



# Study of the abnormal late co-elution phenomenon of low density polyethylene in size exclusion chromatography using high temperature size exclusion chromatography and high temperature asymmetrical flow field-flow fractionation

T. Otte<sup>a,b,\*</sup>, T. Klein<sup>a</sup>, R. Brüll<sup>b</sup>, T. Macko<sup>b</sup>, H. Pasch<sup>c</sup>

<sup>a</sup> Postnova Analytics GmbH, Max-Planck-Straße 14, 86899 Landsberg/Lech, Germany

<sup>b</sup> German Institute for Polymers, Schlossgartenstraße 6, 64289 Darmstadt, Germany

<sup>c</sup> University of Stellenbosch, Department of Chemistry and Polymer Science, Private Bag X1, Matieland 7602, South Africa

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## ABSTRACT

The elution behaviour of linear and branched polyethylene samples in SEC was studied. For the branched samples an abnormal late co-elution of large and small macromolecules manifests itself as an abnormal re-increase of the molar mass and the radius of gyration values detected with multi angle light scattering at high elution volumes in SEC. The late co-elution of small and large macromolecules cannot be explained by the SEC mechanism alone. The influence of several experimental parameters on the late co-elution was studied. It was found that the type of SEC column and the flow rate have a significant influence. The late eluting part of the sample was fractionated and separated by HT-SEC- and HT-AF4-MALS. The different results of both methods have been discussed with the aim to find possible explanations for the late elution. The experiments indicate that especially large branched structures show an increased tendency for the phenomenon.

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## 1. Introduction

The molecular structure is excessively important for the application of polymer materials. Parameters like the degree of branching or the molar mass and their distribution, strongly influence the processability and morphology especially in the case of polyolefin materials [1–5]. For this reason, knowledge of the molecular parameters is essential for product development and quality control [6,7]. Currently, the most common way to analyze linear and branched polyolefins is the application of the high temperature size exclusion chromatography (HT-SEC) [8–15]. This analytical technique is based on the entropy-controlled separation of the polymer molecules according to their hydrodynamics volumes. This leads to an elution of nearly monodisperse molar mass fractions of polydisperse polymers in ideal case [16]. In combination with a multi-angle light scattering detector (MALS) the molar mass and the radius of gyration are accessible. In combination with a concentration detec-

tor, the molar mass distribution and the amount of long chain branching can be calculated [17,18]. An important requirement for a correct analysis is a continuous separation according to the entropy-controlled SEC mechanism and the absence of interactions between the molecules and the stationary phase. Unfortunately, the SEC separation is often disturbed for branched polymers with high molar mass and these macromolecules may co-elute through a SEC analysis together with small molecules. This leads to observation of an abnormal increase of the molar mass and radius of gyration at high elution volumes. This effect was described by several groups [16,19–27]. The phenomenon blocks the correct evaluation of the MMD as well as branching analysis. The following explanations were mostly proposed for the abnormal behaviour: partial entanglement in the porous packing for large macromolecules, limited diffusion of large chains in and out of the SEC pores, sieving in the inter-particle volume, adsorption, presence of small gel particles, molecular topology fractionation or the partial separation according to slalom chromatography.

In this study the late elution effect was investigated using LDPE samples. The influence of different SEC parameters on this effect was proved. New technologies like high temperature asymmetrical flow field-flow fractionation (HT-AF4) and coupling between HT-

\* Corresponding author at: Postnova Analytics GmbH, Max-Planck-Str. 14, 86899 Landsberg, Germany. Tel.: +49 8191428181; fax: +49 8191428175.

E-mail address: [tino.otte@postnova.com](mailto:tino.otte@postnova.com) (T. Otte).

**Table 1**  
Specific values of SEC columns used for separation of the PE-samples.

Column type	Packing material	Particle size ( $\mu\text{m}$ )	Dimensions (mm)	Average pore size ( $\text{\AA}$ ) <sup>a</sup>
PL mixed B	Cross linked SDV	10	7.5 × 360	Mixed bed
PL Olexis	Cross linked SDV	13		Mixed bed
PSS 1	Cross linked SDV	20		$1 \times 10^7$
PSS 2	Cross linked SDV	20		$1 \times 10^5$
PSS 3	Cross linked SDV	20		$1 \times 10^3$
MN 1	Porous silica	10		$3 \times 10^2$
MN 2	Porous silica	7		$1 \times 10^3$

<sup>a</sup> Equivalent-length of a PS molecule.

SEC and HT-AF4 were used to get more detailed information about the influence of the chain-structure and the molar masses on the phenomenon.

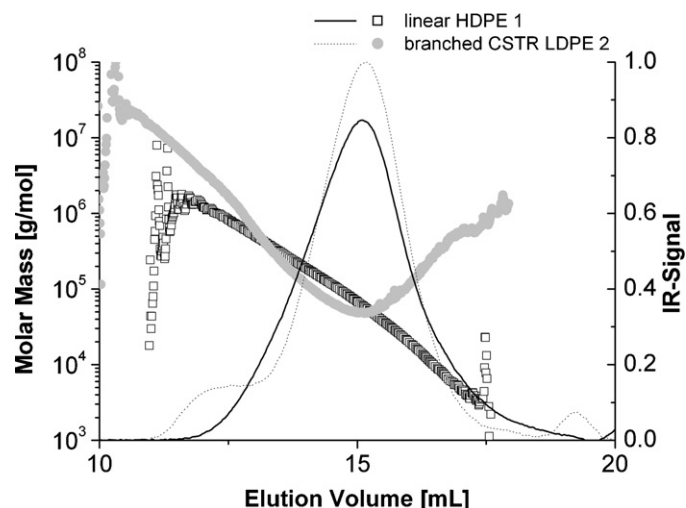
## 2. Experimental

### 2.1. Instrumentation

A PL GPC 220 chromatograph from Polymer Laboratories (Church Stretton, England) was used as a platform for the AF2000 HT-AF4 system from Postnova Analytics. This system allows to perform either HT-SEC or HT-AF4 separations of polyolefins at a temperature of 145 °C in 1,2,4-trichlorobenzene (TCB). The connection between the flow paths was realized by three six-port valves from Valco Instruments (Waterbury, USA). For the most SEC separations two PL mixed B and two PL Olexis SDV-columns were used. In addition, different SDV columns from Polymer Standard Service (PSS, Mainz, Germany) and Silica columns from Macherey-Nagel (Düren, Germany) were used with regard to their influence on the late co-elution effect. The specific values of the columns are given in Table 1.

