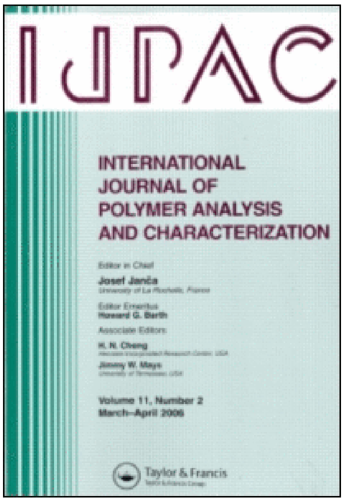

International Journal of Polymer Analysis and Characterization
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713646643

Mechanism of Liquid Chromatography of Macromolecules under Limiting Conditions of Adsorption

Artur Bartkowiak[a,b]; Renata Murgasova[c]; Miroslav Janco[c]; Dusan Berek[c]; Tadeusz Spychaj[a]; David Hunkeler[b]

[a] Polymer Institute, Technical University of Szczecin, Szczecin, Poland [b] Department of Chemistry, Swiss Federal Institute of Technology, Lausanne, Switzerland [c] Polymer Institute, Slovak Academy of Sciences, Bratislava, Slovakia

To cite this Article Bartkowiak, Artur , Murgasova, Renata , Janco, Miroslav , Berek, Dusan , Spychaj, Tadeusz and Hunkeler, David(1999) 'Mechanism of Liquid Chromatography of Macromolecules under Limiting Conditions of Adsorption', International Journal of Polymer Analysis and Characterization, 5: 2, 137 — 155

To link to this Article: DOI: 10.1080/10236669908014180

URL: http://dx.doi.org/10.1080/10236669908014180

Int. J. Polym. Anal. Charact.,
1999 Vol. 5, pp. 137–155
Reprints available directly from the publisher
Photocopying permitted by license only

Mechanism of Liquid Chromatography of Macromolecules under Limiting Conditions of Adsorption

ARTUR BARTKOWIAK[a,b], RENATA MURGASOVA[c], MIROSLAV JANCO[c], DUSAN BEREK[c], TADEUSZ SPYCHAJ[a] and DAVID HUNKELER[b,*]

[a] Polymer Institute, Technical University of Szczecin, ul. Pulaskiego 10, 70 322, Szczecin, Poland; [b] Department of Chemistry, Swiss Federal Institute of Technology, CH-1015 Lausanne, Switzerland; [c] Polymer Institute, Slovak Academy of Sciences, 84 236 Bratislava, Slovakia

(Received 15 May 1998; In final form 28 December 1998)

A mechanistic study of liquid chromatography under limiting conditions of adsorption (LC LCA) is presented. Polystyrene was evaluated in a THF/*n*-hexane binary eluent using wide- and narrow-pore silica gels and styrene/divinylbenzene-based sorbents. The LCA condition was achieved though a balance between the free energies of exclusion and adsorption. Polymer solubility does not influence the retention volume, with LC LCA occurring in the soluble domain of the cloud point diagram. The molecular weight independent retention (LCA) was not found to significantly vary with the mobile phase flow rate, volume and concentration injected. However, the LCA was strongly influenced by the stationary phase surface chemistry and pore size, as well as the temperature. The limiting conditions of adsorption are, therefore, relatively robust for a given polymer–eluent–sorbent combination and depend on the operating and physical characteristics of the chromatograph. Examples of LC LCA conditions are reported herein.

Keywords: Critical conditions; Liquid chromatography; Limiting conditions of adsorption; Point of exclusion–adsorption transition; Poly(methyl methacrylate); Polystyrene; Silica gel

INTRODUCTION

Since Belenkii's pioneering experiments which revealed that entropic and enthalpic separation mechanisms could be offset within a liquid

---

[*] Corresponding author.

chromatographic (LC) arrangement,[1] a series of systems have been investigated at the "critical adsorption point" (LC CAP).[2,3] These methods permit the separation and characterization of functionalized oligomers,[2] block copolymers and polymer blends.[3] Several recent reviews summarize the current potential, weaknesses and experimental protocol of LC coupled techniques[2–7] in addition to providing an extensive tabulation of the polymer–eluent–sorbent systems studied to date.[3,6] In brief, most important coupled LC methods involve the selection of usually a binary mobile phase where a given polymer elution is independent of its molecular size, for a particular stationary phase and temperature. Recent advances in LC CAP include refractive index and density combinations[8] or MALDI-TOF measurements.[9] Mathematical manipulations (deconvolution) and experimental techniques such as orthogonal chromatography[10] have also been applied with limited success.

Limiting Conditions of Solubility

The preceding LC CAP method involves the injection of a polymer in the mobile phase utilized for separation. A variation of this technique, referred to as "liquid chromatography under limiting conditions of solubility" (LC LCS),[6,11] involves the utilization of an injection zone which is a good solvent for the polymer solute while the eluent is, *de facto*, a weak nonsolvent. Under particular limiting conditions the separation proceeds through a microgradient processes of exclusion, precipitation and redissolution. Owing to partial exclusion, the polymer separates from the injection zone, encounters the nonsolvent mobile phase, thereby reducing its coil size, and interacts with the stationary phase. The polymer precipitates and is then redissolved as the injection zone "catches up" to the solute. The net result is that the polymer elutes just in front of the solvent peak, as has been well documented using a variety of DRI, UV and evaporative light scattering detectors.[6,12,13] The LC LCS methodology has also recently been extended to water-soluble polymers.[14]

