

Application of the reversed-phase liquid chromatographic model to describe the retention behaviour of polydisperse macromolecules in gradient and isocratic liquid chromatography

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Abstract

This paper illustrates how conventional models of chromatographic behaviour can be used to predict the separation behaviour of polydisperse macromolecules. Using polystyrene and polymethylmethacrylate homo- and co-polymeric standards, the models were validated by comparing experimental retention behaviour with that predicted by the chromatographic model. The experimental retention time of each of the samples was entered into a spreadsheet application, which calculated the parameters that best described retention (for a given model). When a correlation between the relevant parameters and molecular mass was established, that correlation was used to predict the change in retention behaviour over the molecular-mass range. An expression introduced in a previous paper, to calculate the critical mobile-phase composition of a homopolymer was validated using polystyrene homopolymers. A second expression, which can predict the elution behaviour of copolymers, was also validated. This expression can predict the retention of a copolymer, based solely on the retention of the homopolymeric units that make up the copolymer.

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1. Introduction

The comprehensive characterisation of the molecular distributions present within a polymeric sample is an essential part of predicting and controlling the physical and chemical properties of that polymer. Distributions in synthetic polymers can be multi-dimensional and at the very least will include a molecular-mass distribution (MMD). In the case of

more complex (co)polymers, various other distributions, such as chemical-composition distributions (CCD), functionality-type distributions (FTD), block-length distributions (BLD), branching distributions and tacticity distributions can also be present. Conventional chromatographic techniques for macromolecular separations, such as size-exclusion chromatography (SEC), cannot provide a complete characterisation of such polymers, since they do not distinguish between molecular properties other than size. For complete characterisation, techniques that make use of molecular interactions, such as gradient-elution and isocratic (critical) liquid chromatography

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are more appropriate, since these can separate on the basis of chemical differences other than molecular size [1–3].

Interactive chromatographic techniques are routinely used for the separation of small molecules. However, their application to macromolecules is not so well established, mainly because the mechanisms controlling their retention are not always as straightforward. It is widely accepted that polymers must be regarded as a particular case for liquid chromatography and that there are a number of special features that must be considered [4,5]. These include the molecular radius to pore-diameter ratio, which, when large enough, will induce depletion (exclusion) effects within the column. Multi-site attachment of repeating units can mean that the polymer is either fully sorbed onto the stationary phase or fully desorbed into the mobile phase (i.e. there is no distribution of the molecules between the phases). The transition between these two states can be quite sudden for large polymers and in this case, isocratic chromatography becomes impractical (except at a very specific critical mobile-phase composition, where the monomeric backbone of the polymer no longer influences retention). The kinetics of solubility of the polymer in a given mobile-phase mixture can further complicate the retention behaviour [6]. These factors can ultimately lead to diminished robustness of the polymer-separation method, because they contribute to retention in a way that cannot always be repeated. For example, the dynamics of solubility will depend on sample concentration and sample volume, as well as on molecular mass and the system temperature [2]. Changes in any of these parameters will affect the retention behaviour due to changes in solubility rather than chromatographic interactions. Depletion effects can also occur for high molecular-mass polymers, however, it has been reported that this does not affect the surface area of stationary-phase available for interaction with the macromolecules [7].

In this research, the retention behaviour of a number of polydisperse macromolecules has been fitted to an appropriate chromatographic model (in this case the reversed-phase retention model). A spreadsheet program was used to calculate “best-fit” values to describe retention in terms of the model,

based on experimental data. Details on the format of the spreadsheets and how we can use them to predict retention behaviour have been outlined in a previous paper [8]. In this paper, we will present experimental validation of these predictions by comparing data obtained for (co)polymeric standards with the predictions calculated using the chromatographic model.

In addition, we introduce an expression that describes the retention behaviour of a copolymer in terms of the contribution to retention of each of the monomeric units present. Notably, the expression predicts the retention of a copolymer based solely on the retention behaviour of its component monomeric units. No copolymeric standards are required. We have validated this expression using narrow CCD polystyrene/polymethylmethacrylate copolymers and we demonstrate that there is an excellent agreement between the predicted and actual behaviour of the copolymers.

2. Models in interactive HPLC

2.1. Reversed-phase model

One of the most widely applied models of retention behaviour in interactive HPLC is the so-called reversed-phase model, in which the logarithm of the retention factor is assumed to vary (approximately) linearly with the volume fraction of organic modifier in the mobile phase [11–13], i.e.

$$\ln k = \ln k_0 - S\varphi \quad (1)$$

where φ is the volume fraction of strong solvent in the mobile phase, k_0 is the retention factor of the analyte in 100% of the starting (weak) solvent and S is the solvent strength parameter (a measure of the decrease in $\ln k$ with increasing φ). Once values for $\ln k_0$ and S have been obtained for a given analyte, it is then possible to predict the retention factor of that compound at any mobile-phase composition.

Values for S and $\ln k_0$ can be determined isocratically. Retention factors for a sample are measured at a number of different values of φ . If the model adequately describes retention behaviour, then an approximately linear relationship between $\ln k_0$ and φ will be seen; S is then the negative slope of that line and $\ln k_0$ is the intercept at $\varphi=0$. An example of

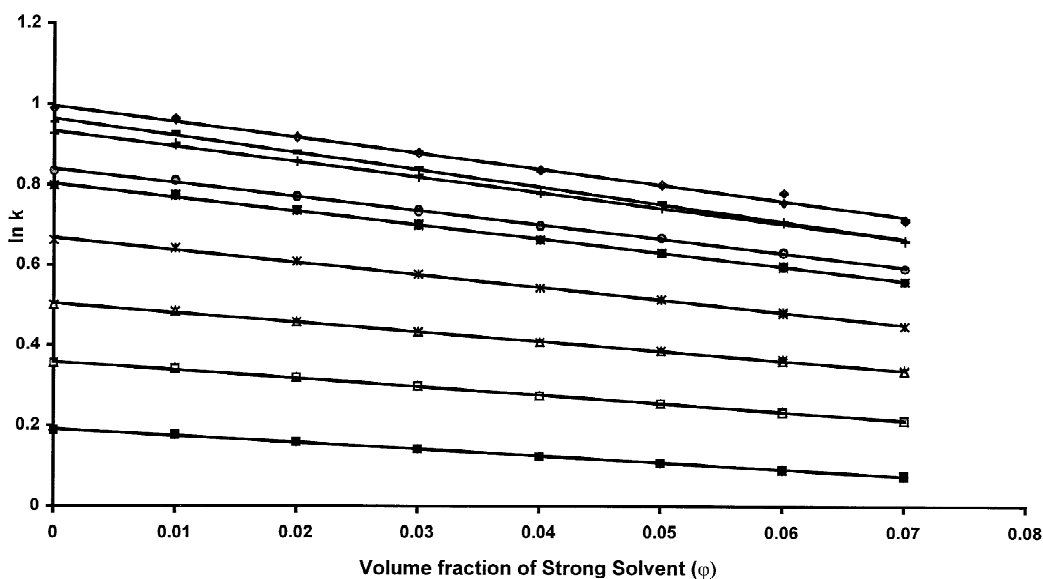


Fig. 1. Semi-logarithmic relationship between the retention factor and the volume fraction of THF for a group of polystyrene oligomers (polystyrene, molecular mass = 580 u, PDI = 1.16).

one such plot for the oligomers of a polystyrene standard (average molecular mass = 580 u, PDI = 1.16) is given in Fig. 1.

