DEVELOPMENT OF TWO-DIMENSIONAL CHROMATOGRAPHIC METHODS FOR THE SEPARATION AND CHARACTERIZATION OF POLYESTERS OF DIFFERENT DEGREES OF BRANCHING

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Publications:

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   "Retention Behaviour of Linear, Branched and Hyperbranched Polyesters in Liquid Chromatography"
   Macromolecules, **43** (2010), 3215-3220.
2. Anna Khalyavina, Falko Schallausky, Hartmut Komber, Michael Al Samman, Wolfgang Radke and Albena Lederer
   "Aromatic – Aliphatic Polyesters with Tailored Degree of Branchings Based on AB/AB_2 and ABB^*/ AB_2 Monomers"
   Macromolecules, **43** (2010), 3268-3276.

Oral presentations:

1. "Separating Polymers of Different Degree of Branching by 2-Dimensional Chromatography"
   SCM-4 (Fourth International Symposium on the Separation and Characterization of Natural and Synthetic Macromolecules),
   28.01.-30.01.2009, Amsterdam, The Netherlands.

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2. "Polymer Characterization of Branched Architectures"
   SCM-4 (Fourth International Symposium on the Separation and Characterization of Natural and Synthetic Macromolecules),
   28.01.-30.01.2009, Amsterdam, The Netherlands.

TABLE OF CONTENT

1 Introduction

Polymers are highly complex multicomponent materials that play an increasingly important role in our daily life. Polymers can be found in many products ranging from ordinary household commodities to speciality polymers in e.g. automobile components or medical drug delivery devices. The demand for synthetic polymers increases constantly and an annual consumption of 300 million tons is expected at the end of this decade [1].

Due to their unique properties and their wide field of applications, polymers are continuously replacing traditional materials such as wood and metal. The wide range of properties is achieved using a large palette of polymeric products, based however on a relative limited number of different monomers.

In contrast to well-defined low molar mass molecules, synthetic polymers are usually disperse due to the randomness of the polymerization processes. Even in the simplest case, the linear homopolymers, heterogeneity in molar mass, i.e. molecules differing in their molar masses, exists. In more complex cases, heterogeneities in chemical composition, functionality or in architecture may also exist beside the one in molar mass. The type and the extent of these heterogeneities can cause significant differences in the final properties of the polymeric materials. The different types of molecular heterogeneity are schematically depicted in Figure 1.1.

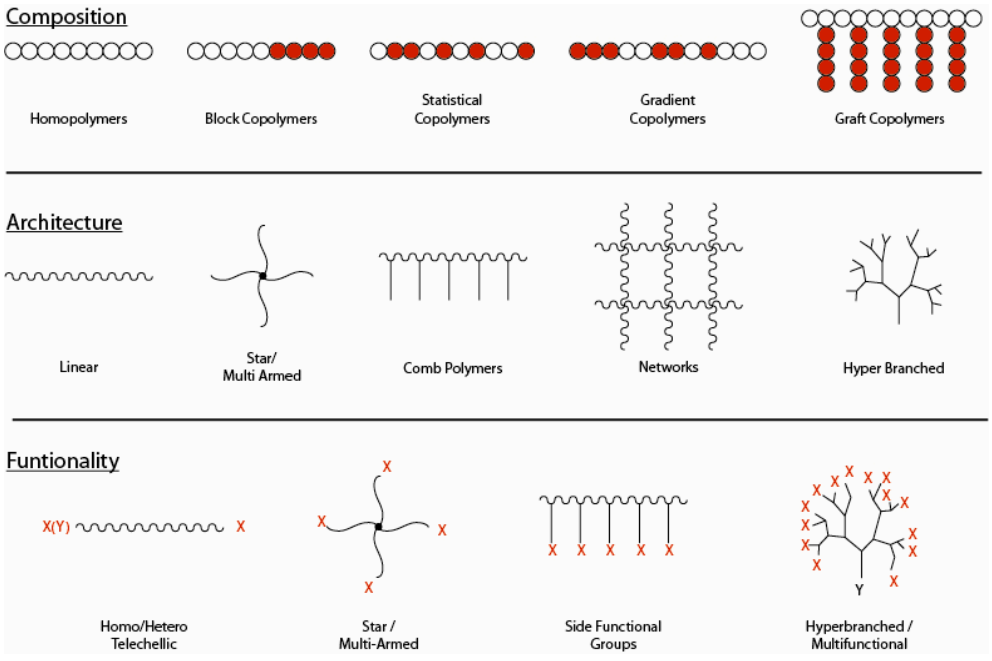


Figure 1.1: Schematic representation of possible molecular structures for polymers in terms of chemical composition, architecture and end-group functionality. Graph taken from [2].

The use of traditional characterization methods of polymer analysis such as infrared spectroscopy or nuclear magnetic resonance (NMR) allows in principle the identification of the types of monomers or functional group present in the sample. In addition such methods might allow for quantifying average compositions, molar masses or functionalities. However, these methods do not yield information on how functional groups, monomers, branching points, etc. are distributed among the different polymer molecules.

In order to characterize and to completely understand the relationship between the molecular structure and the macroscopic properties of such complex polymers, it is necessary to determine not only the average value of a particular property, like the chemical composition or molar mass, but knowledge on the complete complex distribution function is required.

In recent years, high performance liquid chromatography has emerged as the method of choice for the characterization of complex polymer systems [3,4,5]. While Size Exclusion Chromatography (SEC) is the established method for analyzing the molar mass distribution of natural and synthetic polymers [6], other chromatographic techniques have to be used to characterize heterogeneities other than the one in molar mass. Non-SEC separation techniques such as liquid adsorption chromatography (LAC), gradient liquid adsorption chromatography (gradient chromatography) and liquid chromatography at critical conditions (LC-CC) have been proven to be extremely useful for the characterization of complex polymers [7,8]. For example, polymer blends can be separated into their chemically different components [9,10], statistical copolymers can be separated according to their chemical composition [11,12] or the block length distribution of individual blocks in diblock copolymers can be determined by those chromatographic methods. However, the characterization of complex polymers possessing a topological distribution (branched polymers) still remains a major challenge in polymer analysis, even though the increasing applications of branched polymers request for suitable characterization techniques. The conventional techniques for polymer characterization are often based on theories and methods developed for linear polymers and therefore cannot be applied directly to the characterization of branched polymers.

The compact structure of branched polymers and the large number of functional end groups result in new, interesting properties and numerous possible applications. Branched polymers have been used in several applications due to their advanced rheological and mechanical properties in comparison to their linear counterparts [13,14].

Although, there has been considerable progress in the synthesis and characterization of branched polymers so far, their full application potentials will not be used before a complete understanding of the relations between molecular structure and macromolecular properties

can be achieved. The knowledge of the detailed topological characteristics and their effect on functional properties will ultimately allow the design of new tailor made high-performance polymers.

1.1 Aim of the Thesis

The aim of the present thesis was to develop new liquid chromatographic methods for the characterization and separation of branched polyesters according to the degree of branching (DB). The investigated model compounds were based on 4,4-bis(4'-hydroxyphenyl)pentanoic acid and were prepared by polycondensation in solution at the Leibniz Institute of Polymer Research (Dresden). Present characterization techniques for the characterization of linear and branched polyesters do not exist or are limited to yield only average values about topological information of branched polyester such as the average value for the Degree of Branching (DB) of the whole sample. However, for a complete characterization of branched polymers and in order to be able to relate the molecular structure with the macroscopic properties, more information besides the average DB is required.

In the first part of the thesis, it will be experimentally shown that conventional one-dimensional liquid chromatographic methods (SEC-MALLS, gradient chromatography, LAC, LC-CC) are only partially successful in the characterization of branched polyesters. Further chromatographic experiments will be presented to investigate the effect of branching on the retention behaviour of linear and different branched polyesters in enthalpic interaction dominated chromatographic modes. In addition, quantitative results on the enthalpic interactions between the stationary phase and the polymer molecules will be presented. These investigations were performed in order to be able to understand the retention behaviour of linear and branched polymers and therefore to be able to purposefully adjust, predict and optimize the chromatographic conditions in the different chromatographic modes.

In the second part of the thesis, a liquid chromatographic method development for the separation of linear and differently branched polymers will be presented, based on the results in the first chapter. In order to do so, the capabilities of two-dimensional liquid chromatography (e.g. gradient chromatography combined with SEC) were explored. For this purpose, the separation efficiencies of both online and offline two-dimensional liquid chromatography were investigated.

In addition, artificial blends with known amounts of linear and branched polyester were prepared, separated by online 2D chromatography and quantified to evaluate the applicability of the introduced method. Finally, a mathematical approach for the determination of the DB of an unknown polyester sample and for quantifying the dispersity in DB will be introduced.

2 Theoretical Part

2.1 Types of Branched Polymers

Polymers are traditionally classified according to their macromolecular chain architecture into two categories: linear and branched polymers. The description of linear polymers is rather simple. However, there are different ways a branched polymer can be arranged. The most common classes of branched polymers are:

- Star polymers
- Comb polymers
- Crosslinked (network) polymers
- Randomly branched polymers (hyperbranched polymers, long chain branched polymers, etc.)
- Perfectly branched dendrimers

A branched polymer is characterized by the presence of at least one branch point resulting in more than two end groups for the molecule. Many properties of branched polymers are different from those of their linear analogues. Branching effects polymer properties such as crystallinity, melting behaviour, viscoelastic behaviour, solution and melt viscosities [15,16]. These changes in mechanical and rheological properties can be beneficial especially for controlling processability and the performance of such polymers.

The interest on branched polymers and especially on hyperbranched polymers is due to the fact, that they combine some profitable properties of dendrimers (such as: high number of functional groups, compact structure in solution, absence or limited possibility to form entanglement) with the ease of preparation by a one-pot polymerisation.

The main focus of the present PhD thesis will be on randomly branched polymers with a high density of branchpoints. For convenience reason, it will be distinguished between partially branched polymers (DB less than 0.5), hyperbranched polymers (DB around 0.5) and pseudo dendrimers (DB around 1). The use of the expression (hyper)branched polymer will include all three aforementioned types of randomly branched polymers.

2.1.1 Hyperbranched Polymers

Polymers obtained from the statistical polymerization of AB_x monomers ($x \geq 2$), where A and B stands for different functional groups within a monomeric unit, are referred to as hyperbranched polymers. The most common synthetic route is the one-pot polycondensation of a multifunctional monomer (AB_x) with a number of B-groups $x \geq 2$. Using this approach, a wide variety of monomers, such as (3,5-dibromophenyl)boronic acid [17], 3,5-bis(trimethylsiloxy)benzoyl chloride [18], 5-diacetoxybenzoic acid [19] and 2,2–dimethylol propionic acid [20] have been successfully converted to hyperbranched polymers. Besides polycondensation, other techniques [21,22,23,24] for the synthesis of hyperbranched polymers have been described in literature as well.

A schematic representation of the structure of a hyperbranched polymer is shown in figure 2.1.

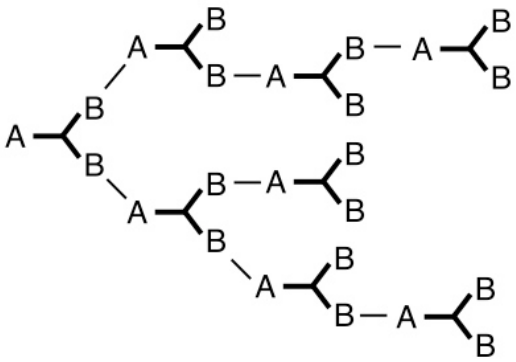


Figure 2.1: Schematic representation of the structure of a hyperbranched polymer obtained from AB_2 monomers.

Hyperbranched polymers exhibit a large number of reactive end groups at its periphical surface, which facilates customization of properties for a wide rage of final products. In contrast to linear polymers, hyperbranched polymers are less prone to form entanglement and to undergo crystallization. Due to the lack of entanglement and their packed structure, hyperbranched polymers show low intrinsic viscosities even at high molar masses [25]. Another common feature of hyperbranched is their high solubility, which is a result of the large number of periphical terminal functional groups. These properties allow large amounts of hyperbranched polymers to be added to polymer blends without or with only a slight increase in the viscosity of the blend. The low cost for synthesizing hyperbranched polymers allows producing them in a large scale for industrial applications. One example of a commercially successful product are hyperbranched aliphatic polyesters marketed under the name BOLTORN© (Perstorp, Sweden). BOLTORN © polymers feature a large number of

primary hydroxyl groups, a densely branched polymer backbone and extensive formulation possibilities. They are frequently used in applications such as:

- Performance additives for flexible polyurethanes foams used in million of cars to improve the firmness of the high-resilience foam articles
- Additives for coatings to improve the performance and to help coating formulators to comply with environmental demands without compromising coating performance.

2.1.2 Molecular Architecture of Hyperbranched Polymers

Unlike perfectly branched dendrimers, where only branched (dendritic, D) and terminal units (T) exist and no linear units (L) are present, all three structural units are present in hyperbranched polymers. Figure 2.2 shows the structural difference between hyperbranched polymers and perfectly branched dendrimers.

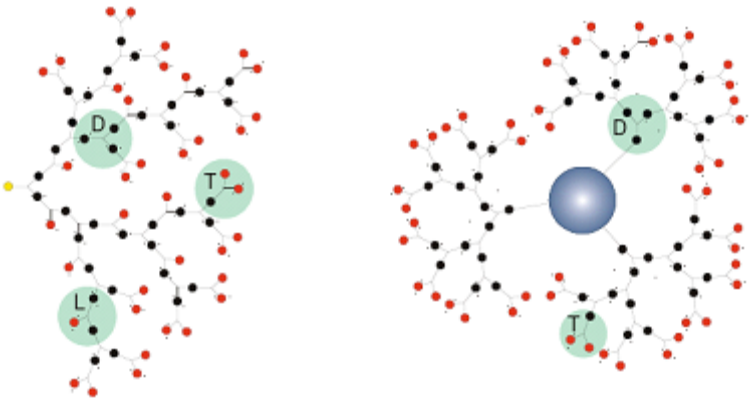


Figure 2.2: Structural difference between hyperbranched polymers and perfectly branched dendrimer. The abbreviations in the figure are: D = dendritic (branched); T = terminal; L = linear. The red dots in the figure represent the unreacted functional groups, while the black dots represent the reacted functional groups. Graph taken from [26].

As can be seen in Figure 2.2, hyperbranched polymers are built up of terminal units having two unreacted functional groups, linear units carrying one unreacted and one reacted functional group and branched (dendritic) units having two reacted functional groups. On the other hand, perfectly branched dendrimers consist only of branched and terminal units, with no linear units incorporated into the polymer structure. The linear segments in the structure of hyperbranched polymers are usually described as defects and it is assumed that their presence has a severe influence on the physical properties of these polymers. In order to be able to

distinguish and to characterize hyperbranched polymers, Fréchet et al. [27] defined the term *DB* (DB) as follows:

$$DB_{Fréchet} = \frac{D+T}{D+T+L} \qquad 2\text{-}1$$

where D, T and L are the number of dendritic (branched), terminal or linear monomer units in the resulting hyperbranched polymer. Equation 2.1 is only valid for branched polymers based on AB_2 monomers and cannot be applied to monomers of type AB_x with $x \geq 2$. In the case of perfectly branched dendrimers, where only branched and terminal units are present, the DB becomes equal to 1. According to equation 2-1, even fully linear structures would possess a DB>0, taking into account that $T = D + 1$. Therefore, Fréchet's approach yields only good results for polymers having a high degree of polymerisation, where the number of the branched units is approximating the number of the terminal units. For low molar masses or only slightly branched polymers, the influence of terminal groups leads to an overestimation of the DB [28,29].

Therefore, Hoelter and Frey [28,29] introduced another equation that is more suitable to calculate the DB for low molar mass hyperbranched polymers:

$$DB_{Frey} = \frac{2D}{2D+L} \qquad 2\text{-}2$$

Another advantage of equation 2-2 is that it does not require determination of the amount of terminal units.

Assuming equal reactivities of all functional groups leads to a final value for DB of 0.5. Experimentally, results obtained for DB are mostly slightly lower, since complete conversion of all B-groups is less likely to be reached. In addition, due to sterical reasons, linear units are less accessible than terminal units, leading to a higher fraction of linear units than expected statistically. A value for DB above 0.5 can be reached, if one modifies the reactivity of the functional groups in linear and terminal units to favour reaction of the functional groups in the linear units. A DB close to 1 (a DB equal to 1 is characteristic for a perfectly branched dendrimer) will be achieved, when there is a very large difference in the reactivities, which favours the reaction of the functional groups of the linear units.

To date, there are two different experimental techniques to determine the DB. The first technique was presented by Fréchet et al. [18] and involves quantitative ^{13}C-NMR-

spectroscopy. The different resonances in the spectrum are assigned to the linear, branched and terminal units. Afterwards, the DB can be calculated from the intensities of the different resonances using equation 2-1 or 2-2, respectively. However, this method is only practical for polymers resulting from monomers, which show distinct differences in the magnetic resonances depending on the substitution pattern.

Another method for the determination of the DB, which is based on digestive methods was presented by Hawker and Kambouris [30]. This technique involves modification of the non-reacted functional groups followed by the complete degradation of the polymeric linkages. Consequently, the degradation products can be separated and identified by capillary techniques. This procedure allowed the determination of the DB for a large number of polymeric systems. Nevertheless, two fundamental chemical requirements have to be fulfilled in order to apply this technique successfully. First, the degradation procedure is not allowed to affect the chemical modified functional groups and secondly the degradation has to result in a complete conversion into the elementary subunits.

Both methods have their limitations and drawbacks, resulting in a large number of systems where the question, whether the materials resemble linear or hyperbranched polymers cannot be finally answered [31,32,33].

The second most important distinction among hyperbranched polymers next to the DB is isomerism. The addition of each monomer unit during the synthesis of a hyperbranched polymer takes place by a random process. Therefore, a large number of geometrical isomers are formed, even for a specific molar mass and DB. This high number of geometrical isomers causes an increase in the heterogeneity. Figure 2.3 shows two different isomers of polymers having identical values for molar mass and DB, but differ in topology.

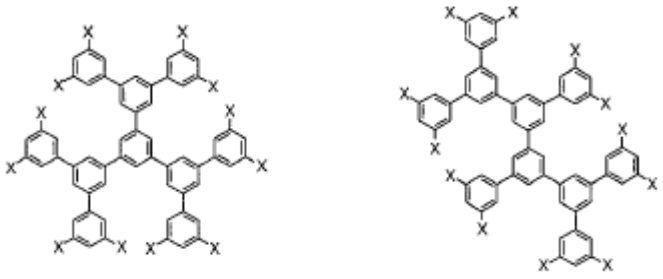


Figure 2.3: Two hyperbranched polymers having the same molar mass and DB, but different topology. Graph taken from [32].

The geometrical variation seems to be neglectable at first sight, but the variation of geometry influences the physical properties in solution as well as the solid-state packing of the polymer.

The variation in packing influences not only the relaxation process but also affects the solubility of the polymer [34].

The quantification of the extent of this topological heterogeneity is difficult compared to the quantification of other molecular parameters such as the molar mass dispersity. Flory showed that the number of possible configurations of a randomly branched polymer can be calculated according to equation 2-3 [35]:

$$Number\ of\ Configurations = \frac{nx!}{(nx - n + 1)!n!} \qquad 2\text{-}3$$

where n equals the degree of polymerization and x is the number of B-groups in the AB_x-unit. It becomes obvious from equation 2-3 that the number of isomers increases with molar mass and with the functionality of the monomeric unit.

There have been some attempts by several research groups to mathematically describe the isomeric structures in dendritic [36] and hyperbranched polymers [37] with more or less success.

2.2 Characterization of Branched Polymers

The existence of different types of heterogeneities in branched polymers, which results from the statistical polymerisation process makes the characterization of branched polymers a non-trivial task. For a complete characterization of a branched polymer, the molar mass and the molar mass distribution, the amount of the branched units and the distribution of the branched units along and among the polymer chains have to be determined.

However, for randomly branched polymers even the determination of the molar mass distribution, for linear polymers a standard problem, is tagged with difficulties. The intricacies arise due to the compact and dense structure of branched polymers, which causes a higher segmental density compared to the linear polymer of same molar mass. Therefore, the determination of the molar mass distribution by application of SEC using a calibration with adequate linear polymer standards can result in strong deviations from the true molar mass. Lederer et al. [38] compared and evaluated the molar masses of hyperbranched poly(ether)amides determined by different SEC-interpretation methods:

- SEC-DRI detection with polyethylene oxide standards
- SEC-DRI detection with polystyrene standards
- SEC-viscosity detection and universal calibration

- SEC coupled with a multiangle laser light scattering detector (MALLS)

Strong deviations were observed between the molar masses determined by the different approaches. Even the data obtained by universal calibration were not consistent with the real molar mass values obtained from SEC-MALLS (which is the method of choice for the determination of the real molar mass of complex architectures). This behaviour was explained by the fact that the universal calibration, which is based on the Flory-Fox equation, cannot be applied to hyperbranched polymers [39]:

$$[\eta] = \phi\left(\frac{R_g^3}{M}\right) \qquad 2\text{-}4$$

where ϕ is the draining factor. For linear polymers, the draining factor ϕ becomes a constant and is therefore not dependent on the size of the molecules [40,41]. However, for hyperbranched polymers, an increasing segment density leads to an increase of the draining parameter, which is contradicting the universality of this relationship [42]. This non-applicability of the Flory-Fox equation for hyperbranched polymers that was previously observed by the authors was also experimentally found for other branched polymer systems [43].

Another persistent problem in the molar mass determination of hyperbranched polymers is caused by the enthalpic interactions between the functional groups of the polymer and the stationary phase, especially for higher molar masses, which has been repeatedly observed by various authors. Due to their very compact and globular structure, a higher number of functional groups are located at the periphical surface of the hyperbranched polymer. Therefore the total amount of enthalpic interaction with the stationary phase is higher compared to the linear analogues. Especially multifunctional polymers show a very strong tendency to interact with the stationary phase. This can result in a shift of the elution volume of the polymer sample, which can falsify the results obtained for the molar mass distribution. Several attempts were undertaken with more or less success to reduce these interactions, e.g. by changing the eluent or the material of the stationary phase, or by adding salt to the eluent [38,44].

Another way to eliminate the problem of interaction with the stationary phase is to use a separation method without a stationary phase known as asymmetric flow field fractionation (AF4) [45,46]. In AF4, separation takes place in an open flow channel. The channel consists of two plates separated by a spacer foil with a typical thickness of 100 to 500 µm. The upper plate is impermeable, while the lower plate is made of a permeable, porous frit material.

A membrane covers the bottom plate to prevent the polymer sample from penetrating the frit or from leaving the channel. Due to the solvent flow, a parabolic flow profile develops within the channel, where the flow rate is lower close to the channel walls as compared to the center of the channel. A second flow which runs perpendicular (cross flow) to the channel flow direction drives the analyte molecules towards the boundary layer of the channel. However, diffusion resulting from Brownian motion counteracts the accumulation of the polymers at the wall. This causes smaller particles with higher diffusion constants to reach in average a higher position in the channel, where the flow is faster. As a consequence, smaller particles are transported faster along the channel than larger particles. Interactions with the membrane do not exist or can be neglected. The main limitation of AF4 is the porous membrane, which is permeable for polymers below ~5000 g/mol. Lederer et al. [47] succeeded in the AF4 separation of an aromatic hyperbranched polyesters with M_W = 161.000 g/mol according to molar mass. In contrast, SEC failed to separate according to size due to strong interactions with the stationary phase for the chemical identical polymer sample having a lower molar mass (M_W = 40.000 g/mol).

For branched polymers below approx. 5000 g/mol, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) seems to be the method of choice for their characterization. MALDI-TOF-MS is a soft ionization technique, allowing the characterization of macromolecules, which tend to be fragmented when ionized by conventional ionization methods [48].

MALDI-TOF-MS has been successfully applied to several branched polymers by various authors [49,50,51]. The limitations for this characterization method are reached when broadly distributed polymer samples or polymers of high molar masses need to be analyzed, since the lower molar mass fractions are preferably activated for desorption. Therefore, the obtained information about the polydispersity of the polymer sample should be interpreted with extreme care. In addition, MALDI-TOF-MS can only distinguish between molecules of different molar masses. Polymers differing in chemical composition or topology but having identical molar masses cannot be distinguished

In order to be able to apply MALDI-TOF-MS on broadly distributed polymers, it is advisable to first separate the polymer samples into narrow fractions using highly sophisticated separation techniques and afterwards determining the molar masses in the fractions using MALDI-TOF-MS. Jaumann et al. [50] used the hyphenation of SEC with MALDI-TOF-MS in order to achieve a complete characterization of branched polyalkoxysiloxanes ranging in molar mass up to 10^6 g/mol. Gooden et al. [52] demonstrated that MALDI-TOF-MS is also

applicable to gain direct access to structural information of hyperbranched polymers. They used MALDI-TOF-MS for the determination of the extent of macrocycle formation during the polymerization and copolymerization of A_2B and A_4B monomers. Nevertheless, MALDI-TOF-MS is mainly limited to narrow distributed fractions and samples of relatively low molar mass. For hyperbranched polymers of higher molar mass, SEC-MALLS is definitely the better choice.

Besides the molar mass distribution, the topology has also to be taken into account for a complete characterization of branched polymers.

One example for a successful chromatographic characterization of star-shaped poly(L-lactide)s (PLA) was performed by Pasch et al. [53]. In a first experimental step, a series of linear PLA's was used to find the chromatographic conditions at which a molar mass independent elution was observed (critical conditions). Afterwards, this critical eluent composition of the linear PLA's was applied to star-shaped PLAs. A linear increase of the elution volume as a function of the number of the arms was observed, which allowed to discriminate between different branched poly(L-lactide)s in a mixture. However, the separation was not based on topology but utilizes the interaction of hydroxyl-groups attached to the ends of the arms. Besides purely experimental works, Radke et al. compared theoretical predictions with the experimental results. They were able to explain the experimental results of Pasch et al. on functionalized star-shaped poly(lactide)s [54].

Another common approach for the characterization of all kinds of branched polymers, which has been developed by Zimm and Stockmayer, is based by the fact that branching decreases the radius of gyration R_g of a polymer chain in comparison to R_g of a linear chain of the same molar mass. This contraction usually is expressed by the contraction factor g [55,56] defined as

$$g = \frac{\left\lfloor R_g^2 \right\rfloor_{br}}{\left\lfloor R_g^2 \right\rfloor_{lin}} \qquad 2\text{-}5$$

where $\left\lfloor R_g^2 \right\rfloor$ is the mean squared radius of gyration, and the subscripts *br* and *lin* refer to the branched and linear polymers at the same molar mass. Since branched polymers have a more compact structure compared to the linear polymers of a given molar mass, the parameter *g* is always less than 1. The Zimm – Stockmayer [57] approach can be used to correlate the contraction factor to a given topology based on the assumption of random flight subchains.

Similarly, the decrease of intrinsic viscosity of a branched molecule can be used to quantify the extent of branching. This is done using the contraction factor g' that is defined as the ratio of the intrinsic viscosities of the linear and branched sample having the same molar mass

$$g' = \frac{\langle \eta \rangle_{br}}{\langle \eta \rangle_{lin}}. \qquad 2\text{-}6$$

Theoretical and experimental studies have shown, that the contraction factor g is related to g' in the following way

$$g' = g^{b} \qquad 2\text{-}7$$

where b is a scaling constant (also called solvent factor in literature). For star-shaped polymers, Zimm theoretically predicted a value of $b = 0.5$, while empirically values between 0.5 and 1.5 are found. For randomly branched polymers, strong variations in the scaling factor b have been observed, depending on the chemical structure and molar mass of the investigated polymer. Once b is known and g' has been determined, the number of branches per molecule can be calculated depending on the branching types from published equations [55,57,58].

The determination of the contraction factor g and the intrinsic contraction factor g' represents a very sophisticated way to characterize branched polymers. In order to minimize complications due to molar mass dispersity, the reduction in size is usually determined by coupling SEC with MALLS [59,60] or viscosity detection [61] or both [62]. However, often suitable linear analogues, required for the determination of g or g', are not available. In addition, the interpretation of g or g' for SEC-fractions is based on the assumption that SEC yields homogeneous fractions. Since branched polymers in general possess heterogeneities in molar mass and topology, coelution of polymers having the same hydrodynamic volumes, but different topologies and molar masses might occur. In those cases the results obtained for molar mass, radius of gyration or intrinsic viscosity for every SEC-fraction have to be interpreted as average values [63,64].

One recent approach to gain information on the extend of heterogeneity within a SEC-slice utilizes the fact that SEC with viscometry detection results in a local number average molar mass, while light scattering detection yields a local weight average molar mass. Thus, by applying both detection systems determination of the local dispersity due to coeluting polymers differing in molar mass should be possible, in principle [65]. However, this approach

cannot overcome the principal problem of coelution of different molecular species and the lack of suitable linear analogues.

In order to overcome the issue of coelution, it seems to be necessary to first effectively separate linear and branched structures from each other before using highly sophisticated detector systems for the complete characterization of the polymer sample.

According to the author's knowledge, no theoretical or experimental studies about the separation of linear and hyperbranched structures based on 4,4-bis(4'-hydroxyphenyl)-pentanoic acid have been performed so far. However, for other topologies, such as long chain branched polymers, star shaped polymers or branched biopolymer, different ways to perform separations of linear and branched topologies can be found in literature.

One approach for separating linear from branched biopolymers makes use of a microfabricated sieve [66]. Lithography is used to etch a matrix of obstacles (1.5 by 6 μm in dimensions) on a silicon chip, which is then sealed to create a "sieve" through which a solution of macromolecules can flow. The macromolecules are supposed to be deflected on the obstacles, such that each species follows a different trajectory, dependent on the size of the macromolecule. The basic applicability of such a device has been demonstrated in recent works of van Oudenaarden and Boxer on lipids [67].

The strength of the system arises from the high progress in lithography of silicon chips, which allows etching regular obstacles on the chip and by the possibility to choose the pattern of the obstacles.

Although no experimental data was presented so far on branched polymers, which proves the validity of this approach for branched polymers, it becomes clear that this approach should work even more effectively for long chain branched polymer, since branched polymer chains should provide additional points of entanglement on the obstacles. However, the dimensions of the matrix would be too large to separate most synthetic polymers. Smaller dimensions would be required to perform separations of synthetic polymers.

A second approach to separate branched and linear polymers involves capillary electrophoresis with a highly viscous gel as entanglement medium. Experiments on linear and star-branched DNA structures revealed that topology has a nontrivial effect on the electrophoretic mobility. Large branched DNA structures became trapped through entanglement with the fixed gel matrix and therefore elute slower than their linear analogues [68].

Smisek et al. [69] used gel electrophoresis with a relatively high viscosity gel as entanglement medium for synthetic polymers (linear sulfonated polystyrene). They found three different

separation regimes depended on their molar mass. In the low molar mass region, the polymer chains were separated in a SEC elution order, since the molar mass of the molecules was too low to entangle with the gel. In the high molar mass region, the separation was governed by an entanglement mechanism. In the intermediate molar mass region, no clear trend could be observed. Experimental works on branched sulfonated polystyrenes revealed a SEC-like elution order, since mainly low molar mass polymers were used. Even if the results are quite promising, an electrophoretically driven separation can only operate with small amounts of synthetic polymers and the polymers need to be charged. Therefore, most synthetic polymers cannot be analyzed by this characterization method.

