Comprehensive characterization of branched polymers

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Chapter 4: Branched Polymers Characterized by Comprehensive Two-Dimensional Separations with Fully Orthogonal Mechanisms

Abstract

Polymer separations under non-conventional conditions have been explored to obtain a separation of long-chain branched polymers from linear polymers with identical hydrodynamic size. In separation media with very narrow flow-through channels (of the same order as the size of the analyte molecules in solution) the separation and the elution order of polymers are strongly affected by the flow rate. At low flow rates the largest polymers are eluted last. At high flow rates they are eluted first. By tuning the channel size and flow rate, conditions can be found were separation becomes independent of molar mass or size. Other differences between polymer molecules are revealed, such as the extent of long-chain branching. This type of separation is referred to as molecular-topology fractionation (MTF) at critical conditions. MTF involves partial deformation of polymer coils in solution. The increased coil density and resistance to deformation can explain the different retention behavior of branched molecules. Much higher efficiency and selectivity were obtained by MTF in columns than with traditional membrane fractionations. MTF in combination with size-exclusion chromatography (SEC) was applied for the separation of branched polymers. Branching selectivity was demonstrated for three- and four-arm “star” polystyrenes of 3 to $5 \times 10^6$ g/mol molar mass. Baseline separation could be obtained between linear polymer, Y-shaped molecules, and X-shaped molecules in a single experiment at constant flow rate. For randomly branched polymers the branching selectivity inevitably results in an envelope of peaks, because it is not possible to fully resolve the huge numbers of different branched and linear polymers of varying molar mass. Separations performed by comprehensive two-dimensional MTF×SEC revealed the presence of branched polymers that could not be discerned with one-dimensional SEC in combination with mass-selective detectors, such as light scattering or viscometry.
4.1 Introduction

Branching that is accidentally or purposefully introduced in (high-molar-mass) polymers may be advantageous. Long chain branching in thermoplastics improves melt strength and flow, which allows for faster processing and unique applications [1]. Through the introduction of branching a favorable balance may be obtained between modulus (i.e. stress-strain relation), viscosity, and elongation behavior [2,3,4]. A major challenge up to this day remains the analysis of long-chain-branched (LCB) polymers, because a distribution of different topologies (or “qualitative geometries”) [5,6] is confounded with a molar-mass distribution (MMD). In many polymer samples both linear molecules and molecules with various degrees of branching are present, depending on the polymerization conditions. Unequivocally demonstrating the properties of branched-polymeric materials can only be achieved by synthesis and physical testing of model compounds with well-described branching topologies [7]. Branching may also be introduced by post-synthesis blending of linear polymers with LCB molecules. Although the positive effects of branched polymers remain after blending with other polymers, no existing techniques can specifically characterize the properties of branched molecules. Recent theories on structure-property relationships for branched polymers emphasize the role of the intra-molecular structure [8]. For material characterization it is, therefore, important that polymers can be separated according to their structure and that molecules can be discriminated based on their topology. However, conventional polymer-analysis techniques cannot be used to perform such separations for LCB polymers. Separations by either branches or end groups based on interaction chromatography [9,10] may be possible for polymers of low to moderate molecular weight with chemically different end groups [11]. High-molecular-weight short-chain-branched (SCB) polyolefins may also be analyzed with gradient liquid chromatography [12]. While spectroscopic techniques offer the best possibility to identify functional groups, they only provide information on population averages of the sample. Hyphenation of size-exclusion chromatography (SEC) with NMR or FTIR spectroscopy has been applied successfully to investigate distributions in terms of functionality [13] or SCB frequency [14]. Studying the extent of LCB using this approach is extremely difficult, because the low frequency of functional groups (i.e. branch points) would require an exceptional sensitivity and dynamic range [15].
Polymers of high molar mass and moderate to low degree of branching are typically separated based on their hydrodynamic size using SEC or field-flow fractionation (FFF), often followed by selective detection using on-line viscometry and/or light scattering [16,17]. Results for branched polymers are commonly reported as so-called contraction ratios based on radius of gyration ($r_g$, Eq. 1, [18]) or intrinsic viscosity ($\eta$, Eq. 2, [19]).

$$g = \left[ \frac{(r_g)_B^2}{(r_g)_L^2} \right]_M$$  \hspace{1cm} (1)

$$g' = \left[ \frac{[\eta]_B}{[\eta]_L} \right]_M$$  \hspace{1cm} (2)

where $B$ refers to branched polymers and $L$ to the linear-equivalent reference polymer and the subscript $M$ indicates molecules of equal mass. LCB has a strong impact on the solution properties of macromolecules, such as the relation between hydrodynamic size, molar mass and intrinsic viscosity. Contraction ratios can be used to estimate the branching frequency and to obtain information on the topology [20] when information on polymer chemistry and linear reference polymers are available. This methodology has developed over the years into the most popular method for LCB analysis [21]. Nevertheless, the separation by SEC is based on the hydrodynamic size of the analyte molecules and is only indirectly related to branching and topology. This is a limitation and a source of errors inherent to this method. As in conventional SEC analysis, a significant bias may be accepted in practice, especially when comparing similar polymers (prepared by identical chemistries). Algorithms exist to estimate the branching frequency for tri- and tetra-functional branching based on the Zimm-Stockmayer theory [18], but failure to separate by topology prevents such an approach from successfully discriminating between branching functionality and frequency. The relationship between molar mass and size in solution is affected by the topology and this implies that a fraction of given size will be polydisperse when a branching distribution is present [22,23].

The ability to separate polymers according to their topology and degree of LCB would clearly benefit the characterization of branched polymers. Potentially interesting for this
purpose are separations that exploit differences in what has been referred to [24] as polymer dynamics in solution. In this study the application of molecular-topology fractionation (MTF) [25,26] has been explored for the separation of branched polymers. This technique is based on the migration of polymers in dilute solution through chromatographic columns with very narrow flow channels. Unlike fractionation under conditions of strong confinement that require the coil to unwind (e.g. reptation), the separation is based on continuous migration of the coiled polymers. This renders MTF less sensitive to clogging of the pores. The size of the polymer in solution has an effect on MTF as well and, therefore, the separation by branching properties will be confounded with the molar-mass distribution. Therefore, comprehensive two-dimensional MTF×SEC (after nomenclature in [27]) separations were used to independently study the selectivities due to branching and due to hydrodynamic size [28]. In the present study key variables, such as the pore size and the flow rate, have been optimized in an attempt to obtain orthogonal separation mechanisms. Accurate information on the pore size will be used to provide a better definition of the MTF separation and predict its application range. Flow-rate gradients have been explored to enhance the applicability of the technique.

4.2 Theory

4.2.1 Separation techniques based on size
Polymers in dilute solution are present as coils. For ideal polymers behaving as perfect random-flight chains the time-averaged coil size scales with the square root of the molar mass. Deviations from this scaling law for real polymers are implied by limited flexibility in the backbone, excluded volume by the chain itself and solvent adsorbed by the coil due to enthalpic interactions [29,30]. A relation where size follows a power law of mass is often still valid across broad molar-mass ranges, provided that composition and structure of the polymer remain constant.

