

## Critical retention behaviour of polymers A study on the influence of some practical parameters

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### Abstract

Liquid chromatography under critical conditions is an important tool for the microstructural characterization of telechelic polymers and block copolymers. Until now, only little information on the practical aspects of this technique is available. The influence of some important practical parameters was investigated, using polystyrene. Critical conditions depend strongly on the type of column packing. The solubility of polymers under critical conditions for different solvent–non-solvent combinations differs to a great extent. For different solvent–non-solvent pairs on reversed-phase systems, a roughly constant eluent strength in terms of the Hildebrand solubility parameter, under critical conditions is found. Temperature can be a useful tool for fine-tuning critical conditions. On normal-phase systems, however, the retention of polystyrene changes non-monotonously with temperature, which limits the use of temperature variations. It is not possible to obtain exact molecular mass independence on any of the investigated systems, which can not be ascribed to chemical differences. This makes the validity of the current theories on critical conditions questionable. Especially for the higher-molecular-mass polystyrenes, peak broadening increases significantly when going from size exclusion conditions to critical conditions. This phenomenon can limit the application of liquid chromatography under critical conditions to a certain molecular mass range. The composition of the solvent in which polystyrenes are dissolved prior to injection, has to be exactly the critical solvent composition, in order to suppress zone splitting as much as possible. For higher-molecular-mass polystyrenes this effect cannot be completely prevented.

**Keywords:** Retention behaviour; critical conditions; Solubility parameters; Temperature effects; Mobile-phase composition; Peak shape; Critical solvent composition; Polymers; Polystyrene

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### 1. Introduction

Mechanical properties of polymers and copolymers are strongly related to their distributions in molecular mass, composition and endgroups. Also the hydrodynamic volume of a coil is strongly

influenced by these factors. These distributions can be determined by liquid-chromatographic methods.

The chromatographic behaviour of polymers can be divided into two different modes: exclusion and adsorption. Critical conditions in the chromatography of polymers are defined as those conditions where entropic exclusion effects are exactly compensated by enthalpic adsorption effects, so the retention is solely governed by small differences in the chemical

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structure of the polymer chains, such as endgroups [1]. Especially when chromatography under critical conditions is combined with size-exclusion chromatography (SEC), information about the distribution of endgroups in relation to the molecular-mass distribution can be obtained [2–8]. It has also been shown that under critical conditions, block-copolymers can be characterized with respect to their block length-distribution [9–21].

Although in some cases it is recognized that chromatography under critical conditions can indeed be very critical and difficult to perform, only little information is available on the real practical aspects of this technique.

The main objective of the present work was to examine the effect of some important variables such as column and eluent type, and temperature. The exact behaviour of polymers under (near) critical conditions was carefully studied and compared with theoretical predictions. Also the effect of solvent composition, in which the polymers were dissolved prior to injection, was investigated. The aim of these experiments was to provide more insight in the practical possibilities and limitations of chromatography under critical conditions. The polymer used in our studies is polystyrene because of its availability as SEC standards over a wide molecular-mass range with low polydispersivity, and because it can be readily dissolved in some commonly used solvents for HPLC.

## 2. Theoretical background

The first theory on the chromatographic behaviour of macromolecules was presented by Cassaca [22]. Based on his approach, Gorbunov and Skvortsov developed a model to describe the behaviour of macromolecules on porous adsorbents [23,24].

The basic idea in their concept was the interaction energy  $-\epsilon$ , which is a measure of the affinity of a monomer unit towards the adsorbent. It depends on the chemical character of the polymer, the type of adsorbent, solvent and the temperature. Below a certain value,  $-\epsilon_{cr}$ , little or no interaction with the substrate takes place. The distribution of macro-

molecules will be governed by entropic exclusion effects. In thermodynamic terms this means that  $\Delta H = 0$  and  $\Delta G = -T\Delta S > 0$ . The exact value of  $\Delta G$  also depends on the hydrodynamic volume of the macromolecules and the pore width. The distribution coefficient  $K_d$  (in RT units) which is related to  $\Delta G$  according to:

$$K_d = \exp(-\Delta G) \quad (1)$$

will be smaller than unity. Under these conditions, a chromatographic system will be in the SEC mode.

When  $-\epsilon$  increases due to a decrease in solvent quality, enthalpic interaction effects will start to contribute to the total distribution of the macromolecules. When the value  $-\epsilon_{cr}$  is exceeded, the distribution will be dominated by these effects and  $\Delta G < 0$ ,  $K_d > 1$ . A chromatographic system under these conditions is in the adsorption mode. Because of the fact that longer molecules possess more monomer units to interact with the adsorbent, they will be stronger retained than smaller molecules. The elution order will be in the direction opposite to that in SEC, meaning that low-molecular-mass species are eluted first.

When  $-\epsilon$  equals  $-\epsilon_{cr}$ , the enthalpic interaction effects are exactly compensated by the entropic exclusion effects. These conditions are called 'critical conditions'. Under these conditions the distribution coefficient  $K_d$  is independent of the chain length:  $\Delta G = 0$ ,  $K_d = 1$ .

