Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 982 (2002) 55–68
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Breakthrough of polymers in interactive liquid chromatography

Xulin Jiang[a,b,*], Aschwin van der Horst[a], Peter J. Schoenmakers[a]

[a]*Polymer-Analysis Group, Department of Chemical Engineering, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands*

[b]*Dutch Polymer Institute, P.O. Box 902, 5600 AX Eindhoven, The Netherlands*

Received 16 May 2002; received in revised form 9 September 2002; accepted 16 September 2002

Abstract

Two separate peaks are observed for narrow polymer standards in both isocratic and gradient HPLC. One peak appears around the solvent front (the ''solvent-plug peak'' or ''breakthrough peak''), whereas the second peak is retained significantly—or even highly. Although the effect has been observed many times before, it has never been rigorously explained. In this paper we provide a detailed explanation for the breakthrough peak. The two completely separate peaks are demonstrated not to represent to different fractions of the sample (e.g., the low- and high-molecular-mass parts of the distribution). Both peaks are representative of the entire polymeric sample for narrow polymer standard. Because the amount of the polymer in the breakthrough peak may vary, the quantitative analysis of the polymers by LC is jeopardized. The effects of the sample solvent, the (initial) mobile phase composition, the injection volume, the injected sample concentration, the column temperature, and the analyte structure and molecular mass on the breakthrough peak were investigated in LC experiments involving standards of polystyrene and poly(methyl methacrylate). Three necessary and sufficient conditions are suggested for the breakthrough phenomenon to be observed. Recommendations to avoid the breakthrough phenomenon are given, culminating in a structured method for selecting the best possible sample solvents.

Keywords: Solvent-plug peak; Breakthrough; Polystryene; Poly(methyl methacrylate)

1. Introduction

Synthetic polymers present many unique separation challenges, because they consist of a distribution of structurally different chains [1]. Due to the solution properties of macromolecules, the liquid chromatography (LC) of specific synthetic polymers is restricted to a narrow selection of solvents. The huge dependence of polymer retention on the mobile phase elution strength often necessitates the use of gradient elution. A typical gradient runs from a non-solvent (or a weak solvent) to a strong solvent. Therefore the solvent in which the sample is dissolved and injected is usually stronger than the mobile phase that surrounds it. This is equally true when the polymers are eluted isocratically [2]. Armstrong and Bui [3], Larmann et al. [4], Glöckner [5], Lochmüller and McGranaghan [6], Schultz and Engelhardt [7], Shalliker et al. [8], Northrop et al. [9], and Philipsen et al. [10], have all observed that single, narrow polystyrene standards of high molecu-

*Corresponding author. Department of Chemical Engineering, University of Amsterdam, Nieuwe Achtergracht 166, 1018 WV Amsterdam, The Netherlands. Fax: +31-20-525-5604.
E-mail address: xljiang@science.uva.nl (X. Jiang).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)01483-8

lar mass eluted as multiple peaks in a binary mobile phase. This ''breakthrough'', ''solvent-plug'' or ''sweep-through'' effect may jeopardize the qualitative and quantitative analysis of polymeric samples. Although the above authors have identified the problem, the significance of the sample solvent is still frequently overlooked, both in scientific literature and, arguably, in common practice.

Lochmüller and McGranaghan [6] reported that for the isocratic elution of polystyrenes in a binary mobile phase mixture of tetrahydrofuran (THF) and water, a fraction of the polymer eluted with the solvent front. They suggested that a mixing chamber between the injector and the column would improve the mixing of the sample with the mobile phase, thus eliminating this anomalous behaviour. Shalliker et al. [11,12] observed double peaks in gradient-elution chromatograms of high-molecular-mass polystyrenes using mobile phases consisting of dichloromethane and methanol. However, when they used a dichloromethane–acetonitrile solvent system, they observed a single, symmetrical peak. They concluded that the amount of polymer eluting together with the sample solvent was considerably reduced when smaller packing particles were used. However, these observations are difficult to interpret, because many parameters (column length, particle size, pore size) were changed simultaneously [11]. Multiple peaks can also be seen in chromatograms of copolymers published in the literature [13–20]. Augenstein and Stickler [17] observed that an additional peak, coinciding with the break-through of the solvent in the case of UV or refractive index (RI) detection, could also be detected with evaporative light-scattering detection (ELSD). Therefore, this additional peak was thought to be due to the polymer sample. They suggested that the breakthrough peak (the additional peak) was due to incomplete precipitation of the injected polymer [17].

In our experiments, the gradient elution of low-molecular-mass (defined as the peak molecular weight, $M_p = 2990$) and high-molecular-mass (M_p 34 500) poly(methyl methacrylate) (PMMA) standards with low polydispersity (PD$<$1.1) produced two separated peaks (with ELSD). A precipitation phenomenon could not have played a role in this case, because the PMMA could be dissolved in the initial mobile phase (consisting of 2% methanol in toluene).

The crucial question is whether the polymer is separated into two fractions that differ in (for example) their molecular masses. If the two fractions differ, the fraction that is eluted at the (physically) correct retention time is not representative of the entire polymeric sample [13]. In that case many incorrect conclusions on the composition and distribution(s) of synthetic polymers may be drawn from LC experiments. If the fractions are both representative of the entire sample, then quantitative analyses will be jeopardized. In the latter case, we would also be left to explain the mechanisms that give rise to the two vastly separated peaks.

We set out to establish a sound explanation for the breakthrough effect, to establish which parameters have a significant effect on this phenomenon and, ultimately, to determine how it can be avoided or overcome. To this end we investigated the sample solvent, the (initial) mobile phase composition, the injection volume, the injected sample concentration, and the effects of the column temperature, of the analyte structure and of the molecular mass on the breakthrough phenomenon.

2. Experimental

2.1. Chemicals

Non-stabilized THF, HPLC grade, was obtained from Biosolve (Valkenswaard, The Netherlands). Toluene and *n*-hexane (both glass-distilled grade), and methanol (HPLC grade), were from Rathburn (Walkerburn, UK). THF was used as obtained (without further purification by, e.g., distillation). Water for use in HPLC was doubly distilled in the laboratory. The polystyrene (PS) and PMMA standards were obtained from Polymer Labs. (Church Stretton, UK). The molecular mass values were supplied by the manufacturer. The PD of all standards was lower than 1.10.

2.2. Equipment

A Waters (Milford, MA, USA) 2690 Alliance liquid chromatography system was used to perform the isocratic LC experiments on PS. This HPLC

instrument contained a built-in auto-injector with a sample loop allowing injection of variable sample volumes, and was equipped with a Waters 996 PDA (photodiode-array detection) system and a Sedex 55 ELSD system (temperature 62 °C, N_2 pressure 2.2 bar). THF was flushed with helium in order to prevent the formation of explosive peroxides. The mobile phase was prepared in-situ using the solvent-mixing capability of the instrument. The data collection and the data analysis were handled by Waters Millennium 3.2 software. The columns used (150 mm×4.6 mm I.D.) were packed in the laboratory with Hypersil silica (3 μm particles, 100 Å pore size; Shandon, Runcorn, UK). The columns used to measure the molecular mass in size-exclusion chromatography (SEC) were three PLgel columns (300 mm×7.6 mm I.D.) with pore sizes of 100, 100, 10^5 Å, respectively.

