Copolymer Characterization by Liquid Chromatography under Limiting Conditions of Adsorption and Solubility

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Limiting conditions of adsorption and solubility have been identified and used to characterize random poly(styrene-*co*-methyl methacrylate)s according to their chemical composition. In particular, a shift in copolymer retention volume, under limiting conditions and in a pure solvent, can be used as a metric of copolymer composition yielding calibration curves over the full range of styrene levels. Liquid chromatography under limiting conditions of adsorption (LC LCA) has advantages over limiting conditions of solubility (LC LCS) due to a reduced peak broadening and the large number of limiting conditions of adsorption already identified. LC LCA can, therefore, be coupled with SEC for the simultaneous identification of copolymer composition and molecular weight distributions.

Keywords: HPLC; Copolymer; Limiting conditions of adsorption; Limiting conditions of solubility; Poly(styrene-*co*-methyl methacrylate)

INTRODUCTION

Liquid chromatography under limiting conditions of solubility (LC LCS),[1,2] involves the balancing of an entropic exclusion mechanism with an enthalpic process which influence polymer solubility and adsorption onto the stationary phase. The creation of an LC LCS condition requires the use of an eluent which is a weak nonsolvent for

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the polymer probe. Given this, an injection zone which is a thermo-dynamically good-solvent for the solute and differs in composition from the mobile phase, must be employed. A typical example, for polystyrene or poly(methyl methacrylate), is the use of THF as a solvent and *n*-hexane as a nonsolvent.[3] Under limiting conditions the separation proceeds through a microgradient processes of exclusion, precipitation and redissolution with the polymer eluting on the "limit" of its solubility. Due to its 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.[1,4] The LC LCS methodology has recently been extended to water-soluble polymers.[5]

Liquid chromatography under limiting conditions of adsorption (LC LCA) utilizes an eluent that strongly promotes the adsorption (ADSORLI) of macromolecules on the column. Indeed, if a polymer solution were injected in the eluent it would be fully retained. 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). 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".[6] As is the case with LC LCS, polymers injected in LC LCA elute with a molar mass independent retention volume. The principal advantage of the limiting condition methods over "critical conditions" is that the molar mass independent retention ranges from oligomers to macromolecules of molecular weights of over one million. This has been a limitation of other methods which combine entropic and enthalpic separation mechanisms that are generally only feasible only for molecules with molecular weights of up to the order of 10^5.[7]

Figure 1 schematizes the LC LCA and LC LCS domains relative to a cloud-point curve. Clearly the LC LCA mechanism operates in the soluble region where adsorption balances exclusion. In contrast, the
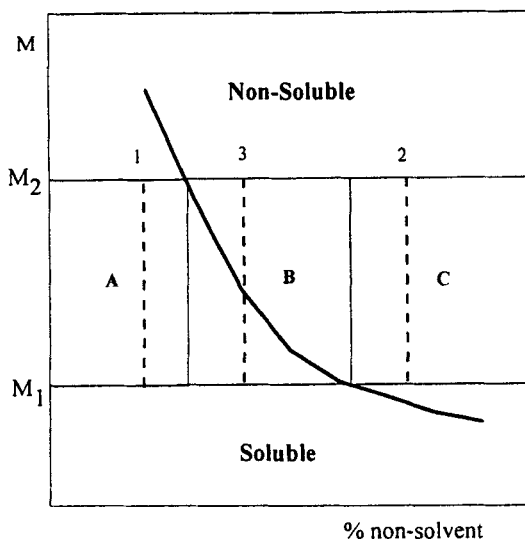
FIGURE 1   A schematic plot of the solubility of polymer standards in a mixed eluent (solvent plus nonsolvent) system, in interactive LC experiments. In domain A, adsorption is the operative enthalpic mechanism which is balanced with exclusion (LC LCA). A typical LC LCA calibration curve is shown by line 1. In domain C the polymer solvent solubility dominates enthalpy. 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 and the calibration curve crosses the cloud-point curve. Note that M_1 an M_2 represent the range where the retention volume is independent of the polymer molecular weight and are, therefore, column specific.

reduced polymer solubility characterized by LC LCS implies that both solubility and adsorption are enthalpic compliments to the entropic steric separation.

