Predicting the chromatographic retention of polymers: Application of the polymer model to poly(styrene/ethylacrylate)copolymers

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ABSTRACT

The retention behavior of a range of statistical poly(styrene/ethylacrylate) copolymers is investigated, in order to determine the possibility to predict retention volumes of these copolymers based on a suitable chromatographic retention model. It was found that the composition of elution in gradient chromatography of the copolymers is closely related to the eluent composition at which, in isocratic chromatography, the transition from elution in adsorption to exclusion mode occurs. For homopolymers this transition takes place at a critical eluent composition at which the molar mass dependence of elution volume vanishes. Thus, similar critical eluent compositions can be defined for statistical copolymers. The existence of a critical eluent composition is further supported by the narrower peak width, indicating that the broad molar mass distribution of the samples does not contribute to the retention volume. It is shown that the existing retention model for homopolymers allows for correct quantitative predictions of retention volumes based on only three appropriate initial experiments. The selection of these initial experiments involves a gradient run and two isocratic experiments, one at the composition of elution calculated from first gradient run and second at a slightly higher eluent strength.

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1. Introduction

In the last decade considerable improvements have been made in the field of macromolecular synthesis. Novel polymerization techniques, especially those termed controlled or living polymerizations, allow synthesizing new macromolecular structures with special microstructures or topologies. However, the increasing structural complexity of the new materials requires analytical tools for their detailed characterization. Size-exclusion chromatography (SEC) is widely used to characterize the molar mass distributions of polymers. Since the separation in SEC is based on the hydrodynamic size of the molecules in solution, SEC can only provide limited information on the distribution in terms of chemical composition or end-group functionality. However, separations by chemical composition, end-group functionality or stereochemistry can be performed when using methods of interaction chromatography depending on the chromatographic experiment [1]. Once a suitable method is developed, separations can be performed in reasonably short times. Unfortunately the retention of macromolecules in interaction chromatography is influenced by molar mass and other structural features like chemical composition, microstructure, functionality, architecture etc. Therefore, considerable time is required for chromatographic method development and optimization.

Today, method development in polymer chromatography is mainly based on trial and error approaches, despite the recent increased knowledge on the factors governing interaction chromatography of polymers. In contrast to polymer chromatography virtual chromatography or computer-aided method development has become an established tool in chromatography of low molar mass compounds. Virtual chromatography applies a suitable retention model, which describes the general dependence of chromatographic retention on experimental variables like mobile phase composition, gradient slope etc. The model contains a few analyte specific parameters [2–6]. If these parameters are known, it is possible to quantitatively predict the changes in retention times under different chromatographic conditions. Application of virtual chromatography allows predicting the retention volumes and therefore the quality of a separation for a large variety of different experimental conditions within a short time using a computer, thereby reducing significantly the number of real experiments required to establish a suitable separation. Unfortunately the models employed in virtual chromatography of low molar mass compounds are often insufficient to describe the retention behavior of macromolecules [7]. However, Skvortsov, Gorbunov and others [8–11] developed a model for polymer chromatography (denoted PM in the following), which adequately describes all common modes of polymer chromatography, i.e. the size exclusion mode (SEC), the adsorption

