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Journal of Chromatography A, 1131 (2006) 130–141
JOURNAL OF CHROMATOGRAPHY A
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Comparison of retention models for polymers 1. Poly(ethylene glycol)s

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Received 25 April 2006; received in revised form 10 July 2006; accepted 18 July 2006
Available online 11 September 2006

Abstract

The suitability of three different retention models to predict the retention times of poly(ethylene glycol)s (PEGs) in gradient and isocratic chromatography was investigated. The models investigated were the linear (LSSM) and the quadratic solvent strength model (QSSM). In addition, a model describing the retention behaviour of polymers was extended to account for gradient elution (PM). It was found that all models are suited to properly predict gradient retention volumes provided the extraction of the analyte specific parameters is performed from gradient experiments as well. The LSSM and QSSM on principle cannot describe retention behaviour under critical or SEC conditions. Since the PM is designed to cover all three modes of polymer chromatography, it is therefore superior to the other models. However, the determination of the analyte specific parameters, which are needed to calibrate the retention behaviour, strongly depend on the suitable selection of initial experiments. A useful strategy for a purposeful selection of these calibration experiments is proposed.

Keywords: Polymer chromatography; Retention models; Size exclusion chromatography; Critical chromatography

1. Introduction

Liquid chromatography has become a powerful tool for the characterization of polymers. Separations can be achieved according to molecular size, functionality or chemical composition, etc. depending on the particular chromatographic experiment [1]. Once a suitable method is established, the chromatographic experiment can be performed in short time allowing chromatographic methods to be used in research laboratories as well as in quality control. However, although the analysis times are short, the time and effort to develop a suitable chromatographic method is high. This is especially true in polymer chromatography, where the retention behaviour is of macromolecules influenced by their molar mass and other types of structural features like chemical composition or functionality. Thus, a method resulting in a good separation for a particular polymer sample might fail if the molar mass or the chemical composition is varied only slightly. Thus, optimization of the chromatographic method for the particular sample is frequently required in polymer chromatography.

For low molar mass compounds, the retention behaviour can often be predicted on the basis of only a few initial experiments using suitable chromatographic models [2–6]. The model describes the general dependence of retention time or retention volume on the eluent composition, which might vary during a gradient experiment. The model contains a small number of analyte specific parameters that have to be determined from a specific number of initial experiments. Once the analyte specific parameters are known, the retention times under different chromatographic conditions can be calculated. Using this procedure, it is possible to calculate the retention times and therefore the quality of a separation for a large variety of experimental conditions within short time on a computer. Doing so, the number of actual experiments is largely reduced, resulting in lower costs and shorter time for method development. However, in polymer chromatography such an approach has not yet become popular, despite considerable improvements in the theory of polymer chromatography during the last decade [7–18]. Schoenmakers and Fitzpatrick investigated the suitability of the linear solvent strength model (LSSM, to be described below) to describe the retention behaviour of polystyrenes (PS) and poly(methyl methacrylate)s (PMMA) in gradient chromatography [16,17]. They were able to show that the LSSM adequately describes the gradient retention of these polymers. From gra-

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doi:10.1016/j.chroma.2006.07.089

dient experiments, the analyte specific model parameters were extracted. By correlating these model parameters with molar mass, it was possible to predict the critical eluent composition at which PS elutes at the same elution volume in isocratic conditions, irrespective of their molar mass. This result is surprising, since for principle reasons the LSSM cannot predict SEC or critically behaviour, as will be discussed later. Fitzpatrick et al. showed that the correlation of the model parameters with molar mass allow predicting the elution behaviour even for other molar masses than those used in the experiments. According to the authors at least nine gradient experiments had to be performed on samples of different molar mass for a proper description. No attempts have been made to predict isocratic elution in adsorption or SEC mode from gradient runs. Brun et al. extended a molecular model describing the isocratic elution behaviour of polymers to gradients [13,15]. According to this work, gradient elution can be described by two molecular parameters, the critical eluent composition and a parameter combining the effect of molar mass and the change of interaction strength with variation in eluent composition. It was shown that high molar mass samples elute irrespective of their molar masses at the same elution volume. This retention time is completely determined by the time at which the composition within the solvent gradient is equal to the critical eluent composition. Due to neglecting the variation of solvent composition along the column, the calculations yield a composition at elution slightly above the critical eluent composition. The corrected equation, however, results for high molar mass samples in elution slightly below or at the critical solvent composition. In the following, comparison is made on the suitability of different retention models to predict the retention behaviour of PEGs under gradient and isocratic chromatographic conditions.

2. Basics of polymer chromatography

The retention volume, V_R, at which an analyte elutes from a chromatographic column, can be described by the general chromatographic equation:

$$V_R = V_i + KV_P \quad (1)$$

where V_i and V_P are the interstitial and pore volume, respectively, while K is the distribution coefficient. The distribution coefficient is defined as the ratio of the analyte concentration in the stationary phase to that in the mobile phase. Due to their large sizes, macromolecules cannot penetrate completely the pores of the stationary phase. Certain conformations of the polymer molecule simply do not fit into the pore. In addition, for a given polymer conformation, the center of gravity cannot access certain regions of the pore volume due to steric exclusion of parts of the molecule with the pore wall. Entering the pore from the free mobile phase therefore causes a loss of entropy. If no or only very weak enthalpic interaction exists between the polymer molecule and the pore wall, the above mentioned steric exclusion will result in a distribution coefficient $K<1$ and therefore in an elution volume $V_R < V_i + V_P$. The mode of chromatography, where the distribution coefficient is determined purely by the steric exclusion of the polymer molecule from the pore, is called size exclusion chromatography (SEC, GPC). Since the size of a linear polymer molecule increases with its molar mass, larger polymer molecules will be stronger excluded from the pores than smaller ones, resulting in a decrease of the distribution coefficient and therefore in a decreasing elution volume with increasing molar mass.

A decrease in the eluent strength results in attractive interactions between the pore walls and the repeating units of the polymer molecule. If this interaction is sufficiently strong, the macromolecule will be retained in the stationary phase and will elute later than the injected solvent band ($K>1$). Since the number of repeating units increases with the molar mass of the polymer molecule, the total interaction energy increases in the same direction. Therefore, the elution volume will increase nearly exponentially with the molar mass of the macromolecule. This molar mass dependence of elution volume characterizes the adsorption mode of chromatography (LAC, liquid adsorption chromatography). The strong dependence of elution volume on molar mass often results in a nearly irreversible adsorption of the macromolecules even for a weak interaction of the repeating units and the stationary phase. Therefore, often gradient methods are applied to systematically vary the adsorption strength.

By varying the eluent composition, it is possible to exactly adjust the eluent strength such that adsorption and size exclusion effects cancel out each other. Under these conditions homopolymers of a given type elute irrespective of their molar mass at the same elution volume, $V_R = V_i + V_P$. This elution behaviour is termed as liquid chromatography under critical conditions of adsorption (LCCC). The eluent composition at which this behaviour is observed is referred to as the critical eluent composition. A schematic depiction of the different molar mass dependences in polymer chromatography is given in Fig. 1. From the previous discussion, it follows that the large size of a macromolecule results in pronounced differences in the retention behaviour as compared to ordinary low molar mass compounds. Therefore, the question arises, whether the retention models used in chromatography of low molar mass compounds

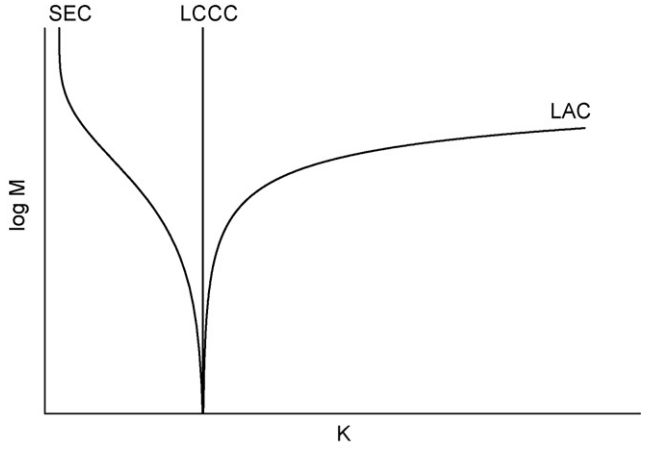

Fig. 1. Schematic representation of the dependences of distribution coefficient, K, on molar mass for size exclusion (SEC), adsorption (LAC) and chromatography under critical conditions of adsorption (LCCC).

are suited to predict retention for polymers and therefore as a tools for method development in polymer chromatography.