Extensive experimental observations,[8–10] and a review of limiting conditions[1] have been reported previously. The present paper seeks to apply LC LCA and LCS to the characterization of copolymers according to composition.

EXPERIMENTAL

Liquid Chromatograph

An L-6000 (Hitachi Instruments, Tokyo, Japan) isocratic pump coupled with an Hitachi L-4000 UV detector operating at a wavelength of

234 nm were utilized in all experiments. A Rheodyne type 7725i valve (Coati, CA, USA) with an injection loop of 20 µL was employed. Chromatograms were collected on a Pentium computer running LaChrom D-7000 Multi HPLC Manager Software (Merck, Germany). The separations involved 1.0 mL/min flowrate, a solute concentration of 1.0 mg/mL with a 2-cm tubing (500 µm ID) connection length between the valve and column.

Mobile and Stationary Phases

Spectranalyzed grade THF (Fisher, Norcross, GA, USA and Merck, Switzerland) and HPLC grade n-hexane (Fisher and Merck) were used as received. A Shodex (JM Science, Grand Island, NY, USA) linear GPC 806L column (0.8 × 30 cm) packed with 10 µm poly(styrene-co-divinylbenzene) particles was employed for the LC LCS experiments. A Tessek (Prague, Czech Republic) SGX-500 column (1 × 25 cm) packed with 10 µm unmodified silica gel was utilized for LC LCA studies. All measurements were thermostated to 25 ± 0.1°C with a Hitachi L-7300 column oven.

Polymers

Polystyrene standards with a molecular weight range of 370–1,400,000 were purchased from American Polymer Standards Corporation (Mentor, Ohio, USA). Poly(methyl methacrylate) standards with a PDI of < 1.1 were obtained from several suppliers. Random poly(styrene-co-methyl methacrylate)s with a molecular weight of 123,000–325,000 and the polydispersity (1.8–2.5) were prepared at the Slovak Academy of Sciences, Bratislava and have been described in a previous publication.[10]

Cloud-Point 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. All measurements

were carried out at a temperature of $23 \pm 1°C$. Samples were agitated with magnetic stirring bars.

RESULTS AND DISCUSSION

Identification of Limiting Conditions of Adsorption and Solubility

The stationary phase plays an important role in the determination of the enthalpic separation under limiting conditions. Figure 2 illustrates that, for a given mobile phase (THF/n-hexane), poly(methyl methacrylate) of various molecular weights can be eluted under either LCA, if an
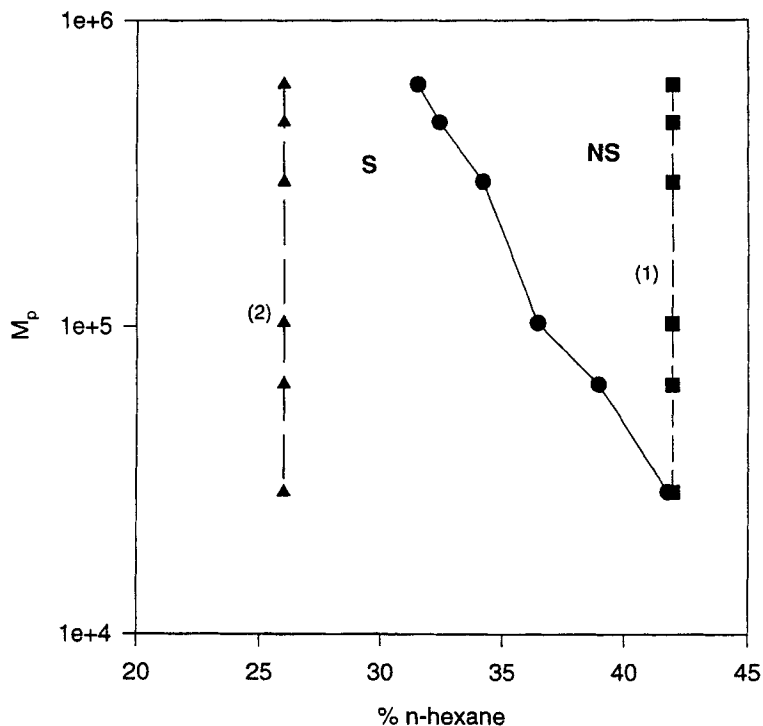
FIGURE 2   A cloud-point curve for poly(methyl methacrylate) in THF/n-hexane. Measurements were performed at a polymer concentration of 1.0 mg/mL. Line 1 designates the LC LCS point, which is clearly in the insoluble domain (obtained using a poly(styrene-divinylbenzene) sorbent). Line 2 designates the LC LCA condition which lies in the soluble region (obtained with a silica sorbent).