mode (LAC) as well as chromatography at critical conditions of adsorption (LCCC). Extensions of the model have been made to describe the isocratic retention behavior of end functionalized linear [9,11,12] and star-shaped [13] homopolymers, linear and star-shaped block copolymers [10,12,14,15] as well as macrocycles [8,16]. The model has also been extended to binary solvent gradient applications resulting in the conclusion that in a linear solvent gradient high molar mass homopolymers elute independent of molar mass, which has been confirmed experimentally [7,17–20]. The eluent composition at the time of elution is close to the critical one, as was shown theoretically and experimentally [7,18–21]. In a theoretical study Brun showed that statistical copolymers too exhibit a critical point, allowing for a molar mass independent elution under isocratic conditions, similar to homopolymers [22]. This result was confirmed by computer simulations [23]. These simulations also confirmed the results of Brun [20,22] that the microstructure affects the interaction with the stationary phase resulting in stronger adsorption for blocky structures as compared to random copolymer structures. As a consequence of the existence of a critical point for statistical copolymers, a molar mass independent elution occurs in gradient chromatography for high molar mass statistical copolymers of a given composition and the eluent composition at elution is close to the critical one [21]. Since the critical point of a statistical copolymer depends on the actual chemical composition of the macromolecules, gradient chromatography allows for separating copolymers according to the chemical composition and therefore for the determination of the chemical heterogeneity distribution. The molar mass independent elution of copolymers and the coincidence of the eluent composition at elution with the critical composition has been proven for chlorinated polyethylenes by Brun [21]. The existing theoretical work on statistical copolymers let assume that the retention of copolymers can be adequately described by the same model as used for homopolymers. However, still more experimental work needs to be done to verify the hypothesis, especially aside from the retention in critical conditions, where retention is influenced by chemical composition and molar mass. In addition, while it has been shown that the PM is capable to quantitatively predict the retention times of homopolymers in all modes of chromatography based on a few purposefully selected initial experiments, to the best of our knowledge, no such investigation has been undertaken until now for statistical copolymers. The present paper therefore investigates the potential of PM to describe the chromatographic retention behavior of statistical copolymers.

2. The retention model for polymers (PM)

2.1. Basic equations of the PM

The following paragraphs will recall the basic equations used to predict the isocratic and gradient retention times or retention volumes. More detailed information is given in [7].

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

$$V_R = V_i + KV_P \tag{1}$$

Here V_R is the retention volume at which an analyte elutes from a chromatographic column. V_i and V_P are the interstitial and pore volume, respectively, while K is the distribution coefficient, which is defined as the ratio of the analyte concentration in the stationary phase to that in the mobile phase. According to the statistical theory of polymers in large slit-like pores ($R < D$), the distribution coefficient, K, can be described by [8]:

$$K = 1 - \frac{4R}{\sqrt{\pi}D} + \frac{2R}{D}\left[\frac{Y(-cR)-1}{cR}\right] \tag{2}$$

$$Y(-cR) = \exp(cR)^2\left[1 - erf(cR)\right] \tag{3}$$

Here R is the root mean squared radius of gyration of the polymer molecule and D is the pore diameter. The interaction parameter, c, describes the interaction strength between the repeating unit and the surface of the stationary phase. A value of $c > 0$ indicates adsorptive conditions, where the enthalpic contributions to the distribution coefficient, as taken into account by the third term in Eq. (2), are dominant. The mode of adsorption chromatography is characterized by an increase of the distribution coefficient with increasing R and therefore with molar mass (M), because of the scaling relation $R \sim M^v$. A value of $c < 0$ characterizes SEC-like elution behavior, where the second term in Eq. (2) becomes dominant and the distribution coefficient decreases with increasing molar mass. At $c = 0$ the entropic and enthalpic contributions to the distribution coefficient compensate each other and the molar mass dependence of the distribution coefficient vanishes. Chromatography at these conditions is referred to as chromatography at the exclusion adsorption transition point (EATP-LC), liquid chromatography at critical conditions of adsorption (LCCC), or simply critical chromatography. At critical conditions the retention volume of a non-functionalized linear homopolymer equals the retention volume of the solvent ($K = 1$).

