Comprehensive characterization of branched polymers
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Chapter 3: Branched-Polymer Separations using Comprehensive Two-Dimensional Molecular-Topology Fractionation × Size-Exclusion Chromatography

Abstract

Branching has a strong influence on the processability and properties of polymers. However, the accurate characterization of branched polymers is genuinely difficult. Branched molecules of a certain molecular weight exhibit the same hydrodynamic volumes as linear molecules of substantially lower weights. Therefore, separation by size-exclusion chromatography (SEC), will result in the co-elution of molecules with different molecular weights and branching characteristics. Chromatographic separation of the polymer molecules in sub-micron channels, known as molecular-topology fractionation (MTF), may provide a better separation based on topological differences among sample molecules. MTF elution volumes depend on both the topology and molar mass. Therefore co-elution of branched molecules with linear molecules of lower molar mass may also occur in this separation. Because SEC and MTF exhibit significantly different selectivity, the best and clearest separations can be achieved by combining the two techniques in a comprehensive two-dimensional (MTF×SEC) separation system. In this work such a system has been used to demonstrate branching-selective separations of star branched polymers and of randomly long-chain branched polymers. Star-shaped polymers were separated from linear polymers above a column-dependent molecular weight or size.
3.1 Introduction

Knowledge of the relationships between polymerization conditions and functional properties of the polymers being formed enables polymer chemists to make materials that are largely optimized for their application. High-performance polymers meet specific needs in the market place. The desired properties of such polymers are typically achieved by optimizing the parameters of the polymerization process. Such an optimization can be performed much more efficiently when key structural parameters affecting polymer properties are understood. Meaningful structure-property relationships can only be developed if the key structural parameters can be measured. In the case of branched polymers, a more detailed description of branching, beyond a basic estimate of the average number of branch points per molecule, is required. Distributions of the molecular properties must be revealed, which requires that the molecules with different degrees of branching be separated, ideally in combination with selective detection techniques. Knowledge of detailed molecular characteristics and their effect on functional properties will ultimately allow the design of high-performance polymers.

Spectroscopic techniques (e.g. Fourier-transform infrared, FTIR, or nuclear magnetic resonance, NMR) and physical measurements (e.g. light scattering or viscometry) are used on a routine basis to characterize the overall (or average) molecular structure of polymers. Using hyphenated techniques (typically combinations of a chromatographic separation with one or more spectroscopic or physical methods) more information concerning the distributed properties may be obtained. Size-exclusion chromatography (SEC) with light scattering and/or viscometry detection is commonly used to characterize long-chain branching (LCB) in high-molecular-weight polymers [1,2,3]. The characterization of LCB in polymers is of particular interest, because of the influence of LCB on processing properties, such as zero-shear viscosity and melt strength. Branching factors based on the Zimm-Stockmayer theory [4] may be calculated when a linear-polymer (reference) sample with identical chemistry or its Mark-Houwink parameters are available. These can subsequently be converted to branching frequencies if assumptions are made regarding the functionality of the branching points and the average branch length. SEC with selective detection is, however, not able to fully characterize branched polymers. Separation by size of the
unperturbed chain in solution yields fractions containing molecules with equal hydrodynamic volumes, but with different topologies and molecular weights. This distribution cannot be characterized by selective detection techniques. For example, light scattering only provides the weight-average molecular weight for the ensemble of chains eluting in each SEC fraction. Molecular-weight polydispersity at a given SEC elution volume was recently confirmed by comparing selective-detection techniques that yielded different types of molecular-weight averages (weight average from light scattering and number average from viscometry, [5]). The authors demonstrated that the so-called local polydispersity was affected by the distribution of the degree of branching and the functionality. NMR is an alternative technique for determining the structure and the frequency of branch points, but the technique has some limitations. A high-field instrument is needed to detect and quantify low levels of LCB, but discrimination of different branch lengths is still not possible when branches are longer than a few carbon atoms [6]. Most importantly, NMR provides only an average number of branches per molecule.

