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Fast determination of critical eluent composition for polymers by gradient chromatography

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Abstract

Gradient chromatography was applied in order to calculate the composition at elution for different methacrylates on normal phase columns. In addition the composition at elution was determined for polyethyleneoxide on a reverse phase column. It is shown that high molar mass polymers elute for a given homopolymer irrespective of their molar mass at the same eluent composition, which varies only slightly with gradient slope. In general the composition at elution in gradient chromatography is expected to be slightly lower than the true critical composition. For high molar mass polymers we found this composition to be close to the critical composition determined by isocratic experiments. The difference between the composition at elution and the true critical composition for a variety of polymethacrylates and for polyethyleneglycol was found to be only between 0.2 and 5%. Thus, after estimating the composition at elution, only a small number of additional isocratic experiments is needed to find the exact critical composition.

Keywords: Homopolymers; Critical composition; Gradient chromatography

1. Introduction

Unlike most natural polymers synthetic ones are polydisperse not only in molar mass but also in chemical composition, functionality and/or topology, enhancing the characterization difficulties accordingly [1]. Liquid chromatography has proven to be one of the most effective methods for the characterization of natural and synthetic polymers. Depending on the strength of the enthalpic interaction between the polymer molecule and the stationary phase, three different molar mass dependences of the elution volume can be distinguished. In ideal size exclusion chromatography (SEC), where no enthalpic interaction exists between the stationary phase and the polymer molecule [2] separation takes place according to the hydrodynamic volume, which can be related to molar mass. Larger molecules elute earlier than smaller ones. After suitable calibration the molecular weight distribution and the molar mass averages can be determined. Liquid adsorption chromatography (LAC) uses the differences in interaction energy between the repeating unit and the stationary phase. Depending on experimental conditions separations according to chemical composition can be achieved [1,3]. For chemically homogeneous samples the interaction strength increases with molar mass, resulting in an opposite molar mass dependence of the elution volume as compared to SEC. In liquid chromatography under critical conditions (LCCC) the retention behaviour of a homopolymer becomes independent of its molar mass [4,5]. The composition of the eluent at which this happens is called the critical composition. Under critical conditions the retention of the polymer is controlled by differences other than molar mass. This mode of polymer chromatography, has become particularly valuable for the analysis of complex polymers with respect to functionality (functionality type distribution) [6], block length distribution [7], separation of polymer blends [8] and stereoregularity [9].

The critical composition at a given temperature is characteristic of a particular polymer–adsorbent–eluent system. Critical conditions have been found for a variety of polymers and have been reviewed recently by Macko et al. [10]. Usually, the search for critical composition involves a large number of isocratic experiments using

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polymer standards of different molar mass [6,11]. Starting from a strong eluent, which results in the SEC-mode, the eluent strength is systematically reduced by adding an adsorption promoting liquid. For each new eluent composition the column has to be carefully equilibrated and the dependence of elution volume on molar mass has to be determined again. The addition of too much of the weak eluent changes the elution behaviour to the LAC mode, which for high molar mass polymers might result in irreversible adsorption. As a result, time consuming flushing and re-equilibration of the column becomes unavoidable. By this trial and error approach an eluent composition is finally found, where the molar mass dependence of the elution volume vanishes.

The critical point, as stated above, refers to a suitable eluent composition at a certain temperature. A different approach to find critical conditions for a particular polymer/adsorbent system is to use either single solvents or solvent mixtures with temperature adjustments [12,13]. Since adsorption can be tuned more precisely by temperature than by eluent mixtures, temperature adjustment allows a more gradual change from size exclusion to adsorption mode. The problem of irreversible adsorption is therefore reduced when starting from a temperature/solvent pair resulting in SEC behaviour. However, temperature changes allow to vary the interaction strength only within a rather narrow range. The selection of a suitable solvent or eluent mixture therefore may also suffer from irreversible adsorption if the eluent strength of the chosen mixture is too weak. Temperature adjustment is therefore used more often in practice to fine-tune the conditions when the critical point has slightly changed due to changes in eluent quality or column performance. However, the capability of temperature to slightly change the interaction strength with the stationary phase has been used by Chang et al. for polymer separations with remarkable resolution [14–16].

As already mentioned, at the critical point polymers elute irrespective of their molar mass. Other routes to achieve a molar mass independent elution are liquid chromatography at the critical point of adsorption or desorption [17]. These techniques use differences in the adsorption strength of the eluent and the sample solvent, which can result in molar mass independent elution of the polymer just before or just behind the solvent band. However, to our knowledge no real separations have yet been described in literature.