Limiting Conditions of Adsorption

Liquid chromatography under limiting conditions of adsorption (LC LCA) utilizes an eluent which more strongly promotes adsorption of macromolecules on the column then in the LC CAP mode. Indeed, if a polymer solution was injected in the eluent, as is the case with LC CAP, it would be fully retained. In contrast, in LC LCA the eluent is a mixture of a good solvent and a weak nonsolvent for the polymer. However, the polymer is dissolved, or injected, in a single desorption promoting liquid (DESORLI). This represents an important difference between LC LCA and LC CAP. In LC LCA the macromolecules travel faster then the injection zone. When the polymer leaves the injection zone it encounters the eluent and is retained by adsorption until it is reached by the DESORLI. It then desorbs and begins to elute again. Eventually an equilibrium is established where the macromolecule elutes from the column just of the leading (front) edge of the injection zone. This has been referred to as "peak compression".[15] As is the case with LC CAP and LC LCS, polymers injected in LC LCA elute with a molecular-weight-independent retention volume. The principal advantage of LC LCA is that it can enable a molecular-weight-independent retention for polymers ranging from oligomers to polymers of over one-million molecular weight. This has been a limitation of the LC CAP method which is generally feasible only for molecules up to a molecular weight of 10^5.

Experimental observations of LC LCA have been reported previously.[5,7] The present paper seeks to mechanistically elucidate the LC LCA concept by determining the sensitivity of the LC LCA condition to variables such as the injection volume, concentration injected, mobile phase flow rate and mixing between the injection zone and eluent. Furthermore, some hitherto unresolved problems, such as the effect of the solubility of macromolecules, as estimated from the cloud point curves, will be assessed. Since measurements to date have been limited to wide-pore silica gel based sorbents, the role of the pore size and the nature of the column packing on the LC LCA condition will also be examined.

Categorization of Coupled LC Techniques

Figure 1 presents a schematized cloud point curve.[14] Clearly in domain A the macromolecule is in the soluble region and the observed molecular weight independent retention point (i.e., LC LCA) is the result of a combination of adsorption, as the enthalpic separation mechanism, with the entropic exclusion process. This is designated by line 1. In contrast, line 2 operates in the nonsoluble portion of the

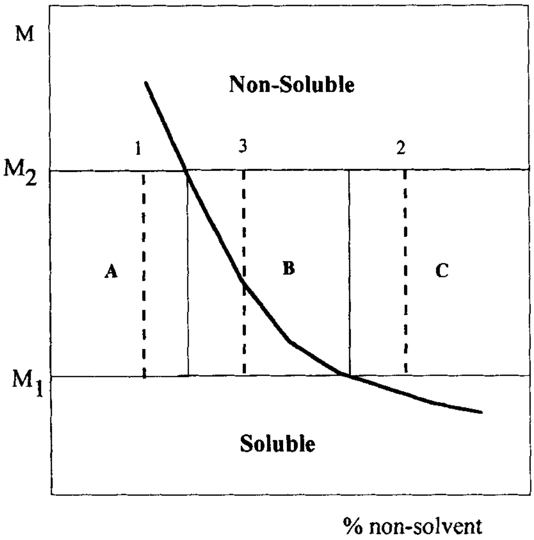

FIGURE 1 A schematic plot of the solubility of polymer standards in a mixed eluent (solvent plus nonsolvent) system, as this relates to interactive liquid chromatography experiments. In domain A, adsorption is the operative enthalpic mechanism which is balanced with exclusion (LC LCA). A typical LC LCA calibration curve is shown by line 1. In domain C the polymer solvent solubility dominates the enthalpy (LC LCS). A typical calibration curve is depicted by line 2. Domain B is a hybrid where the entropic exclusion forces are balanced by the adsorption and solubility (calibration curve, line 3). Note that M_1 an M_2 represent the range where the retention volume in independent of the polymer molecular weight.

cloud point curve where solubility must play a role in the enthalpic mechanism, in addition to adsorption (i.e., LC LCS). Therefore, while LC LCA combines exclusion and adsorption, LC LCS involves exclusion, solubility and adsorption. Since many polymers posses multiple molecular heterogeneity, as manifested in the superposition of molecular weight, chemical composition, branching and functional group distributions, the coupled techniques offer the possibility of separate such complex polymers according to only *one* of their distributions.

EXPERIMENTAL

Mobile and Stationary Phases

Spectranalyzed grade THF (Fisher, Norcross, GA, USA) and HPLC grade *n*-hexane (Fisher) were used as received. A Shodex (JM Science, Grand Island, NY, USA) linear GPC 806L column (0.8 × 30 cm) packed with 10-μm polystyrene-*co*-divinylbenzene particles was employed for all experiments, except where noted. Selected experiments were carried out on an unmodified silica gel column (300 mm in length, 7 mm ID). Wide-pore silica gel particles (SGX 1000 and SGX 500, Tessek, Prague, Czech Republic) had diameters of 5 μm with pore sizes of 100 and 50 nm, respectively.

Liquid Chromatography

An L-6000 (Hitachi Instruments, Tokyo, Japan) pump coupled with an Hitachi L-4000 UV detector operating at a wavelength of 254 nm was utilized in all experiments. A Rheodyne type 7725i valve (Cotati, CA, USA) with an injection loop of 20 μL was employed. Chromatograms were collected on a 486 computer running Viscotek GPC PRO Version 4.01 software (Houston, TX, USA). The standard separation involved 1.0 mL/min flowrate, a solute concentration of 1.0 mg/mL with a 2-cm tubing (500 μm ID) connection between the valve and column, though these were systematically varied in the mechanistic study. Measurements with the narrow-pore silica gel were carried out at 0.5 mL/min. All experiments were performed at ambient temperature (22 ± 1°C).