Chemical differences between the polymer chains, such as functional (end) groups will generally exhibit an affinity towards the substrate, different from that of the monomer unit. As a consequence, the interaction energy  $-\epsilon$  also will be different. Under critical conditions the value of ( $\Delta G$ ) of a functional macromolecule will only be determined by energetic interactions of the functionalities. This means that  $K_d$  values of functional and non-functional macromolecules are different, and only depend on the number and type of the functionalities. As a result, a separation will occur exclusively to functionality [2–8]. Entelis et al. derived expressions for the  $K_d$  values of various functional molecules [1].

When combined with a second separation technique such as SEC, chromatography under critical

conditions can provide unique information about the microstructure of polymers such as the distribution of endgroups in relation to the molecular mass.

Other authors [9–21] have shown that chromatography under critical conditions is also applicable to block-copolymers. The interaction energies of chemically different blocks will mostly differ significantly. So under critical conditions for the A-block of an AB block-copolymer, the presence of the B-block has the same effect as, for instance, an endgroup.  $K_d$  will therefore only be determined by the B-block. When conditions are chosen in such a way that critical conditions for A, are forming exclusion conditions for B, then the elution of the block-copolymer will solely be determined by the block length of B. The resulting SEC curve will thus provide information about the block-length distribution of B.

### 3. Experimental

#### 3.1. Solvents

The solvents used were dichloromethane (DCM), acetonitrile (ACN), *n*-hexane (HEX), water (H<sub>2</sub>O) and toluene (TOL), all LiChrosolv quality from Merck (Darmstadt, Germany) and tetrahydrofuran (THF), HPLC grade from Rathburn (Brunswick Chemie, Amsterdam, Netherlands).

Solvents used for experiments under normal-phase conditions were dried for at least 24 h on a molecular sieve, 0.3 nm, from Merck. During the experiments the solvents were constantly sparged with helium (20 ml/min). All solvent mixtures were made by volumetric mixing by the HPLC pump; no premixes were used unless indicated otherwise.

#### 3.2. Polymer samples

The polymers used for the experiments were narrow standard polystyrenes (PS) from Waters (Milford, MA, USA) ( $M_p$ : 1800, 3600, 8500, 35 000, 110 000, 200 000, 240 000 and 450 000 g/mol;  $M_p$  is defined as the molecular mass at the maximum

(peak) of the molecular mass distribution. Values were supplied by the manufacturer; polydispersity,  $D$ , of all standards <1.10). All samples were dissolved in the eluent, except for the experiments in which the effect of sample solvent was investigated. The injected volume was 5  $\mu$ l.

#### 3.3. Columns

The columns used were Novapak-C<sub>18</sub> (Waters,  $d_p=4$   $\mu$ m, pore size 60 Å, 150×3.9 mm, plate count/m ca. 80 000, Nucleosil-C<sub>18</sub>-100 (Machery-Nagel, Düren, Germany,  $d_p=7$   $\mu$ m, pore size 100 Å, 250×4.0 mm, plate count/m ca. 40 000), Nucleosil-C<sub>18</sub>-4000 (Machery-Nagel,  $d_p=7$   $\mu$ m, pore size 4000 Å, 250×4.0 mm, plate count/m ca. 12 000), PL-gel styrene-divinylbenzene (Polymer Laboratories, Shropshire, UK,  $d_p=10$   $\mu$ m, pore size 100 Å, 300×7.5 mm, plate count/m ca. 60 000),  $\mu$ Styragel styrene-divinylbenzene (Waters,  $d_p=10$   $\mu$ m, pore size 1000 Å, 300×7.8 mm, plate count/m ca. 25 000), Resolve silica (Waters,  $d_p=5$   $\mu$ m, pore size 90 Å, 150×3.9 mm, plate count/m ca. 55 000),  $\mu$ Bondapak-NH<sub>2</sub> (Waters,  $d_p=10$   $\mu$ m, pore size 125 Å, 300×3.9 mm, plate count/m ca. 25 000). The number of theoretical plates was determined for a low-molecular-mass solute under conditions such that  $k'$  was between 5 and 10.

#### 3.4. HPLC equipment

All HPLC experiments were performed, using a Waters 600E 4 solvent gradient pump, and a 717 autosampler from Waters. The detector was a Bruker (Bremen, Germany) variable-wavelength detector, type LC313-I, which was set at 254 nm. The column temperature was controlled, using a thermostat, type Mistral form Spark-Holland (Emmen, Netherlands). Chromatograms were recorded using the Baseline-815 system from Waters. The eluent flow for all experiments was established at 0.5 ml/min.

#### 3.5. Strategy

For the systems investigated, critical conditions were obtained by determining the retention times of

three polystyrene samples at different eluent compositions, starting with 100% solvent. During these experiments, the temperature was held at 30°C. All retention times were measured at least two times. For a properly conditioned system, variations between duplicate experiments did not exceed 0.01 min.

By plotting retention time versus percentage non-solvent (NS) for each of the samples, the critical point was determined from the intersection point of the different curves [25]. This point was called the critical solvent composition (CSC). Because the accuracy of the pump was only 1% NS, the conditions were optimized by varying the temperature (1 or 2°C per step) until retention times of the three polystyrenes coincided as closely as possible.

After each change in percentage NS or in temperature, the system was equilibrated by pumping at least 15 column volumes at a flow-rate of 2 ml/min, followed by pumping at 0.5 ml/min during at least 20 min. By repeated injections of the same sample it was checked whether the system had reached equilibrium.