3. Retention models

3.1. Linear solvent strength model (LSSM)

The most widely used model to predict the retention behaviour of low molar mass compounds is the linear solvent strength model. The LSSM assumes a linear relation between the logarithm of the retention factor, $\log k$, and the eluent composition, Φ. Here Φ denotes the fraction of the strong eluent in the binary eluent mixture. The retention coefficient, k, is defined as [2]:

$$k = \frac{t_R - t_0}{t_0} \tag{2}$$

With t_R and t_0 being the retention time of the analyte and the retention time of a non-retained low molar mass compound, which by multiplying with the flow rate, F, defines the void volume ($V_0 = t_0 \times F$).

According to the LSSM:

$$\log k = \log k_w - S \times \Phi \tag{3}$$

For low molar mass compounds, where eluents are often composed of water and an organic modifier, k_w defines the retention factor of the analyte in pure water. However, the limited solubility of most synthetic polymers prevents using water in polymer chromatography. Despite that, Eq. (3) can still be applied if k_w is interpreted as the retention factor of the analyte at a suitable mobile phase composition where the relation between eluent composition and retention factor (Eq. (3)) is valid.

Using Eq. (3), the analyte specific parameters, k_w and S, can be easily obtained from at least two isocratic runs at different eluent compositions. However, for high molar mass polymers isocratic elution is only possible in a very narrow range of eluent compositions, close to the critical eluent composition, defined above. Therefore, solvent gradients are frequently employed in polymer chromatography. Assuming a linear gradient

$$\Phi(t) = \Phi_0 + bt \tag{4}$$

starting at an eluent composition Φ_0, and increasing linearly with a slope $b = \Delta\Phi/t_G$ the retention time of the analyte at gradient elution is calculated to be [3]

$$t_R = \frac{1}{S \times b} \ln\left(1 + k_0 \times S \times b\left[t_0 - \frac{\tau}{k_0}\right]\right) + t_0 + \tau \tag{5}$$

Here τ is the dwell time, i.e. the time the eluent requires to travel from the mixer to the column head. b is the gradient slope, i.e. the rate of change of eluent composition per unit time and k_0 is the retention factor at Φ_0. Eq. (5) is valid if the analyte elutes within the gradient. Retention times within the dwell time or after ending the gradient can be treated easily. Other gradient shapes can also be approximated by a series of linear gradients having different slopes. However, for the present purpose there is no need for a more detailed description. Eqs. (2)–(5) allow the extraction of the parameters k_0 and S from at least two arbitrarily selected chromatographic experiments (isocratic or gradient). Depending on the set of experiments, parameter extraction might have to be done using non-linear fitting procedures. The fitted parameters can then be used to predict retention times at any experimental conditions.

3.2. Quadratic solvent strength model

The quadratic solvent strength model (QSSM) assumes a quadratic dependence of the logarithm of the retention factor on eluent composition,

$$\log k = A \times \Phi^2 + B \times \Phi + C \tag{6}$$

where A, B and C are the analyte specific model parameters. For linear solvent gradients this results in [3]

$$t_R = \frac{1}{b\sqrt{A}} \mathrm{inverf}\left\{2b\sqrt{\frac{A}{\pi}}\left[t_0 - \frac{\tau}{k_0}\right]\exp\left(\frac{AC - B^2/4}{A}\right) + \mathrm{erf}\left(\Phi_0\sqrt{A} + \frac{B}{2\sqrt{A}}\right)\right\} - \frac{A\Phi_0 + B/2}{Ab} + \tau \tag{7}$$

In Eq. (7), erf and inverf are the error function and the inverse error function, respectively. In contrast to the LSSM, the QSSM has three analyte specific parameters (A, B, C) that have to be determined in order to predict the retention at different chromatographic conditions. Thus, at least three experiments need to be performed. The extraction of these parameters from experimental data has to be performed using non-linear fitting algorithms.

3.3. Polymer model (PM)

Both the LSSM as well as the QSSM are applicable to adsorption chromatography only, i.e. if $k > 0$. For SEC and LCCC, the retention coefficient correspond to $k < 0$ and $k = 0$, respectively. These values of k cannot be described according to Eqs. (3) or (6). In order to develop a suitable model also applicable to SEC and LCCC, we describe the retention of a polymer by the general chromatographic equation (Eq. (1)):

According to the statistical theory of polymers in large slit-like pores ($R < D$) the distribution coefficient, K, can be written as [8]

$$K = 1 + \frac{2R}{D}\left[\frac{Y(-cR) - 1}{cR} - \frac{2}{\sqrt{\pi}}\right] \tag{8}$$
$$Y(-x) = \exp(x^2)[1 - \mathrm{erf}(-x)]$$

With R being the mean square radius of the polymer molecule, D the pore diameter and c the interaction parameter describing the interaction strength between the repeating unit and the surface of the stationary phase. For LAC conditions $c > 0$, while $c < 0$ if an SEC-like elution order is observed. At critical conditions of adsorption $c = 0$. The value of c depends on eluent composition. However, no theoretical description of this dependence exists. In the close vicinity of the critical composition, Φ_c, the dependence

of c on eluent composition can be approximated by a power series, which might be truncated after the linear term. Thus,

$$c = \frac{dc}{d\Phi}(\Phi_c - \Phi) + \dots \quad (9)$$

Beside c also R/D might vary as a function of eluent composition. However, in gradient chromatography polymers are eluting within a very narrow range of eluent compositions, close to Φ_c [13,15,19]. Thus, the effect of Φ on R/D is expected to be neglectable as compared to the effect of Φ on c. Within these approximations, Eqs. (8) and (9) allow calculating the distribution coefficient in isocratic chromatography as a function of the eluent composition. For a linear solvent gradient with slope b, starting from an initial composition Φ_0 (Eq. (4)) the following solution is obtained (see Appendix A)

$$I(Rc_0) - I(Rc_{\text{final}}) = \frac{2Rbt_P}{D}\frac{dRc}{d\Phi}$$
$$I(\varsigma) = \int_0^{\varsigma} \frac{dx}{(Y(-x) - 1)/x - (2/\sqrt{\pi})} \quad (10)$$

with

$$Rc_{\text{final}} = \frac{dRc}{d\Phi}\left(\Phi_c - \Phi_0 - \frac{b \times (V_R - V_i - V_P)}{F}\right)$$
$$Rc_0 = \frac{dRc}{d\Phi}(\Phi_c - \Phi_0) \quad (11)$$

The dependence of the distribution coefficient, K, on the experimental parameters Φ_0 and b is described by Eqs. (8)–(11). The PM includes three analyte specific parameters, viz. R/D, Φ_c and $dRc/d\Phi$. These parameters have to be determined from at least three initial experiments using non-linear fitting procedures.