The tedious and time consuming search for the critical composition is a major drawback of LCCC despite the valuable information it can provide. The search for critical conditions is further complicated by the fact that close to the critical conditions end group effects might show up, resulting in multiple peaks. Therefore the question arises of which peak has to be used for the determination of the dependence of elution volume on molar mass. This problem may exist even when using standards synthesized by controlled polymerization techniques. In addition, it is difficult to obtain well-characterized samples with different molar mass for some polymers. Therefore, a fast, efficient and convenient way to determine the critical composition would be of advantage to improve the use of LCCC.

Instead of plotting the elution volume as a function of molar mass for different eluent compositions, Cools et al. [11] proposed to plot the retention coefficients as a function of eluent composition for different molar mass polymers. The critical composition is found to be the point where the retention curves for the samples intersect. Thus, by this procedure the number of isocratic experiments needed is reduced. The determination of suitable eluent compositions, where isocratic polymer elution is possible, remains a problem, however.

Brun and co-workers [18–20] have shown theoretically and experimentally that gradient chromatography under certain conditions results in a molar mass independent elution close to the critical composition. Unfortunately their paper has not yet attracted as much attention as it should. According to their calculations the eluent composition at which a high molar mass polymer elutes from the column (composition at elution) might be slightly higher than the critical one. This result is due to a minor mistake in their theoretical approach and the correct equations will be given in a subsequent paper [21]. In the present paper it will be shown by simple arguments that the composition at elution cannot exceed the critical one and will approach the critical composition for high molar mass polymers. Experimental data will be presented which support this idea and Brun's assumption of molar mass independent gradient elution of high molar mass polymers.

2. Experimental

2.1. Equipment

All measurements were performed using an Agilent 11,000 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).

2.2. Chromatographic conditions

The injected sample volume was 10 μl. Sample concentrations were 1–2 g/l. Column temperature 35 °C and flow rate was 1 ml/min. Methyl ethyl ketone (MEK), cyclohexane (c-hexane) and methanol (MeOH) were of HPLC grade and used as received. Tetrahydrofuran (THF) was refluxed and distilled from CaH_2. Water was deionized using

Millipore Simplicity 185 (UV) water system (Millipore GmbH, Schwalbach, Germany). Poly(methyl methacrylate) (PMMA), Poly(*n*-butyl methacrylate) (P*n*BMA), Poly(*t*-butyl methacrylate) (P*t*BMA), Poly(decyl methacrylate) (PDMA) and polyethylenglycoles having different molar masses and narrow polydispersities were obtained from PSS Polymer Standards Service GmbH, Mainz, Germany. The molar masses reported are molar masses at the maxima of the molar mass distributions (M_p) as given by the supplier.

The following columns were used:

(A) Nucleosil bare silica, particle size 7 μm, pore diameter 1000 Å, column dimensions $250\times4.0\ \text{mm}^2$ i.d. (Macherey–Nagel, Düren, Germany).
(B) Nucleosil C18, particle size 5 μm, pore diameter 300 Å, column dimensions $250\times4.6\ \text{mm}^2$ i.d. (Macherey–Nagel, Düren, Germany).
(C) Chromolith Si, mesopores 130 Å, 2 μm macropores, $100\times4.6\ \text{mm}^2$ i.d. (Merck KGaA, Darmstadt, Germany).

2.3. Chromatographic measurements

The void volume was estimated by injecting toluene using tetrahydrofuran as eluent. The dwell volume was determined by subtracting the void volume from the onset of the increasing UV-signal due to a linear gradient starting from pure methanol and running to methanol containing 0.3% acetone.

For each polymer standard linear gradients were run from 100% weak to 100% strong eluent, unless otherwise mentioned. The used columns, weak and strong eluents and gradient ranges for the polymers investigated are summarized in Table 1. The highest molar mass of the available standards was used in the gradient experiments to estimate the critical composition. The elution volume (V_g) was taken at the peak maximum. The eluent composition at peak maximum (in terms of strong solvent), $\%B_g$, was calculated using the following equation:

$$\%B_g = (V_g - V_V - V_d)\frac{\Delta\%B_g}{Ft_G} + \%B_0 \qquad (1)$$

where V_v is column void volume, V_d the system dwell volume, $\Delta\%B_g$ the total change in composition (of strong eluent) during the gradient, t_G the gradient time, F the flow rate and $\%B_0$ is the initial composition.

