Journal of Chromatography A, 1217 (2010) 722–730

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Optimisation of ambient and high temperature asymmetric flow field-flow fractionation with dual/multi-angle light scattering and infrared/refractive index detection

T. Otte [a], R. Brüll [a,*], T. Macko [a], H. Pasch [a,1], T. Klein [b]

[a] German Institute for Polymers, Schlossgartenstraße 6, 64289 Darmstadt, Germany
[b] Postnova Analytics GmbH, Max-Planck-Straße 14, 86899 Landsberg/Lech, Germany

ARTICLE INFO

Article history:
Received 7 May 2009
Received in revised form
16 November 2009
Accepted 2 December 2009
Available online 6 December 2009

Keywords:
Flow field-flow fractionation
AF4
Size exclusion chromatography
Shear degradation
Polyolefins

ABSTRACT

Asymmetric flow field-flow fractionation (AF4) enables to analyse polymers with very high molar masses under mild conditions in comparison to size exclusion chromatography (SEC). Conventionally, membranes for AF4 are made from cellulose. Recently, a novel ceramic membrane has been developed which can withstand high temperatures above 130 °C and chlorinated organic solvents, thus making it possible to characterise semicrystalline polyolefins by HT-AF4. Two ceramic membranes and one cellulose membrane were compared with regard to their quality of molar mass separation and the loss of the polymer material through the pores. Separating polystyrene standards as model compounds at different cross-flow gradients the complex relationship between cross-flow velocity, separation efficiency, the molar mass and peak broadening could be elucidated in detail. Moreover, the dependence of signal quality and reproducibility on sample concentration and mass loading was investigated because the evaluation of the obtained fractograms substantially depends on the signal intensities. Finally, the performance of the whole system was tested at high temperature by separating PE reference materials of high molar mass.

1. Introduction

Ultra-high molar mass polyethylene (UHMPE) is used in various applications where mechanical strength, chemical resistance and low weight are required. The presence of UHM-material influences the melt viscosity which is a fundamental parameter to characterise the processability of the polymer [1–3]. For these reasons a precise knowledge about the high molar mass fraction of those polymers is essential. In the case of PE the most common method to determine the molar mass distribution (MMD) is high temperature size exclusion chromatography (HT-SEC). But for UHMPE this technique is not suitable because shear degradation of the UHMW-fraction and an abnormal late elution of large and branched material lead to errors in the SEC results [4–14].

AF4 is an alternative for the separation of ultra-high molar mass macromolecules [15–20]. In this approach the particles or macromolecules are separated according to their hydrodynamic volume. Due to the absence of a stationary phase the shear degradation is significantly reduced in the channel. Fig. 1 shows the separation-principle.

A parabolic flow profile is formed in a narrow channel. The separation is provided by a cross-flow field perpendicular to the injection flow. During the separation process all particles are pushed against the accumulation wall by the cross-flow. The ability of the molecules to diffuse acts against this force and, therefore, the species will diffuse back into the channel. Due to their larger diffusion coefficients the smaller particles will move faster away from the accumulation membrane than the larger ones and as a result the macromolecules will be situated in different flow layers. The elution time depends on the flow velocity of the respective layer in which the polymer molecules are situated [17]. Larger structures are arranged closer to the accumulation wall and as a result they will elute later than the smaller ones. Thus the elution order is reversed to that known from SEC. When AF4 is coupled to a multi-angle light scattering detector in combination with a concentration sensitive detector like RI, UV or IR, it is possible to obtain the molar mass value of an eluted component, the molar mass distribution of the polymer and conformational information.

AF4 coupled to a light scattering detector is well known in literature for ambient temperature application with aqueous and organic solvents [21–27]. The first high-temperature used for the separation of polystyrene (PS) in xylene at 140 °C was published by Miller and Giddings [28] and the first polyolefin separation by HT-AF4 was presented by Mes et al. [29]. Different LDPE, LLDPE and HDPE samples were analysed by HT-AF4, for the latter a newly developed AF4-system with a special ceramic–composite membrane was used

* Corresponding author.
E-mail address: rbruell@dki.tu-darmstadt.de (R. Brüll).
[1] Present address: University of Stellenbosch, Department of Chemistry and Polymer Science, Private Bag X1, Matieland 7602, South Africa.

0021-9673/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.chroma.2009.12.014

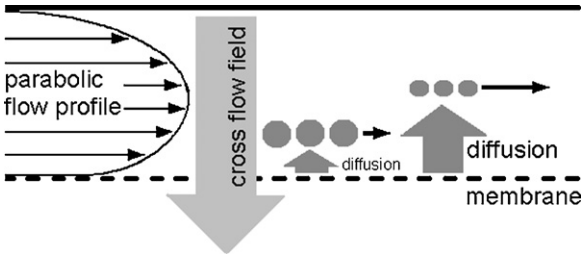

Fig. 1. Cross-section of the AF4-channel, scheme of size separation.

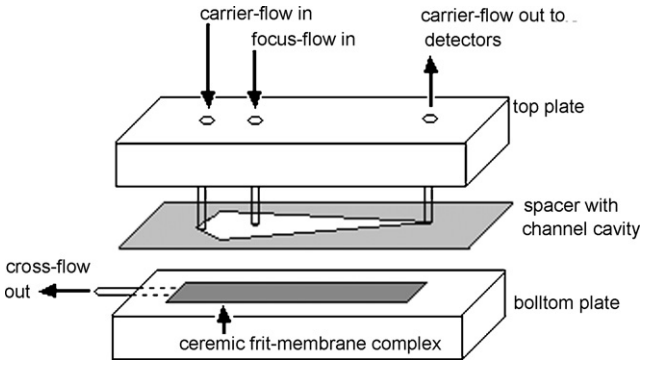

Fig. 2. Scheme of a (HT)-AF4-channel.

which was able to resist the applied temperatures of 145 °C and chlorinated solvents like 1,2,4-trichlorobenzene (TCB). The quality of the membrane is of crucial importance for the separation and it determines the recovery of polymer and the molar mass cut-off.

However, until now little is known about the influence of the membrane, the cross-flow and the sample concentration or injection volume on the separation efficiency, sample recovery and peak broadening.

To ensure successful AF4-measurements it is essential to have information about the separation capability of the membranes used. Therefore, in this work we compare the new high temperature-resistant ceramic composite membrane with a conventional cellulose membrane. Particular attention is given to the loss of material and the separation efficiencies in different channels as well as the quality of the measurements.

