DOI: 10.1002/masy.200551315

Critical Conditions and Limiting Conditions in Liquid Chromatography of Synthetic Polymers

Dušan Berek

Summary: Distinctions between liquid chromatography of synthetic polymers under critical conditions (LC CC) and liquid chromatography under limiting conditions (LC LC) are elucidated. Surface adsorption retention mechanism of macromolecules is employed in the chromatographic systems composed of 10 and 30 nm pore diameter bare silica gels, poly (methyl methacrylate)s of different molar masses, and mixed eluents acetonitrile/dichloromethane of different compositions, and at different temperatures. Increased robustness of the LC LC methods compared to LC CC is confirmed: the LC LC elution behavior is much less sensitive to eluent composition changes compared to LC CC. Still, the LC CC system under study is, surprisingly robust in terms of temperature variations. LC LC methods produce narrow, focused polymer peaks while a peak broadening is observed in LC CC. The results demonstrate importance of sample solvent applied in the isocratic coupled polymer HPLC methods.

Keywords: coupled methods of polymer HPLC; critical conditions and limiting conditions of polymer elution

Introduction

Molecular characterization of *complex polymer systems* exhibiting multiple distributions in their characteristics requires non-traditional methods of high performance liquid chromatography *(polymer HPLC).* These combine entropic *(exclusion)* and enthalpic *(interaction)* retention mechanisms either in the same column *(coupled techniques of polymer HPLC)* or in an on-line tandem of different high performance liquid chromatographic systems *(two- or multi- dimensional polymer HPLC).* The aim of coupled polymer HPLC methods is to either augment or suppress selectivity of separation according to one parameter – it is usually sample molar mass – so that complex polymer species are separated exclusively or at least prevailingly according to another parameter.

Sample elution in coupled methods of polymer HPLC can be performed under isocratic and isothermal conditions – or by applying either a gradient in eluent composition or a temperature gradient. Two different approaches were proposed for the isocratic coupled polymer HPLC. They are based either on the critical conditions or on the limiting conditions of polymer retention.

In this paper, we will compare the critical conditions and the limiting conditions procedures and discuss their similarities and differences.

Critical Conditions Versus Limiting Conditions

Liquid chromatography under critical conditions (LC CC) is the best known isocratic coupled method of polymer HPLC. [1–6] Due to the compensation of molar mass dependence of entropic and enthalpic contributions to polymer retention, sizes of macromolecules do not affect their

---

Laboratory of Liquid Chromatography, Polymer Institute of the Slovak Academy of Sciences, 842 36 Bratislava, Slovakia
E-mail: dusan.berek@savba.sk

retention volumes. Consequently, chemical structure (composition, functionality) or architecture (tacticity) of polymer species can be assessed. Surface or interface adsorption, as well as enthalpic partition (absorption) [7] retention mechanisms can be utilized in LC CC of synthetic, non-charged macromolecules. Accordingly, one speaks about critical conditions of adsorption or about liquid chromatography at critical adsorption point (LC CAP), and about critical conditions of (enthalpic) partition. In both cases, the extent of enthalpy induced retention for a given polymer is controlled with the column packing activity and with the interactions either between eluent and column packing *(solvent strength)* or between eluent and macromolecules *(solvent quality – solvation power).* Solvent strength governs surface adsorption of macromolecules while solvent quality mainly determines the extent of their interface adsorption and enthalpic partition. Solvent strength and solvent quality in polymer HPLC are controlled by eluent nature or temperature. Unfortunately, their fine adjustments are hardly possible in the case of single eluents [8] and therefore mixed, two- or multicomponent eluents are often applied. Polymers dissolved in mixed solvents usually exhibit preferential solvation. One solvent component concentrates in vicinity of polymer chains and concentration of other component(s) raises in bulk solvent. If the HPLC column separates solvated polymer species from their initial bulk solvent, system peaks appear on chromatograms. These are well visible by non-specific detectors [9] and may interfere with the sample peaks if their mutual separation is not complete.

In LC CC, sample is dissolved *in eluent* and as rule macromolecules travel together with their original solvent to elute near the total volume of liquid within column. Therefore, system peaks are usually not (well) separated from the sample peak and may disturb LC CC sample detection.

LC CC was applied to characterization of numerous complex polymers and functional oligomers. [2–6]