active sorbent is employed, or LCS if a more inert stationary phase is utilized. Specifically, when a highly polar silica is employed, the PMMA interacts with the sorbent and the strong, through reversible, adsorption, results in an LC LCA at 26 vol% n-hexane in n-hexane/THF. In contrast, when apolar poly(styrene-divinylbenzene) is employed, adsorption on the polar surface is reduced, relative to the silica gel, and a larger nonsolvent concentration (42 vol% n-hexane) is required to reduce the polymer solubility to a sufficient extent to balance the entropic exclusion process.

Figure 3 illustrates the effect of adding an n-hexane nonsolvent to the calibration curves for polystyrene in THF over a silica sorbent.
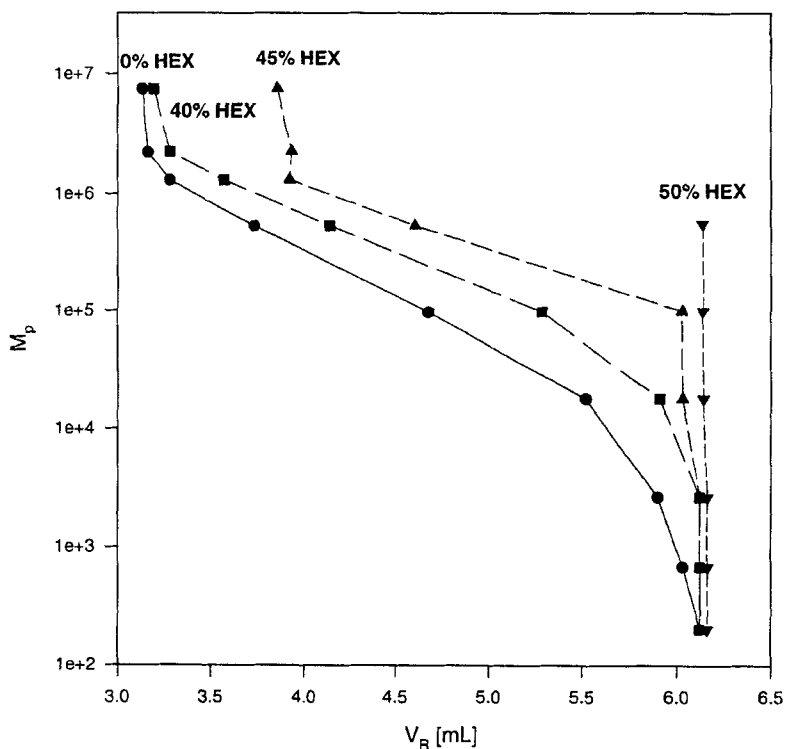

FIGURE 3 A plot of the retention volume of polystyrenes (mL) as a function of molecular weight. The calibration curves for narrow polystyrene standards in a mixed eluent (THF/n-hexane) are shown at various compositions, expressed as vol%. A silica based sorbent was employed.