2. Experimental

2.1. Instrumentation

The AF4 experiments were carried out using an AF2000 instrument from Postnova Analytics (Landsberg/Lech, Germany). The HT-resistant AF4-channel was installed in a PL GPC-220-chromatograph (Polymer Laboratories, Church Stretton, England). The AF2000-system consists of a channel connected to three pumps and a controller module. The channel setup is shown in Fig. 2.

The thickness of the Mylar®-spacer was 350 μm. The plate material was stainless steel. One cellulose membrane (implemented in channel AT) and two ceramic membranes (HT1 and HT2) were used all supplied by Postnova Analytics (Landsberg/Lech Germany). The membranes were declared by Postnova Analytics to have an average cut-off value of 0.3 kg/mol (cellulose) and 50 kg/mol (ceramic), respectively for PS in THF. The ceramic membranes are manufactured from a commercially available raw material which is often heterogeneous. For this reason the membranes were tested by Postnova Analytics before shipping. The flows were provided by two separate pumps (total input pump and focus-pump). The cross-flow was realized by a separate piston pump which is continuously adjustable. A special pump-management system was used to hold a constant detector flow. The output from the channel was connected to a light scattering detector as first device (for ambient temperature measurement: DALLS, type PD2040, Precision Detectors, Bellingham, USA; for high temperature measurement: MALLS, type Heleos 2, Wyatt Technology, Santa Babara, USA). As second component an infrared detector (IR4, Polymerchar, Valencia, Spain) was installed. The last unit was a refractive index detector (RI, Polymer Laboratories, Church Stretton, England). The DALLS was equipped with a 30 mW laser operating at $\lambda = 682$ nm and the scattered light measured at angles of 15° and 90°. The MALLS contained a 120 mW laser operating at $\lambda = 658$ nm. The scattered light was detected at 17 different angles. The HT-AF4-channel was installed in the PL GPC-220-instrument in such a way that it is possible to switch between GPC and F4-mode using three Valco HT-six-port valves (Valco Instruments, Waterbury, USA).

The injection volume was 200 μL. The calibration of the DALLS and the concentration detectors as well as the normalisation of the DALLS and MALLS was done by injecting narrow PS standards with a molar mass of 127 kg/mol at the peak maximum and a polydispersity (PD) of 1.05 (Merck, Darmstadt). The MALLS detector was calibrated with toluene. The inter-detector-delay was corrected by shifting the peak maxima of the PS standard towards the same elution volume for all signals. A specific refractive index increment (dn/dc) for PS in THF at 25 °C of 0.184 mL/g [30] was used. For the PE-measurements in 1,2,4-trichlorobenzene (TCB) at 145 °C a dn/dc of −0.104 mL/g [29] was used. The flow rate at the channel outlet in the SEC-mode and in all detectors was 0.5 mL/min.

2.2. Materials and methods

The PS samples were dissolved and analysed in distilled tetrahydrofuran (THF) containing anti-oxidant (BHT). PS standards (Table 1) with polydispersities (PD) between 1.01 and 1.35 (Merck, Darmstadt, Germany, Polymer Standards Service, Mainz, Germany and Polymer Laboratories, Church Stretton, UK) were used for the AF4 experiments. The sample concentrations were between 1 and

Table 1
Molar mass values of PS standards obtained from AF4-measurements.

$\langle M_w \rangle$ [kg/mol](Producer)	$\langle M_w \rangle$ [kg/mol](AF4)	M_p [kg/mol](Producer)	M_p [kg/mol](AF4)	PD(Producer)	PD(AF4)
–	76	68	77	1.05	1.01
–	128	127	129	1.05	1.02
226	219	246	213	1.06	1.03
–	317	310	313	1.05	1.08
644	645	659	651	1.03	1.02
1070	978	1090	907	1.06	1.08
–	1270	1260	1321	1.06	1.11
–	2594	2750	2691	1.06	1.02
4360	4678	4820	4623	1.14	1.11
8090	8310	8910	7930	1.17	1.09
16800	16237	21100	17510	1.33	1.31

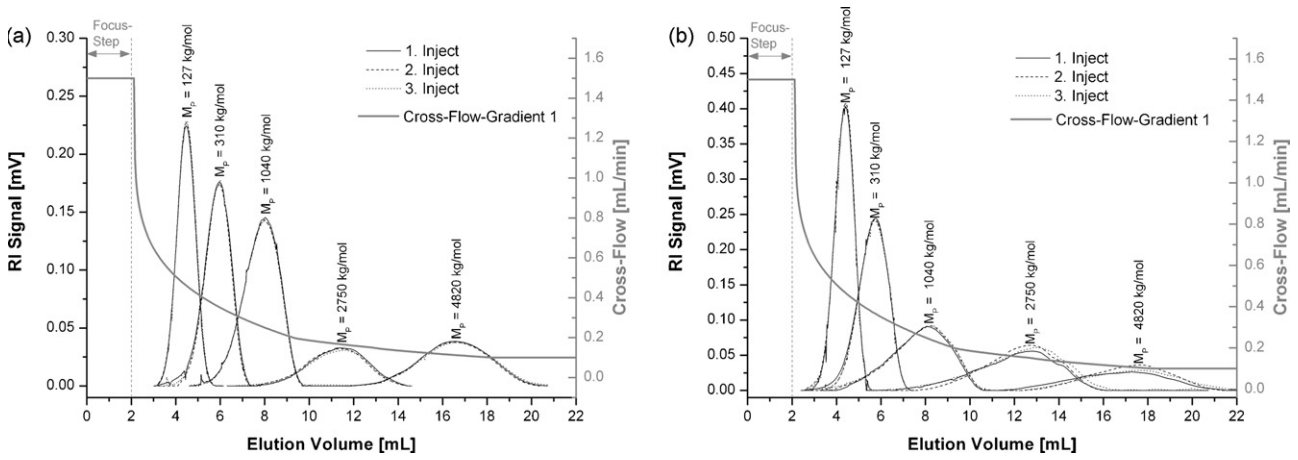

Fig. 3. Overlay of AF4-fractograms of narrow PS standards, the corresponding gradient of the cross-flow is shown. (a) Channel HT1. (b) Channel AT1.

3 mg/mL. The PE-material was dissolved in 1,2,4-trichlorobenzene (TCB) containing 1 mg/mL BHT for 4 h at 160 °C. The measurement was realised at 145 °C in TCB containing 200 ppm BHT.

