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Topological separation of linear and star-shaped polystyrenes by off-line 2D chromatography. Stars having high molar mass arms and quantification of the star fraction

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Abstract

Off-line 2D separations on mixtures of linear and star-shaped polymers were performed using temperature gradient interaction chromatography (TGIC) as a first and SEC as a second dimension. The experiments resulted in clear separations of the linear and star shaped-structures for arm molar masses up to 42,000 g/mol. The resolution is nearly independent of the molar masses of the arms and depends only on the number of star arms. From the 2D chromatograms it is possible to determine the molar mass of the first branched structure, i.e. the three arm star. The evaluation of the relative peak volume allows a reliable estimation of the amount of branched structures in the complex mixture.

Keywords: Chromatography; 2D Chromatography; Star polymers

1. Introduction

Branched polymers have attracted attention from both a scientific and a commercial point of view. The introduction of branches results in a reduction of the melt viscosity, thus, in an improved processability without loss of mechanical properties. The characterization of branched polymers is still a challenge. Branching results in a contraction of the size of the molecules relative to that of the linear analogues of the same molar mass, as has been theoretically shown by Zimm and Stockmayer in 1949 [1]. Even more than 50 years later, branching analysis is still mainly done by viscosity or light scattering experiments on the basis of the Zimm–Stockmayer theory.

A correct application of the Zimm–Stockmayer approach requires light scattering or viscometry experiments on monodisperse fractions. That is why SEC with light scattering or viscometry detection has become popular [2–8]. While it is difficult to characterize the number of branch points in a well defined branched structure, the situation becomes even worse if the branched polymer is heterogeneous or in a mixture with linear molecules. In such cases coelution of different structures within a single SEC slice will severely complicate the analysis of the experiments using molar mass sensitive SEC detectors. The results obtained for molar mass, radius of gyration or intrinsic viscosity for a SEC slice are average values [9–12]. If only small amounts of branched species are in a mixture with linear ones, the size reduction might be too small to be determined experimentally. Therefore, it is necessary to first effectively separate linear and branched structures before using highly sophisticated detector systems for the characterization of the materials.

Two-dimensional chromatography has been found to be a highly effective method for the characterization of complex polymers. In 2D chromatography a first separation is performed according to one parameter, e.g. chemical composition, and the separated fractions are subjected to a second chromatographic separation, e.g. according to molar mass [13–16]. This procedure has been used for the determination of the combined functionality type (FTD) and molar mass distribution (MWD) or the joint distribution of chemical composition (CCD) and molar mass [15–22], which usually cannot be separated by one chromatographic mode alone.

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In a previous article, we have shown that 2D chromatography is capable to separate mixtures of linear and star-shaped polymers purely based on topology. This was due to the fact that in temperature gradient interaction chromatography (TGIC) the elution behaviour of star-shaped and linear polymers was governed by molar mass [23]. Effects of topology could not be detected within the errors given by the experiments. Since gradient chromatography results in a molar mass dependent elution, while elution in SEC is determined by the hydrodynamic volume, separation is possible according to the scheme given in Fig. 1. Here a schematic separation of a mixture of linear and star-shaped polymers is shown. A small eluent fraction of the TGIC-experiment will contain star-shaped and linear polymers having very similar molar masses. The star-shaped polymer has a smaller hydrodynamic volume than the linear species of the same molecular mass. Therefore, the injection of the fraction taken from TGIC into the SEC will result in two peaks, with the peak at higher elution volume corresponding to the star polymer.

The concept as given in Fig. 1 has been proven to work for mixtures of linear and star-shaped polymers having an arm molar mass of 5000 g/mol. However, for higher arm molar masses the separation failed [23]. The present paper extends our 2D approach to star polymers having arm length of more than 5000 g/mol.

The 2D separation of the linear and star-shaped polystyrenes just mentioned has to be distinguished from earlier separations of linear and star-shaped poly(L-lactide)s [22]. The latter separation was due to the different numbers of adsorbing end groups attached to the ends of the arms, while in the former the separation was based on topology only.

2. Experimental section

2.1. Synthesis

Star polymers were synthesized by crosslinking living polystyrene anions with divinylbenzene (DVB) in cyclohexane solution (Fig. 2) [24–26]. This results in star polymers showing a distribution of arms. The polydispersity of the arms is determined by the molar mass distribution of the precursor molecules. In the present case, the precursors prepared had a low polydispersity. However, due to the distribution of the arm number the stars are polydispers with respect to the molar mass. The average number of arms is a complex function of the ratio of the concentration of living anionic chain ends to the DVB concentration, the length of the precursor molecules, the reaction time, etc. The characterization data of the precursors and the stars are given in Tables 1 and 2. Details of synthesis and characterization are given in [23].