## 4. Results and discussion

### 4.1. Effect of the column type on critical conditions

Critical conditions for polystyrene were determined according to the method of Cools et al. [25] on several columns, using various different solvent–non-solvent combinations. An example of this method is given in Fig. 1. The results for the different systems are shown in Table 1. As already has been mentioned, critical conditions were fine-tuned by adjusting the temperature. Differences in percentage of solvent under critical conditions on different phase-systems, caused by temperature differences, are always less than 1% solvent.

From measurements, listed in Table 1, on two Novapak-C<sub>18</sub> columns, differing in column length only, it can be seen that CSC does not depend on column length. Because this means that  $K_d$  is also independent of column length, this result corresponds with theoretical expectations. The observed CSC is also in close agreement with other results

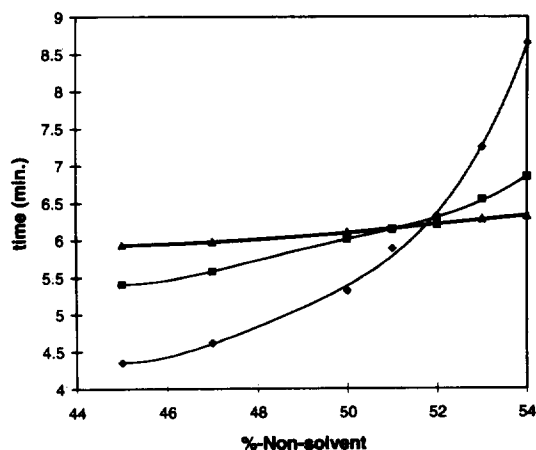


Fig. 1. Determination of critical conditions according to the method of Cools et al. [25]. Column Nucleosil-C<sub>18</sub> (100 Å), eluent DCM–ACN, temperature 25°C, flow 0.5 ml/min, (◆) PS 35 000, (■) PS 8500, (▲) PS 1800.

[25] providing a good indication of the reproducibility of the method.

In Table 1, measurements on Nucleosil-100 and Nucleosil-4000 are shown to result in the same CSC. From this result it can be concluded that  $K_d$  is independent of pore size which is also in agreement with theoretical expectations [23,24].

For the S–NS combination DCM–ACN, the same CSC is found on Novapak-C<sub>18</sub> and on Nucleosil-C<sub>18</sub>. This means that in the reversed-phase (RP) mode, when the same type of derivatized silica from

Table 1  
Critical conditions for polystyrene on different phase systems

Column	Solvent–non-solvent (v/v)	Temperature (°C)
Novapak-C <sub>18</sub> (15 cm)	THF–H <sub>2</sub> O 86:14	58
Novapak-C <sub>18</sub> (30 cm)	THF–H <sub>2</sub> O 86:14	48
Novapak-C <sub>18</sub> (30 cm)	DCM–ACN 58:42	25
Novapak-C <sub>18</sub> (30 cm)	THF–ACN 47:53	35
Nucleosil-C <sub>18</sub> (100 Å)	DCM–ACN 58:42	27
Nucleosil-C <sub>18</sub> (4000 Å)	DCM–ACN 58:42	31
Resolve silica	DCM–HEX 61:39	45
μBondapak NH <sub>2</sub>	DCM–HEX 48:52	25
PL gel (100 Å)	DCM–ACN 50:50	25

different manufacturers is used, CSC does not strongly depend on differences in the type of bare silica or on differences in the derivatisation processes. A significantly different CSC is found, however, when a PL-gel-styrene-DVB column is used. This seems quite logical because a change in chemical nature of column packing will give rise to different column interactions. In the latter case a change in retention mechanism may occur from a partitioning-like ( $C_{18}$ ) to a more adsorption-like (styrene-DVB) mechanism [26]. This will of course strongly influence  $K_d$ .

Further proof for this expectation is provided by the results on two normal-phase columns. A difference in CSC of 13% solvent is found for a Resolve-silica (bare silica) column, as compared with a  $\mu$ Bondapak-NH<sub>2</sub> column. The large difference in CSC can be explained by the fact that on these columns, adsorption effects will be dominated by specific, polar interactions with the  $\pi$ -electrons of polystyrene. Because on Resolve-silica retention is caused by the much more polar silanol groups as compared with the active NH<sub>2</sub> sites on the  $\mu$ Bondapak column, polystyrene is stronger adsorbed on Resolve-silica and more solvent, acting as displacer, is needed to compensate for this. In general, differences in polarity will be more pronounced on normal-phase systems than on reversed-phase systems, in the former case causing larger differences in CSC as a function of specific sorbent type.

#### 4.2. Role of the eluent type

On the same column, critical conditions can be found for different solvent–non-solvent combinations as is demonstrated in Table 1 for the Novapak-C<sub>18</sub> column. A very important difference among these combinations is the solubility of the polymer used. For the THF–H<sub>2</sub>O couple for instance, the highest-molecular-mass polystyrene that could be dissolved was 35 000, whereas for the DCM–ACN combination polystyrenes up to a molecular mass of 450 000 could be dissolved.

For entropic reasons the solubility of polymers in general is mostly restricted to a few ‘good’ solvents.

Under critical conditions, the affinity towards the eluent can not be too high, in order to permit slight adsorption effects which are necessary for the compensation of entropic exclusion effects. Therefore, solubility of polymers under critical conditions will always be limited. From our results, however, it is clear that it is worthwhile trying different S–NS combinations because large differences in solubility can occur.