Polymer Standards

Polystyrene standards with a molecular weight range of 370–1,400,000 g mol^{-1} were obtained from American Polymer Standards Corporation (Mentor, Ohio, USA).

Turbidimetric Measurements

A Bausch and Lomb (New York, NY, USA) Spectronic 20 spectrophotometer operating at 340 nm and ambient temperature was utilized for cloud point measurements. Capped scintillated glass sample vials filled with 2 mL of liquid were employed. Measurements were performed at a polymer concentration of 1.0 mg/mL. Although Elias[16] has reported a dependence of the cloud point curve on the polymer concentration, such a trend was not observed herein over the much narrower, chromatographically relevant, polymer concentration range (1.0–5.0 mg/mL).

Light Scattering

The determination of the average coil size of the polystyrene standards in various THF/*n*-hexane solutions was performed by quasielastic light scattering. The system consisted of Lexel argon-ion laser (2 W) operating at 15 mW and a Brookhaven BI-DS goniometer (Brookhaven Instruments Corporation, Holtsville, NY, USA). Data were acquired for 20 s with a 486 PC containing a Brookhaven BI9000 card. Diameters were then inferred using the Stokes–Einstein relationship from measurements of a mean diffusion coefficient. Measurements were performed at 25°C with disposable presterilized 20-mL glass stationary cells (Fisher Scientific, Norcross, GA, USA).

The refractive indices and viscosities of different compositions are required in order to perform light scattering measurements. Refractive indices were measured using an Abbey ABBE-3L refractometer (Bausch and Lomb, Rochester, NY, USA) while viscosities were determined by measuring the flow time through a capillary in size 25 Cannon-Fenske type calibrated kinematic viscometer tubes (Fisher Scientific, Norcross, GA, USA). Both the refractive index and solution viscosity were found to vary linearly with the percentage *n*-hexane in the THF/*n*-hexane mixture.

RESULTS AND DISCUSSION

Characterization of Polymers in Solvent – Nonsolvent Systems

Figure 2 shows the dependence of the effective coil diameter of a polystyrene standard with a molecular weight of 650,000 as a function of the percentage of *n*-hexane in a THF/*n*-hexane blend. Clearly, and as expected, the coil diameter decreases as the quality of the solvent is reduced (increasing levels of *n*-hexane). These results have implications regarding the interpretation of the LC LCA mechanism. For example, Figure 3 indicates that the LC LCA condition for polystyrene, over wide-pore unmodified silica gel, is 50 vol% *n*-hexane. A comparison

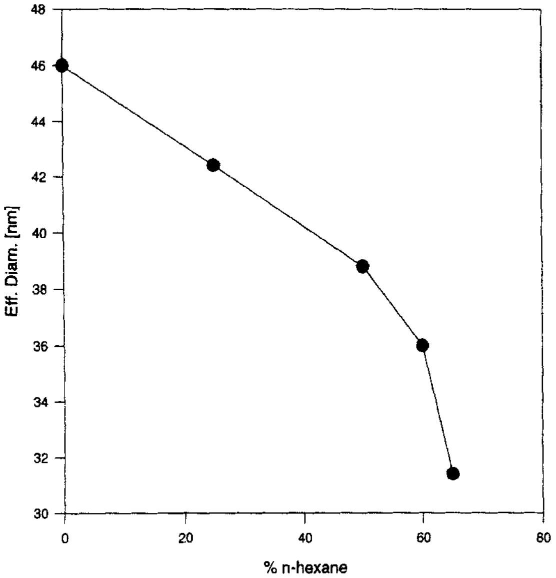

FIGURE 2 The coil size of a polystyrene standard, with a molecular weight of 650,000, as a function of the volume percentage *n*-hexane in a THF/*n*-hexane solvent.

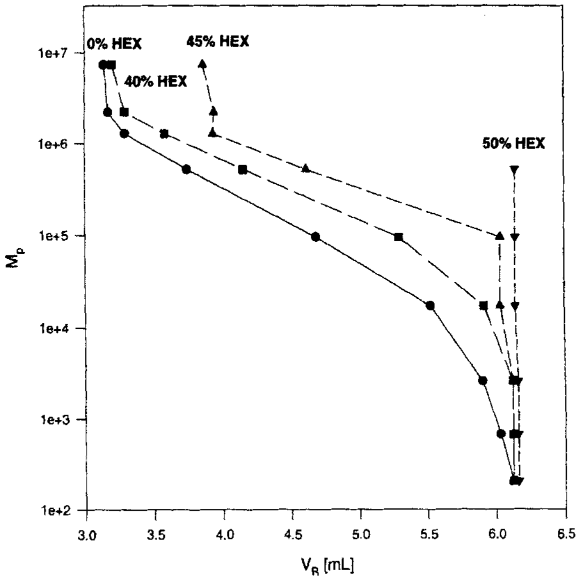

FIGURE 3 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown at various compositions, expressed as volume percentages. The packing material in the column was unmodified silica gel.

of the data in Figures 2 and 3 reveals that at 40 vol% *n*-hexane the calibration curve (Figure 2) is dominated by the entropic free energy term (exclusion) and there is very little shift in the retention volume relative to separations with pure THF as an eluent. This is despite the fact that the coil diameter has been reduced from 46 to 40 nm. This is the first indication that the thermodynamic quality of the eluent does *not* influence the mechanism of LC LCA. This interpretation will be further justified following the presentation of the chromatographic and cloud point data in the following sections.

