Virtual Chromatography for the Effective Method Development in Liquid Chromatography of Polymers

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In the name of Allah, the Gracious, the Merciful

To my parents, wife and sweet little daughter

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Contents

1 Introduction

Synthetic polymer systems play an increasingly important role in our everyday life. They can be found in products ranging from ordinary household commodities to speciality automobile components and drug delivery devices. One important feature of synthetic polymers is the presence of heterogeneities in molecular characteristics due to the randomness of polymerization process. Even in the simplest case, heterogeneity in molar mass is always present giving rise to molar mass distribution (MMD, one-dimensional distribution). In more complex cases, other types of heterogeneities e.g., chemical composition distribution (CCD), functionality type distribution (FTD) or architectural distribution etc. may also be existing (multidimensional distributions) along with the molar mass distribution. The properties and hence the performance of any polymer system is affected by the types and extents of these distributions and slight batch to batch variations in these distributions can cause significant differences in the final properties of a polymeric material. Therefore, in order to ensure the required end-use performance and to establish structure-property relationships, it is often essential to characterize and control the different distributions present in a given polymer.

In recent years, high performance liquid chromatography (HPLC) has emerged as the most effective analysis method for the characterization of distributions in polymer systems [1-3]. While the molar mass distribution of a polymer sample can be determined by size exclusion chromatography (SEC) [4-5], interaction liquid chromatography (iLC) is normally used to characterize distributions other than that in molar mass [2]. For example, polymer blends can be separated by interaction chromatography into their chemically different components [6-8] or statistical copolymers can be separated according to chemical composition [9-12]. This permits a much more effective quality control as compared to the frequently used SEC, which is not able to separate according to chemical composition. Similarly in plastic recyclates different polymer and additive amounts can be quantified [13], making it possible to load the recyclate with only the absolutely necessary quantities of the expensive additives or other fillers in order to obtain the desired end-use properties. Block length distributions of individual blocks in diblock copolymers, which influence the phase behaviour [14-16], can also be determined by chromatographic

methods. Methods of interaction chromatography can also be applied to determine molar mass distributions of homopolymers more accurately as compared to SEC [17, 18].

Separations using HPLC are usually fast [19-22], but only if a suitable separation method is available. However, method development in interaction chromatography is extremely time consuming and expensive as it almost always entails performing a large number of experiments. In order to develop a method for a particular separation problem, a number of parameters have to be adjusted. For a chosen stationary phase, the composition of the mobile phase, the temperature, the flow rate, the gradient slope, the gradient shape etc. must be optimized systematically to get the best resolution for the different components in the sample in the shortest possible analysis time.

In the case of polymer-based samples, the situation becomes even more complicated as the chromatographic retention of polymers is affected by molar mass and chemical composition. Even if a method is suited for a certain polymer class, optimization is frequently required to account for slight changes that may occur in the polymer samples.

Presently, there is no rational strategy for method development in polymer chromatography. Despite an increasing understanding of the mechanisms in polymer chromatography during the last years, method development is still carried out to a large extent purely by employing the empirical “trial and error” approach. Thus, the quality of the produced method is strongly dependant on the personal knowledge and intuition of the chromatographer. These drawbacks make interaction chromatography for polymers less accepted despite the detailed information it can provide.

A potential solution to this problem might be in the use of suitable computer programs just like in the chromatography of small molecules. Several software packages (e.g. DryLab [23], Chromsword [24], ACD LC Simulator [25] to name a few) are commercially available for method development and optimization in the chromatography of low molar mass to high molar mass monodisperse substances. On the basis of a minimum number of simple non-optimized experiments [26-30] (or starting from a predicted experiment on the basis of chemical structure [30, 31]), the

chromatographic behaviour under different experimental conditions can be predicted. This helps to reduce the number of real experiments in the method development procedure. Whether these programs can also be used to predict the chromatographic behaviour of polymers was not known so far. A successful modelling of the chromatographic behaviour of polymers could significantly lower the time and the costs for method development and/or optimization in polymer chromatography. The effects of changing experimental conditions could be studied in short time using computer simulations, allowing the fast and economical development of an optimized separation method. Furthermore, this approach may also permit easily investigating the robustness of a method, i.e. the ability to tolerate small changes in experimental conditions. Small deviations from the optimum conditions (e.g. fluctuations in flow rate, changes in temperature, slight changes in eluent composition etc.) can be selected in the simulations and their influence on the chromatographic separation can be examined without time consuming and expensive experiments.

The present research work examines the suitability of conventional models used in chromatography of small molecules for the predictions in polymer chromatography. However, due to the particular retention behaviour of polymers, a theoretical model available for polymer chromatography was also used for the predictions. This polymer specific model is also extended to account for solvent gradients.

1.1 HPLC of polymers

1.1.1 Methodology in HPLC of polymers – Basic concepts

Chromatographic separations are processes where different substances spend different times on their way through a chromatographic column. The different residence or retention times are caused by the difference in the distribution equilibrium of the solute between the stationary phase and the mobile phase [32]. The well-known distribution coefficient K_d is the ratio of the concentrations of the analyte in the stationary to that in the mobile phase (i.e. $K_d = c_s/c_m$). It is related, thermodynamically, to the free energy difference, ΔG, of the molecules in the two phases. This difference in free energy comprises of enthalpic and entropic contributions [2]. The dependence of K_d on these contributions is given by,

$$\ln K_d = -\frac{\Delta G}{RT} = \frac{-\Delta H + T\Delta S}{RT} \quad 1.1$$

where, R is the gas constant, T the absolute temperature, ΔH and ΔS are the changes in interaction enthalpy and conformational entropy, respectively.

Experimentally, K_d is determined from following equation,

$$K_d = \frac{V_R - V_i}{V_p} \quad 1.2$$

where, V_R is the retention volume of the analyte, V_p the pore volume of the stationary phase and V_i the interstitial volume of the column. Units of time scale can also be used instead of volume units because $t_R = V_R/F$ (F = flow rate).

1.1.1.1 Size exclusion chromatography

In size exclusion chromatography (SEC), ΔS is the dominant factor for K_d. The loss of conformational entropy is due to the steric hindrance of a large flexible chain-like molecule while wandering from the mobile phase into the pores of the stationary phase. In ideal SEC, where no enthalpic interaction (i.e. $\Delta H = 0$) exists between the stationary phase and the polymer molecule [5] separation takes place merely according to the hydrodynamic size of the molecule. Since $\Delta S < 0$, K_d in SEC ranges from $0 - 1$. Larger molecules elute earlier than smaller ones (figure 1.1). After suitable calibration, the molar mass distribution and the molar mass averages of a polymer sample can be determined.

1.1.1.2 Liquid adsorption chromatography

Liquid adsorption chromatography (LAC) is typically employed in the separations of small molecules. Ideally, in this case, $\Delta S = 0$ because of the absence of exclusion effects due to the small molecular size. The enthalpic contribution (ΔH) is due to the attractive interactions (van der Waals forces/London forces and electrostatic forces) of the molecules with the stationary phase. Since ΔH is negative, K_d is greater than 1. The separation takes place due to the differences in the interaction strengths. The strength of interaction between the analyte molecule and the stationary phase can be

controlled by the eluent composition and/or temperature. The retention in LAC of small molecules is typically quantified in terms of the retention factor defined by,

$$k = \frac{V_R - V_0}{V_0} \qquad 1.3$$

where, V_0 is the void volume of the column ($V_p + V_i$), usually determined as the retention volume of a non retained low molar mass substance.

Comparing equations 1.2 and 1.3, the relationship between K_d and k is obtained as follows,

$$K_d = k\frac{V_0}{V_p} + 1 \qquad 1.4$$

The retention behaviour of polymers in LAC is different from that of low molar mass molecules. The large size of polymer molecules may induce exclusion effects. Therefore, the entropic contributions in polymer LAC, even at strong interaction conditions, are always present [2]. However, the effect of entropic contributions is overwhelmed by the far more effective enthalpic contributions ($\Delta S << \Delta H$). Therefore, the overall K_d is always larger than 1.

For homopolymers, the number of interacting groups increases with the molar mass. Thus, the distribution coefficient increases accordingly, resulting in large elution volumes even if the interaction of a single repeating unit with the stationary phase is very weak. This can be explained by the multiple attachment mechanism as proposed by Glöckner [33]. In LAC of polymers, high molar mass polymers elute later than those of lower molar mass (figure 1.1). Hence, the molar mass dependence of retention time in LAC is opposite to SEC. At strong interacting conditions, high molar mass polymers may be irreversibly retained in the column. Consequently, in isocratic elution mode, where the eluent composition remains the same throughout the experiment, the elution of a high molar mass polydisperse polymer sample in reasonable time is only possible in a small range of interaction strength. For this reason, the LAC is often carried out in gradient elution mode, where the interaction strength can be systematically varied by changing the mobile phase composition. In this way, elution of high molar mass polymers can be achieved in a reasonable time.

1.1.1.3 Liquid chromatography at critical conditions

The transition between the above-mentioned two chromatographic modes of SEC and LAC is observed under special conditions, known as critical conditions, where molar mass dependence of retention time vanishes. Critical conditions are obtained by the use of a suitable eluent composition (critical composition). Chromatography at these conditions is known as liquid chromatography at critical conditions of adsorption (LCCC) [2]. The entropic losses due to the exclusion of the polymer molecules from the pores of the stationary phase are exactly compensated by the enthalpic gains due to interaction of molecules with the stationary phase ($T\Delta S = \Delta H$). Therefore, according to equation 1.1, the distribution coefficient of a polymer chain becomes equal to unity irrespective of the molar mass ($K_d = 1$). Under these conditions, non-functional homopolymer chains elute at the same elution volume irrespective of their molar masses (figure 1.1). Since, the elution volume of a complex polymer is not affected by the molar mass of the homopolymer chain, separations according to end groups [34, 35], block length [36-38], architecture [39, 40] etc. can be realized under critical conditions.

Critical conditions have been established for a number of polymers [41]. Despite that, the application of the LCCC is limited, however, by the difficulties in the determination of the critical eluent composition. Thus, a simple, fast and effective method to determine critical conditions for a given polymer would be a substantial advantage to improve the use of LCCC.

1.1.1.4 Gradient liquid chromatography

Since LAC may lead to irreversible adsorption of high molar mass polymer molecules, gradient chromatography is preferably used for separating polymers of very different adsorption strengths. In gradient liquid chromatography (gradient LC) the eluent strength is increased during the chromatographic run, such that K_d decreases and the polymer sample elutes in a reasonable time. In addition, the peaks get sharper and more symmetrical as compared to isocratic elution.

The mechanism of gradient elution in polymer chromatography remains still more difficult to understand as compared to that of isocratic chromatography. From a thermodynamic point of view, both enthalpic and entropic effects are operative in

polymer gradient elution [2]. However, like LAC, the enthalpic effects are more dominant ($T\Delta S << \Delta H$). At the start of gradient, the polymer molecules are adsorbed strongly in the weak initial eluent composition, i.e. $K_d >> 1$. Polymer molecules of high molar mass are more strongly adsorbed than those of lower molar mass. By increasing the eluent strength desorption occurs (K_d decreases) with weakly adsorbed molecules desorbing first. Therefore, lower molar mass molecules elute earlier than those of higher molar masses. At sufficiently high molar masses, a nearly molar mass independent elution is observed (figure 1.1).

The above explanation may often appear to be too simplified in connection with the limited solubility of polymers. Often the initial eluent in polymer gradient elution is a non-solvent for the polymer. In this case, precipitation may occur at the time of injection. The kinetics of the dissolution of the polymer in the eluent may then further complicate the mechanism of gradient elution.

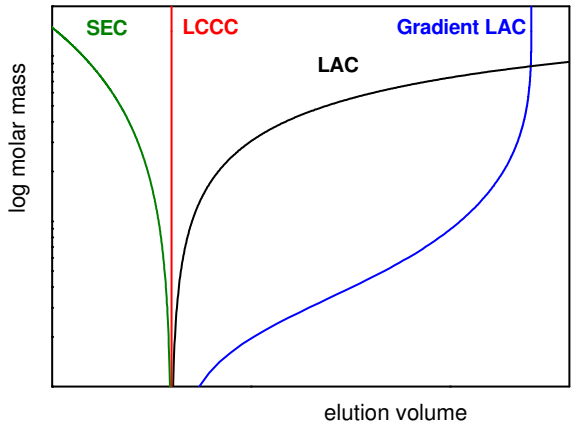


Figure 1.1: Schematic representation of the molar mass dependences of elution volume in polymer liquid chromatography. SEC, LCCC, and LAC modes operate under isocratic conditions of eluent and temperature etc. while in gradient LAC, the eluent strength is changed (weak to strong) with time.

1.2 Method development in polymer HPLC

As mentioned earlier, method development in polymer chromatography is still based on the primitive “trial and error” approach and there exist no well-defined guidelines to proceed successfully through the method development process. The commonsensical route, which is generally adopted or should be adopted, can be illustrated by the flow sheet diagram in Figure 1.2.

First of all, as much information as possible is gathered about the nature of the sample. Based on that information and on the required separation, a proper stationary phase (normal or reversed phase) and eluent is selected and a suitable operating temperature is chosen. This selection is made with the help of the available literature or a guess is made based on the experience of chromatographer, i.e. there are no well-defined rules to be followed for the decision about which stationary phase, eluent and operating temperature should be chosen. For this purpose, there is an obvious need of a database to be established also in the field of polymer chromatography.

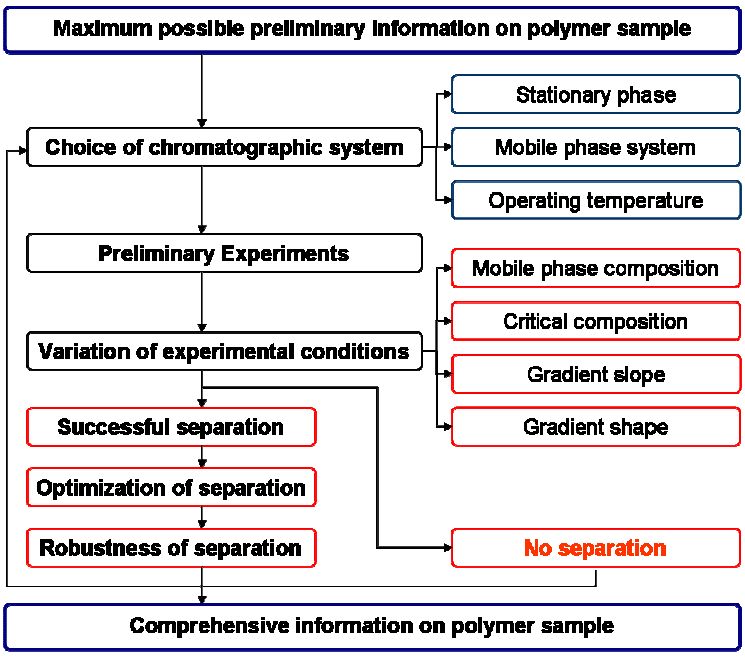


Figure 1.2: A general scheme for the method development in polymer chromatography

The eluent is often composed of two solvents. One component supports adsorption while the other promotes desorption. For a new polymer system, it has to be first determined by experiments, which solvents promote adsorption and desorption. The eluent may also be selected such that the adsorption-desorption equilibrium is obtained by variation of temperature [42, 43]. After the initial decision about the stationary phase and eluent has been made, the retention behaviour of the polymer sample is studied for some initial experiments. Unfortunately, at this stage also, there is no general procedure about which initial experiments to perform. Usually, one starts with arbitrarily chosen initial experiments while the subsequent experiments may not be necessarily rational. This is the area, where there is a huge need of a

general strategy to be followed, i.e. which type of experiments should be initially chosen to extract the most significant information to design the next experiment.

When the separation cannot be obtained even after rigorous experimentation using the chosen eluent system then all the "trial and error" experiments must be repeated for the newly chosen eluent or even for a different stationary phase. If a separation is achieved, optimization of the separation is desired and robustness of the separation method needs to be evaluated.

1.3 Virtual chromatography

The computer-aided predictions of retention behaviour have effectively reduced the number of experiments in the chromatographic method developments of small molecules [26-31]. This approach has been named as virtual chromatography (VC) [30] owing to performing the "trial and error" experiments on computers instead of using real chromatographic systems. This is why it is also an effective and economical tool for teaching chromatography. However, until today, such methods are not applied in polymer chromatography. The fundamental concept of the virtual chromatography approach is schematically represented in Figure 1.3.

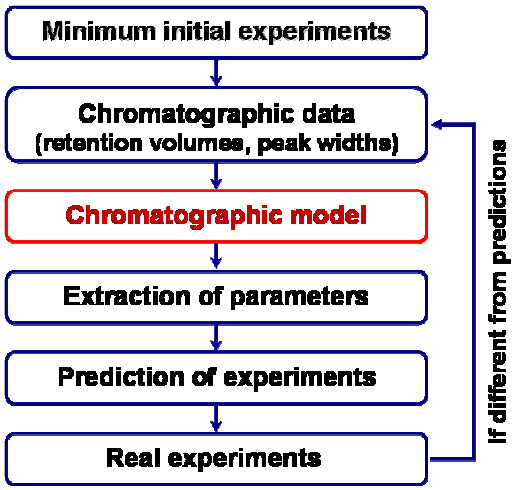


Figure 1.3: Schematic representation of computer assisted method development (virtual chromatography)

The core of the computer-assisted method development is a suitable chromatographic model. A model is described by a set of mathematical equations, which depict the dependence of the analyte's retention volume on the experimental variables i.e. eluent composition, temperature, gradient slope etc. The equations of a model have a

certain number of substance-specific parameters, called model parameters. These parameters are characteristic for the type of analyte under consideration. If the model parameters are known, they can be used to simulate the retention behaviour of the analyte in a variety of experimental conditions. To obtain the model parameters, one performs a minimum number of simple experiments [44, 45], e.g. linear gradients of different slope and/or isocratic experiments at different eluent compositions, from which the retention times of the individual substances are obtained. The number of the initial experiments is determined by the number of the model parameters. The retention times and the values of the corresponding experimental variables are subjected into the suitable chromatographic model. The model parameters of the analyte, which best describe the experimentally determined retention times are extracted by a fitting procedure.

The substance-specific parameters so obtained, can now be used to predict the retention behaviour under a new set of experimental conditions. It means the optimized experimental conditions for a particular separation can be found on the computer by manual or automatic variation of virtual experimental conditions. The computational variation of the experimental conditions is significantly faster than the actual chromatographic experiments. While a chromatographic experiment with sample preparation, column equilibration and actual execution may take several hours and may pose additionally the costs of solvents and equipment use, a virtual experiment needs only a few seconds. When an optimized virtual separation is achieved on the computer, a real experiment under the proposed conditions is carried out. If the experimental results are similar to the predictions then the goal is achieved. If, however, considerable deviations from the predictions are found then the new experiment can be included into the calibration process to improve the determination of the model parameters, which in turn may result in an improved quality of the predictions. This procedure is repeated until a satisfactory agreement of the experimental data with the predictions or the required separation is obtained.

By using the above-mentioned procedure, every new experiment is used to improve the quality of subsequent predictions, using the same model. In other words, the larger the number of experiments, the more reliable would be estimation of the model parameters. If, however, the model is not suitable at all, then accurate

predictions are not possible even though a large number of experiments is used to obtain the model parameters. Thus, the key of the successful application of virtual chromatography is the use of suitable chromatographic models.

1.4 State of the art of virtual chromatography

As far as the chromatography of low molar mass compounds is concerned, virtual chromatographic method has become an essential tool to predict and optimize separations, for example, of pharmaceuticals [46-48], peptides, proteins and metabolites [49, 50], plant extracts [51-54], environmental pollutants [55, 56] etc. There is a growing interest in applying similar methodology to polymer chromatography as well [57-60].

According to Snyder et al. and others [61-65], retention of polymer molecules can be adequately described by conventional chromatographic theory of small molecules. Jandera et al. [66, 67] applied conventional theories of chromatography for reversed phase and normal phase to describe, predict and optimize the isocratic and gradient separation of low molar mass polymers and copolymers in detail. Schoenmakers and Fitzpatrick [68, 69] investigated the suitability of the linear solvent strength theory to describe and predict the retention behavior of polystyrenes and poly(methyl methacrylate)s in different conditions of gradient elution. By correlating the parameters of the theory with the molar mass of the polymers, it was also possible to predict the retention behaviour with respect to the molar mass of the polymers. They were able to predict the critical eluent composition for polystyrene. Their theory has been used to tailor different calibration curves to determine the molar mass distributions of samples by gradient chromatography [70]. However, in order to use their approach, at least nine gradient experiments had to be performed on samples of different molar masses.

Apart from the application of conventional theories for low molar mass analytes, there is a considerable progress in understanding the behaviour of polymers in liquid chromatography [58-60, 71-81]. Gorbunov et al. have written a software package [82] to simulate the separation of mixtures containing up to four polymer components. The retention behaviour of different polymer structures like linear, cyclic, diblocks etc. can be simulated but only for isocratic conditions. An important parameter

characterizing the interaction of the repeating unit of the polymer with the stationary phase is required. However, no procedure for determining this parameter is described. Since only the qualitative retention is predicted, the program cannot be used for method development of real polymer separations. Nevertheless, the software may prove to be a good tool for academic purposes. Trathnigg et al. [58, 78-81] has rigorously validated the polymer specific theory of chromatography for the description and prediction of the retention behaviour of homopolymers and block-copolymers. The interaction parameters for polymers like polyethylene glycol have been determined as a function of the eluent composition [58, 81]. The theory has been used to predict the critical composition and to simulate separations of lower oligomers of polyethylene glycol. However, the procedure of Trathnigg et al. requires standards to be resolved into oligomers, thereby losing its applicability to high molar mass polymers. In addition, their approach requires a large number of experiments. Furthermore, only isocratic elution can be predicted. Brun at al. [83-85] extended this theory to gradients but no attempt has been made to predict quantitatively the retention of polymers in gradient elution. The theory results in a better understanding of the gradient elution of high molar mass polymers. There is, however, a slight error in the final equations of the theory that will be addressed by this thesis.

In short, until now, there have been no real attempts for the quantitative prediction of the retention behaviour of low to high molar mass polymers. No attempts are known to predict separations of polymer mixtures based on the virtual chromatographic approach. In this thesis, an attempt is made to further understand, describe, and predict the retention behaviour of polymers.

1.5 Objective of this thesis

For the reasons stated above, the present study evaluates the possibility of using the virtual chromatography approach in polymer separations. Therefore, this thesis encompass the following objectives,

1. Examining the selected theoretical models of chromatography for their ability to describe the retention behaviour of polymers. This may allow for a deeper understanding of polymer chromatography.

2. If required, extending the models to account for the special features of polymer chromatography

3. Evaluating the models for their appropriateness to predict quantitatively the retention of homopolymers and copolymers both in normal and reversed phase systems in isocratic as well as in gradient conditions. This may require designing a rational strategy to purposefully select the useful experiments so as to obtain maximum information with minimum effort.

4. Investigating the suitability of the models to predict separations, i.e. predicting also the peak widths along with the retention times. Examining the possibility to predict the peak widths of polydisperse homopolymers without any knowledge about their molar mass and polydispersity.

5. Applying the virtual chromatography tool to obtain real polymer separations represented by model mixtures.

2 Theoretical considerations

2.1 Retention models in LC

As described earlier, retention models in liquid chromatography are used to relate the retention of the analyte to the experimental variables. In the present research, we have studied different models, which relate the retention of the solute to the most easily changeable, yet the most effective, of the experimental variables, i.e. the mobile phase composition in isocratic and gradient slope in gradient elution. Since the models are the integral part of this thesis, their description is given below.

2.1.1 Linear Solvent Strength Model

According to the widely employed linear solvent strength model (LSSM) [86-89], the retention factor of an analyte in LAC is described as a linear function of the mobile phase composition, i.e.,

$$\ln k = \ln k_0 - S\Phi \qquad 2.1$$

where, $\ln k$ is the logarithmic retention factor (defined in equation 1.3) of the analyte at the isocratic binary mobile phase composition (Φ). S is the slope of the plot of $\ln k$ vs Φ, where the negative sign indicates that retention decreases with the amount of the strong eluent in the mobile phase. A strong eluent is the solvent, which promotes desorption, while, weak eluent is one which supports adsorption. $\ln k_0$ is the logarithm of the retention factor in the pure weak component of the mobile phase. The LSSM is frequently used in reversed phase chromatography of small molecules where the weak mobile phase component is usually water and the stronger component is an organic solvent. However, in case of polymers, both the components are frequently of organic nature and the stationary phase is not always a reversed phase. Nevertheless, the model can still be applied considering the mobile phase composed of a weak and a strong eluent.

At least two isocratic experiments in different mobile phase compositions are required to determine the parameters $\ln k_0$ and S. These parameters are specific for each analyte, i.e. each molar mass of polymer molecules will have its own set of

parameters. After determining the analyte-specific parameters, the retention in any other mobile phase composition can be predicted.

If analytes of very different adsorption strengths have to be analyzed, gradient elution becomes inevitable. In gradient elution, the mobile phase composition is varied during the chromatographic run in a defined manner, linearly in the simplest case. The relationship between the retention time of the analyte, eluting within the linear gradient, and the gradient variables can be described by equation 2.2 [86-89].

$$t_R = \frac{1}{SG}\ln\left(SGk_{initial}t_0 + 1\right) + t_0 \qquad 2.2$$

where, t_0 is the column dead time, i.e. the time required by mobile phase to travel from injector to the detector, G the gradient slope i.e. the change of mobile phase composition ($\Delta\Phi = \Phi_{final} - \Phi_{initial}$) per unit time ($G = \Delta\Phi/t_G$), $k_{initial}$ the retention factor in the initial mobile phase composition of the gradient. Thus, using equation 2.2 and at least two gradient runs of different gradient slopes, the parameters of the LSSM, i.e. S and $k_{initial}$ can be extracted with the help of non-linear fitting routines. The parameter $\ln k_0$ can be calculated using equation 2.1 after determining the S and $k_{initial}$. The so obtained analyte-specific parameters can then be used to predict the retention behaviour of the analyte in gradients of any shape or in any isocratic mobile phase composition. It should be noted that equation 2.2 assumes that there is no system dwell volume, i.e. volume between mixer and injector. The treatment for this case is given later.

2.1.2 Quadratic Solvent Strength Model

In LAC of low molar mass compounds, sometimes a linear relationship between the $\ln k$ and Φ does not exist. Another model frequently used in LAC of small molecules, accounts for the deviations from linearity. This is done by including a third term in equation 2.1, thus making it a quadratic equation [89]. Due to the quadratic dependence of $\ln k$ on Φ, the name quadratic solvent strength model (QSSM) is used for this model. Thus, for isocratic elution,

$$\ln k = C + B\Phi + A\Phi^2 \qquad 2.3$$

where, A, B, and C are the model parameters specific for each analyte. The C and B parameters have, theoretically, the same meaning as that of ln k_0 and S respectively, in the LSSM. The last term describes the non-linearity in the ln k versus Φ plot. Neglecting the dwell time, the retention time for molecules eluting within a linear gradient can be calculated for QSSM as follows [89],

$$t_R = \frac{1}{G\sqrt{A}} erf^{-1}\left[t_0\left(2G\sqrt{\frac{A}{\pi}}\right)\exp\left(\frac{AC - B^2/4}{A}\right) + erf\left(\Phi_{initial}\sqrt{A} + \frac{B}{2\sqrt{A}}\right)\right] - \frac{A\Phi_{initial} + B/2}{AG} + t_0 \quad 2.4$$

where, *erf*, and erf^{-1} are the error function and the inverse error function, respectively. G is the gradient slope and $\Phi_{initial}$ the mobile phase composition at the gradient start.

Like the LSSM, the parameters of the QSSM (A, B, C) for each analyte can be extracted from isocratic or gradient runs. However, when applying the QSSM, a minimum number of three initial experiments is required, and the non-linear fitting procedure involves a much more complex equation than that of the LSSM when using gradient runs to extract the model parameters.

Hence, using the above mentioned two models the elution behaviour can be predicted, principally, for molecules of any molar mass. However, it should be noted that these models are valid only in adsorption chromatography. These models lose their applicability in the cases when no adsorption with the stationary phase occurs. This is due to the definition of these models, since for $k \leq 0$ ($K_d \leq 1$, according to equation 4) ln k is mathematically not defined.

However, the above-mentioned $k \leq 0$ conditions are frequently encountered in polymer chromatography. In LCCC elution normally occurs at void volume ($k = 0$; $K_d = 1$). The partial exclusion of polymer molecules from the pores of the stationary phase (SEC) results in elution before void volume ($k < 0$; $K_d < 1$). Therefore, a new model has been developed [71-77], which describes all modes of polymer liquid chromatography at isocratic elution.

2.1.3 Polymer Chromatographic Model

This model developed by Russian scientists [71-77] is based on the molecular statistical theory of an ideal polymer chain. Owing to the specificity of the model to polymers, it is named here simply as polymer chromatographic model (PCM).

Equation 1.2 is used as the general equation for expressing the retention in polymer chromatography. The retention time of a polymer molecule is given by,

$$t_R = t_i + K_d t_p . \qquad 2.5$$

where, t_R is the analyte's retention time, t_i the retention time of a completely excluded sample, t_p the retention time of a totally permeated sample (t_0) minus t_i. According to the molecular statistical theory of polymers, the distribution coefficient of polymer molecules in the wide (slit like) pores can be described by the following equation [77].

$$K_d = 1 - \frac{4R}{D\sqrt{\pi}} + \frac{2R}{D}\frac{Y(-cR) - 1}{cR} \qquad 2.6$$

where, $Y(-x) = \exp(x^2)\cdot[1 - erf(-x)]$, *erf* being the error function. R is the radius of gyration of the polymer molecule, D the diameter of the pore, and c is an interaction parameter. The interaction parameter characterizing the strength of the interaction of the repeating unit with the stationary phase depends on the nature of the repeating unit, stationary phase, eluent composition and temperature. It is, however, independent of the degree of polymerization (DP).

The first two terms in equation 2.6 correspond to size exclusion contribution to K_d (K_{SEC}), while the last term represents the contribution of adsorption to K_d (K_{LAC}). The control parameter is cR, such that the last term vanishes for large negative cR and only the parameter R/D then determines retention (SEC condition i.e. $K_d < 1$). It compensates with the second term at the critical point (i.e. $K_d = 1$), where c is equal to zero. Positive values of cR lead to $K_d > 1$. Thus, according to the theory, K_d at a given isocratic mobile phase composition depends on two parameters, R/D and cR.

For practical purposes, the above-mentioned parameters have to be related to the mobile phase composition. The magnitude of c, at a specific temperature, depends strongly on the mobile phase composition [58, 81]. The stronger the mobile phase, the lower will be the value of c. However, there is no theoretical relationship describing how c will change with mobile phase composition. Such a relationship could be established empirically by determining the isocratic retention times in different mobile phases for several standards, differing in molar mass (e.g. a homologous series of oligomers). The interaction parameter, c, [58, 81] can then be determined by a non-linear fitting process.

On the other hand, the change of the interaction parameter, c, with mobile phase composition, Φ, can generally be represented by a power series, which might be truncated after the first term. That is c changes linearly with Φ in the vicinity of critical composition. Thus,

$$c = \frac{dc}{d\Phi}\left(\Phi_c - \Phi\right) + \cdots \qquad 2.7$$

where, $dc/d\Phi$ represents the change in interaction parameter per change of mobile phase composition. Φ_c is the critical mobile phase composition. Beside c, R may also vary with the thermodynamic quality of the mobile phase composition. However, the change in R per change in mobile phase composition is expected to much smaller than that in c, especially when components of mobile phase are solvents for the polymer. Moreover, in adsorption chromatography in isocratic and/or in gradient elution mode, polymers are expected to elute within a very narrow range of mobile phase compositions. For the sake of simplicity, R and thus R/D are assumed to be independent of mobile phase composition. Thus, combining equations 2.5, 2.6 and 2.7, the retention time of a polymer molecule at a given isocratic mobile phase can be described (equation 2.8) by the parameters Φ_c, $Rdc/d\Phi$ and R/D.

$$t_R = t_i + \left[1 - \frac{4R}{D\sqrt{\pi}} + \frac{2R}{D}\frac{Y(-dc/d\Phi\left(\Phi_c - \Phi\right)R) - 1}{dc/d\Phi\left(\Phi_c - \Phi\right)R}\right] \cdot t_p \qquad 2.8$$

Similar to the parameters of LSSM and QSSM, the parameters of PCM can be extracted using non-linear fitting procedure, from the data obtained by at least three

isocratic experiments performed at different mobile phase compositions. In contrast to the parameters of the other two models, the parameters of the PCM also have a physical significance.

Brun et al. [85] have given a quantitative description of gradient elution of polymers by combining the polymer specific theory of chromatography [71] with the classical theory of gradient elution [87]. According to this theory, the elution of high molar mass polymers occurs near the critical mobile phase composition. However, due to a small mistake, the solution given by them is not completely correct, i.e. it describes the elution of the high molar mass polymers to be occurring in an eluent composition slightly higher than the critical composition. Therefore, there is a need for a corrected solution of the model. Moreover, Brun et al. [85] have used a relation for the distribution coefficient, which is only valid in the vicinity of the critical mobile phase composition, instead of the more general equation 2.6. This restriction is reasonable for high molar mass polymers, where LAC is applicable only in the close vicinity of the critical mobile phase composition. The same restriction is valid in gradient elution where elution of high molar mass polymer occurs very close to critical composition [85, 90]. However, the approach developed in this thesis seems more general as it should be valid for a wider range of mobile phase compositions. This is significant for the purpose of this thesis because elution of low molar mass polymer molecules may occur at much weaker mobile phase compositions than the critical one in isocratic and gradient chromatography.

The following equations extend the PCM for elution in linear gradients. The mobile phase composition at the mixer at any time t in a linear gradient is described by the following relationship,

$$\Phi(0,t) = \Phi_{\text{initial}} + G \cdot t \qquad 2.9$$

where, $\Phi(0,t)$ and Φ_{initial} are the instantaneous and the initial mobile phase composition in volume fractions, respectively, and G is the slope of the gradient. Combining 2.7, 2.8 and 2.9 the following solution is obtained after integration:

$$\int_{Rc_{\text{final}}}^{Rc_{\text{initial}}} \frac{dx}{\frac{Y(-x)-1}{x}-\frac{2}{\sqrt{\pi}}} = \frac{2RGt_P}{D}\cdot\frac{dRc}{d\Phi} = I\left(Rc_{\text{initial}}\right)-I\left(Rc_{\text{final}}\right) \qquad 2.10$$

where,

$$\begin{aligned} Rc_{\text{final}} &= R\frac{dc}{d\Phi}\left[\Phi_c-\Phi_{\text{initial}}-G\left(t_R-t_i-t_P\right)\right] \\ Rc_{\text{initial}} &= R\frac{dc}{d\Phi}\left(\Phi_c-\Phi_{\text{initial}}\right) \end{aligned} \qquad 2.11$$

Thus, knowing the three adjustable polymer specific parameters, Φ_c, $Rdc/d\Phi$ and R/D of the PCM, the elution of a polymer molecule in gradient chromatography can be described also in relation to the experimental variables. The three model parameters can be estimated by non-linear fitting procedures similar to the other two models. However, at least three initial gradient experiments differing in gradient slope are required. There is also the possibility of using isocratic experiments (LAC or SEC) along with the gradients to extract the model parameters.

The three models described above were tested for their suitability to describe and predict the chromatographic retention behaviour of polymers in isocratic as well as in gradient elution.

2.2 Multi-step or multi-linear gradients and dwell volume

Often the optimum separation of individual components of a polymer sample can only be achieved by multi-step, multi-linear or curved gradients, which can be approximated by a series of isocratic and linear gradient steps. In such a case, the different components of the sample travel different distances within the column during the different gradient steps. The distances travelled by the compounds depend on the range of eluent strength covered in these steps. Therefore, some components may elute in earlier gradient steps while others elute later. The components eluting in later steps may be either slowly travelling along the column in the preceding eluent compositions or not moving at all. In order to predict the final retention time in such a gradient experiment, the distances within the column travelled by the polymer molecules in individual steps must be calculated first. The following treatment can be

used additionally to correct for the polymer migration within the dwell volume, which is the volume between the gradient mixer and the injector, of the chromatographic system. Due to the dwell volume, any programmed eluent composition reaches the column head with a certain delay. If the initial eluent composition is sufficiently strong, some of the sample components might travel some distance in the column, or may even elute from the column before the actual start of the gradient. This may also be considered as an isocratic step (although unintentional) before the start of actual gradient program. The distances travelled by the polymer molecule within any type of gradient step can be calculated based on the general approach given below. For the sake of clarity, any distance travelled within the column is treated in volume units in the following description.

The situation at the beginning of a certain gradient step can be depicted by the following illustration (figure 2.1), while the situation encountered at the end of the gradient step is depicted in Fig. 2.2.

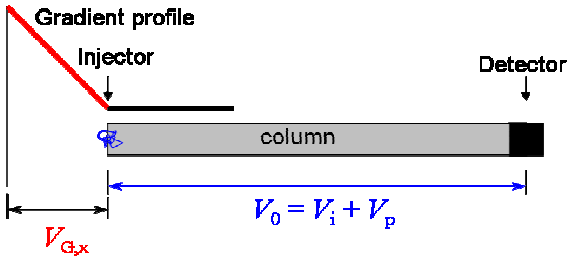


Figure 2.1: The position of gradient and the polymer molecules at the beginning of the gradient step (red).

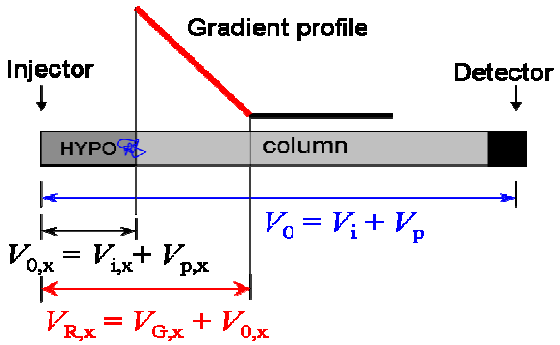


Figure 2.2: The end of the gradient step meets the moving polymer molecules at the end of hypothetical column (hypo)

Within the gradient step, the polymer molecule has migrated a volume $V_{0,x} = V_{i,x} + V_{P,x}$. In other words, $V_{0,x}$ is the distance migrated by the polymer

molecule from the time when the starting composition of the gradient step catches up with the polymer molecule until the composition at the end of the gradient step takes over the moving polymer molecule. A hypothetical column can be defined as the length, which is selected such that the polymer molecule elutes from the hypothetical column, when the end of the gradient reaches the column outlet (column named hypo in figure 2.2). Thus, as can be seen from figure 2.2, the retention volume of the molecule is given generally by $V_{R,x} = V_{G,x} + V_{0,x}$. The length of the hypothetical column, $V_{0,x}$, can now simply be calculated from the equations for gradient and isocratic elution given above, by setting V_R equal to $V_{R,x} = V_{G,x} + V_{0,x}$ and the column length V_0 equal to $V_{0,x}$ in case of LSSM and QSSM. For the PCM, V_i and V_P have to be replaced by $V_{i,x}$ and $V_{P,x}$, respectively. Solving for $V_{0,x}$ or $V_{P,x}$ allows calculating the respective lengths of the hypothetical columns for each gradient step.