The PL mixed B or PL Olexis columns have been applied for the HT-SEC separations because these columns are very frequently used for standard analysis of polyolefins in many laboratories all over the world. The broad size range, the linearity of the calibration curves and the good filtration, which leads to low noise in the light scattering signal, may be the reasons for it. The lower particle size of approximately 10–13  $\mu\text{m}$  compared e.g. to mixed A columns may lead to pronounced shear stress which is also a source of error in addition to the late elution phenomenon. The columns were chosen for comparison of SEC and FFF with the aim to show the real extent of error which can be produced during a routine SEC-MALS analysis.

A HT-AF4 channel obtained from Postnova Analytics was used for the HT-AF4 measurements. The channel has the outer dimensions of 295 × 30 × 60 mm and the internal channel cavity was 275 mm in length with a 350  $\mu\text{m}$  thickness. The channel contains an integrated inert membrane with a nominal pore size of approximately 10 nm. The detection was realized with a HT-MALS Heleos II (Wyatt, Santa Barbara, USA) and with an infrared detector, model IR4, from Polymer Char (Valencia, Spain). The HT-IR4 detector was used for concentration detection. If it is not specially mentioned, the samples were analyzed with a detector flow rate of 0.5 mL/min in SEC and AF4 mode.



**Fig. 1.** Elugram of the mostly linear sample HDPE 1 and the branched sample CSTR-LDPE 2 obtained after SEC separation using two PL mixed B columns. The corresponding molar masses were calculated using MALS + IR detection.

### 2.2. Materials and methods

The HDPE samples 1 and 2 have been obtained from Dow Chemical Company (Terneuzen, Netherlands). The sample HDPE 1 was synthesized by Ziegler Polymerization and HDPE 2 was the reference material NIST NSRM 1496, which contains mainly linear macromolecules. The sample CSTR LDPE 2 is highly branched and was produced by free radical polymerization in a continuously stirred tank reactor (CSTR) under high pressure of approximately 2000 bar and temperatures up to 245 °C. The details of synthesis and rheological data have been reported in Ref. [21].

The PE samples were dissolved for 4 h in 1,2,4-trichlorobenzene (TCB, obtained from Acros, Geel, Belgium) at 160 °C with a concentration of 2 mg/mL. The TCB was stabilized with 1 mg butylated-hydroxytoluene (BHT, obtained from Merck, Hohenbrunn, Germany) per 1 mL of TCB to avoid thermal-oxidative degradation during sample preparation and analysis. In addition, the solvent was flushed with argon gas. No shaking or stirring was applied on the sample to ensure the absence of shear forces in the concentrated polymer solution. A good homogenization was realized by gentle rotation of the vials with the polymer solution inside. The injection volume was 200  $\mu\text{L}$  for all separations. The fractionation of sample LDPE 2 was done during the SEC separation process. The outlet of the columns was collected between an elution volume of 15 and 20 mL for 50 times. All solutions were merged and the polymer was precipitated by adding an excess of methanol (Merck, Hohenbrunn, Germany). After precipitation the solvent and precipitant were removed by filtration and the polymer was dried for 5 h at 50 °C. Prior re-injection the fractionated polymer was re-dissolved with the standard procedure described above. The mass and radius values were calculated from MALS-data using a second order Berry equation.

## 3. Results and discussion

The SEC elugram of a linear HDPE and a branched LDPE is displayed in Fig. 1.

The molar mass of HDPE 1 is continuously decreasing with the elution volume. For the LDPE sample the course of the molar mass runs comparably until an elution volume of approximately 14 mL is reached. At this point values of the molar mass start to increase. This effect is well known for branched polymers of different nature [16,19–27]. A typical explanation for this behaviour, which was

**Table 2**

Average molar mass and radius of gyration obtained after SEC separation of CSTR LDPE 2 calculated from MALS raw data and using a linearly extrapolated calibration curve.

Sample	$M_w$ (kg/mol)	$\langle R_g^2 \rangle_z^{0.5}$ (nm)	PD
CSTR LDPE 2	663	64	15.77
	603 <sup>a</sup>	64 <sup>a</sup>	6.51 <sup>a</sup>

<sup>a</sup> Values for the linear extrapolation of the obtained calibration curve.

already noted above, is the abnormal long retention of strongly branched molecules with high molar mass in the SEC column. These species are co-eluting at high elution volumes together with the molecules of low molar mass [19,25].

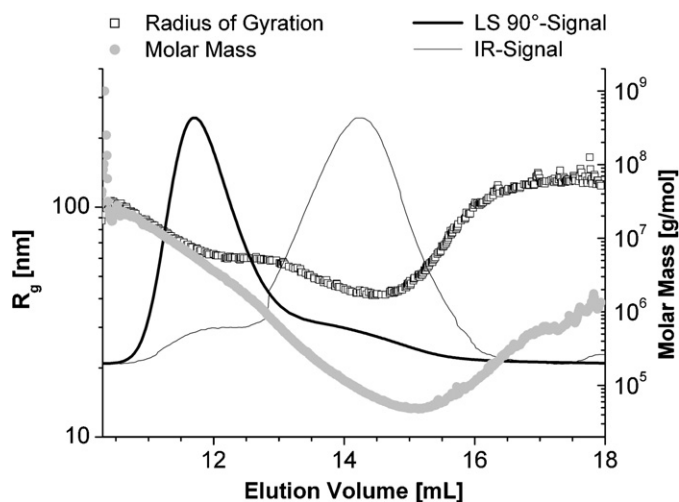
No correct differential molar mass distribution can be calculated from the SEC-MALS data of these samples due to the calibration curve which increases again at high elution volumes. The main requirement for the correct calculation of a differential molar mass distribution is the proper separation of the individual macromolecules into narrow distributed weight fractions. These fractions correspond to each detection point of the MALS. Unfortunately, the polymer fraction in a slice of the elugram will become extremely polydisperse if co-elution with a small amount of high molar mass material occurs. This means that the basis for a correct interpretation of the distribution curve is no longer existent, since there is no correct size-separation of the late eluting part of the sample. For a qualitative comparison of the SEC data with the results from field-flow fractionation, which does not show the late co-elution phenomenon [27,28], it is necessary to get a differential molar mass distribution from the SEC results despite the co-elution phenomenon. Therefore the part of the molar mass elution curve with a constant slope was averaged by a linear fit. The resulting regression line was then extrapolated linearly towards lower molar masses. A calculation of the differential molar mass distribution directly from the re-increasing molar mass versus elution volume curve is not possible because of the missing separation in the late eluting part.

The MMD from extrapolation of the raw-data delivers an apparent differential molar mass distribution, but it has to be taken into account that the late co-elution was neglected. As a result, an important part of the sample is not captured and thus is missing in the distribution. The resulting MMD therefore is not representing the complete polymer sample, but only a fraction of it. The extrapolation procedure will deliver similar results like a polymer-specific calibration [29]. However, until now this method is the only way to get an interpretable and comparable molar mass distribution from the SEC separation of such branched species.