Multi-dimensional separations can be used to study complex polymers that feature more than one distribution simultaneously. In a comprehensive two-dimensional separation system, denoted by the “×” sign, every part of the sample is subjected to two independent mechanisms and the separation obtained in the first dimension is maintained in the final two-dimensional chromatogram) [7]. The peak capacity is increased substantially by comprehensive operation of multi-dimensional separations. However, the separation power is only used efficiently when different selectivity in each separation dimension allows the sample to be separated among its distributions of interest [8,9]. Only in orthogonal separations the retention times in the different dimensions are by definition completely independent (uncorrelated). Although most multi-dimensional separation systems are not orthogonal, confounded distributions that remain unresolved in a single separation step can be separated using two independent separations with different selectivity. Therefore, complex polymers with distributions in distinct molecular properties can successfully be resolved using multi-dimensional systems. Separations by functionality [10] and chemical composition [11] have, for example, been combined with separations according to size using SEC to fully elucidate two mutually dependent distributions.
Branched polymers can also be separated using combinations of independent separations, such as interactive liquid chromatography and SEC, in a comprehensive two-dimensional setup. Selectivity for branched versus linear polymers has resulted from differences in the number of repeat units, number of branch points or size in solution. Star polymers prepared by coupling living polystyrene anions were separated by an off-line combination of temperature-gradient interaction chromatography (TGIC) and SEC [12]. The TGIC separation is thought to be based on molecular weight, while SEC is based on the size of molecules in solution. The relationships between molecular weight and hydrodynamic volume are different for branched and linear polymers allowing separations of differently branched polymers by a combination of both methods. Similar star polymers of lower molecular weights were separated on-line by liquid chromatography at the critical composition in combination with either SEC or TGIC (LCCC×SEC or TGIC×LCCC [13]). In the LCCC separation, branched polymers were separated by interaction of the apolar side-groups at the coupling agent. The techniques described here yielded good separations for branched homo-polymers with numerous branches and chemically different branch points or end groups. High-molecular-weight polymers, with very little long-chain branching (LCB), or without functional groups at the branch points or chain ends of different polarity cannot be separated using these techniques.

For LCB polymers, complete separation may be obtained when the polymer is also separated based on branching parameters. Such a separation has previously been demonstrated on monolithic columns containing sub-micron macro pores [14] and on columns packed with sub-micron particles [15]. Both separation systems featured sub-micron flow channels. Polymers above a stationary-phase dependent molecular weight become retained at low flow rates. Branched polymers were found to elute much later than linear ones of the same molecular weight. This separation method was termed molecular-topology fractionation (MTF) and it was thought to result from the topology-dependent relaxation-time spectrum of polymers in dilute solution [15]. The word topology reflects the geometrical structure of the polymer molecules, more specifically the branch length, frequency and functionality of the branch points. Separation of branched polymers by MTF can only be applied to samples with very narrow molecular-weight distributions, since the degree of polymerization also affects the
retention. Off-line fractionation of LCB polymer by SEC and re-injection of the fractions in MTF was used to demonstrate the differences in selectivity of the two techniques for LCB polymers [15]. Similar to the comprehensive two-dimensional separation systems (described in the previous paragraph) for separating star polymers, samples featuring LCB could be resolved when the separation dimensions display significantly different selectivity towards long-chain branching and hydrodynamic size.

In this paper, the separation of long-chain branched polymers using MTF×SEC will be demonstrated. Knowledge of the relationship between molecular weight, hydrodynamic size and branching will be used to interpret the selectivity in MTF separations. The separation of polymers with similar hydrodynamic size, but different topologies is demonstrated for star polymers with narrow molecular-weight distributions. Results on the separation of randomly long-chain-branched polymers and star polymers will be used to discuss the selectivity of MTF and the applicability MTF×SEC for the separation of complex samples of branched polymers.

