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Comprehensive two-dimensional liquid chromatography of polymers

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Abstract

The need for and the emergence of comprehensive two-dimensional liquid chromatographic separations of synthetic polymers are reviewed in this paper. LC×SEC is shown to be a particularly valuable two-dimensional technique in this domain. An improved (symmetrical) configuration based on a single 10-way switching valve is described. The use of LC×SEC to understand and optimize one-dimensional separations is illustrated, as well as the potential of the technique for the separation and characterization of functional polymers and copolymers.

Keywords: Switching valve; Liquid chromatography, comprehensive two-dimensional; Polymers; Copolymers

1. Introduction

A polymer is not a unique chemical compound. Synthetic (and many natural) polymers are mixtures of (very) many different compounds, the chemical structures of which are typically related, but not identical. The individual molecules in a synthetic polymer are largely built-up from one or a few different repeat units. In order of decreasing importance, the properties of the polymer depend on (i) the type(s) of repeat unit(s) or monomer(s) used, (ii) the average molecular size and structure, and (iii) the variation around the average size and structure. As an example of aspect (i) polystyrene is obviously very different from a polysaccharide. As to aspect (ii) polyethylene waxes (low-molecular mass) behave different from polyethylene foil (high molecular mass). The extent to which the size of molecules varies around the mean (aspect iii) may also have a dramatic effect on the physical properties. A polystyrene sample with a (weight-average) molecular mass of 1000 could be a solid plastic (uniform or narrowly distributed polymer), a sticky rubber (broad molecular-mass distribution), or even a solution of polystyrene in styrene monomer (e.g., a 10% solution of a 10 000 u standard). Variations in the chemical structure, such as the number of functional groups or end groups present, have an equally dramatic effect on the adhesive and reactive properties of the polymer. Clearly, in order to establish relationships between molecular structure and material properties of polymers, we need to obtain information on the average molecular structure, as well as on the distribution around the mean.

Some key distributions that are featured in synthetic polymers are listed in Table 1. There is always a molecular-mass distribution present in synthetic polymers, no matter how brilliant and meticulous their preparation. In fact, chromatographers usually

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Table 1
Important distributions in synthetic polymers

Distribution	Affects[a]
Molecular-weight distribution (MWD)[b]	Material properties (elasticity, strength, etc.)
or molar-mass distribution (MMD)[b]	Processing properties (melt strength, viscosity, etc.)
Chemical-composition distribution (CCD)	Material properties (strength, elasticity, etc.), Morphology
Functionality-type distribution (FTD)	Chemical reactivity, Cross-linking behaviour
Degree of branching distribution (DBD)	Melt strength, Melt viscosity
Block-length distribution (BLD)	Morphology, Elasticity, Thermal properties
Tacticity distribution (TcD)	Crystallinity (hardness, impact strength), Thermal properties

[a] Non-exhaustive listing of some key properties.
[b] Molecular mass is in relative units ($12C=12$); molar mass is in absolute units. IUPAC recommends the use of molecular weight (relative) and molar mass (g/mol).

underestimate the width of polymer distributions. For any molecular-mass distribution the relative standard deviation (σ/M_n) can easily be related to the polydispersity ($D=M_w/M_n$) [1]:

$$\frac{\sigma}{M_n}=\sqrt{(D-1)} \quad (1)$$

Thus, for what polymer chemists would call an extremely narrow distribution ($D=1.05$) we find a relative standard deviation of 22%. If this were a Gaussian chromatographic peak, this number would correspond to 20 theoretical plates, a pathetic number for chromatographers. Generally, chromatographic plate count and polymer dispersity are related by:

$$N=\frac{1}{D-1} \quad (2)$$

The fact that chromatographic peaks are much narrower than polymer distributions is the very reason why chromatography can be used successfully to characterize the latter.

All functional polymers exhibit a functionality-type distribution and all copolymers exhibit chemical-composition distributions (possible exceptions, such as perfectly alternating polyesters, can be classified as homopolymers in this context). The FTD, which features the number of molecules (or fractional mass) with a given number of a given type of functional groups, is extremely narrow if all molecules possess the same end groups and no other functional groups are present in the molecule. However, the FTD evolves into a discrete or semi-continuous distribution if the type(s) and number(s) of end groups start to vary. In a number of cases the FTD is of great significance. The CCD of copolymers typically is in the form of the number of molecules (or fractional mass) versus the ratio of the individual monomers. The other distributions listed in Table 1 are of prevailing importance in some cases, but in other cases they are less relevant. In this paper we will concentrate on the main distributions, MWD, FTD and CCD.

The different distributions are mutually dependent. For example, the larger molecules in a copolymer sample may show a narrow CCD, while the smaller molecules show a greater variation in chemical composition. This means that we cannot fully characterize a polymer sample by measuring the individual distributions.

To characterize a distribution it is necessary to separate the sample. Without a separation only information on averages (average molecular mass, average chemical structure, etc.) can be obtained. The few exceptions to this general rule are rather marginal. For example, rheological measurements allow both the average molecular mass and the width of an MWD to be estimated, but only if an assumption is made as to the shape of the MWD (e.g., a log-normal distribution). Thus, the general rule in polymer analysis is that if you want a distribution, you need a separation.

If more than one distribution exists, more than one separation will almost always be needed. If the distributions are dependent, then we need multi-dimensional separations. In addition, these separations need to be able to differentiate based on the relevant properties of the molecules [2]. If a polymeric sample features an MWD and a CCD, then we

need a two-dimensional separation, in which one step (ideally) distinguishes between molecules of different molecular mass and the other step reveals differences in chemical composition.

1.1. Two-dimensional liquid chromatography

In earlier studies two-dimensional liquid chromatography of polymers was often carried out in an off-line approach [3–15]. Fractions from the first column were collected and re-injected into a second liquid-chromatographic system. This off-line approach had some serious disadvantages. Sample contamination, losses or degradation during solvent evaporation and handling of the fractions were of main concern, as were the analytical repeatability and the labour intensity of the method. To overcome these problems, on-line two-dimensional liquid chromatography based on valve switching was performed [16–18]. ''Heart-cuts'' from the first-dimension were collected in a loop and introduced into the second-dimension column. Only a few fractions from the first-column effluent were analyzed in the second dimension. Thus, such separations were suitable to characterize specific parts (fractions) of a polymeric sample, but not for a complete (''comprehensive'') characterization.

During the 1980s Erni and Frei [19] were probably the first to introduce ''comprehensive'' two-dimensional liquid chromatography, soon followed by Bushey and Jorgenson [20]. In comprehensive two-dimensional LC every fraction from the first dimension is transferred on-line to the second dimension using an automated switching valve [19–23]. Several other research groups, specifically in Germany [24–26], have proceeded to explore two-dimensional separations of polymers.

Following the notation introduced by Blomberg et al. [27], we distinguish between comprehensive two-dimensional liquid chromatography and linear (''heart-cutting'') two-dimensional liquid chromatography by abbreviating the former as LC×LC and the latter as LC–LC. The benefits of comprehensive two-dimensional chromatography are numerous. No sample goes to waste, providing maximum information on minimal amounts of material and allowing rigorous quantitative interpretation of the results. Multiple cuts are usually fractionated for each first-dimensional peak, greatly enhancing the resolving power and peak capacity of the technique. Although LC×LC separations are often lengthy, they do not have to be repeated numerous times to fully characterize a single sample, as is usually the case with LC–LC techniques. No intermediate re-concentration step is necessary and the fractions are not exposed or manually handled, greatly reducing the chances of contamination or (oxidative) degradation.