Clearly at the LC LCA for PMMA (26 vol% *n*-hexane/74 vol% THF) polystyrene elutes in the SEC domain. Therefore, one can separate polystyrene and poly(methyl methacrylate) homopolymers, as has been previously shown.[1] This paper will demonstrate that these LC LCA conditions can also be used to characterize random copolymers according to their composition.

Figure 4 shows the effect of the addition of *n*-hexane to a THF mobile phase on the elution of polystyrene using a column packed with poly(styrene-divinylbenzene) particles. At 42 vol% *n*-hexane/58 vol% THF, an LC LCS for PMMA is identified. However, the polystyrene remains eluted in the exclusion mode at 42% *n*-hexane. Therefore,
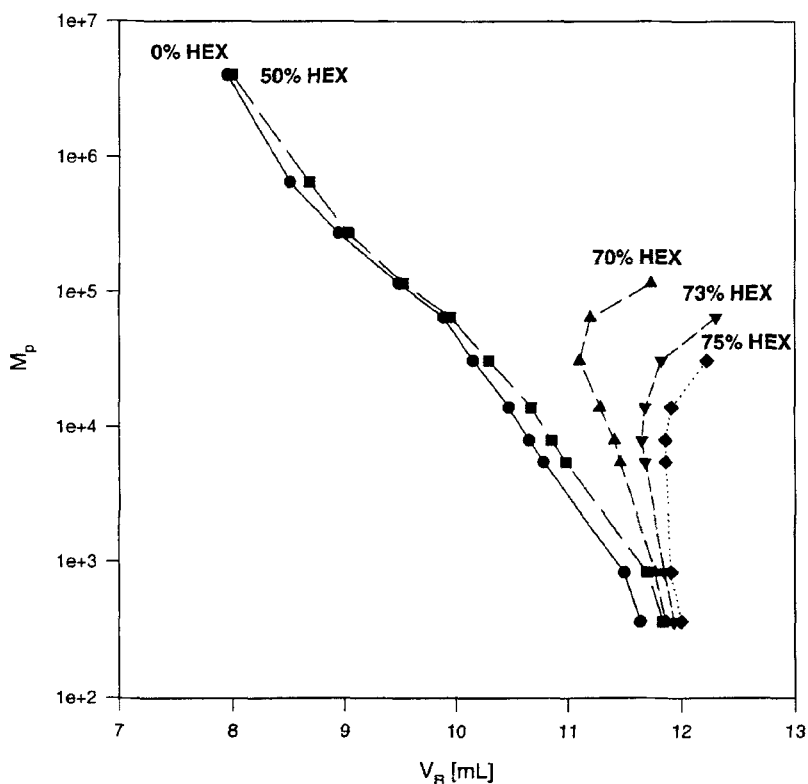

FIGURE 4 A plot of the retention volume of polystyrenes (mL) as a function of molecular weight. The calibration curves for narrow polystyrene standards in a mixed eluent (THF/*n*-hexane) are shown at various compositions, expressed as vol%. A poly(styrene-divinylbenzene) based sorbent was employed.

in principle, both LC LCS and LC LCA can be used for the character-
ization of poly(styrene-*co*-methyl methacrylate) copolymers. These
will be discussed sequentially in the following sections.