In Table 2 the average molar mass values were calculated with and without extrapolation to get information about the error which has to be taken into account.

The lower average molar mass ( $M_w$ ) for the linear extrapolation in Table 2 indicates that a part of the high molar mass molecules partially co-elute with small molecules at high elution times. For the extrapolated curve these molecules are neglected and the molar mass average is decreased by about 10%. The late elution phenomenon affects the molar mass and the radius of gyration. In Fig. 3 the impact on both values is shown.

The course of the curves obtained for the molar mass and radius of gyration in Fig. 2 indicates that the radius of gyration is more sensitive to the co-elution of large structures than the molar mass. The radius already starts to increase at an elution volume of 12 mL while the molar mass shows an increase from a value of 14 mL onwards. Furthermore, the radius shows two significant inflection points which could be an indication for a co-elution of more than one different species. The radius is more sensitive for co-elution of a small amount of large molecules because it is a z-average while the molar mass is a weight average value [25]. Due to the different



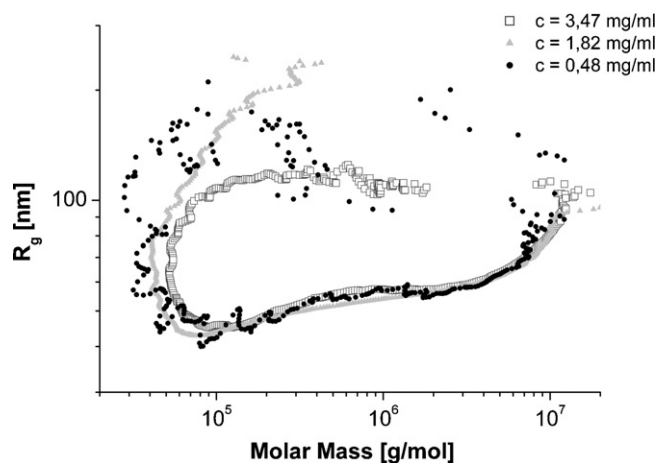
**Fig. 2.** Elugram of CSTR LDPE 2 from HT-SEC-MALS, 2 × PL mixed B column, molar mass and radius of gyration versus elution volume.

sensitivity of both values the conformation plot is pronouncedly falsified by the late co-elution effect. A typical bent in the low molar mass part gets visible (Fig. 3) which prevents the estimation of the correct slope which is needed for examination of the degree of branching of the macromolecules [21].

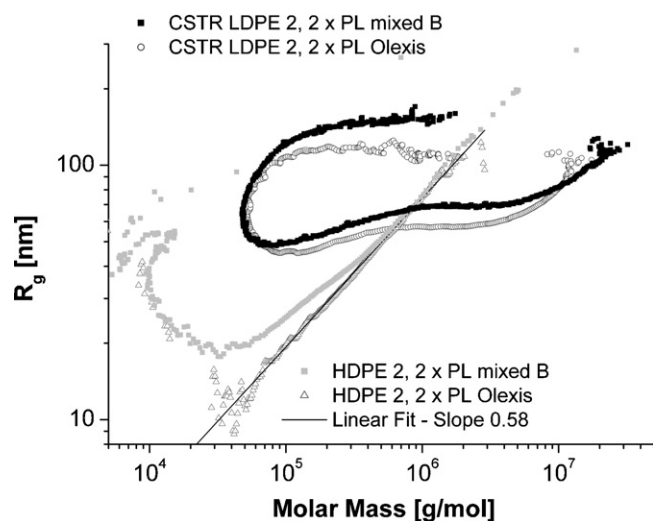
It has been reported in the literature that parameters like flow rate, microgel content, chain structure and the column packing can influence the abnormal late elution behaviour [19,24,25]. Most of the systematic investigations were performed using polymers like PS or PMMA. Also for PE the phenomenon was reported several times [21,27,28], but until now, no systematic study was performed to prove the influence of different parameters on the late elution phenomenon for this material.

The sample CSTR LDPE 2 shows a strong occurrence of the late co-elution. For this reason, this sample was chosen for further investigations. First, the injected concentration was varied with the aim to study its impact on the late co-elution. In Fig. 3 the conformation plots for different concentrations of the sample are shown.

Fig. 3 shows a slight reduction of the curvature for low concentrations. This effect is very weak. The strong scattering of the signal for  $c = 0.48$  mg/mL indicates that the signal to noise ratio has reached a critical value. Thus, a further decrease of the concentration is not possible, because the loss of information would be too



**Fig. 3.** Conformation plots of CSTR LDPE 2 obtained by HT-SEC-MALS at different injected concentrations using 2 PL mixed B columns.



**Fig. 4.** Conformation plot of sample HDPE 2 and CSTR LDPE 2, obtained from HT-SEC-MALS, sample concentration  $c=2$  mg/mL. Used columns are indicated in the figure.

high. However, Fig. 3 clearly indicates that there is a relationship between the concentration and the late co-elution effect.

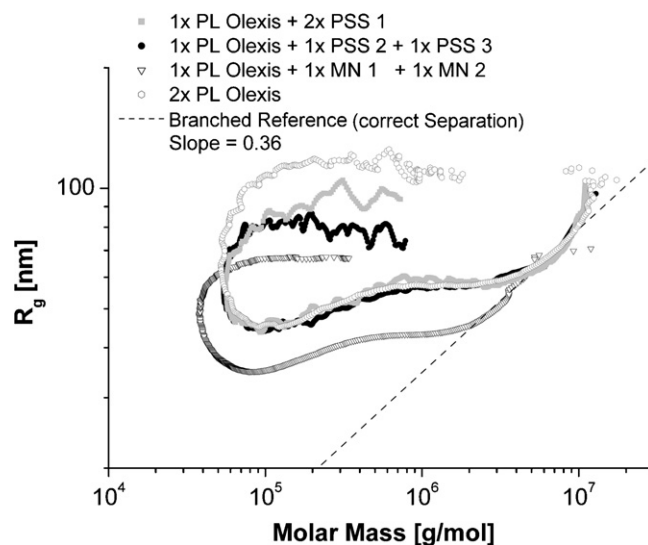
To investigate the influence of the column on the abnormal late elution behaviour, the HDPE sample 2, which is known to be linear, and the highly branched CSTR LDPE 2 were separated using different column sets. The results are shown in Fig. 4.