In LC×LC of polymers several different separation mechanisms can be exploited in the first and second dimensions. The choice for either dimension is dependent on the distributions of interest. If, for example, a molecular-mass distribution (MWD) and a chemical-composition distribution (CCD) are required, one dimension can be ''interactive'' LC, to separate according to chemical composition, and the other dimension can be size-exclusion chromatography (SEC) to separate according to molecular size. Indeed, the combination of LC and SEC is by far the most commonly applied comprehensive two-dimensional separation method for polymers. Both LC×SEC (with LC as first dimension) and SEC×LC (with SEC as first dimension) are feasible and both configurations have their advantages (Table 2). For us, the disadvantages of SEC×LC and the advantages of LC×SEC prevail. To limit the total analysis time in LC×SEC and to preserve the chromatographic separation obtained in the first dimension, it is imperative that the second dimension be fast. Running fast LC gradients in the second dimension is instrumentally difficult and after each gradient the initial conditions need to be reestablished. To perform large numbers of isocratic (critical) chromatographic separations of polymers consecutively with highly repeatable retention times is a practical challenge. For both gradient and isocratic LC connected after a first-dimension SEC separation ''breakthrough'' peaks [28] form an ominous threat. The eluent used for SEC is necessarily very strong, which implies that the second-dimension separation may easily yield large spurious peaks around the solvent front, which contain most of the polymeric molecules and which make quantitative interpretation of the chromatogram completely impossible [28]. The only chance to avoid breakthrough peaks is if the second column is very much more retentive than the

Table 2
Advantages and disadvantages of LC×SEC and SEC×LC

SEC×LC	LC×SEC
Advantages	*Advantages*
• High-resolution SEC possible	• High-resolution (gradient) LC possible
• Possible focussing on top of 2nd-dimension column.	• Choice of detectors (2nd dimension isocratic)
• Possibility to exclude (''heavy'') part of sample	• Finite time of analysis in 2nd dimension
	• Change 1st-dimension LC conditions without need to re-optimize 2nd-dimension conditions
	• LC-system not easily overloaded
Disadvantages	*Disadvantages*
• 2nd-dimension analysis time is not limited	• Limited resolution in (fast) 2nd-dimension SEC
• ''Breakthrough'' peaks in 2nd dimension are hard to avoid	• ''Breakthrough'' peaks in 1st dimension must be avoided
• Gradients in 2nd dimension are highly impractical	
• Overloading and adsorption must be avoided in the 1st dimension	
• Limited choice of detectors	

first column when using the first-dimension eluent. It is hard to envisage such situations in practice.

Thus, we opt for size-exclusion in the second dimension and we need to perform fast SEC analysis, using short columns packed with small particles. A major advantage is the finite separation time (total permeation time) that is associated with SEC. The LC×SEC chromatograms can be protected from confounding signals (''wrap-around'' peaks, baseline disturbances) from previous injections if (i) genuine size-exclusion chromatography is performed, i.e., when adsorption effects are absent, and if (ii) the second-dimension analysis time (the cycle time for the valve switching) exceeds the hold-up time for a totally permeating solute.

For the first-dimensional separation we prefer to use a micro-bore LC column. This ensures comprehensive operation of the system. The alternative is to use a large column and to split the effluent prior to the switching valve. Disadvantages of this latter approach are the large amounts of eluent (and sample) required and the possibility of variations in the split ratio. The latter could easily occur due to the high viscosity of polymer solutions or to system fouling. Quantitative analysis may then easily be jeopardized. Although microcolumns are rarely used for separating polymers, their successful application has been described [29] and they are definitely advantageous for the present purpose.

The first dimension should then be small enough to stay within reasonable limitations on the injection volume for the second-dimension column. The maximum injection volume in the second dimension ($V_{\mathrm{inj,max}}$(II)) and the second-dimension analysis time (t_{max}(II)) determine the maximum flow-rate in the first dimension F_{max}(I) and thus, indirectly, the maximum column diameter (d_{c}(I)).

$$F_{\mathrm{max}}(\mathrm{I}) \leq \frac{V_{\mathrm{inj,max}}(\mathrm{II})}{t_{\mathrm{max}}(\mathrm{II})} \tag{3}$$

The loop size of the switching valve must have a minimum volume for truly comprehensive LC×LC [19]:

$$V_{\mathrm{loop}} \geq F(I) \times t_{\mathrm{max}}(II) \tag{4}$$

For a good reconstruction of the first-dimension chromatogram a high sampling frequency must be used. In discussions on integration methods (e.g., Ref. [30]) no theoretical ''optimum'' for the sampling frequency has been established. The time required for the second-dimension analysis in LC×LC is of the order of several minutes. Faster SEC analysis have been performed by working at uncommonly high linear velocities [31]. Exceptionally fast SEC may be possible in the second-dimension, because the polymer is already properly dissolved into a dilute molecular solution upon leaving the first column. However, the bandwidth in conventional SEC is dominated by the polydispersity PDI of the sample (even in the case of narrow standards). This may not be the case in LC×SEC if the first-dimen-

sion separation induces some molecular-mass-dependent separation. In that case working at high flow-rates may have significant negative effects on the band broadening in the second dimension. In any case, fast size-exclusion chromatography is an extremely important area to explore in the context of comprehensive two-dimensional liquid chromatography separations.

In various papers impressive two-dimensional LC separations have been presented [17,24,26,32,33]. It is clear that the technique has great potential for polymer separations. However, there are still large gaps in our understanding of both one- and two-dimensional polymer separations. One particularly interesting type of separation is the so-called critical chromatography, in which retention is independent of the polymer molecular mass [34]. One of the objectives of the present paper is to demonstrate how we can use comprehensive two-dimensional liquid chromatography (specifically LC×SEC) to improve our understanding of the underlying one-dimensional separations in the critical region.

2. Experimental

2.1. Chemicals

Polystyrene (PS) standards (2 mg/ml) with different molar masses (Table 3) obtained from Pressure Chemical (Pittsburgh, PA, USA) and Polymer Laboratories (Church Stretton, Shropshire, UK) were used to establish the mobile-phase composition (volume fraction of THF in *n*-hexane) at the critical point (ϕ_{cr}) and to illustrate some of the principles of LC×SEC. To demonstrate the possibilities of studying FTDs with LC×SEC around ϕ_{cr}, PS standards with hydroxyl end groups were purchased from Scientific Polymer Products (New York, NY, USA) (Table 3). Styrene-co-MMA random copolymers were prepared at the Technische Universiteit Eindhoven (The Netherlands) by Maarten Staal. Mobile phases used consisted of non-stabilized THF (Biosolve, Valkenswaard, The Netherlands), *n*-hexane p.a. (Acros, Geel, Belgium) in different ratios or non-stabilized THF (Biosolve) in acetonitrile (Biosolve) for gradient LC. As internal standard 1 mg/ml toluene (Acros) was added to the LC (first-dimension) mobile phase. This served as a retention-time marker in the second (SEC-UV) dimension.

2.2. Instrumentation

The isocratic LC system used consisted of a Shimadzu LC-10ADvp solvent-delivery unit (Shimadzu, 's Hertogenbosch, The Netherlands) and a Rheodyne two-position six-way injection valve (Berkeley, CA, USA) equipped with a 1-μl loop. Flow rates of 3 or 4 μl/min of *n*-hexane/THF (different mobile-phase compositions) were used. A

Table 3
Polymer standards used for calibration and optimization of LC×SEC

Polymer:	M_w	M_w/M_n	Supplier:
Polystyrene	1060[a], 1700[a], 2450[a], 5050[a], 7000[a], 11 600[a], 22 000[a], 30 000[b], 76 600[a], 200 000[b], 400 000[b], 900 000[b]	1.11, 1.05, 1.05, 1.05, 1.04, 1.04, 1.03, 1.03, 1.03, 1.08, 1.05, 1.06	[a]Polymer Laboratories (Church Stretton, Shropshire, UK) [b]Pressure Chemical (Pittsburgh, PA, USA)
Polymethylmethacrylate	2900, 6950, 28 300, 127 000, 840 000	1.08, 1.05, 1.04, 1.06, 1.10	Polymer Laboratories (Church Stretton, Shropshire, UK)
S-co-MMA, 20% S	400 000 (29% S)	n.a.	Technical University Eindhoven, The Netherlands
S-co-MMA, 40% S	280 000 (43% S)	n.a.	
S-co-MMA, 60% S	275 000 (59% S)	n.a.	
S-co-MMA, 80% S	250 000 (76% S)	n.a.	
Polystyrene, monohydroxy terminated	10 000, 100 000	1.05, n.a.	Scientific Polymer Products (New York, NY, USA)

n.a., information not available.