It is interesting to compare chromatographic eluent strength for the different S–NS combinations. For reversed-phase systems it is well known that the Hildebrand solubility parameter,  $\delta$ , is a good measure of the eluent strength [27]. The solubility parameter for the CSC using different S–NS combinations on the Novapak-C<sub>18</sub> column are given in Table 2. The  $\delta$  values used for THF, DCM, ACN and H<sub>2</sub>O are 18.6, 19.8, 24.3 and 47.9, respectively [27].

It is obvious that the  $\delta$  values for the different critical solution compositions are in close agreement with each other. The somewhat higher value for THF–H<sub>2</sub>O can probably be explained by the relatively large uncertainty in the  $\delta$  value for water.

Although the similarity in solubility parameters has yet to be confirmed for other polymers, in our opinion this is a very important result, because it offers a possibility of predicting CSC for other S–NS combinations after having determined critical conditions for only one combination. Especially in these cases where, for reasons of solubility of higher-molecular-mass species, different S–NS have to be evaluated, this can mean a large reduction in the number of measurements required.

#### 4.3. Influence of the temperature

Beside varying eluent composition, critical con-

Table 2  
Eluent strength for the CSC of different S–NS combinations on Novapak-C<sub>18</sub>

Solvent–non-solvent	(v/v)	Temperature (°C)	$\delta_m$ (MPa) <sup>0.5</sup>
THF–H <sub>2</sub> O	86:14	48	22.7
DCM–ACN	58:42	25	21.7
THF–ACN	47:53	35	21.6

ditions can also be established by adjusting the temperature. Because adsorption is an exothermic process, for low-molecular-mass solutes an increase in temperature generally speaking will lead to a decrease in retention [27]. Although this behaviour can also be observed for macromolecules, especially for coiled polymers this turns out to be not true in general. For these macromolecules, often an increase of adsorption is observed at elevated temperatures [28]. A chromatographic system under near critical conditions can undergo a transition from the (slight) adsorption mode via critical conditions to the exclusion mode by either raising or lowering the temperature, dependent on whether adsorption decreases or increases with temperature. An example is shown in Fig. 2 where retention times on a Novapak-C<sub>18</sub> column with a DCM-ACN (S-NS) system are plotted as a function of column temperature. A decrease of retention times was observed when the temperature was raised. Besides a decrease of adsorption, this effect can also be caused by improved solubility, which is widely believed to play an important role in reversed-phase chromatography [26].

As can be seen, the three lines in Fig. 2 do not intersect exactly in one point. This phenomenon will be discussed later.

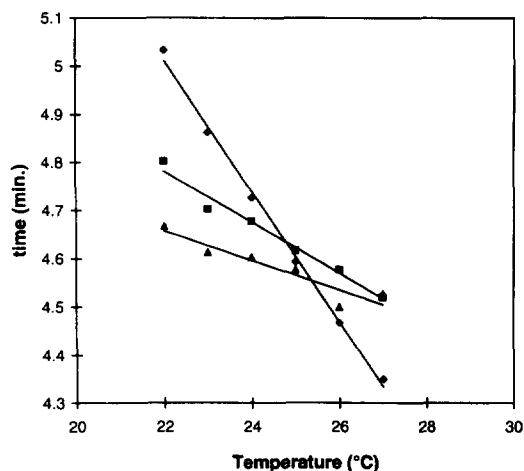


Fig. 2. Retention times of polystyrenes as function of column temperature. Column, Novapak-C<sub>18</sub> (30 cm); eluent, DCM-ACN (58:42); flow, 0.5 ml/min. (◆) PS 35 000, (■) PS 8500, (▲) PS 1800.

Temperature variations are preferred as a mechanism to fine-tune critical conditions over changing the eluent composition. In the latter case it is necessary to allow changes in composition as small as 0.1% solvent. The accuracy of most HPLC pumps being only 1% solvent implies the need of premixes. This makes CSC less reproducible caused by inaccuracies in making the premixes and selective evaporation of the most volatile component.

For low-molecular-mass species, very often a linear dependence is found when the chromatographic capacity factor ( $k'$ ) is plotted against the reciprocal temperature ( $1/T$ ) [27]. At first sight, Fig. 2, where an almost linear dependence is found between retention time (or the equivalent  $k'$ ) and temperature, seems to contradict this general tendency. It must be remembered, however, that the first case holds for chromatographic systems under adsorption conditions, whereas in our situation steric exclusion effects have a significant effect on the total retention. The mechanisms of both modes of chromatography are incomparable, so a difference in temperature dependence might be expected.

From Fig. 2 it is obvious that changes in retention time as a function of temperature are more pronounced at higher molecular mass. This was also observed in all reversed-phase systems investigated (see Table 1). This behaviour can probably be explained by the fact that temperature changes mainly affect the adsorption process. The temperature effect on the hydrodynamic volume and thus on the exclusion behaviour will be limited. Because the retention of large molecules is more influenced by adsorption than that of small molecules, the effect of temperature changes will be more pronounced in the former case.