The sample separation depends on the cross-flow gradient. At the beginning of each measurement a focus stage was applied during the injection procedure. In this procedure the injected molecules are affected by the focus- and cross-flow simultaneously. As a result the zone broadening in the channel is reduced to a minimum [18]. More narrow peaks are the consequence. After the focus-step the regular cross-flow program was applied. For calculation of the molar masses the Berry equation was used [31].

3. Results and discussion

Fig. 3 shows the overlay of fractograms of a blend of PS standards separated in the channel containing a ceramic composite membrane (Fig. 3a) or a cellulose membrane (Fig. 3b).

As illustrated in Fig. 3 the repeatability of the measurements is very good and both types of membrane deliver comparable results with regard to peak broadening and resolution. The width of the peaks increases towards the high molar mass region. This phenomenon is well known for FFF-separation [32–35]. In Fig. 4 the calibration curves for two different ceramic membranes and the cellulose membrane are plotted.

There is no significant difference between the three curves observable. This proves that the separation is independent of the different natures of the analysed membranes. The three M_p/V_E relationships show a decrease of the slope for high molar masses, indicating a higher selectivity for the separation of macromolecules with increased size, which is expected due to the exponential gradient shape with a constant cross-flow value at the end. The lower cross-flow values are affecting especially the high molar mass species due to their lower diffusion coefficients.

For the cellulose membrane the separation of PS standards with much lower molar masses was possible (Fig. 4). A possible explanation for this effect could be that cellulose membranes have a much lower molar mass cut-off than those made from ceramic material. The recovery of different standards is compared for all three channels and as a function of M_p shown in Fig. 5. The material loss in AF4 depends on many parameters such like membrane-quality, cross-flow field duration and field force, interaction abilities of the sample and the carrier flow rate. For this reason the obtained recovery values are only valid for the chosen sample at the specific separation conditions [36–39].

Both ceramic membranes show a much higher molar mass cut-off (12.5 and 34.5 kg/mol) than the cellulose membrane (2.2 kg/mol). The result also demonstrates that the ceramic membranes differ in their quality although both were obtained from the

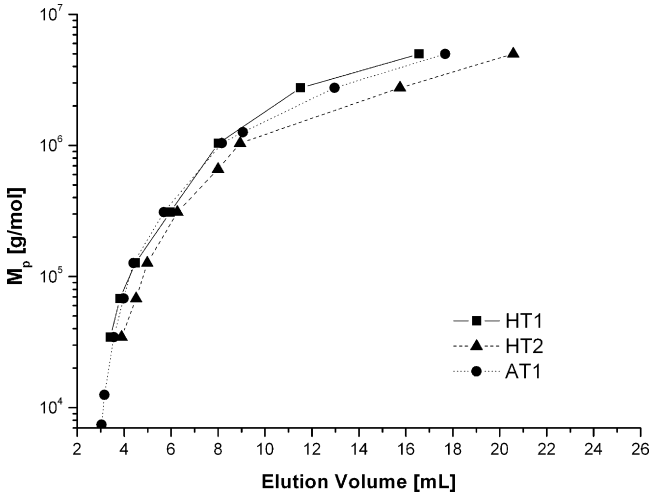

Fig. 4. Calibration curves (PS standards) obtained with channels AT1, HT1 and HT2.

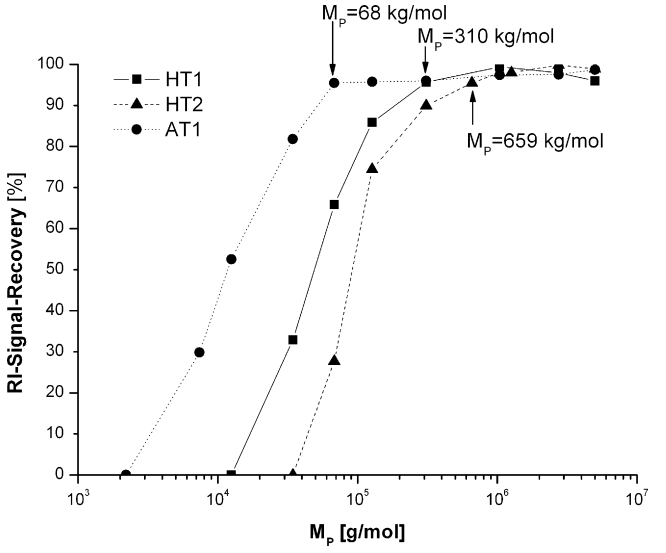

Fig. 5. Recovery of the injected samples (calculated from the RI response) plotted against the molar mass at the peak maximum for the measurements shown in Figs. 3 and 4.

same supplier. The cut-off in AF4 is defined as molar mass value of the first standard which shows no recovery. Also visible is a broad range of molar masses with a recovery lower than 95%. It indicates that there is also a loss of the PS standards with high molar masses. The lowest molar mass of PS which was detected during the AF4 separation with the cellulose membrane was M_p = 7.4 kg/mol. This corresponds to a radius of gyration of R_g = 2.5 nm [9] and requires a minimum pore size of the cellulose membrane in the same range. The specifications given by the supplier are 0.3 kg/mol M_w cut-off and a nominal pore size of 10 nm. For the ceramic membrane the low molar mass range given by the manufacturer was 50 kg/mol for PS. In this case the lowest values obtained were 34.5 kg/mol (channel HT1) and 68 kg/mol (channel HT2). These results indicate a considerable fluctuation in the quality of the ceramic membranes.

As illustrated in Fig. 5, also samples with molar masses above the cut-off range are not fully recovered. The maximum value of the recovery was found to be about 95%. For the channel AT1 this value is constant above a molar mass of M_p = 68 kg/mol. For the ceramic membranes the maximum recoveries were obtained above M_p = 310 kg/mol (HT1) and M_p = 659 kg/mol (HT2). The discussed recovery problems will falsify the calculated molar mass distribution of a polydisperse sample and the corresponding average values. As a result the data obtained from AF4 are only reliable if a constant maximum recovery is reached and all masses are affected to the same extent. As a parameter for characterising the membrane-quality the corresponding molar mass for maximum recovery would be much more suitable. For the used channels this parameter is about M_p = 68 kg/mol for the cellulose membrane and M_p = 310 kg/mol and 659 kg/mol for the ceramic membranes. The large difference between the cut-off and the molar mass for reaching the maximum recovery indicates a broad pore size distribution for all three membranes. Approximately 5% of a polymer sample is not recovered, independent of the molar mass of the sample, for all three channels. In this case the experimentally determined molar mass distributions of polydisperse samples should not be falsified, because all molar masses are affected in the same manner. The detector signal areas decreased due to the reduced recovery of the analysed samples. This may result in a poor signal-to-noise ratio especially for low sample concentrations. Small defects in the sealing of the membrane or some larger pores in the membrane material as well as a broad pore size distribution are probable reasons for the observed phenomena.