The HPLC system used in the gradient LC of PMMA standards consisted of two Gynkotek (Germering, Munich, Germany) Model 300C high-precision pumps and a Rheodyne 7010 injector (Berkeley, CA, USA). The detectors were a variable-wavelength UV–Vis spectrometer (Spectroflow 757, Applied Biosystems, Ramsey, NJ, USA) set at 254 nm and a Varex ELSD II A (Burtonsville, MD, USA). A DuPont Zorbax C_8 column (250 mm×4.6 mm I.D., 5 μm particles, 100 Å pore size; Rockland Technologies, PA, USA) and a laboratory-packed column (150 mm×4.6 mm I.D., 3 μm Hypersil silica particles, 100 Å pore size) were used. The columns were contained in a (Millipore) Waters temperature-control module.

3. Results

Breakthrough peaks have been observed in many different systems [2–22]. Here we chose to study a column/eluent combination that we used for the separation of blends of PMMA and poly(hydroxyethyl methacrylate) (PHEMA) and their copolymers [21]. ELSD is most appropriate for this purpose. This system showed clear breakthrough peaks, allowing the phenomenon to be studied thoroughly. To study quantitative aspects of the breakthrough phenomena, some experiments involving UV detection and PS as the sample are also described.

3.1. Sample solvent

A series of reversed-phase gradient-elution chromatograms were recorded for a PMMA standard (M_p 34 500, PD 1.04), dissolved in mixtures of THF with methanol. The mobile phase composition was programmed from 39.5 to 100% THF in the weak solvent A (water–methanol, 25:75) in 20 min and the C_8 column was used. The flow-rate was 0.6 ml/min. All chromatograms obtained with binary THF–methanol mixtures as sample solvents showed two distinctly different peaks (top four traces in Fig. 1). When the sample solvent was a weak eluent, such as THF–solvent A (50:50) (viz. THF–methanol–water, 50:37.5:12.5; bottom trace), only one peak was observed for PMMA. The peak eluting with a retention time of about 9.5 min was observed with all sample solvents. We believe that this peak represents the true chromatographic retention time of the PMMA standard in the present system (see discussion in Section 4.2), which is why we refer to it as the ''real'' peak. The other peak was approximately unretained. Following Philipsen et al. [10] we refer to this peak as the ''breakthrough peak''. Other authors have observed similar unretained signals and

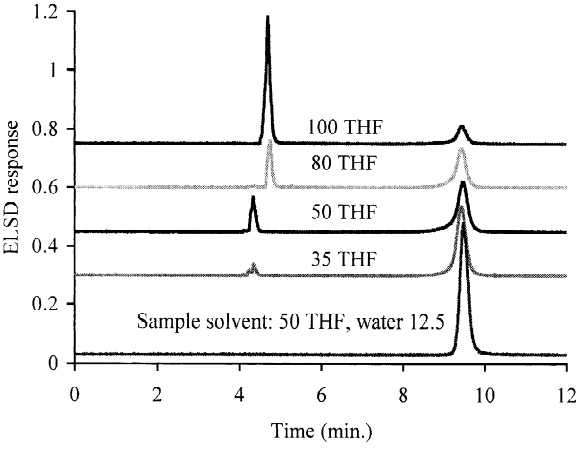

Fig. 1. Chromatograms of PMMA (M_p 34 500, PD 1.04) dissolved in mixtures of methanol, THF (and water) indicated in the figure at 25 °C [from top to bottom sample solvent: pure THF, THF–methanol (80:20), THF–methanol (50:50), THF–methanol (35:35), THF–methanol–water (50:37.5:12.5)]. Detection, ELSD; flow-rate, 0.6 ml/min; injected sample, 20 μl of 1 mg/ml; DuPont Zorbax C_8 column (250 mm×4.6 mm I.D., 5 μm particles, 100 Å pore size); gradient from 39.5% of solvent B (THF) in solvent A (water–methanol, 25:75) to 100% of solvent B (THF) in 20 min.

referred to them as solvent-plug [22] or swept-through peaks [17]. As can be seen from Fig. 1, the size of the breakthrough peak decreases when the strength of the sample solvent decreases (chromatograms from top to bottom). At the same time, the size of the ''real'' (retained) peak increases. Using mixtures of THF and methanol as sample solvents, the occurrence of a breakthrough peak cannot be avoided. The PMMA standards do not dissolve in pure methanol. In the weakest possible injection solvent (about THF–methanol, 35:65) a breakthrough peak is still observed (Fig. 1). A breakthrough peak is not observed when a ternary mixture of THF, methanol, and water is used as the sample solvent. This mixture is a solvent for the PMMA standard, but it is a weaker eluent than the THF–methanol mixtures. We will discuss later (Section 4.2) how a solvent can be selected in order to avoid the breakthrough peak.

As seen from Fig. 1, when the sample solvent consisted of 50:50 or 35:65 THF–methanol, the breakthrough peak elutes somewhat earlier than with pure THF as sample solvent. This indicates that with pure THF as the eluent as is the case in the sample–solvent zone, a greater amount of THF is adsorbed on the stationary phase. Different sample solvents as eluents are known to show different retention times (hold-up time for the column) in reversed-phase liquid chromatography (RPLC) [23]. In normal-phase liquid chromatography (NPLC), we also observed a significant effect of the sample solvent on the occurrence, the size, and the elution time of the breakthrough peak (see discussion in Section 4.2).

3.2. Initial mobile phase composition

To investigate the influence of the initial mobile phase composition on the breakthrough effect, we recorded a series of chromatograms using different initial percentages of the strong solvent B (THF) in the weak solvent A (water–methanol, 25:75). The same PMMA standard (dissolved in THF) and the same reversed-phase gradient-elution system were used as in Fig. 1. The gradient then ran from the indicated composition to 100% of THF in 20 min. As seen in Fig. 2, we observed only one peak when the initial mobile phase contained 35% of THF (65% of mixture A). When the initial mobile phase contained 38 to 40% of THF, we observed two completely separated peaks for PMMA standards. When we subsequently collected these two peaks, evaporated them to dryness, re-dissolved the residues in THF, and injected the resulting solutions in an SEC system, identical molecular-size distributions were found for the material contained in the two peaks (see Section 4.1).