In the case of higher molecular-mass molecules, this type of plot is difficult to obtain, because the range of mobile-phase compositions where there is meaningful retention becomes too narrow, i.e. small changes in the mobile-phase composition lead to excessively large changes in the retention factor (effectively going from a fully retained to a fully unretained state). This is seen graphically as an extremely steep slope in the $\ln k$ vs. φ plot. For this reason, gradient chromatography tends to be the only practical choice for the interactive LC separation of high-molecular-mass molecules.

According to the reversed-phase model, retention in gradient elution chromatography will be a function of the gradient parameters i.e. initial and final mobile-phase compositions, gradient slope B (volume fraction change in solvent composition per unit time) as well as of S and $\ln k_0$. The retention model can be solved for each of the three modes in which the sample can be eluted, i.e. before (isocratic), during or after the gradient [14]. The equations describing retention in these three modes, for the

reversed-phase retention model and assuming a linear gradient profile are given below.

$$t_{\text{before}} = t_m(1 + k_{\text{init}}) \quad \text{[Before]} \quad (2)$$

$$t_{\text{during}} = \frac{1}{SB} \ln(1 + SBk_{\text{init}}) \frac{t_m - t_D}{k_{\text{init}}} + t_m + t_D \quad \text{[During]} \quad (3)$$

$$t_{\text{after}} = k_{\text{final}} \frac{t_m - t_D}{k_{\text{init}}} - \frac{1}{SB} \frac{1 - k_{\text{final}}}{k_{\text{init}}} + t_G + t_m + t_D \quad \text{[After]} \quad (4)$$

t_m and t_D are the column dead time and the system dwell time and k_{init} and k_{final} are the retention factors in the initial and final mobile-phase compositions, respectively. By varying B , it is possible to solve for S and k_{init} (from which k_0 can be calculated), such that the differences between the experimental and the calculated retention times are minimised.

2.2. Using experimental correlations to expand the model

It has been observed experimentally that molecules that form part of a homologous series can exhibit a straight-line correlation between the model parameters S and $\ln k_0$ [15,16]). It has also been seen (again for a homologous series) that there is a correlation between the retention factor and the number of repeat units (e.g. monomeric units) in the molecule i.e. the Martin rule [17]. The solvent-strength parameter S has also been shown to increase with increasing molecular mass [5,18].

When they are seen to apply, these correlations can be useful, because they allow the model to be expanded to describe the changes in the relevant parameters over the molecular-mass range. It is then possible to predict the retention behaviour of a *polydisperse* macromolecular sample under any gradient or isocratic conditions in a given LC system.

3. Experimental

The experiments were carried out on a Waters 2690 liquid chromatograph. Gradient control, data acquisition and data analysis were handled by Waters Millennium 3.2 software. The stationary-phase was Supelco Discovery C₁₈, particle size 5 μm , pore diameter 180 \AA , column dimensions were 150 mm \times 2.1 mm I.D. and column temperature was maintained at 25 $^{\circ}\text{C}$. The solvents were THF (Biosolve, Valkenswaard, The Netherlands) and acetonitrile (Rathburn Chemicals Ltd., Walkerburn, Scotland), both were HPLC grade. The flow-rate was 0.2 ml/min. Samples consisted of low dispersity polystyrene and polymethylmethacrylate standards (Polymer Laboratories, Church Stretton, UK). PS/PMMA copolymers were obtained from the polymer-chemistry group at the Technical University of Eindhoven (TU/e). The sample-injection volume was 5 μl and sample concentrations were 1.5 mg/ml. For the calculation of the model parameters (S and $\ln k_0$), gradient programs from 5 to 95% THF in acetonitrile were run over 20, 45, 60 and 90 min. Detection of the samples was performed with a Waters PDA 996 diode-array detector and a Sedex 55 evaporative light-scattering detector (ELSD). All samples were

run in duplo. Data-modelling spreadsheets were written in Microsoft Excel 97 on a Windows NT operating system.

4. Results and discussion

4.1. Column dead-time and system dwell-time determination

The column dead-time (t_0) was calculated as the time taken for an unretained thiourea peak to reach the detector. The system dwell-time (t_D) was calculated from the time at which a gradient trace reaches half its maximum intensity, minus half the programmed gradient time (t_G), minus any programmed time delay before the start of the gradient, minus the column dead-time (t_0).

4.2. Implementing the models and correlations in a spreadsheet application

The model and its expanded correlations were incorporated into two spreadsheet applications. On the first spreadsheet, experimental data obtained from the chromatographic runs were entered. The spreadsheet was designed to solve for the relevant parameters of a given model, so that there was a minimum sum-of-squares difference between the experimental and the calculated retention times. The differences between experimental and calculated retention times were compared in order to establish whether a particular model could suitably describe retention. When values for the parameters were found where the difference was acceptably small, it was then taken that that model could adequately describe the retention behaviour of that sample. Once parameters were found for a series of standards, it was then established whether any correlation between the parameters themselves and between the parameters and molecular mass could be found.

On the second spreadsheet, these correlations were used to predict retention behaviour under given chromatographic conditions. The correlations that were calculated on the first spreadsheet and the average molecular mass and polydispersity of the sample were entered. An estimate of the efficiency of the system (in terms of plate number) and the

experimentally determined values for t_m and t_D are also required for the calculation of retention time and the construction of the chromatogram and the calibration curve [8].

The chromatographer describes the gradient program in terms of an initial and final composition and the time (t_G) for completion of the gradient (to define an isocratic system, the initial and final mobile phase compositions can be set as equal; the gradient time t_G then becomes irrelevant).

The spread of molecular masses in a given polymeric sample (calculated from its average mass and polydispersity) is split into (up to) 100 separate portions on the spreadsheet. The molecular-mass distribution should be defined to best describe its shape, e.g. a normal distribution or a log normal distribution. It has been seen that a normal distribution can adequately describe the MMD of polystyrene standards [9,10] and it was found to best describe the MMD of the samples used in this study. For each portion of the distribution, values for the relevant parameters are calculated from the observed correlations and a retention time is calculated. The peak width is calculated according to the equation:

$$\sigma = (1 + k_e) \frac{t_m}{\sqrt{N}} G \quad (5)$$

where k_e is the retention factor at the moment of elution, N is a measure of the column efficiency (in terms of plate number) and G is the gradient band-compression factor. N is difficult to calculate for the retention of high molecular-mass polymers since reasonable retention times in the isocratic mode are almost impossible to achieve. We found that with an estimated value of N (~5000 plates), a reasonable agreement between predicted and experimental peak widths was observed. For gradient elution, G is theoretically roughly constant and equal to 0.8 [11]. However, since band compression is not always experimentally seen in gradient chromatography [19], an approximation of $G=1$ was found to be more reasonable in this case.

