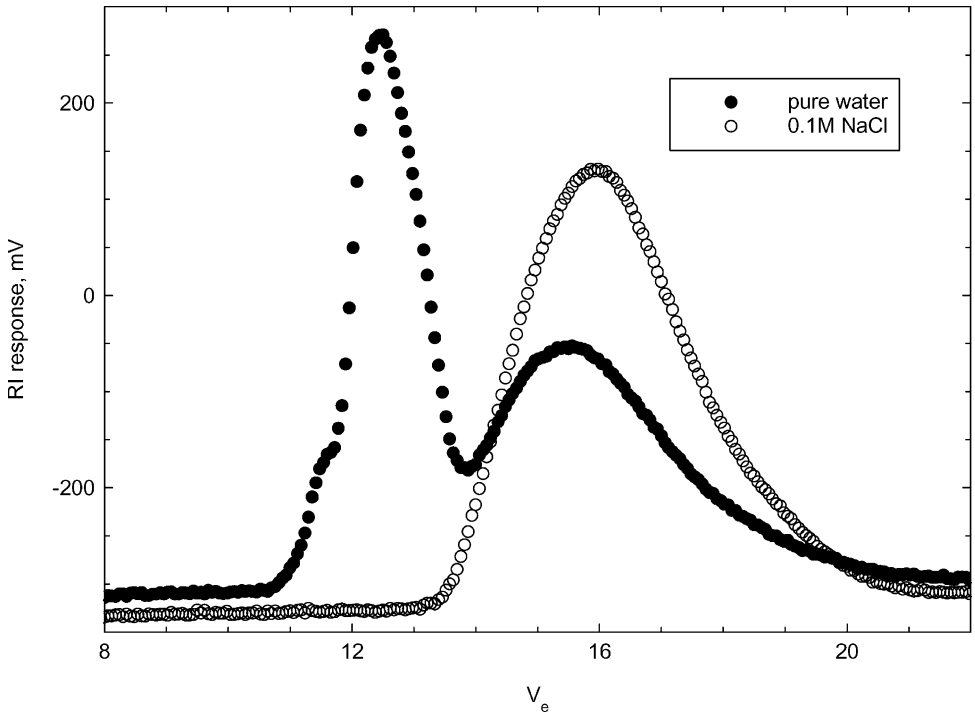

Fig. 6. SEC-LALLS: Ion-exclusion behaviour of HPC LF (Labio column set) in pure water and its suppression in 0.1 M NaCl.

0.02 μm, inorganic Anopore membrane) filter disc (Whatman, Maidstone, England) in an home-made PEEK holder served as in-line mobile phase filter between pump and injector

3. Results and discussion

A comparison of refractometric traces for all three samples studied is presented in Fig. 1. This picture would be obtained if a conventional SEC with single RI detection is used. As RI signals almost coincide, the relevant conclusion would be that all three samples are more or less identical in terms of hydrodynamic size. However, the obvious difference in light scattering response for the samples as seen in Fig. 2, indicates that they are not at all identical in terms of molar mass. It is seen that polymer coils early eluted at the same elution volume (i.e. the same hydrodynamic size) have in the case of HPC L and LM significantly higher molar mass. An indication of increased density of high molecular components of these samples as compared to HPC LF sample is thus obtained. The absence of aggregate peaks in RI records corresponding to L and LM samples means that their mass amount is too low to be seen by mass reflecting RI detection. Their increased density becomes visible only by LS detection as a LS signal is proportional to a product cM and increased molar mass results from higher density at a given elution volume. As RI detection does not see a difference between aggregating (LM, L) and non-aggregating (LF) polymer, other detection techniques reflecting polymer mass, like viscometry, Philpot–Svensson optics in the ultracentrifuge, will not see any difference as well and only LS detection remains to be used.

A fairly low flow rate used in these experiments was found necessary to obtain an optimum resolution of high molar mass sample components as follows from Fig. 3 in the case of TSK column and LALLS detection. Even worse effect of high flow rate was obtained, when Labio column set was used (Fig. 4). It may be argued that LALLS detection is notoriously sensitive to impurities (dust) in the samples and these observations may be an artefact due to LALLS detection. Fig. 5 proves that this is not the case; a quite similar behaviour is seen at 90°, when MALLS detection is used. Again, this flow rate effect becomes hardly visible using only RI detection as a pronounced change take place at very low mass concentrations. An explanation of these observations is obviously related to non-equilibrium in the stationary phase.