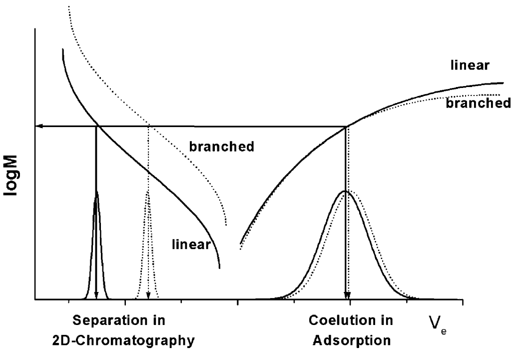

Fig. 1. Schematic representation of a 2D separation of linear and branched polymers.

2.2. Analysis

Gradient chromatography and fractionations were performed on a modular HPLC system consisting of Shimadzu degasser DGU 14A, low pressure gradient former FCV10ALVp, HPLC-pump LC10ADVp, autosampler TSP AS100 equipped with a 100 μl loop, Techlab K4 column oven, Knauer UV/VIS filter photometer and evaporative light-scattering detector (Alltech 500 ELSD). For fractionations the ELSD was replaced by a fraction collector (Advantec SF 2120). Data acquisition and fraction collector control was performed using PSS WINGPC software (PSS Polymer Standards Service GmbH, Mainz, Germany). Reversed phase separations were performed on Nucleosil C18 columns (5 μm, 300 Å, 250×4.6 mm, Macherey and Nagel, Düren, Germany). An eluent mixture of Acetonitrile and THF (48/52% wt/wt) was used at a flow rate of 0.2 ml/min. For the 2D experiments the temperature program involves a 10.5 min isothermal step at 20 °C followed by a 110 min linear temperature rise to 32 °C hold for 10 min. To assure complete elution the temperature was raised linearly to 57.5 °C within 10 min. Typically fractions of 220 μl were collected, the solvent was evaporated and the samples redissolved in THF for subsequent SEC analysis. Care was taken to assure that the added THF amounts were proportional to the sampling sizes of the fractionation step to prevent changes in the relative concentrations.

Subsequent SEC analysis of the fractions was performed on a high resolution column set (PL-Gel mixed B, mixed C, mixed D 30×0.8 cm each) using THF as the eluent. The setup consisted of Waters 510 pump run at 1 ml/min, TSP AS100 autosampler, Waters column oven, Waters 486 UV detector operated at 254 nm and Waters 410 RI-detector.

Table 1
Characterization details of precursor polymers

Precursor	M_n (g/mol)	M_w (g/mol)	M_{peak} (g/mol)
1	4200	4500	4800
2	9600	9900	10,500
3	17,000	20,000	22,500
4	28,000	32,000	34,000
5	36,000	38,000	42,000

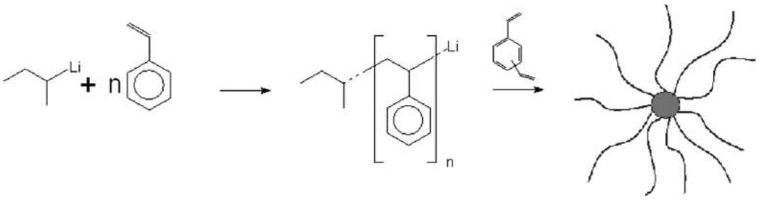

Fig. 2. Schematic representation of the route applied to synthesize the star polymers.

For SEC experiments toluene was used as an internal flow marker, to correct for flow rate fluctuations. SEC calibrations were performed using PS standards (PSS Polymer Standards Service GmbH, Mainz, Germany). Data acquisition and evaluation was performed using PSS WINGPC Software.