The calculated peak heights of each of the portions within the polydisperse sample were summed and automatically plotted as a chromatogram. The reported retention time is the highest point on the peak. The change in retention time along the molecular-mass distribution can be visualised in the molecular

mass versus retention-time calibration-curve, which is also plotted automatically within the excel workbook.

4.3. Reversed-phase model

In order to solve for S and $\ln k_0$ values, a number of standards were run, under four different sets of gradient conditions (i.e. four different B values). Gradient-slope values ranging from approximately 1 to 4.5% per min were programmed for the runs. S and $\ln k_0$ were calculated using the spreadsheet's solver tool. The values calculated are constants for a given sample and in the given LC system (i.e. stationary phase, mobile-phase components and temperature). Shalliker and coworkers [20] have reported that S and $\ln k_0$ values can be gradient-rate dependent when gradients steeper than 2%/min are employed, however, over the gradient range used in this research, no gradient-rate dependence was observed. This was confirmed using Shalliker's method of plotting t'_g/t'_G versus $\log t'_G$ for each of the polymer samples, where t'_g is the retention time of the sample corrected for the column dead-time and the system dwell-time and t'_G is the gradient time from 0 to 100% strong solvent ($t_G/\Delta\varphi$). Fig. 2 shows that there is a linear relationship over the entire gradient-rate range, indicating that S and $\ln k_0$ remain independent of the gradient slope (B) for all the molecular masses studied.

A comparison of the calculated and experimental retention times for polystyrene standards 3250 u, 22 000 u and 160 000 u at the optimum values of S and $\ln k_0$, calculated by the program, is given in Table 1. It can be seen that there is an excellent agreement between experimental and calculated retention times. In most cases, the difference is no more than a couple of seconds. In Table 2, the calculated S and $\ln k_0$ values for each of the polystyrene standards are given, along with the sum of the squared differences (SSQ) between experimental and calculated retention times (SSQ values are the combined error from eight experiments at four gradient-slope values). Once values for the parameters were calculated, the possible correlations were established. Examples of the relationships found for a series of polystyrene standards are given in Fig. 3a, b and c.

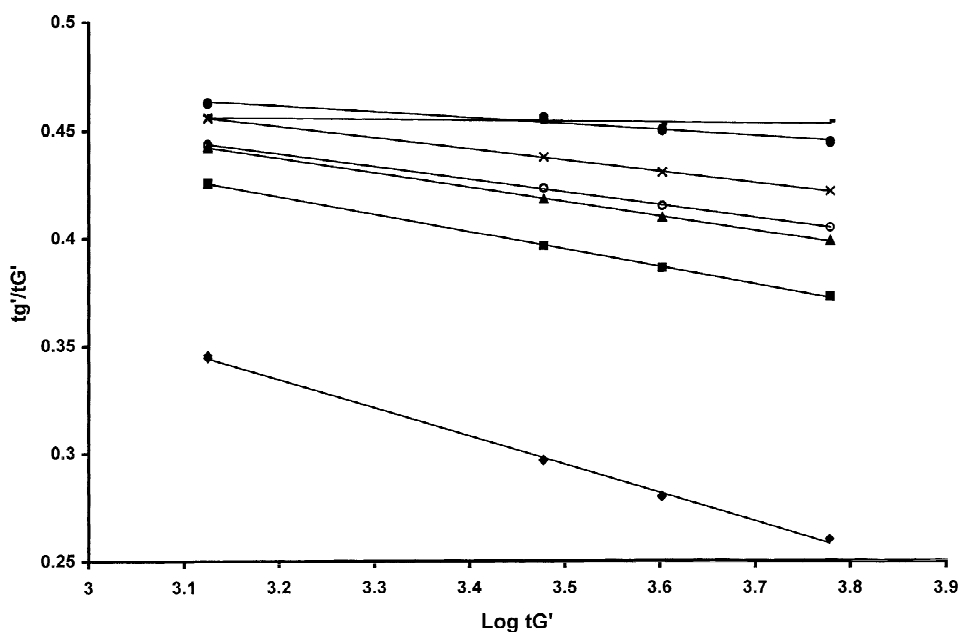


Fig. 2. Determination of the dependence on the mobile-phase gradient-rate when calculating S and $\ln k_0$ values, using the method quoted in reference [20]. Linearity over the entire range indicates that gradient-rates do not influence the calculations. \blacklozenge =PS 3250, \blacksquare =PS 11 600, \blacktriangle =PS 17 600, \circ =PS 22 000, \times =PS 30 000, \blacklozenge =PS 76 600 and \square =PS 325 000.

Table 1

Comparison of predicted and experimental retention times for a number of PS standards; gradient conditions: 5 to 95% THF in ACN in 90 min

Polystyrene molecular mass (u)	Best fit S value	Best fit $\ln k_0$ value	Experimental retention time (s)	Predicted retention time (s)	Difference (s)
3250	18.50	6.48	1978.38	1975.26	3.12
22 000	38.84	17.68	2847.24	2846.87	0.37
160 000	186.31	92.51	3143.88	3146.48	2.60

Table 2

Calculated S and $\ln k_0$ values for a series of polystyrene standards and the calculated error between the experimental and predicted retention times

Polystyrene molecular mass (u)	Best fit S value	Best fit $\ln k_0$ value	SSQ ^a
3250	18.50	6.48	1.69×10^2
11 600	28.89	12.51	1.32×10^1
17 600	35.14	15.87	1.26×10^1
22 000	38.84	17.68	9.88×10^0
30 000	44.47	20.85	6.66×10^0
76 000	72.52	35.26	8.83×10^1
160 000	186.31	92.51	4.78×10^1

^a SSQ error is defined as the squared difference between experimental and calculated retention times (in s).