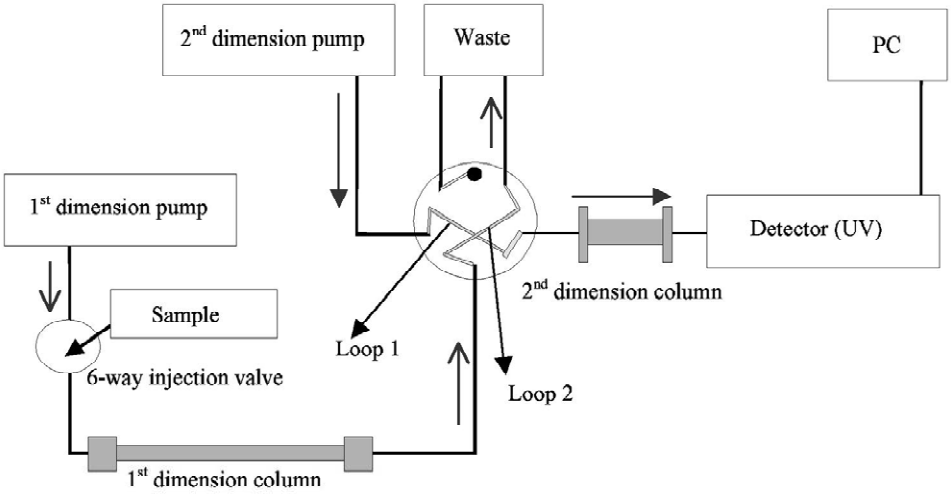


Fig. 1. Experimental set-up for the coupling of LC and SEC with a 10-way air-actuated switching-valve for comprehensive two-dimensional liquid chromatography (LC×LC).

home-packed first-dimension column containing Hypersil ''bare'' silica (ThermoQuest, Breda, The Netherlands) was used (particle diameter 3 μm, specified pore diameter 120 Å, 250 mm length×1.0 mm I.D.). For gradient-LC analysis two Shimadzu LC-10ADvp solvent-delivery units were connected in parallel to a 5-μl high-pressure gradient mixer (Supelco, Zwijndrecht, The Netherlands), yielding a total flow-rate of 4 μl/min (Gradient: 5–70% THF in acetonitrile 0–300 min (40 °C)). A home-packed Nucleosil C_{18} column (150 mm×1.0 mm I.D.; 5-μm particles; Machery Nagel, Düren, Germany) was used. The SEC system consisted of a Kratos Spectroflow 400 pump (ABI, Ramsey, NJ, USA) and a Kratos Spectroflow 757 UV-absorbance detector (ABI) at a wavelength of 254 nm. A 75×4.6 mm I.D. home-packed Mixed-C column (Polymer Laboratories) or a 50×7.5 mm I.D. mixed-E column (Polymer Laboratories) were used with THF at a flow-rate 0.4 ml/min. The LC and SEC systems were coupled (Fig. 1) with an air-actuated VICI two-position 10-way valve (Valco, Schenkon, Switzerland). This valve was operated using a high-speed switching accessory (switching-time of 20 ms using nitrogen) and dual injection loops of equal volume (various sizes between 4 and 40 μl used; see Eq. (4)).

It is relatively straightforward to use an eight-way valve or a 10-way valve equipped with two loops for LC×LC. The two loops can be used alternately to store the effluent from the first-dimension column and to inject it into the second-dimension column (see Ref. [35] for a clear illustration). However, we found that the conventional configuration (shown in

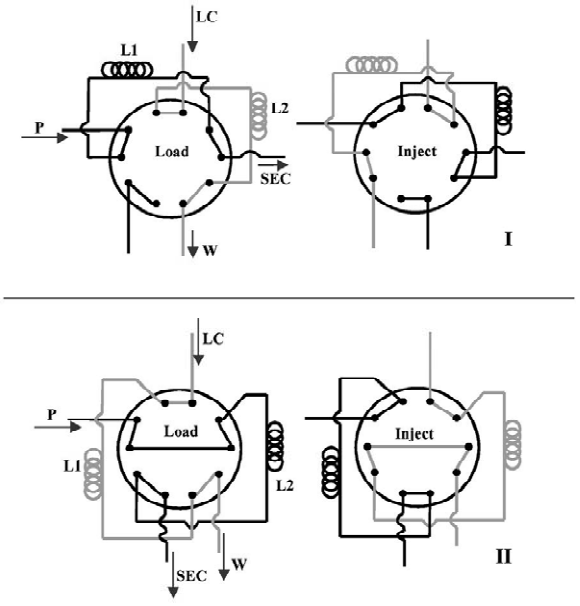


Fig. 2. Two different configurations (I and II) of a 10-way air-actuated switching valve (I, asymmetrical; II, symmetrical) used in LC×SEC; L1, loop 1; L2, loop 2; P, pump; W, waste; LC, from LC; SEC, to SEC. Explanation see text.

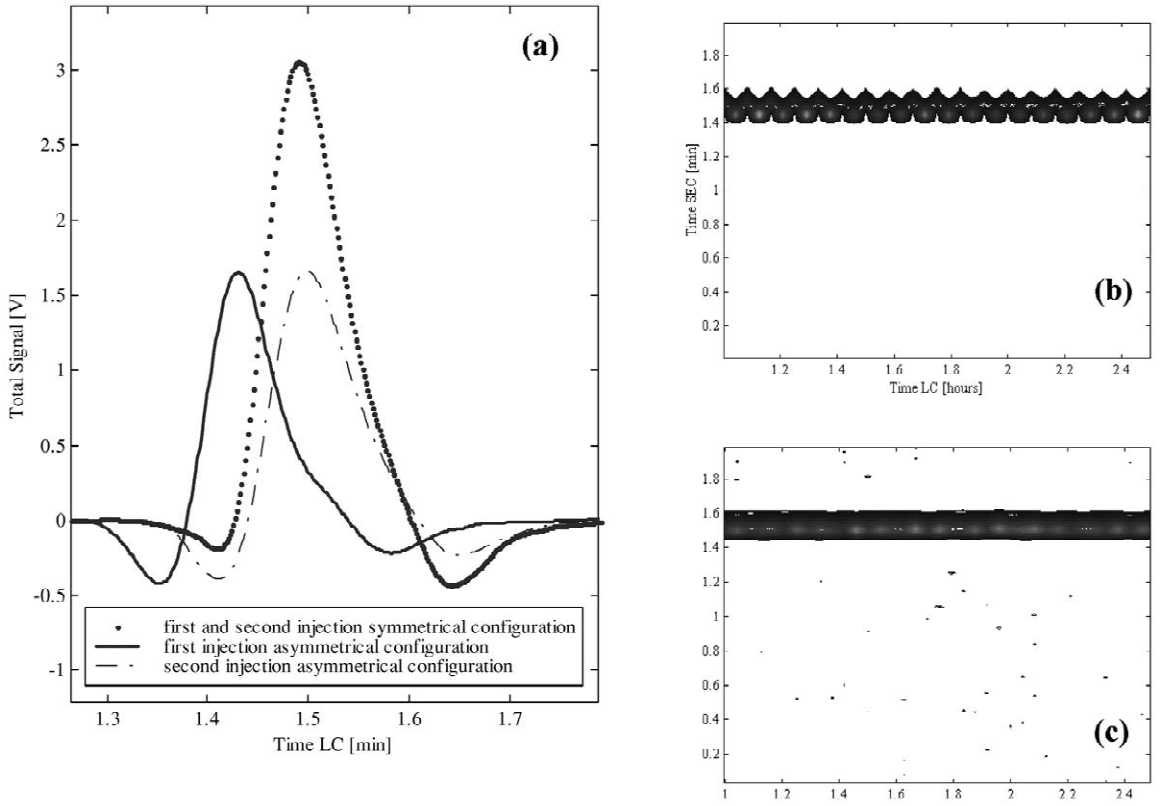


Fig. 3. (a) Extracted LC chromatograms obtained using symmetrical and asymmetrical injection configurations in LC×SEC. (b) Contour plot of toluene; asymmetrical configuration. (c) Contour plot of toluene; symmetrical configuration.