A third approach and one of the most promising attempts was recently introduced for the separation of long chain branched and star shaped polymers under the name molecular topology fractionation (MTF) by D. Meunier and R. Edam [70,71,72]. MTF utilizes monolithic columns with channel diameters in the order of the polymer molecular dimensions as the separation medium. When a polymer migrates through restrictions smaller than its hydrodynamic volume (a tortuous path of molecular dimensions), it must deform and thereby sacrifices entropy. Since branched polymers need to sacrifice more entropy than linear polymer of identical size as it migrated through these restrictions, a separation of topologically different molecules might occur. As a consequence, a separation based on the topology dependent relaxation time spectrum of polymers in dilute solutions follows.

Key parameters in MTF are flow rate, macropore dimensions of the stationary phase and molar mass of the polymer. Meunier et al. observed conventional SEC elution order for higher flow rates (60 and 100 µL/min) for linear polystyrene standards. As the flow rate was decreased, a reversal of elution order could be observed for higher molar masses. The flow rate dependence as well as the molar mass dependence for linear polystyrene standards is depicted in figure 2.4.

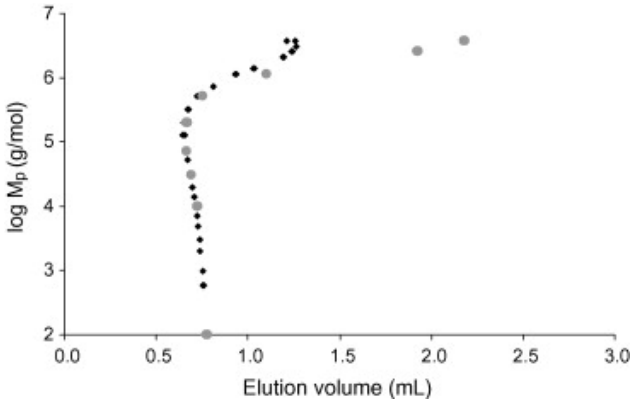


Figure 2.4: MTF calibration curve for linear polystyrene standards obtained at a flow rate of 20 µL/min (♦)and at 10 µL/min (●). Graph taken from [70].

MTF can be used to separate linear and long chain branched molecules, since branched polymers are much more effectively retained than linear ones. However, branching and molar mass contribute to retention in MTF, so that only samples with extremely narrow molar mass distributions can be separated by MTF. Therefore, SEC was coupled to MTF to yield a 2-dimensional separation system, where first a MTF separation was performed and the obtained fractions were automatically injected into a SEC system. Using this 2D-approach, it was possible to demonstrate the branching selective separation of narrowly distributed star-shaped polymers and linear polymers above a critical molar mass that depends on the MTF-column. MTF×SEC was also applied to a broadly distributed polystyrene sample that featured a high degree of long-chain branching. Although some selectivity was observed, the separation may need to be improved if one wants to obtain quantitative results for long-chain branched polymers. In addition, MTF works only for polymer sample above a critical molar mass that depends on the macropore dimension. This dramatically limits the application of MTF.

The fourth approach and the one performed here in this thesis is also based on a two-dimensional liquid chromatographic setup, similar to the combination of MTF×SEC. However, instead of MTF for one dimension, a second conventional chromatographic technique is used. In the first dimension, a separation is performed according to one parameter, e.g. hydrodynamic volume, and the fractions obtained from the first dimension are subjected to a second chromatographic separation e.g., according to molar mass, functionality, etc. [73,74,75].

Im et al. [76] applied 2D-chromatography to linear and branched polystyrenes. Branched polystyrene was prepared by anionic polymerization using *n*-butyl Lithium as initiator and 4-(chlorodimethylsilyl)styrene (CDMSS) as linking reagent [77]. This synthetic approach yields broadly distributed polystyrenes with different number of branches. In the subsequent 2D-chromatographic separation, reversed-phase temperature gradient interaction chromatography (RP-TGIC) was performed in the first dimension and LC-CC was used in the second dimension. The use of RP-TGIC as first dimension resulted in a highly efficient separation according to molar mass, since RP-TGIC is supposed to be more sensitive towards molar mass as compared to conventional SEC [78]. In the second dimension, critical conditions for linear polystyrene were adjusted. Under these conditions the contribution of molar mass on elution volume vanishes and all linear polystyrenes elute at the same elution volume irrespective of their molar mass. If the polymer chains carry different functional groups, then functional end-groups capable to interact with the stationary phase determine the retention

time. Since branched polymers have a large number of end-groups, the retention will be controlled by functionality exclusively. Therefore the application of LC-CC to the TGIC-fractions of the synthesized branched polystyrenes allowed a separation in terms of the number of branches due to the fact that each branch unit acts as an additional functionality. The 2D chromatographic coupling of RP-TGIC x LC-CC yielded good results in the separation of branched polystyrenes with a satisfactory resolution in terms of molar mass and number of branches. However, although a separation according to the number of branches was realized, the separation was based on functionality and not on topology. For chemically identical branched polymer chains, where the branch points do not result in additional retention, a separation would not be efficient by this approach.

Gerber et al. [79,80] realized a 2D-chromatographic separation of mixtures of linear and star-shaped polystyrenes by a combination of temperature-gradient-interaction-chromatography (TGIC) and SEC. Since separation in TGIC is thought to be based on molar mass while separation in SEC is based on hydrodynamic volume, a separation of linear and branched species is possible due to the different ratios between molar mass and hydrodynamic volume. Figure 2.5 shows the online 2D chromatographic separation of a mixture of linear and star-shaped polystyrenes.

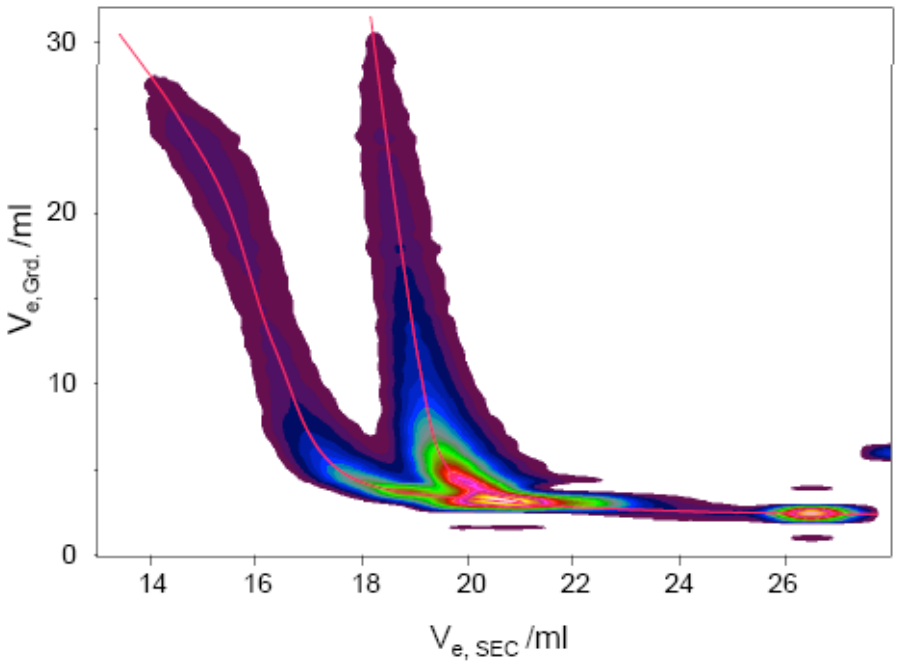


Figure 2.5: Online 2D separation of a mixture of linear and star shaped polystyrenes. Graph taken from [80].

In contrast to the polymer system investigated by Im et al., no strong chemical difference between the branchpoints and the styrene units was present. Hence it can be assumed that a topology dependent separation was performed.

Gorbunov et al. [81] compared the experimental data of Gerber et al. with theoretical results and found the experimental findings to be in good qualitative agreement with the existing theory on polymer chromatography. However, it should be mentioned that the simulated samples of Gorbunov et al. and the experimental ones used by Gerber et al. differ slightly from each other.

The methods of Gerber et al. and Im et al. revealed high potential for separating linear and branched polystyrenes by 2D-chromatography. However, for other topologies the method development is hampered by a lack of well-defined branched model compounds and/or linear polymers with identical chemical structure. Additionally there is a severe lack of knowledge on the retention behaviour of branched polymers in interaction chromatography.

2.3 Liquid Chromatography of Polymers

Any chromatographic separation process is based on the selective distribution of the solute between the mobile phase and the stationary phase of a given chromatographic system [4,82]. The distribution (or partition) coefficient K_d can be used to describe the affinity of a molecule for both phases and thus helps to predict the elution order of the molecules. K_d is defined as the ratio of the analyte concentrations in the stationary ($c_{stat.phas.}$) and mobile phase ($c_{mob.phase.}$), respectively:

$$K_d = \frac{c_{stat.phas.}}{c_{mob.phase.}} \qquad 2\text{-}8$$

The elution volume in liquid chromatography can be described by

$$V_E = V_A + V_p \cdot K_d \qquad 2\text{-}9$$

where V_A is the interstitial volume and V_p the pore volume. K_d is related to the Gibbs free energy difference of the molecules between the two phases and can be described using the following two thermodynamically equations:

$$\Delta G = \Delta H - T\Delta S = -RT \ln K_D \qquad 2\text{-}10$$

$$K_D = \exp\left(\frac{\Delta S}{R} - \frac{\Delta H}{RT}\right) \qquad 2\text{-}11$$

where R is the universal gas constant, T the temperature in Kelvin and ΔH and ΔS are the changes in interaction enthalpy and conformational entropy. In the simplest case the change in Gibbs free energy ΔG can be attributed to two effects:

- When the macromolecule is inside the pore of the stationary phase, it cannot occupy all possible confirmations due to the limited dimensions of the pore. As a consequence, the conformational entropy ΔS decreases.
- When the macromolecule penetrates the pore, it can interact with the surface of the pores. As a consequence, a change in enthalpy ΔH results.

Depending on the chromatographic conditions, the stationary phase and the chemical structure of the macromolecule either enthalpic or entropic contributions or both are operating.

In the general case K_d can be expressed as:

$$K_d = K_{SEC} * K_{LAC} \qquad 2\text{-}12$$

where K_{SEC} is based on entropy changes, while K_{LAC} characterizes the enthalpic interactions. Depending on the magnitude of the enthalpic and the entropic term, three different chromatographic modes can be distinguished, which differ in their dependence of elution volume on molar mass.

- **Size Exclusion Chromatography** (SEC):

In ideal SEC, separation is exclusively governed by conformational entropy (ΔH=0). The elution order in ideal SEC allows polymer of lower molar mass to elute first, while higher molar mass polymer elute later.

- **Liquid Adsorption Chromatography** (LAC):

Retention in ideal LAC is governed by enthalpic interactions (ΔS=0) of the polymer molecules with the functional groups of the stationary phase. The elution volume increases with increasing molar mass in ideal LAC.

- **Liquid Chromatography under Critical Conditions** (LC-CC):

Chromatography under critical conditions is described by a situation, where the enthalpic interactions are exactly compensated by conformational entropy ((ΔH=ΔS). These chromatographic conditions result in a molar mass independent elution of linear non-functionalized homopolymers.

By choosing appropriate chromatographic conditions (stationary phase, mobile phase and temperature) elution in one of the above mentioned chromatographic modes can be realized. The molar mass dependences for the different chromatographic modes in polymer chromatography are presented in figure 2.6.

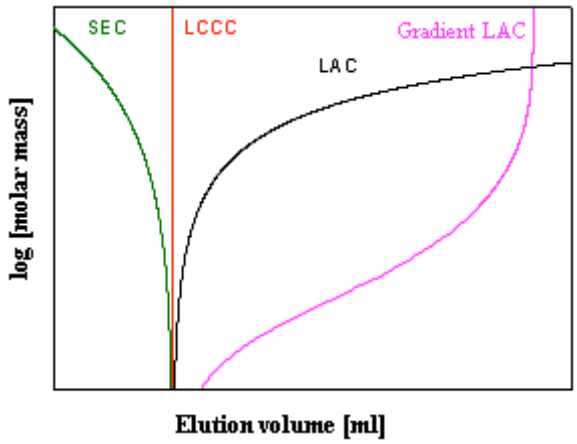


Figure 2.6: Schematic representation of the dependences of molar mass on elution volume in polymer chromatography. SEC, LC-CC and LAC modes operate under isocratic conditions while in gradient chromatography, the eluent composition is changed within the chromatographic run. Graph taken from [83].

2.3.1 Size Exclusion Chromatography (SEC)

SEC is the most widely used chromatographically technique to determine the molar mass as well as the molar mass distribution of a polymer sample.

Separation occurs within a stationary phase commonly consisting of macroporous swollen gel-particles with a characteristic pore size distribution. Normally two or three columns having different pore size distribution are connected in a series to increase the separation range. The mobile phase has to be a good solvent for the polymer and has to prevent any enthalpic interactions between the stationary phase and the polymer molecules.

Due to the absence of enthalpic interactions, the separation in ideal SEC *(*$\Delta H=0$) is solely based on changes of conformational entropy. A macromolecule wandering from the mobile phase into the pore of the stationary phase cannot adopt all possible conformations due to sterical hindrance of a large flexible chain-like molecule. This causes a decrease in conformational entropy when the molecule enters the pore. As a consequence, smaller molecules can enter the pores more easily and spend more time in the pores, increasing their elution volume. Conversely, larger molecules cannot enter the pores so easily and spend less time, if any time at all, in the pores. Therefore larger molecules are eluted faster than smaller ones. Within a homologues series the size of the polymer molecule increases with molar mass. Thus, high molar mass polymers elute earlier than those of lower molar mass. The separation mechanism in SEC is depicted schematically in figure 2.7.

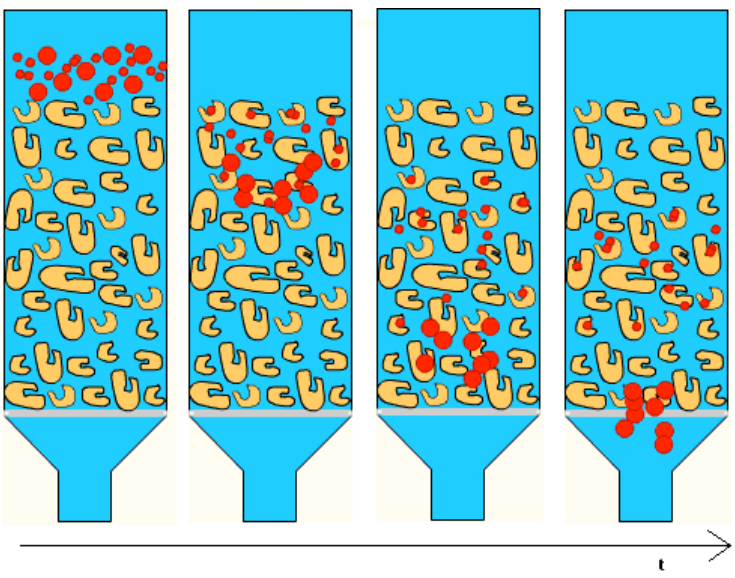


Figure 2.7: Schematic representation of the separation mechanism in a SEC column as schematic sketched by [84].

Since *ΔH=0* applies to ideal SEC, equation 2-10 and 2-11 simplify to:

$$K_D = K_{SEC} = \exp\left(\frac{\Delta S}{R}\right) \qquad (2\text{-}13)$$

Due to the loss of entropy when a macromolecule enters the pore of the stationary phase, the distribution coefficient can take any value between $0 < K_d < 1$. However, one can discuss two limiting cases.

- $K_{SEC} = 0$ relates to a situation, where the macromolecule is too large to penetrate the pores at all (exclusion limit).
- $K_{SEC} = 1$ corresponds to a situation, where the complete pore volume is accessible to the very small macromolecules (separation threshold).

According to equation 2.9, the elution volume V_E in ideal SEC can be described by:

$$V_E = V_A + V_p \cdot K_{SEC} \qquad (2\text{-}14)$$

According to equation 2-14, the separation in ideal SEC takes place in an elution range between V_A and $V_A + V_p$. Figure 2.8 shows the molar mass dependence on elution volume in SEC.

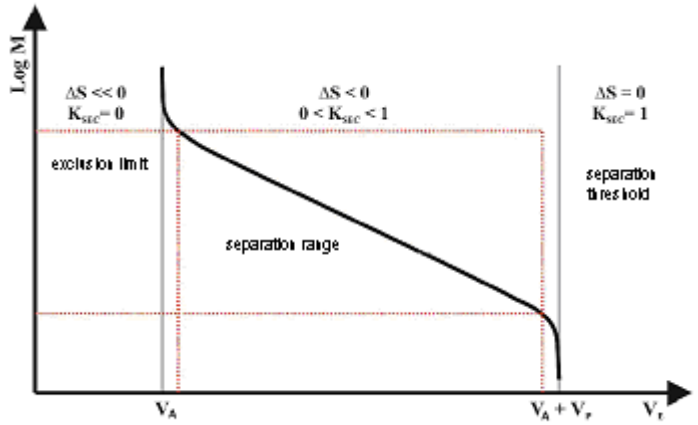


Figure 2.8: Schematic representation of the molar mass dependence on elution volume in SEC. Graph taken from [85].

In the case that enthalpic interactions between the macromolecules and the surface of the stationary phase occur, the distribution coefficient K_D has to be calculated according to 2-12.

2.3.2 Liquid Adsorption Chromatography (LAC)

LAC is the method of choice for the separation and identification of low molar mass molecules. However, LAC can also be applied for the separation of macromolecules. The separation in LAC is mainly governed by differences in enthalpic interactions (van der Waals forces, London forces or electrostatic forces) between the repeating units of the macromolecule and the functional groups of the stationary phase. The entropic term is often not significant compared to the enthalpic term ($\Delta S >> \Delta H$). The strength of the enthalpic interactions can be adjusted by proper selection of the eluent composition and the temperature. In an idealized picture, the entropy changes in LAC are ignored ($\Delta S \approx 0$), so that equation 2-11 simplifies to:

$$K_D = K_{LAC} = \exp\left(-\frac{\Delta H}{RT}\right) \qquad 2\text{-}15$$

In the case of adsorption, ΔH is negative and the partition coefficient K_{LAC} becomes larger than one, thus elution occurs at $Ve > V_A + V_P$.
Depending on the pore size of the stationary phase and the size of the macromolecules, two different cases have to be discussed in LAC of macromolecules.

1. For very large macromolecules and narrow-pore stationary phases, the adsorption of the macromolecules occurs exclusively on the outer surface of the stationary phase. The pores are not accessible to the macromolecules ($K_{SEC} = 0$), so that the pore volume can be neglected for the determination of the elution volume.

$$V_E = V_A + V_{Stat} \cdot K_{LAC} \qquad 2\text{-}16$$

   where V_{Stat} is the volume where the separation can occur in the stationary phase.

2. For stationary phases with large pores, the macromolecules can freely penetrate into the pores ($K_{SEC} = 1$) and the pore volume V_p adds to the interstitial volume. “

$$V_E = V_A + V_p + V_{Stat} \cdot K_{LAC} \qquad 2\text{-}17$$

In real LAC, some pores are accessible for the macromolecules while other pores are not accessible. Therefore, entropic interactions must be assumed in LAC, too.

For homopolymers, the number of interacting groups increases with the molar mass. This can be explained by the multiple attachment mechanism as proposed by Gloeckner [86] explains why high molar mass polymers elute later than those of lower molar mass in LAC. In addition, the multiple attachment mechanism explains the nearly exponential increase in elution volume with molar mass, resulting in a nearly irreversible adsorption on the stationary phase for higher molar mass polymers. Hence, the dependence of molar mass on elution volume is opposite compared to SEC. Consequently, low molar mass polymers elute always before high molar mass polymers of the same chemical composition.

2.3.3 Liquid Chromatography at Critical Conditions (LC-CC)

The transition between the entropy dominated SEC ($\Delta S > \Delta H$) and the enthalpy dominated LAC mode ($\Delta H > \Delta S$) is observed under special conditions. This transition is termed "critical point of adsorption" and relates to a situation where the entropic losses due to the exclusion of the polymer molecules from the pores of the stationary phase are exactly compensated by the enthalpic interactions of the molecule with the stationary phase ($\Delta S = \Delta H$) [87]. According to equation 2-11, the distribution coefficient of a macromolecule becomes unity under these conditions, resulting in a molar mass independent elution for non-functionalized linear homopolymers at the void volume of the stationary phase. The number of repeating units do not contribute to the elution volume, so that the polymer chains are often referred to as being chromatographically "invisible". Because the molar mass does not influence the elution volume under critical conditions, the elution volume is controlled by structural differences other than molar mass. LC-CC has become particularly valuable for the characterization of polymer blends [9], block and graft copolymers [88], end group functionality [89] and stereo regularity [90]. Macko et al. [91] recently summarized the critical conditions for a large number of polymers.

Critical conditions can be realized by adjusting the type and porosity of the stationary phase, the eluent composition and the temperature. However, application of critical chromatography is hampered by the long and tedious adjustment of the critical conditions and the fact that even a slight variation in temperature or eluent composition causes a transition to SEC or LAC-mode. Bashir et al. developed an approach for a simple, fast and effective method to estimate the critical conditions with only a few experiments of gradient chromatography [83].

2.3.4 Gradient Liquid Chromatography (Gradient Chromatography)

The strong dependence of elution volume on molar mass between the repeating unit and the stationary phase often results in incomplete sample recovery in LAC. Consequently, LAC is often carried out in gradient elution mode. The principle of gradient chromatography is to first adsorb the polymer on the stationary phase and to elute the polymer by a gradual increase of the eluent strength. Gradient chromatography can therefore be used to separate polymers of very different adsorption strength. The principles of gradient chromatography have been discussed in detail by various authors [92,93]. In general, gradient elution in polymer chromatography is more difficult to understand compared to isocratic elution. Both enthalpic and entropic interactions contribute to gradient elution to different extent. At the early stage of the gradient run, a weak eluent composition is used, resulting in adsorption of the polymer molecules to the stationary phase (K_d>>1). During the chromatographic run, the eluent strength is increased resulting in decreasing interaction between the repeating units and the stationary phase, accompanied by a decrease of the distribution coefficient K_D. Thus, at sufficient high eluent strength, the macromolecules are eluted from the stationary phase in the order of increasing adsorption strength.

Besides the chemical structure, the molar mass also influences the adsorption strength in gradient chromatography [5]. It is commonly observed in gradient chromatography that low molar mass polymers elute slightly earlier than higher molar mass polymer of identical chemical structure since they consist of a lower number of absorbing units. However, at sufficiently high molar masses, the molar mass dependence on elution volume vanishes and a nearly molar mass independent elution is observed (see figure 2.6).

2.3.5 Two-Dimensional Liquid Chromatography (2D Chromatography)

All previous mentioned one-dimensional chromatographic methods are capable to gain information about only one distribution at a time. However, complex polymers possess more than one distribution, e.g. copolymers can be distributed in molar mass and chemical composition. This can cause coelution of different species within a chromatographic slice in every one-dimensional separation. Therefore, characterization methods that separate by more than one structural parameter are required for a complete characterization of these complex structures.

2D-chromatography combines the separation efficiencies of two different chromatographic methods (dimensions). By using different chromatographic modes and combining them with each other, two-dimensional information on different aspects of molecular heterogeneity can be obtained within one chromatographic run[94,95,96]. For example, the coupling of adsorption chromatography (LAC) with size exclusion chromatography allows the determination of the chemical heterogeneity and the molar mass distribution simultaneously.

Another advantage of 2D-chromatography is a significant enhancement of resolution and peak capacity. Karger [97], Giddings [98] and Guiochon [99] showed that under ideal circumstances, the overall peak capacity ($n_{c,2D}$) becomes more or less the product of the individual peak capacities of the first and second dimension separations (^1n_c and ^2n_c):

$$n_{c,2D} = {}^1n_c \, x \, {}^2n_c \qquad 2\text{-}18$$

Due to its high resolution power, 2D chromatography has become very valuable for the characterization of complex polymers.

2D-chromatography is experimentally realized using a transfer valve equipped with two storage loops. The two storage loops allow collecting the fractions continuously without losses. When the first loop is filled with the efluent of the first dimension, the content of the second loop, a previous fraction, is injected and separated in the second dimension. When the first loop is completely filled with the fraction, the injection valves switches automatically to the opposite position, so that the content of the first loop is injected in the second dimension, while the second loop is filled again. A repeated switching of the transfer valve injects multiple fractions from the first dimension into the second dimension. The operation of the injection valve is directed automatically by a communication software, which is responsible for data collection, storage and processing. A fully automated two-dimensional chromatographic system was developed by Kilz et al. [100]. A schematic setup of an online 2D-chromatography system is shown in figure 2.9.

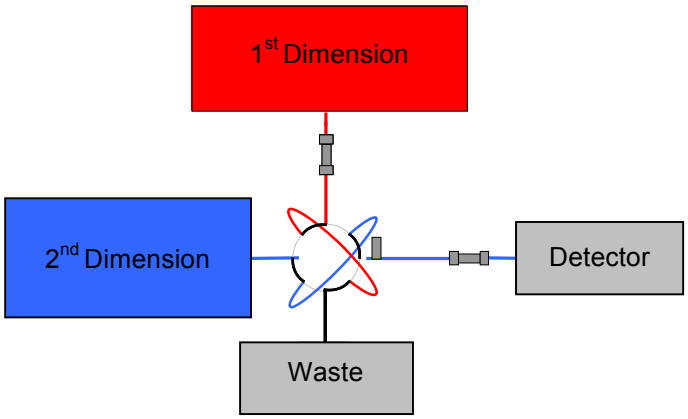


Figure 2.9: Schematic representation of a 2D-LC-system. The red line shows the first dimension solvent flow, the blue line the second dimension. The coupling of the two dimensions (chromatographic modes) is experimental realized by the use of an eight port injection valve with two storage loops. Graph taken from [101].

The most complicated feature of an automated system is the proper adjustment of the flow rates for the first and second dimension. The collection duration for fraction in the first dimension must be equal or larger than the analysis time of the second dimension.

Another important factor of a two dimensional chromatographic separation is the order in which the separations are carried out. In principle any combination of LC techniques is suitable to setup a 2D-chromatographic system. However, the most often reported system corresponds to a coupling of interaction chromatography in the first dimension with size exclusion chromatography in the second dimension [102,103].

There are different ways to present the results of a 2 dimensional separation. A discontinuous presentation (stacked plot) is obtained, when the chromatograms of the second dimension of all fractions are plotted along the elution volume axis of the first dimension (see figure 2.10).

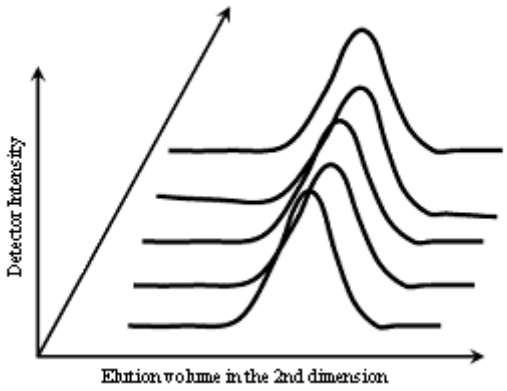


Figure 2.10: Schematic representation of a two-dimensional separation in a discontinuous presentation.

The most sophisticated kind of visualization of the results of a two-dimensional separation is obtained, when a continuous representation is used. In this kind of visualization, the chromatograms of the fractions from the first dimension are drawn in a two dimensional contour plot, similar like a map. In this case, the elution volume of the first dimension is plotted on the y-axis and the elution volume of the second dimension is plotted along the x-axis. The intensity of the detector signal is presented by different colours in the Z-axis. A schematic representation of a two dimensional contour plot of a separation of a polymer blend is shown in figure 2.11.

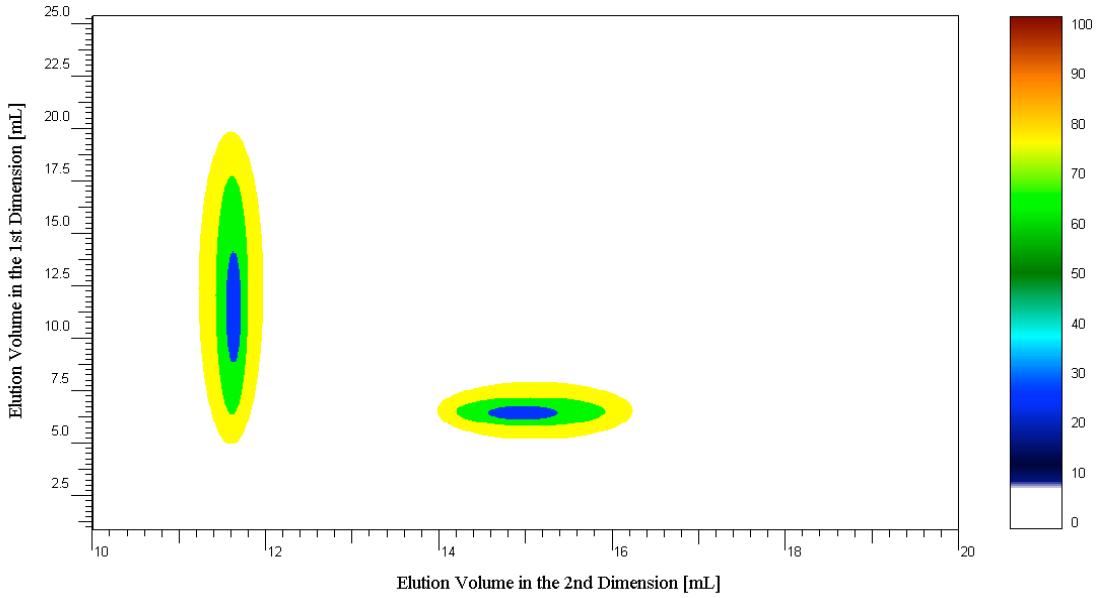


Figure 2.11: Schematic representation of a two-dimensional separation of a polymer blend.

Different reviews were published on the application of 2D chromatography [104,105] allowing to select the most suited chromatographic systems in order to achieve the most efficient characterization and separation.

3 Results and Discussion (Part A)

In the first part of this chapter, a short summary of the synthetic approach to synthesize aromatic-aliphatic polyesters based on 4,4-bis(4'-hydroxyphenyl)pentanoic acid with different DBs but identical chemical structure will be described. Afterwards, conventional one-dimensional liquid chromatographic methods (SEC MALLS, gradient chromatography, etc.) were used for the characterization of these newly synthesized polymer samples.

The main part of this chapter focuses on the investigation of the retention behavior of these newly synthesized linear and branched polymers in enthalpy dominated chromatographic modes, such as LC-CC, LAC and gradient chromatography. The aim of this chapter is to provide a better understanding of the retention behavior of linear and branched polyesters in these chromatographic modes. The gained knowledge about the retention behavior of linear and branched polyesters will hopefully allow adjusting, controlling and optimizing the chromatographic conditions in order to identify whether separations by DB are possible at all and which modes of chromatography are best suited for such separations. The knowledge obtained in the first chapter will be applied to set up a two-dimensional separation, allowing the separation of mixtures of linear and branched polymers by topology and molar mass.