The related non-equilibrium term C^S of Van Deemter equation may be written (Giddings, 1982) as

$$C^S = R(1 - R)d_p^2/30\gamma_s D \quad (2)$$

where $\gamma_s D$ is the effective diffusion coefficient, D being the bulk-solute diffusion coefficient and γ_s is an obstructive factor for the pore network of the support particles. R is the retention ratio, V_m/V_r, where V_m is the interstitial mobile phase volume, V_r is the retention volume, and d_p is the diameter of particles packed in the column. It follows from Fig. 5 that the sample contains macromolecules in molar mass range from 2×10^4 up to 5×10^7. Their bulk

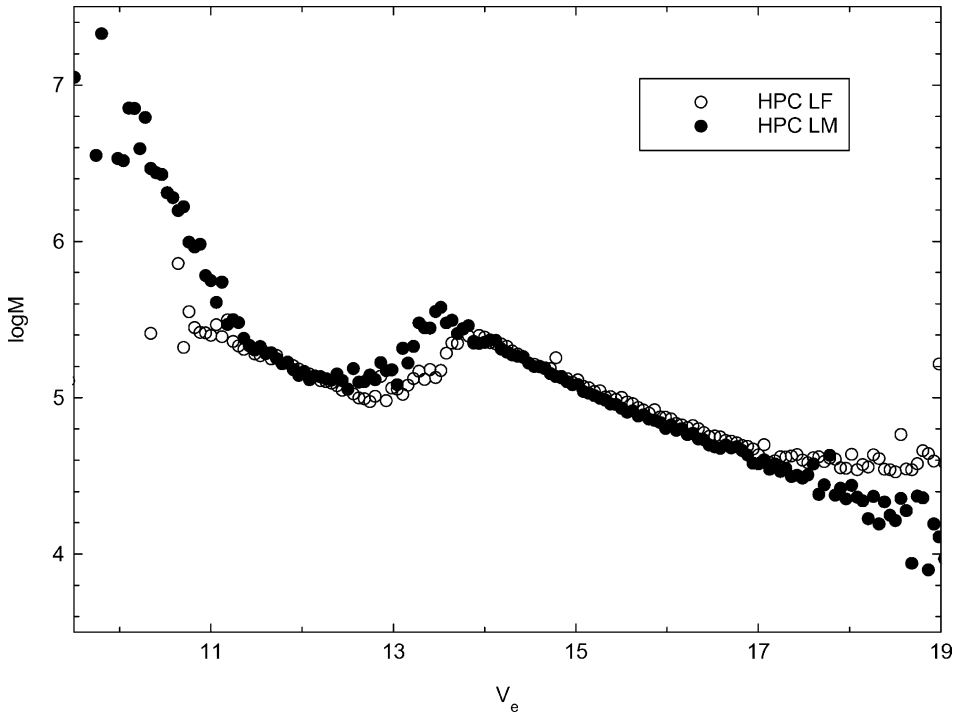

Fig. 7. SEC-LALLS: log M versus V_e curves of HPC LM and LF in ion-exclusion mode (Labio column set) in pure water.

diffusion coefficient D may be estimated to decrease by a factor of 110, if a scaling in a good solvent (Vollmert, 1962) $D \sim M$ is approximated by $D \sim M^{-0.6}$. This means that the of non-equilibrium effect in the stationary phase should increase by two orders of magnitude for the largest macromolecules present in the sample if other variables remain unchanged in Eq. (2). The better resolution obtained in the high flow rate for the 60 cm long TSK GMPW column compared to the 30 cm long $GMPW_{XL}$ (Figs. 3 and 5) is simply due to their length difference. The resolution at 0.11 ml/min seems, however, to be approximately the same. A pronounced loss of resolution at 0.45 ml/min is seen in Fig. 4, when compared to Figs. 3 and 5. As the total length of the Labio column set is 75 cm and particle size 10 μm, it follows from Eq. (2) that the obstruction factor should be larger in this case; most probably poly(-glycidyl methacrylate) particles have a different pore shape (bottleneck) that increases C^S considerably in comparison to TSK columns. This drawback seems to be at least partially compensated; the columns are inexpensive and can survive almost any solvent, being thus suitable as a column of the first choice in the case of samples of unknown purity and/or when proper SEC conditions have to be developed. It is well known that the non-equilibrium in the stationary phase leads to a severe tailing. In our case, tailing should increase when molar mass increases. Large macromolecules should be thus expected to be partially moved to higher elution volumes and increase mainly LS signal. This is visible in Fig. 5; the largest components disappear at the lowest elution volumes in the $\log M = f(V_e)$ curve and a slope of its linear part decreases at 0.45 ml/min. An apparent narrowing of MMD and a decrease of M_w/M_n and M_z/M_w values is the result. An optimised low flow rate, like 0.11 ml/min in our case, seems thus to be necessary in the case of very broad samples having MMD with a long high molar mass tail to avoid bias of results.

