Analyzing the chemical heterogeneity of poly(dimethylsiloxanes) and other polymers: Development and optimization of a polymer HPLC method

Dissertation

zur Erlangung des Doktorgrades der Naturwissenschaften

(Dr. rer. nat)

der Fakultät für Chemie und Pharmazie

der Universität Regensburg

vorgelegt von

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aus **Mitterskirchen**

April 2019

Die vorgelegte Dissertation entstand im Zeitraum von Oktober 2016 bis Januar 2019 in Kooperation des Instituts für Analytische Chemie, Chemo- und Biosensorik der naturwissenschaftlichen Fakultät IV – der Universität Regensburg

und

der Corporate Analytics der Wacker Chemie AG in Burghausen.

Die Arbeit wurde angeleitet von Prof. Dr. habil. Frank-Michael Matysik und durchgeführt unter der Leitung von Dr.-Ing. Thomas Ehmann.

Das Promotionsgesuch wurde eingereicht am: 29.04.2019 Termin des Kolloquiums: 12.07.2019

Den Vorsitz des Prüfungsausschusses übernimmt Prof. Dr. Alkwin Slenczka. Erstgutachter ist Prof. Dr. Frank-Michael Matysik, Zweigutachter ist Prof. Dr. Christian Neusüß und Drittprüfer ist Prof. Dr. Hubert Motschmann.

Die Dissertation enthält entsprechend der Geheimhaltungserklärung der Wacker Chemie AG keine Details bzw. lässt keine Rückschlüsse auf konkrete Produkte von WACKER zu. Auf Grund der Unvereinbarkeit der Geheimhaltungserklärung und der Veröffentlichungspflicht, beschrieben in der Promotionsordnung der Universität Regensburg, wurden in den entsprechenden Abschnitten der Dissertation keine detaillierten Aussagen zu Produkten von WACKER gemacht.

„Ein Chemiker, der kein Physiker ist, ist überhaupt gar nichts.“

„A chemist who is not a physicist is nothing at all.”

Robert Wilhelm Bunsen, 1811 – 1899

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List of publications

Adaption of a parallel-path poly(tetrafluoroethylene) nebulizer to an evaporative light scattering detector: Optimization and application to studies of poly(dimethylsiloxane) oligomers as a model polymer

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik

Journal of Chromatography A 1564 (2018) 214-223.

Abstract

The adaption of a parallel-path poly(tetrafluoroethylene)(PTFE) ICP-nebulizer to an evaporative light scattering detector (ELSD) was realized. This was done by substituting the originally installed concentric glass nebulizer of the ELSD. The performance of both nebulizers was compared regarding nebulizer temperature, evaporator temperature, flow rate of nebulizing gas and flow rate of mobile phase of different solvents using caffeine and poly(dimethylsiloxane) (PDMS) as analytes. Both nebulizers showed similar performances but for the parallel-path PTFE nebulizer the performance was considerably better at low LC flow rates and the nebulizer lifetime was substantially increased. In general, for both nebulizers the highest sensitivity was obtained by applying the lowest possible evaporator temperature in combination with the highest possible nebulizer temperature at preferably low gas flow rates. Besides the optimization of detector parameters, response factors for various PDMS oligomers were determined and the dependency of the detector signal on molar mass of the analytes was studied. The significant improvement regarding long-term stability made the modified ELSD much more robust and saved time and money by reducing the maintenance efforts. Thus, especially in polymer HPLC, associated with a complex matrix situation, the PTFE-based parallel-path nebulizer exhibits attractive characteristics for analytical studies of polymers.

High-Resolution Polymer High Performance Liquid Chromatography: Application of a saw tooth gradient for the separation of various polymers

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik

Journal of Chromatography A 1587 (2019) 88-100.

Abstract

Currently, a lot of research effort in polymer analysis by liquid chromatographic techniques, including size exclusion chromatography, polymer HPLC or liquid chromatography at critical conditions, is done aiming to improve separation performance. In this study, novel gradient protocols were investigated primarily based on gradient polymer elution chromatography (GPEC). Starting with linear gradients and stepwise gradients a new saw tooth gradient profile was developed and optimized. Optimum settings for the saw tooth gradient design were evaluated by design of experiments (DoE) based on Taguchi's methodology for various types of stationary phases. The gain of peak resolution was dependent on the effective gradient step height. The optimized protocol enabled high-resolution polymer HPLC (HRP- HPLC) separations with common HPLC instruments. The quality of separation was evaluated by heart-cut fraction collection of HRP-HPLC and subsequent determination of the individual fractions by SEC or MALDI-ToF mass spectrometry. Finally, different types of polymers, such as PVC, PDMS, PMMA, or PPG, were studied with the new method and a universal applicability was shown.

Separation of linear and cyclic poly(dimethylsiloxanes) with polymer high performance liquid chromatography

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik

Accepted in Monatshefte für Chemie – Chemical Monthly (2019) DOI:10.1007/s00706-019-02389-4.

Abstract

The growing importance of siloxanes in various industrial areas, e.g. health care, cosmetics, automotive and construction industries requires further method development of analysis techniques. In addition, and complementing gas chromatography analysis, a polymer liquid chromatography method for separation of linear and cyclic (poly)dimethylsiloxanes was developed and optimized. By an appropriate choice of

mobile and stationary phase combinations, separations up to 30 monomeric units are achieved. Therefore, various HPLC columns were investigated concerning physical and chemical properties, e.g. pore size, silica base material, and column functionality. Furthermore, solubility properties of siloxanes in adsorption and desorption promoting solvents were investigated and taking these results into account the separation was optimized applying a mixture of methanol: water (75:25, v/v) and acetone. The findings indicate, that precipitation / re-dissolution effects superimposed by adsorption chromatography result in the oligomer separation of up to 30 monomeric units. Besides method development on an analytical scale, linear poly(dimethylsiloxane) oligomers were separated with preparative polymer HPLC. These fractions of single oligomers allow further investigations of different material properties beyond polymer HPLC.

High-Resolution Polymer High Performance Liquid Chromatography: Optimization of the saw tooth gradient profile for various stationary phases and separations on preparative scale

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik

Submitted for publication

Abstract

The recently introduced saw tooth gradient for high-resolution polymer HPLC was optimized and improved in terms of total runtime and separation performance. As a result, increased flow rates enabled reduced runtimes in combination with enhanced peak resolutions. Moreover, the saw tooth gradient profile was further investigated using a saw tooth gradient with a down-to-zero approach which corresponds to an increased height of the negative backward gradient step. Modifying the mobile phase composition allowed two further gradient protocols: a ternary and a three-dimensional setup. Thereby, a ternary saw tooth gradient is characterized by repeating the whole gradient elution with two adequate pairs of adsorption and desorption promoting solvents for mixtures containing diverse polymer components. A three-dimensional saw tooth gradient is determined by combining three different solvents in the gradient elution. In addition to mobile phase modifications, various stationary phases were compared and examined. Applying size exclusion chromatography (SEC) columns for saw tooth gradient polymer elution chromatography enabled the exploitation of two completely different separation mechanisms (SEC and high-resolution polymer HPLC) on one stationary phase. Thus, two-dimensional,

heart-cut coupling of SEC and high-resolution polymer HPLC with only one stationary phase could be achieved. The application of the above-mentioned concept is presented for a silicone oil with a viscosity of 350 mPa·s by using a hybrid HPLC system consisting of a Thermo Fisher Scientific HPLC and an Agilent fraction collector.

Comparison of Molar Mass Determination of Poly(dimethylsiloxanes) by Size Exclusion Chromatography and High-Resolution Polymer High Performance Liquid Chromatography Based on a Saw Tooth Gradient

Bernhard Durner, Beate Scherer, Thomas Ehmann, Frank-Michael Matysik

Accepted in ACS Applied Polymer Materials

Abstract

Polysiloxanes are used in a wide range of application fields and extensive research is currently done to enhance product quality and performance. Therefore, more sophisticated analysis methods are necessary to monitor and support the polymer product optimization. Based on different modes in polymer liquid chromatography, heart-cut two-dimensional polymer HPLC is one powerful analytical approach. Due to different distributions within polymer samples, separations according to chemical heterogeneities, molecular architecture or molar mass differences are possible. With the recently introduced saw tooth gradient protocol a new possibility for determining the polymer (micro-) structure on analytical scale has been developed. Hence, the effect of various stationary phases with different particle base material and chemical modifications were investigated in context of the separation of linear poly(dimethylsiloxane) in a molar mass range from 1000 g·mol^{-1} to 300 000 g·mol^{-1}. The resulting chromatograms allowed a direct correlation between HPLC retention times and molar masses corresponding to separated peaks. Consequently, a detailed analysis of differences in the polymer structure, e.g. fingerprint analysis, is possible.

Miscellaneous

Development and application of a method for ivory dating by analyzing radioisotopes to distinguish legal from illegal ivory

Andreas Schmidberger, Bernhard Durner, David Gehrmeyer and Robert Schupfner

Forensic Science International 289 (2018) 363-367.

Abstract

The age determination of elephant ivory provides necessary and crucial information for all criminal prosecution authorities enforcing the Convention on International Trade in Endangered Species of Wild Fauna and Flora. The knowledge of the age of ivory allows to distinguish between pre-convention, hence legal material and ivory deriving from recent, illegal poaching incidents. The commonly applied method to determine the age of ivory is radiocarbon dating in the form of bomb pulse dating, which however will fade out soon. This work provides an enhancement of the radiocarbon dating method by supplementary determination of the isotope profile of 90-Sr and the two thorium isotopes 228-Th and 232-Th. This combined analysis allows for a precise and unambiguous age determination of ivory. We provided calibration curves for all involved radionuclides by analyzing ivory samples with known age and investigated a new method for the extraction of strontium from ivory.

Investigations on the decomposition of AdBlue urea in the liquid phase at low temperatures by an electrochemically induced pH shift

Peter Braun, Bernhard Durner, Hans-Peter Rabl, Frank-Michael Matysik

Accepted in Monatshefte für Chemie – Chemical Monthly (2019) DOI:10.1007/s00706-019-02406-6.

Abstract

Ammonia-based selective catalytic reduction (SCR) systems are the most widely used technology for reduction of nitrogen oxide emissions from lean-burn engines such as diesel engines. However, at low exhaust temperatures, the SCR process is limited by difficulties in the decomposition of the ammonia precursor urea, which is carried on-board using an aqueous solution “AdBlue”. In this study, the decomposition of AdBlue urea induced by electrical current and the resulting associated pH shifts was investigated in a divided cell configuration in the liquid phase. The decomposition was found to be favoured in both electrochemical compartments, anodic and cathodic, at temperatures of 60 °C – 80 °C compared to a reference without electrochemical treatment. In addition to the determination of ammonia contents, IC/HPLC analyses were carried out for each sample. Different side products such as biuret, nitrate, cyanuric acid, ammelide and others were formed. In the anodic compartment, nitrate formation could be observed, due to oxidation of ammonia at the electrode surface.

Patents

Hochaufgelöste Flüssigchromatographie basierend auf einem Sägezahngradienten

Bernhard Durner, Thomas Ehmann, Wacker Chemie AG

European Patent: PCT/EP2018/064868, application 2018-06-06

Abstract

Die vorliegende Erfindung betrifft ein Verfahren zur Analyse einer Polymerprobe, wobei das Verfahren die Durchführung einer Flüssigchromatographie-Analyse an einer Chromatographiesäule mit einer mobilen Phase enthaltend eine Mischung aus mindestens einem Nichtlösungsmittel (S1) und mindestens einem Lösungsmittel (S2) für die Polymerprobe umfasst, dadurch gekennzeichnet, dass der Volumenanteil an S2 in der mobilen Phase während des Elutionsprozesses stufenweise variiert wird und die Stufen abwechselnd auf- und absteigen.

Conference contributions

Oral Presentations

Konzernanalytikertreffen 2017 Wacker Chemie AG, 15 November 2017, Burghausen
Separation of Linear and Cyclic Poly(dimethylsiloxanes) with Liquid Chromatography
Bernhard Durner

14th International Students Conference "Modern Analytical Chemistry" 2018, 20 to 21 September 2018, Prague
Separation of linear and cyclic poly(dimethylsiloxanes) by interactive chromatography
Bernhard Durner

29. Doktorandenseminar Hohenroda, 6 to 8 January 2019, Hohenroda
Interactive Chromatography for analyzing the Chemical Heterogeneity of Silicones and Poly(vinyl acetate)
Bernhard Durner

Thermo Fisher Scientific Chromatographie Anwenderseminare 2019, 12 March 2019, Wiesbaden
High Resolution Polymer HPLC: A new approach for the separation of various polymers based on the application of a saw tooth gradient
Bernhard Durner

ANAKON 2019, 25 to 28 March 2019, Münster
Anwendungen eines periodischen Sägezahngradienten in der Polymer HPLC
Bernhard Durner

Poster Presentations

ANAKON 2017, 3 to 6 April 2017, Tübingen
Auftrennung linearer und zyklischer Polysiloxane durch interaktive Chromatographie
Bernhard Durner, Thomas Ehmann, and Frank-Michael Matysik

Declaration of collaboration

Most of the theoretical and experimental work presented in this thesis was carried out solely by the author. In some cases, however, the practical implementation of concepts and the performance of measurements were carried out in collaboration with other researchers and individuals. In accordance with *§ 8 Abs. 1 Satz 7 of the Ordnung zum Erwerb des akademischen Grades eines Doktors der Naturwissenschaften (Dr. rer. nat.) an der Universität Regensburg vom 18. Juni 2009 (Änderungssatzung vom 6. Februar 2014)* the declaration of collaboration is given separately in each relevant chapter.

List of abbreviations

Acetonitrile (ACN)

Charged Aerosol Detector (CAD)

Condensation Nucleation Light Scattering Detector (CNLSD)

Critical point of adsorption (CPA)

trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB)

Design of Experiments (DoE)

Diode array detector (DAD)

Evaporative Light Scattering Detector (ELSD)

Fluorescence detector (FLD)

Gas chromatography (GC)

Gel filtration chromatography (GFC)

Gel permeation chromatography (GPC)

Gradient polymer elution chromatography (GPEC)

High Performance Liquid Chromatography (HPLC)

High Performance Precipitation Liquid Chromatography (HPPLC)

High-Resolution Polymer High Performance Liquid Chromatography (HRP-HPLC)

Liquid chromatography (LC)

Liquid chromatography at limiting conditions of adsorption (LC-LCA)

Liquid chromatography at limiting conditions of desorption (LC-LCD)

Liquid adsorption chromatography (LAC)

Liquid chromatography under critical conditions (LCCC)

Multiangle laser light scattering detector (MALLS detector)

Matrix assisted Laser Desorption Ionization - Time of Flight- Mass Spectrometer (MALDI-ToF-MS)

Methanol (MeOH)

Nuclear magnetic resonance spectroscopy (NMR spectroscopy)

operational / performance qualification (OQ/PQ)

Pentafluorophenyl (PFP)

Poly(dimethylsiloxane) (PDMS)

Poly(methylmethacrylate) (PMMA)

Poly(propylene glycol) (PPG)

Polystyrene (PS)

Poly(tetrafluoroethylene) (PTFE)

Poly(vinylchloride) (PVC)

Precipitation- / re-dissolution liquid chromatography (PLC)

Refractive index (RI)

Size exclusion chromatography (SEC)

Standard liter per minute (SLM)

Tetrahydrofuran (THF)

Temperature gradient interaction chromatography (TGIC)

1 Introduction

Responding issues connected to analytical chemistry, is an important aspect in science and industry. A lot of different quality and performance problems are solved through analytical chemistry by choosing the appropriate analysis techniques. **Figure 1.1** gives an overview about the most important steps during the analysis of arbitrary substances. Therefore, it is important to know and control the analytical process [1], including sample collection and preparation, measurement, detection, and data evaluation as well as interpretation. Furthermore, suitable methods must be chosen and if necessary optimized.

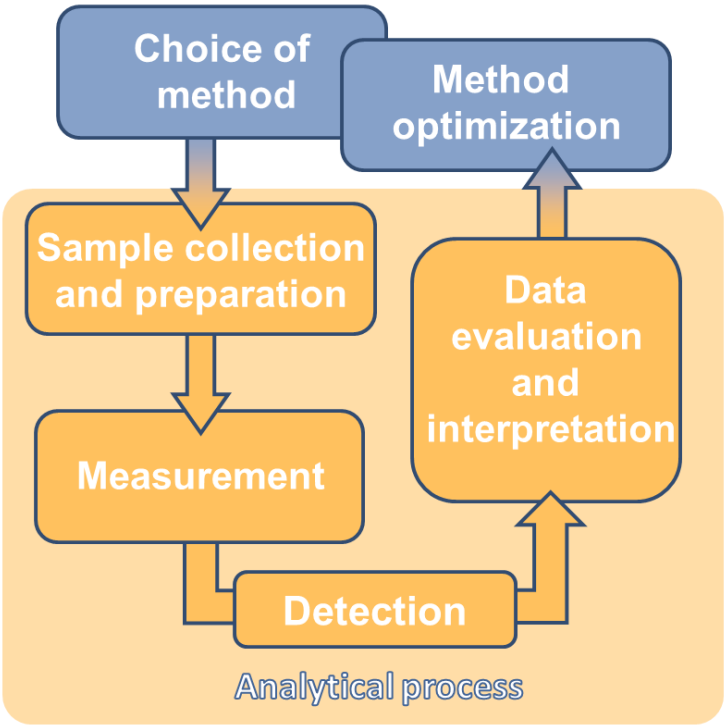

Figure 1.1: *Development of an analytical method within and in context to the analytical process.*

Particularly, complex samples with high amounts of matrix or a lot of different constituents require chromatographic methods. Several molecular and vaporizable analytes can be separated with gas chromatography (GC). However, samples containing high amounts of matrix or high molecular weight compounds are not suited for GC separation and require liquid chromatography (LC) techniques. Consequently, the different steps in and around the analytical process are very important for polymer analysis. As previously described, the choice of a proper method usually is the starting point of research. Thus, if LC is required for polymer analysis, various modes, for example adsorption and size exclusion chromatography or chromatography under critical conditions, are possible [2]. Therefore, it is important

to know as much as possible about the polymer sample and demands of the customer in advance. A first important step during the analytical process is sampling and sample preparation. Particularly, the presence of polymer matrices and product heterogeneities has to be taken in account before or by collecting of the sample. Depending on the type of polymer, different techniques are crucial for a representative sample. Subsequent, polymer sample preparation requires plenty of expertise concerning solubility effects or extraction methods. Even if the sampling was representative and a homogenous sample was taken, the appropriate choice of solvent is important to dissolve the overall polymer. Moreover, even the distribution of the polymer in solution may be an important and error-prone procedure, if polymer chains are degraded by too much stirring because of shear degradation. For example, high molar mass polymers require a long time before being completely dissolved without chain scission [3]. In the next step of the analytical process of polymer liquid chromatography, the successful collected and dissolved polymer sample is separated according to specific macromolecular properties, like molar mass distribution, chemical functionality distribution or a different molecular architecture. In general, depending on physical and chemical interactions different LC modes are worth to be considered and have to be discussed in this study. Furthermore, current separation methods show some limitations making improvements and optimizations necessary. However, successful measurements strongly depend on the capability and properties of the applied detectors. Therefore, every type of detector has its own advantages and disadvantages and further research may be done in choosing the ideal device appropriate for the particular task. Afterwards, the obtained data have to be carefully evaluated and interpreted under considering quality management, robustness and significance. Additionally, after the analytical process is completed, a feedback on the applied method is important and for ensuring competitiveness.

Emerging from the analytical process, robust and innovative polymer LC methods are crucial to analyze polymers. Therefore, various adjustments of stationary and mobile phases as well as instrumental optimizations were already done and have to be made. For example, based on sophisticated approaches of multi-linear or step-wise gradients [4,5] further optimization of the gradient profile will be presented. In each case, the resulting methods should be as simple as possible and give versatile improvements compared to the initial situation.

References

[1] M. Otto (Ed.), Analytische Chemie, 4th ed., Wiley-VCH, Weinheim, 2011.

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2 Basics of polymer liquid chromatography

2.1 Introduction

Polymers are built of a high number of monomers and consequently are statistically distributed in several properties. These distributions cause main differences in the quality of polymer products. Therefore, it is essential to investigate, analyze, and optimize the polymerization reaction. **Figure 2.1** gives an overview about the most important polymer distributions, i.e. molar mass (a), chemical composition (b), molecular architecture (c), and functionality (d). Depending on the polymerization reaction, polymers may be homogeneous in all distributed properties (monodisperse polymers), heterogeneous in only one property (polydisperse polymers) or heterogeneous in more than one property (complex polymers).

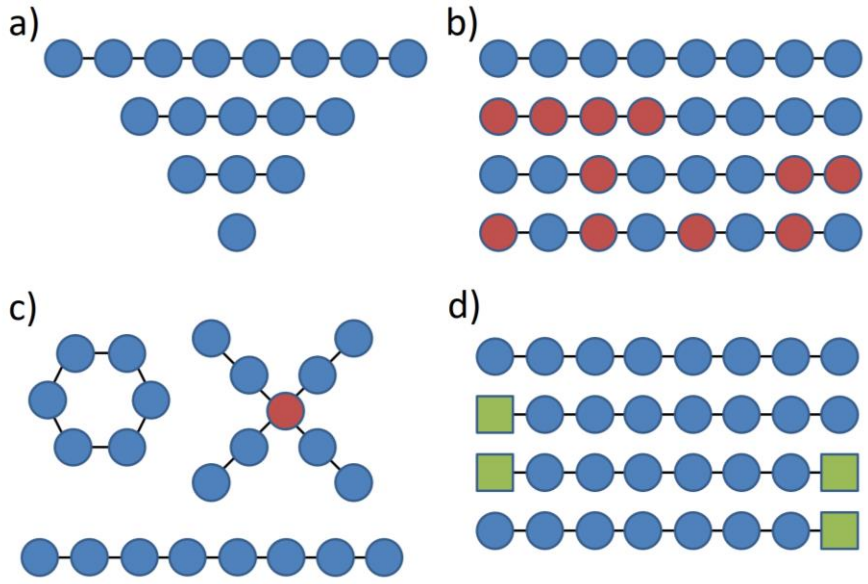

Figure 2.1: *Example for typical polymer heterogeneities:* ***a)*** *molar mass distribution,* ***b)*** *chemical composition distribution,* ***c)*** *molecular architecture differences, and* ***d)*** *chemical functionality distribution.*

At least all synthetic polymers are polydisperse in the molar mass. Thus, analyzing the molar mass distribution is an important issue in liquid chromatography. Besides, as far as two or more monomers with different chemical composition take part in polymerization, chemical heterogeneities appear. As a result, copolymers with defined blocks, alternating sequences or randomly distributed monomer units are built. The analysis of these polydisperse or complex polymers is a challenging task and often more than one separation technique have to be applied [1–5]. Before discussing several typical liquid chromatographic techniques for polymer analysis, a short overview of the investigated polymers of this study is given.

Today, poly(siloxanes) are a very important group of synthetic polymers and are used in various industrial areas, e.g. health care, cosmetics, automotive and construction industries. Since the first synthesis by Müller and Rochow [6], siloxanes are used in a broad variety of different applications, e.g. silicone dispersions, elastomers, resins or rubbers [7–12]. A great deal of research was done on the chemical structure and composition of various poly(siloxanes). The combination of silicon, oxygen, and carbon atoms lead to very specific properties in between inorganic and organic chemistry. As depicted in **Figure 2.2** the number of organic groups *R* in comparison to silicon – oxygen bonds cause the characteristics of the organo-poly(siloxanes). Furthermore, the number of silicon – oxygens bonds determine the functionality of the siloxane basic unit, from monofunctional for one Si-O bond to tetrafunctional for four Si-O bonds. The most important class of silicones is poly(dimethylsiloxane) (PDMS, compare the bifunctional poly(siloxane) in **Figure 2.2**), which contains only methylene groups as organic group and shows a two-dimensional linear structure [11,13].

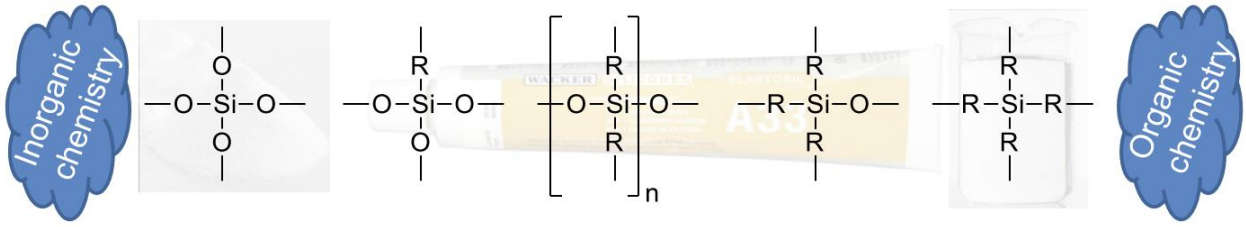

Figure 2.2: *Chemical structure of poly(siloxanes) in between inorganic and organic chemistry, R = organic group.*

Based on their polymer structure, siloxane polymers show high temperature and weather resistance, good dielectric properties, a high film-forming capacity, physiological indifference, and anti-foaming effects. Therefore, poly(siloxanes) are used among other things as sealants, paint additives, marine coatings, cooling liquids in transformers or masonry water repellants. Moreover, various application areas for silicones, e.g. automotive-, energy-, health care-, and construction- industry, are depicted in **Figure 2.3**. Additional information about poly(siloxanes) can be found in [14–25].

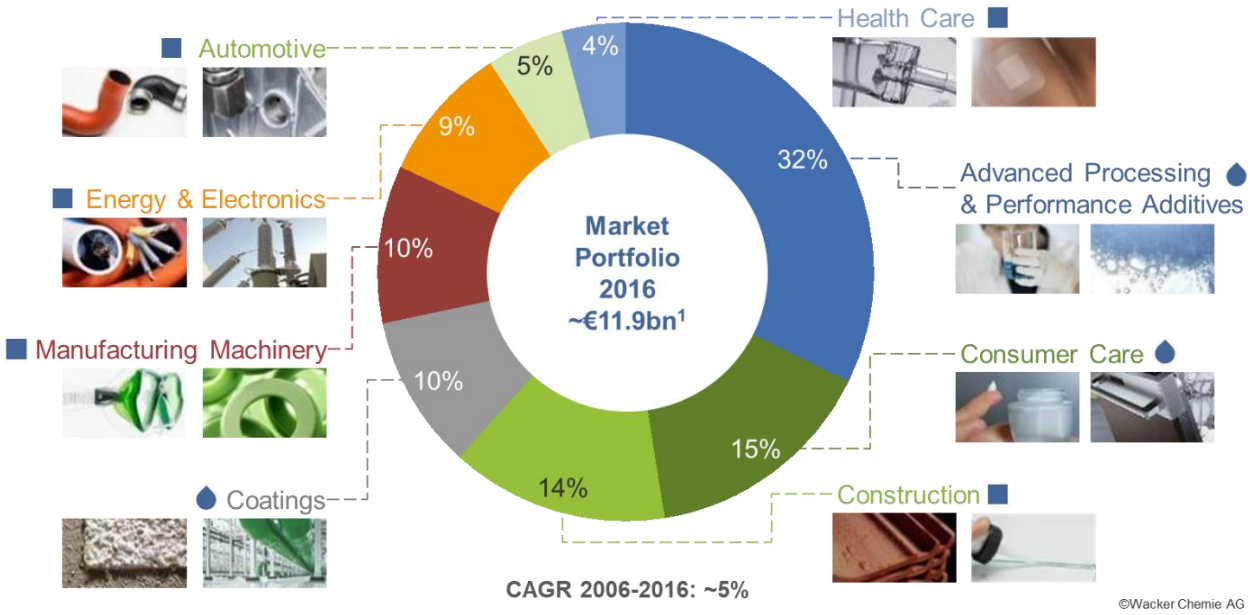

Figure 2.3: *Overview about applications of silicone in industry (out of business unit presentation of Wacker Silicones, 2017).*

Moreover, in this study such homopolymers as poly(methylmethacrylate) (PMMA), poly(propylene glycol) (PPG), polystyrene (PS), and poly(vinylchloride) (PVC) whose structures are depicted in **Figure 2.4**, are investigated.

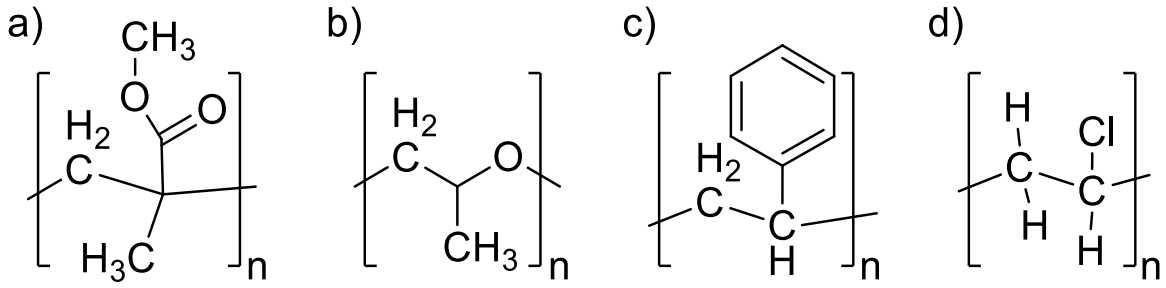

Figure 2.4: *Overview of additionally investigated homopolymers,* ***a)*** *PMMA,* ***b)*** *PPG,* ***c)*** *PS, and* ***d)*** *PVC.*

2.2 Polymer liquid chromatography modes

The heterogeneity of polymers requires different separation techniques in liquid chromatography. Based on molar mass, chemical composition, or molecular architecture distributions of the investigated polymer samples, the main purpose of polymer liquid chromatography is a separation or rather fractionation by molecular properties. Depending on the thermodynamic driving force, a differentiation in size exclusion chromatography, liquid chromatography under critical conditions and liquid adsorption chromatography can be made. For mostly entropy driven separations, size exclusion chromatography is the dominant mode, whereas for prevailing enthalpy effects liquid adsorption chromatography (LAC) is applied. In between both modes when entropic and enthalpic effects balance each other, liquid chromatography at critical conditions (LCCC) becomes possible [26–29]. An elugram showing all three different modes of polymer liquid chromatography is presented in **Figure 2.5**. In SEC mode the molar mass decreases with increasing elution volume and, vice versa, in LAC mode the molar mass increases with increasing elution volume. A more detailed theoretical description including a model for all three modes of polymer liquid chromatography can be found in [28]. In ideal LCCC, the elution of the polymer is independent of molar mass. A more detailed description of these three chromatography modes is given in the following chapters, in which LAC is summarized by the term polymer HPLC.

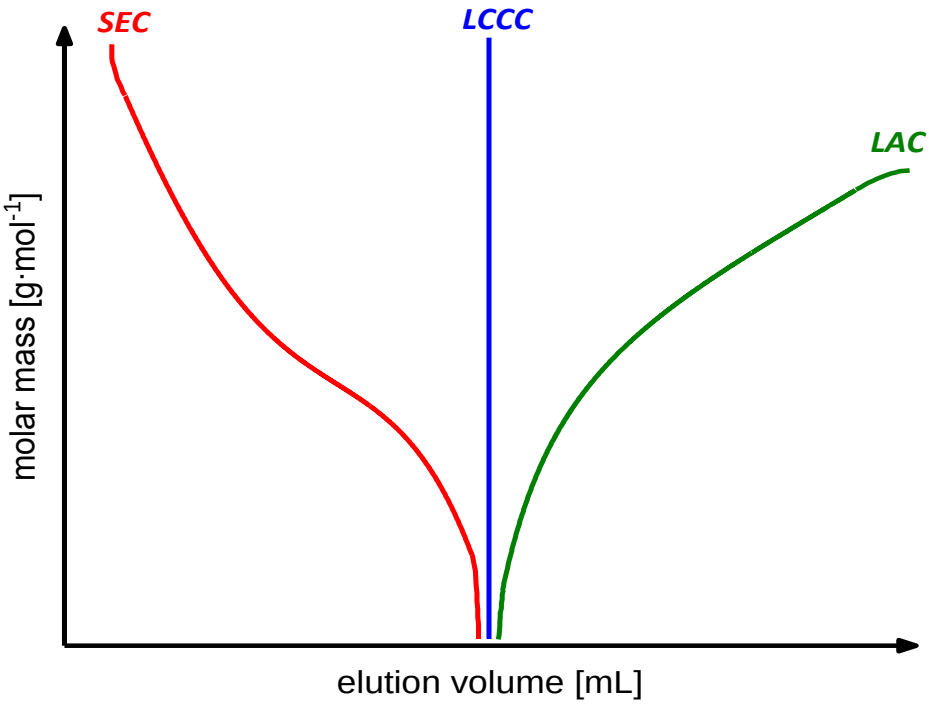

Figure 2.5: *Elugram, showing the three different modes of polymer liquid chromatography.*

2.3 Size exclusion chromatography

SEC is usually the method of choice for determination of the molar mass distributions of macromolecules. For synthetic polymers SEC is also called gel permeation chromatography (GPC) because of the permeation of the analyte molecules into the pores of the stationary phase. For biopolymers separated by aqueous mobile phases, SEC is also referred to as gel filtration chromatography (GFC).

The separation mechanism is based on the size of the macromolecules in the eluent and thus on the hydrodynamic volume of the polymer. The stationary phase provides a packing material with different pore sizes and the mobile phase is ideally a strong solvent for the investigated polymer eliminating any chemical interactions between stationary phase and polymer. Under these conditions, the analytes are separated solely by their size, or to put it another way, the molar mass of the macromolecules. In SEC, high molar mass polymers which cannot penetrate the pores of the stationary phase elute first, or rather complete exclusion of these polymer molecules to the pores occurs (see **Figure 2.6**). At the end of the separation, the eluent which permeates all pores of the column elutes at the dead time. Thus, the separation of polymer samples must be done within the first and only passage through the SEC column. This leads to elevated column lengths and the coupling of more than one column to enhance the separation range and the peak resolution. In contrast to the total stationary phase permeation of the organic solvent, a complete exclusion from all column pores leads to an unseparated polymer mixture. Consequently, the separation range in SEC is primarily determined by the pore size distribution of the used column, the combination of columns with different pore size, and the resulting total length of the column [27,30,31].

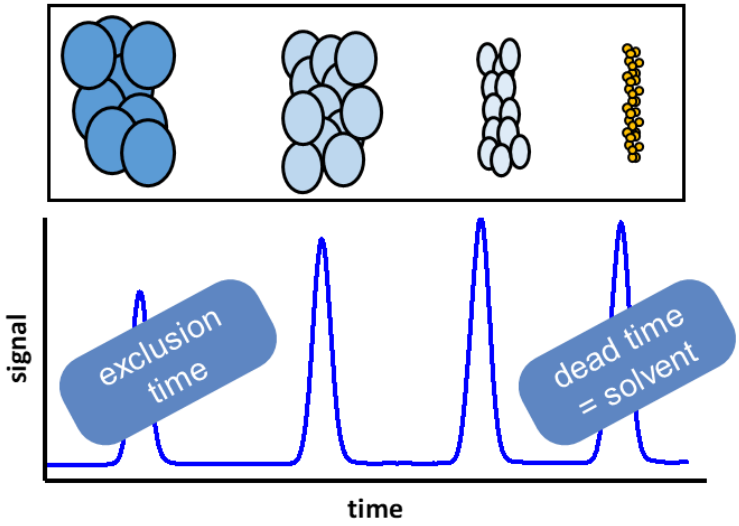

Figure 2.6: *Mechanism in SEC in relation to a SEC elugram; with increasing retention time the molar mass of the eluting macromolecules decreases and at the end of separation the solvent molecules elute at the dead time.*

Additionally, any chemical interactions between analyte and the stationary phase must be avoided, and the mobile phase is solely the carrier transporting the macromolecules through the column. The relation between elution volume or rather retention time and molar mass is usually established by a calibration with monodisperse PS standards over the investigated molar mass range. Generally, it is assumed that the hydrodynamic volume of the PS standards used for calibration acts the same as that of the investigated polymer. Typically, refractive index (RI), multiangle laser light scattering (MALLS), diode array detectors or viscometers are used as detector(s). In case of simultaneous MALLS and RI detection an absolute molar mass determination is possible. Furthermore, combining multiple detectors as RI, MALLS detector and viscometer information about the polymer branching can be obtained by a Mark-Houwink-Sakurada plot [32,33]. The most prominent advantages of SEC include isocratic separation, fast and easy separation and handling, a common relation between molar mass and retention time and a large molar mass range up to 400 million $g \cdot mol^{-1}$. In contrast to that SEC shows only a low molar mass resolution, indirect determination of molar masses via the hydrodynamic volumes in the used eluent, and the possibility of secondary (chemical) interactions between analyte and stationary phase [34]. Further literature and information about operating SEC with polymers is given in [34–41].

2.4 Liquid chromatography at critical conditions

As already shown in **Figure 2.5**, LCCC exhibits a very unique elution behavior. At the point of critical conditions, the steric interactions of the SEC mode counterbalance with attractive adsorption forces of the LAC mode. Consequently, the separation of polymers is independent of the molar mass but depends on chemical modification or functionality. LCCC can be performed by isocratic and gradient elution. Moreover, LCCC is also known as elution at the critical point of adsorption (CPA). For separating block-copolymers consisting of two and more blocks with different chemical functionality, LCCC is extremely valuable. However, adjusting the critical conditions in the separation system can be very tedious and may result in an only slightly robust chromatographic method [28,42,43]. In general, the determination of the CPA is done by adaption of the mobile phase composition and/ or the temperature of the separation system to critical conditions. A combination of CPA and SEC is favorable: The polymer of interest is separated at the CPA while all other components elute in the SEC mode. Combining CPA with LAC is not feasible because the non-polymeric components can irreversibly adsorb to the stationary phase due to the isocratic conditions. Several research groups describe different possibilities of adjusting the critical conditions [44,45].

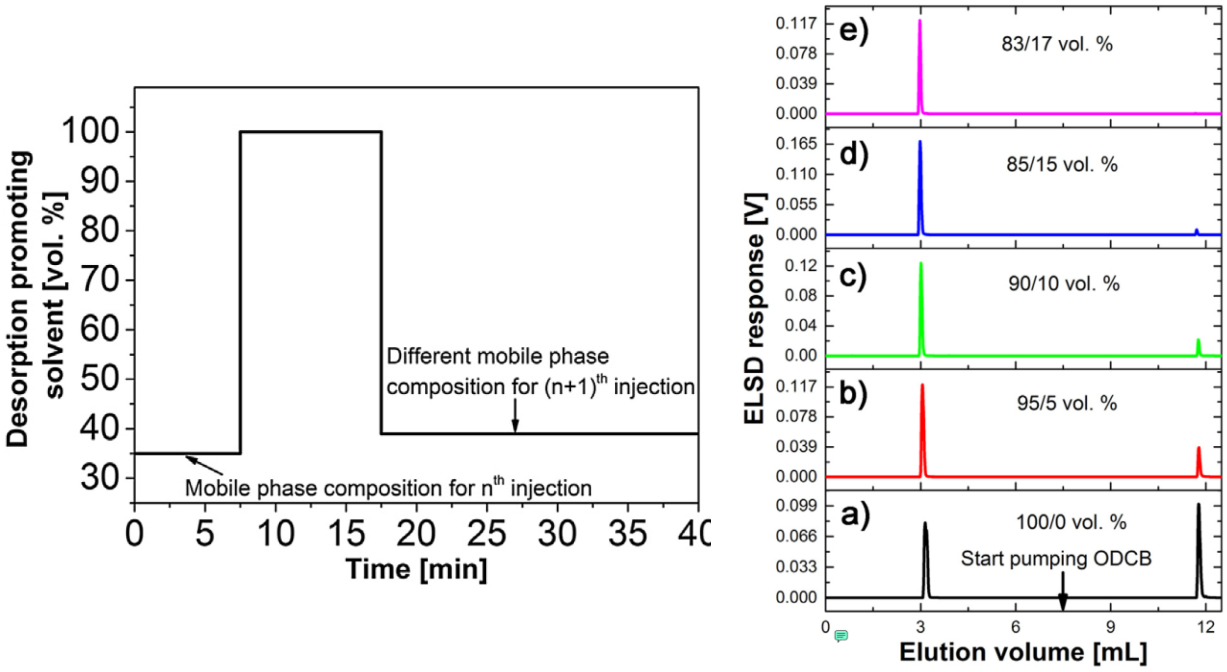

Figure 2.7 left side: *Gradient program for finding the eluent composition for the LCCC;* ***right side:*** *Measurement series for determination of the critical conditions (e) of a isotactic polypropylene sample with the mobile phase 2-octanol/ 1,2-dichlorobenzene at a Thermo Fisher Hypercarb (4.6 x 250 mm) column (both figures out of [44]).*

Bhati et al [44,46] described a very elegant method to determine the CPA of isotactic polypropylene with a mobile phase combination of 2-octanol and 1,2-dichlorobenzene using a Thermo Fisher Hypercarb column (see **Figure 2.7**). Therefore, a high molar mass homopolymer was injected into different isocratic mobile phase combinations, until the complete sample amount eluted in one peak (compare **Figure 2.7**). At these conditions LCCC took place and could be used for separation of real samples. In [47] an overview of various polymers separated by LCCC is given. Some further examples for LCCC of linear and cyclic PDMS are given in [48], for PS and PDMS block copolymers in [49], for PS and poly(ethylene oxide) block copolymers in [50], and for the separation of diverse poly(ethylene glycols) in [51]. Comparing isocratic and gradient CPA, gradient CPA is advantageous because it is a more robust method, the risk of irreversible polymer adsorption is minimized, and the separation does not depend on the column pore size. Nevertheless, LCCC has some limitations, i.e. the lack of sample recovery, high susceptibility to variations of surrounding conditions or disturbance through polymer sample changes [2,28]. In the following, two special separation techniques using the advantages of LCCC are explained.

2.4.1 Barrier techniques

The application of solvent barriers exploiting the transition between two modes of polymer liquid chromatography, enables an isocratic separation according to LCCC. Berek et al [28,52–54] essentially differentiate between liquid chromatography at limiting conditions of adsorption (LC-LCA) and liquid chromatography at limiting conditions of desorption (LC-LCD). In LC-LCA, the mobile phase is composed of a solvent mixture slightly below the CPA (of the polymer) and the composition of the solvent barrier is slightly above the CPA. The injection of the polymer occurs within the solvent barrier. Thus, at the initial solvent composition within the solvent barrier SEC mode enables the polymer to pass the solvent barrier. As soon as critical conditions for the analyte polymer appear, elution in LCCC mode take place and a separation from other sample components is possible. In the second barrier mode LC-LCD, the initial mobile phase composition corresponds to SEC conditions (where the polymer is completely dissolved without the possibility of chemical interactions to the stationary phase) and the barrier composition corresponds to the LCA mode. The polymer injection in the initial mobile phase results in a SEC mechanism at the beginning as long as the macromolecules reach the solvent barrier. At the border area LCCC separates the macromolecules which reach the CPA from the polymer mixture. The complex arrangement of various solvent barriers limits the application of the barrier techniques and causes reproducibility issues [28].

2.4.2 Gradient SEC

Radke et al [54,55] introduced with gradient SEC a further alternative applying mixed polymer liquid chromatography. This approach is based on the previously described barrier techniques. Thus, the dissolved polymer sample is injected on a SEC column in a solvent composition providing LAC for all components. After the injection a solvent gradient towards SEC conditions is started. For each sample compound a suitable CPA is reached in between the transition from LAC to SEC solvent. More or less this separation technique provides an infinite number of solvent barriers for polymer separation. Compared to the barrier techniques an increased reproducibility and an increased system robustness are gained. Nevertheless, this approach is prone to system fluctuations and the small differences of the solvent gradient limits the application to complex real polymer samples. Furthermore, a combination of several SEC columns is necessary and extends the total runtime [28].

2.5 Polymer HPLC

In comparison to a separation according to only one polymer distribution, like molar mass in SEC or chemical functionality in LCCC, polymer HPLC offers various interaction parameters between polymer and mobile/ stationary phase. Therefore, polymer HPLC is often termed as interactive chromatography [27] or interaction polymer (liquid) chromatography [28]. In this study, polymer HPLC is used as name and includes all types of LC shown in **Table 2.1**, except SEC. Liquid adsorption chromatography or gradient polymer elution chromatography (GPEC) are the most common types in polymer HPLC. In addition, precipitation- / re-dissolution chromatography (PLC) or temperature gradient interaction chromatography (TGIC) can also be used as separation techniques. A detailed discussion of LAC and PLC will be given in the subsequent chapters, but as previously discussed, a clear differentiation between both LC types is often sophisticated in practical application.

Table 2.1: *Different types of polymer HPLC compared to SEC, for more detailed information see [5] and [28].*

Type of Liquid chromatography	Separation according to…	Information about molar mass distribution	Information about chemical composition
SEC	… hydrodynamic volume or rather molar mass	✓	✘
LAC, GPEC	… adsorption and partition interactions between polymer and stationary/ mobile phases	✓	✓
LCCC, Gradient elution at the CPA	… chemical composition at critical conditions (molar mass invisibility)	✘	✓
PLC	… different solubilities of the macromolecules	✓	✓
TGIC	… changing system parameters through temperature gradients	✓	✓

Before any polymer HPLC techniques can be applied, dissolving the polymer sample is an important aspect. Thus, the choice of appropriate solvents for the corresponding polymer is crucial for introducing the complete polymer sample into the chromatographic system. Biopolymers or poly(ethylene glycols) are water soluble, but for PMMA, PVC, PDMS or PS stronger organic solvents as tetrahydrofuran (THF) are necessary to completely dissolve the polymer. In some cases, even elevated temperatures or very strong solvents like concentrated acids or protogenic alcohols (e.g. hexafluor-2-propanol) must be applied in

order to dissolve for example polyamides [3,5,56,57]. Moreover, especially polymers of high molecular masses require prolonged dissolution times before a representative sample injection becomes possible [58].

In contrast to SEC, the elution mechanisms in ideal polymer HPLC separate the polymer components according to molar mass and chemical functionality, unaffected by pore size effects or hydrodynamic volume. Furthermore, unlike in SEC, the column dead time or solvent peak marks the starting point of the separation and much more than one column volume is crucial for an appropriate separation. Therefore, the analytes show a different elution order, from low to high molar mass compounds and different chemical functionalities or structures (**Figure 2.8**). In addition, different stationary phases with smaller column lengths and chemical modified silica particles are typically used. As in HPLC of small molecules, analytical columns are used with lengths between 50 and 250 mm and diameters between 2 and 5 mm. Moreover, the same chemical functionalized stationary phases are used in polymer HPLC [27,59–69].

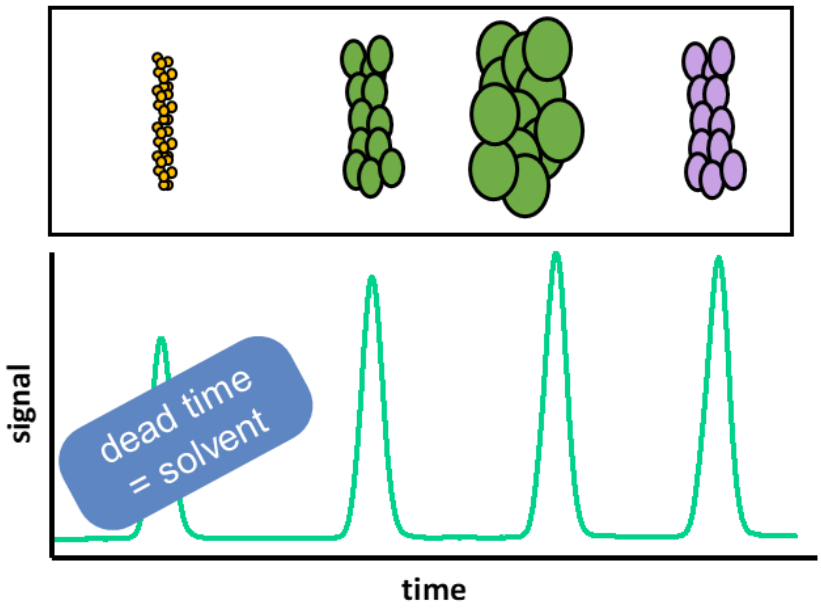

Figure 2.8: *Polymer-HPLC retention mechanism in relation to a chromatogram, the first peak is the dead time or solvent peak, afterwards macromolecules are separated according to molar mass and chemical functionality, whereby for homopolymers an increasing retention time corresponds to an increasing molar mass.*

Comparing isocratic and gradient elution techniques, in polymer HPLC gradients are preferred accelerating the measurement and improving the separation by minimizing band broadening effects [5,28,70,71]. Consequently, in the following section a typical gradient elution is summarized and discussed.

Based on the sample solvents, injection and gradient elution in polymer HPLC (GPEC) follow a different routine compared to HPLC of small molecules. **Figure 2.9** depicts a schematic overview of the major steps in GPEC: Before any sample is introduced on the stationary phase (a) a suitable non-solvent or rather weak solvent, in the following referred to as adsorption promoting solvent, is used for conditioning the column (b). In this surrounding, the injected polymer sample precipitates or is strongly adsorbed on top of the column (c) and the solvent plug originally dissolving the polymer is flushed through the system. Then, a gradient with a second stronger polymer solvent, the desorption promoting solvent, is started to separate the different polymer components (d). After the separation the column is thoroughly flushed with a strong solvent (e) in order to remove remaining polymer components and in a last step the column is re-conditioned to the initial conditions (f) [72–74].

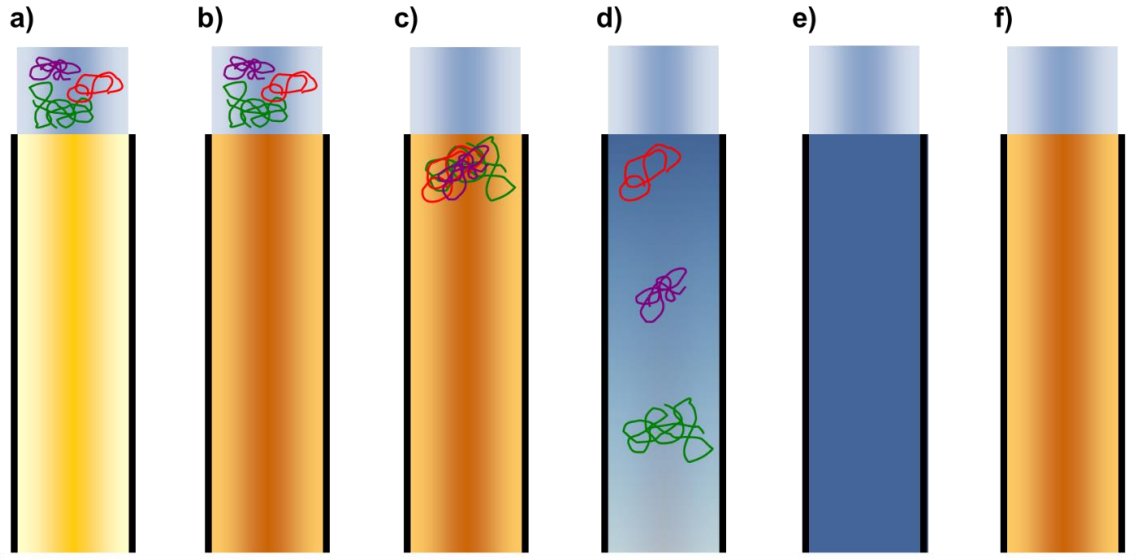

Figure 2.9: *Schematic overview of the major steps in GPEC:* ***a)*** *dissolved polymer sample and unconditioned stationary phase;* ***b)*** *conditioning of stationary phase with weak or non-solvent (adsorption promoting solvent) concerning the investigated polymers;* ***c)*** *injection of the sample on the column – precipitation thereof on top of the column;* ***d)*** *gradient elution through programmed increase of a strong solvent for the polymer (desorption promoting solvent);* ***e)*** *flushing of the column with 100 % desorption promoting solvent;* ***f)*** *re-conditioning of the stationary phase to initial conditions (b).*

Similar to the above-mentioned stationary phases, classical HPLC detectors as diode array detectors (DAD) or fluorescence detectors (FLD) [75,76] are used in polymer HPLC, if the polymer contains chromophoric or fluorescent groups. Furthermore, for isocratic elution, RI detectors may also be used for polymer detection. If neither chromophore groups are present, nor isocratic elution is possible, further much more universal detection principles are inevitable [35]. Applying an Evaporative Light Scattering Detector (ELSD) to polymer analysis allows the use of a gradient protocol and the detection of macromolecules without any functional groups [77–80]. A detailed review of the operation principles and detector optimization is

given in chapter 5, and, thus only basic principles are discussed in this section. For an adequate light scattering detection, at first the LC effluent is nebulized and in a subsequent step the mobile phase is evaporated (**Figure 2.10**). These steps are decisive for the sensitivity and robustness of the whole detection process. Since its invention by Charlesworth [81], the ELSD was optimized to overcome the problems in the detection process and a broad variety of devices were developed by several manufacturers [77]. But nevertheless, some limitations applying ELS detection still remains, for example volatile, vaporable compounds are evaporated with the mobile phase and thus, cannot be detected or clog the concentric nebulizer. A major drawback of the ELSD is its non-linear response which can be approximated by a quadratic calibration curve or more precisely by log-log-transformation of a power function calibration but makes data evaluation tedious. Apart from this, the used mobile phase must be completely volatile within the instrumental settings of the ELSD [78].