3.2 Experimental

3.2.1 Samples and materials

The eluent for MTF and SEC separations was non-stabilized HPLC-grade tetrahydrofuran (THF; Biosolve, Valkenswaard, The Netherlands); it was continuously degassed by purging with helium 5.0 (99.999% Praxair, Vlaardingen, The Netherlands). Sample polymers were dissolved in HPLC-grade THF stabilized with 250 ppm butyl-hydroxylated toluene to prevent degradation by radicals. Narrowly distributed linear polystyrene standards (Polymer Laboratories, Church Stretton, UK) were used to study retention behaviour. These standards were dissolved at concentrations of 0.5 mg/mL. A nominal three-arm star polystyrene sample was obtained from Polymer Source (Dorval, Canada) and used at a concentration of 1.0 mg/mL. This star polymer was synthesized by coupling of anionically polymerized arms with a tri-functional agent (α,α’,α”-trichloromesitylene). The manufacturer specified a nominal molar mass of 1,480 kg/mol for the precursor arms. However, thorough analysis using size-exclusion chromatography with low-angle light scattering and differential viscometry revealed an arm molar-mass closer to 1,250 kg/mol. The sample composition was determined from
the same experiment. Integration of the concentration signal revealed ~5% to be uncoupled precursor, ~45% linear polymer with double the precursor molecular weight and a remainder of three-arm coupling product. A small amount of higher-coupling products as a result of lithium-halide exchange [16,22] was evident from the overall molecular weight of the star-polymer sample as estimated by SEC with light-scattering detection. A four-arm coupling side-product by lithium-halide exchange is expected to have all arms coupled in one functional centre. The concentration of this large-molecule fraction could not be determined quantitatively by SEC. The reaction scheme of the coupling and analysis results have been presented by Meunier et al. [15]. Polystyrene with a high LCB frequency was obtained from the Dow Chemical Company (Midland, MI, USA). The Mark-Houwink plot and information on the molecular-weight distribution of this material can be found in the supplementary information of this article. Details regarding the preparation of this high-LCB sample can be found elsewhere [17].

A custom-made 150 mm × 4.6 mm I.D. column packed with 10-µm 10 Å PLgel particles by Polymer Laboratories was used for fast SEC as the second dimension separation at a flow rate of 750 µL/min. The MTF-column packing consisted of a polydisperse mixture of particles in the range of 0.1 to 1 µm (Admatech, Aichi, Japan). Particle-size-distribution data provided by the supplier revealed that the average particle diameter was 0.5 µm and the half-width of the distribution was about 0.3 µm. The particles were functionalized with C₈ chains to facilitate the packing procedure. A 150 mm × 4.6 mm I.D. column was packed by Diazem (Midland, MI, USA) using an identical procedure as that used previously for packing columns for MTF [15].

3.2.2 Instrumentation and methods

Comprehensive two-dimensional MTF×SEC was performed on a system assembled in-house. Basic components of the system were two LC10ADvp pumps (Shimadzu, ‘s Hertogenbosch, The Netherlands) to perform isocratic separations in the two dimensions, along with an SCL10a system controller (Shimadzu) for interfacing with the data-acquisition computer. Either 10 or 20 µL injections of the samples were performed by a SIL9a autosampler and columns were kept at 50°C in a CTO7 column oven (both from Shimadzu). Detection in MTF×SEC experiments was performed using
Branched-Polymer Separations using Comprehensive 2D MTF × SEC

A Spectroflow 757 (ABI, Ramsey, NJ, USA) UV-absorbance detector equipped with an 8-µl flow cell. Data acquisition was typically performed at 5 Hz, recording the signals of both detectors. A capillary UV detector was used (Linear UVIS 200, Linear Instruments, Reno, NV, USA) to record the data for the calibration curve in Fig. 1. The detector wavelength of UV detectors was set to 260nm, close to the absorption maximum of polystyrene in tetrahydrofuran.

Modulation in comprehensive two-dimensional separations was accomplished with an air-actuated VICI two-position 10-port valve (Valco, Schenkon, Switzerland). This valve was operated using a high-speed switching accessory. The digital valve interface (DVI; Valco) was connected to the SCL10a system controller. The 10-port valve was plumbed for symmetrical dual-loop modulation [11]. Two injection loops of equal volume (43 or 92 µL) were used. From the moment of injection on the MTF column, the 10-port valve was switched either every 2, 2.67 or 4 minutes in order to inject 40 µL from the first dimension (running at 20, 15 or 10 µL/min respectively) at the SEC column. Instrument control and data acquisition were achieved with ClassVP v7.4 build15 software (Shimadzu). Exported data were processed in Matlab v7.3 (The Mathworks, Natick, MA, USA) using in-house written software for data folding and visualization of two-dimensional colour plots.