Effect of the Stationary Phase on LC LCA Conditions

Figure 4 reveals that the point of molecular-weight-independent retention for polystyrene in THF/*n*-hexane is 73 wt% *n*-hexane when styrene/divinylbenzene is employed as a stationary phase. By comparison, the LC LCA point was 50 vol% when a wide-pore unmodified silica gel was used (Figure 3).[†] This indicates that surface interactions are contributing to the enthalpic separation processes at the LC LCA point. The interpretation of Figures 3 and 4 requires the cloud point curve for polystyrene in THF/*n*-hexane (Figure 5). Plotted with the solubility data are the conditions of molecular-weight-independent retention corresponding to silica gel (line 1) and styrene/divinylbenzene (line 2) sorbents. Clearly, line 1 (50 vol% *n*-hexane) lies completely to the left of the cloud point curve and *n*-hexane functions as an adsorption promoting liquid (ADSORLI). One can conclude that, in the polystyrene-THF/*n*-hexane-silica gel system, adsorption and exclusion are the only operative mechanisms and the molecular-weight-independent exclusion is indeed an LC LCA. However, at 73 vol% *n*-hexane is a nonsolvent for polystyrenes with a molecular weight above 150,000, and the molecular-weight-independent retention is a hybrid LC LCA/S phenomenon in the case of polystyrene/divinylbenzene gel.

[†] A direct comparison of the enthalpic interactions in the columns packed with inorganic and organic gels is not possible from this data since the silica gel and styrene/divinylbenzene sorbents had different pore diameters and the columns were of different length and internal diameter.

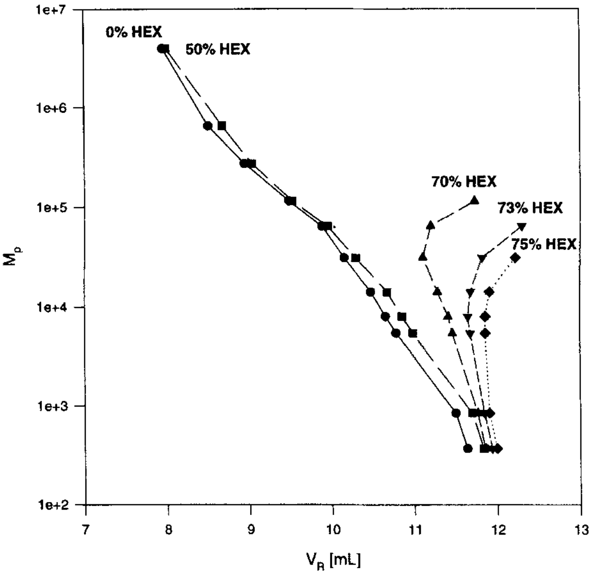

FIGURE 4 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown at various compositions, expressed as volume percentages. The packing material in the column was styrene–divinylbenzene gel.

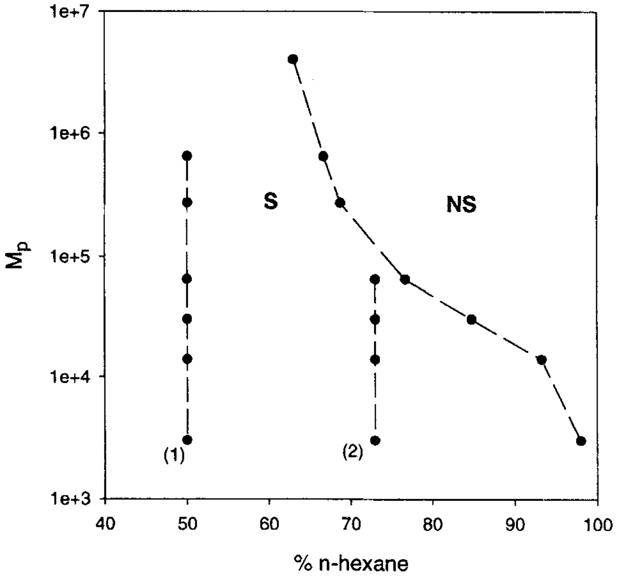

FIGURE 5 A cloud point curve for polystyrene in THF/*n*-hexane. Measurements were performed at a polymer concentration of 1.0 mg/mL. Line (1): silica gel, line (2): polystyrene/divinylbenzene sorbent.

Mechanism of LC LCA

Figure 6 presents a schematic of the exclusion of a polymer solute under limiting conditions of adsorption. The position of the polymer within the injection zone, a desorption promoting liquid (DESORLI), is shown in Figure 6(a). The horizontal line, labeled D_{min}^{22}, represents the minimum level of DESORLI needed to desorb a polymer from the stationary phase at 22°C. At all concentrations of DESORLI below D_{min}^{22} the polymer will be retained on the column. Therefore, the intersection of the D_{min}^{22} line with the injection zone, which itself represents a DESORLI concentration gradient, will define a zone where the polymers can desorb. This is designated by the shaded region in Figure 6(a). Given that polymer molecules move at a velocity faster than DESORLI in the injection zone, due to exclusion, their position within the column ($l_{20\mu\mathrm{L}}^{22}$) will be the maximum attainable without moving to a zone where the DESORLI concentration is less than D_{min}^{22} ($l > l_{20\mu\mathrm{L}}^{22}$).