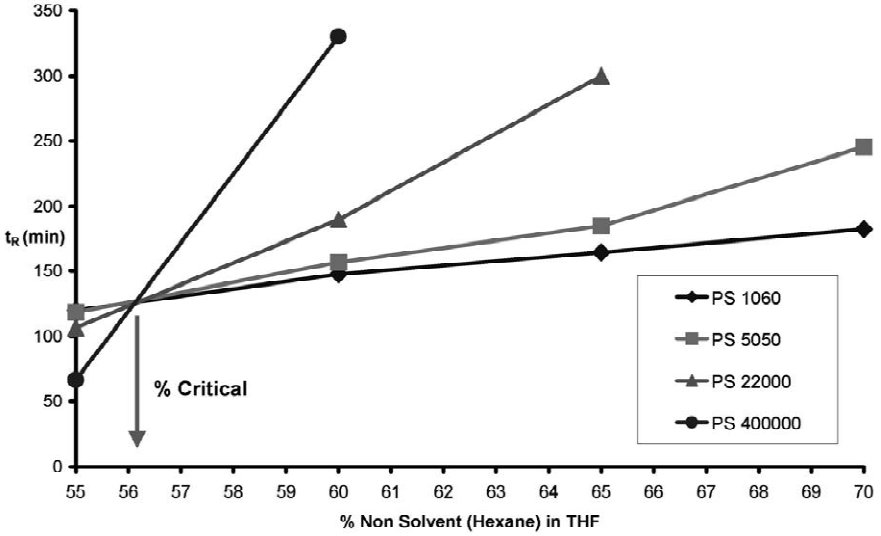


Fig. 4. Determination of the critical composition for polystyrene according to the method of Cools et al. [36]. Column Nucleosil Si 120, d_p = 3 μm, 120 Å, 250×10 mm I.D. eluent THF–hexane, flow 4 μl/min.

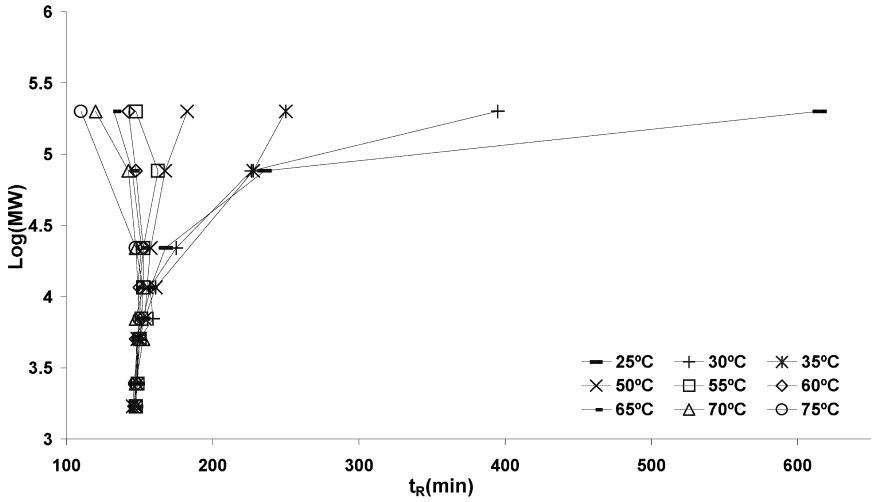


Fig. 5. Retention times (normalized using t_R of PS 1060) of polystyrene as function of molecular mass obtained from LC×SEC experiments at different temperatures. Column Nucleosil Si 120, d_p = 3 μm, 120 Å, 250×10 mm I.D. eluent: hexane–THF (58:42), flow 4 μl/min.

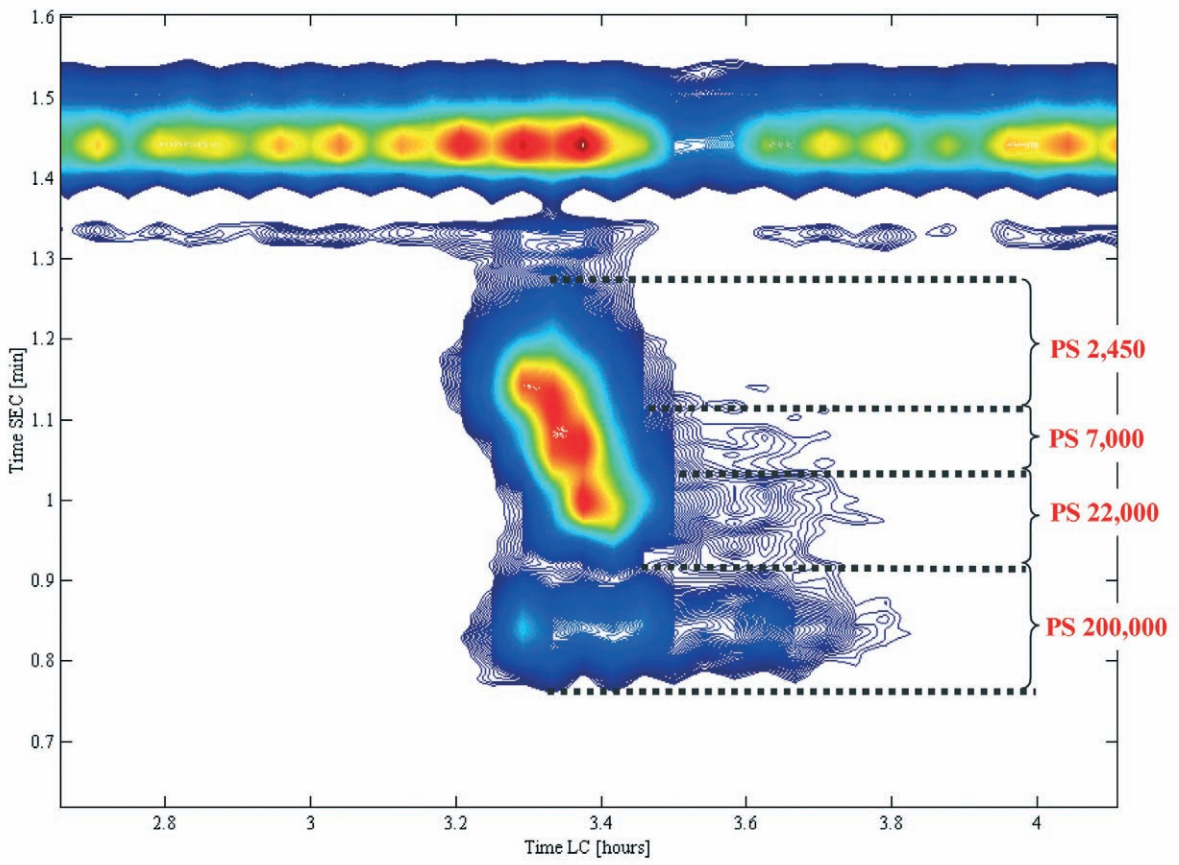


Fig. 6. LC×SEC chromatogram of four PS standards (M_w = 2450; 7000; 22 000 and 200 000) at near-critical conditions (55 °C). μLC column: 250×1.0 mm I.D. 3 μm 120 Å bare silica, 42-58 THF–hexane 4 μl/min. SEC column: 75×4.6 mm I.D. 5 μm Mixed-C, 100% THF 0.6 ml/min.