Isocratic mobile phases of different compositions were delivered by the pump system. The isocratic experiments were performed with at least three different molar mass standards. All runs were performed using duplicate injections.

3. Results and discussions

As already stated in the introduction, three different modes of polymer chromatography can be distinguished. At critical conditions the distribution coefficient $K=1$, meaning that the polymer molecules travel through the column with the velocity of the eluent molecules. In SEC $K<1$ and under adsorbing conditions $K>1$, indicating a faster and slower velocity, respectively, of the polymer molecules as compared to the eluent. In gradient chromatography, a very strong adsorption of the polymer molecules onto the stationary phase takes place in the weak initial eluent composition. With increasing eluent strength desorption occurs. The polymer molecules start moving when an eluent composition of sufficient strength reaches them. Due to the strong molar mass dependence of the retention coefficient desorption occurs only in a narrow region of eluent composition close to the critical one. Lower molar mass polymer molecules, being relatively weakly adsorbed, desorb at an eluent composition well below the critical one while higher molar masses require a stronger eluent to desorb. As the polymer becomes desorbed it will be surrounded by an eluent below the critical composition, i.e. the polymer moves with a velocity lower than that of the eluent. As a consequence it will be surpassed by compositions of increasing eluent strength, resulting in an acceleration of the polymer molecules. However, this acceleration continues as long as the eluent molecules are faster than the polymer molecules. The condition of the polymer velocity being equal to the eluent velocity corresponds to the critical conditions $K=1$. Therefore the polymer molecule can only elute at a composition below or equal to the critical one. The composition at elution from gradient runs therefore provides a lower bound for the critical composition. Higher molar mass molecules are more strongly adsorbed than lower molar mass one. Therefore the

Table 1
Gradient conditions used for the estimation of critical composition

Polymer (M_p (g/mol))	Column	Mobile phase SE/WE[a]	Initial composition	Final composition	Gradient time (min)
PMMA (700,000)	A	THF/Toluene	100% toluene	100% THF	10, 20, 40
PEG (40,000)	B	MeOH/Water	95% water	100% MeOH	30, 60, 90
PMMA (296,000)	C	MEK/c-hexane	100% c-hexane	100% MEK	10, 20, 30, 60
P*n*BMA (240,000)	C	MEK/c-hexane	100% c-hexane	100% MEK	10, 20, 30, 60
P*t*BMA (618,000)	C	MEK/c-hexane	100% c-hexane	100% MEK	10, 20, 30, 60
PDMA (598,000)	C	MEK/c-hexane	100% c-hexane	100% MEK	10, 20, 30, 60

[a] SE, strong eluent; WE, weak eluent.

composition at elution for a high molar mass polymer will be closer to the critical composition than that of a polymer of lower molar mass.

In order to test this hypothesis we run several PMMAs of different molar mass in linear toluene-THF gradients. Fig. 1 shows the chromatograms of PMMA standards of varying molar mass for a 20 min linear gradient. High molar mass polymer samples elute later than those with low molar masses. It can be seen that the peaks of the PMMAs having molar masses of 530,000 g/mol and 700,000 g/mol appear at practically the same elution volume.

The dependence of the elution volume on molar mass can clearly be seen in Fig. 2. It becomes clear that for molar masses above approx. 200,000 g/mol, an elution nearly independent of molar mass is observed. From the elution volume the composition at elution was calculated using Eq. (1). The right axis in Fig. 2 shows the composition at elution versus the molar mass of the standards. It is evident that higher molar masses elute at nearly identical eluent compositions (37% THF), irrespective of their molar mass. In order to determine the effect of gradient slope on the composition at elution, we varied the gradient times keeping all other parameters constant.