The salt content in the mobile phase was checked as a second possible parameter affecting HPC sample behaviour. A correct SEC performance giving MMD averages and distributions identical within the experimental error was

Table 1
Results of SEC-LALLS analyses of HPC on TSK and Labio columns

HPC sample	Columns							
	TSK				Labio			
	M_w	M_n	M_w/M_n	M_z/M_w	M_w	M_n	M_w/M_n	M_z/M_w
LF	111,600	41,800	2.7	3	106,900	46,100	2.3	3
LM	116,800	42,300	2.8	15	116,800	38,100	3.1	12
L	95,300	30,500	3.1	14	99,900	31,300	3.2	17

Table 2
Comparison of SEC-MALLS and SEC-LALLS analyses of HPC samples. Flow rate 0.11 ml/min

HPC sample	Technique								
	SEC-MALLS					SEC-LALLS			
	M_w	M_n	M_w/M_n	M_z/M_w	R_{gz} (nm)	M_w	M_n	M_w/M_a	M_z/M_w
LF	125,000	44,600	2.8	5	31	109,200	43,900	2.5	3
LM	116,000	43,000	2.7	14	40	116,800	40,200	2.9	14
L	97,200	38,900	2.5	17	41	97,600	30,900	3.2	16

obtained, when using 0.1 and 0.01 M NaCl, contrary to the results obtained in pure water (Fig. 6). A large ion-excluded peak around $V_e \sim 12$ ml in Fig. 6 indicates that a substantial part of the sample carries a minute charge. Fig. 7 shows that only ion-exclusion (no aggregation) takes place in pure water; charged coils of the same M are only shifted along elution volume axis. Moreover, all aggregates contained in HPC LM sample seem to be charged as they are almost completely contained in ion-exclusion part of Fig. 7. This behaviour in pure water was already observed in the case of several water-soluble polysaccharides like dextrans, pullulans, hydroxyethyl starch and ethyl(hydroxyethyl) cellulose (Porsch & Sundelöf, 1994; Porsch et al., 1997); most probably mono- and dicarboxy derivatives are again seen here resulting from oxidation of polysaccharide end groups during isolation and/or modification procedures (Porsch & Sundelöf, 1994).

To be sure that ion-exclusion effect is fully absent, 0.1 M NaCl was selected as an optimum mobile phase and all final experiments were run at 0.11 ml/min. A comparison of SEC results obtained with LALLS detection on Labio and TSK columns is presented in Table 1. An agreement within ±5% is obtained for M_w and a difference of about 10% is found for M_n in two cases. A substantial increase of the ratio M_z/M_w adequately reflects a low mass amount of aggregates in NISSO samples clearly visible by light scattering detection.

The average values obtained for both columns were used for a comparison with results of SEC-MALLS technique. This inter-laboratory comparison of SEC-LALLS and SEC-MALLS is summarised in Table 2. The agreement of results in Table 2 is surprisingly good (at least for the authors). Paradoxically, the largest difference in M_w values (13%) is seen in the case of HPC LF sample that should be the easiest one to analyse, as it does not contain any detectable amount of aggregates. The opposite result was expected; it is well known that precision of LS detection becomes poor with

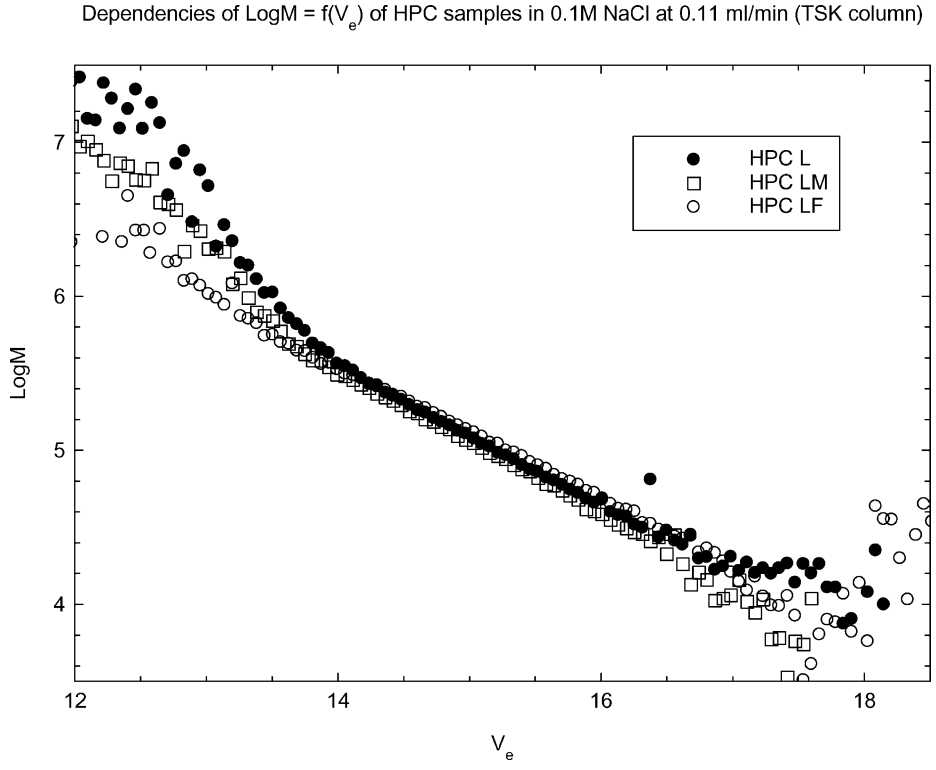

Fig. 8. SEC-LALLS: Dependencies of $\log M = f(V_e)$ of HPC samples in 0.1 M NaCl at 0.11 ml/min (TSK column).