Temperature effects were also examined on normal-phase systems. At first, increasing retention times were found with increasing temperature on a Resolve silica column under near-critical conditions, whereas on the  $\mu$ Bondapak-NH<sub>2</sub> retention times for PS 1800 and PS 8500 decreased and retention times increased for PS 35 000 when the temperature was raised. These results indicate that retention can change non-monotonously as a function of temperature. In order to verify this observation, the temperature range of examination was broadened considera-

bly for the Resolve silica column. Results for this column are plotted in Fig. 3. Since these measurements were carried out at another day, critical conditions were slightly different from former measurements. This caused the exact temperature dependence in the observed temperature window to differ somewhat from the initial observations.

The curve for PS 35 000 surprisingly shows a distinct minimum, but for the other two samples no such point could be found. However, as can be seen from Fig. 3, the slope of the curve of PS 8500 decreases with increasing temperature. Presumably, the curve exhibits a minimum at higher temperatures, but because of the boiling point of the eluent, this could experimentally not be confirmed.

The exact explanation of Fig. 3 is not straightforward but various attempts have been made to explain the observed phenomena.

(1) A decrease of adsorption with increasing temperature is frequently observed, even for low molecular solutes [29]. This can possibly be explained by the desorption of adsorbed trace amounts of water from the (non-aqueous) eluent at higher temperatures, thus generating more active silanol groups available for interactions with the solute [29].

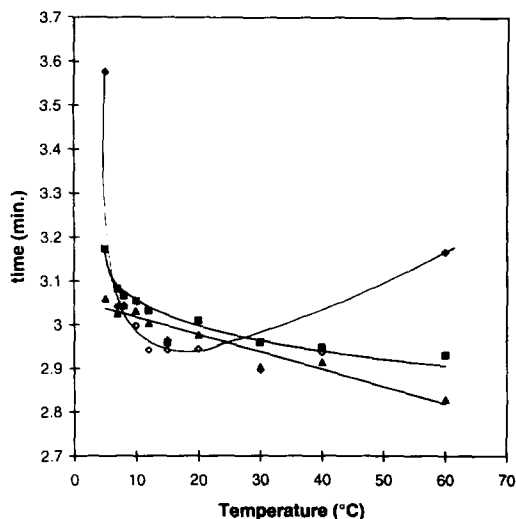


Fig. 3. Retention time as a function of temperature on Resolve silica. Eluent, DCM-ACN (62:38); flow, 0.5 ml/min. (◆) PS 35 000, (■) PS8500, (▲) PS1800.

By Scott and Kucera [30,31] it was shown that on polar column packings, preferential adsorption of the more polar component of an eluent mixture can occur. It is imaginable that at higher temperatures a shift in these adsorption equilibria occurs in such a way that the solute is adsorbed more strongly.

(2) These ideas, however, still leave the appearance of the minimum in the curve of PS 35 000 unexplained. It was considered by us that an increase in hydrodynamic volume at higher temperatures leading to lower retention and an increase in adsorption, that would lead to higher retention, can partially compensate each other, possibly providing a minimum. Therefore, the former effect was experimentally checked by the determination of retention times under SEC conditions at 10°C and 70°C. It was found, however, that retention time differences were only about 0.15 min (picture not shown here), thus providing no explanation for the much larger decrease in the first part of the curve of PS 35 000, which also covers a smaller temperature range.

(3) Therefore adsorption itself must vary non-monotonously with temperature which can be explained by the fact that adsorption of polymers does not proceed isothermally with temperature [28]. Different conformations at different temperatures can lead to differences in coverage of the surface by the polymer, making the change of adsorption with temperature very unpredictable.

It can be concluded that in contrast to reversed-phase systems, on normal-phase systems care must be taken with the variation of temperature to establish critical conditions. Although to our own experience for some polymers this method can even be successful on normal-phase systems; in other cases as e.g. polystyrene, variations in eluent composition have to be preferred.

#### 4.4. Retention behaviour under near-critical conditions

For each investigated system, it was observed that no conditions could be found at which retention times of different polystyrene standards exactly match as is predicted by the theory of Skvortsov and

Gorbunov [23,24]. An example of this observation is given in Fig. 4 where the temperature was varied to establish critical conditions on a Novapak-C<sub>18</sub> column. As can be seen, the behaviour of the two high-molecular-mass samples under near-critical conditions is rather uncontrollable. Small changes in

Peak areas ( $\mu\text{V}\cdot\text{sec}$ )					
Temp. ( $^{\circ}\text{C}$ )	PS1800	PS8500	PS35000	PS102000	PS240000
20	940000	871000	963000	922000	971000
22	943000	864000	973000	924000	968000
23	938000	878000	979000	927000	977000
24	920000	849000	946000	900000	958000
25	911000	885000	946000	900000	989000
40	946000	889800	960000	914000	970000