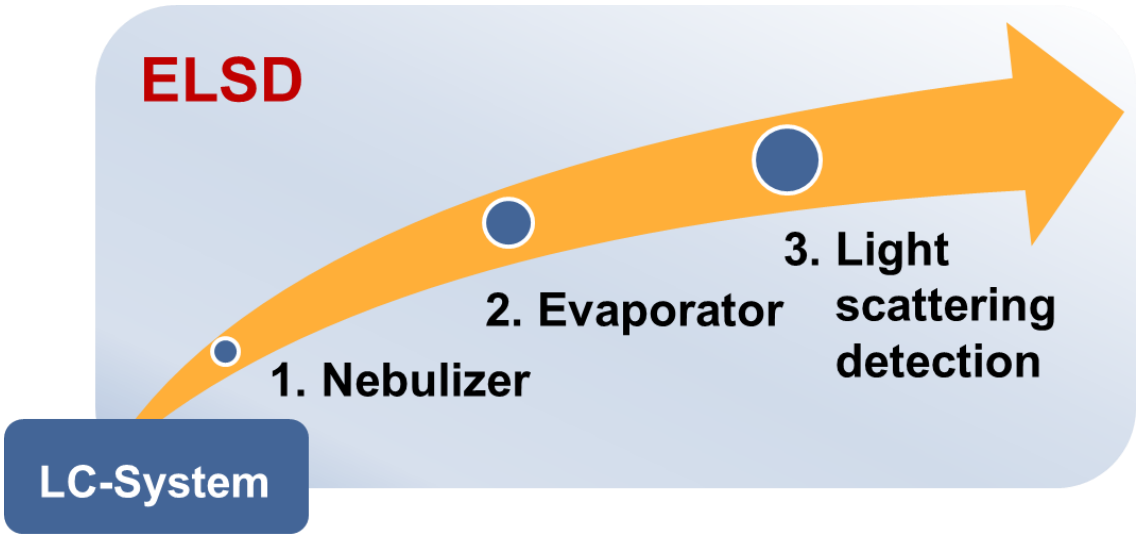

Figure 2.10: *Detection principle of an ELSD, based on three crucial steps – nebulization, evaporation, light scattering detection.*

Adapted from principles of ELSD, a Charged Aerosol Detector (CAD) was invented using a corona discharge needle for transferring charge on dried aerosol particles (**Figure 2.11**). Nitrogen ions are generated by a N_2 gas flow at the corona needle. Afterwards, the nitrogen ions collide with the dried analyte particles in a mixing chamber and a charge transfer to the analyte particle occurs [78,82,83]. Finally, the charged analyte ions hit a perforated plate and are counted by an electrometer measuring the resulting current flow as signal. Compared to ELSD, CAD showed an increased sensitivity and therefore an improved detection limit. However, CAD is more affected by polymer matrix effects and interferences by inappropriate mobile phases, e.g. high amounts of semi- or non-volatile buffer [78]. A further development of the ELSD setup is the condensation nucleation light scattering detector (CNLSD) applying

dried aerosol particles as nucleation sites for liquid condensation. In this process, the aerosol particle size can increase from several nanometers up to 10 µm and, thus, allow a more sensitive light scattering measurement. The major constraints of this detector type are the limited commercial availability of the device and the lack of software drivers for chromatography data systems [78,79].

Figure 2.11: *Overview of the main functional aspects of a CAD.*

Aside from these particle detectors, mass spectrometry can also be applied for detecting synthetic macromolecules. Several applications for polymer LC-MS detections are published in [84,85], but unfortunately uncharged polymers can only be analyzed up to molar masses of 4,000 g·mol^{-1}. Another possibility is matrix assisted laser desorption ionization (MALDI)- MS. One major constraint thereof is the limited possibility in direct coupling to heart cut HPLC because the elution volume of a single peak is normally much larger than the volume which can be directly pipetted on a MALDI target. However, MALDI-MS allows oligomer resolved polymer detection up to a m/ z- ratio of 30,000 [86–88].

Summarizing, **Figure 2.12** shows the most important influencing factors on a successful separation of macromolecules with polymer HPLC. The interaction of mobile phase, stationary phase, and the specific polymer determine the prevailing separation mechanism. For this reason, in the following sections the three main operation modes of polymer HPLC are presented: LAC and PLC are compared with each other and a short overview about gradient temperature interaction chromatography (TGIC) is given which can be an interesting alternative to common LC techniques.

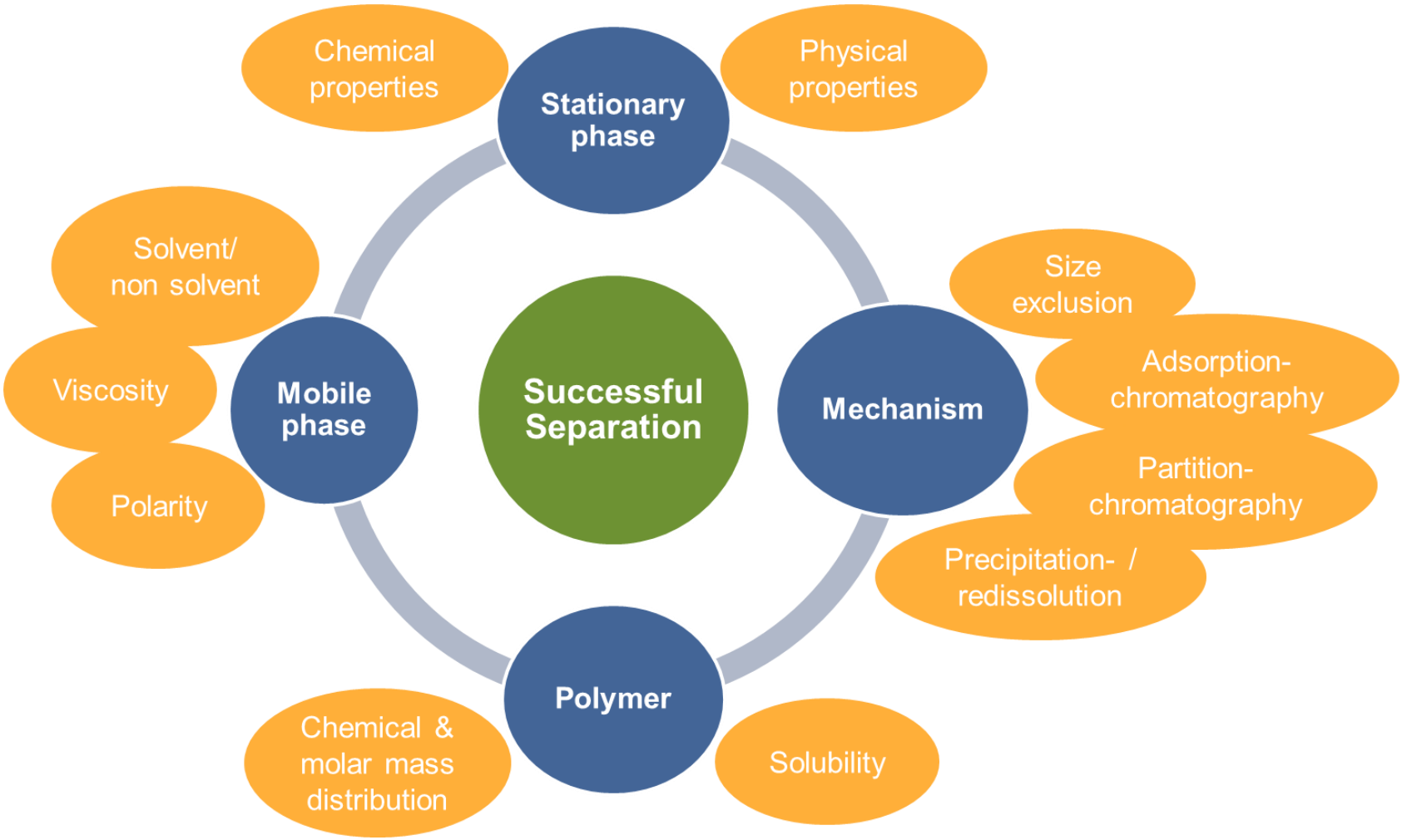

Figure 2.12: *Overview of influence influencing factors for a successful separation in polymer HPLC.*

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2.5.1 Liquid adsorption chromatography

Most of the applications of LAC are performed by gradient elution, because when applying isocratic separation mode, the polymer can irreversibly be adsorbed to the stationary phase. Consequently, LAC is usually performed with mobile phases containing at least two different solvents: an adsorption promoting solvent which is itself a weak or even non-solvent for the polymer and a desorption promoting solvent dissolving the complete polymer sample. In general, with increasing retention time, the molar mass of the oligomers or rather the polymers increase. Particularly for low molecular weight macromolecules, a separation of single oligomers can be achieved by appropriately adjusting of mobile and stationary phases while polymers with increasing molar mass are more or less unresolved or results in broad peaks (**Figure 2.13**). Varying the gradient slope enables the adjustment of the adsorption area to the separation problem. Depending on the CPA, gradient LAC can be performed up to 10 kDa, 100 kDa or 1 MDa. Hence, in contrast to LCCC, reaching the CPA should be avoided for improving the separation [4,5,28].

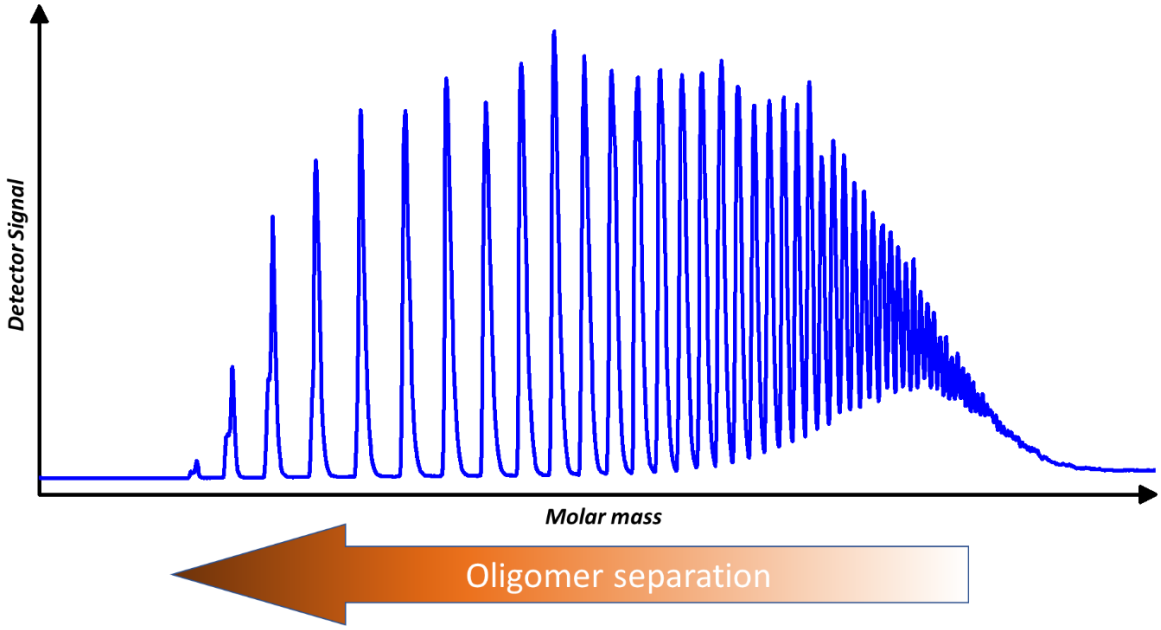

Figure 2.13: *Degree of oligomer separation in LAC in relation to molar mass.*

Compared to HPLC of small molecules, several differences, i.e. small diffusion coefficients of dissolved polymers and nearly the same dimensions of the polymers to the pore sizes of the stationary phase, completely change the chromatographic process resulting in different retention characteristics for macromolecules. Moreover, as long as at least one repetition unit of the polymer backbone is adsorbed to the column the entire polymer is adsorbed. Compared to typical biomolecules in HPLC of small molecules, the polymer solubility is considerably increased and therefore secondary interactions as well

as peak broadening effects through delayed desorption occur [2,3]. Moreover, in LAC the impact of the stationary phase on the separation is significant: Changing the column has an effect on the retention of the polymer. In general, various separation mechanisms (cf. **Figure 2.12**) superimpose each other in real chromatographic systems. Therefore, one further very important mechanism is discussed in the subsequent section.

2.5.2 Precipitation-/ re-dissolution chromatography

The influence of precipitation-/ re-dissolution effects on the separation of polymers was introduced as high performance precipitation liquid chromatography (HPPLC) and applied by Glöckner et al [3,89–95]. As generally described for the injection in GPEC (**Figure 2.9**), in HPPLC or precipitation-/ re-dissolution chromatography (PLC) the polymer precipitates on top of the column. In contrast to LAC, the gradient elution is driven by sequential precipitation and re-dissolution of different polymer components while increasing the content of the stronger solvent for the polymer. The whole process is exclusively driven by solubility effects of the macromolecules (”similia similibus solvuntur”) with changing mobile phase compositions. Staal [72] described the relation between polymer cloud points and the critical elution conditions for the macromolecular sample compounds by reversed phase liquid chromatography. Therefore, the elution order is the same as in LAC: The molar mass increases with increasing retention times. Furthermore, depending on the solubility of the polymer in the appropriate composition of the mobile phase, polymer retention times are independent of the stationary phase [28,72]. As a result thereof, elution at the CAP is impossible in LPC and cannot restrict the separation in the high molecular range [28]. A detailed discussion by German et al [96–98] shows the difficulty in differentiating between LAC and PLC in real chromatography systems. Therefore, they analyzed polyester resins with various stationary phases and showed that, except for crystalline polyester, adsorption chromatography is the dominating effect on separation. Concluding, a true precipitation-/ re-dissolution mechanism occurs rarely in real chromatographic systems [98]. Consequently, polymer HPLC is often operated by a combination of PLC and LAC, while sorption effects predominate in most cases.

2.5.3 Temperature gradient interaction chromatography

A further alternative of polymer HPLC method is called TGIC and was introduced by Chang et al [99,100] by applying a temperature gradient. They separated a mixture of 10 PS standards over a molar mass range from 1,700 to 2,890,000 $g \cdot mol^{-1}$ with a temperature gradient from 0 to 44 °C. Hence, they used a water bath circulator for temperature programming of the column. Moreover, the application to other polymers like PMMA was demonstrated in [101] by a temperature gradient from 10 to 60 °C. With this particular technique, copolymer mixtures or polymers exclusively differing in their molecular architecture are separated [102–104]. The applicability to star shaped or branched PS [105–108] was also demonstrated and even coupling to MALDI mass spectrometry was reported [109–111]. Hutchings [107] reviewed the application of TGIC to byproduct analysis of polymer reactions and summarized miscellaneous examples of the capability of TGIC for microstructure analysis in comparison to SEC. Furthermore, temperature programming can be used to skip between the three main LC modes, LAC, LCCC, and SEC without changing other system parameters [112]. Therefore, TGIC represents an additional powerful alternative in polymer HPLC analysis [28,35]. However, one major drawback is the need to use additional equipment for accurate temperature control and suitable hard- and software implementation.

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3 Experimental

3.1 Chemicals and materials

All used solvents were HPLC grade. Acetone, acetonitrile (ACN), 2-butanol, methanol (MeOH), ethanol, isopropanol, toluene, n-hexane and non-stabilized tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. As cationization reagent sodium trifluoroacetate (Sigma-Aldrich, Darmstadt, Germany) was used and trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) (Sigma-Aldrich) was used as MALDI matrix. All used stationary phases and polymer standards, or samples are separately stated in the appropriate chapters.

3.2 Measurements

For all measurements, a detailed description is given in each chapter. All used instruments were maintained regularly and passed the operational and performance quality control as recommended from the manufacturers.

3.2.1 HPLC

All measurements were performed with the chromatography software package Chromeleon (version 7.2, Thermo Fisher Scientific, Waltham, USA) except from the preparative fractionation of linear and cyclic PDMS in chapter 4, where Open Lab CDS C.01.08 (Agilent, Waldbronn, Germany) was used as software. A part of the investigations on analytical scale was performed on a 1100 series LC System of Agilent with a tetrahydrofuran-resistant 3215α degasser from ERC (Riemerling, Germany) and a 385 ELSD of Agilent equipped with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) (PTFE) nebulizer from Burgener Research (Mississauga, Ontario, Canada) at optimized conditions of 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.2 SLM (standard liter per minute) gas flow (see chapter 5), unless otherwise mentioned. The other part of the measurements was performed with an Ultimate 3000 HPLC system (Thermo Fisher Scientific) equipped with a diode array detector and a modified (PTFE nebulizer) 385 ELSD of Agilent. Preparative HPLC measurements based on linear gradients were performed on an Agilent 1260 Infinity II LC system equipped with a TCC 6000 PSS (Mainz, Germany) column oven, a tetrahydrofuran-resistant PSS degasser, and an Agilent 35900 E analogue/ digital converter and a PL ELS 1000 as detector, unless otherwise mentioned. For fraction collection an Agilent

1260 Infinity II fraction collector equipped with a 40-Funnel-tray (Agilent) and a self-made bottle connector container (**Figure 3.1**) was used.

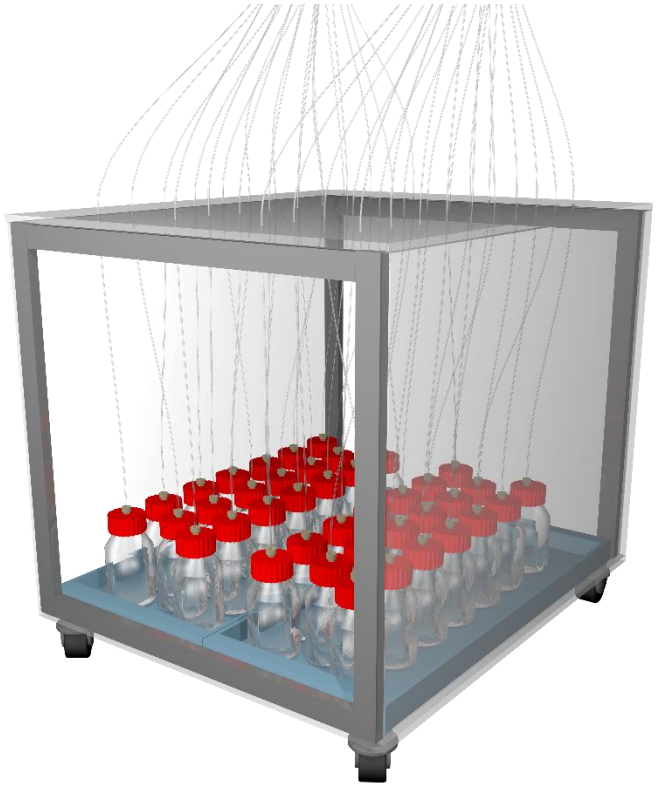

Figure 3.1: *Self-made bottle connector container for up to 40 bottles with various individual bottle volumes. The upturned small tubes are connectable to an Agilent 40-Funnel-tray.*

3.2.2 SEC

All SEC measurements were performed with the software package WinGPC UniChrom (version 8.2, Polymer Standard Services, Mainz, Germany). For all analytical measurements PSS SECcurity GPC1200 systems equipped with RI detectors or coupled to a Shodex (Munich, Germany) RI101 detector. For preparative analysis a PSS SECcurity GPC1200 system equipped with an Agilent 1260 fraction collector or an Agilent 385 ELSD was used.

3.2.3 MALDI-ToF-MS

For polymer analysis a Shimadzu Axima Performance MALDI-ToF-MS (Kratos, Manchester, UK) was used. Measurement control and data evaluation were performed with the software package Shimadzu Biotech MALDI MS (version 2.9, Kratos, Manchester, UK).

4 Separation of linear and cyclic poly(dimethylsiloxanes)

4.1 Separation of linear and cyclic poly(dimethylsiloxanes) with polymer high performance liquid chromatography – Part I

This chapter has been published and adapted from:

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik: in Proceedings of the 14th ISC Conference „Modern Analytical Chemistry": Prague, Karel Nesmerak (ed.), 2018, ISBN 978-7444-059-5.

Measurements and experiments were done solely by the author. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

4.1.1 Abstract

Due to their attractive properties, siloxanes have found many applications in various industrial areas, e.g. cosmetics, health care or construction industries are present in recent years. Therefore, a method for separation of linear and cyclic PDMS, applying liquid chromatographic techniques was developed and optimized. By interactive chromatography, oligomer resolution and separation of linear from cyclic PDMS could be achieved for PDMS with up to 30 monomeric units. Results of investigations of the underlying separation mechanism pointed out that a combination of fractionated-re-dissolution and adsorption effects primarily depending on the adequate choice of the eluent system was essential.

4.1.2 Introduction

Siloxanes are used in a broad variety for different application areas. In general, siloxanes consist of alternating silicone-oxygen bonds in the backbone and different types of functional groups. An important class of siloxanes is poly(dimethylsiloxane) (PDMS) containing only methyl and methylene groups bounded to the polymer backbone. The basic notation of PDMS depends on the nominal number of oxygens bonded to silicon: The basic building blocks M, D, T and Q present one, two, three or four oxygen(s) bonded to silicone, respectively. Therefore, the molecular architecture is clearly defined by the nomenclature, e.g. D4 stands for the cyclic tetramer [1–4].

The unique characteristics of siloxanes, like high flexibility in their backbone, low intermolecular forces between methyl groups or low surface energies make applications in cosmetics, medicine as well as in construction industries very attractive. Especially in case of PDMS, the usage in release agents, antifoams, heat transfer liquids or coatings demonstrate the importance of this type of polymer [5,6]. Concerning applications in pharmaceuticals or medical care products, comprehensive analytical methods are necessary. Therefore, investigations of low molecular weight oligomer, linear and cyclic PDMS are mainly done with gas chromatography [7,8]. Moreover, linear and cyclic PDMS can also be separated with liquid chromatography at critical conditions (LCCC), where the separation only depends on chemical functionalities [9]. A major drawback of LCCC is the high susceptibility to small changes in analytical conditions, e.g. mobile phase composition, temperature changes or small variations of the investigated polymer sample [10]. Apart from LCCC, interactive chromatography, focusing on differences in the chemical structure of macromolecules, is an appropriate alternative. Compared to conventional HPLC, peculiarities like small diffusion coefficients in solution, reduced solubility or a more complex retention mechanism on the stationary phase, occur. Thus, polymer elution is controlled by different types of interactions of various separation mechanisms, caused by adsorption, partition or solubility effects. Consequently, optimizing various parameters in method development, e.g. choice of mobile and stationary phase, LC flow rate, temperature, are necessary for explaining the main separation mechanism [11–13]. The present contribution is concerned with corresponding method developments.

4.1.3 Material and methods

4.1.3.1 Reagents and chemicals

All solvents used were HPLC grade. Acetonitrile, acetone, methanol, ethanol, isopropanol, and non-stabilized tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. All used analytical stationary phases applied in this study are summarized in **Table 4.1**. For fraction collection of single linear and cyclic oligomers a Thermo-Fisher (Waltham, USA) Accucore C30 (150x4.6 mm, 2.6 µm) was used. The used linear and cyclic PDMS samples were obtained from Wacker Chemie AG (Burghausen, Germany). As reference material for linear PDMS a silicone oil with a viscosity of 10 mPa·s and for cyclic PDMS a mixture of D8 – D17 was used.

4.1.3.2 Instrumentation

The investigations were performed on a 1100 series LC system of Agilent (Waldbronn, Germany) with a THF-resistant 3215α degasser from ERC (Riemerling, Germany) and a 385 ELSD of Agilent equipped with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. (Mississauga, Ontario, Canada) at 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.2 SLM (standard liter per minute) gas flow. All test measurements were done with a linear gradient from 100 % A to 100 % B in 40 min, unless otherwise stated. Changing column dimensions, the gradient parameters were adapted to obtain the same effective linear gradient. The final method development was done on an Accucore C30 (50x4.6 mm, 2.6 µm) at a LC flow rate of 2 $mL \cdot min^{-1}$ starting at (methanol: water (75/25, v/v)): acetone 50:50 and ending at 100 % acetone in 160 min. Applying silica beads the stepwise gradient was performed with 5 % step height, 5 min step length with water and acetone as eluent system.

Table 4.1: *Overview of investigated stationary phases for the separation of PDMS; columns were purchased by Agilent (Waldbronn, Germany), Macherey-Nagel (Düren, Germany), MicroSolv Technology Corporation (Leland, USA), Thermo-Fisher (Waltham, USA), Phenomenex (Aschaffenburg, Germany) and YMC (Dinslaken, Germany).*

No.	Manufacturer	Name	Particle type	Dimensions
1	Thermo-Fisher	Accucore C18	2.6 µm, 80 Å	100 x 4.6 mm
2	Thermo-Fisher	Accucore C8	2.6 µm, 80 Å	100 x 4.6 mm
3	Thermo-Fisher	Accucore C30	2.6 µm, 150 Å	50 x 4.6 mm
4	Phenomenex	Kinetex PFP	2.6 µm, 100 Å	100x4.6 mm
5	YMC	Carotenoid C30	3 µm, 80 Å	100x4.6 mm
6	Thermo-Fisher	Accucore C18 aQ	2.6 µm	100x4.6 mm
7	Agilent	Eclipse C18	5 µm, 80 Å	150x4.6 mm
8	Phenomenex	EVO C18	2.6 µm, 100 Å	100x4.6 mm
9	MicroSolv Technology	Cogent Bidentate C18	4.2 µm, 100 Å	150x4.6 mm
10	Macherey-Nagel	Nucleosil 100 C18	5 µm, 100 Å	125x4 mm
11	Macherey-Nagel	Nucleodur Pyramid C18	5 µm, 110 Å	150x4.6 mm
12	Thermo-Fisher	Hypersil BDS C18	2.4 µm, 120 Å	100x4.6 mm
13	Phenomenex	HyperClone BDS C18	5 µm, 130 Å	150x4.6 mm
14	Thermo-Fisher	HyPurity C18	5 µm, 190 Å	150x4.6 mm
15	Macherey-Nagel	Nucleosil C18 EC	5 µm, 50 Å	100x4.6 mm
16	Macherey-Nagel	Nucleosil C18 EC	5 µm, 100 Å	100x4.6 mm
17	Macherey-Nagel	Nucleosil C18 EC	5 µm, 300 Å	150x4.6 mm
18	Macherey-Nagel	Nucleosil C18 EC	7 µm, 1000 Å	150x4.6 mm
19	Self-prepared	Silica beads	75 µm	50x7.0 mm

4.1.4 Results and discussion

4.1.4.1 Optimization of stationary phase

According to common literature for PDMS separation [9] with RP-Polymer-HPLC, acetonitrile as adsorption promoting solvent and THF as desorption promoting solvent were chosen in preliminary experiments. Thus, a C8 stationary phase was selected separating linear and cyclic PDMS (**Figure 4.1**).

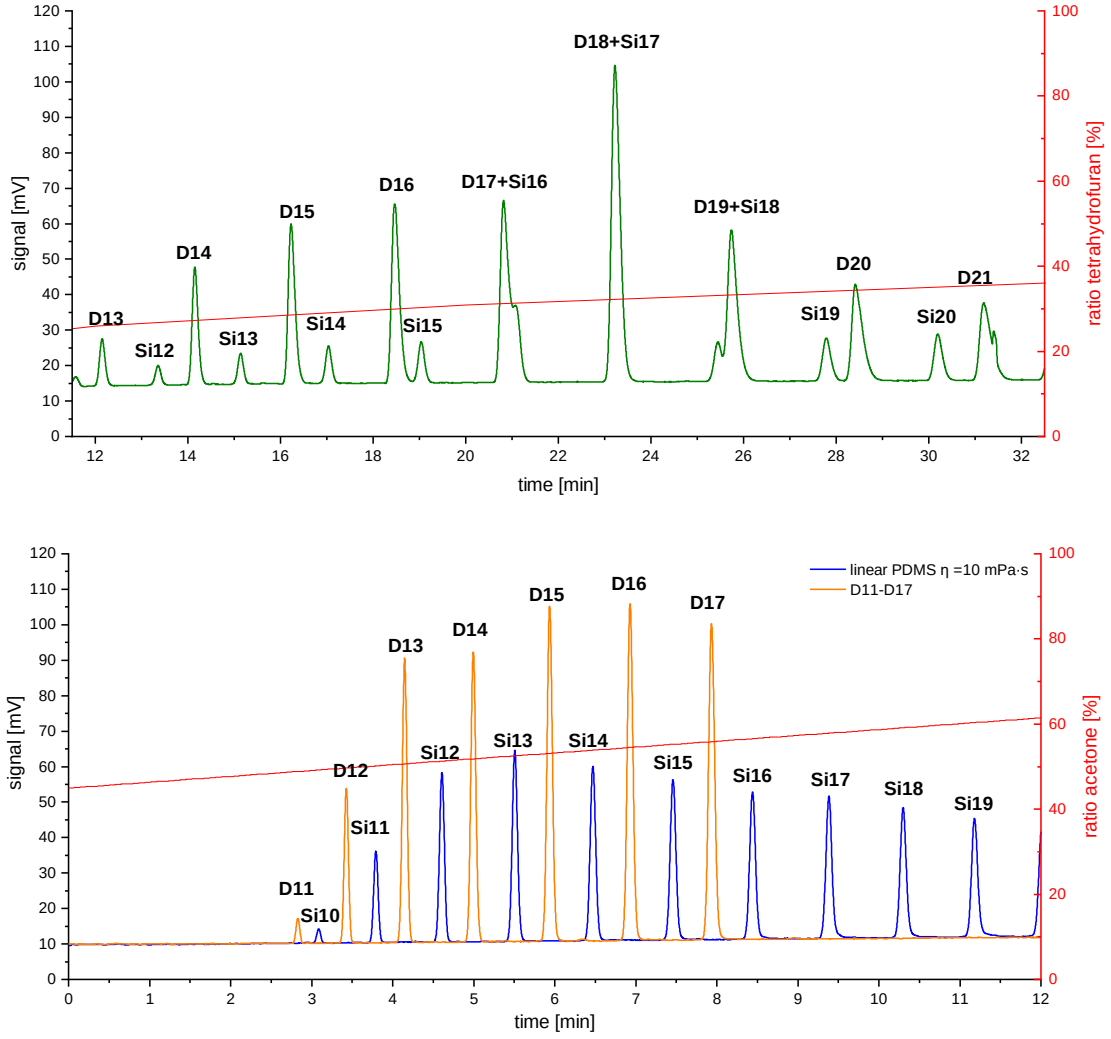

Figure 4.1: *Separation of linear and cyclic PDMS with* ***a)*** *acetonitrile/ THF on an Accucore C8 column (100x4.6 mm, 2.6 µm) and with* ***b)*** *methanol: water (75:25)/ acetone on a Kinetex PFP column (100x4.6 mm, 2.6 µm); cyclic PDMS is annotated as D plus monomoric number and linear PDMS is annotated as Si plus monomeric number.*

The separation performance of this system is limited by repeated peak overlap of linear and cyclic siloxanes. Following a classical HPLC approach, different stationary phases (cf. **Table 4.1**) were tested for improving the separation performance. With a pentafluorophenyl (PFP) column an improvement of the

separation could be achieved by replacing the adsorption promoting solvents from acetonitrile to an adequate mixture of methanol: water (75:25) – the triple bond of acetonitrile prevents the interaction of analyte and stationary phase. Finally, the determination could considerably be improved when using an Accucore C30 stationary phase in combination with the eluent system methanol: water/ acetone (see **Figure 4.2**).

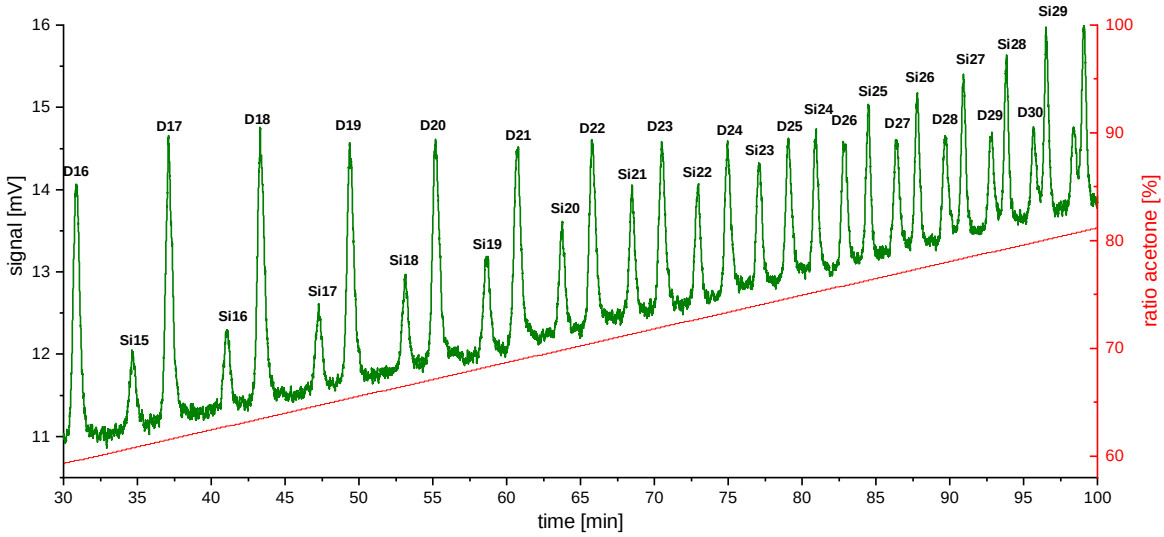

Figure 4.2: *Optimized separation of PDMS applying an Accucore C30 (50x4.6 mm, 2.6 µm) a LC flow rate of 2.0 mL·min^{-1}, methanol: water (75:25) as adsorption promoting solvent and acetone as desorption promoting solvent, the chromatogram in detail highlights the oligomeric separation of linear and cyclic oligomers up to 30 repetition units.*

4.1.4.2 Optimization of mobile phase composition

Using acetonitrile, methanol or water as adsorption promoting solvent and acetone, ethanol, isopropanol or THF as desorption promoting solvent and mixtures thereof, allowed the investigation of various solvent combinations while optimizing the stationary phase for separating PDMS. The choice of an appropriate mobile phase composition interfered with separation improvement in terms of polymer solubility in stationary and mobile phase. Consequently, the originally used eluent system for the PFP column considerably improved the analysis method on other more robust stationary phases, too, e.g. Accucore C30 (cf. **Figure 4.2**). This particular combination of stationary and mobile phases enabled an extended separation range, mainly caused by precipitation-re-dissolution and adsorption of the polymer at the column.

4.1.4.3 Explanation of separation mechanism

According to the aforementioned findings, a more detailed description of the predominant separation mechanism was possible. Particularly, when investigating low molecular weight PDMS (up to 3000 g·mol^{-1}) liquid adsorption chromatography is the prominent separation mode because separation efficiency was highly depending on the applied stationary phase. Apart from this, the significance of well-defined mobile phase composition suggested that an adsorption mechanism is superimposed by a mechanism of precipitation and re-dissolving. Further measurements were performed with a silica beads column (cf. **Figure 4.3**), which showed no useful HPLC separation due to absence of stationary phase modifications. Applying a stepwise gradient, the PDMS (viscosity of 10 mPa·s) polymeric distribution was measured primarily to fraction re-dissolution mechanism. Fractionated re-dissolving elution overlaying HPLC adsorption effects indicated the significance of mobile phase composition. With ideal settings, resolving various molecular weight oligomers become possible.

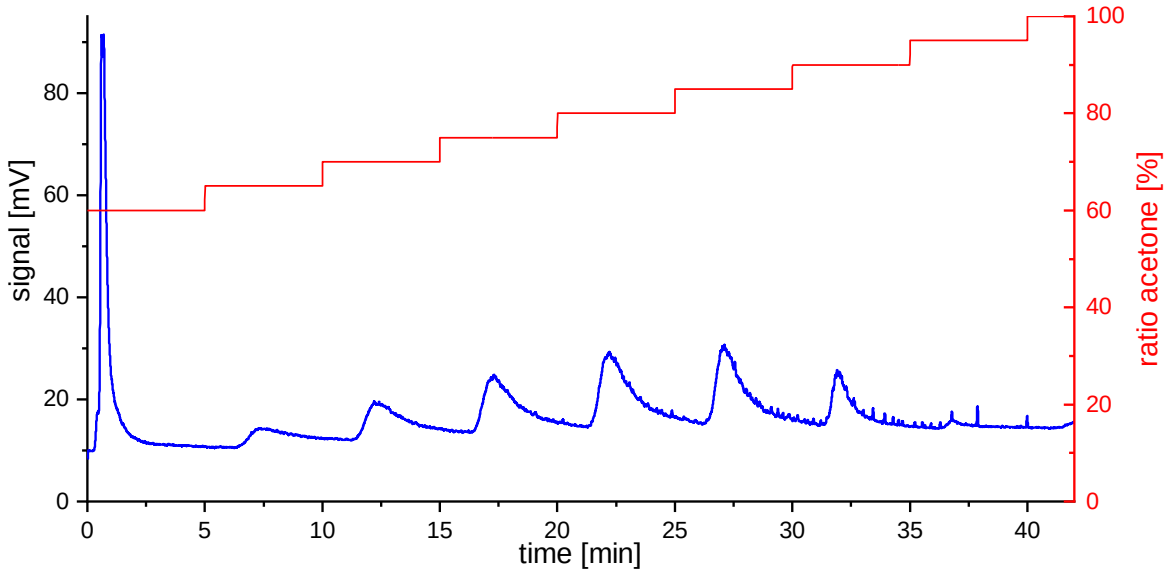

Figure 4.3: *Separation of a silicone oil with a viscosity of 10 mPa·s, containing only linear PDMS oligomers, when applying a stepwise gradient (step length 5 min and step height 5 %) using water and acetone as mobile phase on a silica beads (75 µm) column excluding HPLC adsorption effects of the stationary phase.*

4.1.5 References

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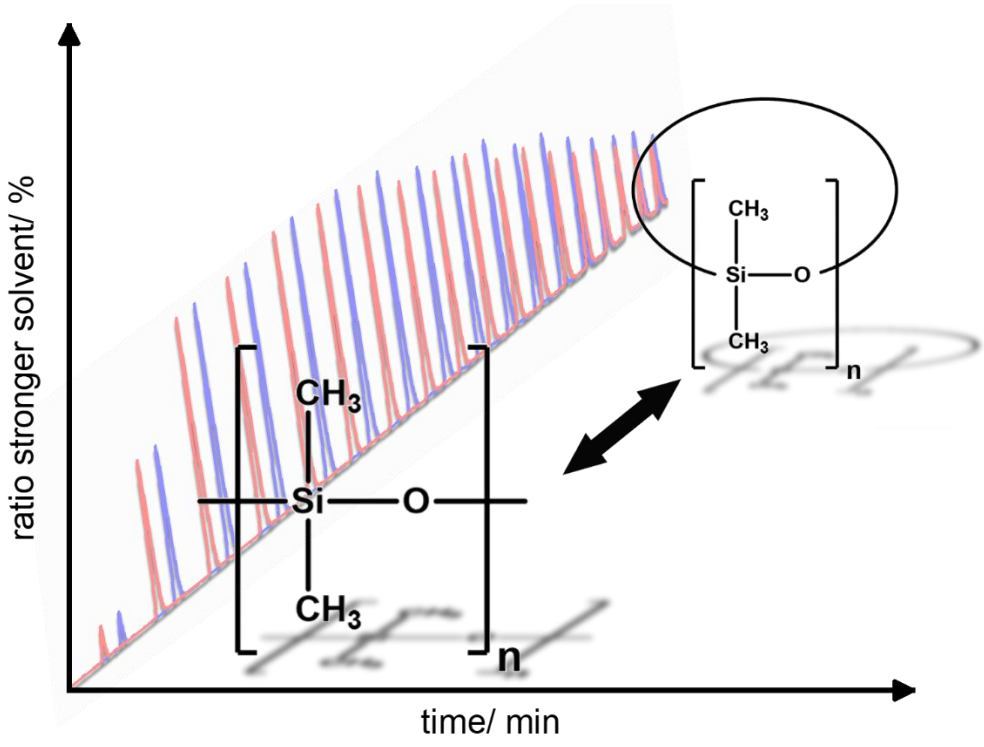
4.2 Separation of linear and cyclic poly(dimethylsiloxanes) with polymer high performance liquid chromatography – Part II



Graphical Abstract: *Separation of linear and cyclic PDMS.*

This chapter has been published and adapted from:

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik, Monatshefte für Chemie – Chemical Monthly, 2019, DOI:10.1007/s00706-019-02389-4.

Measurements and experiments were done solely by the author. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

4.2.1 Abstract

The growing importance of siloxanes in various industrial areas, e.g. health care, cosmetics, automotive and construction industries requires further method development of analysis techniques. In addition, and complementing gas chromatography analysis, a polymer liquid chromatography method for separation of linear and cyclic (poly)dimethylsiloxanes was developed and optimized. By an appropriate choice of mobile and stationary phase combinations, separations up to 30 monomeric units are achieved. Therefore, various HPLC columns were investigated concerning physical and chemical properties, e.g. pore size, silica base material, and column functionality. Furthermore, solubility properties of siloxanes in adsorption and desorption promoting solvents were investigated and taking these results into account the separation was optimized applying a mixture of methanol: water (75:25, v/v) and acetone. The findings indicate, that precipitation / re-dissolution effects superimposed by adsorption chromatography result in the oligomer separation of up to 30 monomeric units. Besides method development on an analytical scale, linear poly(dimethylsiloxane) oligomers were separated with preparative polymer HPLC. These fractions of single oligomers allow further investigations of different material properties beyond polymer HPLC.

4.2.2 Introduction

Siloxanes show a broad variety of application areas, because of their exceptional polymer structure. Various applications in cosmetics, medicine, automotive or construction industry depend on low intermolecular forces between methyl groups, high flexibility in the polymer backbone or low surface energies. The usage in antifoams, shock absorbers or release agents only depicts some examples [1, 2]. In this study, the important class of poly(dimethylsiloxanes) (PDMS) are separated according to its molecular architecture. The PDMS nomenclature depends on the nominal number of oxygen atoms bonded to silicon: the basic building blocks M, D, T, and Q represent one, two, three or four oxygen(s) bonded to silicon, respectively. Consequently, cyclic PDMS are unambiguously described by the amount of [D]-building blocks, e.g. D4 stands for the cyclic tetramer octamethylcyclotetrasiloxane [3, 4]. Additionally, in this publication the short cut "Si" is used with the appropriate number of oligomers as label for linear PDMS oligomers.

Especially for low molecular weight (up to 8 [D]-blocks) linear and cyclic PDMS analysis is predominantly performed with gas chromatography [5, 6]. But with increasing molecular weight liquid chromatographic techniques such as size exclusion chromatography (SEC), liquid chromatography at critical conditions (LCCC) or polymer HPLC become more favorable. SEC provides separation according to hydrodynamic volume or rather molar mass of the investigated polymers. This analytical method is primarily based on changes of entropic interactions of the polymer with the separation system. Therefore, typical SEC stationary phases consist of particles with different pore diameters for achieving successful separation due to differences in molecular size, but any chemical interaction between polymer and stationary phase must be prevented [7–9]. In LCCC, separations solely according to chemical functionalities of polymers are possible. Therefore, enthalpic and entropic energy changes must balance each other for a separation independent of molar mass effects. However, this technique is typically applied for higher molecular weight masses in contrast to the separation of linear and cyclic PDMS oligomers up to 30 [D]-block units as required in this study. Moreover, LCCC compromises some challenges, as e.g. high susceptibility to small changes in analytical conditions or small variations of the investigated polymer sample [12].

In polymer HPLC, the separation is generally based on molar mass differences as well as on variation of chemical functionalities. Compared to HPLC of small molecules, the major differences are small diffusion coefficients and reduced solubility of polymers in solution. Apart from this, polymer elution may occur due to several different separation mechanisms, like adsorption or precipitation / re-dissolution chromatography. The main distinctive feature is the injection of a polymer sample: Assuming an impaired

polymer solubility, the well dissolved sample is injected at a stationary phase pre-conditioned with a typically very weak (or so-called adsorption promoting) solvent. Thus, subsequent to polymer injection, precipitation or at least very strong adsorption on top of the column takes place. Consequently, a programmed gradient is usually used to elute the withheld sample, driven by increasing amounts of desorption promoting solvent [7, 14–17].

The choice of a suitable detector for PDMS is limited because of the lack of chromophores and the need of gradient elution. Therefore, an evaporative light scattering detector (ELSD) is a good choice, permitting gradient elution and a universal detection. Mojsiewicz-Pieńkoswka [22] already described the application of this detection technique for the analysis of PDMS with SEC. Based on these investigations, Durner et al. [13] optimized the detector performance for linear and cyclic PDMS. In this study, method development and optimization for separation of linear and cyclic PDMS oligomers with polymer HPLC is shown.

4.2.3 Material and methods

4.2.3.1 Polymer standards, mobile and stationary phases

All solvents used were HPLC grade. Acetonitrile, acetone, 2-butanol, methanol, and non-stabilized tetrahydrofuran were purchased from Merck (Darmstadt, Germany) and used without further purification. Water were obtained from a Milli-Q-Advantage A10 water system (Merck Millipore). All applied poly(dimethylsiloxane) standards were obtained from Wacker Chemie AG (Burghausen, Germany). The used stationary phases for method development and analytical as well as preparative measurements are summarized in **Table 4.2**. For preparative analysis a mixture of silicone oils with viscosities of 5:10:20 mPa·s at a mixing ratio of 1:1:4 without any solvent dilution, was used.

Table 4.2: *Overview of examined stationary phases for PDMS separation; as annotated the columns were purchased from Agilent (Waldbronn, Germany), Macherey-Nagel (Düren, Germany), MicroSolv Technology Corporation (Leland, USA), Thermo-Fisher (Waltham, USA), Phenomenex (Aschaffenburg, Germany) and YMC (Dinslaken, Germany).*

Manufacturer	Name	Particle type	Dimensions
Thermo-Fisher	Accucore C4	2.6 µm, 150 Å	100 x 4.6 mm
Thermo-Fisher	Accucore C8	2.6 µm, 80 Å	100 x 4.6 mm
Thermo-Fisher	Accucore C18	2.6 µm, 80 Å	100 x 4.6 mm
Thermo-Fisher	Accucore C30	2.6 µm, 150 Å	50 x 4.6 mm
Phenomenex	Kinetex PFP Series: 619128-6 Series: 532053-72 Series: 525802-13	2.6 µm, 100 Å	100 x 4.6 mm
Phenomenex	Kinetex F5 Series: 761360-10 Series: H16-372649	2.6 µm, 100 Å	100 x 4.6 mm
Phenomenex	Luna PFP (2)	5 µm, 100 Å	150 x 4.6 mm
Agilent	Poroshell PFP	2.7 µm, 120 Å	100 x 4.6 mm
YMC	Carotenoid C30	3 µm, 80 Å	100 x 4.6 mm
Thermo-Fisher	Accucore C18 aQ	2.6 µm, 80 Å	100 x 4.6 mm
Agilent	Eclipse C18	5 µm, 80 Å	150 x 4.6 mm
Phenomenex	EVO C18	2.6 µm, 100 Å	100 x 4.6 mm
MicroSolv Technology	Cogent Bidentate C18	4.2 µm, 100 Å	150 x 4.6 mm
Macherey-Nagel	Nucleosil 100 C18	5 µm, 100 Å	125 x 4 mm
Macherey-Nagel	Nucleodur Pyramid C18	5 µm, 110 Å	150 x 4.6 mm
Thermo-Fisher	Hypersil BDS C18	2.4 µm, 120 Å	100 x 4.6 mm
Phenomenex	HyperClone BDS C18	5 µm, 130 Å	150 x 4.6 mm
Thermo-Fisher	HyPurity C18	5 µm, 190 Å	150 x 4.6 mm
Macherey-Nagel	Nucleosil C18 EC	5 µm, 50 Å	100 x 4.6 mm
Macherey-Nagel	Nucleosil C18 EC	5 µm, 100 Å	100 x 4.6 mm
Macherey-Nagel	Nucleosil C18 EC	5 µm, 300 Å	150 x 4.6 mm
Macherey-Nagel	Nucleosil C18 EC	7 µm, 1000 Å	150 x 4.6 mm
Thermo-Fisher	Accucore C30	2.6 µm, 100 Å	150 x 4.6 mm

4.2.3.2 Instrumentation

All investigations on analytical scale were performed on a 1100 series LC System of Agilent (Waldbronn, Germany) with a tetrahydrofuran-resistant 3215α degasser from ERC (Riemerling, Germany) and a 385 ELSD of Agilent equipped with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research (Mississauga, Ontario, Canada) at optimized conditions [13] of 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.2 SLM (standard liter per minute) gas flow, unless otherwise mentioned. Chromeleon 7.2 was used as chromatography software for all measurements. All optimization measurements were performed from 100 % A to 100 % B in a linear gradient of 40 min and a LC flow rate of 1.0 mL·min^{-1} for column dimensions of 100x4.6 mm, 2.6 µm particles, unless otherwise mentioned. For all analytical stationary phases with different dimensions, the gradient settings were adapted to obtain the same effective linear gradient (40 min at 100x4.6 mm column). The optimized method for analytical separation of linear and cyclic PDMS was performed on an Accucore C30 (50x4.6 mm, 2.6 µm) with a linear gradient starting at (methanol: water (75:25, v/v)): acetone 50:50 and ending at 100 % acetone in 160 min and a LC flow rate of 2.0 mL·min^{-1}.

Preparative HPLC measurements were performed on an Agilent 1260 Infinity II LC system equipped with a TCC 6000 PSS (Mainz, Germany) column oven, a tetrahydrofuran-resistant PSS degasser, and an Agilent 35900 E analogue/ digital converter and a PL ELS 1000 as detector. For fraction collection an Agilent 1260 Infinity II fraction collector was used. Open Lab CDS C.01.08 was used as chromatography software. A mixture of methanol: water (75:25, v/v) was used as adsorption promoting solvent and acetone as desorption promoting solvent. The used gradient settings are summarized in **Table 4.3**. For monitoring the purity of the collected fractions, a Bruker (Bremen, Germany) amazon SL ion trap liquid chromatography mass spectrometer, equipped with an electrospray ionization interface, was used.

Table 4.3: *Gradient program for preparative separation of a 1:1:4 mixture of linear PDMS with viscosities of 5, 10, and 20 mPa·s on an Accucore C30 (150x4.6 mm) with methanol: water (75:25, v/v) as solvent A, acetone as solvent B, and THF as flush solvent C.*

time/ min	solvent A/ %	solvent B/ %	solvent C/ %	LC flow rate/ mL·min^{-1}
0.00	50.0	50.0	0.0	2.0
40.00	17.0	83.0	0.0	2.0
90.00	7.0	93.0	0.0	2.0
90.50	0.0	0.0	100.0	2.0
96.00	0.0	0.0	100.0	2.0
96.01	50.0	50.0	0.0	1.0
97.00	50.0	50.0	0.0	2.0
103.00	50.0	50.0	0.0	2.0

4.2.4 Results and discussion

Alkyl chain stationary phases in combination with acetonitrile as adsorption promoting and tetrahydrofuran (THF) as desorption promoting solvent are used for PDMS analysis, based on common reversed phase polymer HPLC [10, 11]. Hence, method development was started comparing four stationary phases with different alkyl chain length (C4 **(a)**, C8 **(b)**, C18 **(c)**, C30 **(d)**, see **Figure 4.4**).

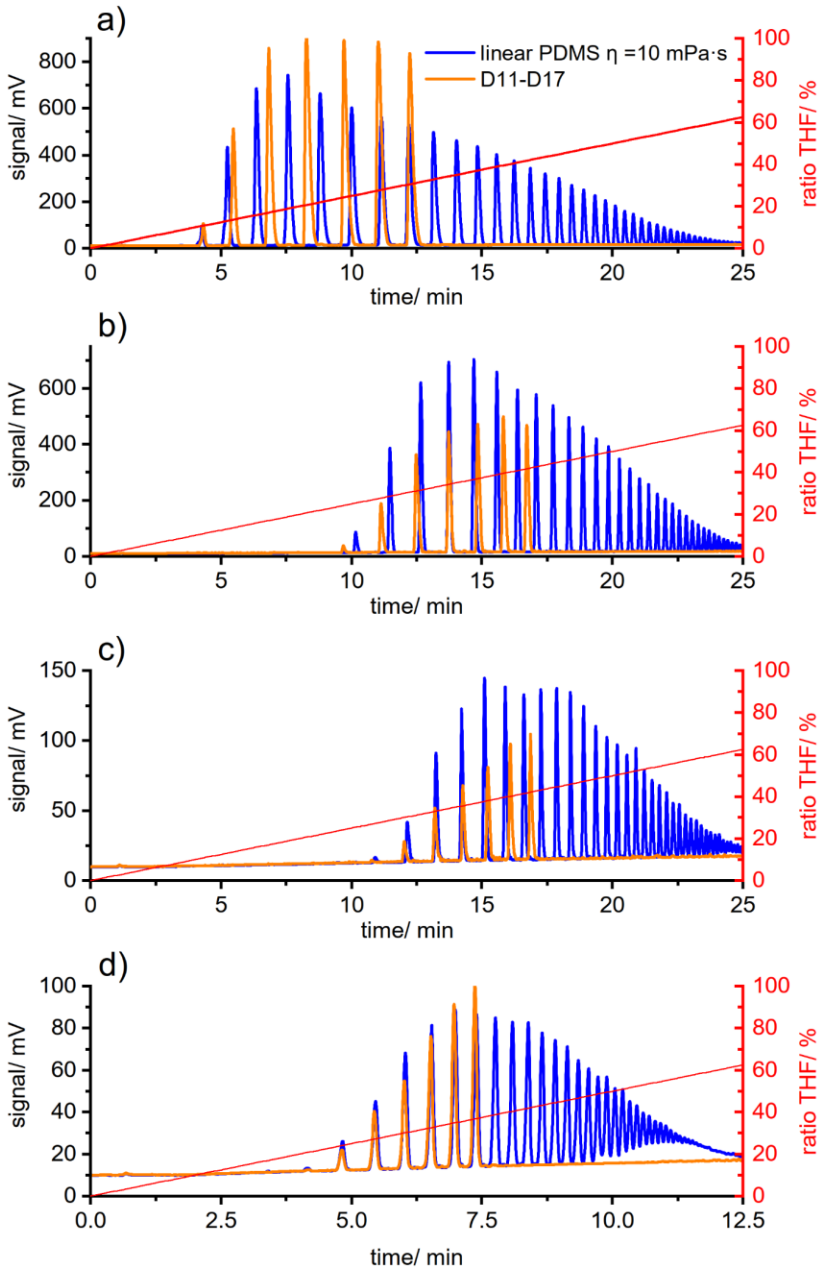


Figure 4.4: *Method development for separating cyclic (orange) from linear (blue) PDMS based on a linear gradient of 40 min with starting from 100 % acetonitrile to 100 % THF on alkyl chain columns:* ***a)*** *C4 column,* ***b)*** *C8 column,* ***c)*** *C18 column, and* ***d)*** *C30 column.*

Apart from the C30 column, all other stationary phases showed at least partial separation of several linear and cyclic oligomers. However, the separation performance in all cases was insufficient. Aside from this, ELS detection of the measurement series (**Figure 4.4 a) – d)**) was performed over a period of three weeks using a concentric glass nebulizer. During this time, a continuous decrease in signal intensity was observed comparing the initially used C4 column and the finally used C30 column. The improvement of ELSD performance was already published elsewhere [13] and as described there, especially for PDMS analysis, the use of an enhanced poly(tetrafluoroethylene) (PTFE) parallel path nebulizer is useful.