Fig. 4 shows that even the most linear HDPE 2 poses a slight curvature of the conformation plot for the separation with the PL mixed B columns. On the other hand, the late elution did not occur with the PL Olexis columns. It seems like the PL Olexis columns have a lower affinity towards abnormal retention of large species in this column. For the LDPE the abnormal increase of the radius seems to start at smaller molar masses if the PL Olexis columns are used for separation. But in this case the difference between both separations is significantly lower compared to the HDPE, which confirms that especially branched molecules show increased abnormal retention in SEC separation.

Both column sets are typically used for SEC analysis of polyolefins. They differ in particle size, pore size distribution and the kind of inlet frits. The material of the stationary phase was cross-linked styrene-divinylbenzene (SDV). Both columns were produced by the same manufacturer but the different internal setups lead to a varying extent of the late elution phenomenon even for HDPE.

Different column sets with varying pore size as well as with different packing material, have been applied for size separation to further investigate the impact of differences in the SEC packing on the elution behaviour of LDPE. Three different column sets were used. For evaluation of the impact of the packing pore-size, four SDV columns (from PSS – Polymer Standards Service, Mainz, Germany) with a very narrow pore-size distribution were tested. Unfortunately, a broad pore-size distribution is needed for a proper SEC-separation, which is necessary to separate the polymer peak from the peak of BHT-stabilizer and the system peak caused by low molar mass impurities in the mobile phase. Thus, a PL Olexis column was chosen as a first device to ensure a proper separation. Moreover, two different silica columns (MN1 and MN2) with narrow distributed pores were attached to the PL Olexis column to test the influence of different packing materials. The results of the experiments are displayed in Fig. 5.

As Fig. 5 shows, there is no significant change of the late elution behaviour for the SDV columns with different pore sizes. On the contrary, the measurements using the MN Silica columns show a difference in the late elution behaviour. The abnormal



**Fig. 5.** Conformation plot of CSTR LDPE 2 separated with different column combinations. Data obtained by MALS detection.

late co-elution effect seems to start at much lower values of the molar mass and radii over 45 nm the  $R_g$ - $M$ -relationship is more reliable. The  $R_g$ - $M$ -relationship seems not to be falsified for very low elution volumes. The relationship of the branched material without co-elution effects, which was evaluated with FFF, is displayed in the figure for a better orientation. FFF is not prone by co-elution and delivers the correct slope also for highly branched material [19,27,28]. While comparing the data obtained with PSS columns with those from the two PL Olexis columns, no significant difference gets visible. The particle size is 20  $\mu\text{m}$  for the PSS and 13  $\mu\text{m}$  for the PL Olexis columns. The low difference between the measurements with different SDV columns indicates that a change of the particle size in the tested range has probably not a strong influence on the late elution phenomenon. However, the MN columns seem to cause a much stronger difference in the elution behaviour and, in addition, the polymer samples seem to undergo increased shear degradation while passing through the silica columns which is strongly indicated by the lower average values of  $M_w$  and  $R_g$  displayed in Table 3. Due to the co-existence of decreased late co-elution and increased shear degradation it is not clear if the change of the elution behaviour was caused by the different packing material or if it is a real consequence of the lower molar masses.

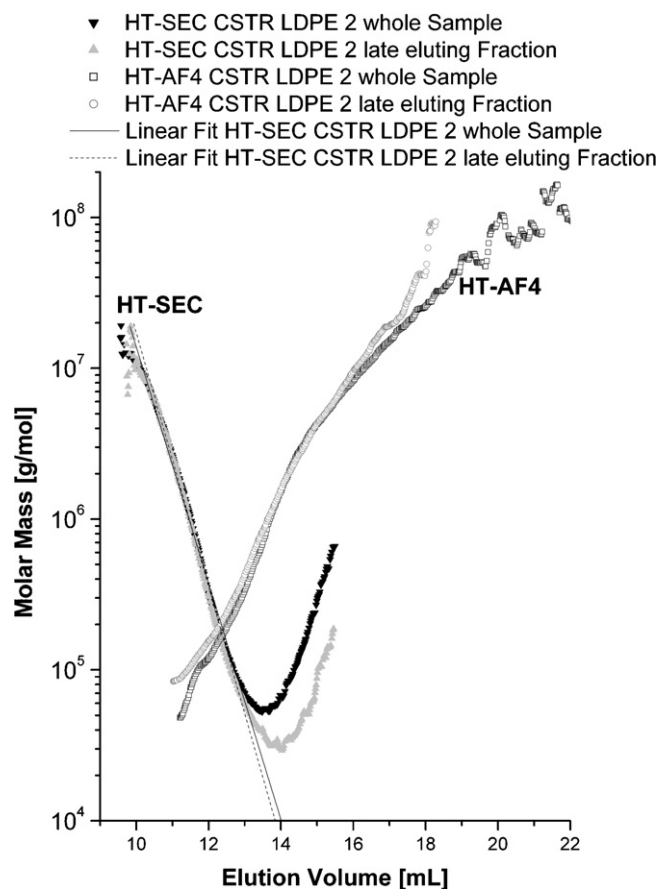
An apparent relationship between shear scission and the late elution phenomenon was found for the different columns. This could be an indication that an increased molar mass of the branched polymer molecules may lead to increased abnormal high retention of those species in the column, which will be visible as amplified late co-elution of high and low molar mass molecules in the elu-gram. For further investigations a better separation technique is needed, which enables to separate macromolecules correctly without co-elution and shear effects.

**Table 3**

Average molar mass and average radius of gyration obtained with SEC separation of sample CSTR LDPE 2 using different column sets.  $M_w$  and  $R_g$  were calculated from MALS raw data.

Columns	$M_w$ (kg/mol)	$\langle R_g^2 \rangle_z^{0.5}$ (nm)
2 x PL Olexis	509	63
1 x PL Olexis + 2 x PSS 1	633	69
1 x PL Olexis + 1 x PSS 2 + 1 x PSS 3	607	65
1 x PL Olexis + 1 x MN 1 + 1 x MN 2	260	48





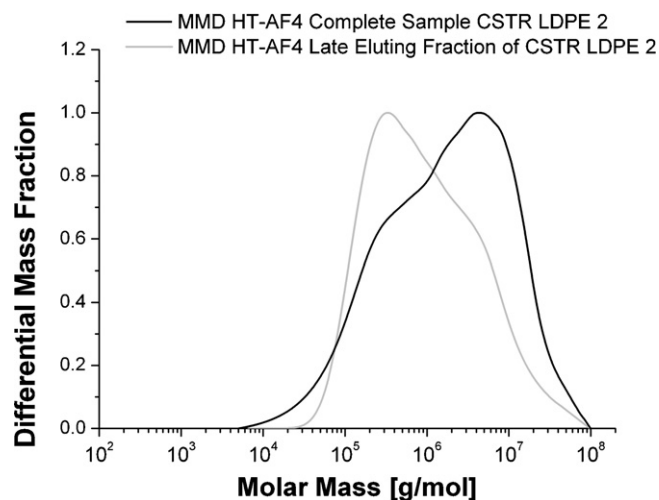
**Fig. 6.** Overlay of HT-AF4 fractograms and HT-SEC elugrams of complete sample CSTR LDPE 2 and of the re-injected late eluting fraction. The molar mass was calculated from MALS data. Two PL Olexis columns were used for SEC separation.