3.1 Synthesis of Linear and Branched Aromatic-Aliphatic Polyesters

One major drawback in the method development for the characterization of branched polymers is the lack of suitable linear and branched model polymers of identical chemical structure, well defined in molar mass and DB. Up to now, the most widely used branched polymer model system, which fulfills these criteria, are polystyrene stars. Such polymers were intensively studied by various authors [70,76,79,80,]. However, it remains unclear whether the developed two-dimensional liquid chromatographic approaches can be applied to other branched polymer systems, due to the topological differences between star shaped polystyrenes and (hyper)branched polyesters.

In order to obtain well defined linear and differently (hyper)branched model polymers (0<DB<100), Khalyavina et al. [106] applied a polycondensation reaction for the synthesis of (hyper)branched polymers [107,108,109,110].

In her PhD-work, Khalyavina synthesized linear and (hyper)branched aromatic-aliphatic polyesters with tailored degrees of branching based on the same AB_2 monomer 4,4-bis(4'-hydroxyphenyl)pentanoic acid using an approach involving combinations of functional group protection and copolymerisation (ABB*/AB_2-approach). Afterwards, all terminal hydroxyl groups were modified with tert-butyldimethylsilyl protecting group to completely prevent interaction by hydrogen bonding between the macromolecules, which have been observed in previous experimental studies for the non protected systems. The synthetic strategy to obtain linear, partially branched and hyperbranched polyesters is depicted in figure 3.1.

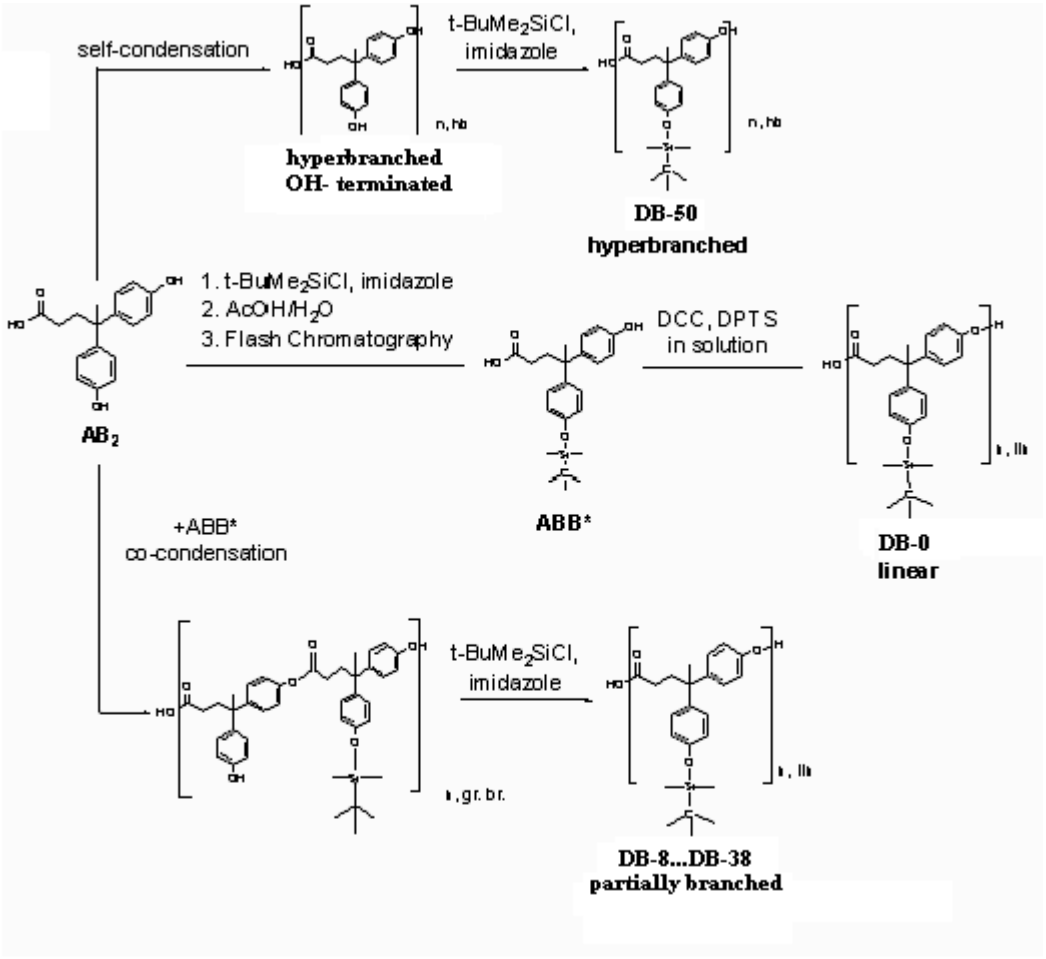


Figure 3.1: Schematic representation of the synthetic strategy for the synthesis of linear, partially branched and hyperbranched polyesters. The complete details of the synthesis are described in [106].

According to this theme, based on the AB_2 monomer, three different species can be synthesized.

- Hyperbranched polymers

- Linear polymers
- Partially branched polymers

The synthesis of hyperbranched polyesters (DB-50) is performed using polycondensation of the AB_2-monomer in solution in the first step. The reactive phenolic groups were consequently fully modified with TBDMS groups in the presence of imidazole.

The synthetic strategy to obtain of linear polyesters (DB-0) required the synthesis of monoprotected ABB^* monomers in the first step and subsequent polycondensation of the ABB^* monomers in presence of DCC and DPTS.

Partially branched polyesters could be obtained by the co-polymerization of the synthesized ABB^* and AB_2 monomers. The targeted DB could be adjusted by different ratios of ABB^* and AB_2 monomers. The reactive phenolic groups were consequently fully modified with TBDMS groups in presence of imidazole.

Polyesters with DB higher than 50 % were also synthesized and named pseudo-dendrimers and similar to dendrimers, they possess a DB of 100%, but a non regular structure [111]. The synthesis of pseudo dendritic polyester samples used the hyperbranched polyester as core molecule and by a series of protection and deprotections steps of the functional groups, the pseudo dendritic polyester DB-100 could be obtained. The details of the synthesis can be found in [106].

These newly synthesized polymers possess several advantages such as adjustable DB and molar mass, identical functional end groups, the absence of aggregation and a high value of the refractive index increments (dn/dc) in common solvents.

Table 3.1 summarizes the DBs of the polymer samples used, as determined by ^{13}C-NMR according to the method described in [106].

Table 3.1: DB for the samples used as given by the IPF Dresden. A DB of 0 % corresponds to a linear sample, while a hyperbranched sample possesses a DB of 50 %. Polymer samples with a DB close to 100 % are called pseudo dendritic polymers, while polymer samples with a DB between 0 – 50 % are termed partially branched polymers.

Sample Code	DB [%]
DB – 0	0
DB – 18	18
DB – 38	38
DB – 50	50
DB – 100	100

At this point, it should be noted that not all chromatographic experiments were conducted with the pseudo dendritic polymer sample DB-100, since this sample was synthesized and received towards the end of the PhD work.

3.2 One Dimensional Chromatographic Separations of Branched Aliphatic Polyesters

First, a SEC-MALLS system was set up for the determination of the molar mass distribution and the dependence of molar mass on elution volume. Afterwards, gradient chromatography, LAC and LC-CC were applied on the model polymers to gain a deeper understanding in the elution behaviour in these chromatographic modes.

3.2.1 SEC MALLS Measurements

Linear and differently branched polyesters were first analyzed by SEC-MALLS in THF to determine the molar mass and the molar mass distribution, respectively. For each polyester sample, both average molar masses M_n and M_w were determined. The results for the SEC-MALLS measurements are listed in table 3.2

Table 3.2: Molar mass averages for linear and differently branched polyesters determined by SEC-MALLS. A refractive index increment (dn/dc) of 0.16 mL/g was used in all measurements. The experimental conditions are given in the experimental part.

Sample Code	M_w [g/mol]	M_n [g/mol]	PDI
DB – 0	29300	19000	1.54
DB – 18	48500	27200	1.78
DB – 38	29200	16500	1.77
DB – 50	39800	25700	1.55
DB – 100	92900	44500	2.09

All samples show weight average molar masses between approximately 30.000 – 50.000 g/mol, besides DB-100 which has approximately twice the molar mass compared to the other samples. The PDIs vary between 1.54 and 2.09, which is in good agreement with literature values for linear polymers synthesized by polycondensation. In contrast, the synthesis of

branched polymers results in general in higher values for the PDIs [112] due to the additional randomness in the polymerization process. However, this phenomenon could not be observed with these experiments.

An overlay of chromatograms for the SEC-MALLS measurements is shown in figure 3.2.

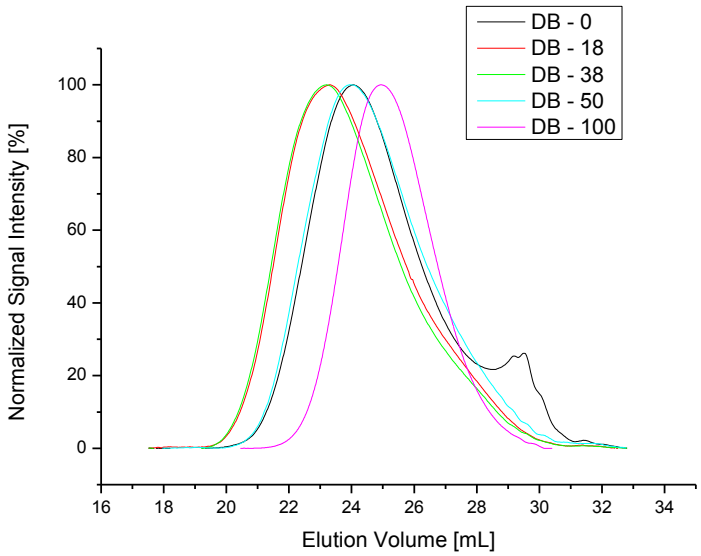


Figure 3.2: Overlay of SEC – chromatograms for linear and several branched polyester samples. Stationary phase: PSS SDV 10^3, 10^5, 10^6 Å (each 300 x 8 mm I.D.), mobile phase: THF, flow rate: 1 mL/min, detection: Refractive Index detector.

The overlay of chromatograms shows that under the given chromatographic conditions, all samples elute in a similar elution range in SEC. In addition, a monomodal distribution and a tailing towards higher elution volumes are observed for all samples. For the linear sample DB-0, a small shoulder towards higher elution volumes can be noticed. The origin of this shoulder will be investigated later within this chapter.

A closer look at the overlay of chromatograms reveals that first the linear and the hyperbranched polymer sample (DB – 0 and DB – 50) co-elute at almost identical elution volumes. The elution volume of the peak maxima is at approximately 23.25 mL. At slightly higher elution volume (approximately 24 mL), coelution of the partially branched polymer sample DB – 18 and DB – 38 occurs. At the end, the pseudo dendrimer DB-100 elutes at an elution volume of approximately 25.20 mL, despite its higher molar mass. It becomes clear from the overlay of SEC chromatogram that under the given chromatographic conditions, no correlation between the DB and the elution volume can be drawn, since both parameters (molar mass and DB) have a direct influence on the hydrodynamic volume of the polymer sample and therefore on the retention behaviour in SEC. The comparison of table 3.2 and figure 3.2 indicates that also no dependence of the elution order and molar mass exists.

Next, the origin of the shoulder that was observed in the chromatogram of the linear species was investigated. MALDI-TOF-MS measurements were performed on fractions of the linear polymer sample DB-0. For the MALDI-TOF measurements, the crude polymer DB-0 was injected several times on the SEC columns and fractions from 25 - 26 mL (main peak) and 29 - 30 mL (shoulder) were collected. After evaporation of the mobile phase, the polymer fractions were redissolved in a solution of the MALDI matrix (10 mg of 1,8,9 Trihydroxy-anthracene in 1 mL of THF). For enhancement of ion formation, LiCl was added to the matrix. The resulting MALDI spectra are shown in figures 3.3 - 3.4.

The spectrum of the main fraction (corresponding to an elution volume of 25-26 mL in the SEC chromatogram shown in figure 3.2) was split up into two ranges (from 1000 – 3000 Da and 3000 – 6500 Da) as shown in figure 3.3a and 3.3b. Figure 3.4 shows the MALDI spectrum of the fraction at higher elution volume (29-30mL).

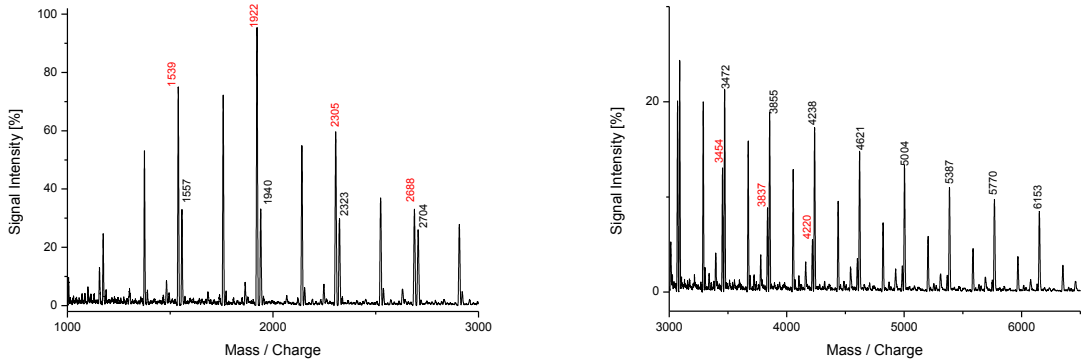


Figure 3.3a and 3.3b: MALDI-TOF mass spectrum of the sample DB-0 in the molar mass range 1000 – 3000 Da (left side) and in the molar mass range of 3000 – 6500 Da (right side) for an elution volume of 25-26 mL. The mass peak corresponding to a possible cyclic structures are marked in red, while the molar masses corresponding to the linear structures are marked in black.

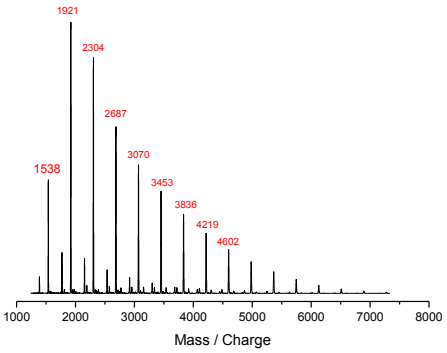


Figure 3.4: MALDI-TOF mass spectrum of the shoulder of the sample DB-0 at an elution volume 29 – 30 mL. The molar masses corresponding to the masses of the cyclic structures are marked in red, while the molar masses corresponding to the linear structures are marked in black.

The assignments of the MALDI signals to different kinds of oligomers were done assuming the formation of lithium adducts. Two series separated by a mass difference of 18 Da can be observed in the spectra. The peaks of both series are separated by a mass difference of 383 Daltons that corresponds to the molar mass of the repeating monomeric unit of 4,4-bis(4'-hydroxyphenyl)pentanoic acid. The molar mass of the red series corresponds to multiples of the repeating unit plus 7 Da. For example, the ion with a molar mass of 1922 Da corresponds to a pentamer of 4,4-bis(4'-hydroxyphenyl)pentanoic acid with a lithium ion (7 Da) attached. Since no residual mass for the distribution marked in red exists, this series can be assigned to the cyclic species. The second distribution, which is marked in black, yields a residual mass of 18 Da and thus can be assigned to the linear species. The difference in the residual masses of 18 Da is exactly the molar mass of water, which is lost when the carboxyl and hydroxyl groups react to form a cyclic structure.

In addition, a third peak distribution is revealed in the MALDI spectrum. This residual mass of this peak series is 215 Da, in addition to the attached lithium ion (7 Daltons). However, these peaks can neither be assigned to the cyclic nor to the linear species and the existence of this distribution is probably due to degradation of the polymer sample during the ionization process.

A comparison of the MALDI spectra of the main peak (figures 3.3a and 3.3b) reveals that in the low molar mass region both linear and cyclic species are present, while for higher molar masses merely linear species and only a few cyclic species are present. In contrast, the MALDI spectra of higher volume fractions reveal only cyclic structures. The fact that the ring formation terminates the polymerization process results in a lower molar mass of the cyclic species as compared to the linear species.

The MALDI measurements on the fractions proved that the existence of the small shoulder in the SEC measurements of the linear polymer is caused by ring formation. This process is more likely to occur for low degrees of polymerization, since the probability of two functional end-groups reacting with each other decreases with increasing molar mass. For molar masses above 5000 Da, no ring formation can be observed at all. Since for our purposes, only molar masses above 5000 Da are important, the small shoulder resulting from the formation of cyclic structures can be neglected.

After the origin of the small shoulder in the SEC measurements for the linear sample DB-0 was clarified, the retention behavior of linear and branched polymers in SEC was investigated. Figure 3.5 shows the dependence of molar mass on elution volume, as

determined by SEC MALLS for all samples. In addition, the corresponding SEC chromatograms are also shown in figure 3.5.

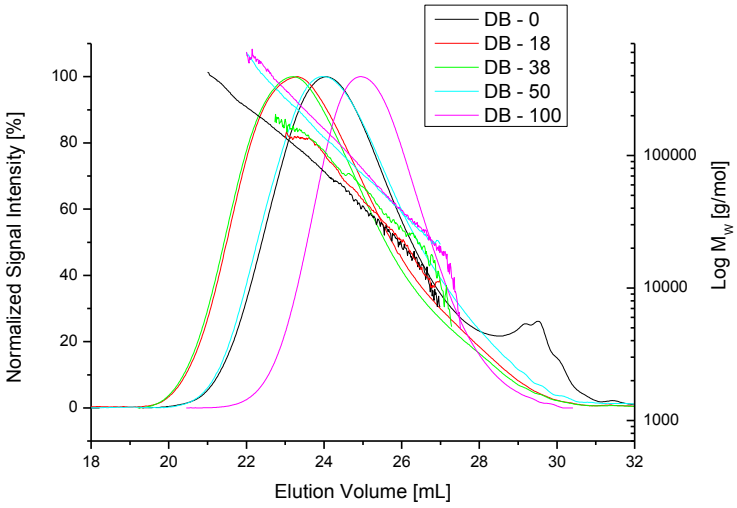


Figure 3.5: Dependence of elution volume on molar mass for linear and several branched polyesters as determined by SEC-MALLS.

The calibration curves in figure 3.5 show that with increasing elution volume the molar mass decreases, as expected for a separation by SEC. A comparison of the calibration curves reveals the following order (from lowest molar mass to elution volume ratio to the highest one): DB-0 → DB-18 →DB – 38 → DB – 50 → DB-100. The fact that the peaks of DB-0 and DB - 50 (also DB-18 and DB-38) in figure 3.5 co-elute at almost identical elution volumes, but possess different molar masses, is related to the different dependence of elution volume on molar mass for different branched samples. An increase in the DB results in a higher molar mass at a given elution volume. This behaviour indicates different hydrodynamic sizes for linear and branched polyesters in solution. Consequently, branched polymers have a more compact structure compared to their linear analogues of identical molar mass [113]. An increase in DB is accompanied with an increase in the compactness of the structure in solution. Due to this more compact structure, branched polymers of equal hydrodynamic size have a higher molar mass compared to linear polymers and therefore elute later in SEC.

Two conclusions can be drawn from the previous SEC-MALLS experiments. First, at a given molar mass, a linear and a branched sample become more and more distinct from each other in their solution properties (size, etc.) with increasing DB. This is in agreement with other branched topologies such as star shaped polymers, where it was found, that the reduction in size is correlated with the number of arms[114]. Secondly, it was shown that no separation based on branching can be realized by SEC. Consequently, the suitability of other modes of chromatography will be evaluated in the following sections.

3.2.2 Gradient Adsorption Liquid Chromatography

Gradient chromatography is the method of choice for the analysis of the chemical composition distribution for complex polymers. The separation in gradient chromatography is based on differences in adsorption strength between the polymer molecules and the stationary phase. Next to a chemical composition dependence, a molar mass dependence is commonly observed in gradient chromatography. Low molar mass polymers of a given chemical composition are less strongly adsorbed than those of higher molar mass. At sufficiently high molar masses, a nearly molar mass independent elution is typically observed.

The influence of topology on the retention behaviour in gradient chromatography was previously investigated by Gerber et al.[79,80] for star shaped polystyrenes. They observed a negligible variation of gradient elution volume with the number of arms and thus on the branching density. Consequently, they assumed that chemically identical but topologically heterogeneous polymer samples show identical retention behavior since there should be no influence of topology on elution volume for a given molar mass.

In order to investigate the influence of the DB of (hyper)branched polymers on the retention behaviour in gradient chromatography conditions for a complete adsorption of the polymer molecules on the stationary phase as well as conditions for complete sample elution had to be determined. After an intensive column and eluent screening it was found that suitable chromatographic conditions for gradient chromatography are obtained on a non-polar C-18 column and using gradients of THF, methanol and acetone.

The chromatographic conditions, resulting in complete adsorption onto the stationary phase could be realized using an eluent composition of 75 % acetone and 25 % methanol. The sample was dissolved in the same eluent composition. An overlay of chromatograms obtained under complete adsorption conditions is shown in figure 3.6. No peaks were observed during isocratic elution in 75 % acetone and 25 % methanol. Therefore the eluent composition was changed in a stepwise fashion to 100% THF after six minutes, which caused complete desorption of the polymer molecules from the stationary phase. In the chromatogram, this can be noticed by the peak at an elution volume around 9 mL.

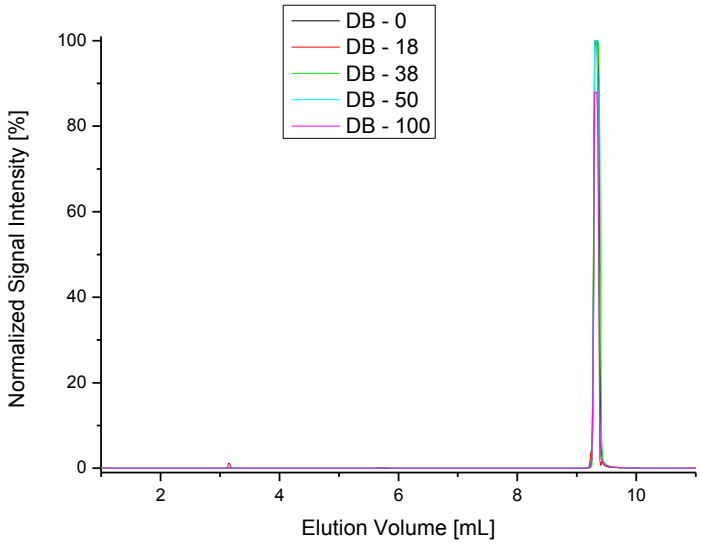


Figure 3.6: Chromatograms for linear and different branched polyester under complete adsorption conditions. After six minutes, the eluent composition was suddenly changed to 100 % THF (complete desorption conditions). Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D.), mobile phase: 75 %THF / 25 % MeOH, flow rate: 1 mL/min, detection: ELSD.

After the chromatographic conditions for a complete adsorption on the stationary phase were identified, the conditions for an interaction free elution were investigated. An interaction free elution was realized in 100 % THF. However, due to convenience reasons, an eluent composition of 75 % THF and 25 % acetone for the interaction free elution was preferred. An overlay of chromatograms for differently branched samples at isocratic elution using an eluent composed of 75 %THF and 25 % acetone is shown in figure 3.7.

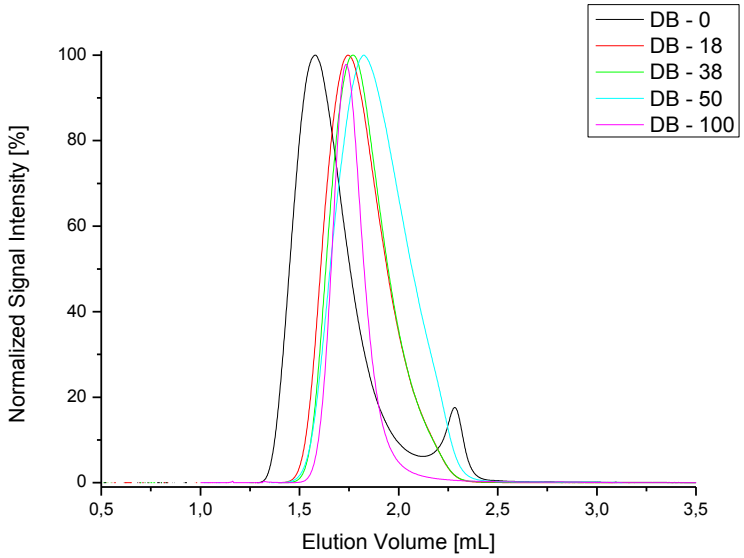


Figure 3.7: Chromatograms of linear of different branched polyester under interaction free conditions. Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D.), mobile phase: 75 %THF / 25 % acetone, flow rate: 1 mL/min, detection: ELSD.

From the overlay of chromatograms, it can be noticed that under the given chromatographic conditions, all samples elute as monomodal peaks (besides DB – 0) at similar elution volume ranges. The origin of the shoulder for DB-0 has been discussed before. All samples elute at an elution volume between 1.3 -2.4 mL. The fact, that the polymer samples elute before the void volume of the stationary phase (V_0 = 2.44 mL) indicates an interaction free elution of the polymer sample in SEC mode, where no or only negligible enthalpic interactions exist between the stationary phase and the analyte molecules. In addition, the eluent composition was changed to 100 % THF after six minutes. However, no peak could be observed after the sudden change in eluent composition, indicating that under the given chromatographic conditions, complete desorption of the polymer molecules from the stationary phase was realized.

Having identified the chromatographic conditions for complete desorption of the polymer sample on the one hand and complete sample adsorption onto the stationary phase on the other hand, allowed performing gradient experiments. A linear gradient of ten minutes ranging from 75 % THF / 25 % methanol to 75 % THF / 25 % acetone was applied. Since the polymer samples were completely soluble in both eluents, the separation can be assumed to be based on adsorption/desorption and not on solubility. Figure 3.8 shows the obtained chromatograms for the different samples in this gradient.

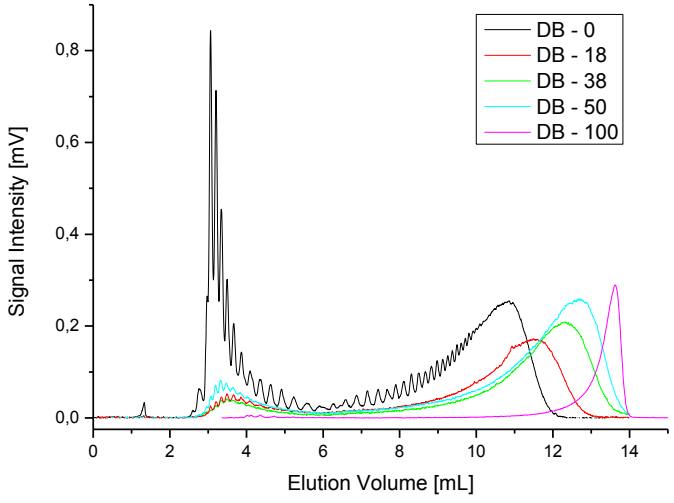


Figure 3.8: Chromatograms of linear and differently branched polyesters in gradient chromatography. Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D.), mobile phase: (THF/acetone/MeOH): 0 - 2 mL (75/0/25); 12 mL (75/25/0);12.01 – 13 mL (100/0/0); 13.01 – 20 mL (75/0/25), flow rate: 1 mL/min, detection: ELSD.

The overlay of chromatograms shows that all samples elute as broad peaks within a similar elution range between 3 and 14 mL. The only exception seems to be the pseudo dendrimer DB-100, which elutes as narrow peak at an elution volume between 12 and 14 mL. The

presence of small well resolved peaks at lower elution volumes indicates a separation according to the degree of polymerization within a given sample. However, this effect decreases with increasing molar mass and with increasing DB. The narrow peak observed for DB-100 could be the consequence of its higher molar masses compared to the other polymer sample. Since the molar mass dependence vanishes in gradient chromatography for higher molar masses, higher molar mass samples are expected to elute in more narrow peaks than lower molar mass samples of the same chemical composition.

The elution volumes of the main peaks increased with increasing DB. If separation in gradient chromatography would be solely governed by molar mass and independent of topology, [79,80] a different elution order would be expected based on the molar masses given in table 3.2. This behaviour might be a first indication that elution in gradient chromatography is governed by both, molar mass and DB, making a separation according to one parameter only impossible, due to coelution of polymer fractions varying in molar mass and DB.

In order to distinguish between the influence of molar mass and DB on the elution volume, homogeneous samples with a distribution in only one dimension (either molar mass or DB) are essential. One way to obtain more homogeneous polymer samples would be 2D-chromatography, where first a fractionation according to one parameter is performed and in a second separation step the separation according to another structural parameter occurs. This 2D-approach will be followed in part B. Another possibility to obtain more homogeneous fractions to investigate the retention behaviour of linear and branched polyester sample in gradient chromatography will be shown in the next section.

3.2.2.1 Retention Behaviour of Linear and Branched Polyesters in Gradient Chromatography

In the last section it was shown, that the retention of linear and differently branched polymers having identical chemical structures in gradient chromatography is influenced by molar mass and topology (DB) and not solely by molar mass, as proposed by Gerber et al. for star shaped polystyrenes.

In order to verify this hypothesis, all samples were repeatedly fractionated using the same gradient conditions as described in figure 3.8, to obtain more homogenous fractions. The first fraction was taken at an elution volume of 2 mL. Subsequent fractions were collected every 0.8 mL. The solvent was removed and the fractions were redissolved in pure THF for

subsequent molar mass determination by SEC-MALLS. The results on the molar masses are summarized in table 3.4.

Table 3.4: Molar masses as obtained by SEC – MALLS on the fractions of linear and differently branched polyesters fractionated by gradient chromatography.

Fraction number	Elution range in gradient chromatography [mL]	M_w/ gmol^{-1}				
		DB - 0	DB - 18	DB - 38	DB-50	DB-50
1	2.8 -3.6	3110	3990	2790	4040	n.a.
2	3.6 – 4.4	5710	5170	4700	4220	n.a.
4	5.2 – 6.0	10220	9150	7620	6830	n.a.
5	6.0 – 6.8	12020	9560	11140	8680	n.a.
6	6.8 – 7.6	14750	12140	10200	9600	n.a.
7	7.6 – 8.4	17890	14590	13700	12090	n.a.
8	8.4 – 9.2	22000	17470	16420	13100	n.a.
9	9.2 – 10. 0	28460	21100	20290	16000	n.a.
10	10.0 – 10.8	37750	26650	25130	19020	14980
11	10.8 – 11.6	45170	37380	32160	23930	21530
12	11.6 – 12.4	n. a.	54630	43750	31940	28950
13	12.4 – 13.2	n. a.	n.a.	n.a.	41610	40270
14	13.2 – 14.0	n. a.	n. a.	n.a.	51030	45830

From the results in table 3.4, the dependences of gradient elution volume on molar mass were established for the different branched samples. These are shown in figure 3.9.