The material loss was investigated using on a broadly distributed technical PS to confirm the results obtained from the separation of PS standards. The bulk sample was separated by AF4 and SEC. In case of the AF4 measurement the cross-flow was collected for several times, concentrated and reinjected into the SEC. The obtained molar mass distributions are overlaid in Fig. 6.

The distribution of the polymer material which passed through the membrane (filtrate) contains molar masses up to 10^6 g/mol which were also detected during the SEC separation of the whole sample. Such high masses are clearly above the cut-off value observed for the HT-2 channel. This supports the observation for the molar mass independent material loss which was found for the narrow PS standards in Fig. 5. The peak maximum of the filtrate is shifted to lower molar masses compared to the SEC measurement of the bulk sample. This is the result of the molar mass dependent loss of small molecules through the pores and leads to an accumulation of the low molar mass content in the filtrate. The distribution obtained from the AF4 separation shows no molar masses below 150 kg/mol. This is in the same range as the cut-off value which was obtained with this channel (Fig. 5). The peak maximum of the MMD obtained with AF4 is almost identical to that from SEC separation of the whole sample (approx. 400 kg/mol). The distributions calculated from SEC measurements of the filtrate and the bulk show a comparable range of the maximum molar mass of approximately

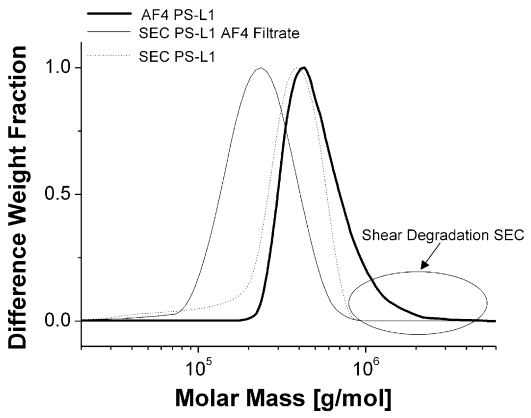

Fig. 6. Overlay of molar mass distributions from SEC and AF4 of a polydisperse PS as well as from SEC of the material collected through the AF4 membrane during separation (filtrate). The results are calculated from light scattering, channel HT2, cross-flow gradient 1.

M_w = 1000 kg/mol. However, the AF4 delivers a maximum molar mass of about 3000 kg/mol for the same sample. These observations strongly indicate that shear degradation occurs when the molecules migrate through the SEC columns.

With the goal to analyse the influence of the cross-flow on the elution behavior three PS standards were separated. After the injection-step different constant cross-flows were applied. The obtained fractograms are shown in Fig. 7.

The fractogram (Fig. 7) shows that a higher cross-flow leads to a longer retention of all PS samples. This effect increases with the molar mass value, i.e., the difference in the retention volumes

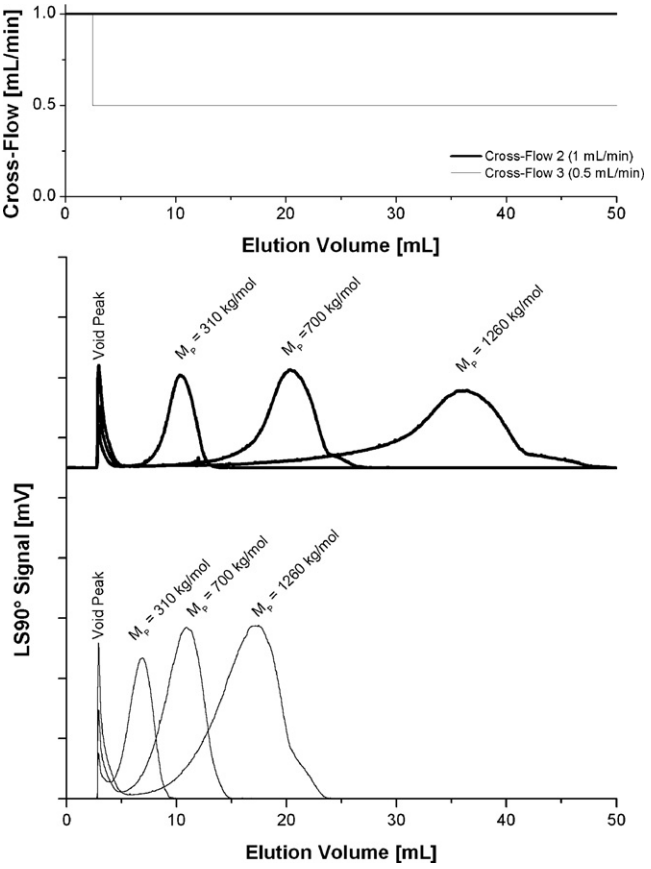

Fig. 7. Influence of a constant cross-flow on the elution behavior of different PS standards, channel HT1.

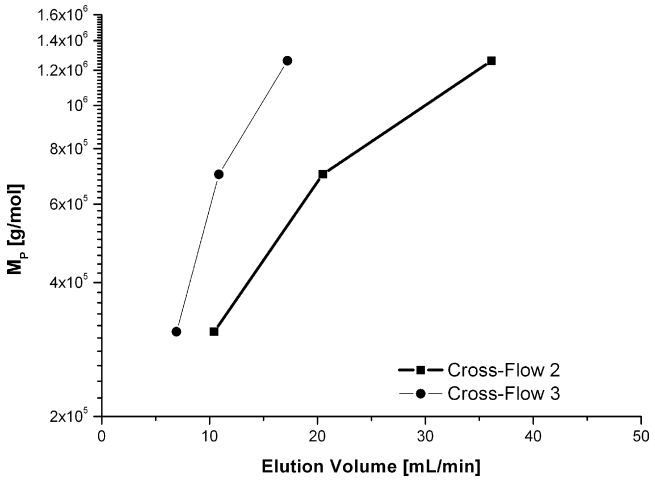

Fig. 8. Calibration curves constructed from the fractograms shown in Fig. 7.

is larger between the PS standards 700 kg/mol and 1290 kg/mol than between the 310 kg/mol and 700 kg/mol (Figs. 7 and 8). Generally the peaks for the cross-flow of 1 mL/min show a strong band broadening.