As an example, the following relation is obtained for a gradient step employing the LSSM equation 2.2,

$$V_{G,x} + V_{0,x} = \frac{1}{SG}\ln\left(SG\,k_{initial}V_{0,x} + 1\right) + V_{0,x} \qquad 2.12$$

Solving for $V_{0,x}$ results in,

$$V_{0,x} = \frac{\exp\left(SGV_{G,x}\right) - 1}{SG\,k_{initial}} \qquad 2.13$$

In complete analogy, the equations for linear gradient steps employing the QSSM (equation 2.14 from equation 2.4) and PCM (equation 2.15 from equation 2.10) can be obtained,

$$V_{0,x} = \frac{erf\left[\left(V_{G,x} + \frac{A\Phi_{initial} + B/2}{AG}\right)G\sqrt{A}\right] - erf\left(\Phi_{initial}\sqrt{A} + \frac{B}{2\sqrt{A}}\right)}{\left(2G\sqrt{\frac{A}{\pi}}\right)\exp\left(\frac{AC - B^2/4}{A}\right)} \qquad 2.14$$

$$V_{P,x} = \frac{I\left(Rc_{initial}\right) - I\left(Rc_{final}\right)}{2R\left(\frac{dc}{d\Phi}\right)\left(\frac{R}{D}\right)G} \qquad 2.15$$

For isocratic steps in case of LSSM and QSSM the following result is obtained from equation 1.3,

$$V_{0,x} = \frac{V_{G,x}}{k} \qquad 2.16$$

For the PCM, $V_{p,x}$ for an isocratic step can be calculated from K_d using following equation,

$$V_{P,x} = \frac{V_{G,x}}{K_d - 1} \qquad 2.17$$

For the PCM, the value of $V_{i,x}$ for isocratic and gradient steps can be calculated from $V_{p,x}$ using equation below,

$$V_{i,x} = \frac{V_i V_{P,x}}{V_P} \qquad 2.18$$

It should be noticed, that the effect of migration of the polymer molecule within the dwell volume can be accounted for by simply setting $V_{G,x} = V_{d.}$

Using these equations, the values of $V_{0,x}$ (or $V_{p,x}$ and $V_{i,x}$) travelled by the polymer in each individual isocratic or gradient step can be calculated and added together to yield the overall column volume travelled by the molecules during all non-eluting steps (for the steps that do not give elution from the original column). At this point the fraction of the original void volume which remains available for the last eluting step has been reduced to $V_{0,e} = V_0 - \Sigma V_{0,x}$ ($\Sigma V_{0,x}$ is the sum of the void volumes of hypothetical columns of all non-eluting steps. The eluting step is thus identified when $\Sigma V_{0,x}$ becomes equal or larger than V_0. The retention volume ($V_{R,e}$) of the eluting step is then calculated in the usual way by setting $V_{0,e}$ equal to the void volume of the column. The final retention volume in a multi-step gradient is finally given by equation 2.19,

$$V_R = \Sigma V_{0,x} + \Sigma V_{G,x} + V_{R,e} \qquad 2.19$$

2.3 Application of chromatographic models to polydisperse samples

The treatment given above is valid for individual polymer molecules with well-defined molar masses. For applying the chromatographic models to heterogeneous polymer samples, it is essential to relate the sample heterogeneity to the models being used. In the simplest case of polydisperse samples heterogeneity means the distribution of polymer molecules with different molar masses. Therefore, the relationship between the molar mass of the polymer molecules and the parameters of the chromatographic model of interest must be established. That means, it is necessary to run a number of experiments with well-defined standards of known molar masses. After the parameters of the model are extracted for each molar mass, correlations can be obtained by plotting the model parameters as function of molar mass. In a similar fashion, calibrations for the other types of heterogeneities can also be established in order to implement their effect on the elution behaviour. In this thesis, emphasis is given only to molar mass heterogeneity.

2.3.1 Application of LSSM to polydisperse homopolymers

The parameters ($\ln k_0$ and S) of LSSM increase with the molar mass of the sample. According to Martin's rule [91], there is a linear relationship between $\ln k$ and the degree of polymerization (DP) or molar mass (M). The same may be valid for the parameter $\ln k_0$. This linearity may be a useful relationship for predicting retention times with respect to molar mass. However, the parameters of the relationships have to be determined by large number of experiments especially when deviations from linearity have been observed [92-94, 70]. Similarly, the parameter S is found to depend exponentially on M ($S = CM^n$) [95]. Thus, generally two calibrations, i.e. $\ln k_0$ vs M and S vs M are needed to describe the retention behaviour of polydisperse samples. A strong empirical correlation between $\ln k_0$ and S parameters has been found for the members of the same series [69, 96, 97]. This correlation has been used to predict the molar mass dependent retention behaviour of polymers [57, 68, 69].

2.3.2 Application of QSSM to polydisperse homopolymers

In the case of QSSM, the situation becomes more complicated as the molar mass dependences of the three parameters need to be established. However, there exist no relationships to describe the molar mass dependence of the QSSM parameters. The C

and *B* parameters should increase with the molar mass like the two parameters of the LSSM. The parameter *A* characterizes the non-linear behaviour of the ln *k* versus Φ relationship but nothing is known about its dependence on molar mass.

2.3.3 Application of PCM to polydisperse homopolymers

As mentioned earlier, unlike the other two conventional models, the parameters of the PCM have physical meanings. Φ_c, the critical eluent composition does not change with the molar mass. The parameter *c* depends only on the nature of repeating unit, not on the degree of polymerization. Thus, the parameter $dc/d\Phi$ is also supposed to be independent of molar mass. The only parameter left to change with molar mass is the radius of gyration, *R*. It is well known that the molar mass dependence of *R* for linear polymer molecules follows a power law, $R \approx M^{\alpha}$ [98], where the value of α depends on the thermodynamic quality of the solvent. Making use of this relation, a linear calibration line is achieved by plotting *R* as a function of molar mass in a log-log plot.

Depending upon the thermodynamic quality of the solvent, the value of the exponent α ranges from $0.5 - 0.6$ for flexible linear polymers of sufficiently high molar mass [99, 100]. However, the exact values of the exponent α are known only for a few polymers. In addition, in liquid chromatography of polymers where the eluents are often composed of mixtures of thermodynamically weak and strong solvents, the value of α might also vary with the eluent composition. On the other hand, the molar mass dependence of *R* must be known to predict the molar mass dependent retention behaviour of polydisperse samples. The value of α might be determined from the chromatographic experiments using samples of different molar masses. However, this would require performing a large number of experiments with sample of different molar masses.

Alternatively, for the sake of simplicity, a value of $\alpha = 0.5$ might be reasonable approximation. This means, after determining the parameter $(R/D)_{ref}$ for a reference molar mass (e.g. at peak maximum), the parameter *R/D* for other molar masses can be calculated using equation 2.20,

$$\frac{R}{D} = \left(\frac{R}{D}\right)_{\text{ref}} \left(\frac{M}{M_{\text{ref}}}\right)^{0.5} \qquad 2.20$$

where, $(R/D)_{\text{ref}}$ corresponds to reference molar mass, M_{ref}. Based on this approach, the retention behaviour of a whole molar mass series can be predicted, in principle, from a single molar mass, i.e. using only three parameters Φ_c, $dc/d\Phi$ and $(R/D)_{\text{ref}}$.

2.4 Band broadening in liquid chromatography of polymers

The distinct chromatographic separation of different components in a mixture not only depends on the difference of their retention times, but also on the extent of peak broadening. Therefore, for the prediction of robust separations, the widths of the peaks of the components in a polymer sample must also be predicted. The peak widths in polymer chromatography may be considered as caused mainly by two types of processes, viz. dispersion effects and the chromatographic selectivity (sample polydispersity).

The observed peak width, assuming a Gaussian peak, of a polydisperse sample in a chromatographic system can be represented by the following equation,

$$\sigma^2_{\text{observed}} = \sigma^2_{\text{PDI}} + \sigma^2_{\text{extra-column}} + \sigma^2_{\text{intra-column}} \qquad 2.21$$

i.e. the variance of the observed peak, $\sigma^2_{\text{observed}}$, is the sum of the variances of the band widths caused by the sample polydispersity (chromatographic selectivity), σ^2_{PDI}, and due to dispersion caused by chromatographic setup outside the column, $\sigma^2_{\text{extra-column}}$, and inside the column, $\sigma^2_{\text{intra-column}}$. It is usually not possible to determine all these contributions independently for a polydisperse sample because it usually cannot be resolved into their individual molecules. However, in some cases, oligomer resolution of low molar mass polymer samples is possible. In such cases, only the last two terms in equation 2.22 describe the peak widths of the individual oligomers.

2.4.1 Band broadening due to sample polydispersity

In polymer chromatography, sample polydispersity can be considered as a major source responsible for peak broadening. The contribution of molar mass

polydispersity on the widths of the observed peak is illustrated in figure 2.3. Each molecule with a different molar mass in a polymer sample will have a different retention volume (except in LCCC), depending upon the mobile phase composition and elution mode. Thus, even for a narrow distributed polymer sample the peak spreads over a wide range of elution volumes.

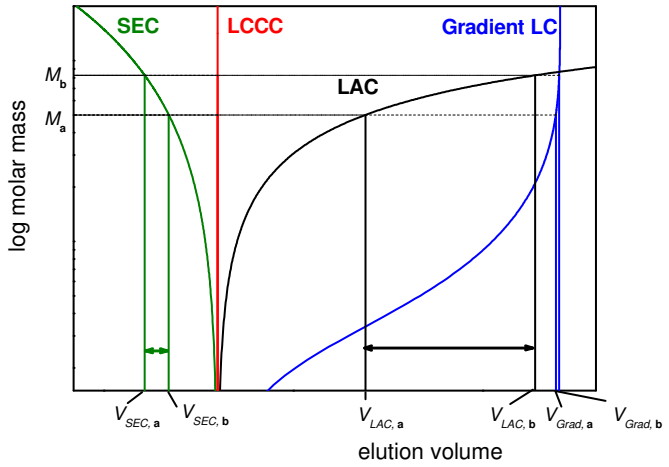


Figure 2.3: Schematic representation of the effect of sample polydispersity on the peak width when eluting in different modes of polymer liquid chromatography. Horizontal lines represent the two molar masses (M_a and M_b) that will elute at two different retention times represented by vertical lines ($V_{SEC\mathbf{a}}$ and $V_{SEC,\mathbf{b}}$ in case of SEC, $V_{LAC,\mathbf{a}}$ and $V_{LAC,\mathbf{b}}$ in case of LAC, $V_{Grad,\mathbf{a}}$ and $V_{Grad,\mathbf{b}}$ in case of gradient LC).

As shown in figure 2.3, the same molar mass (represented by M_a and M_b) range elutes over a much wider range of elution volumes in LAC as compared to SEC mode. This is because of the stronger molar mass dependence of the elution volume in LAC than in SEC. In both LAC and SEC, the molar mass dependence of elution volume is weaker in low molar mass range. Therefore, with all other factors same, the contribution of polydispersity to peak width of low molar mass samples is expected to be lower than that of high molar mass samples. In addition, when the elution is performed in mobile phase compositions close to the critical one, the molar mass dependence of elution volume becomes weak. As a result, the contribution of polydispersity is lower in those conditions. As molar mass independent elution is obtained in critical conditions, polydispersity has no contribution to peak width.

At gradient conditions, in contrast to isocratic LAC, the molar mass dependence is stronger in the low molar mass region (figure 2.3). Thus, larger contributions of polydispersity to peak widths are expected for low molar mass polymer samples. The

higher the molar mass the smaller would be the contribution of polydispersity to peak width and sharper peaks shall be observed.

Thus, the extent of the contribution of sample polydispersity on the total peak width of a polydisperse polymer sample is controlled by its average molar mass, polydispersity and the elution mode. The retention volumes can be predicted for each molecule in that sample using retention models as discussed earlier. Therefore, peak dispersion solely due to sample polydispersity can be predicted.

Apart from the determination of molar mass distributions (where molar mass selectivity is desirable), separations according to chemical composition or end groups etc. are often aimed for in chromatography. Such separations are often performed under conditions where the effect of molar mass polydispersity is minimized. At these conditions, the peak widths of polydisperse polymer samples are influenced also by other factors than just polydispersity in molar mass. Therefore, in practical LC of polymers, instrumental sources of band broadening contributions must also be considered.

2.4.2 Band broadening due to instrumental setup

During a chromatographic run, the initially injected sample band is always subjected to undesirable broadening due to various dynamic and statistical processes occurring in the column and in the extra-column volume consisting of connecting capillaries and detector cells etc. This happens even when the sample is monodisperse (i.e. the same analyte elute over a range of retention volumes). This peak dispersion phenomenon tends to affect adversely the quality of the separation and the related analysis [101].

The lowest possible band broadening due to the instrumental setup is always desirable to achieve effective separations and to obtain correct results in the polymer analysis. While there is considerable literature available how to address and correct dispersion problems in SEC [102-106], there is, to our knowledge, no consistent theory to describe the peak broadening in interaction chromatography of polymers. However, peak broadening in polymer chromatography, caused by the chromatographic setup may be described by the models used in chromatography of

small molecules, although the extent of the broadening factors may be different because of the low diffusion coefficients of large polymer molecules.

2.4.2.1 Extra-column band broadening

Band broadening due to extra-column volume arises by the travelling of sample plug through the connecting lines and detector cells, and the mixing in small dead volumes in the fittings or elsewhere in the chromatograph. This broadening factor may be large. It can be minimized, but it is inevitable. In LAC of small molecules, it tends to be the same for all peaks. However, as can be seen in equation 2.21 the smaller the contribution of band broadening in column, the larger becomes the relative contribution of extra-column band broadening. Thus, the early eluting peaks in isocratic LAC are more strongly affected than the ones eluting later. In polymer chromatography, as mentioned earlier, often the separations of the individual components of a sample occur in the range of small elution volumes where this broadening factor is an important contribution. Therefore, it should be known for optimizing the separation methods. This contribution of extra-column volume to band broadening can be determined by injecting a known volume of the sample into a chromatographic setup after removing the column.

2.4.2.2 Band broadening in column

Band broadening in the column is strongly affected by the nature of the analyte and by the chromatographic conditions. The broadening in column is generally considered to be the result of different phenomena, viz. flow pattern effects (eddy diffusion), axial diffusion, resistance to mass transfer [102]. These phenomena are affected by a number of important factors in relation to polymer chromatography, e.g. molecular diffusion and mobile phase flow rate, retention etc. The resistance to mass transfer becomes larger in adsorbing conditions, and increases with retention. According to classical plate model, the extent of band broadening in the column is related to the efficiency of the separation or the theoretical plate number of the column [86], i.e.

$$\sigma^2 = \frac{t_R^{\ 2}}{N} \qquad 2.22$$

where, N is the classical plate number, and σ^2 is the variance of the observed peak ($\sigma = W_{1/2}/2.35$ for Gaussian peak, where $W_{1/2}$ is the peak width at half height). The lower the t_R or the higher the value of N, the sharper would be the peak. The value of N can be determined from the observed peak width of a monodisperse compound eluted in isocratic conditions. In experiment however, this plate number becomes larger at low t_R suggesting better resolution of the early eluting peaks [107]. Due to this dependence of the classical plate number on the retention time of the peak from which it is determined, another plate number (equation 2.23), was proposed which is independent of the retention time [108].

$$N' = \frac{t_R (t_R - t_0)}{\sigma^2} \qquad 2.23$$

This plate number should be determined from the peaks of high retaining low molar mass substances, which are less affected by other broadening contributions. From equation 2.23 it is clear that this plate number cannot be used to predict peak widths in SEC conditions. In LCCC conditions, equation 2.23 predicts zero contribution of broadening in column. This suggests that the entire band broadening in the column for a monodisperse compound in SEC and LCCC conditions is only due to extra-column band broadening. This, however, is certainly not true since the molecules have to see more volume than the extra-column volume. Therefore, it must be concluded that besides the retention dependent contribution to band broadening (that can be calculated from equation 2.23), there must be a contribution which is effective even for non-retained compounds or for the compounds eluting in SEC conditions. The extent of the contribution of band broadening in column in SEC conditions varies only moderately with the retention time [106, 109]. For the sake of simplicity, the contribution of column band broadening in SEC and LCCC conditions will be considered to be constant in this thesis. The contribution of band broadening in the column, which increases with the retention time, can be predicted using equation 2.23. Thus, the total band broadening in column will then be defined by equation 2.24.

$$\sigma^2_{\text{intra-column}} = \sigma^2_{\text{constant}} + \sigma^2_{\text{variable}} \qquad 2.24$$

The situation becomes different in case of gradient elution where all the peaks elute in weak adsorbing conditions. In contrast to isocratic elution, the peak widths of the monodisperse analytes decrease with increasing retention times in a gradient of particular slope, while for a particular analyte peak width decreases with gradient slope.

2.5 Prediction of retention behaviour of a polydisperse sample

When suitable relationships between the model parameters and the molar mass have been established, the prediction of the elution behaviour of polydisperse samples becomes possible. The non-uniformity, *U*, (*PDI* – 1, where *PDI* is the polydispersity index) of a polymer sample can be related to its number average molar mass by the following equation [110],

$$U = \frac{M_w - M_n}{M_n} = \frac{\sigma_n^{\,2}}{M_n^{\,2}} \qquad 2.25$$

where, *U* is the non-uniformity of polymer sample, and σ^2_n is the variance of the frequency distribution. Thus, using equation 2.25 the range of molar masses contained in a particular sample can be estimated. From the calibrations, the model parameters and hence the retention times for each molar mass can be calculated employing the corresponding chromatographic models (section 2.3). For each monodisperse peak, the effect of dispersion can be calculated. Finally, for each elution volume the concentration contributions of each species are summed up to have the overall chromatogram of the polydisperse polymer sample. Since the prediction of the peak shapes of polydisperse samples seems more facile using the PCM, only the application of this model is described here.

2.5.1 Prediction of peak shapes

In order to estimate the peak shape of a polydisperse sample, the number average molar mass and polydispersity of the sample must be known. However, in some cases these parameters are not available. In the following, proposals are given to predict the peak shapes of polydisperse samples employing the PCM for the two cases, (a) when the molar mass and polydispersity of the sample is known (b) when these are unknown.

2.5.1.1 Case of known molar mass and polydispersity

The standard deviation, σ, of the molar mass distribution (*MMD*) of the polymer samples can be calculated from the knowledge of their number average molar masses and polydispersities using equation 2.25, assuming a normal distribution (in some case log-normal distribution may be a good choice). The *MMD* between $M_n \pm 6\sigma$ (covering 99.7 % of molecules) can be divided into fractions, with each fraction differing from the adjacent exactly by the molar mass of a repeating unit. In order to reduce the calculation time, for the cases where the number of slices exceeds 100, the distribution can be divided into 100 slices differing by a constant mass increment. The values the model parameters must be calculated for each molar mass of the series. For example, in the case of the PCM, the molar mass dependent parameter *R*/*D* for each molar mass of the series can be calculated using equation 2.20, assuming that the extracted $(R/D)_{ref}$ parameter (from the retention times at peak maxima) corresponds to the number average molar mass of the sample. The values of the Φ_c, $dc/d\Phi$ parameters remain constant for all members of series. Retention times for the given chromatographic conditions can be calculated subsequently for each fraction. Thus, the calibration curves are constructed that describe the retention as a function of molar mass covered by that sample. In this way, the effect of the molar mass distribution on the peak profile can be calculated. The shape of the observed peak depends on the relative concentration in the distribution and the shape of the calibration curve.

2.5.1.2 Case of unknown molar mass and polydispersity

The same approach as used in previous section can be used if no information on molar mass and polydispersity is available. First, an arbitrary molar mass is assigned to peak maximum (M_{ref}). Second, an initial trial value for *PDI* is assumed, based for example on the type of polymerization. The rest of the calculations is the same as for case of known molar mass and *PDI*. Additionally, the effect of instrumental broadening is added to obtain total peak broadening (equation 2.21). After performing the three initial experiments needed to extract the model parameters for the peak maximum the PDI can be adjusted until the calculated and experimentally determined peak shapes fit well. Good estimates of *PDI* can be especially obtained for experiments where stronger molar mass dependence of retention times is

expected. It should be mentioned that the arbitrary value of molar mass assigned to peak maximum does not influence the subsequent calculations because only the relative molar masses are used in equation 2.20.

3 Results and discussion

As mentioned earlier, polymer molecules are of large sizes with a large number of interacting units. This feature results in a chromatographic behaviour of polymers quite different from that of low molar mass molecules. The conventional models of liquid chromatography, which are typically valid for well-defined molecules with low molar mass, might however, still be applied to predict the chromatographic behaviour of polymers by assuming that polymers in LAC mode behave similar to small molecules. In the present chapter, the suitability of the different model to predict the retention behaviour of polymers is evaluated.

It is known from literature [80, 81, 111, 112] that polyethylene glycols (PEG) can be separated rather easily into a large number of oligomers up to quite high degrees of polymerization. Therefore, PEG oligomers were used as model compounds. The chromatographic models were tested for their ability to predict retention times for each individual oligomer, i.e. without the influence of the polydispersity of the sample. Later the models were applied to predict the retention behaviour of polymer samples, which cannot be separated into individual oligomers. The models were not only used to predict the retention times, but they also provide a deeper understanding of polymer liquid chromatography, as it will be shown in the following.

3.1 Retention behaviour of PEGs

3.1.1 Retention behaviour of PEGs in isocratic elution

According to the equations of the models used in this study, the retention times (in terms of logarithmic retention factor (equation 2.1 and 2.3) or interaction parameter (equation 2.7)) of the molecules are related to the eluent composition. In order to examine the appropriateness of the models, the dependence of retention time on eluent composition must be established. For this purpose, the isocratic elution behaviour of PEG was investigated at different isocratic eluent compositions of water and methanol on a reverse phase column at 35°C (column A, see experimental section). Water and methanol were used as adsorption and desorption promoting solvents, and shall be referred to as weak and strong eluent components, respectively.

Isocratic elution of PEGs with oligomer resolution is feasible only in a small range of eluent compositions. For example, the elution of PEG 1000 (see table 5.1 for detailed properties of the used samples) with appropriate oligomer resolution was possible only in a range of 54/46 - 51/49 v/v water/methanol. Figure 3.1 shows the chromatogram of PEG 1000 eluted in 54/46 v/v water/methanol. As can be seen, about 18 peaks can be identified, each corresponding to a single oligomer. Although the early eluting peaks are sharper than those eluting later, yet the resolution of the early eluting peaks is lower than those of eluting later. This is due to the smaller selectivity, i.e. the separation ratio defined as the ratio of the retention factors of two adjacent peaks ($\alpha_{B/A} = k_B/k_A$), at low retention times. It can be inferred from here that both the retention time, i.e. peak positions and the peak widths are required to predict the separations.

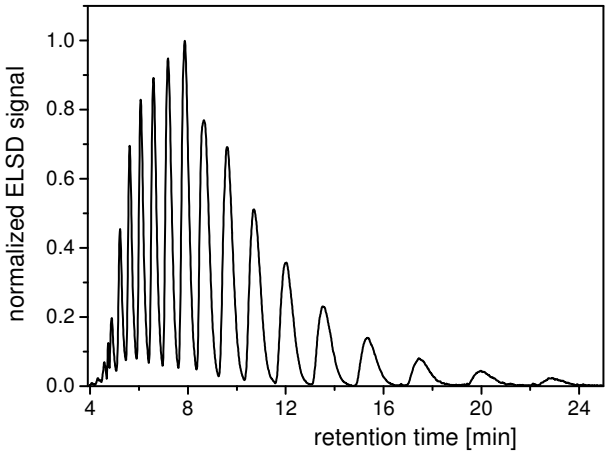


Figure 3.1: Chromatogram of PEG 1000 g/mol obtained by isocratic elution in 54/46 v/v water/MeOH; stationary phase: Nucleosil C18, column A (see experimental section); flow rate: 1 ml/min; column temperature: 35°C; Detector: ELSD

From chromatograms like the one given in figure 3.1, the retention times corresponding to each oligomer, in different eluent compositions, were determined. The assignment of DP to the peaks was made by comparing the retention times of standard polyethylene glycol compounds. The dependence of retention times on DP in four different eluent compositions are given in figure 3.2.

From figure 3.2, one can see that the retention time increases strongly with the DP, thus, higher molar mass molecules may no longer elute from the column at a certain weak eluent composition. However, the retention times can be decreased by increasing the strength of the eluent, i.e. by adding a higher amount of methanol.

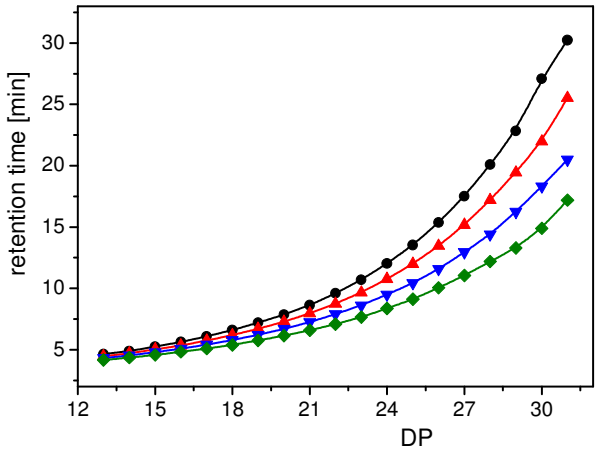


Figure 3.2: Dependence of retention times of PEG oligomer on the degree of polymerization, DP, for different eluent compositions, 54/46 (●), 53/47 (▲), 52/48 (▼), 51/49 v/v (◆) water/methanol. Other chromatographic conditions same as in figure 3.1

From the retention times in figure 3.2, retention factors (k) were calculated using equation 1.3. The values of the logarithmic retention factor (ln k) are plotted against DP in figure 3.3 and 3.4. As shown in figure 3.3, a linear dependence is observed for the higher oligomers. This linear dependence of ln k versus DP is commonly known as Martin's rule [91]. This relationship can be important as the dependence of retention time on molar mass may be easily predicted. However, as can be seen in figure 3.4, the lines show deviations from linearity in the region of low degree of polymerization and therefore for the lower values of ln k. The stronger the eluent strength, the larger is the deviation.

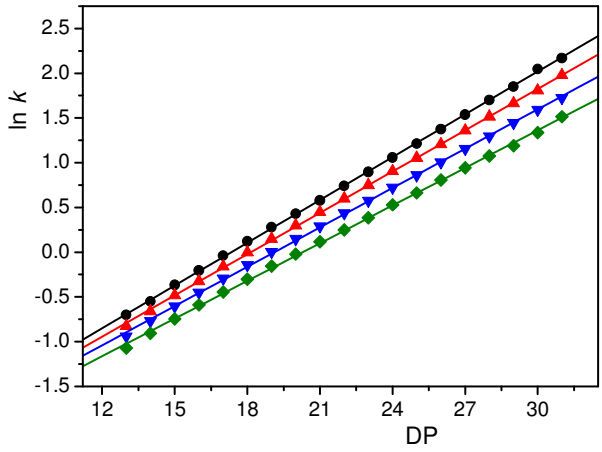


Figure 3.3: Dependence of logarithmic retention factors of PEG oligomers on DP for different eluent compositions, 54/46 (●), 53/47 (▲), 52/48 (▼), 51/49 v/v (◆) water/methanol. Other chromatographic conditions same as in figure 3.1

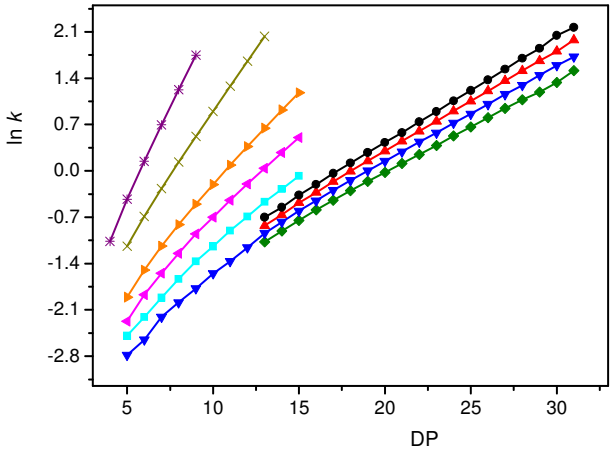


Figure 3.4: Logarithmic retention factors of PEG oligomers as a function of DP for different eluent compositions, 79/21, 72/28, 64/36, 60/40, 56/44, 54/46, 53/47, 52/48, and 51/49 v/v water/MeOH (top to bottom). Other chromatographic conditions same as in figure 3.1

The molecular statistical theory of polymer adsorption in wide pores shows that a linear relationship is expected only in conditions of strong interaction between the polymer molecules and the stationary phase [94]. These conditions may be realized for oligomers with a high DP but might not been reached for low DP. However, elution of high molar mass molecules is possible only in weak interaction conditions obtained by the use of stronger eluent.

In order to establish the molar mass dependence of retention for high molar mass PEGs, isocratic experiments were performed also for PEGs with relatively high molar masses in different water/MeOH mixtures. These samples could not be resolved into oligomers. The retention times were determined at the peak maxima. The experimental retention times of PEGs with different average molar masses are depicted in figure 3.5 against the methanol content of the eluent. The highest retention times are observed at eluent compositions with low content of MeOH. Higher molar masses cannot be eluted completely from the column at these compositions. The completeness of elution was judged by flushing the column with 100 % MeOH when the ELSD signal returned to baseline. When an additional peak was observed at flushing, the elution at the corresponding eluent composition was regarded to be incomplete. In figure 3.5, only those experiments are included that resulted in complete elution.

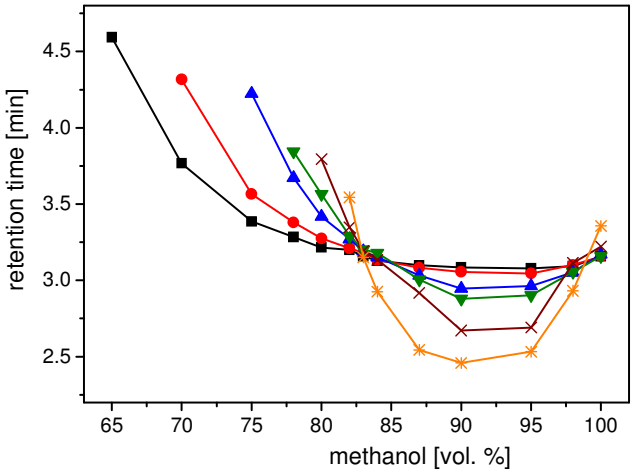


Figure 3.5: Dependence of retention times on the methanol content of eluent for high molar mass PEG standards; M_p = 2000 (■), 3000 (●), 6000 (▲), 12000 (▼), 23000 (×), 40000 (✳) g/mol. Other chromatographic conditions same as in figure 3.1

It is obvious that the retention times strongly depend on eluent composition. They are first decreasing with the increase in MeOH content of eluent. This dependence is more pronounced for higher molar masses. For example, complete elution of PEG 2000 is possible only in eluent mixtures with more than 65 % MeOH, while PEG 23000 is eluting only in compositions containing at least 80 % MeOH. Thus, the higher the molar mass of the sample, the closer it elutes to the void volume of the column in order to elute completely. As can be seen, the curves of different molar masses are merging at a MeOH content of about 83 %. This eluent composition, where the molar mass dependence of retention time vanishes, is referred to as the critical composition. With MeOH contents higher than the critical composition the elution order is reversed and higher molar masses elute earlier than lower ones, i.e. SEC like behaviour is observed. The retention times continue to decrease until a MeOH content of approximately 90 %. At even higher MeOH contents, an unexpected increase in the retention time is observed and the different curves merge again at approximately 98 % MeOH. This indicates the existence of second critical point. Such behaviour of PEG on silica based reversed phases has been previously observed with other eluents by Trathnigg [78]. According to their explanation, the behaviour at low MeOH content is due to interaction of ethylene units of PEG chain with the C-18 chains of reversed phase, while the unusual behaviour above 90 % MeOH may be due to the interaction of oxygen atoms in the PEG chain with residual

silanol groups of the stationary phase. The existence of two critical points for another polymer system has also been reported by Berek [113].

Since only one critical point can be dealt with by the polymer specific model given in chapter 2, the following discussion is restricted to the range of eluent composition, which results in normal reverse phase behaviour, i.e. to MeOH contents of less than 90 %.

3.1.1.1 Conventional LC models and PEG retention in isocratic elution

In order to evaluate the appropriateness of conventional chromatographic models (i.e. LSSM and QSSM) to describe the retention behaviour of PEG, the dependences of the logarithmic retention factors on the eluent composition was established. The plots of ln k versus the methanol content of the eluent (Φ) for PEG oligomers of DP equal to 15, 20, 25, and 30 are given in figure 3.6. As can be seen, the data for each oligomer can be fitted by a linear dependence with a negative slope, showing that retention is decreasing with the strength of the eluent. The intercept at zero MeOH content gives the value of ln k_0, the retention factor in the pure weak eluent, i.e. water in this case. Thus, LSSM appears to be a valid model within the composition range studied for these oligomers.

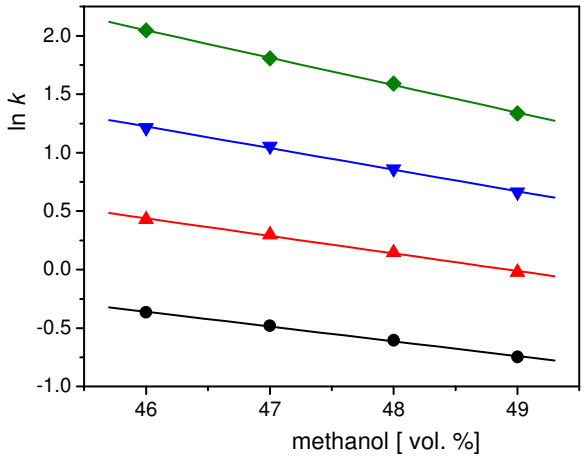


Figure 3.6: Dependence of logarithmic retention factors (ln k) on the eluent composition for different oligomers, DP = 15 (●), 20 (▲), 25 (▼), 30 (◆). Data are fitted with a linear relation. Chromatographic conditions same as in figure 3.1

A non-linear dependence of ln k on eluent composition is observed however, when the retention factors of lower PEG oligomers are analyzed over a wider range of eluent compositions. The ln k vs Φ curves of four oligomers are given in figure 3.7.

As can be seen, the data points deviate from a straight line and can only be well described by a quadratic relation. Hence, the QSSM seems more suitable than the LSSM to describe the elution behaviour of lower PEG oligomers, over a wider range of eluent compositions.

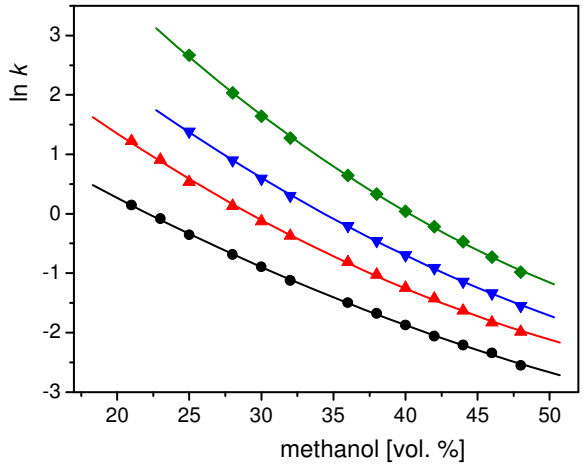


Figure 3.7: Dependence of logarithmic retention factors (ln *k*) on the eluent composition for different oligomers, DP = 6 (●), 8 (▲), 10 (▼), 13 (◆). Data are fitted with a quadratic equation. Chromatographic conditions same as in figure 3.1

3.1.1.2 PCM and retention behaviour of PEG in isocratic elution

As mentioned in chapter 2, LSSM and QSSM are suitable to describe the retention behaviour of polymers in LAC mode of chromatography only, i.e. LCCC and SEC behaviour cannot be described in principle. On the other hand, using the PCM the retention of polymers in all three modes of polymer chromatography (LAC, LCCC and SEC) could be described. According to the PCM, the retention behaviour of a polymer in isocratic mode of elution is described by the parameters R/D and cR. Often the pore size (D) of the stationary phase is known (the values of pore size provided by manufacturer was used in all calculations). As mentioned, R describes the size (radius of gyration) of the polymer coil, and can be estimated from its molar mass, M, by using the relation [58, 99],

$$R = M^{0.5} L \qquad 3.1$$

Thus, equation 2.6 can be rewritten as follows,

$$K_d = 1 - \frac{L}{D}\left[\frac{4M^{0.5}}{\sqrt{\pi}} - \frac{2}{cL}\left(Y(-cLM^{0.5}) - 1\right)\right] \qquad 3.2$$

where, L is referred to as the Kuhn length or polymer flexibility parameter. For PEG, the value of L equal to 0.079 (nm) has been used by Trathnigg et al. [58]. Knowing this value for the case of PEG, the only unknown parameter in equation 3.2 is the interaction parameter, c. While the parameter L and hence, R are supposed to be independent of eluent composition, the parameter c is changing.

The values of c for each eluent composition can be determined from a plot of K_d (or t_R) versus DP using equation 3.2 via a non-linear fitting procedure. Such a plot for the oligomers of PEG is given in figure 3.8.

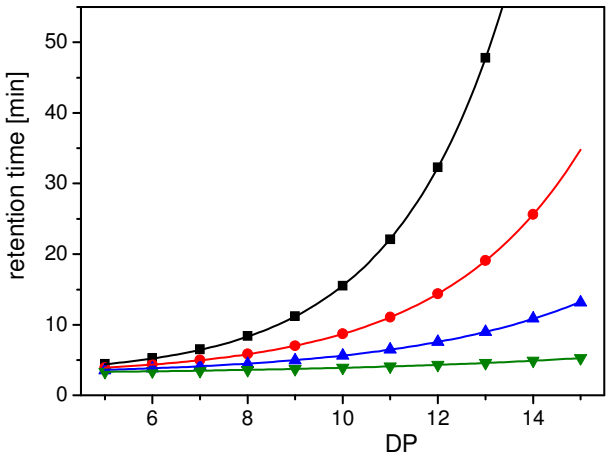


Figure 3.8: Dependence of retention times on oligomer DP at different isocratic eluent compositions of water/MeOH; 75/25 (■), 70/30 (●), 64/36 (▲), and 54/46 v/v (▼). Chromatographic conditions same as in figure 3.1. The lines indicate the fits obtained using equation 3.2. Fitting constants: t_p = 1.56 min, t_i = 1.54 min, D = 30 nm, L = 0.079.

The symbols in figure 3.8 represent the experimentally obtained retention times in four different eluent compositions, while the solid lines are the fitted curves according to equation 3.2 using the values of c that gives the best possible description of the experimental data over the whole series. As can be seen, there is a good agreement between the theory and the experiment. In this way, the values of c can be extracted for each eluent composition. The so obtained values of c for eleven different eluent compositions are plotted against the methanol content of the eluent in figure 3.9.

As can be seen, the lower the content of methanol the higher the value of c and the stronger is the adsorption. Within the experimental region of eluent composition, the dependence of c on the methanol content is linear. The linearity of c vs. Φ

relationship shows the validity of equation 2.7. The red line in figure 3.9 is obtained by fitting the data with a linear function. The linear dependence of c on Φ implies that the eluent composition at which c becomes zero can easily be obtained from such a relation. This eluent composition corresponds to the critical composition (Φ_c). The value of Φ_c determined by extrapolating to $c = 0$ is found to be 24/76 v/v water/MeOH, which is in reasonable agreement with the experimentally determined critical composition (17/83 v/v water/MeOH). The slope of the line yields the important parameter ($dc/d\Phi$), which describes the change of c per change in eluent composition. Thus, knowing Φ_c and $dc/d\Phi$, c in any of the eluent composition can be determined.