4. Experimental

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).

4.1. Chromatographic conditions

The injected sample volume was 10 μL. Sample concentrations were 1–2 g/L. Column temperature 35 °C and flow rate was 1 mL/min. Mixtures of methanol (MeOH), Chromasolv, HPLC grade, and water, deionized using Millipore Simplicity 185 (UV) water system (Millipore GmbH, Schwalbach, Germany), were used as mobile phase. The stationary phase was Nucleosil C18, particle size 5 μm, pore diameter 300 Å, column dimensions 250 mm × 4.6 mm I.D. (Macherey–Nagel, Düren, Germany). Polyethylenglycols (PEG) having different molar masses and narrow polydispersities were obtained from PSS Polymer Standards Service GmbH, Mainz, Germany. Table 1 gives the molar masses at the peak maximum of the distribution (M_P), the weight average molar mass, M_w, and the polydispersity, D for these standards. In addition technical PEGs were obtained from Hüls AG, Marl, Germany.

Table 1
Molar masses, polydispersities and suppliers of the samples used

M_P (g/mol)	M_w (g/mol)	D	Supplier
PEG (200)			Hüls
PEG (400)			Hüls
PEG (1000)			Hüls
PEG (2010)	1960	1.03	PSS
PEG (3120)	3060	1.03	PSS
PEG (6240)	6000	1.03	PSS
PEG (12,000)	11200	1.51	PSS
PEG (23,000)	22500	1.60	PSS
PEG (40,000)	41500	1.14	PSS

The void volume was estimated from the retention volume of toluene using tetrahydrofuran as eluent. The dwell volume was determined 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.

Isocratic mobile phases of different compositions were delivered by the pump system. All runs were performed using duplicate injections.

Data evaluations were performed by spreadsheet calculations using Origin™ software. Extractions of the analyte specific parameters was carried out by applying the Origin's Levenberg–Marquardt routine to self-written scripts.

5. Results and discussion

In order to determine the suitability of the different models to predict the chromatographic behaviour of PEGs, gradient experiments were performed under conditions that allow separating PEGs into individual oligomers. Fig. 2 shows the chro-

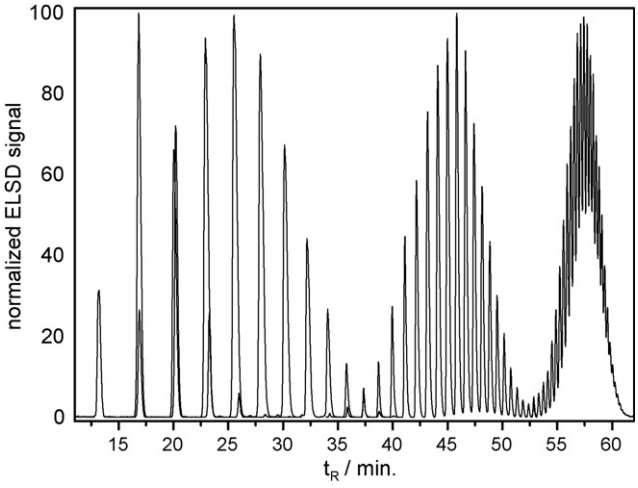

Fig. 2. Gradient chromatograms of PEG 200, PEG 400, PEG 1000, PEG 1960 using a linear gradient from 5 to 100% MeOH in 90 min.

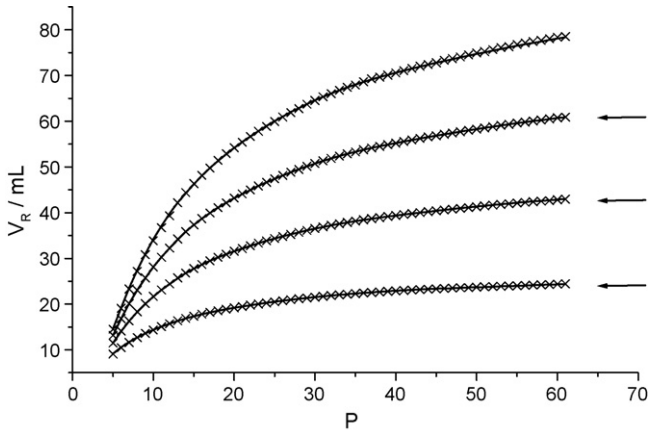

Fig. 3. Comparison of experimentally determined (lines) and predicted retention volumes using PM (×) for PEGs. The arrows indicate the gradients used for calibration. From upper to lower curve: t_G = 120, 90, 60, 30 min.

matograms of PEGs of various molar masses in 90-min linear gradients ranging from 5 to 100% MeOH. As can be seen, a good separation into individual oligomers is obtained, allowing precise determination of the retention times for each single oligomer. The analyte specific parameters were determined (calibration) from 30 and 90-minute gradients for the LSSM and from 30, 60 and 90-minute gradients for the other models, respectively. Using these calibrations, the retentions times for other gradients were calculated according to the equations given above.

Fig. 3 shows the comparison of the experimentally determined retention times with those predicted by the PM for gradients from 5 to 100% methanol as a function of degree of polymerization, *P*. The calculated and experimentally determined retentions times differ by less than 1% in all cases. Figures of similar accuracy were obtained for the LSSM and QSSM. Predictions for gradients ranging from 5 to 100% methanol, differing in gradient slope were also analyzed using calibrations with different sets of two or three gradients. In addition predictions for other composition ranges and slopes were investigated. For all models the errors between the predictions and the experiments are also less than 1%. Therefore, a nearly perfect agreement of predicted and experimentally determined retention times is observed for all oligomers in all cases investigated, irrespective of the retention model used. Exceptions are only those experiments where Φ_0 is strong enough to result in elution within the dwell volume. In such cases, higher errors were observed. The investigations were extended to higher molar mass PEGs, which could not be resolved into individual oligomers. For the higher molar mass PEGs 5–100% methanol gradients with run times of 5, 10, 20, 30, 60 and 90 min were carried out. The parameters were extracted using the three longest gradients for the QSSM or the PM and the 30 and 90 min gradients for the LSSM. Fig. 4 shows the box-plots of the % deviation between the calculated and experimentally determined retention times for those gradients that have not been used for the calibrations. It can be seen that the deviations between the experimental retention times and the predictions are very low, similar to the results on the oligomers. While for the LSSM and the PM a nearly perfect

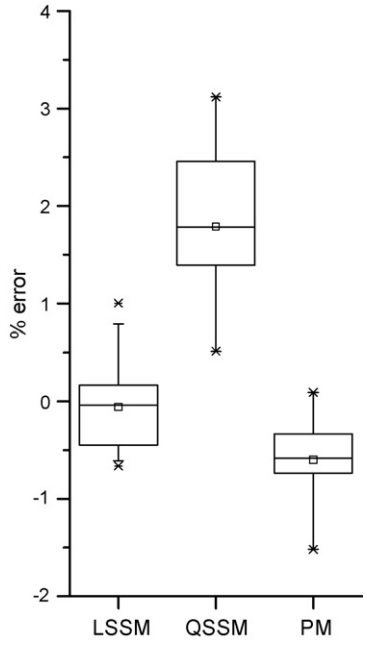

Fig. 4. Box-plots of the percentage deviation between predicted and experimentally determined gradient retention times of PEGs having molar masses of 2010, 3120, 6240, 12,000, 23,000, 40,000 g/mol. Estimation of analyte specific parameters from gradient runs.

match is found, the QSSM slightly overestimates the retention times. However, the differences are still very low and we can conclude that a precise prediction of gradient retention times is possible by all three models, provided that the analyte specific parameters were extracted from gradient runs as well.