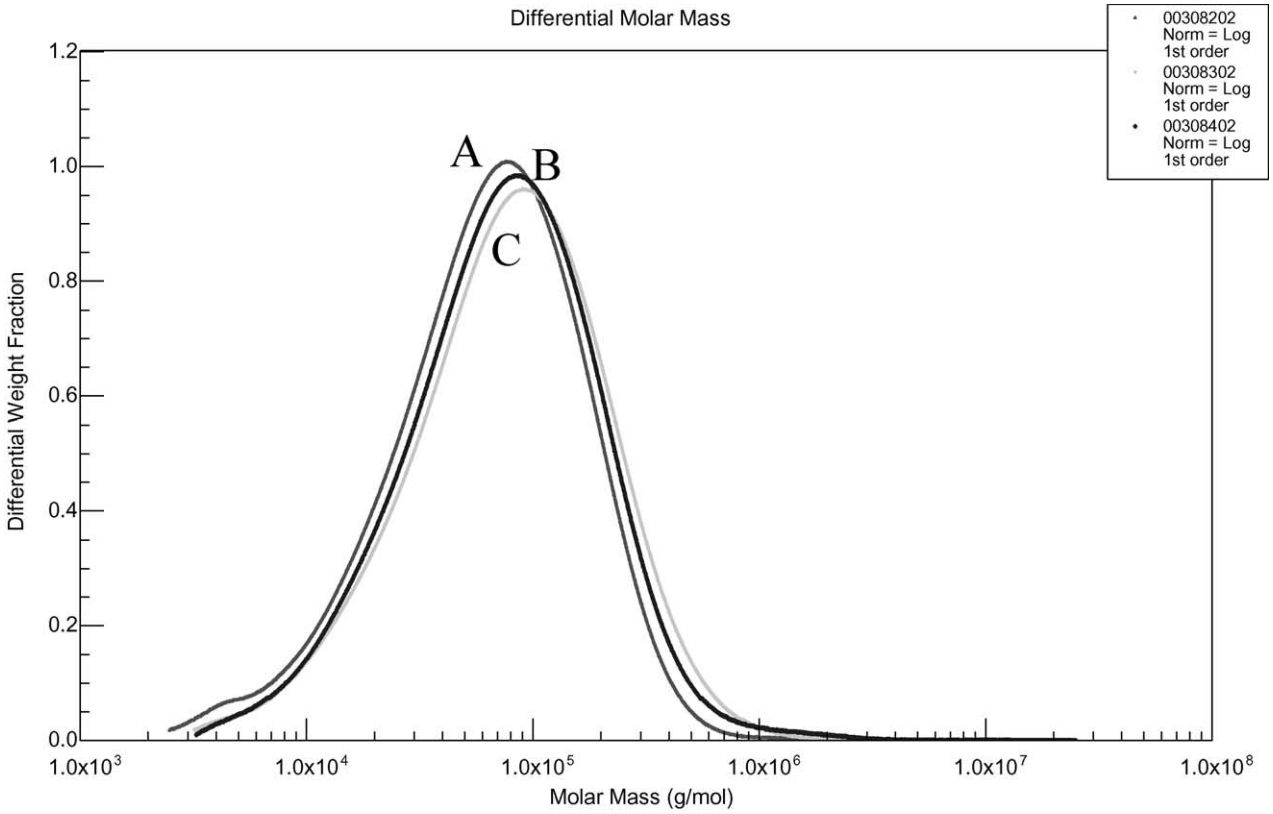

Fig. 9. SEC-MALLS: MMDs for HPC L (A), LM (B) and LF (C) obtained at 0.11 ml/min.