In Figs. 7 and 8 it is shown that a larger cross-flow increases the retention time. The enhanced separation of high molar masses with a higher cross-flow and the selectivity of AF4 for high molar masses are well known from literature [32–35,40]. The band broadening which occurs for larger cross-flow rates (Fig. 7) can be explained as an effect of statistic diffusion of the macromolecules into any direction of the channel [17]. An explanation for the strong peak broadening especially for high molar masses is given by the transversal diffusion. Molecules of the same size will be situated in different flow layers which results in enhanced peak broadening [41,42]. The influence of concentration and injection volume on the AF4 separation of a PS blend is illustrated in Fig. 9.

Fig. 9 shows that the peak broadening increases with increasing concentration or injection volume. The concentration profiles of the molecules having the same size broaden when the amount of macromolecules in the channel is increased. This is the consequence of the limited capacity in the individual flow layers. The wider concentration profiles finally will result in an overloading effect which manifests as broadening of the detected peaks [43]. An increased amount of polymer inside the channel also leads to a shift of the peaks towards higher elution volume (Fig. 9). This effect is more pronounced for the high molar mass species. A possible explanation can be that the overlap concentration was partly exceeded or the large species have not been completely dissolved. Both would result in larger structures which contain more than one macromolecule. Such big structures will be stronger retained by the cross-flow and as a result the average retention volume of the peaks increases. For a decrease of the peak broadening and the peak shift at high concentrations or injection volumes the cross-flow gradient or the channel setup has to be optimised.

Taking the mechanism into account the effect of band broadening will be most pronounced for monodisperse samples. The elution of polydisperse samples, where the number of macromolecules with identical molar mass is significantly smaller, will be much less affected.

In Fig. 9(a) spikes are visible in the LS-signals whereas the corresponding RI-signals in Fig. 9(b) do not show any abnormality. The intensity of the spikes depends on the injected sample volume and concentration. No similar effect has been reported for AF4 until now. The smooth RI-signal indicates that the spikes in the LS-signal are not a result of a disturbed flow, e.g. induced by high local viscosities. A possible explanation could be the presence of a minute amount of large aggregates which contain multiple polymer chains. It can be speculated that these aggregates result from incomplete dissolution [44]. The increased retention volume of the peaks with high concentration (Fig. 9) supports this theory. The formation of aggregates during the separation process as a result from high local concentrations inside the AF4-channel for example during the focus-step could be an alternative explanation. Also viscous effects and flow irregularities directly inside in the light scattering flow cell might be considered.

As shown previously (Figs. 7 and 8) the distance between the separated PS standards as well as the peak broadening increase with the velocity of the cross-flow. Therefore, this might be a way to optimise the separation. Fig. 10 shows the separation of three PS standards using different gradients of the cross-flow.

All PS standards are shifted towards higher elution volume when the slope of the cross-flow gradient is increased. For the PS 310 and PS 1260 kg/mol an increase of the slope improves the separation while the standards of 8090 and 1260 kg/mol elute closer to each other. The material loss, which was already described, is recognizable as a decrease of the peak areas at an increased gradient slope. Particularly the low molar mass standard is affected because the small molecules are passing the membrane. The stronger retention of the standards is caused by the increased cross-flow force which acts over a longer time. As a result all molecules are forced closer to

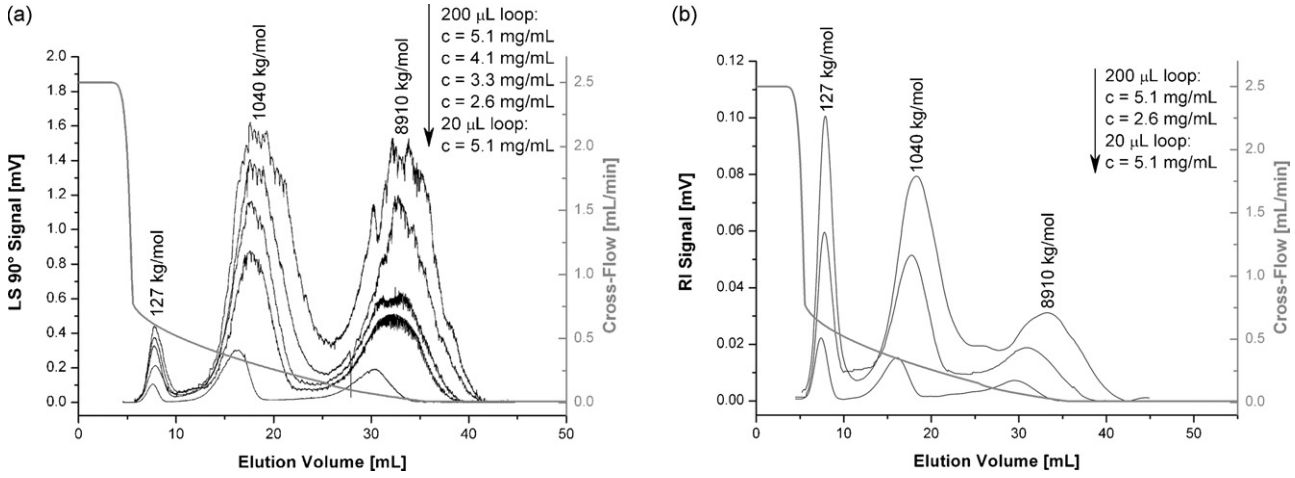

Fig. 9. Overlay of fractograms of a PS blend concentrations of the blends and injection volumes were varied, channel HT2. (a) DALLS-signals 90° angle. (b) RI-signals of three measurements of (a).