3.3 Results and discussion

3.3.1 Calibration curve for molecular-topology-fractionation column
Linear polystyrene standards with a well-defined molecular-weight distribution (MWD) are readily available, in contrast to well-characterized branched polymers with high molecular weights. Therefore, linear polystyrenes were used to determine retention behaviour in MTF as a function of molecular weight. The elution volume at the peak maximum is plotted against the logarithm of the peak molecular weight in Fig. 1. Reversal of the curve is observed around 200 kg/mol. The molecular weight where such a reversal occurs will be referred to as the critical molecular weight M_{crit} for reversal. The elution order for polymers below M_{crit} was consistent with that observed for polystyrenes separated on columns packed with 1-µm, non-porous particles and can be explained as hydrodynamic chromatography [18]. The interest in MTF stems from the
elution region above $M_{\text{crit}}$, because in this range the selectivity for branching (molecular topology) has been observed [14, 15]. Although branched molecules are more effectively retained than linear ones (section 3.2), the flow-rate effect on retention of linear molecules may be used as a benchmark for the MTF selectivity of the column.

Retention times of linear polymers above $M_{\text{crit}}$ were measured at two different flow rates. The influence of flow rate on retention volume is much larger for high molar masses, i.e. above $M_{\text{crit}}$. Elution-order reversal has also been observed for polystyrene standards in hydrodynamic chromatography (HDC) on columns packed with 1-µm non-porous particles [18], but in that case the effect is very much smaller than observed for MTF. In HDC the reversal in the calibration curve has been explained by shear deformation of the polymers in solution [18]. After such a deformation the radius of the polymer molecules perpendicular to the direction of flow is effectively smaller compared to its unperturbed state. As a result the deformed molecules can get closer to the channel walls, where the linear velocity is lower, and elute later from HDC columns. Reversal due to polymer deformation is expected to be observed most strongly at high shear and thus high flow rates. This is indeed observed in HDC, but not in the present MTF system, where the effect is strongest at the lowest flow rates (Fig. 1). Our calibration curves (molecular weight vs. elution volume) are thus not in agreement with HDC data. However, our results are in agreement with observations in previous MTF studies [14]. Thus, the separation mechanisms in HDC and MTF are based on different principles.

![Fig. 1. MTF calibration curve for linear polystyrenes; • obtained at a flow rate of 20 µL/min, • at 10 µL/min.](image)
One important difference between the more conventional chromatographic separation techniques (SEC, HDC, or field-flow fractionation, FFF) and MTF is the aspect ratio ($\lambda$), defined as the ratio of the effective radius of the polymer in solution and the radius of the channel that it is migrating through. Hydrodynamic separation techniques (such as HDC) are typically operated below $\lambda = 0.2$ [19], whereas branching selectivity in MTF is obtained only at values of $\lambda$ that exceed this value. HDC theory predicts that for large values of $\lambda$ the forces resulting from rotation and solvent lagging (inertia) will reduce the migration rate of the polymer, ultimately resulting in elution volumes greater than the column volume. Shear alignment or deformation at such high values of $\lambda$ may be responsible for decreasing retention with increasing flow rate for large polymers (above $M_{\text{crit}}$). However, this is unlikely in MTF considering that the linear velocity of the mobile phase is several times lower for MTF than in HDC with 1-µm particles [18].

A quantitative comparison of $\lambda$ values for the different separation systems cannot be made, because absolute values of the average diameter of the flow-through channels are hard to obtain for the column used in this study. The flow path in particle packed beds is much more complex than that in open-tubular channels, for which HDC theory was derived. Successful attempts to relate hydrodynamic retention in particle packed beds with retention in capillaries were made by using the hydraulic radius to define the interstitial channel diameter [18, 20, 21]. However, the polydisperse packing material of the present MTF column complicates the use of the classical concepts. Stationary phases with well-defined channel parameters will have to be used to for a robust comparison of HDC and MTF in terms of the aspect ratio.