An alternative to LC CC represent procedures designated *liquid chromatography under limiting conditions* (LC LC). [3,10] Similar to critical conditions, entropic and enthalpic contributions to sample retention mutually compensate also under limiting conditions and macromolecules of different molar masses elute together. LC LC methods belong to the so called *barrier polymer HPLC* approaches. [3,10] They employ different transport rates of small molecules of eluent or of auxiliary compounds, which permeate the packing pores and of macromolecules, which are partially or fully excluded from the pores. Progression of small molecules is slow compared to elution of macromolecules in the otherwise identical system. Appropriately chosen small molecules may promote adsorption, enthalpic partition or precipitation of macromolecules and thus create a slowly moving barrier within column, which hinders fast transport of macromolecules. The barrier can be either a continuous phase (eluent) [3,10–12] or a short pulse of appropriate substance, which is injected together with polymer species or just in front of sample solution. [3,13,14] So far, mainly the nonsolvent and the adsorptive barriers were studied in detail. In the latter case, the corresponding methods are denoted liquid chromatography under limiting conditions of adsorption LC LCA [3,10–12] and liquid chromatography under limiting conditions of desorption (LC LCD). [3,13] In LC LCA, eluent is an adsorptive barrier, an *adsorli*, while sample solvent promotes desorption, it is a *desorli*. As result, macromolecules stay confined during their entire travel along column within the zone of their original solvent. Advantageously, sample solvent in LC LCA is the desorli component of eluent so that the solvent demixing effects are avoided. On the contrary, LC LCD eluent is a *desorli* and the barrier is formed either by the *adsorli* sample solvent or by a pulse of an appropriate *adsorli* introduced into column immediately before sample. Here again, the barrier is usually formed by the adsorli component of eluent. Polymer species of different interactivities can be easily separated applying optimized LC LC systems. [12,14] More interactive macromolecules are slowed down by the barrier while less interactive species break-through the barrier to be eluted in the exclusion mode. In both LC LC approaches the decelerated macromolecules elute within the barrier boundary and this prevents polymer detection with the non-specific detectors such as differential refractometers.

As mentioned, in both LC CC and LC LC sample species of the same nature are eluted in the domain of total volume of liquid situated within column and irrespectively of their molar masses or molecular dimensions. Still there are clear differences between these two liquid chromatographic modes. LC LC covers a broad molar mass range up to over thousands of $kg \cdot mol^{-1}$, independently of the column packing pore sizes. [9–14] At the same time, application of LC CC to very high molar masses may be problematic. [15–18] The LC CC peaks of high molar mass polymers are often slightly to extensively broadened in comparison with peaks obtained for the same polymer and in the same column but working under purely exclusion conditions. [17,18] On the contrary, the LC LC peaks are narrow due to focusing effects. [3,14] Unlike LC CC, the LC LC columns may allow overloading and especially high sample concentrations can be applied in LC LCA. [14] This allows molecular characterization of minor components ($\leq 1\%$) in polymer blends. Moreover, LC LC procedures are intrinsically more robust than LC CC. LC LC behavior may be obeyed in a rather wide range of eluent compositions, [13] while LC CC is often sensitive or even very sensitive toward minute changes in eluent composition. [2,15–17] This makes LC LC less vulnerable to experimental errors and, correspondingly, more user – friendly.

Though the above similarities and differences of LC CC and LC LC approaches were discussed in several papers, [3,9,13] the LC LC methods are still sometimes confused with LC CC. For example, Jiang et al [19,20] recently published interesting separations of non-functional poly(methyl methacrylate)s (PMMA) and poly(n-butyl acrylate)s from their hydroxyl- and carboxy- terminated analogues, respectively on bare silica gel. They designated their procedure critical HPLC though their samples were injected in an adsorli dichloromethane into mixed eluents comprising of dichloromethane and acetonitrile at a composition, which promoted desorption of the main component of the analyzed mixture. Such experimental conditions correspond to a typical LC LCD system. Sample volume applied by Jiang et al [19,20] was, however, rather small, only 10 μL for a column of 150 × 4.6 mm. It cannot be excluded that the successively diluting barrier of sample solvent could not effectively hamper fast progression of macromolecules along entire length of column. In this case a mixed mode of polymer HPLC, namely LC LCD/SEC would be operative. This can explain some unexpected results presented in the paper. [20]

The elution behavior of non-functionalized poly(methyl methacrylate)s was investigated in this present study. The chromatographic system was similar to that applied in [19,20] with bare silica gel column packings and acetonitrile/dichloromethane mixed eluents. However, various sample solvents were used in order to demonstrate distinctions between the LC CC and LC LC approaches.

Experimental Part

The HPLC apparatus consisted of the pump Model 64 (Knauer, Berlin, Germany) operated at 1 mL min^{-1}, of the manual sample injection valve Model 7725 (Rheodyne, Cotati, CA, USA) provided with the 50 μL sample loop and of the evaporative light scattering detector DDL-21 (Eurosep, Cergy-Saint-Pontoise, France). Polymer samples were dissolved and injected either in the particular eluent, or in pure acetonitrile, or in pure dichloromethane. Sample concentration was 1 ± 0.1 mg mL^{-1}. Unless otherwise stated, column temperature was kept at 30 ± 0.1 °C with help of a custom made column – oven with a duplex wall connected to a water thermostat. The pump was operated at ambient temperature but both eluent and sample solutions were preheated before entering the column. The resettability of pump, and the actual flow rate was controlled by a burette situated at the column outlet. The data were processed by means of the software Chroma (Chromtech, Graz, Austria). It should be noted that a 10 % increase in apparent retention time compared to the real time-scale was unintentionally generated by computer in the course of all experiments.