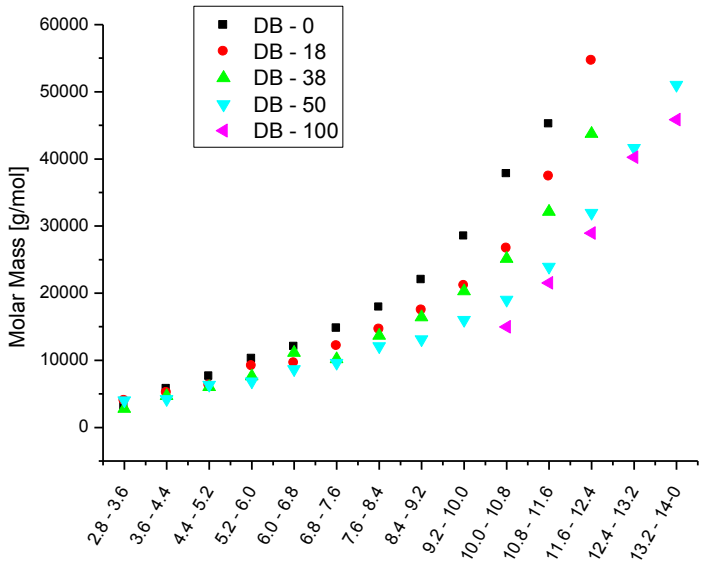


Figure 3.9: Dependences of gradient elution volume on molar mass for polyesters of different DB.

From table 3.4 and figure 3.9 it becomes clear that an increase in molar mass results in later elution of the fractions. However, with increasing molar mass the variation in elution volume becomes less pronounced, indicating that the molar mass dependence vanishes for high molar mass samples. Such behaviour is commonly observed in gradient chromatography of polymers[115,116]. Additionally, it is obvious that samples of different DB follow different calibration curves, indicating that elution is influenced by DB as well as by molar mass. Upon comparing the different molar mass dependences it becomes clear that at a given molar mass, samples of higher DB elute later than those of lower DB.

At the early stage of a gradient run (low eluent strength) adsorption of all polymer molecules onto the stationary phase takes place due to enthalpic interactions between the stationary phase and the repeating units. With increasing eluent strength, the polymer molecules desorb from the stationary phase and start moving. For high molar mass polymers, desorption from the stationary phase occurs only in a narrow region of eluent composition close to the critical one [83,116]. The gradient experiments showed that elution of high molar mass polymers increases with DB. This is a first indication that the critical eluent composition might also depend on DB. Hence, more densely branched polymer samples, which possess stronger affinity to the column material require stronger eluent compositions to desorb than those of lower DB.

Since linear and (hyper)branched polymers of a given molar mass consist of the same number and types of structural units (phenyl groups, silyl groups, ester linking, etc.), the reason for the dependence of DB on elution volume can not result from differences in the chemical structure, but must be related to topological effects.

A possible explanation might be the more compact structure of branched polymers compared to their linear analogues of the same molar mass [113]. As shown by the SEC-MALLS measurements, an increase in the DB is accompanied with an increase in the compactness (segment density) of the structure. The topological difference causes a higher density of functional groups at the periphery of the polymer coil enabling more repeating units to interact with the stationary phase. In addition, the higher density of functional groups on the peripheral surface enhances neighborhood effects (triple contacts) due to the sterical reasons. Consequently, not a linear but a more steep relationship between the total amount of enthalpic interactions and the DB exists.

That should be the reason why in the gradient experiments the linear polymer molecules elute first, followed by the partially branched and hyperbranched polymer molecules and ending

with the pseudo dendrimers. The influence of the DB seems to be more pronounced than the influence of molar mass on the elution volume.

These findings are in contrast to the experimental works on star-shaped polymers by Gerber et al.[79,80]. They observed a negligible variation of gradient elution volume with the number of arms and thus on the branching density. However, the sorbent-solvent systems as well as the chemical and topological structures of branched polymers in the work of Gerber et al. differ strongly from those used in the present investigation. In the work of Gerber et al., star shaped polystyrenes with different numbers of arms were used. Though the segment density increases with the number of arms as well, the probability of neighbourhood contacts is less pronounced. Therefore, the effect of the number of arms on the gradient elution volume is negligible in their system.

The observed differences in the elution volume on molar mass for samples differing in DB let one assume that also in other enthalpic interaction dominated chromatographic modes a dependence of elution volume on DB might exist. To verify this hypothesis the retention behaviour of linear and differently branched polymer samples was investigated under critical conditions and in adsorption mode, respectively.

3.2.2.2 Retention Behaviour under Critical Conditions and in Adsorption Mode

In order to investigate the retention behaviour of linear and differently branched polyesters under critical conditions and in adsorption mode, all polymer samples were repeatedly fractionated by SEC in order to obtain fractions of narrow molar mass distribution but comparable DB. Subsequently, the fractions were dried and characterized by SEC-MALLS to obtain their true molar mass. A summary of the characterization details is given in table 3.5.

Table 3.5: Molar masses of the SEC-fractions of linear and differently branched polyesters as determined by SEC – MALLS.

Fraction number	M_w of DB - 0	M_w of DB - 18	M_w of DB - 38	M_w of DB-50
1	45200	55700	59400	78200
2	34780	43500	48400	60300
3	31100	33100	35600	43300
4	24400	25100	28000	33200
5	20100	20300	21100	22200
6	14300	17100	18800	16200
7	10400	14500	15300	10200

As expected for a fractionation in SEC, the molar mass decreases with increasing elution volume (corresponding to an increase in the fraction number) within a polymer sample. For a given fraction number, the molar mass increases with DB, since higher branched polymers possess a more compact structure compared to less branched polymers.

In order to establish the critical conditions for the linear polymer DB-0, fractions of DB-0 were injected using different eluent compositions. The eluent composition, where a molar mass independent elution is observed corresponds to the critical eluent composition. In figure 3.10, the elution volumes at the peak maxima of several fractions of DB-0 are plotted for different isocratic conditions.

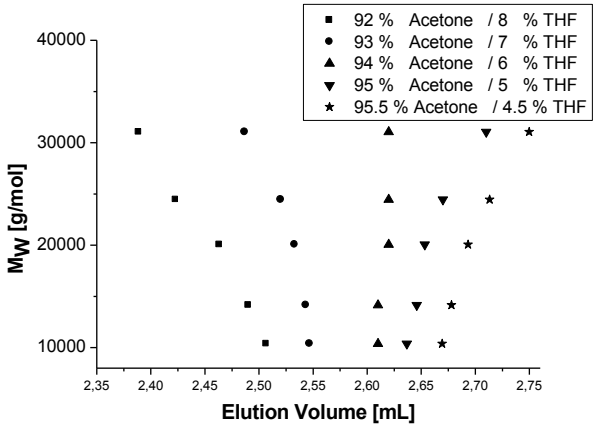


Figure 3.10. Dependences of molar mass on elution volume for fractions of linear polyester (DB-0) at various eluent compositions.

As can be seen in Figure 3.10 an eluent composition containing more than 6% THF results in an increase of the elution volume with decreasing molar mass, indicating an SEC-like elution order. In contrast, a THF content of less than 6% results in the typical LAC-like increase of the elution volume with increasing molar mass. A nearly molar mass independent isocratic

elution can be observed at an eluent composition of 94 % acetone and 6 % THF. Hence, this eluent composition can be defined as the critical one.

According to theoretical predictions and experimental results of various authors on linear and star shaped polymer, the critical composition at a given temperature is characteristic for a particular polymer - stationary phase - mobile phase system and independent on topology [117,118,119].

However, based on our results in gradient chromatography, it can be assumed that this is not valid for linear and branched polyesters differing in their DB. If critical conditions would be independent on topology, all samples should elute in rather narrow peaks at the same elution volume, irrespective of molar mass and DB.

In order to test this assumption, isocratic experiments were performed on all fractions of the differently branched samples at an eluent composition of 94 % acetone and 6 % THF, which corresponds to the critical conditions of the linear polyester.

For the low molar mass fractions of different DB (fraction no 5), an overlay of chromatograms under critical conditions is depicted in Figure 3.11.

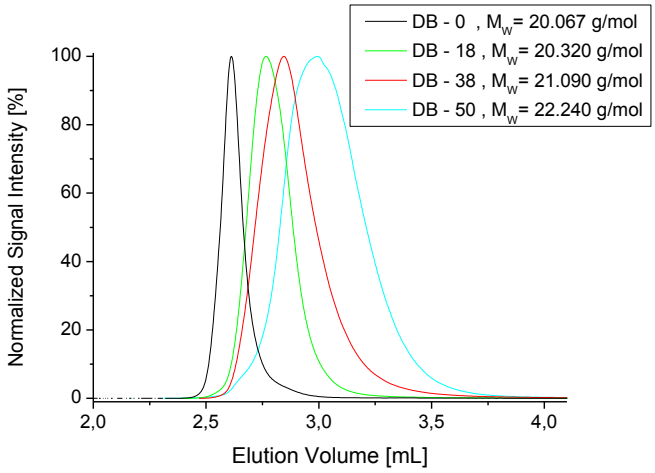


Figure 3.11: Overlay of chromatograms for linear and various branched polyesters for identical SEC elution volumes (fraction no 5) at critical eluent composition (94 % THF / 6 % acetone) of the linear polymer. Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D.), mobile phase: THF/acetone: 94%/6%, flow rate: 1 mL/min, detection: ELSD.

As can be seen from Figure 3.11, linear and different branched polymers elute at different elution volumes under the given chromatographic conditions. The linear sample elutes as narrow peak close to the void volume of the column, as expected for elution in LC-CC. The elution volume for the branched samples increases with increasing DB. The slightly different molar masses of the different fractions should have no or only little influence on the retention behaviour.

A further look at the peak width reveals that higher values of DB result in broader peaks. This phenomenon is probably due to two reasons. First, the total amount of enthalpic interactions

between the branched polymer sample and the stationary phase are more pronounced (as previous discussed) compared to the linear sample for a given molar mass. Thus, the exclusion effect is overcompensated by the enthalpic contribution, resulting for branched polymers in a molar mass dependent elution in adsorption mode at the critical conditions of the linear one. This molar mass dependence on the elution volume results in peak broadening due to the molar mass heterogeneity of the SEC slice. The increase in DB is accompanied with stronger adsorption of the polymer sample on the stationary phase, resulting in peak broadening especially for higher branched polymer samples.

Secondly, branched polymer samples consist of many different topological isomers having the same hydrodynamic volume co-eluting in SEC. These species have identical hydrodynamic volumes but differ slightly in topology and molar mass and will therefore elute at different elution volumes, when injected under critical conditions of linear polymer. These two phenomena contribute to the peak widths of the branched samples.

To evaluate the retention behaviour for all fractions at LC-CC of the linear sample, a plot of elution volumes against the molar mass for the fraction was constructed and is shown in Figure 3.12.

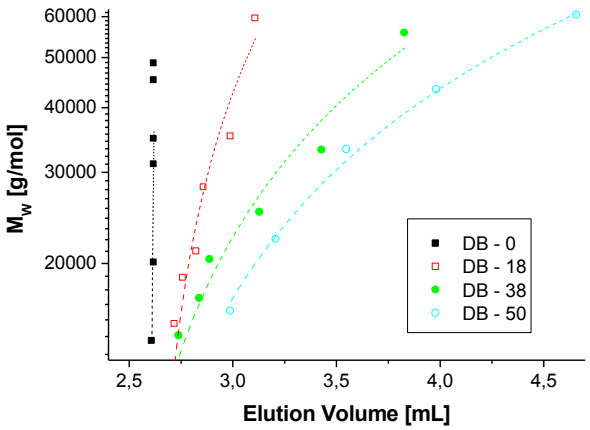


Figure 3.12: Plot of log M_W versus peak elution volume for linear and branched polymers at critical conditions of the linear polyester (94 % acetone and 6 % THF). Molar masses were determined by SEC-MALLS.

Figure 3.12 clearly illustrates that the linear and branched polymer samples of a given molar mass exhibit different elution volumes under the same chromatographic conditions. While the linear polymer samples elute independent of molar mass, an increasing elution volume with increasing molar mass is observed for the branched polymers. For very high molar masses, the enthalpic interactions between the stationary phase and the branched polymer molecules become so strong that the branched sample is not eluting at all from the stationary phase.

Furthermore, Figure 3.12 shows that at a given molar mass samples with higher DBs exhibit longer elution volume. This again can be explained by the higher density of functional groups on the surface of branched polymers, which enhances the total amount of enthalpic interactions with the stationary phase, resulting in longer elution volumes.

These results clearly demonstrate that retention in gradient chromatography and under critical conditions is not exclusively governed by the chemical nature but are also dependent on the topology to a certain degree.

A closer look at figure 3.11 and figure 3.12 reveals a potential strategy for separating linear from branched polymers using a combination of SEC and LC-CC. Under critical condition, the linear polymer sample DB-0 elutes independent of molar mass at the void volume of the column, while the branched samples elute later according to their DB. Highly branched polyester samples of higher molar masses elute under these conditions not at all from the stationary phase and only a change in eluent composition allows their desorption from the stationary phase. If one takes narrow fractions collected by SEC fractionation and injects them under critical conditions, then the linear sample should elute first while the branched sample should elute later or not at all from the stationary phase. In principle, this 2D approach should result in the separation of linear and branched polyesters. This approach was not further explored in this PhD thesis, but kept in mind for future experimental studies.

In the following section, the influence of temperature on the retention behaviour of linear and different branched polyesters was investigated in order to explore the origin for the different retention behaviour of linear and branched polyester in liquid interaction chromatography.

3.2.3 The Effect of Temperature on the Retention Behaviour of Linear and Branched Polymers

The previous sections have shown that polymers of identical molar mass but different DB elute at different elution volumes in gradient chromatography and at critical conditions of the linear polymer sample. This was explained assuming that branched polymers exhibit a higher number of contacts with the stationary phase, due to the more compact and globular structure, where more functional groups can be found on the periphical surface. In addition, more distinctive neighbourhood effects are assumed for higher branched polymer samples. However, no real evidence was given to support this assumption. In order to clarify the origin of the differences in elution volume, temperature dependent isocratic experiments were

performed. A change in temperature should have a strong effect on the retention behaviour in all chromatographic modes which are dominated by enthalpic interactions, while retention in chromatographic modes dominated by entropic interactions such as SEC are hardly influenced by temperature changes. According to our assumption, the total amount of enthalpic interactions of a linear polymer with the stationary phase should be weaker compared to the branched analogue. Therefore, a change in temperature should have a small influence on the retention behaviour of a linear polymer in interaction chromatography, while a significant effect on the retention behaviour is expected for a branched polymer of identical chemical structure and molar mass. In addition, it can be assumed, that the higher the DB, the stronger is the effect of the temperature change on the retention behaviour.
In order to test this hypothesis, gradient and isocratic experiments were performed at various temperatures.

3.2.3.1 The Influence of Temperature on the Retention Behaviour in Gradient Chromatography

In order to understand the influence of temperature on the retention behaviour of linear and branched polymers in gradient chromatography, it is useful to assume to have a blend of both species of identical molar mass and chemical structure. Since both species consist of the same number of repeating units, i.e. adsorbing groups, the only difference between the two species is the arrangement of the segments within the polymer structure, i.e. the segment density. This difference will be responsible for the different number of interaction contacts with the stationary phase and thus for differences in the total enthalpic interactions.
In a gradient experiment on such a blend of a linear and a hyperbranched polyester, two separated peaks are expected to occur as can be predicted from Figure 3.9 (for higher molar masses). The first peak should belong to the linear polyester DB-0, while the second peak corresponds to the hyperbranched polyester DB-50. Since branched polymers are expected to provide a higher amount of enthalpic interactions with the stationary phase, a variation in temperature should have a stronger influence on the elution volume of the branched polymer as compared to the linear counterpart. As a result, an increase of temperature is expected to cause the linear species to elute slightly earlier, but the branched polymer to elute much earlier. Hence, the two peaks should approach each other, causing the separation efficiency to decrease. On the other hand, a decrease in temperature should increase the separation efficiency.

In order to test the hypothesis, a blend of linear and crude hyperbranched polymer sample was fractionated several times by SEC in order to obtain sufficient amounts of the individual fractions. Afterwards, a slightly modified gradient was applied for one of the higher molar mass fractions. The modified gradient started at an eluent composition of 75 % acetone / 25 % methanol and ended after ten minutes in 100 % THF. The chromatogram obtained under these chromatographic conditions at a temperature of 5 °C is depicted in Figure 3.13.

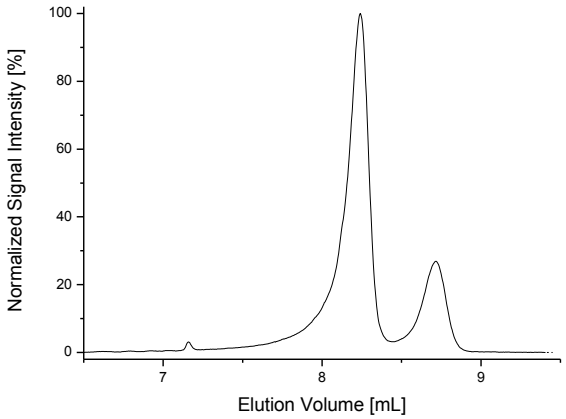


Figure 3.13: Chromatogram for a high molar mass SEC-fraction of a blend of DB-0 and DB-50 in a linear 10 min gradient at 5°C. Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D.), mobile phase: (THF/acetone/MeOH): 0 - 2 mL (75/0/25); 12 mL (100/0/0; 12.01 – 20 mL (75/0/25), flow rate: 1 mL/min, detection: ELSD.

Two peaks can be observed in the chromatogram shown in figure 3.13, where the first peak corresponds to the linear species while the second peak corresponds to the hyperbranched polymer sample. A clear baseline separation between the linear and the hyperbranched polymer sample can be noticed. The chromatogram is consistent with Figure 3.10, where it was shown that for higher molar masses a DB dependent elution in gradient chromatography is observed, which causes different elution volumes for linear and hyperbranched polymers.
In order to study the effect of temperature on the resolution, gradient experiments at various temperatures between 5 and 55°C were performed using the same gradient conditions. The elution volumes for the peak maxima and their differences are listed in Table 3.6.

Table 3.6: Peak elution volumes in gradient chromatography for different temperatures for a fraction of linear and hyperbranched polymer of identical hydrodynamic volume.

Temperature [°C]	Elution volume of peak no 1 (DB-0) [mL]	Elution volume of peak no 2 (DB-50) [mL]	Difference in elution volume between peak no 1 and no 2 [mL]
5	8.24	8.71	0.47
15	8.04	8.51	0.465
25	7.68	8.14	0.46
35	7.59	8.05	0.46
45	7.39	7.85	0.46
55	7.15	7.60	0.45

As expected the elution volumes for the linear and the hyperbranched polymer decreases with increasing temperature. In addition, the decrease in elution volume for the hyperbranched sample is slightly more pronounced compared to the linear sample, such that the differences in elution volume between the two samples decreases with increasing temperature. In Figure 3.14, the difference in elution volume between the two peaks is plotted for various temperatures.

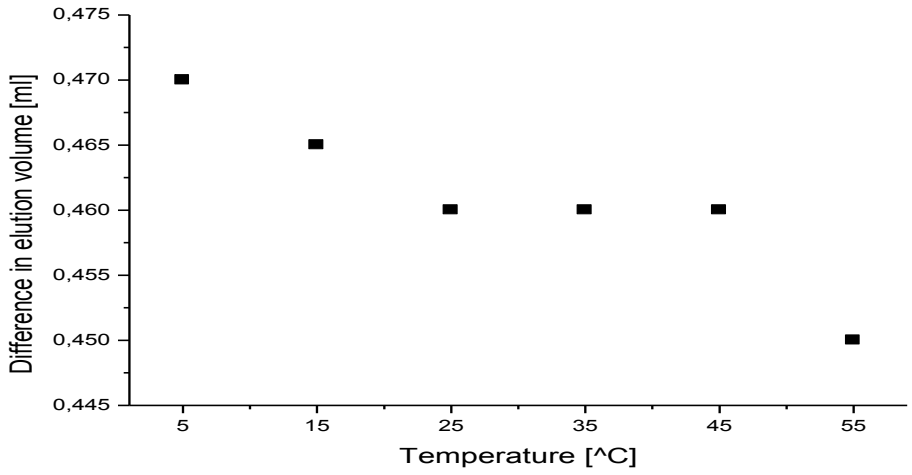


Figure 3.14: Dependence of the difference in elution volume between the two peak maxima in gradient chromatography on temperature for a blend of linear and hyperbranched polymers of the same hydrodynamic volume.

The variation of the peak separation of the two peaks is not very pronounced, but can clearly be identified in Figure 3.14. An increase in temperature causes the two peak maxima to become closer, while lowering the temperature causes the two peaks to elute further apart from each other. The results support the aforementioned hypothesis, that mainly the enthalpic

interactions between the polymer molecules and the stationary phase are responsible for the different elution behaviour of linear and branched polymers in gradient chromatography.
In the next chapter, the influence of temperature on linear and (hyper)branched polyester under isocratic conditions (LAC and LC-CC) will be investigated to strengthen the assumption.

3.2.3.2 The Influence of Temperature on the Retention Behaviour of Linear Polymers under Isocratic Conditions

In the previous section it was shown that the temperature has a non-negligible effect on the retention behaviour of linear and (hyper)branched polyester in gradient chromatography. Since gradient chromatography is an enthalpy driven separation method, other enthalpy driven separation methods such as LAC and LC-CC should also be affected by changes in temperature in a similar way. Due to the assumed higher number of interaction contacts of the branched polymer sample, a shift in temperature is expected to have a stronger influence on highly branched polymers than on lower branched samples.
First, the influence of temperature on the retention behaviour on linear polymer samples under isocratic conditions was investigated. Therefore, isocratic temperature experiments at an eluent composition of 94 % acetone and 6 % THF were performed. This eluent composition corresponds to the critical eluent composition of DB-0 at a temperature of 25°C. Figure 3.15 shows the dependence of elution volume on molar mass for DB-0 at different temperatures.

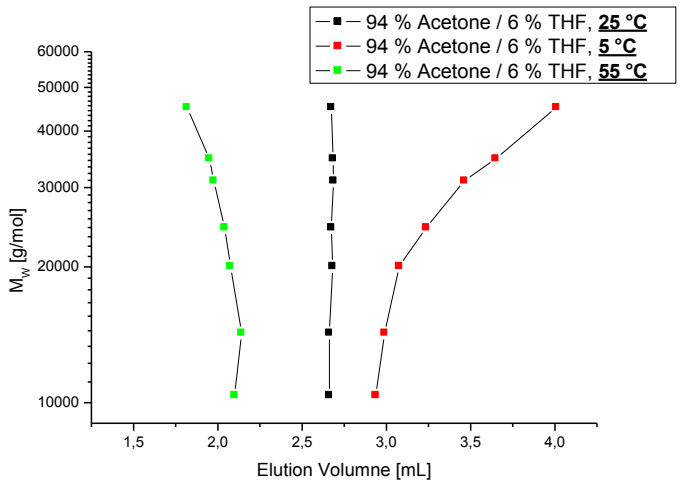


Figure 3.15: Dependence of molar mass on elution volume for fractions of linear polyester (DB-0) at three different temperatures (5°C, 25°C and 55°C).

The dependence of molar mass on elution volume for fractions of the linear polyester DB-0 is plotted in figure 3.15 for different column temperatures. A nearly molar mass independent isocratic elution can be observed at a column temperature of 25°C. A temperature rise to 55°C enhances the strength of the eluent, resulting in an SEC-like elution order. In contrast, a temperature decrease to 5°C results in a typical LAC-like elution order, indicated by an increase in elution volume with molar mass. The experiments showed that a change in temperature has also a significant influence on the retention behaviour of linear polyesters, even under isocratic conditions.

In the following section, the influence of temperature on the retention behaviour of linear and branched polyester under isocratic conditions will be used for the quantification of the enthalpic interactions between the polymer molecules and the stationary phase.

3.2.3.3 Determination of the Enthalpic Interactions between Differently Branched Polymers and the Stationary Phase

In the previous section it was shown that temperature has a significant influence on the elution of linear polyesters under isocratic conditions. In this section, the influence of temperature on the elution of linear and branched polymers will be used in order to determine enthalpic interactions for linear and branched polyester. As previously mentioned, the retention behaviour of macromolecules in ideal LAC can be described by the following equations:

$$V_E = V_A + V_{Stat} \cdot K_{LAC} \quad \rightarrow \quad K_{LAC} = \frac{V_E - V_A}{V_{Stat}} \qquad 2\text{-}14$$

$$K_{LAC} = \exp\left(-\frac{\Delta H}{RT}\right) \quad \rightarrow \quad \log K_{LAC} = -\frac{\Delta H}{RT} \qquad 2\text{-}15$$

According to equation 2-14 and 2-15, the enthalpic change ΔH can be determined from the slope by plotting $\log K_{LAC}$ against $1/T$. If our previous assumptions are correct, the change in enthalpy is expected to increase with DB for a given molar mass.

In order to test this hypothesis, the retention behaviour of SEC fractions of the linear sample DB-0, the hyperbranched sample DB-50 and the pseudo dendrimer DB-100 was studied. It is worth mentioning that the experiments were performed with samples of less than 10.000 g/mol. High molar mass sample do not elute at all from the stationary phase without a

change of the eluent composition due to the strong increase in elution volume with molar mass. The characterization details for the fractions that were used for these experiments are given in table 3.7.

Table 3.7: Characterization details of the SEC fraction used in the temperature experiments determined by SEC-MALLS.

	M_W for Fraction 1 [g/mol]	M_W for Fraction 2 [g/mol]	M_W for Fraction 3 [g/mol]	M_W for Fraction 4 [g/mol]	M_W for Fraction 5 [g/mol]
DB – 0	8760	7160	5970	5210	4870
DB - 50	9480	7620	6110	5370	5410
DB-100	9700	8950	6890	6360	5900

Isocratic experiments were performed at an eluent composition of 94 % acetone and 6 % THF at different temperatures. From the chromatograms the peak maxima were determined. The elution volumes for the peak maxima at various temperatures are listed in table 3.8a, 3.8b and 3.8c for DB -0, DB – 50 and DB – 100.

Table 3.8a: Elution volumes of the peak maxima for fractions of DB-0 at various temperatures.

Temperature [K]	Elution volume of Fraction 1 [mL]	Elution volume of Fraction 2 [mL]	Elution volume of Fraction 3 [mL]	Elution volume of Fraction 4 [mL]	Elution volume of Fraction 5 [mL]
328	2.55	2.54	2.54	2.53	2.51
318	2.60	2.59	2.59	2.58	2.57
308	2.65	2.65	2.65	2.65	2.65
298	2.72	2.74	2.74	2.74	2.75
288	2.81	2.83	2.85	2.87	2.89
278	-	-	-	-	-

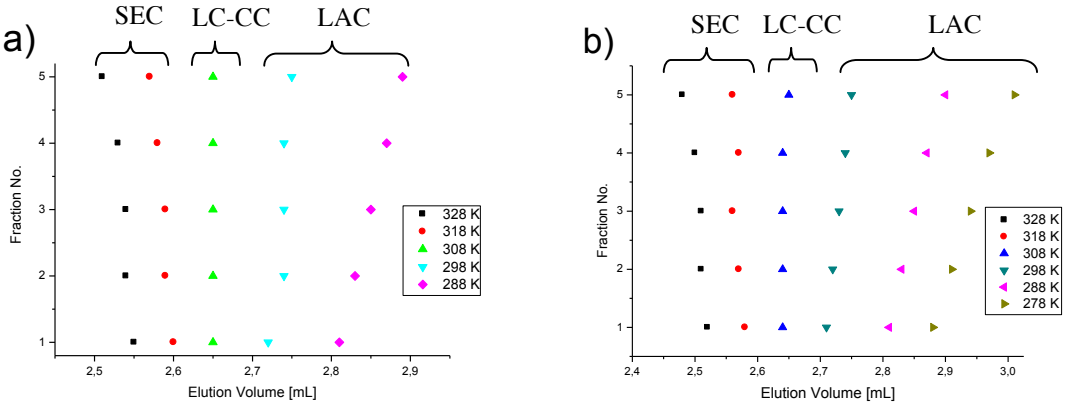
Table 3.8b: Elution volumes of the peak maxima for fractions of DB-50 at various temperatures.

Temperature [K]	Elution volume of Fraction 1 [mL]	Elution volume of Fraction 2 [mL]	Elution volume of Fraction 3 [mL]	Elution volume of Fraction 4 [mL]	Elution volume of Fraction 5 [mL]
328	2.52	2.51	2.51	2.50	2.48
318	2.58	2.57	2.56	2.57	2.56
308	2.64	2.64	2.64	2.64	2.65
298	2.71	2.72	2.73	2.74	2.75
288	2.81	2.83	2.85	2.87	2.90
278	2.88	2.91	2.94	2.97	3.01

Table 3.8c: Elution volume of the peak maxima for fractions of DB-100 at various temperatures.

Temp. [°K]	Temperature [K]	Elution volume of Fraction 1 [mL]	Elution volume of Fraction 2 [mL]	Elution volume of Fraction 3 [mL]	Elution volume of Fraction 4 [mL]
328	2.55	2.55	2.56	2.56	2.57
318	2.61	2.62	2.63	2.64	2.65
308	2.68	2.69	2.71	2.73	2.76
298	2.76	2.78	2.81	2.87	2.91
288	2.86	2.90	2.96	3.03	3.08
278	2.94	2.99	3.07	3.17	3.30

Graphical representations of the dependences of elution volume on molar mass are given in figure 3.16a, 3.16b and 3.16c for DB – 0, DB -50 and DB – 100 for various temperatures.



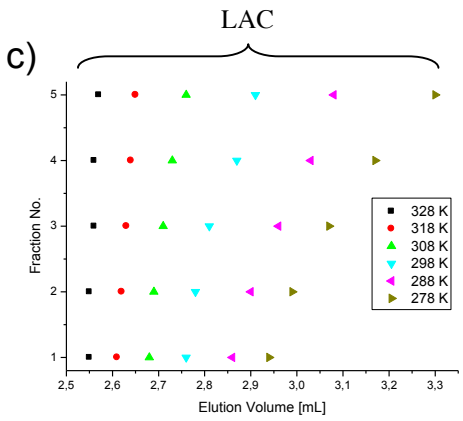


Figure 3.16a (upper left), 3.16b (upper right) and 3.16c (bottom): Dependences of elution volume on molar mass (fraction no. respectively) for different temperatures at an eluent composition of 94 % acetone and 6 % THF for DB - 0 (upper left), DB – 50 (upper right) and DB-100.

An interesting elution behaviour can be noticed from the elution volumes of the peak maxima as plotted in figure 3.16a and 3.16b. For fractions of DB-0 and DB-50, it can be distinguished between the elution order at lower and higher temperatures. For lower temperatures (278 K – 308 K), the elution volume increases with molar mass, showing a typical elution in LAC mode. When the temperature raises over 308 K, a change in the elution order can be noticed. In that case, an increase in molar mass causes the polymer fraction to elute earlier, which is typical for an elution in SEC mode. The pseudo dendrimer DB-100 elutes under the given chromatographic conditions always in LAC mode, independent of the column temperature. A special case occurs for the linear polymer sample DB-0 at 308 K, where a molar mass independent elution order can be noticed, which is typical for an elution in LC-CC. Similar retention behaviour can be observed for DB-50 at 308 K and DB-100 at 328 K, where only a slight variation in elution volume with increasing molar mass exists.

In order to quantify the extend of enthalpic interactions between the stationary phase and the polymer molecules, the previous determined elution volumes at various temperatures were used to construct plots of $\log K_{LAC}$ versus $1/T$. The values for V_A and V_{Stat} were determined to be 1.41 mL and 1.02 mL respectively. These plots are shown in figure 3.17.