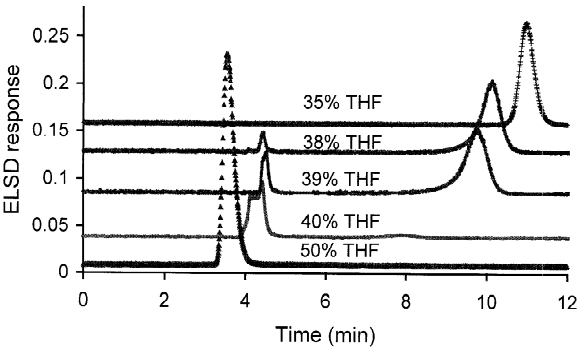

Fig. 2. Chromatograms of PMMA (M_p 34 500, PD 1.04) in LC gradients with different initial compositions at 25 °C. Detection, ELSD; flow-rate, 0.6 ml/min; injected sample, 20 μl of 2 mg/ml PMMA solutions in THF; DuPont Zorbax C_8 column (250 mm× 4.6 mm I.D., 5 μm particles, 100 Å pore size); gradient from initial percentages of solvent B (THF) in solvent A (water–methanol, 25:75) as shown in the figure to 100% of solvent B (THF) in 20 min.

As seen in Fig. 2, the retention time of the second peak (retention peak) increased with decreasing THF composition in the initial mobile phase. This is due to the change in the gradient slope. The lower the THF content in the initial mobile phase, the longer it takes until the mobile phase composition reaches the elution composition of the PMMA standard. The location of the breakthrough peak is also affected by the initial composition. If the initial mobile phase is very strong (above the critical composition, e.g., 50% THF), then the polymer sample may be eluted in the size-exclusion mode before the column dead time, t_0. In this case, only one (partially) excluded peak can be observed.

3.3. Injection volume

To investigate the effect of the injection volume on the breakthrough phenomenon, we recorded a series of chromatograms for the same PMMA stan-

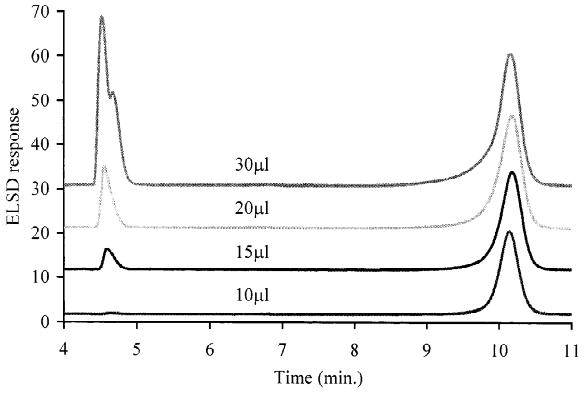

Fig. 3. Effect of the injection volume on the breakthrough phenomenon for PMMA (M_p 34 500, PD 1.04) in reversed-phase gradient-elution LC with THF as the sample solvent at 25 °C. Detection, ELSD; flow-rate, 0.6 ml/min; 1 mg/ml of sample dissolved in THF; DuPont Zorbax C_8 column (250 mm×4.6 mm I.D., 5 μm particles, 100 Å pore size); gradient from 38% of solvent B (THF) in solvent A (water–methanol, 25:75) to 100% of solvent B (THF) in 20 min.

dard as used in Figs. 1 and 2 using one of the reversed-phase gradients also used in Fig. 2 (gradient from 38 to 100% of solvent B, THF, in weak solvent A, water–methanol, 25:75, in 20 min; C_8 column; flow-rate 0.6 ml/min). It is clearly seen in Fig. 3 that the size of the breakthrough peak increased sharply when the injection volume was increased from 10 to 30 μl. If the injection volume is large, the breakthrough peak is asymmetrical, with a sharp front and a slower tail. When the injection volume becomes very large, we even observe double peaks around the breakthrough volume.

Because the PMMA sample cannot be detected with UV detection with THF in the mobile phase, a PS standard was used to quantitatively investigate the effects of the injection volume and of the sample concentration on the breakthrough phenomenon. To minimize the effect of mobile phase UV absorption, isocratic elution was used instead of gradient elution. We recorded a series of isocratic chromatograms of PS standards (M_p 11 600, PD 1.03) with THF as the sample solvent and 25% THF in hexane as the mobile phase. The laboratory-packed silica column was used and the flow-rate was 1.0 ml/min. The injection volume was varied from 12 to 40 μl. The sample concentration was constant. As shown in Fig. 4, we observed two peaks in every chromatogram. One was the breakthrough peak, eluting near t_0; the other was the real retention peak, which was always

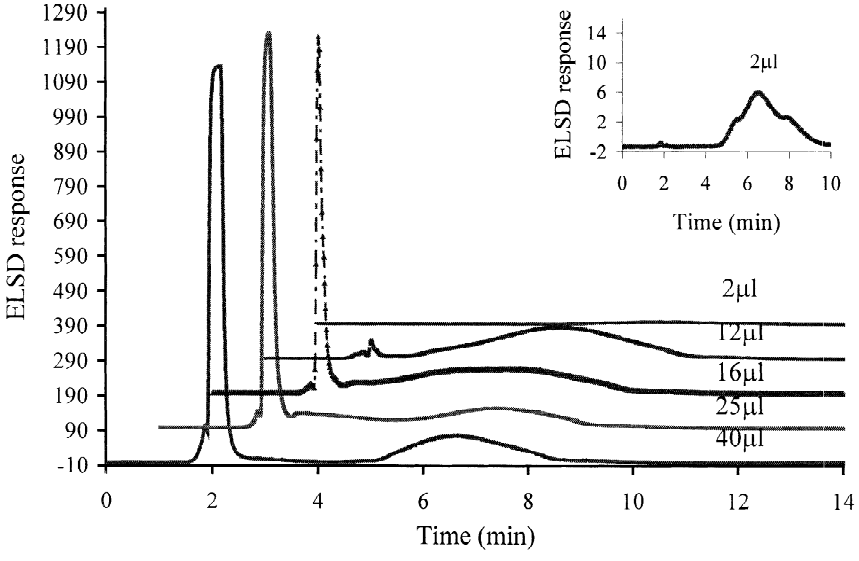

Fig. 4. Effect of the injection volume on the breakthrough peak for the isocratic elution of a PS (M_p 11 600, PD 1.03) standard on a laboratory-packed column (150 mm×4.6 mm I.D., 3 μm Hypersil silica particles, 100 Å pore size) at 25 °C. Detection, ELSD; flow-rate, 1 ml/min; mobile phase, 25% THF in hexane; constant sample concentration of 5 mg/ml dissolved in THF. The inserted part shows the enlarged 2-μl-injection-volume chromatogram. (Note: successive chromatograms are shifted by 1 min and 100 ELSD units).