The equilibrium size of polymers in solution provides a robust basis for SEC and HDC separations. Wall exclusion from the stationary-phase surface based on coil-size of dissolved polymers is the driving force in both separations (steric exclusion). In SEC smaller polymers selectively populate the stagnant volume in narrow pores by diffusion
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from adjacent convective channels [31,32]. In packed beds with porous particles the porous properties can be optimized to provide selective exclusion and convective transport takes place in the interstitial space between the particles. In HDC large polymers are excluded from the slow-moving solvent layers near the walls in convective pores [33,34,35]. Selectivity scales with the aspect-ratio ($\lambda$) that relates the size of the solute molecules (radius $r$) to the size of the flow-through channel (radius $r_c$) as $\lambda = r / r_c$. Besides steric exclusion, which increases the migration rate for larger polymers, hydrodynamic interactions in convective flow will affect the polymer migration rate. For most flexible-chain polymers with $M > 10$ kg/mol the polymer coil behaves as if impermeable to flow (i.e. non-draining behavior) under the mild conditions of SEC or viscometry [16,36,37]. Friction in shear flow due to non-draining behavior of the analyte will generally result in retardation and counteract the exclusion effect at higher $\lambda$ values [38,39,40]. Hydrodynamic interaction is specific to the conditions used and depends on both $\lambda$ and absolute size. A detailed breakdown of hydrodynamic effects for polymers in shear flow has been provided by Tijssen et al. [41] and Stegeman et al. [42]. Migration in HDC can be described using Eq. 3 where migration rate ($\tau$) is defined as residence time of the polymer ($t_p$) relative to the residence time of a flow marker ($t_m$) and expressed as a function of $\lambda$ and a constant $C$ describing hydrodynamic interaction.

$$\tau = \frac{t_p}{t_m} = \frac{1}{1+2\lambda-C\lambda^2}$$

4.2.2 Deformation of polymers in solution

Under HDC conditions shear stress on the solute may become significant and result in flow-rate-dependent elution behavior [42]. The effective size ($r$) may decrease when the molecules are subjected to shear stress above a certain threshold that may be related to the Deborah number ($De$) [24,43]. $De$ is defined as the product of effective elongation ($\dot{\varepsilon}$) and the longest relaxation time of the polymer ($\tau_{rel}$). The effect of elongation may become detectable in HDC for Deborah numbers exceeding 0.1, while for $De > 0.5$ severe elongation may occur. Separation in this latter domain does result in elution behavior that differs significantly from that of HDC and is referred to as slalom chromatography (SC) [44]. Deborah numbers can be calculated using either molar mass
(Eq. 4) or radius of gyration (Eq. 5), depending on the experimental conditions and available data on the polymer [24,45].

\[ De = \dot{\varepsilon} \tau_{rel} = K_{deb} \frac{\nu}{d_p} \frac{0.42 \eta_s [\eta] M}{RT} \]  

(Eq. 4)

\[ De = \dot{\varepsilon} \tau_{rel} = K_{deb} \frac{\nu}{d_p} \frac{6.12 \Phi \eta_s r_g^3}{RT} \]  

(Eq. 5)

\( K_{deb} \) is a constant related to the geometry of the pores (with a typical value of 6), \( \nu \) is the superficial solvent velocity, \( d_p \) the particle size of the packing, \( \Phi \) the Flory-Fox parameter, \( \eta_s \) the solvent viscosity, \( [\eta] \) the polymer intrinsic viscosity, \( r_g \) the polymer radius of gyration, \( R \) the gas constant and \( T \) the absolute temperature. The Flory-Fox equation (Eq. 6) is used to transform Eq. 4 into Eq. 5.

\[ [\eta] M = 6^{3/2} \Phi r_g^3 \]  

(Eq. 6)

It is important to note that the Flory-Fox parameter is a measure of hydrodynamic interaction and depends on solvent conditions, molar mass and branching of the polymer. For example, values ranging from \( \Phi = 1.8 \times 10^{23} \text{ mol}^{-1} \) for linear polymers to \( \Phi = 3.5 \times 10^{23} \text{ mol}^{-1} \) for branched polymers have been observed, while at \( 0 \)-conditions (indicated by the subscript 0) a value of \( \Phi_0 = 2.8 \times 10^{23} \text{ mol}^{-1} \) is common [46,47]. When studying molecules of varying topology in good solvents we prefer to use Eq. 4, avoiding the assumptions inherent to Eq. 5.

A problem with the application of Deborah numbers for branched polymers is in the treatment of polymer relaxation. The polymer relaxation \( \tau_{rel} \) (Eq. 7) is based on a model by Zimm for a chain of beads connected by ideal springs [48,49].

\[ \tau_{rel} = C_Z \frac{\eta_s [\eta] M}{RT} \]  

(Eq. 7)

The constant \( C_Z \) corrects for hydrodynamic interaction (e.g. draining behavior) of the polymer. Branching and topology effects are not included in the model and correct treatment should therefore not be assumed. Notice that according to Eq. 7 relaxation for polymers with identical hydrodynamic volumes is identical. Linear and branched
polymers in dilute solution can be expected to respond differently to stress. It is known that branching leads to increased segment density and reduced internal freedom of motion for random-coil polymers in solution \[50,51\]. The entropy decrease resulting from deformation of a branched molecule will be greater than that for the deformation of a linear polymer. Therefore, branched polymers are expected to deform less than linear polymers under identical stress. A size-dependent separation may then be applied to achieve branching selectivity, dependent on the level of stress on the molecules (\textit{i.e.} on the flow rate).

The aspect ratio and Deborah number provide convenient metrics to compare separation techniques based on size and dynamic properties of the polymers. In Fig. 1 techniques are indicated at $\lambda$ and $De$ values corresponding to typical operating conditions. Arrows in the insert indicate how $\lambda$ and $De$ values respond to changes in the separation conditions, such as increasing the flow rate and decreasing the particle size ($d_p$). Molar mass has an indirect effect on both $\lambda$ and $De$ through its impact on the radius. Ultra-high-pressure SEC separations are by definition operated at higher flow rates and with smaller particles. Complex multi-mode separations result for samples with high molar mass \[52\]. Some uncertainty is introduced in the assessment of elongation for $\lambda > 0.3$ (shaded region in Fig. 1), because shear stress experienced by the polymer is no longer continuous and its elongation character is reduced. In Poiseuille flow shear stress will mainly affect the periphery of the coiled polymer with $\lambda > 0.3$ and rotation is largely prohibited. This situation no longer meets the assumptions in calculation of Deborah numbers \textit{i.e.} steady elongation against relaxation of the entire coil, but it is expected that deformation as a result of shear forces will still occur. Hydrodynamic interaction of polymers is significant for $\lambda > 0.3$ and MTF separations can be obtained under these conditions. The upper limit of $\lambda$ for MTF separations is not strictly defined. The dashed line in Fig.1 puts an approximate limit at $\lambda = 1$, but MTF separations with linear polymers up to $\lambda = 2$ have been performed.
4.2.3 Reptation

Separation techniques which are performed at conditions associated with MTF are reviewed for similarities that can explain the topology sensitivity obtained at different flow rates. Different mechanisms can be identified for polymer migration through strongly confining media i.e. $\lambda > 1$. Sieving, entropic-barrier transport, and reptation can be distinguished based on channel geometry and rigidity of the solute [53]. Random-coil polymers in dilute solution with equilibrium sizes larger than the flow-through channels through which they migrate will be continuously squeezed into a stretched conformation. This condition is most similar to the tube-like diffusion path of molecules as described in the theory of reptation [41,54]. This model finds its origin in the description of the rheological behavior of melted polymers, where it is used to describe motion, diffusion, and viscosity successfully [7,55]. Modifications to the theory have been described for polymers in dilute solution, specifically for the case of separating polymers by their degree of branching [56]. The barrier energy and critical flux required to overcome the osmotic pressure under strong and weak confinement was derived for ideal-star and randomly branched chains. Shortcomings of the theory impose serious
limitations on its applicability for real chains. For critical-flux calculations the flow needed to overcome diffusion of a polymer segment is considered, while treating the molecule as free-draining \( i.e. \) interaction of each polymer segment with flow is not affected by other segments in the molecule). Because non-draining conditions are more likely for real chains, shear inside the coil domain will be low, resulting in smaller drag forces on the chains in comparison with free-draining conditions. Thus, flow-induced migration through strongly confining pores, resulting in unwinding of the polymer and in molar-mass insensitivity for linear chains, will not take place for real polymers. It was later pointed out that the critical flux for linear and branched chains under strong confinement will be identical and cannot be used to achieve separation, because of the progressive nature of the drag force once the first segments of a chain have entered the pore [57].