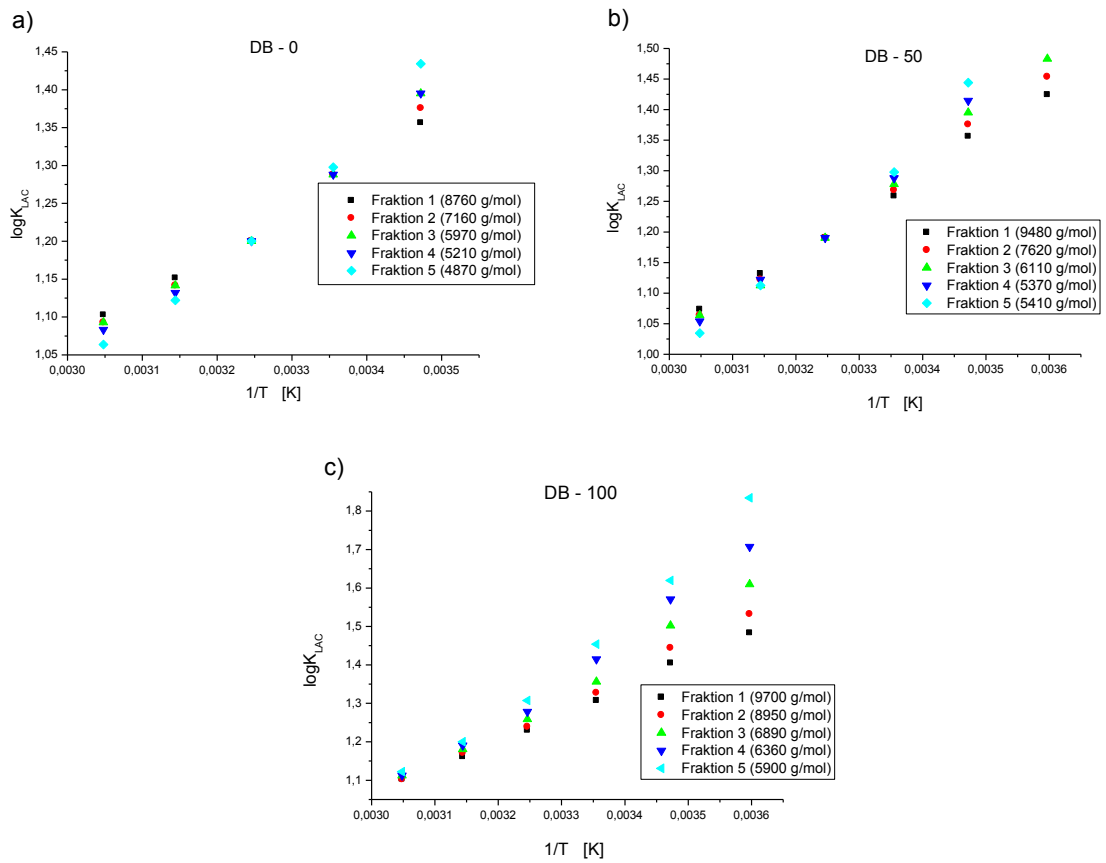


Figure 3.17a, 3.17 b and 3.17c: Plot of $\log K_{LAC}$ against $1/T$ for DB - 0 (upper left), DB – 50 (upper right) and DB-100. From the slope of the values, the value for the enthalpic interactions ΔH between the stationary phase and the polymer molecules can be determined. For the calculation of ΔH , no entropic interactions were assumed ($\Delta S = 0$). Therefore ΔS was set equal to zero, so that the slope goes through the origin of the graph.

From the slopes of the plot of $\log K_{LAC}$ against $1/T$, the enthalpic interactions ΔH between the stationary phase and the polymer molecules was extracted. Hereby, ΔS was set equal zero, so that the slope goes through the origin, since no entropic interactions should occur in ideal LAC. The resulting values for ΔH are listed in table 3.10.

Table 3.10: $-\Delta H/R$ for fractions of linear and branched polyester sample.

	$-\Delta H/R$ Fraction 1	$-\Delta H/R$ Fraction 2	$-\Delta H/R$ Fraction 3	$-\Delta H/R$ Fraction 4	$-\Delta H/R$ Fraction 5
DB – 0	374	376	377	375	377
DB – 50	375	377	380	383	386
DB – 100	388	395	405	419	433

A graphical representation of the data in table 3.10 is given in figure 3.18.

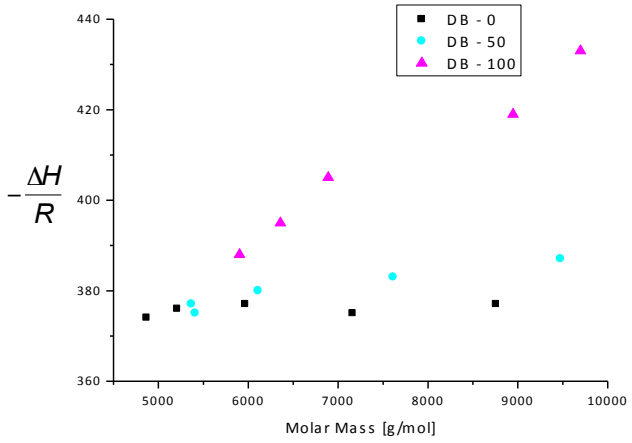


Figure 3.18: Plot of $-\Delta H/R$ against the molar mass for DB-0, DB-50 and DB-100.

Besides some scattering in the low molar mass region, the plot clearly shows that the enthalpic interactions between the polymer sample and the stationary phase increase with molar mass for a given DB. In addition, there seems to be a tendency to a higher slope with increasing DB, indicating a stronger increase in the enthalpic interactions with increasing DB. The scattering of the data in the low molar mass region is probably due to the experimental error in molar masses obtained by SEC-MALLS. Another factor causing similar values for the enthalpic interactions in the low molar mass region, is the small variation in the absolute number of branched units between low and high branched polymer samples However, for molar masses higher than 10.000 g/mol, the enthalpic interactions clearly increases with DB at a given molar mass.

Finally, the entropic interactions were neglected in the calculations, which should also have a small influence on the accuracy of the plot.

The temperature experiments showed that temperature has a significant influence on the retention behavior of linear polyester in enthalpic dominated chromatographic methods with a stronger influence on the retention behavior of branched samples. In addition, the investigation proved that with increasing DB and with increasing molar mass, the enthalpic interactions between the polymer sample and the stationary phase become stronger.

The performed experiments and the resulting temperature dependences for linear and branched polymers might open new opportunities to adjust the temperature in order to fine tune the enthalpic adsorption strength between the stationary phase and the polymer

molecules. This will ultimately allow the optimization of the separation efficiency in these enthalpic interaction driven chromatographic modes.

In the next part of this PhD-thesis, we will make use of the previous findings on the retention behaviour of linear and branched polymers in liquid chromatography in order to develop a two dimensional chromatographic method, allowing to perform separations according to the DB and molar mass.

4 Result and Discussion (Part B)

The previous chapters have shown that conventional one-dimensional liquid chromatography is not capable for the separation of branched polymers differing in molar mass and DB. Especially for samples consisting of linear polymer chains blended with branched polymer chains it seems to be necessary to first effectively separate the different topologies from each other before analyzing them. Otherwise, coelution of polymer chains differing in molar mass and DB might occur, causing the results (molar mass, radius of gyration, etc.) of a fraction to be average values. Since conventional one-dimensional chromatography in general is not prosperous for the separation of blends of linear and branched polymer chains, two-dimensional liquid chromatographic methods need to be developed. The basis for setting up such a two dimensional liquid chromatographic system was developed in the previous chapter, where the retention behaviour of branched polymers was investigated in SEC, LAC, LCCC and gradient chromatography.

Based on the information gained in the previous experimental studies, a two dimensional system combining size exclusion chromatography and gradient chromatography was selected as the most promising choice. Both chromatographic methods possess different retention behaviours and their combination should allow in principle a separation of linear and branched polymer chains according to the concept schematically shown in figure 4.1. In figure 4.1, we assume to have a blend of a linear and branched species of the same chemical structure and similar hydrodynamic size distribution. Due to the similar hydrodynamic size distribution we expect to find two different co-eluting polymer species at a given SEC elution volume, when running a SEC experiment. Since at a given hydrodynamic size the molar mass of the branched polymer is higher than that of the corresponding linear polymer coelution of a linear fraction of lower molecular weight and a branched fraction of higher molecular weight (left side of figure 4.1) is expected to occur. In a chromatographic experiment, the narrow fractions of coeluting species are subjected to gradient chromatography that is based on another chromatographic mode with different retention behaviour than SEC. As shown in the previous chapter the linear polymer chains at a given molar mass are less strongly absorbed than the branched polymer chains and retention increases with molar mass. Consequently, the species coeluting in SEC are expected to elute at different retention times in the gradient separation, where the first peak corresponds to the linear species, while the second peak corresponds to the branched species (right side of figure 4.1). Thus, by performing the

gradient separation for every SEC-slice a complete separation of linear and branched molecules should result.

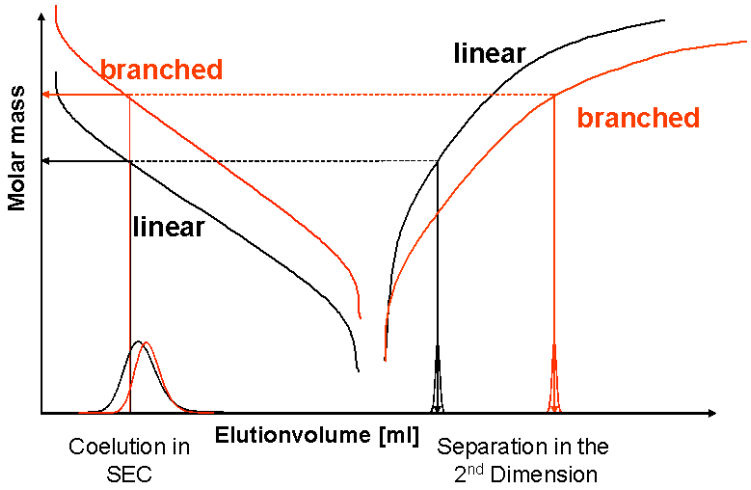


Figure 4.1: Schematic representation of the 2-dimensional concept to separate linear and branched polymers [79,80].

The introduced two-dimensional concept for the separation of linear and branched polyester used SEC in the first dimension while gradient chromatography is applied in the second dimension. However, a reverse of the separation order should also be possible in principle and should have no impact on the separation efficiency.

4.1 Offline Two-Dimensional Liquid Chromatographical Separation (SEC x Gradient)

In order to test the suitability of the 2D-approach, offline two-dimensional experiments were performed, using SEC in the first dimension, while gradient chromatography was applied in the second dimension.

Multiple injections of the crude polymer samples into the first dimension allowed the collection of linear and branched fractions in sufficient amounts for subsequent gradient analysis. Fractions of 250 µL each were taken at different retentions times during the SEC experiments (first dimension) using a fraction collector. An overlay of the SEC chromatograms for DB – 0 and DB – 50 is shown in figure 4.2.

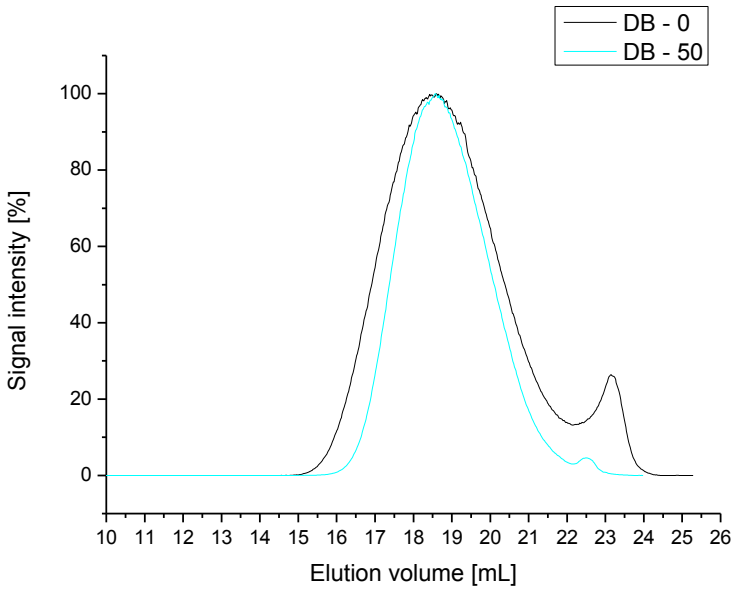


Figure 4.2: Overlay of SEC – chromatograms for DB – 0 and DB – 50. Stationary phase: PSS SDV 10^5, 10^6 Å (each 300 x 8 mm I.D.), mobile phase: THF, flow rate: 1 mL/min, detection: Refractive index detector.

The first fraction was taken at an elution volume of 17 mL. Subsequently, the SEC fractions were analyzed by gradient chromatography.

Since branched polymers have a more compact structure and therefore a higher molar mass compared to their linear analogue at the same SEC elution volume, they are expected to exhibit stronger enthalpic interactions with the stationary phase and therefore elute later than the linear polymer in gradient chromatography. Consequently, a fraction of a blend of linear and branched polymer chains should result in two distinct peaks, where the first peak represents the linear polymer sample while the later eluting peak belongs to the branched polymer sample.

In order to test this hypothesis, a ten minute gradients starting from 75 % / 25% acetone / methanol to 75 % / 25% acetone / THF was applied to the individual SEC-fractions. Overlays of gradient chromatograms for the SEC fractions of the linear sample DB-0 and the hyperbranched sample DB-50 are shown in figure 4.3a and figure 4.3b, respectively.

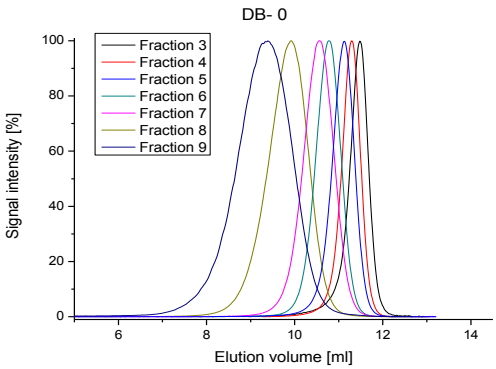


Figure 4.3a: Overlay of gradient chromatograms for different SEC fractions of the linear sample (DB-0) Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 4.6 mm I.D.), mobile (THF/acetone/MeOH): 0 - 2 mL (0/75/25);12 mL (25/75/0);12.01 – 13 mL (100/0/0);13.01 – 20 mL (0/75/25), flow rate: 1 mL/min, detection: ELSD.

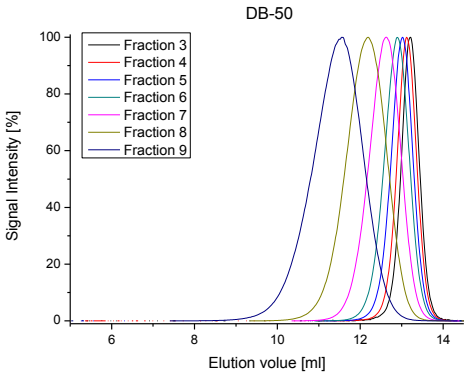


Figure 4.3b: Overlay of gradient chromatograms for different SEC fractions of the hyperbranched sample (DB – 50). Stationary phase: Nucleosil C18 columns (5µm, 300 Å, 4.6 mm I.D.), mobile (THF/acetone/MeOH): 0 - 2 mL (0/75/25);12 mL (25/75/0);12.01 – 13 mL (100/0/0);13.01 – 20 mL (0/75/25), flow rate: 1 mL/min, detection: ELSD.

In both figures, a dependence of gradient elution volume on SEC elution volume (which increases with fraction number) can be observed. While low molar mass fractions (higher SEC fraction number) elute first, fractions of higher molar masses are eluting later. This behaviour is quite common for linear polymers eluting in gradient chromatography and has also been discussed in section 2.3.4.

A comparison of the elution volumes of the linear and the branched polymers at the same fraction number (identical SEC elution volume, respectively) reveals that the linear fractions always elute before the corresponding hyperbranched fractions of identical hydrodynamic volume. This phenomenon is caused by the fact that at equal hydrodynamic elution volumes, branched polymers posses a higher molar mass compared to their linear counterparts due to their more compact structure. Consequently, a later elution of higher branched polymer samples in gradient chromatography is observed.

In addition, it can be seen from the overlays that the widths of the peaks increase with decreasing molar mass. This behaviour was already discussed in figure 2.6, where it was shown that in gradient chromatography a strong dependence of molar mass on elution volume exists for lower molar masses. This strong dependence of elution volume on molar mass results in broader peaks in gradient chromatography for fractions of low molar mass. For higher molar masses this dependence declines until above a certain molar mass the molar mass dependence on elution volume vanishes totally. Thus, the molar mass dispersity of the fraction does not account any longer to peak broadening.

In order to evaluate the retention behaviour of linear, partially branched and hyperbranched polyester fractions in gradient chromatography, the elution volume at the peak maxima in

gradient chromatography (second dimension) are plotted versus the fraction number from SEC (first dimension) in figure 4.4.

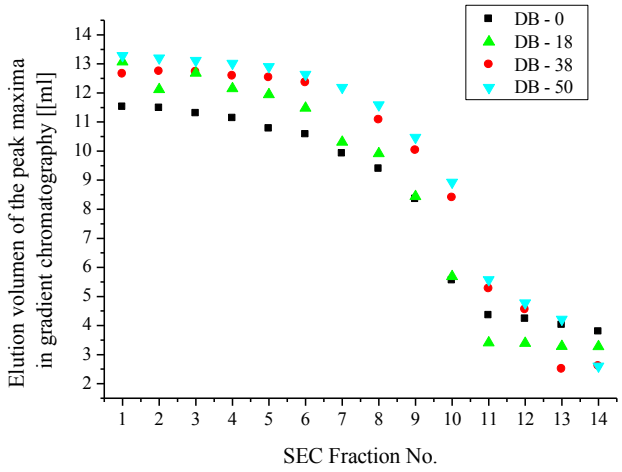


Figure 4.4: Dependence of gradient elution volume on SEC fraction no. for the linear, partially branched and the hyperbranched polymer sample in gradient chromatography.

As can be seen in figure 4.4 polyester fractions of the same SEC-fraction number (that means identical hydrodynamic size) elute at different gradient elution volumes. Two different regions can be identified in the plot. For higher molar masses (left side of the figure, lower SEC fraction numbers) the gradient elution volume increases with DB. In addition, the difference in gradient elution volume for samples differing in DB is more pronounced for lower SEC fraction numbers (higher molar masses) than for higher SEC fraction number (lower molar masses). Thus, for high SEC fraction numbers an increase in DB does not result in a pronounced change of gradient elution volume. In addition, at low molar masses the widths of the peaks become very broad for the linear polymer and the branched polymer samples, making the separation of linear and branched samples impossible.

An impression of the resolution that could be obtained in the high and low molar mass region for the linear and hyperbranched sample is shown in 4.5a for the high molar mass fraction no. 6 and in figure 4.5b for the low molar mass fraction no. 11, respectively.

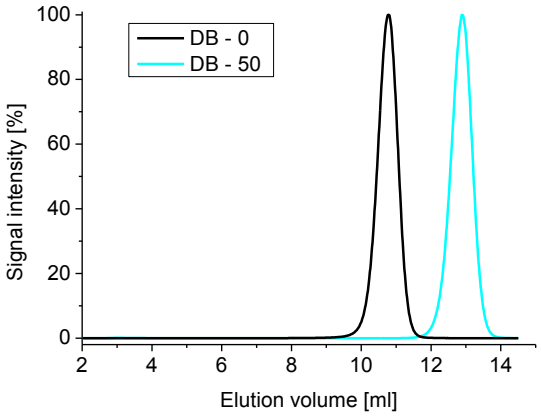


Figure 4.5a: Overlay of chromatograms for SEC fractions no. 6 of DB-0 and DB-50 in gradient chromatography.

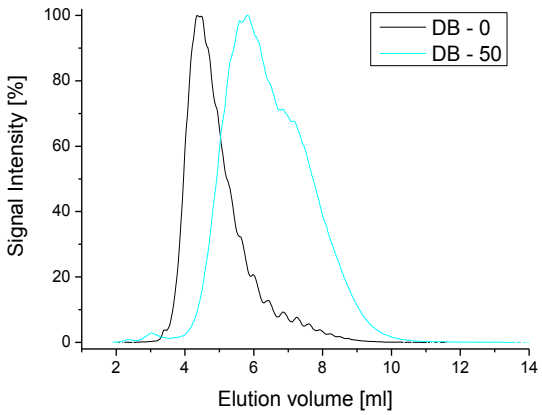


Figure 4.5b: Overlay of chromatograms for SEC fractions no. 11 of DB-0 and DB-50 in gradient chromatography.

In both figures, two peaks can be observed, where the first peak corresponds to the linear fraction while the second peak is due to the hyperbranched polymer. The difference in elution volume is approximately 2.2 mL for the sample of higher molar mass and approximately 1.4 mL for the lower molar mass fraction. This indicates that the two peaks approach each other as the molar mass decreases. However, the resolution is determined by the differences in retention times and peak width. Figure 4.5a shows two narrow distributed peaks resulting from the gradient experiment in the high molar mass region. Therefore a clear baseline separation between the linear and the hyperbranched polymer sample is expected. In contrast, for the low molar mass region (figure 4.5b) two broad peaks are observed, which in a real separation would decrease the resolution between the linear and the branched polymer fractions. The reason for the differences in peak widths is found in the dependence of molar mass on elution volume in gradient chromatography, which vanishes for higher molar masses. The offline 2D experiments on the individual fractions showed, that a separation of a blend of linear and branched polyesters should be possible, at least for higher molar masses. Furthermore, it is expected that the separation efficiency increases as the difference in DB between the two samples increases. In contrast, lower molar mass polymer blends or polymer blends where the difference in DB is not very pronounced can probably not be completely separated by offline 2D chromatography, due to the lower separation efficiency and larger peak widths.

In order to investigate the potential for a real separation of linear and branched polymers, DB-0 and DB-50 were solution blended and separated by offline 2D chromatography. The results will be presented in the next section.

4.1.1 Separation of an Artificial Blend of DB-0 and DB-50 by Offline 2D-Chromatography

In the previous experiments, fractions obtained by SEC fractionation for differently branched polyesters were analysed by offline 2D chromatography. The elution behaviour was compared by overlaying the individual chromatograms. However, this does not prove that a real separation is possible. Therefore, an artificial blend of the linear polymer sample DB-0 and the hyperbranched polymer sample DB-50 was prepared to test our 2D approach for a real system, truly heterogeneous with respect to molar mass and DB. The blend of the linear and the hyperbranched polyester was fractionated by SEC several times. All SEC-fractions were subsequently injected into gradient chromatography. A stacked plot of the chromatograms of the gradient runs for selected fractions is shown in figure 4.6

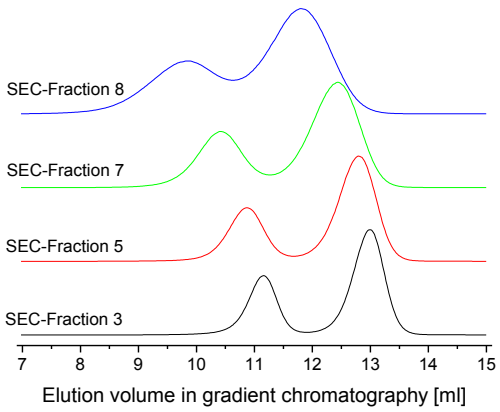


Figure 4.6: Stacked plot of chromatograms for selected SEC-fractions of a mixture of linear and hyperbranched polymer sample in gradient chromatography.

As expected, each chromatogram in figure 4.6 clearly reveals two distinct peaks. The peak at lower elution volumes can be assigned to the linear species, while the peak at higher elution volumes corresponds to the hyperbranched polymer sample DB – 50.

The comparison of the chromatograms reveals that the two peaks become broader with increasing SEC fraction number, i.e. with decreasing molar mass. Consequently, a baseline separation is only possible up to SEC fraction no. 5. For higher SEC fractions, the peak broadening results in a decreased resolution. Thus, the separation of a true blend proofs the results of the preceding section, where fractions of the branched and linear polymer were analyzed separately.

From the previous results, it becomes clear that the separation of linear and branched polyesters is not a trivial task. For higher molar masses, a complete separation of a mixture of

linear and hyperbranched polymers can be obtained using the offline 2D approach. However, for lower molar masses, a separation of linear and hyperbranched polymers cannot be archived by the introduced offline 2D liquid chromatographical approach.
In order to accomplish a better separation for blends of linear and branched polymers in the low molar mass region, the separation efficiency has to be increased. One way to achieve this is by taking narrower fractions in the first dimension that are subsequently subjected to a separation in the second dimension.
Since offline two-dimensional chromatography was only partially successful in the separation of linear and branched polymer chains in the low molar mass system and in order to quantify the amounts of linear and branched polymer chains in a blend of both, the possibilities of online two-dimensional chromatography were explored within the next chapter.

4.2 Online Two Dimensional Liquid Chromatographically Method Development

In the previous chapter, it was shown that offline two-dimensional chromatography allows the separation of a mixture of linear from branched polymers in the high molar mass region. With decreasing molar mass, the difference in elution volume decreases while the peaks become broader, making a separation more difficult. As previously mentioned, one possible way to increase the separation efficiency is by online 2D chromatography, where the volume of the fractions transferred from the first dimension into the second dimension and consequently the heterogeneity of the corresponding fraction, is reduced. Therefore, the possibility of a fully automated two-dimensional system will be explored within this chapter.
Via a storage loop system, fractions from the first separation step are automatically transferred into the second separation system. The operation of the column switching device is automatically driven by the software. Errors due to human failure can be excluded in that case. Another advantage of online two-dimensional chromatography is that in contrast to offline two-dimensional chromatography, where a time consuming fractionation and preparation step is required, online two-dimensional chromatography can be performed within a few hours.
In principle, there are two ways to set up an online two-dimensional liquid chromatography system. Either SEC can be performed in first dimension and gradient chromatography in the second dimension (similar to the offline experiments) or the other way around. The most

reported approaches apply gradient chromatography in the first and SEC in the second dimension[102,103] due to the following advantages:

- In case that SEC is used in the first dimension, the injected sample is completely dissolved in a strong solvent when the fraction is transferred to the second dimension. This might cause breakthrough effects, where parts of the injected polymer sample is not adsorbed onto the stationary phase but migrate through the column within the injection band without retention, making an accurate separation impossible [120].
- Sample loads on HPLC columns can be much higher compared to SEC columns[4].
- A larger number of parameters (eluent, type of stationary phase, gradient slope and temperature) in gradient chromatography allow a better fine tuning of the separation to achieve more homogeneous fractions.
- An equilibration of the stationary phase in gradient chromatography is needed after every chromatographic run. This equilibration substantially increases the duration of a gradient experiment. In case that gradient chromatography would be applied in the second dimension, the flow rate in the first dimension has to be adjusted and might be too low to be properly controlled by the pump.

Therefore gradient chromatography was performed in the first dimension on a Nucleosil 300-5 C-18 column (250 x 4.6 mm). In order to achieve a complete sample transfer from the first into the second dimension, the transfer loop has to be filled with the effluent of the first dimension in the same time required to perform the analysis of the fractions in the second dimension. Thus, the first dimension has to be slow, while the analysis time for the second dimension has to be reduced as far as possible. Therefore, the flow rate in the first dimension was reduced to 0.025 mL/min in order to fill the 100 µL transfer loops in the 4 minutes required to perform one SEC (second dimension) run. In contrast to the offline measurements, a slightly modified gradient was used (see table 4.1) for the gradient experiments to obtain better results.

Table 4.1: Eluent composition of the modified gradient used in the first dimension.

T (min)	0	40	160	320	480
% THF	0	0	5	15	30
% MeOH	30	30	25	15	0
% Acetone	70	70	70	70	70

The second dimension separation was performed on two SEC columns (PSS SDV 10^5 Å, 30 x 8 cm and PSS SDV 10^6 Å, 30 x 0.8 cm). The flow rate was adjusted to 2 mL/min. ELSD was used to detect the polymer species after the SEC separation.

The calibration curve for the 2nd dimension was constructed using 9 polystyrene calibration standards in the molar mass range between 1.800 – 1.312.700 g/mol to determine the molar masses in the 2D contour plots. The calibration curve is shown in figure 4.7.

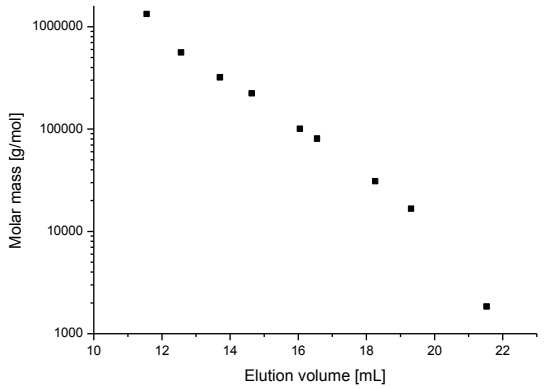


Figure 4.7: Polystyrene calibration plot for 2nd dimension (PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm); mobile phase: THF; flow rate: 2.0 mL/min; detection: ELSD.

The total analysis time for a chromatographic run in the second dimension is 11 min, which corresponds to an elution volume of 22 mL at a flow rate of 2 mL/min.

In the first dimension (gradient chromatography) of the two-dimensional system, complete elution is realized after 12 mL, which corresponds to an analysis time of 480 min at a flow rate of 0.025 mL/min. Since fractions of 100 µL were transferred into the second dimension, 120 SEC measurements are required for a comprehensive 2D analysis. In order to decrease the total analysis time, it is useful to inject the next fraction into the SEC analysis before the analysis of the previous fraction has been completed. This is possible, since no sample component can elute before the interstitial volume in SEC [101]. Therefore, the previously injected fraction does not interfere with the next injected fractions. By using this procedure which is referred to as overlaid injections, the fractions get transferred into the second dimension every 4 min instead of 11 min. This procedure reduced the total separation time for

a comprehensive 2D measurement to 480 min as compared to a total analysis time of 1320 min without overlaid injections.

In the following figures, the obtained 2D-contour plots will be shown. The gradient elution volume is represented on the Y-axis whereas the molar mass from SEC is plotted on the X-axis. First, 2D-measurements of the homopolymers DB-0, DB-18, DB-38, DB-50 and DB-100 are shown. Afterwards blends of homopolymers of linear and several branched polymers will be discussed in order to investigate the separation capabilities of the online two-dimensional blends of polymers approach.

In particular, it was investigated whether an online two-dimensional separation yields better results compared to the offline experiment. In addition, quantification of the amount of linear and branched material in a blend of both species was performed. Finally, the 2D measurements of the samples of known DB were used in order to establish a calibration curve, which might allow the determination of the DB and DB distribution of an unknown sample.

4.2.1 Online Two Dimensional Chromatography on Linear and Branched Polyesters

In the following figures, contour plots of DB – 0, DB – 18, DB – 38, DB – 50 and DB - 100 will be presented and discussed briefly.