### 3.3.2 Branched-polymer separations

Because branched polymers and linear polymers of the same hydrodynamic size co-elute in SEC, triple-detection SEC can only be used to obtain the average number of branches per molecule at any given elution volume. Therefore, branching properties cannot be fully characterized when polymers are separated by hydrodynamic size only. Comprehensive two-dimensional separation by hydrodynamic size and by branching properties will be used to demonstrate this point. MTF is used in the first dimension to fractionate polymers that vary in molecular weight and/or branching properties. This choice for MTF in the first dimension is dictated by the experimental conditions. MTF
is operated at flow rates between 10 and 20 µL/min on a 150 mm × 4.6 mm I.D. column, resulting in analysis times of one or several hours. Therefore, MTF is convenient as a (slow) first-dimension separation, but it cannot be applied as a (fast) second-dimension separation. In MTF×SEC, 120 fractions of 40 µL each were collected using the two-way 10-port switching valve. These fractions were injected and analysed in real time on a fast SEC column. The same number of fractions was collected, irrespective of the first-dimension flow rate. The time used for collection and second-dimension separation was adapted to the first-dimension flow rate. By keeping the number of fractions and the second-dimension flow rate constant, we were able to directly compare the resulting chromatograms in terms of (MTF) resolution in relation to the hydrodynamic size (SEC retention volume) of the molecules.

A sample of a star polymer, prepared by coupling anionically polymerized linear polystyrene [22], was used for our studies. Because the PS-precursors possess a very narrow molecular-weight distribution, the sample exhibits a nearly discrete relationship between molecular weight and topology (the number of branches connected to the coupling point in the molecule). The molecular weight of the PS-precursor was determined to be 1,250 kg/mol [15]. In the star synthesis of the star polymers, coupling was performed by reaction of the living ends of the precursor polymers with a trifunctional coupling agent. Besides a three-arm star molecule, some unreacted linear polymer remains and linear two-arm polymers are formed, as well as some higher order coupling products. The presence of a four-arm star was first demonstrated using one-dimensional MTF with low-angle-light-scattering detection [15]. More details on the synthesis and composition of the star polymer can be found in the experimental section. Linear polymers with molar masses comparable to the coupling products of the precursor polymer were injected for reference purposes.

Coupling of one, two or three precursor polymers (arms) used for the star-polymer sample would result in the peaks as observed in figure 2a, b and c if the MTF separation would be based solely on molecular weight. In the chromatogram of the star sample in Fig. 3a, which was obtained using identical experimental conditions, two peaks (1.60 and 2.68 mL) are observed at MTF elution volumes higher than any of the peaks observed in Fig. 2. The elution volumes corresponding to peak-maxima have been
summarized in Table 1 for both dimensions. Because retention of branched molecules is expected to be higher in MTF, it can be tentatively concluded that the peak in Fig. 3a at $V_{MTF} = 1.6 \text{ mL}$ is due to the three-arm star-shaped coupling product. Comparison of $V_{SEC}$ values suggests that the hydrodynamic size of the three-arm star is close to that of the linear polymer of 2,536 kg/mol. Both the increased retention in MTF and the decreased elution volume in size-exclusion chromatography compared to linear polymer of identical mass can be explained by branching. The last peak eluting in Fig. 3a is consistent with a higher order star as the peak has nearly the same SEC retention volume as the three-arm star, but is retained much longer in the MTF column. The probability of such products being formed decreases with increasing functionality, but the presence of a peak corresponding to the four-arm coupling product is clearly visible in Fig. 3a.

At lower MTF flow rates the components of the star-polymer sample were better separated. This is illustrated in Fig. 3b and 3c where flow rates of 15 and 10 $\mu$L/min were used. At low flow rates the calibration curves in MTF become less steep (Fig. 1) and branched components are retained much longer. Because of the very slow molecular diffusion of high molecular-weight polymers we do not anticipate increased band broadening at very low flow rates. Indeed, peak broadening is hardly affected by the long residence time in the MTF column (Fig. 3). Comparison of the peak widths in Fig. 3 with those of the linear polystyrene standards (with $M_w/M_n$ 1.03 – 1.04) in Fig. 2 shows that the peak widths of branched polymers are not significantly greater than those of narrowly distributed linear standards. The observed broadening may be due to the limited efficiency of the column. Furthermore, it is known that overloading occurs easily in HDC. Therefore, overloading may also be a threat when using MTF. The low porosity ($\epsilon = 0.3$) of the column may well aggravate the loadability issues.