For a given stationary phase and temperature, the value of the interaction parameter c strongly depends on the eluent composition Φ. Assuming a linear relation between c and Φ, the following solution was obtained for the retention volume of a linear homopolymer in a gradient of linearly changing eluent composition [7]:

$$I(Rc_0) - I(Rc_{\text{final}}) = \frac{2RbV_P}{F \times D}\frac{dRc}{d\Phi} \tag{4}$$

$$I(\varsigma) = \int_0^\varsigma \frac{dcR}{(Y(-cR)-1)/cR - (2/\sqrt{\pi})} \tag{5}$$

Here F is the flow rate and $b = \Delta\Phi/t_G$ is the gradient slope. $I(Rc_0)$ and $I(Rc_{\text{final}})$ indicate the values of the integral defined in Eq. (5) at $\varsigma = Rc_0$ and $\varsigma = Rc_{\text{final}}$, respectively, where

$$Rc_{\text{final}} - \frac{dRc}{d\Phi}\left(\Phi_c - \Phi_0 - \frac{b \times (V_R - V_i - V_p)}{F}\right) \tag{6}$$

$$Rc_0 = \frac{dRc}{d\Phi}(\Phi_c - \Phi_0) \tag{7}$$

c_0 and c_{final} are the values of the interaction parameter at the beginning and end of the gradient, Φ_0 and Φ_c are the eluent composition at the start of the gradient and the critical eluent composition, respectively. The term $dRc/d\Phi$ describes the change of the interaction parameter with eluent composition. It should be noted that a similar problem has been treated by Brun for stationary phases having small pores ($D < R$)[19,20]. Both cases result in the conclusion that high molar mass homopolymers elute independent of molar mass at eluent compositions close to the critical one, allowing estimating critical conditions from a simple gradient experiment. Lower molar mass polymers elute at lower retention times at eluent compositions resulting in adsorption mode if applied in isocratic experiments.

2.2. Determination of model parameters

It has been shown that the equations given above are suited to quantitatively predict the chromatographic retention volumes of non-functionalized linear homopolymers in all modes of polymer chromatography. The retention volumes depend on the experimentally known parameters Φ_0 and b as well as on three analyte specific

parameters, vis. R/D, Φ_c and $dRc/d\Phi$. These analyte specific parameters need to be determined from at least three initial experiments, the selection of which is crucial for the quality of the determined parameters and therefore for the accuracy of the subsequent predictions of the elution volumes. For homopolymers the following procedure was found to represent a systematic approach to select suitable initial experiments [7].

(1) Run a linear gradient and calculate the mobile phase composition at the volume of elution (Φ_g)
(2) Perform an isocratic run at the composition, Φ_g, determined in step 1
(3) If step 2 results in elution under adsorbing conditions (i.e. $V_R > V_i + V_P$), perform a third run using a slightly stronger mobile phase composition
(4) If step 2 results in elution under strong SEC conditions (i.e. $V_R < V_i + V_P$), perform a third run at slightly weaker mobile phase.

One might argue that step 4 violates the above statement that gradient elution will occur either at the critical eluent composition or at a weaker one. However, due to the experimental uncertainties e.g. in the determination of void, dwell and pore volumes and the sudden change in elution mode close to the critical point, elution in SEC mode in step 2 might result experimentally.

Once the model parameters R/D, Φ_c and $dRc/d\Phi$ have been determined from the initial runs using non-linear fitting procedures, predictions of retention volumes can be made for any chromatographic condition given by variation in mobile phase composition, including non-linear gradients.

The above procedure has been found to be suited for linear homopolymers [7,18]. Its suitability to statistical copolymers will be investigated in the present manuscript.

3. Experimental

3.1. HPLC-setup

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).

3.2. Materials and methods

The SEA copolymers with low chemical heterogeneity were synthesized by radical copolymerizations to conversions below 10% [24]. Details on the synthesis and characterization can be found in [24,25]. Table 1 lists the molar masses and compositions.

Mixtures of acetonitrile (AN, HPLC grade, Acros Organics) and tetrahydrofuran (THF, refluxed and distilled from CaH_2) were used as mobile phase. The stationary phase was Nucleosil C18, particle size 7 μm, pore diameter 1000 Å, column dimensions 250 mm × 4.0 mm i.d. (Macherey-Nagel, Düren, Germany). The samples were prepared in AN/THF (50/50) at concentrations of 1–2 g/L. The injected sample volume was 10 μL. Column temperature was 35 °C and flow rate 1 mL/min. Isocratic mobile phases of different compositions were delivered by the pump system. All runs were performed using at least duplicate injections.