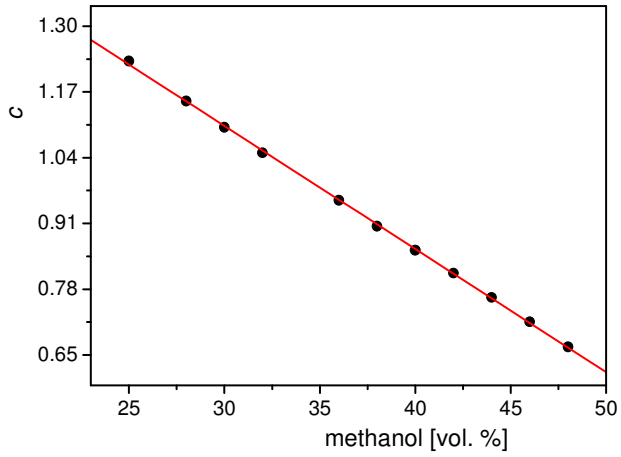


Figure 3.9: The interaction parameter, c, as a function of eluent composition, obtained by non-linear least square fitting of equation 3.2 to the retention time data of PEG oligomers. Chromatographic conditions same as in figure 3.1. Fitting parameters: same as in figure 3.8

As mentioned earlier, the control parameter in PCM is cR, the total interaction strength of a polymer chain with the stationary phase. Knowing the relationship of c versus Φ, and the value of R, the dependence of cR parameter on the eluent composition can be described for each molar mass of polymer. Figure 3.10 shows the calculated values of cR as a function of MeOH content of the eluent based on the c vs. Φ relationship in figure 3.9 for different values of R calculated using equation 3.1. As can be seen, the higher the molar mass, the higher the slope of line. This indicates that the total interaction strength of higher molar masses is very sensitive to changes in the mobile phase. High molar mass polymer molecules may even become irreversibly retained on the column if the experiment is performed in an inappropriate eluent composition. It can be concluded that using the PCM with

equation 3.1 the isocratic retention as a function of mobile phase composition as well as molar mass can be described.

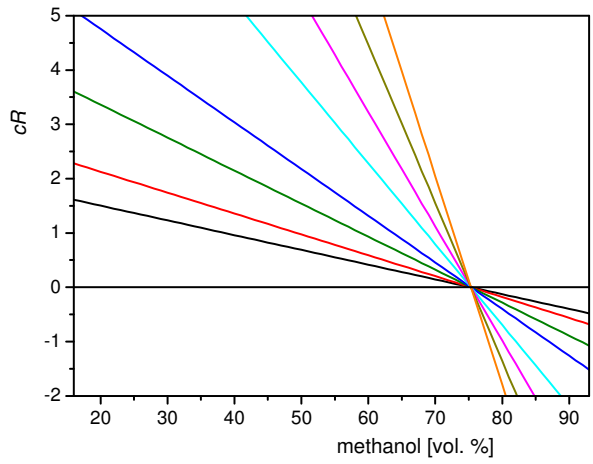


Figure 3.10: Dependence of the parameter *cR* on MeOH content of the eluent, calculated for various values of *R* corresponding to molar masses, *M* = 200 (—), 400 (—), 1000 (—), 2000 (—), 6000 (—), 12000 (—), 23000 (—), and 40000 (—). Calculation based on *c* vs. Φ dependence in figure 3.9 and equation 3.1.

In the previous discussion, a known relationship between *R* and *M* was used keeping the value of *L* constant (equation 3.1). However, the value of *L* is not known for every polymer. When the value of *L* is unknown, the values of *c* and *L* may be fitted simultaneously to the dependences of K_d or t_R on DP for a given eluent composition. It should be noted that the quality of the fit, estimated by the errors between the experimental and calculated retention times, is much better if *L* is allowed to vary than when it is kept constant. This is due to the greater flexibility if the two parameters are varied. This fitting results in *cL* and *L* parameters. The values of *cL* and *L* so obtained (from PEG 400) are plotted against the eluent composition in figure 3.11.

As can be seen, parameter *cL* decreases with MeOH content of eluent in a well-defined linear fashion. The value of *L* (and therefore the value of *R*) is showing an unexpected decreasing trend with increasing MeOH content. The reason of this behaviour may lie in the low molar masses of the oligomers that may not be described accurately by Gaussian chains. It is also possible that the Kuhn segment length, *L*, varies with eluent composition. This trend is in accordance with the results of Baran et al. [114] that *R* may change with the thermodynamic quality of eluent. For

the sake of simplicity, L parameter would be considered independent of changes in eluent compositions.

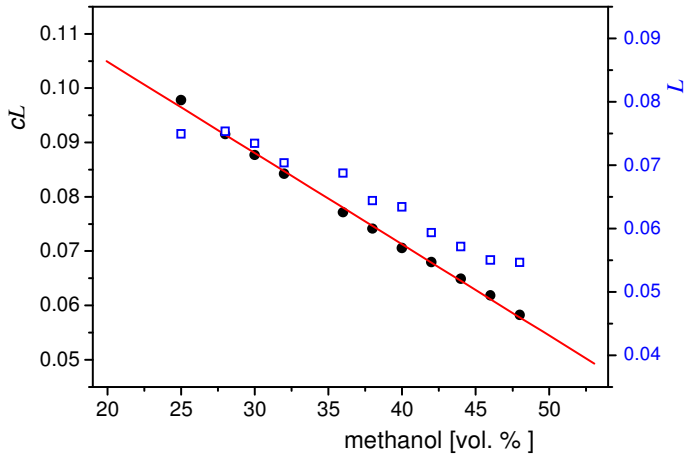


Figure 3.11: Dependence of the cL (●), (fitted with straight-line equation) and L (□) on the eluent composition. Values were obtained by fitting equations 3.2 to the retention data for PEG oligomers using non-linear least square fitting procedure. Fitting constants: t_p = 1.56 min, t_i = 1.54 min, D = 30 nm.

The above discussion shows that a linear dependence of c or cR (when both c and L parameters are determined from experiments) on Φ is an appropriate assumption which is the pre-requisite for the extension of the PCM for gradient elution.

3.1.2 Retention behaviour of PEG in gradient elution

As already shown, isocratic elution of high molar mass polymers in LAC mode is possible only in a narrow range of eluent composition, close to the critical composition. For this reason, gradient elution is often used in chromatography of polymers. The following discussion evaluates the suitability of different chromatographic models to describe the retention behaviour of PEGs during gradient elution. The gradient experiments were performed using the same stationary phase (column A, see experimental section) and mobile phase (water/MeOH) system at 35°C as in the case of isocratic elution. Several PEG standards were subjected to solvent gradients of different slopes and different initial and final eluent compositions. An overlay of chromatograms of PEGs of different molar mass for 90 minutes linear gradient ranging from 5 to 100 % MeOH in water is shown in figure 3.12.

As can be seen, a large number of PEG oligomers can be separated in a 90 minute gradient. This picture also highlights the superiority of gradient elution over isocratic elution. More than 55 oligomers could be clearly separated in about 60 minutes only. Early eluting peaks are better resolved and the late eluting peaks show less broadening as compared to isocratic elution (figure 3.1). This shows that gradient elution is an appropriate choice when analytes of very different interaction strengths have to be separated, e.g. polydisperse polymer samples. As it will be shown later, the gradient elution is also helpful to obtain the information about the eluent compositions allowing for isocratic elution.

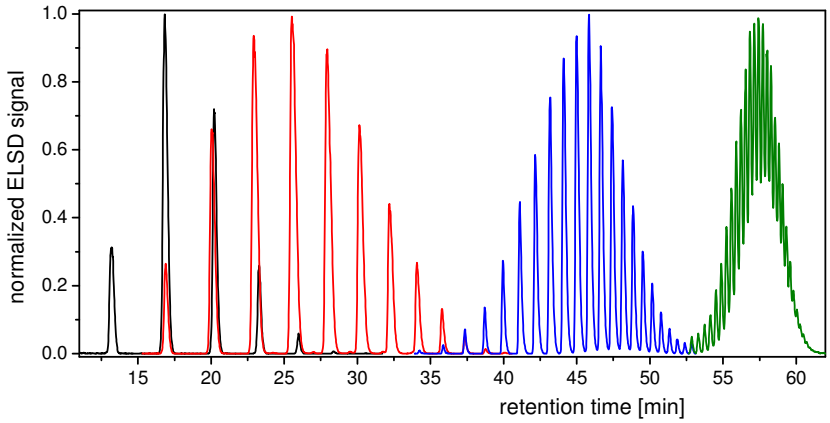


Figure 3.12: Overlay of chromatograms of PEG 200 (—), 400 (—), 1000 (—), and 2000 (—), obtained by gradient elution from 5 - 100 % MeOH/water in 90 minutes. First peak corresponds to DP = 5 as determined by comparing the retention times of standard PEG compound. Other chromatographic conditions same as in figure 3.1

The retention times of the different oligomers eluted in gradients of different slopes were determined from the chromatograms. The dependence of retention times on DP in linear gradients with different slopes is depicted in figure 3.13. Here the data of PEG oligomers are given along with the data of high molar mass PEGs, which could not be resolved into individual oligomers. The continuity in the curves provides good comparison of the retention behaviour of low and high molar mass PEGs. This shows that there is no abrupt transition in the retention behaviour of low and high molar mass PEGs.

Figure 3.13 illustrate that the retention time of polymers in gradient elution strongly depend on degree of polymerization. The higher the degree of polymerization, the larger is the retention time. However, with increasing DP, the molar mass dependence of retention time becomes weaker, as shown by the decreasing slope of the retention time curve. This means that the molar mass selectivity decreases with

molar mass. The selectivity is influenced, however, by the gradient slope, with the molar mass dependence of the retention time being more pronounced for the longer gradients. This shows that to a certain limit, molar mass selectivity can be enhanced by performing elution with gradients of more shallow slopes.

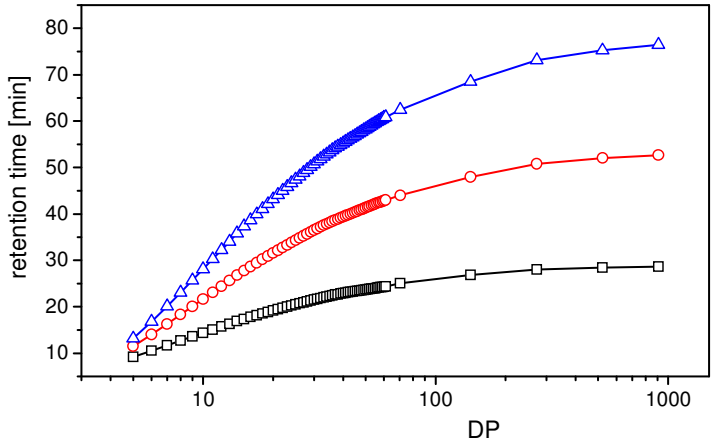


Figure 3.13: Dependence of retention times of individual PEG oligomers and polydisperse high molar mass PEGs on DP for gradient elution from 5 to 100 % MeOH against water in 30 (□), 60 (○), and 90 (△) minutes. Other chromatographic conditions same as in figure 3.1

3.1.2.1 Description of gradient elution of PEG by the models of LC

As has been shown in the preceding chapter, the LC models can be used to describe how the retention of an analyte changes with eluent composition in isocratic chromatography. The same model parameters that describe the retention behaviour in isocratic elution should determine gradient elution as well (equations 2.2, 2.4, 2.10 – 2.11).

Therefore, the model parameters might also be determined from gradient experiments, however, by using non-linear fitting procedures. Figure 3.14 shows one such example where the model parameters for all the three LC models are adjusted to the retention data of a PEG oligomer having DP = 20. The retention times in four linear gradients of different lengths are plotted against gradient time and fitted by equation 2.2, 2.4 and 2.10 – 2.11. The nearly perfect agreement between the experimental and calculated retention times reflects the suitability of all three models to describe accurately the gradient elution of PEGs. The following discussion attempts to explain the gradient elution with respect to the studied models.

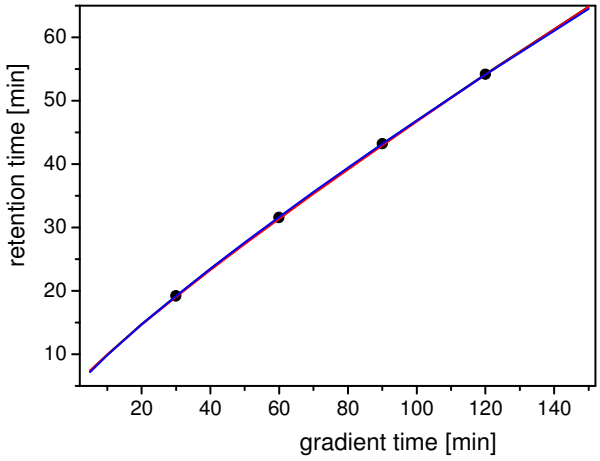


Figure 3.14: Dependence of retention times of a PEG oligomer (DP = 20) on gradient length (linear 5 to 100 % MeOH against water). The gradient equations of LSSM (—), QSSM (—), and PCM (—) were used to fit the data. Other chromatographic conditions same as in figure 3.1

3.1.2.1.1 Understanding gradient elution of PEG by conventional LC models

The gradient elution can be explained on the basis of LSSM and QSSM as follows. During gradient elution, the analyte's k value varies over a wide range. At the beginning, it is large and decreases as the strength of the mobile phase is increased. Thus, the values of k directly calculated from the gradient retention times do not provide information about all the retention factors experienced during a gradient elution. However, the average of all the values of k, the analyte molecule experience during its way through the column, k_{ave}, can be calculated using the equation given by Snyder et al. (equation 3.3) [115]. Similarly, Φ_{ave}, the average composition that molecules experience during the gradient experiment can also be directly calculated from LSSM equations given below [116].

$$k_{ave} = \frac{1}{1.15b} \qquad 3.3$$

$$\Phi_{ave} = \Phi_0 + \left[V_R - V_0 - V_D - 0.3\left(\frac{V_0}{b}\right) \right] \frac{\Delta\Phi}{F t_G} \qquad 3.4$$

where, $b = \frac{\Delta\Phi S V_0}{F t_G}$

V_R is the retention volume, V_0 the column void volume, V_D delay volume, F the flow rate, $\Delta\Phi$ the change in eluent composition during the gradient, and t_G the gradient

time. By plotting ln k_{ave} versus Φ_{ave}, the validity of a particular model (LSSM or QSSM) to describe the gradient as well as isocratic retention can be determined.

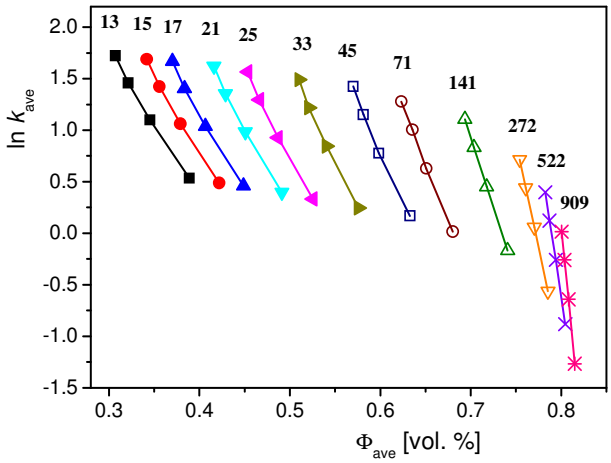


Figure 3.15: Dependence of average logarithmic retention factors (ln k_{ave}) on the average methanol content (Φ_{ave}) during gradient elution for PEGs of different DPs. Gradients: 5 to 100 % MeOH against water in 30, 60, 90, and 120 (bottom to top) minutes. The numbers represent the DP of the PEGs. Other chromatographic conditions same as in figure 3.1

Figure 3.15 shows the plots of ln k_{ave} versus Φ_{ave} for different low and high molar mass PEGs calculated from four gradient runs having different slopes (range 5 – 100 % MeOH in 30, 60, 90, 120 min.). Oligomers with lower DP are eluted at lower methanol contents and experience larger k_{ave} as compared to PEGs with higher DP. The lower the DP, the larger is the Φ_{ave} range that molecules experience during elution in gradients of different slopes. For example, compare the range of Φ_{ave} experienced by DP 13 (30 – 38 % MeOH) with that by DP 909 (80 – 81.5 % MeOH) in gradients of four different slopes. This proves that high molar mass samples elute only in a very narrow range of mobile phase composition. The same is valid for the range of ln k_{ave}. For a fixed molar mass, the longer the gradient the lower is Φ_{ave} and the higher is the value of k_{ave}. The lines for high molar mass PEGs have higher slopes that indicate that high molar mass PEGs will either be adsorbed strongly or be suddenly desorbed at a specific mobile phase composition.

If the LSSM model is valid, the plot of ln k_{ave} versus Φ_{ave} should be linear. As can be seen in figure 3.15 this is not true for the lower oligomers. This shows that LSSM is not a valid model for these cases. The non-linearity is not observable for oligomers of higher DP. Thus, the LSSM may be a suitable choice for high molar masses. These observations are in good agreement well to the isocratic elution behaviour

discussed in section 3.1.1. Thus, both isocratic and gradient elution can be explained with the help of conventional models of LC.

There is a clear transition from the QSS behaviour to the LSS behaviour in relation to molar mass and eluent composition. Since the models are empirical, they provide no reasonable explanation for the cause of this transition. Since PCM is based on the theory of polymer, it is expected to give more insight into the retention behaviour in gradient elution.

3.1.2.1.2 Understanding gradient elution of PEGs using PCM

In order to investigate the validity of the PCM to describe the retention behaviour of polymers at gradient elution, different linear gradient experiments of PEGs were analyzed with respect to the PCM. Mobile phase system for PEG was the same as given earlier and the retention time data is given in figure 3.13. As can be seen in figure 3.13, there is a limiting value of retention time for each gradient as can be seen at the high molar mass end. That means all higher molar masses would be eluting at these limiting retention times.

In order to relate the gradient retention times of different PEGs to the eluent composition, the eluent composition at the time of elution, Φ_g, was calculated from the retention times and the corresponding gradient program using the following equation.

$$\Phi_g = (V_R - V_0 - V_D)\frac{\Delta\Phi}{Ft_G} + \Phi_{initial} \qquad 3.5$$

The compositions at elution for PEGs in gradients of different slopes are plotted versus the molar mass of the PEGs in figure 3.16. As can be seen the higher the molar mass, the higher the methanol content in the eluent composition at elution. Similar to figure 3.15 it can be seen that the range of composition at elution for low molar mass PEGs eluting in gradients of different slopes is larger than that for higher PEGs. The higher molar mass PGEs elute in a very limited range of eluent composition. Evidently, there is a limiting value of eluent composition at the time of elution regardless of the gradient slope. All PEGs are eluting before this eluent composition is reached during the gradient experiment. The difference in molar mass

tends to vanish at this eluent composition. It is important to note at this point that the eluent composition that results in molar mass independent elution in isocratic mode is referred to as the critical eluent composition.

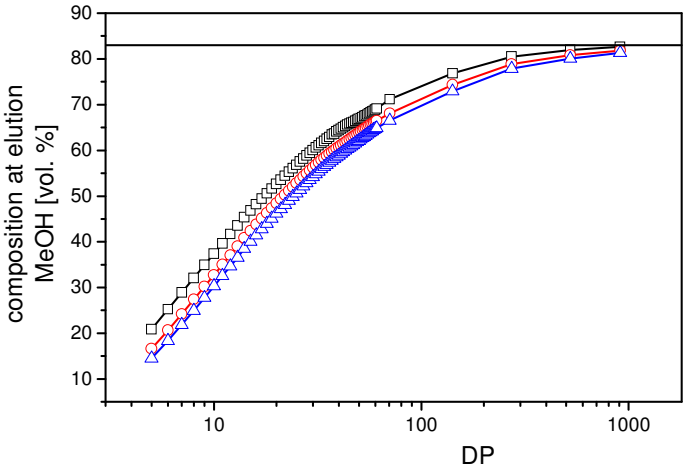


Figure 3.16: Composition at elution (% MeOH) for PEG as a function of DP. Gradient: linear 5 to 100 % MeOH against water in 30 (□), 60 (○), and 90 (△) minutes. The solid horizontal line indicates the limit of eluent composition at which highest molar mass PEG reaches the detector.

This elution behaviour of polymers at gradient conditions can be explained as follows: In gradient experiments, the mobile phase strength is varied over time. A very strong adsorption of the polymer molecules on the stationary phase takes place at the weak initial mobile phase composition. That is, the total interaction strength, cR, is very large ($K_d >> 1$). With increasing mobile phase strength desorption occurs. The polymer molecules start moving when a mobile phase composition of sufficient strength reaches them. Lower molar mass polymer molecules, being only weakly adsorbed (lower values of cR, figure 3.10), desorb at a much weaker mobile phase composition while higher molar mass polymer molecules require a stronger mobile phase to desorb (high values of cR, figure 3.10). As the polymer molecules are desorbed, they are surrounded by a mobile phase composition in which the polymer molecules experience weak interactions with the stationary phase ($\Delta H < T\Delta S$) and thus, move with a velocity lower than that of the mobile phase ($K_d > 1$). Consequently, they are surpassed by mobile phase compositions of increasing strength, resulting in a continuous acceleration of the polymer molecules. This acceleration continues as long as the mobile phase molecules are faster than the polymer molecules. During this course, low molar mass polymer molecules may elute from the column, while for high molar mass polymer molecules finally a

situation is reached where the velocity of polymer molecules become equal to the mobile phase velocity ($K_d = 1$).

It should be mentioned that the velocity of polymer molecules cannot be faster than that of mobile phase, i.e. overall elution may never be dominated by exclusion. This is due to the presence of a weaker mobile phase composition ahead of the moving polymer molecules. Whenever a polymer molecule would try to become faster than the mobile phase ($K_d < 1$), it would meet that weaker mobile phase composition and would slow down. At the same time, the polymer molecules are being pushed by the strong mobile phase composition coming from behind to move faster. Therefore, the only choice left for a polymer molecule is to move slower than or at velocity equal to the mobile phase. The condition, in which the mobile phase and polymer molecules has the same velocity, corresponds to critical conditions where the compensation of enthalpic and entropic effects occurs ($T\Delta S = \Delta H$). From the above discussion, it follows that this compensation can also be achieved in the gradient elution. This forms the basis for a new concept of polymer separation at the critical point of adsorption during a gradient elution put forward by Brun et al. [83-85]. According to this concept, the separation of high molar polymers in gradient chromatography takes place independent of molar mass. However, according to the calculations of Brun et al., the elution of high molar mass polymer samples occurs at compositions slightly higher than the critical. This is the result of neglecting the variation of the mobile phase composition along the column. According to the corrected equations of PCM given in chapter 2 (where also a more general relationship for K_d has been used), the polymer molecule can only elute at a mobile phase composition below or equal to the critical one. Therefore, the composition at elution from gradient runs sets an lower limit for Φ_c i.e. all polymer molecules elute at lower or equal eluent strength than Φ_c [90]. Since higher molar mass polymer molecules are more strongly adsorbed than lower molar mass molecules, the composition at elution for high molar mass polymer molecules is closer to the critical mobile phase composition than for polymers of lower molar mass.

3.1.3 Prediction of retention behaviour of PEGs

The preceding chapters have shown that the selected conventional chromatographic models as well as the polymer specific model are suitable to describe the retention

behaviour of polymers whenever they are applicable. However, it is yet to be evaluated if they are able not only to describe but also to predict the retention behaviour based on a minimum number of experiments. Therefore, the studied models are investigated further to predict the retention times, in both gradient and isocratic modes of elution. The initial experiments are named in the following as the calibration experiments as discussed in sections 2.1.1 – 2.1.3. The minimum number of calibration experiments needed to model the retention times is two for LSSM and three for QSSM and PCM. The analyte specific parameters of the models that best describe the calibration experiments were determined using non-linear least square (NLS) fitting. Once the model parameters were successfully determined, predictions of the retention times were made using these parameters. For the purpose of extraction of model parameters and predictions of the retention times, scripts based on the model equations were written in Origin's LabTalk language. The predictions were made for two polymer systems, firstly for oligomers of PEG that have relatively low molar masses and are free from the complications of polydispersity, secondly for high molar mass PEGs that cannot be resolved into oligomers.

3.1.2.1 Isocratic to isocratic prediction

In isocratic to isocratic predictions, the parameters extracted from isocratic experiments were used to predict the retention times at different isocratic conditions. Only PEG 200 and 400 could be eluted isocratically with sufficient resolution to identify the individual oligomers, over a relatively wide range of MeOH contents in the eluent. Here, the predictions for PEG 400 oligomers are given as an example. Two isocratic runs at 70/30 and 64/36 v/v water/MeOH were used as calibration experiments in case of LSSM, while three runs at 72/28, 70/30 and 64/36 v/v water/MeOH were used to extract the parameters of the QSSM and PCM for each oligomer.

Figure 3.17 illustrates the fitting procedure for different oligomers using PCM. The retention times obtained from calibration runs were first plotted against MeOH content. Then the fitting was performed using Origin's NLS fitter. Fitting is started with speculated parameter values. Best values can then be found by iterations. It can be seen in figure 3.17 that a good fit of the calibration data can be achieved.

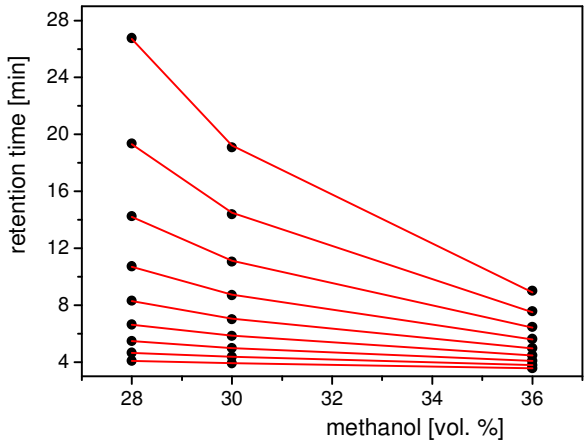


Figure 3.17: Fit of the PCM (—) to three isocratic experiments (●) to extract the three parameters of the PCM for oligomers of PEG with DP = 5 – 13 (bottom to top). Chromatographic conditions same as in figure 3.1

The calibration parameters of the models were then used to calculate the retention times at other MeOH contents in the range of 25 – 48 % MeOH. The MeOH content of 32 % lies within the calibration range while for compositions with 25 % MeOH (weaker eluent) and with MeOH contents higher than 36 % (stronger eluent), predictions have to be made outside the calibrated region by extrapolation.

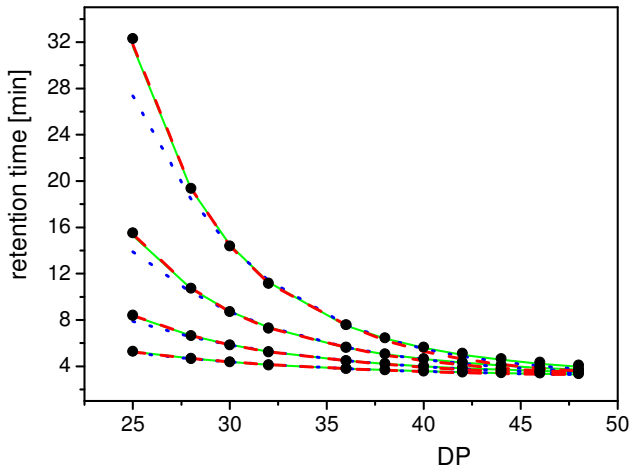


Figure 3.18: Comparison of isocratic retention time predicted by LSSM (····), QSSM (--), PCM (—), with those obtained experimentally (●) for different oligomers of PEG with DP = 6, 8, 10, and 12 (bottom to top). Calibration experiments for parameter extraction: isocratic experiments at 70/30, 64/36 for LSSM; 72/28, 70/30, and 64/36 v/v water/MeOH for QSSM or PCM. Other chromatographic conditions same as in figure 3.1

Figure 3.18 provides a visual comparison of retention times predicted by the three models with the ones obtained experimentally. A good agreement is found for the experiment and the predictions in the case of QSSM as well as PCM. The LSSM

predictions however, deviate from the experimental results at stronger adsorbing conditions. Because of the low retention times, the differences between the predictions and experiments are difficult to see for weak adsorption conditions. In order to quantify and compare the accuracy of the model predictions, the absolute percent relative errors were calculated using the following equation,

$$Error = \sqrt{\left(\frac{t_{R\,\mathrm{predicted}} - t_{R\,\mathrm{experimental}}}{t_{R\,\mathrm{experimental}}} \times 100 \right)^2} \qquad 3.6$$

The errors for the oligomers retention times are plotted in figure 3.19 versus the methanol content as bar graph. The arrows indicate the eluent compositions that were used for calibration. Very small or even zero errors for the calibration compositions prove the quality of the fit.

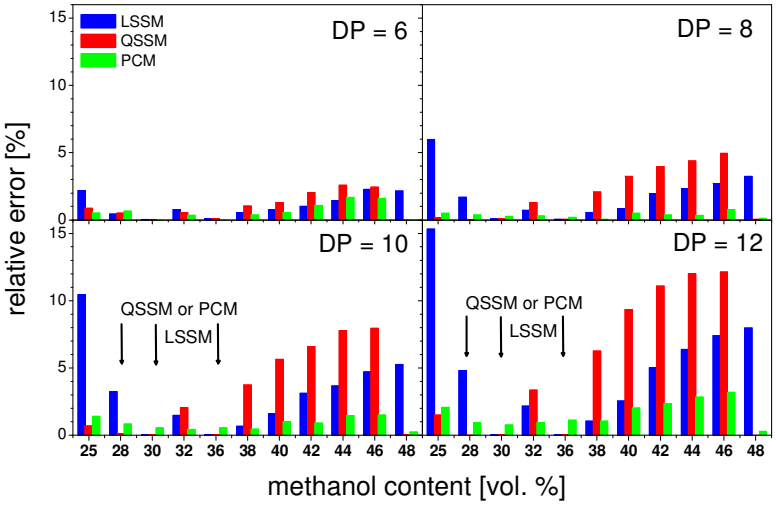


Figure 3.19: Comparison of % relative errors (square root of squared relative error) as a function of MeOH content in mobile phase for the predicted isocratic retention times of selected PEG oligomers (from the data in figure 3.18).

It can be seen that the highest errors of up to 15 % are observed for the predictions of LSSM at 75/25 v/v water/MeOH for oligomer of higher DP. Errors of QSSM and PCM for these compositions are less than 2 %. The lower errors may be attributed to the greater flexibility provided by the three adjustable parameters. As expected, within the calibrated region i.e. at 68/32 v/v water/MeOH, all the models predict approximately the same with low errors (less than 3 %), although the errors increase with increasing DP. Extrapolation to stronger eluents results in larger errors for the

predictions of the LSSM and the QSSM, with QSSM producing higher errors. The PCM model, however, results in predictions having much lower errors (less than 4 %) in all eluents for each oligomer. From the above given results, it can be concluded easily that PCM is the best model to predict isocratic retention times from isocratic calibration experiments. However, since isocratic elution is not very easy to perform for high molar mass polymers, this mode of prediction may not be suitable for high molar mass polymers.

3.1.2.2 Gradient to gradient predictions

Due to the problems in performing isocratic chromatography experiments, gradient elution is the first choice in polymer chromatography. Therefore, the models were also investigated for their suitability to predict retention time in gradient experiments. In gradient to gradient mode, gradient experiments were used to extract the model parameters and predictions of retention times for other gradient conditions were performed. A larger number of linear gradient experiments with different ranges and slopes has been performed to assess the suitability of the models to predict the retention times in gradient conditions. Parameters of the models were extracted using the NLS fitter in Origin. As an example, the retention times predicted by the three models and the ones determined experimentally for polyethylene glycol oligomers of DP = 5 – 61 in linear gradients of 5 to 100 % MeOH against water are shown in figure 3.20.

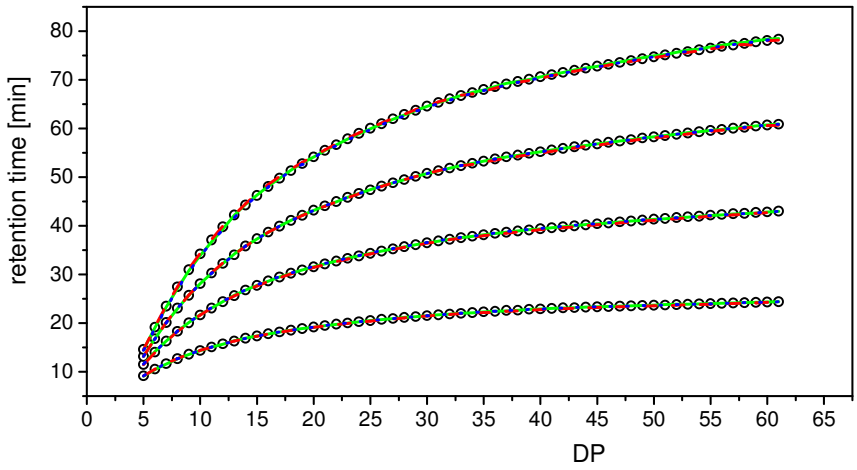


Figure 3.20: Comparison of gradient retention times predicted by LSSM (····), QSSM (– –), PCM (—), with the ones obtained experimentally (○) for gradients of 5 to 100 % MeOH against water with durations of 30, 60, 90, 120 minutes (bottom to top) as a function of DP for PEG oligomers. Calibration experiments: Gradients of over 30, 90 minutes for LSSM, and 30, 60, and 90 minutes for QSSM or PCM. Other chromatographic conditions same as in figure 3.1

In the case of the LSSM, gradients of 30 and 90 minutes were used to extract the model parameters. Gradient experiments of the same composition range but of 60 and 120 minutes length were predicted. The predicted experiments therefore lie within and outside the calibration range of the gradient time, respectively. Excellent visual agreement between the experimentally determined and simulated retention times is found for both cases.

In case of the QSSM and the PCM three gradients runs were used for calibration, i.e. the parameters of each model for each oligomer were extracted from the data of 5 – 100 % linear gradients over 30, 60, and 90 minutes. The predictions were made for the gradient experiment of 120 minutes that lies outside the calibration. It can be easily concluded from the comparison of the predicted and experimental results given in figure 3.20 that both QSSM and PCM are also suitable to predict accurately the gradient retention times from gradient experiments. The performance of the models is quantitatively evaluated by calculating the square root of squared relative deviations from the experimentally determined retention times. The comparison of the errors produced by all three models to predict the retention times of a range of PEG oligomers for the 120 minute gradient is given in figure 3.21. As can be seen, the errors are less than 2 % for all the models and all oligomers.

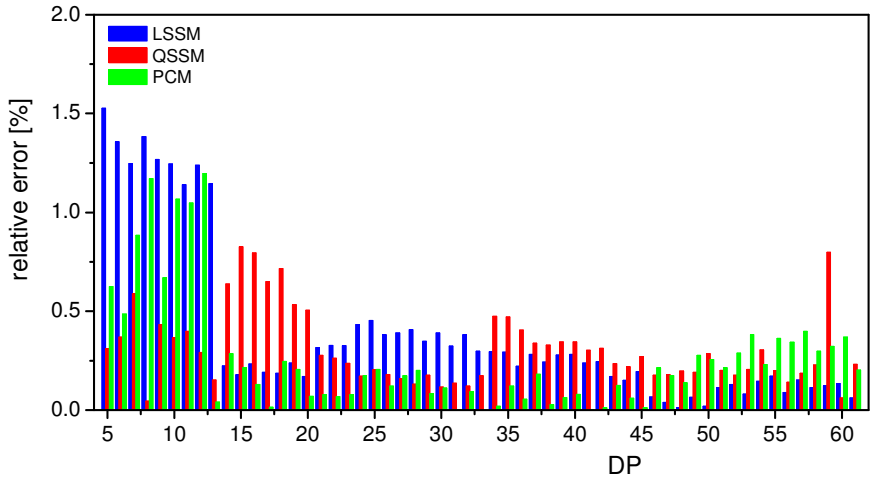


Figure 3.21: Comparison of % relative errors in retention time predictions made by three models for 5 to 100 % MeOH gradient against water in 120 minutes as function of DP of PEG oligomers.

Various combinations of gradients for the calibration experiments were used to check their effect on the parameter extraction and the subsequent predictions. Similarly, gradients of various ranges, i.e. 20 – 90 %, 40 – 90 %, 50 – 80 % MeOH, at different

slopes were also investigated. Every combination tested resulted in predictions with errors less than 1 % for more than 98 % of all data points as long as the calibration is performed with the same gradient range. In cases where the initial eluent composition was stronger than 20 % MeOH, quite large errors (up to 25 %) were found for the early eluting oligomers when the calibration was performed with 5 – 100 % gradients. The strong initial eluent composition causes the isocratic movement of the lower oligomers during the system dwell time. As will be shown in the next section, the reason for the large errors in these cases is related to the poor quality of the predictions of isocratic retention times from gradient calibrations.

In order to examine the applicability of the models to predict the retention behaviour of high molar mass polymers, gradient to gradient predictions were also performed for high molar mass PEGs. For this purpose linear gradients were run. The retention times were determined from the peak maxima. Similar to the oligomers, two or three gradients with different slopes were used for calibration for LSSM or QSSM and PCM, respectively. The predictions were made for other gradient slopes. The comparison of the predicted and experimentally obtained gradient retention times and the corresponding errors are given in figure 3.22 and 3.23, respectively.

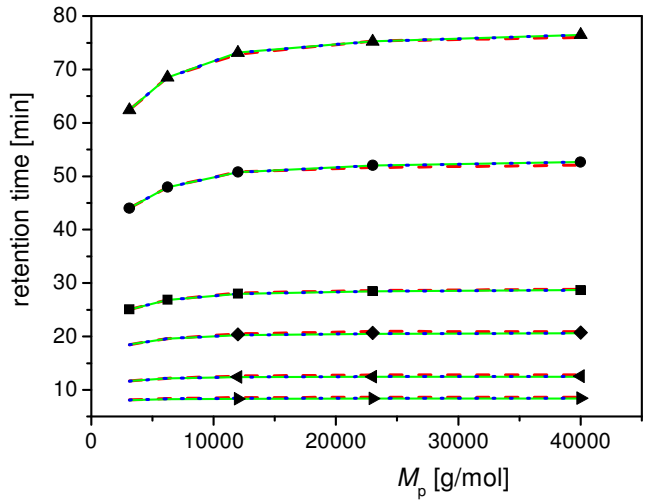


Figure 3.22: Comparison of gradient retention times predicted by LSSM (····), QSSM (--), PCM (—), with the ones obtained experimentally (●) as a function of molar mass of PEG. Gradient: 5 to 100 % MeOH against water with durations of 5, 10, 20, 30, 60, 90 minutes (bottom to top). Calibration experiments: Gradients of 5 to 100 % MeOH against water over 30, 90 for LSSM, and 30, 60, and 90 minutes for QSSM or PCM. Other chromatographic conditions same as in figure 3.1

As can be seen, there is again an excellent agreement between the predictions of all three models and the experiments. The errors are less than 3 % in all cases. These

results show that all three models studied can be used to predict accurately the gradient retention times of low as well as high molar mass PEG samples when the calibration is performed using gradient experiments.

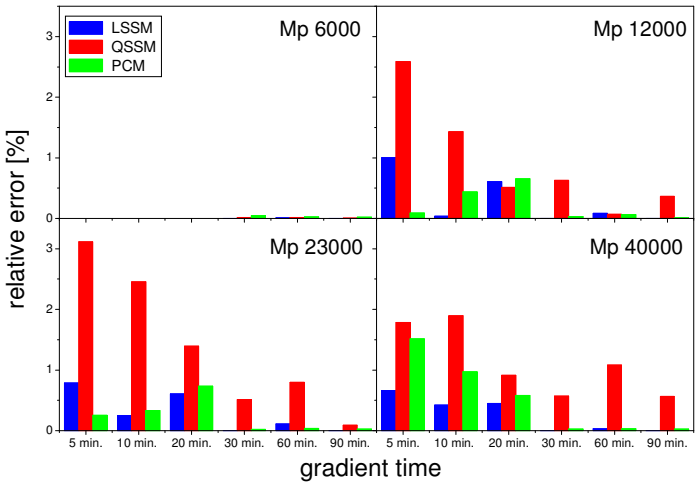


Figure 3.23: Comparison of % relative errors (square root of squared relative error) in gradient retention times of high molar mass PEG samples predicted by the three used models as function gradient time, from the data of figure 3.22

3.1.2.3 Gradient to isocratic predictions

In this case, the model parameters were extracted from the necessary number of gradient experiments, while isocratic retention times were predicted. The prediction of isocratic retention time is important due to many reasons, e.g. when dealing with the elution in the dwell time of the chromatographic system. In addition, some separations may require gradients with isocratic steps, or simply sometimes isocratic elution is desirable as in LCCC. On the other hand, gradient experiments can be performed relatively easily for unknown polymers samples. Therefore, gradient elution may be a good starting point even for the isocratic method development. In principle, a suitable model should be able to predict isocratic retention times from gradient data. In the following, the suitability of each described model is tested to make gradient to isocratic predictions for PEGs.