Columns 250 × 4 mm were slurry packed with Biospher SI-100 and SI-300 bare silicagels from Labio, Prague, Czech Republic. Their nominal pore diameters were 10 and 30 nm, respectively.

Analytical grade solvents were used as eluents, or eluent components viz. acetonitrile (ACN) from Merck, Darmstadt, Germany, dichloromethane (DCM) from Centralchem, Bratislava, Slovakia, and tetrahydrofuran (THF) from Polskie Odczynniki Chemiczne S.A., Gliwice, Poland. Solvents were vacuum distilled immediately before use. THF was stabilized by 0.22 wt. % of di-t-butyl p-cresol and DCM by 0.015 vol. % of amylene. Mixed eluents were prepared by weighing the components and the control of eluent composition was in range of 0.1 wt. %.

The poly(methyl methacrylate)s (PMMA), prepared by anionic polymerization, were gifts from Dr. W. Wunderlich, Röhm, Darmstadt, Germany and from Dr. J. Herz of Institut Sadron, Strasbourg, France (M ranged from 1.1 to 613 $\text{kg} \cdot \text{mol}^{-1}$). The polymers exhibited low degree of stereoregularity and narrow to medium molar mass distributions. [21] The polystyrene standards were purchased from Pressure Co., Pittsburgh, PA, USA. Their molar masses ranged from 0.7 to 498 $\text{kg} \cdot \text{mol}^{-1}$.

Acetonitrile (ACN) is a polar, rather strong solvent, with the solvent strength parameter according to Snyder recalculated for silica gel, $\varepsilon^0 = 0.5$. [22] ACN is a very poor, theta solvent for PMMA with the exponent of the Kuhn-Mark-Houwink-Sakurada viscosity law $a = 0.50$ between 30 and 45 °C. [23] The difference in experimental theta temperatures for PMMA in ACN published by various authors is remarkable.

Dichloromethane is a weak, low polarity solvent, with $\varepsilon^0 = 0.32$. [22] It promotes adsorption of PMMA on free silanols of silica gel column packing. DCM is a thermodynamically good solvent for PMMA (a values lie between 0.68 and 0.72). [23]

For all polymers used, the peak retention volumes (V_R) could be well identified. After each set of experiments the retained macromolecules were removed from the columns by an overnight action of a displacer, tetrahydrofuran. Columns were re-equilibrated with 20 volumes of the fresh eluent before the next series of measurements. Most experiments were done twice and the V_R values were averaged. The repeatability of retention volumes was better than 3 %.

Results and Discussion

Recently, it was confirmed that acetonitrile is a medium efficient desorli for poly(methyl methacrylate)s from bare silica gel. [24] The universal ''calibration'' dependences of log V_h vs. V_R, where V_h is the hydrodynamic volume of macromolecules in eluent [25] for PMMA in ACN almost coincided with those obtained in THF. However, the universal calibration dependences, which take into account changes of polymer coil dimensions in solvents of different thermodynamic quality, were for PMMA in both ACN and THF slightly shifted to higher retention volumes compared to the non-adsorbing system THF/polystyrene applying the same silica gel column. DCM is an efficient adsorli for PMMA in connection with bare silica gel. Samples of PMMA were fully retained within column by adsorption from this eluent irrespective of their molar masses.

Dependences of log M vs. V_R, where M is the most abundant (''peak'') sample molar mass, obtained with silica gels 10

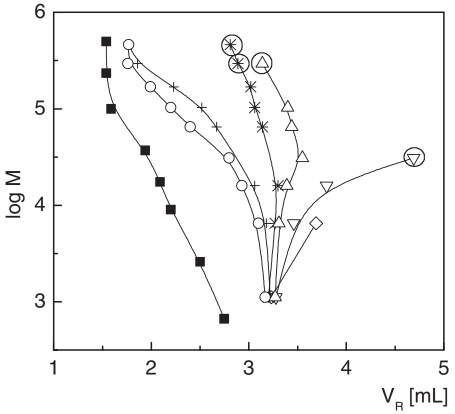

Figure 1.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-100. Compositions of eluents are in wt. % of ACN: (○) 60; (+) 50; (*) 46; (△) 45; (▽) 41, and (◇) 36. **Sample solvent was eluent.** The datapoints (■) are for PS; eluent and sample solvent was THF. Additional circles denote the peaks, for which the sample recovery (peak area) was reduced more than about 25 %.