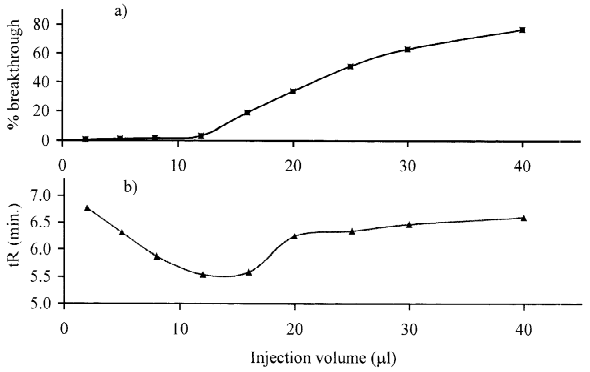

Fig. 5. (a) The relative area of the breakthrough peak and (b) the retention time of the ''real'' retention peak detected by UV at 254 nm for different injection volumes. LC conditions and PS standard used as in Fig. 4.

very broad under isocratic conditions. The online UV spectra recorded for these two peaks were found to be almost identical and appeared to indicate the dominant presence of PS (obvious absorption bands at 261.5 nm in the UV spectrum). It is also apparent from Fig. 4 that the relative size of the breakthrough peak increased as the injection volume increased. As can be seen in Fig. 5a, when the injected volume exceeded about 12 μl, the relative area of the breakthrough peak increased sharply. At the same time, the observed elution time for the real retained peak showed a distinct minimum (Fig. 5b). This can be explained by a gradual diminishing of the breakthrough effect at the top of the column (cf. Fig. 8 below) and the real retention peaks are broadened.

To investigate whether the sample-size effect can be eliminated, we injected the same amount (40 μg) of PS in different volumes and concentrations (Table 1). The results again showed that the size of the breakthrough peak increased as the injection volume increased. The retention time of the second analyte peak again showed a minimum at intermediate injection volumes. It should be noted that the observed peaks were very broad in all these isocratic chromatograms. Because ELSD is not convenient for quantitative analysis, the quantitative results in Table 1 are based on the UV response at 254 nm (UV254). However, the ELSD response is shown as an indication of the presence of polymer in the peak. There is some variation in the observed total UV areas in Table 1. In part this is related to variations in the injected volume and to inaccuracies in the (approximate) concentrations specified. Also, these data refer to isocratic experiments, where the real-retention peaks are very broad.

3.4. Sample concentration

In order to investigate the effect of the polymer concentration injected on the breakthrough peak, we measured the ratio of the areas of the two peaks for a series of injections of equal volume, but with different concentrations, using the same PS standard and same conditions as in Figs. 4 and 5 and Table 1. Table 2 shows that the fractional area of the breakthrough peak decreases with increasing sample concentration, but that this effect is small until very high concentrations (20 mg/ml) are reached. It should be noted that the observed peaks are very broad in all these cases.

3.5. Column temperature

The effect of the temperature was studied from a series of reversed-phase gradient-elution chromato-

Table 1
Effect of the injection volume on the breakthrough peak for the isocratic elution of a PS (M_p 11 600, PD 1.03) standard

	Injection volume (μl)				
	40	20	8	4	2
Polymer concentration (mg/ml)	1	2	5	10	20
Total area (UV254)	1361	1444	1659	1545	2058
First peak area (%) (UV254)	74	65	2	1	1
Time for first peak (min) (UV254)	1.9	1.9	1.9	1.9	2.0
Time for second peak (min) (UV254)	7.1	6.8	6.0	6.6	6.8
First peak area (%) (ELSD)	94	93	0.2	0.2	0.2

Conditions as in Fig. 4, except the polymer concentration and injection volume as indicated.

Table 2
Absolute and relative areas (arbitrary units) of the breakthrough peaks and the real retained peaks for different injected concentrations of a PS (M_p 11 600, PD 1.03) standard

	Polymer concentration (mg/ml)				
	1	2	5	10	20
Total area (UV254)	851	1444	3563	6621	13 887
First peak area (%) (UV254)	67	65	65	63	14
Time for first peak (min) (UV254)	1.9	1.9	2.0	2.0	2.0
Time for second peak (min) (UV254)	6.9	6.8	6.6	6.2	4.9
First peak area (%) (ELSD)	97	93	84	74	8

Isocratic conditions and column as in Fig. 4. Sample volume 20 μl for all injections.

grams recorded at different column temperatures using the same PMMA standard as in Figs. 1 and 2. The same C_8 column was used, but the flow-rate was slightly lower (0.5 ml/min). The gradient ran from 30 to 100% of solvent B (THF) in solvent A (methanol–water, 75:25) in 20 min. As shown in Fig. 6, the retention time of the real PMMA peak decreased with increasing column temperature. Concomitantly, the column temperature was seen to influence the size of the breakthrough peak. At room temperature (25 °C), only one peak was observed. However, when the column temperature was increased, the magnitude of the observed breakthrough peak increased, while the height of the second peak (''real'' retention peak) decreased.

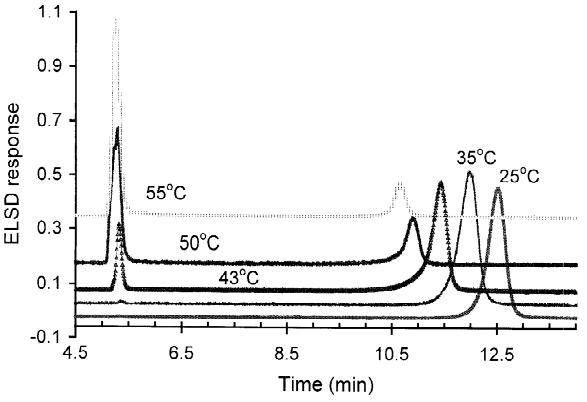

Fig. 6. Effect of the temperature on the retention time of PMMA (M_p 34 500, PD 1.04) in gradient RPLC. Injection volume, 20 μl; detection, ELSD; flow-rate, 0.5 ml/min; sample concentration, 5 mg/ml in THF; DuPont Zorbax C_8 column (250 mm×4.6 mm I.D., 5 μm particles, 100 Å pore size); gradient from 30 to 100% of solvent B (THF) in solvent A (water–methanol, 25:75) in 20 min.