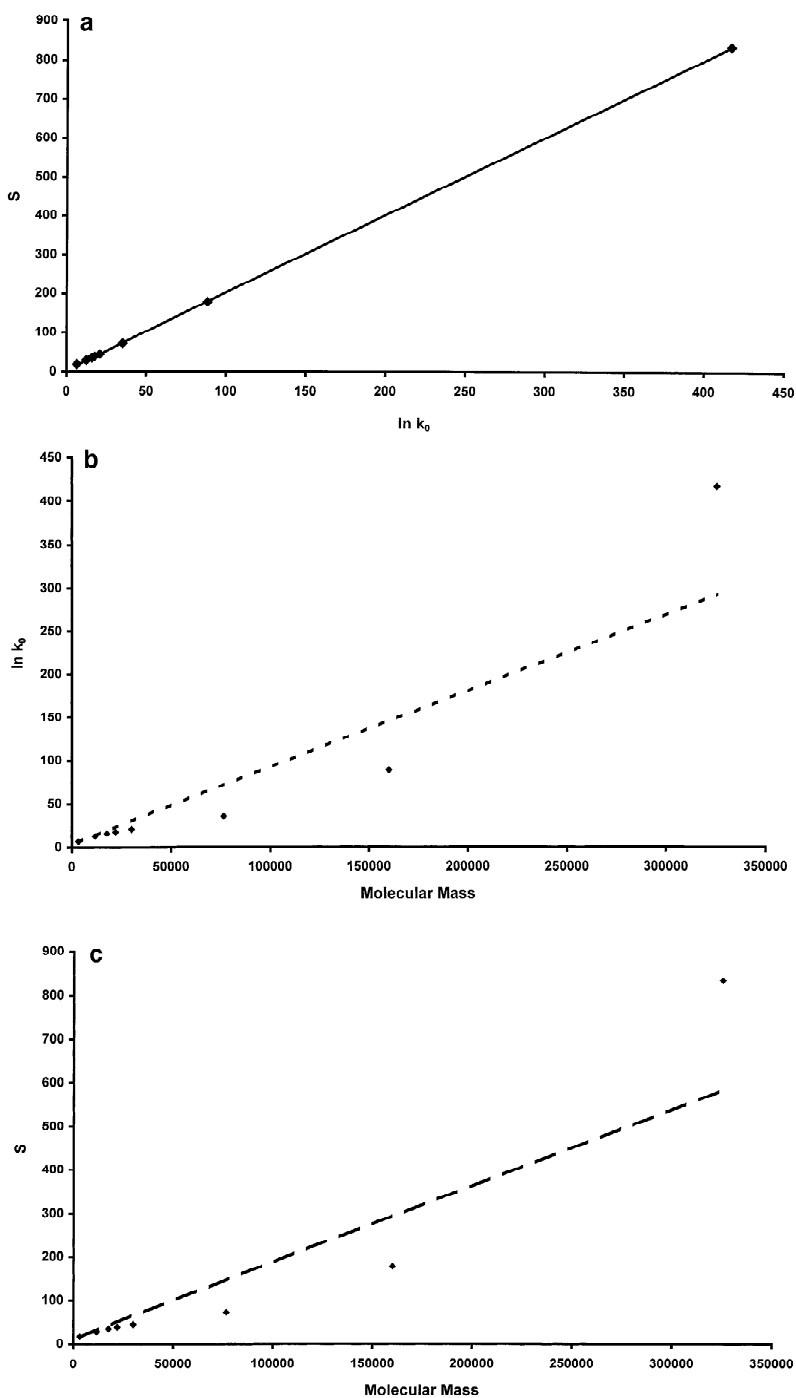


Fig. 3. (a) Correlation between the reversed-phase model parameters S and $\ln k_0$ for a series of polystyrene standards. Equation of the line: $y = 1.9825x + 4.8586$. (b) Correlation between $\ln k_0$ and molecular mass for a series of polystyrene standards. Dashed line corresponds to the best-fit line calculated, assuming a linear correlation between S , $\ln k_0$ and molecular mass. Equation of the line: $y = 0.0009x + 3.1777$. (c) Correlation between S and molecular mass for a series of polystyrene standards. Dashed line corresponds to the best-fit line calculated assuming a linear correlation between S , $\ln k_0$ and molecular mass. Equation of the line: $y = 0.0018x + 11.158$.

It was seen for low-molecular-mass-polystyrenes and polymethylmethacrylates, in a reversed-phase system, that there was a reasonably linear correlation between molecular mass and S and molecular mass and $\ln k_0$. However, at higher molecular-mass values, the calculated “best-fit” values for S and $\ln k_0$ tended to deviate quite significantly from the line. While this may initially seem to be a failing of the assumed correlations, it is important to remember that at high values of S , the slope of the $\ln k$ vs. φ relationship is very steep. A steep slope indicates that the transition from a fully retained to a fully unretained state is quite sudden and occurs over a very narrow range of mobile-phase compositions. Although higher molecular-mass molecules can have values of S and $\ln k_0$ that do not exhibit a linear correlation with molecular mass, in practice, these values are so high that changing them to fit the linear molecular mass correlation does not lead to significantly different retention behaviour. In terms of calculating “best-fit” values for S and $\ln k_0$ for higher molecular-mass molecules, there is a broad range of steep slopes and intercepts that can reasonably predict their retention behaviour. The key factor

is that the slope *and* intercept i.e. S and $\ln k_0$ should change concomitantly, i.e. the parameters must not deviate from the S vs. $\ln k_0$ line, otherwise significant errors in predicted retention times can occur.

In order to prevent the higher molecular-mass polymers from overly (and incorrectly) influencing the “best-fit” equations for the molecular-mass correlations, the lines were re-calculated by assuming linear relationships between S and molecular mass and $\ln k_0$ and molecular mass. The equations for the lines were then simultaneously calculated based on the best agreement of predicted and experimental retention behaviour over the entire molecular-mass range. These correlations are represented as the dashed lines in Fig. 3b and c.

Using these correlations, the model was expanded to describe the chromatographic behaviour (i.e. retention time and peak width) of polystyrene and PMMA of any molecular mass within the relevant mass range. S and $\ln k_0$ values were calculated for (up to) 100 individual portions of the distribution and a chromatogram of the overall separation was constructed, based on the gradient parameters provided by the chromatographer. Fig. 4 is a comparison of