Figure 3.24 compares the isocratic retention times of four different PEG oligomers at four different MeOH contents, predicted by LSSM (calibration; 5 to 100 % MeOH gradients of 30 and 90 minutes), QSSM and PCM (calibration; 5 to 100 % MeOH gradients of 30, 60 and 90 minutes) with those obtained experimentally. As can be seen, all models predict at least the correct retention behaviour, i.e. the retention

decreases with the eluent strength and increases with DP. The accuracy of the models in quantitatively predicting the retention times is revealed from figure 3.25, where the square root of squared relative errors are plotted versus MeOH content of the eluent for different PEG oligomers.

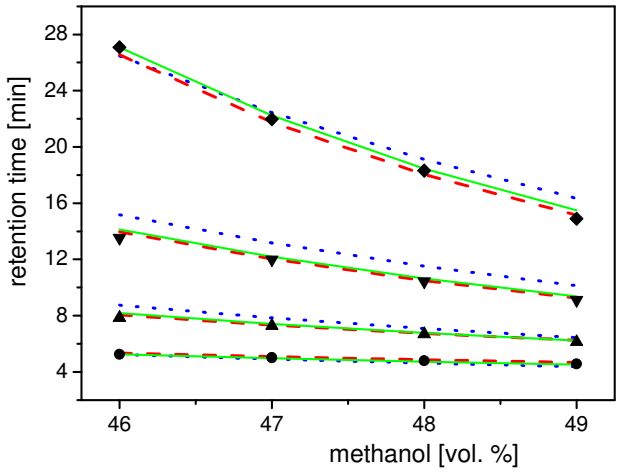


Figure 3.24: Comparison of isocratic retention times predicted by LSSM (····), QSSM (--), PCM (—), with the ones obtained experimentally (●) for PEG oligomers of DP = 15, 20, 25, and 30 (bottom to top) as a function mobile phase composition. Calibration experiments: Gradients of 5 to 100 % MeOH against water for 30, 90 (LSSM), and 30, 60, 90 (QSSM or PCM) minutes. Other chromatographic conditions same as in figure 3.1

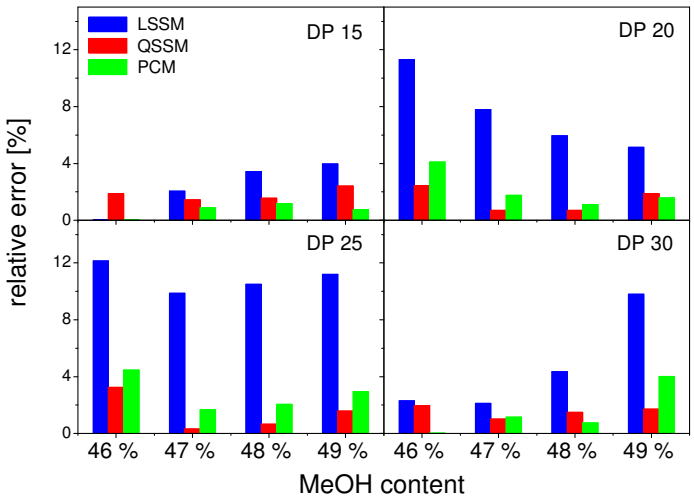


Figure 3.25: Comparison of % relative errors (square root of squared relative error) of isocratic retention time for some of PEG oligomers from the data in figure 3.24. Predictions made by three models as a function of MeOH content in mobile phase.

As can be seen, the errors in case of the LSSM prediction are highest, i.e. 6 – 8 % (with the maximum errors up to 15 % for some oligomers). The other two models show significantly lower average errors of up to 3 % only (with maximum of 5 %). The larger deviations of LSSM predictions might at first be attributed to the usage of

only two gradient experiments instead of three in case of the QSSM and PCM. However, using three gradient experiments for calibration does not improve the quality of prediction. This shows that the model itself is not suitable to predict the isocratic retention times from gradient calibration. Moreover, gradient experiments provide information about retention in a rather narrow range of eluent composition as compared to the isocratic experiments [117]. The predictions of isocratic retention times, therefore, require extrapolations, which may result in large errors even if the model is suitable. This might also be a reason of the larger errors in the LSSM predictions. The last origin of errors may also explain the somewhat higher errors in the gradient to isocratic predictions of QSSM and PCM as compared to the gradient to gradient predictions. From the above results, it can be concluded that only QSSM and PCM are suitable to predict the isocratic retention time from gradient data.

The models were also applied to predict isocratic retention of high molar mass PEGs using gradient calibration. The calculated retention times at different MeOH contents are compared with the ones determined experimentally in figure 3.26.

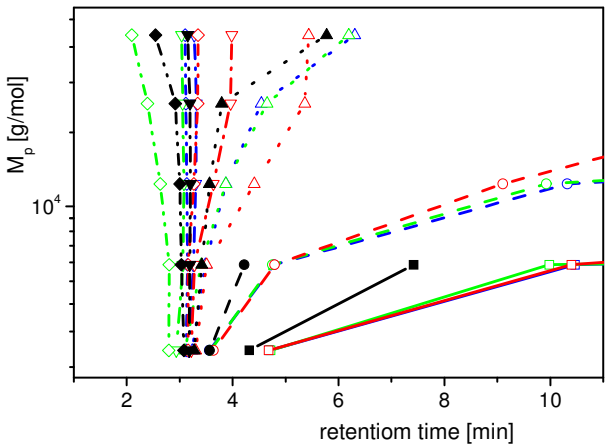


Figure 3.26: Isocratic retention times predicted by LSSM (blue), QSSM (red), PCM (green), as compared to the ones obtained experimentally (black) for high molar mass PEGs at different mobile phase compositions, 30/70 (■), 25/75 (●), 20/80 (▲), 17/83 (▼), 13/87 v/v water/MeOH (◆). Calibration experiments: gradients of 5 to 100 % MeOH against water for 30, 90 (LSSM), and 30, 60, 90 (QSSM or PCM) minutes. Other chromatographic conditions same as in figure 3.1

This illustration exposes significant differences between the experiments and calculations based on all three models. The deviations are more pronounced at lower MeOH contents, i.e. in LAC. In addition, it becomes visible that for higher MeOH contents the experimental elution behaviour changes from LAC behavior (below 17/83 v/v water/MeOH, the critical composition) to SEC behavior (at higher MeOH

contents). Neither the LSSM nor the QSSM can account for this transition in elution behavior, because, as mentioned earlier, ln k in the equations of these models is not defined for $k \leq 0$ (see equations 2.1 and 2.3). On the contrary, the PCM gives at least a correct qualitative picture and the transition between these two chromatographic modes is observed at the correct eluent composition. This also shows that PCM can be used to predict the critical compositions from a minimum number of experiments using one single sample only. The large errors in the isocratic retention prediction of PCM for high molar mass samples at LAC and SEC condition are due to the inaccuracy in the determination of the model parameters, since the quality of the extracted PCM parameters depends significantly on molar mass of the polymer sample and the type of experiments used for their extraction, as will be shown later.

3.1.2.4 Isocratic to gradient predictions

As mentioned earlier, it is tedious to establish conditions for isocratic experiments for polymer samples. Therefore, isocratic experiments as initial experiments are not a practical choice. However, for the comprehensive evaluation of the models, the predictions of gradient retention times from isocratic data were also analyzed. For the PEG oligomers of DP in the range of 14 - 31, isocratic experiments at 46/54 and 48/52 v/v water/MeOH were used for the LSSM parameter extraction while compositions of 46/54, 47/53 and 48/52 v/v water/MeOH were used for QSSM and PCM. Predictions for 5 to 100 % MeOH gradient experiments of 30, 60, 90, and 120 minutes durations were made. For the QSSM no calculation of the gradient retention times was possible. This is due to the fact that the parameter A in equation 2.4 (describing the non-linear relation between ln k and Φ) is in the denominator of the QSSM gradient equation. This creates problems in the calculations of gradient retention times when the value of A is close to zero, which is actually the case here.

A comparison of the predicted and experimentally determined retention times for different oligomers is given in figure 3.27. There are significant deviations in the low molar mass region for both cases, although the predictions of PCM (errors up to 10 %) are better than that of LSSM (errors up to 22 %). The predictions for the longest gradient (120 min.) are worst. The large errors can be attributed to the larger experimental errors in the isocratic experiments that result in the larger errors in the parameter extraction.

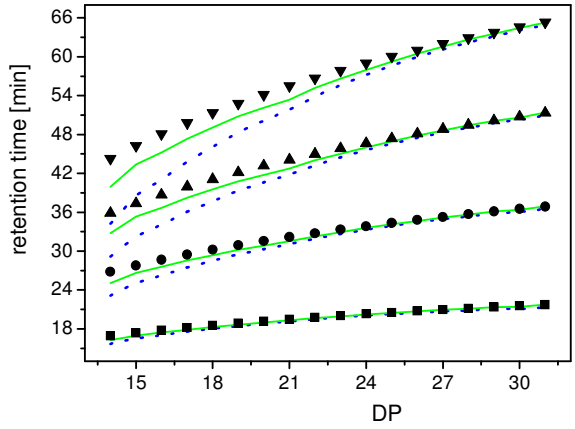


Figure 3.27: Gradient retention times of PEG as a function of DP of oligomers predicted by LSSM (┈┈), PCM (—), in comparison to those obtained experimentally. Gradient: of 5 to 100 % MeOH against water. Gradient length: 30 (■), 60 (●), 90 (▲), and 120 (▼) minutes. Calibration: Isocratic experiments at 53/47, 51/49 (LSSM), and 53/47, 52/48, and 51/49 v/v water/MeOH (PCM). Other chromatographic conditions same as in figure 3.1

For high molar mass PEGs, isocratic experiments at 80/20, 82/18, and 83/17 v/v water/MeOH were used to extract the model parameters. The predictions of the gradient retention times for high molar mass PEGs are compared with the experiments in figure 3.28. Here also large errors are observed, which decrease for higher molar masses. This behaviour is similar to the oligomers. However, the two cases cannot be simply compared because the calibrations were made using isocratic experiments that cover completely different composition domains.

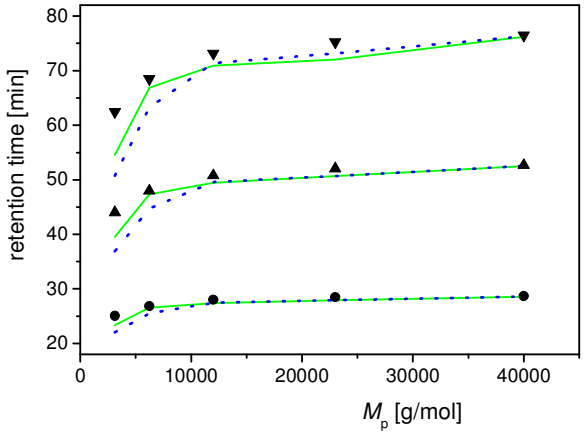


Figure 3.28: Gradient retention times of high molar mass PEGs predicted by LSSM (┈┈), PCM (—), in comparison to those obtained experimentally as a function of molar mass. Gradients: 5 to 100 % MeOH against water. Gradient lengths: 30 (■), 60 (●), 90 (▲), and 120 (▼) minutes. Calibration: Isocratic experiments at 53/47, 51/49 (LSSM), and 53/47, 52/48, and 51/49 v/v water/MeOH (PCM). Other chromatographic conditions same as in figure 3.1

A summary of the above given results is given figure 3.29. Here, the performances of the models to predict the retention behaviour in different modes of prediction are compared. The box-plots were constructed from percentage deviations for the whole range of PEG oligomers.

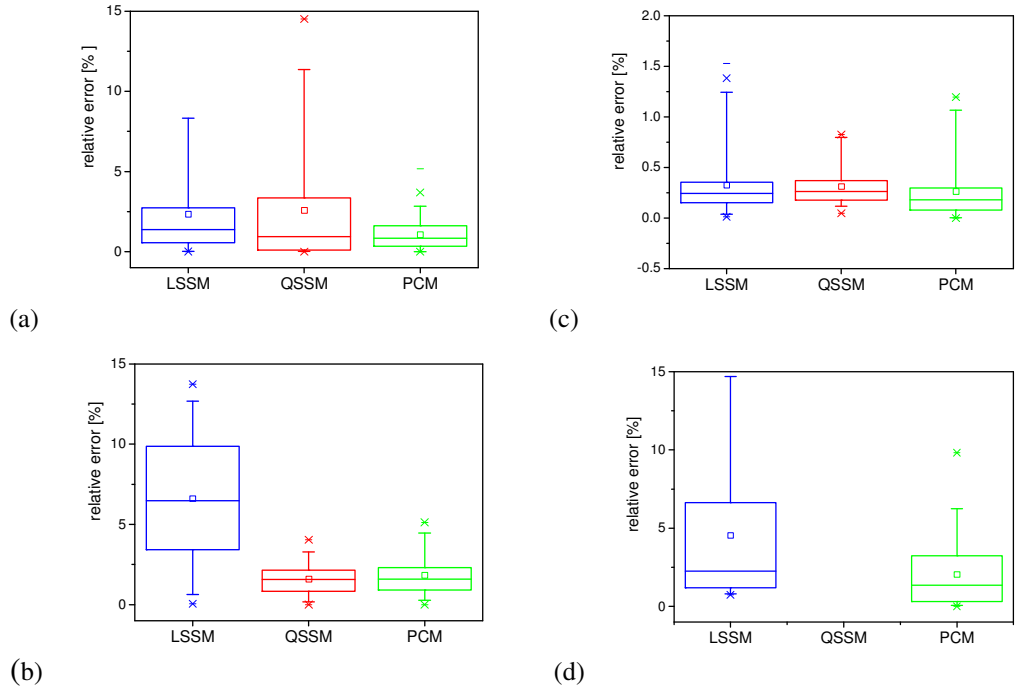


Figure 3.29: Comparison of the overall performance of the used models to predict the retention times of the PEG oligomers in different modes of data transfer, (a) isocratic-isocratic, (b) gradient-isocratic, (c) gradient-gradient (note the difference in scale), and (d) isocratic-gradient (QSSM was not able to predict). The boxes are generated from the % relative errors (square root of squared errors) in all experiments performed in each mode.

Box-plots provide the excellent visual summary of the error distributions. A typical box-plot consists of a box that contains the 50 % of all the values, with the upper and lower edges indicating 75th and 25th percentile, respectively. The horizontal line within the box represents the median of the data, which, when not equidistant from the edges of the box, shows that the data are skewed. The symbol inside the box represents the mean, the symbols outside the extremes. From figure 3.29, it can be easily concluded that PCM is the most suitable model for predicting the retention times of PEG in any of the prediction modes. Although the performance of the other two models is also good in some cases, yet they cannot be used to predict the retention behaviour in LCCC and SEC mode for principle reasons. On the other hand, PCM is applicable to all the modes of liquid chromatography of polymers. Therefore, PCM will be further investigated for the more precise prediction of the retention times.

3.2 Understanding and improving the quality of prediction

The preceding chapters have shown that the PCM is the most suitable model for prediction of retention prediction of PEGs in various modes of polymer liquid chromatography. However, the accuracy of predictions is poor when predictions are made from one elution mode to the other especially for high molar mass PEGs. In the following discussion, the possible causes of the poor predictions and possibilities to improve the accuracy of the predictions will be evaluated in relation to the PCM.

3.2.1 Identifying the sources of errors

When predicting retention behaviour of polymers, gradient experiments should be the preferable initial measurements to determine the model parameters. If a model is valid, then the parameter extraction and hence the quality of predictions should not be influenced by the type of the initial experiments, provided that there are no experimental errors. However, as it is shown in the preceding sections, the models predict the best when the elution mode of the calibration and predicted experiments is same, e.g. gradient to gradient prediction is better than gradient to isocratic prediction. There may be several reasons for the observed larger errors where the elution modes of calibration and prediction are different.

The different sources for the errors may be,

(i) The model itself, i.e. whether the model is valid or not

(ii) The experimental errors

(iii) Inaccuracy in the extracted model parameters

A non-linear dependence of c or cR on Φ, which could be a reason of larger errors, can be ruled out at least for PEG oligomers (section 3.1.1.1, figure 3.9 and figure 3.11). The validity of this result can be verified further by the extrapolation of c or cR for PEG 400 and PEG 1000 to the zero value, which results in an estimate of the critical composition with a deviation of less than 1 %. This linear dependence might be especially true for high molar masses PEGs because of the small range of c or cR that allows an elution from the column at all. Thus, any non-linearity, even if it exists, should have practically no effect for high molar masses. Thus, it can be

concluded that the errors observed for predictions using the PCM are not related to a failure of the model. Rather they are arising either from errors in the experimental measurements or from the uncertainties associated with the extraction of the parameters.

The experimental uncertainties may contribute significantly to the errors in the predictions. One source of experimental error particular to polymer chromatography is the difficulty in assigning the representative retention time to the peaks. The peak maxima that are often used to determine the retention times of polydisperse samples do not represent the same molar mass in different modes of elution because of the non-linear shape of retention curve with respect to molar mass. This problem is more severe in isocratic LAC. Thus, the incorrect determination of the retention time would appear as error in the predictions of the model. The effect of experimental errors on the predictions can be reduced by using suitable selection of experiments for calibration that cover a larger variation of experimental variable, e.g. isocratic experiments at widely different eluent compositions [118, 119].

The uncertainties in the extracted model parameters may also be an important reason for the large errors in the isocratic predictions. The fitting process often does not result in the true model parameters because the extraction of the parameters depend on several factors, e.g. the fitting procedure, the number of multiple local minima, the initializing values of the parameters, the number of model parameters and the number as well as the type of the experimental runs. Usually, the uncertainties in determination of parameter values become higher with increasing number of parameters to be extracted.

The last two sources of errors are interconnected. Even if error free experimental data would have been available, the non-linear fitting procedures may not be able to extract the true parameters due to the possible existence of local minima. These local minima may result in inaccurately extracted model parameters upon selection of inappropriate parameter values for the initialization of the non-linear fitting procedure. On the other hand, the experimental errors would not allow for accurate extraction of the model parameters even if the fitting procedure would be robust. Often the predictions require extrapolations outside the calibrated region of eluent composition. The errors in the calibration experiments may lead to considerable

errors in extrapolated predictions because of the direct propagation of the experimental errors into the extracted parameters. A purposeful selection of the initial experiments may be helpful in reducing the uncertainties in the extracted parameters. For this purpose, the following discussion evaluates the possibility to identify the initial experiments that provide the best information about the model parameters.

3.2.2 Quality of PCM parameters extracted from gradient calibration

The large errors in the predictions of isocratic retention times for high molar mass PEGs with gradient calibration using the PCM can be understood by looking at figure 3.30. In this graph, the PCM parameters providing the best fit to the experimental retention times (with less than 1 % residuals for almost all samples) of PEGs are plotted against the molar mass. The values have been extracted from three gradient experiments.

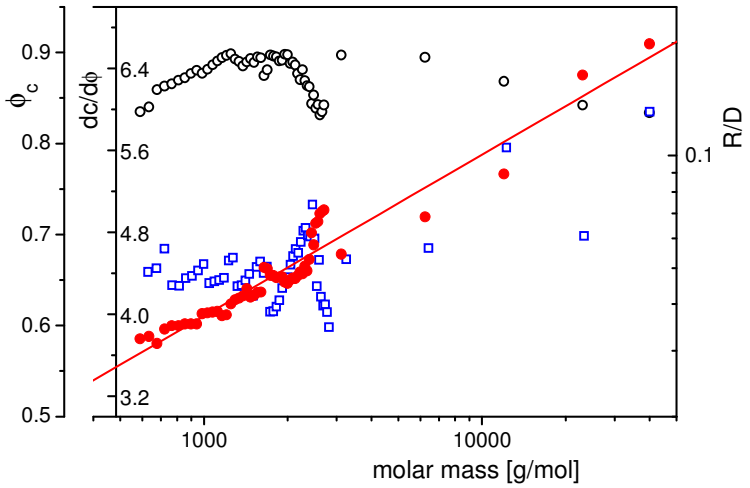


Figure 3.30: The PCM parameters, Φ_c (○), $dc/d\Phi$ (□), R/D (●), of PEGs extracted from three gradient experiments as calibration runs. Red line is a linear fit (log Y = -2.73 + 0.43 log X) to R/D versus molar mass plot.

As can be seen in figure 3.30, the parameter Φ_c (critical composition) clearly shows deviations, between 80 – 90 % MeOH in the eluent. The deviation of the critical composition predicted from gradient experiments as compared to the true critical eluent composition (17/83 v/v water/MeOH) is larger for low molar mass samples than for higher molar masses. The reason for the correct prediction of critical composition from high molar masses may be that high molar mass molecules elute in a gradient at a composition close to the critical one (section 3.1.2.1.2, figure 3.16).

One of the reasons for the observed scattering of the extracted Φ_c for low molar masses may be that the lower molar mass molecules during gradient elution experience only eluent compositions much lower than the critical one, corresponding to certain positive values of *cR* (figure 3.10, section 3.1.1.1). Therefore, the prediction of the critical composition requires extrapolation to zero value of *cR* over a significantly larger range of eluent compositions, as compared to high molar mass polymers. Consequently, depending upon the extent of experimental errors, the values of critical composition determined from low molar mass PEGs show larger errors in addition to scattering as compared to when determined from higher molar masses. Therefore, it may follow that the true critical composition can never be estimated accurately from low molar mass samples using only gradient experiments. However, the deviations of the critical composition are not very important for the predictions of retention of low molar mass polymer molecules. This is because the critical retention behaviour of low molar mass polymers is described by a range of eluent compositions instead of one specific eluent composition, i.e. there exists a critical region of eluent composition around the critical point [77]. The higher the molar mass, the narrower is the range of critical region. That means, for higher molar masses the critical point has to be determined more accurately. Fortunately, gradient elution of high molar mass polymers allows the extraction of true critical composition as they elute close to critical eluent composition.

The estimated values of *R/D* increase with molar mass. An exponent value of 0.42 is found for the scaling behaviour of *R/D* on *M*. This value is slightly lower than the expected value of 0.5 for a Gaussian coil. As will be discussed later (chapter 3.8), a good fit of experimental data can also be obtained for value of 0.5 or 0.6, indicating that the quality of the fit does not depend strongly on the value of the exponent. Although the absolute values of *R/D* can still be in error, the molar mass dependence of *R/D* indicates that the values of *R/D* extracted only from gradient experiments are not completely meaningless. For the parameter $dc/d\Phi$ no systematic variation with molar mass can be found. The values scatter significantly, indicating that reliable estimation of this parameter is not possible from gradient experiments only.

The scattering in the extracted parameters of PCM and the large errors of the isocratic predictions in LAC and SEC mode suggests that the parameters extracted

from only gradient experiments are not appropriate enough to predict the retention behaviour of PEGs comprehensively. This conclusion questions the general appropriateness of the PCM for polymer retention prediction. Therefore, in order to test further the suitability of PCM, it was fitted to all gradient and isocratic experiments for different molar masses of PEGs. The fitting errors are plotted as box-plots for different molar masses in figure 3.31. As can be seen, the PCM describes the retention for 50 % percent of the experiments with less than 2 – 3 % error. For 95 % of all experiments the errors are less than 5 % only. This shows that both gradient and isocratic retention data of PEG can be described by PCM with reasonable accuracy provided appropriate parameters have been selected.

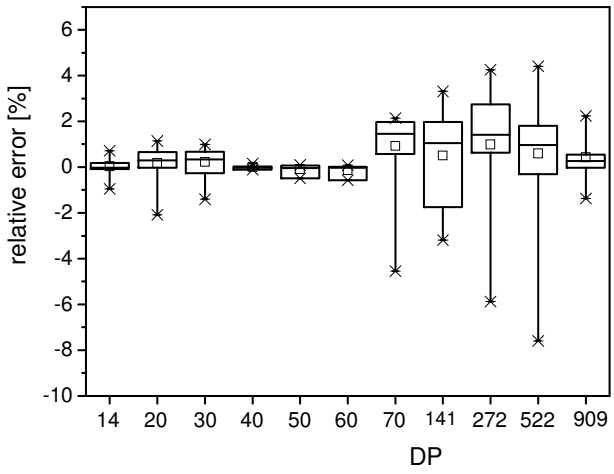


Figure 3.31: Box-plots of % deviations between PCM prediction and experiment for a variety of molar masses of PEG. Fitting was performed using all gradient and isocratic experiments for a particular molar mass.

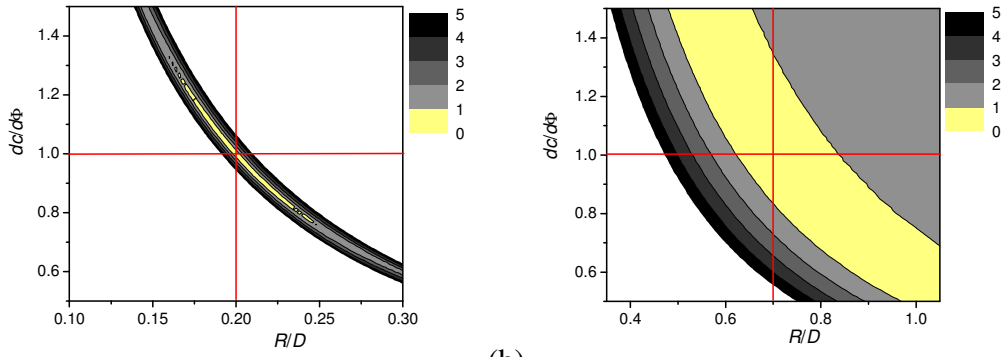
In order to test further whether reliable parameter extraction only from gradient experiments is possible, and to demonstrate the effect of the experimental errors or the non-linear fitting procedure, calculations with simulated data were performed. For this purpose, data for gradient and isocratic elution were simulated using the parameters values; $\Phi_c = 0.8$, $dc/d\Phi = 1$, and $R/D = 0.2$ - 0.7. The values of R/D used here represent the molar mass range of approximately 5000 – 60000 g/mol for PEGs (equation 3.1). The value of $dc/d\Phi = 1$ gives reasonable retention times for the used values of R/D. The column parameters were taken as; $t_i = 1$, $t_p = 2$, $D = 30$.

The parameters of the PCM were extracted from the error free simulated data of three gradient experiments (0 – 100 % strong solvent in 10, 30 and 60 minutes). It

was not possible to return the original parameters even after several iterations and initializations of the fitting process. The extracted parameters depend on the starting values used to initialize the non-linear fitting process. The higher the values of the original R/D parameter, the larger were the deviations of the extracted parameters from the original ones. However, the critical composition was extracted with reasonable accuracy for high *R/D* values in agreement with the experimental results (section 3.1.2.3, figure 3.26). The extracted parameter sets were used to predict the retention behaviour for assumed gradient and isocratic experiments. These predictions were compared with the retention behaviour simulated using the original parameters. It was found that the extracted parameters were good enough to predict gradient elution for both the small and large values of *R*/*D*. However, considerable deviations were found for the prediction of the isocratic retention behaviour, especially for high values of *R*/*D* that correspond to high molar masses.

The inability to return the original parameters back even from fitting the error free gradient data can be understood by looking at the error landscape of the model. The examples of the error landscape for one small and one high value of *R/D* parameter are given in figure 3.32. In the figure 3.32, the cumulative percent relative errors between the simulated retention times of three gradient runs and those obtained by variation of $dc/d\Phi$ and *R*/*D* for a fixed value of Φ_c, are plotted as a function of $dc/d\Phi$ and *R*/*D*. The original values of parameters were selected as $\Phi_c = 0.8$, $dc/d\Phi = 1$, and $R/D = 0.2$ in figure 3.32a and $\Phi_c = 0.8$, $dc/d\Phi = 1$, and $R/D = 0.7$ in figure 3.32b. Since critical composition can be predicted with reasonable accuracy from gradient experiments, the parameter Φ_c was fixed to original value. The values of $dc/d\Phi$ were varied between 0.5 – 1.5, while *R*/*D* varied from 0.1 – 0.3, and 0.35 – 1.05 in case of *R/D* = 0.2 and 0.7, respectively (±10 % deviation from the original value). The errors were calculated by comparing the calculated retention time with the retention times obtained from original parameters. As can be seen, there exist no sharp minima in the error landscapes obtained for both small and large *R*/*D* original values. Instead, each graph shows a broad valley corresponding to a range of $dc/d\Phi$, *R/D* pairs resulting in similar magnitudes of errors. Even for the value of Φ_c fixed to original, the valley in case of high *R*/*D* is very broad, i.e. there is a large number of $dc/d\Phi$ and *R*/*D* combinations giving cumulative errors up to one percent only. However, in case of high *R/D* values a variation of only 2 to 3 % of the original $dc/d\Phi$ and *R/D*

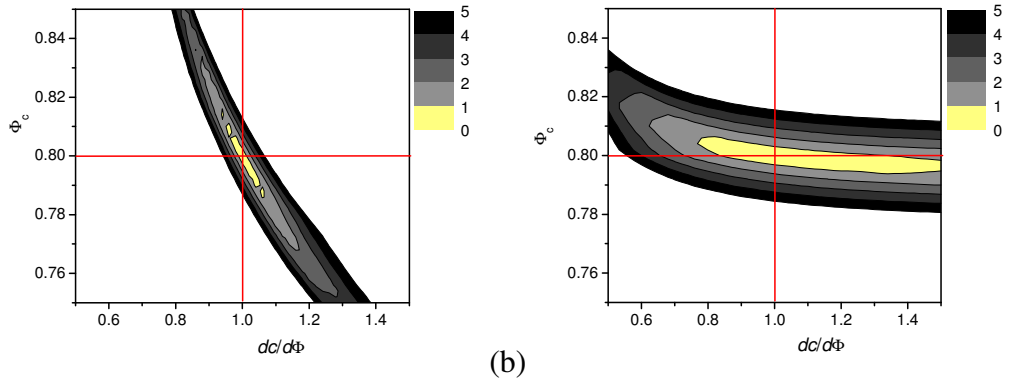
parameters, which resulted in cumulative errors of less than 1 % in case of gradient prediction (figure 3.32b), produces errors of up to 10 % for predictions of even moderate isocratic retention times. For *R/D* = 0.2 instead, the same variation of the original parameters causes errors of up to only 3 % when predicting isocratic retention times for the same range of eluent compositions.



(a) (b)

Figure 3.32: Error landscapes of PCM obtained from the three simulated error free gradient runs for two different values of *R/D* used for simulation (0.2 (a) and 0.7 (b) corresponding to low and high molar mass PEGs). The parameter Φ_c was kept constant (equal to 0.8) while the parameters *R/D* and *dc/d*Φ were varied. Red lines represent the original parameters. See text for details.

When the parameter *R/D* was fixed at the original values and Φ_c and *dc/d*Φ were varied, slightly different pictures were obtained (figure 3.33). As can be seen in figure 3.33, a relatively sharp valley is observed for the case of small original *R/D* value. However, still a large number of highly correlated Φ_c and *dc/d*Φ values describes the gradient elution equally well. This is in accordance with the experimental results (figure 3.30) that the true critical composition cannot be extracted from gradient experiments using the low molar mass samples. However, the inaccuracy of the extracted parameters is not very important for low molar masses as indicated by the good results obtained for the retention time predictions for oligomers both in gradient and isocratic mode using gradient calibration. For high original value of *R/D*, a broader valley is observed covering a very large range of *dc/d*Φ values for a small range of Φ_c. This result supports the observation that Φ_c can be easily determined from gradient experiments of high molar mass polymers. However, only gradient experiments are not suitable to extract the value of parameter *dc/d*Φ with reasonable accuracy. For high molar mass samples, even a small uncertainty in *dc/d*Φ (or *R/D*) parameter results in large errors in isocratic predictions.



(a) (b)

Figure 3.33: Error landscapes of PCM obtained from the three simulated error free gradient runs for two different values of *R/D* (0.2 (a) and 0.7 (b) corresponding to low and high molar mass PEGs). The parameter *R/D* was kept constant while the parameters Φ_c and $dc/d\Phi$ were varied. Red lines represent the original parameters. See text for details.

The results of the calculations given above clearly shows that the use of gradient runs only is not a good choice to extract the parameters of the PCM, even for error free retention data. The condition may become even worse when dealing with experimental data. Thus, it can be concluded that the errors in predictions of isocratic retention time for high molar masses may be mainly due to inaccurate parameter determination resulting from improper selection of calibration runs. The parameter extraction and hence the quality of the predictions of isocratic retention times might be improved by a proper selection of the starting experiments.

3.2.3 Improving the quality of PCM prediction – influence of initial runs

One way to reduce the errors in isocratic elution might be to use isocratic experiments for calibration, preferably over widely different eluent compositions. However, this is not possible for high molar mass polymers, because the elution is only possible very close to critical or in SEC conditions. Instead, combinations of gradient and isocratic experiments could be used for calibration. This may help to improve the quality of the extracted parameters. Therefore, in order to investigate how different starting experiments affect the quality of predictions, the PCM was applied to predict the retention times for a range of PEG standards using calibrations with different combinations isocratic and/or gradient experiments. The combinations of the initial experiments used for the calibrations, are tabulated below (table 3.1).

Table 3.1: Combination of different gradient and isocratic experiments for the prediction of PEG retention

Calibration Nr.	Gradient (t_G min.)[1]	Isocratic (water/MeOH v/v)[2]	Line code
1		20/80, 18/82, 10/90	———
2	30, 60, 90		– – –
3	30	20/80, 18/82	·····
4	30	20/80, 10/90	–·–·–
5	60	20/80, 10/90	–··
6	90	20/80, 10/90	–·–·–

[1] gradient experiments: 5 – 100 % MeOH against water linear over time given
[2] isocratic experiments: at given eluent compositions

In figure 3.34, the predictions for the retention times of PEGs of two different molar masses are compared with the experimental results. As can be seen, the predictions in gradient elution agree well with the experimental data, independent of the types of calibration experiments. This is in agreement with the previous results that the gradient elution can be accurately predicted. This is due to the fact that gradient retention of the high molar mass PEGs is mainly determined by the critical eluent composition. The excellent prediction of the gradient retention times shows that the used experiments are suitable enough to estimate this parameter accurately, except in the case where only isocratic experiments have been used. In contrast to gradient elution, clear differences between the predictions of the isocratic experiments from different calibration are observed for PEG 23000. While for PEG 40000 all calibrations result in similar predictions. The large deviations between the predicted and experimental curves of isocratic retention times are due to the abnormal behaviour of the PEGs at eluent compositions above 10/90 v/v water/MeOH. In almost all cases, best agreements of isocratic experiments (below 10/90 v/v water/MeOH) were obtained with calibration number 1 i.e. a set of three isocratic experiments (two isocratic experiments in the LAC, and one in the SEC mode). However, the use of this combination is not very practical for high molar mass polymer samples.

It can be seen in figure 3.34 that the results obtained using the combination of gradient and isocratic experiments are better than using only gradient experiments or only isocratic experiments for calibration. The combination 3 (see table 3.1), i.e. one gradient and two isocratic experiments seems to provide better predictions of the gradient and isocratic retention times for both molar masses. All other similar combinations (one gradient, one LAC and one SEC run) provide good predictions

only for PEG 40000. These results show that the suitability of any calibration using the mixed experiments cannot be clearly decided from the experimental data. However, the simulated data can be used to deal with this problem. In addition, there are no guidelines how to select the starting gradient and isocratic experiments. In the following discussion, guidelines for the selection of initial experiments are proposed and evaluated on the basis of simulated retention data.

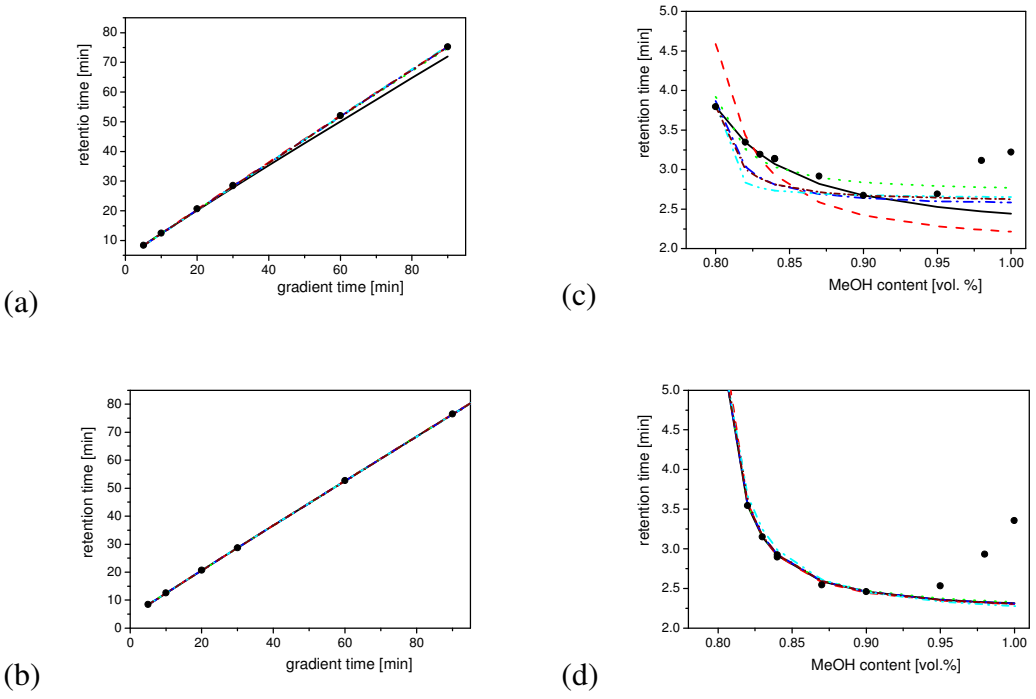


Figure 3.34: Comparison of experimental retention times (●) at gradient (a-b) and isocratic (c-d) elution with predicted retention times (see table 3.1 for line code) for different sets of starting experiments (calibrations). Samples: PEG 23000 (a and c) and PEG 40000 (b and d).

3.2.4 A protocol for the purposeful selection of calibration experiments

A purposeful approach for the selection of proper starting experiments for the PCM parameter extraction process is proposed in the following. The ease of the experiment suggests using gradient first. From the gradient experiment, an initial estimate of critical composition can be obtained. It can be assumed that isocratic experiments in the SEC and LAC mode are influenced to different degrees by the parameters R/D and $dc/d\Phi$. Thus, these two types of experiments should also be chosen for the calibration. The estimation of the eluent compositions, at which these experiments can be run, results from the first gradient experiment. Usually, the composition at elution is expected to result in weak LAC conditions (section 3.1.2.1.2 and section 3.3.1) while a slightly stronger eluent should result in elution in

SEC mode. In order to test the procedure for the purposeful selection of the starting experiments, simulations were accomplished.

For the simulation of retention data, the following parameters of the PCM were selected; $\Phi_c = 0.8$, $dc/d\Phi = 1$ and $R/D = 0.1$. The column parameters were selected as; $D = 30$ nm, $t_i = 1$ and $t_p = 2$ minutes. Using this set of parameters, "error free" retention times were calculated for different isocratic and gradient conditions. In order to simulate the effect of experimental uncertainties, errors taken from a Gaussian distribution having a 5 % standard deviation were added to the error free values. The so obtained retention times were treated as the "experimentally determined" data to test the protocol for selection of suitable set of initial experiments.