The HT-AF4-technique is able to separate polymer molecules inside an empty channel, without a stationary phase which could interact with the sample molecules. For this reason the HT-AF4 was used to further investigate the influence of the molar mass on the late co-elution phenomenon.

The sample CSTR LDPE 2 was separated with HT-SEC and HT-AF4 and, in addition, the late eluting part from SEC was collected at an elution volume of 15 mL and onwards where the molar mass starts to re-increase (Figs. 1 and 2). The collection was repeated 50 times. Following, all fractions were merged and the polymer was precipitated with methanol. Subsequently, the precipitated polymer was dried and dissolved in TCB with a concentration of 2 mg/mL. The dissolved fraction was injected in HT-AF4 and HT-SEC again. The resulting elugrams and fractograms obtained with both separation methods are displayed in Fig. 6.

As visible in Fig. 6, the separation of the late eluting fraction by HT-AF4 confirms that large macromolecules with molar masses of above  $10^6$  g/mol are present in the late co-elution part of the sample. While the HT-SEC separation shows the co-elution effect, the HT-AF4 delivers a proper separation for both samples, the original and the late co-eluting fraction. Moreover, the molar masses from SEC do not reach such high maximum values as they were detected after HT-AF4 separation. The macromolecules with very high  $M_w$  are either degraded in the column or they are masked in the late co-eluting part of the sample by the co-eluting smaller molecules.

It is well known that shear degradation occurs in the packing and the frits of SEC columns and as a result the molar mass of macromolecules decreases [11,30–36].



**Fig. 7.** Differential molar mass distribution of sample CSTR LDPE 2 and the late eluting fraction, both calculated from HT-AF4-MALS data. Please note that there is a loss of small molecules below 150 kg/mol present due to the larger pore size of the ceramic membrane employed inside the HT-AF4 channel. For this reason only the masses over this value should be incorporated.

In Fig. 7 the molar mass curve of the whole sample CSTR LDPE 2 increases up to a value of approximately  $10^8$  g/mol while the corresponding SEC run shows only maximum masses of approximately  $10^7$  g/mol. Shear degradation in the SEC columns and maybe abnormal long retention of the ultra-high molar mass fraction could be the reasons for this difference.

The maximum masses in the HT-AF4 fractogram of the re-injected late eluting fraction are slightly reduced (approximately  $4 \times 10^7$  g/mol) compared with those of the whole sample fractionated with HT-AF4 (approximately  $10^8$  g/mol). Also, the differential molar mass distributions from HT-AF4 analysis of CSTR LDPE 2 and the late eluting fraction in Fig. 7 show the presence of molar mass degradation for the late eluting fraction. A significant amount of the upper molar masses is missing in the corresponding MMD. A reason for the difference could be the additional shear degradation of the molecules inside the late eluting fraction as a result of the first passage of the whole sample through the SEC columns.

What is interesting about these data is that there are very high molar mass molecules present in the late eluting fraction, which normally should only contain masses below 50 kg/mol. Moreover, the masses detected by HT-AF4 are significantly higher than the maximal mass values from both SEC separations (Fig. 6). This is a strong indication that there are very large macromolecules present in the sample which preferably undergo an abnormal high retention in SEC and consequently they will elute late, together with the smaller molecules. As a result, these molecules get invisible in the SEC elugram because they co-elute together with an excess of smaller structures at high elution times.

The late eluting fraction of CSTR LDPE 2 was also re-injected in HT-SEC. As it can be seen in Fig. 6, there is also a late co-elution visible for this sample. But the effect is significantly lower compared with the whole sample. A possible explanation could be the different molar mass of both samples. The late eluting fraction has already passed the column set. As a result shear degradation leads to a decreased molar mass. This is confirmed by the average values of mass and radius at a flow rate of 0.5 mL/min for both samples in Table 4. A relationship between molar mass and late elution was already indicated by the results from the different column sets. The destroyed ultra-high molar mass chains in the late eluting fraction are absent at high elution volumes and as a result the curvature is decreased.