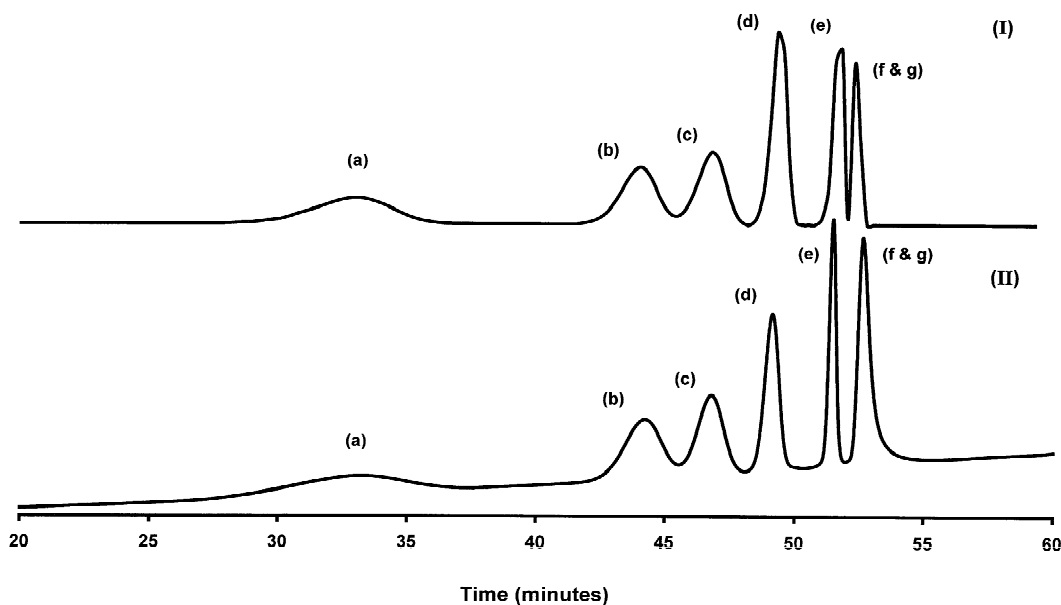


Fig. 4. Comparison between the predicted (I) and experimental (II) retention times for a series of polystyrene standards; (a) PS 3,250, (b) PS 11 600, (c) PS 17 600, (d) PS 30 000, (e) PS 76 600, (f) PS 160 000 and (g) PS 325 000. Gradient conditions: 5 to 95% THF in ACN over 90 min.

the predicted and experimental retention times of seven polystyrene standards in a gradient separation. The standards range in molecular mass from 3250 u to 325 000 u and so represent the entire range studied. In general, there is excellent agreement between the experimental and predicted traces. In some cases, the values used for polydispersity in the predicted chromatograms were lower than those quoted by the manufacturer, however this is not surprising, as it has been reported in other investigations that the polydispersity of polymeric samples can often be significantly lower than the values quoted by the manufacturers [10]. As molecular mass increases, resolution is seen (and predicted) to decrease. There is very little separation between the 76 600 u standard and higher molecular masses and the 160 000 u and 325 000 u standards co-elute.

4.4. Determination of the critical point using the reversed-phase model and the S vs. $\ln k_0$ correlation

The critical composition is defined as the mobile-phase composition at which the separation is independent of the molar mass of the macromolecule. This occurs when the free energy contribution of the monomer (ΔG_m) is zero and (since $\Delta G = \Delta H - T \Delta S$) corresponds to the point where the enthalpic and entropic contributions of the monomer exactly compensate [21].

Chromatography at the critical mobile-phase composition has many potential applications in polymer chromatography. Polydisperse macromolecules can be separated on the basis of chemical differences rather than on differences in molecular mass. For example, separation may be according to the number of functional groups on the molecule or, in the case of a copolymer, according to the non-critical monomer(s) on the polymer backbone [22,23].

The critical point is equivalent to the intersection point on the $\ln k$ versus φ (volume fraction of strong solvent) plot for the reversed-phase model. A simulated example of this (based on experimental data) is shown in Fig. 5a. At this point, the retention factor of a homopolymer will no longer depend on the length of the polymeric chain and any retention will only be due to interaction of the end-groups.

Determining the intersection point from an ex-

perimental plot of $\ln k$ vs. φ can be difficult because experimental error can cause scattering of the lines so that no exact point can be established (Fig. 5b). A more convenient way to estimate the critical point is to incorporate the S vs. $\ln k_0$ correlation i.e.

$$S = p + q \ln k_0 \quad (6)$$

into the RP model (Eq. (1)). Retention can then be described only in terms of $\ln k_0$, i.e.

$$\ln k = \ln k_0(1 - q\varphi) - p\varphi \quad (7)$$

At a given composition $\varphi_{\text{crit}} = 1/q$ (i.e. the inverse of the slope of the S vs. $\ln k_0$ correlation), all k values will be equal, regardless of the value of $\ln k_0$. This composition then corresponds to the critical mobile-phase composition for that particular homopolymeric series. By the same reasoning, the logarithm of the critical retention factor ($\ln k_{\text{cr}}$) will be the negative intercept divided by the slope (of the S vs. $\ln k_0$ correlation). A more detailed explanation of this estimation is given in a previous paper [8].

From the slope of the correlation shown in Fig. 3a, the critical composition was calculated as 50.44% with a retention factor at the critical composition of 0.086. Fig. 6 shows experimental chromatograms for a mixture of four polystyrene standards (2100, 7000, 76 000 and 325 000 u) at 49, 50 and 51%. Retention shifts from the sorption mode at 49%, to approximately critical at 50% and finally to exclusion at 51%. The prediction of the model is thus quite accurate in this case. True critical conditions can be realised using temperature as a “fine-tune” parameter [24]. Since the critical point for polymers is usually found by tedious trial and error experiments, prediction using this method constitutes a significant saving in time and resources for the chromatographer.

4.5. Separation of a PS/PMMA copolymer according to its styrene fraction

Characterisation of complex macromolecules such as copolymers can be challenging, because retention in this case can be based on both molecular mass and chemical composition. Without appropriate standards, it is difficult to deconvolute any single distribution from the resulting chromatogram. In this