As already stated in the introduction, isocratic LAC of high molar mass polymers is possible only within an narrow range of eluent compositions close to the critical one. Thus, establishing suitable isocratic conditions is a time consuming task. In addition, isocratic steps might be required in multi-step gradients to achieve a desired separation. Thus, the precise prediction of isocratic retention times from gradient experiments would be advantageous. We therefore predicted isocratic retention times using the parameters extracted from gradient experiments and compared those with the retention times obtained experimentally. The results for the LSSM, QSSM and the PM obtained for PEG oligomers in are given in Fig. 5. The QSSM and the PM predictions show good agreement with the experiment with average and maximum deviations of 1.2 and 5% or 1.5 and 4%, respectively. Significantly larger deviations with average and maximum deviations of 5 and 14% are found for the predictions of the LSSM.

Similar to the investigations on oligomers, the isocratic retention times for high molar mass PEGs were determined and compared with the predictions of all three models based on the analyte specific parameters extracted from gradient experiments. As for high molar mass polymers, isocratic elution in adsorption mode was only possible in close vicinity to the critical eluent composition. Therefore, isocratic experiments were performed in MeOH/H_2O mixtures between 65/35 and 100/0. A peculiar behaviour is found on the dependence of the iso-

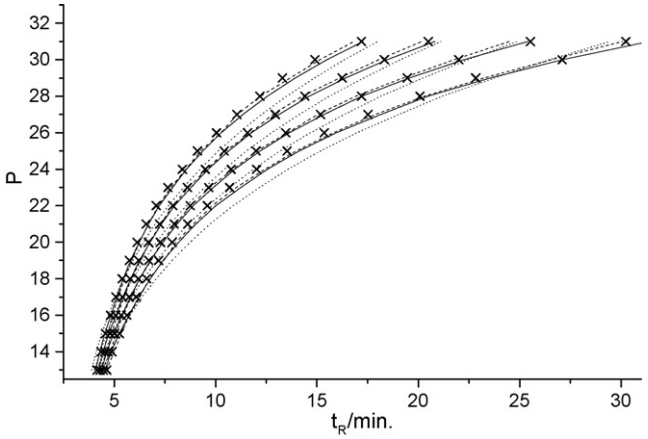

Fig. 5. Comparison of experimental isocratic retention times (lines) of PEG oligomers with predictions by LSSM (dotted), QSSM (dashed) and PM (solid). Analyte specific parameters obtained from linear gradients (30, 90 min for LSSM, 30, 60, 90 min for QSSM and PM). Eluent compositions: MeOH/H_2O, 54/46, 53/47, 52/48, 51/49 (from top to bottom).

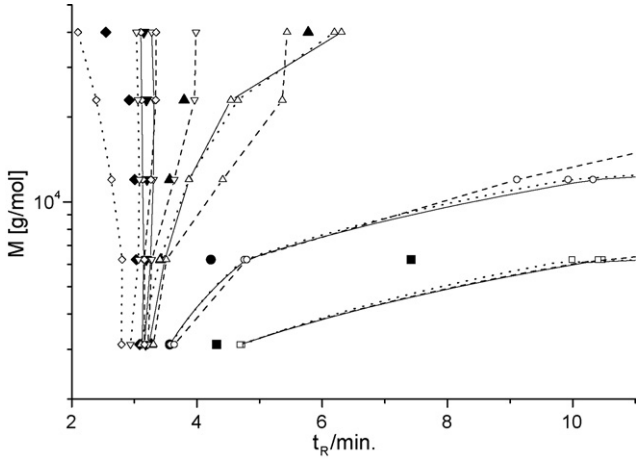

Fig. 7. Comparison of experimental (solid symbols) and calculated (lines with open symbols) isocratic retention times for high molar mass PEGs in different MeOH/water compositions ((■) 70/30, (●) 75/25, (▲) 80/29, (▼) 83/17, (♦) 87/13). LSSM (solid lines, open symbols), QSSM (dashed lines, open symbols), PM (dotted lines, open symbols). Calibration of analyte specific parameters by linear gradients.

cratic retention times on MeOH content for different high molar mass PEGs (Fig. 6). At low MeOH contents, the retention times decrease with increasing MeOH content. The higher the molar mass, the stronger is the decrease in elution time. At a MeOH content of 83% the curves for different molar masses merge. This behaviour indicates the critical conditions, where the elution time does not depend on molar mass. A further increase of the MeOH content reverses the elution order with higher molar mass samples eluting at lower elution times than those of lower molar mass, i.e. an SEC like elution order is observed. Only the PM is capable to describe these two modes of chromatography. However, at MeOH contents of more than 90% the retention times decrease again and the different curves merge once more at a MEOH content of approximately 98%, indicating a second critical point. Such a behaviour for PEGs has been found for other mobile phases on RP stationary phases as well and has been attributed to the interaction of residual silanol groups of the stationary phase with the oxygen atoms of the PEO chain, while the ethylene structures interact with the C_{18}-chains in the usual

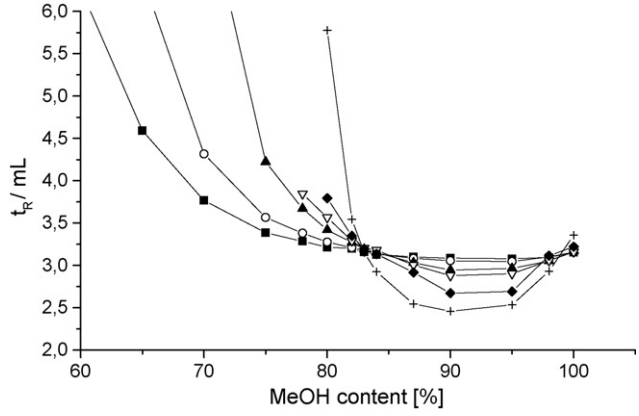

Fig. 6. Dependence of elution time on MeOH content in isocratic chromatography of high molar mass PEGs. M = 2010 (■), 3210 (◯), 6240 (▲), 12,000 (▽), 23,000 (♦), 40,000 (+) g/mol.

reverse phase mode [20,21]. The PM as used above does not account properly for two different critical points. Assuming that both types of interacting groups interact independently of each other, it is possible to describe the U-shaped curves by a combination of two columns having different critical compositions and different dc/dΦ values. Beside *R/D* an additional parameter describing the relative lengths of the two columns has to be introduced. Thus, at least six parameters would have to be extracted from the experimental data to finally quantitatively describe the observed behaviour. The uncertainty introduced by fitting such a large number of parameters would most likely not improve the predictions. We therefore restrict the following discussion to the results of isocratic experiments at MeOH content $\Phi < 90\%$, where standard reverse phase behaviour is observed.