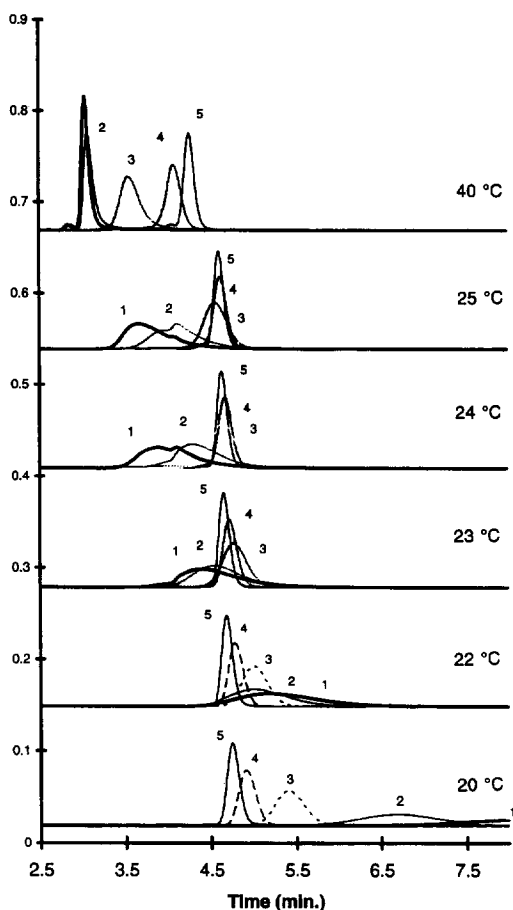


Fig. 4. Retention behaviour as a function of temperature under near-critical conditions. Column, Novapak-C<sub>18</sub> (30 cm); eluent, DCM-ACN (58:42); flow, 0.5 ml/min. Peaks: 1=PS 240 000, 2=PS 110 000, 3=PS 35 000, 4=PS 8500, 5=PS 1800.

temperature causes the retention behaviour to alter abruptly from adsorption to exclusion, or vice versa.

This behaviour can be explained by the fact that the molar masses of both polystyrenes are outside the linear range of selective permeation as can be seen from Fig. 5, in which SEC curves are given of different applied columns. Caused by the fact that little or no pore volume is available to these polymers, the equilibrium between adsorption and exclusion cannot be established properly. Use of columns with larger pores can prevent this problem, as could be concluded from experiments where a 4000 Å column was used (picture not shown here). From this result it becomes clear that the pore size must be chosen such that all polymers of interest lie within the linear part of the SEC calibration curve.

In Fig. 2 retention times of the three lowest-molecular-mass polystyrenes are plotted versus temperature. It is obvious that no temperature can be found at which the different retention times exactly match. The same result was obtained when the eluent composition was varied to establish critical conditions on a  $\mu$ Bondapak-NH<sub>2</sub> column (picture not shown). These observations cannot be explained by

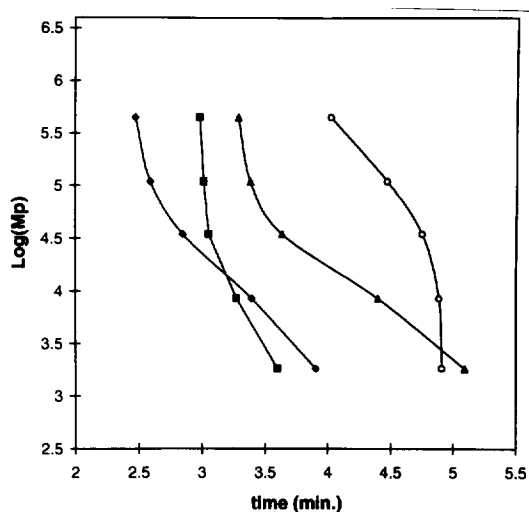


Fig. 5. SEC calibration curves of different used columns. Eluent, THF; flow, 0.5 ml/min; temperature, 30°C. (◆) Nucleosil-C<sub>18</sub>-100, (■) Novapak-C<sub>18</sub>, (▲)  $\mu$ Bondapak-NH<sub>2</sub>, (○) Nucleosil-C<sub>18</sub>-4000.



the fact that the molecular masses lie outside the linear SEC range of the column, as can be seen from Fig. 5. Another possibility might be the presence of small chemical differences in the standards, such as other initiator endgroups, although this is not very likely because the different standard polystyrenes were purchased from the same manufacturer. When this would be the case, however, the elution order of the different samples would have to be reversed when a normal-phase system was used instead of a reversed-phase system. The same polarity difference that would cause PS8500 to elute later on the  $C_{18}$  column as compared to the other two samples, would lead to an earlier elution on, for instance, the  $\mu$ Bondapak-NH<sub>2</sub> column. Instead of this expected behaviour, however, the same elution order was observed on both types of systems. So the fact that retention times under critical conditions are not exactly the same might be an indication that the present theories on the behaviour of macromolecules on porous media are imperfect.

According to the model of Skvortsov and Gorbunov [23] the chromatographic distribution coefficient,  $K_d$ , equals unity under critical conditions. The capacity factor  $k'$ , which is related to  $K_d$  by Eq. 2, must be equal to zero then, meaning that polymers under these conditions should elute exactly at the column void volume  $V_0$ . This seems to be in contradiction with our observations. Although the exact value of  $V_0$  is difficult to determine [32], it is generally accepted that the baseline disturbance caused by the elution of the injected solvent, roughly coincides with  $V_0$ . From the chromatograms in Fig. 4 it becomes clear that the polystyrenes under near-critical conditions elute significantly later than this baseline disturbance, which means that  $K_d$  must be greater than unity. Qualitatively this might be explained as follows. Under critical conditions the change in conformational entropy is exactly compensated by the change in adsorption enthalpy. The entropy change for a polymer in a porous medium will always have a small but positive value. This means that the enthalpy change must also be positive, causing the polymers to be moderately retained by the column, which provides an explanation of our observations [33].