In the weak-confinement case deformation and moderate stretching are considered for polymers in solution. Most theoretical work takes into account constriction by the pore walls alone, whereas for real polymers hydrodynamic interactions are present as well. The forces induced by solvent shear on the polymer contribute to deformation and can be controlled externally by adjusting the flow rate. Polycarbonate or mica filtration membranes prepared by fast-atom bombardment and track-etching have been used to study reptation of synthetic polymers. Flow-rate-dependent rejection of polymers with equilibrium dimensions close to or larger than the membrane pores has been described by Long and Anderson [58]. They confirmed that polymer migration through pores in a mica membrane at higher flow rates was due to deformation rather than degradation for the range \( 1 < \lambda < 2 \). Selective rejection of branched-polymers was considered in a follow-up article [59]. A greater rejection of comb and star polymers relative to linear polymers was attributed to deformability of the polymers. Unfortunately, the branched samples used in this study had different molecular weights and high dispersities. Challenges with membrane separations are the limited separation efficiency and low sample capacity (concentrations of 160 ppm w/w had to be used). It was observed that high concentrations (above the overlap limit) were needed for polymers with \( \lambda \approx 1 \) to diffuse through the membrane when using osmotic pressure only [60,61]. Therefore concentration build-up on membranes in flow-driven separations may present a problem, because this would alter the migration behavior of the polymer [62]. The
results obtained for flow-rate dependent polymer rejection are most relevant to the separations considered here, since the conditions and findings are in good agreement with the observations for molecular-topology fractionation (MTF).

4.2.4 Calibration curves and separation of deformed-polymers

The impact of polymer deformation on migration can be very different depending on the separation technique and corresponding conditions. Whether a useful separation may be obtained for polymers with different deformability is assessed by comparing the calibration curves as a function of polymer equilibrium size (Fig. 2). SEC (pore exclusion) and HDC (wall exclusion) separate non-deformed macromolecules and result in decreasing residence times for analyte molecules with increasing size. SEC by definition takes place in pores that are not subject to convective transport. Pores in stationary phases for SEC are typically smaller than the flow-through channels and may be optimized independently from the flow-through-channel size. The channel size (related to the particle diameter) may vary, but \( \lambda \) is generally below 0.1 for polymers separated by SEC. Modern phases offer both high porosity, which ensures a broad separation window \((e.g. \ 0.5 < \tau < 1)\), and a high mechanical strength, which allows separation at higher flow rates. In case high-molar-mass polymers are separated using small particles \((i.e. \ small \ flow-through \ channels)\) \( \lambda \) may be high enough for a seamless transition into HDC to occur \([63,64]\). A continuous SEC-HDC separation is the result, with the largest polymers eluting first. However, if \( \lambda \) increases beyond about 0.35 a strong reversal of the HDC calibration curve is observed (Fig. 2). Unlike rigid particles, which will be significantly retarded for \( \lambda > 0.4 \), polymers will respond to shear stress either through deformation or degradation. Deformation is broadly defined and may comprise many effects, such as compression, elongation or increased flow draining of the coil periphery. In the MTF region \((above \ \lambda \approx 0.35; \ see \ Fig.2)\) the behavior of non-rigid polymers may be used to discriminate between different architectures. Deformation of polymers results in departure from the HDC calibration curve, which applies for rigid solutes, with \( \lambda \) corresponding to the non-deformed-polymer. All molecules will be deformed, but linear molecules more so than branched ones. The linear molecules, therefore, elute earlier (indicated by the gray horizontal arrows in Fig. 2).
Hydrodynamic interactions of polymers play an important role in flow-induced deformation. This is supported by the observation that linear polymers with equilibrium dimensions of the order of the channel size elute much faster at increased flow rates [65]. In the absence of deformation the polymer lags the solvent in its surrounding due to non-draining behavior (i.e. impermeability to flow). Especially in monoliths or packed beds with highly interconnected flow-through channels this results in \( \tau > 1 \) for polymers with \( \lambda \approx 1 \). At elevated flow rate \( \tau = 0.7 \) was observed, which is possible only by depletion of the slower-moving solvent layers near the stationary phase surface (Fig. 3). The effect does not occur for smaller polymers that do not have sufficient hydrodynamic interaction relative to the fast internal relaxation, rotation and translational diffusion. Only for large polymers will a higher shear strain near the surface result in a higher internal stress, thereby making conformations that occupy this region unfavorable. To think of this effect as stress-induced diffusion or stress-induced deformation depends on whether or not migration of the entire coil perpendicular to the direction of flow is achieved. Separation due to stress-induced deformation is a more-accurate description for the aspect ratios considered in MTF.
Higher entropic elasticity of branched polymers was suggested as a qualitative explanation for topology selectivity. Different possible interpretations of polymer deformation prevent a more accurate description at this moment. Deformation may be explained as selective population of polymer conformations. After all, the spherical equilibrium dimensions represent the average of many instantaneous aspherical conformations at a time scale longer than the longest relaxation time of the polymer [66,67]. This supports the entropic nature of (stress-induced) deformation. Differences in flow permeability are reflected in the viscosity-shielding ratio [21], the Flory-Fox parameter, and ratios of viscosity radius and radius of gyration [16] in Poiseuille flow under traditional separation conditions. Most published work on deformation under flow conditions focuses on the coil-stretch transition in elongational flow. The shear-rate dependent coil-stretch transition for DNA was found to agree very well with predictions based on Brownian dynamics for random coils [68]. Experiments on polystyrene, however, demonstrated that the extended length under flow conditions did not exceed twice the radius of gyration and was generally lower than predicted [69,70]. Experimental evidence from light scattering and birefringence measurements on the absence of a coil-stretch transition for polystyrene were later suggested to be biased and not selective [68,71]. Furthermore, it was suggested that shear levels were simply too low for the polymers considered and the experiments therefore failed to provide conclusive results.
In slalom chromatography (SC) coil-stretch transition interferes with the accelerated migration by wall-exclusion of the polymer. Stretched molecules are retarded in the tortuous interstitial space by frequent conformational changes and changes in flow direction through the stationary phase packing. In the SC region, the extent of deformation could potentially result in a different elution volume for linear and branched molecules. Successful application of SC is unlikely, because it is expected that the selectivity (length of the arrows in Fig. 2) is limited. Conditions where wall-exclusion and slalom chromatography co-exist and their effects on migration cancel out do not exist. Instead it was observed that flow rate could be used to obtain coil-stretch transition for different molar masses in agreement with Deborah-number calculations. Once this transition was reached elution volumes increased quickly [52].

4.3 Experimental

4.3.1 Chemicals and materials

The eluent for MTF and SEC separations was non-stabilized tetrahydrofuran (THF, HPLC-grade, Biosolve, Valkenswaard, The Netherlands). The eluent was degassed by purging with helium 5.0 (99.999% Praxair, Vlaardingen, The Netherlands). Sample polymers were dissolved in THF stabilized with 250 mg/L butyl-hydroxylated toluene to prevent degradation by radicals. Narrowly distributed linear polystyrene standards (Polymer Laboratories, Church Stretton, UK) with molar masses ($M$) between 1,990 and $3.74 \times 10^6$ g/mol with polydispersity indices (PDI) no larger than 1.05 were used to study retention behaviour. These standards were dissolved at concentrations between 0.1 and 1 mg/mL. A nominal “three-arm star” (or Y-shaped) polystyrene sample was obtained from Polymer Source (Dorval, Canada) and used at a concentration of 1.0 mg/mL. Synthesis of the “star” polymer was by coupling of anionically polymerized arms with a tri-functional agent ($\alpha,\alpha',\alpha''$-trichloromesitylene). Analysis using SEC with light scattering and viscometry revealed an arm molar-mass of 1,250 kg/mol and a composition of 5% uncoupled precursor, 45% linear two-arm coupling product and a remainder of three-arm coupling product [26]. Suspected side products were a four-arm star polymer formed by lithium-halide exchange with all arms coupled in one functional centre (X-shaped) [72,73]. The concentration of this large-molecule fraction could not be determined quantitatively by SEC.
Polystyrenes with broad molar-mass distributions were obtained from the Dow Chemical Company (Midland, MI, USA). Dow polystyrene 1683 (weight-average molar mass \((M_w)\) 250 kg/mol, PDI 2.5) was used as a linear reference material. LCB polystyrene \((M_w) 810\) kg/mol, PDI 3.3, SEC-MALLS [28]) and low-LCB polystyrene \((M_w) 310\) kg/mol, PDI 5) were used for analysis of branched polymers. Long-chain branching at high-molar mass is confirmed by the reduced intrinsic viscosity in the Mark-Houwink plot (Fig. 7). The LCB polystyrenes were prepared by coupling of polystyryl anions with di- and tri-functional benzyl chlorides as published elsewhere [74]. Comb polystyrene (kindly donated by Dr. C. Fernyhough, University of Sheffield) has a backbone molar mass of around 200 kg/mol and approximately 30 randomly placed branches of 70 kg/mol. The synthesis technique of the comb polymer has been described in [75].