and 30 nm at various eluent compositions and for PMMA samples dissolved and injected in particular eluents are shown in Figures 1 and 2. For comparison, the same dependences for polystyrenes in pure THF eluent and using THF sample solvent are plotted. As usual, the critical adsorption point and resulting vertical log M vs. V_R course is better defined with the wide-pore column packing material. In the latter case also an increase of V_R for low molar mass polymer species at critical adsorption point compared to the exclusion mode is more pronounced. A strongly reduced sample recovery is observed for high molar mass species. Corresponding data points are denoted by additional circles. Full sample retention appears for higher molar masses in the area of eluent compositions where adsorption retention mechanism prevails. The critical adsorption point for Biospher SI-100 lies at about 45 wt.% of ACN and it is situated at about 43 wt.% of ACN for Biospher SI-300. This indicates a slightly higher adsorptivity of Biospher SI-100 compared to Biospher SI-300. The small difference in the column packing activity may reflect increased both activity of surface silanols and packing surface available for adsorption in Biospher SI-100. A high selectivity SEC-like separation area can be observed near the critical adsorption point for higher molar masses (see for example results for eluents containing 50 and 60 wt. % of ACN for Biospher SI-100, as well as 45 and 46 wt. % of ACN for Biospher SI-300). This phenomenon is designated "enthalpy assisted SEC". [26] Medium sensitivity of critical adsorption point toward eluent composition is observed for both silica gel column packings. It can be estimated that a transition from the adsorption prevailing elution mode to the exclusion prevailing mode takes place within about 2 to 3 wt. % change in eluent composition. This is a somewhat wider eluent composition range than usual for LC CC with the adsorption driven enthalpic polymer retention (LC CAP).

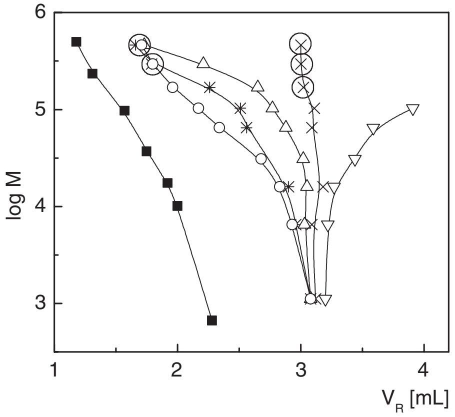

Figure 2.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-300. Compositions of eluents are in wt. % of ACN. (○) 60; (*) 46; (△) 45; (×) 43, and (▽) 41. **Sample solvent was eluent.** The datapoints (■) are for PS; eluent and sample solvent was THF. Additional circles denote the peaks, for which the sample recovery (peak area) was reduced more than about 25 %.

The effect of sample solvent on polymer elution is demonstrated in Figures 3 to 6. A vertical course of log M vs. V_R dependences is attained in the ACN sample solvent (LC LCA behavior), while adsorption prevails if sample solvent was eluent (Figures 3 and 5).

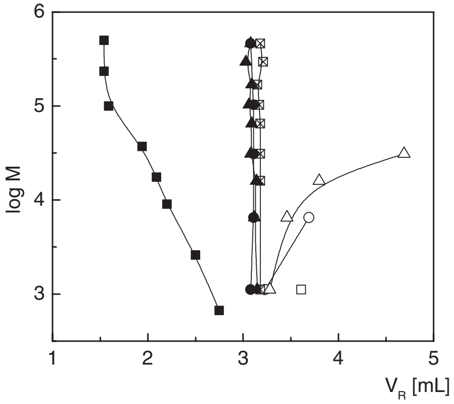

Figure 3.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-100. Compositions of eluents are in wt. % of ACN: (▲) 41; (●) 36, and (☒) 30. **Sample solvent was ACN**. For comparison, retention volumes obtained for samples injected in eluent are depicted: (△) 41; (○) 36, and (□) 30. The datapoints (■) are for PS in pure THF. Only PMMA with the lowest $M = 1.1\ \mathrm{kg \cdot mol^{-1}}$ was eluted in eluent containing 30 wt. % of ACN. Notice that LC LCA behavior appeared in the adsorption promoting eluents.

Similarly, molar mass independent elution is observed for the DCM sample solvent in the LC LCD systems but exclusion prevails in the same eluents if these were used as sample solvents (Figures 4 and 6). The increased robustness of both LC LCA (Figures 3 and 5) and LC LCD (Figures 4 and 6) in comparison with LC CC is clearly demonstrated because a molar mass independent retention is observed in a rather wide range of eluent compositions.[13] This again confirms user-friendliness of optimized LC LC procedures.

The effect of temperature on the LC CC elution of PMMA in the ACN/DCM mixed eluent containing 45 and 43 wt. % of ACN for SI-100 and SI-300, respectively is shown in Figures 7 and 8.