Fig. 7 compares the predicted isocratic retention times of high molar mass PEGs with those determined experimentally. The calibrations of the analyte specific parameters were performed using gradient experiments. It can be seen that significant differences exist between the calculated and predicted retention times for all three chromatographic models. These differences are more pronounced at lower MeOH contents, i.e. at stronger adsorption strengths. In addition, it becomes evident that for MeOH contents lower than 83% an increase in retention time with molar mass, i.e. LAC behaviour, is observed, while at MeOH contents above 83% SEC-like behaviour is found. At a MeOH content of 83%, a molar mass independent elution time is established, indicating critical conditions for PEGs. Neither the LSSM nor QSSM can account for this change in elution behaviour. As mentioned in the introduction, this is inherent in the mathematical formulation of the undefined logarithm for $k \leq 0$. In contrast to the LSSM and the QSSM, the PM gives at least a correct qualitative picture of the elution behaviour in all three modes of polymer chromatography. Although the PM shows significant deviations at SEC and LAC conditions, yet the transition between these two chromatographic modes

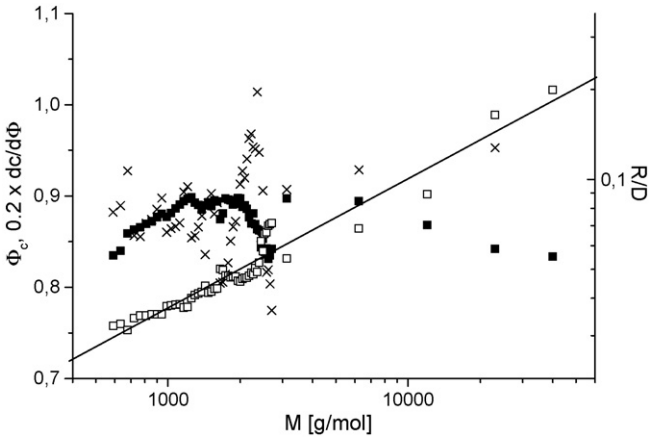

Fig. 8. Analyte specific parameters of PM determined from gradient runs *R/D* (□), Φ_c (■) and d*c*/dΦ (×) for PEGs as a function of molar mass.

is observed at the correct eluent composition. The unexpected deviations in SEC and LAC-mode might therefore reflect the low precision of the parameters d*c*/dΦ and *R/D* in the parameter extraction process.

In order to get a better understanding of this behaviour, the parameters of the PM, which best describe the experimental retention times from three gradient runs, are plotted as a function of molar mass in Fig. 8. The deviations between experimental and predicted retention times for the parameter sets were less than 1% in all cases. As can be seen, the critical composition predicted from the gradient data, varies between 80 and 90% MeOH content of the mobile phase. The deviation of the critical composition calculated from gradient experiments as compared to the true critical eluent composition (83% MeOH) is larger for low molar mass samples than for higher molar masses. The explanation might be that high molar mass molecules elute in a composition close to the critical composition, while lower molar mass molecules experience only solvent compositions much lower than the critical one during gradient elution. This can be understood from Fig. 9, where the eluent composition at gradient elution is plotted versus degree of polymerization. A limiting

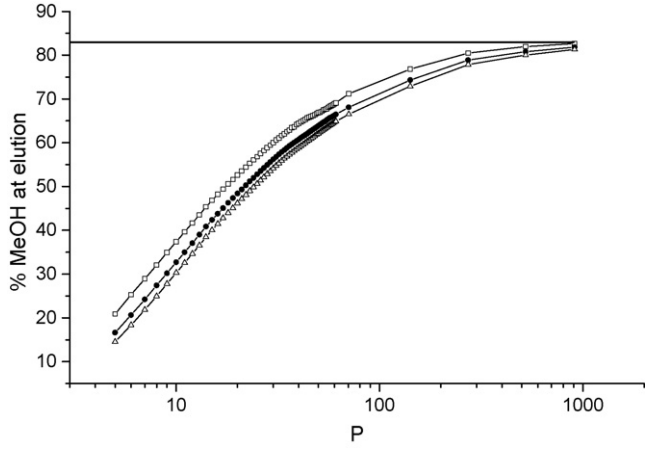

Fig. 9. Eluent composition at elution for PEGs as a function of degree of polymerization at gradient times of t_G = 30 (□), 60 (●) and 90 (△) min. The solid horizontal line corresponds to the critical composition.

value, which is very close to the critical composition determined by isocratic experiments, is approached for high degrees of polymerization. This is in agreement with calculations by Brun et al. [13,15] and the experimental findings that the critical composition can be effectively estimated from a single gradient run using a single high molar mass polymer [19]. During gradient elution low molar mass polymers experience only eluent compositions of much lower eluent strengths than the critical composition, corresponding to certain positive values of *cR*. Therefore, the prediction of the critical composition requires the extrapolation to zero value of *cR* over a significantly larger range of mobile phase compositions, as compared to high molar mass polymers. As a consequence the errors in the determination of the critical composition are larger for lower molar masses than for higher ones. While the critical eluent composition for high molar mass polymers can be predicted quite accurately, the errors are significant for lower molar masses. The situation is different for the other parameters of the PM. The estimated values of *R/D* increase with molar mass (Fig. 8). For the scaling behaviour of *R/D* on *M* an exponent of 0.42 is found, slightly lower than the value of 0.5, expected for a Gaussian coil. Although the absolute values of *R/D* still can be in error, this indicates that the values of *R/D* extracted from gradient experiments only are not completely meaningless. As will be discussed later, a good fit of experimental data can also be obtained for a fixed value of $a = 0.5$, indicating that the quality of the fit does not depend strongly on the value of *a*. For the parameter d*c*/dΦ, no systematic variation with molar mass can be found. The values scatter significantly, indicating that reliable estimation of this parameter only from gradient experiments is not possible. One possible explanation for the impossibility of reliable estimates of d*c*/dΦ might be that the assumption of a linear dependence of *c* (or *cR*) on Φ is not valid, questioning applicability of the PM in general. However, as will be shown below, the proper selection of the model parameters allow description of the elution behaviour for a wide variety of experimental gradient and isocratic conditions and large range of molar masses. This indicates that the PM is suitable to predict the retention behaviour of PEGs. Therefore, the explanation of the poor predictions of the isocratic experiments is not a general failure of the model, but might be due to the parameter extraction itself. In order to test whether reliable parameter extraction only from gradient experiments is possible, retention times were calculated using a given set of model parameters of the PM. Using these retention times and the corresponding gradient conditions we tried to determine the parameters by non-linear fitting. However, even from the simulated error free gradient experiments, the original parameters could not be obtained. The extracted parameters depend on the starting values used to initialize the non-linear fitting process. This indicates the existence of a number of local minima. The absolute minimum, i.e. the correct parameter values can be found by performing the initialization several times. However, such an approach is undesirable from a practical point of view. In addition, the experimental errors also contribute to the uncertainty of the extracted parameters. All these factors contribute to the fact that the model parameters extracted using merely three gradient runs are not very reliable although they are good for gra-

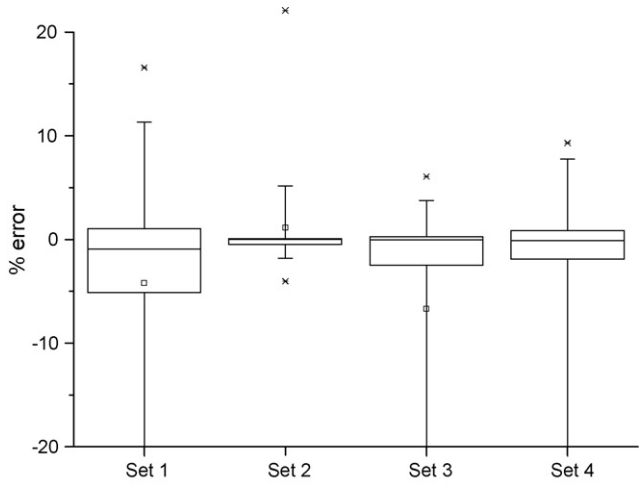

Fig. 10. Comparison of the percentage deviations between experimental and PM model based calculated isocratic retention times for high molar mass PEGs. The box-plots correspond to different sets if initial experiments used for calibration. Set 1: linear gradients of $t_G = 30$, 60, 90 min, set 2: isocratic data at 80, 82 and 90% MeOH; set 3: isocratic data at 80 and 90% MeOH and a linear gradient of $t_G = 30$ min; set 4: isocratic data at 78 and 80% MeOH and a linear gradient of $t_G = 30$ min.

dient predictions. For high molar mass polymers this is easily understood. Since high molar mass polymers elute at an eluent composition very close to Φ_c, irrespective of gradient slope, the only information needed to predict the retention times during gradient elution is Φ_c. In other words, irrespective of the values of *R/D* and d*c*/dΦ the predictions of gradient retention times are of similar precision, as long as Φ_c is correct. Since the other parameters have no significant impact on the gradient retention times, their reliable extraction is impossible.