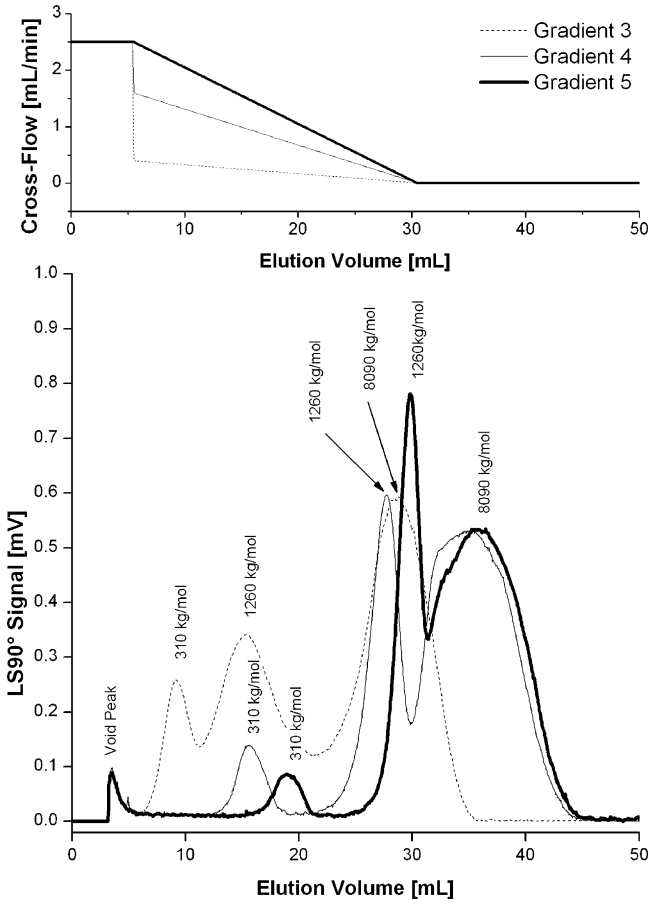

Fig. 10. Influence of different linear cross-flow gradients on the elution behavior of a blend of three PS standards, channel HT2.

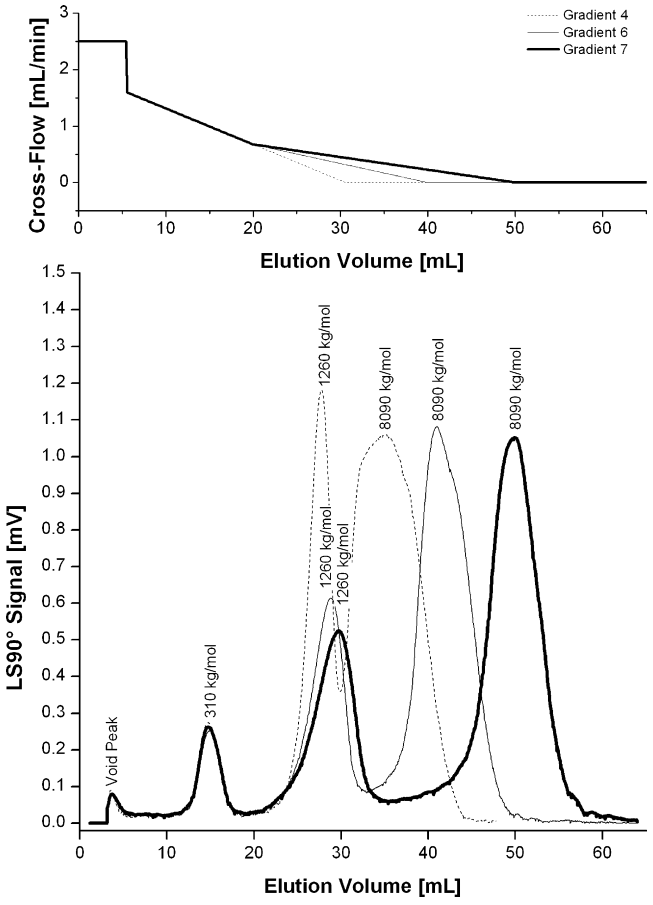

Fig. 11. Influence of different two steps cross-flow gradients on the separation of a blend of three PS standards, channel HT2.

the accumulation membrane and will in consequence be situated in a flow layer with a lower flow velocity as it was already explained. A possible explanation for the missing shift of the largest standard could be that the cross-flow had already reached when the largest standard started to elute. The missing field will result in a poor separation between the two biggest standards because the PS 8090 will not show an increased retention for a steeper gradient. As a result the high molar mass standards converge when increasing the gradient from 4 to 5 (Fig. 10).

The gradient can be a valuable tool to improve the separation. The effect of introducing an additional slope is shown in Fig. 11.

The change of the slope in the gradient after elution of the PS 310 kg/mol enables an enhancement of the retention for the two high molar mass standards without influencing the elution volume of the low molar mass standard. The increasing separation between the two standards indicates that the separation takes place according to the regular FFF mechanism. As a result both standards are situated in flow layers with different flow velocity which results in different elution volumes. An elongated influence of the cross-flow results in an increased difference in the elution volume of both standards. It was shown in Fig. 11 that there is a possibility to further increase the separation of PS 1260 and PS 8090 kg/mol by reducing the slope and simultaneously elongating the duration gradient. This indicates that the limit of separation for gradient 4 and 5 in Fig. 10 is due to the missing cross-flow. Fig. 11 demonstrates that the cross-flow is a very sensitive tool and that even a very small variation of the cross-flow leads to a greatly improved separation for the both largest PS standards.

The measurements above demonstrate the advantage of an adjustable cross-flow program which offers the possibility to adapt the calibration curve of a channel for the sample to be analysed. Furthermore, the gradient can be tuned with the aim to optimise separation in the region of particular interest. Thus AF4 offers larger flexibility than SEC with its fixed calibration curve for the chosen columns.

To evaluate the precision of the AF4-measurements various PS standards were analysed and the calculated average molar masses were compared with those from the producer (Table 1). The calculated MMDs are shown in Fig. 12.

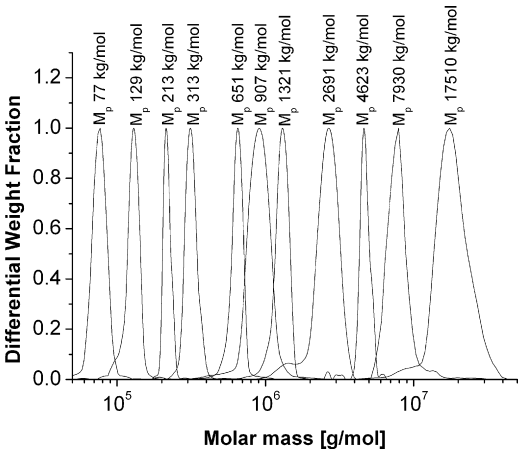

Fig. 12. MMDs of PS standards obtained from AF4-mesurements with channel HT1. The calculated mass values are shown in Table 1. Examples of the belonging Fractograms are shown in Fig. 3a.