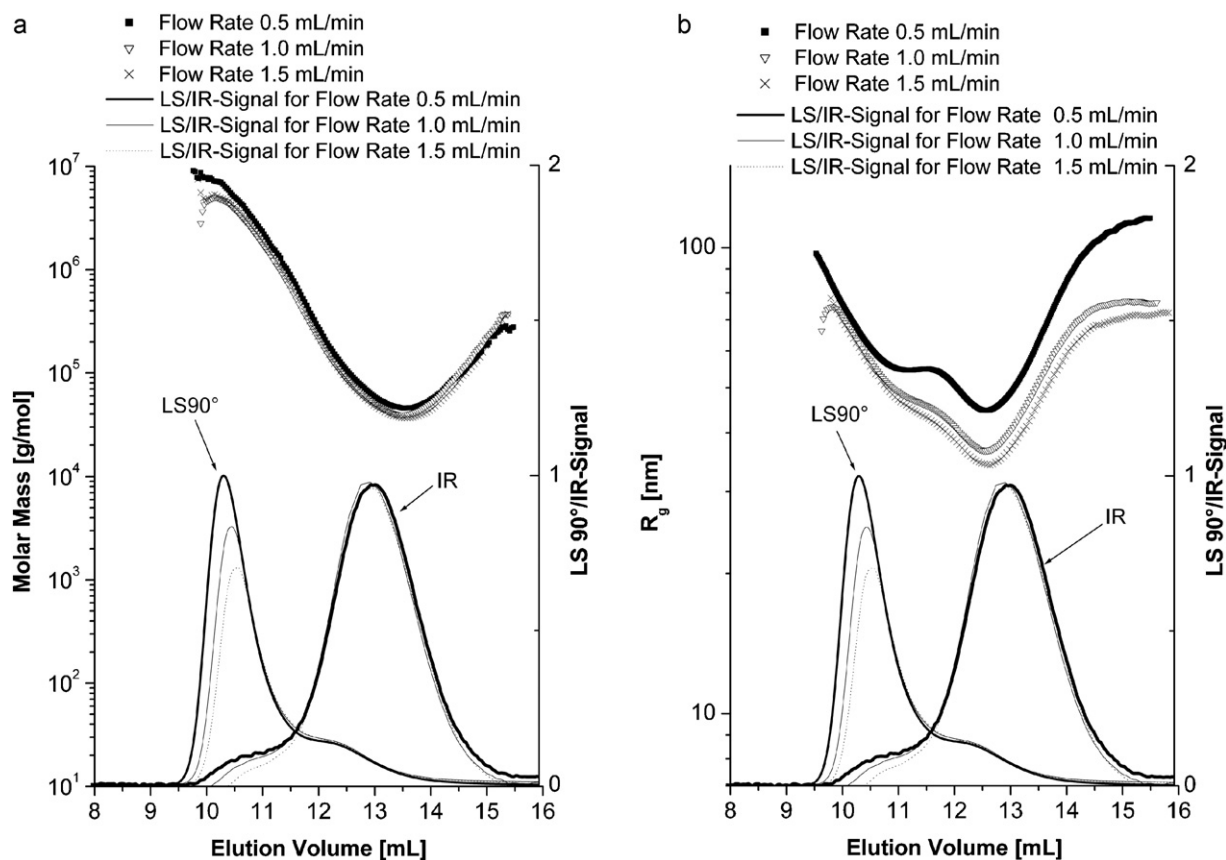


Fig. 8. Elugram of CSTR LDPE 2 from HT-SEC-MALS overlay of molar mass overlay of radius of gyration.

However, also the HT-SEC results confirm that there are large molecules inside the fraction, because the detected mass reaches the same value of  $10^7$  g/mol like it was visible for the separation of the whole sample.

For further investigation of the influence of shear degradation on the late elution behaviour, the whole sample CSTR LDPE 2 and the late eluting fraction were separated with SEC at different flow rates. In addition, the flow rate was reported to have a significant influence on the late elution of branched PS-molecules [19,24]. The whole sample CSTR LDPE 2 and the late eluting fraction have been separated at different flow rates using two PL Olexis SEC columns. The obtained elugrams and conformation plots are displayed in Figs. 9 and 10(a) and (b), the molar mass and radius average values are given in Table 4.

The sample LDPE CSRTR 2 shows a decrease of the abnormal late co-elution behaviour with increasing flow rate (Fig. 8(a) and

(b)). The change of the radius of gyration is more pronounced than those of the molar mass. Since the detected molar mass is a weight average in case of co-elution, it is not as sensitive to the presence of large macromolecules as the radius, which is a z-average for poly-disperse fractions. In Fig. 9, the impact of the reduced co-elution on the conformation plot is shown. The atypical curvature is less pronounced for high flow rates and the re-increase of the radius starts at lower molar mass values for both samples. This result is in contrast to the observations reported for e.g. star shaped polymers or branched PS [19,24]. A possible reason for the flow-rate dependent behaviour may be the degradation of the high masses with increased flow rate. Since the late elution phenomenon seems to be mass-dependent there will be a decreased co-elution if the masses are degraded inside the columns. Another explanation could be that a part of the sample material was not eluting from the column at high flow rates.

The  $R_g$ - $M$  relationship of the late eluting fraction seems to be more affected by the increase of the flow rate (Fig. 9(b)). The curvature is strongly decreased for high flow rates. For a flow velocity of 2.0 mL/min hardly any falsification is visible.

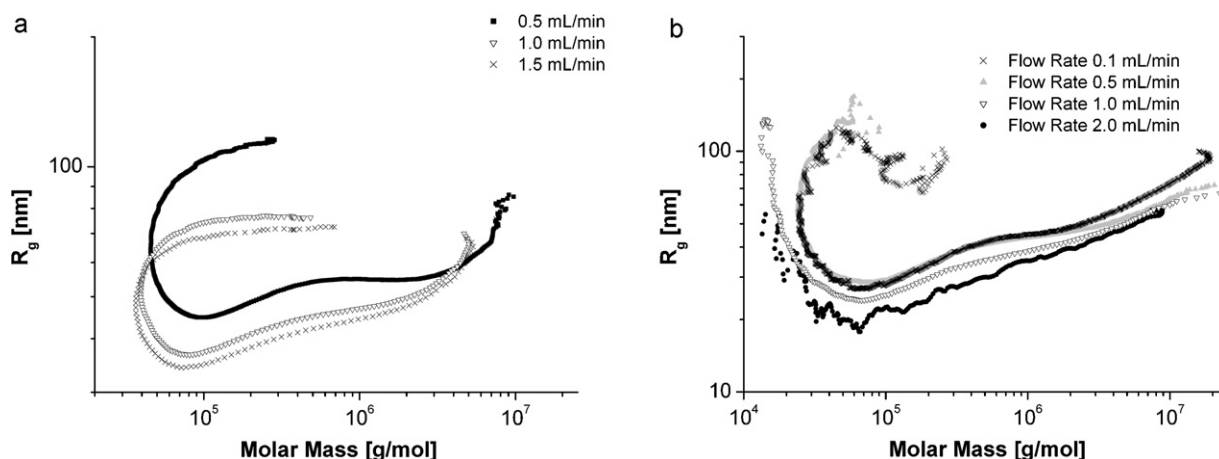
On the contrary, for the whole sample CSTR LDPE 2 the curvature is also decreased, but even at a flow rate of 1.5 mL/min the late co-elution is still visible. It seems that the late eluting fraction does not show such a strong tendency towards late elution like the whole sample. As a result the increase of the flow rate will lead to a much straighter  $R_g$ - $M$  curve.

Fig. 8(a) and (b) proves that the light scattering signal shows a decreased high molar mass shoulder for high flow rates. This is an indication for amplified shear degradation caused by the high flow rate [11,30–36]. The upper molar mass material is partly destroyed and as a consequence the light scattering signal is decreased. It is

Table 4

Average values of molar mass and radius of gyration values at different flow rates calculated from MALS data. SEC performed with 2 × PL Olexis columns.

Sample	Flow rate (mL/min)	$M_w \times 10^3$ (g/mol)	$\langle R_g^2 \rangle_z^{0.5}$ (nm)
CSTR LDPE 2	0.5	509	63
	1	340	54
	1.5	297	51
Late eluting fraction of CSTR LDPE 2	0.1	514	71
	0.3	390	61
	0.5	348	54
	1	270	45
	2	229	41



**Fig. 9.**  $R_g$ - $M$ -relationship, separation with HT-SEC-MALS at different flow rates,  $2 \times$  PL Olexis column. Sample CSTR LDPE 2 late co-eluting fraction obtained from sample CSTR LDPE 2.

well known that shear degradation is especially pronounced for macromolecules with very high molar masses [27,28]. The molar mass average values in Table 4 support this proposal. For both samples a significant decrease in molar mass average is visible, which is caused by an increased flow velocity.