Fig. 3 shows the dependence of composition at elution on gradient slope for various molar masses. It is evident that an increase in gradient slope enhances the amount of the strong eluent in the composition at elution. The dependence on gradient slope vanishes for high molar masses. This behaviour can be explained as follows: The polymer molecules, depending on their molar mass, start moving before they are caught up by the critical composition, which is somewhere in the gradient profile behind the sample molecules. However, their velocity is lower than that of the eluent. In order to catch the sample molecules by the critical composition in the column, the gradient slope has to be suitably higher. Otherwise the molecules are fast enough to elute from the column. This happens in the case of lower molar mass molecules where the critical composition in a gradient cannot meet them before they exit the column. However, with the increase in gradient slope the composition at elution becomes closer to the actual critical composition. As the molar mass becomes higher, the movement of the sample molecules starts in a stronger eluent composition closer to the critical one. Thus the critical composition may catch up with the polymer somewhere within the column. When this happens the elution of the molecules occurs at the critical composition. In this case, the velocity of the polymer molecules is fast enough (being closer or identical to that of eluent, i.e. $K \approx 1$) that the gradient slope has practically no effect on the composition at elution. According to our assumptions above, this eluent composition should be close to the actual critical composition.

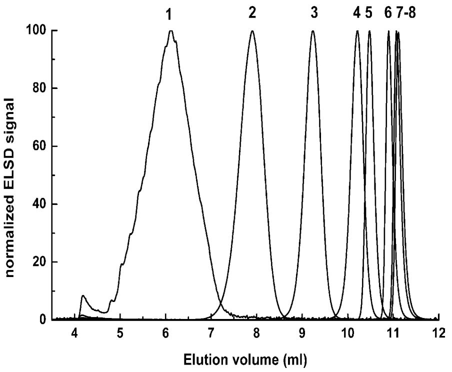

Fig. 1. Overlay of chromatograms for PMMA standards having different molar mass, M_p. (1) 1210 g/mol, (2) 3500 g/mol, (3) 10,900 g/mol, (4) 30,500 g/mol, (5) 60,000 g/mol, (6) 240,000 g/mol, (7) 530,000 g/mol, (8) 700,000 g/mol, for a linear 20 min gradient from 100% toluene to 100% THF. detector: ELSD, column: A.

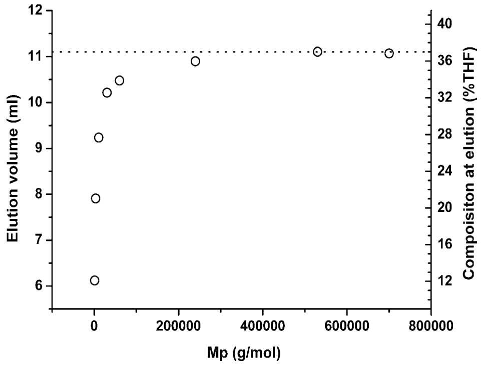

Fig. 2. Elution volume and composition at elution (%THF) at peak maxima as a function of molar mass of PMMA standards. The dotted line shows the critical composition as obtained by isocratic runs. Chromatographic conditions: see Fig. 1.

In order to check this hypothesis, the critical composition was determined using isocratic runs of PMMAs having different molar masses. Since the composition at elution is assumed to give a lower bound for the critical composition we chose the highest composition at elution of the PMMA

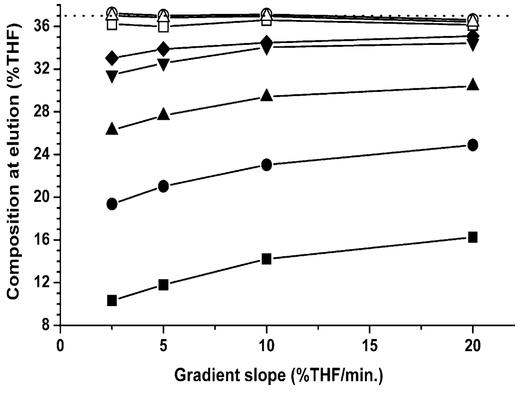

Fig. 3. Composition at elution (% THF in toluene) as a function of gradient slope for PMMA standards of different molar mass, M_p. (■) 1020 g/mol, (●) 3500 g/mol, (▲) 10,900 g/mol, (▼) 30,500 g/mol, (◆) 60,000 g/mol, (□) 240,000 g/mol, (○) 530,000 g/mol, (△) 700,000 g/mol. The dotted line shows the critical composition as obtained by isocratic runs. Chromatographic conditions as in Fig. 1.

700,000 g/mol i.e. 37% THF in toluene (Fig. 3) for the first isocratic runs of four different PMMAs.

As can be seen in Fig. 4, all standards have practically the same elution volume in this eluent composition with only a slight tendency towards the adsorption mode, which can actually be ignored. Isocratic runs were also performed at eluent compositions one percent above (38% THF) and one percent below (36% THF) the composition at elution which resulted in clear SEC and LAC mode, respectively (Fig. 4). It should be noted that PMMA 700,000 g/mol does not elute in one percent weaker eluent composition than the composition at elution.