Copolymer Characterization Under Limiting Conditions of Solubility

Figure 5 shows chromatograms for poly(styrene-*co*-methyl methacry-
late)s as a function of copolymer composition. The experiments were
performed under LC LCS conditions for PMMA (42 vol% *n*-hexane/
58 vol% THF). A shift to lower retention volumes, relative to pure
PMMA, is observed as the content of styrene in the copolymer
increases. This is reasonable since, at LC LCS for PMMA polystyrene
elutes in the SEC mode and one would expect copolymers high in
styrene to move faster through the column. Figure 5 also illustrates
that for copolymers with high PMMA contents, peak broadening
and polymer retention in the column are more pronounced. This is
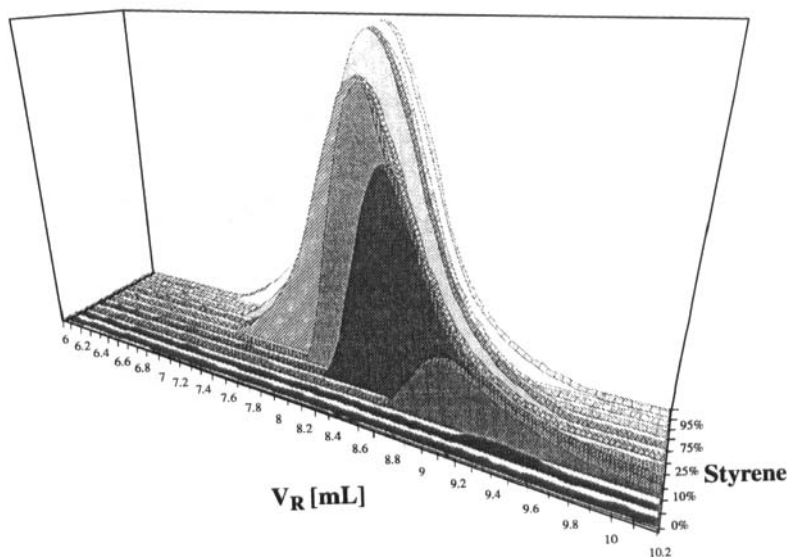

FIGURE 5   Chromatograms for a series of random poly(styrene-*co*-methyl meth-
acrylate)s separated at the LC LCS for PMMA (42 vol% *n*-hexane/58 vol% THF). Peak
broadening, associated with a strong copolymer retention within the column, is evident.

Peak distance   D
Ratio of peak areas S_2/S_1
Ratio of peak heights H_2/H_1

good solvent

LCA or LCS

H_1

H_2

S_1
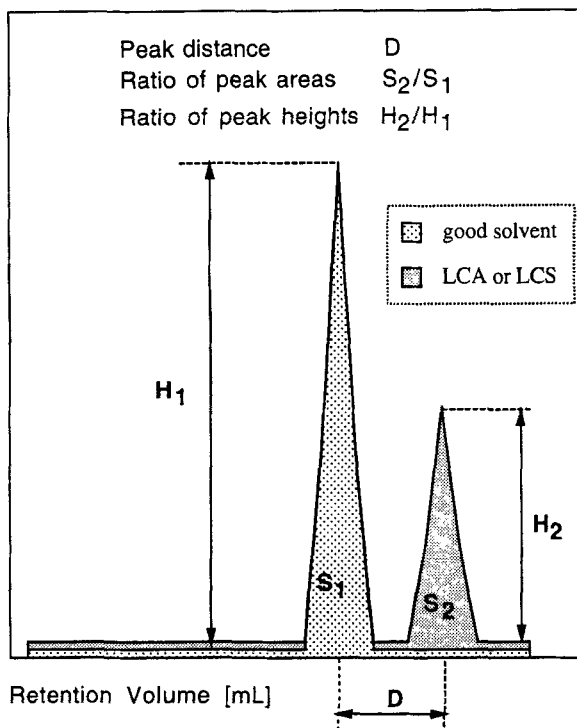
S_2

Retention Volume [mL]   D

FIGURE 6   A schematic describing the reduction in peak area, peak height, as well as the difference in retention volume, relative to pure SEC, for a polymer eluting under limiting conditions.

diagrammed schematically in Figure 6 where three metrics, the peak height, peak area and difference in retention volume of the copolymer under limiting conditions and in a pure solvent, can be used to distinguish copolymers according to their composition.