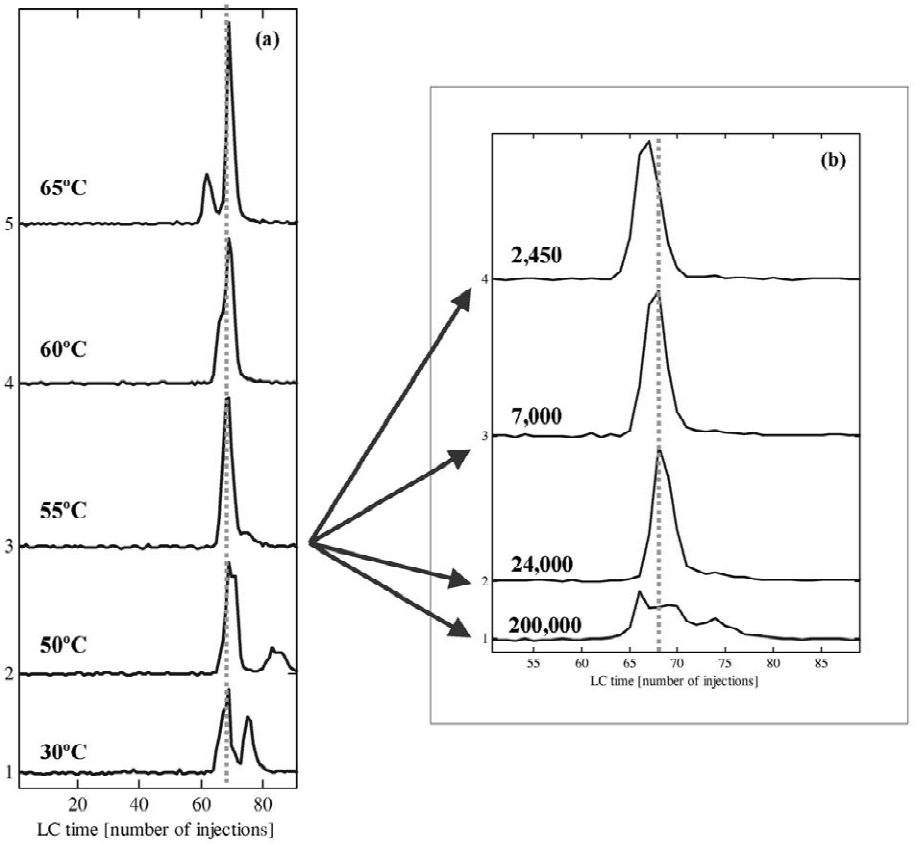


Fig. 7. (a) One-dimensional projections of the chromatograms in the LC dimension at different temperatures. (b) One-dimensional chromatograms (cuts) of four PS standards at critical conditions (same conditions as Fig. 6.).

the top half of Fig. 2) was not compatible with truly comprehensive two-dimensional liquid chromatography. This is illustrated in Fig. 3. The retention times observed for the toluene peaks are seen to differ significantly for the two loops. The reason for this is that the two loops are used differently. During the injection of the fraction into the second column, one of the loops is emptied in the forward-flush mode, the other loop in the back-flush mode. Because it is our desire to work quantitatively and thus comprehensively, the size of the loop must be at least as large as the volume of the collected fraction. Because of the parabolic flow profile in the loop the loop size must be significantly larger than the volume of the fraction for true comprehensiveness. This results in significantly different retention times for toluene (and all subsequent peaks) for the two loops (Fig. 3a) and to an undulation effect on the signals observed in LC×LC contour plots (1 mg/ml of toluene added to the first-dimension mobile phase; see Fig. 3b).

In Fig. 2b (bottom) an alternative, symmetrical configuration (II) is described. It can be seen in Fig. 3a,c that this configuration does yield identical retention times and peak shapes for the odd and even injections and thus a regular horizontal band for the toluene peak. This configuration allows partial filling of the loop, truly comprehensive operation and accurate quantitation.

2.3. Instrument control

A personal computer with Windows NT was equipped with a Keithley KNM-DCV 12 Smartlink

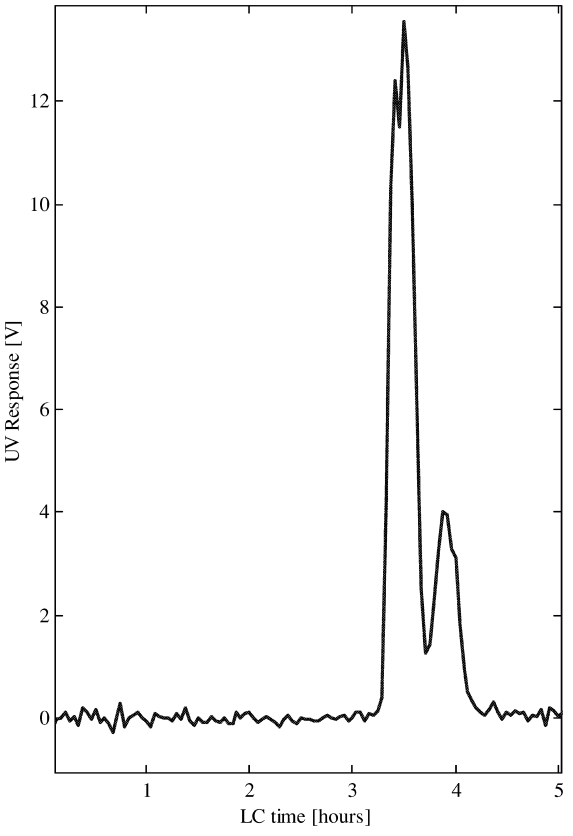
interface (Cleveland, OH, USA). Two-dimensional plots and distribution data where calculated with an in-house program written in a Matlab (Natick, MA, USA) software environment. This program enabled us to register and control the valve-switching time for the two-dimensional separation. The program options also allowed us to extract LC and SEC chromatograms at any positions in the LC×SEC contour-plot. Furthermore, the software was able to carry out quantification by computing peak volumes for specified retention ranges and to calculate MWDs and CCDs for different polymers. The latter is subject to calibration data provided by the user (polymer composition-dependent SEC calibration curves and retention versus mobile-phase composition curves for LC). Improved calibration procedures are the subject of further research.

3. Results and discussion

3.1. Using LC×SEC to study critical chromatography

Critical conditions for PS were established by recording a series of LC×SEC chromatograms of four PS standards (M_w = 1060, 5050, 22 000, and 400 000) with different mobile-phase compositions in the first dimension. Starting with 100% solvent (THF), the mobile phase was changed after each set of duplicate experiments, increasing the concentration of non-solvent (*n*-hexane) by 5% in each step. With the selected first-dimension column and flow-rate, the retention time of an unretained compound was approximately 2 h. Fractions of the first-dimension (''heart-cuts'') were taken every 2.5 min. The (room) temperature was kept constant during the analysis at ca. 20 °C. Each experiment was performed in duplicate. The observed retention times for the PS standards were plotted against the percentage of non-solvent (Fig. 4), following the method of Cools et al. [36]. This figure provides a clear indication of the critical composition at room temperature for PS on the present Hypersil-silica column (ϕ_{cr} = 56% *n*-hexane in non-stabilized THF). The retention time at the critical composition is about 120 min.

Since temperature is said to play an important role in critical chromatography [8,11,37–49], it was kept constant during the above characterization of polystyrene standards. Once an approximate critical composition had been established using the procedure described above (Fig. 4), fine tuning was performed by varying the temperature. From measurements shown in Fig. 5, it can be seen that the best critical conditions (smallest dependence of



(a)

Fig. 8. (a) One-dimensional projection of the LC separation of a mixture of PS standards (M_w = 2450, 7000, 22 000 and 200 000) and PS-OH (M_w = 10 000 and 100 000) at critical conditions for PS. μLC-column: 250×1.0 mm I.D. 3 μm 120 Å bare silica, 42/58% THF–hexane 4 μl/min (55 °C). SEC column: 75×4.6 mm I.D. Mixed-C, 100% THF 0.6 ml/min. (b) A two-dimensional LC×SEC chromatogram of a mixture of PS standards (M_w = 2450, 7000, 22 000 and 200 000) and PS-OH (M_w = 10 000 and 100 000) at critical conditions for PS. μLC-column: 250×1.0 mm I.D. 3 μm 120 Å bare silica, 42/58% THF–hexane 4 μl/min (55 °C). SEC column: 75×4.6 mm I.D. Mixed-C, 100% THF 0.6 ml/min.