4.3.2 Instrumentation and operating conditions

HPLC experiments were performed on a Shimadzu LC system (‘s Hertogenbosch, The Netherlands) consisting of a system-controller (SCL10a), two micro-pumps (LC10ADvp), a column oven (CTO7), autosampler (SIL9a), UV detector (SPD10AVvp) and right-angle laser light scattering (RALLS) detector model LD600 (Viscotek, Houston, TX, USA). UV detection was performed simultaneously at 260 nm and 214 nm. Modulation for comprehensive two-dimensional separation was performed with a VICI two-position 10-port valve (Valco, Schenkon, Switzerland) with a high-speed switching accessory and digital valve interface. The 10-port valve was plumbed for symmetrical dual-loop modulation [76]. From the moment of injection on the 1D column, the 10-port valve was switched at regular intervals between 1 and 3 minutes in order to inject first-dimension effluent on the 2D 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.

Triple-detection SEC was performed using the Shimadzu LC system plumbed for 1D chromatography. The detection array consisted of a UV detector (SPD10AVvp), RALLS detector (LD 600) and chip-based on-line viscometer (Polymer Laboratories /
Micronit, Enschede, The Netherlands). Data acquisition and processing was performed with PLCirrus (Polymer Laboratories, v3.0) based on triple-detection SEC principles [1].

### 4.3.3 Columns and experimental conditions

Wide-bore columns (4.6-mm I.D.) were used for all separations. The sample volume injected on the 1D column was between 5 and 25 μL. Monolithic columns with narrow macropore sizes were prepared in columns of 100, 150 and 250 mm length according to the procedure published previously [65]. Custom-made columns (Polymer Laboratories) were used for fast 2D SEC. A 150-mm long column packed with 10-μm 10^6 Å PLgel particles (V₀ = 1.9 mL) was used at 750 μL/min. Two 100-mm long columns packed with 5-μm 10^5 Å PLgel particles were used in series only for the 2D separation in HDC×SEC at 600 μL/min.

Chromatograms in the HDC×SEC mode (Fig. 5, Fig. S-1) were acquired with two 150 mm 1D monolithic column in series (V₀ = 3.1 mL) at 10 μL/min. 160 2D chromatograms were obtained during a runtime of 480 minutes at 50°C. Flow-rate studies in MTF×SEC (Fig. 6) were performed on a 250 mm monolithic 1D column (V₀ = 2.6 mL) with D_pore 126 nm and a 150 mm 10^6 Å 2D column, with both columns operated at 40°C. 200 2D chromatograms were obtained by injection of 30 μL 1D effluent fractions at a modulation interval matched to the 1D flow rate. MTF×SEC at conditions with minimal molar mass selectivity (Fig. 7) was achieved using two 150 mm monolithic columns (D_pore = 126 nm, 30 μL/min) and a 150 mm 10^6 Å 2D column. 80 2D chromatograms were obtained during a runtime of 120 minutes at 50°C.

Light scattering was used in most 2D experiments. It allows for overlapped 2D injections, because a solvent signal is not present. This is beneficial in experiments where higher 1D flow rates are used and the time required to complete the 2D separation is rate limiting. More second-dimension chromatograms can be obtained when using the RALLS signal.

Triple-detection SEC was performed at room temperature with a flow rate of 0.3 mL/min. A set of two minimix B (10-μm particles) and one minimix C (5-μm particles) columns (each 250 mm long) was used.
4.4 Results and discussion

4.4.1 Flow-rate effect for columns with different pore size

Monolithic columns with well-controlled macropore sizes were prepared by in-situ polymerization of polystyrene and divinylbenzene. In thermodynamically favorable or ‘good’ solvents, such as tetrahydrofuran for polystyrene, polymers readily dissolve and can be separated free from enthalpic interaction with the column. The selectivity for hydrodynamic separations on monolithic columns has been studied for linear polymers [65]. A flow-rate effect on migration rate was observed and stress-induced diffusion (SID) was presented as the mechanism responsible for this effect when separating synthetic polymers in macropores close to unperturbed-polymer dimensions. The hypothesis of SID playing an important role in MTF separations will be tested with the results obtained in this work on branched-polymer separations. After presenting the results we provide a detailed discussion on the mechanism.

Channel dimensions of the columns used in the present work have been optimized for MTF separation. Separation by a HDC mechanism according to unperturbed-polymer dimensions was obtained at low flow rates. This is demonstrated by the calibration curves obtained with narrow-molar-mass polystyrenes for columns with pore sizes \( D_{\text{pore}} \) ranging from 160 nm down to 75 nm (Fig. 4, Table 1). At 20 \( \mu \text{L}/\text{min} \) reversal of the calibration curve is observed for high molar masses. This is in agreement with HDC separation of rigid solutes (solid spheres) where hydrodynamic interaction at \( \lambda > 0.4 \) will reduce the migration rate. The retardation for high- molar-mass polymers is generally reduced when the flow rate is increased to 50 \( \mu \text{L}/\text{min} \). This was also observed for micro-porous membranes where the rejection of large polymers decreased at higher flow rates [58]. Calibration curves in Fig. 4 indicate that only the hydrodynamic effects for \( \lambda \) approaching 1 are affected, because the selectivity for molecular weights below the point of reversal is maintained. Wall exclusion-effects and coil dimensions that induce these effects apparently have not changed for polymers below the reversal molar mass. Under certain conditions the molar-mass selectivity diminishes above the reversal point. Hydrodynamic interactions and calibration-curve reversal depend on the aspect ratio \( \lambda \), rather than on the molar mass. Thus, we should preferably speak of a reversal (or critical) size. In practice, we may refer to a critical molar mass. In the present case
this is a linear-polystyrene-equivalent molar mass. The effective radius for wall-exclusion separations [77] is used in calculating $\lambda$ and is given in Eq. 8 for polystyrene in THF [78].

$$r_{eff} = \frac{\sqrt{\pi}}{2} d_g = \frac{\sqrt{\pi}}{2} 0.0118 M^{0.600}$$  \hspace{1cm} (8)

In MTF experiments it has been observed that branched polymers are ‘retained’ longer than linear polymers for $\lambda > 0.4$ [41,28]. At optimized experimental conditions a separation may then be performed where linear polymers above a critical molar mass co-elute, while branched materials elute later from the column and co-elute with linear polymers of lower molecular weight. In a comprehensive MTF×SEC separation the co-eluting species can be separated and distributions in terms of hydrodynamic size and branching will be obtained.