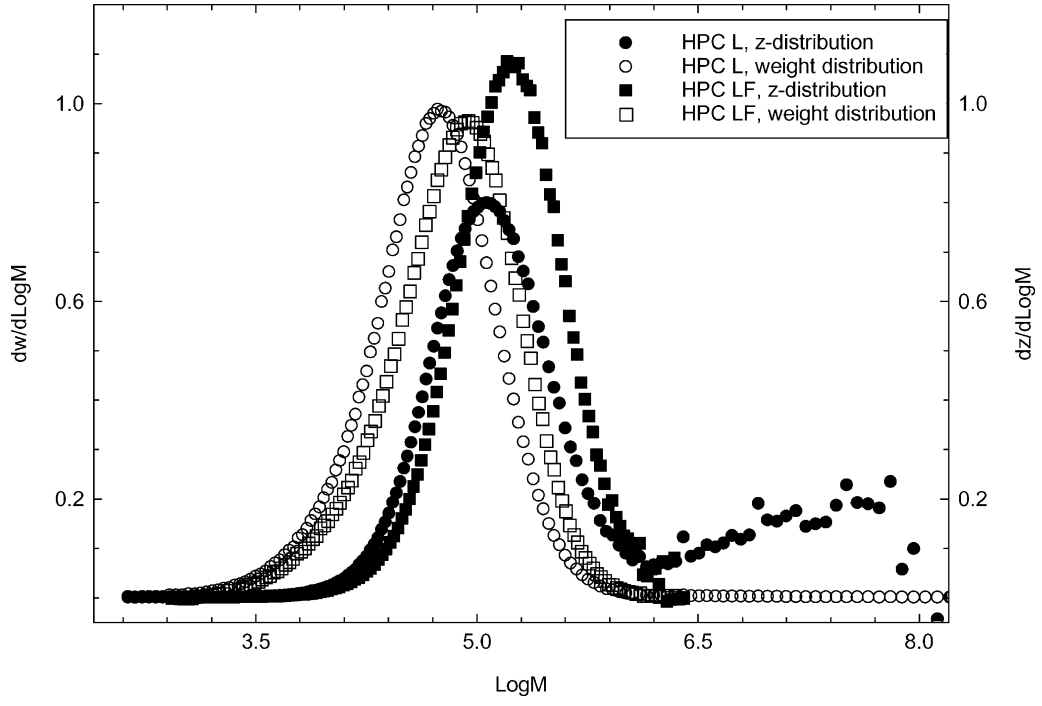

Fig. 10. SEC-LALLS: Weight defined and intensity (*z*-) defined MMD of HPC LF and L.

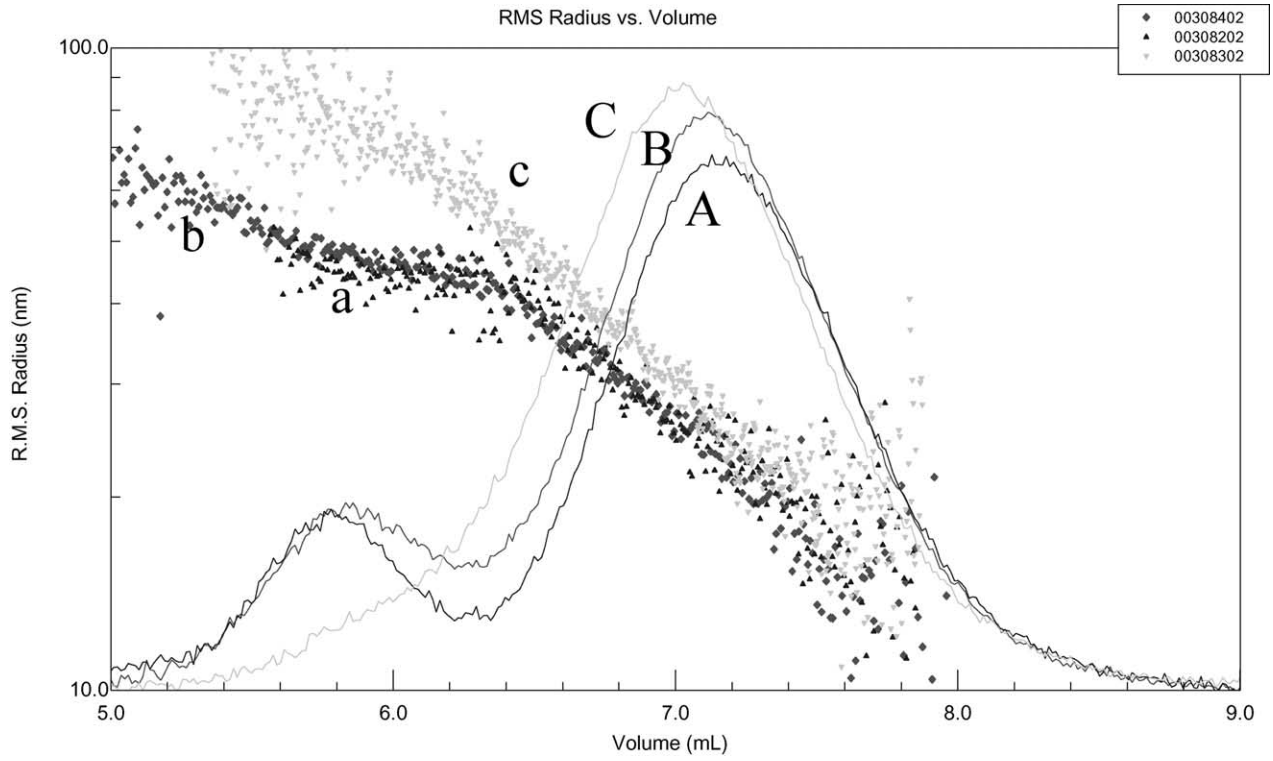

Fig. 11. SEC-MALLS: MALLS elution profiles for HPC L (A), LM (B) and LF (C) at a flow rate of 0.11 ml/min. The radii of gyration versus V_e for HPC L (a), LM (b) and LF (c) are superimposed.