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

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. The values of dwell volume and void volume were used to calculate the eluent composition at the time of elution [17].

Data evaluations were performed by spreadsheet calculations using OriginTM software (OriginLab Corporation, Northampton, USA). Extractions of the analyte specific parameters were carried out via self-written scripts using Origin's non-linear fitting routine which employs a Levenberg–Marquardt algorithm. The integrant of Eq. (5) tends to infinity as cR approaches zero. However, since only the difference of the integral enters into Eq. (4), it is sufficient to numerically evaluate the integral starting from an arbitrary but reasonable low value cR. On the other hand, the integral asymptotically approaches a constant value for large ς. For these reasons the integral in Eq. (5) was evaluated numerically in the range $\underline{\varsigma} = 0.02$–2.

4. Results and discussion

The retention behavior of the copolymers was investigated in linear gradients of different duration ranging from ACN to THF. The retention volumes increased nearly linearly with gradient time. According to step1 of the procedure for parameter extraction, the compositions at elution, Φ_g, were calculated and are plotted versus gradient slope in Fig. 1. The fraction of THF required for desorption

Table 1
Average molar masses and compositions of SEA copolymers having narrow chemical composition distributions (CCD).

Sample	M_w (g/mol)[a]	M_n (g/mol)[a]	Styrene content (mol%)	Symbol
PEA	90,000	45,000	0	□
PSEA 1	271,000	148,000	40	●
PSEA 2	310,000	166,000	54	△
PSEA 3	330,000	174,000	66	▼
PSEA 4	364,000	189,000	78	◇
PS	240,000	130,000	100	◄

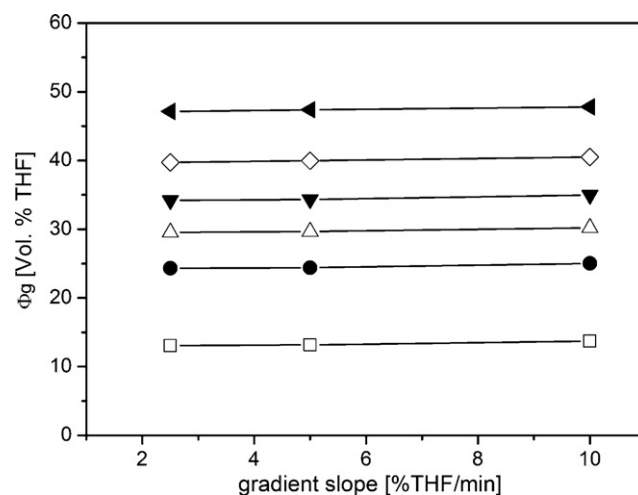[a] Molar masses determined relative to polystyrene standards.

Fig. 1. Composition at elution, Φ_g, as a function of gradient slope for SEA copolymers of different compositions, 0 (□), 40 (●), 54 (△), 66 (▼), 78 (◇), 100 (◄) mol% styrene. Gradient: linear from 100% ACN to 100% THF.