Monolithic columns with three different flow-through-pore sizes were considered for MTF in MTF×SEC separations. Small pores are required to obtain $\lambda$ values high enough to allow MTF separations of polymers below 1000 kg/mol. A special test was designed to establish the molecular weight at the point of reversal in the calibration curve. Broad-MWD polystyrene was analyzed in a comprehensive two-dimensional separation with the monolith in the first dimension (1D) and a regular SEC column in the second dimension (2D). Separation in the ‘HDC×SEC-mode’ was obtained for monolith 7 (see Table 3) with $D_{pore} = 126$ nm at a flow rate of 10 $\mu$L/min (Fig. 5). The ionol peak originating from the sample solvent marks the void volume in both the 1st and 2nd dimensions at $\tau = 1.00$ and $V_{sec} = 2.4$ mL, respectively. The earliest eluting polymeric material from the monolith ($\tau = 0.69$) was used to determine the critical molar mass of reversal. $M_{crit}$ (Table 1) is the peak molar mass in the 2D SEC separation of the first fraction containing polymer. Two-dimensional separations for monolith 8 and 9, as well as the calibration curve for the 2D SEC separation are presented in section 4.6.1 of the Appendix. Calibration-curve reversal for separation at 10 $\mu$L/min occurs at roughly identical values of $\lambda$ (Table 1), which are very close to the expected value of 0.37 based on the Dimarzio-Guttman retention model for HDC of unperturbed polymers (Eq. 3 with $C = 2.7$).
Fig. 4. Calibration curves for narrow polystyrene standards obtained on monoliths at 20 µL/min (a) and 50µL/min (b).
 Comprehensive-two-dimensional chromatography is highly useful for exploring the migration behavior in MTF. Not only does it provide more information than a calibration curve based on the injection of narrow-MWD standards, it also allows the characterization of samples with a high polydispersity. The 2D chromatogram gives a qualitative impression of the calibration curve in a single experiment (Fig. 5).

Therefore, the flow-rate effect was examined by MTF×SEC with 1D flow rates \( F \) between 10 and 75 \( \mu \text{L/min} \). A shorter 2D column with packing optimized for separation of large polymers was used (supplementary information, S2). Linear polymers with molecular weights above \( M_{\text{crit}} \) were separated on a monolith with \( D_{\text{pore}} = 126 \) nm at 10 \( \mu \text{L/min} \) (Fig. 6a) and at 30 \( \mu \text{L/min} \) (Fig. 6b). A sample of three polystyrene standards of 1.37\( \times 10^6 \), 2.56\( \times 10^6 \) and 3.74\( \times 10^6 \) g/mol was used, which for this monolith corresponds to aspect ratios in the range 0.80 < \( \lambda \) < 1.46. Higher flow rates result in a transition from

\[ \text{Fig. 5. Calibration plot obtained by MTF×SEC for a broad standard } F = 10 \ \mu\text{L/min} \text{ (PS1683; } M_n = 250 \text{ kg/mol, PDI = 2.5) using monolith 7 in the first dimension.} \]

\[ \text{Table 1. Reversal molar masses for monoliths determined by HDC×SEC at } 10 \ \mu\text{L/min.} \]

<table>
<thead>
<tr>
<th>Monolithic ID</th>
<th>( D_{\text{pore}} ) (nm)</th>
<th>( M_{\text{crit}} ) (kg/mol)</th>
<th>( \lambda )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>160</td>
<td>[700]*</td>
<td>[0.4]*</td>
</tr>
<tr>
<td>7</td>
<td>126</td>
<td>355</td>
<td>0.36</td>
</tr>
<tr>
<td>8</td>
<td>104</td>
<td>260</td>
<td>0.36</td>
</tr>
<tr>
<td>9</td>
<td>75</td>
<td>207</td>
<td>0.39</td>
</tr>
</tbody>
</table>

*Approximate values obtained from calibration curve at 20 \( \mu\text{L/min} \)
polymers separated under equilibrium conditions (HDC) to polymers separated in a
deformed state (MTF) (Fig. 6, Fig. S-4). The three peaks corresponding to the narrow
standards merge into a single peak in 1D and molecular-weight independence is
achieved for these high-molecular-weight materials. For the monolith with $D_{\text{pore}} = 126$
nm a flow rate of $F = 30 \text{ µL/min}$ was used in subsequent experiments to suppress the
molar-mass selectivity. A similar suppression of molar-mass selectivity above $M_{\text{crit}}$ was
obtained with a $D_{\text{pore}} = 104$ nm monolith, but at a higher flow rate. In 2D experiments
with this column ($L = 100$ mm) the lowest degree of molar-mass selectivity was
obtained for $F = 50 \text{ µL/min}$ (Fig. S-6). The calibration curves in Fig. 4b confirm this
trend, but do reveal little retention above $M_{\text{crit}}$. The small 1D column volume is limiting
the number of 2D chromatograms and thus the resolution of the 2D experiment.
However, the use of long columns with narrow pores is experimentally challenging. The
pressure drop that is required for operating longer monolithic columns with $D_{\text{pore}} = 104$
nm or smaller is so high as to cause irreversible damage by compression of the
stationary phase. Additional results on MTF×SEC at various flow rates for both column
types are presented in the supplementary information, section S3.

![Fig. 6. A mixture of three narrow-MMD polystyrenes separated on an MTF column with $D_{\text{pore}} = 126$ nm at different flow rates. (a) 10 µL/min (b) 30 µL/min.](image)

### 4.4.2 Branched-polymer separations

Conditions for MTF separation with minimal molar-mass selectivity for linear polymers
above $M_{\text{crit}}$ were established. Branched polymers with different topology were analyzed
at those conditions using MTF×SEC to assess branching-selectivity in MTF (Fig. 7). A
linear reference prepared by mixing Dow1683 with the narrow standards from Fig. 6
was used to cover a wide molar-mass range. Only little retardation for the highest
molar-mass polymers was observed in Fig. 7a. Such small difference may be the result of inaccuracy of the flow or the porous properties of the column (similar columns were used, see section 4.3.3). Star-branched polystyrene prepared by coupling of 1250 kg/mol linear ‘arms’ was mixed with the linear reference. Base-line separation of the 3-arm (Y-shaped) and 4-arm (X-shaped) branched coupling products from the linear materials is obtained (Fig. 7b). The linear 2-arm coupling product and single arm polystyrene elute together with the linear materials. The 4-arm star, which is a by-product from the coupling reaction, is base-line separated from the 3-arm star. This separation provides a dramatic demonstration of the separation of high molar-mass, branched polymers with only a single branching point from linear polymers with a broad range in molar mass and hydrodynamic size. An overview of the elution volumes in both dimensions is given in Table 2. Calibration of the ²D separation with linear polymers was used to calculate $\lambda$ values for the branched material eluting from the MTF column (supplementary information, S2).

Retardation of polymers with higher branching frequency was observed already at smaller hydrodynamic size relative to long-chain branched (LCB) star polymer. Random long-chain branched material (LCB PS, Fig. 7c) and a comb polymer with controlled long-chain branching (Fig. 7d) were separated at the same conditions as those used in Fig. 7a and 7b. The results of analyses by SEC with triple detection are provided in Fig. 8 for reference. For both the separations of LCB PS and Comb polymers by MTF×SEC a tail is observed in the MTF direction. This is the expected result for the LCB PS, because here the degree of branching increases with molecular weight. In case of the comb the tail may reflect a cross-linking byproduct of the synthesis. The comb itself ($V_{MTF} = 2.48$ mL) is hardly retained, most likely because its aspect ratio ($\lambda$) is too low for significant hydrodynamic interactions to occur. In the UV chromatogram a small amount of polystyrene with a smaller hydrodynamic size than the bulk of the sample can also be observed (Fig. S-7). This material is not separated from the bulk in the ²D SEC separation or in the triple-detection analysis. Most likely this is a uncoupled linear precursor that has remained in the comb sample as a side-product.
The branched materials retarded in MTF have different hydrodynamic sizes depending on their topology. In Fig. 9 an overlay with 2D-SEC peak maxima from Fig. 7 is presented on top of the linear reference polymer. LCB and comb polymers start to be retained at lower hydrodynamic sizes than the star polymers. This implies that stress-induced deformation effects are less effective in counteracting the hydrodynamic effects for polymers with higher degree of branching. A plausible explanation is that polymers with higher segment density are less susceptible to deformation. Segment density in solution is inversely related to intrinsic viscosity, which is given in Fig. 8. Lower intrinsic viscosity for polymers above log $M = 6$ correlates well with the lower $\lambda$ for material retarded in MTF (Table 2).