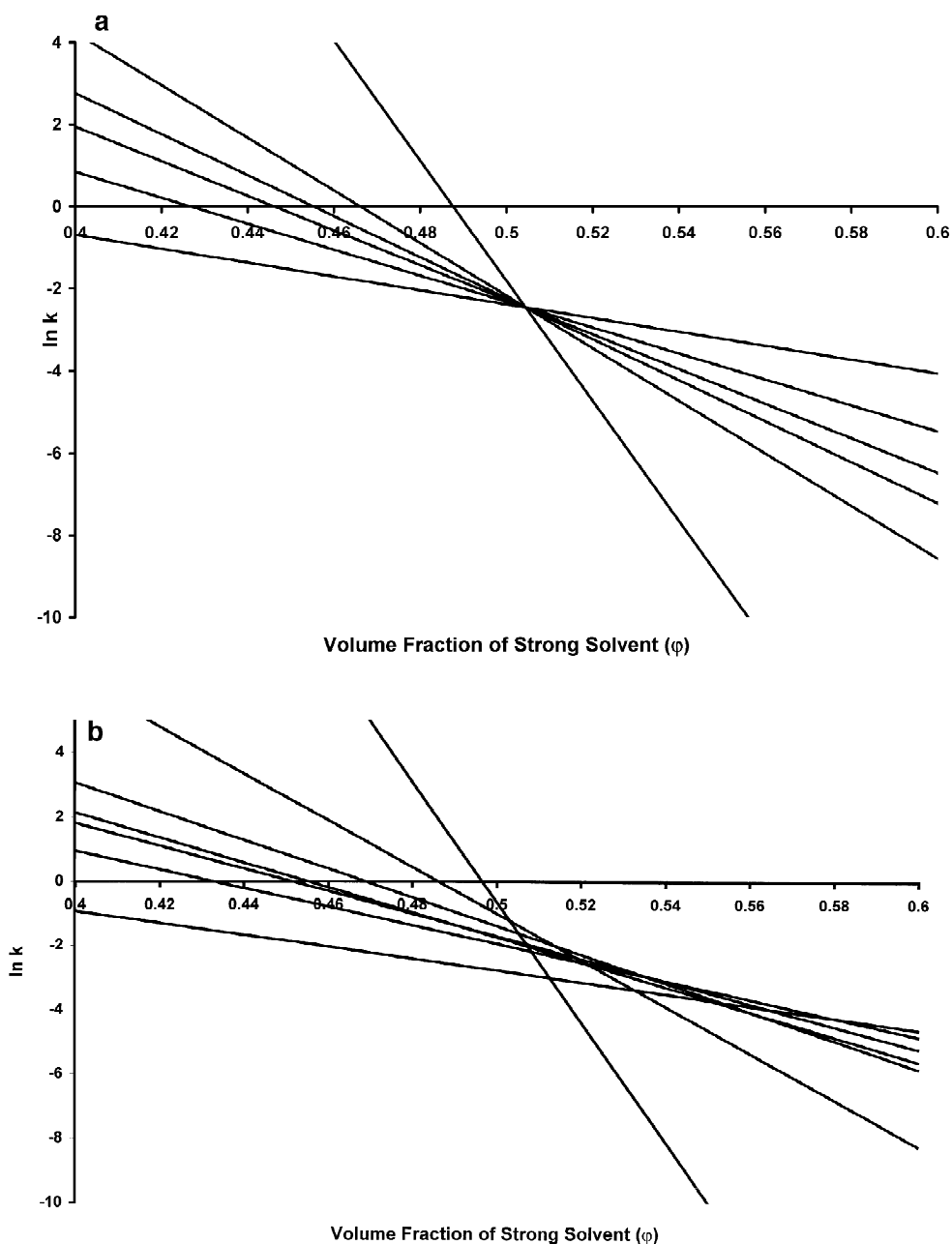


Fig. 5. (a) Simulated retention factor (k') vs. mobile-phase composition (ϕ) for a series of polystyrene homopolymers. (b) Experimental retention factor (k') vs. mobile-phase composition (ϕ) for a series of polystyrene homopolymers (constructed from the calculated values of S and $\ln k_0$).

case, it can help to model the retention behaviour of the homopolymeric standards, so that their contribution to retention can be understood and therefore

controlled. For the characterisation of a PS/PMMA copolymer, the retention behaviour of PS and PMMA standards on a reversed-phase column and in

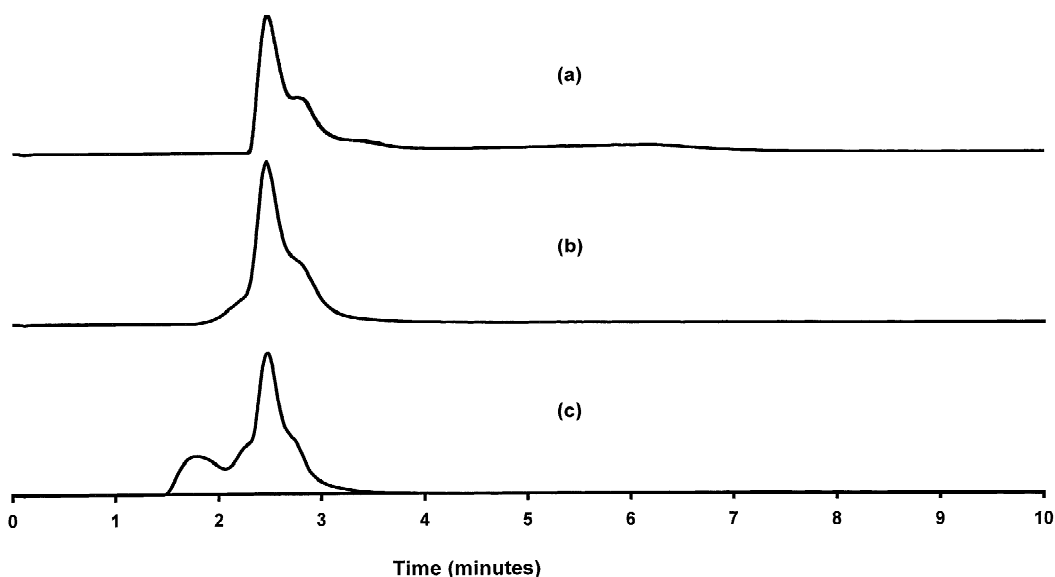


Fig. 6. Stacked chromatograms around the predicted critical point of polystyrene. The transition from a primarily enthalpic to a primarily entropic based retention mechanism is seen as the mobile-phase strength increases from 49% THF (Fig. 4a) to 50% THF (Fig. 4b) to 50% THF (Fig. 4c).

an ACN–THF mobile phase was modelled by calculating the relevant parameters for the RP model and establishing the correlation between the parameters and molecular mass. The S vs. molecular mass and $\ln k_0$ vs. molecular mass correlations for both PS and PMMA are shown in Fig. 7a and b. The S vs. molecular mass correlation indicates that S values are slightly lower for PMMA than for PS (at equivalent masses), indicating a slightly more gradual decrease in the retention factor over a given change in solvent strength. The slope of the $\ln k_0$ vs. molecular mass correlation for PMMA is significantly lower than that of PS. In practical terms, the correlations show that PMMA is less retained in this system than PS. By choosing the appropriate mobile-phase conditions, the separation can be optimised so that only the PS portion of the macromolecule interacts with the stationary phase. Once the solvent strength has reached a certain (critical) composition, PMMA will no longer significantly contribute to retention. The influence of molecular mass (of both PMMA and PS) on retention can also be understood (and therefore controlled). The calibration curves resulting from a mobile-phase gradient from 10 to 60% THF in ACN in 45 min are shown in Fig. 8.

The lower trace is calculated for a broad high-molecular-mass PMMA homopolymer (PDI=2, $M_p=350\,000$ u) and the upper trace corresponds to the equivalent (in terms of MMD) PS homopolymer. The curves show the molecular-mass (in)dependence of the retention of the two homopolymers under these conditions. In the lower molecular-mass region, some influence of the molecular mass of PS on retention remains, however, once the mass increases past $\sim 70\,000$ u, this effect becomes negligible. Under these conditions the separation should only be dependent on the fraction of polystyrene in the copolymer. This type of molecular-mass independent separation has previously been termed pseudo-critical chromatography [25].

4.6. Predicting the retention of copolymers

By applying the reversed-phase model for retention and assuming linear correlations between S and $\ln k_0$ and between $\ln k_0$ and molecular mass, an expression can be derived for the retention behaviour of a copolymer in terms of the contribution to retention of each of the monomeric units present.