3. Results and discussion

Fig. 3 shows the previously determined dependence of elution volume on molar mass for star-shaped polymers of various arm length under identical TGIC conditions The graph was created using a 2D chromatographic setup. TGIC experiments on star polymers of different arm length were run in the first dimension and the eluting fractions of the stars were on-line injected into the SEC. SEC calibration using linear standards allowed the determination of the apparent molar mass for each fraction. A previously established correlation between the apparent and the true molar mass was used to calculate the true molar mass for each star polymer fraction. Thus, for each elution volume of the TGIC elution volume the true molar mass could be assigned. From Fig. 3 it becomes clear that elution is determined by the total molar mass of the star [23]. No effect of the molar mass of the arms is observed. The only exception is the star polymer having the lowest arm molar mass. It is exactly this star-shaped polymer that could be separated previously in an on-line 2D experiment from the linear polystyrene [23]. Because this star polymer shows a stronger retention than the other star polymers, the 2D experiment is expected to result in a better separation from linear polymers than for the other stars. However, no general reason exists why the given separation strategy according to Fig. 1 should fail for mixtures of linear and star polymers of higher arm molar mass.

It was therefore assumed that simply a lack of resolution in the SEC experiments causes the 2D experiment to fail. In order to test this hypothesis a TGIC separation was performed on a mixture of linear polystyrene standards and a star polymer having an arm molar mass of 10,500 g/mol. Fractions of 250 µl each were taken at different retention times of the TGIC experiment (first dimension) using a fraction collector. These fractions were subsequently analysed off-line on a high resolution SEC column set. Since the resolution of the SEC column set is higher than the resolution of the single column used in the previous on-line 2D experiments a separation of the fractions was expected to occur. The SEC chromatograms of the different fraction are given in Fig. 4. Each chromatogram clearly shows two different peaks with the separation of the peaks increasing with gradient retention time, i.e. with increasing molar mass. Since both peaks are expected to have similar molar mass, the peak at the higher SEC elution volume has to be assigned to the star polymer, due to the lower hydrodynamic volume of the branched molecule as compared to a linear one. The change in elution volume with gradient retention time is more pronounced for the linear species than for the star polymer, indicating the lower slope of the SEC calibration curve for linear polymers than for star polymers. The increase in molar mass for the star polymer is accomplished by an increasing number of arms, which results in a much weaker increase in hydrodynamic volume as compared to an increase in arm molar mass at constant arm number. As a consequence of the different slopes of the SEC calibration curves the separation of the peaks increase

Table 2
Synthesis and characterization details of star polymers synthesized

Precursor	%DVB[a]	M_n (g/mol)	M_w (g/mol)	M_{peak} (g/mol)	f_{peak}[b]	Residual precursor (%)[c]
1	12.5	79,000	107,000	100,000	24	5.2
2	12.5	450,000	610,000	461,000	48	2.6
3	12.5	390,000	463,000	460,000	27	9.0
4	12.5	529,000	605,000	618,000	18	16.0
5	12.5	680,000	799,000	810,000	19	13.6

[a] Weight fraction of divinylbenzene (DVB) used in synthesis.

[b] f_{peak}, arm number at peak maximum. $f_{peak}=M_{peak}/M_{n,arm}$.

[c] Obtained from relative area of RI-Signal.

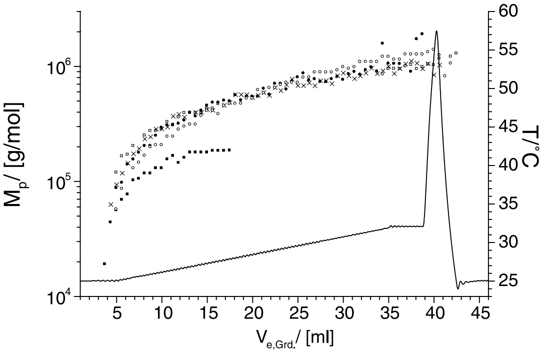

Fig. 3. Dependence of gradient elution volume on molar mass for star-shaped polymers of different arm length ((■) $M_P = 4800$ g/mol; (□) $M_P = 10{,}500$ g/mol; (×) $M_P = 22{,}500$ g/mol; (○) $M_P = 34{,}000$ g/mol; (●) $M_P = 42{,}000$ g/mol). (Nucleosil, C18, 5 μm, 300 Å, 25×0.46 cm^2. THF/AN = (50/50% wt/wt). Flow rate 0.1 ml/min [23].

with molar mass. The increasing difference of the hydrodynamic radii of the branched and linear polymers with increasing number of arms provides a better separation at a give molar mass (Fig. 4).