If the preceding interpretation is correct, one should observe a shift in the polymer retention as a function of the volume injected. Figure 6(b) shows that, as the size of the injection zone is increased (to 100 μL), the amount of DESORLI in the injection zone above D_{min}^{22} is larger. This leads to a increase in the size of the zone where the polymer can desorb (shading). Therefore, the instantaneous position of the polymer within the column is also greater ($l_{100\mu\mathrm{L}}^{22} > l_{20\mu\mathrm{L}}^{22}$) when larger injection zones are employed (100 versus 20 μL). The validity of this mechanism will be evaluated in the following sections of the discussion.

(*i*) *Effect of the Volume Injected* A series of experiments have been performed in order to test the proposed mechanism for LC LCA as well as to determine if the LC LCA condition is constant for a given polymer/eluent, or if they are dependent on the chromatographic conditions such as the mobile phase characteristics, injection volumes and concentrations and stationary phase properties. Figure 7 illustrates the effect of the volume injected. This experiment was actuated by varying the size of the injection loop between 5 and 100 μL. Evidently, a smaller volume injected corresponds to a smaller injection zone, with a concentration of DESORLI lower than that required to desorb the polymer ($[\mathrm{DESORLI}] < D_{\mathrm{min}}^{22}$), as is shown in Figure 6(c). This is observed experimentally in Figure 7 where the highest molecular weight samples

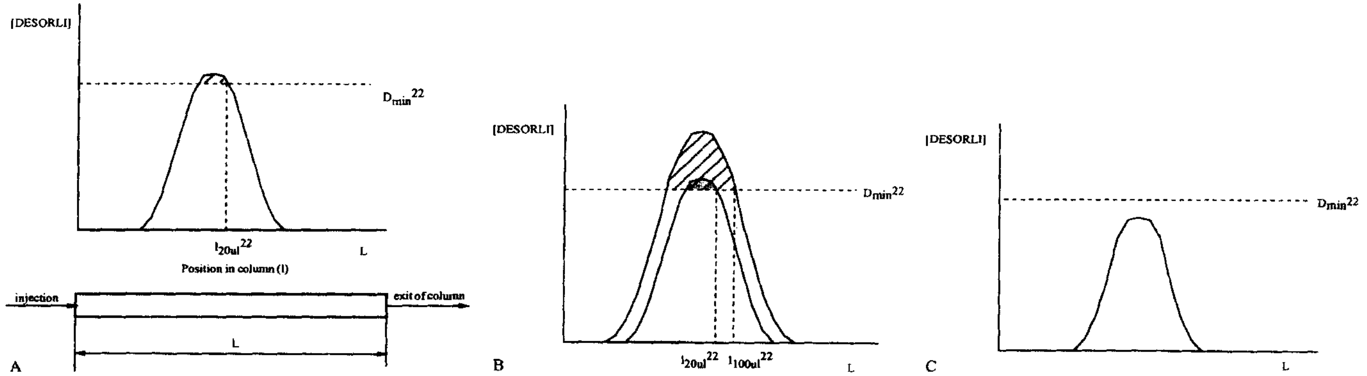

FIGURE 6 Schematic of the influence of zone broadening on the polymer exclusion in LC LCA. The concentration of the desorption promoting liquid in the injection zone [DESORLI] is plotted as a function of the position within the column (*l*). The total length of the column is given by the symbol *L*. The position in the column (*l*) is an instantaneous value. At a given time, a smaller value of *l* corresponds to a slower elution and, ultimately, a larger retention time when the polymer is eluted completely from the column ($l = L$). D_{min}^{22} corresponds to the minimum concentration of DESORLI needed to desorb an adsorbed polymer, at a given temperature, in this case 22°C. (a): for a 20-μL injection of DESORLI the instantaneous position of the polymer in the column is given by $l_{20\mu\text{L}}^{22}$, (b): when 100 μL of DESORLI is injected the volume is still sufficient to desorb polymer molecules from the sorbent and results in an instantaneous position of the polymer in the column of $l_{100\mu\text{L}}^{22}$ (c): the 5-μL injected DESORLI volume in insufficient to desorb polymer molecules from the sorbent.

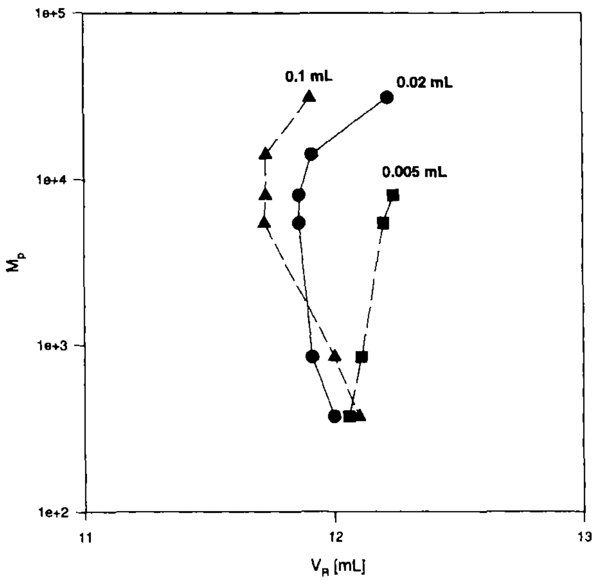

FIGURE 7 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown as a function of the volume injected (mL). The packing material in the column was styrene- divinylbenzene gel.

are not eluted when a 5 μL injection loop is used. In contrast when a 100 μL injection loop is employed, the effect of ADSORLI dilution on the injection zone is strongly diminished, since a much higher quantity of DESORLI (THF) was injected. Therefore, in Figure 6(b) the [DESORLI] is much greater then $D^{22}_{\rm min}$, reducing surface interactions. This increases the instantaneous position of the polymer within the column ($l^{22}_{100\mu L} > l^{22}_{20\mu L}$), reducing the retention volume, and causing a shift in the calibration curve toward the SEC domain, as is observed experimentally in Figure 7.