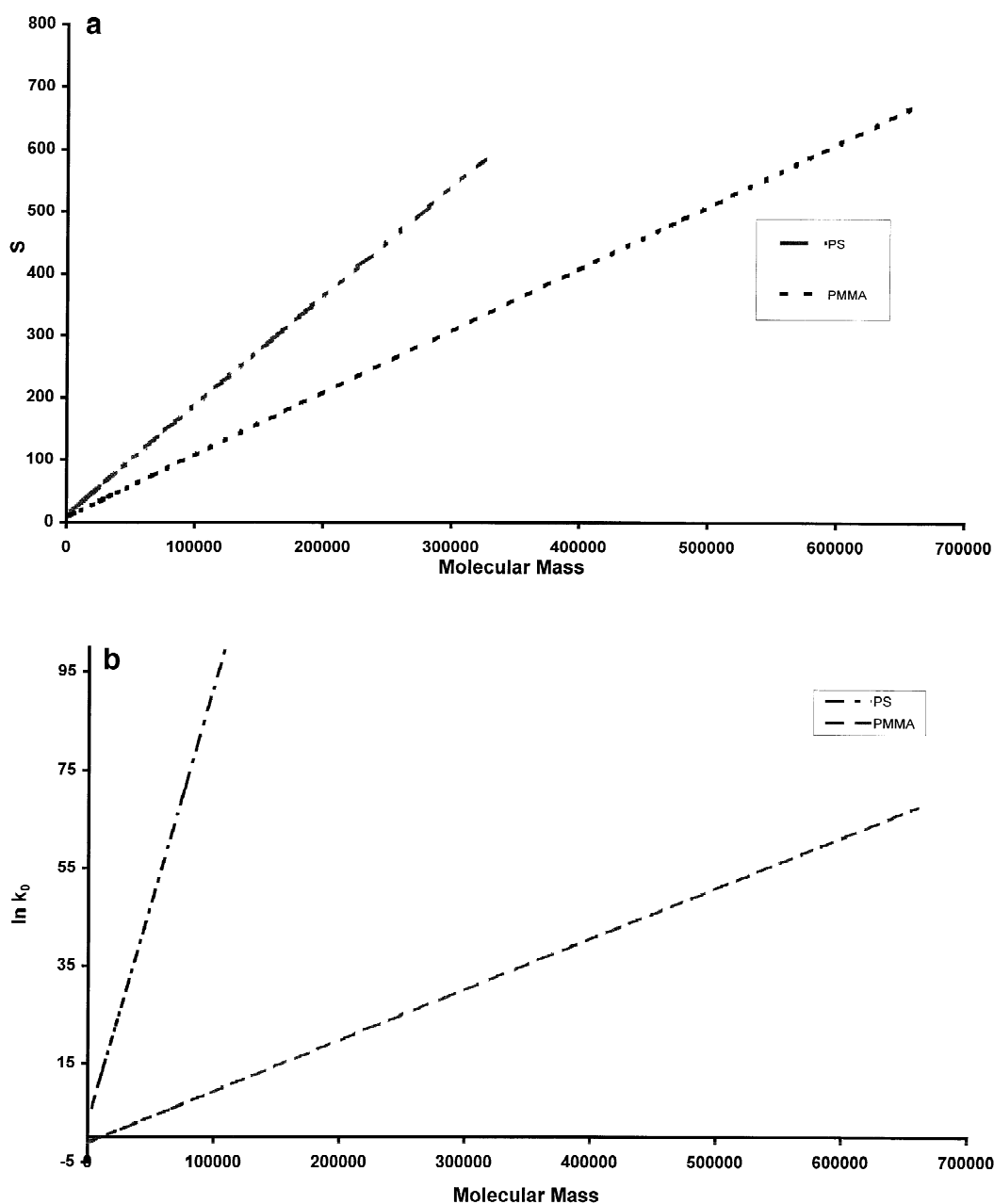


Fig. 7. (a) S vs. molecular mass correlations for polystyrene and polymethylmethacrylate. (b) $\ln k_0$ vs. molecular mass correlations for polystyrene and polymethylmethacrylate.

$$\varphi_{\text{crit}} = \frac{b_I(1 - X_{II}) + b_{II}X_{II}}{q_I b_I(1 - X_{II}) + q_{II} b_{II} X_{II}} \quad (8)$$

where b_I and b_{II} are the slopes of the $\ln k_0$ vs. molecular mass correlation for monomer I and

monomer II, q_I and q_{II} are the slopes of the S vs. $\ln k_0$ correlations of monomer I and monomer II and X_{II} is the mass fraction of monomeric unit II in the copolymer. The derivation of this expression is given in Appendix A.

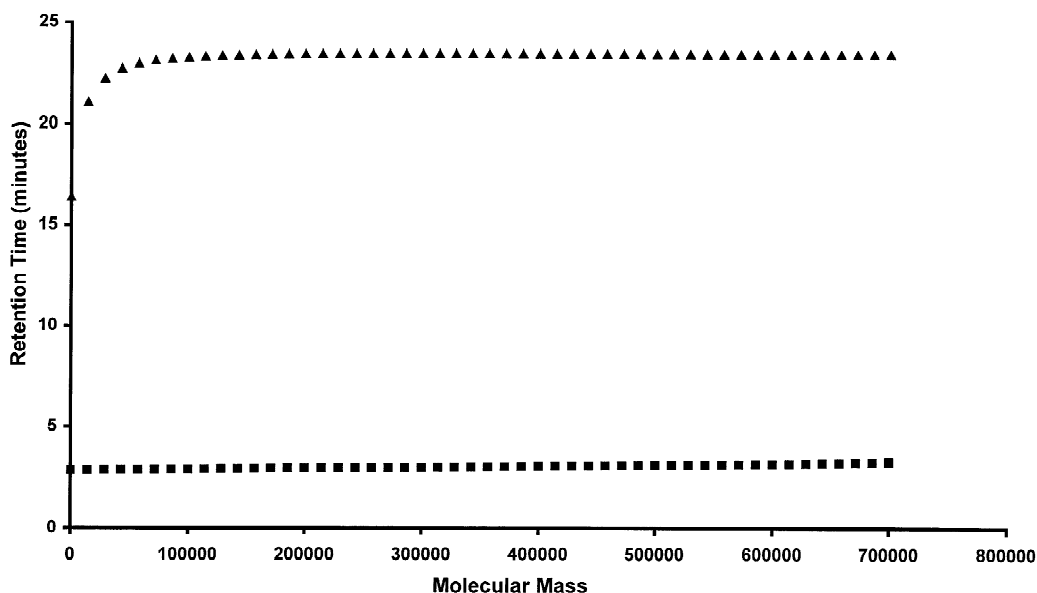


Fig. 8. Simulated calibration curves for high-dispersity polystyrene and polymethylmethacrylate; ■ = PMMA, ▲ = PS. Gradient conditions: 10 to 60% THF in ACN over 45 min.