A 20 °C change in temperature practically did not affect the critical elution behavior of PMMA. It can be concluded that this LC CC system is surprisingly robust as to the effect of temperature. In many LC CC systems, already a few degrees of centigrade difference may cause

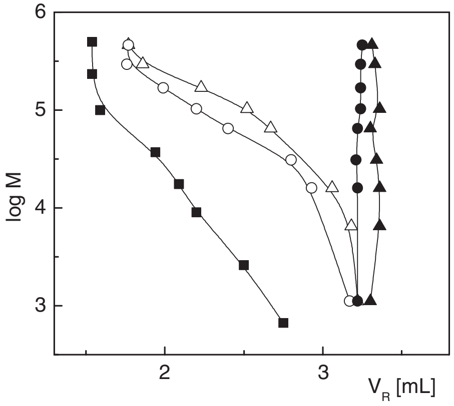

Figure 4.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-100. Compositions of eluents are in wt. % of ACN: (●) 60, and (▲) 50. **Sample solvent was DCM**. For comparison, retention volumes obtained for samples injected in eluent are depicted: (○) 60, and (△) 50. The datapoints (■) are for PS is pure THF. Only PMMA with the lowest $M = 1.1\ \mathrm{kg \cdot mol^{-1}}$ was eluted in eluent containing 30 wt. % of ACN. Notice that LC LCD behavior appeared in the desorption promoting eluents.

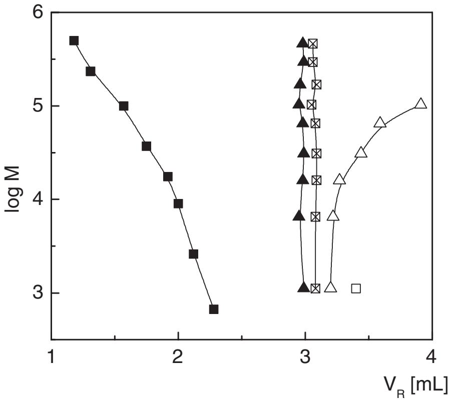

Figure 5.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-300. Compositions of eluents are in wt. % of ACN: (▲) 41, and (☒) 30. **Sample solvent was ACN**. For comparison, retention volumes obtained for samples injected in eluent are depicted: (△) 41, and (□) 30. Only PMMA with the lowest $M = 1.1\ \mathrm{kg \cdot mol^{-1}}$ was eluted in eluent containing 30 wt. % of ACN if sample solvent was eluent. The datapoints (■) are for PS in pure THF. Notice that the LC LCA behavior appeared in the adsorli eluents.

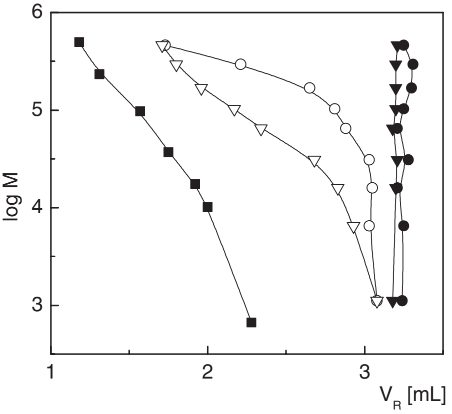

Figure 6.
Dependences of log M vs. V_R for PMMA samples in mixed eluents ACN/DCM obtained with Biospher SI-300. Compositions of eluents are in wt. % of ACN: (▼) 60; and (●) 45. **Sample solvent was DCM**. For comparison, retention volumes obtained for samples injected in eluent are depicted: (▽) 60, and (○) 45. Datapoints (■) are for PS in pure THF. Notice that the LC LCD behavior appeared in the desorli eluents.

transition from adsorption to exclusion mode of elution. [15,18] The increased sensitivity of critical conditions to temperature is observed in the systems where polymer solubility strongly affects its retention that is where interphase adsorption and/or enthalpic partition of macromolecules is the leading enthalpic retention mechanism. [5]

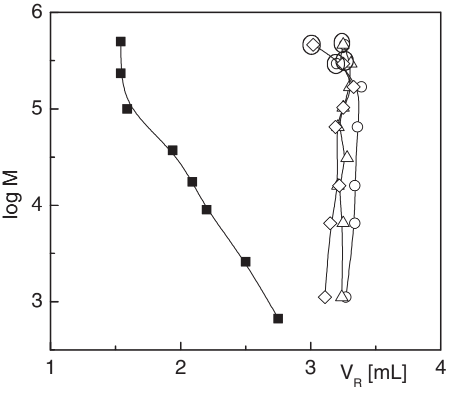

Figure 7.
Effect of temperature on the LC CC dependences of log M vs. V_R for PMMA samples obtained with Biospher SI-100. Mixed eluent ACN/DCM containing 45 wt. % of ACN. **Samples were injected in eluent**. Temperatures were (○) 20 °C; (△) 30 °C, and (◇) 40 °C. (■) is the dependence for PS in THF eluent at 30 °C.

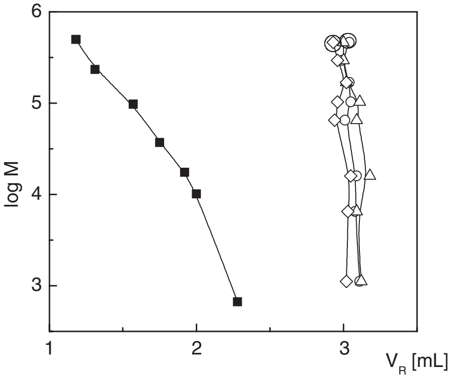

Figure 8.
Effect of temperature on the LC CC dependences of log M vs. V_R for PMMA samples obtained with Biospher SI-300. Mixed eluent ACN/DCM containing 43 wt. % of ACN. **Samples were injected in eluent**. Temperatures were (○) 20 °C; (△) 30 °C, and (◇) 40 °C. (■) is the dependence for PS in THF eluent at 30 °C.