increases with increasing fraction of styrene in the copolymer, as expected for a reversed phase column. In addition, the THF fraction of the eluent is nearly independent of gradient slope, i.e. the elution occurs for each copolymer at a specific eluent composition, which for homopolymers has been shown to be close to the critical eluent composition [7,17–20]. For homopolymers it is quite simple to identify the critical eluent composition from the molar mass dependence of elution volume at different eluent compositions. However, the situation is more difficult for copolymers, since the identification of the critical eluent composition – if it exists at all- would require samples of different molar mass having exactly the same composition and a low chemical heterogeneity. If the composition at gradient elution for the copolymer would be identical to the critical eluent composition, similar to the case of homopolymers, isocratic elution of the respective SAE copolymers at lower THF contents in eluent than the ones identified in Fig. 1 should result in adsorption mode, i.e. at elution volumes higher than the dead volume of the column. In contrast, isocratic elution at higher THF contents should result in elution before the dead volume, whereas the isocratic elution at the composition of gradient elution is expected to result in elution at or very close to the dead volume of the column. 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 volumes obtained by these isocratic experiments are given in Fig. 2. For all samples, isocratic experiments at the composition at elution (vertical dotted lines in Fig. 2) resulted in retention volumes very close to or identical to the column dead volume (horizontal solid line in Fig. 2). At eluent compositions of only 1% lower THF content, retention volumes larger than the column dead volume are observed, indicating elution in adsorption mode. A further decrease of the THF content resulted in incomplete isocratic elution, i.e. a significant loss of recovery of the samples (represented by encircled symbols in Fig. 2). This was confirmed by observing the appearance of additional peaks upon flushing the column with pure THF. The limited recovery in the isocratic elution of copolymers poses a special problem because the retention in LAC is affected by both the molar mass and the chemical composition. As a consequence, isocratic LAC of chemically heterogeneous copolymers might not be of any practical use, since even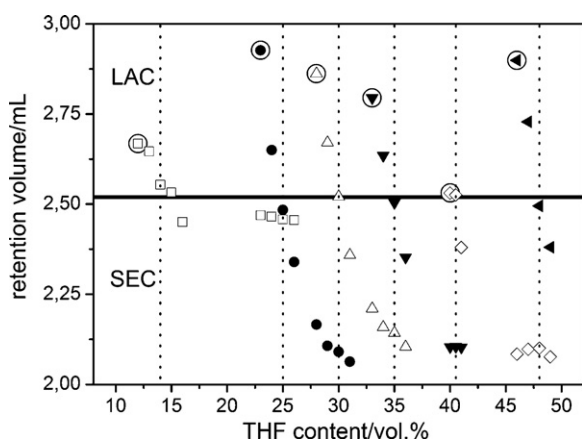 if the critical composition is realized for a particular copolymer composition, chains of other compositions might elute in adsorption mode, i.e. with a limited recovery. On the other hand, at THF contents only 1% higher than the compositions at elution, retention volumes smaller than column dead volume were observed indicating elution in SEC mode. A further increase in the THF content results in an additional decrease of the retention volumes until a limiting retention volume is approached. It can be noticed that this limiting volume for the pure PEA is higher than for the other samples. This is due to lower molar mass of the PEA (see Table 1). This transition from LAC to SEC mode, indirectly verifies the identical nature of critical eluent composition and eluent composition at gradient elution.

In addition to the changes in retention volumes, the observed variations of peak widths with changing eluent composition are another indication of the transition from adsorption to size exclusion mode. As an example, a comparison of the chromatograms for the SEA copolymer containing 66 mol% styrene obtained under different isocratic eluent compositions is given in Fig. 3. 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 expected to be homogenous with respect to chemical composition, but heterogeneous with respect to molar mass (having molar mass polydispersity of approximately 1.9), the narrow peak at 65/35, v/v ACN/THF indicates that no molar mass effect is present, i.e. that this composition 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 as already stated by Brun [21].

From the observations outlined above, it appears that statistical copolymers qualitatively behave similar to homopolymers. Thus, the given retention model might also be used for statistical copolymers without any additional modification. In order to test this hypothesis, the retention model was applied to predict the retention volumes of SEA copolymers in gradient and isocratic elution, on the basis of minimum number of initial experiments, as it was shown for homopolymers [7,18].