Using the results of these runs, plots of elution volume as a function of eluent composition (Cools plot) were created and are shown in Fig. 5. The lines for different molar masses intersect close to 37% THF, indicating a good agreement of the composition at elution and the critical composition. The critical composition found by us coincides nicely with results of Berek in a similar system [17]. All this supports our assumptions given above.

The same approach was used on a reversed phase column for PEG using methanol and water as the strong and weak eluent, respectively. PEG of molar mass 40,000 g/mol was used to get an estimate from three linear gradients. As can be seen in Fig. 6, the composition at elution varies only slightly with gradient slope.

According to our above assumption and the results on the PMMAs we assume that the composition at elution from the faster gradient (82.6% MeOH) should be closer to the actual critical composition. Thus, a composition of 83% MeOH in water was taken to perform the first isocratic experiments with three different PEG standards, which elute independent of molar mass at this composition (Fig. 7). In addition runs were performed at 80 and 90% MeOH, which resulted in adsorption and exclusion mode, respectively (Fig. 7). The dependence of elution volume on eluent composition for the PEGs chosen is depicted in Fig. 8. The critical composition, as determined by the crossing of the lines corresponding to different molar mass, is 83% MeOH. This value is in good agreement with the estimation from the eluting composition of the gradient runs (82.6%).

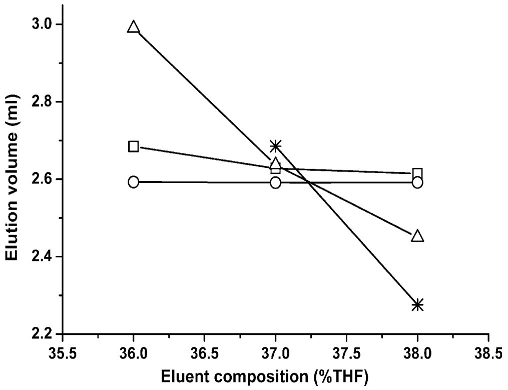

Fig. 5. Dependence of elution volume on eluent composition for PMMAs of different molar mass, M_p; (○) 3500 g/mol, (□) 30,500 g/mol, (△) 240,000 g/mol, (*) 700,000 g/mol, Detector: ELSD, Column: A.

Finally we used the approach for the determination of critical composition for PMMA, P*n*BMA, P*t*BMA and PDMA on a monolithic silica column. The results are given in Table 2. Again very good agreement is found between the compositions at elution and the critical compositions obtained from isocratic runs. Only in the case of P*n*BMA a difference above 4% is observed. With the exception of poly(decyl methacrylate) all compositions at elution are slightly below the critical composition, as expected from our simple arguments. Closer inspection of Table 2 reveals that for lower amounts (<50%) of the strong eluent the deviations between the composition at elution and the true critical compositions are higher than for higher ones (> 50%). In order to determine the composition at elution with an error of 1% the retention volume has to be determined with the same accuracy (Eq. (1)), which becomes more difficult the lower is the retention time. In addition, the larger errors found are for the different poly(methacrylates) with gradients running from cyclohexane to MEK. Cyclohexane is a non-solvent for the poly(methacrylates), resulting in precipitation of the polymer at the beginning of the gradient. As discussed by Brun [18,20] as long as the

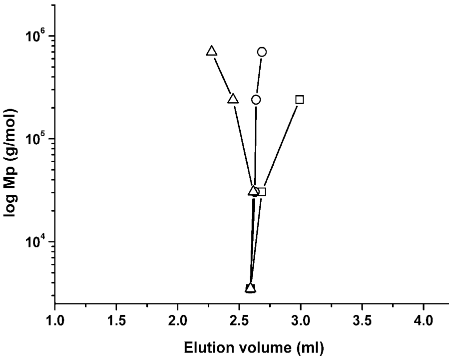

Fig. 4. Dependence of elution volume on molar mass for PMMAs at different isocratic eluent compositions, (□) 36%, (○) 37%, (△) 38% THF, Detector: ELSD, Column: A.