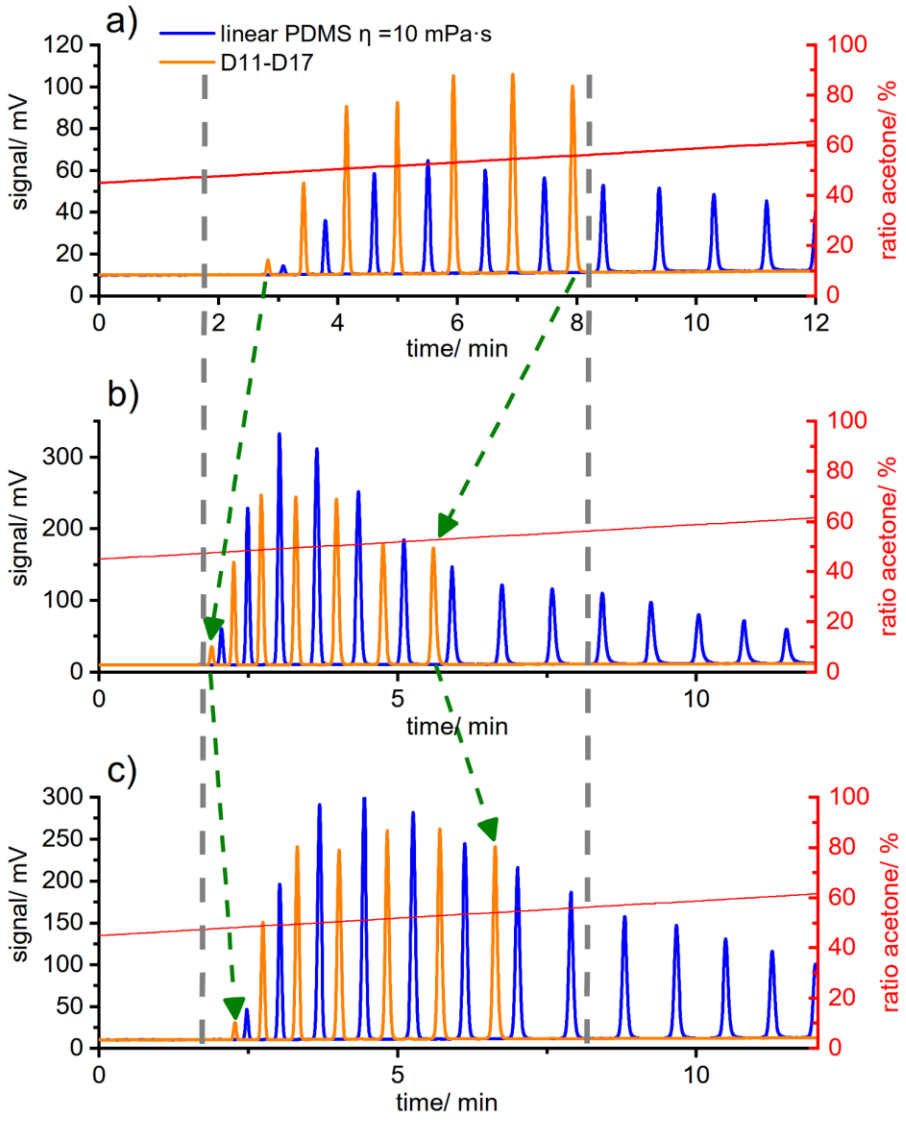


Figure 4.5: *Comparison of three batches of Phenomenex PFP columns (100x4.6 mm) with methanol: water (75:25, v/v) as adsorption promoting solvent and acetone as desorption promoting solvent:* ***a)*** *series 619128-6,* ***b)*** *series 532053-72, and* ***c)*** *series 528502-13; cyclic PDMS orange and linear PDMS blue.*

Consequently, for all further measurements an enhanced PTFE nebulizer was used improving the detector long-term stability and signal intensity. As shown in [18], for PDMS analysis an optimization in oligomer separation could be achieved by applying a C8 column and by using a convex gradient with extended total runtime. Whereby, even this improvement showed repeated changes in the elution order of linear and cyclic oligomers. For this reason, a change in the separation system concerning stationary and mobile phase was necessary. Applying a pentafluorophenyl column seemed appropriate because of its very different selectivity and the possibility of stereoisomer separation [19]. Beside electron donor / acceptor interactions, presumably the π-π interactions of the aromatic pentafluorophenyl group led to a change of the separation of linear and cyclic PDMS oligomers (**Figure 4.5**).

Acetonitrile with its triple bond blocks this π-π stacking effects and had to be replaced with a different proper adsorption promoting solvent. Thus, a mixture of methanol and water (75/25, v/v) was used to adjust a similar elution strength as pure acetonitrile [20, 21]. Furthermore, in place of THF, acetone was found to be a suitable desorption promoting solvent when using pentafluorophenyl columns. These modifications of the separation system showed increased separation performance without crossed elution of linear and cyclic PDMS up to 17 [D] repetition units. Beside the improved separation result, a major drawback using a Phenomenex Kinetex PFP column was the stationary phase's batch-to-batch reproducibility (see **Figure 4.5 a) - c)**). Applying other pentafluorophenyl columns from other manufactures, e.g. Agilent Poroshell PFP, Phenomenx Luna PFP (2) or Phenomenex Kinetex F5 resulted in a decreased separation performance. Thus, further optimization had to be done and the lack of separation performance comparing various pentafluorophenyl columns showed that presumably not only the modification of the stationary phase determined the separation. Because of these findings, modifications of the mobile phase components were investigated applying linear alkyl chain columns.

Furthermore, the LC flow rate was increased from 1.0 $mL \cdot min^{-1}$ to 2.0 $mL \cdot min^{-1}$ decreasing run time without losing separation performance. This change showed a substantial improvement in peak separation when using an Accucore C30 column (see **Figure 4.6 b)** and **c)**). **Figure 4.6 b)** and **c)** only differ in the gradient slope of acetone (i.e. the acetone volume ratio change per minute) from $12.5 \cdot 10^{-3}$ min^{-1} for **b)** to $3.125 \cdot 10^{-3}$ min^{-1} for **c)**. Consequently, beside the increase in separation performance, the flattening of the gradient slope led to an increased measurement time. Nevertheless, an effective gradient runtime of 160 min (**Figure 4.6 c)**) was a good compromise between total runtime and separation performance.

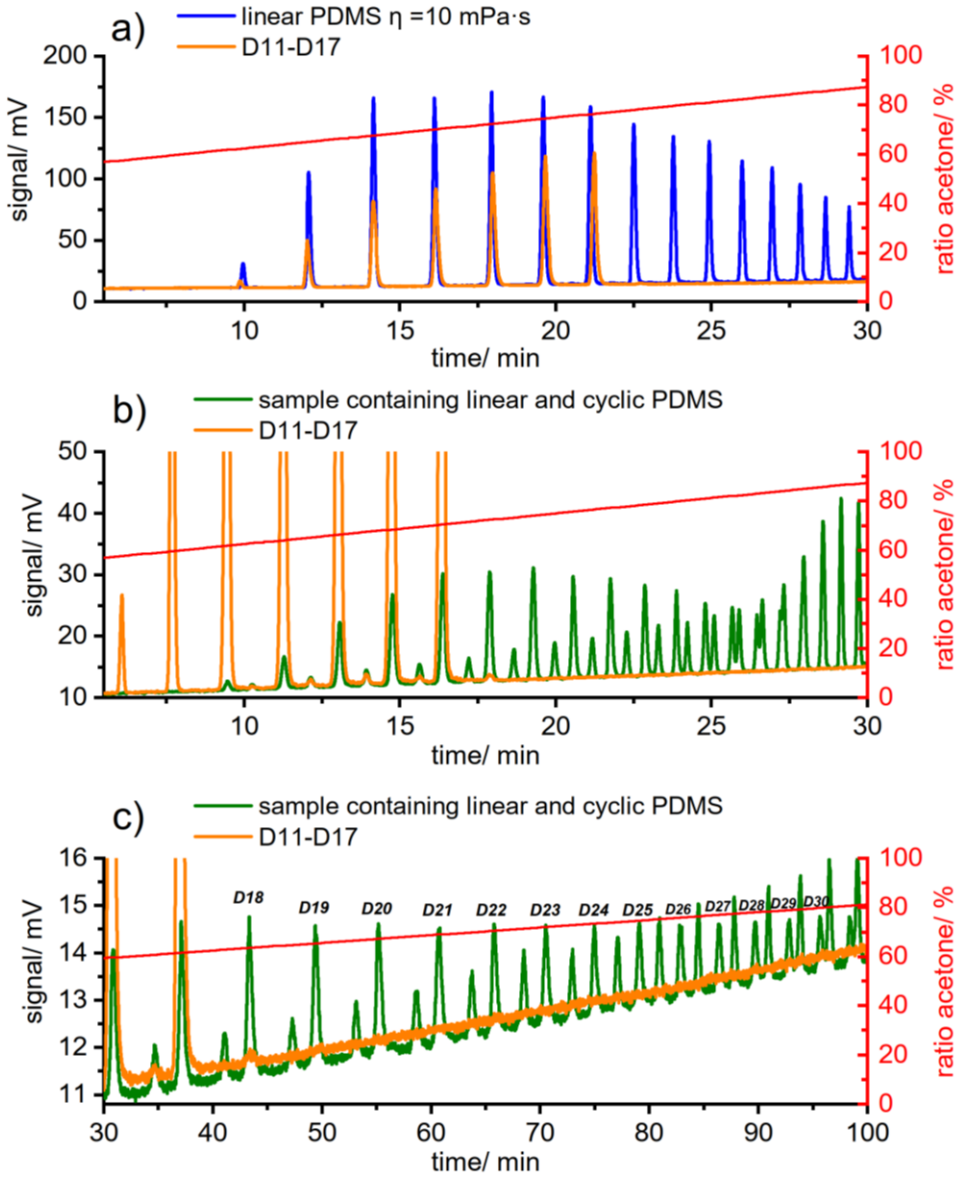


Figure 4.6: *Comparison of two C30 columns with methanol : water (75:25, v/v) as adsorption promoting solvent and acetone as desorption promoting solvent:* ***a)*** *YMC carotenoid C30 (100x4.6 mm) and* ***b)*** *Accucore C30 (50x4.6 mm) with an effective linear gradient of 40 min duration;* ***c)*** *Accucore C30 with an effective linear gradient of 160 min and a LC flow rate of 2.0 mL·min^{-1}.*

The comparison to another C30 column (YMC Carotenoid C30, 100x4.6 mm, **Figure 4.6 a)**) showed no separation of cyclic and linear oligomers at all. This result revealed, that apart from the chemical modification of the stationary phase, presumably the manufacturing process itself and the type of particles may have an important impact.

Consequently, additional stationary phases (**Figure 4.7**, **Table 4.2**) were compared to investigate separation differences and dependencies on further column characterizing parameters, e.g. particle diameter or C18 base silica material. Each subsequent measurement was carried out with methanol:

water (75:25, v/v) as adsorption promoting solvent, acetone as desorption promoting solvent, and an effective linear gradient of 40 min (on a column with 50x4.6 mm, 2.6 µm) adapted to the different column dimensions, respectively. As quality criterion for separation, the last baseline separated cyclic and linear PDMS oligomer was used. The comparison of C18 columns showed no considerable tendencies regarding particle diameter or other column specific parameters - only larger column pore sizes seemed to be having an effect (e.g. Hypersil BDS C18, HyPurity C18, Accucore C30). Even the comparison of various C18 columns with Accucore C30, Accucore C4 or a pentafluorophenyl column showed no direct correlation. As consequence, minor differences in the physics and chemistry of the column could result in major differences in the separation of polymer HPLC. Beside this, three stationary phases – Hypersil BDS C18 (100x4.6 mm), HyPurity C18 (150x4.6 mm) and Accucore C30 (50x4.6 mm) showed the best separation results. Comparing these three stationary phases with each other regarding total runtime, the Accucore C30 was shorter in length and, consequently, offered shorter runtimes. Nonetheless, all three stationary phases provided a good separation performance for linear and cyclic PDMS oligomers.

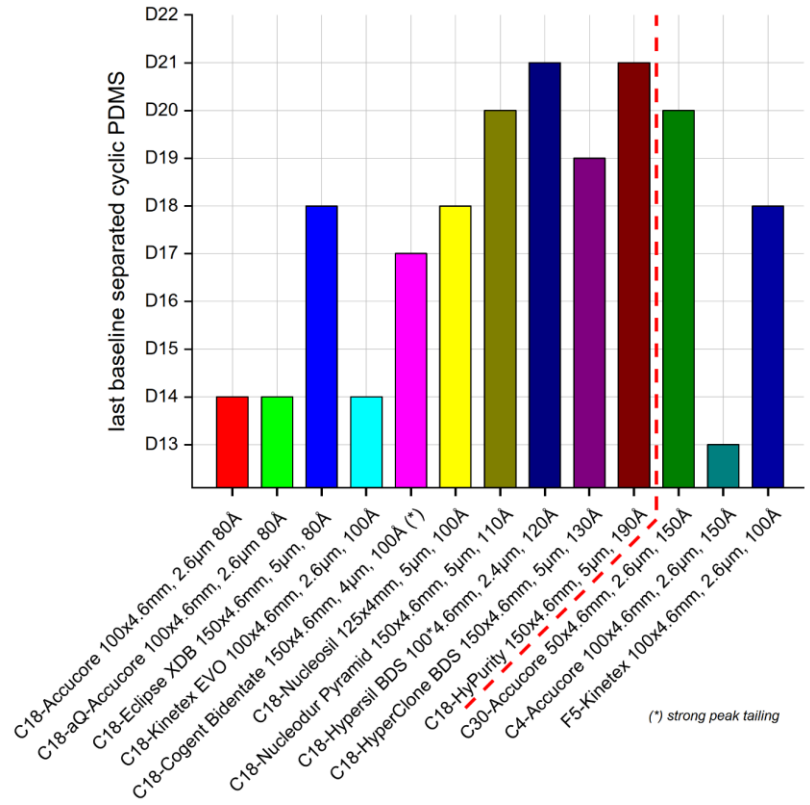


Figure 4.7: *Separation performance of various stationary phases, assessed according to the last separated pair of cyclic and linear PDMS oligomers, all measurements were performed applying an effective linear gradient of 40 min with methanol: water (75:25, v/v) as adsorption promoting solvent and acetone as desorption promoting solvent.*

For further investigations of the influence of column pore size on separation quality, an additional measurement series was performed using four C18 columns from the same manufacturer (Macherey-Nagel) while only varying mean pore sizes from 50 up to 1000 Å (see **Table 4.4**).

Table 4.4: *Extended overview of tested columns from Macherey-Nagel concerning pore size effects; all columns were purchased in April 2017.*

Column name	mean pore size/ Å	Carbon content/ %	surface area silica/ $m^2 \cdot g^{-1}$	binding density/ $\mu mol \cdot m^{-2}$
Nucleosil C18 EC	50	14.5	420	1.70
Nucleosil C18 EC	100	15.0	350	2.11
Nucleosil C18 EC	300	6.5	100	3.20
Nucleosil C18 EC	1000	1.0	25	1.97

Except of mean pore size, the column's carbon content *C%*, silica surface area *S* and binding density d_b were under investigation, too. Calculation of the binding density was done according to equation (1) [23] taking the molar mass M and number of carbon atoms per ligand n_c into account.

$$d_b[\mu mol/m^2] = \frac{10^6\ C\%}{1200\ n_c - C\%\ (M-1)} \cdot \frac{1}{S} \qquad (1)$$

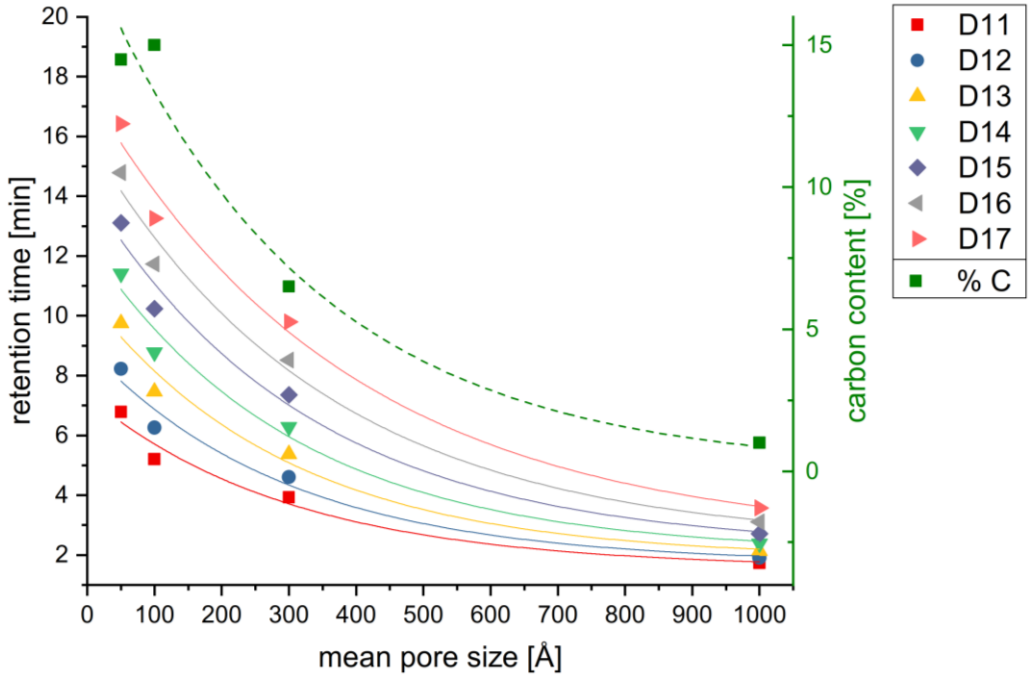


Figure 4.8: *Dependence of the retention time of cyclic PDMS oligomers (D11-D17) on the mean pore size; the right y-axis depicts the characteristics of carbon content in context to mean pore size. The measurements were done with methanol: water (75:25, v/v) as adsorption promoting solvent and acetone as desorption promoting solvent and an effective linear gradient of 40 min on four different Nucleosil C18 EC columns described in* ***Table 4.4****.*

Equation (1) showed that mean pore size, carbon content, and surface area were depending on each other for different columns. **Figure 4.8** depicts a plot comparing the retention times of cyclic PDMS with respect to the mean pore size. Generally, with increasing pore size the retention times of the oligomers decreased, while the separation performance in case of 1000 Å was found insufficient for cyclic oligomers. Thus, the separation performance decreased with increasing pore size. However, it was not possible to determine whether the separation quality was affected by pore size or other parameters.

Like the choice of stationary phase, as already discussed, the optimization of the mobile phase composition had decisive influence on the separation quality. Therefore, the combination of various common HPLC solvents were investigated. Using pure solvents, like acetonitrile as adsorption promoting solvent or THF as desorption promoting solvent, led to insufficient separation of linear and cyclic PDMS oligomers, as depicted in **Figure 4.4**. For the examined low molecular weight PDMS samples, methanol was a partial solvent and therefore, using pure methanol as adsorption promoting solvent was not an option. Furthermore, water was a too strong non-solvent and resulted in longer retention times for PDMS in combination with a minimization of the elution range. Taking these results and the aforementioned constraints of pentafluorophenyl columns for acetonitrile into account, a solvent mixture of methanol and water with a mixing ratio of 75:25 (v/v) was found considerably improving the separation quality (see **Figure 4.6**). Additionally, the desorption promoting solvent THF showed overtightened dissolving properties so that a less stronger solvent was needed for low molecular mass PDMS. For PDMS with up to 30 [D-] repetition units, acetone was found to be an appropriate compromise. Substituting acetone with the next higher homologue 2-butanone (containing one methyl group more) showed a considerable decrease in separation of oligomers (see **Figure 4.9 c)**).

All subsequently discussed data referring to pre-mixed mobile phase components present the best separation result for the appropriate pair of solvent mixtures. **Figure 4.9 a)** depicts an inadequate separation quality by using a mixture of acetonitrile and water (50:50, v/v) in combination with acetone. Substituting the acetonitrile- water- mixture with acetone and water (75:25, v/v) in combination with pure acetone as desorption promoting solvent resulted in a partial separation of linear and cyclic PDMS oligomers (**Figure 4.9 c)**). However, none of these modifications of the mobile phase composition could improve the performance compared to methanol: water (75:25, v/v) and acetone at a C30 column.

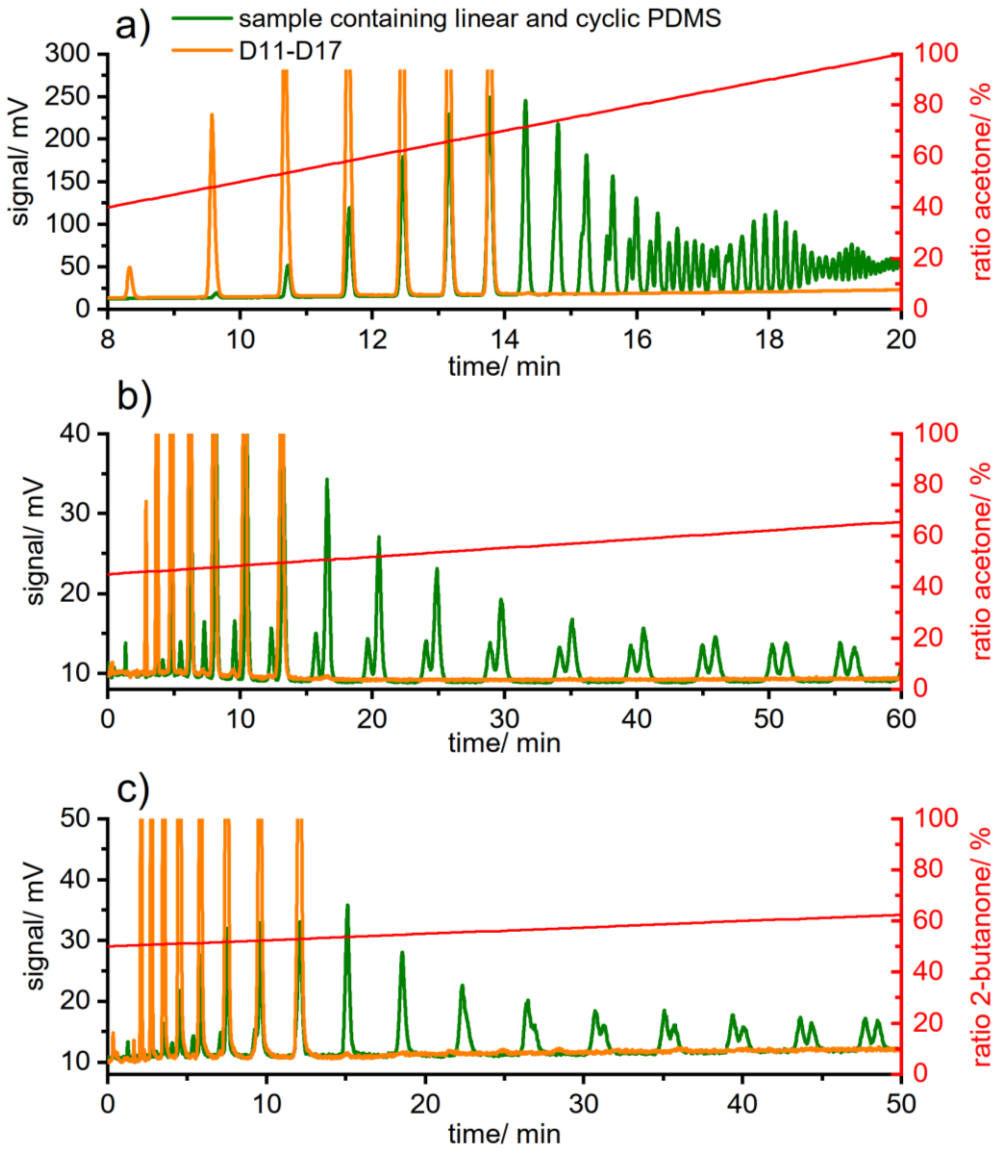


Figure 4.9: *Variation of mobile phase components (adsorption promoting solvent | desorption promoting solvent) on a Accucore C30 (50x4.6 mm) column:* ***a)*** *acetonitrile: water (50:50, v/v) | acetone,* ***b)*** *acetone: water (75:25, v/v) | acetone,* ***c)*** *methanol: water (75:25, v/v) | 2-butanone; cyclic PDMS orange, mixture of linear and cyclic PDMS green.*

Regarding the separation mechanism, polymer liquid chromatography provides various interactions between polymer and stationary phase, e.g. size exclusion effects, adsorption chromatography or precipitation / re-dissolution chromatography [17]. Based on the comparison of column pore size and the parameters carbon content and surface area, the influence of actual size exclusion effects should be circumstantial. As mentioned in [17, 24–26] an unambiguous differentiation between adsorption and precipitation / re-dissolution effects was difficult for the investigated low molecular weight PDMS oligomers. In ideal precipitation / re-dissolution chromatography altering the stationary phase should not result in different retention times for a polymer. As shown by comparing various stationary phases, the

separation performance remarkably differs for cyclic and linear PDMS oligomers. Thus, adsorption chromatography had a major impact on oligomer separation of PDMS. However, the superordinate influence of the mobile phase composition indicated a slight overlay with precipitation / re-dissolution effects.

In addition to analytical separation of PDMS oligomers, preparative polymer HPLC was used to fractionate pure linear PDMS oligomers. Therefore, a mixture of silicone oils, containing linear oligomers only, with viscosities of 5:10:20 mPa·s at a mixing ratio of 1:1:4, without any solvent was directly injected on an Accucore C30 column (150x4.6 mm). Again, a mixture of methanol: water (75:25, v/v) was used as adsorption promoting solvent and THF as desorption promoting solvent with a multi-linear gradient shown in **Table 4.3**. After injecting 1.0 mL of the PDMS mixture, linear PDMS oligomers from Si9 up to Si48 (depicted in **Table 4.5**) were obtained by fraction collection. The purity of each fraction was analyzed with LC-MS and for most fractions purities above 95 or 99 % were found. The major impurities were hydroxy terminated linear PDMS and low amounts of cyclic silicones. The results of semi-preparative studies showed a good applicability of the analytical separation approach. Moreover, combining preparative polymer HPLC with other analytical methods may improve the understanding of varying behaviors of different linear PDMS oligomers in future investigations.

Table 4.5: *Amount of linear PDMS oligomers fractionated by preparative HPLC on an Accucore C30 column (150x4.6 mm) and the corresponding purity determined by LC-MS.*

Name	Amount/ mg	LC-MS purity/ %
Si9	7.2	> 99
Si10	15.5	> 99
Si11	20.2	> 99
Si12	24.6	> 99
Si13	29.9	50
Si14	28.9	> 99
Si15	24.9	> 99
Si16	22.4	> 99
Si17	19.9	> 99
Si18	18.4	> 99
Si19	16.5	50
Si20	17.5	50
Si21	17.7	75
Si22	15.5	90
Si23	19.2	95
Si24	17.7	> 95
Si25	17.2	> 95
Si26	16.2	> 95
Si27	15.5	> 99
Si28	15.0	> 95
Si29	14.1	> 95
Si30	13.1	> 95
Si31	13.0	> 95
Si32	12.1	> 95
Si33	12.5	> 99
Si34	9.9	> 99
Si35	9.9	> 99
Si36	9.5	> 99
Si37	8.9	> 99
Si38	7.9	> 99
Si39	8.1	> 99
Si40	7.2	> 99
Si41	7.2	> 99
Si42	5.3	> 99
Si43	5.9	> 99
Si44	5.7	> 99
Si45	4.7	> 99
Si46	4.4	> 99
Si47	4.6	> 99
Si48	4.3	> 99

4.2.5 Conclusion

A polymer HPLC method for separation of linear and cyclic PDMS was developed and optimized. The new method offers complementary information to gas chromatography for low molecular weight PDMS oligomers and expanded the analytical range of baseline separated linear and cyclic oligomers up to 30 [D-] block units. Therefore, stationary phase and mobile phase were optimized investigating several parameters. On the one hand, the chemical and physical properties, like pore size, carbon content, silica basis material or phase modification of the stationary phase were adjusted achieving an efficient separation system. On the other hand, various mobile phase compositions showed the dependence on oligomer separation regarding minor changes in polymer solubility and elution strength. Taking all parameters together, the separation mechanism may be primarily driven by adsorption effects superimposed with solubility or rather precipitation / re-dissolution effects. In addition to the analytical method development, preparative separation of linear PDMS oligomers was investigated and the appropriate oligomers were isolated for the first time. Fractions containing only a single oligomer were obtained allowing further investigations of these isolated species with other analytical techniques, such as mass spectrometry and nuclear magnetic resonance spectrometry.

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5 Adaption of a parallel-path poly(tetrafluorethylene) nebulizer to an evaporative light scattering detector: Optimization and application to studies of poly(dimethylsiloxane) oligomers as a model polymer

This chapter has been published and adapted from:

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik, Journal of Chromatography A, 1564, 2018 214-223.

Measurements and experiments were done solely by the author. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

5.1 Abstract

The adaption of a parallel-path poly(tetrafluoroethylene)(PTFE) ICP-nebulizer to an evaporative light scattering detector (ELSD) was realized. This was done by substituting the originally installed concentric glass nebulizer of the ELSD. The performance of both nebulizers was compared regarding nebulizer temperature, evaporator temperature, flow rate of nebulizing gas and flow rate of mobile phase of different solvents using caffeine and poly(dimethylsiloxane) (PDMS) as analytes. Both nebulizers showed similar performances but for the parallel-path PTFE nebulizer the performance was considerably better at low LC flow rates and the nebulizer lifetime was substantially increased. In general, for both nebulizers the highest sensitivity was obtained by applying the lowest possible evaporator temperature in combination with the highest possible nebulizer temperature at preferably low gas flow rates. Besides the optimization of detector parameters, response factors for various PDMS oligomers were determined and the dependency of the detector signal on molar mass of the analytes was studied. The significant improvement regarding long-term stability made the modified ELSD much more robust and saved time and money by reducing the maintenance efforts. Thus, especially in polymer HPLC, associated with a complex matrix situation, the PTFE-based parallel-path nebulizer exhibits attractive characteristics for analytical studies of polymers.

5.2 Introduction

With evaporative light scattering detectors (ELSD), it is possible to detect analytes without chromophores. Therefore, this universal HPLC detector, exhibiting good suitability for gradient elution protocols, is an attractive alternative to the typically used diode array detector in HPLC or refractive index detector in size exclusion chromatography (SEC). An important field of application is the investigation of polymers [1,2]. The use of ELSD for studies of carbohydrates [3], poly(dimethylsiloxane) [4] or poly(ethylene glycol) [5,6] is already well established. Nevertheless, the ELSD also has some constraints, i.e. the non-linear dependence of the ELSD signal on sample concentration and restriction to volatile or semi volatile mobile phases and correspondingly to analytes which are not vaporable through the detection process [7,8]. For understanding the potentials and limitations of the device a detailed analysis of the process associated with this detection principle is necessary. The signal generation is determined by the following three steps: Initially the liquid effluent from liquid chromatography is nebulized, followed by evaporation of the mobile phas and finally the remaining analyte particles are transferred to a measurement cell where a light beam is scattered by the latter and detected with a photomultiplier [9–12].

Mojsiewicz-Pienkowska [13] classified four groups of factors which influence the signal response of an ELSD: (i) parameters determining the separation procedure (e.g. flow and composition of mobile phase); (ii) parameters which can be modified by the user without influencing the actual separation in liquid chromatography (e.g. detector temperatures, nebulizer type); (iii) chemical and physical properties of the investigated analyte (e.g. molecular weight, vapor pressure) and (iv) types of different nebulization gases (e.g. different heat conductivities [14]). The most important parameters which can be varied for optimization of the detector response are factors one and two. Thus, the nebulizer and evaporator temperature, ELSD gas flow, type of nebulizer and the detection parameters of the scattered light are the aspects the analytical chemist should properly adjust because the complexity of the detector is mostly determined by these interactions [15,16]. Additionally, parameters like the choice of mobile phase and flow rate also affect the ELSD response significantly.

In the initial detection step, the effluent of the LC system gets nebulized to a primary aerosol, which shows a narrow polydispersity and particle distribution [11]. The amount of nebulization gas has to be adjusted carefully to generate an ideal aerosol, consisting of the majority of analyte particles and adequate amount of mobile phase. Furthermore, the aerosol formation should be robust and thus generate a flat baseline with low noise. For organic solvents, a low gas flow is usually preferred. As soon as the mobile phase contains small amounts of water, a higher gas flow or evaporator temperature is necessary for complete

nebulization of the droplets. An appropriate and uniform aerosol is an important prerequisite for a robust detector performance. After nebulization, the remaining mobile phase in the primary aerosol is evaporated in a heated drift tube. In ideal case only analyte particles pass the drift tube. Small aerosol droplets facilitate an efficient evaporation because of their larger surface-to-volume ratio [7] compared to large droplets.

Apart from this, special attention needs to be paid to prevent the evaporation of easily vaporizable analytes. For these substances, it might be advisable to decrease the evaporation temperature if possible in order to obtain sufficient detector response. After evaporation of the mobile phase, the primary aerosol is essentially changed to an aerosol containing only large analyte particles that ideally scatter light. In general, three different kinds of light scattering are considered: reflection-refraction, Rayleigh and Mie scattering. The entire scattering process is dominated by the wavelength of the incident light beam and the particle diameter d. Rayleigh scattering occurs from particles which are much smaller than the wavelength of the incident light while scattering by reflection and refraction is dominated by larger particles. In between, Mie scattering, which is slightly asymmetric in comparison to Rayleigh scattering, occurs. The asymmetry in direction of propagation leads to decreased light intensity in the light measuring angle [15]. At a ratio of d/ of about 3.5 the detector sensitivity is in an optimum [17]. A further comprehensive theoretical discussion is beyond the scope of this work and can be found elsewhere [15–18]. An unfavorable ratio of surface area to volume results in a reduced detector performance. Monochromatic light sources, like LED or laser with a short wavelength radiation are used in the most cases to obtain an appropriate detector signal. In conclusion, scattering processes due to particle distributions result in a non-linear behavior of the ELSD [7,8,17].

In addition to parameters discussed above, the mobile phase also affects the detection process. Thus, different optimizations for improvement of the signal intensity are described. Stolyhwo et al. [14] and Mathews et al. [19] proposed an adaption of ELSD parameters during gradient elution, due to the change of mobile phase composition. The complex process of signal generation leads to various optimized settings for different solvents, but to our knowledge, it is not completely understood yet which solvent parameter is significant for an alteration in sensitivity. Viscosity, density, surface tension, vapor pressure or boiling point (summarized in **Table 5.1**) might influence the detection process [12].

The investigation of analytes like PDMS is commonly done with ELSD and could be used as typical model substance in polymer HPLC, and thus a detailed analysis of these species shows the influences of molar mass and polymer matrix. In the recent years, applications of PDMS in various areas, e.g. in cosmetics, medicine or pharmacy showed increasing importance. Siloxanes are used as detergents, coating excipients in various pharmaceuticals or as implants [22–24].

***Table 5.1**: Overview of selected data of relevant solvents; *) data taken from [20], all other data out of [21].*

Solvent	Viscosity (T=25 °C) [mPa·s]	Density (T=30 °C) [g·mL^{-1}]	Vapor pressure (T=25 °C) [kPa]	Boiling temperature [°C]	Surface tension (T=25°C) [mN·m^{-1}]
Acetonitrile	0.369	0.7707	11.9	81.6	28.66
Tetrahydrofuran	0.456	0.8833	21.6	66.0	26.4 *)
Acetone	0.306	0.7796	30.8	56.1	22.71
Methanol	0.544	0.7815	16.9	64.5	22.17

With polymer HPLC, a detailed analysis of silicone oils and a separation of several particular oligomers are possible. Furthermore, detailed information about the influence of molar mass and polymer matrix on the detection process is obtained. In a recent publication, Mojsiewicz-Pienkowska [13] analyzed linear siloxanes of different viscosities with SEC and investigated the influence of flow rate of nebulizer gas, temperature of drift tube and flow rate of mobile phase on the ELSD signal. Based on these results, further investigations can contribute to a more detailed understanding of this polymer type.

Since its invention by Charlesworth [15], various types of ELSDs were investigated. As each manufacturer uses a slightly different setup of the detector, it is difficult to compare different detector models. Reports on comparative characterization are very rare [2,7,8]. The nebulization step is a very crucial point and especially in case of polymer analysis the issue of decreasing signal occurs, when high amounts of sample matrix prevail. In this case, polymer matrix includes both, other polymers than the analyte polymer and high molecular weight constituents of the investigated polymer. The challenge of these types of matrices occurs at the tip of the nebulizer where small amounts thereof remain as fragments and may lead to nebulizer clogging after several measurement series. Especially in case of a concentric glass nebulizer which is installed in some commercially available ELSDs, polymer sample components can clog the tip of the thin capillary within the nebulizer. In order to overcome these problems, a more robust type of nebulizer is desirable. The comparison with different nebulization-based techniques, as for example inductively coupled plasma mass spectrometry (ICP-MS), shows similar concerns regarding high amounts of matrix. For ICP-based techniques, it is recommended to use a parallel-path poly(tetrafluoroethylene) (PTFE) nebulizer in place of a concentric glass nebulizer to avoid capillary clogging [26–29]. For the

compatibility with ELSD, the given dimensions of the nebulizer have to be considered as well as its shape, diameter and range of flow rate. These constraints were fulfilled by a parallel path PTFE nebulizer developed by Burgener [30–32]. In the present work, a comparison of both nebulizers is done with caffeine as model analyte and PDMS as an important practical application in the field of polymer analysis. Detector parameters were optimized for both ELSD configurations. Furthermore, the application of the parallel-path PTFE nebulizer for PDMS analysis and ELSD response factors concerning different molar mass oligomers were determined.

5.3 Material and methods

5.3.1 LC system and ELSD

All used solvents were HPLC grade. Acetonitrile (ACN), acetone, methanol (MeOH) and non-stabilized tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. The measurements were performed on an 1100 series LC system of Agilent (Waldbronn, Germany) with a THF-resistant 3215α degasser from ERC (Riemerling, Germany) and diode array detector at 272 nm detection wavelength for caffeine. An Agilent 385 evaporative light scattering detector was used. Nitrogen was used as carrier gas and the gas flow was set to 1.2 SLM (standard liter per minute) unless otherwise mentioned. Two different types of nebulizer, the Agilent concentric glass nebulizer (**Figure 5.1 a**) and an enhanced parallel-path MiraMist® PTFE nebulizer (**Figure 5.1 b**) from Burgener Research Inc. (Mississauga, Ontario, Canada), purchased from AHF Analysentechnik AG (Tübingen, Germany), were used. The signal generation of the ELSD could be tuned regarding three parameters: (i) evaporator temperature from 10 to 80 °C, (ii) nebulizer temperature from 25 to 90 °C and (iii) a gas flow from 0.9 to 3.25 SLM.

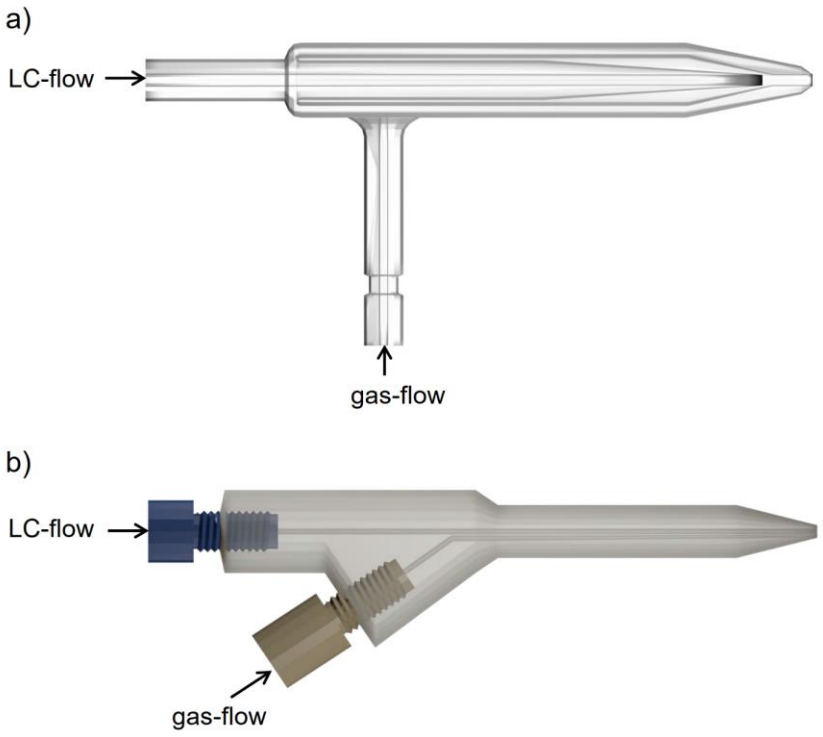

Figure 5.1: *Schematic overview of nebulizer configurations used in this study:* ***a)*** *concentric glass nebulizer and* ***b)*** *parallel-path PTFE nebulizer, designed with software package Blender based on pictures of the nebulizers.*

5.3.2 Stationary phases

A restriction capillary (15 m length and 0.18 mm ID from OQ/PQ Kit) from Thermo Fisher Scientific (Waltham, USA) was used for all measurements with caffeine and the ELSD optimization with PDMS. The use of a capillary simulated real polymer measurement conditions concerning pressure and peak width. Further, a Thermo Fisher Scientific (Waltham, USA) Accucore C30 column (50 x 4.6 mm and 2.6 µm particle size superficially porous particles) was used for all analytical studies of PDMS and an Accucore C4 column (100 x 4.6 mm and 2.6 µm particle size) was used for the endurance tests of the investigated nebulizers. The column oven temperature was set to 30 °C.

5.3.3 Caffeine analysis

Caffeine of HPLC-grade quality was purchased from Sigma-Aldrich (Darmstadt, Germany) and used as a 500 $\mu g \cdot mL^{-1}$ solution in the respective solvent. The injection volume was 2 µL for all measurements and the verification with DAD showed uncertainties (n=5) below 0.5 %.

5.3.4 PDMS analysis

PDMS samples of different viscosities (5, 10, 20, 50, 100, 1000 and 10000 mPa·s) were obtained from Wacker Chemie AG (Burghausen, Germany). All measurements with the restriction capillary were done with 3 µL injection volume at a sample concentration of 0.5 $mg \cdot mL^{-1}$ in THF. For gradient separation on the C30 column, eluent A consisted of 75 % methanol and 25 % water and eluent B was 100 % acetone. The gradient started at 50 % B with a gradient slope of $3.125 \cdot 10^{-3}$ min^{-1}. The calibration standard Si16 (see **Table 5.2**) was a fraction of silicone oil with a viscosity of 10 mPa·s and a purity higher than 99 % was determined with LC-MS. The analyzed oligomers Si12 to Si50 were part of three PDMS samples with viscosities of 10, 20 and 50 mPa·s.

Table 5.2: *Calibration data for Si16, concentrations and corresponding signal areas; RSD for calibration 2.5 %.*

concentration [$\mu g \cdot mL^{-1}$]	signal area [mV·min]	RSD (n=3) [%]
6.6	0.0460	2.14
23.1	0.2948	1.48
45.3	0.7651	1.64
73.0	1.3861	1.73
217.7	6.6052	1.32
473.6	22.9891	1.58
811.3	55.1592	1.28
920.1	68.1832	1.17

5.4 Results and discussion

Two different types of nebulizer (illustrated in **Figure 5.1**) were studied and the responses of the respective ELSD configurations were optimized and compared. The conventional concentric glass nebulizer showed good performance at low amounts of sample matrix, but for polymer samples, the signal decreased. It was expected that a parallel-path PTFE nebulizer should be less affected by matrices in long-term usage [29]. This is especially important for polymer analysis, where high amounts of sample matrix are present as explained above. Comparison and evaluation of both types of nebulizer are important because to our knowledge this is the first time that the integration of a parallel-path PTFE nebulizer into an Agilent ELSD is reported. Therefore, at first caffeine, which is easily available, readily soluble in various solvents and used for operational and performance qualification of Agilent ELSDs, was used to compare general, analyte independent, performance characteristics. Second, PDMS in high amount of polymer matrix was investigated concerning long term stability of the detector response and detector optimization. All presented data points were measured in triplicate and presented as mean value unless otherwise stated. In addition to the two types of nebulizer, three detector parameters namely evaporator temperature, nebulizer temperature and gas flow as well as the effect of various solvents and LC flow rates on the detector response were studied.

5.4.1 Caffeine measurements

The effect of flow rate of the nebulizer gas on the signal response of caffeine is depicted in **Figure 5.2 a)** for THF. A similar behavior is observed for other organic solvents, e. g. acetone. The result that the peak area or signal-to-noise ratio decreases with increasing flow rate of the nebulizer gas is in accordance with published data [6,13,14]. In this respect, the investigated parallel-path PTFE nebulizer and the concentric glass nebulizer showed similar performances.

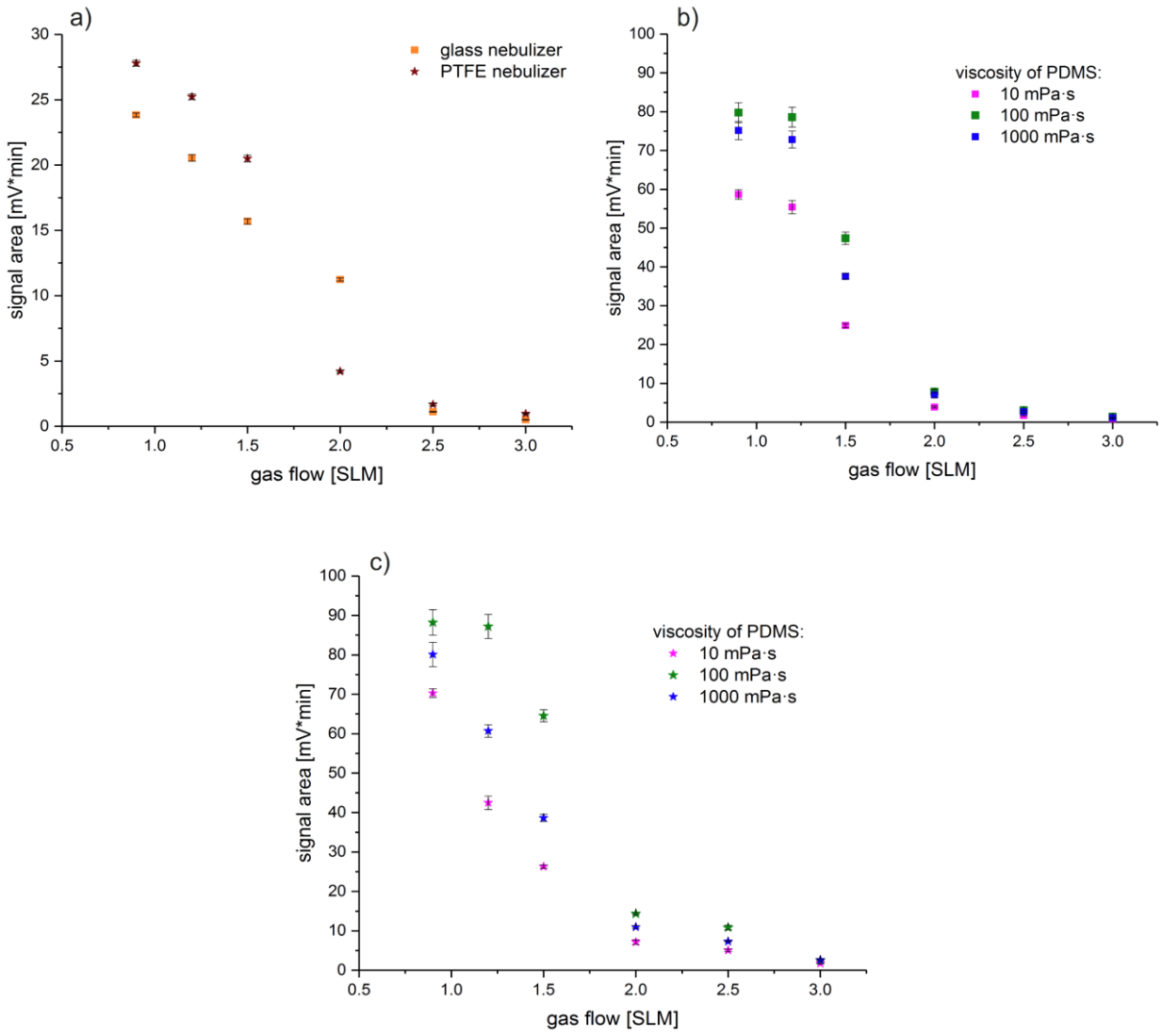

Figure 5.2: a) *Influence of flow rate of nebulizer gas on the peak area of caffeine with THF as mobile phase for concentric glass and parallel-path PTFE nebulizer; influence of flow rate of nebulizer gas on the peak area of PDMS of 10 mPa·s, 100 mPa·s, and 1000 mPa·s for* ***b)*** *concentric glass nebulizer and* ***c)*** *parallel-path PTFE nebulizer; error bars (n=5) are indicated;* T_E *= 30 °C,* T_N *= 60 °C, and the LC flow rate was 1 mL·min*$^{-1}$*.*

For all subsequent measurements (of caffeine and later PDMS) the flow rate of the nebulizer gas was set to 1.2 SLM (standard liter per minute) because higher gas flow rates generated smaller aerosol particles and therefore a reduced scattering signal. Lower gas flow rates prevented the formation of a proper aerosol by changing the detector temperatures in extreme (low evaporator and high nebulizer temperature) regions.

More complicated results were expected for the variation of evaporator and nebulizer temperatures, because both temperatures are not independent from each other. The results for acetone **(a)**, methanol **(b)**, tetrahydrofuran **(c)**, and acetonitrile **(d)** are presented in **Figure 5.3** and showed generally very similar trends.