A linear gradient from 0 – 100 % of solvent B over 10 minutes was selected as the first starting experiment. The error free retention time was calculated to be 10.43 min., while the experimentally determined retention time was found to be 10.37 min. From the experimental retention time of the first experiment, the eluent composition at the time of elution was calculated to be 73.72 % B. The isocratic experiment performed at this eluent composition is expected to result in elution within a reasonable retention time in LAC conditions. Therefore, an isocratic experiment at 74 % B was simulated. The "error free" and "experimentally determined" retention times were 3.41 and 3.43 min., respectively. As expected, this experiment corresponds to LAC mode (since $t_R > t_i + t_p$). Therefore, the third experiment at an isocratic eluent composition only 3 % higher i.e. 77 % B is expected to result in elution in SEC mode. The "error free" and "experimentally determined" retention times were found to be 3.15 and 3.09 min. These retention volumes still correspond to adsorbing conditions. Using these "experimentally determined" data as the three initial experiments, the analyte specific parameters of PCM were extracted by non-linear fitting. The result obtained was $\Phi_c = 0.784$, $dc/d\Phi = 0.347$, $R/D = 0.225$. The residuals of the fitted curve were less than 0.5 %. Using these parameters, a prediction was made for an isocratic experiment that should result in retention time in SEC conditions ($t_R < t_i + t_p$). According to the extracted parameter Φ_c, eluent compositions of more than 78 % B were expected to result in SEC like elution. Therefore, an isocratic experiment at 82 % was predicted

and the retention time compared with the "experimentally determined" value. The predicted retention time was 2.79 min. as compared to the "experimentally obtained" 2.98 min. (error free 2.93 min.). Thus, a reasonable agreement (with error -6.3 %) was found between the experiment and the predicted values using the proposed approach. This run can be used to improve further the quality of the extracted parameters. Thus, the fitting process was restarted with four runs. Excellent agreement between the extracted ($\Phi_c = 0.805$, $dc/d\Phi = 1.033$, $R/D = 0.091$) and original parameters is found just after the fourth runs that allows for the reliable prediction at other experimental conditions. Figure 3.35 shows a comparison of the theoretical i.e. "error free" and the predicted (based on three experiments) dependence of elution volume on % B along with the "experimentally determined" data used for predictions. The line predicted using only the gradient calibration (three gradients of t_G 10, 20, and 40 min.) is also shown for comparison.

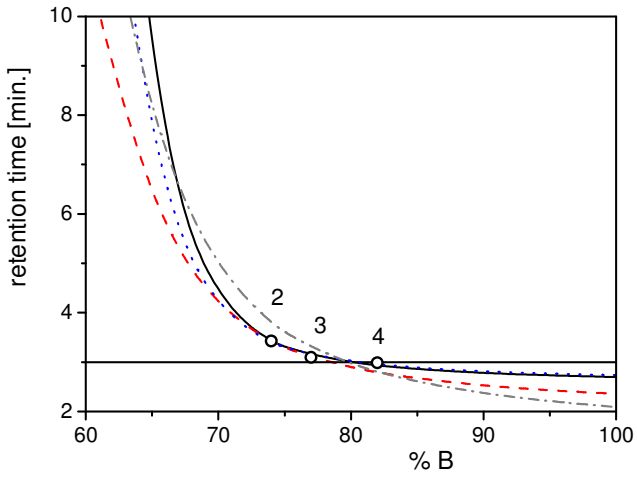


Figure 3.35: Comparison of true (black solid) and predicted retention time as a function of % B for different sets of calibration experiments; three gradients (grey dotted-dashed), one gradient (t_G = 10 min.) and two isocratic experiments (red dashed), one gradient and three isocratic runs (dotted blue). The solid symbols represent the "experimental" data points. The numbers represents the order in which experiments were performed. Solid horizontal line represents the t_0.

It can be observed that the estimate of the critical composition from three gradient runs is reasonably good. The predictions in both the LAC and SEC mode are worse. Considerable improvements can be observed by including isocratic experiments. Only at the stronger adsorption conditions some deviations are seen which may be further reduced if another isocratic experiment may be performed at suitable conditions of eluent composition. The suitable conditions, which should allow for

elution within reasonable time, can be estimated from the preceding experiments using PCM.

Based on above results, the following procedure represents a systematic approach to select suitable initial experiments.

1. Run a linear gradient and determine the eluent composition at the time of elution
2. Perform an isocratic run at the composition determined in step 1
3. If step 2 results in an elution under adsorbing conditions, perform a third run using a slightly stronger eluent
4. If step 2 results in a run under SEC conditions, perform a third run at slightly weaker eluent.

In order to prove further that the suggested selection of initial experiments is a suitable choice for the PCM parameter extraction, the error landscape of PCM based on the suggested combination of initial experiments was constructed. For this purpose, the same set of parameters as in figure 3.32b was used to simulate error free retention times for one gradient and two isocratic experiments that were purposefully selected as proposed above.

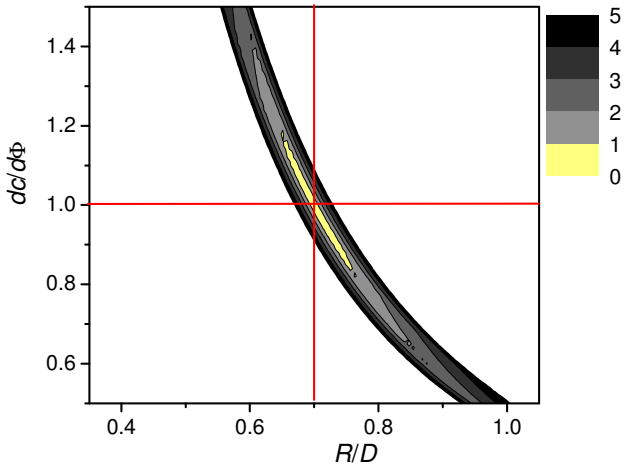


Figure 3.36: Error landscapes of PCM obtained from the three simulated error free experiments (0 – 100 % B) and isocratic (at 79 and 82 % B) runs. The parameters values: $\Phi_c = 0.8$, $R/D = 0.7$, $dc/d\Phi = 1$, $t_i = 1$, $t_p = 2$, $D = 30$. The parameter Φ_c was kept constant while the parameters R/D and $dc/d\Phi$ were varied up to ± 10 % of the original value. Red lines represent the original parameters.

Figure 3.36 presents the error plot obtained for varying parameters $dc/d\Phi$ and R/D at a fixed value of the parameter Φ_c. As can be seen, the valley of $dc/d\Phi$ and R/D values giving up to 1 % error is greatly reduced as compared to that in figure 3.32b where only gradient experiments were used. In other words, the combination of gradient and isocratic experiments significantly reduces the number of parameter sets describing the experimental data, making the parameter extraction more reliable.

3.3 Retention behaviour of poly(methyl methacrylate)s

The previous chapters have shown that PCM is the most appropriate among the studied models not only for the description but also for the prediction of retention behaviour of PEGs. However, it is not yet clear whether this model is suitable for the description and prediction of retention behaviour of homopolymers in general. Therefore, the studies were extended to other homopolymers. For this purpose, poly(methyl methacrylate)s (PMMAs) were chosen. Retention behaviour of PMMA standards of a range of molar masses was determined on a normal phase column (column B, see experimental section) at 35°C using a mobile phase system composed of toluene and THF as adsorption and desorption promoting eluent components, respectively. Since gradient elution should be the first choice while dealing with the high molar mass polymers, the following discussion evaluates the PCM for gradient elution of PMMAs.

3.3.1 Gradient elution of PMMAs and PCM

Figure 3.37 shows the chromatograms of PMMA standards of different molar masses obtained using a linear 20 minutes gradient from 100 % toluene to 100 % THF. As expected, low molar mass polymer samples elute earlier than high molar mass samples. It can be seen that the difference between the retention times (determined from peak maxima) of adjacent peaks is larger for lower molar mass samples than for the higher molar masses and the peaks of the PMMAs having molar masses of 530000 g/mole and 700000 g/mole elute practically at the same retention time. Thus, the same behaviour is observed for both PEG and PMMA, which indicates that this behaviour is general for homopolymers in linear gradients.

The dependence of the retention time on molar mass in gradient chromatography of PMMA is shown in figure 3.38. It is evident that an elution nearly independent of molar mass is observed for molar masses above approx. 200000 g/mole. The right axis of figure 3.38 shows the composition at elution calculated using equation 3.5 (section 3.1.2.1.2) versus the molar mass of the PMMA standards. Clearly the higher molar masses elute at nearly identical mobile phase compositions (63/37 v/v toluene/THF), irrespective of their molar mass.

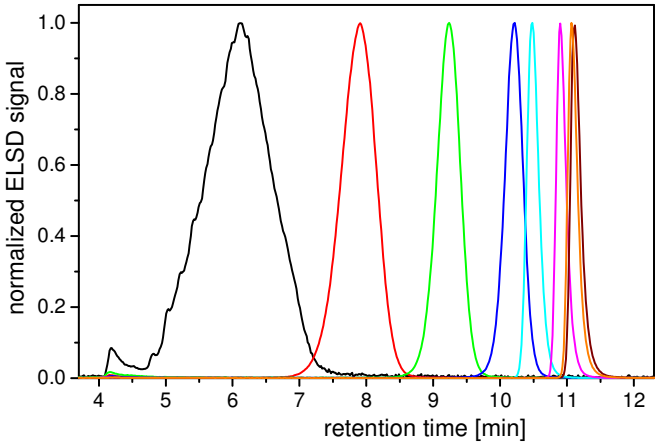


Figure 3.37: Overlay of chromatograms obtained for PMMA standards of different molar masses, M_p = 1020 (black), 3500 (red), 10900 (green), 30500 (blue), 60000 (cyan), 240000 (magenta), 530000 (brown), 700000 g/mol (orange) in a 20 minutes linear gradient of 100 % toluene to 100 % THF. Column: B (see experimental section); Temperature 35°C; flow rate: 1 mL/min.

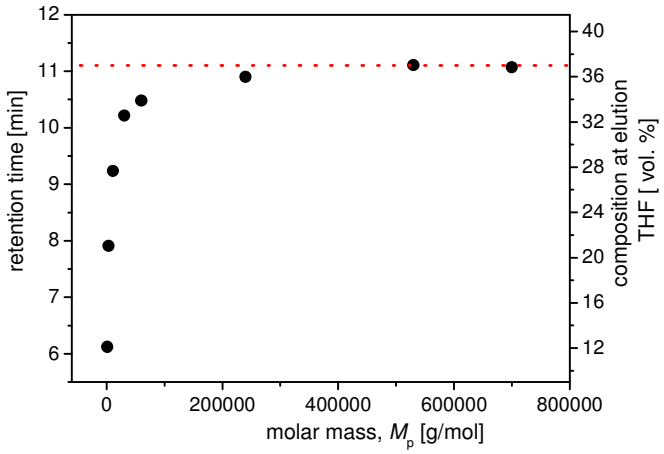


Figure 3.38: Retention time and composition at elution (% THF) at peak maximum as a function of molar mass of PMMA standards. The dotted line shows the critical composition as obtained by isocratic experiments. Other chromatographic conditions same as in figure 3.37

According to the arguments given in the discussion of PEG elution behaviour, this composition should be very close to the critical mobile phase composition. In order to prove this, the critical composition of PMMA was determined conventionally by isocratic experiments in different mobile phase compositions using PMMA standards having different molar masses. The molar mass dependences of the retention times in different mobile phase compositions are given in figure 3.39.

Clearly, the transition from adsorbing conditions (in THF < 37 %) to size exclusion conditions (in THF > 37 %) can be realized at a mobile phase composition around 63/37 v/v toluene/THF. The critical composition so obtained is also represented by

the dotted line in figure 3.38, which appears to be the limiting composition at gradient elution. This is in agreement with the published results of Brun et al. [85] and for PEGs (section 3.1.2.1.2). It will be shown later that this phenomenon allows for the fast estimation of critical mobile phase composition (section 3.4).

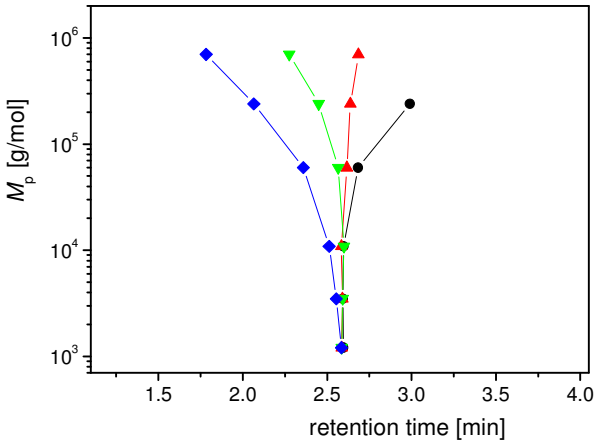


Figure 3.39: Molar mass dependence of retention times for PMMA in different isocratic mobile phase compositions, 0/100 (◆), 62/38 (▼), 63/37 (▲) and 64/36 (●) v/v toluene/THF. Other chromatographic conditions same as in figure 3.37

In order to determine the effect of gradient slope on the composition at elution, the gradient times were varied keeping all other parameters constant. Figure 3.40 shows the dependence of the composition at elution on gradient slope for various molar masses of PMMA standards. It is evident that an increase in gradient slope results in a higher amount of the desorption promoting mobile phase component. This dependence of composition at elution on gradient slope vanishes for high molar masses.

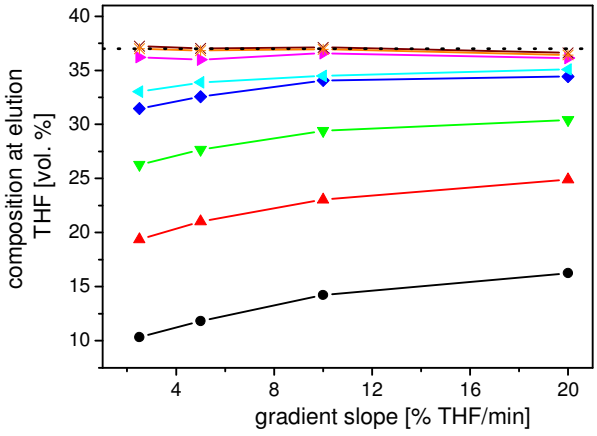


Figure 3.40: Composition at elution (% THF in toluene) as a function of gradient slope for PMMA standards of different molar masses, M_p = 1020 (●), 3500 (▲), 10900 (▼), 30500 (◆), 60000 (◀), 240000 (▶), 530000 (×), 700000 g/mol (✳). The dotted line shows the critical composition as obtained by isocratic experiments. Chromatographic conditions same as in figure 3.37

The dependence of composition at elution on the gradient slope can be explained as follows: The polymer molecules, depending on their molar mass, start moving before they are caught up by the critical mobile phase composition, which is somewhere in the gradient profile behind the sample molecules. However, the velocity of the polymer molecules is lower than that of the mobile phase. In order to catch the sample molecules by the critical mobile phase composition within the column, the gradient slope has to be suitable. Otherwise, the polymer molecules are fast enough to elute from the column before they are caught up by Φ_c. This happens in the case of lower molar mass polymer molecules, which are already desorbed at lower eluent strength than the higher molar masses. Thus, the critical mobile phase composition in a gradient cannot surpass the polymer molecules before they exit the column. However, with an increase in gradient slope the composition at elution becomes closer to the actual critical mobile phase composition. This is because the time required by a certain mobile phase composition to reach the polymer molecule is shorter in a gradient of higher slope than in a gradient of lower slope. As the molar mass becomes higher, the migration of the sample molecules starts at a mobile phase composition closer to the critical one. Thus, the critical mobile phase composition may catch up with the polymer somewhere within the column. When this happens, the elution of the molecules occurs at the critical mobile phase composition. In this case, the gradient slope has practically no effect on the composition at elution. As it will be shown later, this behaviour can be used to quickly determine the critical eluent composition.

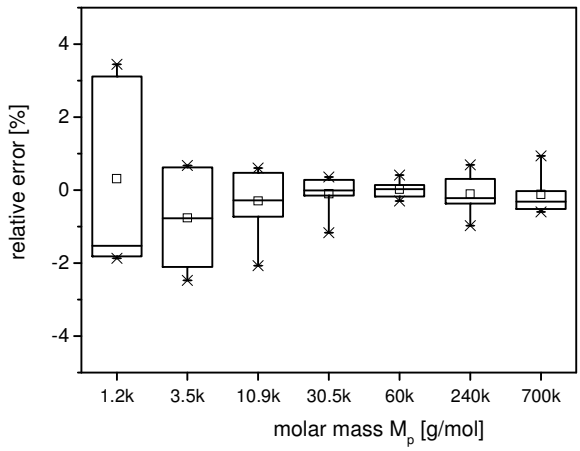


Figure 3.41: Comparison of box-plots of the % deviations of fitting the PCM to all the available gradient and isocratic retention data of PMMA standards having different masses.

In order to investigate the general suitability of the PCM to describe the complete retention behaviour of PMMAs, the PCM was fitted to all isocratic (except 0/100 v/v toluene/THF) and gradient retention data for every PMMA standard investigated. The residuals of the fit are given in figure 3.41 as box-plots. As can be seen, the residuals for all samples are less than 4 %. This shows that the PCM can be used to describe accurately the retention behaviour of PMMA of any molar mass in gradient as well as isocratic modes of elution.

3.3.2 Prediction of retention behaviour of poly(methyl methacrylates)

After testing the PCM to describe the retention of PMMAs, the suitability of the model to predict the retention behaviour of PMMAs was evaluated.

3.3.2.1 Gradient to gradient prediction

In order to verify the results obtained for PEGs, first the predictions of the gradient retention times were carried out using the gradient calibration. That means linear gradients ranging from 0 to 100 % THF against toluene within 10, 20 and 40 minutes were used for the parameter extraction. After extracting the model parameters (Φ_c, $dc/d\Phi$, R/D) for each sample, the predictions of retention times for other gradient conditions were made and the deviations from real experiments were determined as percent relative errors.

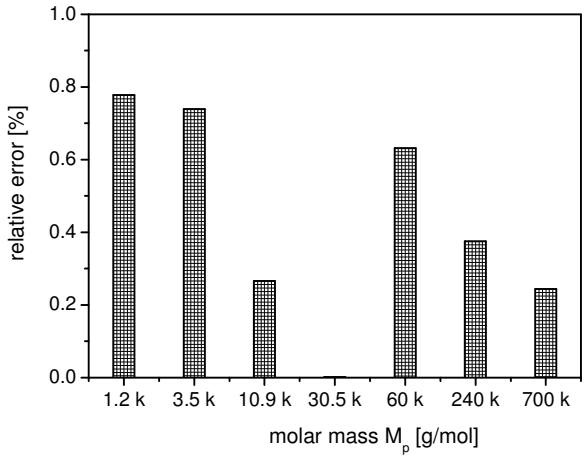


Figure 3.42: Comparison of the % relative deviations for the gradient to gradient prediction using the PCM. Prediction: 100 % toluene – 100 % THF gradient linear in 5 minutes. Calibration: 100 % toluene – 100 % THF linear gradients over 10, 20, and 40 minutes.

Figure 3.42 compares the deviations of the model predictions from the experimentally found retention times for a linear gradient over 5 minutes for

different molar masses of PMMA. As expected from the results of PEGs, only minute errors, i.e. less than 1 %, are found for the prediction of all molar masses.

3.3.2.2 Gradient to isocratic prediction

The same parameters of the model used for gradient calculations were also used for predicting the retention times for PMMAs at different isocratic eluent compositions. When the predictions are compared with the experimentally obtained retention times, errors up to 15 % are found (figure 3.43). In figure 3.43, no errors are shown for PMMA 240k and 700k at eluent composition of 0/100 v/v toluene/THF and for PMMA 700k at 67/36 v/v toluene/THF. The reason for 0/100 v/v toluene/THF is the inability to obtain any value for retention time from the used set of parameters, while for 67/36 v/v toluene/THF it was not possible to obtain representative experimental retention time due to incomplete elution. The somewhat larger errors obtained in other cases are in agreement with the results obtained for PEGs. It was found that the gradient experiments alone are suitable to estimate the parameter Φ_c only. The other two parameters ($dc/d\Phi$, R/D) extracted from just the gradient experiments were not suitable for isocratic retention times (section 3.2.2). The higher errors in predictions of isocratic retention times for PMMAs further support the earlier observations. It can be concluded that the calibration only with gradient experiments is not suitable for the prediction of isocratic retention times of polymers in general.

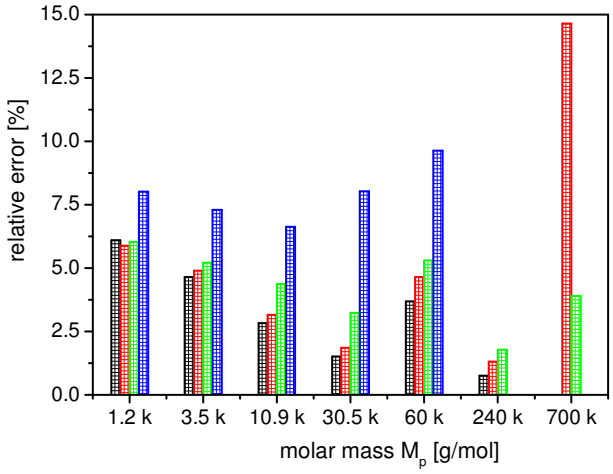


Figure 3.43: Comparison of the % relative deviations for the gradient to isocratic retention predictions using the PCM at different mobile phase compositions, 64/36 (black), 63/37 (red), 62/38 (green), 0/100 v/v toluene/THF (blue). Calibration same as in case of figure 3.42

As has been shown, the errors in prediction arising from the fitting and from experimental errors can be reduced by a proper selection of initial experiments.

Therefore, for the case of PMMAs also, the initial experiments were purposefully selected to apply the PCM for isocratic retention predictions. Thus, when two isocratic experiments are used along with just one gradient experiment for the PCM parameter extraction, the isocratic predictions are significantly improved for the experiments in LAC or SEC depending upon the selection of the experimental runs. Although only one gradient experiment is used for this calibration of the PCM, the gradient predictions are not affected very much. The results of the predictions using this calibration are given in table 3.2 and 3.3.

Table 3.2: Gradient (100 % toluene to 100 % THF linear) retention times of PMMAs predicted by PCM, using the calibration of one gradient (100 % toluene to 100 % THF linear in 20 min.) and two isocratic experiments (at 63/37 and 62/38 v/v toluene/THF), in comparison to experiment

t_G[1]	5 min.		10 min.		40 min.	
M_p (g/mol)	Predicted (min.)	% deviation	Predicted (min.)	% deviation	Predicted (min.)	% deviation
1200	4.64	2.88	5.24	2.14	7.34	-6.26
3500	5.01	1.21	6.07	0.10	11.08	-3.23
10900	5.25	0.57	6.65	0.15	14.04	-1.27
30500	5.43	0.18	7.07	-0.56	16.23	-0.37
60000	5.45	-0.18	7.17	0.28	16.9	-0.12
240000	5.5	-0.18	7.33	-0.41	17.83	-1.98
700000	5.49	-0.54	7.35	-0.66	18.42	-0.49

[1] Gradient time

Table 3.3: Isocratic retention times of PMMAs predicted by PCM, using the calibration of one gradient (100 % toluene to 100 % THF linear in 20 min.) and two isocratic experiments (at 63/37 and 62/38 v/v toluene/THF), in comparison to experiment

Toluene/THF	64/36 v/v		62/38 v/v		0/100 v/v	
M_p (g/mol)	Predicted (min.)	% deviation	Predicted (min.)	% deviation	Predicted (min.)	% deviation
1200	2.59	-0.38	2.58	-0.39	2.48	-3.87
3500	2.61	0.77	2.58	-0.39	2.47	-3.14
10900	2.62	0.77	2.58	-0.77	2.45	-2.39
30500	2.68	0	2.6	-0.38	2.44	-0.41
60000	2.68	0	2.57	0	2.41	2.12
240000	2.91	-2.68	2.45	0	1.82	-12.08
700000	4.23	--	2.28	0	--	--

From the above results, it can be concluded that the PCM can be used to predict the complete retention behaviour of both PEGs and PMMAs equally well. The prediction of isocratic retention times can be improved by the purposeful selection of calibration experiments. This shows that the PCM is an appropriate model to describe and predict the retention behaviour of homopolymers in general.

3.4 Fast estimation of critical eluent composition for polymers using gradient experiments

It follows from the discussions in the preceding chapters that gradient elution, in general, yields the information about the eluent compositions, which allows for the isocratic elution of homopolymers in adsorbing conditions. Since the composition at elution for a high molar mass polymer sample approaches the critical eluent composition, it provides an excellent estimate of the critical composition. Thus, gradient elution of high molar mass polymers might be used to determine the critical eluent composition of a polymer stationary/mobile phase system rapidly and efficiently. Establishing critical conditions otherwise is a very time consuming task. In the following, the application of this method to determine the critical compositions for different polymer stationary/eluent systems is discussed.

Since high molar mass polymers elute close to the critical eluent composition, different gradient experiments having linear slopes were evaluated for the highest molar mass PMMA sample (700000 g/mol) as an example. The composition at elution was calculated from the retention times using equation 3.5. Since the composition at elution may vary with the gradient slope, the gradient of highest slope was chosen to estimate the critical composition. In the present case, however, the gradient slope has no effect on the composition at elution (figure 3.40, section 3.3.1). Thus, this composition is expected to be very close to critical. Next, isocratic experiments were performed with a minimum of three molar mass standards at the eluent composition calculated from the gradient experiments. In addition, isocratic experiments were performed using eluents with one or two percent more or less of the desorbing component (THF) than the composition at elution. The results of these three runs were then used to determine the exact critical composition by Cools' plotting method [120]. In a Cools' plot, the retention times are plotted against the eluent composition. The critical eluent composition is determined from the point where the curves of all molar masses intersect. The Cools' plot for PMMA is given in figure 3.44. The lines for different molar masses intersect close to the composition at elution (62/37 v/v toluene/THF), indicating a good agreement between the estimated and the exact critical eluent composition (62.8/37.2 v/v toluene/THF). The

critical eluent composition found here coincides nicely with results of Berek in a similar system [121].

As can be seen, an almost molar mass independent elution occurs for a composition 63/37 v/v toluene/THF (see also figure 3.39, section 3.3.1), which is identical to the composition at elution calculated from the gradient experiments. In 62/38 and 64/36 v/v toluene/THF, size exclusion and strong adsorption behaviour is observed, respectively. It should be noted that PMMA 700k could not be eluted isocratically in 64/36 v/v toluene/THF due to the strong adsorption.

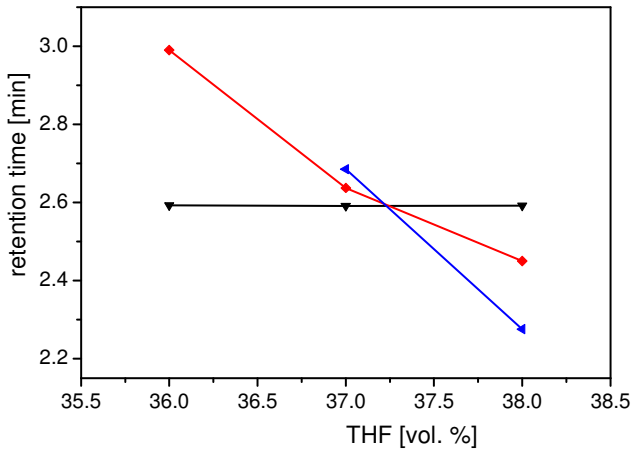


Figure 3.44: Dependence of retention time on mobile phase composition for PMMAs with molar masses, $M_p = 3500$ (▼), 240000 (◆), 700000 g/mol (▲),(Cools Plot). Other chromatographic conditions same as in figure 3.37 (section 3.3.1)

The same approach was used on a reversed phase column (column A, see experimental section) for PEG using MeOH/water. PEG of the highest molar mass available (40,000 g/mole) was used to get an estimate of the critical composition. Since the molar mass of used PEG is relatively low, three linear gradients were used to determine the effect of gradient slope.

As can be seen in figure 3.45, the composition at elution varies only slightly with gradient slope. According to the forgoing discussion, the composition at elution for the fastest gradient (17.4/82.6 v/v water/MeOH) is expected to be closest to the actual critical eluent composition. Thus, a composition of 83 % MeOH in water was taken to perform the first isocratic experiments with three different PEG standards, which elute independent of molar mass at this composition (figure 3.46). Thus, the critical composition determined by isocratic experiments is in excellent agreement with the estimation from the gradients. In addition, runs were performed at 20/80 and

10/90 v/v water/MeOH, which, as expected, resulted in adsorption and exclusion mode, respectively. The dependence of elution volume on eluent composition for the PEGs chosen is depicted in figure 3.46.

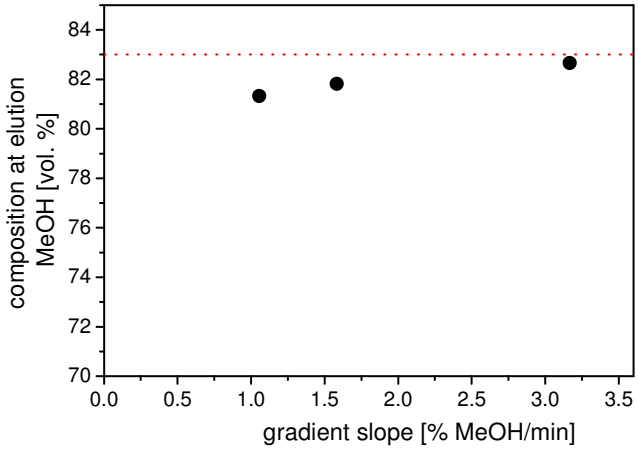


Figure 3.45: Composition at elution (% MeOH) of PEG, 40000 g/mole as a function of gradient slope (●). The dotted line shows the estimated critical composition. Other chromatographic conditions same as in figure 3.1 (section 3.1.1)

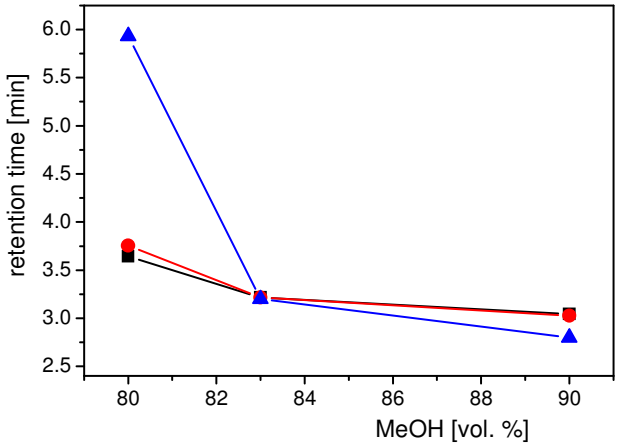


Figure 3.46: Dependence of retention time on mobile phase composition for PEG with molar masses, M_p = 12000 (■), 23000 (●), 40000 g/mol (▲), (Cools Plot). Other chromatographic conditions same as in figure 3.1 (section 3.1.1)

Finally, in order to further validate the approach, determinations of critical compositions for PMMA, poly(*n*-butyl methacrylate) (PnBMA), poly(*t*-butyl methacrylate) (PtBMA) and poly(decyl methacrylate) (PDMA) on a monolithic silica column (column C, see experimental section) were performed using the same procedure. The results are given in table 3.4. Again very good agreement is found between the compositions at elution and the critical compositions obtained from

isocratic runs. Only in the case of PnBMA a difference above 4 % is observed. As expected, with the exception of PDMA all compositions at elution are slightly below the critical composition.

Table 3.4: Critical compositions of eluent (% volume of strong eluent) estimated from gradients in comparison with actual critical composition determined by isocratic measurements

Polymer	column	Molar Mass (*Mp* (g/mol))	Estimated composition (% THF)	Found composition (% THF)	Difference (% THF)
PMMA	Column B	700000	37.00	37.20	0.2
PEG	Column A	40000	82.60	83.00	0.4
PMMA	Column C	296000	70.40	70.60	0.2
PnBMA	Column C	240000	13.55	17.90	4.4
PtBMA	Column C	618000	16.95	18.60	1.7
PDMA	Column C	598000	5.37	4.00	1.4

Closer inspection of table 3.4 reveals that for lower amounts (< 50%) of the strong eluent component the deviations between the composition estimated from gradients and the true critical compositions are higher than for higher ones (> 50%). In order to determine the composition at elution with an error of 1% the retention time has to be determined with the same accuracy, which becomes more difficult at lower retention times. In addition, the larger errors are found for the different poly(methacrylates) with gradients running from cyclohexane to MEK. Cyclohexane is a non-solvent for the poly(methacrylates), which results in precipitation of the polymer at the beginning of the gradient. As discussed by Brun [78] as long as the composition at the precipitation threshold is weaker than the critical composition, gradient elution still results in a composition at elution close to the critical one. However, if solubility can only be achieved above the critical composition, critical conditions cannot be found for the system under investigation.

In our systems, critical conditions could be established by isocratic runs indicating that the precipitation threshold must be lower than the critical composition. However, if redissolution during the gradient is slow and the critical composition is close to the precipitation threshold, the polymer might elute at a composition higher than the critical one. The kinetics of dissolution therefore might be responsible for the unexpectedly higher percentage of MEK at the composition at elution found for poly(decyl methacrylate). It may also attribute to the larger errors observed at low percentages of the strong solvent.

Despite these problems, the good agreement between the compositions at elution and the critical composition illustrates the efficiency and accuracy of the present approach. Based on the above given results, the following general strategy of finding the critical composition is, therefore, proposed;

1. Run one to three linear gradients with different slopes, e.g. 0 – 100 % strong eluent component in 10, 20, and 40 min for a single high molar mass sample and calculate the composition at elution.

2. Perform isocratic runs with a minimum of three standards at the calculated highest composition at elution and at a composition a few percent higher in the strong eluent. If a strong dependence of composition at elution on the gradient slope is observed in step 1, then the difference between the composition at elution and that for the second isocratic run should be larger than if a weak dependence is observed.

3. Plot the elution volume versus isocratic eluent composition for the different molar masses (Cools plot). The eluent composition at the intersection point corresponds to the critical composition. One more isocratic experiment can be performed at this eluent composition to verify the results.

3.5 Virtual chromatography for the separation of homopolymer blend

The ability of the PCM to predict the retention behaviour of different homopolymers in different modes of liquid chromatography suggests that the model can be used to simulate real chromatographic separations of homopolymers. In order to verify this hypothesis, a model mixture composed of poly(n-butyl acrylat) (PnBA), poly(t-butyl acrylat) (PtBA) and two PMMAs of different molecular weights was selected (table 3.5). It was tested whether virtual chromatography (VC) can be used to predict suitable conditions for the separation of this blend on a normal phase column (column B).

Table 3.5: The homopolymers and their molar masses used as the components in a model blend

Sample	Polymer	M_p (g/mole)	Colour code
PnBA 22.6k	Poly(*n*-butyl acrylate)	22600	Black
PtBA 210k	Poly(*t*-butyl acrylate)	210000	Red
PMMA 24.4k	Poly(methyl methacrylate)	24400	Green
PMMA 263k	Poly(methyl methacrylate)	263000	Blue

As mentioned earlier, for the separation of samples composed of components having widely different interaction strengths gradient elution is the practical choice. Thus, a gradient run of 100 % toluene to 100 % THF in 10 minutes was performed as a starting experiment for each component. The overlay of the chromatograms of the components is shown in figure 3.47. As can be seen, the PMMAs of different molar masses are well separated indicating a strong molar mass dependence of retention time in this gradient. PnBA and PtBA being less polar than PMMA elute earlier in the same gradient. The two polyacrylates are eluting at only slightly different retention times forming the critical peak pair (defined as the two peaks with the least resolution in a chromatographic separation). Therefore, the selectivity at the given chromatographic conditions, defined by t_R^{PtBA}/t_R^{PnBA}, is too low to result in a useful separation. In order to improve the resolution, the selectivity has to be increased. In addition, the separation of the PMMAs may also be tailored. How this could be done is analyzed using virtual chromatography. For this purpose, two additional experiments are required.

For a rational selection of these experiments, the composition at gradient elution for each of the components was calculated from the first gradient experiment. Since the

composition at elution for PtBA and PnBA were very close to each other (4.0 and 4.7 % THF in toluene, respectively), the second run performed for these components was a linear gradient from 3 – 8 % THF against toluene in 5 minutes, which covers an eluent range around the composition at elution of both components. The third experiment was performed isocratically at 5 % THF for both PtBA and PnBA

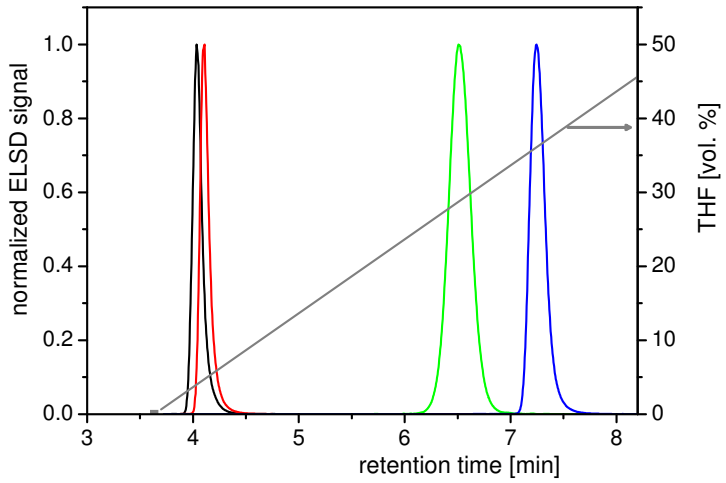


Figure 3.47: Overlay of chromatograms of the components of the model mixture in a 100 % toluene – 100 % THF linear gradient over 10 minutes. See table 3.5 for colour code. Grey line is the composition profile at detector. Column: B (see experimental section); Temperature: 35°C; Flow rate: 1 ml/min.; detection: ELSD.

For PMMA 24.4k and PMMA 263k, the second experiments were performed isocratically at 29 (composition at elution 28.8 % THF) and 36 % THF (composition at elution 36.1 % THF), respectively. The third run for both PMMAs was carried out isocratically at 40 % THF. The retention times obtained from these experiments were put into the spreadsheet program along with the corresponding values of variables and the parameters of PCM for each component were extracted using Origin's fitting tool. The critical eluent composition as obtained from fitting was 3.5 % THF in toluene for PnBA, 4.8 % THF for PnBA and 34.8 % THF and 34.9 % THF from PMMA 24.4k and PMMA 263k, respectively.

Once the model parameters were at hand, "trial and error" experiments were performed on a computer to increase the separation between the poly(butyl acrylates). It is known, that the selectivity in gradient chromatography can be increased by decreasing the gradient slope just as it can be done by decreasing the eluent strength at isocratic elution. Therefore, the retention times for each component

were calculated for linear gradients of different slopes. A difference of one minute between the peak positions of PnBA (at approx. 7 min.) and PtBA (at approx. 8 min.) was predicted for an 80 minutes linear gradient from 100 % toluene to 100 % THF. In this gradient, the last PMMA peak is expected to elute at approximately 32 minutes. This shows that a separation of components can be obtained by using flatter linear gradient but at the cost of a long analysis time. Another disadvantage of the flatter gradients is that they result in broadening of the peaks due to the more pronounced molar mass selectivity in gradients of lower slopes. This may cause difficulties in separation and detection. The effect of molar mass on the peak widths and hence on the separation will be discussed in chapter 3.8.

A better solution is to use gradients of different shapes. A gradient program may be composed of either linear gradient steps with different slopes or the isocratic steps. Designing an optimum gradient program involves time-consuming “trail and error” experimentation, which however, can be easily handled via computer using virtual chromatography. Thus, in order to achieve appropriate separation of PnBA and PtBA and to optimize the retention times for the PMMAs, the selection of the initial composition of the gradient and the shape of the gradient was chosen by “trial and error” experiments on the computer. One multi-step gradient that predicts a slightly better resolution of the critical pair is given in figure 3.48 as an example of the computer based “trial and error” approach.