It therefore seems useful to select other types of starting experiments in order to obtain better estimates for *R/D* and d*c*/dΦ and thus more reliable predictions. While gradient experiments most strongly depend on Φ_c, it can be expected that isocratic experiments at SEC and adsorbing conditions will depend on the parameters *R/D* and *c*, thus via Eq.(9) on d*c*/dΦ, to different degrees. In order to prove this hypothesis, parameter extraction was performed using different sets of isocratic and gradient experiments. Using the so obtained parameters, predictions were made and compared with the experimental results.

Similar to Fig. 7, strong deviations were found for strong adsorbing conditions in all cases, while the agreement between predictions and experimental data in weak adsorbing or under SEC-conditions depends on the selection of the calibration experiments. Fig. 10 compares the box-plots for the percentage deviations between the experimental and calculated retention times based on the PM for different sets of calibration experiments. While the calibration based purely on gradient experiments show quite large differences even for small deviations from the critical composition (set 1 and Fig. 7), the most reliable results are obtained if only isocratic runs are used for calibration (Fig. 10, set 2). However, from a practical point of view the selection of isocratic runs at weakly adsorbing, weak SEC and critical conditions is only possible when a large number of additional experiments are performed first. Reasonable agreement of prediction and experiment was found, however, if a single gradient run in addition with two isocratic experiments (SEC and LAC or two LAC experiments) was used for calibration (set 3 and set 4). Therefore, for the proper selection of initial experiments, a systematic approach is proposed below, based on additional support by simulations. For the simulations a hypothetical set of PM parameters and column parameters $D = 30$, $\Phi_c = 0.8$, $R/D = 0.5$, dc/d$\Phi = 0.2$, $V_i = 1.0$ and $V_P = 1.5$ mL was selected. For 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, a random error taken from a Gaussian distribution having a 5% standard deviation was added to the respective error free elution volumes. The so defined values were treated as experimental results, to develop a protocol for selecting a suitable set of initial experiments. The artificial error of 5% is large compared to the errors in retention times found experimentally. However, if the calculations result in a reasonable agreement even for errors of this magnitude, smaller errors can easily be tolerated.

As the first initial experiment, a 20 min linear gradient from 0 to 100% of solvent B was simulated. The error free retention time was calculated to be 13.27 min, while the "experimentally determined" retention time (i.e. the retention time with the statistical error) was found to be 13.79 min. From this retention time, the eluent composition at the time of elution was calculated to be 56% B. Since a polymer molecule is expected to elute at a composition slightly below or at the critical composition, an isocratic experiment performed at the composition of gradient elution is expected to result in a reasonable retention time. Therefore, an isocratic experiment at 56% B was simulated. The error free and "experimentally" determined retention times were 4.64 and 4.96 min, respectively. Thus, the experimentally determined retention time is larger than retention time of the pure solvent and therefore corresponds to an experiment under adsorbing conditions. Since it was expected that the most reliable information on the parameter *R/D* would result from an additional experiment in SEC mode, the conditions for the third experiment were therefore selected such that SEC like elution was expected to result. An eluent of 61% B was selected, rendering error free and "experimental" retention times of 3.93 and 3.86 min for the third experiment. However, these retention times still correspond to adsorbing conditions. Using the so selected three initial experiments, the analyte specific parameters were extracted by non-linear fitting. The resulting parameter set was: $\Phi_c = 0.78$, $R/D = 0.44$; dc/d$\Phi = 0.28$. In order to get information on the quality of the extracted parameters a prediction was made. The isocratic experiments performed so far resulted in adsorbing conditions we therefore selected conditions which should result in SEC behaviour. Since the critical composition was estimated to be $\Phi_c = 0.78$, SEC like elution was expected at higher % B than $\Phi = 0.78$. Thus, an isocratic experiment at $\Phi = 82\%$ of the strong solvent was simulated, resulting in 2.41 and 2.32 min for the true and "experimentally determined" retention times, respectively. From the extracted parameters, a retention time of 2.31 min was expected for these conditions. Thus, a nearly perfect agreement was found between the "exper-

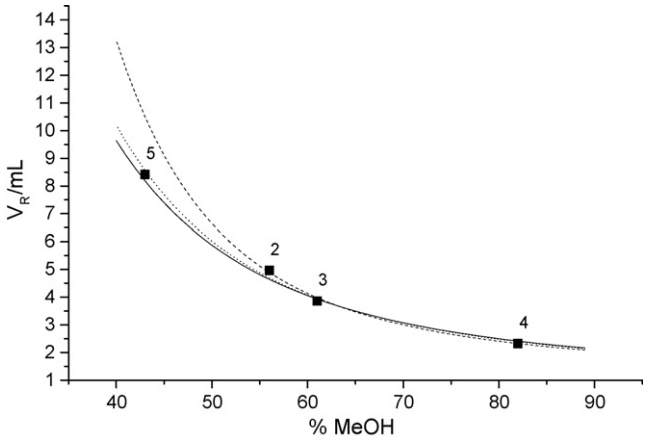

Fig. 11. Comparison of isocratic dependences of elution volume on MeOH content. Solid line: error free, dashed: predicted from one gradient ($t_G = 20$ min) and two isocratic experiments, dotted: predicted from one gradient and four isocratic runs. The solid symbols represent the "experimental" data points, labels indicate the number of the isocratic run.

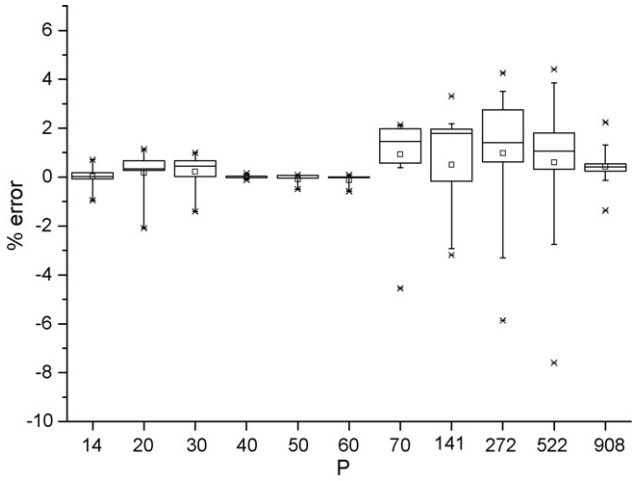

Fig. 12. Box-Plots of relative deviations between experimentally determined and calculated retention volumes for all isocratic and gradient experiments for PEGs of different degree of polymerization.

iment" and the prediction for the given approach. Fig. 11 shows a comparison of the true (i.e. the curve based on the parameter set $\Phi_c = 0.8$, $R/D = 0.5$; $dc/d\Phi = 0.2$) and the predicted (based on three experiments) dependence of elution volume on MeOH content in comparison to the "experimental" data points. It can be observed that the experimental data points are better described by the predicted curve than by the true curve. This seems to be a contradiction at first glimpse. However, this apparent contradiction is due to the errors associated with each "experimental" data point. If predications have to be made for very weak eluents, it is advisable to select a reasonable retention time of, e.g. 10.5 min based on the given parameter set. This retention time is expected to occur for a composition of 43% B. The true and experimental retention times were found to be 8.41 and 8.18 min, respectively. Using now all six runs, the parameters were estimated to be $\Phi_c = 0.79$, $R/D = 0.49$, $dc/d\Phi = 0.21$.