First, linear gradients ranging from 100% ACN to 100% THF in 5, 10, and 20 min were used to extract the model parameters of the retention model. Then predictions were made for other gradient durations. The predicted retention volumes were compared with the experimental ones and the relative errors were calculated to determine the quality of predictions.
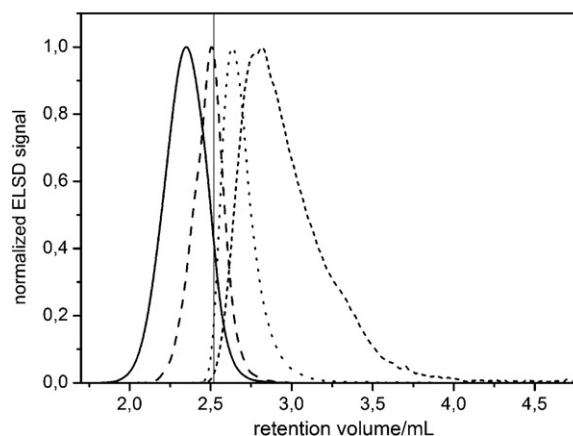

Fig. 2. Retention volumes in isocratic experiments as a function of THF content of eluent for SEA copolymers of different compositions; 0 (□), 40 (●), 54 (△), 66 (▼), 78 (◇), 100 (◀) mol% styrene. Encircled symbols are those where incomplete elution is observed. The black horizontal line represents the column dead volume where the elution is expected for the respective critical condition of the copolymer. Vertical dotted lines indicate the compositions at elution calculated from the gradient experiments.

Fig. 3. Overlay of peaks of a copolymer with 66 mol% styrene obtained at different isocratic eluent compositions (left to right 64/36, 65/35, 66/34, 67/33, v/v ACN/THF) close to the composition at elution in gradient chromatography (65.1/34.9, v/v ACN/THF). The vertical dashed line indicates the column dead volume.
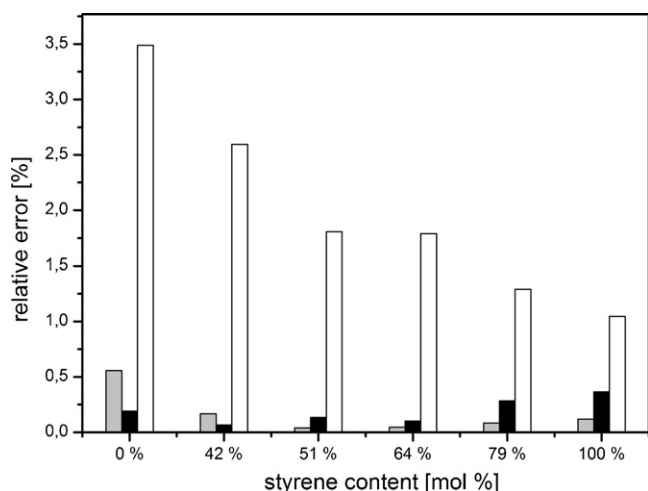
Fig. 4. Comparison of percent relative errors in gradient to gradient retention prediction for statistical copolymers of styrene and ethyl acrylate. Prediction: linear gradients from 100% ACN to 100% THF in 2.5 (grey), 5 (black), and 40 min (white). Calibration: linear gradients from 100% ACN to 100% THF 5, 10, and 20 min.

Fig. 4 shows the percent relative deviations of the predictions from the experimental retention volumes for PEA, PS and different copolymer compositions as a function of styrene content in the samples. The errors for all cases are below 3.5%. This maximum error is observed for predictions for gradient durations outside the calibrated range of gradient times. Thus, with the aid of the given retention model accurate predictions of gradient retention behavior of statistical copolymers are possible using linear gradient retention volume data.