low M part of the distribution for broad samples, where the LS signal approaches baseline level. The same should apply for RI signal that corresponds to high M part of the distribution in the case of HPC L and LM samples. Detailed analysis of experimental errors of combined LS–RI detections was published elsewhere (Reed, 1995). From the point of view of recent round robin tests of SEC (Barth, Boyes, & Jackson, 1998) performed with polystyrenes and poly(methyl methacrylates) in tetrahydrofurane, where differences of M_w 7–10% and of M_n 16–18% were found, the agreement found here thus seems quite satisfactory. The more so as SEC in aqueous mobile phase is well known to be more difficult to perform properly, when compared to organic solvents (Christensen et al., 2001). Moreover, at least HPC L and LM polymers probably represent extremely difficult samples owing to the presence of aggregates.

The observed agreement of results using LALLS and MALLS detection thus indicates that a frequent objection against LALLS detection, i.e. the overestimation of molar mass due to the use of low detection angle, does not apply here. At the same time, it can be concluded that the sometimes-questioned angular extrapolation necessary in MALLS technique is reliable even in the case of aggregate containing polymers.

$\log M = f(V_e)$ curves of all three samples studied are presented in Fig. 7. Deviations of $\log M \sim V_e$ dependencies from a common line appear only in high M part, that one corresponding to HPC LF sample being almost linear (Fig. 8).

The column used is manufactured to provide linear calibration $\log M \sim V_e$ for a polymer obeying power law in terms of size against M^a, hence, the content of more dense structures in HPC LF sample appears to be almost negligible. On the other hand, a significant presence of more dense particles is found in HPC L and LM samples. Larger M at the same elution volume observed for HPC L, when compared to HPC LM means the presence of even denser such aggregates.

MMDs of all three polymers studied are presented in Fig. 9. Only minor differences are seen among samples, as the content of aggregates on a mass scale is very low. Displayed MMDs $x(M)$ are defined as a weight fraction of molecules having molar mass in an interval M, $M + dM$. A z-defined MMD $z(M)$ gives a z-fraction (weight fraction $\times M$) in the same interval. This distribution, called intensity distribution due to scaling of scattered intensity with cM, is well known to be obtained from dynamic light scattering experiments (Chu, 1974), describing the distribution of diffusion coefficients in polydisperse systems. When weight defined MMDs of HPC LF and L samples are transformed to the z-scale (Fig. 10), a second maximum appears (cM may increase even when c decreases) and the difference in the presence of aggregates becomes quite pronounced. Let us note that intensity (z-) defined bimodal particle size distributions,