3.6. Effect of analyte structure and molecular mass

Chromatograms were recorded for a series of PMMA standards of different molecular mass and one PS standard (M_p 11 600, PD 1.03) under the same conditions as those of Fig. 3. As shown in Fig. 7, low-molecular-mass PMMA standards gave rise to a very large breakthrough peak. A PMMA standard with a high molecular mass only yielded a small breakthrough peak, whereas very-high-molecular-mass PMMA standards (≥500 000) and the PS standard (M_p 11 600, PD 1.03) did not give rise to a breakthrough peak (not shown). Thus, the occurrence of a breakthrough peak and the ratio of the areas of the breakthrough peak and the real retention peak depend not only on the LC conditions, but also on the molecular mass and chemical structure of the analyte polymers. As seen in Fig. 7, low-molecular-mass samples (below 34 500) showed relative short

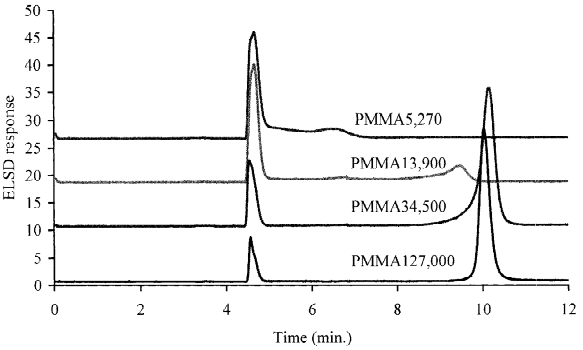

Fig. 7. Effect of PMMA molecular mass indicated in the figure on the breakthrough peak in gradient RPLC with THF as the sample solvent at 25 °C. Injection volume, 20 μl; LC conditions as in Fig. 3.

retention times, which strongly depended on molecular mass. In contrast, PMMA samples with molecular masses of 34 500, 67 000 (not shown), and 127 000, showed practically the same retention time. This is all in agreement with contemporary studies on the retention behaviour of polymers in LC, with the high-molecular-mass standards being eluted at approximately the critical composition for PMMA [24]. The retention time of the 127 000 PMMA sample was less than that of the 34 500 PMMA sample. This may well be due to a size-exclusion effect. In gradient elution, when the polymers are eluted near the critical point, high-molecular-mass polymers may be effected by SEC phenomena and may be eluted somewhat earlier [24]. However, when the molecular mass of PMMA was higher than 480 000, the peaks shifted to somewhat higher retention times (around 11 min). At this point other factors will start playing a role, such as the diameter of the pores (100 Å) in relation to the effective radius of the analyte molecules.

4. Discussion

4.1. Nature of the two peaks

In the chromatographic experiments described above, narrow polymer standards produced two completely separate peaks. The position of the first peak was near t_0, the dead time of the column. As will be explained below (Fig. 8), we expect the polymer-breakthrough to coincide with the beginning of the solvent peak. The actual peak maximum of the breakthrough peak may occur a bit earlier than that of the solvent peak. The exact time of elution of the latter is somewhat dependent on the actual mobile-phase composition [23]. When using UV detection this first peak is often obscured by a strong solvent peak and therefore overlooked. In a detailed quantitative study Glöckner [13] assumed that the peaks eluted around the solvent peak formed part of the polymer sample, but since he only used UV detection he could not prove conclusively that the first peak represented a true polymer. He concluded that it was a ''crucial question'' whether both peaks were representative of the entire polymer, the alternative

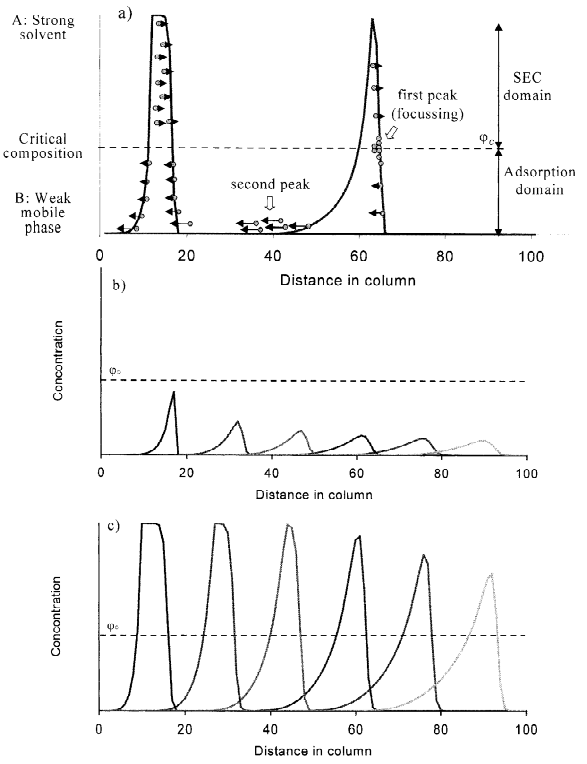

Fig. 8. Simulated representations of the solvent zone in the column. The horizontal dashed line represents the critical composition (φ_c). (a) A focusing of the polymer molecules within the solvent zone at the exact location of the critical point is expected and polymers in the tail of the solvent plug will be retained properly, giving rise to the second (''real retention'') peak. (b) When both the sample solvent and the mobile phase are weaker than the critical composition, we are in the adsorption mode and the polymer will be retained on the column, producing only one peak. (c) The percentage of A solvent in the solvent zone will decrease and the width of the solvent zone will increase along the column.

hypothesis being that the polymer was separated in two different fractions (e.g., one fraction of low molecular mass and one of high molecular mass).

In our experiments we used both UV detection and ELSD. A blank injection of pure solvent did not give rise to a peak on the ELSD trace. If the sample solvent was different from the mobile phase, the UV detector yielded a large solvent peak. The two peaks observed for the PMMA and PS standards were seen with both detectors. The PDA signals for the two separate peaks of PS featured almost identical UV spectra (PMMA does not strongly absorb in the UV

region). We then collected two effluent fractions for the PMMA standard (M_p=34 500, PD=1.04) corresponding to the two peaks. After evaporation of the eluent, the non-volatile analytes were re-dissolved and injected into an SEC system. The result (not shown) indicated that the two fractions obtained from a single standard had identical molecular mass distributions. When we re-injected the collected peaks into the original LC system (C_8 column, see Section 3.3), we again obtained two peaks for each of the fractions under the same gradient conditions. Therefore, we may conclude unequivocally that the two vastly separated peaks do not represent different parts of the (narrowly distributed) sample. Rather, the material responsible for either peak is representative of the entire polymeric sample. It should be noted that the above experiments concerned a very narrow standard. If a (very) broad polymer sample or a copolymer sample is injected, we may easily obtain two separate peaks that represent different parts of the sample (see Section 3.6).

4.2. Explanation for the breakthrough phenomenon

The solvent zone as it may exist in the column after the injection of the sample is shown in Fig. 8. The sample solvent A is assumed to be a strong solvent and the mobile phase B is assumed to be a weak solvent or non-solvent. Mixtures of A and B should have an eluent strength somewhere between that of A and B. At some composition (φ_c) the migration velocity of polymers without strongly interacting end groups is approximately the same as that of the solvent. This is known as the critical composition, where—by definition—retention is independent of molecular mass and all members of a polymeric series co-elute. The horizontal dashed line in Fig. 8 represents the critical composition (φ_c).