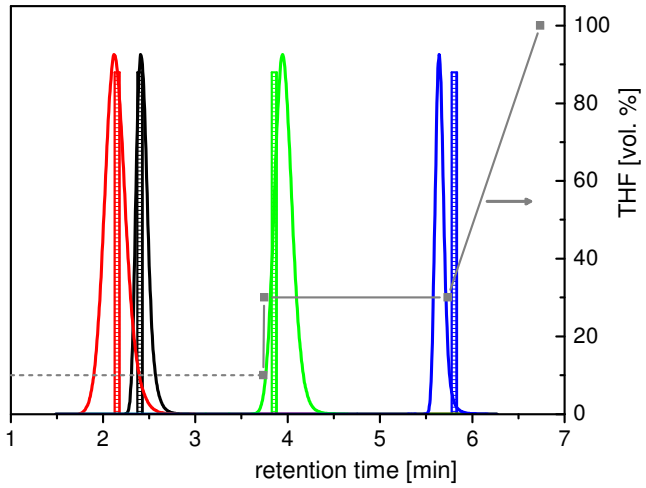


Figure 3.48: Separation of blend of different polyacrylates and PMMAs predicted using PCM (vertical bars) as compared to experiment (peaks). See table 3.5 for colour code. Gradient profile: 0 min 10 %, 0.1 – 2 min 30 %, 3 min 100 % THF against toluene. Grey lines represent the composition profile at the detector. Other condition same as in figure 3.47.

As can be seen, the first two peaks are expected to elute isocratically within the initial mobile phase composition at the retention times smaller than the column dead time. Note the reversal of the peaks as compared to the initial gradient experiment (figure 3.47). This is because these peaks are now eluting in SEC mode. The proposed gradient should also result in a reduction of the analysis time. The chromatograms of the components in the real experiments using the proposed gradient are also shown for comparison. One can see, there is good agreement for the retention times of the prediction and the experiment, with errors in retention times less than 3 % only. However, the separation of critical peak pair is not sufficient due to the broadening of the peaks in the real experiment. The simulation of peak broadening effects will be discussed later in chapter 3.8.

By further experimentation on the computer using the PCM, a separation of all components with approximately the same peak distance can be obtained. The gradient required a profile composed of isocratic steps as shown in figure 3.49. The experimentally obtained chromatograms of the components for the proposed gradient are given for comparison. As can be seen, a base line separation of all the components can be achieved in reality. There is an excellent quantitative agreement between the predicted and experimental retention times with the exception of the second peak where the error is up to 6 %. Errors in retention times for all other components were less than 2.2 %.

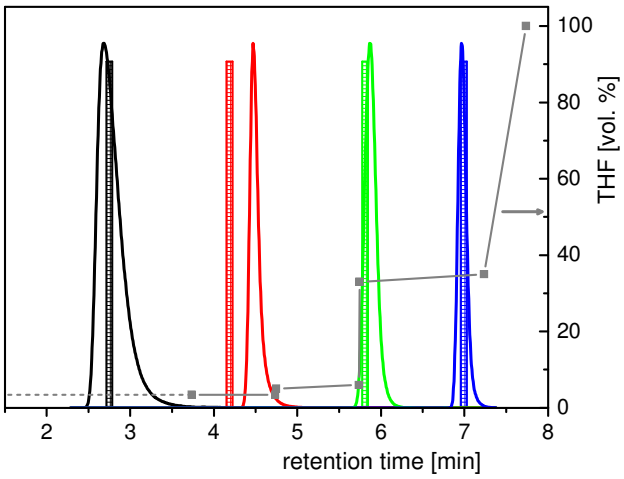


Figure 3.49: Optimized separation of the model blend of different polyacrylates and PMMAs predicted by PCM (vertical bars) in comparison to experiment (peaks in overlaid chromatograms). See table 3.5 for colour code. Gradient profile: 0 – 1 min 3.4 %, 1.1 min 5 %, 2 min 6 %, 2.1 min 33 %, 3.5 min 35 %, 4 min 100 % THF against toluene. Grey lines represent the composition profile at the detector. Other condition same as in figure 3.47.

The above given separation was achieved based on only three purposefully selected initial experiments. This example illustrates the advantage of the computer-assisted method development over the generally followed empirical “trial and error” approach. Thus, the virtual chromatography approach can be used to optimize separations of homopolymers. Whether the approach can also be used for copolymer separations will be investigated in the following chapters.

3.6 Retention behavior of statistical copolymers

According to the theory [83, 84], copolymers having the random distribution of interacting segments along the chain (statistical copolymers) should chromatographically behave like homopolymers. In order to verify whether the chromatographic models under investigation can also be applied to predict the retention behaviour of statistical copolymers, styrene/ethyl-acrylate (SEA) copolymers were examined. Copolymers with narrow chemical heterogeneity having different chemical compositions were prepared by radical copolymerizations to low conversions. The chemical compositions and molar masses, as given in table 3.6, were determined using NMR and SEC analysis, respectively [21]. Similar to the homopolymers PEG and PMMA the elution behaviour of these copolymers was determined at gradient and isocratic elution.

Table 3.6: Average molar masses and compositions of SEA copolymers having narrow chemical composition distributions (CCD)

Sample	M_w (g/mol)*	M_n (g/mol)*	Styrene content (mole %)	Colour code
PEA	ca. 90000	ca. 45000	0	Black
PSEA 1	270540	148316	42	Red
PSEA 2	310378	166160	51	Green
PSEA 3	329942	173584	64	Blue
PSEA 4	364088	188484	79	Cyan
PS	ca. 240000	ca.130000	100	Pink

* Molar masses determined relative to polystyrenes

3.6.1 Gradient elution of random SEA copolymers

The retention behavior of SEA copolymers as well as poly(ethyl acrylate) (PEA) and polystyrene (PS) homopolymers at gradient elution was determined on a reversed phase column (column D, see experimental section) using an eluent system composed of acetonitrile (ACN) and THF. The ACN behaves as adsorption promoting solvent for PEA and as a non-solvent for PS, while THF supports dissolution and desorption of both polymers. Thus, 100 %ACN to 100 % THF gradient experiments with different slopes were performed for all copolymer and the corresponding homopolymer samples. The retention times determined for each sample are plotted as a function of gradient time in figure 3.50. PEA, due to its high polarity elutes first on the reversed phase stationary phase, while the retention times of the copolymers increase with increasing styrene content. PS being the least polar

sample is the most strongly retained. Similar to homopolymers, the retention times of the copolymers increase linearly with the gradient time.

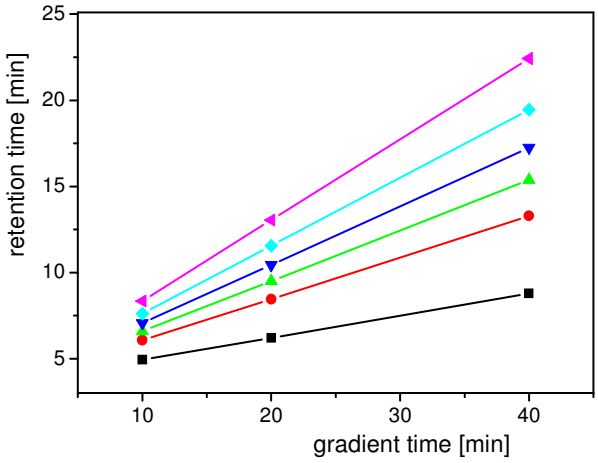


Figure 3.50: Gradient retention times of SEA copolymers (in a linear 100 % ACN to 100 % THF gradient) as a function of gradient time. Copolymer compositions: 0 (■), 42 (●), 51 (▲), 64 (▼), 79 (◆), 100 (◀) mol % styrene. Column: D (see experimental section), Flow rate: 1 ml/min, temperature: 35°C.

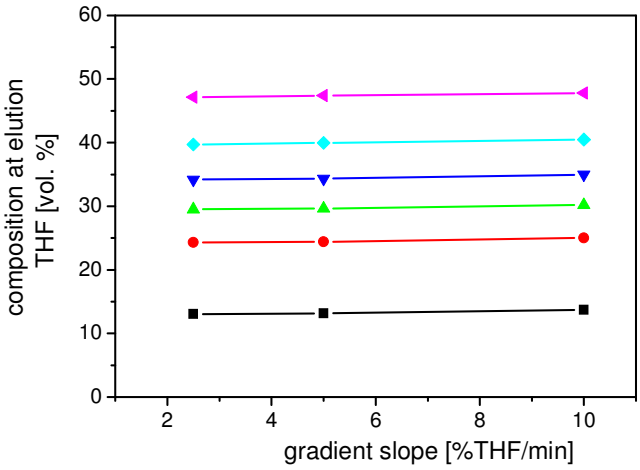


Figure 3.51: Composition at elution as a function of gradient slope for SEA copolymers of different compositions, 0 (■), 42 (●), 51 (▲), 64 (▼), 79 (◆), 100 (◀) mol % styrene. Gradient: linear from 100 % ACN to 100 % THF. Column: D (see experimental section), Flow rate: 1 ml/min, temperature: 35 °C.

From the gradient retention times, the compositions at elution for each copolymer sample were calculated. Figure 3.51 shows the composition at elution as a function of gradient slope. It can be seen that the elution of each sample occurs at only one specific eluent composition. For high molar mass homopolymers, as shown earlier, such behaviour suggests that elution occurs close to the critical composition. Thus, the composition at elution for PEA and PS may be regarded as the critical

composition. Whether the same is valid for statistical copolymers too, will be discussed in the following section.

3.6.2 Isocratic elution of random SEA copolymers

The existence of critical compositions for the copolymers cannot be simply justified. The critical point can be described for a particular chemically homogenous polymer, while the copolymer samples are generally chemically heterogeneous. However, according to the theoretical considerations of Brun [83, 84], a critical point exists for chemically homogeneous statistical copolymers if the adsorbing units are statistically independent of each other. The necessary conditions are that the length of the macromolecule and the pore diameter of the stationary phase have to be larger than the correlation length of the monomers. The copolymers under investigation meet both of these requirements as they have high molar masses and are analyzed using large pore size columns. Thus, it can be assumed that critical conditions exist for each of the used copolymers. However, an experimental verification is not easy because this would require the preparation of copolymers of low chemical heterogeneity with identical chemical composition but different molar masses.

On the other hand, a good estimate of the critical composition was obtained from gradient elution in case of homopolymers. It can be seen in figure 3.51 that PEA is eluting at a composition of about 86/14 v/v ACN/THF. The amount of THF at elution increases with the styrene content. Polystyrene elutes at the highest THF content (approx. 52/48 v/v ACN/THF). From the independence of these compositions on the gradient slope and from the arguments of PCM, these compositions are expected to be very close to the critical compositions for each polymer/copolymer sample. In order to verify this hypothesis, isocratic experiments were performed at various eluent compositions close to the compositions of elution estimated from the gradient experiments.

The retention times obtained by these isocratic experiments are given in figure 3.52. For all samples, isocratic experiments at the estimated composition (vertical dotted lines in figure 3.52) resulted in retention times very close to or identical to column dead time (horizontal solid line in figure 3.52). Eluent compositions of only 1 % less THF than this composition resulted in retention times larger than the column dead

time, indicating elution in adsorption mode. A further decrease of the THF content resulted in incomplete isocratic elution of the samples, as observed by the appearance of additional peak upon flushing the column with pure THF. Therefore, the isocratic elution of copolymers poses special difficulty because the retention in LAC is affected by both the molar mass and the chemical composition. The retention times of the compositions resulting in incomplete elution of the samples are represented by encircled symbols in figure 3.52. On the other hand, at THF content 1 % higher than the composition at elution, retention times smaller than column dead time were obtained indicating SEC conditions. A further increase in the THF content results in additional decrease in the retention times until a limiting value of retention time is approached. The transitions from LAC to SEC mode, indirectly illustrates the presence of a critical point, obtainable at the composition easily estimated from gradient elution.

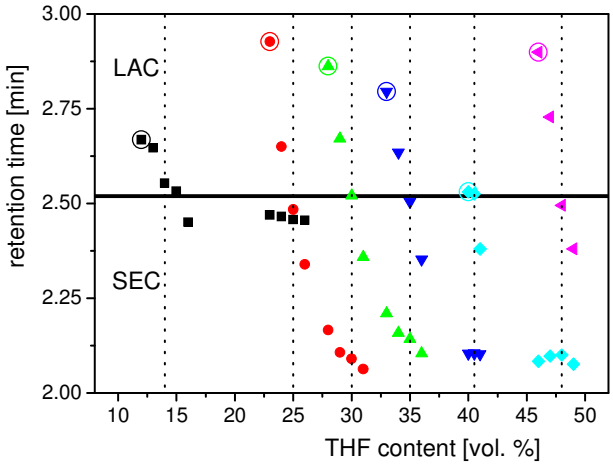


Figure 3.52: Retention times in isocratic experiments as a function of THF content of eluent for SEA copolymers of different compositions; 0 (■), 42 (●), 51 (▲), 64 (▼), 79 (◆), 100 (◀) mol % styrene. Encircled symbols are those where incomplete elution is observed. The black horizontal line represents the column dead time where the elution is expected for the respective critical condition of the copolymer. Vertical dotted lines indicate the compositions at elution calculated from gradient experiments. Chromatographic conditions same as in figure 3.50.

Besides the variation of peak positions, changes of peak widths are observed with changing eluent composition. As an example, a comparison of the chromatograms obtained under different isocratic eluent compositions is given in figure 3.53 for the SEA copolymer containing 64 mol % styrene. It can clearly be seen that the chromatogram obtained from the experiment in 65/35 v/v ACN/THF, which is identical to the composition at elution determined from gradient experiments, shows the narrowest peak. The use of any other eluent composition increases peak width.

Since the sample is homogenous with respect to chemical composition, but heterogeneous with respect to molar mass, the smaller peak width for the sample eluted in the composition at elution as compared to any other eluent composition additionally supports that the composition of elution is very close to the critical eluent composition. Thus, it can be concluded that the gradient experiments provide a very good estimate of the critical composition also for statistical copolymers. This method, therefore, allows the determination of critical conditions for statistical copolymers for the first time.

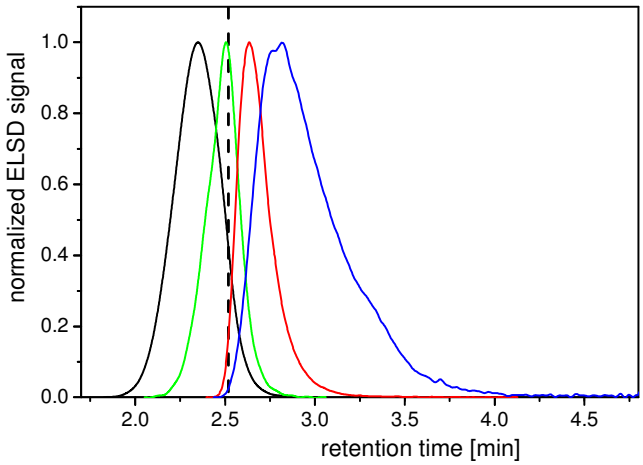


Figure 3.53: Overlay of peaks of a copolymer with 79 mol % styrene obtained at different isocratic eluent compositions, 64/36 (—), 65/35 (—), 66/34 (—), 67/33 v/v ACN/THF (—) around composition at elution (65.1/34.9 v/v ACN/THF) calculated from gradient. Vertical dashed line indicates the column dead time.

3.6.3 Predicting the retention behaviour of random SEA copolymer

Keeping the above given results in mind, it can be concluded that statistical copolymers qualitatively behave similar to homopolymers. Thus, the PCM can be used for statistical copolymers too without any additional modification. In order to test whether also quantitative agreement can be obtained, the PCM was applied to predict the retention times of SEA copolymers in gradient and isocratic elution, on the basis of minimum number of initial experiments, as it was done for homopolymers.

3.6.3.1 Gradient to gradient prediction

Similar to homopolymers, gradients with a range of 100 % ACN to 100 % THF linear over 5, 10, and 20 minutes were used for the calibration to extract the model

parameters of PCM. The predictions were made for other gradient conditions. The predicted retention times were compared with the experimental ones and the relative errors were calculated.

Figure 3.54 shows percent relative deviations of the PCM predictions from experiment for PEA, PS and different copolymer compositions as a function of styrene content in the samples. One can see that the errors for all cases are very small with largest errors of only 3.5 % in the case of PEA for the longest gradient. This example indicates that the PCM permits accurate gradient to gradient predictions of statistical copolymers as well. How accurate isocratic retention times using the gradient calibration can be predicted for copolymers is investigated next.

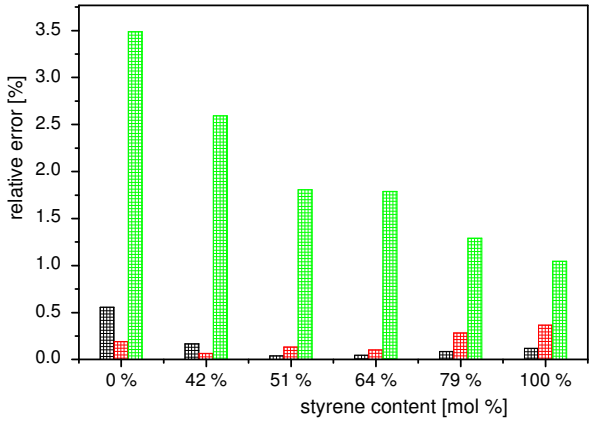


Figure 3.54: Comparison of percent relative errors in gradient to gradient retention prediction for statistical copolymers of styrene and ethyl acrylate. Prediction: linear gradients from 100 % ACN to 100 % THF in 2.5 (black), 5 (red), and 40 minutes (green) using PCM. Calibration: linear gradients from 100 % ACN to 100 % THF 5, 10, and 20 minutes. Chromatographic conditions same as in figure 3.50.

3.6.3.2 Gradient to isocratic prediction

As for homopolymers, the parameters extracted from gradient experiments can be used also to predict the isocratic retention of the above-mentioned series of statistical copolymers. The predictions are compared with the experimental results in figure 3.55, where percent relative deviations are shown as box plots. It can be recognized that 50 % of all errors range up to approximately 10 %. These results are similar to those for the homopolymers where the isocratic retention time predictions from only gradient experiments resulted in considerably larger errors as compared to the gradient to gradient predictions. As mentioned earlier, the reason for this observation is the inaccuracy of the parameters R/D and $dc/d\Phi$ for high molar mass polymers

extracted from gradient experiments alone. However, according to the previously established methodology for homopolymers, the isocratic predictions often can be significantly improved by including isocratic experiments along with one starting gradient experiment into the calibration.

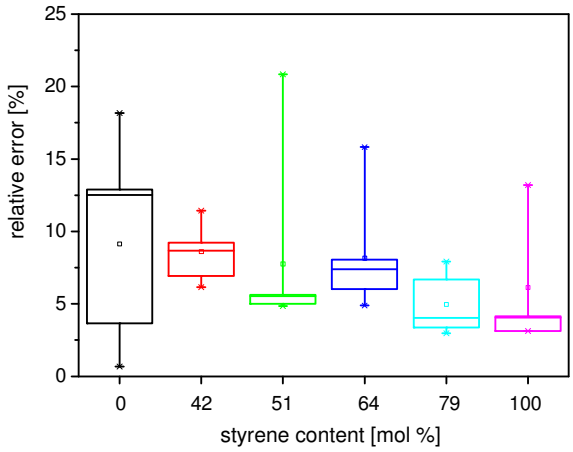


Figure 3.55: Box plots of the percent relative errors in retention times for isocratic elution of SEA copolymers as predicted by the PCM. Calibration: same as in figure 3.54.

3.6.3.3 Isocratic retention prediction from gradient and isocratic experiments

In order to investigate the effect of combining isocratic and gradient experiments for parameter extraction on the quality of the isocratic predictions, two different sets of experimental data were used for the calibration. For this purpose, the selection of initial experiments was performed similar to homopolymers. Therefore, the first run used was a linear gradient from 100 % ACN to 100 % THF in 10 minutes. For each copolymer sample, the retention time was determined at the peak maximum and the composition at elution calculated. The isocratic experiments were performed at the composition at elution and at eluent compositions with slightly higher THF contents than the compositions at elution. The details of the three initial experiments for all samples of the copolymer series are tabulated in table 3.7.

Table 3.7: Calibrations used for prediction of isocratic retention times of SEA copolymers

Sample	**calibration 1** t_G* **; ACN/THF**; ACN/THF****	**calibration 2** t_G* **, ACN/THF**; ACN/THF****
PEA	10 min.; 86/14; 84/16	10 min.; 86/14; 77/23
PSEA 1	10 min.; 75/25; 74/26	10 min.; 75/25; 72/28
PSEA 2	10 min.; 70/30; 69/31	10 min.; 70/30; 67/33
PSEA 3	10 min.; 65/35; 64/36	10 min.; 65/35; 60/40
PSEA 4	10 min.; 59.5/40.5; 59/41	10 min.; 59.5/40.5; 52/48
PS	10 min.; 52/48; 51/49	10 min.; 52/48; 51/49

* Gradient time in minutes for 100 % ACN to 100 % THF

** Isocratic eluent compositions in v/v

Using these experiments, the substance-specific parameters of the PCM were determined and the retention times for different isocratic experiments were calculated. The results are shown in figure 3.56 where the comparison of the experimental and predicted retention times is given. As can be seen, for the first calibration (dotted lines), a very good agreement exists between the prediction and experiment close to column dead time, i.e. at the critical compositions of the copolymers. The low errors reflect the accuracy in predicting the critical compositions.

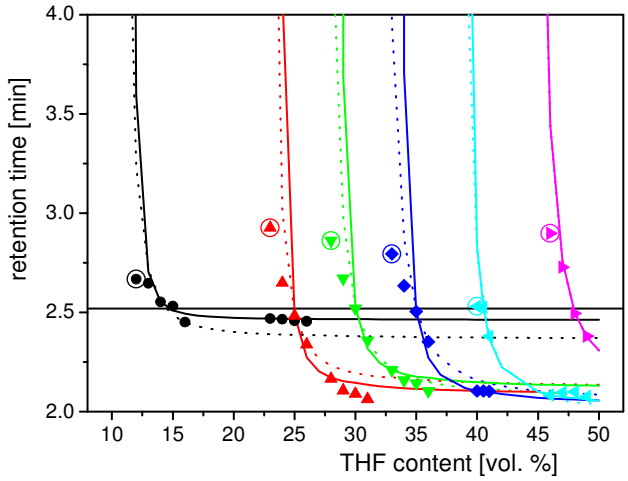


Figure 3.56: Comparison of the experimental (symbols) and predicted isocratic retention times as a function of THF content of the eluent for SEA copolymers of different compositions, 0 (■), 42 (●), 51 (▲), 64 (▼), 79 (◆), 100 (◀) mol % styrene. Prediction model: PCM; Calibration: 1 (dotted lines) and calibration 2 (solid lines) (see table 3.7).

As can be seen in figure 3.56, larger deviations are found for retention times corresponding to lower and higher THF contents of the eluent, i.e. in LAC and SEC mode, respectively. These errors are not specific to copolymers, as the same is observed for the homopolymers in this series of samples. It should be mentioned that the largest experimental retention times (encircled symbols) are those where the samples are not completely eluted. Instead, the eluting fraction may have either lower molar mass or lower styrene content than the average of the actual sample. Thus, their retention times might not be representative of the complete sample. This means that the average retention time of the actual sample is higher than that reported here, as has been predicted by the model. In addition, even if the peak is eluted completely, the peak maximum in different interaction conditions, due to the non-linear molar mass dependence of retention times, does not correspond to the same molar mass for which the retention time is predicted. This may results in the

apparent errors in the prediction. It is also possible that the dependence of the interaction parameter on eluent composition is not linear for a wide range especially in SEC conditions. This non-linearity may result in large errors in prediction.

In order to improve the predictive quality in the SEC mode, isocratic experiments with higher amounts of THF were used in the second calibration (table 3.7). As can be seen in figure 3.56, the predictions in the SEC mode are significantly improved (solid lines). This shows that accurate results can be obtained when the experiments to be predicted and experiments used for calibration are of the same nature.

In order to test the general suitability of PCM for statistical copolymers, the model was fitted to all the experimental data obtained from gradient and isocratic experiments. All the data of each copolymer can be fitted with reasonable accuracy by just three model parameters with errors of less than 4 %, as shown in figure 3.57, where percent errors are given for each sample as box-plots. The good agreement for a large variety of experimental conditions and different samples again confirms the suitability of the PCM to describe the chromatographic behavior of statistical copolymers.

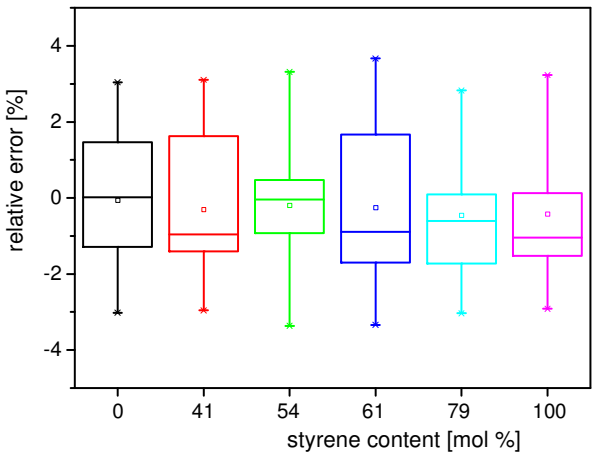


Figure 3.57: Box-plots of errors when fitting the PCM to the experimental retention times of styrene-ethyl acrylate copolymers for gradient and isocratic experiments.

3.6.4 Gradient method development for the chemical composition distribution analysis of SEA copolymers

After establishing that the PCM can be used to describe and accurately predict the retention times of the SEA copolymers, attempts were made to use the model to develop a separation method for a real application, e.g. for the determination of the chemical composition distributions by separating these copolymers. The analysis of chemical heterogeneity along with molar mass heterogeneity is important in order to understand the physical properties of such polymers. The compositional heterogeneity of the copolymers arises during the statistical copolymerization of a mixture of two monomers, due to the tendency of one monomer to be preferentially incorporated into the growing copolymer chains. When a monomer becomes preferentially incorporated into the copolymer, its content in the monomer mixture is reduced with respect to the second monomer. As a result, the instantaneous composition of the monomer mixture changes with time and this in turn results in change of the instantaneous copolymer composition. This means that the average composition of copolymer chains also changes as the conversion increases. The copolymer so produced is composed of chains having different chemical compositions. Thus, the copolymers that are typically prepared by copolymerization reaction to high conversions are heterogeneous with respect to chemical composition, in contrast to those produced by low conversion copolymerizations. Spectroscopic methods (such as IR and NMR) permit only the determination of the average composition of the copolymers. On the other hand, liquid chromatography, as shown above, allows separating the copolymers (here SEA copolymers) according to chemical composition. However, the determination of the chemical heterogeneity by chromatographic analysis requires the use of a calibration, i.e. the knowledge of dependence of retention time on chemical composition. For this purpose, well-defined samples with different composition but narrow chemical composition distributions (CCD) are required (low conversion copolymer samples). If the copolymer composition is x, then the relationship between the concentration signal $s(t_R)$ and the CCD $w(x)$ is given by,

$$w(x) = s(t_R)\frac{dt_R}{dx} \qquad 3.7$$

Thus, the CCD function of the copolymers can be determined from the chromatograms and the slope of the dependence of retention time on chemical composition. Often the dependence of retention time on chemical composition is not linear. A nonlinear relationship between retention time and copolymer composition, i.e. a varying value dt_R/dx, make the calculation of the concentration from the detector signal more difficult, because retention time intervals of same width correspond to different intervals of the copolymer composition in this case. In order to avoid these difficulties, a linear correlation between retention time and copolymer composition is highly desirable.

In case of SEA copolymers, the calibration obtained using linear solvent gradients is not linear over the whole range of copolymer composition, as shown in figure 3.58. As can be seen, the copolymer having a styrene content of approximately 40 % is not on the line of the other samples. This behaviour is reproducible in all gradients of different lengths. This shows that this deviation from straight line is not related to the experimental or personal error. Moreover, the errors in the composition determination by spectroscopic analysis are far lower to be responsible for this behaviour. Using the calibration curve in figure 3.58 for determination of CCD would require, therefore, additional data points in the composition range near 40 % styrene. The alternative is to use a gradient that may result in a linear calibration curve. However, establishing such a gradient is not very economical if done on a real chromatographic system, as it may require a large number of experiments.

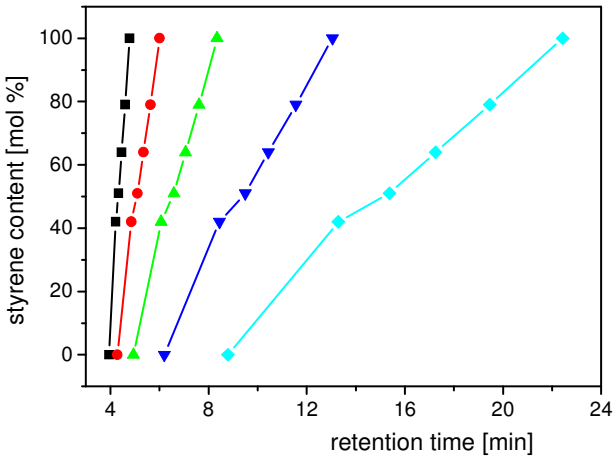


Figure 3.58: Dependence of gradient retention times of SEA copolymers on the styrene content for linear gradients from 100 % ACN to 100 % THF over 2.5 (■), 5 (●), 10 (▲), 20 (▼), and 40 (◆) minutes. Chromatographic conditions same as in figure 3.50.

Therefore, a large number of experiments with different gradient shapes were easily simulated on computer employing the PCM parameters extracted for each copolymer sample using the three initial runs that were used in calibration 2 in table 3.7. Finally, a gradient program was found at computer, which was expected to result in a linear relationship between the copolymer composition and the retention time. The parameters obtained from the gradient calibration in section 3.6.3.1 or from the calibration 1 from table 3.7 also provide the same results.

Figure 3.59 shows the proposed gradient program that consists of several steps. The retention times from simulations, fitted with a straight-line equation, are also plotted along with the actual experimental results for this gradient. As can be seen, the complex multi-step non-linear gradient that was easily obtained by the virtual chromatography approach in fact results in an almost perfect linear relationship between retention time and copolymer composition. The errors between the calculated and experimentally determined retention times are less than 1 % with exception of the pure poly(ethyl acrylate). The deviations for this, however, are only about 5 %. The reason of this error for the retention time of poly(ethyl acrylate) is not related to the model itself. It is attributed to a small change of the column dead time, which affects the early eluting peak stronger than the late eluting ones. Virtual chromatography may also be very efficient in tailoring the analysis times, e.g. in this example, the whole separation is achieved within 8 minutes.

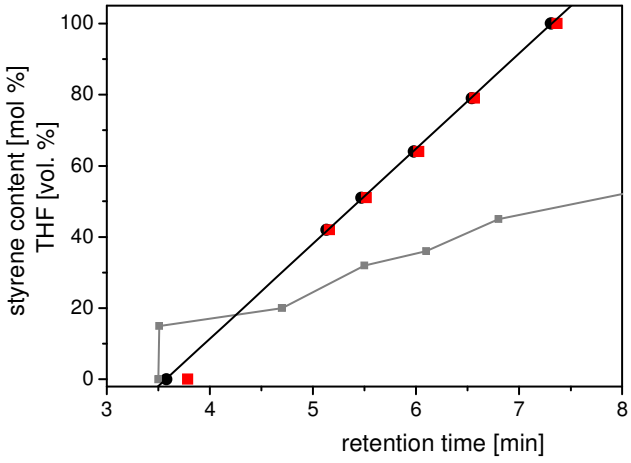


Figure 3.59: Predicted (●) and experimentally found (■) linear dependence of copolymer retention time on the styrene content using the given gradient profile (grey line) run on a normal analytical column (column D, see experimental section). The black line is the linear fit to the predicted retention times. Flow rate: 1 ml/min. Other chromatographic condition same as in figure 3.50.

The same approach was also used to develop a similar method for a short high throughput reverse phase column (column E, see experimental section) for the fast analysis of chemical heterogeneity. A gradient that gives the separation within three minutes with a linear dependence of retention time on chemical composition was designed on the computer, for a flow rate of 1 ml/min. In order to reduce the analysis time further, the predicted gradient was run at 2 ml/min flow rate. The experimentally obtained calibration along with the gradient profile is given in figure 3.60. Again, the calibration can be fitted perfectly by straight-line equation. The low analysis time of just over 2 minutes reflects the effectiveness and efficiency of the virtual chromatography approach in method development. Since the molar masses of the samples were high enough to cause their elution at critical compositions, the decrease in retention times when using a flow rate of 2 ml/min instead of 1 ml/min is expected to be only due to the corresponding decrease in the system delay time and column dead time. As can be seen, there is a small constant deviation of the predicted retention times from the ones obtained experimentally. This deviation may be attributed to the inaccuracy in the determination of the system's dwell volume and/or the column dead volume.

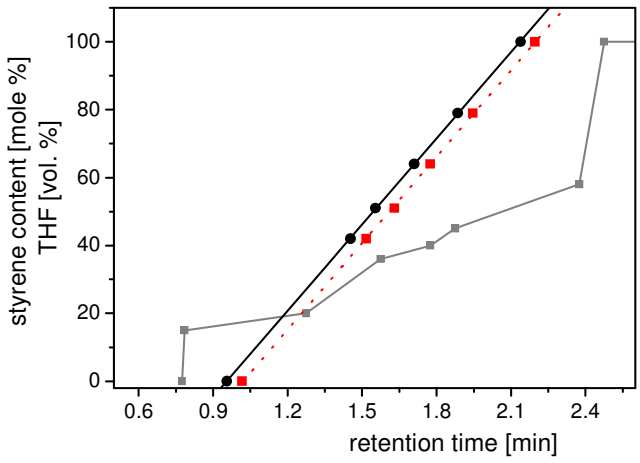


Figure 3.60: Predicted (●) and experimentally found (■) linear dependence of copolymer retention time on the styrene content using given gradient profile (grey line) run on a high throughput column (Column E, see experimental section). The black solid and red dotted lines are the linear fits to the predicted and found retention times, respectively. Flow rate: 2 ml/min. Other chromatographic condition same as in figure 3.50.

The examples given here show that virtual chromatography provides a simple procedure for the optimization of a polymer separation according to the specific requirements, e.g. optimized resolution or optimized analysis time.

3.7 Prediction of the retention behavior of segmented copolymers - Graft copolymers of butadiene and methyl methacrylate

In the preceding sections, the PCM was validated with homopolymers and statistical copolymers, both behaving chromatographically in the same fashion. The PCM is based on the molecular statistical theory of homopolymers and is applicable, in principle, only to homopolymers and copolymers that behave like homopolymers i.e. alternating and statistical copolymers. On the other hand, copolymers like block or graft copolymers are expected to behave quite differently than homopolymers, because the correlation length of the adsorbing repeating units is too large to allow for a statistical independent interaction with the stationary phase [83, 84]. That is why no critical eluent conditions can be defined for these copolymers. In order to predict the retention behaviour of these polymers, the PCM should be modified. The PCM, e.g., when extended for block copolymers [59, 78, 79] has at least six parameters that have to be extracted from experiments. The number of parameters for graft copolymers may be even higher since not only the molar mass of the backbone and side chain but also the number of chains affects the retention behaviour. Usually, the higher the number of parameters, the larger is the number of experiments required to extract these parameters, which is beyond the scope of present research. Therefore, in this section it is investigated whether the PCM in its present form can be used to predict the retention behaviour of segmented copolymers also. For this purpose, graft copolymers composed of polybutadiene (PB) and PMMA were examined.

The copolymers used were prepared by radical grafting reaction of MMA onto a PB backbone. The reaction product was composed of the copolymer and the two corresponding homopolymers. The separation of PB was obtained by a linear gradient from 100 % toluene to 100 % THF on a bare silica stationary phase (column B, see experimental section). Under these conditions, PB elutes in SEC conditions at 100 % toluene, while graft copolymer and PMMA homopolymer co-elute close to the critical composition of PMMA. Two fractions of graft copolymer having different MMA content were obtained by repeated fractionation of a PB free graft copolymer sample at the critical conditions of PMMA. The PB free sample was obtained by repeated fractionations using a linear gradient ranging from toluene to THF. The collected fractions were expected to have narrow chemical composition

and molar mass distributions. The qualitative analysis of chemical composition and molar mass was performed by FTIR spectroscopy and SEC experiments on the fractions. The properties of two fractions obtained are given in table 3.8

Table 3.8: Properties of graft copolymer fractions

Sample	PMMA content *	molar mass, M_p (g/mol) **	Colour code
Fraction 1 (F-I)	Low	700000	Black
Fraction 2 (F-2)	High	85000	Red

* Qualitative composition by FTIR spectroscopy based on the ratio of absorption bands characteristic of PMMA and PB, i.e. at 1730 cm^{-1}/990 cm^{-1} ($C{=}O_{stretching}$/$C{=}C_{bending}$), respectively.
** Molar masses at peak maximum obtained using PMMA calibration in pure THF

Linear gradient experiments were performed with these samples. The conditions of the gradient experiments and the obtained retention times are given in table 3.9. As can be seen from the experimental results, sample F-I elutes slightly earlier than F-II in faster gradients, even though its molar mass is considerably higher than that of F-I. This shows that the elution is determined by MMA content, not by molar mass. The two fractions of graft copolymers were simply taken as two different unknown samples and the PCM was applied for predictions similar to homopolymers. As in the previous studies, gradient to gradient predictions were made for these two samples. The gradients used for calibration were linear from 100 % toluene to 100 % THF in 10, 20 and 40 minutes. The calculated retentions are given in table 3.9. The absolute values of the relative errors against experiment number (see table 3.9) are given in figure 3.61 as bar graphs.

Table 3.9: Prediction of gradient retention times for graft copolymers of PB and PMMA using gradient experiments as starting runs (100 % toluene – 100 % THF in 10, 20, and 40 minutes)

Exp. Nr.	Range [1] THF	t_G [2]	F-I Retention times (min.)		F-II Retention times (min.)	
			Experiment	Prediction	Experiment	Prediction
1	0 -100 %	5	5.15	5.29	5.30	5.33
2	0 -100 %	10	6.72	6.84	6.87	6.93
3	0 -100 %	20	9.83	9.95	10.14	10.10
4	0 -100 %	40	16.24	16.16	16.36	16.37
5	0 -100 %	80	28.94	28.55	28.48	28.71
6	28 – 38 %	5	4.50	4.50	4.78	4.67
7	28 – 48 %	5	5.49	5.26	5.62	5.47

[1] Linear gradient at flow rate of 1ml/min on column B at 35°C;
[2] Gradient time

As can be seen in figure 3.61, there is good agreement between the PCM prediction and the experiment. The errors in all predictions are less than 3 %, with the exception for F-I in experiment number 7 where the error is just above 4 %. From this example,

it can be concluded that the PCM in its non-modified form is suitable to predict the retention behaviour of the graft copolymers as far as gradient to gradient predictions are concerned. This result can be justified since in the present case both copolymer fractions are eluting within a composition range of only 5 % of THF in gradients of widely different slopes. This shows that the elution of graft copolymers occurs at a specific eluent composition in gradient experiments, which depends on the content of adsorbing segments. Once this composition of eluent (in terms of parameter Φ_c) is known, the gradient retention times with reasonable accuracy can be predicted.

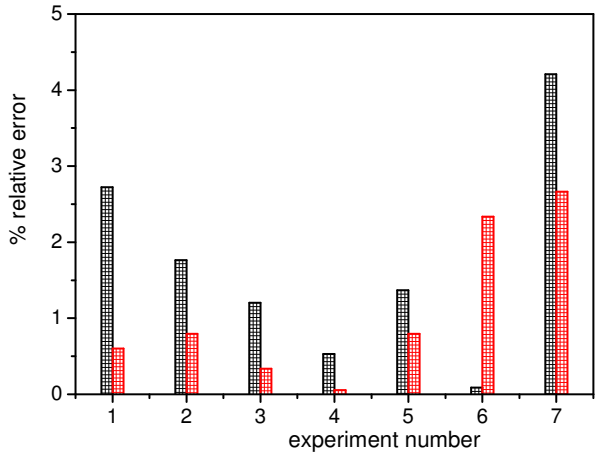


Figure 3.61: Percent error in the PCM predictions of gradient retention times for graft copolymer fractions, F-I (black) and F-II (red) using gradient calibration.