Important differences between LC CC and LC LC behavior lie also in peak broadening and in sample recovery. As shown in the paper, [17,18] LC CC peaks were substantially broadened and their areas, which reflected sample recovery, were dramatically reduced. Both peak broadening and sample recovery reduction increased with increasing molar mass of polymers and with decreasing pore size of the column packing. On the contrary, marked peak focusing was observed in several LC LC systems [3,14] because macromolecules accumulated on the barrier edge. LC LC peak widths were less dependent of the sample molar mass and often remained almost constant over a wide range of injected sample volumes. [14] Typical examples of peak shapes and sizes for constant injected volume and almost constant injected sample concentration are displayed in Figures 9 to 13.

Figures 9 and 10 illustrate the effect of sample molar mass and pore diameter on

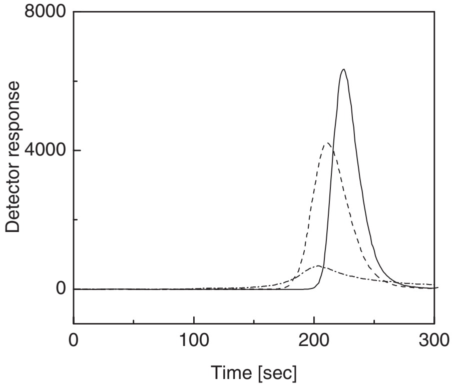

Figure 9.
Dependence of LC CC peak sizes and shapes on sample molar masses. Column packing was Biospher SI-100 and mixed eluent ACN/DCM contained 45 wt. % of ACN. **Samples with similar concentrations were injected in eluent** and their molar masses in kg · mol^{-1} were: (—) 6.5; (- - -) 65, and (-.-) 461 kg · mol^{-1}.

the peak sizes and shapes under critical conditions, while Figure 11 shows the effect of temperature on the LC CC peak shapes.

It is evident from Figures 9 and 10 that the peak areas decrease and peak widths increase with increasing sample molar masses. This effect is especially well pronounced for large, macromolecules, which would be fully excluded from the packing pores in absence of adsorption. Figure 11 demonstrates a relatively small influence of column temperature on the peak widths under LC CC conditions. It may reflect low temperature effect on the overall polymer adsorption. On the other hand, sample recovery increases with raising temperature.

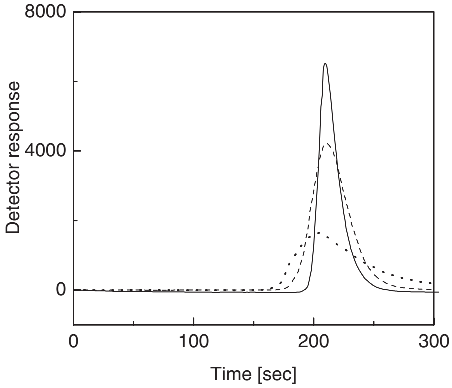

Figure 10.
Dependence of LC CC peak sizes and shapes on sample molar masses. Column packing was Biospher SI-300 and mixed eluent ACN/DCM contained 43 wt. % of ACN. **Samples with similar concentrations were injected in eluent** and their molar masses in kg · mol^{-1} were: (—) 6.5; (- - -) 65, and (. . . . . .) 461 kg · mol^{-1}.

Figures 12 and 13 display typical peaks, which were obtained in the LC LCA and LC LCD modes. The front parts of peaks are rather sharp due to focusing effect of barriers. The peak tailing is caused by the exclusion effects taking part in the desorli zone for LC LCA and in eluent for LC LCD. Still, the difference in peak widths between LC CC and LC LC is evident, especially for higher molar mass species. Reduced sample recovery for high molar mass samples was observed not only in the LC CC but also in the LC LCA mode [27] while recovery remained very high in the LC LCD mode. [27] Present results support conclusions from paper.[26]

Extensive peak focusing can be observed also in chromatograms published by Jiang et al. [19,20] The non-functionalized PMMA and poly(n-butyl acrylates), which eluted under LC LCD or nearly LC LCD conditions, produced much narrower peaks than the functionalized macromolecules eluted in the slightly prevailing adsorption mode.