Therefore, the following proposed 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 elution under adsorbing conditions, perform a third run using a slightly stronger eluent composition.
4. If step 2 results in elution under SEC conditions, perform a third run at slightly weaker eluent (1%).

Finally, it was investigated whether all experiments on PEGs can be fitted with a single parameter set, irrespective of the type of experiment (isocratic or gradient). In order to do so, all gradient as well as all isocratic experiments at MeOH content <90% for a single molar mass were fitted. The relative deviations between the calculated and the experimental data are represented as box-plots in Fig. 12. It becomes clear that more than 95% of all data points show a deviation of less than 5%, 50% show a deviation of less than 3%, indicating that the PM allows a good quantitative description of the retention behaviour of PEGs in gradient and isocratic elution. This strongly supports that the errors observed in Figs. 7 and 10 are not due to the generally poor description of chromatography by the PM, but to a poor parameter extraction. In addition Fig. 12 indicates that the parameter extraction and therefore the description of the chromatographic behaviour are significantly improved by the use of isocratic experiments in the parameter extraction process. The larger scattering at higher molar masses might be due to the increasing peak width in isocratic elution with increasing molar mass, resulting in a larger uncertainty in the precise determination of the peak maximum. In addition, peak maxima determined at different chromatographic conditions (especially different isocratic conditions) correspond to different degrees of polymerization. This is due to the different slopes of the calibration curves of molar mass versus elution volume at different chromatographic conditions.

Apparently, the PM describes the elution behaviour for each degree of polymerization. However, Fig. 12 was derived by separately fitting all experiments associated with a particular molar mass. The parameters Φ_c and $dc/d\Phi$ of the PM are expected to be independent of molar mass. In contrast the parameter R/D describes the molar mass dependence of the elution volume. The radius, R, is assumed obey the scaling relation of typical Gaussian coils, $R \sim M^{1/2}$. Thus, fitting of all experiments, irrespective of molar mass, was performed with a single parameter set. The PEG sample having a molar mass of $M = 40{,}000$ g/mol was selected as reference sample. The parameters R/D for other molar masses were calculated according to

$$\frac{R}{D} = \left(\frac{R}{D}\right)_{\text{ref}} \left(\frac{M}{M_{\text{ref}}}\right)^{1/2} \tag{12}$$

By this procedure, it is possible to fit all experiments irrespective of molar mass or experiment (isocratic or gradient) with a single three parameter set, Φ_c, $dc/d\Phi$ and $(R/D)_{\text{ref}}$. A total of more than 360 experiments were used for the fitting procedure. Fig. 13 shows the histogram of the % deviation between exper-

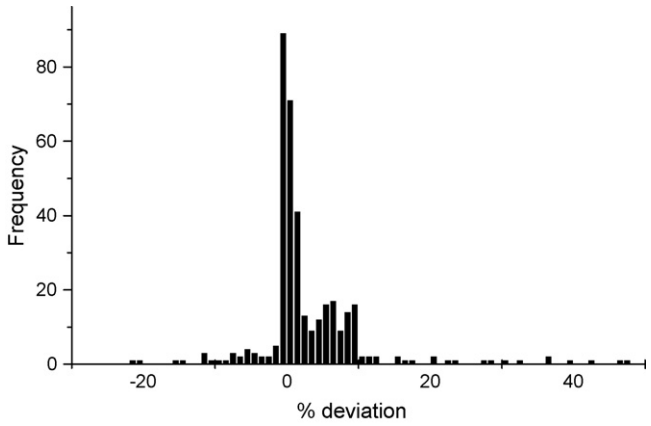

Fig. 13. Histogram of % deviation obtained for all PEG samples and experiments. All experiments and errors were fitted to result in $\Phi_c = 0.83$, $dc/d\Phi = 3.98$ and $(R/D)_{ref} = 0.29$.

imental retention volumes and those calculated by fitting the PM to all experimental data points. The majority (66%) of all data points show deviations of less than 5%. Fig. 14 shows the percentage deviation between the fitted and the experimental data points as a function of degree of polymerization. It can be observed that the larger errors are found especially in the region of low degree of polymerization ($P < 10$), especially for gradient experiments. This might be due to the fact, that for low degrees of polymerization the molar masses might be too low to describe the molecules by Gaussian chains. On the other hand for high degrees of polymerization that can be described by coil-like structures, the PM describes the elution behaviour of PEGs over a wide range of experimental conditions.

Accepting the suitability of the PM to quantitatively describe the retention behaviour of polymers it becomes possible to systematically study the errors of the LSSM and QSSM predictions. As has already been stated above, on principle neither the LSSM nor the QSSM can describe elution in SEC or under critical conditions. However, it has been proposed that these models might be applicable if the void volume is redefined to account for the large size of the polymer [22]. The void volume is typically determined from the retention volume of a non-retained low molar mass molecule. In order to account for the large size the macromolecule the void volume should be redefined as the retention volume of the non-retained macromolecule, i.e. for conditions, where no interaction between the macromolecule and the stationary phase exists, thus it would be identical to the elution volume in pure SEC conditions. However, its determination would require additional experiments to determine this elution volume and to assure that no interaction exists between the polymer and the stationary phase. An alternative is to treat the void volume of the macromolecule as an additional adjustable parameter in LSSM and QSSM. In order to calculate the error associated with the use of the LSSM and to prove whether the approach of an adjustable void volume is suitable, the following investigation was performed. First, the analyte specific parameters of the PM were estimated using all gradient and isocratic runs with MeOH content <90% for PEGs having 23,000 and 40,000 g/mol. Using these parameters, retention volumes for isocratic experiments at various eluent compositions were calculated using the PM. These retention volumes were assumed to correctly describe the retention behaviour of the PEGs, which according to Fig. 12 is true within approximately 2–3%. The obtained data therefore represent error free experiments based on realistic parameters of the PM. From these data sets, the parameters of the LSSM were estimated, once in the classical way, using the void volume of a low molar mass substance and secondly allowing for an adjustable void volume in the fitting process. From the so obtained parameters the retention volumes were predicted for the conventional and the modified LSSM. Fig. 15 shows the deviations of the retention volume predictions. Unacceptably high errors were found for both samples if the void volume of a low molar mass compound was used. The adjustable void volume approach significantly reduces the errors. However, for a molar mass of 40,000 g/mol the error still is too high to be acceptable, despite the fact that isocratic experiments were used for both, parameter extraction and prediction of retention times. This clearly shows that for high molar mass samples only the PM is capable to properly describe and predict

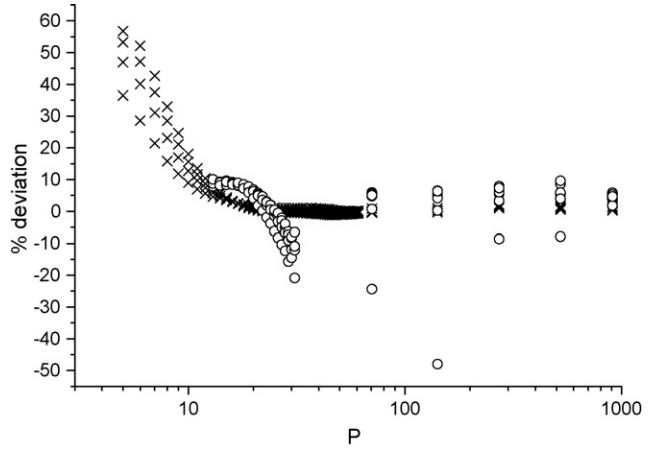

Fig. 14. Percentage error of all experiments (◯: isocratic, ×: gradients) as function of degree of polymerization. All experiments and errors were fitted to result in $\Phi_c = 0.83$, $dc/d\Phi = 3.98$ and $(R/D)_{ref} = 0.29$.