According to these results a 2D separation should be possible using a SEC column set with high separation efficiency as second dimension. We therefore fractionated several mixtures of the stars and linear polystyrenes by TGIC and analysed all fractions off-line by SEC. Since the elution volume of the first dimension is known from the fractionation it is possible to construct a complete off-line 2D separation from the individual SEC chromatograms. Two-dimensional chromatograms for such mixtures containing stars having an arm molar mass 10,500 and 42,000 g/mol are shown in Figs. 5 and 6, respectively. In both cases different elution regions can be found for the star-shaped and linear fractions, with the separation improving with increase in gradient retention time. The extrapolations over the peak maxima merge at SEC retention volumes of approximately 24.5 and 22 ml, corresponding to linear polystyrene molar masses of 350,000 and 130,000 g/mol, respectively. These values closely correspond to the expected molar masses of the first branched species, the three arm stars. From these figures it appears as if the separation is worse for the higher arm molar mass than for the lower one. However, assuming that TGIC separates according to molar mass only, it should be kept in mind that at a given gradient elution volume a short arm star has a higher number of arms than a star of higher arm molar mass. This means that for a given molar mass the difference in hydrodynamic size between the coeluting star-shaped and linear molecules becomes larger the higher the number of star arms, i.e. the lower the molar mass of the arms. Therefore, the separation in SEC improves with increasing arm number.

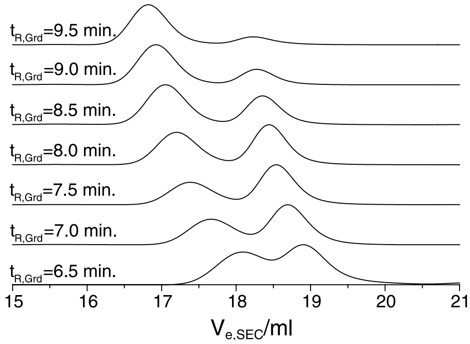

Fig. 4. Individual SEC-traces of fractions taken at different retention times of the TGIC experiment. The polymer mixture consists of polystyrene standards and star polymers having an arm molar mass of $M_{arm} = 10{,}500$ g/mol.

Comparing the SEC elution volumes of the linear polystyrene of molar mass 580,000 g/mol and the star polymer having arm molar mass of 42,000 g/mol at the same gradient elution volume we observe a difference in SEC elution volume of approximately 1.2 ml (Fig. 6). From the molar mass of the arms it follows that this star has approximately 14 arms. The 14 arm star with an arm molar mass of 10,500 g/mol is expected to coelute with a linear polystyrene of molar mass 147,000 g/mol. In Fig. 5 we estimate by interpolation for this molar mass a difference in SEC elution volume of approximately 1.5 ml. Thus, the similar number of arms results in a similar separation in the 2D experiment.

From Figs. 5 and 6 one might notice a shift of approximately 2 ml in the gradient elution volume for the high molar mass polystyrene standard. The observed difference in retention time corresponds to a temperature difference of approximately one degree and can easily be explained by small differences in the solvent quality for the two runs. High molar mass polymers will elute in gradient chromatography very close to their critical conditions [27–29]. These can be established either by eluent composition or by temperature. Therefore, a small change in the eluent composition has to be counterbalanced by a small temperature shift in TGIC.

Since the 2D chromatography allows identifying the molar mass of the first branched species, it should be possible to determine the amount of star-shaped polymers in a mixture with linear ones. The synthesis of the star polymers was performed by crosslinking polystyrene anions with divinylbenzene. It is known that non-reacted double bonds remain within the core. These double bonds change the UV spectrum and enhance the extinction coefficient of the star relative to that of linear polystyrene. This prevents the use of the UV detector for quantification of the relative amounts of the branched species. Thus, the quantitative evaluation was performed using the RI-signal, which, however, is less sensitive.

In order to quantify the amount of star-shaped polymer in the mixture with linear polystyrene we created artificial mixtures of linear and star-shaped polystyrenes of known compositions. The amount of residual precursor polymer in the crude star polymers was taken into account when calculating the star polymer fraction. The mixtures were