Figure 7 shows the change in peak height and area as a function of the styrene content of the copolymer. A type of "calibration curve" can be established which is sensitive over a limited copolymer composition range (0–30 wt% styrene). A more useful indicator of copolymer composition is the retention volume difference, which is shown in Figure 8. At LC LCS, the peak distance (retention volume difference between the copolymer eluting under LC LCS and in a pure solvent) correlates with copolymer composition. However, by employing a solvent
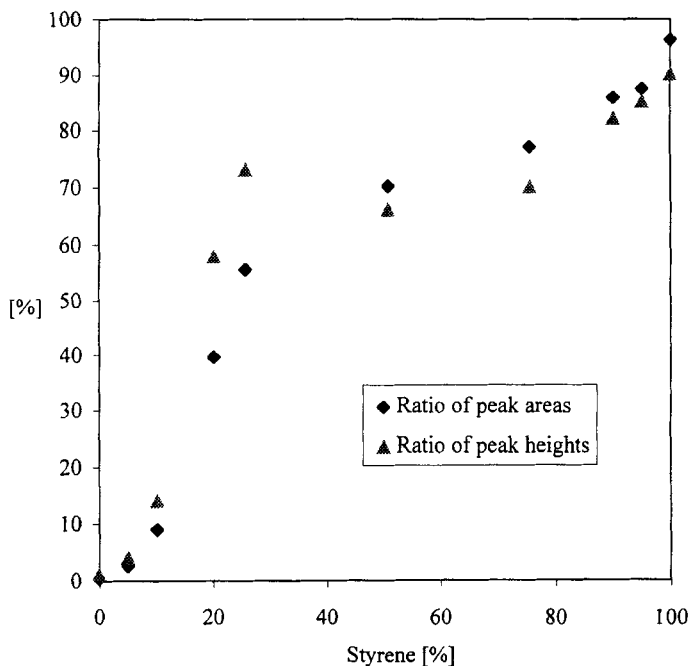
FIGURE 7   A plot of the peak area and peak height ratio of a poly(styrene-*co*-methyl methacrylate) copolymer at the LC LCS of PMMA as a function of the molar fraction of styrene in a poly(styrene-*co*-methyl methacrylate) polymer. Measurements were performed at 42 vol% *n*-hexane in THF/*n*-hexane.

of lower thermodynamic quality (higher concentration of the *n*-hexane nonsolvent) the useful range of this "calibration curve" can be extended to 50% polystyrene. This implies that "near limiting" conditions are actually preferable for copolymer characterization then limiting conditions, eliminating the need to identify the LC LCS precisely prior to copolymer characterization, thereby saving considerable methods development time. It should also be mentioned that even though polymer is retained on the column during LC LCS, Berek *et al.*[11] have shown that this can be easily flushed off with an injection of good solvent. Furthermore, the adsorption of polymer corresponding to 200 injections under LC LCS is insufficient to shift the calibration curve. Therefore, although peak broadening, and the lack of a "calibration curve" extending over the entire copolymer composition are concerns related to the LC LCS method, if employed for copolymer
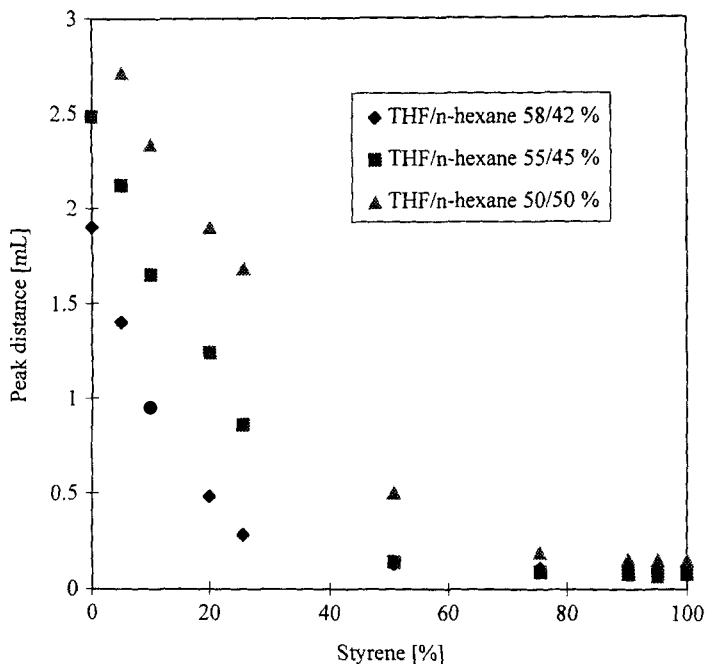
FIGURE 8 A plot of the difference in the retention volume (peak distance) of a poly(styrene-*co*-methyl methacrylate) copolymer under LC LCS of PMMA, relative to that under SEC (pure THF). The peak distance is a function of the molar fraction of styrene in the poly(styrene-*co*-methyl methacrylate) copolymer.