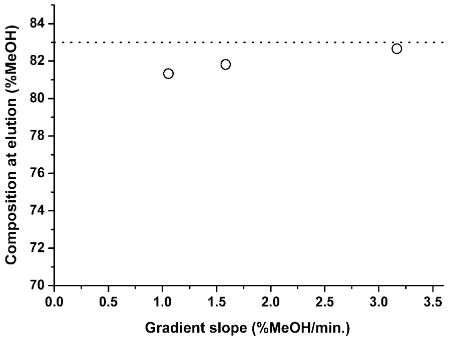

Fig. 6. Composition at elution (% methanol) of PEG, M_p 40,000 g/mol as a function of gradient slope. The dotted line shows the critical composition as determined from isocratic runs. Detector: ELSD, Column: B.

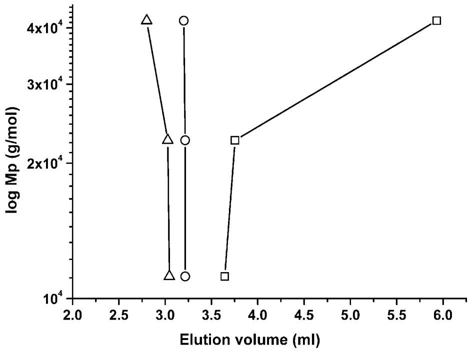

Fig. 7. Dependence of elution volume on molar mass of PEGs at different eluent compositions, (□) 80%, (○) 83%, (△) 90% methanol, Detector: ELSD, Column: B.

composition (in terms of percentage of strong solvent) at the precipitation threshold is lower than the critical composition, gradient elution should still result in a composition at elution close to the critical one. If solubility can only be achieved above the critical composition, critical conditions cannot be found for the system under investigation. In our systems critical conditions could be established by isocratic runs, indicating that the precipitation threshold must be lower than the critical composition. However, if redissolution during the gradient is slow and the critical composition is close to the precipitation threshold, the polymer might elute at a composition higher than the critical one. The kinetics of dissolution therefore might explain the unexpectedly higher percentage of MEK at the composition at elution found for poly(decyl methacrylate). It may also attribute to the larger errors observed at low percentages of strong solvent. Despite these problems the good agreement between the compositions at elution and the critical composition illustrates the efficiency and accuracy of the present approach.

Based on these results we therefore, propose the following general strategy of finding the critical composition:

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

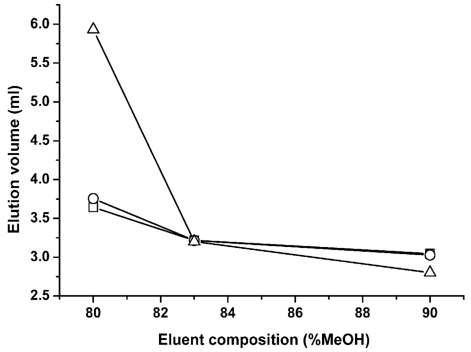

Fig. 8. Dependence of elution volume on eluent composition for different molar masses (M_p) of PEGs (□) 12,000 g/mol, (○) 23,000 g/mol, (△) 40,000 g/mol, Detector: ELSD, Column: B.

Table 2
Critical compositions of eluent (in terms of % amount of strong eluent) estimated from gradients in comparison with actual critical composition determined by isocratic measurements

Polymer	Molar Mass (M_p (g/mol))	Composition at elution (%)	Found composition (%)	Difference (%)
PMMA	700,000	37.00	37.20	0.20
PEG	40,000	82.60	83.00	0.40
PMMA	296,000	70.40	70.60	0.20
P*n*BMA	240,000	13.55	17.90	4.35
P*t*BMA	618,000	16.95	18.60	1.65
PDMA	598,000	5.37	4.00	1.37

2. Perform isocratic runs with a minimum of three standards at the estimated composition and at a composition a few percent higher in the strong eluent. If a strong dependence of composition at elution on the gradient slope is observed in step 1, then the difference between the composition at elution and that for the second isocratic run should be larger than if a weak dependence is observed. This is due to the fact that strong dependences on gradient slope are found for lower molar mass polymers which elute at lower compositions.
3. Plot the elution volume versus isocratic eluent composition for the different molar masses (Cools plot). The eluent composition at the intersection point will correspond to the critical composition. One more isocratic experiment can be performed at this eluent composition to verify the results.

4. Conclusions

We have applied linear solvent gradients to estimate the critical composition for different polymer and stationary–mobile phase systems. For high molar mass polymers the composition at elution is close to the critical composition. Using isocratic runs of polymers having different molar masses at the composition at elution and another composition slightly stronger than that, the critical composition can be determined with a minimum of experiments.

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