The critical (i.e. molecular-mass independent) mobile-phase composition of a copolymer is calculated as a function of the mass fraction of one of its monomeric units. The expression assumes that retention follows the RP-model and that there are linear correlations between both S and $\ln k_0$ and molecular mass. The critical mobile-phase composition for a copolymer of a given chemical composition then becomes a function of the slope of the two correlations (q and b). Since polymers of sufficiently high molecular mass will be eluted (from a gradient) at their critical mobile-phase composition, a plot of volume-fraction of strong solvent at the point of elution vs. fraction monomer should follow the predicted trend.

The elution behaviour of four narrow CCD copolymers of varying chemical compositions was

studied. The polymers were synthesised in a controlled polymerisation and have a narrow and well-defined chemical-composition distribution. The chemical composition (found from elemental analysis) and the molecular mass (relative to polystyrene) and polydispersity are given in Table 3. Since the molecular mass of the copolymeric standards is high, S values for the samples will be high enough to cause a sudden transition between fully retained and fully unretained around the critical mobile-phase composition. It is therefore reasonable to assume that the copolymers will be eluted at their critical point.

An overlay of the predicted elution pattern of a PS/PMMA copolymer (based solely on correlations calculated for the homopolymers) and the actual elution pattern for the standard copolymers is shown in Fig. 9. The volume fraction of strong solvent in

Table 3
Calculated molecular masses and fraction styrene in the standard PS/PMMA copolymers

PS/PMMA Standard copolymers	M_n (g/mol)	M_w (g/mol)	Fraction styrene
1	185 000	400 000	0.29
2	130 000	280 000	0.43
3	120 000	275 000	0.59
4	110 000	250st000	0.76

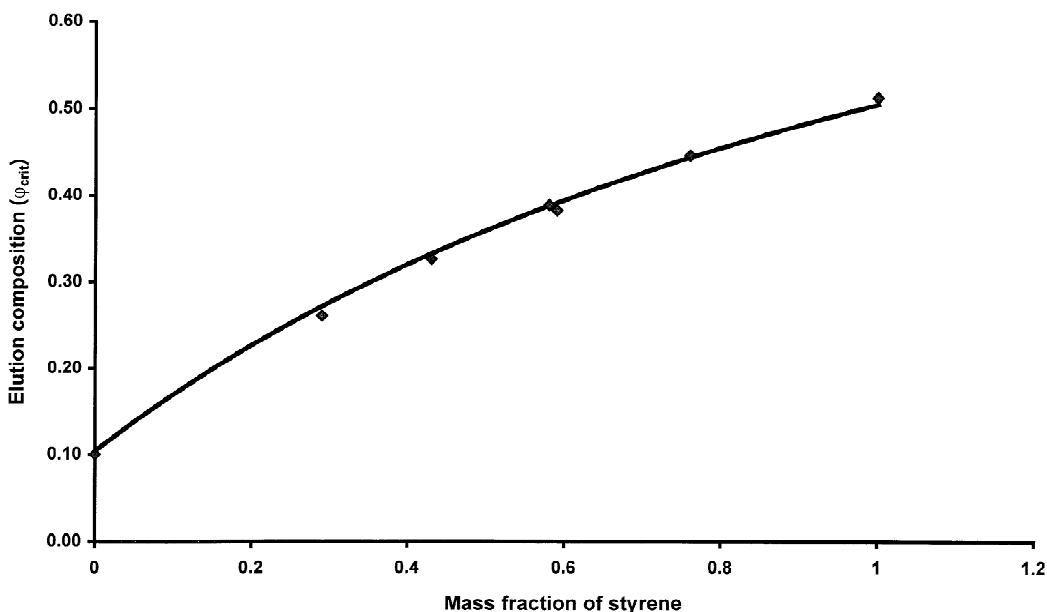


Fig. 9. Comparison of the predicted elution behaviour of PS/PMMA copolymers with the experimental results.

the mobile-phase at the point of elution (φ) was calculated from the experimental retention time, by subtracting the time delays due to t_m and t_D and then adding the calculated volume fraction increase of the strong solvent over that time to its concentration at the start of the gradient. Excellent agreement between the predicted and the experimental elution compositions was seen.

5. Conclusions

Retention models used to describe the behaviour of small molecules in LC can also be applied to macromolecules. By applying an appropriate model to a polymer separation, the chromatographer can better understand and control the separation of a given sample. The critical mobile-phase composition of a homopolymer can easily be calculated using the model. The retention behaviour of high-molecular-mass copolymers can be predicted solely on the basis of the retention of the component homopolymers.

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Appendix A

It is assumed that:

The reversed-phase model applies i.e.

$$\ln k = \ln k_0 - S\varphi \quad (\text{A.1})$$

There is a linear correlation between S and $\ln k_0$, i.e.

$$S = p + q \ln k_0 \quad (\text{A.2})$$

There is a linear correlation between $\ln k_0$ and molecular mass (MM), i.e. $\ln k_0 = a + b(MM)$ (A.3)

The molecular mass is high enough that there is a sudden transition between fully retained and fully eluted states (around the critical point).

Incorporating Eqs. (A.2) and (A.3) into Eq. (A.1) gives the following expression for $\ln k$:

$$\ln k = a + bMM - p\varphi - qa\varphi - qb\varphi MM$$

For a copolymer consisting of two different monomeric units (I and II), the retention of both units can be added to give the overall retention of the copolymer:

$$\ln k_{\text{copolymer}} = a_{\text{I}} + a_{\text{II}} + (b_{\text{I}}\xi + b_{\text{II}})MM_{\text{II}} \\ - (p_{\text{I}} + p_{\text{II}})\varphi - (q_{\text{I}}a_{\text{I}} + q_{\text{II}}a_{\text{II}})\varphi \\ - (q_{\text{I}}b_{\text{I}}\xi + q_{\text{II}}b_{\text{II}})\varphi MM_{\text{II}}$$

where

$$\xi = \frac{MM_{\text{I}}}{MM_{\text{II}}}$$

At the critical point, molecular mass will no longer influence retention and so: $(b_{\text{I}}\xi + b_{\text{II}} - (q_{\text{I}}b_{\text{I}}\xi + q_{\text{II}}b_{\text{II}})\varphi_{\text{crit}})MM_{\text{II}} = 0$ and therefore:

$$\varphi_{\text{crit}} = \frac{b_{\text{I}}\xi + b_{\text{II}}}{q_{\text{I}}b_{\text{I}}\xi + q_{\text{II}}b_{\text{II}}}$$

Expressing the molecular mass of each monomeric unit as a fraction of the mass of the copolymer, the mass fraction of M_{II} will be:

$$\frac{M_{\text{II}}}{M_{\text{II}}\xi + M_{\text{I}}} = X_{\text{II}} = \frac{1}{1 + \xi}$$

and so the critical mobile-phase composition of the copolymer will be:

$$\varphi_{\text{crit}} = \frac{b_{\text{I}}(1 - X_{\text{II}}) + b_{\text{II}}X_{\text{II}}}{q_{\text{I}}b_{\text{I}}(1 - X_{\text{II}}) + q_{\text{II}}b_{\text{II}}X_{\text{II}}}$$

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