Whether the PCM is suitable to predict the retention of graft copolymers for isocratic elution too, is explored below. For this purpose, isocratic experiments were performed at the compositions estimated from the gradients. The above used model parameters extracted from the gradient experiments were employed to calculate the isocratic retention times at the eluent compositions give in table 3.10. The experimental retention times are also given for comparison.

As can be seen in table 3.10, the two samples are eluting in SEC mode or at the column dead time at the selected eluent compositions, depending upon their PMMA content. The magnitude of the relative errors reflects the quality of predictions. It can be seen that the model provides inaccurate predictions. However, the predictions are at least qualitatively true. The large errors in this case may be attributed primarily to the uncertainty of the model parameters extracted from gradients, similar to the behaviour of the homopolymers.

Table 3.10: Prediction of isocratic retention times for graft copolymers of PB and PMMA. Gradient calibration: same as in table 3.9

sample	Eluent composition (Toluene/THF v/v)	t_R (min.) experimental	t_R (min.) PCM prediction	% error PCM
F-I	65/35	1.80	1.22	-32.33
	63/37	1.84	1.15	-37.41
	0/100	1.73	--	
F-II	65/35	2.53	2.06	-18.74
	63/37	2.41	1.97	-18.23
	0/100	2.31	--	

For homopolymers, it was shown that the accuracy of the PCM predictions at isocratic elution could be significantly improved by using isocratic experiments along with gradient experiments for calibration. Whether this is also valid for the graft copolymers was investigated. For this purpose, the parameters of PCM were extracted by using two isocratic experiments at eluent compositions of 65/35 and 0/100 v/v toluene/THF mixtures along with a linear gradient of 100 % toluene to 100 % THF over 10 minutes (calibration 1) or two gradients of same range but with 10 and 80 minutes length (calibration 2). The results are summarized in table 3.11.

Table 3.11: Prediction of isocratic retention times from gradient and isocratic experiments as calibration runs for graft copolymers of PB and PMMA

sample	Toluene/THF[1]	$t_R$exp.[2]	$t_R$pred.[3] Calibration 1	$t_R$pred.[4] Calibration 2	% error Calibration 1	% error Calibration 2
F-I	65/35	1.80	1.96	2.43	8.6	35.13
	63/37	1.84	1.89	2.33	2.82	26.87
	0/100	1.73	1.68	--	-3.02	
F-II	65/35	2.53	2.58	2.47	2.00	-2.32
	63/37	2.41	2.58	2.41	2.76	-0.07
	0/100	2.31	2.58	2.29	0	-1.19

[1] Isocratic eluent compositions in v/v;
[2] Retention times obtained from experiments;
[3] Retention times predicted by PCM using calibration 1 (10 minutes linear gradient from 100 % toluene to 100 % THF and isocratic runs at 65/35 and 0/100 v/v toluene/THF);
[4] Retention times predicted by PCM using calibration 2 (10 and 80 minutes linear gradients from 100 % toluene to 100 % THF and isocratic runs at 65/35 and 0/100 v/v toluene/THF)

One can see that including the isocratic experiments leads to the improvement in the prediction for the graft copolymer having the high MMA content (F-II). For the sample with lower MMA content (F-I), the results are not consistent. As can be seen, the predictions are even worse when using four calibrating runs (calibration 2). This is attributed to the inability to calculate isocratic retention times at 0/100 v/v toluene/THF in the fitting process for this combination of experiments. That means,

the calibration 2 is actually based on two gradients and one isocratic runs. Thus, this again results in the same magnitude of errors as if using three gradient experiments as calibration. From the comparison of the errors, it appears as if the graft copolymer containing high content of MMA behaves much like PMMA homopolymer. The graft copolymer with low amount of PMMA behaves quite differently. This difference in the behaviour of the two graft copolymers with different MMA content may be attributed to architectural differences between the two. The results presented here suggest that the isocratic retention behaviour of segmented copolymers cannot be described with as good accuracy as for gradient predictions using the PCM in the present form.

In order to investigate further the validity of the PCM to describe the retention behaviour of graft copolymers, the model was fitted to all the available data of gradient and isocratic experiments for the two samples F-I and F-II. The extents of the inaccuracy of the retention times calculated using the PCM is compared with the help of box-plots in Figure 3.62.

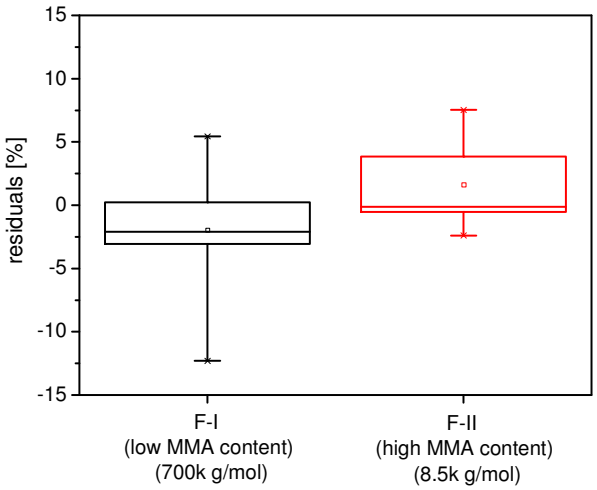


Figure 3.62: Box-plots for the residuals of fitting the PCM to all the available isocratic and gradient retention data PB-g-PMMA copolymer fractions, F-I (black), F-II (red). The boxes represent the extent of deviation in terms of percent relative errors between the fitted and experimental retention times.

It can be seen that the PCM model can be fitted very well to the experimental retention data of both fractions. The retention times of both fractions F-I and F-II can be reasonably described by PCM. Here the 50 % of all error values range only up to 3 % and 5 % (shown as the box) with only the 12 % and 8 % error at 95 percentile for F-I and F-II, respectively. The data points with larger errors are due to the errors

in isocratic predictions, which may also be the consequence of the poor quality of the fit or the selection of the initial experiments. These results are in accordance with the results of the isocratic predictions given above (table 3.11). This shows that the PCM is not a correct model for segmented copolymers, yet it is sufficient to describe their retention behaviour at gradient as well as isocratic conditions with reasonable accuracy. However, it may still be true that additional difficulties may arise due to the different nature of the graft copolymer samples.

At the end of this chapter, it can be safely concluded that the PCM is a suitable model for the description of the retention behaviour of homopolymers as well as statistical copolymers. Accurate predictions of peak positions can be made on the basis of a minimum number of starting experiments. The model can also be used to predict accurately gradient retention times of graft copolymers, however, not their isocratic elution behaviour.

3.8 Retention behaviour of polydisperse samples

The previous sections dealt with the prediction of the peak positions of monodisperse polymers. However, for prediction of a real separation the peak widths must also be predicted. This is especially true in case of synthetic polymers because their polydispersity may have significant effect on the peak width. That means retention times with respect to molar mass must be predicted. Therefore, this chapter focuses on the prediction of peak widths of polydisperse samples. In addition, the influence of peak broadening on the quality of the separation will be discussed with the help of examples of separations of model mixtures.

The PCM, which has been found to be the best model for the prediction of gradient as well isocratic retention behaviour, was further explored for its usefulness to include molar mass effects that would allow for the prediction of retention behaviour of polymers with respect to molar mass. As mentioned earlier in chapter 2 (section 2.3.3), if equation 2.20 holds (i.e. $\alpha = 0.5$), the retention behaviour of a whole molar mass series in a polydisperse sample can be predicted, in principle, from a single molar mass, i.e. using only three parameters Φ_c, $dc/d\Phi$ and $(R/D)_{ref}$.

The validity of equation 2.20 was tested for different homopolymers. For this purpose, the PCM along with equation 2.20 was fitted to a large number of gradient and isocratic experiments for a large number of PEGs (oligomers with DP = 5 – 61 and high molar mass of polydisperse samples), PMMA and polystyrene (PS) standards having different molar masses. The residuals of this fitting are plotted against the DP for PEGs in figure 3.63, and against molar mass of PMMA and PS in figure 3.64.

As can be seen in figure 3.63, as far as gradient elution is concerned, small deviations (less than 3 %) between the calculated and experimental retention times are found for oligomers with DP > 10. This shows that PCM together with equation 2.20 describes the gradient retention behaviour of high DP of PEGs with reasonable accuracy. This is also true for high molar mass PEGs, which cannot be separated into oligomers. However, larger errors are observed for the lower oligomers. The reason might be that the radius of gyration to molar mass correlation of lower oligomers cannot be described accurately by the scaling relation based on Gaussian chain

model. In the case of isocratic elution, no relationship between the extent of errors and DP can be observed. The deviations for isocratic elution are high (up to 8 %) for about half of the studied oligomers, even for oligomers for which lower errors are observed in case of gradient elution. One reason for the larger deviations for the isocratic experiments may be the experimental errors. Another explanation may be simply the lack of best fit because the fitting to a large data set becomes complicated.

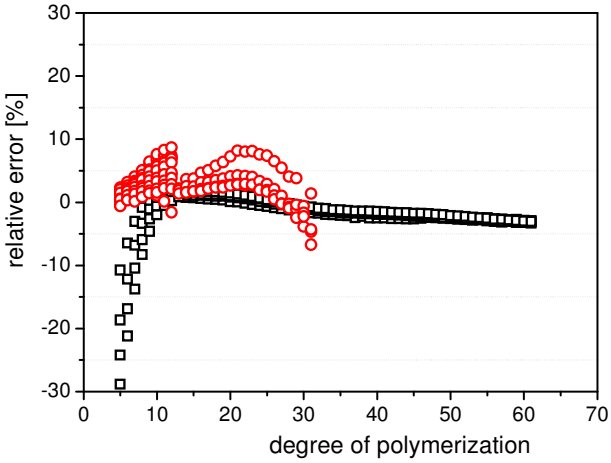
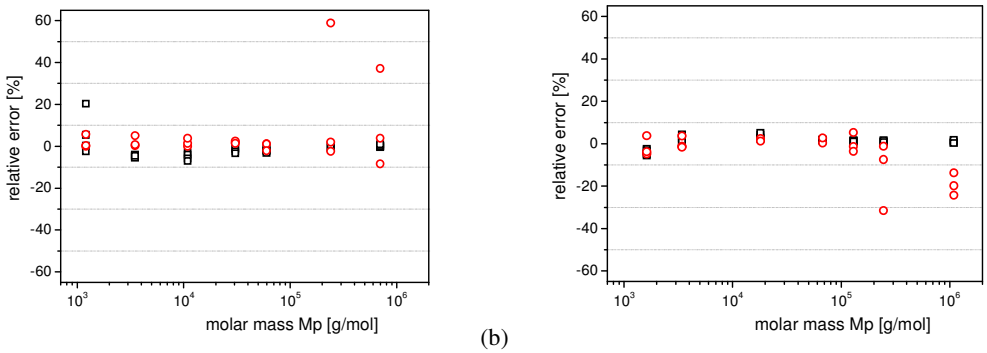


Figure 3.63: Relative errors between calculated and experimental retention times for PEG oligomers obtained from gradient (□) and isocratic (○) experiments versus DP. Model parameters: $\Phi_c = 0.8258$, $dc/d\Phi = 4.0908$, and $(R/D)_{ref} = 0.0702$.



(a) (b)

Figure 3.64: Residuals of fitting PCM to retention times from gradient (□) and isocratic (○) experiments for PMMA (a) and PS (b) standards of different molar masses. Model parameters: PMMA ($\Phi_c = 0.3700$, $dc/d\Phi = 5.8596$, and $(R/D)_{ref} = 0.0624$); PS ($\Phi_c = 0.4860$, $dc/d\Phi = 3.7549$, and $(R/D)_{ref} = 0.2258$).

Almost similar trends are found for other polymers, i.e. PMMA and PS (figure 3.64). Again, larger deviations for gradient elution are found only for PMMA at low molar mass range. For isocratic elution, however, no such systematic dependence can be described. Larger errors are found for elution in strong eluents for both of the polymers in the high molar mass range. This might not be related to the wrong

scaling of *R/D* parameter but due to non-linear dependence of the *c* vs Φ for large Φ_c - Φ.

Figure 3.65 shows the box-plot summary of the errors in fitting the PCM with equation 2.20 to all the molar masses of the used polymeric systems. It can be seen that the model is able to describe the gradient retention of polymers in general with only less than 2 % deviations for the 50 % of the data points while 95 % of them show only errors up to only 5 %. In case of isocratic experiments, for 50 % and 95 % of the data points the errors are still lower than 4 and 17 %, respectively. It should be stressed that only three PCM parameters describe the full molar mass dependent retention behaviour for each polymer system over wide range of chromatographic conditions. It should be mentioned that similar qualities of the fits were obtained when the same retention data fitted with the PCM using values of α = 0.4 or α = 0.6. Similarly, approximately the same range of errors between the calculated and experimental retention times was found when the value of α is extracted along with the other three parameters of the PCM. This shows that value of α does not influence strongly the quality of predictions. Consequently, the determination of α from chromatographic data may not provide reasonable values.

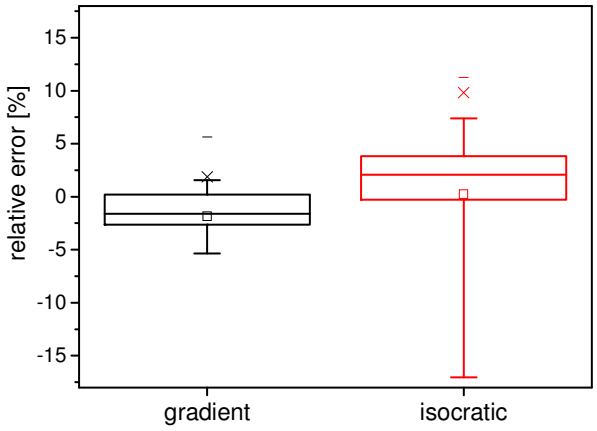


Figure 3.65: Box-plot summary for the errors in fitting the PCM including equation 2.20 to all gradient and isocratic data of homopolymers (PEG, PMMA, PS) by PCM. The reference molar masses were 12000, 60000, and 130000 for PEG, PMMA, and PS respectively.

From the above discussion, it follows that in principle, three experiments of a single sample should be sufficient to predict the retention behaviour of polymers with respect to molar mass with the reasonable accuracy especially for the short range of molar masses. Whether the PCM with scaling method for *R/D* parameter (equation

2.20) as used above is valuable enough to predict the peak shapes reasonably will be analyzed in the following.

3.8.1 Peak widths in polymer liquid chromatography

As mentioned in chapter 2, there are two types of peak broadening factors in polymer chromatography. First, peak broadening due to sample polydispersity and second peak broadening caused by the experimental setup.

When molar mass effects are present, the polydispersity of the sample can hamper the separations according to chemical composition or the separation may become entirely unfeasible in some cases. That is why it is very important to predict the effect of peak broadening due to polydispersity. Only LCCC or gradient conditions where no or low molar mass dependences of elution volumes are observed may be suitable for the separation according the differences of the polymer components other than that in molar mass. In LCCC, which operates in isocratic conditions, no molar mass selectivity is observed and a narrow peak is expected for a chemically homogeneous polymer sample. How the widths of the peaks of polymer samples vary in gradient LC will be investigated in the following section.

3.8.1.1 Peak widths in gradient chromatography of polymers

In order to study the variation in peak width of a polymer sample in gradient chromatography, peak widths for a series of PS standards differing in average molar mass but with approximately the same polydispersity were determined using different gradient conditions.

Figure 3.66 shows the peak widths determined at half peak height as a function of molar mass in gradients of four different slopes. As can be seen, the largest peak widths are found for low molar mass samples. This is the result of the stronger dependence of retention time on molar mass for low molar mass samples (see also figure 3.16, section 3.1.2.1.2 and 3.38, section 3.3.1). Since, the higher the slope of the gradient, the lower is the molar mass dependence of retention time, peak width decreases with increasing gradient slope. Above a certain molar mass (60000 g/mol in the present case) the peak widths are nearly independent of molar mass. For high molar mass samples, the peak widths are nearly independent of gradient steepness

also. This is due to the molar mass independence of retention time observed for high molar mass samples especially in steeper gradients. This means, similar to LCCC, the sample polydispersity has practically no effect on peak broadening for the above-mentioned conditions. Therefore, it can be concluded that the peak widths observed in these cases are caused mainly by instrumental broadening. This shows that for high molar masses, the gradient elution can be used to separate polymer samples exclusively according to chemical composition.

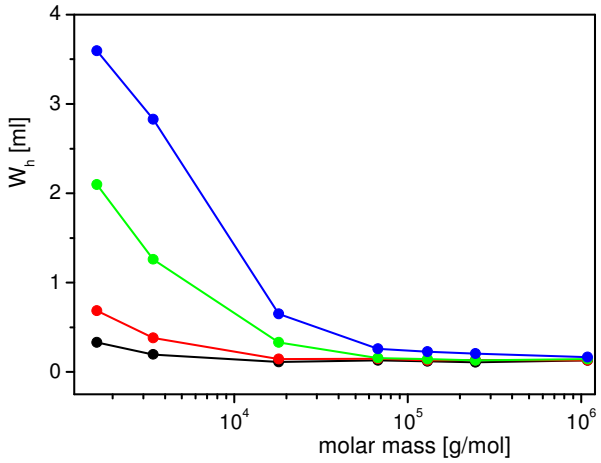


Figure 3.66: Peak widths at half height for PS standards versus molar mass as observed in gradient experiments. Gradients: linear from 100 % ACN to 100 % THF in 5 (black), 10 (red), 20 (green) and 40 (blue) minutes, plotted against molar mass. Column: D (see experimental section)

3.8.2 Prediction of peak shapes of polydisperse samples

From the discussion in the introductory section of this chapter, it is expected that the chromatogram of a polymer sample can be adequately predicted using the PCM model accounting for the molar mass dependence of retention times.

3.8.2.1 Prediction of peak widths at isocratic elution

Usually the extent of band broadening for monodisperse compounds in the column is related to classical number of theoretical plates (N) by equation 2.22 (section 2.4.2.2). However, it is known that the plate number calculated in that way depends on retention time [107]. In order to study the dependence of N on retention time, the retention data of PEG oligomers for different isocratic conditions were analyzed. The values of N for each oligomer peak were calculated from the observed peak widths at half height. The values of the plate number, N', defined in equation 2.23 (section 2.4.2.2) were also determined. The values of both plate numbers are plotted against

the retention time in figure 3.67. It can be seen that N decreases significantly with the retention time while the values of N' vary much less as compared to N. The upward curvature at lower and higher retention times may be due the lower accuracy in the determination of the peak widths of the smaller peaks at those retention times. Thus, if the prediction of peak broadening due to the column is made based on N, it will strongly depend upon the sample used to determine N. However, this problem can be avoided using N'. Therefore, this plate number is used in the following calculations. One additional isocratic experiment is required to determine this parameter from the peak width of a strongly retained low molar mass compound.

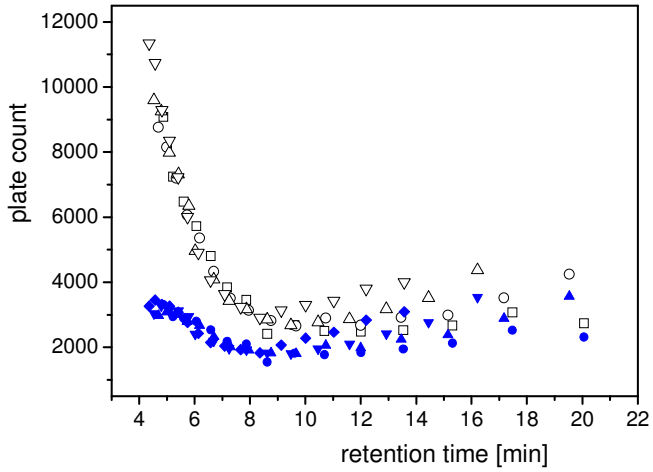


Figure 3.67: Comparison of two different theoretical plate numbers, N (hollow symbols) and N' (filled symbols) as a function of retention time, calculated from the isocratic elution data of PEG oligomers at different eluent compositions on column A. Eluent compositions: 54/46 (■), 53/47 (●), 52/48 (▲), 51/49 v/v water/MeOH (▼). Flow rate 0.5 ml/min. Temperature 35°C.

From equation 2.23, it can be noticed that N' is not applicable in conditions where elution occurs at or below the column dead time, i.e. in LCCC or SEC mode. Therefore, band broadening in column is considered as composed of two types as mentioned in chapter 2 (equation 2.24, section 2.4.2.2). The first one is a constant contribution arising from traveling of the molecules through the void volume of the column. This band broadening contribution and that of the extra-column volume of the chromatographic system was determined, together by injecting a known volume of toluene solution using THF as eluent. This means, this parameter can be calculated from the same experiment, which is used to determine volume between injector to detector including the column. The second contribution to band broadening in the column, which increases with the retention time, was predicted using equation 2.23.

The value of N' was determined using the isocratic experiments with PEG oligomers (see experimental section 4.1).

As an example, the retention behaviour of PEG 1000 was simulated. To obtain an overall chromatogram of a polydisperse sample, first the approach described in section 2.5.1 was employed to determine the contribution of polydispersity to peak widths. Thus, the retention time for each oligomer of the series was calculated using the PCM parameters determined at peak maximum and a value of $\alpha = 0.5$. The *MMD* was approximated by a Gaussian distribution with the approximated value of *PDI*. Next, the contribution of instrumental broadening was calculated for each uniform component eluting in isocratic conditions using the determined contributions to peak broadening mentioned above in this section 3.8.1. The final chromatogram is a summation of all peaks. The simulated chromatogram is shown in figure 3.68. The chromatogram obtained experimentally is also given for comparison.

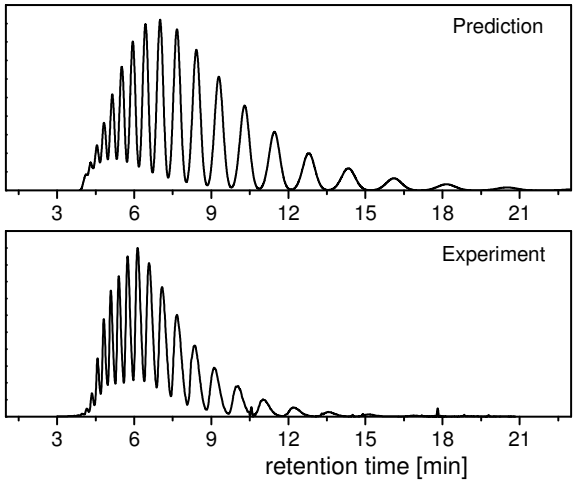


Figure 3.68: Comparison of predicted (top) and experimentally obtained (bottom) chromatogram for the isocratic elution of PEG 1000 at 51/49 v/v water/MeOH on column A. Calibration: Linear gradients from 5 – 100 % MeOH against water in 10, 30 and 90 minutes. Used Parameters: Mn = 1030 g/mol ($\Phi_c = 83.25$, $dc/d\Phi = 2.73$, $R/D_{ref} = 0.064$), PDI = 1.03, $N' = 2500$. Case of known molar mass and polydispersity.

Figure 3.68 shows that there exists a reasonable agreement between the calculation and experiment. Thus, the proposed method allows a reasonable estimation of the retention range of the polydisperse sample. In addition, reasonable estimation of tailing and the loss in resolution at low retention times in isocratic conditions can be predicted. The predicted chromatogram, however, is slightly broader than the one experimentally obtained. This may be due to several reasons. The first reason may be

the approximation of molar mass distribution using a Gaussian frequency distribution. Second, the value of $\alpha = 0.5$ may not be accurate, which also affects the relative position of the oligomer peaks. Third, an evaporative light scattering detector was used in experiment, whose response to concentration is known to be non-linear [122, 123]. The first two factors cause errors in the range of retention times. The second factor, in addition, causes the systematic errors in the retention times of the individual oligomers. The first and third factors cause errors in the intensities of the peaks. Keeping these sources of errors in mind, the agreement of the predicted and experimental chromatograms in figure 3.68 is quite reasonable.

3.8.2.2 Prediction of peak widths in gradient elution

The same approach as in previous section was also applied to gradient elution except that equation 2.23 as such is not applicable for gradient elution. The first approximation may be to consider the peak broadening in column to be constant during gradient conditions, i.e. peak widths are independent of the gradient retention time or gradient slope. However, in practice the peaks for monodisperse samples eluting later in the column are sharper than those eluting earlier. In addition, the peaks of monodisperse samples are broader in shallow gradients than in steeper ones. A simple approach can be applied to calculate the peak widths at gradient elution still using equation 2.23. According to this approach, the peak width of a solute in gradient elution is approximated by the peak width as if the solute elutes isocratically in the eluent composition at the time of elution in the gradient. A similar approach has also already been used to predict the peaks widths of low molar mass compounds in gradient elution [124]. Therefore, the prediction of gradient chromatogram was consisted of the following steps: First, the retention time for each oligomer of the series, approximated by Gaussian distribution, was calculated using the PCM parameters for the individual peaks. Second, for each oligomer the composition at elution was calculated using equation 3.5 (section 3.1.2.1.2). Third, isocratic retention times corresponding to composition at elution was calculated using equation 2.8 (section 2.1.3) for each oligomer. Fourth, the retention times calculated in the third step were used to calculate peak widths using equations 2.23 and 2.24 (section 2.4.2.2). Finally, the peaks for each oligomer were summed up to obtain a complete chromatogram.

Figure 3.69 shows a gradient chromatogram of PEG 1000 in which the peak width for each PEG oligomer was calculated using the above-mentioned procedure. The experimentally obtained chromatogram is given for comparison. As can be seen there is a good agreement between the predicted and experimental retention range. In addition, as with the experiment, decreasing peak widths of the oligomers with retention time and fronting of the overall chromatogram is predicted. The examples given here show that the approach gives a reasonable estimate of peak widths for both isocratic and gradient elution.

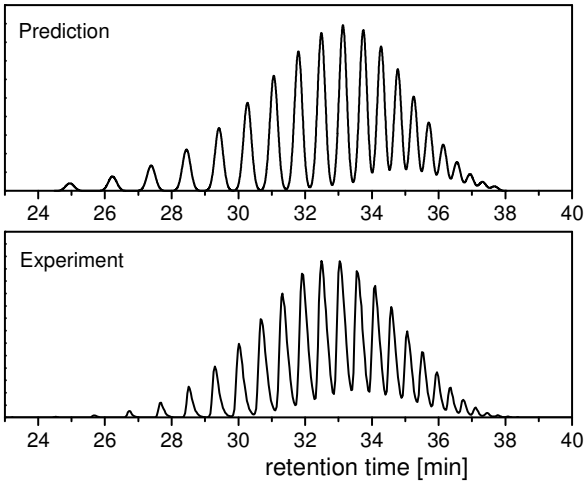


Figure 3.69: Comparison of predicted (top) and experimentally obtained (bottom) chromatogram for the elution of PEG 1000 in a linear gradient of 5 – 100 % MeOH against water over 60 minutes on column A. Parameters used are same as in figure 3.68.

3.8.3 Predicting the separation of a homopolymer blend

In order to test the approach incorporating the peak broadening factors, a separation of model blend of different homopolymers was used as test case, as discussed below.

3.8.3.1 Case study of a three-component blend

As discussed in the previous sections, molar mass effects should be absent or minimized in order to separate a mixture of components exclusively according to chemical composition. Therefore, for a three-component blend, LCCC where one component elutes independent of molar mass while the other components elute in SEC or LAC mode, respectively, might be the best choice. However, if the component eluting in LAC is of high molar mass, then the analysis time may become exceptionally long or the component may even be irreversibly retained on the column. Since high molar mass polymers elute very close to the critical eluent

composition, gradient elution may be a more suitable choice for such situations. In this experiment, the gradient profile can be programmed to deliver the critical compositions for each component to reduce the analysis time. Thus, once critical compositions are known, the separation of a complex mixture according to chemical composition can be easily predicted. In other words, robust and fast separations can be achieved when the molar masses of the sample components are sufficiently high.

In order to prove the above statement, the separation of a blend composed of three high molar mass components, i.e. PMMA, PtBA and PS was predicted and compared with the experiment. The molar masses and polydispersities of the polymers used are given in table 3.12.

Table 3.12: Molar masses and polydispersities of the blend components

Component	Polymer	Abbreviation	M_n (g/mol)	*PDI*	Colour code
I	Poly(methyl ethacrylate)	PMMA	829000	1.04	Black
II	Poly(*t*-butyl acrylate)	PtBA	839000	1.08	green
III	Polystyrene	PS	1010000	1.06	Red

Figure 3.70 shows the chromatograms for an initial gradient experiment on a reversed phase column. As can be seen, PMMA being the most polar component elutes very early, PtBA elutes much later, just before PS. This separation is achieved in 9 minutes.

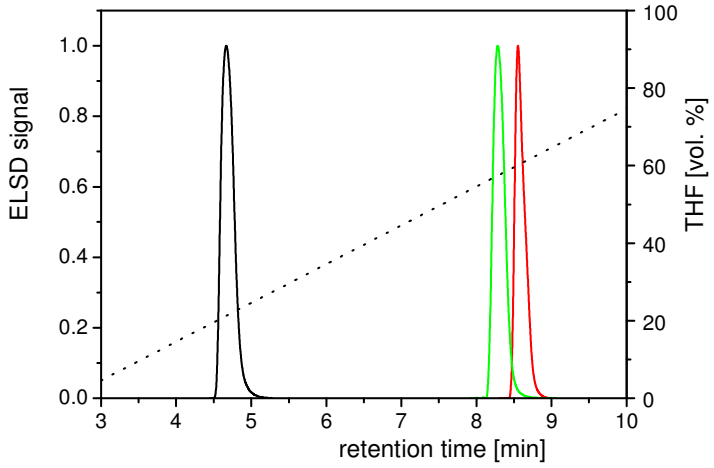


Figure 3.70: Overlay of chromatograms of PMMA (black), PtBA (green), and PS (red) obtained at a linear gradient of 100 % ACN – 100 % THF over 10 min as a first calibration run. Column: D. Flow rate: 1 ml/min. Temperature 35°C.

The second and third experiments were rationally selected according to the general rules derived in previous sections. Thus, isocratic experiments were performed at the

eluent compositions of 90/10 and 89/11 (for PMMA), 53/47 and 50/50 (for PtBA), and 50/50 and 47/53 v/v ACN/THF (for PS). The PCM parameters were extracted for each component. An optimized separation might be achieved by employing a gradient consisting of different steps. This type of gradient combines the greater selectivity with the shorter run time. A number of such experiments were simulated on computer. A step gradient that gives optimized separation of all three components was predicted. In this example, known values of molar mass and *PDI* were used to simulate the effect of polydispersity on band broadening.

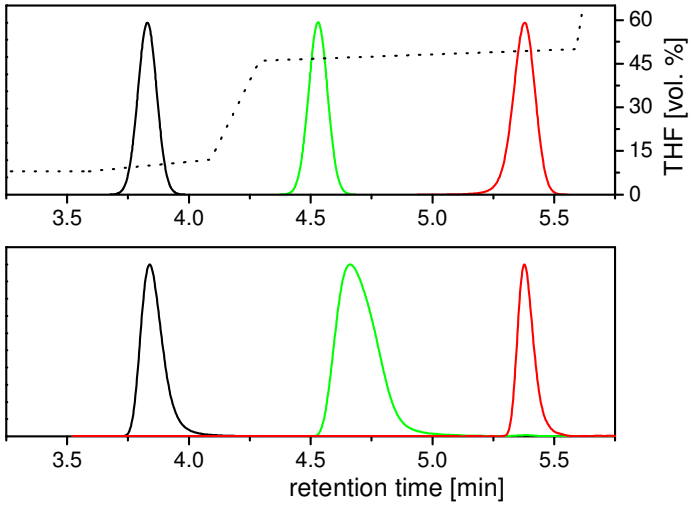


Figure 3.71: Predicted (top) separation of blend of PMMA (black), PtBA (green), and PS (red) obtained using a step gradient. Gradient profile: 0 – 0.5 min 8 – 12 %, 0.7 min 46 %, 2 min 50 %, 2.1 – 4 min 100 % THF against ACN, in comparison to real experiment. Chromatographic conditions same as in figure 3.70.

The results of the prediction and real experiment for the studied blend of high molar mass components are compared in figure 3.71. As can be seen, a good agreement between the predicted and experimental results, not only for the peak positions but also for the peak widths, is found. However, the experimentally observed peak width for PtBA is larger than the prediction. It is unlikely that the peak width in case of PtBA is due to the effect of polydispersity, since the molar mass of PtBA is too high to expect a significant molar mass effect on retention time. The broadening of PtBA peak might be the result of structurally different polymer molecules, e.g. molecules differing in tacticity or end groups etc. Such effects have not been included in the PCM. Peak widths of the other two are mainly due to the instrumental dispersion.

3.8.3.2 Case of two-component blend

As another example, the case of a model blend composed of two homopolymers having similar polarities and lower molar masses is considered. The characteristics of the components of this blend are given in table 3.13. The contributions of molar mass polydispersities to the peak widths were calculated using the known values of M_n and *PDI* unless it is mentioned otherwise.

Table 3.13: Molar masses and polydispersities of the blend components

Component	Polymer	Abbreviation	M_n (g/mol)	*PDI*	Colour code
I	poly(*n*-butyl acrylate)	PnBA	27600	1.13	Black
II	poly(*t*-butyl acrylate)	PtBA	92100	1.19	Red

Figure 3.72 shows that the two components are practically co-eluting in a linear gradient from 100 % ACN to 100 % THF over 10 minutes as a first step of the method development procedure. The slight difference in the peak positions suggests that there is some selectivity between the two components, although only one peak would have been observed if a mixture of both would have been injected.

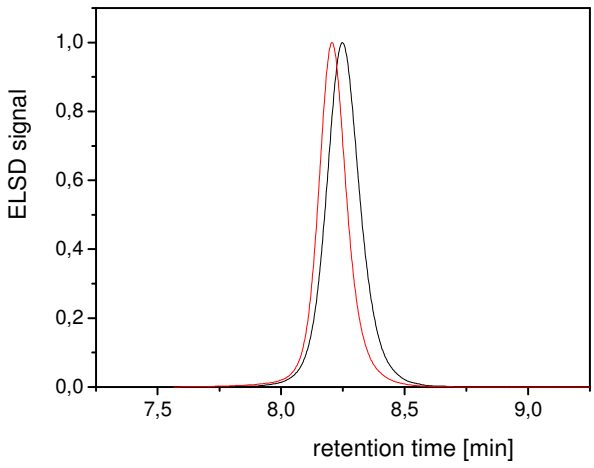


Figure 3.72: Overlay of chromatograms of PnBA (black) and PtBA (red). Gradient: 100 % ACN to 100 % THF linear over 10 min. as a first calibration run. Column: D. flow rate: 1 ml/min. Temperature: 35°C

The compositions at elution were calculated to be 53.4/46.6 and 53.8/46.2 v/v ACN/THF for PnBA and PtBA from the gradient run. The compositions at elution of both components differ by 0.5 % THF only. Following the rational strategy to select suitable initial experiments, the second and third runs were performed isocratically at

54/46 and 49/51 v/v ACN/THF, respectively. Figure 3.73 shows the overlays of the chromatograms for the two components at the two isocratic conditions.

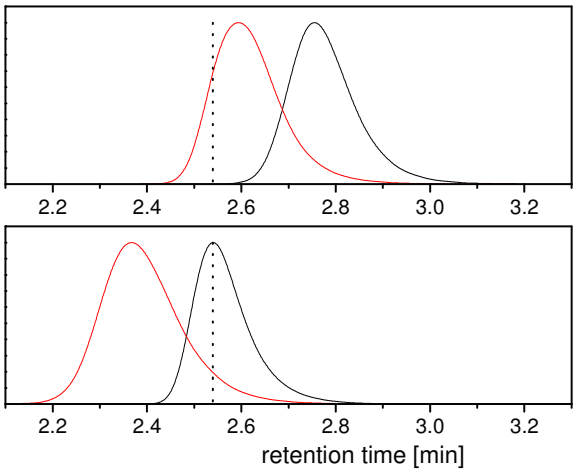


Figure 3.73: Overlay of chromatograms of PnBA (black) and PtBA (red) obtained at isocratic 54/46 (top) and 49/51 v/v ACN/THF (bottom), as 2nd and 3rd calibration run, respectively. Vertical line represents the column dead time. Chromatographic conditions were the same as in figure 3.72

As expected, both components can be eluted isocratically at the compositions at elution, estimated from the gradient experiment. The elution of both samples later than column dead time indicates that they are eluting in LAC mode. This shows that the eluent composition used for first isocratic experiment is not the critical composition for any of the components. PnBA is eluting later than PtBA although it has a lower molar mass as compared to the PtBA. This shows that the composition at elution is a weaker eluent for PnBA as compared to PtBA. In the second isocratic experiment, the elution of the PtBA occurs in SEC mode (elution at retention time lower than column dead time), while PnBA elutes very close to column dead time. Although, higher selectivity for the components is seen in the two initial isocratic experiments, yet they cannot be separated to baseline.

In the following, it will be discussed whether there is a possibility to achieve a baseline separation of the components of this blend, using virtual chromatography. For this purpose, the retention data from the three initial runs were used to extract the PCM parameters at the peak maximum. Peak positions with regard to the peak maxima were first predicted assuming the components to be monodisperse.

As it is known that the selectivity can be improved by increasing the gradient length, linear gradients of shallow slopes were simulated. As far as the peak maxima are

concerned, a better separation can be achieved but on the cost of longer analysis time. The isocratic experiment at 59/41 v/v ACN/THF should provide a useful selectivity along with a short run time as shown in figure 3.74.

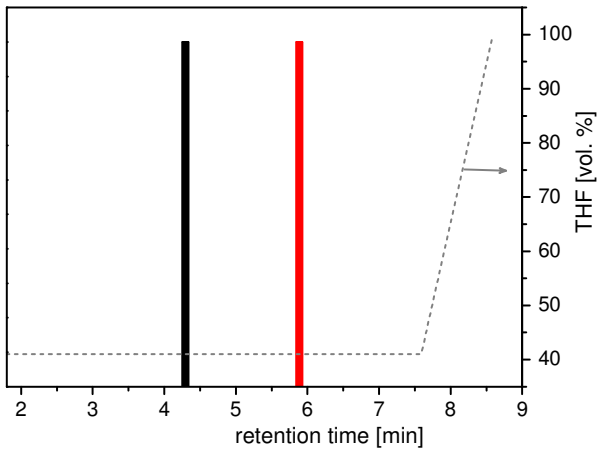


Figure 3.74: Predicted separation of blend of PnBA (black) and PtBA (red) using isocratic experiment. PCM parameters: PnBA, $\Phi_c = 50.44$, $dc/d\Phi = 1.97$, $(R/D)_{ref} = 0.078$; PtBA $\Phi_c = 46.87$, $dc/d\Phi = 2.22$, $(R/D)_{ref} = 0.1174$

Since the blend components are polydisperse, the effect of sample polydispersity must be included. For this purpose, the general strategy described in section 2.5.1.1 was used, employing the molar masses (M_n) and *PDI* values for both components. The concepts described in section 3.8.2.1 were used to account for the instrumental broadening. Thus, the overall retention behaviour of both components was simulated for the isocratic experiment with a gradient step in the end, as given above in figure 3.74. The comparison of simulated chromatograms with the experimental ones is given in figure 3.75. It can be seen that an adverse effect of molar mass polydispersity on the separation is predicted. According to prediction, a part of PnBA elutes early in the isocratic step, but as a very broad peak while a second minor part corresponding to the high molar mass fraction is expected to elute in the gradient step. Similarly, a part of the PtBA sample elutes isocratically along with PnBA, but the major part is eluting in the gradient step. A similar prediction results were obtained (figure 3.75, middle) using *PDI* values that result in good agreements between the simulated and the experimental peak widths for initial experiments. It is interesting to note that quite similar results are obtained when the experiments are performed in reality (figure 3.75, bottom). It is important to realize that the injection of both components would result in two distinct peaks. This might have been

erroneously interpreted as a true separation of the components without additional knowledge. Such a separation however, is not achieved in reality. The long tailing of the PnBA sample would not be seen by the detector unless a gradient step is run.