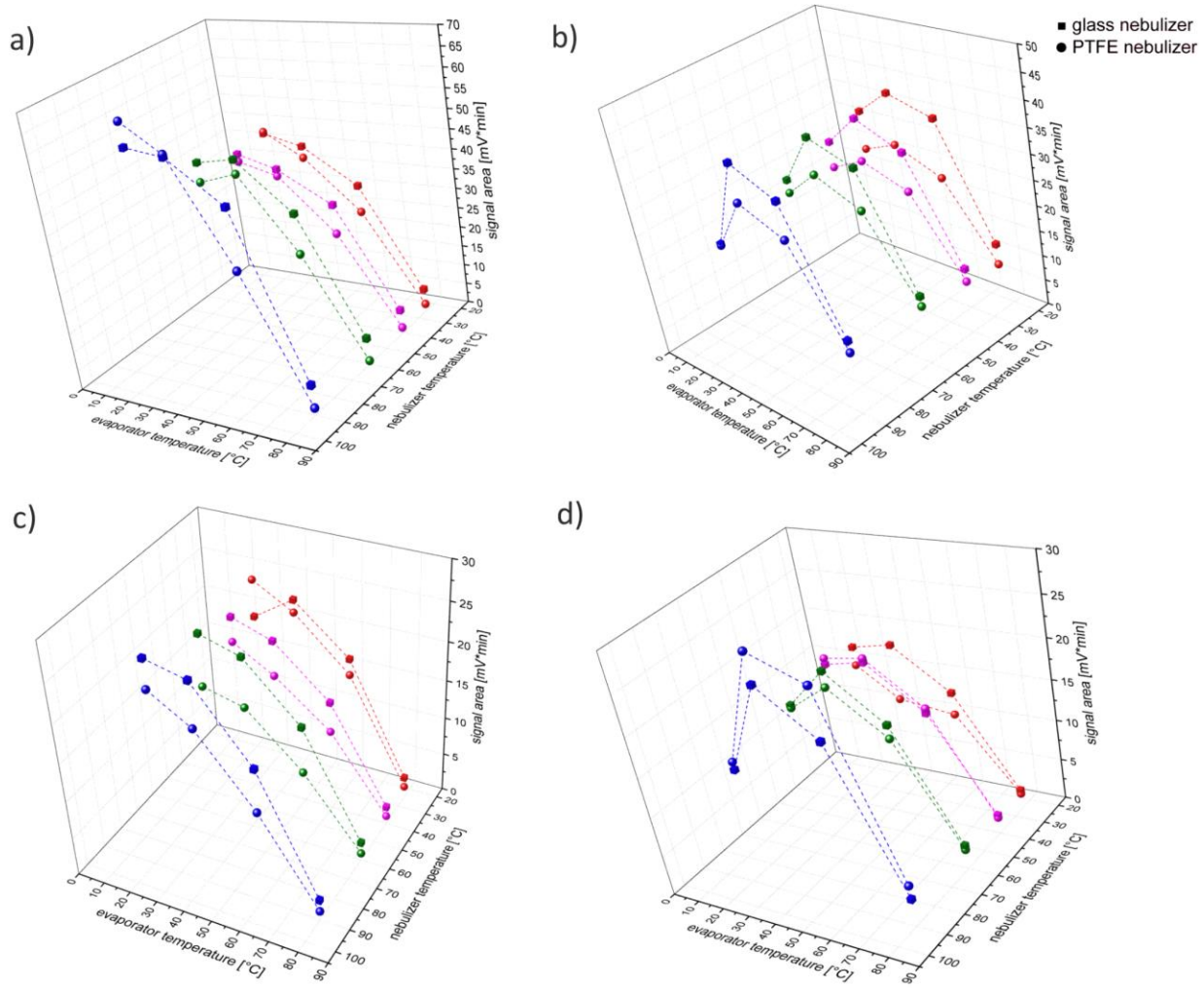

Figure 5.3: *Dependence of caffeine peak area on different settings for nebulizer- and evaporator-temperature for* ***a)*** *acetone,* ***b)*** *methanol,* ***c)*** *THF, and* ***d)*** *acetonitrile; cubes and spheres represent the glass nebulizer and PTFE nebulizer, respectively; different settings for nebulizer temperature TN are color-coded and connected by dotted lines (red: T_N = 20 °C, pink: T_N = 40 °C, green: T_N = 60 °C, blue: T_N = 90 °C); LC flow rate was 1 mL·min^{-1} and gas flow rate was 1.2 SLM.*

For each set of different nebulizer temperatures one color was chosen and the orientation of the 3D diagrams was adapted to give a better overview. A more detailed overview of the diagrams is given as separate contour plots (**Figure 5.4** for parallel-path PTFE nebulizer and **Figure 5.5** for concentric glass nebulizer).

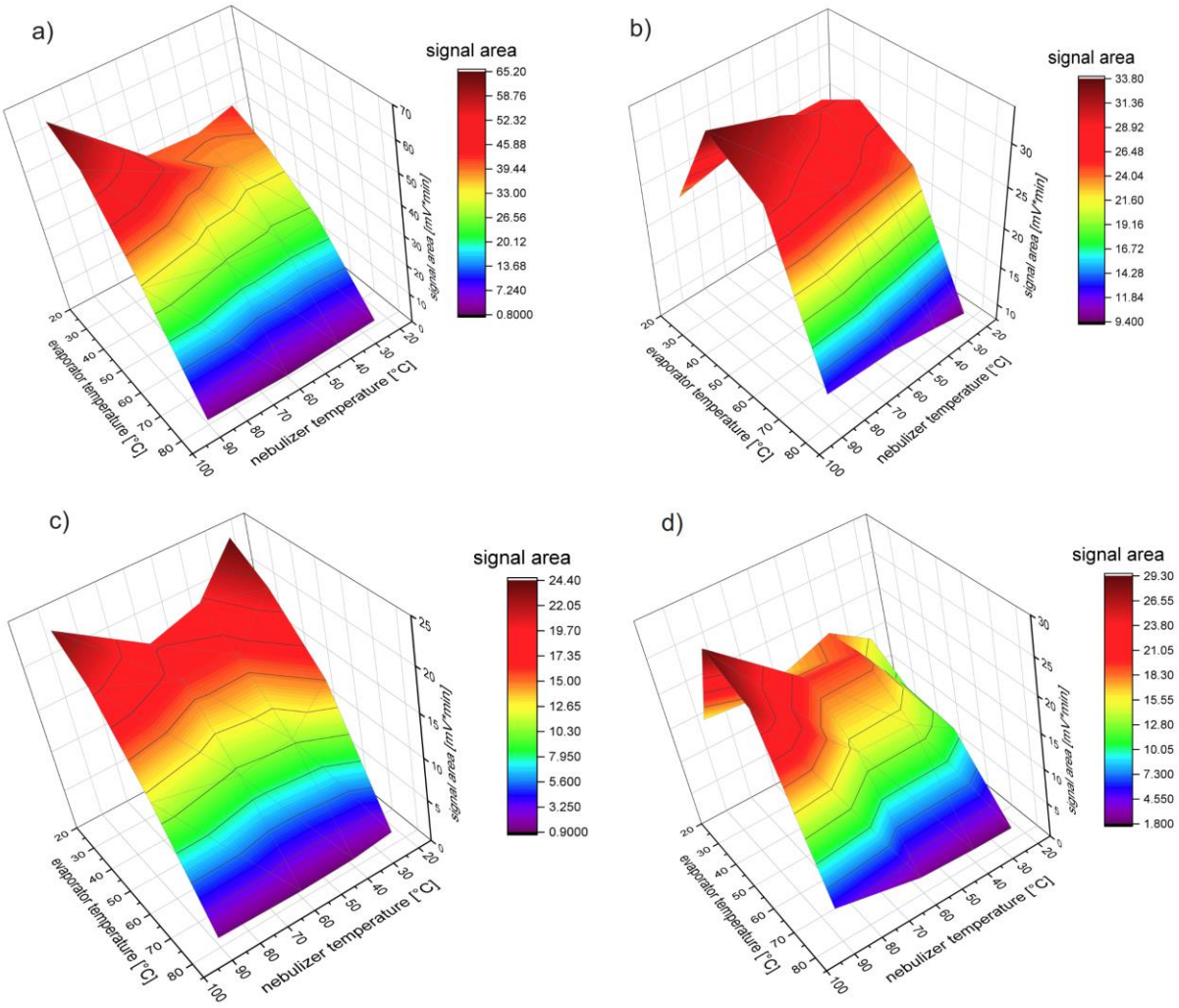

Figure 5.4: *Dependence of caffeine peak area on different settings for nebulizer- and evaporator-temperature for* ***a)*** *acetone,* ***b)*** *methanol,* ***c)*** *THF, and* ***d)*** *acetonitrile for parallel-path PTFE nebulizer shown as a contour plot; LC flow rate was 1 mL·min^{-1} and gas flow rate was 1.2 SLM.*

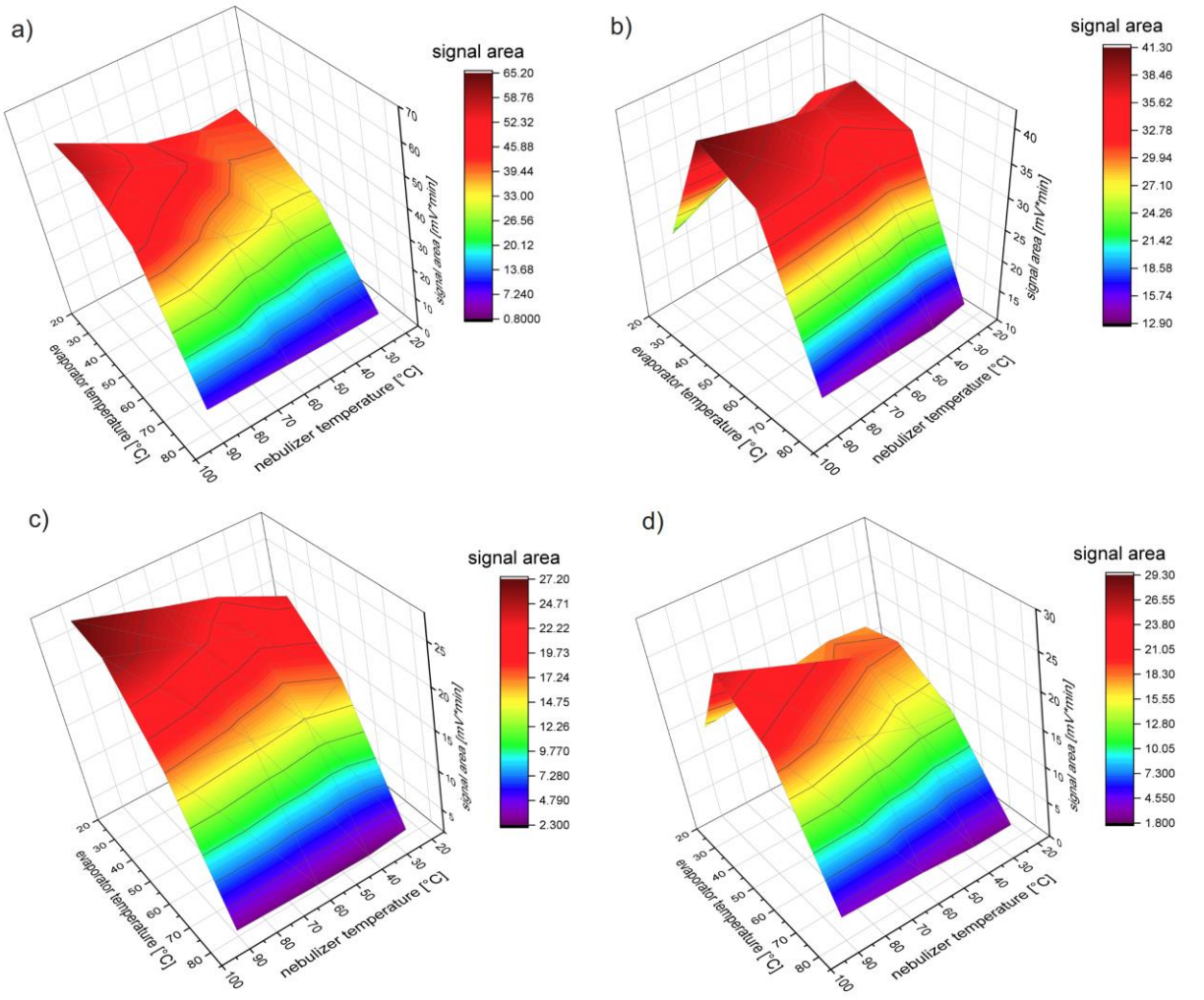

Figure 5.5: *Dependence of caffeine peak area on different settings for nebulizer- and evaporator-temperature for* ***a)*** *acetone,* ***b)*** *methanol,* ***c)*** *THF, and* ***d)*** *acetonitrile for concentric glass nebulizer shown as a contour plot; LC flow rate was 1 mL·min^{-1} and gas flow rate was 1.2 SLM.*

The maximum peak area for methanol and acetonitrile was slightly different from THF and acetone, where the maximum signal was obtained for the lowest evaporator temperature and the highest nebulizer temperature. But these are only general trends. Considering absolute values, there are significant differences regarding the kind of solvent. Acetone and methanol showed considerably larger signals than acetonitrile and THF. This result emphasizes the importance of choice of a proper mobile phase or mobile phase composition for optimum detector performance. According to **Table 5.1** it can be assumed that the differences of various solvents are caused by differences in the surface tension. Nevertheless, reducing the solvent composition to only one parameter seems to be an oversimplified approach when considering comprehensive publications concerning the effect of the mobile phase on response of the ELSD [11,18,19]. Thus, further research will be necessary to understand the complex behavior. According to the present

investigations there are no significant differences in the behavior of concentric glass nebulizer and parallel-path PTFE nebulizer concerning detector temperature settings. These findings showed that in case of molecules like caffeine there are no disadvantages of the investigated parallel-path PTFE nebulizer in comparison to the original concentric glass nebulizer.

In further experiments the LC flow rate was modified in a range from 0.1 to 2.0 mL min^{-1}. For both nebulizers, the results for caffeine were very similar to the measurements with the later discussed PDMS samples (see section 5.4.2.1). The parallel-path PTFE nebulizer showed the most sensitive peak signal for a flow rate of 0.1 mL min^{-1} at the minimum detector response of the concentric glass nebulizer. For both nebulizers a similar behavior was observed in the range from 1.0 to 2.0 mL min^{-1}. Replacing the concentric glass nebulizer with a parallel-path PTFE nebulizer, low LC flow rates led to a much-improved detector response and therefore the application in miniaturized devices might be possible.

5.4.2 PDMS measurements

In analogy to the measurements of caffeine described above, evaporator temperature, nebulizer temperature and ELSD gas flow were optimized. Furthermore, the influences of LC flow rate and different PDMS viscosities on the detector signal were determined. Finally, the separation of different PDMS oligomers and long-term performance of both nebulizers were studied.

5.4.2.1 ELSD optimization

Based on the results of caffeine measurements, the detector optimization was done in a similar way. Only two solvents (acetone and THF) were tested, because of a reduced solubility of the used PDMS (viscosity 10 mPa s) in comparison to caffeine. Variation of the ELSD gas flow showed the same tendencies for PDMS (**Figure 5.2 b)** and **c)**) as for caffeine, which is in further accordance to literature. Thus, a low gas flow is usually recommended for good detector performance. **Figure 5.6** represents an overview of different temperature settings. Compared to caffeine, PDMS again showed very similar tendencies, so that a low evaporator temperature in combination with a high nebulizer temperature resulted in the largest peak area. In detail, acetone as mobile phase again showed a slightly better performance than THF. The comparison between caffeine and PDMS led to a similar analyte-independent, but solvent-dependent behavior for the ELSD and both types of nebulizers.

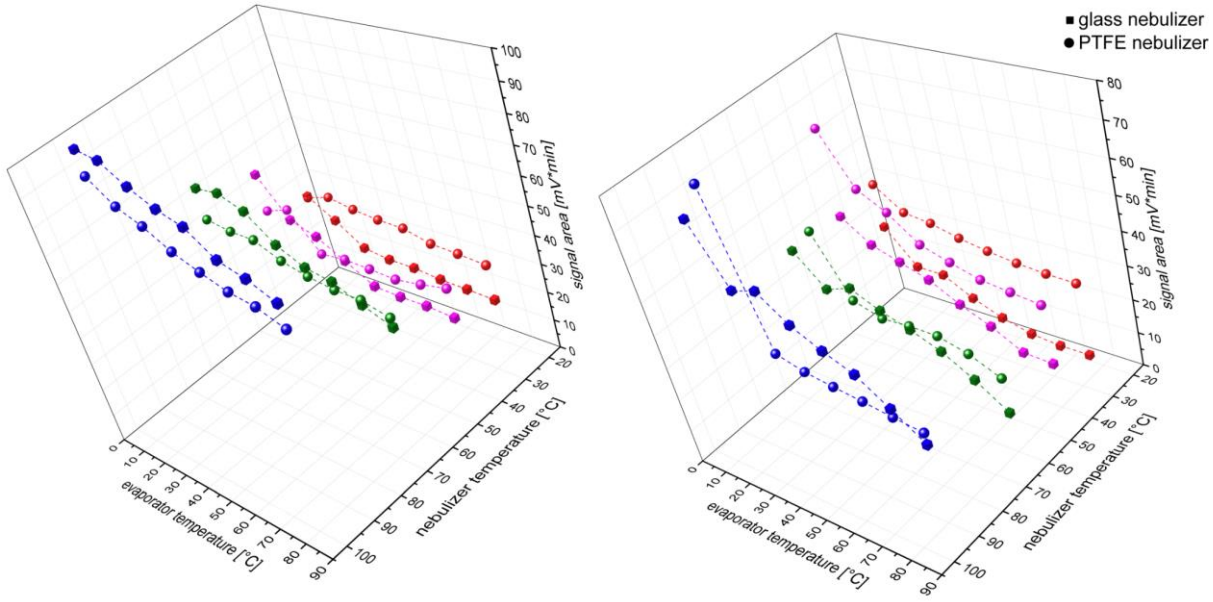

Figure 5.6: *Dependence of PDMS peak area on different settings for nebulizer and evaporator temperature for acetone* ***(left)*** *and THF* ***(right)****; different settings for nebulizer temperature are color-coded (see* ***Figure 5.3****) and connected by dotted lines; LC flow rate was 1 mL·min^{-1} and gas flow rate was 1.2 SLM.*

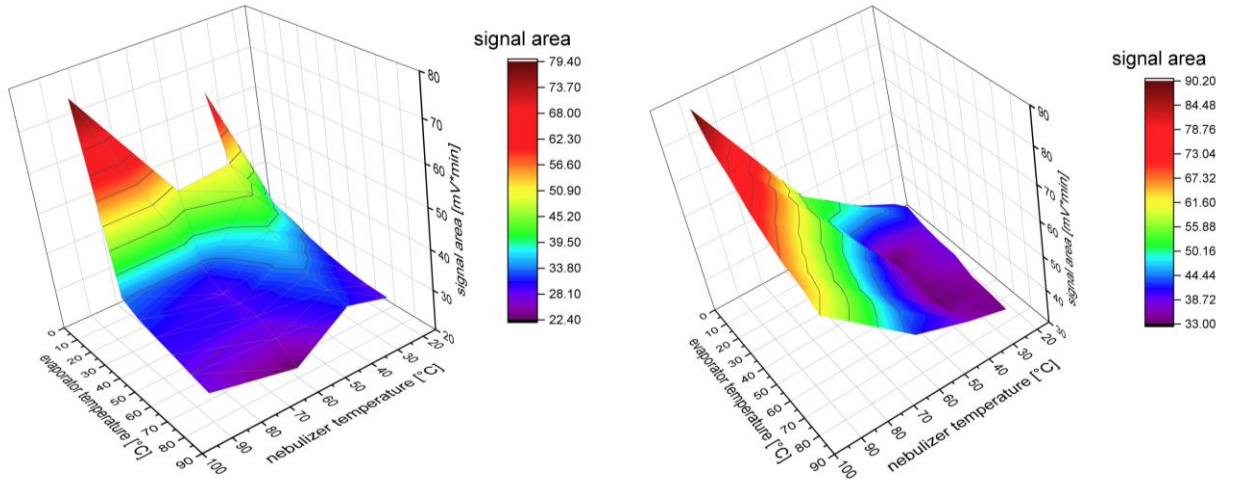

Figure 5.7: *Dependence of PDMS peak area on different settings for nebulizer and evaporator temperature for acetone* ***(left)*** *and THF* ***(right)*** *for parallel-path PTFE nebulizer; LC flow rate was 1 mL·min-1 and gas flow rate was 1.2 SLM.*

The additional contour plots (**Figure 5.7** for parallel-path PTFE nebulizer and **Figure 5.8** for concentric glass nebulizer) show these conclusions in a more detailed way. Operating the detector near its limits of nebulizer and evaporator temperature showed some combinations for THF (T_N = 90 °C, T_E = 10 °C and T_N = 60 °C, T_E = 10 °C) where the generation of a proper aerosol was not possible due to large effluent solvent droplets. These limitations showed that beside a good detector response a proper aerosol formation was of particular importance for a robust detector performance.

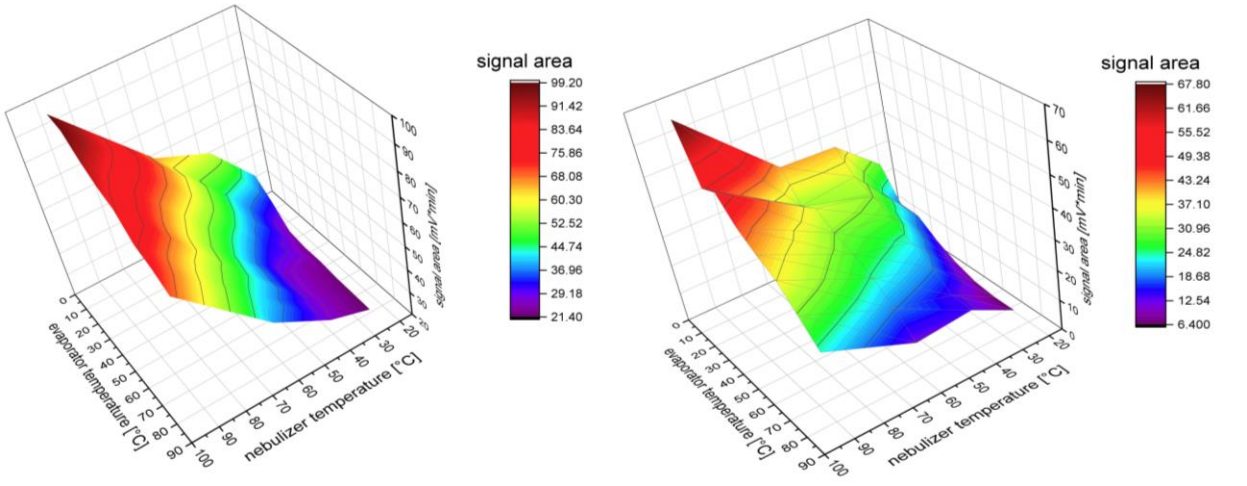

Figure 5.8: *Dependence of PDMS peak area on different settings for nebulizer and evaporator temperature for acetone* ***(left)*** *and THF* ***(right)*** *for concentric glass nebulizer; LC flow rate was 1 mL·min^{-1} and gas flow rate was 1.2 SLM.*

Similar to the measurements with caffeine, the LC flow rates had a different effect on parallel-path PTFE and concentric glass nebulizer, which resulted in a better signal of the former for low flow rates below 0.3 mL min^{-1} (**Figure 5.9**). As mentioned above, miniaturized LC systems would be ideally suited for combinations with a detector offering high sensitivity at low flow rates, but on the other hand LC flow rate is in a category of parameters which are not freely adjustable in all analyses. The ideal parameters in case of acetone as mobile phase were T_E = 10 °C and T_N = 90 °C and in case of THF T_E = 20 °C and T_N = 90 °C.

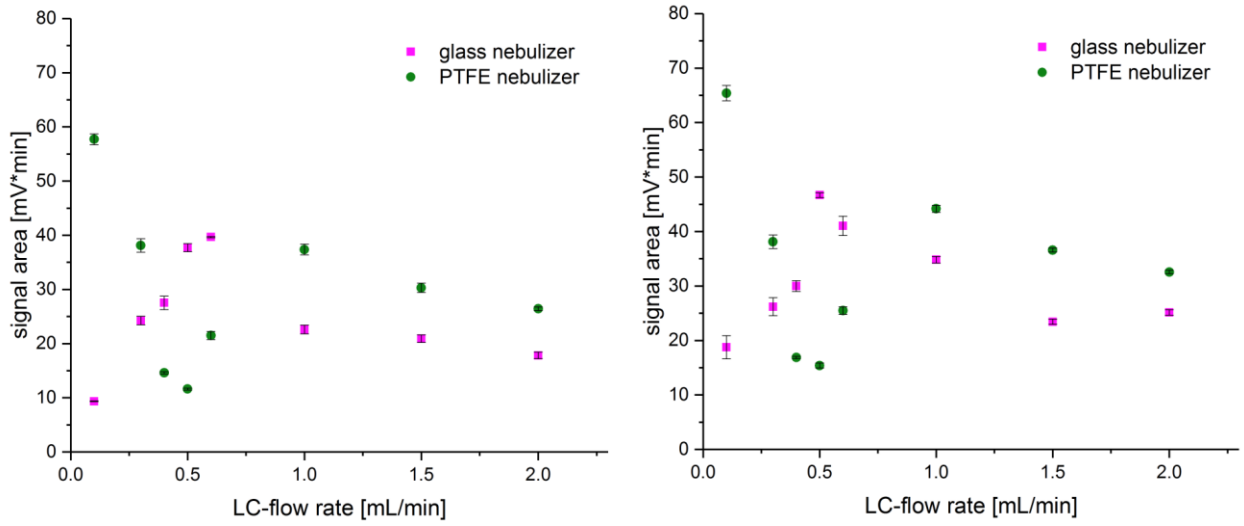

Figure 5.9: *Dependence of the ELSD signal for PDMS with viscosity of 10 mPa·s* ***(left side)*** *and with viscosity of 100 mPa·s* ***(right side)*** *on LC flow rate of THF using a concentric glass nebulizer and a parallel-path PTFE nebulizer, respectively. ELSD settings: evaporator temperature 25 °C, nebulizer temperature 40 °C and gas flow rate 1.2 SLM.*

Beside variations of detector settings and solvents, PDMS samples with different viscosities (**Figure 5.2 b)** and **c)** and **Table 5.3**) were used to study the influence of molecular weight on the detector response. For viscosities ranging from 5 to 100 mPa s the signal intensity increased continuously, which originated from a decreasing content of vaporable PDMS. Above a viscosity of 100 mPa s the slight decrease of the detector signal can be attributed to a reduced number of particles for light scattering. Furthermore, as mentioned in the introduction, the variation of particle diameter is associated with different type of light scattering. For the study of PDMS a molar mass-dependent detector response was found over a broad molar mass range. Therefore, the detection with comparable response characteristics over an extended mass range is challenging, and universal calibration is not possible. Consequently, detailed studies concerning separated oligomer PDMS species and their corresponding ELSD responses were undertaken. Apart from these considerations, the comparison of both nebulizer types showed a better performance for the parallel-path PTFE nebulizer, except in the case of silicone oil of viscosity of 5 mPa s. Thus, for

polymer samples the use of a parallel-path PTFE nebulizer was found an advantageous alternative to a concentric glass nebulizer due to the improved sensitivity.

Table 5.3: *The influence of PDMS viscosity on the ELSD signal and type of nebulizer, sample concentration of each type of silicone oil (in THF) was 500 µg·mL^{-1}, ELSD settings: evaporator temperature = 25 °C and nebulizer temperature = 40 °C and gas flow = 1.2 SLM, LC flow rate 1.0 mL·min^{-1}.*

		Signal area [mV·min]			
Viscosity [mPa·s]	**Mean molar Mass [g·mol^{-1}]**	**Glass nebulizer**	**RSD (n=5)**	**PTFE nebulizer**	**RSD (n=5)**
5	1100	22.48	± 1.93	17.62	± 1.68
10	1700	32.61	± 1.58	37.37	± 1.57
100	9700	34.82	± 1.24	44.17	± 1.21
1000	31900	27.88	± 2.16	41.53	± 2.11
10000	69300	25.3	± 1.88	38.67	± 2.48

5.4.2.2 Comparison of nebulizer long-term stability

Investigations regarding the long-term stability of both nebulizer types were done under continuous operation and typical requirements of polymer HPLC. Therefore, aliquots of the same sample were measured after different time intervals. In **Figure 5.10**, repeated chromatographic recordings of PDMS separations are shown for the concentric glass nebulizer after 280 h and for parallel-path PTFE nebulizer after 2000 h operation hours between the two depicted chromatograms.

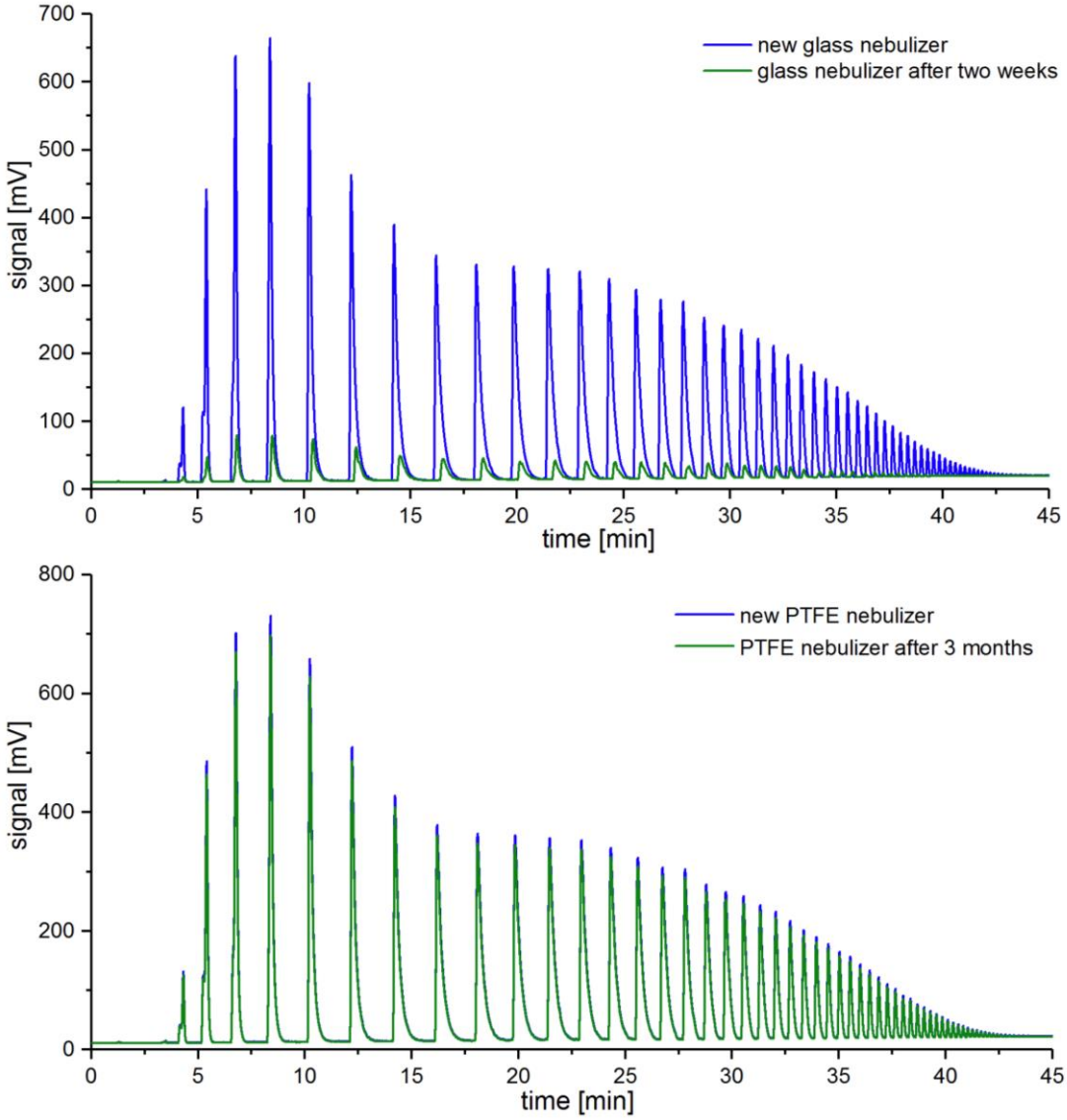

Figure 5.10: *Long-term stability of concentric glass* ***(above)*** *and parallel-path PTFE* ***(below)*** *nebulizer in polymer HPLC, separation of a silicone oil with viscosity of 10 mPa·s; ELSD settings: evaporator temperature 25 °C, nebulizer temperature 40 °C and gas flow rate 1.2 SLM; LC flow rate 1 mL·min^{-1}.*

In case of the parallel-path PTFE nebulizer almost no loss in performance was found after this extended period of operation. In contrast, measurements with the concentric glass nebulizer led to clearly reduced response behavior after a much shorter time interval. The concentric glass nebulizer could be reactivated temporarily by flushing and washing the dismounted nebulizer with strong organic solvents and peroxyacetic acid. But this cleaning method was not always successful and required considerable efforts. The parallel-path PTFE nebulizer was in continuous use over a period of three months without any loss in detection performance independently from molecular mass of the used PDMS samples and might be operated even longer. The strongly diminished performance of the concentric glass nebulizer was a consequence of partial clogging of the sample capillary within the tip of the nebulizer. The alternate spray technology with two different paths for LC flow and gas flow of the parallel path PTFE nebulizer in combination with a change of basis material from glass to PTFE led to an increased robustness of the ELSD. Thus, especially in case of polymer samples this adaption of the ICP parallel-path PTFE nebulizer to an ELSD device is a promising instrumental development for the field of polymer HPLC. According to these findings, the improvement of long-term stability of the ELSD is attributed by the exchange of the nebulizer type. It can be assumed that a) the material exchange from glass to PTFE and b) the different spray technology lead to this enhancement.

5.4.2.3 PDMS oligomer analysis

For detailed analysis of PDMS an Accucore C30 column was used to separate individual oligomers with a mobile phase combination of methanol: water (75:25, v/v) and acetone. Therefore, it was important to consider influences of small amounts of water on the nebulization and evaporation processes of the ELSD. Ideal settings for pure organic solvents prevent a robust detection with proper sensitivity. To adapt the settings for use with water, the manufacturer [9] advised an increase of evaporator temperature or an increase of gas flow. In this work, the evaporator temperature was optimized for aqueous conditions and the use of the parallel-path PTFE nebulizer in analogy to the above described procedure. Thus, for a proper ELSD performance an evaporator temperature of 40 °C and a gas flow of 1.2 SLM was found suitable in combination with a nebulizer temperature of 90 °C.

To assign the individual PDMS species, the number of monomer units was indicated along with the symbol "Si" representing the linear structure of the PDMS oligomers. In a first measurement series, the isolated pure Si16 was used for detector calibration in a concentration range from 10 g mL^{-1} up to 1000 g mL^{-1}. The expected nonlinear calibration plot with a second degree polynomial fit is shown in **Figure 5.11**. According to the regression model and the six-sigma method, relative standard deviation for the calibration curve was 2.5 %, the limit of detection was 10 g mL^{-1} and the limit of quantification (LOQ) was 40 g mL^{-1}.

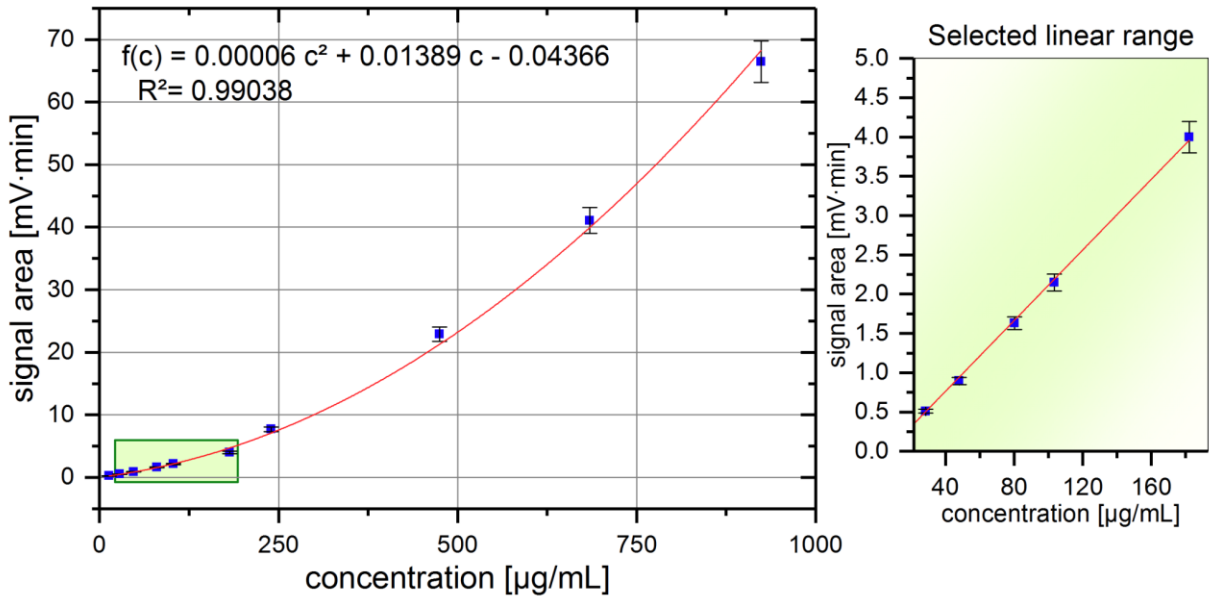

Figure 5.11: *Calibration plot for Si16,* ***left side*** *second degree polynomial fit over the whole concentration range of calibration and* ***right side*** *extracted linear area for response factor determination.*

For determination of the response factors for the respective analytes, the slope of the linear regression of a small linear dynamic detector range from the LOQ up to 180 g mL^{-1} was used (see right side **Figure 5.11**). Other possible fits such as using the power function or approaches to determine the response factors from the quadratic calibration curve showed higher uncertainties than this simple method. With these criteria, the determination of all response factors from Si11 to Si50 (**Figure 5.12**) were determined to compare detector sensitivity for this range of PDMS oligomers. The narrowing of the dynamic range to these concentrations was done according to aspects of trace analysis and for reasons of comparability.

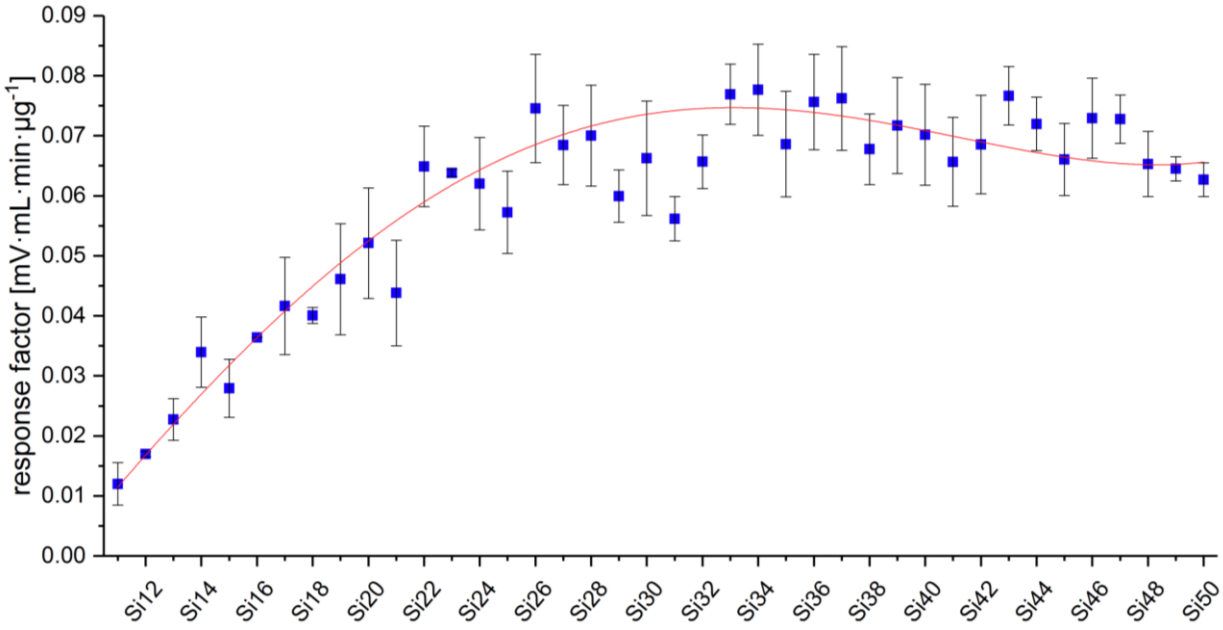

Figure 5.12: *Response factors for different PDMS oligomers from Si11 to Si50. Error bars (n=3) are calculated from linear regression; the red fit is a forth order polynomial fit curve ($y = 8.3 \cdot 10^{-8} x^4 + 8.0 \cdot 10^{-6} x^3 + 1.5 \cdot 10^{-4} x^2 + 1.5 \cdot 10^{-2} x - 4.7 \cdot 10^{-2}$).*

The evaluation of determined response factors (**Figure 5.12**) showed an increase in sensitivity from Si11 to Si22. For higher oligomers a more or less equal response factor was found. This behavior agreed with the results for non-oligomer separated PDMS of different viscosities shown above (**Table 5.3**). This molar mass-independent response allowed a universal calibration and therefore lesser complexity in the molar mass range from Si24 to Si50. Furthermore, these observations showed that a universal calibration curve for ELSD is not ideal because of different detector sensitivity particular for analytes which are highly vaporable. Beside this, different light scattering phenomena depending on particle size and diameter might change the signal intensities of different molecular weight samples as mentioned above.

5.5 Conclusion

Various factors influencing the signal response of an Agilent ELSD, i.e. evaporator temperature and nebulizer temperature, flow rate of ELSD gas, LC flow rate and different mobile phases were investigated. In general, temperature and gas flow settings of the ELSD showed similar trends for various solvents. The analytes caffeine as model system and PDMS as real sample were studied and showed similar behavior regarding the optimization of detector parameters. The highest signal intensity could be obtained when applying the highest possible nebulizer temperature at lowest possible evaporator temperature with a low gas flow rate for the nebulizer.

Comparison of a concentric glass nebulizer and a parallel-path PTFE nebulizer showed a similar performance concerning the optimization of the above-mentioned parameters. However, for LC flow rates below 0.3 mL min^{-1} the parallel-path PTFE nebulizer exhibited an improved sensitivity. In addition, ELSD with an integrated parallel-path PTFE nebulizer enabled much better long-term stability in case of studies of PDMS samples with complex matrices than the concentric glass nebulizer. Thus, the use of a parallel-path PTFE nebulizer increased the robustness of the detector in daily operation. An increase of evaporator temperature was necessary in presence of low amounts of water in the mobile phase. PDMS samples with various viscosities (and thus differing regarding the mean molar masses) showed that the volatility of the compounds in the detection process had a superordinate effect on peak area. Only if the analyte remained in the aerosol, other influencing factors like mobile phase composition or nebulizer type became more important.

The newly introduced parallel-path PTFE nebulizer showed quite good results in case of polymer matrices and for the first time individual response factors for various PDMS oligomers were determined. PDMS oligomers had a nearly constant response factor for species above Si22 which allowed a universal calibration in this molar mass range. In contrast, it was necessary to perform calibrations for PDMS oligomers of lower molecular weight separately. The same conclusion has to be drawn for samples containing large amounts of (compared to the investigated oligomers) of PDMS with very high molecular mass, where different physical aspects of light scattering predominate. In summary, it can be concluded that the substitution of a concentric glass nebulizer of a conventional Agilent ELSD by a parallel-path PTFE nebulizer originally developed for ICP applications by Burgener enables attractive performance characteristics for polymer analysis. The main advantage is the superior long-term stability, especially in presence of complex sample matrices.

5.6 References

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6 High-resolution polymer HPLC

6.1 High-Resolution Polymer High Performance Liquid Chromatography: Application of a saw tooth gradient for the separation of various polymers

This chapter has been published and adapted from:

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik, Journal of Chromatography A, 1587, 2019 88-100.

Measurements and experiments were done solely by the author. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

6.1.1 Abstract

Currently, a lot of research effort in polymer analysis by liquid chromatographic techniques, including size exclusion chromatography (SEC), polymer HPLC or liquid chromatography at critical conditions, is done aiming to improve separation performance. In this study, novel gradient protocols were investigated primarily based on gradient polymer elution chromatography (GPEC). Starting with linear gradients and stepwise gradients a new periodic saw tooth gradient profile was developed and optimized. Optimum settings for the saw tooth gradient design were evaluated by design of experiments (DoE) based on Taguchi's methodology for various types of stationary phases. The gain of peak resolution was dependent on the effective gradient step height. The optimized protocol enabled high-resolution polymer HPLC (HRP-HPLC) separations with common HPLC instruments. The quality of separation was evaluated by heart-cut fraction collection of HRP-HPLC and subsequent determination of the individual fractions by SEC or MALDI-ToF mass spectrometry. Finally, different types of polymers, such as PVC, PDMS, PMMA, or PPG, were studied with the new method and a universal applicability was shown.

6.1.2 Introduction

The investigation of polymers, with heterogeneous composition in more than one distribution property, is a challenging task. Applying various types of liquid chromatographic techniques, separations according to molar mass, chemical composition or polymeric architecture can be achieved. In recent years, different approaches for the separation of complex polymeric materials were used, e.g. isocratic or gradient SEC as well as liquid chromatography at critical conditions (LCCC) [1,2]. The connection between these different modes of polymer separation techniques can be explained by thermodynamic treatment [3–6]: Under ideal SEC conditions the separation depends on entropy changes only, while under ideal liquid adsorption chromatography (LAC) conditions the separation only depends on enthalpy changes. In SEC, polymers are separated due to their different hydrodynamic volumes in a solvent and, thus, a molecular mass distribution can be obtained. Therefore, no interaction between the polymer and the stationary phase should occur or virtually be minimized. In LAC, polymer analysis is mainly determined by interactions between analyte, mobile and stationary phase. Therefore, a variety of different parameters must be adjusted. If enthalpic and entropic energy changes equalize each other and thus the change of free Gibbs energy becomes zero critical conditions are realized. At critical conditions, LCCC for isocratic elution or critical point of adsorption (CPA) for gradient elution, molar mass does not contribute to retention volume, enabling separations solely based on differences in chemical composition. Compared to LCCC, applying gradient elution for separation of polymers at critical conditions provides a separation system which is not terminated by pore size of the stationary phase [1,4,6]. However, each method has its own advantages and disadvantages. The constraints of SEC include for example secondary enthalpy driven interactions or indirect molar mass determination by measuring the hydrodynamic volume of the polymer. Furthermore, depending on the pore volume and molecular weight of investigated samples, low separation performance with broad peaks may occur [6]. Nevertheless, SEC provides an enhanced resolution especially in the high molecular mass range of polymers, compared to gradient LAC where at the critical point of adsorption no separation due to molar mass differences is possible [1].

As well as in SEC, liquid chromatography at critical conditions, shows disadvantages in reproducibility, susceptibility to fluctuations, sample recovery or overall application to minor changes of the sample matrices. Beside this, in polymer HPLC, optimizing the parameters of measurement is often difficult concerning the choice of proper stationary and mobile phase combinations, e.g. adsorption promoting and desorption promoting solvents or a retention promoting column for the investigated polymer. Furthermore, the diversity of various separation parameters makes method development challenging and time consuming [6–9].

Compared to HPLC of small molecules, polymer HPLC especially differs in terms of small diffusion coefficients of the constituents in solution and a different retention mechanism of polymers on the stationary phase. A further difference between macromolecules and small molecules is the objective of the chromatographic separation, whereby in HPLC of small molecules, the exact identification and quantification is in focus and in polymer HPLC, the fractionation of macromolecules based on various polymer characteristics, e.g. molar mass distribution, size in diluted solution, chemical distribution, or chain structure, prevails. In addition, polymer retention lasts as long as at least one repetition unit of the polymer is adsorbed to the stationary phase. Unlike in HPLC of small molecules, polymers must be dissolved in very strong solvents, e.g. THF or toluene, considering reversed phase polymer HPLC. Consequently, solubility effects become important in addition to adsorption and partition phenomena. The injection of the dissolved polymers at the (usually strong adsorption promoting) initial conditions in gradient polymer elution chromatography (GPEC) result in precipitation or strong adsorption of the analytes on the head of the column [1,10–12]. With increasing amounts of desorption promoting solvent, the (homo-)polymers elute in reversed elution order compared to SEC, from low to high molar masses, at least as long as the critical point of adsorption is not reached. Therefore, it is not only sufficient that a solvent is a strong solvent for the investigated polymer. Additionally, the separation system (mobile and stationary phase) must provide desorption promoting characteristics for the used strong polymer solvent. Apart from molar mass differences, chemical functionalities cause an additional separation, especially dominating in the low molecular mass region [3,13]. The separation occurs predominantly according to adsorption effects to the stationary phase and precipitation effects depending only on the solubility in the mobile phase. In addition to LAC, Glöckner et al [7] termed separations without adsorption effects to the stationary phase high performance precipitation liquid chromatography (HPPLC). Staal [14] showed the similarities between cloud point determination by turbidimetric titration and the precipitation- / re-dissolution processes in the different steps of the chromatographic separation for reversed phase systems. In the first step, the polymer is dissolved in a strong solvent (1), and then the precipitation of the dissolved polymer on the column head occurs (2) in combination with the adsorption to the stationary phase (3). By attaining a suitable solvent combination between solvent and non-solvent, e.g. the cloud-point of polymer, the precipitated polymer is re-dissolved (4) but remains adsorbed to the stationary phase (5). In the final step, the complete elution of the fully dissolved polymer occurs from the stationary phase (6). German et al [13,15,16] showed in a series of papers the differences between precipitation- / re-dissolution and adsorption mechanisms by analyzing polyesters. For crystalline polyester, a clear

dependence on precipitation- / re-dissolution mechanism could be shown, as for all other studies adsorption effects dominated or at least supported the separation.

An overview of different possible setups for gradient elution is shown by Deyl [17] and Jandera [18]. For a first approach, the slope variation of a linear gradient is a good choice and may sometimes lead to multilinear gradients enhancing the separation. Therefore, Nikitas et al [19–23] presented various approaches for optimizing multilinear gradients. Moreover, software packages such as DryLab or PREGA use similar theoretical concepts [24]. As a consequence, concave and convex gradient shapes might also be a useful alternative [25]. Furthermore, especially for various types of macromolecules, a step gradient improves peak resolution and separation performance. The analysis of azeotropic and low-conversion poly(styrene-stat-2-methoxyethyl methacrylate) [26], styrene acrylonitrile copolymers [27], lignin [28] or humic like substances [29,30] was improved through different types of step gradients. Applying a step gradient, various isocratic steps result in an improved peak resolution, which cannot usually be achieved by a linear gradient. A further improvement of this gradient profile was presented by Kajdan et al [31] for ion chromatographic separation of recombinant proteins and by Spranger et al [32] for the separation of humic like substances with RP-HPLC. They used a kind of spiked gradient profile, where in an additional step the elution promoting solvent was reduced at the end of the original gradient step. This modification results in a much better separation performance than in previously described gradient profiles. Beside this, Morris et al [33] optimized with a saw tooth like gradient the separation of complex protein mixtures.

As the application of step gradients in polymer separation showed pretty good results [27,34], further investigations concerning the shape of the gradient profile are promising for improving the separation. In this report, a novel gradient protocol for the separation of polymers, which allows each individual solvent composition to perform its unique re-dissolution ability, is evolved. With a saw tooth gradient protocol, the separation performance significantly increased. Therefore, the aim of this work was the development of a preferably universal saw tooth gradient protocol which allows high-resolution polymer HPLC (HRP-HPLC) of different types of polymers over a broad molecular weight range.

6.1.3 Material and methods

6.1.3.1 Software

The programming of the saw tooth gradient was possible over the entire gradient range from 0 to 100 % desorption promoting solvent with the chromatographic data system Chromeleon (Thermo-Fisher Scientific, version 7.2), as other investigated chromatographic software packages are limited in the number of possible entries of the gradient table. Moreover, currently, a complete saw tooth gradient ranging from 0 to 100 % with the corresponding steps can be achieved in combination with Thermo Fisher Scientific (Waltham, USA) HPLC pumps. The Agilent driver module of Chromeleon limits the gradient entries to 69 and Agilent ChemStation Version C limits the gradient entries to 100 for an Agilent (Waldbronn, Germany) HPLC system, while the limitation of WinGPC UniChrom 8.2 (Polymer Standard Service) is 161 entries. These limitations made it difficult to use the full potential of the saw tooth profile and, therefore, using an Agilent fraction collector only a small region of interest could be fractionated.

6.1.3.2 Hardware

6.1.3.2.1 LC systems and detectors

The optimization of the saw tooth gradient with design of experiments (DoE) by Taguchi was done on a Thermo Fisher Scientific (Waltham, USA) Vanquish UHPLC with UV detection at 215 nm and a 385 ELSD, equipped with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. (Mississauga, Ontario, Canada) at 40 °C evaporator temperature, 90 °C nebulizer temperature, and a gas flow of 1.2 SLM (standard liter per minute, see [35]). The investigated parameters for the optimization of the saw tooth gradient profile by DoE are given in **Table 6.1** and were performed from 0 % THF (100 % methanol) to 100 % THF (0 % methanol) considering different types of stationary phases. The other analytical measurements of different types of polymers were performed on an Ultimate 3000 HPLC of Thermo Fisher Scientic with the modified 385 ELSD.

Table 6.1: *Investigated parameters for the design of experiment according to Taguchi's L16 (4^5) approach.*

	Group	**1**	**2**	**3**	**4**	**5**
		Poroshell C18 50x4.6 mm, 2.7 µm	Poroshell C18 100x4.6 mm, 2.7 µm	Hypersil BDS C18 100x4.6 mm, 2.4 µm	Luna C18 100x4.6 mm, 5 µm	Hypersil Gold C18 aQ 100x10 mm, 5 µm
Label	**Parameter**	**Level 1**	**Level 2**	**Level 3**		**Level 4**
A	height of the negative backward gradient step [%]	3.0	6.0	9.0		12.0
B	effective step height [%]	0.2	0.5	0.8		1.0
C	retardation of negative slope	0.5	1.0	2.0		3.0
D	lower plateau	0.5	1.0	2.0		3.0
E	retardation of positive slope	0.1	0.5	1.0		2.0

6.1.3.2.2 Studies of the real shape of the gradient profile

The actual gradient profile was measured with an Ultimate 3000 diode array detector at 265 nm, following the recommendation of Thermo Fisher Scientific for operational/ performance qualification (OQ/PQ) for gradient accuracy with 100 % pure water as starting condition against 0.1 % acetone in water [36]. As test columns an Agilent Poroshell C18 EC (50x4.6 mm, 2.7 µm), an Agilent Poroshell C18 SB (150x4.6 mm, 2.7 µm), and a restriction capillary of 15 m length and 0.18 mm ID from OQ/PQ Kit of Thermo Fisher Scientific were used.