**4.4.3 Selectivity for branched polymers**

A better look at the separation conditions is needed to understand the separation selectivity for branched polymers. We will assume that polymers with higher segment densities than linear polymer will deform to a lesser extent under stress and, therefore, resemble HDC behaviour. Material eluting later than linear polymers above $M_{\text{crit}}$ at $\lambda > 0.4$ will have a higher segment density and/or a larger hydrodynamic size. In the case of 3- and 4-arm star polymers the elution is affected by segment density only, because hydrodynamic size (e.g. $\lambda$) is identical. For the randomly branched LCB polymer branching and hydrodynamic size both increase, indicating that molar mass increases for material eluting later from the MTF column. However, when segment density is too high to allow for deformation and hydrodynamic size is large then polymers may elute very late or not at all. The tail for both LCB and comb polystyrene is indicative of very dense polymer (possibly crosslinked) that is not completely eluting. Applicability of the present separation conditions is limited to conditions that allow polymers to elute from the MTF column within reasonable time. This may be achieved by using MTF columns with pore size matched to polymers of interest or by changing the conditions to make also more dense polymers at higher $\lambda$ elute.

While material eluting slowly from the MTF column can be explained as material with exceptional high coil density, it can also be argued that this is the result of polymer degradation. The light-scattering signal from RALLS divided by a concentration signal (UV) was used to estimate molar-mass changes for material eluting from the MTF
High angular dependence of the RALLS signal makes it impossible to quantitatively compare LS/UV ratios without correction for angular dependence, which strongly depends on polymer size. However, it is possible to use the LS/UV ratio for a qualitative comparison when dealing with polymers of identical size (e.g., eluting at identical $t_r$). The LS/UV ratio for polymers in Fig. 7 is presented in Fig. S-7. In all cases material eluting later from the MTF column was found to have a higher LS/UV ratio, which is indicative of higher molar mass. MTF×SEC-MALLS was performed to obtain accurate molar mass following the separation of LCB PS on the monolithic column with $D_{pore} = 104$ nm (Supplementary Information, S5). The gradual increase in molar mass was confirmed and material up to the highest mass present in this polymer as measured in Fig. 8 was found back in fractions eluting later from the MTF column.

![Fig. 7. 2D Chromatograms of polystyrene polymers separated by MTF×SEC with 1/F 30 μL/min.](image)

(a) Linear polymers 20 – 3740 kg/mol; (b) linear and star polymers; (c) LCB PS; (d) comb PS.
Chapter 4

Table 2. Elution volume and aspect ratio for polymers of different topology in MTF×SEC ($V_{\text{MTF}} = 3.1$ mL).

<table>
<thead>
<tr>
<th>Topology</th>
<th>$V_{\text{max}}$ (mL)</th>
<th>$\tau$</th>
<th>$V_{\text{max}}$ (mL)</th>
<th>$\lambda$</th>
<th>$\tau$</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-arm star</td>
<td>3.02</td>
<td>0.97</td>
<td>1.28</td>
<td>0.88</td>
<td>0.78</td>
</tr>
<tr>
<td>4-arm star</td>
<td>4.41</td>
<td>1.42</td>
<td>1.28</td>
<td>0.87</td>
<td>0.78</td>
</tr>
<tr>
<td>LCB</td>
<td>3.38</td>
<td>1.09</td>
<td>1.38</td>
<td>0.55</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>3.60</td>
<td>1.16</td>
<td>1.37</td>
<td>0.56</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>4.37</td>
<td>1.41</td>
<td>1.36</td>
<td>0.58</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>5.09</td>
<td>1.64</td>
<td>1.35</td>
<td>0.60</td>
<td>0.77</td>
</tr>
<tr>
<td>Comb</td>
<td>2.48</td>
<td>0.80</td>
<td>1.42</td>
<td>0.43</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>3.38</td>
<td>1.09</td>
<td>1.41</td>
<td>0.47</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>5.04</td>
<td>1.63</td>
<td>1.38</td>
<td>0.54</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Fig. 8. Mark-Houwink plot of linear and branched polystyrene samples. Dow PS1683 (1), star [26] (2), low-LCB PS (3), LCB PS (4), comb (5).
4.4.4 Effect of flow rate on migration of branched polymers

The flow rate applied in MTF separation can be used to change migration behavior for branched polymers in a similar way as it was used to obtain molecular-weight independent elution for linear polymers. MTF×SEC experiments were performed with MTF flow rates up to 75 µL/min on the star polymer and two LCB polymers with different degrees of branching (Supplementary information, S6). At 75 µL/min the star polymer and the polymer with a low degree of LCB could be completely eluted within a single column volume. These results support that flow rate can be used to control migration rates for both linear and branched polymers and influence their recovery. Only polystyrene with material that is highly branched or even cross-linked did not readily elute at these conditions.

Polymers with a low degree of branching are not well separated in the MTF separation at higher flow rates. In order to obtain good separation for materials subject to much different extent of retardation on the MTF column a flow-rate gradient may be applied. It was observed in experiments at constant flow rate that little separation was obtained for polymers eluting after the column void volume ($V_0$). An MTF×SEC experiment was performed in which the flow rate was increased gradually from 10 µL/min to 20 µL/min between 150 and 250 minutes or once the elution volume approached $V_0$ (Fig. 10). A
complete separation resulted, in which well-resolved peaks were observed for the single-arm precursor up to the 4-arm star. Even a high molar-mass coupling product can be discerned, which has a slightly smaller hydrodynamic size. The elution of highly-branched materials with a smaller hydrodynamic size than earlier eluting material was also observed in constant flow rate experiments for three-arm star polymer (Fig. S-9) and LCB polystyrene (Fig. S-10).

![MTF×SEC separation of star-polymer sample using a flow-rate gradient](image)

**Fig. 10.** MTF×SEC separation of star-polymer sample using a flow-rate gradient; (1) unreacted single-arm polymer (2) two-arm linear coupling product (3) 3-arm star polymer (4) 4-arm star polymer (5) higher coupling product.

The star polymer separated in Fig. 10 could even be fractionated by topology in a 1D-MTF experiment. Such a separation is unlikely for other branched polymers under the conditions used for this separation, because linear and branched polymers may co-elute as a result of the low flow rate. Operating at conditions with minimal selectivity for linear polymers provides the most powerful application. The optimal experiment would therefore start at conditions with minimal selectivity for linear chains. Once linear materials have eluted the flow rate may be ramped up to elute branched material with higher segment density from the column.
4.4.5 Effect of temperature on migration of polymers in MTF

The effect of flow rate on selectivity in MTF is very clear and can be understood. It provides direct control over the levels of shear and stress that polymers in solution are subjected to. Temperature as a parameter to control deformation was not considered in detail, but it may certainly be used for this purpose considering the low speed of separation. Higher temperatures induce faster polymer relaxation ($\tau_{rel}$, eq. 7) through faster Brownian motion and lower solvent viscosity. The migration of polymer above $M_{crit}$ will be slower at higher temperatures because of less deformation. This trend was confirmed in a comparison of linear and star polymers separated at both 50 °C and room temperature. However, the effect of temperature on retention proved rather small, even at low flow rates (Supplementary section S7). Great changes in polymer relaxation times cannot be achieved within the limits of practical separation conditions. Flow rate is a more effective parameter, exerting a greater effect on migration and selectivity.