The experiments have shown that the flow rate has an influence on the abnormal late co-elution of branched material. An increase of the flow rate always leads to shear scission in case of PE material. For this reason, it cannot clearly be distinguished if the change of the flow velocity influences the mechanism of the abnormal retention or if the changes are a consequence of the decreased size/mass of the molecules after degradation.

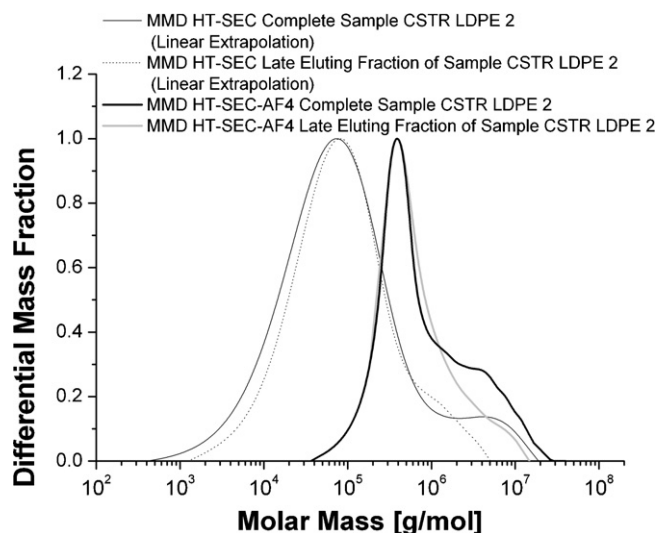
However, also for the separations with different columns a change of the late co-elution was observed (Figs. 4 and 5). In Table 2 it is visible, that the molar mass decreases with decreasing size of the column filling particles. In the same way the late co-elution was reduced. This is a second indication for a molar mass dependence of the abnormal late elution effect.

If the late co-elution phenomenon is amplified for high molar masses, there should be an increased amount of high molar mass molecules visible for the late eluting fraction. Polyethylene is very sensitive to degradation and thus the external fractionation of the late eluting fraction leads to additional degradation during the re-dissolution process at high temperature. For a better comparison of the MMDs an online separation with FFF, directly after the SEC separation, was performed. An additional benefit of this method is that the MMD for the whole sample from online-HT-SEC-AF4 coupling shows the same shear degradation like the MMD direct from HT-SEC but no co-elution will be present. This means the quantitative comparison of the masses with and without co-eluting material will be more reliable.

The HT-SEC columns were arranged between the injection valve and the inlet of the HT-AF4 channel. The special sample focusing technology in HT-AF4 allows the injection of high sample volumes into the AF4 channel without band broadening effects [37,38]. After the LDPE peak has left the SEC column, the whole peak will be focused inside the AF4 channel. After focusing, the material will be separated again by the cross-flow force without the possibility to interact with a stationary phase. Thus the coupling technique of both methods allows a direct separation of the polymer which is coming out of the column without abnormal co-elution but with the primary shear degradation offset from the passage through the SEC columns. As a result, the real amount of the molar masses with and without the late co-elution part can be determined. In Fig. 10 the molar mass distributions from linear extrapolations of the HT-SEC and from the HT-SEC-AF4 coupling are displayed.

The MMDs obtained using the linear extrapolation of the HT-SEC data, neglect the late elution of larger molecules. The comparison with the MMDs obtained from the HT-SEC-AF4 coupling makes the additional large molecules visible, which are masked at high elution volumes in case of direct evaluation of the SEC raw data. While coupling both methods, the shear effect, which is always present after SEC separation, will be more comparable for the complete sample and for the fraction. Both samples have now already passed the SEC columns prior AF4 analysis. Note that the curves from AF4 separation are falsified in the low molar mass region below approximately 150 kg/mol, which is the cut-off value of the used ceramic membrane. The extrapolated curves in Fig. 10 represent the results without abnormal late co-elution, while the curves from the HT-SEC-AF4 coupling represent also the molecules which were strongly retained due to the abnormal interaction with the stationary phase. There are higher masses visible in both MMDs from the HT-SEC-AF4 coupling. This proves that especially high molar mass molecules are concentrated in the late co-eluting fraction.

In addition, there is also a systematic difference visible in the MMDs of the complete sample and of the late eluting fraction for both separation methods. This means that the molar mass of the re-injected fraction is always lower than those of the complete



**Fig. 10.** Differential molar mass distribution of CSTR LDPE 2 and the late eluting fraction from HT-SEC- and HT-SEC-AF4-MALS. The curves from SEC were calculated by linear extrapolation of the  $M_w$ - versus  $V_E$ -curve towards low molar masses.

sample which can be explained with the additional shear stress of the re-injected sample during the additional passage through the columns. Also the re-dissolution process of the late eluting fraction may cause supplemental thermal degradation.

The high molar mass shoulder of the late eluting fraction is decreased in comparison with the MMD of the complete sample independently from the separation method. The fraction has passed the column two times and the additional separation leads to increased shear stress. As a result, the maximum molar masses will be smaller than for the complete sample.

All experiments indicate a molar mass dependence of the late co-elution mechanism in HT-SEC. The variation of different SEC parameters like concentration of the sample, packing material, pore and particle size or the flow rate had an impact on the late co-elution behaviour. However, there was shear degradation simultaneously present in a different extent, except for the concentration variation. Consequently, the molar mass also changed with variation of the SEC parameters. The online SEC-AF4 measurements have confirmed that there is an ultra-high molar mass fraction present in the late eluting part of the sample, which is not visible in traditional SEC-MALS analysis due to the overlay with low molar mass molecules. The coupling between SEC and AF4 verifies that the differences in molar mass were not due to the lower shear degradation in AF4, because the same shear-offset was present for both separations.

The results indicate that there might be a second retention mechanism present during the SEC separation process, which is highly sensitive to branched species of very high molar mass inside the analyzed LDPE.

The accumulation of the high masses inside the late eluting fractions indicates a reversed elution order compared with the normal SEC-mechanism. Until now, there are only a few possible mechanisms described in the literature which may occur simultaneously with the regular SEC separation process inside the column.