To assess whether shear degradation was occurring in the system, polymer elution was studied as MTF flow rate was varied. It was speculated that at relatively high flow rates in MTF shear-induced degradation (i.e. chain scission) of the polymer could occur. Because even partial degradation of the polymer is likely to have a significant effect on the hydrodynamic-size distribution of the polymer, MTF×SEC provides information on the likelihood of chain scission. Neither increase in SEC elution volume nor tailing in
the SEC dimension towards lower molecular weights was observed as MTF flow rate was increased. Thus, polymer molecules do not appear to be shear degraded as a result of MTF separation. Shear degradation in the SEC separation has been addressed in the supporting information of this article. No significant evidence for shear degradation that might impair the integrity of MTF × SEC was found. It is much more difficult to establish whether or not the polymer molecules are deformed during the MTF separation. If they are, it is likely that the molecules relax to their unperturbed shapes before they are analysed by SEC or characterized by light scattering.

Table 1. Peak maxima in MTF and SEC for different first-dimension flow rates.

<table>
<thead>
<tr>
<th>V_{max} (mL)</th>
<th>MTF 20 µL/min</th>
<th>MTF 15 µL/min</th>
<th>MTF 10 µL/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,373 kg/mol</td>
<td>1.08</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>2,536 kg/mol</td>
<td>1.24</td>
<td>1.35</td>
<td></td>
</tr>
<tr>
<td>3,742 kg/mol</td>
<td>1.24</td>
<td>1.31</td>
<td></td>
</tr>
<tr>
<td>1-arm linear polymer</td>
<td>1.00</td>
<td>1.47</td>
<td>1.12</td>
</tr>
<tr>
<td>2-arm linear polymer</td>
<td>1.16</td>
<td>1.37</td>
<td>1.40</td>
</tr>
<tr>
<td>1.60</td>
<td>1.34</td>
<td>2.12</td>
<td>1.34</td>
</tr>
<tr>
<td>4-arm star polymer</td>
<td>2.68</td>
<td>1.33</td>
<td>&gt; 3.5</td>
</tr>
</tbody>
</table>

Another example of the separation of a branched polymer is presented by the separation of a broadly distributed polystyrene (PDI = 3.3, see supplementary information) with random long-chain branching (LCB) in Fig. 4a. For a sample with a considerable degree of LCB (MTF flow rate 20 µL/min) a low-concentration tail towards higher MTF elution volume is observed which is considerably different from that of the three-arm star polymer sample. However, the star sample contained discrete populations of branched species which could be separated into discrete peaks in the MTF separation. On the other hand, the broadly distributed PS contains a nearly continuous distribution of branched components varying in the number of branch points and branch lengths. The fact that peaks in the MTF separation tail to larger elution volumes, while the SEC separation becomes constants, suggests that this distribution of branching may result in separation in the MTF direction. Elution of this material in the SEC dimension was compared to that of the linear polymers shown in Fig. 2. The peak maximum of the
Branched-Polymer Separations using Comprehensive 2D MTF × SEC

branched PS in SEC for an MTF elution volume of 1.24 mL was 1.47 mL. This is considerably different from the 1.35 mL and 1.31 mL that were found for linear polymer (table 1).

Fig. 2. (left). Comprehensive two-dimensional MTF×SEC of linear polystyrene standards with MTF at 20 µL/min. Nominal molecular weights (a) Mₚ 1373 kg/mol, (b) 2536 kg/mol, (c) 3742 kg/mol, (a)/(b)/(c) 126.7 kg/mol (internal-reference peak in top-left corner).