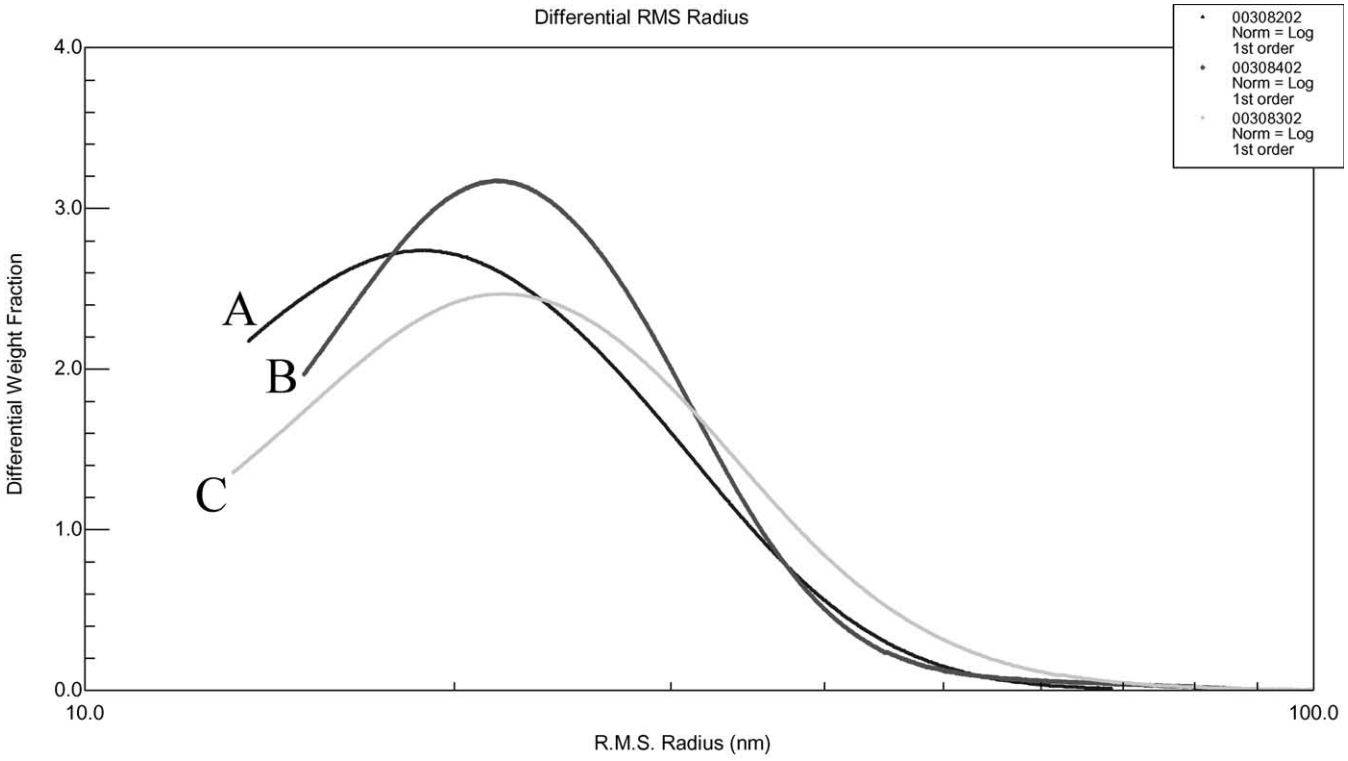

Fig. 12. SEC-MALLS: The distribution of radii of gyration obtained for HPC L (A), LM (B) and LF (C). Flow rate 0.11 ml/min.

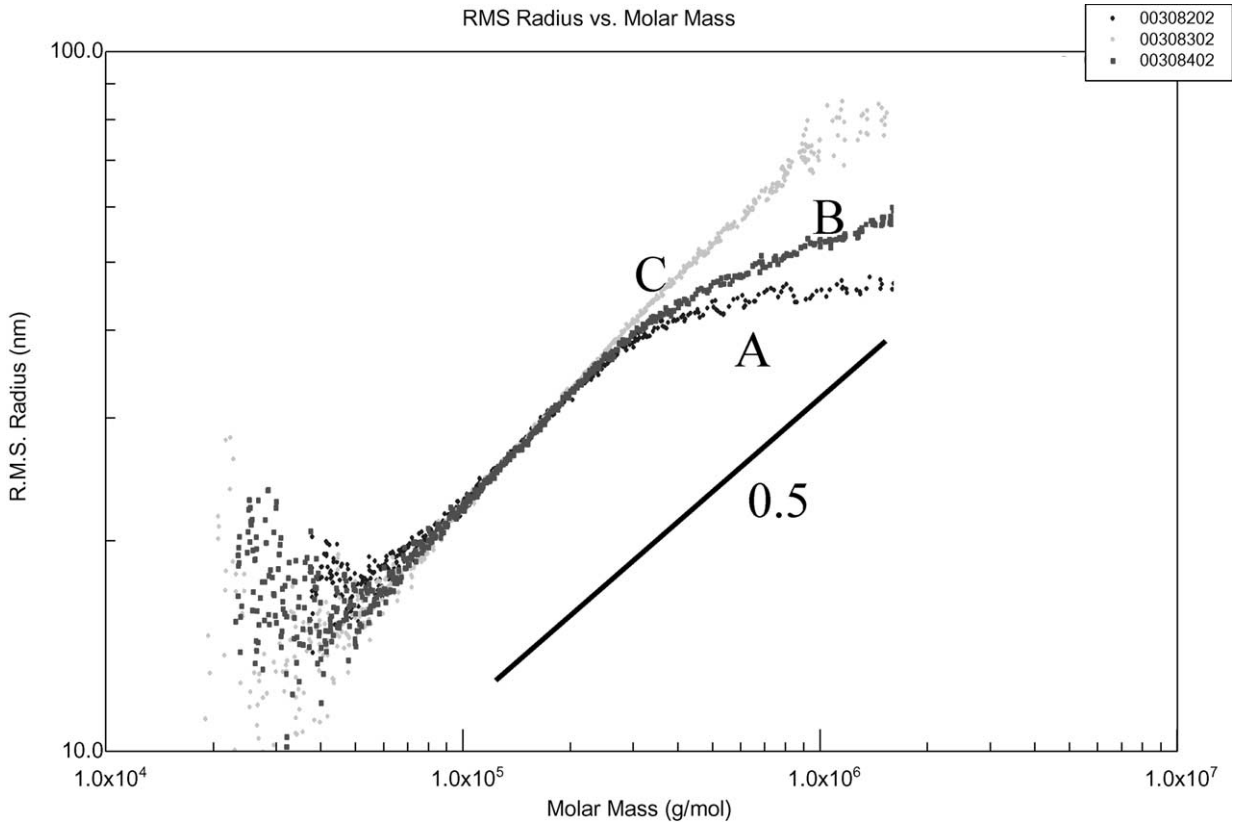

Fig. 13. SEC-MALLS: Conformation plots for HPC L (A), LM (B) and LF (C). A line with the slope of 0.5, corresponding to a random coil conformation, is included.

almost always obtained in dynamic light scattering of water-soluble polymers, look very similar to z-defined MMDs in Fig. 10.