4.5 Conclusions

Molecular-topology fractionation (MTF) is a term used to denote separations of branched and linear polymers as a result of selective deformation. MTF separations combine characteristics of HDC and reptation. Polymers were separated on monolithic column with flow-through channels only slightly larger than the polymer itself. At such conditions polymer molecules experience strong hydrodynamic interactions, resulting in deformation and increased migration rates. MTF separations were used successfully to fractionate polymers by their topology in a comprehensive two-dimensional separation with size-exclusion chromatography (SEC) in the second dimension (MTF×SEC). Selectivity based on topology is introduced through faster relaxation of branched polymers subject to shear deformation. At optimized conditions the effects of hydrodynamic interaction and deformation for linear polymers compensate for each other. Such conditions allows for an orthogonal separation of polymers by their hydrodynamic size and branching properties above $M_{crit}$.

Branched polystyrenes with $\lambda$ between 0.4 and 0.9 were separated from linear polymers with identical hydrodynamic size. A relation was observed between the aspect ratio of branched polymers retarded in MTF and coil density in solution. The absence of migration effects resulting from polymer properties other than topology with an effect
on coil density was not rigorously validated in this study. It would be interesting to assess the impact of chemical composition and short-chain branching on migration for application of MTF to polyolefins and polar synthetic polymers. Several results support the hypothesis of molecular relaxation as the decisive property for selectivity in MTF, such as increased retention above $M_{\text{crit}}$ at higher temperature or the higher flow rates needed for molar-mass-independent elution from monoliths with narrower flow-through channels.

MTF×SEC was used to investigate MTF separations preferably on long 1D columns for better separation efficiency. Lengthy experiments were the result and comprehensive on-line coupling with SEC proved impossible without a significant sacrifice in terms of separation efficiency. For practical purposes off-line fractionation may be used once the separation conditions are known [26]. A simple SEC-MTF experiment with MTF performed at conditions with minimal molar mass selectivity above $M_{\text{crit}}$ can be used for a fast and inexpensive screening for branched material. In another approach a short MTF column may be used to obtain the fraction with branched material and linear molecules below $M_{\text{crit}}$. A high-resolution SEC experiment with selective detection may than be used to characterize only the branched material free from co-eluting linear material.

A challenge for the application of MTF is still the lack of commercially available stationary phases with suitable flow-through-channel dimensions. High diffusion and fast relaxation of synthetic polymers require an aspect ratio ($\lambda$) close to unity for branched molecules. Extension of deformation-selective separation of branched polymers towards either larger polymers or polymers with longer relaxation times may be possible even when using commercially available HDC columns. Starches and bio-polymers may satisfy this criterion and provide interesting cases [79].
4.6 Appendix

4.6.1 Comprehensive HDC×SEC experiment

Monolithic columns with different pore sizes were used to obtain chromatograms at identical conditions (see Table 1). The chromatograms obtained using each monolith in the first dimension for HDC×SEC are presented in Fig. S-1.

The sample was 0.8 mg/mL PS1683 + 0.1 mg/mL 2MDa narrow-standard PS in THF. Two 100 mm × 4.6 mm I.D. column packed with 5-µm 105 Å PLgel were used in the second dimension. Accurate molar-mass calibration was performed by injection of four mixtures, each with three narrow-MMD PS standards. Calibration samples were injected at 30 min intervals with $F = 10 \mu$L/min. Elution volumes corresponding to peak maxima were used to construct a third order polynomial fit (Fig. S-2). This calibration data were used to calculate $M_{crit}$ for each monolithic phase (Table 1).

$$y = -2.5x^3 + 11.8x^2 - 20.6x + 18.1$$

Fig. S-2. Narrow MMD PS standards separated using HDC×SEC (a) and calibration of the second-dimension SEC separation (b).
4.6.2 Second-dimension calibration for MTF×SEC

For MTF×SEC experiments a 2D column with a high exclusion limit (10-µm 10^6 Å PLgel particles, 150 × 4.6 mm I.D.) was used to prevent overloading and anomalous-elution behavior in the 2D-SEC separation. Accurate molar-mass calibration was performed by injection of four mixtures, each with three narrow-MMD PS standards. Peak maxima from the UV signal were used to construct the calibration curve (Fig. S-3). The polynomial equation fitted to the calibration curve was used to calculate polymer size corresponding to each elution volume in order to calculate λ values (Eq. 8 / Table 2).

![Fig. S-3. Calibration experiment and calibration curve for second-dimension of MTF×SEC separations.](image)

4.6.3 Flow-rate effect in MTF×SEC

The transition from an HDC to an MTF-type separation at critical conditions is demonstrated in Fig. S-4 using a comprehensive two-dimensional experiment. Linear and star-branched polymers were separated on a 250-mm long monolithic column with $D_{pore} = 126$ nm at 10, 15, 20 and 30 µL/min (Fig. S-4). A sample containing three linear polystyrene standards of $1.37 \cdot 10^6$, $2.56 \cdot 10^6$ and $3.74 \cdot 10^6$ g/mol was used, as well as a nominal “three-arm star” (or Y-shaped) polystyrene sample obtained from Polymer Source (Dorval, Canada; see section 4.3.1).

A particularly challenging aspect of studies into flow-rate effects is the transfer of all 1D effluent to the 2D separation. The flow rate and injected amount in the 3D were kept identical to maximize the comparability. 200 Injections of 30 µL each were transferred to the 2D in all chromatograms, irrespective of the 1D flow.
For the chromatogram with $F = 30 \mu\text{L/min}$ only one minute is available for each 2D chromatogram. An experimental problem is the overlap or wrap-around in the UV signal for these short sampling intervals. Signals near the void volume of the 2D column interfere with the high-molar-mass peaks in the subsequent 2D chromatogram.

Experiments were performed with RALLS detection using 150 mm 1D columns to study selectivity at higher flow rates for linear polymers. The sample was a mixture of nine narrow-MMD linear PS standards in the range 200 – 3740 kg/mol. Separations on monolithic material 7 were performed with $F$ between 75 and 10 µL/min (Fig. S-5). A 1D flow rate higher than 33 µL/min does not significantly change the selectivity or bring additional advantages other than analysis-time reduction. Such high flow rates are not practical for comprehensive 2D separations, because adjustments to the second dimension are required to deal with the larger 1D flow. These will result in either a reduced separation efficiency or a lower sensitivity. For $F$ of 50 and 75 µL/min part of the 1D effluent is lost between 2D injections.

**Fig. S-4.** Transition of the 1D separation mode from HDC to MTF. Linear polymer at 10 (a), 15 (b), 20 (c) and 30 µL/min $F$ (d); three-arm “star” polymer at 10 (e), 15 (f), 20 (g) and 30 µL/min $F$ (h).
Flow-rate effects were also investigated for a 100 mm monolith with smaller pores (Fig. S-6). Molar-mass selectivity can be suppressed at comparable or slightly higher flow rates relative to a $D_p = 126$ nm monolith. At a flow rate in between 33 and 50 µL/min a separation can be obtained with minimal molar-mass selectivity. The narrow-pores of this monolith induced higher operating pressures. A backpressure of 13 MPa was measured for separation at 50 µL/min with THF at 50°C.
Fig. S-6. Flow-rate effect for linear polystyrene on a 100 × 4.6 mm I.D. monolith with $D_{pore} = 104$ nm.

$F = 50$ (a), 33 (b), 22 (c), 15 (d), and 10 (e) µl/min.