Break-out and break-through phenomena resulting in split peaks appeared in the LC LCD systems containing a very narrow pore (6 nm) silica packing material, high molar mass PMMA samples, and toluene adsorli plus THF desorli eluents if sample was injected in pure toluene. [14] This problem was solved by applying a tandem injection procedure, in which samples dissolved in eluent were introduced immediately after an appropriately wide zone of a pure adsorli. It seems that the break-out and break-through phenomena are less important for the packings with wider pores and for samples with lower molar masses. They were observed neither in the present systems nor in the previous studies. [13,19,20]

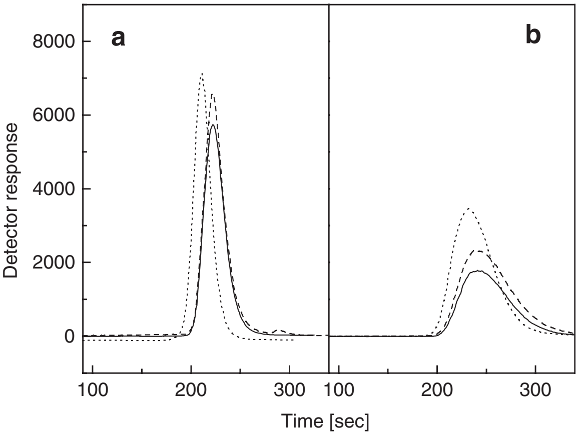

Figure 11.
Chromatographic traces of PMMA 1.1 kg · mol^{-1} (a) and 31 kg · mol^{-1} (b) eluted in the LC CC mode from column Biospher SI-100 at various temperatures: (—) 20 °C; (- - -) 30 °C, and (. . .) 40 °C. Mixed eluent ACN/DCM contained 45 wt. % of ACN. **Samples with similar concentrations were injected in eluent.**

Conclusions

An important role was demonstrated of sample solvent in the isocratic coupled methods of polymer HPLC combining exclusion and adsorption retention mechanisms. In dependence on the sample solvent strength, interactivity of eluent must be adjusted to obtain molar mass independent polymer elution. The direct compensation of entropic (exclusion) and enthalpic (adsorption) contributions to retention volumes is attained in the eluent with critical composition, which is used also as the sample solvent. This is the principle of liquid chromatography of synthetic polymers under critical conditions (LC CC). The indirect entropy/enthalpy compensation utilizes slowly moving liquid barrier on which macromolecules accumulate near the critical adsorption point. The barrier is formed by adsorption promoting

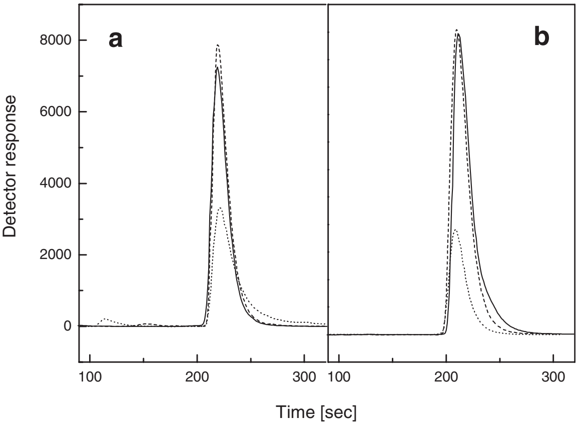

Figure 12.
Chromatographic traces of PMMA with different molar masses: (—) 1.1; (- - -) 65, and (. . .) 461 kg · mol^{-1}. Column was packed with Biospher SI-100 and mixed eluents ACN/DCM contained 60 wt. % ACN (a) and 41 wt. % ACN (b). **Samples with similar concentrations were injected in DCM** (LC LCD mode) **(a) or in ACN** (LC LCA mode) **(b). For the sake of clarity the peaks for M = 461 kg · mol^{-1} were electronically reduced by 50%.**

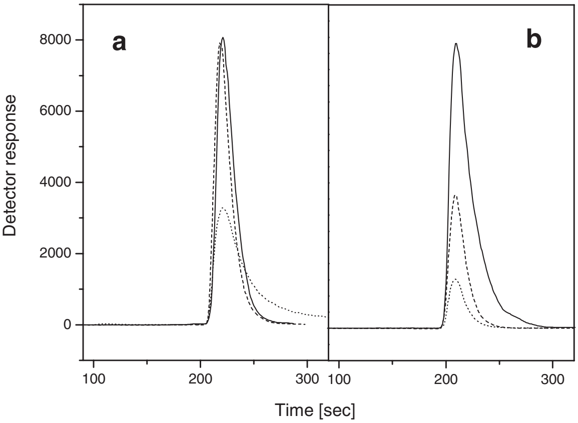

Figure 13.
Chromatographic traces of PMMA with different molar masses: (—) 6.5; (- - -) 65; and (. . .) 461 kg · mol^{-1}. Column was packed with Biospher SI-300 and mixed eluents ACN/DCM contained 60 wt. % ACN (a) and 41 wt. % ACN (b). **Samples were injected in DCM** (LC LCD mode) **(a) or in ACN** (LC LCA mode) **(b). For the sake of clarity the peaks for M = 461 kg · mol^{-1} were electronically reduced by 50 %**.