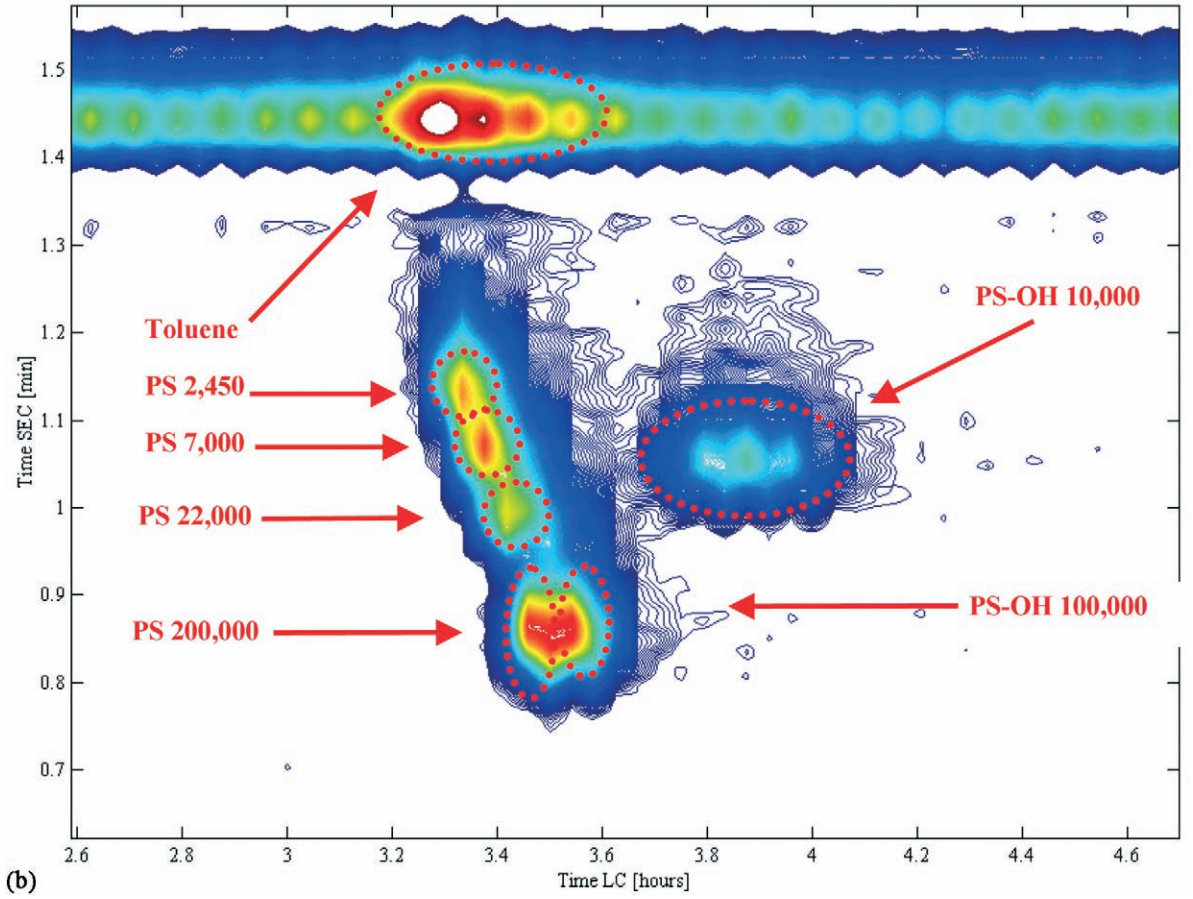


Fig. 8. (*continued*)

retention on molecular mass) for PS at a ϕ_{cr} of *n*-hexane–THF (58:42) were achieved at a temperature of 55 °C (for comparable results see Refs. [28,50,51]). An LC×SEC chromatogram of four PS standards at these critical conditions is shown in Fig. 6. The power of two-dimensional LC×SEC in the study of the critical behaviour of PS is the extra information obtained on the effect of molecular mass influence on retention. It can be clearly seen in Fig. 6 that some variation of retention with molecular mass pertains. This can also be seen in Fig. 5, which is constructed based on the LC×SEC retention data. In the range of relatively low-molecular masses retention is seen to increase slightly with M_w. This suggests that we are just on the adsorption side of the critical point. However, the largest standard (M_w = 200 000) is seen to elute earlier than the smaller standards. It is also shown to give rise to substantial tailing. The probable explanation for these observations is that the largest standard is almost completely excluded from the pores of the stationary phase. Around the total exclusion volume tailing peaks are usually observed [52]. The explanation is reasonable given the specified pore diameter (120 Å, given by the column manufacturer) and the calculated size of the molecules of a M_w = 200 000 PS standard in THF ($r_g \approx 200$ Å; see Ref. [52]). The mobile-phase composition puts us slightly on the adsorption side, but the surface area available to excluded molecules is very small, so that exclusion is the dominant effect at high M_w.

None of this information would have been available from one-dimensional chromatography on a broad (polydisperse) sample, as is evident from the

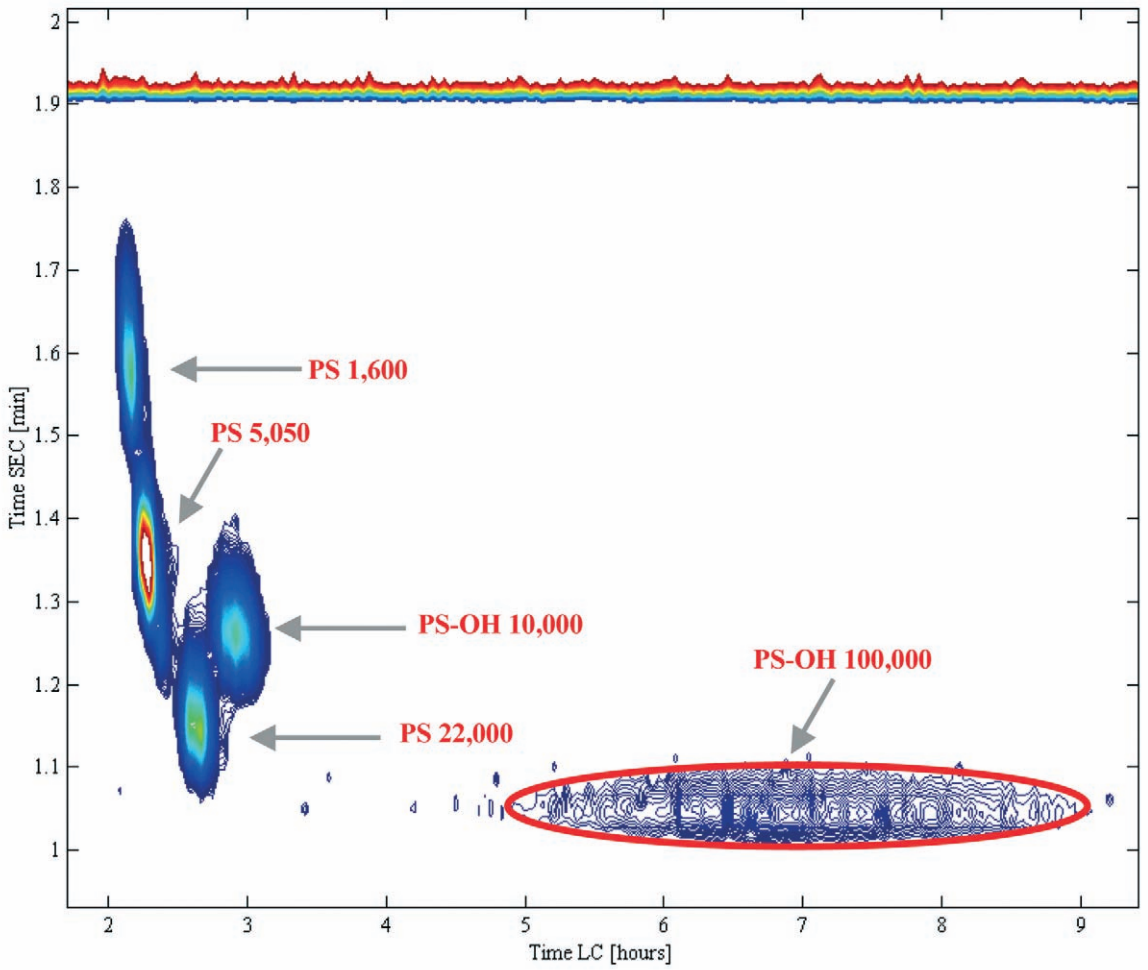


Fig. 9. LC×SEC chromatogram of a mixture of PS standards (M_w = 1060, 5050 and 22 000) and PS-OH (M_w = 10 000 and 100 000) at near critical conditions. μLC column: 250×1.0 mm I.D. 3 μm 120 Å bare silica, 40-60 THF–hexane 3 μl/min (25 °C). SEC column: 50×7.5 mm I.D. 3 μm Mixed-E, 100% THF 0.8 ml/min.