6.1.3.2.3 Semi preparative LC systems

Fraction collection of PVC was performed on an Agilent 1100 series LC system with a THF resistant 3115α degasser from ERC (Riemerling, Germany) equipped with an Agilent fraction collector. For adjusting the separation pattern at an Agilent Poroshell C18 EC (50x4.6 mm), a 385 ELSD modified with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. (Mississauga, Ontario, Canada) was used at 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.2 SLM gas flow. The LC flow rate was set to 1.0 mL·min^{-1}, and the injection volume was 10 µL (1 mg absolute sample amount). The saw tooth gradient was started at 26 % THF and 74 % methanol and ended at 56 % THF and 44 % methanol with an effective step height of 2 % and a height of the negative backward gradient step of 9 %. After each separation, the column was flushed with 100 % THF for 8 min in order to remove not eluted polymer from the column. For sufficient amount of sample per fraction, 50 injections were performed. The SEC measurements were performed with an Agilent 1260 SEC system and a Shodex (Munich, Germany) RI101 detector on a set of four Styragel® columns (HR1, HR3, HR4 and HR5, Waters, Eschborn, Germany) and THF as eluent at 1.2 mL·min^{-1}.

Fraction collection of PS was performed on a 1260 series LC system of Agilent with a THF resistant 3115α degasser from ERC (Riemerling, Germany). UV detection was performed at a wavelength of 215 nm. A Poroshell C18 EC (50x4.6 mm) was used as stationary phase. The gradient profile was started at 0 % THF and 100 % methanol, within 15 min a linear gradient was set to 31 % THF and then the actual saw tooth gradient started with an effective step height of 0.2 % and 9 % height of the negative backward gradient step up to a final concentration of 39 % THF and 61 % methanol. After each separation, the column was flushed with 100 % THF for 8 min in order to remove not eluted polymer from the column. A flow rate of 1.0 mL·min^{-1} and an injection volume of 15 µL (0.75 mg absolute sample amount) were applied. To get sufficient sample amount per fraction 100 runs were performed. For evaluation of the collected fractions MALDI-ToF-MS measurements were performed on a Shimadzu Axima Performance MALDI-ToF-MS

(Kratos, Manchester, UK). As cationization reagent a solution of 100 mol·L^{-1} sodium trifluoroacetate (Sigma-Aldrich, Darmstadt, Germany) in THF was used and 10 mg·mL^{-1} trans-2-[3-(4-tert-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) (Sigma-Aldrich) was used as MALDI matrix. Various mixing ratios between sample solution: matrix solution: cationization solution of 10:10:1, 10:20:10, 10:50:1 and 10:100:1 (v/v/v) were used in order to obtain an appropriate spectrum because the actual concentrations of the collected fractions were unknown.

Fraction collection of PDMS with a viscosity of 350 mPa·s was performed on a 1260 series LC system from Agilent with a THF resistant 3115α degasser from ERC. For adjusting the separation pattern, a 385 ELSD modified with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. was used at 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.2 SLM gas flow. An Accucore C18 (50x4.6 mm) column was used, the LC flow rate was set to 1.0 mL·min^{-1} and 0.4 mg sample amount were injected 100 times, to get sufficient amount of sample per fraction, for separation with a saw tooth gradient (effective step height 1.0 %, effective step length 1.50 min) and a linear gradient with methanol and THF as mobile phase components, respectively. The multilinear gradient was started at 100 % methanol and reached 30 % THF after 15 min, 65 % THF after 78 min, and 100 % THF after 79 min. Each fraction (16 fractions, starting from 37 min up to 61 min) was collected within 1.50 min intervals, for both gradients. For fractionation evaluation, MALDI-ToF-MS measurements were performed according to the above-mentioned protocol, but in place of sodium trifluoroacetate, silver trifluoroacetate was used as cationization reagent.

6.1.3.3 Stationary phases

For preparative fraction collection of PS and PVC, an Agilent Poroshell C18 EC (50x4.6 mm, 2.7 µm) was used. For the DoE approach (see **Table 6.2**) an Agilent Poroshell C18 EC (50x4.6 mm, 2.7 µm), an Agilent Poroshell C18 EC (100x4.6 mm, 2.7 µm), a Thermo Fisher Scientific Hypersil BDS C18 (100x4.6 mm, 2.4 µm), a Thermo Fisher Scientific Hypersil Gold C18 aQ (100x10 mm, 5 µm), and a Phenomenex (Torrance, USA) Luna C18 (100x4.6 mm, 5 µm) were used as superimposed group for the parameters of the saw tooth gradient profile. The investigations of various polymer standards were done under optimized gradient conditions on a Thermo Fisher Scientific Accucore C18 (50x4.6 mm, 2.6 µm) and an Agilent Poroshell HILIC (50x4.6 mm, 2.7 µm).

Table 6.2: *DoE confirmation experiments for different stationary phases for the optimum shape settings of the saw tooth gradient.*

Column	A [%]	B [%]	C	D	E
Poroshell C18 50x4.6 mm, 2.7 µm	6.0	0.2	1.0	3.0	2.0
Poroshell C18 100x4.6 mm, 2.7 µm	6.0	0.2	0.5	2.0	2.0
Hypersil BDS C18 100x4.6 mm, 2.4 µm	6.0	0.2	1.0	3.0	2.0
Luna C18 100x4.6 mm, 5 µm	6.0	0.2	1.0	3.0	2.0
Hypersil Gold C18 aQ 100x10 mm, 5 µm	6.0	0.2	0.5	0.5	2.0
ideal settings independently of column type	6.0	0.2	1.0	3.0	2.0

6.1.3.4 Polymer samples and chemicals

All solvents used were HPLC grade. Acetone, acetonitrile (ACN), methanol (MeOH), toluene, n-hexane, and non-stabilized tetrahydrofuran (THF) were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. The analyzed polymer standards and samples are summarized in **Table 6.3**. PS 19 600 standard was purchased from PSS (Mainz, Germany), PDMS standards were obtained from Wacker Chemie AG (Burghausen, Germany), PPG standards were purchased from American Polymer Standards Corporation (Mentor, OH, USA) and all other standards listed in **Table 6.3** were purchased from Agilent (Church Stretton, UK).

Table 6.3: *Overview of used polymer standards.*

Polymer	M_p [g·mol^{-1}]	Polydispersity
PS 8995	8995	1.03
PS 19600	19600	1.02
PVC 23900	23900	1.21
PVC 45400	45400	1.30
PVC 92100	92100	1.32
PVC 202000	202000	1.34
PDMS 1300	1300	1.34
PDMS 2000	2000	1.42
PDMS 5400	5400	1.67
PDMS 8300	8300	1.83
PDMS 20700	20700	3.02
PDMS 36500	36500	2.98
PDMS 71200	71200	4.35
PDMS 130000	130000	6.09
PDMS 250000	250000	10.94
PMMA 19700	19700	1.09
PMMA 107000	107000	1.1
PMMA 690000	690000	1.09
PMMA 1600000	1600000	1.33
PPG 4850	4850	1.10
PPG 13300	13300	1.14
PPG 19600	19600	1.25
PPG 27100	27100	1.61

6.1.4 Results and discussion

Beginning with linear and stepwise gradients, the development of a saw tooth like gradient profile was done for improving separation performance of polymer HPLC. For optimization of the new gradient profile, design of experiments (DoE) according to Taguchi's approach were applied. Additionally, the limitations of the concept regarding laminar flow profiles and therefore mixing accuracy were evaluated. The separation performance of the saw tooth gradient was studied by heart-cut fraction collection with subsequent MALDI-ToF-MS or SEC measurements of each fraction. Finally, the universal application of this high-resolution polymer HPLC (HRP-HPLC) approach to various types of polymers, e.g. PS, PVC, PMMA, PDMS, and PPG, was demonstrated.

6.1.4.1 Fundamental studies of the saw tooth gradient

6.1.4.1.1 Development – from linear gradient to saw tooth gradient

As aforementioned, the resolution of HPLC for polymer analysis especially in the high molecular mass region is limited. In adsorption dominated gradient separation, no separation according to molecular mass is achievable above the point of critical adsorption. Only by means of a dominating precipitation- / re-dissolution mechanism or by absence of a point of critical adsorption further separation in this higher molecular mass range are possible [1]. Our current research is primarily directed to the yet unresolved, or rather poorly resolved molecular mass range above low molecular oligomer separation. In case of the investigated PVC standards (molar masses in peak maximum from 23,900 g·mol^{-1} up to 202,000 g·mol^{-1}, see **Table 6.3**) with linear gradients in polymer HPLC, a poorly resolved peak could be measured. The multimodalities in the peak were also present on other stationary phases, other solvent combinations or at injecting lower sample amounts. Therefore, even by optimizing mobile and stationary phases, no significant improvements were possible. Comparing a high-resolution stepwise gradient with 0.2 % step height with a linear gradient starting from 100 % MeOH to finally 100 % THF did not show any appreciable differences (see **Figure 6.1 a) + b)** for PVC 45,400).

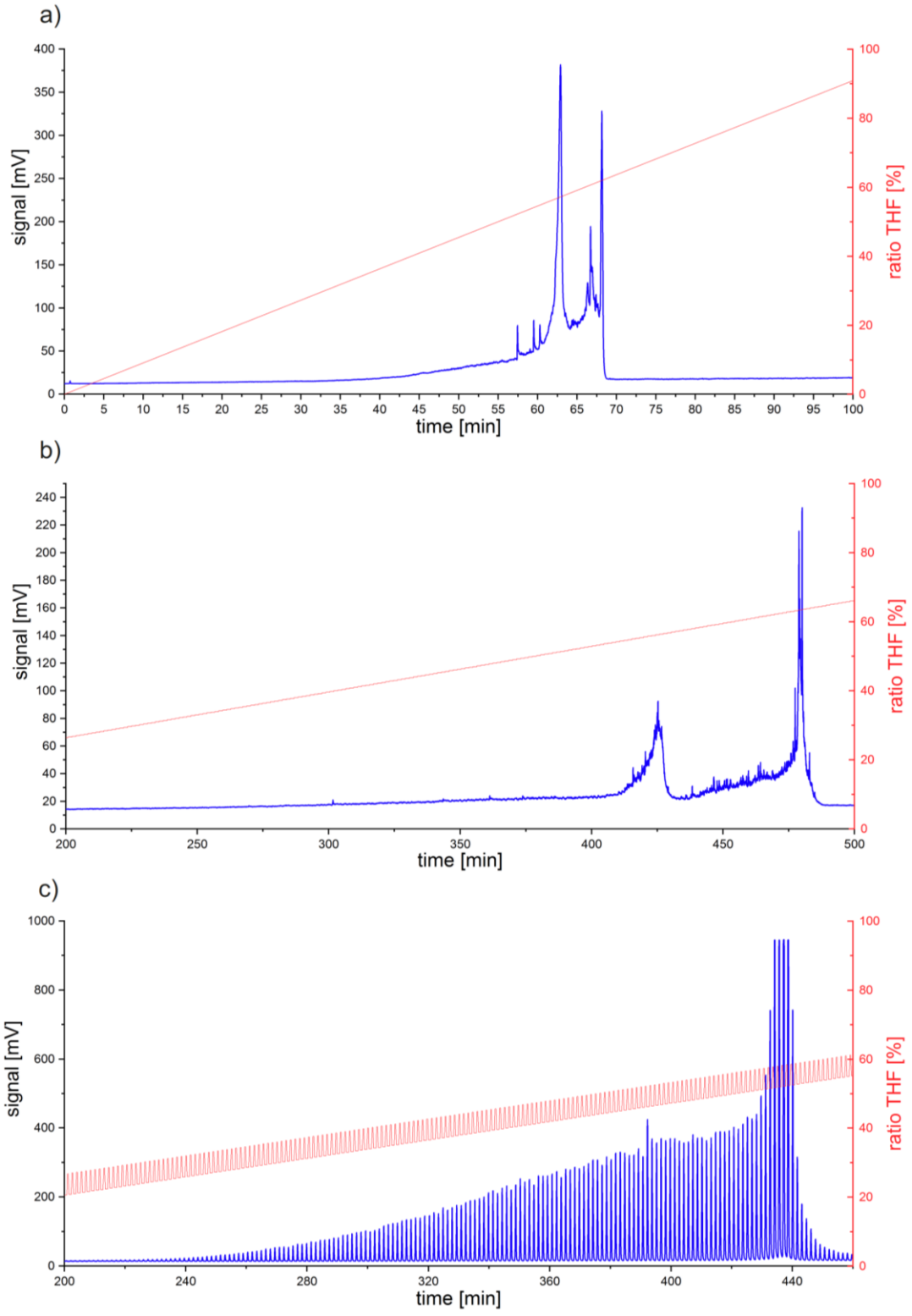


Figure 6.1: *Development of saw tooth gradient profile for the separation of PVC 45400 on an Accucore C18 (50x4.6 mm, 2.6 µm) column with MeOH as weaker or rather non-solvent and THF as desorption promoting solvent; detection with ELSD; chromatograms corresponding to* ***a)*** *a linear gradient,* ***b)*** *a stepwise gradient with step length of 1.5 min and step height of 0.2 %, and* ***c)*** *a saw tooth gradient with effective step length of 1.5 min and effective step height of 0.2 %.*

Applying the saw tooth gradient, where in an additionally step a negative gradient slope interrupts the elution of the polymer, resolution was significantly improved. The back and forth strategy of the gradient profile led to repeated fractionated elution steps, enabling selective elution of different polymer fractions. Therefore, the improvement of resolution, which was highly reproducible (compare, e.g. the set of measurements described in chapter 6.1.4.2.1 for preparative studies), was achieved to get a more detailed overview of the investigated polymer samples. An actual oligomer separation was not the primary target. According to these experimental results, two main questions arose:

1) Was this a real separation result or the recording of artefacts corresponding to the chosen gradient profile?

2) What is the optimum adjustment of the saw tooth profile and where are the limitations?

A detailed answer to the first question will be given in chapter 6.1.4.2.1 and briefly, it is a HPLC-like separation from low to high molecular masses. For the second question, further considerations were necessary which were regarded in a DoE by gradient specific parameters. Furthermore, the accuracy of mixing system and gradient profiles were examined.

6.1.4.1.2 Investigation of the gradient profile of the saw tooth approach

6.1.4.1.2.1 Design of experiments according to the methodology of Taguchi

The optimization measurements were carried out with a PS standard of molar mass at peak maximum of 19 600 g mol^{-1}. Methanol was used as weaker or rather non-solvent and THF as strong or desorption promoting solvent with a gradient range from 0.0 to 100.0 % THF (100.0 to 0.0 % MeOH). Five different types of C18 columns were used varying in length, internal diameter, particle size and total porous particles as well as superficially porous particles (see **Table 6.1**).

Using DoE, instead of one-factor-at-a-time offers the advantage of simultaneously varying several parameters and, thus, reducing the number of necessary experiments [37,38]. According to Taguchi's transformation of the response value into a signal-to-noise value, the variability of the different types of columns was included in the evaluation resulting in a higher reliability and optimization. Particularly, the advantage of Taguchi's approach is the reduced number of experiments necessary for considering the investigated parameters at different levels when compared to other approaches used in chemometrics. Furthermore, in Taguchi's approach the influence of a disturbance on the system is minimized without eliminating its reason. Further information of Taguchi's methodology is given in [39–41].

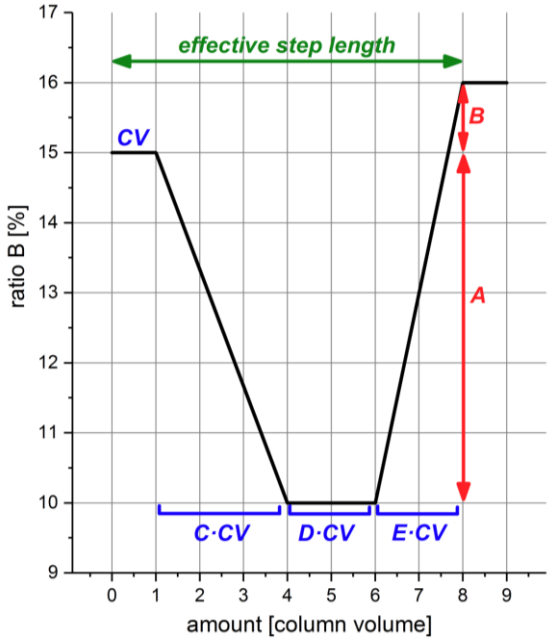


Figure 6.2: *Scheme of a general saw tooth gradient protocol, presented at one explicit effective step length and described by the amounts of column volumes (CV). Parameter A [in %] represents the height of the negative backward gradient step for the drop of the mobile phase composition, B [in %] represents the effective step height of the saw tooth gradient; the variation in several regions of the saw tooth shape is described by parameters C, D and E, as retardation of the height of the negative backward step, duration of the lower plateau, and retardation of the positive slope, respectively.*

Figure 6.2 gives an overview of the investigated and optimized parameters. The parameters A and B describe the ratio of desorption promoting solvent THF in the different steps of the saw tooth profile: A depicts the height of the negative backward gradient step, and B the effective step height between the consecutive upper plateaus. Initially, the experiments started based on interstitial column volumes using the column volume (CV) as scale up / down factor. The parameters C, D, and E determine the step length of the retardation of the height of the negative backward step, the one of the lower plateau and the one of the retardation of the positive slope, respectively. The investigated parameters and corresponding levels of Taguchi's L16 (45) are summarized in **Table 6.1**. Data evaluation based on number of peaks detected by the experiment, lowest peak resolution, asymmetry and peak width at half height of the highest peak. The confirmation experiments considering the five investigated stationary C18 phases are presented in **Table 6.2**. The confirmation experiments considered each column alone as well as all columns together resulting in a set of parameters, which are independent of column dimension, particle size, or type. These observations were in good agreement with the literature [1,13]. The prominent response factors for the saw tooth gradient profile are number of discriminable peaks, the larger the better, peak resolution, also larger the better because the inherent limited resolution of polymer peaks, and peak asymmetry, which should be around one. As depicted in **Table 6.2** the height of the negative backward gradient step (parameter A), effective step height (parameter B) and retardation of positive slope (parameter E) show the same behavior at all investigated columns. Parameter B, the effective step height alone, dominates the number of peaks and the peak height while parameter A accounts for asymmetry and resolution. Furthermore, the retardation of the positive slope (parameter E) was ideal at its highest investigated level of one column volume while the retardation of the height of the negative backward step (parameter C) should be one column volume. The length of the lower plateau (parameter D) shows the greatest variability, especially in considering analytical and semi-preparative columns. However, due to the analyses of variances (ANOVA), this parameter is only of minor significance for the saw tooth gradient profile and, thus, can be arbitrarily chosen. Although not considered by the DoE, the time of measurement should always be kept as short as possible maintaining a reasonable peak resolution. The number of steps applied in the saw tooth gradient directly affects the run time: If high resolution with maximum number of peaks is sought, the corresponding run time will be rather long. For the investigated types of columns, a set of parameters can be chosen being independent of the type of particles and column dimensions allowing a nearly universal approach. Based on these results, it is assumed that the back- and forth change in solvent composition, which caused the fractionated elution, is in general the most effective parameter for the enhancement of the separation.

6.1.4.1.2.2 Constraints by sample loading per injection

The saw tooth gradient basically depends on consistency of the programmed gradient profile to the actual gradient because of mixing accuracy and the system diffusion. The actual gradient profile was evaluated by measurements based on 0.1 % acetone in water against pure water at 265 nm detection wavelength. In a first approach, the effect of various column dimensions compared to a restriction capillary of 15 m x 180 µm was studied concerning the actual gradient profile. On basis of a very symmetric (which means each gradient step is of equal duration) saw tooth gradient a pretty good correlation or rather similar shape between calculated and effective profile was found, independently of the column dimensions or pathway within the LC system (**Figure 6.3 a)**). The major drawback of such a saw tooth gradient was its very poor separation performance. If the parameter settings obtained in a DoE are optimal, a more asymmetric gradient profile is necessary for a better separation performance. Applying a generic optimal gradient setting (**Figure 6.3 b) + c)**) by only varying the effective step length (cf. **Figure 6.1**) the overlay between calculated and actual gradient showed distinct deviations. **Figure 6.3 b) + c)** depict the impact of effective step height on the match or mismatch between both curves. No considerable dependence of the effective step height was noticeable between both measurement series (**Figure 6.3 b)** vs. **Figure 6.3 c**)). At effective step lengths above 0.60 min (chromatograms IV – VI) the alignment between actual and calculated gradient curve at the right edge (at increasing positive slope) improved as well as the separation performance. Besides an appropriate congruence of theoretical and practical gradient profile, the analysis time should possibly be shortened. Thus, the gradient setup IV in **Figure 6.3** represented a good compromise between profile alignment and analysis time.

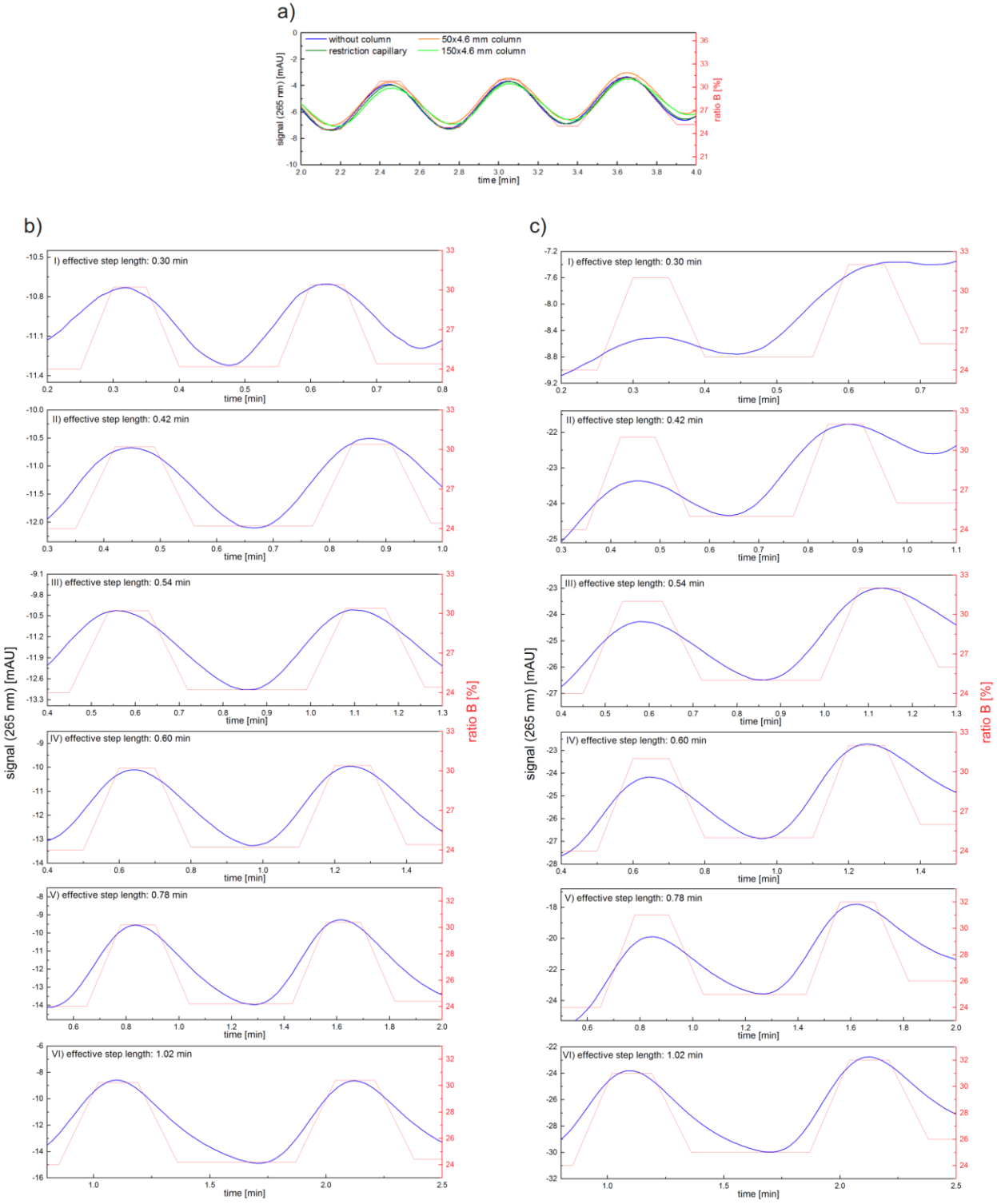


Figure 6.3: *Comparison between programmed saw tooth profile and real gradient shape at different columns and step lengths of the saw tooth profile;* ***a)*** *shows the overlay of the programmed almost symmetric (each step of similar length) saw tooth gradient with a restriction capillary, a 50x4.6 mm column, a 150x4.6 mm column and without column, the distinctions depended on the effective step length between the programmed and the actual gradient profile are shown for* ***b)*** *0.2 % and* ***c)*** *1.0 % effective step height; measurements were done according to the PQ/OQ of Thermo Fisher Scientific [36] for gradient accuracy.*

A further important influencing factor on peak resolution is the amount of polymer sample. In **Figure 6.4** the impact of injection volume and, thus the sample amount of PDMS is shown. Similar results were found while varying sample concentration at constant injection volume. The height of the small double-headed arrow in **Figure 6.4** represents a qualifier of separation performance. For injection volumes up to 5 µL or absolute sample amounts up to 100 µg nearly baseline resolved polymer peaks were obtained on the investigated stationary phase, while for higher sample amounts a prolonged effective step length would be necessary.

As conclusion, sample concentration or respectively absolute sample amount had to be adjusted carefully to the saw tooth gradient profile to achieve a good separation performance in the shortest possible analysis time. In addition to general overloading effects, caused by oversized injection volumes or absolute sample amounts with respect to stationary or mobile phase, it is important to avoid mass overloading and volume overloading with respect to the gradient profile.

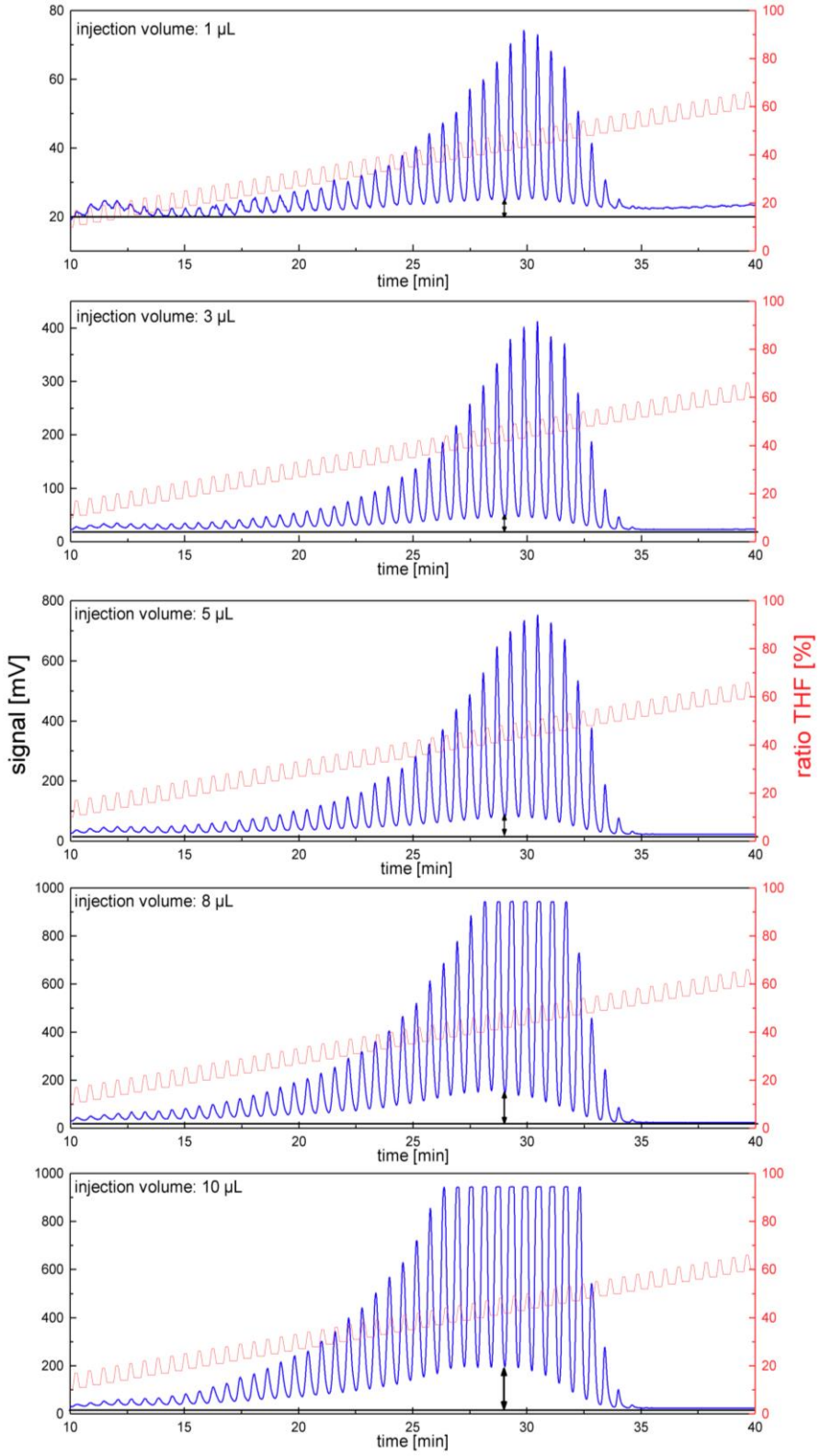


Figure 6.4: *Variation of sample amount and influence on the separation performance of the saw tooth gradient, showed for PDMS of viscosity of 1000 mPa·s and an injected sample concentration of 20 mg·mL^{-1}; Measurements were done with MeOH as weaker or rather non-solvent and THF as desorption promoting solvent on a Poroshell HILIC (50x4.6 mm, 2.6 µm).*

6.1.4.2 Application to polymer samples

With the developed and optimized saw tooth gradient, several polymer standards, e.g. PS, PVC, PMMA, PDMS, and PPG were studied. PS, PVC, and PDMS were further investigated by fraction collection evaluating the degree of separation of the applied saw tooth gradient profile while for the other polymers just the applicability was demonstrated. Methanol and THF were used as eluents; acetonitrile as weak or rather non-solvent did not show any significant advantage compared to methanol. Substituting THF as desorption promoting solvent was not investigated because of extraordinary dissolving properties of THF for the used polymers.

6.1.4.2.1 Preparative HRP-HPLC

For fraction collection by heart-cut technique, polymeric standards were chosen with a polydispersity of about 1.1. to 1.5. Re-analyzing each single fraction showed that separation depended on molecular mass differences: The low molecular mass analytes elute first and with elution time the molar masses increase.

6.1.4.2.1.1 Heart-cut HRP-HPLC + SEC for PVC analysis

As depicted in **Figure 6.5** PVC was fractionated on a Poroshell C18 EC with methanol and THF as eluents. The effective step height of the saw tooth gradient was 2.0 %, simplifying the gradient profile and reducing the necessary time as well as overcoming the restraints due to limited entries in the gradient timetable for the used LC system.

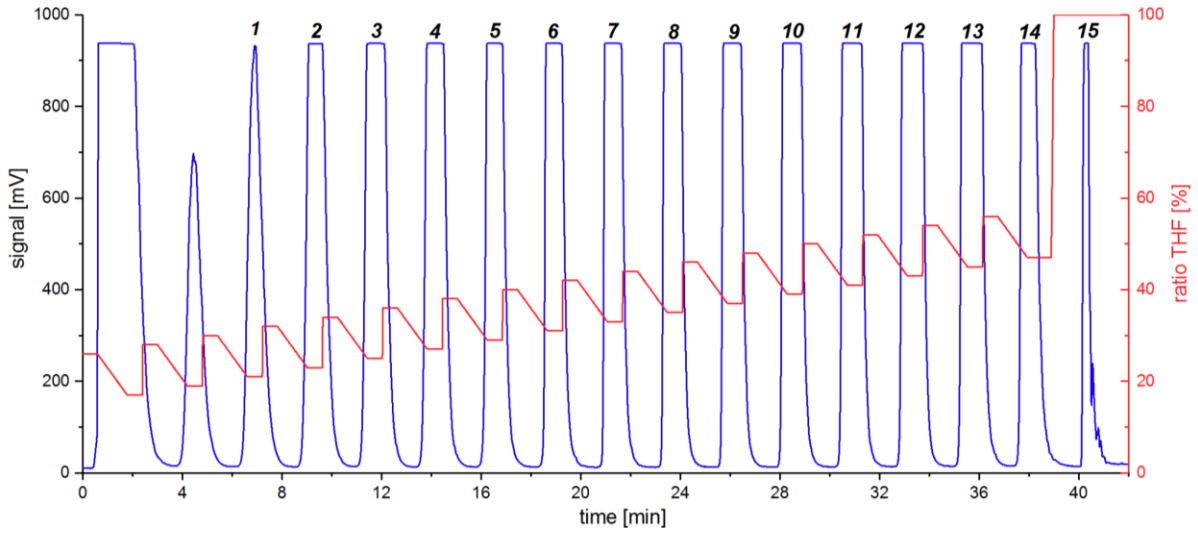


Figure 6.5: *Chromatogram and cutting pattern for preparative PVC analysis, PVC 23900 (c= 115 mg·mL-1) was analyzed with a saw tooth gradient of 2.0 % effective step height, because of the limitation to only 69 gradient time table entries for the used HPLC pump; analysis was done with MeOH as weaker or rather non-solvent and THF as desorption promoting solvent on a Poroshell 50x4.6 mm Poroshell C18 EC; fractions 1 to 15 were analyzed with SEC.*

Afterwards, the 15 collected fractions were investigated by SEC and the results are presented in **Table 6.4**. Compared to the original PVC sample of a specified molar mass of M_p = 23,900 g·mol^{-1}, the polydispersity of all fractions became narrower from fraction 1 up to fraction 15. These results proved that the HRP-HPLC separation of the investigated homopolymer only depended on the molecular weight. As consequence, the separation using the saw tooth gradient profile is a real separation and not caused by artefacts (to answer question 1 from section 6.1.4.1.1). This novel approach to the analysis of synthetic polymers considerably improved the separation performance for polymeric samples.

Table 6.4: *Results of SEC measurements after fraction collection with the saw tooth gradient of PVC 23 900 (determined on a set of Waters Styragel® (HR1, HR3, HR4 and HR5) with THF as eluent at 1.2 mL·min^{-1}).*

Sample name	M_w [g·mol^{-1}]	M_n [g·mol^{-1}]	Polydispersity	M_z [g·mol^{-1}]	M_p [g·mol^{-1}]
PVC 23900	26 000	20 600	1.26	30 500	28 100
F1	18 600	17 100	1.08	19 900	18 400
F2	20 300	18 500	1.10	21 900	20 700
F3	22 900	20 600	1.11	24 800	23 800
F4	24 900	22 200	1.12	27 400	26 100
F5	26 400	23 200	1.14	29 300	27 000
F6	27 000	23 600	1.14	30 100	27 600
F7	27 700	24 200	1.15	30 900	28 200
F8	28 400	24 700	1.15	31 700	29 000
F9	28 800	25 100	1.15	32 300	29 300
F10	29 400	25 600	1.15	33 000	30 200
F11	29 900	25 800	1.16	33 700	30 700
F12	29 900	25 700	1.16	33 800	30 700
F13	30 500	26 300	1.16	34 400	31 300
F14	30 600	26 400	1.16	34 500	31 300
F15	31 600	27 200	1.16	36 200	32 500

6.1.4.2.1.2 Heart-cut HRP-HPLC + MALDI-ToF-MS for poly(styrene) analysis

For the separation of PS, an extended saw tooth gradient with an effective step height of 0.2 % was chosen. Throughout 20 injections of PS with molar mass of M_p = 8995 g·mol^{-1}, (50 mg·mL^{-1}, 15 µL) a continuous increase of column backpressure occurred. Flushing the column with THF, toluene, or n-hexane as well as flushing the column in reversed direction overnight did not reduce the high backpressure. Thus, it was assumed that the analytical stationary phase was not an ideal choice for semi-preparative HPLC measurements with such high sample loads on the column. The same characteristics were observed for the later (chapter 6.1.4.2.2) mentioned PDMS sample with an analytical Accucore C18 column. Therefore, further research will be done with different types of stationary phases, which are more suitable for preparative separation. **Figure 6.6** depicts the chromatogram obtained with the corresponding saw tooth gradient and the collected fractions, which were measured by MALDI-ToF-MS (**Table 6.5** and **Figure 6.7** to **Figure 6.10**).

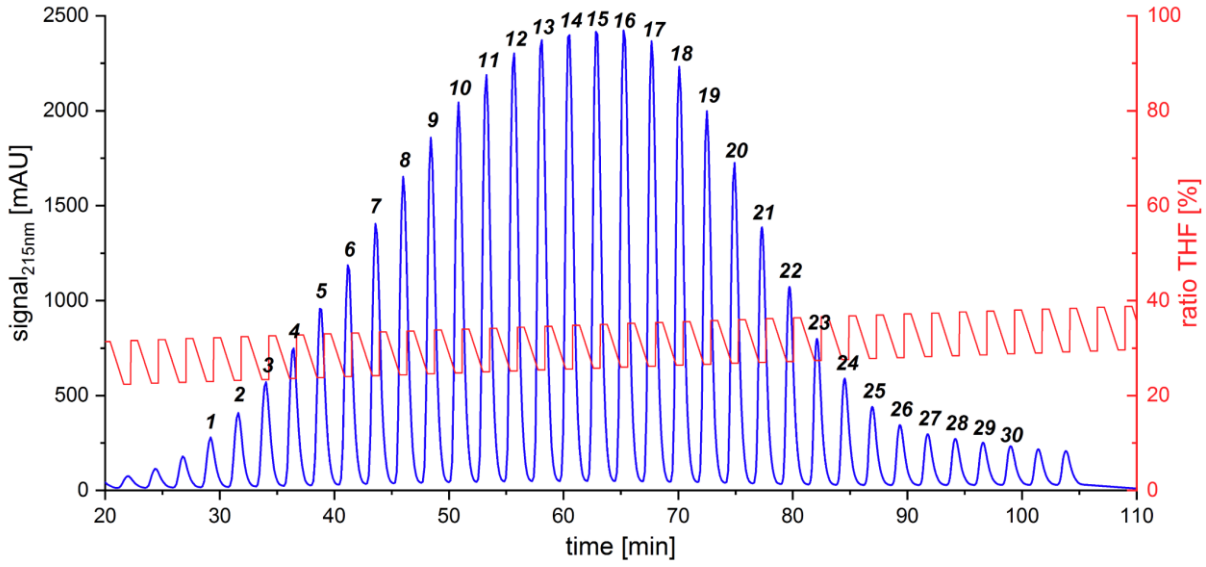


Figure 6.6: *Chromatogram and cutting pattern for preparative PS analysis, PS 8995 (c= 50 mg·mL^{-1}) was analyzed with a saw tooth gradient of 0.2 % effective step height, limited by the given mixing accuracy of the used HPLC pump; analysis was done with MeOH as weaker or rather non-solvent and THF as desorption promoting solvent on a Poroshell C18 EC (50x4.6 mm, 2.7 µm); fractions 1 to 30 were analyzed with MALDI-ToF-MS.*

Compared to the SEC measurements for PVC analysis, where polydispersity might be overestimated through band broadening effects, MALDI-ToF-MS often leads to under estimating polydispersity due to discrimination by the ionization process [42]. In this study, beside these differences, both techniques led to the same conclusion of decreasing polydispersities by fractionation with a saw tooth gradient. Beginning from fraction one to twenty the molar mass continuously increased, while this was not found for higher fraction numbers. Presumably, the separation performance was reduced because of the use of

the analytical stationary phase, which was not ideal for semi-preparative separation with the applied system. Especially the used high sample amounts may have caused these problems. Nevertheless, by heart-cut fraction collection it was proven that PVC and PS were separated due to their differences in molar mass caused by the same principle of separation.

Table 6.5: *MALDI-ToF-MS-Results of fraction collection of PS8995 after HRP-HPLC.*

Sample name	M_w [g·mol^{-1}]	M_n [g·mol^{-1}]	Poly-dispersity	M_z [g·mol^{-1}]	M_p [g·mol^{-1}]
FC01	7427	7385	1.0057	7469	7453
FC02	7589	7550	1.0051	7627	7662
FC03	7726	7690	1.0048	7763	7664
FC04	7873	7838	1.0045	7910	7873
FC05	8044	8009	1.0043	8079	7975
FC06	8214	8180	1.0042	8249	8183
FC07	8272	8241	1.0038	8303	8186
FC08	8404	8374	1.0035	8434	8290
FC09	8497	8467	1.0035	8528	8288
FC10	8709	8681	1.0033	8738	8497
FC11	8844	8816	1.0031	8871	8600
FC12	8860	8833	1.0031	8888	8704
FC13	9077	9051	1.0029	9103	9019
FC14	9166	9136	1.0033	9197	9125
FC15	9370	9340	1.0031	9398	9331
FC16	9499	9468	1.0032	9528	9644
FC17	9639	9604	1.0036	9671	9855
FC18	9713	9669	1.0046	9754	9956
FC19	9912	9860	1.0053	9961	10270
FC20	10009	9944	1.0065	10069	10478
FC21	10073	9994	1.0079	10146	10374
FC22	9799	9689	1.0114	9905	9643
FC23	9882	9753	1.0131	10003	9643
FC24	9629	9504	1.0131	9753	9122
FC25	9426	9311	1.0123	9541	9331
FC26	9160	9070	1.0099	9249	8917
FC27	9174	9090	1.0092	9258	9125
FC28	9307	9216	1.0099	9396	9226
FC29	9146	9064	1.0091	9227	9123
FC30	9244	9154	1.0098	9331	9120
PS 8995	8939	8855	1.0094	9020	8914

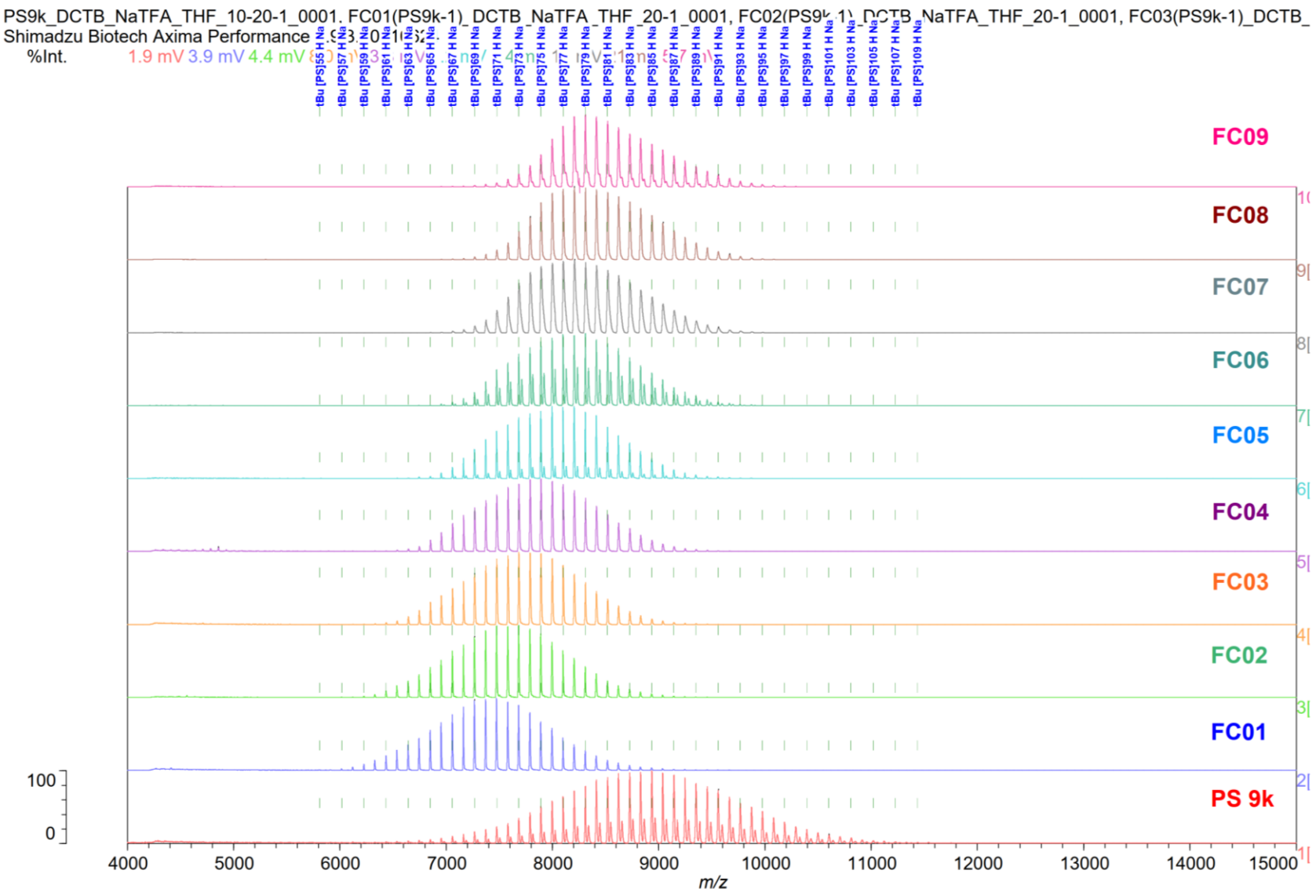


Figure 6.7: *MALDI-ToF-MS spectra of PS 8895 fractions FC 01 – 09 from HRP-HPLC separation, compared to the original PS 8895 standard (PS 9k).*

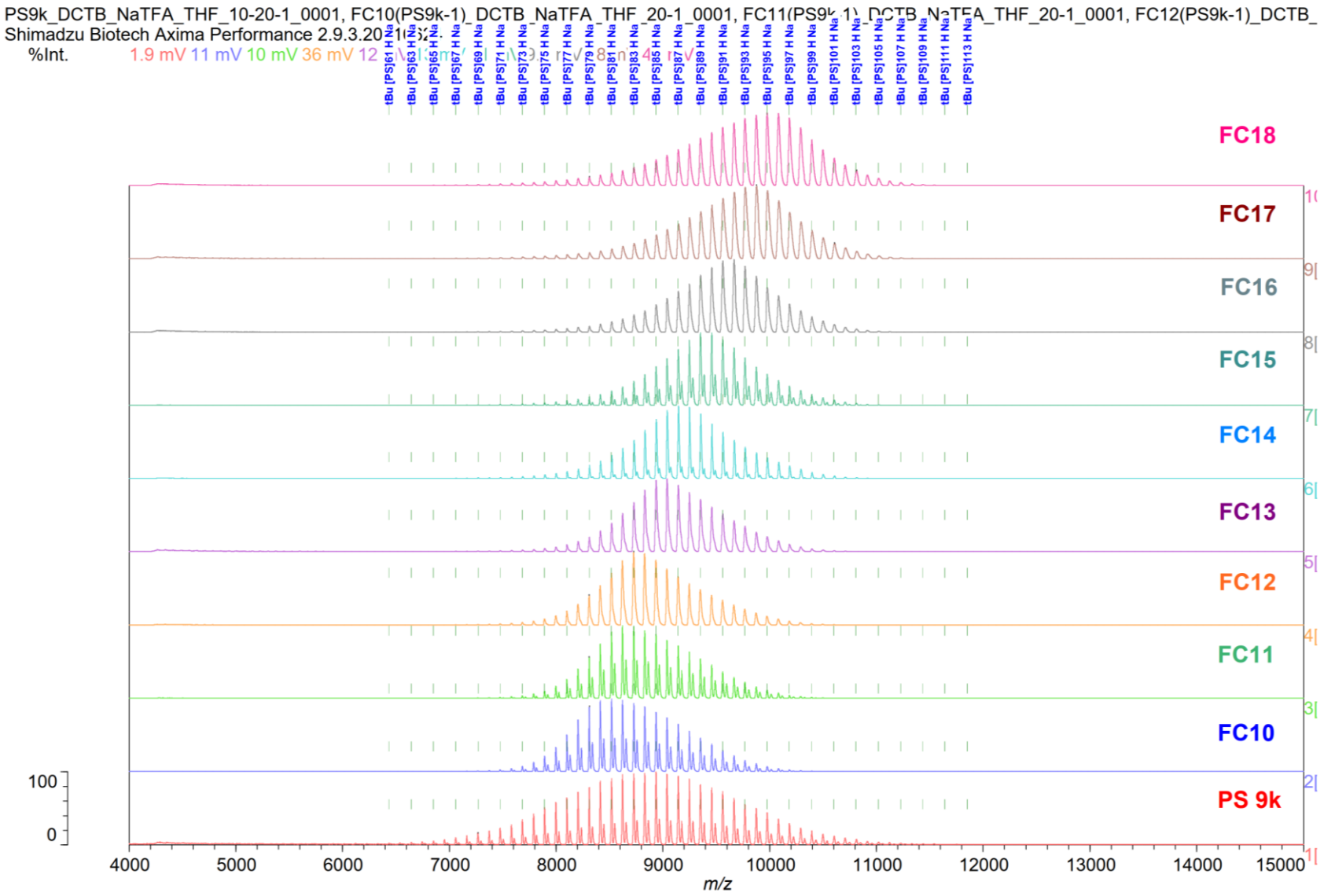


Figure 6.8: *MALDI-ToF-MS spectra of PS 8895 fractions FC 10 – 18 from HRP-HPLC separation, compared to the original PS 8895 standard (PS 9k).*

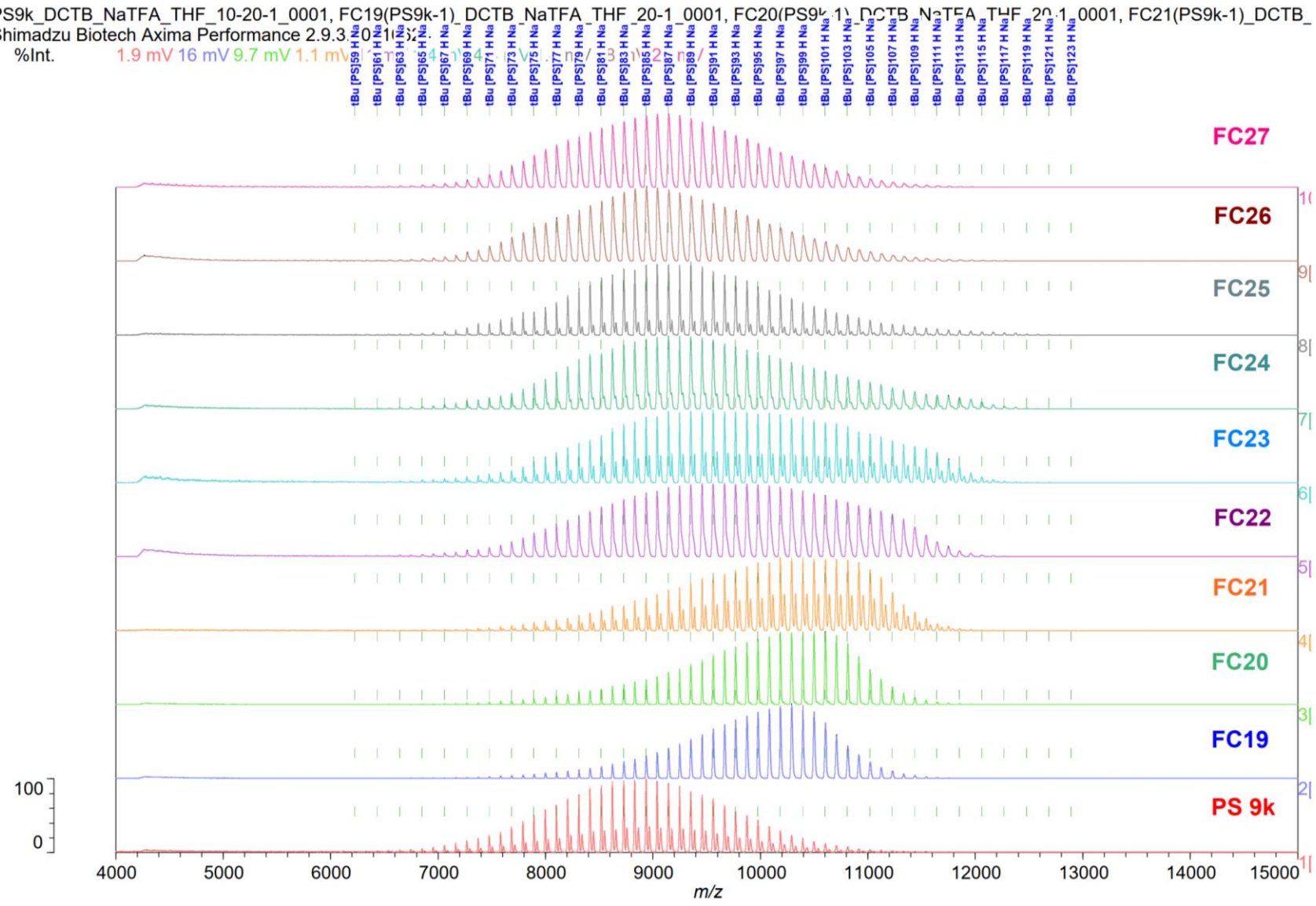


Figure 6.9: *MALDI-ToF-MS spectra of PS 8895 fractions FC 19 – 27 from HRP-HPLC separation, compared to the original PS 8895 standard (PS 9k).*

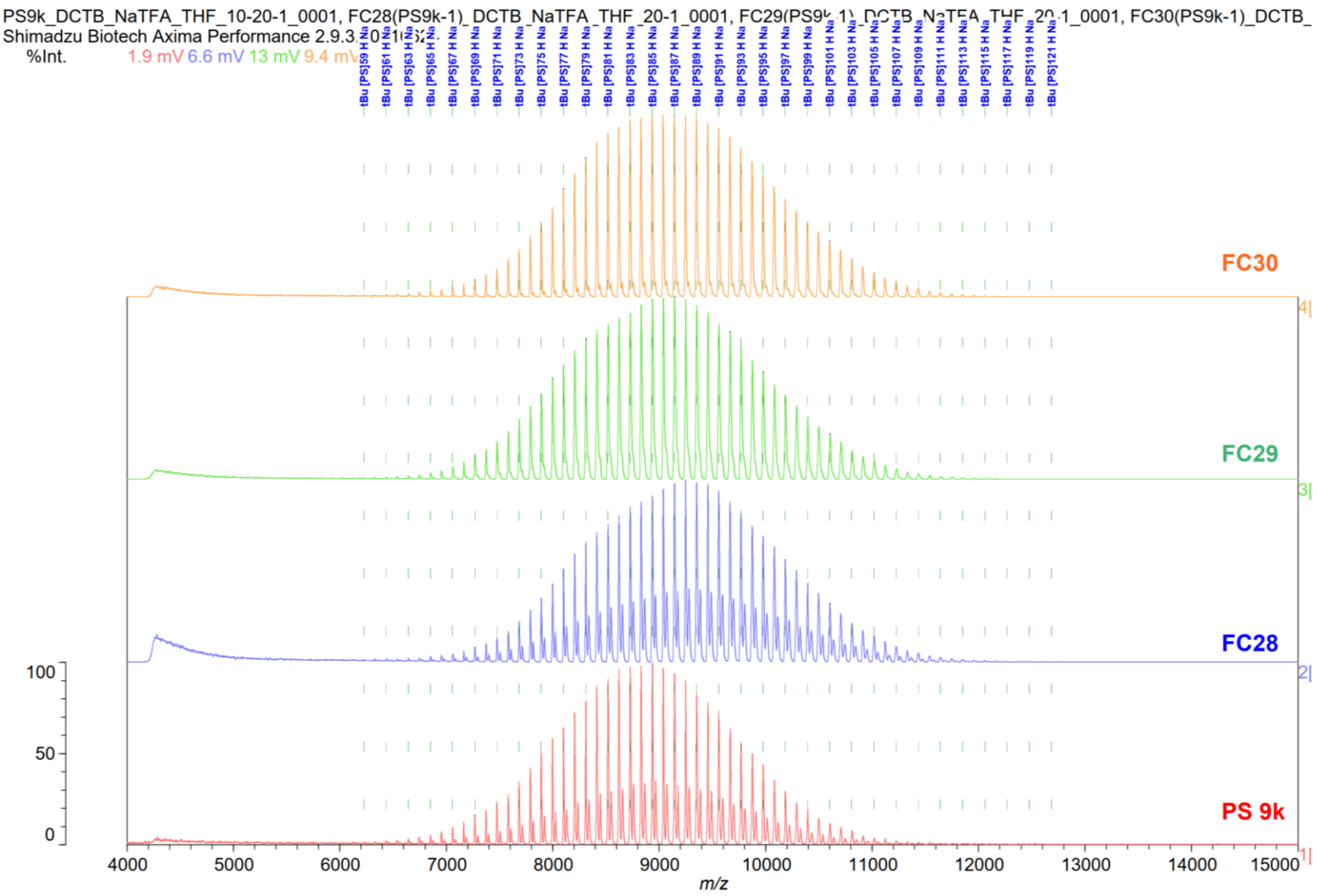


Figure 6.10: *MALDI-ToF-MS spectra of PS 8895 fractions FC 28 – 30 from HRP-HPLC separation, compared to the original PS 8895 standard (PS 9k).*

6.1.4.2.2 Application to various polymer types

All measurements of **Figure 6.11** and **Figure 6.12** were performed with a reduced resolution associated with choosing 1 % effective step height in the saw tooth gradient reducing the runtime. An economical approach is first to apply a saw tooth gradient with low resolution for obtaining an overview and then applying a gradient with higher resolution if necessary. For several polymers, e.g. PVC (**Figure 6.11 a)**), PMMA (**Figure 6.11 b)**), PDMS (**Figure 6.11 c)**) and PPG (**Figure 6.11 d)**), screening measurements were performed.