TABLE I Summary of limiting conditions identified for polystyrene and poly(methyl methacrylate) in THF/*n*-hexane for various stationary phases

Separation mechanism	Stationary phase	Limiting condition (*vol% n-hexane in n-hexane/THF*)	
		Poly(methyl methacrylate)	Polystyrene
LC LCA	silica gel	26	50
LC LCA	poly(styrene-divinylbenzene)	—	74
LC LCS	poly(styrene-divinylbenzene)	42	—

characterization, the polymer retention is not foreseen as a difficulty, either experimentally or in terms of data repeatability and interpretation. Table I summarizes the limiting conditions observed for polystyrene and poly(methyl methacrylate) in *n*-hexane/THF over silica and organic gel-based stationary phases.

Copolymer Characterization under
Limiting Conditions of Adsorption

Figure 9 shows the chromatograms for poly(styrene-*co*-methyl methacrylate)s of various compositions at the LC LCA for PMMA (26 vol% *n*-hexane/74 vol% THF). The peak height and area are quite similar to that of pure PMMA, in contrast to that observed in LC LCS. Therefore, the best metric to correlate with copolymer composition is the retention volume. Figure 10 illustrates the tendency in retention difference between the copolymer eluting under LCA conditions and in pure solvent (THF). A monotonous trend is observed over the complete composition range. Therefore, in the case of poly(styrene-*co*-methyl methacrylate), LC LCA is advantageous relative to LC LCS due to the significantly lower peak broadening. LC LCA conditions have also
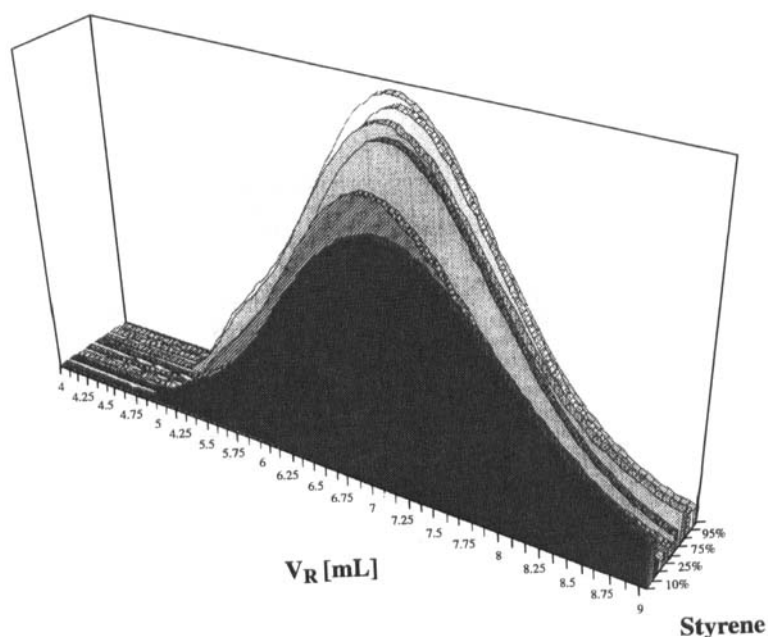