Next, the parameters extracted from gradient experiments were applied to predict isocratic retention for the series of statistical copolymers. Fig. 5 shows box plots of the percent relative deviations to the experimental retention volumes. 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 volume predictions from only gradient experiments resulted in considerably larger errors as compared to the gradient to gradient predictions. This is due to the inaccuracy of the parameters R/D and $dc/d\Phi$ extracted for high molar mass polymers from gradient experiments only. However, according to the previously established methodology for homopolymers, the isocratic
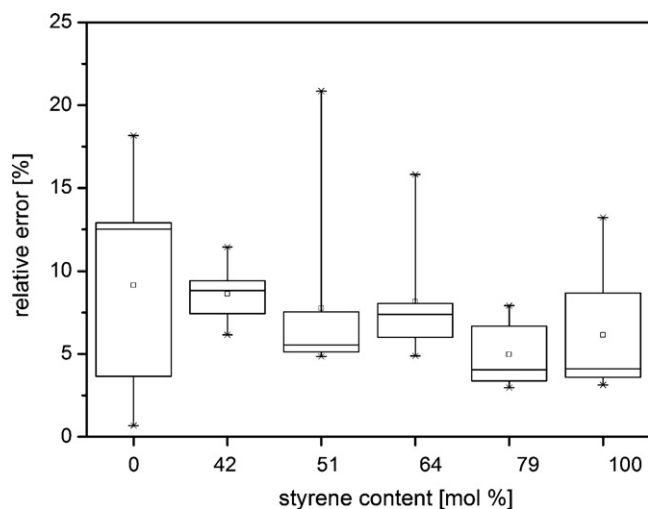
Table 2
Calibrations used for prediction of isocratic retention volumes of SEA copolymers.

Sample	Calibration 1 t_G; ACN/THF[a]; ACN/THF[b]	Calibration 2 t_G; ACN/THF[a]; ACN/THF[b]
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

[a] Composition at elution for a 10 min linear gradient from 100% ACN to 100% THF.
[b] Isocratic eluent compositions in v/v.

predictions often can be significantly improved by including isocratic experiments along with one starting gradient experiment into the calibration of the model. Therefore, both isocratic and gradient experiments were used to extract the final model parameters.

Two different sets of experimental data were used for the calibration. First the composition at elution was calculated for each sample from a linear 10 min gradient from 100% ACN to 100% THF. Then isocratic experiments were performed for every copolymer at the calculated compositions at elution and at one eluent composition of slightly higher THF content. The details of the three initial experiments for all samples of the copolymer series are tabulated in Table 2.

Using these experiments, the model parameters were determined for each sample and the retention volumes for different isocratic experiments were calculated. Fig. 6 shows a comparison of the experimental and predicted retention volumes. As can be seen, for the first calibration (dotted lines), a very good agreement exists between the prediction and experiment close to column dead volume, i.e. at the critical compositions of the copolymers. The small difference between the lines reflects the accuracy in predicting the critical compositions.

Larger deviations are observed for retention volumes 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 volumes (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 volumes
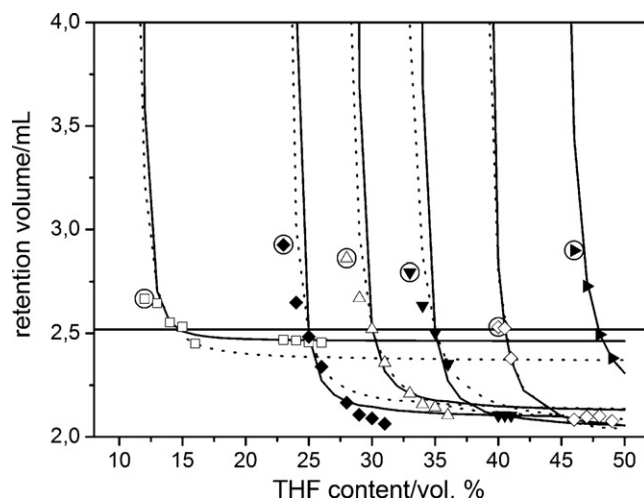

Fig. 5. Box plots of the percent relative errors in retention volumes for isocratic elution of SEA copolymers as predicted. Calibration: same as in Fig. 4.