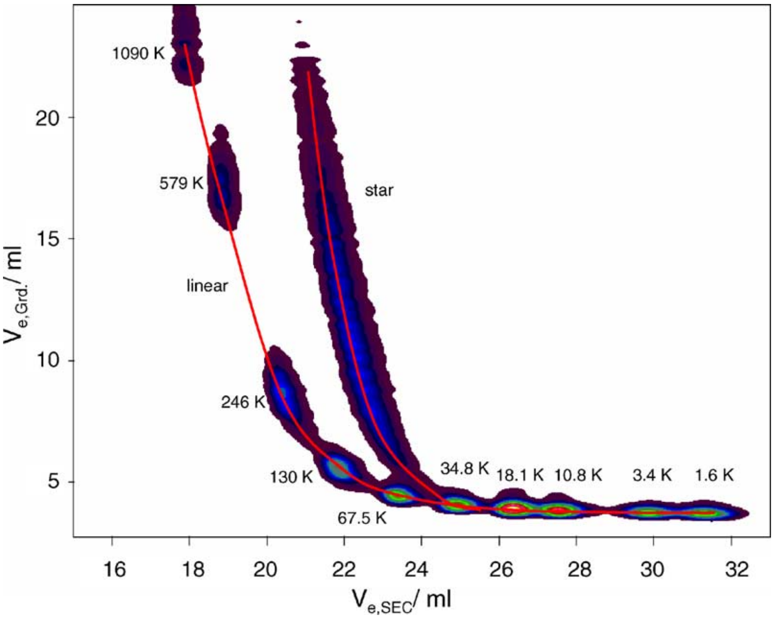

Fig. 5. Off-line 2D separation of a mixture of star polymers having an arm molar mass $M_{arm}=10{,}500$ g/mol and linear polystyrene standards.

separated by 2D chromatography and the weight fractions for the star polymers were calculated from the relative peak volume of the star polymer fraction to the total peak volume of the polymer. The comparison of the true and experimentally obtained star polymer fractions is given in Table 3. Although the agreement is not perfect, 2D chromatography gives a rather good estimation of the weight fraction of branched material. The deviations are probably due to the weak separation at low number of arms, where the linear and star fractions overlap in the 2D chromatogram (Fig. 4). It might be possible to increase the accuracy by deconvolution of the individual SEC chromatograms. However, the determination of the fraction of branched material in such a complex mixture would be very difficult by other means like SEC-viscometry or SEC-light scattering.

4. Conclusions

Using off-line 2D chromatography it was possible to separate mixtures of linear and star-shaped polymers having

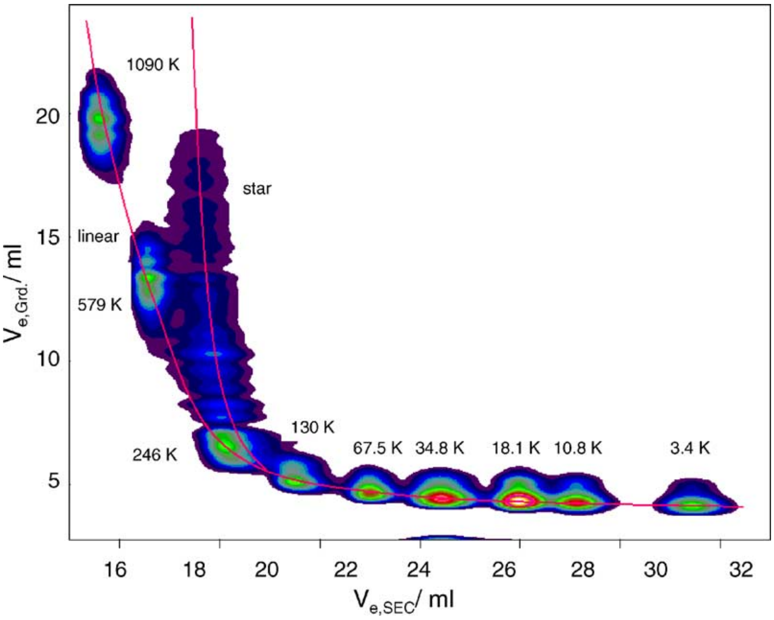

Fig. 6. Off-line 2D separation of a mixture of star polymers having an arm molar mass $M_{arm}=42{,}000$ g/mol and linear polystyrene standards.

Table 3
Calculated, w_{star}, and experimentally found, $w_{star,2D}$, weight fractions of stars for mixtures of linear and star-shaped polystyrenes

M_{arm} (g/mol)	w_{star} (%)[a]	$w_{star,2D}$ (%)
10,500	48	39
10,500	15	17
22,500	45	37
34,000	42	36
42,000	42	46

[a] Calculation corrected for residual precursor in crude star polymer.

arm molar masses of up to 42,000 g/mol. This clearly shows that 2D chromatography is a suitable method for the separation of different topologies. However, the small differences in the SEC elution volume between branched and linear structures having identical molar mass require a high separation efficiency in the second dimension (SEC). Therefore, such separations might not be possible on-line in some cases, but require off-line separations on high resolution SEC column sets. 2D separations allow obtaining reliable estimates for the amount of the branched species in the complex mixture, which is difficult to obtain by other means.

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