If the concentration of strong solvent in the mobile phase is higher than the value of φ_c, we are in the SEC mode and only one peak will be observed. If both the sample solvent and the mobile phase are weaker than the critical composition, we are in the adsorption mode and the polymer will be retained on the column, again producing only one peak (Fig. 8b). The retention factor (k_i) of this peak is related to the thermodynamic partition coefficient of the analyte across the two phases ($K_{c,i}$) by the conventional relationship:

$$k_i = \frac{c_{i,s}}{c_{i,m}} \cdot \frac{V_s}{V_m} = K_{c,i} \cdot \frac{V_s}{V_m}$$

where $c_{i,s}$ is the equilibrium concentration of the analyte in the stationary phase (s), and $c_{i,m}$ is its equilibrium concentration in the mobile phase (m). V_s and V_m are the respective volumes of the two phases in the chromatographic column. Because this second peak obeys the conventional laws of chromatography, we have referred to it as the ''real retention peak'' in the present paper.

Only when the mobile phase is weaker than the critical composition and the sample solvent is stronger is it possible to observe the breakthrough effect.

How do the two peaks come about? As shown in Fig. 8a, when the polymer sample is injected into the column, polymer will be present throughout the sample zone. Those molecules that are ahead of the solvent plug will move more slowly (the mobile phase is a weak eluent), so that they will soon be caught up by the solvent front. Polymers present in the centre of the solvent plug will experience exclusion conditions and they will move faster than the solvent, until they reach the critical point. Thus, we expect a focusing of the polymer molecules within this solvent zone at the exact location of the critical point (Fig. 8a). However, those polymer molecules that are in the tail of the solvent plug, where the composition is below the critical value, will rapidly fall behind the solvent plug and they will be retained properly, giving rise to the second (''real retention'') peak (Fig. 8a).

Due to kinetic diffusion and other dispersive processes, the percentage of A solvent in the solvent zone will decrease and the extent (width) of the solvent zone will increase along the column as shown in Fig. 8b and c. If the solvent plug is dispersed (or the composition at its ''peak'' falls below the critical value) the polymer molecules will be gradually left behind and the two peaks will not be completely separated (cf. the isocratic LC experiments shown in Fig. 4). When the injection volume increases (cf. Section 3.3), the solvent zone stays essentially intact and a long plug of strong solvent

will leave the column, increasing the size of the breakthrough peak. If the polymer concentration in the injected sample increases, a longer zone of strong solvent will be required to push the (viscous) plug of concentrated polymer solution through the column. A greater fraction of the polymer is likely to fall behind and the solvent plug may break up more easily. Therefore, when the polymer concentration is increased without increasing the volume of strong solvent injected, the breakthrough peak becomes smaller relative to the ''real'' retention peak (cf. Table 2).

One tentative explanation for the observations in Fig. 5 may be that at injection volumes smaller than about 12 μl only small patches or droplets of the injection solvent reach the detector without being diluted by the mobile phase to a (maximum) concentration below the critical composition. Large volumes (>about 12 μl) may result in a single, coherent plug of the injection solvent reaching the end of the column. The analyte molecules have a greater chance to ''escape'' from a diffuse (droplet) solvent plug than from a coherent one, resulting in a peak with a retention time in between that of the real retention peak and the breakthrough peak. The excessive peak broadening observed for the analyte peaks especially at intermediate injection volumes (between 10 and 20 μl; see Fig. 4) provides some support for such a model.

Different polymers (e.g., PS, PMMA) have different critical compositions. Thus, it is easy to understand that the breakthrough phenomenon depends on the chemical composition of a copolymer. As to the molecular mass dependence shown in Fig. 7, one of the possible reasons is that the viscosity of the polymer in solvent zone increases with the molecular mass. As was the case when increasing the polymer concentration (Table 2), a longer zone of strong solvent will be required to push the viscous polymer-solution plug through the column. A greater fraction of the polymer is likely to fall behind the solvent plug. Another reason is that the retention factor increases with increasing molecular mass. The greater the retention factor, the lower the tendency for the analyte peak to be distorted when the injection solvent is stronger than the mobile phase [22].

Thus, an essential condition that needs to be fulfilled for a breakthrough peak to be observed is that the solvent plug stays intact until the end of the column and that the eluent strength in the centre of this solvent zone remains stronger than the critical value (Fig. 8c). In summary, the three necessary conditions for separating a single, narrow (or even monodisperse) polymer standard into two vastly separated peaks are:

(1) The mobile phase should be weaker than the critical composition.

(2) The sample solvent should be stronger than the critical composition.

(3) A plug of sample solvent with a concentration higher than the critical composition should remain intact throughout the column.

Although these conditions seem rather restrictive, they are, unfortunately, often met in practice. If we wish to separate polymers according to their chemical composition (ratio of different monomers) or functionality (number and type of end groups or functional groups), we must rely on interactions between the polymer and the stationary and mobile phases, rather than on exclusion effects. Thus, condition 1 will be met. Condition 2 is hard to avoid in practice, because it is very much easier to inject a polymer in a strong solvent than in a weak solvent. Especially under gradient-elution conditions, the initial mobile phase tends to be a weak solvent, which is not suitable for preparing and injecting samples. The third condition is likely to be met with contemporary HPLC systems and columns, both of which have been designed with the intention to minimize zone dispersion. The breakthrough phenomenon may be circumvented by inserting a mixer after the injector [6], but this does lead to additional band broadening. It is, therefore, not an attractive option from the perspective of chromatographic separation.

Nguyen and Berek [25] measured the zones of the injection solvent as they left the column. The sample solvent appeared to always overload the column (250 mm×4.6 mm I.D.) for injection volumes of 10 μl, corresponding to about 10 000 μg of solvent [26]. A zone of pure injection solvent (''solvent plug'') pertains throughout the column. However, the conditions for the solvent zone to remain intact are affected by the packing of the column, the viscosity of the mobile phase, the polarity difference between the components of the injection solvent and the

mobile phase, the kinetic diffusion of the polymer sample, etc.

A very important factor is the location of the critical point (composition). When the column temperature changes, the critical point will change and the breakthrough peak will vary as shown in Fig. 6.

There are several ways in which the occurrence of breakthrough peaks can be avoided. As discussed above, condition 1 cannot be avoided. However, conditions 2 and 3 can be avoided by a judicious choice of conditions and sample solvents. Fundamentally, it is best to use the mobile phase (or an even weaker solvent) as the sample solvent. The injection of turbid samples, containing finely dispersed (rather than dissolved) polymer in weak solvents, reputedly yields good results in polymer LC [27,28]. In any case the weakest possible solvent (whether weaker or stronger than the mobile phase) should be used in the smallest possible volume. It must be noted that the sample solvent is not necessarily a (mixture of) eluent component(s). It is quite possible that a third solvent can be found which (i) dissolves the polymer, (ii) mixes with the (initial) eluent and (iii) is a relatively weak eluent itself. According to the conventional liquid–solid chromatography (LSC) retention model of competitive adsorption [29], desirable sample solvents interact favourably with the polymer, but not with the stationary surface.