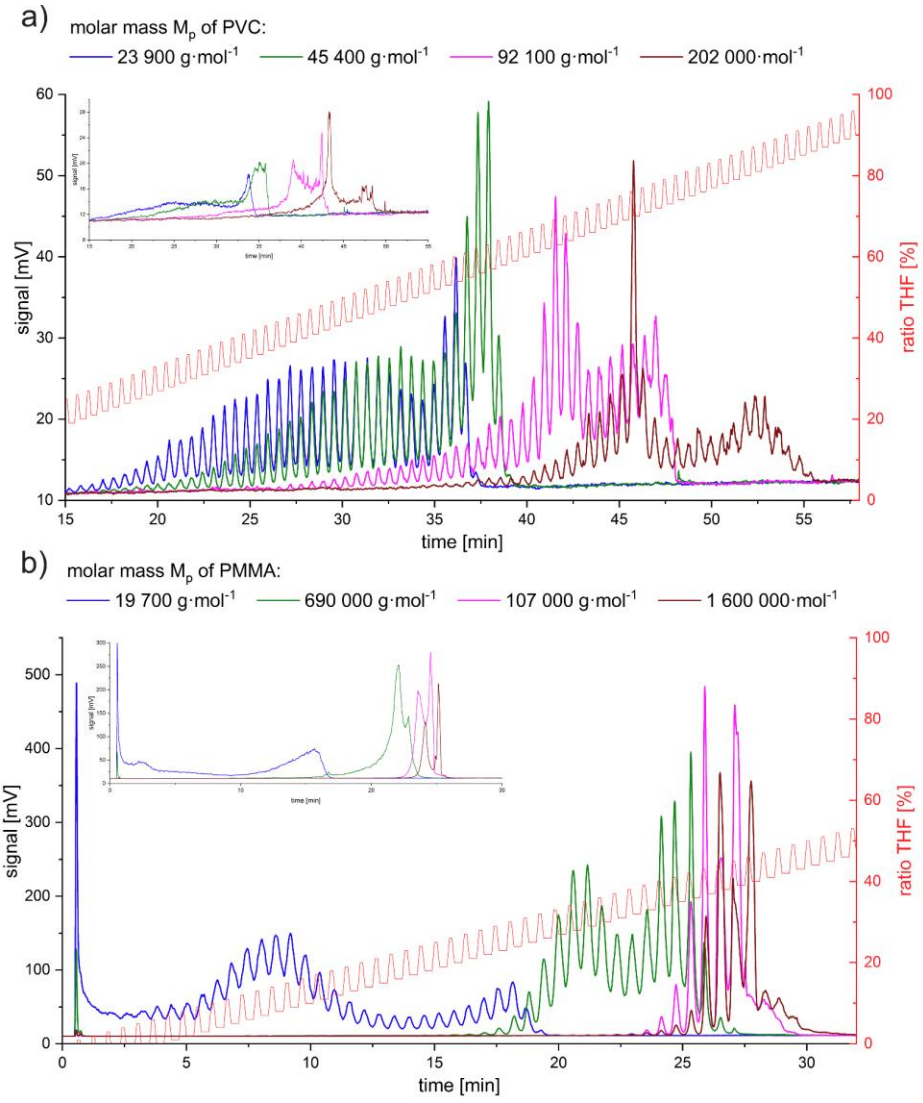
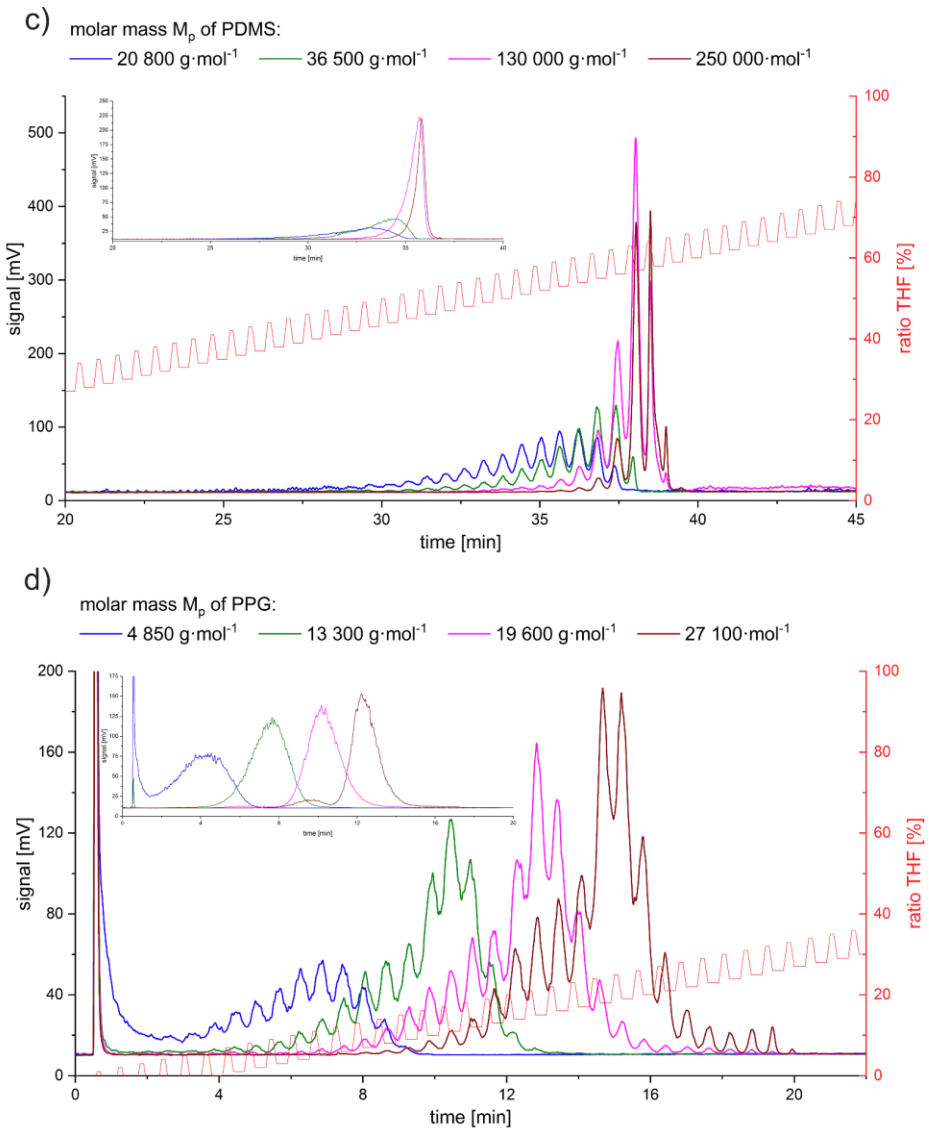


Figure 6.11: *Application of screening saw tooth gradients (effective step length of 0.6 min, effective step height 1.0 %) for separation of various polymer types over a broad molecular weight range, the picture-in-picture chromatogram shows the separation with application of a standard linear gradient; HRP-HPLC applied to* ***a)*** *PVC,* ***b)*** *PMMA,* ***c)*** *PDMS, and* ***d)*** *PPG on an Accucore C18 (50x4.6 mm, 2.6 µm) column with MeOH as weaker or rather non-solvent and THF as desorption promoting solvent.*

- Figure 6.11 continued -



In each case, the determination was done on an Accucore C18 column with methanol as weak or rather non-solvent and THF as desorption promoting solvent. In future measurement series, the influence of different adsorption promoting solvents with respect to the dissolving property of the investigated polymers will be further investigated. For comparison, the inset on the left-hand side of **Figure 6.11** shows the separation performance of a linear gradient, respectively.

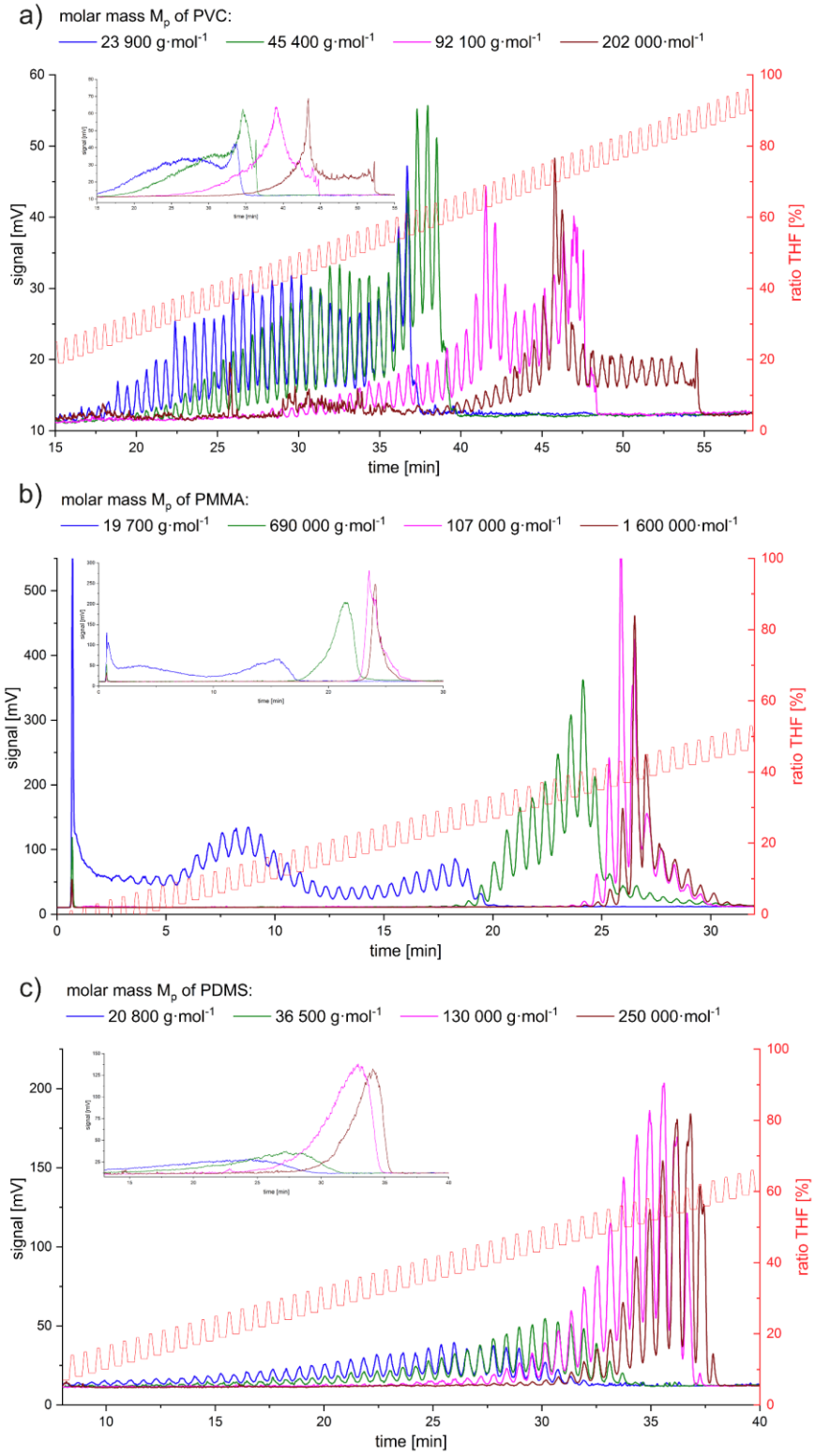


Figure 6.12: *Application of screening saw tooth gradient for separation of various polymer types over a broad molecular weight range, the picture-in-picture chromatogram shows the separation with application of a standard linear gradient; HRP-HPLC applied to* ***a)*** *PVC,* ***b)*** *PMMA, and* ***c)*** *PDMS on a Poroshell HILIC (50x4.6 mm, 2.7 µm) column with MeOH as adsorption promoting solvent and THF as desorption promoting solvent.*

Evaluating the performance of preparative linear and saw tooth gradients on analytical columns, PDMS with an average molar mass of 20,800 g·mol^{-1} was fractionated with a screening saw tooth gradient of 1.0 % effective step height and a corresponding linear gradient. Subsequent analysis of the fractions at the maximum of the original PDMS distribution (about M_p= 22,000 g·mol^{-1}) with MALDI-ToF-MS showed even for the screening saw tooth gradient an improved separation performance (fraction 13, **Figure 6.13**). Apart from a more symmetric polymer distribution, the saw tooth gradient resulted in a better separation, particularly at the boundaries (compare the inlets in **Figure 6.13**) of the polymer distribution. For the fraction of the linear gradient, the mass resolution was decreased over the whole mass spectrum. Furthermore, in the mass range between 24,000 and 25,000 g·mol^{-1} a shoulder in the distribution occurred. This might be caused by a more unprecise fractionation at the linear gradient compared to the saw tooth gradient. Based on these results, further research with semi-preparative equipment is planned to show further applicability of the combination of saw tooth gradient and heart-cut two-dimensional liquid chromatography.

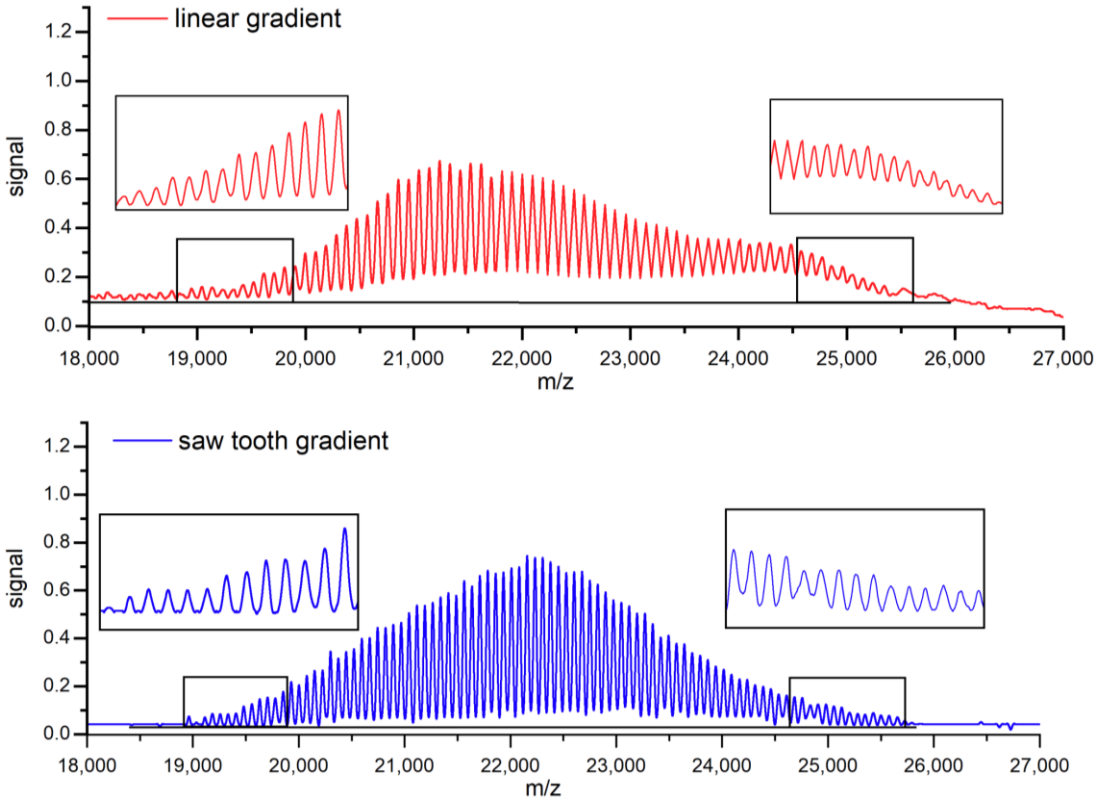


Figure 6.13: *MALDI-ToF-MS spectra of fractionated PDMS samples with mean molecular weights of 22 000 g·mol^{-1} for separation with a linear gradient (**above**) and for separation with a saw tooth gradient (**below**), both mass spectra show the same fraction 13; the inlets give a detailed overview about the boundaries of the polymer distribution; silver trifluoroacetate was used as cationization agent in a DCTB matrix. The comparison shows a broader polymer distribution from 18 000 up to 27 000 g·mol^{-1} for the linear gradient.*

The main contribution of the stationary phase is primarily its capability of retention of the analyte. Replacing a C18 column by a Poroshell HILIC column (c.f. **Figure 6.12**) PVC, PMMA and PDMS showed the same separation behavior. Interestingly, PPG could not be separated on a HILIC column applying the same conditions because of missing retention. However, comparing C18 with HILIC for HRP-HPLC, peak resolution of various polymers corresponded to each other and showed the minor significance of stationary phase in HRP-HPLC. Adapting an appropriate separation system, e.g. eluent combination, nearly each homopolymer can be separated in a distinct peak distribution. In fractionating polymers of the same kind, the distribution can be simplified for further investigations with other techniques such as mass spectrometry or size exclusion chromatography. Particularly, for the molar mass range greater than 200,000 g mol^{-1} an unprecedented separation performance regarding peak resolution was achieved by HRP-HPLC (cf. **Figure 6.14**).

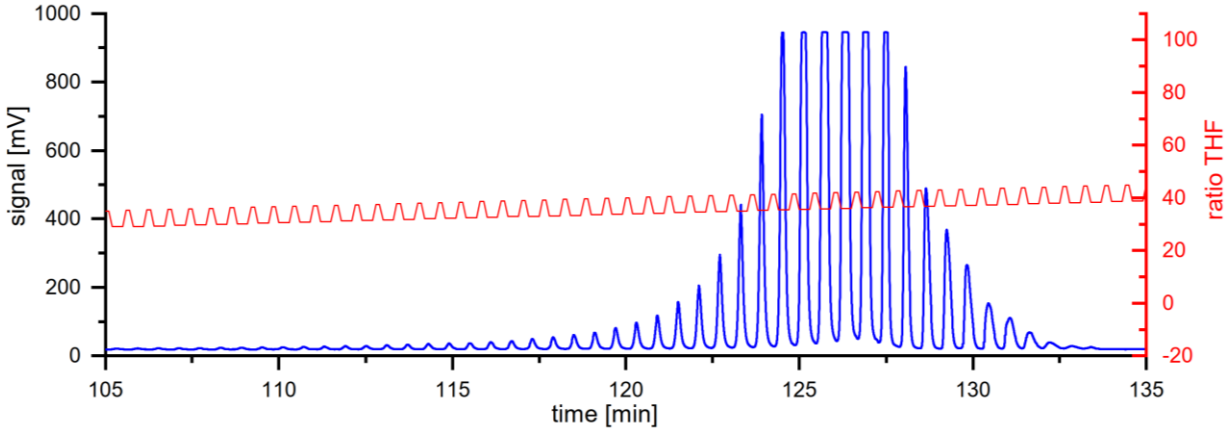


Figure 6.14: *High-resolution saw tooth gradient applied to PMMA 1 600 000 on an Accucore C18 (50x4.6 mm, 2.6 µm) with methanol as weaker or rather non-solvent and THF as desorption promoting solvent.*

6.1.5 Conclusion

Based on GPEC and HPPLC a novel technique termed high-resolution polymer HPLC (HRP-HPLC) was introduced. The HRP-HPLC is based on the application of a saw tooth gradient profile, which was developed, optimized, and validated for analysis of polymers. The profile of an optimum saw tooth gradient was evaluated by design of experiments. Special attention has to be taken choosing the appropriate sample amount for injection because peak resolution and effective step length of the gradient profile depend on the sample concentration. Regarding analysis time, a screening approach with reduced run time and resolution or a high-resolution approach with an extended run time can be chosen by only adjusting the effective step height of the gradient profile. Compared to common liquid chromatographic methods such as SEC, HRP-HPLC is characterized by a superior resolution especially in the high molecular mass range. Despite the highly increased resolution, the new gradient technique currently does not allow a separation of single oligomers. However, the number of oligomers per single saw tooth gradient step could be considerably reduced through the fractionated elution. A major constraint of typical chromatographic software packages is the possibility of generating gradient tables with up to 2000 entries for exploitation of the entire potential of this technique – from 100 % weaker or non-solvent to 100 % stronger or desorption promoting solvent with 0.2 % effective step height. Presently, this approach for example is possible with Chromeleon 7.2.2 in combination with HPLC systems from Thermo Fisher Scientific. Preparative HRP-HPLC on analytical columns showed some limitations concerning sample amount, runtime, and column overloading. Therefore, further improvements of the preparative measurements are in progress. The universal applicability of HRP-HPLC was demonstrated by the separation of various types of polymers, e.g. PVC, PDMS, PMMA, or PPG, using a conventional “ordinary” HPLC system. In conclusion, the newly developed HRP-HPLC paves the way for comprehensive studies of polymeric materials.

6.1.6 References

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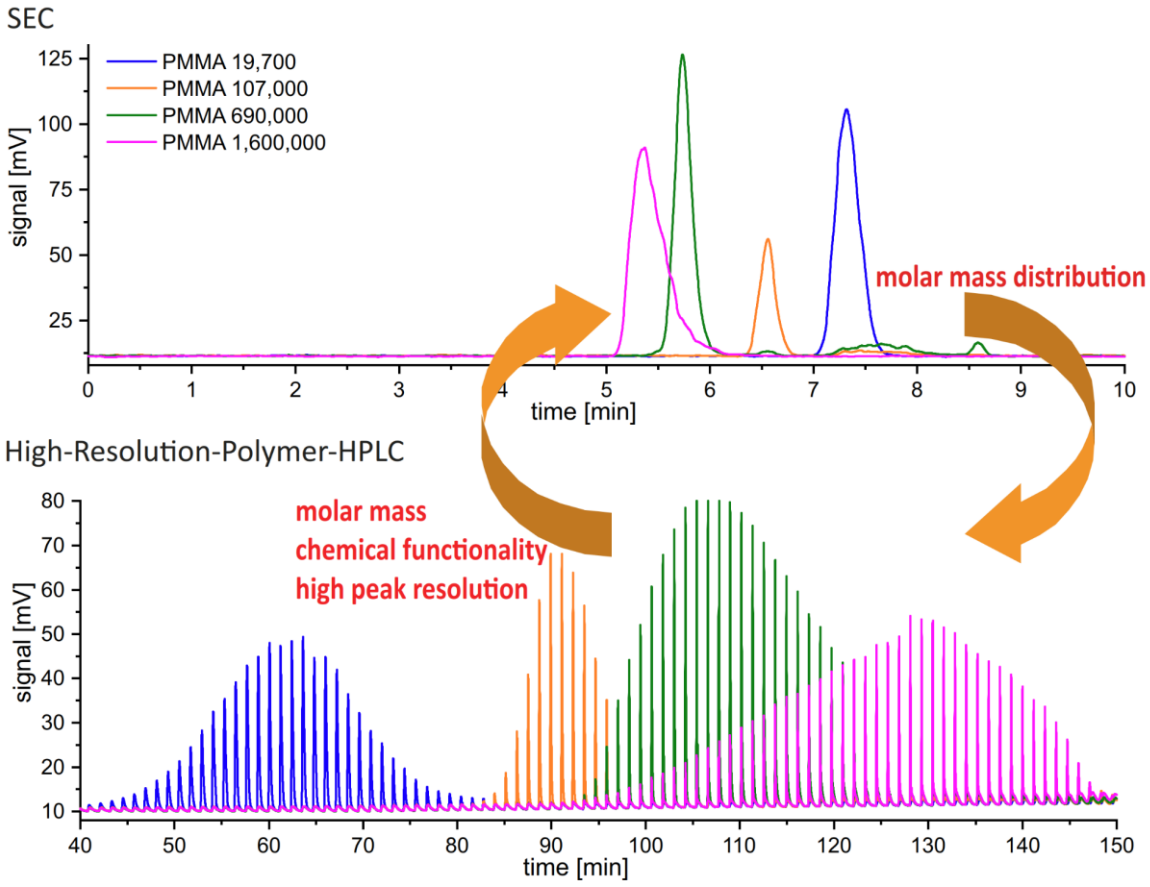
6.2 High-Resolution Polymer High Performance Liquid Chromatography: Optimization of the saw tooth gradient profile for various stationary phases and separations on preparative scale



Graphical Abstract: *One column – two separation mechanisms; SEC vs. HRP-HPLC.*

This chapter has been submitted for publication.

Bernhard Durner, Thomas Ehmann, Frank-Michael Matysik.

Measurements and experiments were done solely by the author. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

6.2.1 Abstract

The recently introduced saw tooth gradient protocol for high-resolution polymer HPLC was further improved and optimized in terms of total runtime and separation performance. As a result, increased flow rates enabled drastically reduced runtimes in combination with enhanced peak resolutions. Moreover, the saw tooth gradient profile was further investigated using a saw tooth gradient with a down-to-zero approach concerning the height of the negative backward gradient step. Modifying the mobile phase composition enabled two further gradient protocols: a ternary, and a three-dimensional approach, respectively. Thereby, a ternary saw tooth gradient was realized by repeating the whole gradient elution with two adequate pairs of adsorption and desorption promoting solvents for mixtures containing diverse polymer components. A three-dimensional saw tooth gradient was established by combining three different solvents in the gradient elution. In addition to mobile phase modifications, various stationary phases were compared and examined. Applying size exclusion chromatography (SEC) columns for saw tooth gradient polymer elution chromatography enabled the exploitation of two completely different separation mechanisms (SEC and high-resolution polymer HPLC) on one stationary phase. Thus, two-dimensional, heart-cut coupling of SEC and high-resolution polymer HPLC with only one stationary phase could be achieved. The application of the above-mentioned concept and its highly attractive performance characteristics are demonstrated for a silicone oil with a viscosity of 350 mPa·s by using a hybrid HPLC system coupled to a fraction collector.

6.2.2 Introduction

The application of polymers in various areas, like in pharmaceutical or in medical care products, automotive industry, and construction business, depicts their relevance. Considering liquid chromatographic separation techniques, particularly size exclusion chromatography (SEC) is used for the determination of molecular weight distributions. Apart from this mainly entropy-controlled separation technique avoiding any chemical interactions between polymer and stationary phase, the field of interaction polymer chromatography provides different separation interactions [1–6]. At the critical point of adsorption, enthalpy and entropy changes equalize each other and enable the separation of polymer mixtures according to their chemical functionalities without molar mass influences. The method of liquid chromatography at critical conditions (LCCC) shows a high potential for separation of copolymers [7–9] and enables subsequent separation techniques as barrier technique or gradient SEC [1, 10]. These techniques provide a high separation performance if the chromatographic system is properly adjusted and perturbations on the sensitive system can be minimized. Apart from SEC and LCCC, polymer HPLC [13–15], which is primarily driven by enthalpic interactions, enables the separation based on molar mass and chemical functionality distributions or differences in polymer architecture within a single chromatographic system [16]. In general, the interaction of these different separation mechanisms may complicate the choice of a suitable combination of stationary and mobile phases. Consequently, most separations in polymer HPLC are performed as gradient polymer elution chromatography (GPEC) by using at least one adsorption promoting solvent and one desorption promoting solvent. In our recent work [11], we developed and optimized a new gradient concept based on a saw tooth gradient (see **Figure 6.15**). The application of a saw tooth gradient protocol allowed an enhanced peak resolution and improvement of separation quality. The optimization of the gradient profile was achieved by design of experiments (DoE) [19–21]. Based on these results, several aspects like the choice of stationary and mobile phase, LC flow rate or measurements on a (semi-) preparative scale required additional investigation.

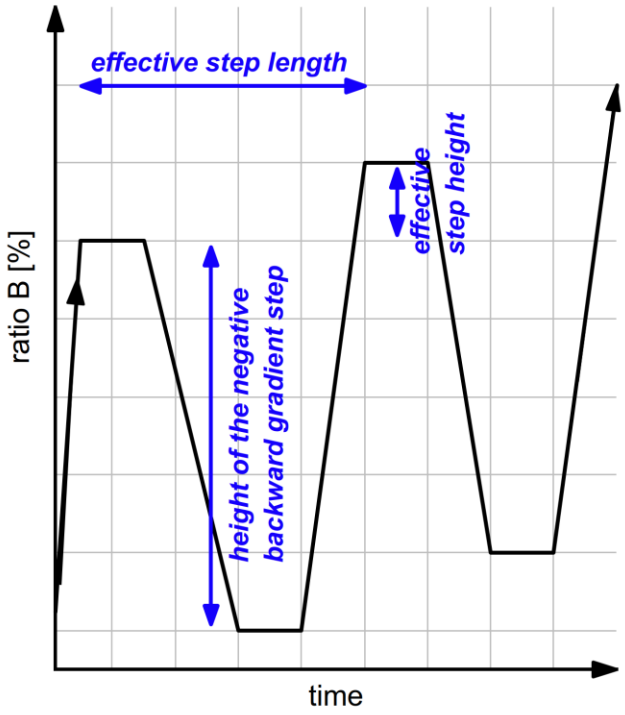


Figure 6.15: *Scheme of a saw tooth gradient design; the predominant parameters effective step height, step length and the height of the negative backward gradient step are annotated.*

The up-scaling from analytical to semi-preparative columns provides the possibility of heart-cut two dimensional liquid chromatographic separations with subsequent analysis of the collected fractions. In polymer analysis, particularly the coupling with MALDI mass spectrometry [12] or NMR spectroscopy [17] enhance the information about (micro-)structure and composition of the analyzed polymers or polymer mixtures. Up-scaling from analytical to preparative polymer HPLC often requires long runtimes and expensive, time-consuming method development. Thus, additional investigations of high-resolution polymer HPLC applying saw tooth gradients may result in protocols simplifying the overall process.

6.2.3 Material and methods

As reported recently [11], programming a saw tooth gradient over a range from 0 to 100 % desorption promoting solvent was possible without any limitations with the chromatographic data system Chromeleon (Thermo Fisher Scientific, version 7.2), in contrast to other investigated software packages limited in the number of possible entries in the gradient time table. Furthermore, comparing the driver configuration between Agilent and Thermo Fisher Scientific HPLC pumps, gradient programming without limited number of entries in the gradient time table was only possible for pumps from Thermo Fisher Scientific.

6.2.3.1 Mobile phase components and polymer standards

All used solvents were HPLC grade. Acetone, acetonitrile, methanol, non-stabilized tetrahydrofuran (THF), and toluene were purchased from Merck (Darmstadt, Germany) and used without further purification. Water of a Milli-Q-Advantage A10 water system (Merck Millipore) was used. Poly(dimethylsilioxane) (PDMS) standards with viscosities of 350 mPa·s and 1000 mPa·s were obtained from Wacker Chemie AG (Burghausen, Germany). The used poly(methyl methacrylate) (PMMA), poly(vinyl chloride) (PVC) and poly(propylene glycol) (PPG) were purchased from Agilent (Church Stretton, UK). All used polymer standards were dissolved in THF.

6.2.3.2 Optimization of LC flow rate

The effect of various LC flow rates was measured with a Thermo Fisher Scientific (Waltham, USA) Ultimate 3000 HPLC system equipped with a binary pump. For detection, an Agilent (Waldbronn, Germany) 385 ELSD modified with an enhanced parallel-path MiraMist® poly(tetrafluoroethylene) nebulizer from Burgener Research Inc. (Mississauga, Ontario, Canada) at 40 °C evaporator temperature, 90 °C nebulizer temperature and 1.6 SLM (standard liter per minute) gas flow was used [18]. The measurements were done with an Agilent Poroshell HILIC (50x4.6 mm, 2.7 µm) and a silicone oil of 1000 mPa·s (Wacker Chemie AG, Burghausen, Germany). The saw tooth gradient was programmed with an effective step height of 0.2 % and a height of the negative backward gradient step of 6.0 % (compare [11]) and LC flow rates of one, two, and three $mL \cdot min^{-1}$.

6.2.3.3 Evaluation measurements of various saw tooth gradient profiles

For comparison of three different discussed saw tooth profiles, a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump, the above-mentioned modified Agilent 385 ELSD and a Thermo Fisher Scientific Accucore C18 (50x4.6 mm, 2.6 µm) were used. In each case the saw tooth gradients were performed with an effective step height of 1.0 % and an effective step length of 0.2 min. The height of the negative backward gradient step was 6.0 % or the maximally possible value of 100 % and for the three-dimensional saw tooth gradient in each step during the height of the negative backward gradient step the mobile phase composition was changed to water instead of methanol followed by a reconditioning step back to a mixture of THF and methanol before switching to the positive slope. A PMMA standard with an average molecular weight of 107,000 $g \cdot mol^{-1}$ at a concentration of 20 $mg \cdot mL^{-1}$ was used as analyte.

The setup of the ternary saw tooth gradient setup separating PMMA (19,700 $g \cdot mol^{-1}$), polypropylene glycol (PPG, 18,000 $g \cdot mol^{-1}$), and PDMS (18,600 $g \cdot mol^{-1}$) at a concentration of 20 $mg \cdot mL^{-1}$ respectively, consisted of two different saw tooth gradient approaches: Starting at 100 % methanol (adsorption promoting solvent) a first saw tooth gradient with an effective step length of 0.6 min, an effective step height of 1.0 % and a height of the negative backward gradient step of 6.0 % were performed with acetone as desorption promoting solvent (elution of PMMA und PPG). Subsequently, at 100 % acetone the same saw tooth gradient was used but with THF as a stronger desorption promoting solvent (elution of PDMS) while acetone acted as an adsorption promoting solvent.

6.2.3.4 Comparison of different LC columns

Various types of liquid chromatographic stationary phases were compared in terms of separating PMMA, PDMS, PVC, and PPG at a concentration of 20 $mg \cdot mL^{-1}$ respectively. **Table 6.6** gives an overview of the specific data. The measurements were performed on a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump and a modified Agilent 385 ELSD. Each polymer was separately measured on each column with a linear gradient starting from 100 % methanol and finishing at 100 % THF.

Table 6.6: *Overview of used LC columns separating PMMA, PVC, PDMS, and PPG with an average molecular mass of approximately 20 kDa, the categories are an indicator for elution order of the different polymers:* ***category 1*** *- PMMA-PPG-PDMS-PVC,* ***category 2*** *- PMMA-PPG-PVC-PDMS,* ***category 3*** *- PPG-PMMA-PDMS-PVC,* ***category 4*** *- PDMS-PMMA-PPG (PVC irreversibly remained at the column), and* ***category 5*** *- PPG-PDMS-PMMA-PVC.*

Category	Manufacturer	Name	Dimensions, particle type	Evaluation
1	ZirChrom	Diamond Bond C18	150x4.6 mm, 5 µm, fully porous	zircon based, carbon coated, extended pH range
1	Phenomenex	Luna Omega PS C18	150x4.6 mm, 5 µm, fully porous, 100 Å	mixed mode C18 with positive charge for additional ionic interactions
1	Tosoh	TSK Gel Boronate 5PW	75x7.5 mm, 10 µm, Polymer	polymer based, m-aminophenylboronate coated, for affinity chromatography
2	Merck	Chromolith Performance RP C18	100x4.6 mm, monolithic column	monolithic column
2	Microsolv Technology	Cogent Bidentate C18	150x4.6 mm, 4.2 µm, fully porous, 100 Å	no silanol activity, C18 chain directly to Silica-hydrid surface bonded
3	Agilent	PLRP-S	50x4.6 mm, 10 µm, polymer, 4000 Å	polymer based, for biomolecule separation
3	Thermo-Fisher	Omnipac Pax-500	250x4.6 mm, 8.5 µm, polymer	mixed mode polymer phase with anion exchange interactions
3	Thermo-Fisher	Accucore C18	50x4.6 mm, 2.6 µm, core shell, 80 Å	typical C18 column, non-polar interactions
3	Phenomenex	Kinetex F5	50x4.6 mm, 2.6 µm, core shell, 100 Å	pentafluorophenyl column for steric separations
3	Phenomenex	Kinetex Biphenyl	50x4.6 mm, 2.6 µm, core shell, 100 Å	hydrophobic, aromatic and polar interactions
3	Phenomenex	Luna Phenylhexyl	50x4.6 mm, 5 µm, fully porous	hydrophobic, aromatic interactions
4	Thermo- Fisher	Hypercarb	100x4.6 mm, porous graphitic carbon	graphitic carbon
5	Agilent	PL gel Mixed C	300x7.5 mm, 5 µm, polymer	SEC column, molar mass range 200 to 2,000,000 g·mol-1

6.2.3.5 HRP-HPLC using a SEC column

SEC measurements were performed with a Polymer Standards Service (Mainz, Germany) SECcurity GPC 1200 system equipped with a refractive index detector. Data evaluation was done with the SEC software package WinGPC UniChrom 8.2. For polymer HPLC, a Thermo Fisher Scientific Ultimate 3000 HPLC system equipped with a quaternary pump and the modified Agilent 385 ELSD was used. An Agilent PL gel Mixed C column (300x7.5 mm, 5 µm) was used for the separation of PMMA standards with an average molecular weight of 19,700 g·mol^{-1}, 107,700 g·mol^{-1}, 690,000 g·mol^{-1}, and 1,600,000 g·mol^{-1}, with THF as eluent in SEC mode and acetonitrile as adsorption promoting solvent and THF as desorption promoting solvent in polymer HPLC mode.

6.2.3.6 Preparative HRP-HPLC

Preparative HRP-HPLC within the entire gradient range was achieved by coupling a Thermo Fisher Scientific Ultimate 3000 HPLC (with a binary pump (1), autosampler (2) and column oven (3)) to an Agilent 1260 fraction collector (5) equipped with an Agilent LAN Interface Card as depicted in **Figure 6.16**.

Figure 6.16: *Instrumental setup of a preparative LC system by coupling a Thermo Fisher Scientific HPLC consisting of a binary pump (1), an autosampler (2) and a column oven (3) either with an Agilent ELSD (4) for measurement of the cutting pattern or an Agilent fraction collector (5) in combination with an Agilent pump (6) and autosampler (7) for preparative separation. The whole installation is controlled by the software package Chromeleon (8).*

Additionally, a proper communication of both LC systems via the chromatographic software package Chromeleon had to be established. For receiving a trigger signal from the Thermo Fisher Scientific HPLC necessary for the Agilent fraction collector, the entire configuration required an additional Agilent pump (6) and an additional Agilent autosampler (7). Regarding software control (8), two autosampler caused problems and thus the Agilent autosampler could easily be "deactivated" in the script editor of Chromeleon by allocating the value "1" using the command: "LCSystem.ALS.Position". Furthermore, the flow rate of the Agilent pump was set to 0.0 $mL \cdot min^{-1}$ and the acquisition of the pump pressure had to be activated. Further details referring to this hybrid HPLC are given in the results and discussion part.

Preparative HPLC of a silicone oil with viscosity of 350 mPa·s required in the first step, a fraction collector offset corrected detection run, with the ELSD (4) for determining an adequate cutting pattern. In a second step, instead of the ELSD, the fraction collector was connected to the column outlet and the fraction collection was started without detection. For these measurements, methanol was used as adsorption promoting solvent and THF as desorption promoting solvent. The saw tooth gradient had an effective step height of 1.0 %, an effective step length of 6 min and a height of the negative backward gradient step of 100 % (saw tooth gradient down-to-zero). A Thermo Fisher Scientific Hypersil Gold C18 aQ (100x10 mm, 5 µm) was used as stationary phase. After the preparative separation, each single fraction was measured with a Polymer Standards Service SECcurity GPC 1200 system equipped with a refractive index detector applying a set of three Agilent PlGel MiniMIX-C columns (250x4.6 mm, 5 µm) at a eluent flow rate of 0.3 $mL \cdot min^{-1}$.

6.2.4 Results and discussion

Based on the previously reported results [11] further optimization of high-resolution polymer HPLC using a saw tooth gradient was performed. Therefore, the influence of varying LC flow rates and modified saw tooth gradient profiles on the separation quality were investigated. Furthermore, various types of LC columns were tested, resulting in a comparison of two separation mechanisms (SEC and HRP-HPLC) on the same SEC stationary phase. To overcome the constraints in preparative HRP-HPLC, like limited entries in the gradient time table of Agilent pumps (see [11]), an optimized configuration of a preparative LC system for PDMS analysis was applied.

6.2.4.1 Optimization of LC flow rate

As stated in [11], employing a saw tooth gradient with a low effective step height required long runtimes for high peak resolutions. Apart from the actual shape of the single gradient steps as an important parameter in saw tooth gradient design, the optimization of the effective step length could shorten total runtime without deteriorating peak resolution. Consequently, increasing the LC flow rate was a good compromise between short runtime and high peak resolution (see **Figure 6.17**). As far as the actual peak separation is retained by the saw tooth gradient, no significant decline in peak resolution was observed. Thus, an increase of the flow rate by a factor of three reduced the runtime to one third – for the shown example of PDMS from 165 min to 55 min – without changing the peak resolution. The disadvantage of increasing backpressure could easily be managed, because particle and column dimensions showed only minor impact on the separation efficiency and thus the application of short columns with suitable diameters was possible [11].

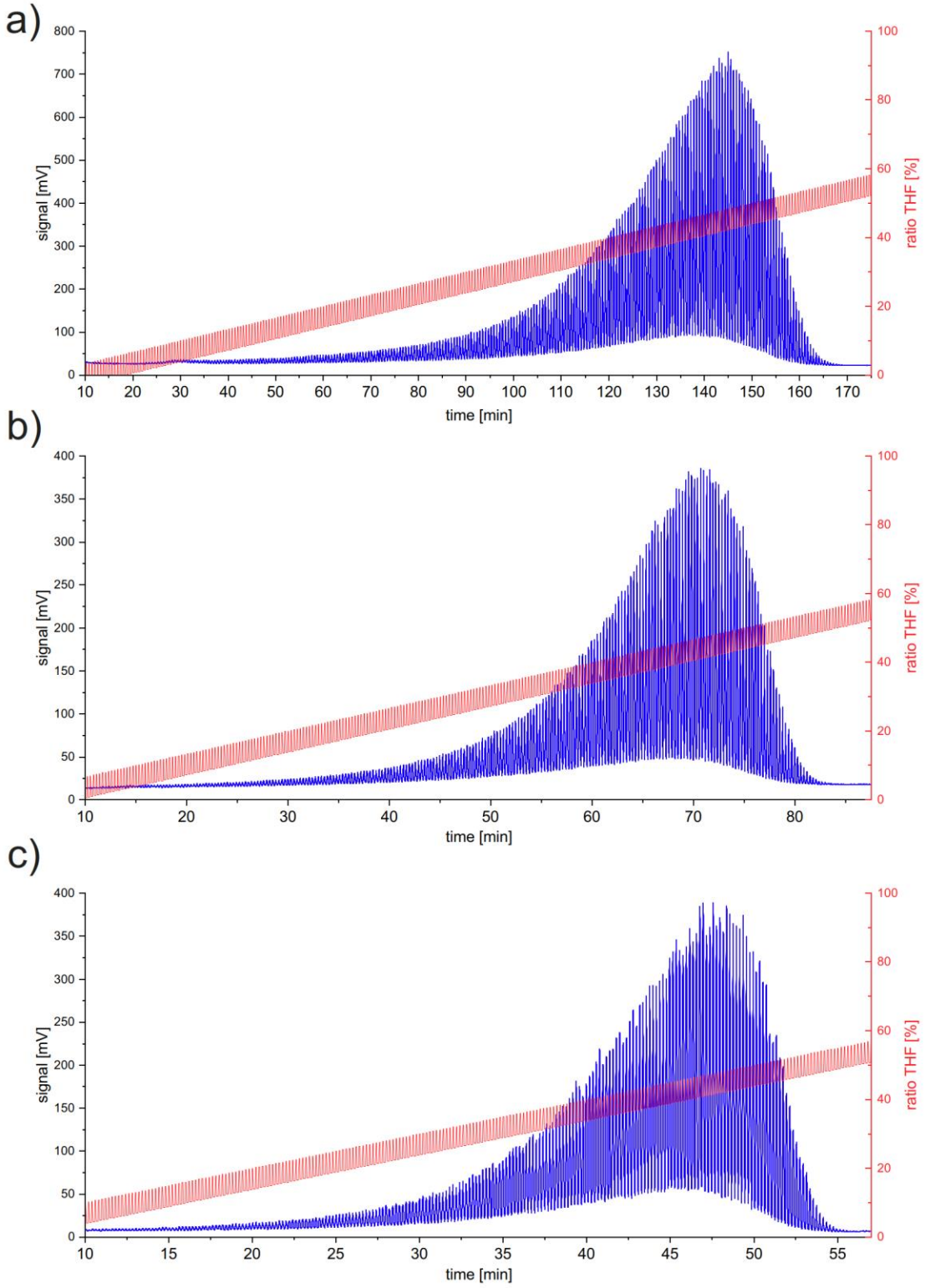


Figure 6.17: *HRP-HPLC measurement of PMMA with average molecular weight of 107,000 g·mol^{-1} on a Poroshell HILIC (50x4.6 mm, 2.7 µm) using methanol as adsorption promoting solvent and THF as desorption promoting solvent and a saw tooth gradient with an effective step height of 0.2 % and a height of the negative backward gradient step of 6.0 %; the figure depicts the effect of varying flow rate of* ***a)*** *1 mL·min^{-1},* ***b)*** *2 mL·min^{-1}, and* ***c)*** *3 mL·min^{-1} on the total runtime and peak separation.*

6.2.4.2 Development and evaluation of various saw tooth gradient profiles

Apart from a standard saw tooth approach, the number of used solvents and the variation of the height of the negative backward gradient step to higher values allowed different "critical" mobile phase compositions when reaching the elution point of the polymer.

6.2.4.2.1 Saw tooth gradient down-to-zero

In accordance with design of experiments (DoE) due to Taguchi's approach for optimizing the gradient shape in our previous research [11], an additional response optimization combining different integration modes was performed to analyze the influence of the height of the negative backward gradient step on separation performance. Therefore, the tallest peak height difference in between the integration mode of perpendicular line dropping and valley-to-valley integration mode was calculated for the entire DoE (see **Table 6.7**).

Table 6.7: *Calculated values for peak height difference between the integration modes of perpendicular line dropping and valley-to-valley setting; the DoE number refers to the DoE described in [11].*

Number in DoE	height of the negative backward gradient step [%]	Peak height difference [mAU]
1	3.0	624.2
2	3.0	1090.0
3	3.0	1226.0
4	3.0	873.4
5	6.0	0.2
6	6.0	277.6
7	6.0	335.2
8	6.0	578.9
9	9.0	0.2
10	9.0	135.8
11	9.0	8.6
12	9.0	14.9
13	12.0	0.2
14	12.0	0.2
15	12.0	0.2
16	12.0	0.4

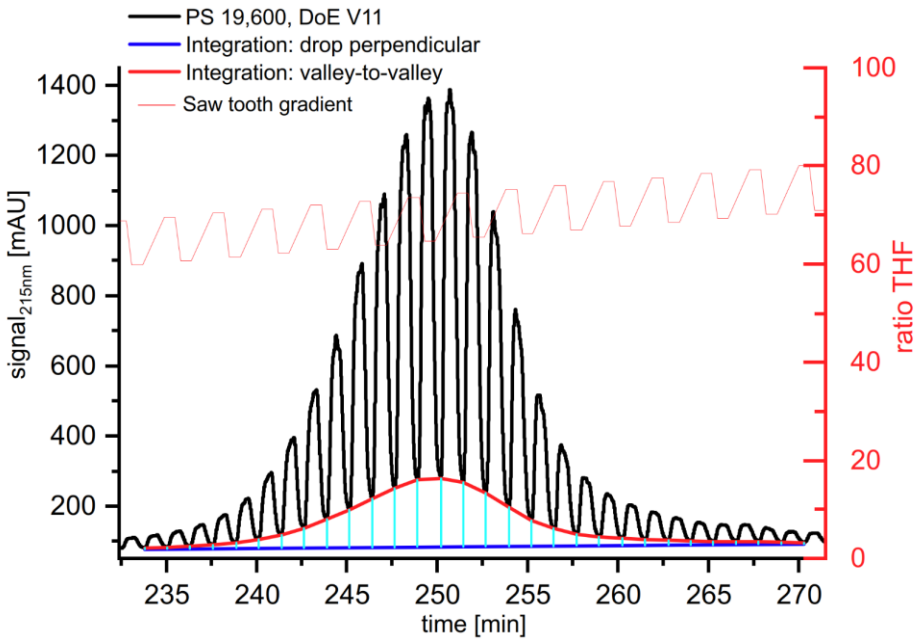


Figure 6.18: *Chromatogram (**black curve**) of measurement PS 19,600, V11 of DoE cf. [11] for comparison of two integration modes: "drop perpendicular" (**blue line**) and "valley-to-valley" (**red line**); For this measurement a Poroshell C18 (50x4.6 mm, 2.7 µm) with methanol as adsorption promoting solvent and THF as desorption promoting solvent was used while detecting at 215 nm.*

Figure 6.18 depicts an example chromatogram used for data evaluation. In summary, minimizing peak height differences indicated the best baseline separation. The DoE evaluation showed that an increasing height of the negative backward gradient step reduced the peak height difference and therefore resulted in an improved baseline separation of the peaks.

Based on these results, the height of the negative backward gradient step was further investigated and maximized to a value of 100.0 % corresponding to an amount of 0.0 % desorption promoting solvent, termed as "saw tooth gradient down-to-zero", in the backward direction of the saw tooth profile. **Figure 6.19 a)** shows the difference between a saw tooth gradient with 6.0 % height of the negative backward gradient step (blue line) and a down-to-zero saw tooth gradient (pink line) for investigating a PMMA standard of an average molecular weight of 107,000 g·mol^{-1} at an Accucore C18 column. The latter showed an enhanced peak resolution and an increased retention time, even though the effective step length remained the same. Furthermore, the pink chromatogram depicts an increased peak tailing, which might be a consequence of a highly shortened equilibration time after reaching the next higher plateau. Thus, changing the height of the negative backward gradient step additionally to flow rate and effective step height is an important opportunity for improving separation efficiency. Nevertheless, further research will be necessary to further optimize the gradient setup with these parameters.