FIGURE 9   Chromatograms for a series of random poly(styrene-*co*-methyl methacrylate)s separated at LC LCA for PMMA (26 vol% THF/74 vol% *n*-hexane). Relative to LC LCS (Figure 5), the peak broadening and copolymer retention within the column are minimal.
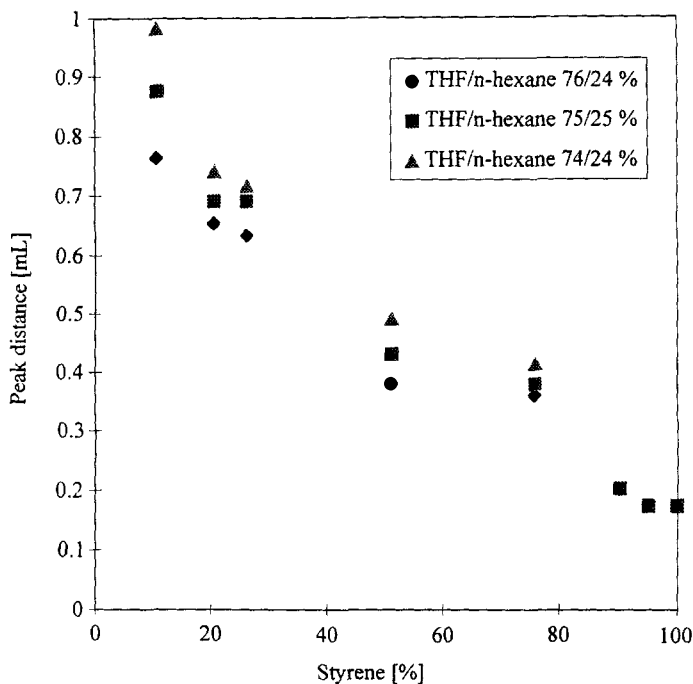
FIGURE 10 A plot of the difference in the retention volume (D) of a poly(styrene-co-methyl methacrylate) copolymer under LC LCA of PMMA, relative to that under SEC, as a function of the molar fraction of styrene in a poly(styrene-co-methyl methacrylate) polymer. The peak distance axis is logarithmic [1 + log(D)].

been identified for several polymer-mobile phase compositions[1] rendering it more likely for widescale copolymer characterization than LC LCS for which conditions have been, at least thus far, not extensively observed. Table II summarizes the advantages and difficulties associated with the two limiting condition methods evaluated herein for copolymer characterization.

CONCLUSIONS AND FUTURE WORK

The experiments conducted to date have been carried out on single columns. One would expect that if multiple columns were employed, with higher plate counts, the resolution for copolymer characterization

TABLE II A comparison of limiting conditions of adsorption and limiting conditions of solubility for the characterization of poly(styrene-co-methyl methacrylate)

Property of chromatogram	Separation conditions	
	LCA	LCS
Peak broadening	Minimal	Significant
Difference in retention volume in a good solvent and under limiting conditions	Linear dependence between retention volume difference and copolymer composition over the whole composition range	Linear dependence between retention volume difference and copolymer composition up to 40 wt% styrene
Ratio of peak areas under limiting condition and using a good solvent	no dependence	Curvilinear dependence between peak area ratio and copolymer composition over the whole composition range
Ratio of peak heights under limiting condition and using a good solvent	no dependence	Curvilinear dependence between peak height ratio and copolymer composition up to 30 wt% styrene

could be improved. Furthermore, the application of limiting conditions whereby polymers from oligomers to molecular weights of over one million are eluted at the same retention volume permits a separation according to composition exclusively, effectively decoupling the composition and molecular weight distributions. The subsequent off-line and on-line coupling of LC LCA with an SEC column, to simultaneously resolve composition and molecular weight, a longstanding goal in polymer characterization, will be attempted. The extension of the limiting condition methods to other polymers will also be carried out, along with a thermodynamic modeling of the mechanism of separation.

Acknowledgments

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