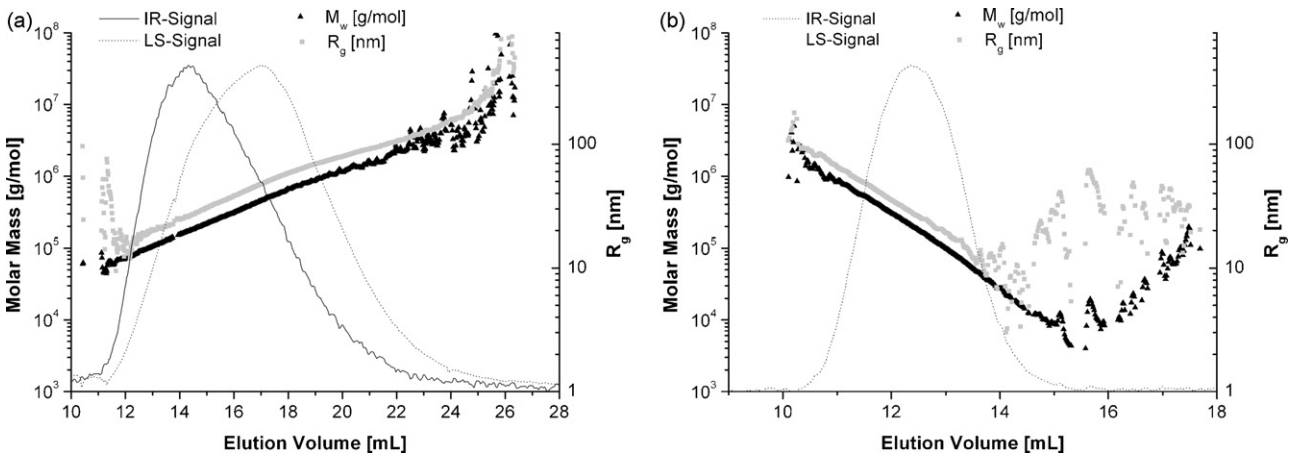

Fig. 13. (a) Fractogram of NIST SRM 1496 from HT-AF4-separation, channel HT-1, molar mass and R_g calculated from light scattering data overlaid. (b) Elugram of NIST SRM 1496 from HT-SEC, 2 PL Olexis columns, molar mass and R_g calculated from light scattering data overlaid.

As it could be expected, due to the low PD of the PS standards, the obtained molar mass distribution curves are narrow. The peak broadening which is visible in the corresponding fractograms (Fig. 3a) does not influence the molar mass distribution and the calculated average values. The calculated values of the weight average molar mass (M_w), molar mass at the peak maximum (M_p) and polydispersity (PD) are in good agreement with the producer values (Table 1).

After the model studies of the AF4-system with known PS standards at ambient temperature the instrument was heated to 145 °C using TCB as solvent. To prove the comparability between HT-SEC and HT-AF4 measurement the HDPE reference material NIST SRM 1496 was separated by both techniques. The fractogram (a) and the corresponding elugram (b) together with the values for the molar mass and the radius of gyration are shown in Fig. 13.

The comparison between the results from HT-AF4 and HT-SEC in Fig. 13 shows that HT-AF4 offers the possibility to detect much higher molar masses and radii than HT-SEC. Unfortunately the low molar mass material is lost due to the high cut-off of the ceramic membrane. For this reason the molar mass and R_g curves detected by HT-SEC show much lower values than visible in HT-AF4. Both separation methods deliver an almost linear molar mass and radius calibration curve for this unbranched material. In Fig. 14 the differential molar mass distributions (MMD) and the conformation plots from both methods are overlaid.

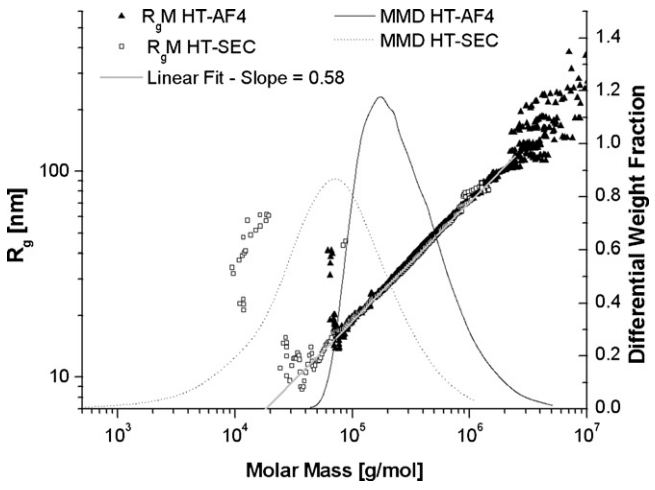

Fig. 14. Conformations plot and differential molar mass distribution (MMD) from HT-SEC and HT-AF4 of NIST SRM 1496.

Clearly the MMD from HT-AF4 shows a much larger portion of high molar mass material than HT-SEC and masses above 1000 kg/mol are visible which cannot be identified in HT-SEC. Both conformation curves show a straight linear nature which is expected for a linear polymer and are congruent with a slope of 0.58, which is very close to the theoretical value of 0.588 for a linear polymer in a good solvent [45]. The molar mass cut-off of the ceramic membrane causes a loss of most of the polymer molecules with a molar mass below 100 kg/mol in the HT-AF4 run while the HT-SEC measurement shows also the smaller molecules. To demonstrate the full capability of HT-AF4 vs. HT-SEC a second NIST reference was analysed, which is known to have bimodal light scattering signal caused by a small amount of ultra-high molar mass material. The sample is known to be strongly affected by shear degradation in HT-SEC [46,47]. In Fig. 15 the fractogram (a) and the corresponding elugram (b) of this sample are displayed.

HT-AF4 clearly shows higher radii and molar masses as well as a much more pronounced light scattering peak in the high molar mass area than HT-SEC. In the HT-SEC measurement an abnormal effect becomes visible: both the radius of gyration and the molar mass increase toward high elution volumes, leading to a curvature of the calibration curves calculated from light scattering data. This phenomenon has been reported for long chain branched polymer samples and is mostly explained by the late co-elution of a small fraction of strongly branched molecules [4,8,9,48].

In Fig. 15 it is visible that the light scattering peak in HT-AF4 is much more pronounced than in HT-SEC. The calculation of the mass and radius values confirms this indication. Very high molar mass values and radii were obtained in HT-AF4 which cannot be detected by HT-SEC due to the shear degradation in the columns and frits. In Fig. 16 the conformation plot and the MMD are shown for HT-AF4 and HT-SEC separation of the sample.