(*ii*) *Effect of Temperature* The effect of temperature on the chromatographic separation of macromolecules is complicated. However, we offer the following tentative explanation of the role of temperature on LC LCA. If, at higher temperatures, the adsorption is reduced, then a lower concentration of DESORLI will be required to keep the adsorbed polymer molecules "moving along" the column. This is shown schematically in Figure 8 where the concentration of DESORLI required to desorb macromolecules at 40°C (D^{40}_{min}) is less than that at 22°C (D^{22}_{min}). As in Figure 6, the polymer molecule cannot pass the front of the injection zone, since the concentration of DESORLI is less then the minimum required for desorption (D^{22}_{min}). Therefore, if adsorption is reduced at higher temperatures (40°C versus 22°C) less DESORLI is required to desorb the polymer and the chains will move at a higher velocity through the column relative to the case at lower temperatures ($l^{40}_{20\mu\text{L}} > l^{22}_{20\mu\text{L}}$). This faster elution will correspond to a reduced retention time as is experimentally observed in Figure 9 by the shift from LC LCA conditions at 22°C towards the SEC mode at higher temperatures (40°C).

(*iii*) *Effect of Concentration Injected* Figure 10 illustrates the effect of the concentration injected on the LC LCA. One would expect a larger injected concentration to lead to more polymer peak broadening and an increase in the speed at which the polymer moves through the column (see Figure 6(a) and (b)). This would correspond to a reduced retention volume, as is experimentally observed. Therefore, the effect of increasing the concentration injected is similar to

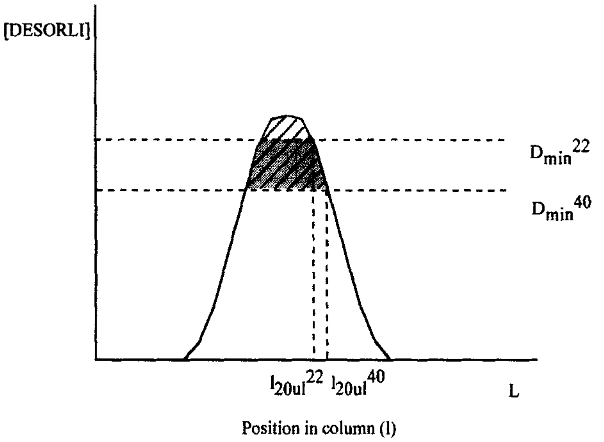

FIGURE 8 Schematic of the influence of zone broadening on the polymer exclusion in LC LCA. The concentration of the desorption promoting liquid in the injection zone [DESORLI] is plotted as a function of the position within the column (l). The total length of the column is given by the symbol L. D^{22}_{min} and D^{40}_{min} correspond to the minimum concentration of DESORLI needed to desorb an adsorbed polymer at 22°C and 40°C, respectively. Similarly, $l^{22}_{20\mu\text{L}}$ and $l^{40}_{20\mu\text{L}}$ correspond to the instantaneous position of the polymer within the column at 22°C and 40°C using a 20-μL injection loop.

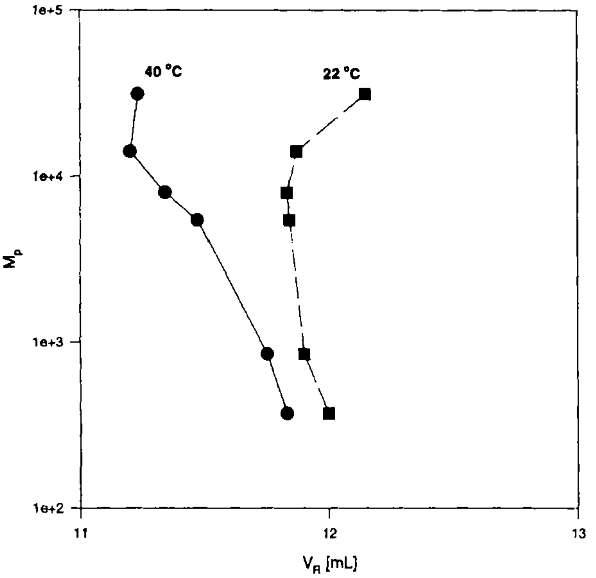

FIGURE 9 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown as a function of the temperature. The packing material in the column was styrene -divinylbenzene gel.

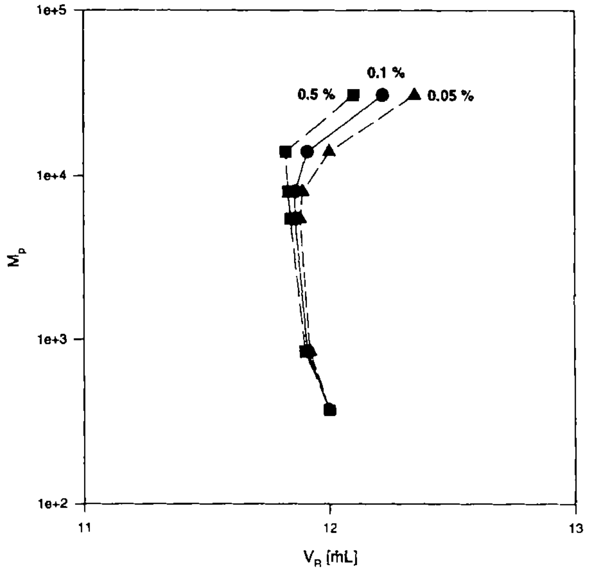

FIGURE 10 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown as a function of the concentration of polymer injected (mg/mL). The packing material in the column was styrene–divinylbenzene gel.

that of larger volumes injected (Figure 7) with the shift to the SEC mode as the amount of polymer injected increases.