Fig. 3. (right). Comprehensive two-dimensional MTF×SEC of linear and star-branched polymers. (a) MTF at 20 µL/min, (b) MTF at 15 µL/min, (c) MTF at 10 µL/min.
Fig. 4. Separations of a polystyrene sample with a broad MWD and a high degree of LCB
(a) MTF×SEC, (b) SEC×SEC

An important question is whether or not the samples are fully eluted from the MTF column (featuring sub-μm flow-through channels). Recovery may be negatively affected if the flow rate is decreased in order to increase resolution. At 15 μL/min the four-arm star polymer is no longer observed to elute within about five column volumes (Fig. 2b). The chromatograms in Fig. 2 were integrated over MTF volumes from 0.5 to 3.5 mL and SEC volumes from 1.15 to 1.7 mL. Compared to MTF performed at 20 μL/min, only 89% of the sample was eluted at 15 μL/min in the same retention window. For 10 μL/min this relative recovery drops to 80%. The sample that is not recovered is expected to elute after the elution window as a result of increased retention. The separation was not extended long enough to observe all the branched polymers at flow rates below 20 μL/min. Therefore, the run length was increased in subsequent experiments for recovery studies (Fig. 5a). Furthermore, these experiments were all performed at 20 μL/min.

Fig. 5. Separations of the star-polymer sample (a) MTF×SEC, (b) SEC×SEC
To assess absolute sample recovery in the MTF×SEC system, the MTF column was replaced by a SEC column ($V_0 = 1.2 \text{ mL}$; Fig. 4b and 5b). The experimental conditions were equal to those for MTF×SEC, except that the first-dimension separation was run until only 1.5 times the total permeation volume had passed through the first-dimension SEC column. No polymer is expected to elute after this point. This showed that in the MTF×SEC set-up linear polymers with high molecular weight (Fig. 2) and the LCB polystyrene (Fig. 4) were all recovered quantitatively (> 95%). The star polymer sample was recovered for 92% (average of triplicate MTF×SEC measurements) when integrated over MTF volumes from 0.5 to 4.7 mL (Fig. 5a). When the integration was extended to a volume of 7.2 mL the recovery was also found to be quantitative (>95%).

### 3.4 Conclusions

Molecular-topology fractionation (MTF) provides branching-selective separation. The technique can be used to separate molecules according to their degree of long-chain branching, but only for samples with extremely narrow molecular-weight distributions. In all other cases, the effect of branching on retention in MTF is confounded with the effect of molecular weight. A solution to this problem has been found by combining MTF and size-exclusion chromatography into a comprehensive two-dimensional separation system. This MTF×SEC technique was used to clearly demonstrate the branching-selective separation obtained by MTF for a sample of (narrowly distributed) star-shaped polystyrenes. MTF×SEC was also applied to a broadly distributed polystyrene sample that featured a high degree of long-chain branching (LCB). Although some selectivity was observed, the separation may need to be improved if we are to obtain quantitative measures for LCB. However, even in the present, immature state, the fractionation of LCB polymers may prove to be useful in predicting rheological properties of polymers.

Only high-molecular-weight polymers were separated using MTF for this study. The range of applicability of MTF is limited to the range above the reversal molecular weight ($M_{\text{crit}}$), which in turn depends on the diameter of the flow-through-channels. Several improvements are foreseen in the near future. The presently used MTF column was packed with polydisperse particles. It is difficult to obtain such columns, let alone
pack them reproducibly. Even the repeatability of nominally identical columns is poor. It is also difficult to use these columns to perform fundamental studies on MTF, because accurate information regarding the size of the inter-particle (flow-through) channels is cannot be obtained. Because it is difficult to accurately characterize the channel dimensions in these packed columns, the relationship of the former with elution behaviour is challenging to evaluate. Columns packed with mono-disperse particles are needed to sensibly compare hydrodynamic chromatography (HDC) and MTF. However, because the two techniques operate in different regimes of the aspect ratio (size of molecules compared with that of the flow channels) and because the effects of changes in the flow rate were found to be opposite, we believe that HDC and MTF are based on different separation mechanisms. Columns that are well packed with uniform sub-micron particles are also difficult to obtain. Monolithic stationary phases with well-characterized sub-micron flow-through pores may be a viable alternative. The use of such monolithic columns for MTF separations will be reported elsewhere.

3.5 Appendix

*MTF column parameters*

The column volume and efficiency were determined by injection of 5 µL of a 1000-ppm solution of ethylbenzene. At a flow rate of 20 µL/min THF and a column-oven temperature of 50°C ethylbenzene eluted at 38.1 minutes. Therefore, the MTF column volume ($V_0$) was 762 µL, the efficiency of the separation was 3400 plates per meter. The porosity of the packing was calculated by dividing the ethylbenzene elution volume by the theoretically calculated volume of the empty column (150 x 4.6 mm) and was $\varepsilon = 0.30$.