one-dimensional projection(s) of the chromatograms in the LC dimension (shown as Fig. 7a) and the slices shown as Fig. 7b. From the series of chromatograms at different temperatures (Fig. 7a) the only sensible observation is that the peak observed for the entire sample is narrowest at 55 °C. If individual standards are available they can be injected separately. In that case, a number of (short) one-dimensional runs provide the same information as a single (long) comprehensive two-dimensional liquid chromatogram. By comparing the chromatograms of narrow standards with those of the mixture it may also be deduced that perfect critical conditions have not been achieved. A residual variation of retention with M_w can be observed in Fig.7b. The LC×SEC chromatogram reveals that further trial-and-error work will not result in better (more-critical) conditions. Rather, it suggests that a column (packing material) with larger pores should be selected in combination with a slightly stronger eluent. In most practical situations narrow standards are not available. Thus, LC×SEC is shown to be a highly useful

tool to establish critical conditions and to study LC around the critical point of adsorption.

3.2. Functionality-type distributions

The LC×SEC separation of PS standards with one hydroxyl end group (PS-OH) gave remarkable insights in the analysis of polymers according to FTD and MMD simultaneously. As shown in Fig. 8a, a mixture of non-functional and mono-functional standards yields two clearly separated peaks. It is tempting to conclude that the first peak represents the non-functional standards and the second one the mono-functional ones. This is the expected elution order, due to the enhanced interaction of the OH end group with the silica surface. Indeed, a small mono-functional PS–OH standard ($M_w = 10\,000$) does elute under the second peak. However, the comprehensive two-dimensional liquid chromatogram in Fig. 8b shows that a large mono-functional PS–OH standard ($M_w = 100\,000$) virtually coincides with a non-functional PS standard ($M_w = 200\,000$) on the first-dimension axis. The explanation is the same as that provided above. The high-M_w standards are (almost) completely excluded from the pores, leaving very little surface area for adsorption-based separation.

Fig. 9 shows that the various non-functional and mono-functional standards can be separated on the present LC×SEC system using near-critical conditions in the first dimension. Under these conditions the large standards are strongly adsorbed. Now it is somewhat of an advantage that the high-M_w standards are excluded, so that their retention does not become prohibitively long. However, a very broad peak is observed for the $M_w = 100\,000$ PS-OH standard. Again, a stationary phase with larger pores may ultimately lead to better separation conditions.

3.3. Chemical-composition distributions of copolymers

Chemically inhomogeneous copolymers, which exhibit a broad chemical-composition distribution, are often analyzed using gradient-elution LC [10,36,37]. Separating polymers according to their CCD using gradient LC gives only one distribution and the drastic assumption has to be made that retention is independent of M_w (in case of gradient elution this is referred to as pseudo-critical chromatography). The one-dimensional LC chromatogram of a mixture of five PS standards, five PMMA standards and four S-co-MMA copolymers (extracted from the two-dimensional LC×SEC-ELSD contour plot of Fig. 11a) is shown as Fig. 10. The LC-chromatogram shows six peaks. Because we have standard materials available, we can verify that these are separated according to chemical composition. Without the availability of standards, this could be confirmed with an informative detection device, such as an IR [26,32,53] or NMR spectrometer [32,54]. Fig. 11a shows an LC×SEC chromatogram obtained using ELSD detection. Fig. 11b is based on UV detection at 254 nm. In this case the former detector is more sensitive, yielding less-noisy signals, and is selective towards non-volatile analytes. Thus, no signal arising from the gradient solvents used in the first dimension is observed in Fig. 11a around t_0 (top of the figure). PMMA homopolymer shows hardly any UV absorbance. Thus, only the high-M_w PMMA standards are seen as small peaks on the left of Fig. 11b. ELSD detection is notoriously non-linear. UV detection allows better quantitation, but it is specific to chromophors and the response is a strong function of the chemical composition.

The injection solvent used for the separations in

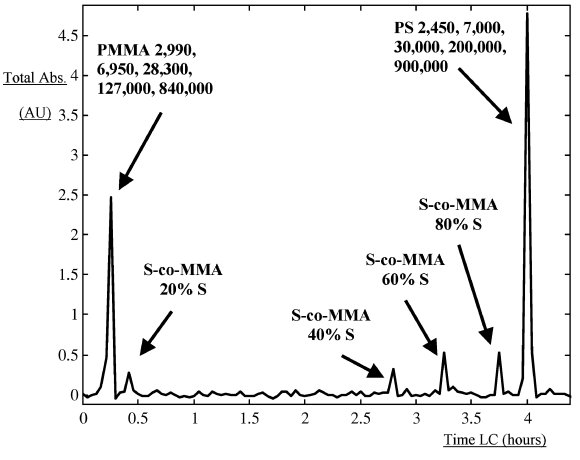
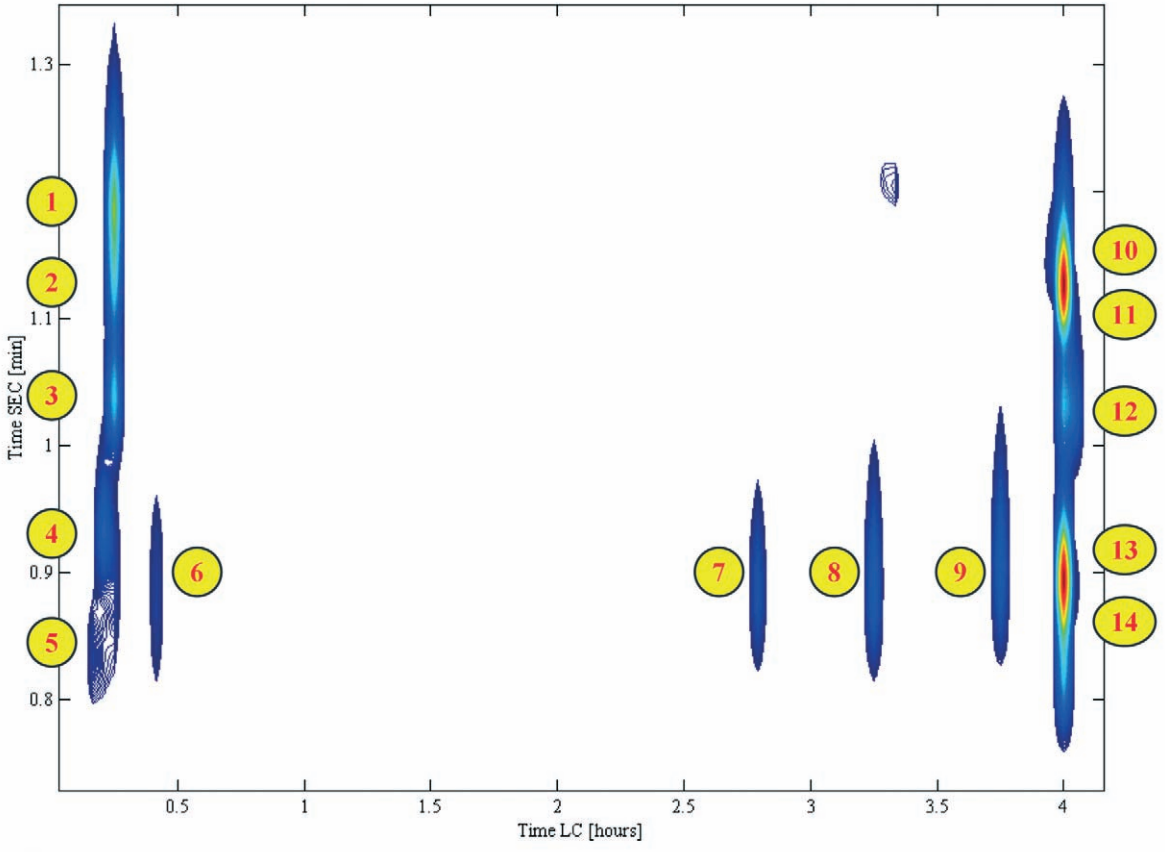


Fig. 10. Extracted LC–ELSD chromatogram of a mixture of 5 PMMA, 5 PS and 4 S-co-MMA standards (see Fig. 11a for conditions).