Meunier and Smith found a topological fractionation process which will cause reversed elution order for branched species and which separates preferably according to the branching structure [39]. Linear PS was also separated in monolithic columns and a reversal of the elution order of high molar mass molecules from low towards high elution volume was shown for very low flow rates. In addition, a higher selectivity of the effect for branched species was demonstrated. In later publications this molecular topology fractionation (MTF) was optimized and used in 2D-analysis for the separation of complex species with different branching architecture [40,41]. The MTF mechanism occurs if there are pores or pathways inside the columns which have similar diameter as the dimension of the macromolecules. For the measurements mostly monolithic columns were used which contain macro- and mesopores of different sizes [42–46]. Also, in a SEC column various pores of different sizes are existent. It seems to be possible that some of these structures are very small with dimensions in the range of the molecular size. Thus, the abnormal high retention of branched or high molar mass species, which was observed for the LDPE, could be due to a MTF mechanism which overlays the regular SEC separation. Also the increase of the phenomenon at low flow rates would be in agreement with the results published in the MTF papers. The small number of narrow channels in SEC in comparison to monolithic columns could be the reason that only a small amount of the sample is separated in reversed order.

Another flow-dependent separation mechanism with reversed elution order is the coil-stretch transition during HDC [47–52] or SEC separation of macromolecules [53]. In 1989 it was reported that larger DNA-fragments show a reversed elution order compared with normal SEC mode. It was claimed that the high molar mass fragments were stretched due to the high flow rate. There-

fore, they are too large for penetration of the pores so that they have to pass through the inter-particle volume of the spherical particle packing which can form very thin channels. The explanation given for the reverse retention behaviour of the large molecules was that the inter-particle channels are very loopy so that the stretched molecules have to turn very frequently and as a result they will need a longer time to pass the column compared with the regular separated molecules. The phenomenon was reported to be sensitive in regard to mass, flow rate and solvent viscosity [54,55]. The so called slalom chromatography-effect was also described for HDC separation of DNA [56]. Later the new separation mode was investigated on the basis of PS material [57,58]. A reversal of the elution order was found for very high molar mass molecules in mono- and polydisperse samples using HDC columns with small packaging particles and high flow rates for separation. It was demonstrated that the impact of slalom chromatography transition increases with increasing molar mass, flow rate and decreasing particle size. The abnormal late elution of the LDPE leads to similar chromatograms like they were shown for the separation according to slalom chromatography. However, the late co-elution of LDPE increased with increasing particle size and decreasing flow rate. This difference indicates that coil-stretch transition may not be the reason for the described late co-elution phenomenon. A final answer cannot be given at this point because in this work the flow-rate dependence of the abnormal late elution seemed to be related to the degradation of the molecules. In addition there is no evidence for the presence of interstitial space of macromolecular dimensions in the column which would be necessary for a HDC mechanism.

A different possibility for the increased retention of the macromolecules with high molar mass could be the presence of enthalpic interactions between the macromolecules and the column packing material. It was shown recently that also polyolefins may be separated by adsorption liquid chromatography [59–64] despite the fact that they do not have functional groups. Adsorption of polyolefins from TCB on specific zeolite stationary phases was observed at temperatures of 140 °C. The retention mechanism was described as an adsorption process in the very thin pores of the zeolite stationary phase. The chain ends of the PE molecules penetrate these narrow pores and are adsorbed. The pore diameter is in the size range of the molecules and as a result the solvent molecules are not able to penetrate into pores which are occupied by the adsorbed chains and no desorption of the polymer was observed.

Also in case of the SDV or silica gel column packing material in the SEC columns, very narrow pores could be present or the inter particle zones may have the same dimensions. There will be a large surface area of the polymer in contact with the packaging material, if a PE chain enters the constricted space. As a result, increased interaction could be possible which may cause abnormal long retention of the polymer chains. This would also be an explanation for the dependency between late co-elution and molar mass or branching. Branched molecules have many side-chains which may be able to penetrate the narrow pores or inter-particle zones. Highly branched molecules of increased molar mass pose an increased number of side chains compared with a similar branched molecule of low molar mass. Both can result into higher retention which will be visible as abnormal late elution of preferably large, branched structures.

Recently, the adsorption of polyolefins with different molar mass and short chain branching in a hypercarb® column was presented by Macko et al. [65]. In this paper it is shown that branched polyolefin co-polymers and also linear polyolefins are adsorbed in the column. The adsorption tendency increases with molar mass and branching. Short chain branching are also present in LDPE due to the polymerization mechanism. The described results show that adsorption of polyolefins can have different characteristics and may be also overlaid to a normal SEC separation.



#### 4. Conclusions

It was shown that in SEC separation of branched polyolefins a high molar mass part of the samples is strongly retarded in the column which leads to a late co-elution of the large species with properly separated small molecules. Due to their different physical nature, the effect is more pronouncedly observable for the values of  $R_g$  than for  $M_w$ . For polydisperse fractions the molar mass is a weight average value while  $R_g$  is z-averaged. As a result,  $R_g$  will be more sensitive to presence of small amounts of large macromolecules eluting from a column. The co-elution prevents a correct calculation of the MMD and the detection of branching because the conformation plot is falsified by the different sensitivity to both molar mass and radius of gyration. The presence of different species in the late eluting fraction was verified by fractionation and re-injection of the late eluting part of the sample. New separation techniques like HT-AF4 allow the correct separation of highly branched macromolecules. For this reason the HT-AF4-technique was used for quantification and size determination of the co-eluting molecules. It was shown that especially high molar mass macromolecules are cumulated inside the late eluting fraction. This indicates a special sensitivity of the phenomenon for large branched material, which was often proposed in the literature before. In addition, the influence of different SEC parameters on the late co-elution was tested. The modification of flow rate and column packing material was always resulting in the change of the molar mass of the eluted polymer. It was found that increased flow rates as well as decreased concentration and particle size of the column packing led to a decreased co-elution phenomenon and decreased molar values.

Different alternative separation mechanisms were proved to be in accordance with the observed results of this study. Only the MTF fractionation or possibly adsorption would be in agreement with the relationship between increasing molar mass or branching and the amplified late co-elution effect. The manuscript supports the possibility of adsorption processes involved in SEC-separations. Due to the used stabilizers in polyolefin analysis also a modification of the SDV-column by e.g. degraded stabilizer molecules may be an additional parameter for a possible adsorption process.

Finally, it was shown that most of the observed limitations of SEC can be overcome by using the AF4 technique. Only the relatively large pore size of the employed HT-AF4 membranes was a limitation of the method. During the publishing process of the data a new ceramic membrane with a lower cut-off became available which now allows complete analysis of the most UHM samples. In an upcoming paper first HT-AF4 measurements with this new membrane will be presented.

The study showed that it is possible to decrease the late elution phenomenon as well as shear degradation down to a certain point, but it was not possible to fully eliminate this effect to compensate the immense drawback of SEC. It seems that the packing of the columns itself is the major problem which can only be solved by the use of HT-AF4 or other FFF techniques which enable separation of macromolecules without any employment of stationary phase.

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