Further studies on the chromatographic behaviour

of other types of polymers under near-critical conditions may indicate whether our observations hold more generally, thus necessitating adaptation of the current theories [23,24] on critical conditions.

#### 4.5. Peak broadening under near-critical conditions

From Fig. 4 it becomes obvious that peak broadening dramatically increases when the retention mode changes from exclusion to adsorption, especially for the higher-molecular-mass polystyrenes. The processes contributing to peak broadening are generally divided into the Eddy diffusion, axial diffusion and mass transport. Peak broadening in liquid chromatography is mainly governed by mass transport. In turn, this process can be subdivided into transport in the mobile phase, diffusion from the mobile phase to the stagnant mobile phase and diffusion into the stagnant mobile phase [27]. The theoretical plate height  $H_c$  can be expressed as a function of these three processes according to:

$$H_c = [H_{cm}] + [H_{csm}] + [H_{cs}] \quad (2a)$$

$$H_c = [C_m d_p^2 u / D_m] + [C_{sm} d_p^2 u / D_m] + [C_s d_f^2 u / D_s] \quad (2b)$$

in which  $D_m$  and  $D_s$  represent the diffusion coefficients in the mobile and stationary phases,  $u$  is the linear velocity of the mobile phase and  $C_m$ ,  $C_{sm}$  and  $C_s$  are plate height coefficients.

In the exclusion mode the third term hardly contributes to the peak broadening.  $D_s$  and  $D_m$  will decrease with increasing molecular mass, giving rise to broader peaks. This can be observed by comparing the peaks due to the three lower-molecular-mass polystyrenes in the chromatogram at 40°C from Fig. 4. The higher-molar-mass polystyrenes, however, lie beyond the linear SEC region of the column. The contribution of the second term from Eq. 2 will also be very small in these cases, thus giving rise to narrower peaks as compared to the lower-mass polystyrenes.

When adsorption starts to contribute to the total retention, peak broadening significantly increases as can also be observed in Fig. 4. This can probably be ascribed to the fact that the third term from Eq. 2

starts to contribute to the total broadening. It is remarkable, however, that under near critical conditions, where  $K_d$  for all molecular masses has about the same value, peak broadening for the high-molecular mass polystyrenes by far exceeds the broadening for the lower-mass samples. Two possible reasons for this observation can be given, of which the first, the decrease of  $D_s$  and  $D_m$  with increasing molecular mass, has already been mentioned. Secondly, it should be considered that the contribution of adsorption to the total retention under critical conditions, increases with molecular mass, because more exclusion has to be compensated. In that case, broader peaks may be caused by the increased importance of  $C_s$ .

For a few different systems, peak broadening under near-critical conditions was determined. The results presented in terms of the reduced plate height, are shown in Table 3. It is obvious that peak broadening depends on several different parameters.

By comparing the results for the two Novapak- $C_{18}$  columns it becomes clear that the reduced plate height decreases with increasing column length. This can only be explained by the reduced relative influence of extra-column broadening, when a longer column is used. The contribution to broadening of dead volume of injector, capillaries and detector increases for solutes having low  $k'$  values. Under near-critical conditions  $k'$  approaches zero, implying that these broadening effects will have a significant influence on the total peak broadening. Obviously the use of larger-volume columns can reduce this influence.

The applied S–NS combination also affects the peak width, which is probably caused by differences in diffusion coefficients. Of three tested couples, the THF– $H_2O$  combination, being the most viscous eluent, showed the largest back pressure. This is in agreement with the observation that for this combination the largest value for the reduced plate height is found.

The use of a column with larger pores reduces peak broadening especially for the higher-molecular-mass polystyrenes. A reasonable explanation for this observation in terms of Eq. 2 cannot be given yet. Qualitatively this might be due, however, to the fact that for the case of larger pore size less exclusion effects have to be compensated by adsorption, thus reducing the influence of this parameter on the total retention. Because adsorption causes peak broadening, peak width decreases with increasing pore size.

To our knowledge, the relatively large influence of peak broadening effects under critical conditions, especially for higher-molecular-mass polymers, has not been reported so far. Separation with respect to functionality can become very complicated due to this effect, because excessive broadening effects can easily cause zones to overlap, due to different functionalities. Because the theories on critical conditions [23,24] have been derived from thermodynamics, and thus only are able to predict peak maxima, peak broadening due to kinetic effects is not taken into account. Extension of these theories as to include also kinetic effects can provide more insight in the possibilities and limitations of chromatography under critical conditions.

Table 3  
Reduced plate height of polystyrenes under near-critical conditions on different separation systems

Column	Solvent–non-solvent	$N$	Reduced plate height ( $h$ )				
			PS 1800	PS 8500	PS 35 000	PS 110 000	PS 240 000
Novapak- $C_{18}$ (15 cm)	THF– $H_2O$	13 000	14	23	79	–	–
Novapak- $C_{18}$ (30 cm)	THF– $H_2O$	23 000	9	22	75	–	–
Novapak- $C_{18}$ (30 cm)	DCM–ACN	23 000	9	18	63	–	–
Novapak- $C_{18}$ (30 cm)	THF–ACN	23 000	9	18	53	–	–
Nucleosil- $C_{18}$ (100 Å)	DCM–ACN	10 000	6	9	19	61	168
Nucleosil- $C_{18}$ (4000 Å)	DCM–ACN	3000	6	7	8	11	15

$N$  represents the number of theoretical plates.