6.2.4.2.2 3D saw tooth gradient

Besides the aforementioned technical parameters, a change in the dissolution power of the mobile phase composition also could be realized by using a third solvent during the gradient setup. Therefore, water was used as an additional, much stronger adsorption promoting solvent than methanol and as desorption promoting solvent THF was used for analysis of PMMA with an average molecular weight of 107,000 g·mol^{-1} (**Figure 6.19 a)**, green line). This 3D gradient setup is characterized by an additional step in gradient programming, whereby the backward direction of desorption promoting solvent is fortified by simultaneously increasing the water content as stronger adsorption promoting solvent compared to methanol (**Table 6.8**).

Table 6.8: *Explanation of a three-dimensional gradient setup combining water, methanol, and THF as mobile phase components, the first column describes the appropriate step during the gradient programming.*

Gradient step	**Ratio water [%]**	**Ratio methanol [%]**	**Ratio THF y_n [%]**
upper plateau	0.0	100-y_1	y_1
height of the negative backward gradient step	↑	0.0	↓
first half of lower plateau	100-y_2	0.0	y_2
second half of lower plateau	0.0	100-y_2	y_2
positive slope	0.0	100-y_3	y_3

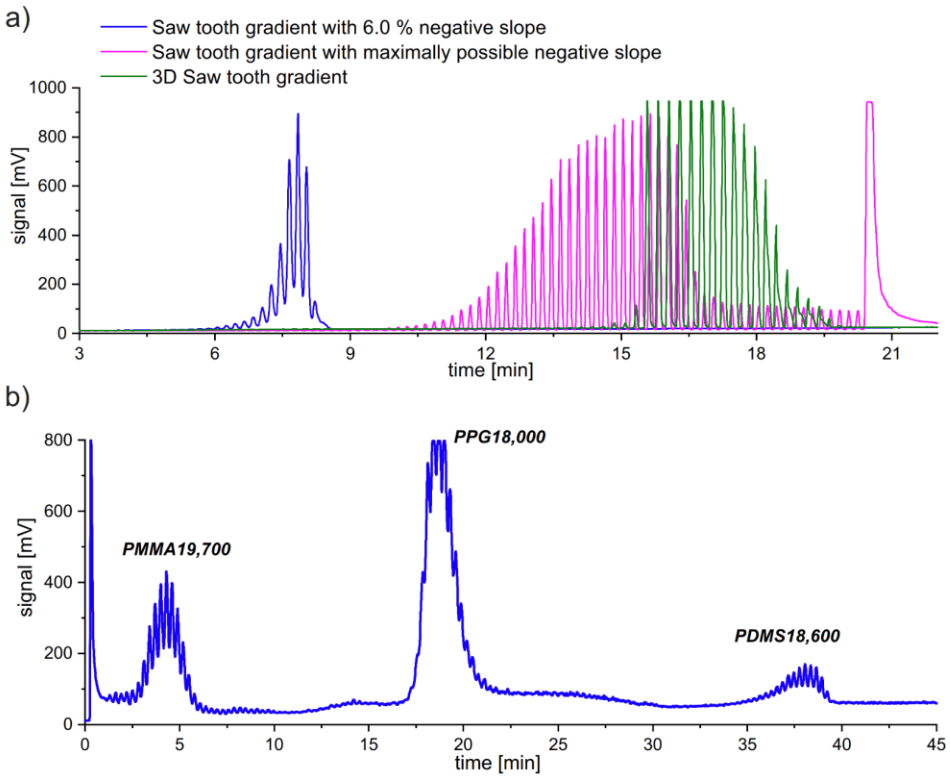


Figure 6.19: a) *Comparison of different saw tooth profiles with effective step height of 1.0 % effective step length of 0.2 min and flow rate of 3.0 mL·min^{-1} for separating PMMA with an average molecular weight of 107,000 g·mol^{-1}; the gradients differ concerning a height of the negative backward gradient step of 6.0 % (**blue curve**), the maximum height of the negative backward gradient step of 100.0 % (**pink curve**) and a three dimensional saw tooth gradient with a mobile phase combination of water, methanol and THF (**green curve**); **b)** ternary saw tooth gradient separating PMMA with an average molecular weight of 19,700 g·mol^{-1}, PPG with an average molecular weight of 18,000 g·mol^{-1}, and PDMS with an average molecular weight of 18,600 g·mol^{-1}, and a concentration of 20 mg·mL^{-1} respectively, at an Accucore C18 (50x4.6 mm, 2.6 µm); from 0 to 30 min methanol was used as adsorption promoting solvent in combination with acetone as desorption promoting solvent and then acetone was used as adsorption promoting solvent in combination with THF as desorption promoting solvent.*

6.2.4.2.3 Ternary saw tooth gradient

A further option using more than two mobile phase components is depicted in **Figure 6.19 b)** for simultaneously separating PMMA, PPG, and PDMS (each with an average molecular weight of 20 kDa) with an Accucore C18 column. Therefore, in the first half of the analysis (0 – 30 min) a combination of methanol as adsorption promoting solvent for all three investigated polymers and acetone as desorption promoting solvent for PMMA and PPG was used. As soon as 100 % of acetone was reached, THF was used as desorption promoting solvent for the remaining PDMS. Using this ternary saw tooth gradient setup, a change in the solvent strength of the mobile phase enabled an enhanced separation of the examined polymers within a single analysis. For analysis of complex polymer mixtures, the repeated application of a saw tooth gradient might considerably shorten the runtime.

6.2.4.3 Application of HRP-HPLC to different LC stationary phases

Additionally to an optimized gradient profile, changing the stationary phase affected the polymer adsorption and thus the polymer elution. In contrast to classical HPLC separations, previous measurements showed that a change of particle size and column dimensions had only minor effects on separation efficiency. Hence, various liquid chromatographic columns with great differences in the stationary phase chemistry were evaluated for separating PMMA, PDMS, PVC, and PPG with similar average molecular weights. **Table 6.6** gives a detailed overview of the used stationary phases. Unless otherwise mentioned, methanol was used as adsorption promoting solvent and THF as desorption promoting solvent in an ordinary linear gradient reducing total runtime.

6.2.4.3.1 Comparison of classical HPLC stationary phases

As aforementioned, for columns used in HRP-HPLC the most important factor is the adsorption of the polymer and therefore the retention of the investigated polymers. For this reason, the columns were categorized due to the elution order of the polymers as main differentiating factor (**Table 6.6**). Columns of category 1 showed an elution order of PMMA-PPG-PDMS-PVC, category 2 of PMMA- PPG-PVC-PDMS, category 3 of PPG-PMMA-PDMS-PVC, category 4 of PDMS-PMMA-PPG and category 5 of PPG-PDMS-PMMA-PVC. In addition to peak position in the chromatogram, the peak shape was a further key parameter when comparing various stationary phases.

Figure 6.20 summarizes the stationary phases of each column category with the best overall peak shape and peak separation. For PDMS and PVC, the separation efficiency of the ZirChrom Diamond Bond C18 (**Figure 6.20 a)**) was quite impressive compared to all other investigated columns. Comparing the performance of a Luna Omega PS C18 and a TSK Gel Boronate to the Diamond Bond C18, the separation between the polymers and the peak shapes declined. **Figure 6.20 b)**) depicts the polymer separation using a monolithic Chromolith Performance RP C18 column with a partial co-elution of PDMS and PVC. Applying a Cogent Bidentate C18 column, the separation efficiency further decreased. Most of the examined stationary phases featured an elution order of category 3 of which the Agilent PLRP-S polymer-based column showed the best separation performance (**Figure 6.20 c)**). Comparing elution order and separation power of the examined C18 columns a broad variety occurred within the similar modified alkyl chain stationary phases. These results show that the stationary base material and fabrication of the particles had a larger impact on the polymer separation than the modification of the phases.

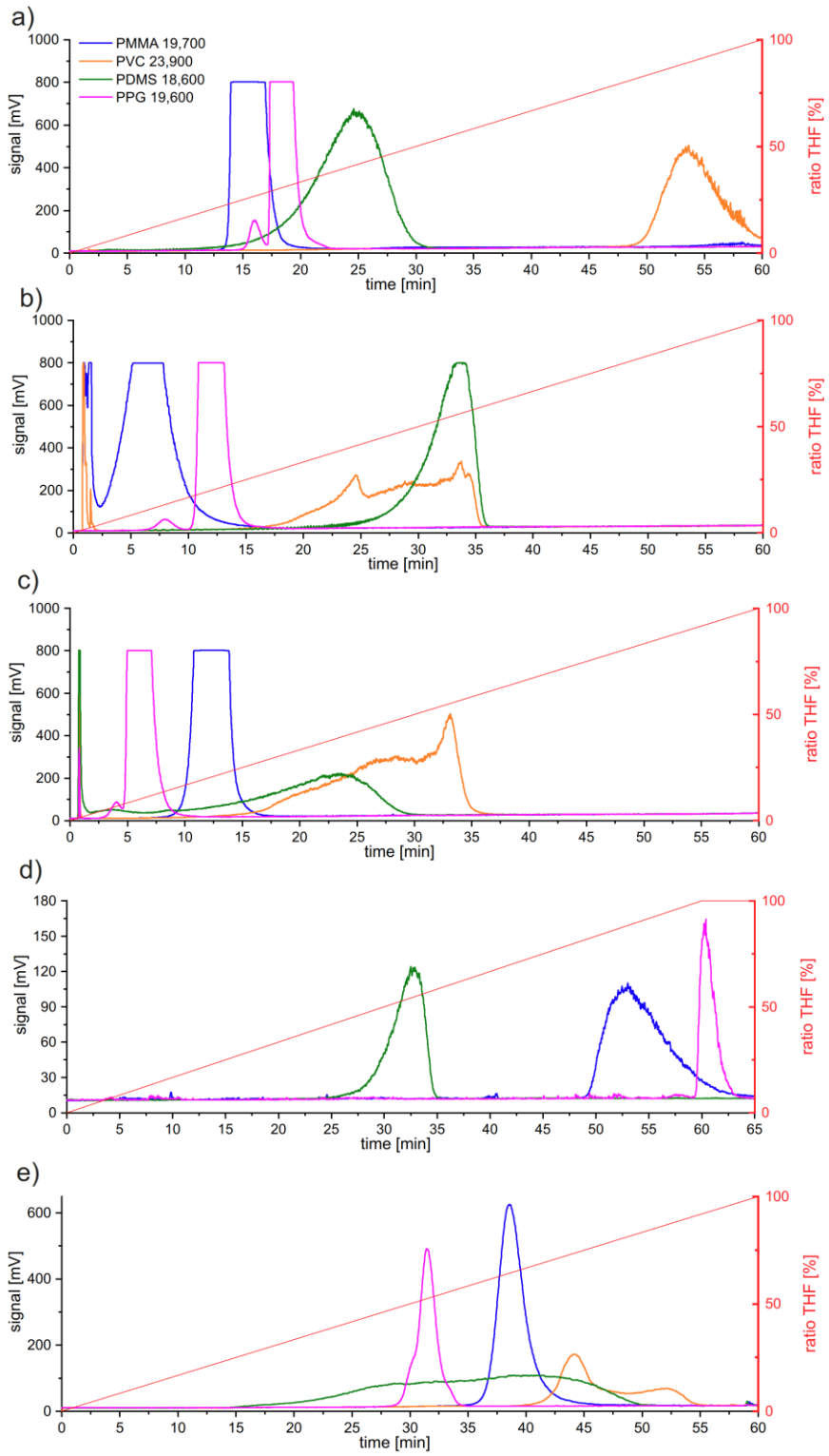


Figure 6.20: *Separations of PMMA (blue line), PVC (orange line), PDMS (green line), and PPG (pink line) with an average molecular mass of approximately 20 kDa and a concentration of 20 mg·mL^{-1} respectively, methanol as adsorption promoting solvent, THF as desorption promoting solvent (linear gradient) at **a)** a Diamond Bond C18 (150x4.6 mm, 5 µm), **b)** a Chromolith RP C18 (100x4.6 mm, monolithic), **c)** a PLRP-S (50x4.6 mm, 10 µm, polymer material),**d)** a Hypercarb (100x4.6 mm, porous graphitic carbon), and **e)** a PLgel Mixed C (300x7.5 mm, 5 µm, SEC column).*

Using a Hypercarb, a porous graphitic carbon column, the elution order was inverted, so that the polar PPG elutes last (**Figure 6.20 d)**). Furthermore, PVC irreversibly remained on the stationary phase and even intense flushing with THF at elevated temperatures (up to 60 °C) could not desorb the polymer. In comparison to columns of categories 1 to 3, porous graphitic carbon offered a significantly different polymer elution. Another interesting application of polymer HPLC is shown in **Figure 6.20 e)**, applying a PL gel Mixed C SEC column as stationary phase. Apart from a very broad polymer distribution of PDMS, PMMA, PVC, and PPG could also be separated. Moreover, the separation of three different polymer types with similar molecular masses with gradient HPLC allowed the application of a further separation mechanism without using another stationary phase in combination with conventional SEC.

6.2.4.3.2 One column – two separation mechanisms

For a more detailed investigation of the two separation mechanisms employing a SEC column, e.g. PL gel Mixed C, 300x7.5 mm, a set of four PMMA standards with average molecular weights of 19,700 g·mol^{-1}, 107,000 g·mol^{-1}, 690,000 g·mol^{-1}, and 1,600,000 g·mol^{-1} were analyzed. SEC measurements were performed with THF as eluent (**Figure 6.21 a)**) and for HRP-HPLC acetonitrile was used as adsorption promoting solvent and THF as desorption promoting solvent (**Figure 6.21 b)**).

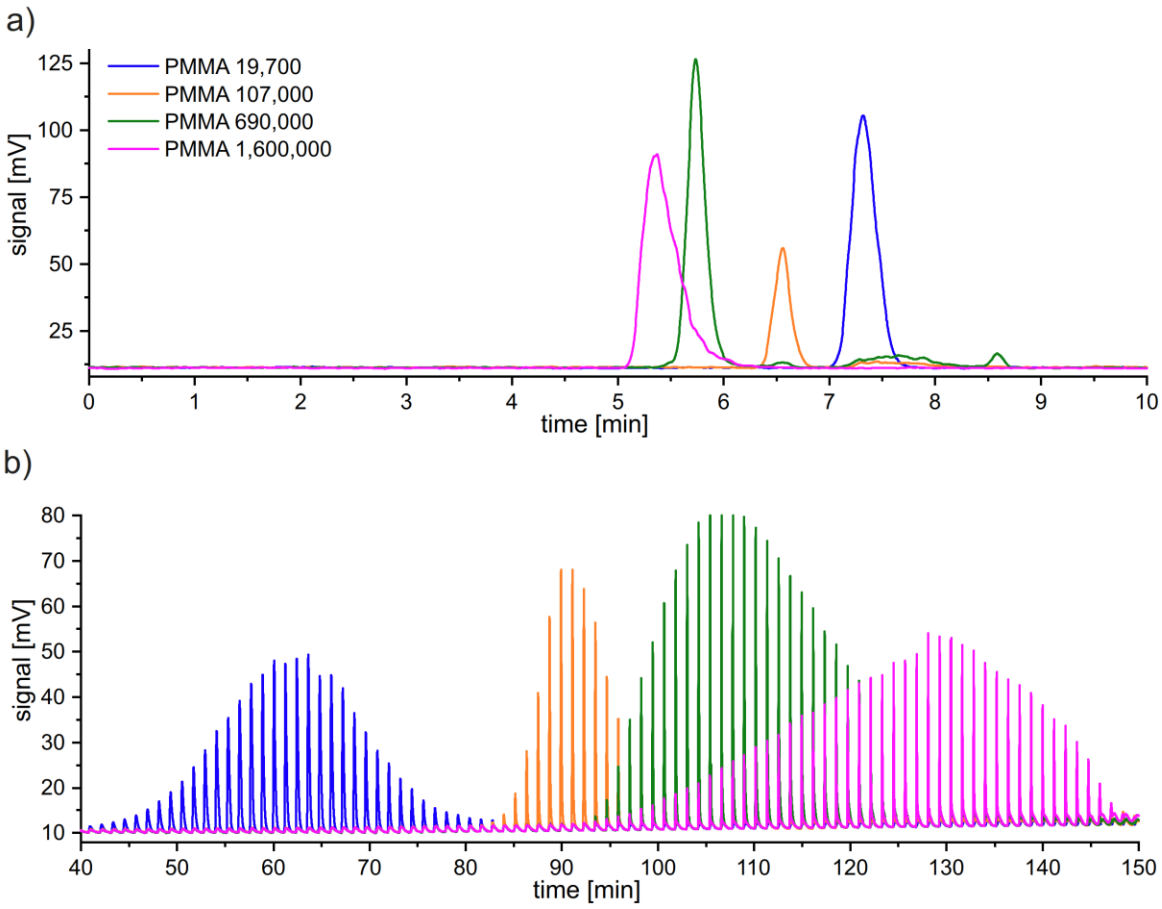


Figure 6.21: a) *SEC elugrams of various PMMA standards at a flow rate of 1.0 mL·min^{-1} and THF as mobile phase;* ***b)*** *HRP-HPLC chromatograms of the same PMMA standards employing a saw tooth gradient of 6.0 % height of the negative backward gradient step, 1.2 min effective step length, and 0.2 % effective step height with acetonitrile as adsorption promoting solvent and THF as desorption promoting solvent.*

In normal SEC mode the polymers were separated due to their solvation volume, i.e. average molar mass distributions, while the elution order is from high molecular weight to low molecular weight polymer in accordance with size exclusion mechanism [22]. In contrast when applying HRP-HPLC, the polymer distributions were divided in several distinct peaks for each sample. Furthermore, molar mass increased with increasing retention time. Comparing both separation modes, HRP-HPLC gave a more detailed insight to the polymer distribution, while SEC offered the molar mass distribution of a polymer applying e.g. conventional calibration by polystyrene standards. Combining both techniques yields more information about the chemical composition and/ or the molar mass distribution of polymer samples and, especially for complex polymer mixtures (see the preceding section), a more detailed overview by applying only one stationary phase becomes possible.

6.2.4.4 Optimization of preparative HRP-HPLC

For an evaluation of separation performance and for coupling with other analytical methods, a two-dimensional approach employing a saw tooth gradient in the first dimension would be beneficial. One major drawback was the limited number of gradient steps being allowed in the gradient table of chromatographic software packages. This was overcome by coupling a Thermo Fisher Scientific pump with an Agilent faction collector using Chromeleon as chromatographic software. For a proper control, two HPLC systems, one from Thermo Fisher Scientific and one from Agilent were integrated as one instrument in the software. Whereby, neither the Agilent pump nor the Agilent autosampler had to be work active in the actual separation nor had to be fully functional. The only request to these additional components was a proper electronic signal for the software. Running two autosamplers within one configuration was possible without any problems if in the script editor of Chromeleon the command for the Agilent autosampler "LCSystem.ALS.Position" was set to "1". The flow rate of the Agilent pump was set to 0.0 mL·min^{-1} but the pressure signal had to be recorded for a proper run control. Additional care had to be taken in the sequence table of Chromeleon: No entry specified as "blank" was allowed and the instrument could not be automatically set in standby condition. When considering these peculiarities, an Agilent fraction collector can be combined with a Thermo Fisher Scientific LC system employing gradient tables with more than 2000 entries. By this hybrid HPLC system and a down-to-zero saw tooth gradient, preparative separations and fraction collection could be achieved. For this purpose, a Hypersil Gold C18 aQ (100x10 mm, 5 µm) was employed with methanol as adsorption promoting solvent and THF as desorption promoting solvent separating a silicone oil with a viscosity of 350 mPa·s. The pattern used for fraction collection was determined by ELS detection and is depicted in **Figure 6.22**.

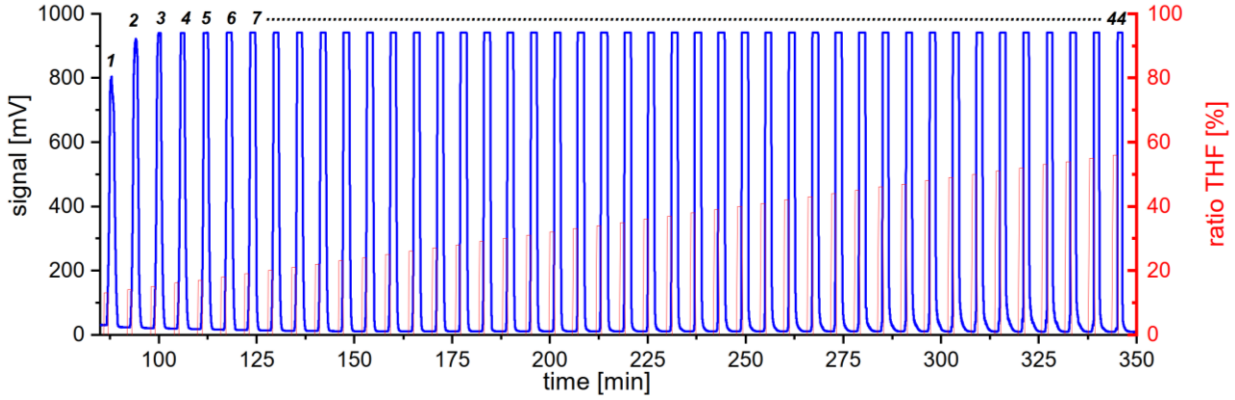


Figure 6.22: *Measurement for preparative separation of PDMS with a viscosity of 350 mPa·s (absolute sample amount 165 mg per run, fraction collection of two runs) on a Hypersil Gold C18 aQ column (100x10 mm, 5 µm) with methanol as adsorption promoting and THF as desorption promoting solvent; the added numbers correspond to the collected fractions.*

Forty-four fractions were collected and afterwards the molar mass distribution of each fraction was determined by SEC. **Table 6.9** gives an overview about the collected weight of each fraction, its number average molar mass, weight average molar mass, average molar mass at peak maximum and the polydispersities. The molar masses rose with increasing number of fraction collection. The polydispersities of the fractions were all approximately 1.10 compared to 2.01 as the polydispersity of the original PDMS sample. **Figure 6.23** depicts the corresponding elugrams of SEC measured on a set of three PL gel MiniMIX-C (250x4.6 mm, 5 µm) columns using toluene as eluent and the RI signals were normalized. The plotted peak widths correspond not only to the polydispersity (cf. **Table 6.9**) but also to the intensity of the signal which do not affect peak position. A set of 10 fractions was converted by color encoding for a better overview in **Figure 6.23** including the original, non-fractionated silicone oil of a viscosity of 350 mPa·s as black elugram. Based on heart cut fraction collection by HRP-HPLC employing a saw tooth gradient and subsequent measurement by SEC an exceptional peak resolution could be achieved in the first dimension.

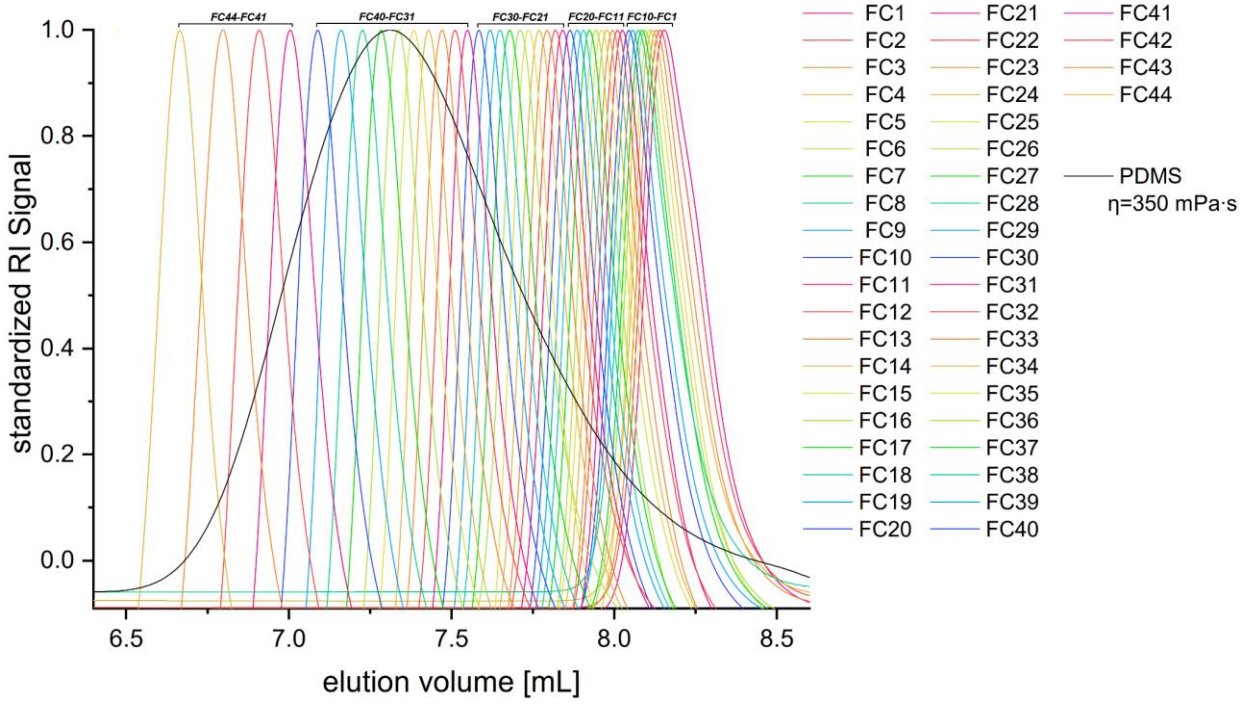


Figure 6.23: *SEC elugrams of PDMS fractions 1 – 44 using three PL gel MiniMIX-C (250x4.6 mm, 5 µm) columns at a flow rate of 0.3 mL·min^{-1} with toluene as eluent and a normalization of the RI signal, the different fractions are color encoded from red to blue with increasing molar mass or fraction number; the black curve shows the elugrams of the non-fractionated PDMS with a viscosity of 350 mPa·s.*

Table 6.9: *SEC results of the preparative HRP-HPLC separation of PDMS with viscosity of 350 mPa·s, the values for the number average molar mass M_n, weight average molar mass M_w, and average molar mass at peak maximum M_p were measured with an RI detector and calculated with the software WinGPC Unichrom 8.2; the fraction number refers to those of* ***Figure 6.22****.*

	weight of fraction [mg]	**M_n [g·mol^{-1}]**	**M_w [g·mol^{-1}]**	**M_p [g·mol^{-1}]**	**Poly-dispersity**
original sample	----	9460	19000	18600	2.01
FC 1	0.1	2390	2580	2880	1.08
FC 2	0.1	2460	2660	2990	1.08
FC 3	0.2	2630	2770	3060	1.05
FC 4	0.3	2690	2850	3150	1.06
FC 5	0.3	2690	2900	3210	1.08
FC 6	0.5	2780	2980	3310	1.07
FC 7	0.5	2790	3030	3380	1.09
FC 8	0.1	2910	3120	3450	1.07
FC 9	0.7	3050	3260	3570	1.07
FC 10	0.1	3110	3340	3670	1.07
FC 11	0.5	3290	3510	3850	1.07
FC 12	0.0	3420	3630	3990	1.06
FC 13	1.0	3540	3760	4130	1.06
FC 14	0.9	3670	3910	4300	1.07
FC 15	1.1	3840	4070	4410	1.06
FC 16	1.2	3970	4230	4630	1.07
FC 17	1.2	4170	4420	4820	1.06
FC 18	1.7	4360	4620	5030	1.06
FC 19	1.5	4550	4820	5220	1.06
FC 20	1.5	4690	5040	5500	1.07
FC 21	1.7	5020	5320	5780	1.06
FC 22	2.0	5210	5580	6040	1.07
FC 23	2.0	5550	5900	6450	1.06
FC 24	2.8	5860	6220	6810	1.06
FC 25	2.6	6250	6660	7230	1.07
FC 26	1.8	6620	7050	7740	1.06
FC 27	3.4	7090	7560	8220	1.07
FC 28	3.8	7530	8050	8870	1.07
FC 29	4.3	8040	8610	9470	1.07
FC 30	5.3	8650	9280	10200	1.07
FC 31	5.8	9340	10000	11000	1.07
FC 32	6.3	10200	10900	12000	1.07
FC 33	10.1	11100	11900	13100	1.07
FC 34	8.7	12200	13100	14300	1.07
FC 35	8.0	13400	14400	15800	1.07
FC 36	11.6	15000	16000	17600	1.07
FC 37	13.2	16800	18000	19800	1.07
FC 38	14.8	19000	20500	22300	1.08
FC 39	21.7	21700	23500	25800	1.08
FC 40	19.5	25400	27600	30300	1.09
FC 41	17.7	30300	33200	36300	1.10
FC 42	21.8	38000	41300	44600	1.09
FC 43	20.4	48000	53000	57300	1.10
FC 44	15.2	60100	70200	76200	1.17

6.2.5 Conclusion

The application of a saw tooth gradient for gradient polymer elution chromatography showed additional promising results. Optimization of flow rate, gradient profile, and mobile phase compositions revealed further peculiarities and benefits of the recently introduced saw tooth gradient profile. Based on these results, fingerprint analysis of complex polymer mixtures becomes possible with a high peak resolution. Employing preparative fraction collection further investigations of polymers by SEC, MALDI-ToF-MS or NMR are possible while simplifying complex polymer samples. Due to the high-resolution power of HRP-HPLC using a saw tooth gradient, analyses of polymer (micro-) structures and complex mixtures are comprehensively realized.

Furthermore, a comparison of various stationary phases gave an overview over associated separation performance and elution orders, which are an important basis for an optimal polymer separation. As recently shown [11], the column length had only minor impact on the performance of the saw tooth gradient and especially in combination with elevated LC flow rates, the use of shorter columns became favorable. Therefore, further research with short monolithic columns, as presented by Maksimova et al. [23] for the analysis of various polymers, would presumably be a good combination to HRP-HPLC.

Choosing a SEC column as stationary phase for high-resolution polymer HPLC allowed the application of two completely different separation mechanisms on the same chromatography column, i.e. SEC and HRP-HPLC mode. In this way two-dimensional liquid chromatography was enabled with one column. As an exemplary application, the highly resolved separation of PDMS was demonstrated. Finally, the optimized saw tooth gradient concept constitutes a robust alternative to common polymer liquid chromatography techniques and represents a good basis for the analysis of various polymers and complex polymer mixtures.

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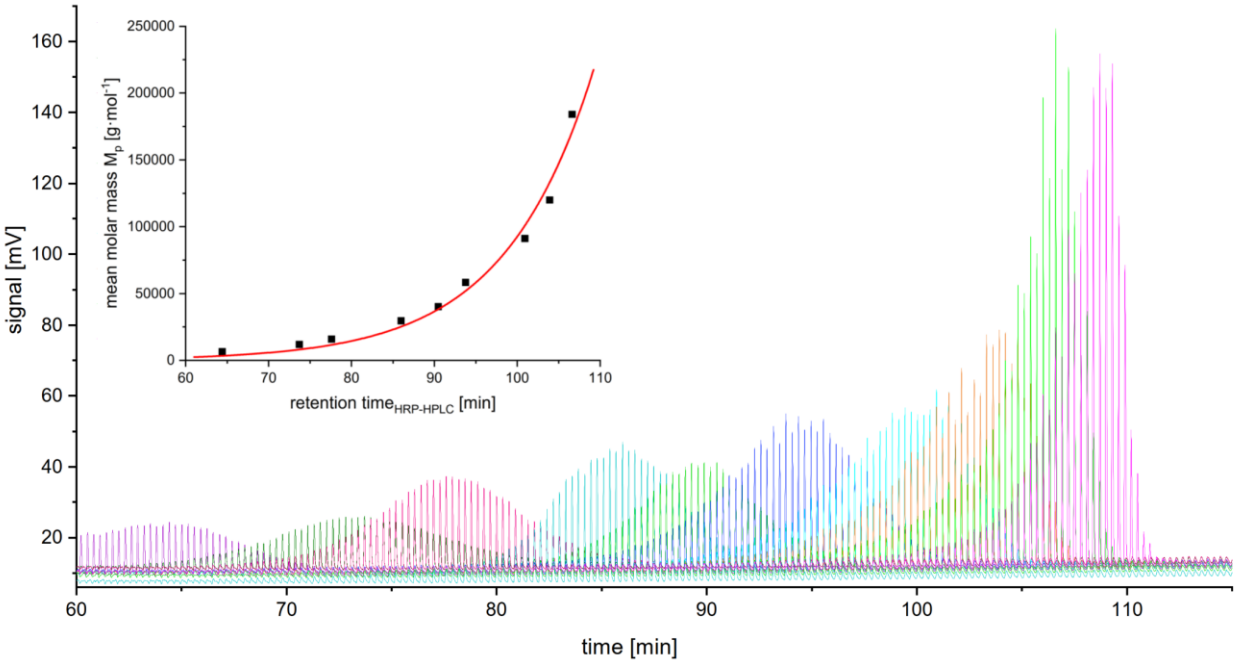
6.3 Comparison of Molar Mass Determination of Poly(dimethylsiloxanes) by Size Exclusion Chromatography and High-Resolution Polymer High Performance Liquid Chromatography Based on a Saw Tooth Gradient



Graphical Abstract: *Correlation between polymer molar masses and retention times in HRP-HPLC.*

This chapter has been accepted in ACS Applied Polymer Materials

Bernhard Durner, Beate Scherer, Thomas Ehmann, Frank-Michael Matysik

Measurements and experiments such as polymer HPLC analysis were done solely by the author. Experimental work regarding SEC was done by Beate Scherer at the business unit Technologie Werkstoff- und Verfahrensanalytik (TI-323) of the BMW group in Dingolfing. The authors contributed equally for interpretation and writing. The research was done under supervision and guidance of Dr. Thomas Ehmann and Prof. Dr. Frank-Michael Matysik.

6.3.1 Abstract

Polysiloxanes are used in a wide range of application fields and extensive research is currently done to enhance product quality and performance. Therefore, more sophisticated analysis methods are necessary to monitor and support the polymer product optimization. Based on different modes in polymer liquid chromatography, heart-cut two-dimensional polymer HPLC is one powerful analytical approach. Due to different distributions within polymer samples, separations according to chemical heterogeneities, molecular architecture or molar mass differences are possible. With the recently introduced saw tooth gradient protocol a new possibility for determining the polymer (micro-) structure on analytical scale has been developed. Hence, the effect of various stationary phases with different particle base material and chemical modifications were investigated in context of the separation of linear poly(dimethylsiloxane) in a molar mass range from 1000 g·mol^{-1} to 300 000 g·mol^{-1}. The resulting chromatograms allowed a direct correlation between HPLC retention times and molar masses corresponding to separated peaks. Consequently, a detailed analysis of differences in the polymer structure, e.g. fingerprint analysis, is possible.

6.3.2 Introduction

Since their first synthesis by Müller and Rochow [1], siloxanes are applied in a broad variety of different application areas, e.g. automotive, electronic, construction or health care industries. The versatile use of siloxanes stem from their inorganic and organic character of the polymer backbone, depending on modification of the number and type of organofunctional groups at the side chains. Based on the microscopic conditions and the crosslinking density, siloxanes are distinguished in silicone dispersions, elastomers, resins or rubbers [2–7]. One of the most important representatives of this polymer class is poly(dimethylsiloxane) (PDMS) containing silicon and oxygen atoms as well as methylene groups in the backbone. Therefore, PDMS are used as release agents, heat transfer liquids, antifoams, coatings or in the pharmaceutical or medical industry [8]. Further information about siloxane polymers and their applications are given in [9–15].

As most synthetic polymers, siloxanes vary in some properties like molecular weight, chemical composition and chain architecture. Therefore, it is necessary to obtain detailed information about these characteristics [16]. Various analytical methods can be applied including size exclusion chromatography (SEC), liquid chromatography at critical conditions (LCCC), and polymer HPLC [17,18]. Regarding thermodynamics, SEC is ideally dominated by entropic effects without any impact of chemical interactions. In polymer HPLC or more specifically liquid adsorption chromatography (LAC), the separation is driven by enthalpic interactions. For LCCC, enthalpic and entropic interactions equalize each other and allow a separation independent of the molar mass of the investigated polymer. Each technique has its own advantages and disadvantages; however, this study is focused comparing SEC and polymer HPLC. Further information concerning LCCC can be found in [19–22]. Typical detectors used in SEC and polymer HPLC are refractive index (RI) or/ and multi angle laser light scattering (MALLS) detectors in isocratic elution or evaporative light scattering detectors (ELSD) for gradient elution [23–26]. Moreover, the coupling to matrix assisted laser desorption ionization time of flight mass spectrometry can also be performed [27,28].

In SEC the hydrodynamic volume is assumed to be proportional to the molecular weight enabling the determination of molar mass distributions. SEC is performed either isocratically [29,30] which is the more common case or by applying a solvent gradient [31]. One drawback of SEC is the low resolution due to the pore volume of the separation column resulting in broad peaks. Furthermore, enthalpic interactions cannot often be completely excluded tending to inaccurate molecular weight-distributions. Nevertheless, SEC can offer enhanced resolution especially in the high-molecular mass range when compared to liquid

adsorption chromatography (LAC) [26,32,33]. In order to enhance the performance of polymer HPLC, we recently introduced a newly developed saw tooth gradient protocol [34]. Applying a saw tooth gradient profile to polymer separations allows highly resolved analytical and (semi-) preparative measurements. Therefore, the back and forth of the saw tooth gradient enables a very detailed characterization of various polymer samples.

Often a combination of the above modes of polymer liquid chromatography is used for an enhanced separation, especially in case of polymer HPLC. Therefore, heart-cut techniques of e.g. polymer HPLC or LCCC coupled to SEC are a powerful approach [32]. Combining with high-resolution polymer HPLC (HRP-HPLC), a very powerful preparative fractionation is achieved. Compared to classical preparative HPLC approaches [35,36], the method upscale based on a saw tooth gradient is simplified. Therefore, the optimized gradient profile can be used similar in analytical and (semi-) preparative systems, only the effective step length must be adjusted. Furthermore, concentration and volume overloading can easily be adapted to the separation system, as far as for gradient polymer elution chromatography (GPEC) the injection always occurs in 100 % adsorption promoting or rather non-solvent. Thus, peak broadening effects can be avoided. Therefore, we recently [37] optimized a hybrid HPLC system which allowed the overall application of the saw tooth gradient and analyzed a PDMS sample with a mean molar mass of approximately 20 000 $g \cdot mol^{-1}$. In this study, the investigated molar mass range is extended and the impact of different stationary phases on separation performance was analyzed. Additionally, after fraction collection of a polydisperse PDMS sample, the molar masses of the single fractions were correlated to retention times in polymer HPLC.

6.3.3 Material and methods

6.3.3.1 Mobile phase compounds and polymer standards

All used solvents were HPLC grade, except from toluene (analytical grade > 99.9 %). Acetonitrile, methanol, non-stabilized tetrahydrofuran (THF), and toluene were purchased from Merck (Darmstadt, Germany) and were used without further purification. All poly(dimethylsiloxane) (PDMS) standards with viscosities of 5 mPa·s, 20 mPa·s, 200 mPa·s, 8000 mPa·s, and 1 000 000 mPa·s, were obtained from Wacker Chemie AG (Burghausen, Germany). The mixtures of various PDMS for preparative HRP-HPLC contained 5 mPa·s, 20 mPa·s, 200 mPa·s, 8000 mPa·s, and 1 000 000 mPa·s in a ratio of 1:3:4:4:4 dissolved in THF with an overall PDMS concentration of 100 mg·mL^{-1} resulting in a molar mass distribution of a polydispersity of 18. The linear PDMS oligomers of Si10, Si22, Si30, and Si40 were isolated as described previously [38]. In this study, a concentration of 1 mg·mL^{-1} for each in THF dissolved linear PDMS oligomer was used.

6.3.3.2 Preparative HRP-HPLC based on a saw tooth gradient

Preparative HRP-HPLC was performed by coupling a Thermo Fisher Scientific (Waltham, USA) Ultimate 3000 HPLC (with binary pump, autosampler, and column oven) to an Agilent (Waldbronn, Germany) 1260 fraction collector equipped with an Agilent LAN Interface Card. For determination of the cutting pattern an Agilent 385 evaporative light scattering detector (ELSD) modified with a parallel path poly(tetrafluoroethylene) (PTFE) nebulizer was used (40 °C evaporator temperature, 90 °C nebulizer temperature and a gas flow rate of 1.6 SLM [25]). For fraction collection, an additional (but inactive) Agilent 1100 HPLC pump and autosampler were necessary. The Agilent autosampler had to be deactivated in the script editor of the chromatographic data system Chromeleon (Thermo Fisher Scientific, version 7.2) by using the command “LCSystem.ALS.Position” set to value of “1”.

For the preparative separations, acetonitrile was used as adsorption promoting or rather non-solvent (in the following referred to as adsorption promoting solvent independently of influences of precipitation and re-dissolution effects) and THF as desorption promoting solvent. The first set of fraction collection was performed on a Hypersil Gold C18 aQ (100x10 mm, 5 µm) with an overall fractionated amount of 1200 mg PDMS (24 injections at 50 mg). Thus, the injection volume was 100 µL. The used saw tooth gradient had an effective step length of 2.25 min, an effective step height of 1.0 %, a height of the negative backward gradient step of 40 %, and a LC flow rate of 4.0 $mL \cdot min^{-1}$. The second set of fraction collection was performed on an Agilent PL Gel Mixed C SEC column (300x5 mm, 5 µm) with an overall fractionated amount of 800 mg PDMS (16 injections at 50 mg). The injection volume again was 100 µL. The used saw tooth gradient had an effective step length of 3.0 min, an effective step height of 1.0 %, a height of the negative backward gradient step of 100 %, and a LC flow rate of 3.0 $mL \cdot min^{-1}$.

6.3.3.3 SEC equipment and measurements

For conventional calibration by a refractive index (RI) detector a set of Agilent polystyrene standards between 580 and 2 698 000 g·mol^{-1} was used with a concentration of 0.50 % w/v. All SEC measurements were performed according to ISO 16014-3 and ISO 16014-5 [39,40]. A chromatography system Agilent Series 1260 Infinity equipped with an isocratic pump, a 4-channel degasser (PSS, Mainz, Germany), an autosampler, and a column oven (T = 45 °C, Waters, Eschborn, Germany) was used. The RI detector Optilab Tr-EX and the multi-angle laser light scattering (MALLS) detector Dawn HELEOS II were manufactured by Wyatt Technologies (Santa Barbara, USA). The Optilab Tr-EX detector was set to 45 °C. All SEC measurements and data evaluation were performed by using ASTRA 7 and HPLC Manager software (Wyatt Technologies, Santa Barbara, USA). The light scattering measurements and the measurements for the conventional calibration were performed with an injection volume of 20 µL, a flow rate of 0.3 mL·min^{-1}, toluene as eluent, and a sample concentration of 3 mg·mL^{-1}. An Agilent PL Gel MiniMIX-C Guard column (50x4.6 mm, 5 µm) and three Agilent PL Gel MiniMIX C columns (250x4.6 mm, 5 µm) were used as stationary phase.

6.3.3.4 Analytical HRP-HPLC based on a highly resolved saw tooth gradient

The analytical HRP-HPLC measurements were performed on a Thermo Fisher Scientific Ultimate 3000 HPLC (quaternary pump, autosampler, column oven) equipped with an Agilent 385 ELSD modified with a parallel path PTFE nebulizer (see above). Methanol was used as adsorption promoting solvent and THF as desorption promoting solvent. The saw tooth gradient (for methodical details see ref. [34]), with an effective step height of 0.2 %, an effective step length of 0.3 min, a height of the negative backward gradient step of 40 %, and a LC flow rate of 3.0 mL·min^{-1}, started at 100 % methanol and ended at 100 % THF. The measurements were performed on a Thermo Fisher Scientific Accucore C18 (50x4.6 mm, 2.6 µm) and a ZirChrom (Anoka, USA) Diamondbond C18 (150x4.6 mm, 5 µm). The injection volumes were 8 µL for each analyzed sample.

6.3.4 Results and discussion

In addition to the previously reported results [34], further investigations with preparative high-resolution polymer HPLC (HRP-HPLC) based on a saw tooth gradient were performed. With a model mixture of linear PDMS composed of silicone oils with different viscosities and thus molar masses, the separation capability of the saw tooth gradient is discussed. Furthermore, re-analysis of samples fractionated by a high-resolution saw tooth gradient on two appropriate stationary phases (refer to [37]) allowed the correlation of average molar mass at peak maximum to retention times of HRP-HPLC.

6.3.4.1 Preparative HRP-HPLC for PDMS fractionation and SEC data evaluation

Comparing two completely different stationary phases, the separation efficiency of the used saw tooth gradients is discussed. Initially, a Hypersil Gold C18 aQ (100x10 mm, 5 µm) was used to separate the PDMS mixture specified above in 22 consecutive fractions (**Figure 6.24**). Therefore, acetonitrile was used as adsorption promoting or rather non-solvent for the investigated PDMS mixture and THF as desorption promoting solvent. The polymer sample was introduced in the separation system by multiple injection (5 times) of 100 µL of the above PDMS mixture at a low flow rate of 0.2 mL·min^{-1} in 100 % of adsorption promoting solvent. The advantage of this injection procedure is that the injection loop had not to be changed. Thus, volume overloading effects were reduced, and the peak width remained small because the sample precipitated or was strongly adsorbed on the column head. This procedure was very beneficial for scaling up from analytical [34] to (semi-) preparative saw tooth gradients.

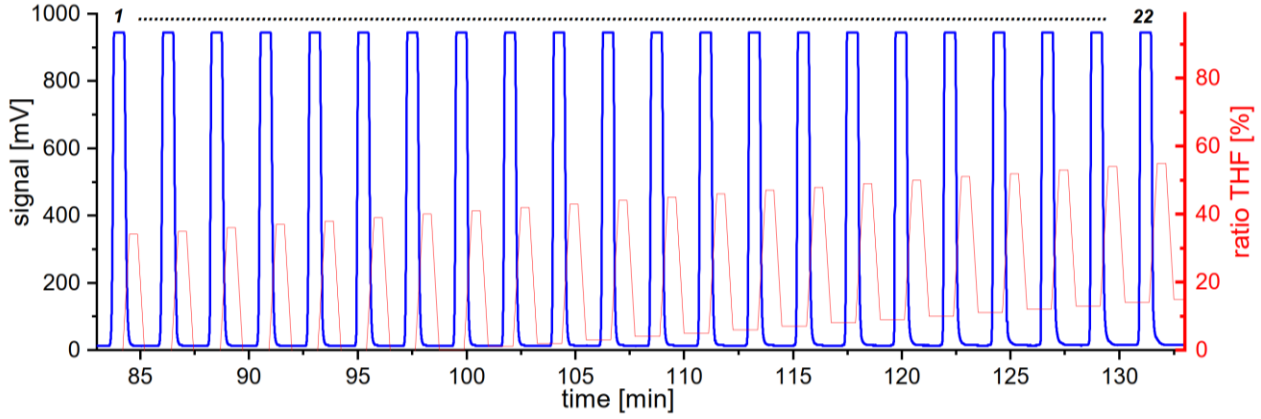


Figure 6.24: *Measurement for preparative separation of a linear PDMS sample mixture containing silicone oils with viscosities of 5 mPa·s, 20 mPa·s, 200 mPa·s, 8000 mPa·s, 1 000 000 mPa·s, dissolved in THF with a mixing ratio of 1:3:4:4:4 and an overall PDMS concentration of 100 mg·mL^{-1}. A Hypersil Gold C18 aQ column (100x10 mm, 5 µm), acetonitrile as non-solvent, and THF as desorption promoting solvent were used with a saw tooth gradient of 40 % height of the negative backward gradient step, 1 % effective step height, and 2.25 min effective step length. The collected fractions are numbered from 1 to 22.*

The sample amount per fraction is depicted in **Table 6.10**. Furthermore, an overview of the number average molecular weight M_n, the weight average molecular weight M_w, the average molar mass at peak maximum M_p, and the polydispersity (M_w/M_n) of fractions 1 to 22 is also given. Elugrams of each fraction were evaluated by means of conventional calibration as equivalents of polystyrene standards using only the refractive index detector and retention times (see **Table 6.10**). Moreover, the absolute masses were determined by using multi-angle laser light scattering (MALLS) detection with RI detection for determining the concentration for each fraction. These results are also given in **Table 6.10** as MALLS results. Therefore, 100 % recovery is assumed for calculating the distinct refractive index increments dn/dc from the RI signals. In calculating the absolute molar mass dn/dc is used as square in the corresponding equation emphasizing the importance of reliable values for dn/dc. In general, SEC measurements show a continuous increasing molar mass with increasing fraction number. But by evaluation based on absolute molar mass determination the number and weight average molecular masses do not consistently increased from fractions 1 up to 22. Referring to the average molecular masses the polydispersity index also fluctuated. However, the M_p values of MALLS and RI increased throughout from fraction 1 up to fraction 22 with just one exception of fraction 2 when assessed by MALLS detection. One possible reason why the molar masses obtained by MALLS were not monotonously increasing, e.g. at fraction 2, 9, 10, 12, and 19, could be caused by the inappropriate assumption of 100 % mass recovery and its impact on the dn/dc calculation. Nevertheless, values evaluated by conventional calibration show continuous increase of M_w, M_n und M_p values. Regarding polydispersity fractions 1 up to 17 are highly monodisperse with values ranging from 1.04 to 1.07. While polydispersity indices of fraction 18, 19, 20, and 21 showed a remarkable increase followed by the more than doubled polydispersity index of fraction 22 compared to 21. This could be seen as indicator that the column used for fraction collection probably had to be optimized to achieve an ideal separation.

The RI detector is the most common detector in SEC as most of the polymers can be detected. If standards for mass calibration of the respective polymer are available, the assessed molar masses directly refer to the investigated polymer. If not, the molar mass averages are determined as equivalents of certain available polymer standards e.g. polystyrene or polymethylmethacrylate. For PDMS toluene is used as eluent because THF and PDMS are nearly isorefractive resulting in a very poor signal [41]. In **Figure 6.25** the normalized signals obtained by MALLS (a) and RI-detection (b) are depicted. Each fraction is color coded. The peak maxima of lower fractions are located directly next to each other. The peak maxima of the first fractions are very close to each other and with increasing fraction number the distance slightly increases. But fraction 21 and 22 showed an atypical behavior. Therefore, for subsequent measurements

only the RI detector was used assessing the molar mass distributions. The large distance between the peak maximum of fraction 20 and fraction 21 could be explained by the failure of the applied stationary phase in appropriately separating the high molecular mass components of the sample by the saw tooth gradient approach. It could be assumed that fraction 21 and 22 eluted very close to the point of critical adsorption. This limiting condition for the application of a preparative saw tooth gradient could be overcome by applying a different stationary phase. Additionally, for the analyzed PDMS mixture RI detection was sufficient and was solely used in subsequent measurements.

Table 6.10: *Results of the SEC measurements of the first set of fraction collection (compare* ***Figure 6.24****), SEC was performed with 3 MiniMix C SEC columns (250x4.6 mm) and toluene as eluent; the values for the weight average molar mass M_w, the number average molar mass M_n, and the average molar mass at peak maximum M_p were evaluated by an RI and a MALLS detector.*

Fraction number	Weight of fraction [mg]	M_w [g·mol^{-1}]		M_n [g·mol^{-1}]		M_p [g·mol^{-1}]		Polydispersity	
		MALLS	RI	MALLS	RI	MALLS	RI	MALLS	RI
FC1	5.5	4,600	4,300	4,500	4,200	4,400	4,600	1.02	1.04
FC2	5.8	4,300	5,200	4,200	4,900	4,300	5,500	1.01	1.05
FC3	5.8	4,600	5,400	4,600	5,200	4,600	5,800	1.01	1.04
FC4	6.2	4,800	5,600	4,800	5,400	5,000	6,000	1.01	1.04
FC5	6.1	4,900	5,900	4,800	5,600	5,100	6,300	1.01	1.05
FC6	6.4	5,000	6,100	4,900	5,900	5,200	6,600	1.02	1.04
FC7	6.7	5,600	6,500	5,600	6,200	5,600	7,000	1.00	1.04
FC8	7.0	6,700	6,800	6,300	6,400	6,200	7,300	1.08	1.05
FC9	7.4	6,600	7,200	6,400	6,900	6,500	7,800	1.04	1.05
FC10	8.0	6,200	7,800	5,900	7,500	6,600	8,400	1.05	1.05
FC11	8.8.	8,000	8,300	7,800	7,900	7,600	9,100	1.04	1.06
FC12	9.3	7,400	9,000	7,100	8,500	7,900	9,800	1.04	1.06
FC13	10.1	8,000	9,700	7,800	9,100	8,400	10,500	1.02	1.06
FC14	11.4	8,600	10,600	8,300	9,900	9,200	11,500	1.03	1.07
FC15	12.4	9,900	11,700	9,700	11,000	10,400	12,600	1.02	1.06
FC16	13.9	10,700	12,700	10,000	11,900	11,600	13,800	1.07	1.07
FC17	15.3	12,100	14,000	11,000	13,100	13,200	15,200	1.10	1.07
FC18	17.2	15,500	15,500	15,000	13,200	15,300	17,100	1.03	1.18
FC19	19.0	14,700	17,600	13,200	16,000	16,300	19,300	1.11	1.10
FC20	21.2	20,100	20,100	19,800	15,100	20,300	22,400	1.01	1.34
FC21	83.0	162,700	161,700	119,600	102,800	125,800	125,600	1.36	1.57
FC22	81.4	335,000	269,000	128,400	82,100	499,100	392,900	2.61	3.27

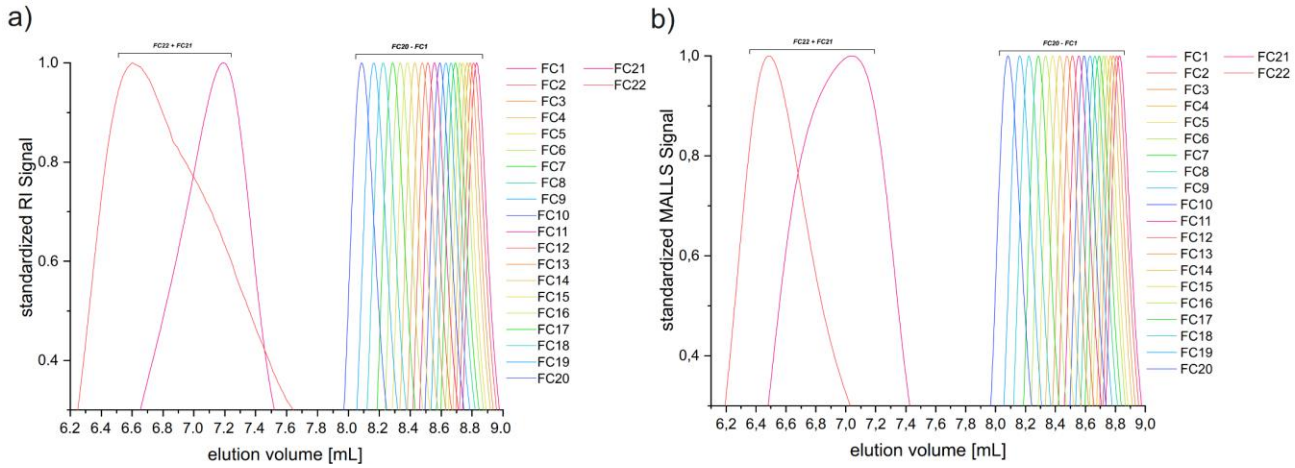


Figure 6.25: *SEC results of the preparative separation depicted in* ***Figure 6.24****, each elugram shows a standardized y axis for* ***a)*** *the RI signal and* ***b)*** *the MALLS signal; the fraction numbers are shown and are color coded in the legend.*

In a following preparative HRP-HPLC separation (**Figure 6.26**) a PL gel Mixed C (300x5 mm) SEC column was used with acetonitrile and THF as mobile phase components again. The different base material of the stationary phase increased the working range for the separation based on the saw tooth gradient. As already depicted in [37], operating a saw tooth gradient on a SEC column worked pretty well. Fraction collection was performed for 37 fractions, with several time jumps covering certain ranges of the total distribution of the synthetic PDMS mixture. Additionally, a saw tooth gradient down-to-zero (with 100 % height of the negative backward gradient step) was applied to enhance the separation performance. Compared to the aforementioned fraction collection, more peaks could be resolved and collected.