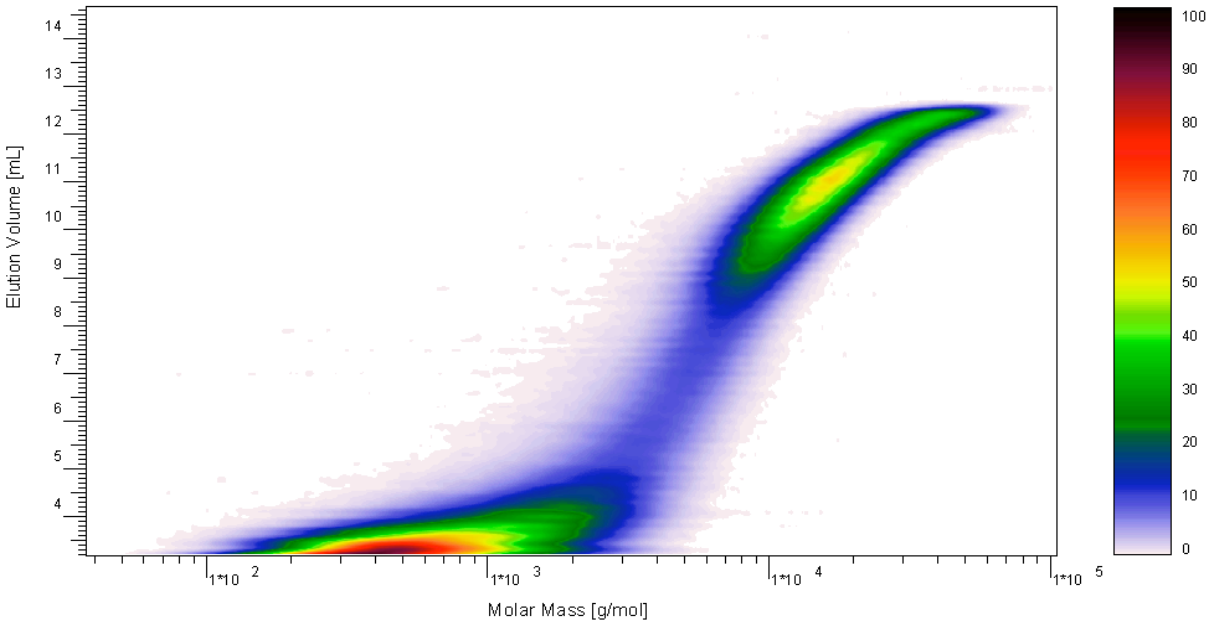


Figure 4.8: 2D-LC contour plot of the linear polyester DB-0, 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

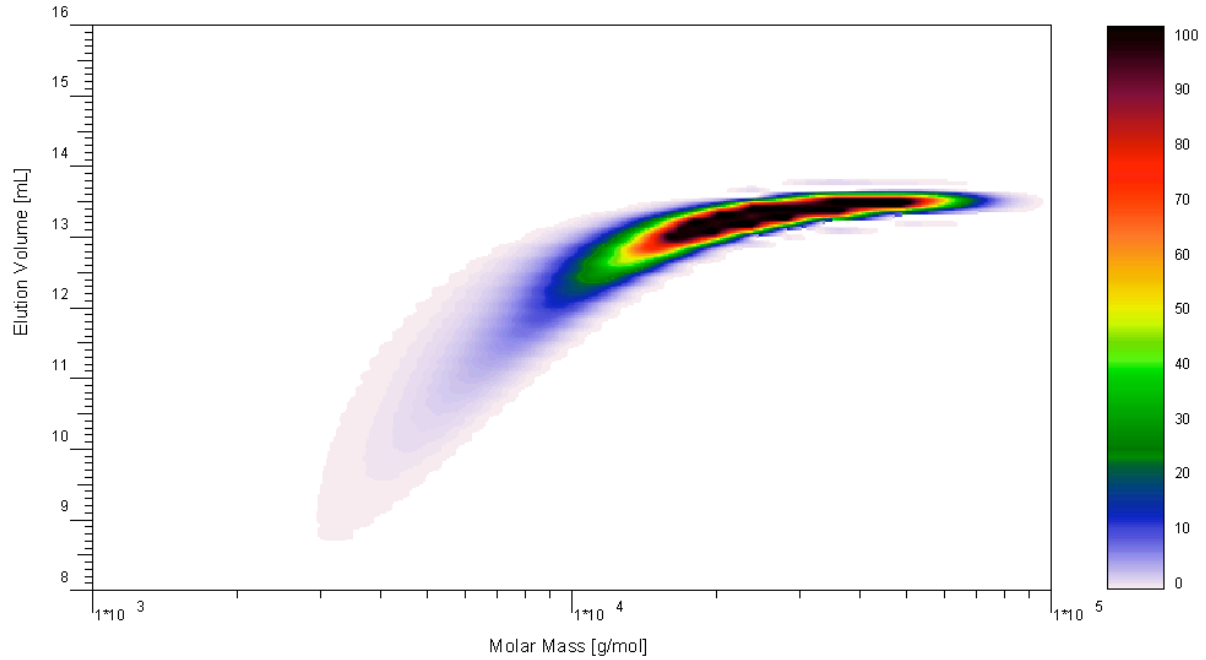


Figure 4.9: 2D-LC contour plot of the partial branched polyester DB-18, 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

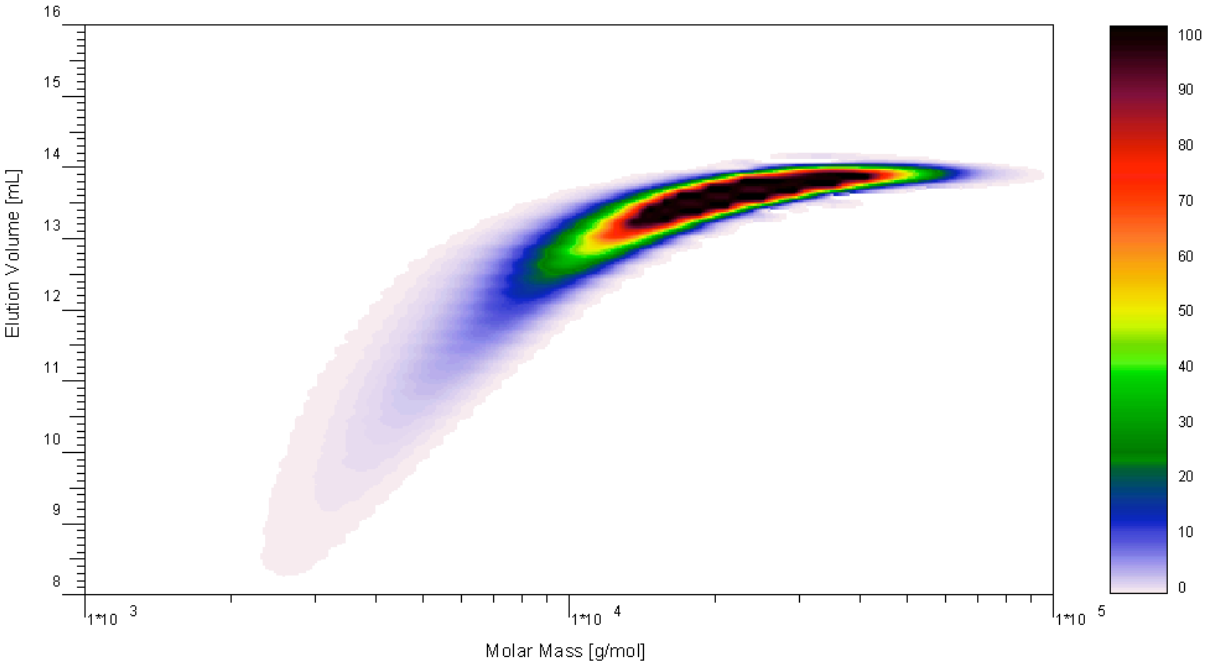


Figure 4.10: 2D-LC contour plot of the partial branched polyester DB-38. 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

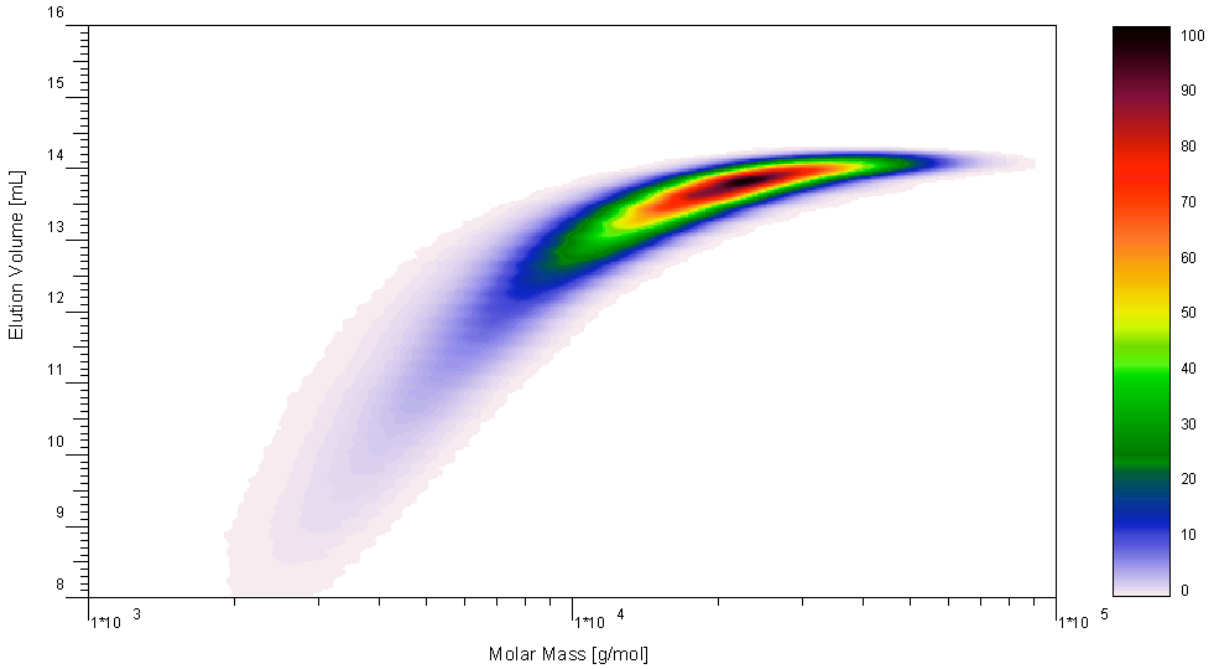


Figure 4.11: 2D-LC contour plot of the hyperbranched polyester DB-50, 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

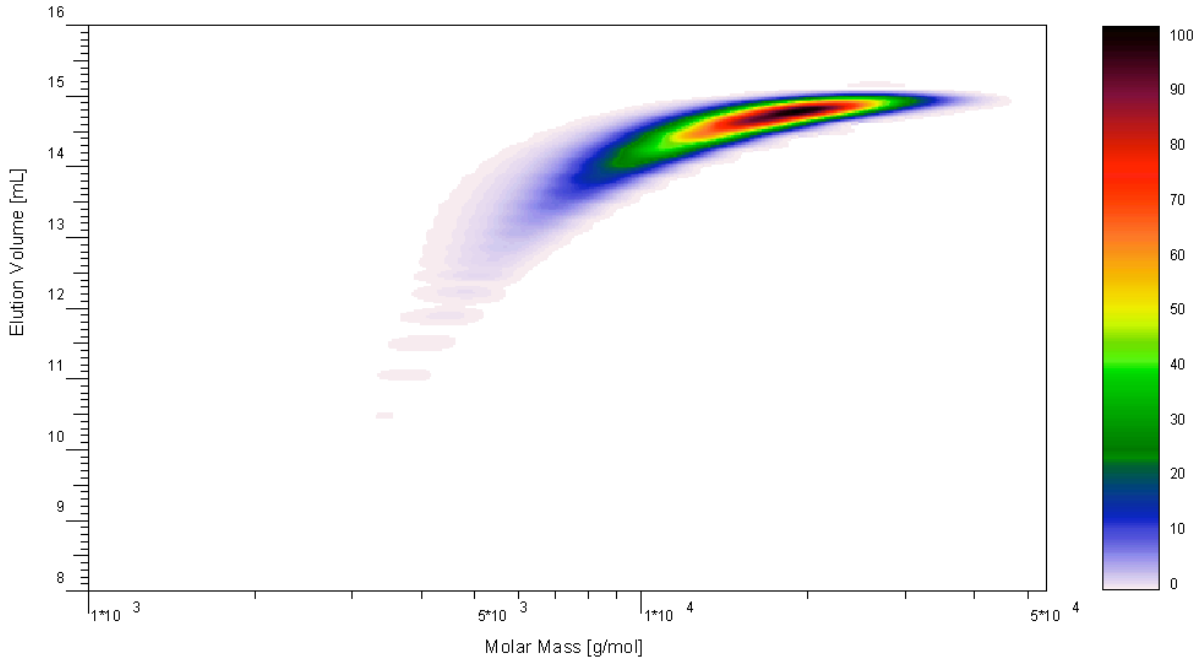


Figure 4.12: 2D-LC contour plot of the highly branched polyester DB-100, 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

Figure 4.8 – 4.12 show the elution of linear and different branched using the previously mentioned conditions for online two-dimensional chromatography. A similar shape of the peaks in the contour plot can be noticed for the differently branched polymer samples. The curved peaks for the polymer samples are directly related to the molar mass dependence in gradient chromatography. The molar mass dependence of elution volume at low molar masses vanishes in the high molar mass region causing the peak to approach a constant elution volume in gradient chromatography (y-axis).

A closer look at the linear polymer sample DB-0 reveals that the sample exhibits two maxima, while all branched samples show only a single maximum. For the linear sample the first maximum is found in the low molar mass region and is caused by the cyclic species due to ring formation of the low molar mass chains, as discussed in the chapter 3.2.1. The second maximum corresponds to the expected linear polymer species of higher molar mass. The partial cut of the first peak is caused by the fact that no separation can occur before approximately 3 mL in gradient chromatography, which corresponds to the death volume of the stationary phase in the first dimension.

Comparison of all contour plots shows that all polyester samples elute in a similar elution range in the first and second dimension. A similar elution behavior was expected since the polymer samples are based on identical functional groups and differ only in their topology and molar mass. However, a slight shift towards higher gradient elution volumes (y-axis) can be observed with increasing DB, indicating that an increase in DB is accompanied by a stronger adsorption of the polymer sample on the stationary phase.

For all samples, the peak widths with respect to the y-axis at a given SEC elution volume (x-axis) is rather narrow in the high molar mass region. This indicates a low topological heterogeneity of the SEC slices at high molar masses. This is beneficial for the separation of blends of linear and branched polymer samples. In the low molar mass region, the elution time in the second dimension is influenced by both, molar mass and DB. Therefore no conclusion can be drawn on the heterogeneity of a SEC slice without further calibration.

In order to test the possibilities for true separations of two different topologies in online 2D chromatography, blends of linear and differently branched polymer samples were prepared and investigated in the following section.

4.2.2 Blends of Linear and Branched Polyester Samples

The previous section showed that under the given chromatographic conditions linear and differently branched homopolymer samples elute at different positions in 2D-chromatography, depending on DB and molar mass. In this section, artificial blends of linear and branched samples (DB – 0 + DB-18, DB – 0 + DB-38, DB – 0 + DB-50, DB – 0 + DB-100) were prepared and analysed by online 2D chromatography. Similar experiments had been already performed offline. The previously performed offline experiments (section 4.1.1) revealed that in the high molar mass region, a separation between linear and branched polymer samples can be achieved, while in the low molar mass region, the separation efficiency decreases. This lower separation efficiency in offline 2D chromatography at lower molar masses might partially be caused by the large sample volumes transferred from the first dimension into the second dimension.

For that reason, online 2D experiments were performed, where fractions of lower volume are transferred from the first into the second dimension. The use of more narrow gradient fractions might result in a better separation of the linear and branched polymers as compared to offline 2D experiments.

In the following figures, the contour plots for online 2D chromatographic experiments are presented and discussed for the blends of the linear and the differently branched polymers.

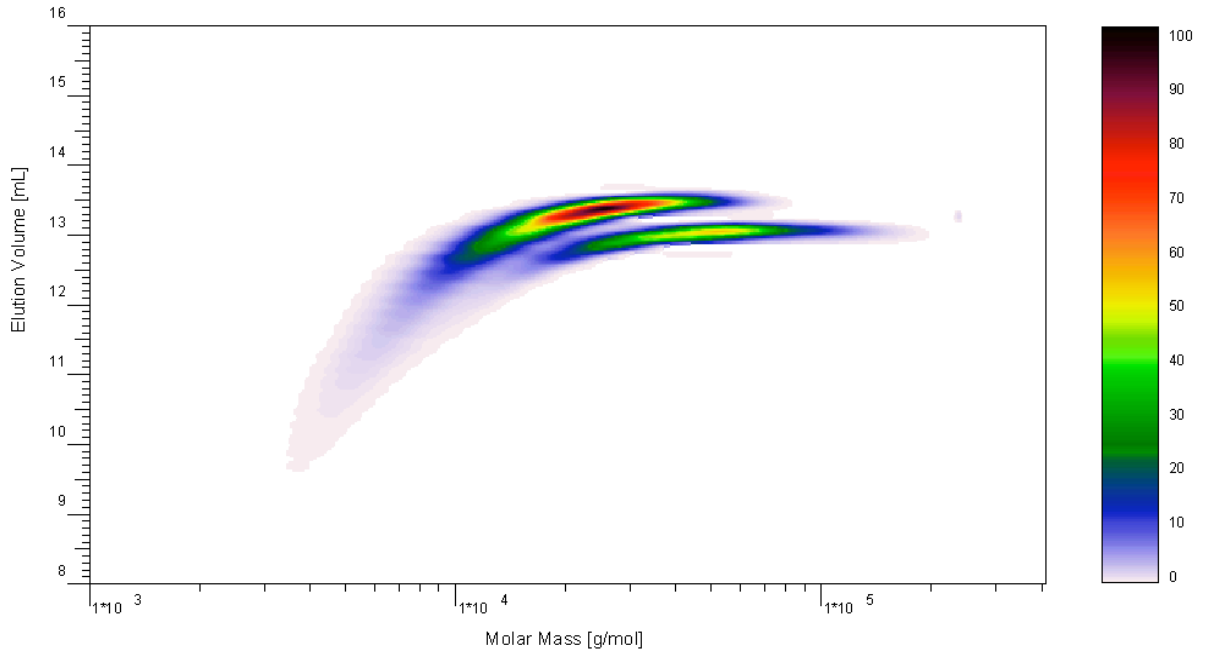


Figure 4.13: 2D-LC contour plot of a blend of DB-0 and DB-18. 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

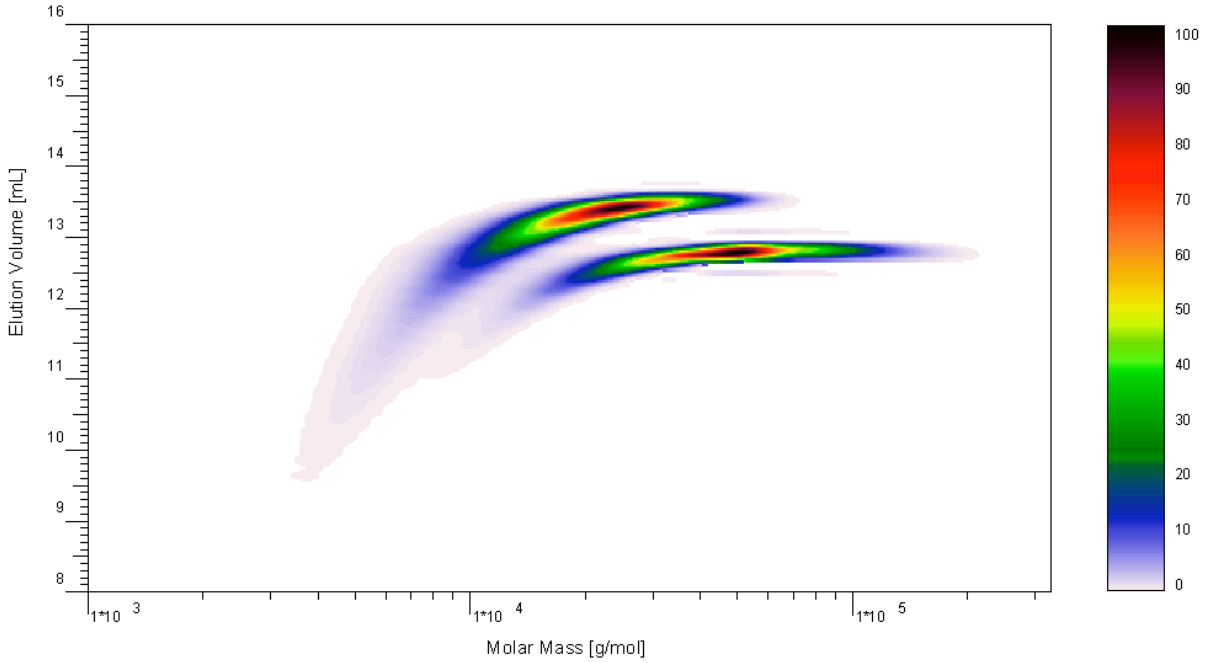


Figure 4.14: 2D-LC contour plot of a blend of DB-0 and DB-38, 1st Dimension: Gradient given in tab 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

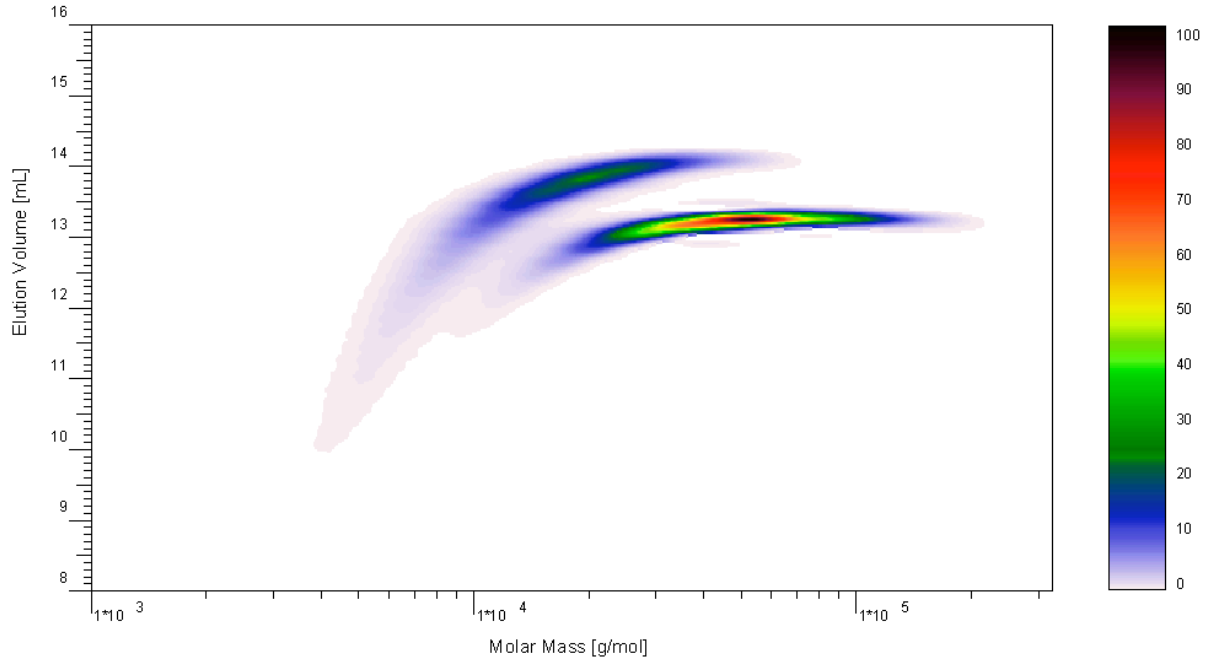


Figure 4.15: 2D-LC contour plot of a blend of DB-0 and DB-50, 1st Dimension: Gradient at given in 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

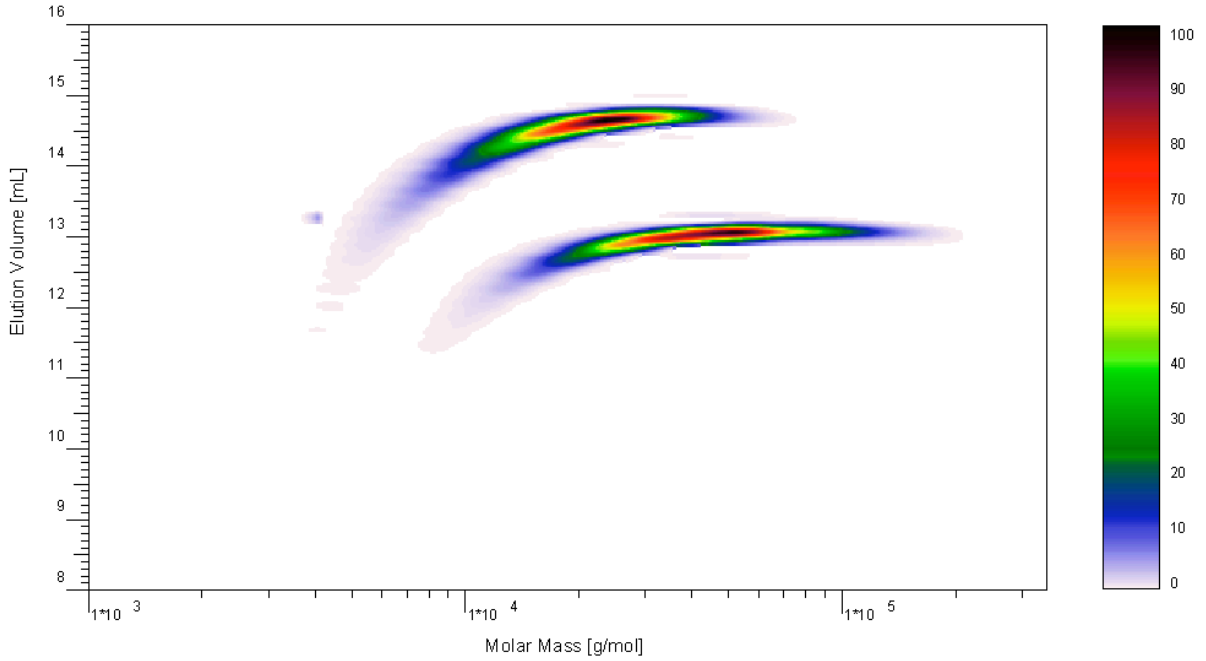


Figure 4.16: 2D-LC contour plot of a blend of DB-0 and DB-100, 1st Dimension: Gradient given in table 4.1 at 0.025 mL/min on Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D.); 2nd Dimension: SEC in THF at 2.0 mL/min on PSS SDV, 5µm, 10^5 Å, 30 x 8cm and PSS SDV, 5µm, 10^6 Å, 30 x 0.8 cm; Detection: ELSD.

In all contour plots clearly two peaks can be distinguished. The first peak at lower gradient elution volume represents the linear polyester, while the second peak at higher elution volume is due to the branched polyester. Interestingly the peak for the cyclic product that was observed in the 2D-plot of the pure sample DB-0, could not be observed when the linear species was mixed with the branched one. However, this phenomenon that can probably be explained by the low concentration of the cyclic species and was not further investigated.
A direct comparison of all contour plots reveals the following trends.

- The higher the DB of the branched polymer the better is the separation of the two peaks. Therefore, a separation can more easily be performed on polymer blends that differ strongly in DB.
- For lower molar masses, the separation efficiency between linear and branched samples is low. However, with increasing molar mass, the difference in elution volume increases. This should allow the separation of linear and branched polymer samples in the high molar mass region, while in the low molar mass region, the separation becomes less effective. The exception is the mixture of DB – 0 and DB – 100, where a separation can be realized for both, the low and the high molar mass region.

From the previous results, it can be concluded that 2D chromatography is a reliable and quick method allowing the complete separation of mixtures of linear and branched polyesters, provided the molar masses of both species exceed approximate 20.000 – 40.000 g/mol, depending on the difference in DB. However, for molar masses below 20.000 g/mol the loss in separation efficiency causes a dramatic decrease in the resolution between the two peaks, making a separation less effective. The reason for this phenomenon will be discussed in detail in one of the following sections (4.2.4).

One way to increase the resolution in the low molar mass region between the linear and the branched polymer sample is by using a set of high resolution SEC columns designed for low molecular weights. These columns are therefore a must for a successful and complete separation in the low molar mass region.

In the next section, the possibilities to quantify the amount of linear and branched species in a blend will be discussed.

4.2.3 Quantification of the Amount of Branched Material in Blends of Linear and Branched Polyesters

The previous section has shown, that 2D chromatography allows a reasonable separation of linear and branched polymer species in blends of both components at least at higher molar masses. Consequently, it should be possible to quantify the amount of the branched species in a blend of linear and branched species. In order to test this hypothesis, blends of linear and hyperbranched polyesters having well defined and known compositions (see table 4.2) were prepared and separated by online 2D chromatography. The amount of each species was calculated from the relative peak volume of the hyperbranched polymer fraction. The corresponding contour plots for the different blends as well as the integration limits for the linear and branched fractions are shown in figure 4.17a, figure 4.17b and figure 4.17c.

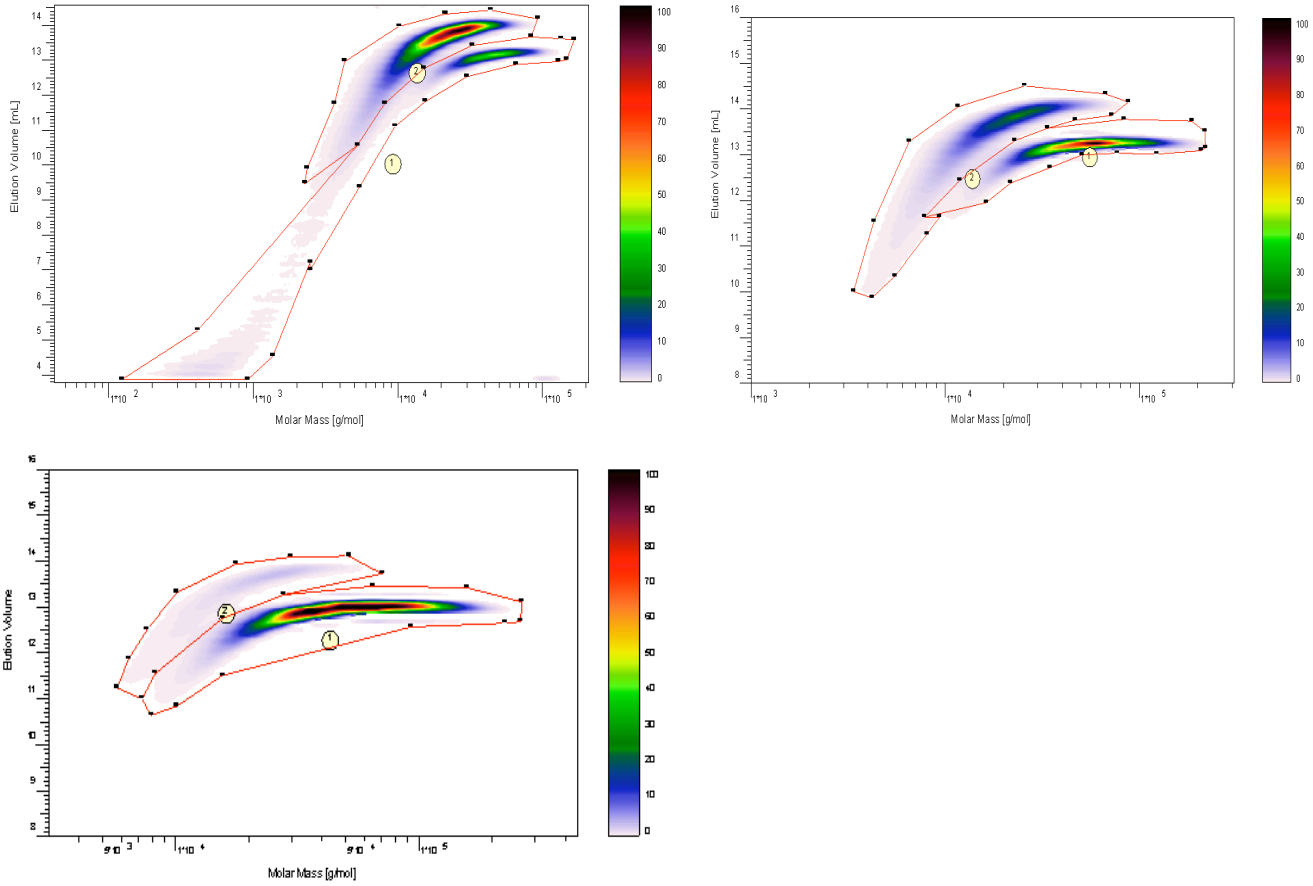


Figure 4.17a (upper left), 4.17b (upper right) and 4.17c (bottom): 2D contour plots and integration limits for blends of linear and hyperbranched polyesters.