4.6.4 MTF×SEC at orthogonal conditions

All detector signals for the separations shown in Fig. 7 (see section 4.4.3) are presented in Fig. S-7. The detector array consisted of a Shimadzu dual-wavelength UV detector and a Viscotek right-angle laser light scattering (RALLS) detector coupled in series. In the bottom row the light-scattering signal divided by the UV absorption signal at 214 nm is presented to give an indication of changes in molar mass. The angular dependence for 90° light scattering is significant for the polymers considered here. Regardless of the reduced scattering intensity for large solutes, the signal is most sensitive for high molar-mass polymers. A comparison of the LS/UV ratio at identical $t_r$ yields a qualitative indication of molar-mass changes. The ratio is sensitive to inter-detector delay and inter-detector band broadening.
Fig. S-7. Polystyrene separated by MTF×SEC at 30 µL/min; consecutive detector signals from top to bottom: UV 260nm, UV 214nm, 90° light scattering and light scattering / UV 214nm ratio (indicated near color bar). (a) linear polymers (b) linear and star polymers (c) LCB polymer $M_n$ 810 kg/mol (d) comb polymer
4.6.5 **MTF×SEC-UV/MALLS on long-chain-branched polystyrene**

An experiment was performed where the RALLS detector in the MTF×SEC-UV/RALLS setup was replaced by a MALLS detector. For the 1D a 150 mm × 4.6 mm I.D. column with $D_{pore} = 104$ nm monolith (monolith 8) was used with $^1F = 30\mu$L/min. The 2D was identical to other MTF×SEC experiments (150 mm × 4.6 mm I.D. 10 µm $10^6$ PLgel with $^2F = 750 \mu$L/min). 25 µL of 1 mg/mL LCBps were injected. Fractions of 90 µL each were transferred to the 2D separation. Results of this experiment are presented in Fig. S-8.

![MTF×SEC-UV/MALLS plots](image)

**Fig. S-8.** 2D plots for UV absorption (a) and the 90° light-scattering signal (b), as well as molar mass for MTF fractions from the MTF×SEC-UV/MALLS experiment between 1.1 and 1.9 mL in the 1D (c).
The signal from the MALLS detector was sufficient to calculate molar-masses, but not for calculating $R_g$. Therefore, a conformation plot with results of the different fractions could not be created. The increase in molar mass for materials eluting at the same hydrodynamic volume (in the 2D) supports the hypothesis that material eluting later from the MTF column has an increasing degree of branching.

### 4.6.6 Selectivity in MTF as a function of flow rate

MTF×SEC Experiments were performed with linear (Fig. S-5) and branched polymers (Fig. S-9 through Fig. S-11) at different 1D flow rates. A 150 mm × 4.6 mm I.D. column with $D_{pore}$ 126nm with $V_0 = 1.65$ mL was used. Experimental conditions are presented in Table S-1.

Red +++ was added as a marker for 2D-peak maxima to 2D chromatograms of branched samples as a visual aid to help establish whether material is still eluting from the 1D column.

**Fig. S-9.** “Star” polymer separated at different flow rates in 1D MTF.
75 (a), 50 (b), 33 (c), 22 (d), 15(e) and 10 µL/min (f)
Branched-Polymers Characterized by Comprehensive 2D Separations with Fully Orthogonal Mechanisms

Fig. S-10. LCB Polymer separated at different flow rates in 1D MTF. 75 (a), 50 (b), 33 (c), 22 (d), 15(e) and 10 µL/min (f).

Fig. S-11. Polymer with little LCB separated at different flow rates in 1D MTF. 75 (a), 50 (b), 33 (c), 22 (d), 15(e) and 10 µL/min (f).

A problem with the 2D pump prevented completion of the experiment at 10 µl/min (f).
Table S-1.: Experimental conditions for the separations in Fig. S-9, S-10 and S-11.

<table>
<thead>
<tr>
<th>1D flowrate (µL/min)</th>
<th>2D time (min)</th>
<th>2D injection volume (µL)</th>
<th>Total time min. (2D chromatograms)</th>
<th>Total volume 1D (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>1.33</td>
<td>100*</td>
<td>80 (60)</td>
<td>6</td>
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<tr>
<td>50</td>
<td>1.33</td>
<td>66.6*</td>
<td>120 (90)</td>
<td>6</td>
</tr>
<tr>
<td>33</td>
<td>1.33</td>
<td>34</td>
<td>180 (135)</td>
<td>6</td>
</tr>
<tr>
<td>22</td>
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<td>30</td>
<td>266 (200)</td>
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<td>15</td>
<td>2</td>
<td>30</td>
<td>400 (200)</td>
<td>6</td>
</tr>
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<td>10</td>
<td>3</td>
<td>30</td>
<td>600 (200)</td>
<td>6</td>
</tr>
</tbody>
</table>

* Incomplete sampling is expected due to the use of 45 µL transfer loops.

4.6.7 Effect of temperature on MTF×SEC separations

Separations of linear and star-branched polystyrene polymers by MTF×SEC were performed under identical conditions, but at room temperature and 50°C different temperatures. For the 1D a 100 mm × 4.6 mm I.D. column with $D_{pore} = 104$ nm (monolith 8) was used with $^1F = 10$ µL/min. The 2D was a 250 mm × 4.6 mm I.D., 10 µm Mini-mixed B column with $^2F = 600$ µL/min. 25 µL of sample solution were injected. 60 Consecutive fractions of 50 µL were transferred to the 2D.

2D Chromatograms of the separations are provided in Fig. 12 and Fig. 13. Dimensionless elution volume ($\tau$) is provided for a convenient comparison of elution volumes in the first-dimension (table S-2) and the second-dimension (table S-3).

Table S-2.: First-dimension temperature dependence of polymer separations in Fig. S-12 and S-13

<table>
<thead>
<tr>
<th>Label</th>
<th>Sample</th>
<th>Elution volume MTF (mL)</th>
<th>$\tau_{25^\circ C}$</th>
<th>$\tau_{50^\circ C}$</th>
<th>$\tau_{25^\circ C}$</th>
<th>$\tau_{50^\circ C}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ionol</td>
<td>1.1</td>
<td>1.1</td>
<td>1</td>
<td>1</td>
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</tr>
<tr>
<td>2</td>
<td>9.9 kg/mol PS</td>
<td>1.05</td>
<td>1.05</td>
<td>0.95</td>
<td>0.95</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>197 kg/mol PS</td>
<td>0.8</td>
<td>0.8</td>
<td>0.73</td>
<td>0.73</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>3742 kg/mol PS</td>
<td>1.25</td>
<td>1.4</td>
<td>1.14</td>
<td>1.27</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ionol</td>
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<td>1.15</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2-arm linear PS</td>
<td>1.2</td>
<td>1.3</td>
<td>1.09</td>
<td>1.18</td>
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</tr>
<tr>
<td>7</td>
<td>3-arm “Star” PS</td>
<td>1.65</td>
<td>1.95</td>
<td>1.50</td>
<td>1.77</td>
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</tr>
</tbody>
</table>
Table S-3.: Second-dimension temperature dependence of polymer separations in Fig. S-12 and S-13

<table>
<thead>
<tr>
<th>Label</th>
<th>Sample</th>
<th>Elution volume SEC (mL)</th>
<th>τSEC</th>
<th>25°C</th>
<th>50°C</th>
<th>25°C</th>
<th>50°C</th>
</tr>
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<td>0.86</td>
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<tr>
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<td>197 kg/mol PS</td>
<td>2.21</td>
<td>0.73</td>
<td>0.73</td>
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<td></td>
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</tr>
<tr>
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<td>3742 kg/mol PS</td>
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<td>0.60</td>
<td>0.60</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>ionol</td>
<td>3.18</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>6</td>
<td>2-arm linear PS</td>
<td>1.87</td>
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<td>3-arm “Star” PS</td>
<td>1.84</td>
<td>0.61</td>
<td>0.61</td>
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<td></td>
</tr>
</tbody>
</table>

**Fig. S-12.** Room-temperature separation of narrow-MMD linear standards (a) and a three-arm “Star” Polymer (b).

**Fig. S-13.** Separation of narrow-MMD PS standards (a) and a three-arm “Star” Polymer (b) at 50°C.
Chapter 4

References

[64] Uliyanchenko, SEC-HDC demonstration, choose article where this is shown