The MMD from HT-AF4 clearly shows that the high molar mass part of the sample is much more pronounced than in HT-SEC. Due to the relatively large pores of the HT-membrane the low molar mass part of the sample (<100 kg/mol) is not visible in HT-AF4. The comparison of the conformation plots shows that HT-AF4 delivers more information about the chain structure of the high molar mass tail. NIST SRM 1476 has a strongly branched high molar mass part and the HT-AF4 measurement indicates that the high degree of branching starts at clearly higher molar masses than observed by HT-SEC. The reason could be a falsification of the MMD by shear degradation of the high molar mass macromolecules in HT-SEC. Furthermore the late elution phenomenon in HT-SEC leads to a curvature of the conformation plot in the low molar mass area due to the co-elution of large and small molecules which falsifies the branching infor-

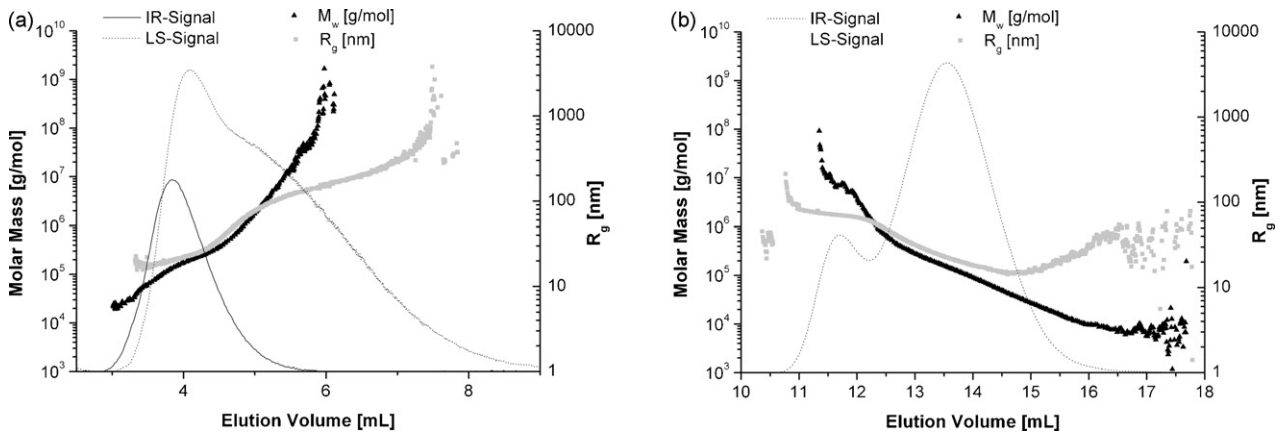

Fig. 15. (a) Fractogram of NIST SRM 1476 from HT-AF4-separation, channel HT-1, molar mass and R_g calculated from light scattering data overlaid, channel HT-1. (b) Elugram of NIST SRM 1476 from HT-SEC, 2 PL Olexis columns, molar mass and R_g calculated from light scattering data overlaid, channel HT-1.

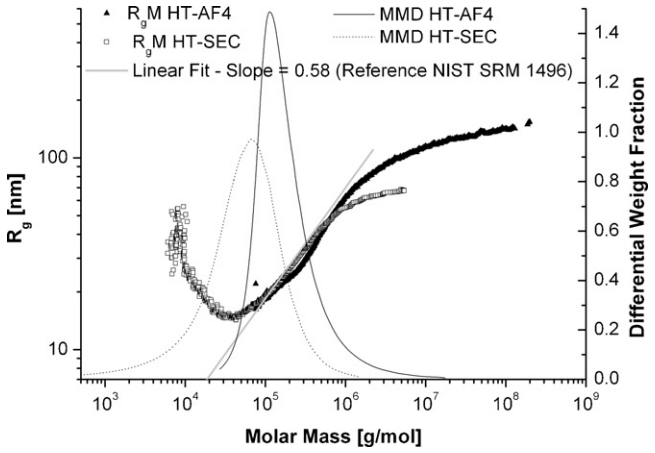

Fig. 16. Conformations plot and differential molar mass distribution from HT-SEC and HT-AF4 of NIST SRM 1476.

mation of this part of the sample. In HT-AF4 such effect was not observed due to the missing stationary phase in the channel and the different separation mechanism.

4. Conclusions

Two newly developed ceramic membranes for AF4 have been compared with a typical cellulose membrane under conditions of AF4. Series of polystyrene standards in tetrahydrofuran at room temperature have been analysed. The molar mass cut-off for the ceramic membranes was found to be larger compared to that for cellulose. This is a major disadvantage of the tested ceramic membranes. The mechanical stability, the chemical and heat resistance of the ceramic membranes, however, enable to perform AF4 analysis in solvents and at temperatures, where the cellulose membrane fails (for example, at temperatures above 140 °C in TCB). The separation of macromolecules with low molar masses by AF4 is not complete, because the small molecules are lost through the membrane. As a result it is not possible to exchange SEC by AF4 – rather AF4 is a complementary method in the range of very high molar masses, where SEC often fails and SEC is a complementary method to AF4 in the range of smaller molar masses, where recovery of AF4 is very low.

The low shear forces and the separation of very large macromolecules are the most important advantages of this method and a pronounced plus compared to SEC. The steric AF4-mode may complicate the separation. For this reason it will be useful to investigate the transition from regular to steric AF4 in the future with the aim to avoid the discussed separation problems.

We have demonstrated that the cross-flow programming allows to optimise the separation and to minimise the broadening effects. The observed overloading problems were resolved by choosing the adequate concentrations. Furthermore, the variable cross-flow allows for adjusting the elution behavior of the analysed macromolecules with different molar masses. The gradient can be tuned with the aim to optimise separation in a region of particular interest. Thus AF4 offers higher flexibility than SEC can provide with its fixed calibration curve for a given column set.

Finally the system was tested at high temperature and it was shown that HT-AF4 works properly and delivers the correct slope for the R_g–M relationship of a linear HDPE NIST standard. Moreover it was also shown that HT-AF4 delivers a lot of new information of the high molar mass part and the chain structure for the linear and a bimodal long chain branched NIST reference. The abnormal late elution phenomenon is missing in HT-AF4. This leads to additional information about the chain structure in the low molar mass part in the conformation plot. Although the ceramic as well as the cellulose membranes are suitable for AF4-measurements at different temperatures, the development of membranes with much smaller pores and a narrower pore size distribution, will be necessary to obtain complete information about the MMD of a sample and to explore the potential of AF4 to full capacity.

Acknowledgements

The financial support of the BMBF (project: 03X2006) and Basell Polyolefins (Frankfurt, Germany) as well as the technical support of Mrs. Moldenhauer and Mr. Welz (Postnova Analytics, Landsberg/Lech, Germany) are greatly acknowledged. The discussions with Drs. Mes, de Jonge (Dow, Terneuzen, Netherlands) and Podzimek (SYNPO, Pardubice, Czech Republic) are highly appreciated.

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