For example, in RPLC a relatively weak eluent should have a high polarity parameter (P', Ref. [30], p. 285). Examples include water (10.2), dimethylsulfoxide (DMSO, 7.2), dimethylfomamide (DMF, 6.4), acetonitrile (5.8), and methanol (5.1). Of all these, only DMSO and DMF are good solvents for PMMA. The same PMMA standard and the same RPLC conditions as in Fig. 1 were used in the following examples. When pure DMSO or pure DMF was used as the sample solvent, no breakthrough was observed. As shown in Fig. 1 (bottom trace), also no breakthrough was observed when the sample solvent contained water (a very weak eluent)–methanol–THF (12.5:37.5:50).

Fig. 9 illustrates a concept for selecting suitable sample solvents. Ideal injection solvents dissolve the sample easily (right-hand-side of the figure), but are poor eluents (bottom half of the figure). The best solvents to avoid breakthrough effects are thus

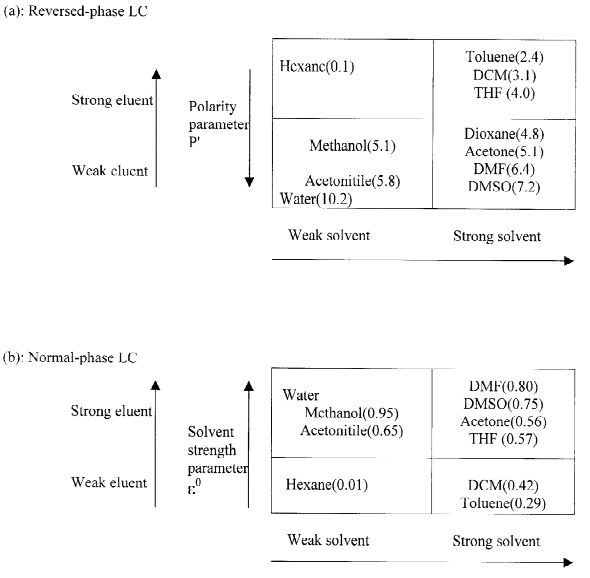

Fig. 9. Solvent selection scheme for PMMA: when the injection solvent is a strong solvent for the polymer and a strong eluent (top-right corner), a breakthrough peak will appear; the injection solvent should be a strong solvent for the sample and a weak eluent (bottom-right corner) to avoid the breakthrough phenomenon. (a) Reversed-phase LC. (b) Normal-phase LC.

situated in the bottom-right corner of the diagrams in Fig. 9. Fig. 9a shows the relevant characteristics for a number of solvents for PMMA in RPLC. The polarity parameter (P') [30] is used to indicate the solvent strength. Qualitative information on the quality of the suggested solvents for dissolving PMMA was obtained from Ref. [31]. Fig. 9b applies to NPLC. Snyder's eluent-strength parameter (ϵ^0, Ref. [30], p. 365) is now plotted on the vertical axis. Fig. 10 provides evidence for the usefulness of Fig. 9a to select an appropriate injection solvent for the PMMA samples of Fig. 1. Usually, a strong sample solvent is selected that is also a strong eluent, such as THF (top right corner in Fig. 9a). The presence of THF in the sample solvent ensures sample solubility and compatibility with the eluent. When adding sample solvents from the top left corner in Fig. 9a to THF, large breakthrough peaks are observed, as exemplified by the mixture of hexane–THF (33:67) (Fig. 10a). Hexane is a non-solvent for PMMA. Because the polarity parameter of hexane is very low (0.1), it is a strong eluent in RPLC. Selecting a solvent from the bottom left corner in Fig. 9a leads

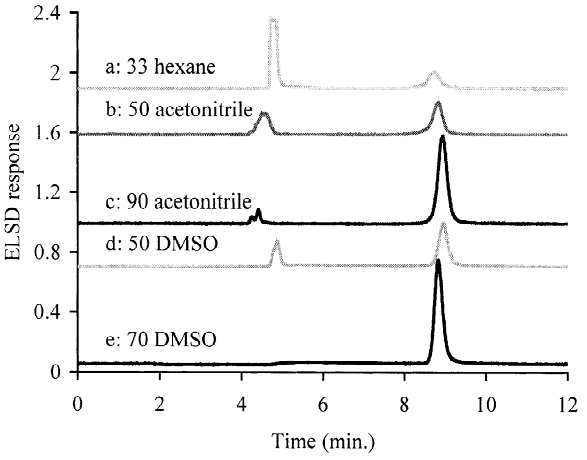

Fig. 10. Chromatograms of PMMA (M_p 34 500, PD 1.04) dissolved in mixtures of THF and solvent according to Fig. 9a indicated in the figure at 25 °C [from top to bottom, sample solvent: a=THF–hexane (67:33), b=THF–acetonitrile (50:50), c=THF–DMSO (50:50), d=THF–acetonitrile (10:90), e=THF–DMSO (30:70)]. LC conditions and PMMA standard used as in Fig. 1.

to less breakthrough. Thus, mixtures of acetonitrile and THF lead to better results (Fig. 10b and c). However, using mixtures of THF and acetonitrile as sample solvents, the occurrence of a breakthrough peak could not be avoided. The PMMA standards do not dissolve in pure acetonitrile. In the weakest possible injection solvent (about THF–acetonitrile, 10:90) a breakthrough peak was still observed. The best results can be obtained with solvents from the bottom right corner in Fig. 9a, such as DMSO (Fig. 10d and e). No breakthrough peak was observed with DMSO–THF (70:30) as the sample solvent, but breakthrough did occur when the sample solvent contained 50% or more of THF in DMSO. Pure DMSO is a good solvent for PMMA and it does not show any breakthrough. However, it is less attractive due to its high viscosity.

To demonstrate the usefulness of Fig. 9b, a series of NPLC chromatograms were recorded for a PMMA standard (M_p 5270, PD 1.06) dissolved in various mixtures of toluene and methanol. A 20-μl volume of 5 mg/ml sample solution was injected. The laboratory-packed silica column was used and the flow-rate was 0.9 ml/min. The mobile-phase composition was programmed from 2 to 100% methanol in toluene in 20 min. When the sample solvent was pure toluene (bottom-right corner in Fig. 9b), only one peak was observed for PMMA with a retention time of about 4.5 min (not shown). Solvents in the top-left corner are least attractive. When a significant amount (≥20%) of methanol was added to toluene severe breakthrough was observed. When dichloromethane (another sample from the bottom-right corner) was used as the sample solvent, no breakthrough was observed.

Injecting the same amount of polymer, a small injection volume and a relatively high analyte concentration are favourable from the point of view of avoiding breakthrough peaks. In isocratic separations, we cannot vary the mobile phase, as its composition is dictated by the desired separation. In gradient-elution experiments the use of weak initial solvents is recommended. The weaker the initial solvent, the more effectively the solvent plug can be dispersed during the early stages of the experiment. This effect can be consciously exploited by using the ''sandwich'' injection method proposed by Mengerink et al. [32]. Measures to enhance mixing between the (initial) mobile phase and the sample solution [6] reduce the risk of breakthrough peaks. However, they may result in additional band broadening, especially in the case of isocratic LC.