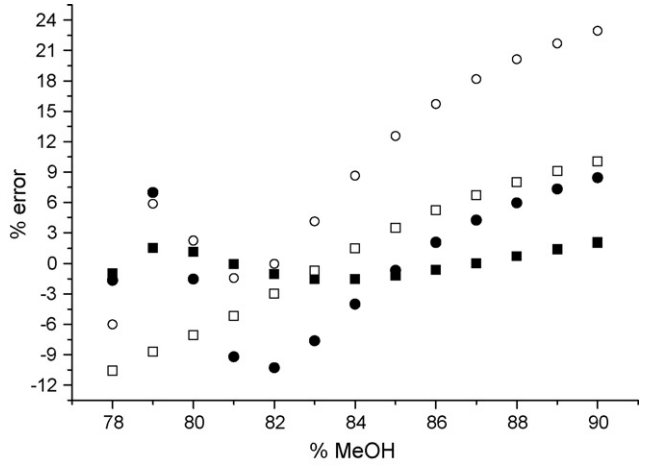

Fig. 15. Percentage deviation isocratic retention volumes of the LSSM for fixed (open symbols) and variable adjustable void volume (closed symbols) from simulated data based on the PM for PEG 23,000 (□■) and PEG 40,000 (◯●).

the isocratic retention behaviour. The addition of an additional model parameter, through the use of the QSSM with or without adjustable void volume does not change the results significantly.

6. Conclusions

It was shown that the LSSM and the QSSM allow to predict gradient retention volumes if the determination of the analyte specific parameters is done by gradient experiments. However, both models cannot adequately describe the isocratic retention behaviour. This is due to the fact that the description of the logarithmic retention factor fails if critical conditions are approached, which correspond to a value of $k=0$. The use of an adjustable void volume to account for the large size of the macromolecules does not solve this problem. In contrast to the two classical models the PM is designed to account properly for LAC, LCCC and SEC mode of chromatography. However, the capability of the PM to predict the chromatographic behaviour of polymers crucially depends on the suitable selection of the initial experiments, used for the determination of the analyte specific parameters. Based on simulations an experimental protocol is proposed which allows for a purposeful selection of the initial experiments.

Acknowledgement

"Financial support from the Federal Ministry of Economics and Technology [Bundesministerium für Wirtschaft und Technologie (BMWi)] through the Federation of Industrial Cooperative Research Associations "Otto von Guericke" [Arbeitsgemeinschaft industrieller Forschungsvereinigungen "Otto von Guericke"e.V. (AiF) (AiF-No. 13701 N) is gratefully acknowledged".

Appendix A

The elution volume in chromatography can be described by the general chromatographic equation

$$V_R = V_i + KV_P$$
$$t_R = t_i + Kt_P \tag{A1}$$

where V_R, V_i, and V_P are the elution volume, interstitial and pore volume of the column under investigation, while K is the distribution coefficient for the polymer. By dividing by the constant flow, F, we can also write the equation in time space rather than volume space. t_R is the retention time required, while t_i and t_P are the times, which needed to replace the eluent in the interstitial volume and pores, respectively.

The time required per unit length is the reciprocal velocity of the polymer molecule, i.e.

$$\frac{dt}{dx} = \frac{t_R}{L} = \frac{t_i + Kt_P}{L} \tag{A2}$$

The retention time for the polymer is obtained from (A2) by integrating from the time $t=0$, corresponding to $x=0$ to the retention time t_R corresponding to the column length L. However, for a solvent gradient K is a function of the solvent composition surrounding the analyte. This composition will change with time and distance from the column inlet.

According to the statistical theory of polymers in large slit-like pores ($R<D$) the distribution coefficient, K, can be written as

$$K = 1 + \frac{2R}{D}\left[\frac{Y(-cR)-1}{cR} - \frac{2}{\sqrt{\pi}}\right]$$
$$Y(-x) = \exp(x^2)[1 - \mathrm{erf}(-x)] \tag{A3}$$

here R, D and c are the radius of gyration, the pore diameter and the interaction parameter, respectively. In order to correlate the interaction parameter, c, with the solvent composition, Φ, the unknown function $c(\Phi)$ is approximated for small values of c, i.e. close to the critical solvent composition, Φ_c, by a power series, which is truncated after the first term.

$$c = \frac{dc}{d\Phi}(\Phi_c - \Phi) + \ldots \tag{A4}$$

The parameter R/D might vary with solvent composition via variation in R or D or both. However, during gradient experiments high molar mass polymers elute in a very narrow range of solvent compositions close to the critical one. Thus, the effect of solvent composition in retention volume due to the variation in R/D is expected to be much smaller than the effect of varying eluent composition due to a change in c. Combining (A2)–(A4) yields the dependence of the reciprocal velocity of the polymer molecule with solvent composition. Due to the adsorption of the polymer molecules, their velocity is lower or equal to that of the solvent in gradient chromatography. Thus, upon changing the solvent composition at the column inlet the polymer molecule will experience different solvent compositions during its way through the column. The solvent composition which surrounds the molecule is a function of time, t, and distance x from the column inlet ($\Phi = \Phi(t, x)$).

Since the velocity of the solvent front is given by $L/(t_i + t_P)$, the composition at a time t found at the distance x from the column inlet has entered the column at a time $t - \Delta t = t - x(t_i + t_P)/L$. Thus,

$$\Phi(x, t) = \Phi\left(x = 0, \quad t - \frac{x(t_i + t_P)}{L}\right) \tag{A5}$$

The composition introduced at a time t into the column is given by

$$\Phi(0, t) = \Phi_0 + bt \tag{A6}$$

where Φ_0 is the initial composition of the solvent and $b = d\Phi/dt$ is the rate of change in eluent composition.

Combining (A4)–(A6) results in

$$Rc = R\frac{dc}{d\Phi}\left[\Phi_0 - \Phi_c + bt - \frac{bx(t_i + t_P)}{L}\right] \tag{A7}$$

Taking the derivative with respect to x it follows

$$\frac{dRc}{dx} = R\frac{dc}{d\Phi}\left[b\frac{dt}{dx} - \frac{b(t_i + t_P)}{L}\right] \tag{A8}$$

Using (A2) and (A8) results in the following differential equation

$$\frac{\mathrm{d}Rc}{\mathrm{d}x} = \frac{Bt_\mathrm{P}}{L}\frac{\mathrm{d}Rc}{\mathrm{d}\Phi}[K-1] \tag{A9}$$

Upon combination with (A3) the solution to this differential equation is given by:

$$\begin{aligned} I(Rc_0) - I(Rc_\mathrm{final}) &= \frac{2Rbt_\mathrm{P}}{D}\frac{\mathrm{d}Rc}{\mathrm{d}\Phi} \\ I(\varsigma) &= \int_0^{\varsigma} \frac{\mathrm{d}x}{(Y(-x)-1)/x - (2/\sqrt{\pi})} = \end{aligned} \tag{A10}$$

where

$$\begin{aligned} Rc_\mathrm{final} &= \frac{\mathrm{d}Rc}{\mathrm{d}\Phi}(\Phi_c - \Phi_0 - B(t_\mathrm{R} - t_\mathrm{i} - t_\mathrm{P})) \\ Rc_0 &= \frac{\mathrm{d}Rc}{\mathrm{d}\Phi}(\Phi_c - \Phi_0) \end{aligned} \tag{A11}$$

(A10) and (A11) are identical to Eqs. (10) and (A11).

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