*Characterization of long-chain-branched polystyrene sample*

The long-chain-branched (LCB) polystyrene sample was characterized using size-exclusion chromatography (SEC) with multi-angle light scattering (MALS) and on-line viscometry detection. The SEC columns used were three mixed-B columns (300x7.5 mm each; 10-µm particles) from Polymer Labs (Church Stretton, UK). Stabilized THF
(J.T. Baker, Deventer, The Netherlands) was used as the mobile phase at a flow rate of 1 mL/min.

The weight-average molecular weight of the LCB polymer (measured by SEC-MALS) was 810 kg/mol with a polydispersity of PDI = 3.3. The presence of long-chain branching at high molecular weights is illustrated by the reduction in intrinsic viscosity in the Mark-Houwink plot in Fig. 1.

![Mark-Houwink plot](image1)

**Fig. S1.** Mark-Houwink plot for the long-chain branched polystyrene used in this article and a linear reference polystyrene polymer.

**Verification of SEC at elevated flow rates**

SEC was performed at two and a half times the recommended flow rate in the MTF×SEC experiment. The impact of separation at elevated flow rates was validated by performing SEC separations comparable to those in a two-dimensional experiment at the recommended and elevated flow rates.

SEC was performed at 300 and 750 µL/min on a 150 mm x 4.6 mm I.D. column with 10-µm PLgel particles with a pore size of 10^6 Å. The porosity of the frits in this column was 5 µm. The mobile phase was non-stabilized tetrahydrofuran (Biosolve, Valkenswaard, The Netherlands) and separations were performed at 50°C. The injection volume was 5 µL of polymer solution. Four samples and one blank were injected in duplicate. Polystyrenes with peak molecular weights of 7,450, 3,742, 2,536 and 1,373 kg/mol (Polymer laboratories, Church Stretton, UK) were dissolved in tetrahydrofuran individually at a concentration of 0.1 mg/mL. The samples with 3,742, 2,536 and 1,373
kg/mol polystyrene were all used with an additional 0.2 mg/mL of 126.7 kg/mol polystyrene (reference standard). All four samples and the blank contained approximately 250 ppm butyl-hydroxylated toluene to prevent degradation by radicals. Detection was performed using a Spectroflow 757 (ABI, Ramsey, NJ, USA) UV-absorbance detector equipped with an 8-µl flow cell and set for detection at 260 nm.

The chromatograms have been overlaid in Fig. S2. An x-axis multiplier was chosen to have this x-axis display the elution volume. Peak assignment for Fig. S2 from left to right: 7,450, 3,742, 2,536, 1,373 and 126.7 kg/mol linear polystyrene, ionol, injection-solvent related peak.

For all high-molecular-weight PS polymers a shift towards higher elution volume is observed when separated at 750 µL/min. This shift is small compared to the separation of the individual standards and therefore has a small effect on the separation. The shift in elution volume may possibly be explained by the slow diffusion of high-molecular-weight polymers, being responsible for incomplete inclusion of the polymer in the pores of the packing material. If any shear degradation were to occur, this would be expected to result in significant tailing and changes in the peak shape. Only the peak front of the 7,450 kg/mol appears to be deformed at 750 µL/min. Absolute molecular-weight determination techniques, such as low-angle laser light scattering, can be used to discriminate between poor chromatography or shear degradation. Because fast SEC is not used for absolute-molecular-weight determination, this discussion is beyond the

![Fig. S2. UV absorbance of high molecular weight polystyrenes at 750 µL/min (offset 0 mAU) and 300 µL/min (offset 15 mAU). Retention times are annotated in red for 7,450 kg/mol PS, ionol and an injection-solvent related peak.](image-url)
scope of the present article. Based on the results in Fig. S2 we conclude that peak
elution volumes for polystyrene polymers up to a molecular weight of 7,450 kg/mol
may be used to compare hydrodynamic size parameters at 750 µL/min. under the
conditions used for MTF×SEC.

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