#### 4.6. Zone splitting

Small differences in composition between eluent and polymer solvent can lead to severe disturbances in the elution of polymers under near-critical conditions. An example is shown in Fig. 6. Although the system is still in the exclusion mode, a difference of 8% solvent causes PS 35 000 to elute in two chromatographic zones. This zone splitting disappears when the polymer is dissolved in the exact eluent composition.

From the chromatography of polymers it is well known that when a polymer is dissolved in a composition containing more solvent than the eluent, an effect called breakthrough can occur, causing a part of the sample to elute unretained due to insufficient mixing of the injection plug with the eluent [34]. It is remarkable that under near-critical conditions much smaller differences in percentage of solvent can lead to disturbances in the elution. To our experience even differences as small as 1% can cause zone splitting. It must be considered, however, that under near-critical conditions the average ve-

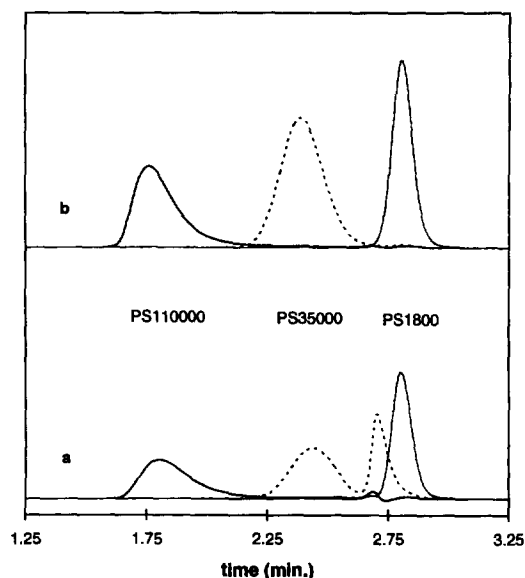


Fig. 6. Zone splitting caused by a difference between eluent and solvent composition. Solvent composition, DCM-HEX 55:45 (a), 63:37 (b). Column, Resolve silica; eluent, DCM-HEX (63:37); flow, 0.5 ml/min; thick line=PS 110 000, dotted line=PS 35 000, thin line=PS 1800.

locity of migration of the solvent nearly equals that of the polymer. This causes a continuous disturbance of the distribution of the polymer between the mobile and stationary phase which, under these conditions, is already very 'critical'.

In some cases zone-splitting effects occur even when the polymer is dissolved in the eluent composition. This is especially the case for the higher-molecular-mass polymers which, due to the broad peaks, co-elute with the void volume. An example of this can be seen in the chromatogram at 25°C in Fig. 4, in which the elution profile of PS 110 000 is anomalous, even when the chromatogram is corrected for the baseline disturbance at  $V_0$ . This effect becomes less pronounced when the difference between retention volume of polymers under critical conditions and  $V_0$  increases (results not shown here).

This is possibly caused by small system disturbances due to the injection, migrating with eluent speed and thus, under near-critical conditions, with the same average velocity as the polymers. Therefore zone splitting, especially for the higher-molecular-mass polymers cannot be completely suppressed by dissolving these in the eluent composition! To prevent misinterpretation, for instance in the case of a separation according to functionality differences, this effect has been taken into account when judging the results of chromatographic experiments under near-critical conditions.

#### 5. Conclusions

CSC has been shown to depend strongly on the type of column packing. The observed differences could be related in a logical way to polarity differences. The pore size has no influence on CSC. On the same column, critical conditions could be established for different S-NS combinations. The solubility of the investigated polystyrenes in these couples differed to a great extent with respect to the molar-mass range. Under reversed-phase conditions the eluent strength at CSC for polystyrene expressed as the solubility parameter  $\delta$ , was shown to have about the same value for three S-NS combinations. Temperature variation was demonstrated to be a

good tool for fine-tuning critical conditions especially on reversed-phase systems. On normal-phase systems retention changes of polystyrenes were shown to vary non-monotonously with temperature which in some cases can limit the usefulness of temperature variations. A probable explanation for this phenomenon might be non-isosteric adsorption behaviour of polymers. For polymers lying outside the linear SEC range of the used column, critical conditions could not be properly established. In none of the cases investigated, it was possible to obtain an exact molecular-mass independence of retention times under critical conditions. Retention time differences could not be ascribed to inaccuracies in the measurements or to chemical differences, thus making the correctness of current theories on critical conditions questionable. Peak broadening of polymers was shown to increase significantly when going from SEC to critical conditions. The effect becomes worse with increasing molecular mass, probably due to differences in diffusion coefficients and the increasing contribution of adsorption to the total retention under critical conditions. The use of larger pore column packings reduces peak broadening. Until now, little attention has been paid to this phenomenon which, in our opinion, can limit the molecular-mass range of application under critical conditions. It was demonstrated that the solvent used to dissolve the polymers must have exactly the same composition as the eluent to reduce zone splitting as much as possible. For higher-molecular-mass polymers exhibiting broader peaks which partly co-elute with  $V_0$ , zone splitting could not be completely avoided.

In our opinion liquid chromatography under critical conditions is a feasible technique which can provide unique information on polymeric microstructures in special cases. Some 'critical' aspects, however, seem to have been underestimated until now. Further research can give more insight in possibilities and limitations of this useful technique.

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