Conclusions

In the chromatographic experiments described above, narrow polymer standards produced two completely separate peaks. These two peaks showed identical molecular mass distributions upon re-injection. They are both representative of the entire polymeric (narrow-standard) sample. However, whether the breakthrough peak exists (and how large the ratio of the breakthrough peak to the real retention peak is) depends not only on the LC conditions, but also on the molecular mass and chemical composition of the analyte polymer. This implies that if a (very) broad polymer sample or a copolymer sample is injected, we may easily obtain

two separate peaks that represent different parts of the sample.

The sample solvent, the (initial) mobile-phase composition, the injection volume, the injected sample concentration, and the effect of the column temperature on the breakthrough phenomenon were investigated in the LC of PS and PMMA. The results showed that the breakthrough peak increased as the injection volume, the column temperature, the strength of the sample solvent injected, and the strength of the initial mobile phase in gradient LC increased, or as the polymer concentration decreased.

An explanation for breakthrough phenomenon in the polymer LC was proposed and discussed in detail. The three necessary conditions for separating a single, narrow (or even monodisperse) polymer standard into two vastly separated peaks are:

(1) The mobile phase should be weaker than the critical composition.

(2) The sample solvent should be stronger than the critical composition.

(3) A plug of sample solvent should remain intact throughout the column.

In order to avoid the breakthrough phenomenon, the chromatographer should:

(i) Use an injection solvent that is as weak an eluent as possible. (a) In case of RPLC a solvent with a high polarity parameter (P') should be selected as the sample solvent (or as one of the components of the injection solvent); (b) in the case of NPLC a solvent with a low solvent-strength parameter (ϵ^0) should be selected as the sample solvent (or as one of the components of the injection solvent).

(ii) Inject (relatively) high concentrations in (relatively) small volume.

(iii) (a) In the case of gradient elution, use the weakest possible initial solvent; (b) in the case of isocratic elution use the ''sandwich'' injection method [32] when necessary, i.e., when the above remedies (ia and ib) fail to yield satisfactory results.

For the case of PMMA, the rules for selecting sample solvents in RPLC and NPLC have been summarized in simple diagrams (Fig. 9), the validity of which has been demonstrated by selecting suitable sample solvents for RPLC (DMF or DMSO) and NPLC (dichloromethane or toluene) separations. To prepare similar figures for other polymers, only some (qualitative) information on the solubility of the polymer in various solvents is required.

Acknowledgements

This project is funded by the Dutch Polymer Institute (DPI project 205). We thank Fiona Fitzpatrick (University of Amsterdam) and Professor Rob van der Linde (Eindhoven University of Technology) for their cooperation.

References

[1] T.C. Schunk, J. Chromatogr. A 656 (1993) 591.
[2] D. Berek, Prog. Polym. Sci. 25 (2000) 873.
[3] D.W. Armstrong, K.H. Bui, Anal. Chem. 54 (1982) 706.
[4] J.P. Larmann, J.J. Destefano, A.P. Goldberg, R.W. Stout, L.R. Snyder, M.A. Stadalius, J. Chromatogr. 255 (1983) 163.
[5] G. Glöckner, Chromatographia 25 (1988) 854.
[6] C.H. Lochmüller, M.B. McGranaghan, Anal. Chem. 61 (1989) 2449.
[7] R. Schultz, H. Engelhardt, Chromatographia 29 (1990) 205.
[8] R.A. Shalliker, P.E. Kavanagh, I.M. Russell, J. Chromatogr. 543 (1991) 157.
[9] D.M. Northrop, D.E. Martire, R.P.W. Scott, Anal. Chem. 64 (1992) 16.
[10] H.J.A. Philipsen, B. Klumperman, A.M. van Herk, A.L. German, J. Chromatogr. A 727 (1996) 13.
[11] R.A. Shalliker, P.E. Kavanagh, I.M. Russell, J. Chromatogr. 558 (1991) 440.
[12] R.A. Shalliker, P.E. Kavanagh, Chromatographia 44 (1997) 421.
[13] G. Glöckner, Chromatographia 23 (1987) 517.
[14] G. Glöckner, M. Stickler, W. Wunderlich, Fresensius Z. Anal. Chem. 328 (1987) 76.
[15] G. Glöckner, M. Stickler, W. Wunderlich, Fresensius Z. Anal. Chem. 330 (1988) 46.
[16] S. Mori, J. Appl. Polym. Sci. 38 (1989) 95.
[17] M. Augenstein, M. Stickler, Makromol. Chem. 191 (1990) 415.
[18] S. Mori, J. Chromatogr. 541 (1991) 375.
[19] T.M. Zimina, J.J. Kever, E.Y. Melenevskaya, A.F. Fell, J. Chromatogr. 593 (1992) 233.
[20] M. Petro, F. Svec, J.M.J. Fréchet, S.A. Haque, H.-C. Wang, J. Polym. Sci. A 35 (1997) 1173.
[21] X.-L. Jiang, P.J. Schoenmakers, manuscript in preparation.
[22] N.E. Hoffman, S.-L. Pan, A.M. Rustum, J. Chromatogr. 465 (1989) 189.
[23] G.E. Berendsen, P.J. Schoenmakers, L. De Galan, J. Liq. Chromatogr. 3 (1980) 1669.
[24] Y. Brun, J. Liq. Chromatogr. Rel. Technol. 22 (1999) 3067.

[25] S.H. Nguyen, D. Berek, J. Polym. Sci. A 37 (1999) 267.
[26] G. Glöckner, Gradient HPLC of Copolymers and Chromatographic Cross-Fractionation, Springer-Verlag, Berlin, Heidelberg, 1991.
[27] F.A. Buijtenhuijs, Akzo Nobel Chemicals Research, Arnhem, personal communication.
[28] P.J.C.H. Cools, Ph.D. Thesis, Eindhoven University of Technology, Eindhoven, 1999.
[29] L.R. Snyder, Principles of Adsorption Chromatography, Marcel Dekker, New York, 1968.
[30] L.R. Snyder, J.J. Kirkland, Introduction to Modern Liquid Chromatography, 2nd ed., Wiley, New York, 1979.
[31] D.R. Bloch, in: J. Brandrup, E.H. Immergut, E.A. Grulke (Eds.), Polymer Handbook (VII/497), 4th ed., Wiley, New York, 1999.
[32] Y. Mengerink, R. Peters, M. Kerkhoff, J. Hellenbrand, H. Omloo, J. Andrien, M. Vestjens, Sj. Van der Wal, J. Chromatogr. A 876 (2000) 37.