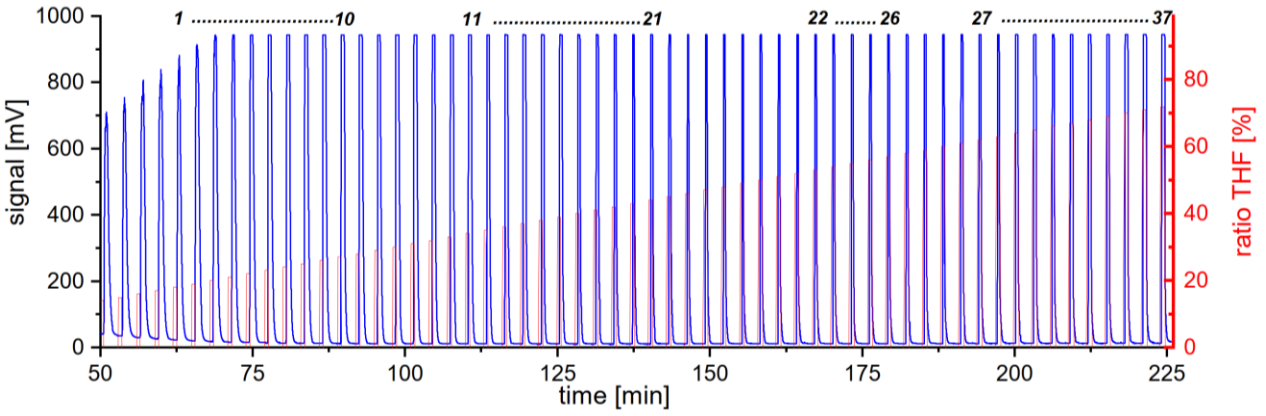


Figure 6.26: *Chromatogram of preparative separation of a synthetic mixture of linear PDMS containing silicone oils with viscosities of 5 mPa·s, 20 mPa·s, 200 mPa·s, 8000 mPa·s, 1 000 000 mPa·s, dissolved in THF with a mixing ratio of 1:3:4:4:4 and an overall PDMS concentration of 100 mg·mL^{-1}. A PL gel Mixed C SEC column (300x5 mm), acetonitrile as non-solvent, and THF as desorption promoting solvent were used with a saw tooth gradient of 100 % height of the negative backward gradient step, 1 % effective step height, and 3.0 min effective step length. The collected fractions are assigned from 1 to 37 over the chromatogram.*

The molar mass distribution of SEC (**Figure 6.27**) showed the same gaps in the molar mass distribution as depicted in the cutting pattern (**Figure 6.26**). Similar to the first set of fraction collection, the molar masses increased with increasing fraction number, but with an improved peak resolution for high molecular components. Particularly, for fraction numbers 27 to 37 the Mixed C column showed an enhanced separation performance suggesting that the critical point of adsorption was shifted to even higher molecular masses. In summary, a Mixed C SEC column offered an improved separation range compared to Hypersil Gold C18 aQ for applying a saw tooth gradient on extremely polydisperse synthetic PDMS mixtures.

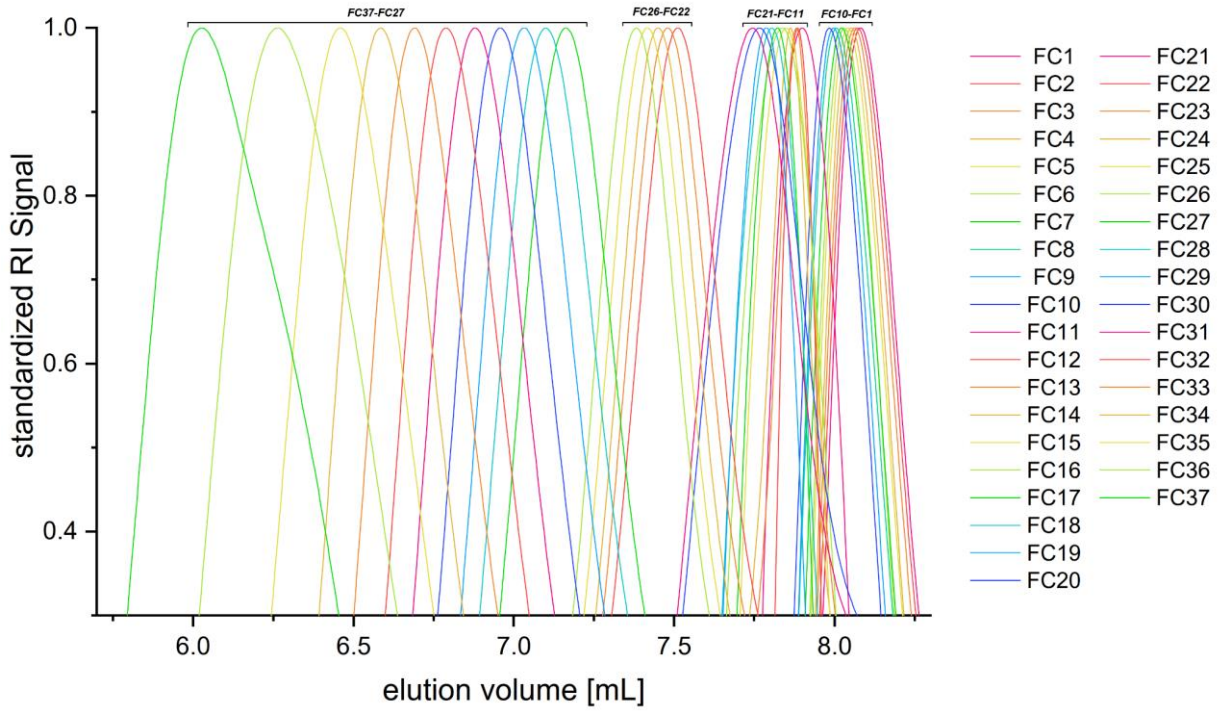


Figure 6.27: *SEC results for the preparative separation depicted in* ***Figure 6.26****, the y axis shows the standardized RI signal and the fraction numbering correlates to the numbers in* ***Figure 6.26****.*

The results for weight average molar mass M_w, the number average molar mass M_n, and the average molar mass at peak maximum M_p are presented in **Table 6.11**. Compared to the Hypersil Gold C18 aQ, the values for M_w, M_n, and M_p showed a monotonous rise with the fraction number. M_p starts from 3400 g·mol^{-1} at fraction 1 and ends at 311000 g·mol^{-1} for fraction 37, M_w from 2900 g·mol^{-1} to 231000 g·mol^{-1} and M_n from 2500 g·mol^{-1} to 170 400 g·mol^{-1}.

Table 6.11: *Results of the SEC measurements of the second fraction collection (compare **Figure 6.26**), SEC was performed with 3 MiniMix C SEC columns (250x4.6 mm) and toluene as eluent; the values for the weight average molar mass M_w, the number average molar mass M_n, and the average molar mass at peak maximum M_p were evaluated by an RI detector.*

Fraction number	Weight of fraction [mg]	M_w [g·mol^{-1}]	M_n [g·mol^{-1}]	M_p [g·mol^{-1}]
FC1	1.6	2900	2500	3400
FC2	1.5	3000	2500	3500
FC3	1.6	3100	2600	3600
FC4	1.9	3100	2700	3600
FC5	1.7	3200	2700	3700
FC6	2.1	3300	2800	3800
FC7	1.7	3400	2800	3900
FC8	1.5	3500	2900	4000
FC9	2.4	3500	2900	4100
FC10	2.3	3600	3000	4200
FC11	3.0	4400	3500	5100
FC12	4.9	4600	3700	5300
FC13	4.5	4600	3700	5300
FC14	5.4	4900	3800	5500
FC15	2.7	5000	3900	5700
FC16	1.9	5200	4000	5900
FC17	3.3	5400	4200	6100
FC18	2.3	5500	4400	6300
FC19	2.7	5700	4600	6500
FC20	2.6	6000	4900	6800
FC21	2.7	6200	5000	7100
FC22	4.4	10000	7700	12000
FC23	4.4	10800	8600	12800
FC24	5.3	11500	9500	13800
FC25	4.4	12400	10200	14800
FC26	4.3	13200	10300	15900
FC27	7.0	25500	20600	25800
FC28	5.8	26500	21800	29500
FC29	8.5	29100	23900	34100
FC30	9.5	33100	25700	40200
FC31	9.7	39600	29600	48100
FC32	9.8	47600	36800	58400
FC33	10.6	63300	47800	71900
FC34	11.7	73800	56700	91100
FC35	11.7	99700	77300	120000
FC36	11.8	144800	110700	184000
FC37	17.2	231000	170400	311000

6.3.4.2 Correlation of average molar masses to HRP-HPLC retention times

Based on the measurement series on the PL gel Mixed C column, re-analysis of the fractionated PDMS samples by applying a high resolution saw tooth gradient (0.2 % effective step height) was performed on a ZirChrom Diamondbond C18 column (**Figure 6.28**) showing a good separation performance for PDMS over a broad molar mass range in previous measurements [37]. Under these conditions, no chromatography under critical conditions occurred in the investigated molar mass range. Thus, even the highest masses of PDMS (up to 300 000 g·mol^{-1}) could be separated with good resolution.

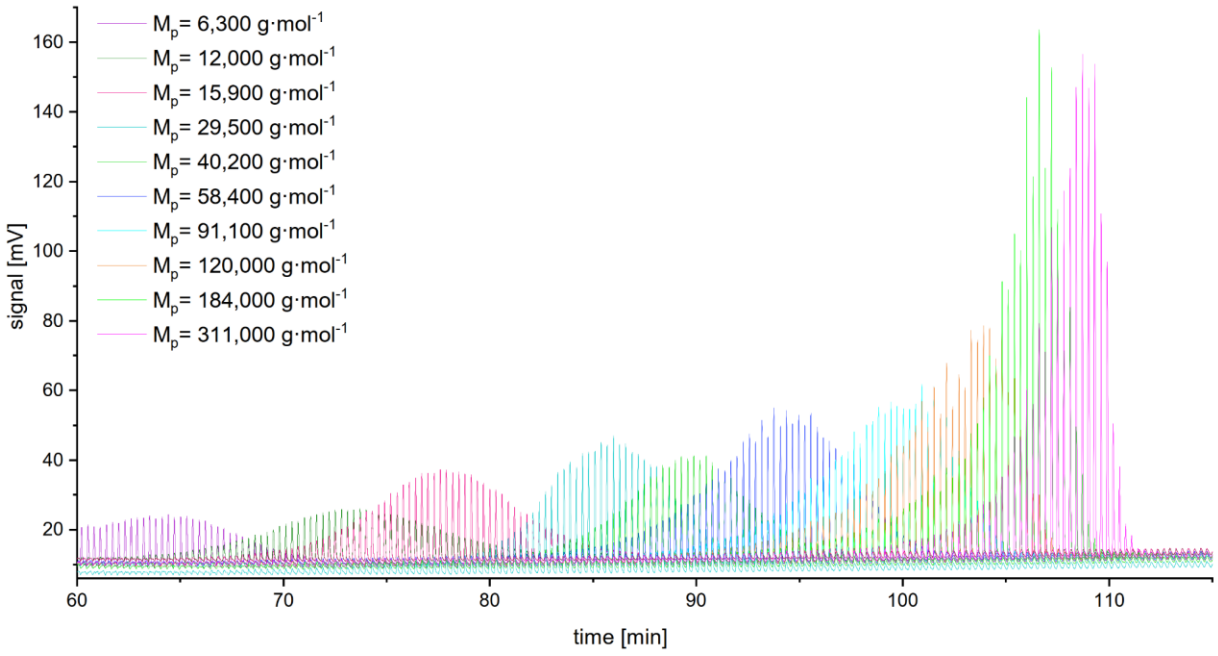


Figure 6.28: *Correlation of average molar masses of the fractionated PDMS sample to retention times in HRP-HPLC by performing a saw tooth gradient (effective step height: 0.2 %, effective step length: 0.3 min, height of the negative backward gradient step: 40 %) with methanol as adsorption promoting solvent and THF as desorption promoting solvent applying a ZirChrom Diamondbond C18 column (150x4.6 mm, 5 µm).*

In addition to this high molar mass separation, an Accucore C18 column (**Figure 6.29**) was chosen in order to analyze the low molar mass PDMS which were insufficient separated (extremely broad distribution) on the Diamondbond C18. For all fractionated samples down to fraction 1 a good separation was achievable. Furthermore, linear PDMS oligomer standards Si10, Si22, Si30, and Si40 were used to show the limits of the separation based on the applied saw tooth gradient. Furthermore, applying these oligomer standards with exactly defined molar masses to the separation were in very good accordance with the average molar mass corresponding to peak maximum values obtained from SEC. However, peak splitting of the pure oligomer samples showed the limitation of the saw tooth gradient for low molar mass compounds. Peak splitting originated from mass overloading of the single gradient steps. Consequently, either a saw tooth gradient with an extended effective step length or with a reduced sample amount would be necessary. Apart from that, referring to the single oligomers the molar masses of HRP-HPLC correlated pretty good to these of SEC, even for the lower mass range. Generally, for molar masses up to 2000 g mol^{-1} other separation techniques are preferred as shown in the separation of cyclic from linear PDMS [38,42].

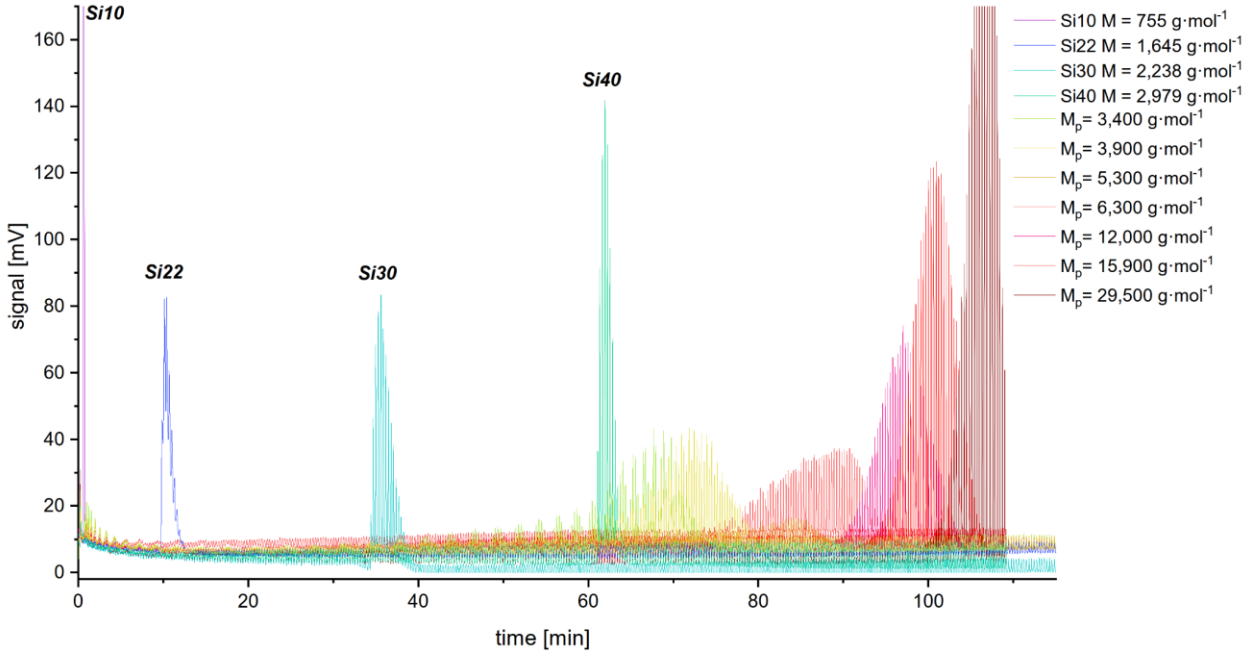


Figure 6.29: *Correlation of average molar masses of the fractionated PDMS sample and linear PDMS oligomers Si10, Si22, Si30, and Si40 to HRP-HPLC retention times by applying a saw tooth gradient (effective step height: 0.2 %, effective step length: 0.3 min, height of the negative backward gradient step: 40 %) with methanol as adsorption promoting solvent and THF as desorption promoting solvent using a Thermo Fisher Scientific Accucore C18 column (50x4.6 mm, 2.6 µm).*

Based on analytical HRP-HPLC applying a high-resolution saw tooth gradient, the molar masses of each PDMS fraction was correlated to retention times in HRP-HPLC. Thus, the molar mass at peak maximum obtained by SEC was related to the highest peak of the peak distribution and the corresponding retention time was determined. **Table 6.12** summarizes molar masses at peak maximum and the retention times. Employing an Accucore C18 and a Diamondbond C18 as stationary phases, the mass range between 6000 g·mol^{-1} and 16000 g·mol^{-1} overlapped, thus, by combining both stationary phases a molar mass range from < 1000 g·mol^{-1} up to 300 000 g·mol^{-1} could be covered.

Table 6.12: *Correlation of HRP-HPLC retention time to average molar mass at peak maximum M_p for several fractionated PDMS samples and linear PDMS oligomers Si10, Si22, Si40, and Si48 isolated as described in [38]. For covering the complete molar mass range two HPLC columns, Accucore C18 (50x4.6 mm) and Diamondbond C18 (150x4.6 mm), were used for analytical measurements.*

HPLC Column	Sample name	M_p [g·mol^{-1}]	Retention time [min]
Accucore C18	Si10	755	0.65
	Si22	1645	10.12
	Si30	2238	35.60
	Si40	2979	61.96
	Si48	3572	70.95
	FC1	3400	67.66
	FC7	3900	71.85
	FC12	5300	83.54
	FC18	6300	89.53
	FC22	12000	97.62
	FC26	15900	100.92
Diamondbond C18	FC18	6300	64.42
	FC22	12000	73.71
	FC26	15900	77.60
	FC28	29500	85.99
	FC30	40200	90.48
	FC32	58400	93.78
	FC34	91100	100.94
	FC35	120000	103.93
	FC36	184000	106.62
	FC37	311000	108.71

Based on the values of **Table 6.12** a calibration model for the Diamondbond C18 (**Figure 6.30 a**) and for the Accucore C18 (**Figure 6.30 b**) was established correlating the retention times to molar masses. For the Diamondbond C18 column, the best fit was an exponential curve with the following equation ($M_p = 8.62 \cdot \exp(0.0928 \cdot t)$), and for the Accucore C18 column a fifth-order polynomial equation ($M_p = 3.1 \cdot 10^{-5} \cdot t^5 + 6.5 \cdot 10^{-3} \cdot t^4 + 0.5 \cdot t^3 - 16.5 \cdot x^2 + 237.4 \cdot x + 567.5$) was an appropriate fit model. Based on these models, a more thorough characterization of investigated PDMS samples could be generated. For instance, small differences in the polymer distributions of various samples could be compared with respect to the exact molar masses. However, as shown by employing two different stationary phases, the shape and the type of the fitting curve highly depends on the type of the stationary phase. Presumably, changes in the composition of the mobile phase and the temperature setting also influence the fit function. Therefore, choosing the overall separation system in advance is helpful to keep the efforts as small as possible. Comparing the molar mass determination in SEC and HRP-HPLC, the major advantage of SEC is the nearly polymer independent conventional calibration when applying the polymer equivalent approach. But with HRP-HPLC an essentially improved resolution of the polymer distribution is achieved facilitating the distinction of small differences within polymer samples. For correlating molar mass to retention time in HRP-HPLC, the type of the polymer has to be known as well as the chemical functionality. Anyway, the measurements in HRP-HPLC are highly reproducible and only showed minor uncertainties in determining the retention times.

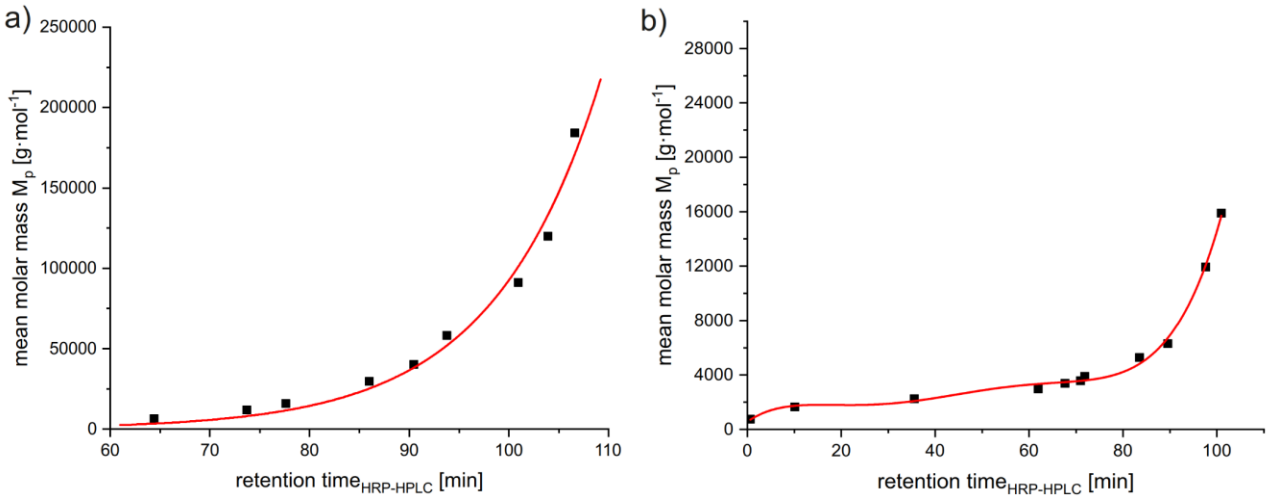


Figure 6.30: *Calibration curves for average molar mass correlation to retention time in HRP-HPLC.* ***a)*** *exponential fit* $[M_p = 8.62 \cdot \exp(0.0928 \cdot t)]$ *in the high molar mass range on a Diamondbond C18 column,* ***b)*** *fifth order polynomial fit* $[M_p = 3.1 \cdot 10^{-5} \cdot t^5 + 6.5 \cdot 10^{-3} \cdot t^4 + 0.5 \cdot t^3 - 16.5 \cdot x^2 + 237.4 \cdot x + 567.5]$ *in the low molar mass range on an Accucore C18 column; the uncertainties and the resulting error bars for each data point (3 repetitions) are smaller than the data symbols.*

6.3.5 Conclusion

Two sets of preparative HRP-HPLC measurements for PDMS were compared with respect to separation performance of the used saw tooth gradient and impact of the used stationary phases. Therefore, based on heart-cut preparative HRP-HPLC, using a PL gel Mixed C column allowed a considerably improved separation result. By re-analyzing the collected fractions with SEC, a consecutive increase of molar mass with the fraction number was observable. Molar masses obtained by absolute molar mass evaluation applying a MALLS detector showed some deviation from a monotonous increase presumably due to uncertainties in calculation dn/dc for each fraction. When assessing the molar masses by an RI detector and conventional calibration as equivalents of polystyrene the results steadily increased from fraction to fraction. Consequently, for the second set of fraction collection by the saw tooth gradient only RI detection and evaluation was applied for re-analyzing with SEC. A constant increase of molecular weight due to the fraction number and a correlation between molecular weights and retention times were obtained.

Re-analysis with a high resolution saw tooth gradient showed good results over the whole investigated molar mass range. Consequently, two different stationary phases, an Accucore C18 and a Diamondbond C18, were used for covering either the low or the high molar mass range. The Diamondbond C18 column showed a superior separation performance for the high molar mass range because the CPA did not affect the separation. The correlation between molar masses and retention times in HRP-HPLC enabled an enhanced characterization of polymers. Based on the calibration model, information about the polymer (micro-) structure was measurable and comparable especially for high molar mass polymer samples. Comparing with SEC one drawback is the susceptibility of HRP-HPLC to simultaneous changes of chemical and molar mass distribution of the investigated polymers. Thus, for each kind of polymer differing in more than one molecular distribution property, a proprietary optimization is necessary. Nevertheless, the high resolution of HRP-HPLC up to molar masses of 300 000 $g \cdot mol^{-1}$ and the possibility to generated quantitative results at molecular level make this technique very attractive.

6.3.6 References

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7 Conclusion and Outlook

In this study, PDMS was intensely investigated with different methods of polymer HPLC. In chapter 4, the separation of low molar mass linear and cyclic PDMS was optimized by adjusting the mobile and stationary phases. Hence, the predominant impact of an appropriate mobile phase revealed the importance of solubility and adsorption-/ desorption promoting solvents on separation of PDMS with varying molecular architecture. Thus, the separation from eight up to 30 monomer units or corresponding to a molar mass range from 600 g·mol^{-1} to 2,500 g·mol^{-1}, was successfully adjusted in the low molar mass regime. For higher molar mass of PDMS or of other polymers, only broad distributed peaks were obtained by the same approach. Thus, the separation of higher molar mass polymer required a different proceeding (cf. **Figure 7.1**).

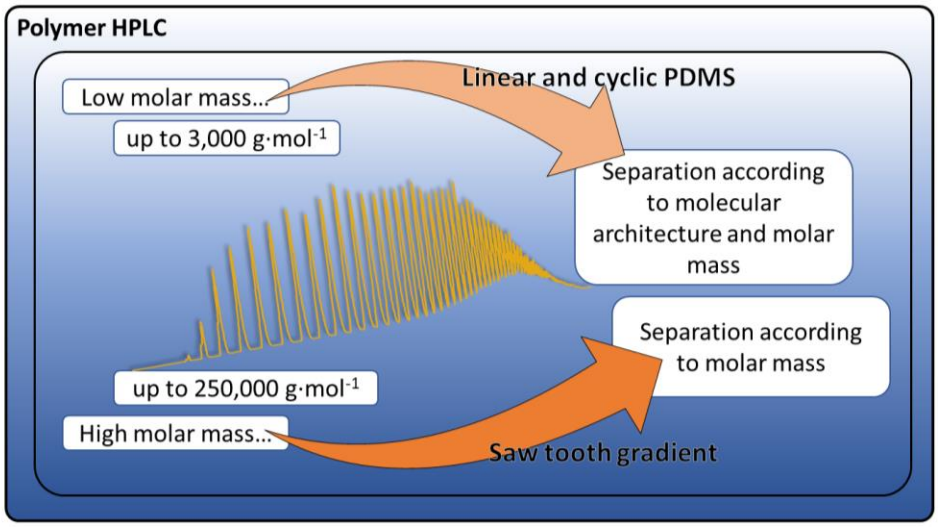

Figure 7.1: *Analysis of PDMS with polymer HPLC over a broad molar mass range.*

As discussed in the introduction, evaluating the analytical process (cf. **Figure 1.1**) in combination with continuous method optimization, allowed the development of a new gradient technique. For this reason, a saw tooth gradient (chapter 6) was introduced for obtaining high resolved polymer peaks, depending on the polymer type, up to a molar mass of 1,600,000 g·mol^{-1}. **Figure 7.2** provides an overview of the complete range of PDMS with viscosities from 10 to 1,000,000 mPa·s which can be analyzed. Compared to the common application of SEC for analysis of these polymer samples, it could be demonstrated that with an appropriate adjustment of the profile shape, effective step length, step height, the height of the negative backward step, stationary phase, and mobile phase composition a universal applicable technique was invented.

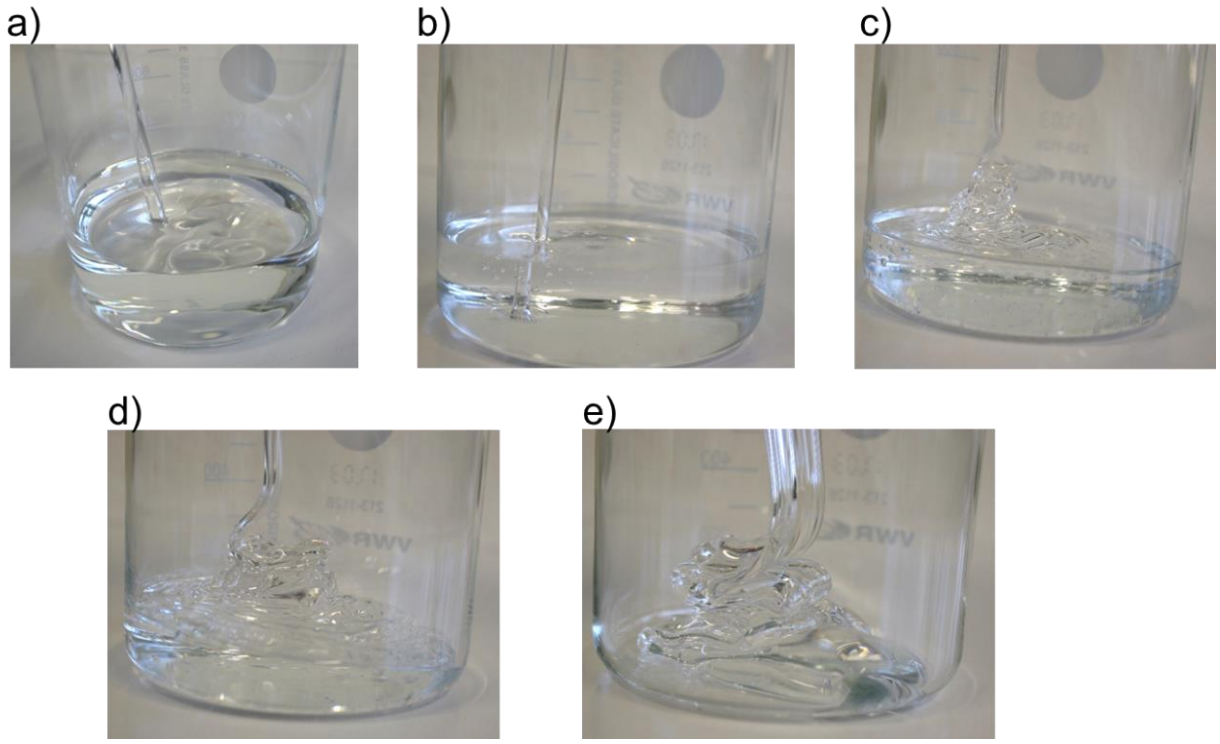

Figure 7.2: *Comparison of PDMS silicone oils with viscosities of* ***a)*** *10 mPa·s,* ***b)*** *1,000 m Pa·s,* ***c)*** *20,000 m Pa·s,* ***d)*** *60,000 m Pa·s, and* ***e)*** *1,000,000 m Pa·s (self-captured pictures).*

Apart from method development, a powerful and robust detector is an important prerequisite for the analytical process (cf. **Figure 1.1**). Therefore, the optimization of ELSD (chapter 5) was an essential step for these investigations. Replacing the originally installed concentric glass nebulizer by a parallel-path PTFE nebulizer substantially improved the detector performance and long-term stability. Particularly for PDMS analysis this was decisive in order to prevent clogging of the capillary within the concentric glass nebulizer. Based on this optimization of the detector further improvements concerning the measurement could be achieved. Concluding, the separation of low molar mass PDMS oligomers as well as the analysis of high molar mass PDMS by applying HRP-HPLC based on a saw tooth gradient was achieved in this study.

Nevertheless, several aspects should be further investigated: The results when applying different stationary phases supposed to couple various short columns with different phase selectivity for enhancing the separation of complex polymer formulations. For preparative HRP-HPLC, a mathematical model for calculating the optimum saw tooth profile depending on the geometry of the applied stationary phase considerably simplifies the result transfer from analytical to (semi-)preparative columns. Applying the saw tooth gradient to more than one SEC column with different pore size distribution should be an interesting extension of the technique regarding peak resolution. Moreover, the direct coupling of HRP-HPLC to

MALDI-ToF-MS or the off-line combination of both techniques could improve the detection of high molar mass polymers; the more monodisperse a higher molar mass polymeric sample is the higher is the probability that MALDI-ToF-MS can detect the sample as resolved oligomers. Finally, the saw tooth approach should be applied to other types of polymers which are not investigated in this study and should be transferred to other chromatographic techniques, i.e. GC, ion chromatography, or micro- or nano-HPLC.

8 Summary

Polymers are used versatile and ubiquitous as raw material for plastics, fibers, elastomers, rubbers, textiles, adhesives, and packaging. In this study, the determination of these materials with polymer liquid chromatographic techniques was applied, improved, and optimized. Beginning from the analysis of low and high molar mass poly(siloxanes) or silicones, the findings were also applied to various other polymers, e.g. poly(vinylchloride) or poly(methylmethacrylate). For low molar mass poly(dimethylsiloxanes) (PDMS), a baseline resolved separation of linear and cyclic oligomers up to 30 monomer units was developed and optimized by adapting mobile and stationary phases. For high molar mass PDMS (up to 250,000 $g \cdot mol^{-1}$), a new saw tooth gradient design was invented, enabling high-resolution measurements. In addition to and as enhancement to size exclusion chromatography, the new high-resolution polymer HPLC (HRP-HPLC) allows the assignment of retention times to the corresponding molar masses in HPLC for single resolved peaks of (complex) polymer samples. The shape of the saw tooth gradient and further significant parameters as effective step length and height are optimized by Design of Experiments (DoE). Various other polymers, e.g. poly(vinylchloride), poly(styrene), poly(methylmethacrylate), and poly(propylene glycol) were investigated with HRP-HPLC by only adapting the stationary and mobile phase. Additionally, the performance of an evaporative light scattering detector (ELSD) was improved by implementing a new parallel-path poly(tetrafluoroethylene) nebulizer instead of the originally used concentric glass nebulizer. Thus, a significant improvement of the long-term stability and sensitivity was achieved for polymer analysis. Particularly, the invention, development, and optimization of the sawtooth gradient design results to a substantial improvement in (micro) structure elucidation of especially high molar mass polymers. Moreover, (semi) preparative fraction collection of various polymers enable the off- and online coupling to other powerful analytical techniques like MALDI mass spectrometry or NMR spectroscopy.

9 Zusammenfassung in deutscher Sprache

Die vielfältige und ubiquitäre Anwendung von Polymeren als Rohstoffe für Kunststoffe, Fasern, Elastomere, Kautschuke, Textilien, Klebstoffen oder Verpackungen zeigt die Wichtigkeit dieser Stoffklasse. In der vorliegenden Arbeit wurden deshalb flüssigchromatographische Verfahren zur Verbesserung und Vereinfachung der Analyse dieser Werkstoffe weiterentwickelt und optimiert. Ausgehend von der Gruppe der Poly(siloxane) bzw. Silicone wurden Untersuchungen mit Polymer HPLC sowohl im niedermolekularen als auch im hochmolekularen Massenbereich durchgeführt. Für cyclische und lineare niedermolekulare Poly(dimethylsiloxane) (PDMS) -Oligomere ist eine Methode ausgearbeitet worden, die durch Anpassung von stationärer und mobiler Phase eine basisliniengetrennte Oligomeren- Auftrennung, bis zu 30 Wiederholeinheiten ermöglicht. Durch die Entwicklung eines neuartigen Sägezahngradienten-Profils konnte auch im hochmolekularen Massenbereich – für PDMS bis zu 250.000 $g \cdot mol^{-1}$ eine hohe Auflösung der Polymerverteilung erreicht werden. Ergänzend zur Größenausschlusschromatographie (SEC) können mit dieser neuen Gradiententechnik auch in der HPLC den einzelnen dezidierten Polymerpeaks Retentionszeiten und Molmassen zugeordnet werden und somit detailliertere Aussagen über die Zusammensetzung komplexer Polymerproben gewonnen werden. Mit statistischer Versuchsplanung wurde die Form des Sägezahngradienten-Profils sowie die effektive Stufenhöhe und effektive Stufenlänge optimiert. Die universelle und vielseitige Anwendbarkeit der hochaufgelösten Polymer HPLC (HRP-HPLC) ist dadurch gezeigt worden, dass durch Anpassung der stationären und der mobilen Phase auch weitere Polymere, wie z.B. Poly(vinylchlorid), Poly(styrol), Poly(methylmethacrylat) oder Poly(propylenglykol) untersucht werden konnten. Darüber hinaus ist der verdampfende Lichtstreudetektor (ELSD) optimiert worden, so dass dieser als universell einsetzbarer und robuster Detektor für die Polymer HPLC zur Verfügung steht. Dazu wurde der vom Hersteller verbaute konzentrische Glaszerstäuber durch einen Parallelfluss- Poly(tetrafluoroethylen)- Zerstäuber ersetzt und anschließend wurden die einzelnen Detektorparameter optimiert. Dadurch konnten Langzeitstabilität und Empfindlichkeit entscheidend verbessert werden. Besonders durch die Entwicklung und Optimierung des Sägezahngradienten können die analytische Trennung von hochmolekularen Polymeren verbessert und auch Messungen im (semi-) präparativen Maßstab durchgeführt werden, die eine Kopplung mit weiteren Methoden wie MALDI Massenspektrometrie oder NMR Spektroskopie ermöglichen. Dadurch können insbesondere die (Mikro) Strukturen komplexer Polymerproben detaillierter untersucht werden.

Appendix A: Separation of linear and cyclic PDMS

Universität Regensburg

[1]Wacker Chemie AG, Burghausen
[2]Institute of Analytical Chemistry, Chemo- and Biosensors
University of Regensburg

Separation of cyclic and linear polydimethylsiloxanes with interactive chromatography

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Motivation

The separation of higher molecular cyclic and linear polydimethylsiloxanes (PDMS) with polymer high performance liquid chromatography (HPLC) is of great importance for the product quality. In addition, the elucidation of the separation mechanism (between size exclusion and classic liquid chromatography) will provide an important contribution to understanding the analysis of higher molecular silicones. The separation of a wide range of different PDMS consisting of 7 up to 40 monomer units by means of HPLC will close a gap to analysis of PDMS with gas chromatography, which is limited to a maximum of 8 monomers at normal injection mode.

Mechanism of separation:

Understanding the effects that are responsible for the separation of PDMS is of greatest importance. The possible separation mechanisms in polymer HPLC are based on classic HPLC as well as basic size exclusion effects. The results show a typical HPLC separation after the sample injection. Figure 1 demonstrates the correlation of flow and gradient slope in accordance to the separation. Measurements with different mobile phases on alkyl chain RP columns (C4-C30) also support this conclusions. Another consequence is the possible optimization of PDMS separation on a pentafluorophenyl (PFP)-phase (Figure 2). In this case, the stereoselectivity of the PFP column towards the analytes plays an important role. The fluorophenyl ring has an electron withdrawing effect on the analytes. Furthermore, the rigid aromatic ring is responsible for stereoselective separations. Hydrophobic interaction forces and hydrogen bonds play only a minor role in the retention process.

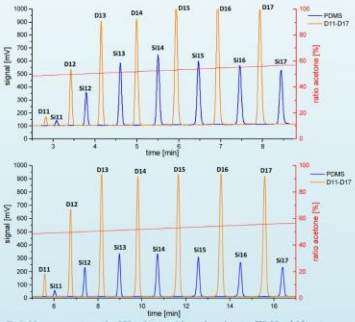


Fig 1: Measurements on the PFP-column, with methanol-water (75:25, v/v)/ acetone, above: 2 mL/min, 40 min; below: 1 mL/min, 80 min.

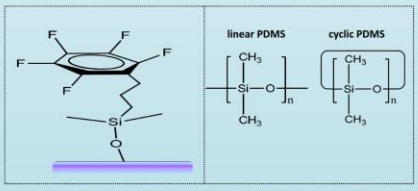


Fig 2: Structure of stationary phase and analytes (degree of polymerisation = n).

Experimental

An Agilent 1100 series HPLC-system equipped with an 385 Agilent evaporative light scattering detector (ELSD) was used for the measurements. In the experiments, a Kinetex PFP 100 Å 100 x 4.6 mm, 2.6 µm (Phenomenex) and different alkyl chain columns (C4, C8, C18, C30: Accucore 100 x 4.6 mm, 2.6 µm, ThermoFisher) were used. As standard reference for linear PDMS (abbreviated "Si") a silicone oil (viscosity 10 mPa·s, c = 5 mg/mL in THF) was used. For cyclic PDMS (abbreviated "D") a mixture of pure standard substances was used.

Summary

The results of our recent research suggest that the separation of cyclic and linear PDMS is based on classic HPLC. The used PFP column constitutes a very good fractionation of PDMS. The extension of gradient duration can result in a further enhancement of separation.

Further research interests deal with extension of the separation range to lower homologs (like D7/Si7). These results will be compared to the gas chromatographic measurements. The aim is to develop a LC method over a wide range of PDMS, so that the analysis of homologs from D7/Si7 up to D40/Si40 can become possible.

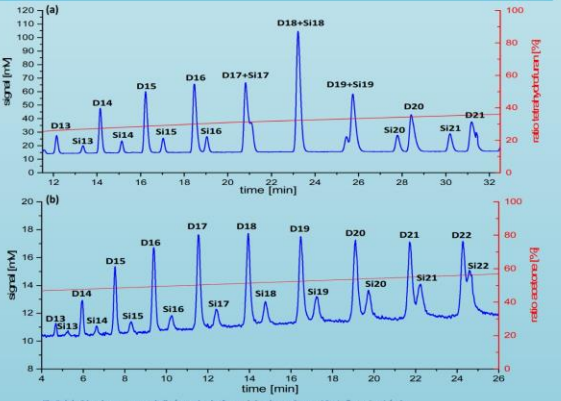
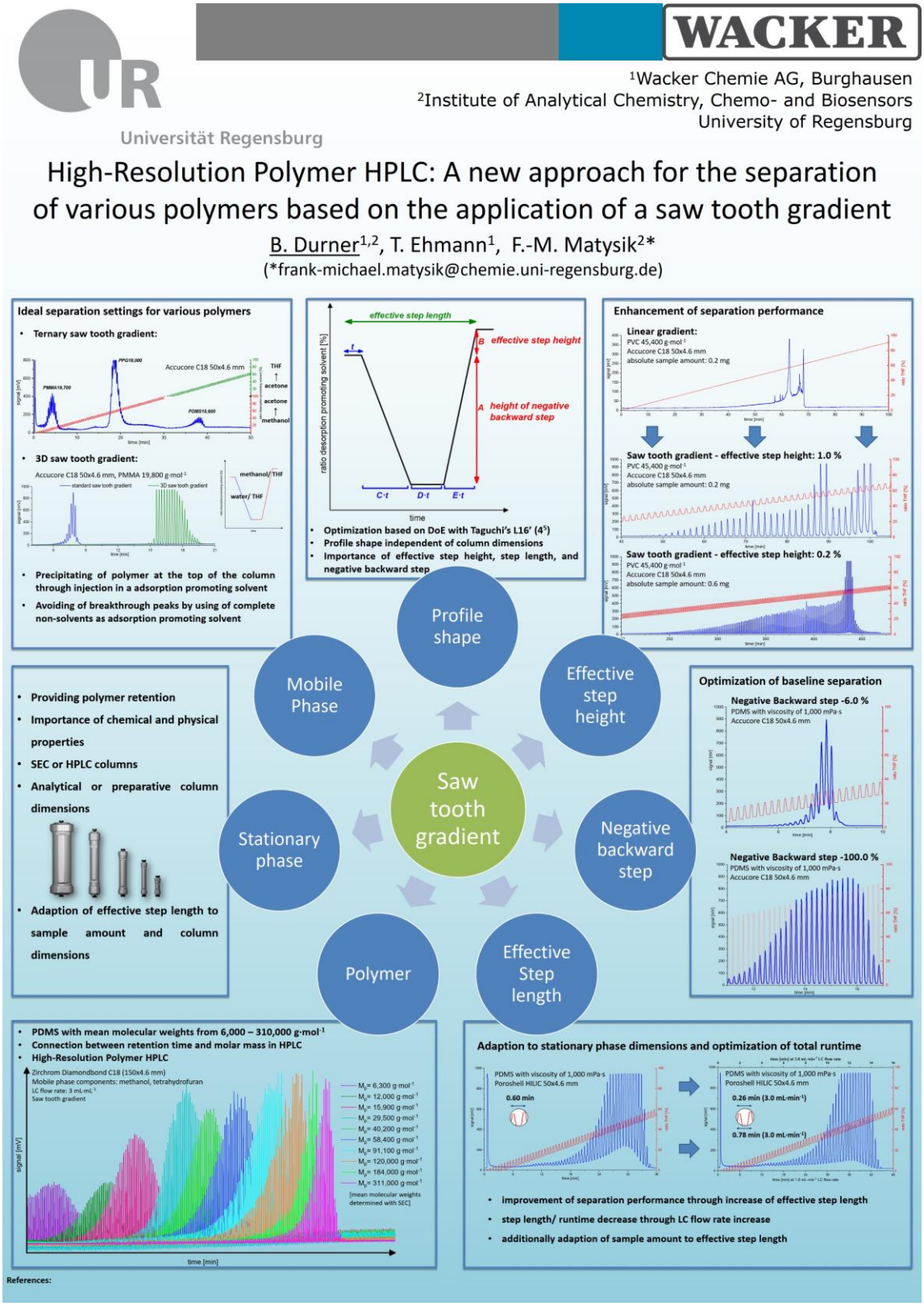


Fig 3 (a): C4-column, acetonitrile/ tetrahydrofuran, injection volume: 10 µL, flow: 1 mL/min,
(b): PFP-column, methanol-water (75:25, v/v)/ acetone, injection volume: 4 µL, flow: 2 mL/min.

Appendix B: Optimization of the saw tooth gradient



Appendix C: Applications of a saw tooth gradient

Universität Regensburg

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[2]Institute of Analytical Chemistry, Chemo- and Biosensors
University of Regensburg

High-Resolution Polymer HPLC: A new approach for the separation of WACKER polymers based on the application of a saw tooth gradient

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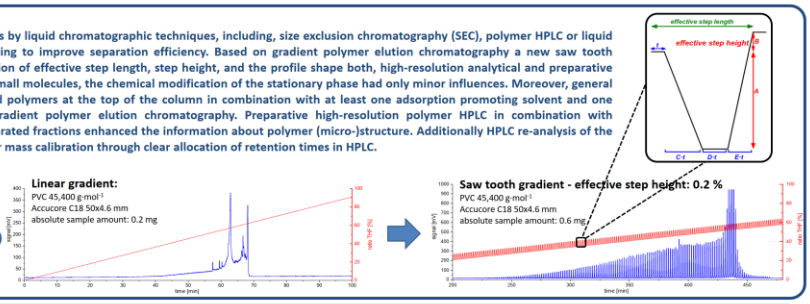
Abstract:

Currently, a lot of research effort in polymer analysis by liquid chromatographic techniques, including, size exclusion chromatography (SEC), polymer HPLC or liquid chromatography at critical conditions, is done aiming to improve separation efficiency. Based on gradient polymer elution chromatography a new saw tooth gradient profile was developed. With the optimization of effective step length, step height, and the profile shape both, high-resolution analytical and preparative HPLC separations were possible. Unlike in HPLC of small molecules, the chemical modification of the stationary phase had only minor influences. Moreover, general retention or rather precipitation of the investigated polymers at the top of the column in combination with at least one adsorption promoting solvent and one desorption promoting solvent were crucial for gradient polymer elution chromatography. Preparative high-resolution polymer HPLC in combination with subsequent SEC, NMR or MALDI analysis of the separated fractions enhanced the information about polymer (micro-)structure. Additionally HPLC re-analysis of the different molecular weight fractions allowed a molar mass calibration through clear allocation of retention times in HPLC.

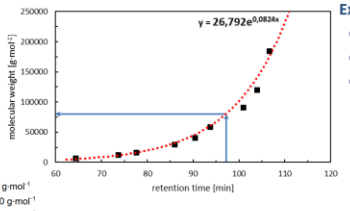
Highlights:

- High-Resolution separation performance
- Universal approach
- Application to various polymers (PDMS, PVOH,...)
- Analytical or preparative scale
- Quantitative and qualitative analysis



Application 1: Poly(dimethylsiloxanes)

- PDMS with mean molecular weights from 6,000 – 310,000 g·mol^{-1}
- Connection between retention time and molar mass in HPLC
- Qualitative and quantitative analysis of PDMS
- High-resolution method for polymer analysis and product re-formulation
- Application to various other polymers (PMMA, PS, PVC, PPG) possible



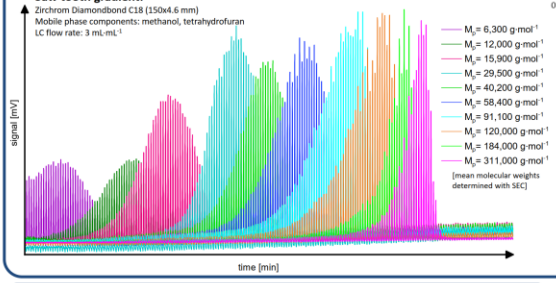
Example:

- Unknown linear PDMS sample
- Retention time: 97.0 min
- Mean molar mass: 79,300 g·mol^{-1}

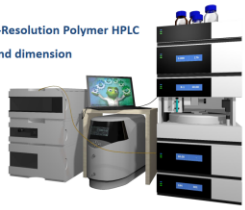
- high resolution
- fast
- unequivocal

Saw tooth gradient:

Zirchrom Diamondbond C18 (150x4.6 mm)
Mobile phase components: methanol, tetrahydrofuran
LC flow rate: 3 mL·mL^{-1}

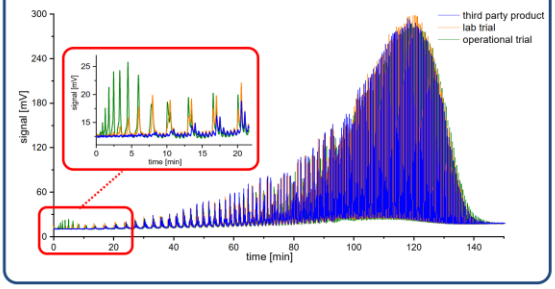


- Combination of preparative and analytical High-Resolution Polymer HPLC
- Coupling to NMR, MALDI, SEC, HPLC in the second dimension
- Alternate high-resolution method to SEC
- Online molar mass characterization beyond the typical mass spectrometry range



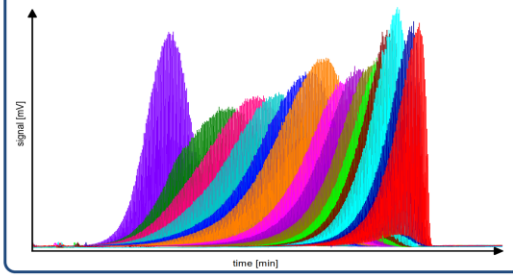
Application 2: Fingerprint analysis

- Considerable differences at low molecular weight range of sample distribution
- Extraction of region of interest
- Identification and structure elucidation with subsequent LC- and GC-MS analysis



Application 3: Structure elucidation based on chemical functionality

- Connection between retention time and chemical functionality
- Qualitative and quantitative determination
- Analysis of microstructure of polymer samples
- High reproducibility and reliability



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Eidesstattliche Erklärung

Ich habe die Arbeit selbstständig verfasst, keine anderen als die angegebenen Quellen und Hilfsmittel benutzt und bisher keiner anderen Prüfungsbehörde vorgelegt. Von den in §27 Abs. 5 vorgesehenen Rechtsfolgen habe ich Kenntnis genommen.

Regensburg, 29.04.2019 …….

(Bernhard Durner)