(*iv*) *Effect of Connection Length and Flowrate* Figure 11 illustrates the effect of the connection length between the injection loop and the column on the LC LCA condition. As is expected, a larger connection length increases the time between the injector and the column causing a shift to higher retention volumes. The shift is not, however, equal for all molecular weights with higher molecular weight polystyrenes retained for longer times within the column when the connection length is increased.

Figure 12 demonstrates the negligible effect of flow rate on the LC LCA condition. At the high flow rates the balance between exclusion and adsorption is slightly shifted in favor of the latter, particularly for polystyrenes with higher molecular weights. However, larger flow rates typically increase peak broadening which would slightly increase the speed at which the polymer passes through the column (Figure 6(b)),

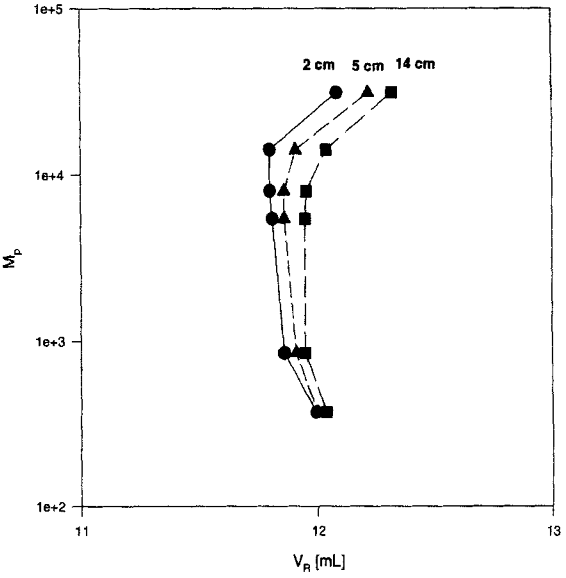

FIGURE 11 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown as a function of the connection length (cm) between the injection loop and column. The packing material in the column was styrene-divinylbenzene gel.

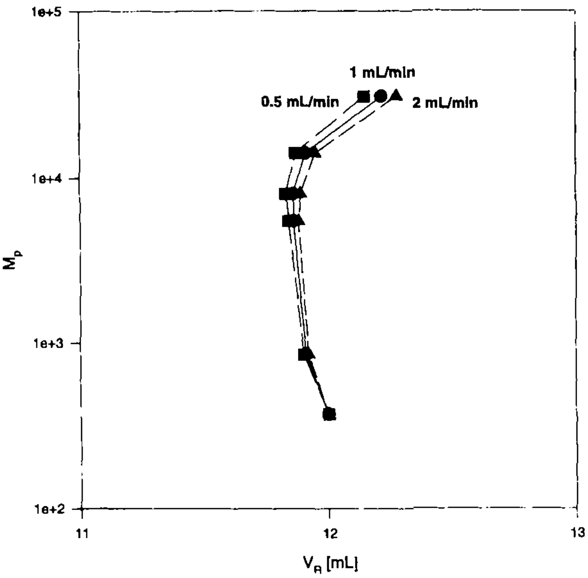

FIGURE 12 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown as a function of the mobile phase flow rate (mL/min). The packing material in the column was styrene–divinylbenzene gel.

lowering its retention volume. Therefore, secondary phenomena may be occurring at high flow rates. For example, the separation process may not be at equilibrium and could be kinetically controlled.

(*v*) *Effect of Sorbent Pore Size on LC LCA* Figure 13 identifies an LC LCA at 64 vol% *n*-hexane for polystyrene in THF/*n*-hexane using a 50-nm pore silica gel sorbent. A comparison of this with retention independent exclusion point observed with 100-nm pore silica gel (Figure 3: 50 vol% *n*-hexane) indicates an effect of pore size. However, differences in the surface chemistry, and hence the adsorptive properties, of the two silica gels could provide an alternative explanation for the differences in the LC LCA conditions.

CONCLUSIONS

This paper sought to mechanistically evaluate liquid chromatography under limiting conditions of adsorption. Further, it aimed to answer a

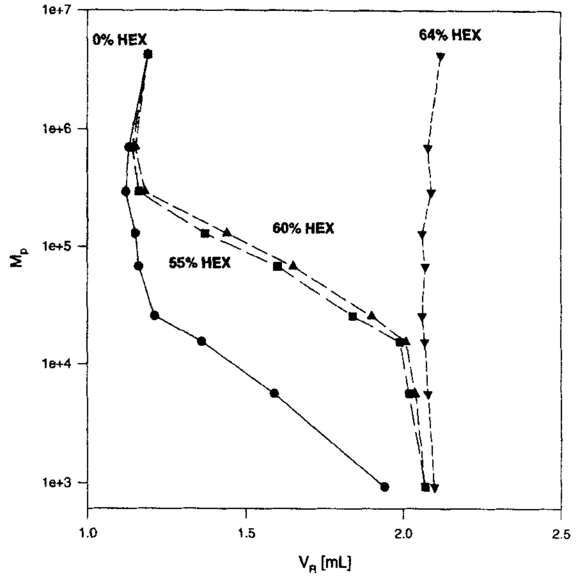

FIGURE 13 A plot of the molecular weight as a function of the retention volume (mL). The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown at various compositions, expressed as volume percentages. The packing material in the column was narrow-pore (50 nm) unmodified silica gel.