As expected, two peaks are observed in all contour plots, with the peak at lower elution volumes (y-axis) corresponding to DB-0 (linear), while the second peak at higher elution volume is assigned to due to DB-50.

In figure 4.17a, the cyclic structure can be noticed in the contour plot at lower elution volumes, which vanishes when the amount of the linear species was increased. However, the reason for this phenomenon was not further investigated within this PhD thesis, as merely the low molar mass region was affected by the cyclic structure.

A comparison of the three figures shows, that in the lower molar mass region the peaks overlap, while for higher molar masses a good resolution between the linear and branched polyester is obtained. A good agreement between the composition of linear and branched polymer sample that were weighted in (see table 4.2) and the corresponding peak intensities can be noticed in all contour plots. In the first contour plot (figure 4.17a), the blend was composed of 22 % (wt) DB-0 and 78 % (wt) DB-50. Therefore, the peak representing the linear fractions (lower gradient elution volume) is less visible, while the spot representing the hyperbranched fraction shows a higher intensity. The opposite behaviour can be seen in figure

4.17c, where a blend composition of 84.73 % DB-0 and 15.27 % DB-50 was used. In figure 4.17b, the intensity of both peaks is similar, since equal amounts of linear and hyperbranched sample were used.

Next, quantification of the relative amount was performed assuming the relative peak volumes to represent the relative concentrations. A comparison of the true and experimentally obtained amount of the branched polymer fractions is given in table 4.2.

Table 4.2: Comparison of the true and the experimentally determined compositions for blends of linear and hyperbranched polyesters.

	DB-0, true **[%]**	DB-50, true **[%]**	DB-0, exp. **[%]**	DB-50, exp. **[%]**
Figure 4.17a	21.98	78.02	22	76
Figure 4.17b	48.73	51.27	39	58
Figure 4.17c	84.73	15.27	94	5

For the first composition (figure 12a), a very good agreement between the experimental results and the true composition was achieved with only a very slight deviation. For the other two artificial blends, the deviation between the values was stronger. The origin of the deviation is probably due to the following reasons:

- The quantification of the fractions was performed using an ELS-detector, which is known to be non-linear in concentration. That means, that ELSD sensitivity is dependent on a large number of factors, such as e.g. analyte structure (size, architecture and molar mass) and mobile phase composition. In case of the present 2D-LC system, the detector evaporates always the same mobile phase, i.e. THF, as it is placed in the second dimension (SEC) separation. However the separated peaks obtained by 2D-LC are heterogeneous in molar mass and architecture. A topology or molar mass dependence of the ELSD response might cause this deviation. The results of the quantification must hence be taken carefully. A calibration of the detector with suitable references has to be conducted in order to properly quantify all present species. However, no other detection method such as an UV detector or RI detector can be used due to the experimental conditions during a online 2D measurement, making ELS detection the only suitable detection method.
- As previous mentioned, the separation in the low molar mass region is not very efficient, due to the low number of branches at low molar masses. Therefore the linear and hyperbranched fractions partially overlap in the 2D chromatogram. One way to

overcome this problem might be by deconvolution of the individual SEC chromatograms.

- The integration limits (as shown in figure 4.17a, 4.17b and 4.17c), which have to be set for the quantification of the linear and the branched species slightly, differ from each other in the figures. Since the integration limits cannot automatically be set by the software and have to be set by the user in the 2D software, a small variation occurs. This results in slightly different integration limits for the different blends, which influence the final amounts for the quantification.

Even if the quantification for the individual species is not perfect, 2D-chromatography yields at least reliable estimates for the composition of blends of linear and branched species. In addition, to the author's knowledge no other technique currently exits, which allows the separation and quantification of linear and branched polyesters.

In summary, it was shown that 2D chromatography allows the separation and quantification of linear and hyperbranched polyesters in the high molar mass region. In the low molar mass region, the difference in elution volumes between the linear and the hyperbranched polyester vanishes, making a complete separation impossible. The reason for this will be investigated within the next section.

4.2.4 Resolution in the Low Molar Mass Region

In the previous section it was shown that online 2D chromatography allows the separation of linear and hyperbranched polymer sample in the high molar mass region but not for lower molar masses. Therefore, it can be assumed that the hydrodynamic volume of the branched polyesters approaches the hydrodynamic volume of the linear polyester as the molar mass decreases.

One possible explanation might be a variation in DB as a function of molar mass for the branched samples, resulting in a lower DB for lower molar masses and a higher DB at the high molar mass end of the chromatogram. If this assumption would be valid, the hyperbranched polymer of average DB of 50 % should have a DB slightly above 50 % for higher molar masses, while a DB lower than 50% should be found in the low molar mass region. Then, the hydrodynamic volume in solution of the hyperbranched polymer should approach the size of the linear one in the low molar mass region. Such a variation of DB

could not be identified using standard NMR characterization, since NMR only provides an average DB of the whole polymer sample.

Consequently, the hyperbranched polyester DB - 50 was fractionated by repeated SEC runs on an analytical column set. Afterwards, the percentages of linear, branched and terminal structural units were determined by ^{1}H-NMR and the DB was calculated for each fraction. The results are given in Table 4.3.

Table 4.3: Calculated DB according to Fréchet and Frey for several low molar mass SEC-fractions determined by ^{1}H-NMR.

Fraction no.	Dendritic units [%]	Linear units [%]	Terminal units [%]	$DB_{Fréchet}$ [%]	DB_{Frey} [%]
SEC Fraction No. 14	24.69	43.86	31.45	56	53
SEC Fraction No. 13	18.85	53.28	27.86	47	41
SEC Fraction No. 12	25.92	47.64	26.44	52	52
SEC Fraction No. 11	27.52	46.97	25.50	53	54
SEC Fraction No. 10	28.51	43.01	28.47	57	57
SEC Fraction No. 9	27.35	42.74	29.91	57	56
DB-50 (non fractionated)	23.05	48.95	28	51	49

The results in table 4.3 show no strong and especially no systematic variation of degree of branching with the fraction number, i.e. with decreasing molar mass. This is in contrast to the work of Falko[108] who observed a slight increase in DB with molar mass. This discrepancy might arise from differences in the fractionation method. Falko et al. used an elution fractionation, which is based on differences in solubility, while the separation in SEC is based on differences in the hydrodynamic volume.

However, despite the slight discrepancies in results, it becomes clear that the slight variation in DB with molar mass cannot explain the lack of separation efficiency in the low molar mass region.

Another possible explanation for the insufficient separation in the low molar mass region might be that even for a constant DB the reduction of hydrodynamic size of the branched molecule of a given molar mass depends not only on DB, but on molar mass as well.

The correlation of molar mass, number of branched and size reduction can be estimated based on the fundamental theoretical work of Zimm and Stockmeyer. They developed equations

relating the ratio of the mean square radii of the branched molecule to the one of the corresponding linear polymer of the same molar mass for different branched architectures, e.g. stars, or statistically branched polymers. For statistically branched polymers, the radius of gyration was found to decrease with the number of branched units in the polymer for a given molar mass. Since the radius of gyration is directly correlated with the hydrodynamic volume, which is the basis of separation in SEC, branched polymers with similar radius of gyration cannot be separated from each other in SEC. As for a given DB the number of branched units decreases with decreasing molar mass, the separation of linear and branched structures in SEC is less pronounced for lower molar masses.

This will be become clearer from table 4.4, where a low molar mass hyperbranched polyester with a molar mass of approximately 9.000 Da will be compared with a high molar mass hyperbranched polyester with a molar mass of approximately 80.000 Da.

Table 4.4: Calculation of the contraction factor *g* for DB-50 in the low and high molar mass region.

DB-50, Molar mass: ~9.000 Da	DB-50, Molar mass: ~80.000 Da
• **Monomer unit:** 382 g/mol	• **Monomer unit:** 382 g/mol
→ Degree of Polymerization: ~23	→ Degree of Polymerization: ~200
• **Degree of Branching:** 50 %	• **Degree of Branching:** 50 %
→ 6 terminal units → 5 branched units → 12 linear units	→ 51 terminal units → 50 branched units → 102 linear units
• **Functionality:** 3	• **Functionality:** 3
→ $g = 0.698$	→ $g = 0.318$
$g = \frac{\langle R^2_{br} \rangle}{\langle R^2_{lin} \rangle}$ → $\underline{R_{br} = 0.834 \cdot R_{lin}}$	$g = \frac{\langle R^2_{br} \rangle}{\langle R^2_{lin} \rangle}$ → $\underline{R_{br} = 0.56 \cdot R_{lin}}$

A hyperbranched polyester with a molar mass around 9.000 Da and a DB of 50 % consists of 6 terminal units, 5 branched units and 12 linear units. As a functionality of three is assumed

for an AB_2 – polymer, a contraction factor g of 0.698 is calculated for the randomly branched polymer based on the Zimm and Stockmayer theory. From the definition of g it follows that the branched polyester has approximately 83 % the size (radius of gyration) of the corresponding linear polymer.

The same calculation for hyperbranched polyester with a molar mass of approximately 80.000 g/mol and DB= 50% results in a g-value of 0.318, indicating that hyperbranched polyester has only about half the size (56 %) the size the corresponding linear analogue of the same molar mass.

The above calculations show that the absolute number of branch points and not the DB is mainly responsible for the size reduction of the branched polymer. For a fixed DB, the number of branch points decreases with decreasing molar mass and consequently the size reduction as well. Since for a given molar mass the difference in size of the branched and linear polyester defines the separation in the SEC run, the performed calculations provide a suitable explanation, why the separation between linear and branched polymer fails in the low molar mass region.

In order to overcome this general problem, a high resolution SEC column set is required for the low molar mass region. Another way to separate linear and branched molar mass in the low molar mass region could possibly be achieved using a different separation mechanism instead of SEC.

In the next section, the analysed polymer samples will be used to construct a calibration curve, which might allow the determination of the DB of an unknown polymer sample. More importantly, it will allow the determination of the DB distribution, which might be used to describe the dispersity in topology of a branched polymer sample.

4.2.5 Construction of a 2D Chromatographic Calibration

The previous section has shown that the peak position in the first dimension in the 2D-experiment depends on both DB and molar mass. In order to be able to determine the DB and the DB distribution of a polymer sample from the two-dimensional chromatograms, a suitable calibration is required.

In figures 4.8 – 4.12, it can be noticed that all samples elute as curved peaks in the contour plots. This curvature is due to the dependence of elution volume on molar mass in gradient chromatography (first dimension). This dependence decreases with increasing molar mass,

resulting in a DB dependent limiting value for the elution volume, which will be called limiting volume V_{lim}. The limiting volume can be determined by plotting the elution volume of the first dimension as a function of the molar mass relative to polystyrene as shown in figure 4.18.

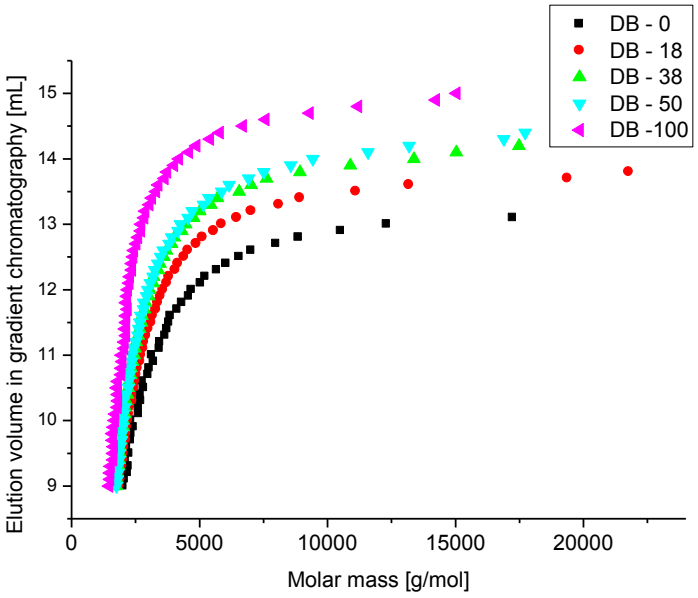


Figure 4.18: Plot of molar mass against gradient elution volume.

Figure 4.18 clearly shows that the limiting volume depends on the DB of the polymer sample. The higher the DB of the polymer sample, the higher is the limiting volume. The dependence of elution volume on (polystyrene equivalent) molar mass might be approximated by the following equation:

$$V_{Gra} = V_{\lim} * [1 - \exp(-(M) * a)] \qquad 4\text{-}1$$

where V_{Gra} corresponds to the gradient elution volume, V_{lim} corresponds to the limiting elution volume at high molar masses and the parameter a describes the curvature.

In the following plot (figure 4.19), equation 4-1 was fitted to the experimental data in order to determine the limiting values V_{lim} and the parameters a. In order to ease the subsequent calculations, the parameter a was assumed to be independent of DB and thus, identical for all samples.

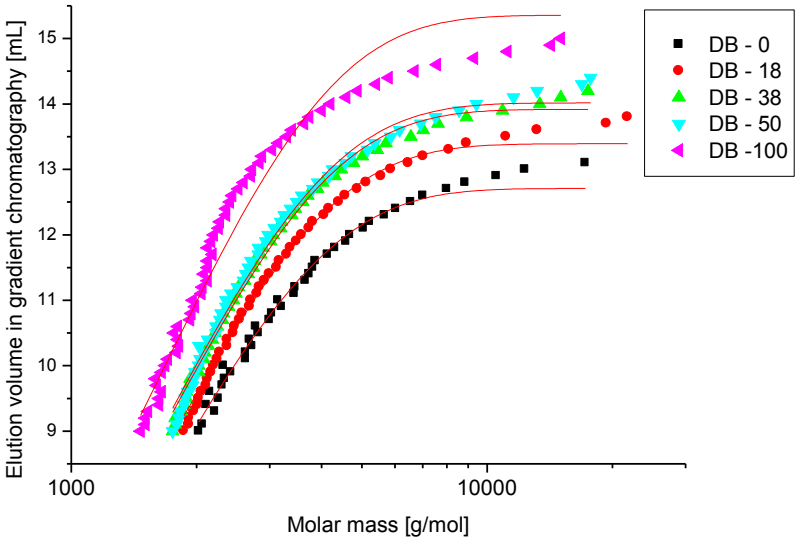


Figure 4.19: Determination of the limiting value and the parameter *a*. The parameter *a* was kept identical for all samples.

Figure 4.19 show that a reasonable agreement exists between the fitted curves and the experimental data. The obtained values for the parameter a and the limiting volumes V_{lim} are listed in table 4.5.

Table 4.5: Results for the limiting volume V_{lim} and the parameter *a* as determined by non-linear curve fitting of equation 4.1.

Sample	Limiting volume V_{lim}	Parameter *a* (kept identical for all samples)	Regression coefficient R^2
DB – 0	12.70	0.000627	0.9883
DB – 18	13.39		0.9931
DB – 38	13.92		0.9936
DB – 50	14.01		0.9901
DB -100	15.35		0.9614

In the following figure, the dependence of V_{limit} is plotted against DB.

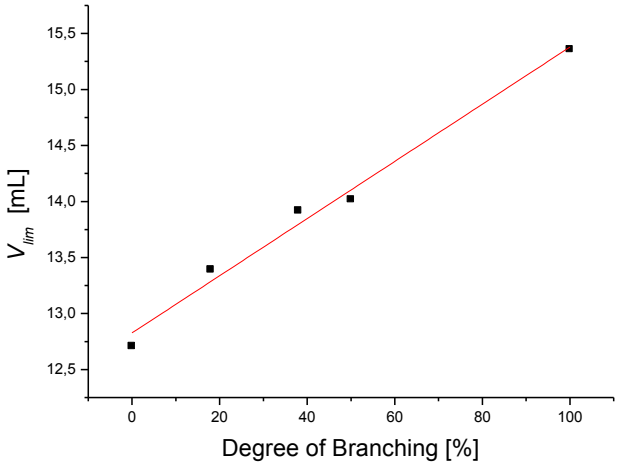


Figure 4.20: Plot of V_{lim} versus DB. The data points can be approximated to the following equation: $y = 12.82 + 0.0252 * DB$.

A nearly linear dependence of V_{lim} on DB is observed, which can be approximated by:

$$V_{lim} = 12.82 + 0.0252 * DB \qquad 4\text{-}2$$

Combining Equation 4-1 and 4-2 results in:

$$DB = \frac{\frac{V_{Gra}}{1 - \exp(-(M) * 0{,}000627)} - 12.82}{0.0255} \qquad 4\text{-}3$$

This equation allows to estimate the DB for each fraction in the two dimensional chromatogram from the elution volume of the first dimension V_{Gra} and the elution volume of the SEC, via the PS calibration curve. Knowing the DB for each fraction it is possible to calculate the DB distribution and thereof averages of DB.

In the next section, first the validity of the previous approach will be investigated for the calculation of the DB of an unknown sample and consequently compared to the results from NMR spectroscopy.

4.2.6 Determination of the Degree of Branching of an Unknown Sample

In the previous section, an approach for the determination of the DB and the DB distribution from the elution volumes of a 2D experiment was derived. In this section, this approach will be used to determine the DB of an unknown sample and consequently the results will be

compared to results determined by NMR spectroscopy. In case that the validity of the previous approach is approved, a small adjustment will allow the determination of the DB distribution, which can be correlated with the extend of the topological heterogeneity of a branched polymer sample.

In Figure 4.21, the contour plot of a branched polyester with an unknown DB is shown. The only known information about the sample was that it was fractionated several times, so that a narrow distributed (in molar mass and topology) polyester sample is expected.

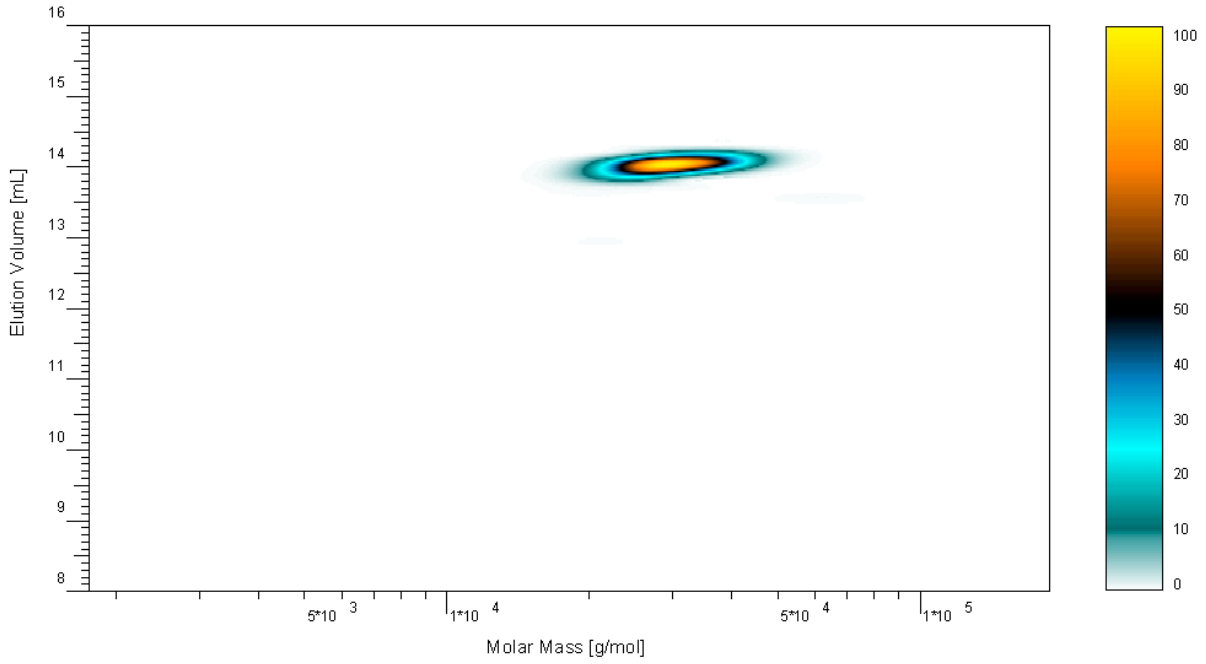


Figure 4.21: 2D-LC contour plot of a branched polyester DB-X with unknown DB, 1st Dimension: Gradient at 0.025 mL/min on Nucleosil 300-5 C18; 2nd Dimension: SEC with THF at 2.0 mL/min on two PSS SDV 10^5 Å and PSS SDV 10^6 Å; Detection: ELSD.

As previous expected, the sample DB-X is represented by a narrow peak in the contour plot. From the peak width, it can be assumed that the sample is narrowly distributed in molar mass and DB. Since the spot does not show the typical curved elution behavior like the other polyester samples, it can be assumed that the sample is either located in the higher molar mass range, where no molar mass dependence on elution volume exist or that the sample consist of a very low heterogeneity in molar mass.

In order to calculate the average DB of the unknown polymer sample, first the average DB_i of each individual gradient fraction has to be calculated. Therefore, equation 4-3 can be used to determine DB_j of each individual SEC slice of the gradient fraction (i.e. for each position in the two dimensional chromatogram). Subsequently, equation 4-4 can be used to calculate the weight average DB_i from the individual DB_j as follows:

$$DB_i = \frac{\sum DB_j * c_j}{\sum c_j}, \qquad 4\text{-}4$$

where c_j corresponds to the signal intensity of the individual SEC slice *j*, for the fractions of gradient chromatography. It has to be mentioned that due to the mathematical approach of equation 4-3 and the experimental error, physically meaningless results for DB_j below 0 and above 100 were received. However, for the calculation of DB_i, only the SEC slices were used, which delivered a DB_j between 0 and 100. All other SEC slices were discarded.

The results of DB_i for the different gradient elution volumes are given in table 4.6. In addition, the values for the peak intensities of the different gradient fractions, i.e. the total peak area of the corresponding SEC-chromatograms are also given.

Table 4.6: Calculated DB_i and peak intensities for different gradient elution volumes in the first dimension.

Gradient elution volume (first dimension)	DB_i	Peak Intensity $c_i = \sum_j c_{ij}$
13.7 – 13.8 mL	35.4	0.08289
13.8 – 13.9 mL	41.4	0.08643
13.9 – 14.0 mL	43.7	0.4426
14.0 – 14.1 mL	47.2	2.55064
14.1 – 14.2 mL	50.8	8.78677
14.2 – 14.3 mL	54.7	12.78781
14.3 – 14.4 mL	58.6	5.76522
14.4 – 14.5 mL	62.5	0.81434
14.5 – 14.6 mL	66.5	0.62153

A variation in DB_i with increasing gradient elution volumes can be identified from table 4.6. The main peak intensity is located at a gradient elution volume between 14.1 – 14.2 mL and 14.2 – 14.3 mL, which corresponds to a DB_i of 50.8 and 54.7 respectively.

In order to calculate the weight average DB_W of the whole sample, equation 4-5 can be used:

$$DB_W = \frac{\sum DB_i * c_i}{\sum c_i} \qquad 4\text{-}5$$

where c_i corresponds to the peak intensity at different gradient elution volumes. By the use of equation 4-5, a DB of 53.5 could be determined for the polymer sample DB-X. In order to test

the accuracy of the chromatographic approach, ^{1}H-NMR-spectroscopy was performed on the sample to determine the DB of DB-X. Table 4.7 compares the DB-values determined by the different approaches for the sample of unknown DB.

Table 4.7: Comparison of the DB obtained online 2D-chromatography and ^{1}H-NMR.

	2D liquid chromatographic method	^{1}H-NMR
Calculated DB	53.5	47.3

A very reasonable agreement between the DB determined by the introduced 2D liquid chromatographically method and the spectroscopic method can be observed. The DB determined by 2D liquid chromatography is slightly higher than the DB determined by ^{1}H-NMR. Possible reasons for this discrepancy are:

1. The detection of the different branched polyester was performed using an ELS-detector. However, since ELSD sensitivity is dependent on a large number of factors such as topology, molar mass, concentration and mobile phase composition, the values for the signal intensity of each slice and the peak intensity must be taken carefully. Another detector, which shows no dependence of molar mass, topology, etc. in the response factor should be more accurate for the determination of the DB.
2. For the calculation of DB_i only values of DB_j were used, which resulted in physically meaningful values between 0 and 100. Other SEC slices were discarded from the calculation of DB_i . However, even when the signal intensity of these neglected values was low, they have an impact on DB_W of the whole sample, which was not considered in the calculation.
3. As previous mentioned, the spectroscopic determination of the DB was performed by ^{1}H-NMR. In literature [106,108] the determination is suggested to be performed by quantitative ^{13}C-NMR in order to obtain more precise results. Both spectroscopic techniques reveal three peaks for linear, branched and terminal units, which however slightly overlap with each other in ^{1}H-NMR. Consequently, a deconvolution of the three peaks was necessary which decreases the accuracy of the method. For a more precise determination of the DB, ^{13}C-NMR has to be used on the sample. However, due to the small amount of sample that was available, no reliable, quantitative ^{13}C-NMR measurements could be performed.

The previous example showed that the developed method is able to determine the DB of an unknown sample with reliable results as compared to other spectroscopic methods.
The advantages of the introduced method are that no expensive equipment (such as NMR spectroscopy) is necessary, which require also special trained personal to run the instrument and to interpret the data. In addition, in contrast to NMR spectroscopy which delivers only average values for the DB, the previous approach should be able to determine the extend of the topological heterogeneity of a branched polymer sample.
In order to test this hypothesis, the dispersity in DB between a homopolymer and a polymer blend will be compared with each other in the next section.

4.2.7 Quantification of the Extend of Topological Heterogeneity

In the previous section, a method for the determination of the DB of an unknown polyester sample was successfully presented. In this section, a mathematical approach for characterizing and quantifying the degree of topological heterogeneity will be introduced. The standard deviation of the DB with respect to its weight average value will be used to quantify the topological dispersity of the sample. A high value in the standard deviation correlates to a high dispersity in topology. Consequently, a single not blended homopolymer is supposed to have a lower value for the standard deviation as compared to a polymer blend made of several of samples differing in DB.
In order to test this hypothesis, the standard deviation of the partially branched polyester sample DB-38 will be compared to a blend composed of almost equal amounts of DB – 0, DB – 18, DB – 38 and DB – 50. The standard deviation of the DB can be calculated according to:

$$\sigma = \sqrt{\frac{\sum_{i}^{GRA}\sum_{j}^{SEC} c_{ij} * \left[DB_{ij} - DB_W\right]^2}{\sum_{j}^{GRA}\sum_{i}^{SEC} c_{ij}}} \qquad 4\text{-}6$$

where DB_W corresponds to the weight average DB of the whole sample calculated according to equation 4-4 and equation 4-5.
As previously mentioned, only the SEC slices were used in the second dimension, which resulted in a DB_i between 0 and 100. All other SEC slices were not used for the calculation of

DB_i. In addition, for the determination of the DB and the standard variation only every 5th fraction was considered from the first dimension. For more precise results, every fraction has to be considered in the calculation. However, since we were only interested to prove the concept of this approach, only every fifth fraction was used for the calculation of DB_W and σ. The results of DB_W and σ are listed in table 4.8.

Table 4.8: Comparison of the DB and the standard deviation for DB-38 and a polymer blend. For the calculations of the polymer blend, equal amounts of the compositions were assumed.

	DB – 38	**Polymer blend**
DB_W by ^{13}C-NMR spectroscopy as determined by the IPF Dresden	38	not measured
DB_W as determined by 2D chromatography	43	36
Calculated σ as determined by 2D chromatography	12	22

For the partially branched homopolymer sample DB-38, a good correlation between the DB determined by NMR-spectroscopy and our two-dimensional approach can be noticed. For the polymer blend, which is composed of DB-0, DB – 18, DB- 38 and DB – 50, a DB of 36 was calculated from the chromatogram. This value is significantly higher than expected, since a value of 26.5 is expected if all components are present in the same amount. However, the slightly higher value in DB can be traced back to higher amounts of higher branched samples in the polymer blend. For the standard deviation, which is supposed to be an index for the dispersity in DB of the sample, a plot as shown in figure 4.22 can be set up.

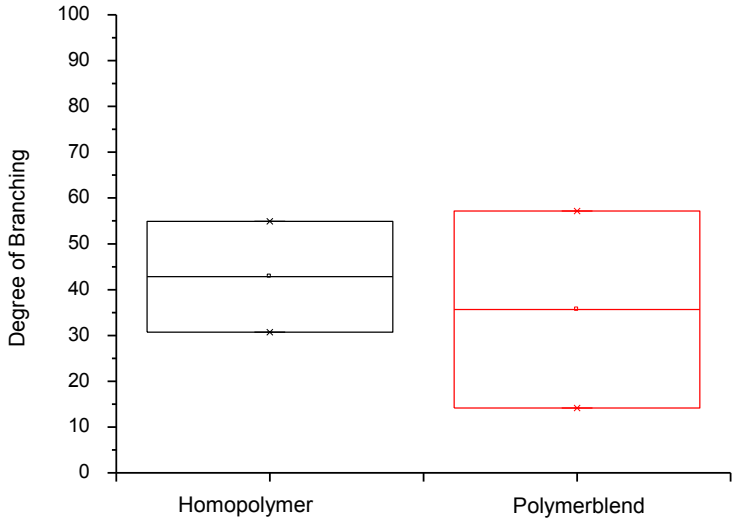


Figure 4.22: Plot of the results in DB_W and σ for DB-38 and the polymer blend. In the box, the calculated DB_W is represented by the line in the middle, while the upper and lower lines belong to $DB_W \pm \sigma$.

The plot in figure 4.22 shows an approximately two times bigger box for the polymer blend as compared to the not blended homopolymer. Therefore, a two times higher topological heterogeneity is expected for the polymer blend as compared to the not blended homopolymer. This behaviour was previously expected, since the homopolymer is composed of only one polymer sample, while the polymer blend is composed of four polymer samples. Consequently, the polymer blend contains more different topologies, resulting in a higher value for the standard deviation as compared to the homopolymer.