Figs. 10 and 11 was dichloromethane (DCM), which is a solvent for both PS and PMMA, but a weak eluent on bare silica. As a consequence [28], no breakthrough peaks were observed. LC×SEC is an excellent tool to study the phenomena of breakthrough peaks.

In principle, the chromatograms in Fig. 11 contain all the information required to determine a complete two-dimensional CCD×MWD distribution. The LC-axis (*x*-axes in the contour-plot) corresponds to the CCD. Fitzpatrick et al. [55] have already shown that fundamental relationships may be established between the chemical compositions of these S-co-MMA copolymers and their LC retention times. Only information on homopolymer standards was used to compute such a calibration curve. The SEC-axis (*y*-axes in the contour-plot) corresponds to the size of the PS, PMMA and S-co-MMA molecules in solution. Unfortunately, it is still quite difficult to convert this information to accurate molecular masses for copolymers. Interpolation of the SEC calibration curves for the respective homopolymers may be very dangerous [56]. At this stage we compute MWDs of the S-co-MMA relative to PS or PMMA standards. This procedure if often used in the SEC of copolymers. It yields reasonably precise, but inaccurate results. Despite the remaining calibration issues, LC×SEC yields a much better characterization of



(a)

Fig. 11. (a) LC×SEC-ELSD contour plot of a mixture of PMMA 2900 (1), 6950 (2), 28 300 (3), 127 000 (4), 840 000 (5); S-co-MMA 20% S (6), 40% S (7), 60% S (8), 80% S (9); PS 2450 (10), 7000 (11), 30 000 (12), 200 000 (13) and 900 000 (14). LC: C_{18}-column; flow 4 μl/min; gradient 5–70% THF in acetonitrile 0–300 min (40 °C). SEC: Mixed-C-column; flow 0.6 ml/min THF. (b) LC×SEC-UV contour plot of a mixture of PMMA, S-co-MMA and PS (see (a) for conditions).

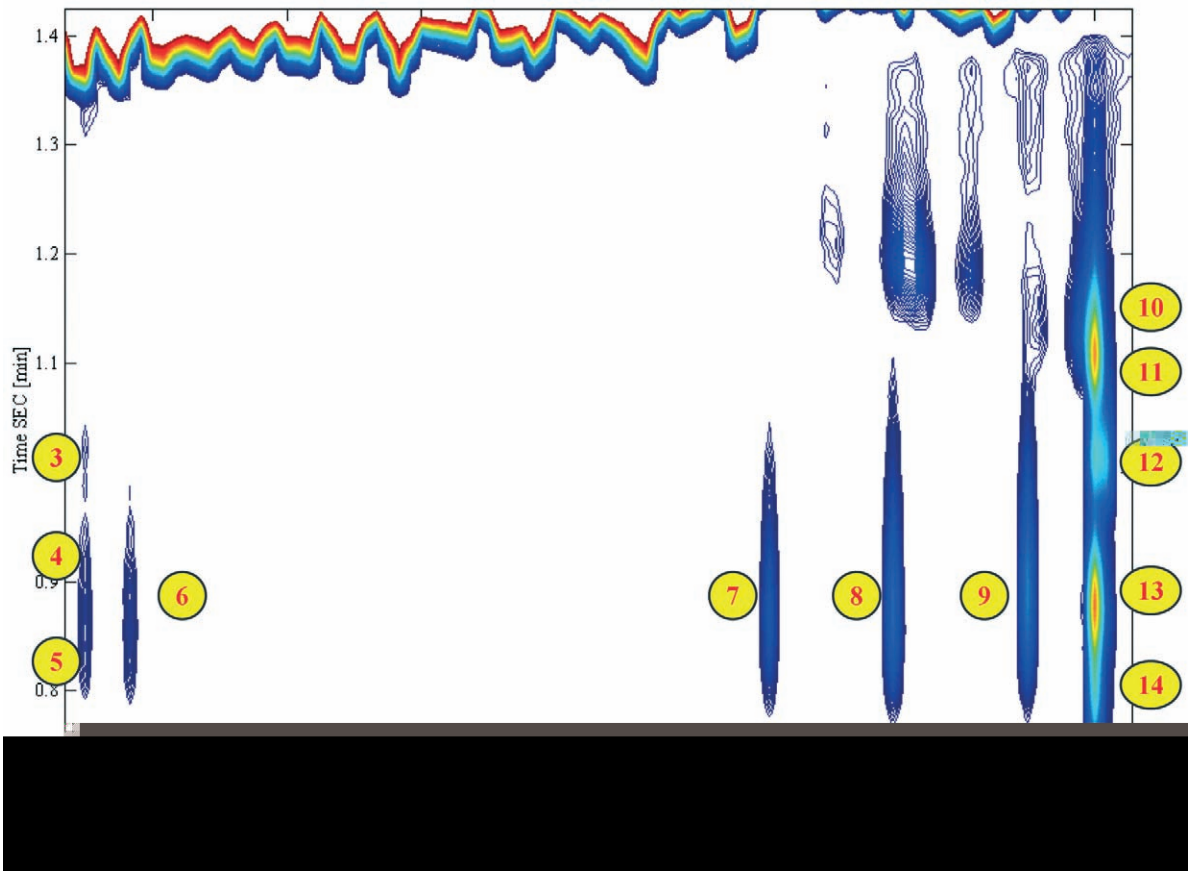


Fig. 11. (*continued*)

complex copolymers than either of the two separation techniques separately.

4. Conclusions

Synthetic polymers are very complex mixtures, which often feature several distributions simultaneously. In order to characterize such distributions, multi-dimensional separations are essential. LC×SEC is an eminently suitable comprehensive two-dimensional liquid-chromatographic technique, which has significantly matured in a number of research laboratories. In order to perform quantitative LC×SEC fully comprehensive operation must be stressed. This has been achieved by implementing a miniaturized separation column (1 mm I.D.) in the first dimension and by a new, symmetrical configuration of a 10-way switching valve.

The usefulness of LC×SEC for the separation and characterization of synthetic polymers has been demonstrated by a study of retention behaviour in the vicinity of the critical conditions, and by studying the separation of functional polystyrenes and of copolymers of polystyrene and PMMA. LC×SEC yields much more and more-reliable information on retention mechanisms, functionality-type distributions and combined chemical-composition and molecular-mass distributions than either of the individual one-dimensional separation techniques.

Comprehensive two-dimensional liquid chromatography in general and LC×SEC in particular will greatly benefit from faster second-dimension separations. Currently, we perform many experiments with a second-dimension analysis time of about 2 or

2.5 min and a total run time of about 4 h. By reducing the second-dimension analysis time to 30 s or less, LC×SEC may become an acceptable tool for (industrial) polymer-analysis laboratories.

Much work is still needed to accurately convert LC retention data to functionality or chemical composition and, especially, to convert SEC retention data to molecular masses for complex copolymers.

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