question which has not yet been posed regarding LC LCA and LC CAP: is the molecular-weight-independent retention condition sensitive to the operating conditions and characteristics of the stationary phase employed? The answer, based on the data presented herein for polystyrene in THF/*n*-hexane, appears to be that the LC LCA condition is influenced by the stationary phase but not extensively by the operational conditions. While the LC LCA point is relatively independent of the volume injected, concentration and mobile phase flow rate, it is highly dependent on the chemistry and possibly also on the pore size of the stationary phase, as well as the temperature. These results are summarized in Table I and have implications to users of the LC LCA methodology. Specifically, a published LC LCA condition can be duplicated over the normalized chromatographic conditions. However, and by no means the current practice, authors should specify the operational conditions used to identify LC LCA including mobile and stationary phases employed, temperature, the flowrate,

TABLE I Summary of the dependence of the LC LCA condition on chromatographic variables

Chromatographic variable	*Effect on the LC LCA condition*	*Explanation*	*Recommendation*
Volume injected	Small	1. Changes size of injection zone and diffusion of ADSORLI 2. Alters balance between exclusion and adsorption	LC LCA will occur for any reasonable injection volume. To increase molecular weight range for limiting conditions, larger injected volumes may be useful
Concentration injected	Small	Alters balance between exclusion and adsorption	LC LCA will occur for any reasonable injected concentration
Connection length (injection loop to column)	Minimal	Small shift in "calibration curve" due to change in retention volume. Adsorption is stronger for higher molecular weights due to ADSORLI mixing with the injection zone	Minimize the dead volume between the injection loop and column to avoid mixing and peak broadening. Applicable to LC LCA measurements.
Flow rate	Minimal	The balance between exclusion and adsorption is shifted in favor of the latter	LC LCA will occur for any reasonable flow rate
Stationary phase surface chemistry	Large	Sorbent surface chemistry controls the polymer adsorption	Use noninteractive stationary phases
Stationary phase porosity	Small	Small pores sizes can alter the balance between exclusion and adsorption which defines the LC LCA	Use a suitably porous column which does not exclude the polymers of interest
Temperature	Large	1. Reduction in adsorption with increase in mobile phase temperature 2. Shifts into the SEC mode	1. Thermostat column 2. Give temperature when quoting limiting conditions

injection volume and concentration, as well as the connection length between the injector and column.

The utility of limiting conditions for the characterization of polymer blends,[13] the tacticity of homopolymers[12] and random copolymers[17] has been demonstrated. Future experiments should determine if LC LCA can help resolve longstanding problems in liquid chromatography, such as the simultaneous characterization of the composition, functionality and molecular weight distributions by coupling LC LCA (composition) with LC CAP (functionality) and SEC (molecular weight).

Acknowledgments

This work was supported by the US-Slovak Scientific Technical Cooperation, Project 007-95.

References

[1] B.G. Belenkii, E.S. Gankina, M.B. Tennikov and L.Z. Vilenchik (1978). *Dokl. Akad. Nauk SSSR*, **231**, 1147; *J. Chromatogr.*, **147**, 99.

[2] S.G. Entelis, V.V. Evreinov and A.V. Gorshkov (1987). *Adv. Polym. Sci.*, **76**, 129.

[3] H. Pasch (1997). *Adv. Polym. Sci.*, **128**, 1.

[4] D. Berek (1996). *Makromol. Symp.*, **110**, 33.

[5] D. Berek, *Macromol. Symp.* (submitted).

[6] D. Hunkeler, T. Macko and D. Berek (1993). In *Chromatography of Polymers: Characterization of SEC and FFF*, Provder, T. (Ed.) (American Chemical Society, Washington, D.C.); *Polym. Mat. Sci. Eng.*, **65**, 101 (1991).

[7] D. Berek (1996). Proc. Simposio Latino Americano, Mar del Plata.

[8] B. Thrathnigg and X. Yan (1993). *J. Chromatogr.*, **33**, 467.

[9] H. Pasch and K. Rode (1995). *J. Chromatogr.*, **699**, 21.

[10] S.T. Balke and R.D. Patel (1983). In *Polymer Characterization: Spectroscopic, Chromatographic and Physical Instrumental Methods*, Carver, C.D. (Ed.); (American Chemical Society, Washington, D.C.).

[11] D. Hunkeler, M. Janco, V.V. Guryanova and D. Berek (1995). In *Chromatographic Characterization of Polymers*, Provder, T., Barth, H.G. and Urban, M. (eds.) (American Chemical Society, Washington, D.C.).

[12] D. Berek, M. Janco, K. Hatada and T. Kitayama (1994). *Polym. Bull.*, **32**, 629.

[13] D. Hunkeler, M. Janco and D. Berek (1996). In *Strategies in Size Exclusion Chromatography*, Dubin, P. and Potschka, M. (Eds.) (American Chemical Society, Washington, D.C.).

[14] A. Bartkowiak, D. Hunkeler, D. Berek and T. Spychaj (1998). *J. Appl. Polym. Sci.*, **69**, 2549.

[15] D. Berek and D. Hunkeler, *Macromolecules* (submitted).

[16] H.G. Elias, *Makromol. Chem.*, **33**, 140 (1959); **50**, 1 (1961); **78**, 72 (1964).

[17] A. Bartkowiak and D. Hunkeler (1988). In *Hyphenated Techniques*, Provder, T. (Ed.) (American Chemical Society, Washington, D.C.) (in press).