The radius of gyration is directly determined from the angular dependence of the scattered light and is thus only accessible from multi-angle measurements. As the intercepts of the angular extrapolations give M_w in a good agreement with LALLS experiments (which give the intercepts directly), it is believed that linear Berry fits of the angular dependencies are reliable as well. In Fig. 11, the radii of gyration versus elution volume are plotted for the three HPC samples. For the HPC LF sample, the slope of the obtained radii is almost constant within the limits of the peak, whereas for L and LM, there is an abrupt shift in the slope of the radii at $V_e = 6$ ml. Thus, the lower slope is obtained for the first part of the bimodal peak, whereas the steeper slope is obtained for the main peak and coincides with the radii data from the LF sample. These results may seem contradictory to the observation of dramatically increasing slope of the molar mass versus elution volume discussed previously. However, an increasing tendency for molar mass at the same time as a decreasing for the radius of gyration further confirms the compact shape of the components present in the high molar mass part of the L and LM distributions.

The radii distributions for the three samples are depicted in Fig. 12. The LF sample has the broadest distribution and the obtained R_{gz} value (Table 2) is consequently somewhat higher, 41 nm, than for the other samples. Again, the lower R_{gz} values in relation to the molar mass observed for especially for L in comparison to LF suggest conformational differences between the samples.

The conformation plot of R_g against M presented in Fig. 13 illustrates directly different structure (density) of aggregates in the L and LM samples and very low if none presence of such structures in the LF sample. The deviations from a common line appear earlier 300,000 g/mol and R_g increases much less for HPC L and LM samples with increasing molar mass than for HPC LF sample. This one may be said to be composed from a series of homopolymers as a correct scaling between R_g and M is obtained. The slope is 0.52, in agreement with what is expected for a random coil polymer. A maximum deviation from this slope is found for the L sample indicating an even more pronounced aggregation at the same molar mass compared to LM. More detailed discussion of deviations among the samples in terms of slope changes at molar mass above a million does not seem relevant here as large errors in M can be expected owing to very low concentration signal in the related part of RI trace.

These results appear to be fully compatible with Burchard's results (Burchard & Schulz, 1989; Schulz & Burchard, 1993) obtained for cellulose-2,5-acetate, cellulose tricarbanilate and some water-soluble cellulose derivatives. A picture of aggregates observed termed fringed micelle, i.e. micro-crystalline non-modified cellulose core (insoluble, highly aggregated, possibly having some memory of the partly crystalline cellulose fibre structure) with a hairy region of derivatised chains of different length seems to apply here as well. The onset of aggregate density variations takes place at a similar molar mass in our case and a small increase of R_g is seen without a loss of separation. A good separation of aggregates found for our samples means that a dimension $R_\eta = ([\eta]M)^{1/3}$ decisive for SEC separation (Potschka, 1987) must sufficiently increase. A decrease of a ratio R_g/R_η can be thus expected for HPC aggregates similarly to a decrease of Burchard's ρ-parameter (Schulz & Burchard, 1989), $\rho = R_g/R_h$, having on mind that, in general, $R_\eta \neq R_h$, but both these dimensions are hydrodynamic ones.

Recently, it was suggested (Thuresson & Lindman, 1999) that, in the case of ethyl(hydroxyethyl) cellulose, aggregates might form in a solution (instead of being rests of unsubstituted fibre structure) and remain long-lived due to formation of micro-crystallites formed by an assembly of unsubstituted domains on polymer chains. This concept is difficult to apply here, among others, as our samples are more or less highly substituted by just one substituent.

A great similarity between our HPC solutions and other polysaccharide solutions (Burchard & Vogel, 2000) supports the conclusion that fringed micelles might be a general feature of water-soluble derivatives. This is not so surprising if we take into the account that the first step in almost all cellulose modifications is a formation of alkali cellulose. A various content of aggregates found in our samples thus can be traced to originate in different conditions in alkali hydroxide dissolution procedure of a native cellulose starting material. Various native cellulose materials, being formed from amorphous, as well as micro-crystalline parts, greatly differ in structure depending on their origin (Fink, Hofmann, & Purz, 1990). The use of another native cellulose (easier to homogenise by alkali) to produce HPC LF polymer might be a convenient explanation of the absence of aggregates in this polymer.

Acknowledgements

B.P. wishes to thank AstraZeneca R&D Mölndal for financial support. Mrs Eva-Lotta Ekberg and Jiřina Nováková are gratefully acknowledged for experimental assistance.

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