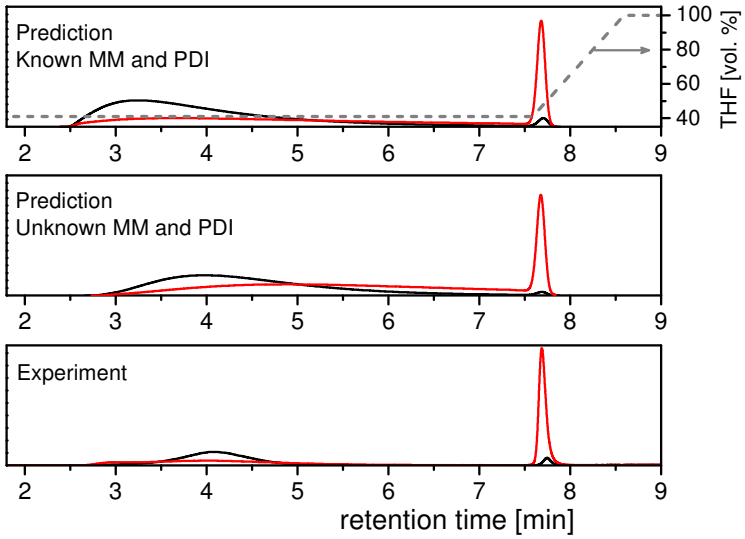


Figure 3.75: Predicted separation of blend of PnBA (black) and PtBA (red) in comparison to that obtained in the real experiment. Gradient: 0 – 4 min 41 % THF, 5 – 7 min 100 % THF against ACN. Used parameters: Top figure: PnBA, M_n = 27600 g/mol (Φ_c = 50.44, $dc/d\Phi$ = 1.97, $(R/D)_{ref}$ = 0.078), PDI = 1.13; PtBA, M_n = 92100 g/mol (Φ_c = 46.87, $dc/d\Phi$ = 2.22, $(R/D)_{ref}$ = 0.1174), PDI = 1.19; N' = 2500. Middle figure: molar mass = unknown, PDI = 1.05. Other chromatographic conditions were same as in figure 3.72

In the above example, both components elute at LAC conditions. However, LCCC has been shown to be a good choice for separation of blend components [6-8]. Predictions using the PCM accounting for band broadening showed that keeping one component in critical conditions while the other component elutes in SEC or LAC mode does not provide a better separation than obtained in the two initial isocratic experiments (figure 3.73), where the second isocratic experiment is already performed at critical conditions for PnBA. This is due to the presence of low molar mass molecules that do not give rise to strong exclusion or strong adsorption on the used column at the critical composition for the other polymer. The construction of the calibration curves, i.e. the molar mass dependences of retention time are very helpful to judge the applicability of a separation for particular range of molar masses of the samples. Figure 3.76 shows a solution to the present separation problem that is expected to give best separation. In this experiment, one component (PtBA) elutes in SEC mode while the other (PnBA) elutes under LAC conditions. The figure shows the overlay of the expected chromatograms under these conditions along with the

calibration curves for the two components. From the comparison of the ranges of retention times covered by the calibration curves and the corresponding peaks of two components in figure 3.76, it can be concluded that the width of the PtBA peak is mainly due to molar mass selectivity, while the peak of PnBA is adversely affected by the undesirable instrumental band broadening. It can be seen that the peaks overlap for a significant range of molar masses, i.e. below 30000 g/mol for PnBA and below 75000 g/mol for PtBA. These molar masses are represented by the horizontal lines in figure 3.76, which correspond to the retention times of the overlapping fractions of the peaks that are represented by the vertical lines.

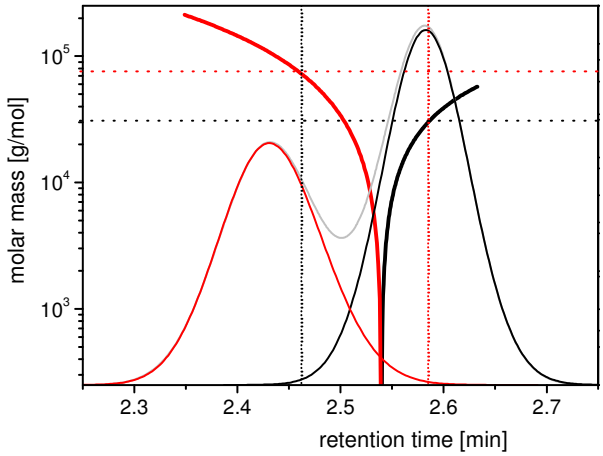


Figure 3.76: Overlay of simulated chromatograms of PnBA (black) and PtBA (red), with a summed peak (light grey) at an isocratic eluent of 51/49 v/v ACN/THF. The molar mass dependence of retention time is shown by solid lines. Horizontal lines highlight the overlapping molar masses of PnBA (black) and PtBA (red) determined from the corners of two peaks (vertical lines). Used parameters: same as in figure 3.74

A better separation, however, will be obtained by the same experiment using a small pore size column. Figure 3.77 shows the overlay of the chromatograms simulated for a 10 nm pore size of the stationary phase instead of 100 nm used in the experiments presented in this section. As can be seen the two components can be separated almost to baseline due to shifting the PtBA peak towards smaller retention times as a consequence of the smaller pore size of the stationary phase. In addition, the PnBA peak is also shifted but towards the larger retention times due to the larger value of *R/D*. Since the experimental conditions are very close to the critical condition of PnBA, only weak variation in peak position for the PnBA is predicted.

The presented case study of the blend clearly shows that influences of peak broadening due to molar mass distribution as well as instrumental broadening must be considered to predict polymer separations.

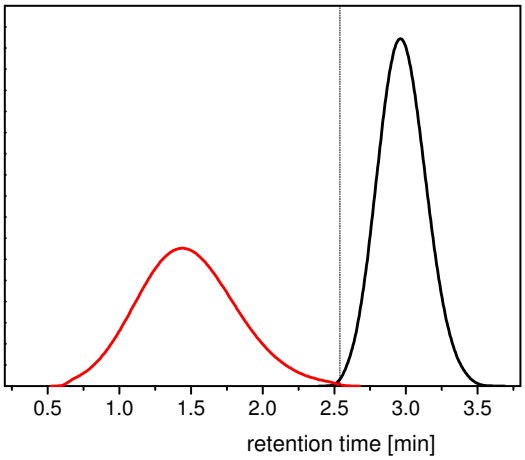


Figure 3.77: Overlay of simulated chromatograms of PnBA (black) and PtBA (red at an isocratic eluent of 51/49 v/v ACN/THF on a stationary phase with pore size 10 nm instead of 100 nm as used in figure 3.76. Vertical dashed line represents the column dead time. All other conditions same as in figure 3.76.

The examples of separations given in the preceding sections show that virtual chromatography can be very effective in predicting separations of polydisperse samples. Thus, it is a suitable tool to verify quickly whether a given column and eluent system is applicable to result in a robust separation. The use of virtual chromatography would significantly reduce efforts, time, expenditure of expensive consumables and the wear of instruments caused by unnecessary experimentations.

4 Summary and conclusions

Despite the recent advances in the understanding of polymer liquid chromatography, method development is still carried out using the "trial and error" methodology. This makes the method development in polymer chromatography a tedious, time consuming and expensive task. This thesis attempts to develop a rational strategy to ease the method development process in polymer chromatography. In this regard, the quantitative prediction of the retention behaviour of synthetic polymers is a significant element and gains increasing attention of polymer chromatographers. The successful prediction of retention allows developing fast and cost-cutting chromatographic methods. Therefore, in this thesis, the focus is given for the first time to computer assisted method development in polymer liquid chromatography based on a minimum number of experiments.

For this purpose, two conventional chromatographic models (LSSM and QSSM) that are frequently used in liquid chromatography of small molecules and one model specific for polymers (PCM) were applied to describe and predict the retention behaviour of polymers as a function of eluent composition, gradient time, gradient shape etc. The PCM was extended to account for linear and multi-step gradients. An application in Origin software was written that can be used to predict retention times in gradients of virtually any shape.

All the studied models can only be applied to predict the retention behaviour of monodisperse analyte molecules. This requirement is fulfilled by PEGs, which can be easily separated into a large number of individual oligomers up to relatively high molar masses. Thus, PEG oligomers were used as the model polymer system to test the suitability of the models to predict the retention times. The investigations accomplished in this thesis showed that not all examined models are equally appropriate to predict the retention behaviour of PEGs. The accuracy of predictions depends on the mode of elution as well as on the type of calibration experiments. However, all three models predict the gradient elution of PEGs with large accuracy if the calibration is constructed by gradient experiments. Only the QSSM and the PCM were able to predict the isocratic retention times of PEG oligomers in LAC conditions with reasonable accuracy when calibration is made from isocratic or

gradient experiments. However, calibration using isocratic experiments is not very practical because of the difficulties in establishing conditions allowing for isocratic elution, especially for high molar mass polymers that elute only very close to critical or in SEC conditions. It was shown that both the LSSM and the QSSM for principle reasons could not describe the retention behaviour at the critical point of adsorption or in SEC conditions, two very important modes of polymer chromatography. Only the PCM is able to describe the retention times in all the three modes of polymer chromatography. Being the most appropriate model to describe the retention behaviour of the model polymers the PCM was selected for further studies.

The quality of the PCM predictions for the isocratic retention times of PEGs depend strongly on the choice of suitable starting experiments. It was found that the most purposeful calibration consists of one gradient experiment and two isocratic experiments, one in LAC and the other in SEC conditions. With the help of simulations, a rational strategy to select these experiments was developed for the first time. According to this approach, the first experiment should be a linear gradient. From this experiment, the eluent composition at the time of the elution is calculated. The calculated eluent composition is used to perform the first isocratic experiment. According to theoretical considerations, this experiment should lead either to elution in adsorption mode or to elution under critical conditions of adsorption. On basis of the retention behaviour of the first isocratic experiment, the second isocratic experiment is carried out in a slightly stronger eluent than used in the first isocratic experiment to obtain elution in SEC conditions. The retention data of these three rationally selected starting experiments permit the extraction of the PCM parameters with reasonable accuracy allowing reliable predictions in both the isocratic and gradient elution mode. The results obtained for PEGs could be verified for PMMAs using similar investigations with polydisperse PMMA standards. This indicates that the developed strategy is generally applicable to homopolymers.

It was shown that gradient elution of high molar mass polymers occurs very close to the critical composition. This can be explained easily on the basis of the PCM. The close relationship between composition at elution and the critical composition was utilized for fast estimations of critical eluent compositions of different homo-polymers for different stationary phase/mobile phase combinations gradient

experiments. The higher the molar mass of the polymer, the more precise was the estimate of the critical composition. This fast estimation of critical composition for polymers reflects the benefit of application of chromatographic theory to accelerate the method development process for polymers.

The suitability of the PCM to predict retention times was demonstrated also for real separations. As an example, a separation for a model blend composed of poly(n-butyl acrylate), poly(t-butyl acrylate) and two poly(methyl methacrylate)s was carried out. Using the virtual chromatography approach a multi-step gradient could be developed in a short time, which resulted in a baseline separation of the four components.

In addition to homopolymers, the investigations of the suitability of the PCM were extended to describe and predict the retention behaviour of copolymers. It was shown that statistical copolymers of styrene and ethyl acrylate (SEA copolymers) behave chromatographically similar to homopolymers and the PCM is equally suitable to describe and predict the retention times as a function of eluent composition and gradient slope. Similar to high molar mass homopolymers, the composition at elution of high molar masses for statistical copolymers in a gradient experiment corresponds very well with the critical eluent composition. This behaviour results in a simple procedure for the determination of the critical composition for statistical copolymers for the first time. With the help of virtual chromatography employing the PCM, it was possible to develop gradients that resulted in a linear dependence of retention time on the chemical composition of the statistical SEA copolymers. This linear calibration facilitates the computation of the chemical composition distribution from the chromatograms. The extension of the studies to the graft copolymers composed of polybutadiene and poly(methyl methacrylate) showed that also their retention behavior in gradient elution can be described reasonably by the PCM. This is surprising because theoretically even block copolymers can be described only by at least six parameters instead of just three used in the PCM.

The PCM was extended to incorporate the effect of band broadening due to molar mass polydispersity of the polymer samples. According to theoretical considerations, only one parameter of the PCM, *R/D*, changes with the molar mass of the polymer molecules. A simple scaling relationship describing the dependence of *R/D* on molar

mass was used. It was shown that even no information about the molar mass or polydispersity of the sample is required, yet the complete retention behaviour of the polydisperse samples in various experimental conditions (varying eluent composition, gradient slope, and gradient shape etc.) can still be predicted with good accuracy. Using the examples of blends, the influence of sample polydispersity on the resolution of the blend components was demonstrated. The problem is severe especially in the low molar mass range where the molar mass strongly affects retention times. It was found that the effect of instrumental broadening must also be incorporated to predict real separations when weak or no influence of sample polydispersity exists. In order to include instrumental broadening one additional isocratic experiment with a strongly adsorbing low molar mass substance is required. A general approach to predict the effect of instrumental broadening in the gradient elution is used. According to this approach, a decrease in the peak widths with retention times is predicted for the monodisperse analytes eluting in a linear gradient, while for one analyte the peak widths increases with increasing gradient slope. Since elution of high molar mass polymers in adsorption chromatography is possible only in conditions where molar mass of the samples has little influence on the peak widths, the separation of high molar mass polymers is relatively easy and can be optimized easily.

The results obtained in this thesis show that the PCM is the most suitable chromatographic model for the prediction of the retention behavior of variety of polymers. Therefore, a fast and purposeful method development in polymer chromatography is possible employing the virtual chromatographic method. Using this approach suitability of a certain chromatographic system to obtain optimum separations can be evaluated in short time. This can save substantially the precious time of a chromatographer for screening the mobile phases and stationary phases.

5 Zusammenfassung

Moderne Kunststoffe sind komplexe Materialien. Zur Erreichung eines gewünschten Eigenschaftsprofils werden Homopolymere, Copolymere und funktionalisierte Polymere alleine oder als Blends verwendet. Die aus mehreren Komponenten bestehenden Werkstoffe sind überaus komplex, aber auch schon beim Einsatz nur eines einzigen Polymeren treten Heterogenitäten bezüglich der Funktionalität, der chemischen Zusammensetzung, der Topologie oder des Molekulargewichtes auf. Zur Charakterisierung derartig komplexer Produkte, z.B. für das Erstellen von Struktur-Eigenschaftsbeziehungen, für die Qualitätssicherung oder zum Nachweis von Patentrechtsverletzungen, sind Methoden der Flüssigkeitschromatographie, und hier vor allem der Wechselwirkungschromatographie besonders geeignet. Diese erlauben nicht nur die Bestimmung der mittleren Zusammensetzungen oder Funktionalitäten, sondern der kompletten zugehörigen Verteilungsfunktionen. Ihre Verwendung für ein spezielles Trennproblem setzt jedoch die Existenz einer geeigneten chromatographischen Methode voraus. Während jedoch die Durchführung der chromatographischen Messungen einfach und schnell ist, kann sich – je nach Problemstellung - die chromatographische Methodenentwicklung bzw. Methodenoptimierung schwierig, zeit- und kostenaufwendig gestalten.

Im Bereich der Chromatographie niedermolekularer Substanzen werden aus diesen Gründen bei der Methodenentwicklung teilweise schon Computerprogramme eingesetzt, die es erlauben, nach Durchführung weniger „Kalibrierexperimente“ das chromatographische Verhalten des zu trennenden Substanzgemisches unter weiter veränderten chromatographischen Bedingungen vorherzusagen. Den zentralen Punkt einer solchen computerunterstützten Methodenentwicklung bildet ein Retentionsmodell, welches die Abhängigkeit der Retentionszeiten von den experimentellen Variablen, wie Temperatur, Eluentenzusammensetzung, Gradientensteilheit usw. grundsätzlich beschreibt, dabei jedoch eine Anzahl anpassbarer und substanzspezifischer Parameter enthält. Ausgehend von einer minimalen Anzahl an Start- oder Kalibrierexperimenten ermittelt man die Retentionszeiten der einzelnen Substanzen. Die experimentellen Parameter sowie die daraus resultierenden Retentionszeiten werden anschließend dem Modell zur Verfügung gestellt. Die Modellparameter der einzelnen Substanzen werden derartig

angepasst, dass eine möglichst gute Übereinstimmung der berechneten und der experimentell ermittelten Retentionszeiten erreicht wird (Kalibration). Die so gewonnenen substanzspezifischen Parameter können nun verwendet werden, um vorherzusagen, wie Trennungen unter anderen experimentellen Bedingungen aussehen sollten. Durch Variation der experimentellen Parameter am Computer können so in kürzester Zeit eine große Anzahl an unterschiedlichsten Experimenten simuliert und damit optimale chromatographische Bedingungen für die jeweils durchzuführende Trennung gefunden werden (Virtuelle Chromatographie).

Obwohl in den letzten Jahren erhebliche Fortschritte im Verständnis des Retentionsverhaltens von Polymeren gemacht wurden, erfolgt die Methodenentwicklung im Bereich der Polymerchromatographie dennoch immer noch weitgehend empirisch nach den dem „Trial and Error“-Verfahren. Hierdurch bleibt die chromatographische Methodenentwicklung für Polymere eine schwierige, zeitraubende und damit kostenintensive Aufgabe. In der vorliegenden Arbeit wurde daher der Versuch unternommen, eine rationale Strategie zur Erleichterung dieser Aufgabe zu entwickeln.

Auf Grund der zentralen Stellung, welche die Retentionsmodelle in der virtuellen Chromatographie einnehmen, war es daher das Ziel der vorliegenden Arbeit, Retentionsmodelle und experimentelle Strategien zu bewerten und zu entwickeln, die eine verlässliche Vorhersage der Retentionszeiten von Polymeren basierend auf wenigen Kalibrierexperimenten erlauben.

Zu diesem Zwecke wurden zwei konventionelle chromatographische Retentionsmodelle (LSSM und QSSM), welche häufig im Bereich der Chromatographie niedermolekularer Verbindungen verwendet werden, sowie ein Modell, welches die polymerspezifischen Besonderheiten der Flüssigkeitschromatographie berücksichtigt (PCM), angewandt, um das chromatographische Verhalten von Polymeren als Funktion der Eluentenzusammensetzung sowie der Gradientendauer und –form zu beschreiben und auch vorherzusagen Das PCM wurde dabei im Verlaufe dieser Arbeit erweitert, um auch für lineare und Stufengradienten anwendbar zu sein. Schließlich wurden Anstrengungen unternommen, auch die Effekte der Polydispersität und der instrumentellen Bandenverbreiterung auf die Peakformen zu erfassen

Für die gestellte Aufgabe wurde zunächst das Retentionverhalten niedermolekularer Polyethylenglykole (PEGs) untersucht, welches in die einzelnen Oligomere getrennt werden können. Durch Verwendung linearer Gradienten unterschiedlicher Steilheit sowie isokratischer Experimente konnten für die einzelnen Oligomeren Kalibrierparameter ermittelt und damit Vorhersagen zum Verhalten auch unter veränderten chromatographischen Bedingungen gemacht werden. Die Qualität der Voraussagen wurde an Hand der Abweichungen zwischen den Vorhersagen und dem tatsächlichen Experiment bewertet. Es zeigte sich, dass alle drei Modelle für PEGs hervorragend das chromatographische Verhalten in Gradienten vorhersagen, sofern die Kalibration ebenfalls mittels Gradientenmessungen erfolgte. Hingegen können weder das LSSM noch das QSSM das isokratische Verhalten von PEGs hinreichend exakt beschreiben. Hierfür sind prinzipielle Unzulänglichkeiten der Modellannahmen verantwortlich, die eine Vorhersage der Elution im Größenausschlussmodus (SEC) oder in der Nähe des kritischen Punktes der Adsorption (LCCC) unmöglich machen. Gerade diese Elutionsmodi sind aber im Bereich Polymerchromatographie von enormer Wichtigkeit. Das PCM hingegen ist prinzipiell in der Lage, aller Modi der Polymerchromatographie qualitativ zu beschreiben.

Die Genauigkeit der Vorhersage hängt dabei jedoch empfindlich von der Wahl geeigneter Kalibrierexperimente ab. Aus Simulationen konnte gefolgert werden, dass offenbar die besten Voraussagen erhalten werden, wenn man als Startexperimente ein Gradienten- sowie zwei isokratische Experimente verwendet. Dabei sollte ein isokratisches Experiment möglichst zu einer Elution im Adsorptions-, das zweite zu einer Elution im Größenausschlussmodus führen. Da aber gerade das Auffinden geeigneter isokratischer Elutionsbedingungen im Adsorptionsmodus ein schwieriges Problem darstellt, wurde eine allgemeine experimentelle Vorgehensweise zur Auswahl der Kalibrierexperimente entwickelt. Gemäß dieser Strategie wird zunächst ein lineares Gradientenexperiment durchgeführt. Aus diesem Experiment kann die Eluentenzusammensetzung zum Zeitpunkt der Elution ermittelt werden. Bei dieser sollte eine Elution unter schwach adsorbierenden oder unter kritischen Bedingungen und somit eine vollständige Elution der Proben erfolgen. Daher sollte bei dieser Zusammensetzung das erste isokratische Experiment durchgeführt werden. Auf der Basis dieses ersten isokratischen Experimentes wird dann das zweite isokratische Experiment unter Verwendung eines geringfügig stärkeren Eluenten durchgeführt,

wodurch eine Elution unter SEC-Bedingungen resultieren sollte. Damit stehen drei sinnvoll gewählte Kalibrierexperimente zur Verfügung die eine verlässliche Bestimmung der Modellparameter erlauben.

Um zu überprüfen, ob die an den PEGs erhaltenen Ergebnisse allgemeine Gültigkeit haben, wurden anschließend Untersuchungen an Polymethylmethacrylaten (PMMA) durchgeführt. Diese Untersuchungen bestätigten die an den PEGs gefundenen Befunde und belegen so ihren universellen Charakter.

Die an den Homopolymeren gewonnenen Erkenntnisse wurden anschließend genutzt, um mittels virtueller Chromatographie die Bedingungen für die Trennung eines Blends aus Poly(*n*-butylmethacrylat), Poly(*t*-butylmethacrylat) und zweier PMMAs mit unterschiedlichen mittleren Molekulargewichten zu erarbeiten. Basierend auf der allgemeinen Strategie zur Auswahl der Startexperimente wurden die Kalibrierparameter der unterschiedlichen Substanzen ermittelt und damit ein mehrstufiger Gradient entwickelt, der theoretisch eine gute Trennung des Polymerengemisches ergeben sollte. Diese konnte auch im Experiment realisiert werden. Die Retentionszeiten korrespondierten dabei sehr gut mit der Vorhersage.

Zur Überprüfung der Fragestellung, ob das PCM und die entwickelte Vorgehensweise zur Bestimmung der Modellparameter auch das Retentionsverhalten statistischer Copolymere zufriedenstellend widergibt, wurde das chromatographische Verhalten von Copolymeren aus Styrol und Ethylacrylat analysiert. Zum Einsatz kamen dabei Copolymere unterschiedlicher Zusammensetzung, die nur eine geringe chemische Heterogenität aufwiesen. Auch bei den Copolymeren konnte das isokratische Verhalten durch das PCM zufriedenstellend wiedergegeben werden. Um auch eine Aussage über die Praxistauglichkeit des PCM für Trennungen von Copolymeren zu machen, wurde mittels der virtuellen Chromatographie ein Gradient entwickelt, der zu einer linearen Beziehung zwischen dem Retentionsvolumen und der Copolymerzusammensetzung führen sollte. Ein solcher Gradient würde die Umrechnung des erhaltenen Chromatogramms in eine Heterogenitätsverteilung deutlich erleichtern. Die experimentelle Überprüfung des entwickelten multilinearen Gradienten ergab tatsächlich den vorhergesagten linearen Zusammenhang zwischen Elutionsvolumen und Copolymerzusammensetzung und belegt damit eine hervorragende Übereinstimmung zwischen dem Experiment und der Vorhersage.

Im Verlaufe der Untersuchungen an den Homo- und statistischen Copolymeren wurde festgestellt, dass diese bei der Gradientenelution nahezu unabhängig von der Gradientensteilheit immer bei einer Zusammensetzung eluieren, die sehr nahe an der Zusammensetzung am kritischen Punkt der Adsorption liegt. Hierdurch kann mit einem einzigen Gradientenexperiment an einer einzigen hochmolekularen Probe die kritische Eluentenzusammensetzung mit geringem Fehler abgeschätzt werden.

Statistische Copolymere sollten sich in ihrem chromatographischen Verhalten wie Homopolymere verhalten, wenn die Korrelationslängen der Monomereinheiten deutlich geringer sind als die Abstände zwischen den adsorbierten Polymerbereichen. Für segmentierte Copolymere ist diese Annahme jedoch i.A. nicht gerechtfertigt. Um zu untersuchen, ob das PCM dennoch auch für segmentierte Copolymere sinnvolle Vorhersagen liefert, wurden Untersuchungen an Pfropfcopolymeren aus PMMA und Polybutadien durchgeführt.

Durch analytische Fraktionierung mittels Gradientenchromatographie wurden dazu zunächst zwei Pfropffraktionen mit unterschiedlicher chemischer Zusammensetzung erhalten. Diese wurden für die weiteren chromatographischen Untersuchungen genutzt. Ebenso wie zuvor bei den Homo- und statistischen Copolymeren wurde zunächst analysiert, wie gut die Vorhersagen durch das PCM gelingen. Entgegen der Annahme wurde festgestellt, dass selbst für segmentierte Pfropfcopolymere verlässliche Vorhersagen gewonnen werden können, wenn man für die Kalibration sinnvoll gewählte Kombinationen aus isokratischen und Gradientenexperimenten verwendet.

Gemäß den obigen Ausführungen ist das PCM bei geeigneter Wahl der Kalibrierexperimente sehr gut in der Lage, die Retentionszeiten unterschiedlichster Polymerer vorherzusagen. Zur vollständigen Vorhersage einer chromatographischen Trennung ist es jedoch notwendig, die durch die Probendispersität und die instrumentellen Bedingungen hervorgerufene Bandenverbreiterungen zu berücksichtigen.

Dies gelang für den Effekt der Polydispersität durch eine Erweiterung des PCM. Gemäß den Voraussetzungen des PCM hängt nur die Molekülgröße R vom Molekulargewicht des Polymeren ab. Zur Beschreibung dieser Abhängigkeit genügt eine einfache Skalierungsrelation. Es konnte gezeigt werden, dass mit diesem

einfachen Ansatz, die Peakform mit guter Genauigkeit beschrieben werden kann, selbst wenn keine Informationen zur Polydispersität vorliegen. Der Einfluss der Polydispersität auf die Auflösung von Polymerblends wurde analysiert und diskutiert.

Die Berücksichtigung des Einflusses der instrumentellen Bandenverbreiterung in Gradienten gelang durch einen Ansatz, welcher bislang nur für niedermolekulare Substanzen Anwendung fand. Dabei wird angenommen, dass die Peakbreite unter Gradientenbedingungen gleich derjenigen ist, die resultiert, wenn die Probe bei der Eluentenzusammensetzung zum Zeitpunkt der Gradientenelution isokratisch chromatographiert wird.

Die Ergebnisse der vorliegenden Arbeit belegen, dass von den untersuchten Retentionsmodellen das PCM am besten geeignet ist, das Retentionsverhalten verschiedenster Polymere zu beschreiben. Hierdurch ist eine schnelle und zielgerichtete Methodenentwicklung unter Verwendung der virtuellen Chromatographie möglich. Mittels virtueller Chromatographie ist es auch möglich, schnell die generelle Eignung eines chromatographischen Systems für eine gegebene Trennaufgabe zu bewerten. Hierdurch lässt sich der Aufwand für die Auswahl geeigneter stationärer und mobiler Phasen erheblich reduzieren.

6 Experimental

6.1 Equipment

All measurements were performed using an Agilent 1100 series HPLC system (Agilent Technologies GmbH, Böblingen, Germany) consisting of vacuum degasser (G1322A), quaternary pump (G1311A), auto-sampler (G1313A), column oven (G1316A), and variable wavelength UV-detector (G1314A). In addition an evaporative light scattering detector (ELS 1000, Polymer Laboratories Inc. church Stretton, England) was used. Data collection and processing was performed using PSS WinGPC version 6 software (PSS Polymer Standards Service, Mainz, Germany).

The void and interstitial volumes of the columns were estimated by injecting toluene and a high molar mass polystyrene standard (PS 2570000), respectively, using pure tetrahydrofuran (THF) as eluent. Pore volumes were taken as the void volume subtracted by interstitial volume.

The dwell volume was determined to be 1.05 ml by subtracting the void volume from the onset of the increasing UV-signal due to a linear gradient starting from pure methanol and running to methanol containing 0.3 % acetone.

The isocratic experiment used to determine column void volume was also used to calculate the extent of instrumental broadening including the column. The number of theoretical plates (*N*') for the columns was determined from the isocratic experiment of PEG 1000 in 54/46 v/v water/methanol as eluent (from the widths at half heights of late eluting peaks).

All fittings, predictions, and calculations were performed using Origin® Software (OriginLab Corporation, Northampton, USA) employing self-written scripts in Origin's LabTalk programming language.

6.2 Chromatographic conditions

For all samples, eluents or the starting eluent in gradients were used as solvent for injection. Sample concentrations were 1 – 2 g/L. The injected sample volume was

10 – 20 µl. Column temperature 35°C and flow rate was 1 ml/min unless otherwise mentioned. All experiments were performed using duplicate injections.

6.3 Polymers samples, Solvent/Eluents, and Columns

6.3.1 Polymer samples

Polyethylene glycol (PEG), Polystyrene (PS), Poly(methyl methacrylate) (PMMA), Poly(n-butyl acrylate) (PnBA), Poly(t-butyl acrylate) (PtBA), Poly(n-butyl methacrylate) (PnBMA), Poly(t-butyl methacrylate) (PtBMA) and Poly(decyl methacrylate) (PDMA) having different molar masses and narrow polydispersities were obtained from PSS Polymer Standards Service GmbH, Mainz, Germany, unless otherwise mentioned. The molar masses and polydispersities of the PEG, PMMA, and PS samples used in the present studies are given in tables 4.1 – 4.3. Properties of all other samples can be found in the sections where they are reported.

Table 4.1: Molar masses and polydispersities of the PEG samples used in this thesis.

Name	M_p **g/mol**	M_w **g/mol**	***PDI***	**Supplier**
PEG 200	200	200	~ 6 oligomers	Hüls
PEG 400	400	400	~ 10 oligomers	Hüls
PEG 1000	1000	1000	1.03	Hüls
PEG 2010	2010	1960	1.03	PSS
PEG 3120	3120	3060	1.03	PSS
PEG 6240	6240	6000	1.03	PSS
PEG 12000	12000	11200	1.51	PSS
PEG 23000	23000	22500	1.60	PSS
PEG 40000	40000	41500	1.14	PSS

Table 4.2: Molar masses and polydispersities of used PMMA standards.

Name	M_p **g/mol**	M_w **g/mol**	***PDI***
PMMA 1.2k	1210	1070	1.20
PMMA 3.5k	3500	3600	1.10
PMMA 10.9k	10900	10600	1.06
PMMA 30.5k	30500	29000	1.06
PMMA 60.0k	60000	60000	1.03
PMMA 240.0k	240000	-	1.04
PMMA 700.0k	700000	640000	1.10

Table 4.3: Molar masses and polydispersities of used PS standards.

Name	M_p g/mol	M_w g/mol	*PDI*
PS 1620	1620	1560	1.1
PS 3420	3420	3470	1.06
PS 18100	18100	17900	1.03
PS 67500	67500	65000	1.02
PS 130000	130000	125000	1.04
PS 246000	246000	226000	1.06
PS 579000	579000	564000	1.04
PS 1040000	1040000	1000000	1.03
PS 2570000	2570000	2530000	1.04

6.3.2 Solvents

Following solvents were used as received:

Acetonitril (ACN), HPLC grade, Acros Organics,

Cyclohexane (c-hexane), HPLC grade, Fisher Chemicals,

Methanol (MeOH), HPLC Grade, Chromasolv,

Methyl ethyl ketone (MEK), HPLC grade, Acros Organics,

Toluene, HPLC grade, Acros Organics.

Tetrahydrofuran (THF) was refluxed and distilled from CaH_2.

Distilled water was further deionized (conductivity 0.054 µS/cm) using Millipore Simplicity 185 (UV) water system (Millipore GmbH, Schwalbach, Germany).

Isocratic eluents of different compositions were delivered by the pump system.

6.3.3 Columns

The following columns were used:

A. Nucleosil C18;
   Particle size 5 µm, pore diameter 300 Å, column dimensions 250×4.6 mm i.d. (Macherey–Nagel, Düren, Germany). Void volume 3.10 ml, interstitial volume 1.54 ml.

B. Nucleosil bare Si;
Particle size 7 µm, pore diameter 1000 Å, column dimensions 250×4.0 mm i.d. (Macherey–Nagel, Düren, Germany). Void volume 2.59 ml, interstitial volume 1.29 ml.

C. Chromolith Si,
Mesopores 130 Å, macropores 2 µm, 100×4.6 mm i.d. (Merck KGaA, Darmstadt, Germany). Void volume 1.50 ml.

D. Nucleosil C18,
Particle size 7 µm, pore diameter 1000 Å, column dimensions 250×4.0 mm i.d. (Macherey–Nagel, Düren, Germany). Void volume 2.54 ml, interstitial volume 1.30 ml.

E. High-throughput Luna C18,
Particle size 3 µm, pore diameter 100 Å, column dimensions 35×4.0 mm i.d. (Phenomenex, Germany). Void volume 0.55 ml, interstitial volume 0.30 ml.

6.4 Parameter extraction

The parameters of the used models were extracted using Origin's non-linear fitting tool, which employ Levenberg-Marquardt algorithm for the iterative process. The first step in a fitting process was to plot the data against the experimental variable, e.g., eluent composition or gradient slope. The values of the known parameters such as, pore size, pore volume and interstitial volume of the column etc. were provided to the program. After initializing the values of the unknown parameters (ln k_0, S for LSSM; A, B, C for QSSM; and Φ_c, dc/dΦ, R/D for PCM), the iterative process was carried out until the calculated curve matches the experimental data well. The quality of the fit was monitored by observing the residuals during the iterative process. The best-fit values of the model parameters produced deviations of less than 1 % in most cases.

7 List of abbreviations/symbols

A, B, and C	The analyte-specific parameters of quadratic solvent strength model
ACN	Acetonitrile
α	Exponent of radius of gyration vs. molar mass relationship
c	Interaction parameter
CCD	Chemical composition distribution
c_{final}	Interaction parameter in final mobile phase composition of a gradient
$c_{initial}$	Interaction parameter in initial mobile phase composition of a gradient
D	diameter of the pores of stationary phase
$dc/d\Phi$	The change in interaction parameter per change of mobile phase composition
DP	Degree of polymerization
ΔG	Free energy difference
ΔH	Change in interaction enthalpy
ΔS	Change in conformational entropy
$\Delta\Phi$	Change in mobile phase composition during a gradient
ELSD	Evaporative light scattering detector
erf	Error function
erf^{-1}	Inverse error function
F	Flow rate
FTD	Functionality type distribution
FTIR	Fourier transform Infrared
Φ	Mobile phase composition
Φ_{ave}	Average composition that molecules experience during a gradient experiment
Φ_c	Critical mobile phase composition
$\Phi_{initial}$	mobile phase composition at the start of gradient
Φ_{final}	mobile phase composition at the end of gradient

G	The gradient slope i.e. the change of mobile phase composition per unit time G = ΔΦ/tG
HPLC	High performance liquid chromatography
iLC	Interaction liquid chromatography
k	Retention factor
k_{ave}	Average retention factor of the analyte molecule in a linear gradient
K_d	Distribution coefficient
$k_{initial}$	Retention factor in the initial mobile phase composition of a gradient
K_{LAC}	Contribution of adsorption to distribution coefficient
K_{SEC}	Contribution of size exclusion to distribution coefficient
L	Kuhn length or polymer flexibility parameter
LAC	Liquid adsorption chromatography
LC	Liquid chromatography
LCCC	Liquid chromatography at critical conditions of adsorption
ln k	Logarithmic retention factor
ln k_0	Logarithm of the retention factor in the pure weak component of the mobile phase
LSSM	Linear solvent strength model
MEK	Methyl ethyl ketone
MeOH	Methanol
min	Minute
MMA	Methyl methacrylate
M	Molar mass
MMD	Molar mass distribution
M_n	Number average molar mass
M_p	Molar mass at the peak maximum of MMD
M_{ref}	Reference molar mass
M_w	Weight average molar mass
N	Classical number of theoretical plates in column
N'	Number of theoretical plates in column independent of retention time

NLS	Non-linear least square
nm	Nanometer
NMR	Nuclear magnetic resonance
PCM	Polymer chromatographic model
PDI	Polydispersity
PDMA	Poly(decyl methacrylate)
PEA	Poly(ethyl acrylate)
PEG	Polyethylene glycol
PMMA	Poly(methyl methacrylate)
PnBA	Poly(n-butyl acrylate)
PnBMA	Poly(n-butyl methacrylate)
PS	Polystyrene
PtBA	Poly(t-butyl acrylate)
PtBMA	Poly(t-butyl methacrylate)
% B	Amount of good solvent in a binary mobile phase composition
QSSM	Quadratic solvent strength model
R	Radius of gyration
$(R/D)_{ref}$	Radius of gyration for a reference molar mass
S	LSSM parameter, the slope of the plot of ln k vs. mobile phase composition
SEA	Poly(styrene-co-ethyl acrylate)
SEC	Size exclusion chromatography
$\sigma^2_{extra\text{-}column}$	Dispersion caused by chromatographic setup outside the column
$\sigma^2_{intra\text{-}column}$	Dispersion in the column
$\sigma^2_{observed}$	Variance of the observed peak
σ^2_{PDI}	Variances of the peak caused by the sample polydispersity
σ^2_n	Variance of the frequency distribution
T	Absolute temperature,
t	Time

t_0	Column dead time
t_G	Gradient time
THF	Tetrahydrofuran
t_i	Retention time of a completely excluded sample
t_p	Retention time of a totally permeated sample minus t_i
t_R	Retention time
U	Non-uniformity
V_0	Void volume of the column
$V_{0,e}$	Void volume available of eluting step in a multi-step gradient
$V_{0,x}$	Void volume of the hypothetical column migrated by the polymer molecule in a non-eluting step in a multi-step gradient
VC	Virtual chromatography
$V_{d.}$	Dwell volume of chromatograph
$V_{G,x}$	Length of a step in a multi-step gradient
V_i	Interstitial volume of the column
$V_{i,x}$	Interstitial volume of the hypothetical column migrated by the polymer molecule in a non-eluting step in a multi-step gradient
V_p	Pore volume of the stationary phase
$V_{P,x}$	Pore volume of the hypothetical column migrated by the polymer molecule in a non-eluting step in a multi-step gradient
V_R	Retention volume
$V_{R,e}$	Retention volume in the eluting step in a multi-step gradient
$W_{1/2}$	Peak width at half height

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Eidesstattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich meine Dissertation selbstständig und nur mit den angegebenen Hilfsmitteln angefertigt und noch keinen Promotionsversuch unternommen habe.

Mubasher Ahmed Bashir
Darmstadt, den 12.12.2006