The previous calculation showed, that the calculation of DB of an unknown polymer sample after calibration with appropriate calibration standards with our two-dimensional approach is possible in principle. More interestingly, for the first time in literature, an informative value for the DB distribution can be determined for polymer samples and compared to each other in order to have an index for the topological heterogeneity of the polymer sample. The knowledge of the detailed topological characteristics and their effect on functional properties will ultimately allow the design of new tailor made high-performance polymers.

5 Experimental Part

a) Chromatographic Equipment:

High Performance Liquid Chromatography (HPLC) measurements were performed using an Agilent 1100 series HPLC system (Agilent Technologies GmbH, Boeblingen, Germany) consisting of a vacuum degasser (G1322A), quaternary pump (G1311A), auto-sampler (G1313A), column oven (G1316A) and variable wavelength UV-detector (G1314A). In addition an evaporative light scattering detector (ELS 1000, Polymer Laboratories Inc. Church Stretton, England) was used. The following operating parameters were applied for the ELSD: Gas flow 1.4 L/min, nebulizer temperature 80°C, evaporator temperature 120°C.

For the SEC – Multi Angle Laser Light Scattering (MALLS) experiments a system composed of a Waters 515 pump (Waters, Milford, USA), a TSP AS100 auto sampler, a Waters column oven, a Waters 486 UV detector operated at 254 nm, a Waters 410 RI-detector and a DAWN DSP light scattering detector (Wyatt Technology, Santa Barbara, USA) was used.

Data collection and processing for SEC and HPLC were performed using PSS WinGPC version 7 software (Polymer Standards Service, Mainz, Germany). For data acquisition and evaluation of SEC-MALLS experiments Astra version 4.73 (Wyatt Technology, Santa Barbara, USA) was used. The MALLS instrument was calibrated using pure toluene assuming a Rayleigh ratio of $9.78 \cdot 10^{-6}$ cm^{-1} at 690 nm. The refractive index increment (was determined for all samples using the Waters RI detector and assuming 100 % mass recovery. Subsequently an average value for the refractive index increment of 0.16 cm^3/g was assumed for all polyester samples. No correction was applied to correct the refractive index increment for the different wavelengths of RI and MALLS instrument.

For the online 2D setup, the detector in the standard HPLC setup was replaced by an eight port two position transfer valve (VICI Valco, Swizerland), which acted as the injector for the second dimension (SEC). The valve was equipped with two 100 µL sample loops. Care was taken to assure that both loops had identical volume. The second dimension consisted of a Shimadzu LC-10ATvp pump). For detection, an evaporative light scattering detector (ELS 1000, Polymer Laboratories Inc. Church Stretton, England) was used with the same experimental parameter as for the HPLC measurements. Data collection and processing for 2D chromatography was performed using PSS WinGPC version 7 software (Polymer Standards Service, Mainz, Germany)

b) Chromatographic Conditions:

The injected sample volume for gradient chromatography and LC-CC analysis was 10 µL, sample concentrations were 1–2 g/L, column temperature was 25 °C and the flow rate was set to 1 mL/min unless mentioned otherwise. The experiments were performed on a single Nucleosil C18 columns (5µm, 300 Å, 250 x 4.6 mm I.D., Macherey–Nagel, Düren, Germany).

For the SEC and SEC-MALLS analysis an injection volume of 118 µL, a sample concentration of 1-2 g/L, a column temperature of 35 °C and a flow rate of 1 mL/min THF was used, unless mentioned otherwise. SEC-MALLS analysis was performed on a high resolution column set bought from Polymer Standards Service GmbH, Mainz, Germany (SDV 5µm 10^6 Å, SDV 5µm 10^5 Å, SDV 5µm 1000 Å, 300 x 8 mm I.D.). SEC fractionation was performed on a single SEC column (SDV 5µm 10^5 Å, 300 x 8 mm I.D.,) of the same manufacturer.

For the online 2D setup (HPLC x SEC) a Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D., Macherey–Nagel, Düren, Germany) was used in the first dimension and a set of two SEC columns (PSS SDV 5µm 10^6 Å and PSS SDV 5µm 10^5 Å) in the second dimension. An injection volume of 100 µL, a concentration of 10-12 g/L, a column temperature of 25 °C and a flow rate of 0.025 mL/min in the first dimension and 2 mL / min in the second dimension were applied.

For the offline 2D chromatographically setup (SEC x HPLC), a set of two SEC columns (PSS SDV 5µm 10^6 Å and PSS SDV 5µm 10^5 Å) was used in the first dimension and a Nucleosil C18 column (5µm, 300 Å, 250 x 4.6 mm I.D., Macherey–Nagel, Düren, Germany) in the second dimension. An injection volume of 100 µL, a concentration of 10-12 g/L, a column temperature of 25 °C and a flow rate of 1 mL/min in the first dimension and second dimension were used.

c) Solvents:

Acetone and methanol (VWR, Leuven, Belgium) were of HPLC grade and used as received.

Tetrahydrofuran (THF) was refluxed and distilled over calciumhydrid (CaH_2).

d) Polymer Standards:

All narrow distributed polymer standards of polystyrene (PS) are synthesized and distributed by Polymer Standards Service GmbH (Mainz, Germany)

e) Linear and Branched Polyester Samples:

All samples were synthesized by Anna Khalyavina during her PHD work at the Leibniz Institut of Polymer Research Dresden. Details of the synthesis and the determination of the DB are given in [106].

f) Molar Mass Analysis by MALDI-ToF-MS:

The spectra were recorded on a Shimadzu Biotech Axima TOF^2 MALDI instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 3377 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight-path-linear, mass high (20 kV acceleration voltage), 100 – 150 pulses per spectrum.
The samples were dissolved in THF (4 mg/mL). The sample solutions were mixed with equal volume of THF solution (10 mg/mL THF) with the matrix (1,8,9 Trihydroxy-Anthracen). To enhance ion formation LiCl was added to the matrix. 0.5 – 1 µl of the resulting solution were placed on the MALDI target. After evaporation of the solvent the MALDI target was introduced into the spectrometer.

g) NMR:

The ^{1}H-NMR spectra were acquired using a Mercury-VX 400 spectrometer (Varian Inc., Palo Alto, USA) with a 5 mm nuc4-probe and an observing frequency of 400.11 MHz. Further parameters were as follow: 45° pulse, 32 k data points (corresponding to an acquisition time of 2.6 s at a spectral width of 6.4 kHz), relaxation delay of 5 s and a total of 128 scans. Fourier transformation was done after zero filling the data to 32 k time domain points and an exponential filtering of 0.3 Hz. Phase and baseline correction were done manually. The evaluation of the spectra was done by fitting the region of interest (1.8 ppm to 1.4 ppm) using the “dmfit programme” (D. Massiot, CRMHT-CNRS, France).

The samples were dissolved in deuterated chloroform ($CDCl_3$). The software from Varian (VNMRJ 1.1D) was used for data acquisition. Processing and spectra correction were carried out using the software Bruker Topspin 1.3.

6 Summary and Conclusions

The demand for new and innovative products with new or improved properties leads to the synthesis of a large variety of new complex polymer structures. One way to synthesize structures with new or improved properties is the introduction of branches. The introduction of branches results however in an another type of heterogeneity besides the most commonly known according to molar mass, making the characterization of these complex structures more complicated.

However, there is a need for the characterization of these complex structures, since the knowledge of the detailed topological characteristics and their effect on material properties will ultimately allow the design of new tailor made high-performance polymers. Thus, the necessity for new characterization methods grows. These methods should allow understanding the molecular structures of such polymer products in order to relate them to the observed macroscopic properties.

The aim of the present thesis was to develop new liquid chromatographic methods for the characterization of the degree of branching (DB) and DB distribution of branched polyesters in order to gain a better understanding on how branching influences the structure-property relationship of polymeric materials. Present analytical techniques for the characterization of linear and branched polyesters do not exist or are limited to yield only average values on e.g. the DB of the whole sample. However, for a detailed characterization of branched polymers, more information than the average DB has to be obtained. Therefore, the focus on the method development within this PhD thesis was on conventional and multidimensional liquid chromatographic techniques, which allow a more detailed characterization of linear and branched polymers.

By using different chromatographic techniques within this PhD thesis, the following results could be achieved:

1. Using SEC-MALLS it was shown that samples of different DB having a broad molar mass distribution elute at similar elution volumes in SEC, even if they exhibit different molar masses. This coelution of polymer species differing in molar mass and DB makes separations of linear and branched polyesters solely by SEC impossible. Further SEC – MALLS experiments confirmed that branched polyesters of different DB exhibit different calibration curves in SEC. The calibration curves of more highly branched samples are shifted to higher molar masses at identical SEC elution volume. The shift in the calibration curves reflects the

more compact structure of the branched molecules as compared to the linear analogue of identical molar mass.

2. Since no separation could be achieved by SEC, gradient chromatography was applied. A significant dependence of DB on retention volume for differently branched polyesters of identical molar mass was observed. An increase in DB causes an increase in gradient elution volume. Since linear and branched polyesters of a given molar mass are chemically identical, the reason for the dependence of DB on elution volume has to be related to topological effects. A possible explanation might be that the more compact structure of the branched molecules causes a higher density of functional groups at the periphery of the polymer coil enabling more repeating units to interact with the stationary phase. In addition, the higher density of functional groups on the peripheral surface enhances neighbourhood effects. Consequently for samples of identical DB but different in molar masses, the elution volume increases with molar mass. That means that elution in gradient chromatography is influenced by both factors, molar mass and DB. This makes a separation of samples that are heterogeneous in molar mass and DB by gradient chromatography alone impossible.

3. The observed dependence of elution volume on molar mass for samples differing in DB in gradient chromatography let to the assumption that also in other enthalpy-dominated chromatographic modes such as LC-CC and LAC, a dependence of elution volume on DB might be observed. Therefore, critical conditions were established for the linear polyester. To investigate the effect of DB on retention at the critical conditions of the linear polyester, the samples of different DB were fractionated to obtain fractions of identical DB but differing in molar mass. These fractions were injected at the critical conditions of the linear polyester. It was observed that linear and branched fractions of a given molar mass exhibit different elution volumes. While the linear polymer samples elute independent of molar mass, an increasing elution volume with increasing molar mass was observed for a constant DB. For very high molar masses, the enthalpic interactions between the stationary phase and the branched polymer molecules become so strong that the branched samples are not eluting at all from the stationary phase. Additionally for a given molar mass the elution volume increases with DB. These results indicate that retention in absorption chromatography and under critical conditions depend on the topology of the polymer.

4. In order to investigate the origin of the differences in retention behaviour of the linear and branched samples, temperature dependent gradient and isocratic experiments were performed. Hereby, it was observed that changes in temperature have a stronger influence on the retention volume for higher branched samples compared to the less branched ones. This is due to the fact that at a given molar mass, the enthalpic interactions between the stationary phase and the polymer sample are more pronounced for the higher branched samples than for lower branched ones. Since branched and linear molecules are made of identical chemical composition and therefore have the same interaction energy per monomeric unit, branched polymers must have more contacts with the stationary phase in total, due to the higher total enthalpic interaction energy between the stationary phase and the polymer molecule.
In addition, the influence of temperature on the elution of linear and branched polymers was used to quantify the enthalpic interactions for linear and branched polyester. Despite some scattering in the low molar mass region, the experiments proved that the enthalpic interactions between the polymer sample and the stationary phase increase with molar mass and DB. For branched samples identical in molar mass but differing in DB, an increase in the enthalpic interactions between the polymer and the stationary phase was confirmed with increasing DB.

5. Based on the information gained in the previous experiments, it could be concluded that conventional one-dimensional liquid chromatography is not prosperous to achieve a complete separation of linear and branched polymer samples heterogeneous in molar mass and DB. Therefore, the separation capabilities of two-dimensional chromatography were explored. Preliminary tests revealed that a combination of Size Exclusion Chromatography with gradient chromatography is best suited to separate linear and branched polymers both heterogeneous in molar mass and DB.
Offline 2D chromatographic experiments on the crude polymers and on a blend of linear and hyperbranched polymer samples showed that a separation of linear and branched polyesters is possible in principle. For high molar masses, the separation between the two structures is more pronounced than for lower molar masses and the separation results in two narrowly distributed peaks. Furthermore, the separation efficiency increases as the difference in DB between the two samples increases. In contrast, low molar mass polymer blends with a small difference in DB can not be separated by offline 2D chromatography, since both peaks elute at similar elution volumes.

6. Having shown by offline separations that two-dimensional chromatography is suited for the separation of linear and branched polymers, online experiments were performed in order to enhance the separation efficiency especially in the low molar mass region. From the online experiments on blends of linear and branched samples the following general trends were identified.

- The higher the DB of the branched polymer the better is the separation efficiency of the two peaks. Therefore, a separation can more easily be performed on polymer blends that differ strongly in DB.
- For lower molar masses the resolution is lower than for the higher molar mass region Therefore, baseline separations can be achieved only in the high molar mass region, while in the low molar mass region, the peaks for the branched and linear samples merge.

The limited resolution in the low molar mass region despite identical DB was explained based on theoretical works of Zimm and Stockmayer. For samples of identical DB the absolute number of branch points increases with increasing molar mass, resulting in a more pronounced reduction in hydrodynamic size as compared to the linear analogue of same molar mass. Consequently, for a given molar mass the difference in SEC elution volume decreases with decreasing molar mass

7. Online 2D chromatography was successfully applied to quantify the amount of the branched polymer in blends of a branched polymer of DB – 50 and the linear samples (DB – 0). Three artificial blends were prepared and analysed. It was found, that the quantification by online 2D chromatography gives reasonable results, which are in good correlation with the true composition of the artificial mixture. Even though the quantification for the individual species is not perfect, 2D chromatography results in reliable estimates for the amount of the linear and branched species in blends of both. To the best of the author's knowledge, no other technique currently exits allowing quantification of the amount of branched materials in such mixtures.

8. Finally, a strategy was developed, allowing the determination of the DB of an unknown polymer sample from the 2D chromatogram. The strategy consists of calibrating the 2D chromatographic system with respect to molar mass and DB. The performed calibration

allowed the determination of the DB of an unknown sample from the elution volumes of both chromatographic dimensions.

In order to evaluate the accuracy of the 2D liquid chromatographic approach, the DB of an unknown sample was determined and compared to the value obtained by ^{1}H-NMR-spectroscopic measurements. A good agreement between the DB determined from 2D liquid chromatography and by NMR spectroscopy was achieved.

In contrast to spectroscopic techniques, the 2D chromatographic approach allowed the determination of the standard deviation of the DB, which can be used as a measure for the topological heterogeneity of the sample. A large standard deviation correlates to a high dispersity in topology. This concept was successfully proven on a partially branched polyester sample and blends of samples having different DB. The blend revealed a much higher standard deviation as compared to the individual sample.

In conclusion, it has been demonstrated that two-dimensional liquid chromatography provides a new method for the characterization of branched polymers. It allows separating samples by DB and molar mass and therefore quantification of the amount of branched materials within a blend with the corresponding linear material. In contrast to existing methods, 2D chromatography allows quantification of the extend of topological heterogeneity of branched material. 2D chromatography provides a more detailed knowledge of the topological characteristics of a polymer sample and its effect on material properties. This will ultimately allow the design of new tailor-made branched high-performance polymers.

7 Zusammenfassung und Ausblick

Der Bedarf an innovativen Produkten mit neuen, verbesserten oder kombinierten Eigenschaften führt zur Entwicklung von immer komplexeren Polymerstrukturen. Eine Möglichkeit zur Synthese von Polymerstrukturen mit neuen oder verbesserten Eigenschaften liegt in der Einführung von Verzweigungen. Die Einführung von Verzweigungen führt zu einer weiteren Verteilung neben der immer vorhandenen Molmassenverteilung, wodurch die Charakterisierung dieser komplexen Polymeren weiter verkompliziert wird.
Neuartige und fortschrittliche Methoden aus dem Bereich der Polymercharakterisierung ermöglichen die Bestimmung der unterschiedlichen Polymerheterogenitäten in komplexen Polymeren. Jedoch sind im Bereich der Methodenentwicklung für die Charakterisierung verzweigter Polymere und deren Heterogenitäten kaum nennenswerte Erfolge vorzuweisen. Dies, obwohl der Bedarf an derartigen Charakterisierungstechniken für verzweigte Polymere stetig ansteigt. Für ein detailliertes Verständnis der molekularen Struktur und um in der Lage zu sein Struktur-Eigenschaftsbeziehungen aufzustellen, sind daher neue und bessere Charakterisierungsverfahren notwendig. Nur so können gezielt die notwendigen Parameter bei der Synthese variiert werden um letztendlich Produkte mit optimierten Eigenschaften zu erhalten.
Das Ziel der vorliegenden Arbeit war daher die Entwicklung von flüssigchromatographischen Methoden zur Trennung von verzweigten Polymeren aufgrund unterschiedlicher Verzweigungsgrade. Im ersten Teil der Arbeit lag der Schwerpunkt zunächst darin ein Verständnis über das Elutionsverhalten verzweigter Polymere in unterschiedlichen chromatographischen Modi zu gewinnen. Basierend auf diesen Kenntnissen wurde eine zweidimensionale flüssigchromatographische Trennmethode entwickelt, die eine Trennung von Polymeren unterschiedlicher Verzweigungsgrade ermöglicht.

Die Ergebnisse der vorliegenden Arbeit können wie folgt zusammengefasst werden:

1. Mithilfe der Größenausschlusschromatographie, gekoppelt mit einem Lichtstreudektor konnte gezeigt werden, dass unterschiedlich verzweigte Polymere mit voneinander abweichenden Molmassen bei ähnlichen Elutionsvolumina von der stationäen Phase eluieren. Aufgrund der Coelution von Polymerketten, die sich sowohl in ihrer Molmasse als auch im DB unterscheiden, ist eine reine SEC-Trennung von linearen und verzweigten Proben nicht möglich. Die SEC – MALLS Experimente bestätigten, dass unterschiedlich verzweigte

Polymerproben unterschiedlichen Kalibrationskurven in der SEC folgen, was auf verschiedene Knäueldichten der Polymerketten in Lösung hinweist. Dabei verursacht eine Erhöhung des Verzweigunggrades eine dichtere, kompaktere Knäuelform der Polymerkette, wodurch die Kalibrationskurve in Richtung höherer Molmassen verschoben wird.

2. Da mittels SEC keine Trennung zwischen linearen und verzweigten Polymeren erzielt werden konnte, wurde das Potential der Gradientenchromatographie zur Trennung nach Verzweigungsgrad untersucht. Dabei konnte eine deutliche Abhängigkeit des Elutionsvolumens vom Verzweigungsgrad beobachtet werden. Eine Erhöhung des Verzweigungsgrades resultierte für Polymere gleicher Molmasse in einer späteren Elution im Gradienten. Da das Elutionsverhalten beim Vorliegen enthalpischer Wechselwirkungen auch von der Molmasse beeinflusst wird, kann angenommen werden, dass die Elution von linearen und verzweigten Polymeren in der Gradientenchromatographie sowohl von der Molmasse als auch vom Verzweigungsgrad abhängt. Dies sollte eine Trennung nach dem Verzweigungsgrad unmöglich machen, da es, ebenso wir in der SEC, zur Coelution von Polymerketten kommt, die sich in der Molmasse und im Verzweigungsgrad unterscheiden.
Zusätzliche Gradientenexperimente an Fraktionen von linearen und verzweigten Polymerproben belegten diese Annahme. Eine mögliche Erklärung für die spätere Elution der stärker verzweigten Polymere im Gradienten kann die unterschiedlich kompakte Struktur in Lösung sein. Da eine Erhöhung des Verzweigungsgrads den Polymerradius schrumpfen lässt, steigt auch die Dichte an wechselwirkenden Gruppen an der Oberfläche des Knäuels mit zunehmendem Verzweigungsgrad an. Die hierdurch resultierende stärkere Wechselwirkung mit der stationären Phase führt zu einer verzögerten Elution, welche aufgrund möglicher Nachbarschaftseffekte noch weiter verzögert wird. Diese Nachbarschaftseffekte können aufgrund der hohen Dichte an funktionellen Gruppen an der Polymeroberfläche entstehen, wodurch bei der Wechselwirkung der funktionellen Gruppen des Polymeren mit der stationären Phase auch immer naheliegende weitere funktionelle Gruppen der Polymerkette mit der stationären Phase in Wechselwirkung treten können (auch Doppel-, Dreifachkontakte genannt).
Aufgrund der durchgeführten Experimente konnte bestätigt werden, dass das Elutionsverhalten von unterschiedlich verzweigten Polyestern in der Gradienten-chromatographie sowohl von der Molmasse auch als vom Verzweigungsgrad beeinflusst wird. Dadurch kann eine Trennung nach nur einer Heterogenität (entweder nur nach Molmasse oder nur nach DB) nicht durch die Gradientenchromatographie realisiert werden.

3. Die beobachtete Abhängigkeit des Elutionsvolumens vom Verzweigungsgrad in der Gradientenchromatographie ließ vermuten, dass auch in anderen flüssigchromatographischen Trennmodi mit enthalpischen Wechselwirkungen wie LC-CC und LAC, eine ähnliche Abhängigkeit des Elutionvolumens vom Verzweigungsgrad zu finden ist. Zur Überprüfung dieser Annahme wurden nach SEC-Fraktionierung der linearen Probe die kritischen Bedingungen des linearen Polyesters experimentell bestimmt. Anschließend wurde das Elutionsverhalten von SEC-Fraktionen der verzweigten Proben unter diesen chromatographischen Bedingungen untersucht. Während die Fraktionen des linearen Polymeren molmassenunabhängig eluierten, konnte für die verzweigten Proben ein Anstieg des Elutionsvolumen mit steigender Molmasse und steigendem Verzweigungsgrad beobachtet werden. Für stark verzweigte Proben bei hohen Molmassen wurde dabei die Retention so stark, dass keine Elution oder nur eine unvollständige Elution beobachtet wurde. Diese Ergebnisse zeigen, dass das Elutionsverhalten von Polymeren in der Adsorptionschromatographie und unter kritischen Bedindungen nicht nur von der chemischen Struktur und der Molmasse beeinflusst wird, sondern auch von der Anordnung der wechselwirkenden Gruppen innerhalb der Polymerkette (Topologie).

4. Zur Untersuchung des unterschiedlichen Elutionsverhaltens von linearen und verzweigten Polymeren wurden temperaturabhängige chromatographische Experimente unter isokratischen Bedingungen und unter Verwendung von Lösungsmittelgradienten durchgeführt. Entsprechend der gemachten Annahmen sollte eine Temperaturänderung eine größere Auswirkung auf die höher verzweigten Proben zeigen als auf die weniger verzweigten und linearen Proben. Dies kann dadurch erklärt werden, dass bei einer bestimmten Molmasse die enthalpischen Wechselwirkungen zwischen der stationären Phase und den Polymermolekülen bei wenig verzweigten Proben geringer ausgeprägt sein sollten als bei stärker verzweigten Proben. Weiterhin erlaubten die temperaturabhängigen Messungen, die Wechselwirkungsenthalpien für lineare und verzweigte molmassenabhängig zu quantifizieren. Trotz einer Streuung der Messwerte im niedermolekularen Bereich zeigten diese Experimente, dass die enthalpischen Wechselwirkungen zwischen der stationären Phase und der Polymerprobe mit zunehmender Molmasse steigen. Für unterschiedlich verzweigte Polymere mit identischen Molmassen konnte ein Anstieg der enthalpischen Wechselwirkung mit steigendem Verzweigungsgrad beobachtet werden.

5. Basierend auf den Erkenntnissen welche anhand der vorhergehenden Messungen gesammelt wurden, konnte geschlossen werden, dass eindimensionale flüssigchromatographische Methoden nicht in der Lage sind, eine Trennung unterschiedlicher verzweigter Polymere zu erzielen. Aus diesem Grunde wurden die Möglichkeiten einer zweidimensionalen flüssigchromatographischen Trennmethode untersucht..
Offline 2D-Experimente an den Ausgangsproben und an einem Polymerblend bestehend aus einer linearen und einer hyperverzweigten Probe zeigten, dass im hochmolekularen Bereich eine Trennung von linearen und verzweigten Polyestern erzielt werden kann. Hingegen war die Trenneffizienz im niedermolekularen Bereich niedrig, so dass hier keine Trennung erzielt werden konnte. Des Weiteren konnte für die Polymerblends gezeigt werden, dass die Trenneffizienz mit zunehmender Verzweigungsgraddifferenz zwischen den beiden Polymerproben zunimmt. Dies führt dazu, dass vor allem niedermolekulare Polymerblends mit kleiner Differenz im Verzweigungsgrad nicht durch offline 2D-Chromatographie getrennt werden können.

6. Nachdem die Validität eines 2-dimensionalen Ansatzes überzeugend demonstriert war, wurden zur Optimierung der Trennleistung vor allem im niedermolekularen Bereich online 2D-Experimente an Blends aus verzweigten und linearen Proben durchgeführt. Dabei konnten die folgenden Trends beobachtet werden.

- Je größer der Unterschied im Verzweigungsgrad der Blendkomponenten, desto besser ist die Auflösung der zwei Peaks. Daher kann eine Trennung leichter für stark unterschiedlich verzweigte Polymerproben erzielt werden.
- Im niedermolekularen Bereich ist die Auflösung zwischen der linearen und verzweigten Fraktion nur sehr niedrig. Sie verbessert jedoch mit zunehmender Molmasse. Daher lassen sich Trennungen zwischen linearen und verzweigten Proben mit konstantem DB besonders erfolgreich im hochmolekularen Bereich erzielen

Dieses Verhalten konnte basierend auf den theoretischen Arbeiten von Zimm und Stockmayer damit begründet werden, dass für den Erfolg der 2D-Trennung nicht primär der Verzweigungsgrad verantwortlich ist, sondern die absolute Anzahl an verzweigten Einheiten in der Polymerkette, welche bei konstanten DB mit fallendem Molekulargewicht abnimmt. Somit unterscheiden sich bei einem gegebenen Molekulargewicht die Molekülgrößen der linearen und verzweigten Polymerketten im niedermolekularen Bereich nur gering. Daher

können die in der ersten Dimension coeluierenden Fraktionen mit einer normal auflösenden SEC-Trennsäule nicht getrennt werden, da die resultierende Differenz im Elutionsvolumen in der SEC zu gering ist. Eine Verbesserung der Auflösung könnte unter Verwendung eines hochauflösendes SEC-Säulensatzes, welches für niedermolekulare Polymere ausgelegt ist, gelingen.

7. Nachdem eine Trennung von linearen und verzweigten Polymerketten erreicht werden konnte, wurde der zweidimensionale Ansatz zur Quantifizierung der Anteile an verzweigten Polymeren in Blends unterschiedlicher Zusammensetzung aus linearen und hyperverzweigten Polymeren verwendet.
Hierbei konnte gezeigt werden, dass die Quantifizierung durch online 2D-Chromatographie Ergebnisse liefert, die mit der Zusammensetzung der eingewogenen Polymere sinnvoll übereinstimmt.. Daher kann festgehalten werden, dass es mit Hilfe der 2D-Chromatgrographie erstmalig gelingt, realistisch die Menge an verzweigten und linearen Polymerketten in einer Polymermischung abzuschätzen. Nach dem Wissen des Autors gibt es momentan keine andere analytische Methode, die in der Lage ist, den Anteil von linearen und verzweigten Polymerstrukturen in einer Mischung zu quantifizieren.

8. Schließlich wurde ein Ansatz entwickelt, der die Bestimmung des mittleren Verzweigungsgrads und der Verzweigungsgradverteilung einer unbekannten Probe aus dem Kontourplot erlaubt. Hierzu wurde eine Kalibrationskurve konstruiert, so dass zu jedem Punkt des zweidimensionalen Chromatogrammes der DB bestimmt werden kann. Dieser Ansatz wurde anhand der Bestimmung des Verzweigungsgrads einer unbekannten Probe verifiziert.
Das experimentell bestimmte Ergebnis der zweidimensionalen flüssigchromatographischen Auswertung wurde anschließend mit dem Wert aus der ^{1}H-NMR verglichen. Dabei konnte eine gute Übereinstimmung des Wertes für den Verzweigungsgrad aus beiden Messmethoden erzielt werden.
Neben der Bestimmung des mittleren Verzweigungsgrades erlaubt dieser 2D-Ansatz auch die Bestimmung der Standardabweichung des Verzweigungsgrades. Diese kann als Maß für die topologische Dispersität der Probe angesehen werden. Eine große Standardabweichung entspricht einer topologisch sehr dispersen Polymerprobe. Zur Überprüfung der Hypothese wurde die Standardabweichung eines teilverzweigten Polyesters mit einem mittleren

Verzweigungsgrad von DB=0.38 mit der Standabweichung eines Polymerblends bestehend aus DB=0, DB=0.18, DB=0.38 und DB=0.50 verglichen. Dabei konnte für den Polymerblend eine fast doppelt so große Standardabweichung wie für das teilverzweigte Polyester DB – 38 bestimmt werden.

Mittels des in dieser Arbeit entwickelten chromatographischen Ansatzes ist man somit in der Lage, detailiertere Informationen über verzweigte Polymere zu erhalten, als es vorher möglich war. Erstmalig ist es möglich, das Ausmaß der topologischen Heterogenität für verzweigte Polymere abzuschätzen. Damit ergeben sich neuartige Möglichkeiten zur Kontrolle der Struktur verzweigter Polymere und somit zur gezielten Synthese maßgeschneiderter Hochleistungspolymere.

8 List of Abbreviation and Symbols

2D-LC	Two-Dimensional Liquid Chromatography
AF4	Asymmetric field flow fractionation
Da	Dalton
DP	Degree of polymerization
DB	Degree of branching
$\frac{d_n}{d_c}$	Refractive index increment
ΔG	Free Gibbs energy difference
ΔH	Change in interaction enthalpy
ΔS	Change in conformational entropy
ELSD	Evaporative light scattering detector
g	Contraction factor
HPLC	High performance liquid chromatography
k	Retention factor
K_d	Distribution coefficient
K_{LAC}	Contribution of adsorption to the distribution coefficient
K_{SEC}	Contribution of size exclusion to distribution coefficient
LAC	Liquid adsorption chromatography
LC	Liquid chromatography
LC-CC	Liquid chromatography at critical conditions
MALDI	Matrix assisted laser desorption / ionization
MALLS	Multiple angle laser light scattering
MeOH	Methanol
M_n	Number average molar mass
MTF	Molecular topology fractionation
M_w	Weight average molar mass
nm	Nanometer
NMR	Nuclear Magnetic Resonance
PDI	Polydispersity
PS	Polystyrene
R	Gas constant
R_g	Radius of gyration

RI	Refractive Index
RP	Reverse Phase
SEC	Size exclusion chromatography
THF	Tetrahydrofuran
TGIC	Temperature gradient interaction chromatography
TOF-MS	Time of flight mass spectrometer
V_A	Interstitial volume of the stationary phase
V_E	Elution volume
Vp	Pore volume of the stationary phase

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Eidesstattliche Erklärung

Ich erkläre hiermit Eides Statt, daß ich meine Dissertation selbstständig und nur mit den angegebenen Hilfsmitteln angefertigt habe.

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Erklärung

Ich erkläre hiermit, noch keinen Promotionsversuch unternommen zu haben.

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