Fig. 6. Comparison of the experimental (symbols) and predicted isocratic retention volumes as a function of THF content of the eluent for SEA copolymers of different compositions, 0 (□), 40 (●), 54 (△), 66 (▼), 78 (◇), 100 (◄) mol% styrene. Calibration: 1 (dotted lines) and calibration 2 (solid lines) (see Table 2).
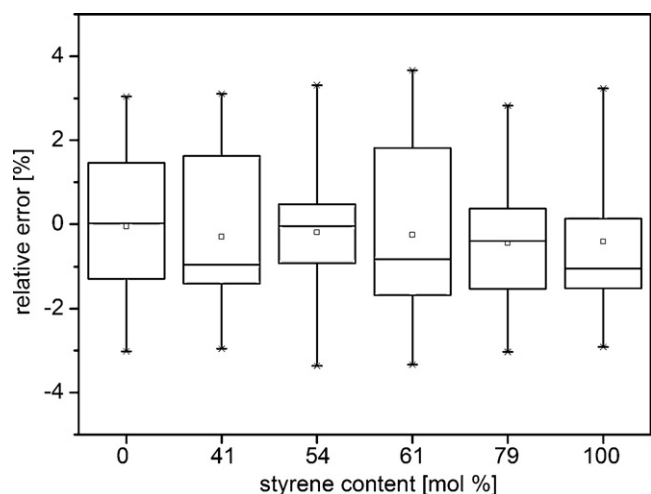
Fig. 7. Box-plots of errors when fitting the retention model to the experimental retention volumes of SEA copolymers for gradient and isocratic experiments.

might not be representative of the complete sample. This means that the average retention volume of the actual sample is higher than that reported here, as has been predicted by the model. As a consequence the use of isocratic runs at adsorbing conditions to extract the model parameters is discouraged. 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 volumes, does not correspond to the same molar mass for which the retention volume is predicted. This may contribute to the deviations observed. Another source of error might be a possible non-linear dependence of the interaction parameter on eluent composition for a wide range especially in SEC conditions.

In order to improve the quality of the predictions a wider range of isocratic eluent compositions was selected using isocratic experiments with higher amounts of THF second calibration in (Table 2). As can be seen in Fig. 6, the predictions in the SEC mode are significantly improved (solid lines). This shows that significant improvements in the predictions are possible by suitable selection of the experiments used to extract the model parameters.

In order to test the general suitability of the retention model for statistical copolymers, the model was fitted to all experimental data obtained from gradient and isocratic experiments. The data of each copolymer can be fitted with good accuracy by just three model parameters as shown in Fig. 7, where percent deviations of experimental and predicted retention volumes are shown for each sample as box-plots. For 50% of all experiments the retention volumes differ by less than 2%. No retention volume differences higher than 4% were observed. This good agreement for a large variety of experimental conditions and different samples clearly confirms the suitability of the retention model to describe the chromatographic behavior of statistical copolymers.

It should be noticed that method development applying the PM relies on samples of low chemical heterogeneity. This does not indicate a failure of the model for heterogeneous samples. The samples with narrow chemical heterogeneity (but over a full range of chemical compositions for SEA copolymers) were deliberately used to

partially decouple the influence of chemical composition on retention. Retention in the isocratic experiments in particular, is strongly influenced by both molar mass and chemical composition, resulting in coelution of different macromolecular structures which complicates the elution behavior. Nevertheless, the chromatographic separation conditions developed using virtual chromatography based on PM can be applied to chemically broadly distributed samples as well, after knowing the relation between the chemical composition and critical eluent composition [25].

5. Conclusions

The above investigation has shown that the retention behavior of SAE copolymers in isocratic and gradient experiments can be adequately depicted by the described retention model for polymers. The required model parameters to perform the retention volume predictions can be extracted using one gradient run from which the compositions of two additional isocratic experiments can be systematically selected. In addition, similar to homopolymers, the gradient experiments on statistical copolymers allowed to reasonably estimating the critical eluent composition of copolymers.

Acknowledgements

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