(adsorli) eluent and sample is injected in a desorli (liquid chromatography under limiting conditions of adsorption – LC LCA). On the contrary, a zone of adsorli creates a barrier in liquid chromatography under limiting conditions of desorption (LC LCD). In this case, sample is either dissolved and injected in an adsorli solvent or the adsorli zone is introduced just before sample solution. LC CC peaks are often broadened and their size is decreased probably because of slow kinetics of desorption. One part of sample may be fully adsorbed within column. In contrast, LC LC elution produces narrowed peaks and a high sample recovery is attained in the optimized LC LCD systems. [27] LC LC procedures are rather robust as to temperature and eluent composition changes. Variations in eluent composition as high as 10 wt. % do not affect the retention mode. The LC CC system comprising PMMA, bare silica gel and ACN/DCM eluents exhibits a medium sensitivity toward eluent composition and a low sensitivity toward temperature variations.

Acknowledgements: This work was supported by the Slovak Grant Agency Vega, project 2-301 123, as well as by the Slovak Grant Agency APVT 51-013 204. The author thanks Drs. W. Wunderlich and J. Herz for samples of poly(methyl methacrylate)s, and Mrs. J. Tarbajovska for her excellent technical assistance.

[1] B.G. Belenkii, E.S. Gankina, M.B. Tennikov, L.Z. Vilenchik, *J. Chromatogr., A* **1978**, *147*, 99
[2] H. Pasch, B. Trathnigg, *HPLC of polymers*, Springer, Berlin, **1998**
[3] D. Berek, *Prog. Polym. Sci.*, **2000**, *25*, 873
[4] T. Chang, *Adv. Polym. Sci.*, **2003**, *163*, 1
[5] T. Macko, D. Hunkeler, *Adv. Polym. Sci.*, **2003**, *163*, 61
[6] H.J.A. Philipsen, *J. Chromatogr. A*, **2004**, *1037*, 329
[7] D. Berek, *Chromatographia* **2003**, *57*, S-45
[8] T. Macko, D. Hunkeler, D. Berek, *Macromolecules* **2002**, *35*, 1797
[9] D. Berek, T. Bleha, T., Z. Pevna, *J. Chromatogr. Sci.*, **1976**, *14*, 560
[10] D. Berek, "Two-Dimensional Liquid Chromatography of Synthetic Macromolecules", In: C.-s. Wu, *Handbook of Size Exclusion Chromatography an Related Techniques*, Marcel Dekker, New York, **2004**
[11] D. Berek, "Coupled procedures in liquid chromatography of macromolecules", In: *Proc. Simp. Latino-Americano des Polimeros*, Mar del Plata, **1996**, 37
[12] D. Berek, D. Hunkeler, *J. Liq. Rel. Technol.*, **1999**, *22*, 2867
[13] D. Berek, *Macromolecules* **1998**, *31*, 8517
[14] M. Snauko, D. Berek, accepted in *J. Chromatogr. A.*
[15] H.J.A. Philipsen, B. Klumperman, A.M. van Herk, A.L. German, *J. Chromatogr. A*, **1996**, *727*, 13
[16] D. Berek, *Macromol. Symp.*, **1996**, *110*, 33
[17] D. Berek, M. Janco, G.R. Meira, *J. Polym. Sci. A Polym. Chem.*, **1998**, *36*, 1363
[18] P.P. Nefedov, T.I. Zhmakhina, *Vysokomol. Sojed. (Moscow)*, **1984**, *A23*, 276

[19] X. Jiang, V. Lima, P.J. Schoenmakers, *J. Chromatogr. A*, **2003**, *1018*, 19

[20] X. Jiang, P.J. Schoenmakers, X. Lou, V. Lima, J.L.J. van Bongen, J. Brokken-Zijp, *J. Chromatogr. A*, **2004**, *1055*, 123

[21] D. Berek, M. Janco, M., K. Hatada, T. Kitayama, *Polym. Bull.*, **1994**, *32*, 629

[22] L.R. Snyder, J.L. Glajch, J.J. Kirkland, Practical HPLC Method Development, Wiley, New York, **1988** (values for alumina recalculated by R. Gant, Sigma-Aldrich Chromatography Products, Milwaukee, Wisconsin, USA)

[23] M. Kurata, Y. Tsunashima, "Viscosity Molecular Weight Relationships and Unperturbed Dimensions of Linear Chain Molecules", In: E.H. Brandrup, E.H. Immergut, E.A. Grulke, A. Abe, D.R. Bloch, eds., *Polym. Handbook*, 4th edition, Wiley, New York, **1999**

[24] D. Berek, *Macromolecules* **2004**, *37*, 6096

[25] H. Benoit, Z. Grubisic, P. Rempp, D. Decker, J.-G. Zilliox, *J. Chim. Phys.*, **1966**, *33*, 1507

[26] D. Berek, *Macromol. Symp.*, **2004**, *216*, 145

[27] M. Snauko, D. Berek, *Chromatographia* **2003**, *57*, S-55