Characterization of Random Copolymers by Liquid Chromatography under Limiting Conditions of Adsorption

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Abstract: Liquid chromatography under limiting conditions of adsorption (LC LCA) can be considered as a new tool to perform, simultaneously, molar mass and composition determination of statistical copolymers and to separate copolymer blends as a function of copolymer composition or microstructure. The suitability of LC LCA has been demonstrated for two series of statistical copolymers, poly(styrene-*co*-methyl methacrylate), poly(S/MMA), varying either in molar mass or composition. The binary eluent consisted of a mixture of *n*-hexane and THF, containing either 26 or 30 vol.% of *n*-hexane, respectively, for *low* and *high* LC LCA. Two parameters, the ratio of peak area over height and the ratio of peak area of chromatograms obtained by LC LCA and size exclusion chromatography (SEC), were employed to evaluate statistical copolymer elution through LC LCA. From the peak analyses, macromolecule recovery was complete over the entire composition and the molar mass ranges. LC LCA and SEC were further compared, and off-line fractionation was subsequently performed using a combination of both methods, with different statistical copolymer solutions having narrow, broad, and bimodal composition distributions.

INTRODUCTION

Over the past two decades, various chromatographic methods have been developed that have employed solvent mixtures as eluents. Both isocratic[1] and gradient-based liquid chromatography[2,3] have been utilized to characterize oligomer,[4] polymer[5,6] or copolymer blend,[7] statistical,[2] block,[8,9] and graft copolymers[10] as well as to separate homopolymers according to their tacticity.[11,12] Based on the different elution behavior of homopolymers with respect to the eluent composition (exclusion, transition, adsorption),[1] several attempts have been made to develop isocratic elution methods for the determination of statistical or block copolymer composition. For example, liquid adsorption chromatography (LAC) has been studied by Mori[7] and was applied to analyze the chemical composition of statistical poly(styrene-co-methyl methacrylate) copolymers. So-called "critical conditions" have also been defined[13] and found to lead to an elution of homopolymers independent of their molar mass. In such experiments, the eluent corresponds to a mixture of a thermodynamically good solvent and a non-solvent for the macromolecules. In this case, the eluent has the same composition as the solvent used to solubilize the sample. These conditions have been applied to copolymer characterization in terms of molar mass[14] and composition. It seems that one part of the block copolymer becomes "invisible"[8] due to thermodynamic compensation.[15] Recently, similar methods have been defined as "limiting conditions". They differ from the previous "critical conditions" in that the macromolecules are dissolved and injected in a good solvent while the eluent remains a mixture composed of a good solvent and a non-solvent.

Liquid chromatography (LC) under limiting conditions can be subdivided according to the solubility of the homopolymer in the eluent and to the eluent interaction with the stationary phase. These subdivisions are LC under limiting conditions of solubility (LC LCS),[16,17] LC under limiting conditions of desorption (LC LCD),[18] and LC under limiting conditions of adsorption (LC LCA).[17,19,20] Limiting conditions are defined as the binary eluent (solvent/non-solvent) composition where the retention volume of the homopolymer is molar mass independent. The eluent is a mixture of adsorption- and desorption-promoting liquids, so-called adsorli and desorli.[21] In the case of LC LCA, the macromolecules are injected dissolved in the desorli, which is also a good solvent, and the polymer chains are soluble in the eluent (adsorli). Indeed, the vertical calibration curve (retention volume versus molar mass) of the homopolymer is located under the cloud-point curve, i.e., in the solubility zone first observed by Bartkowiak et al.[17,20] The difference in nature between eluent and injection solvent influences the elution mechanism of this method, as it has been previously described.[18] As the macromolecules

move faster than the injection zone (desorli), when the polymers "leave" the injection zone, they encounter the eluent (adsorli) and are retained by adsorption on the stationary phase, until they are reached by the slower moving desorli. As a consequence, the macromolecules desorb and begin to elute again. This process of exclusion, adsorption, desorption, and redissolution occurs until an equilibrium is established where the macromolecules elute on the front (leading edge) of the injection zone,[18] on the limit of their adsorption.

Previous studies related to LC methods, and most particularly to LC LCA, have shown the possibility of evaluating the copolymer composition and the composition distribution.[17,22] Prior to applying LC LCA to the analysis of poly(styrene-*co*-methyl methacrylate) (poly (S/MMA)) polymers, the convenient eluent composition must be identified with the corresponding homopolymers. Using a mixture of THF/ *n*-hexane containing between 26 and 30 vol.% of *n*-hexane, poly(methyl methacrylate) homopolymer is eluted independently of molar mass and poly(styrene) homopolymer is eluted according to a size exclusion chromatography (SEC) process.[20] In this work, two conditions have been then applied to separate statistical poly(S/MMA) copolymers: *low* and *high* LC LCA, corresponding to 26 and 30 vol.% of *n*-hexane, respectively. In order to decouple both molar mass and composition effects, two series of model copolymers, varying either in molar mass or in composition, have been synthesized and characterized. With these model compounds, a calibration curve was established relating the peak distance (PD) to the copolymer composition.[22] The PD was defined as the difference of the retention volumes obtained from SEC and LC LCA[23] and is related to the copolymer composition according to the following empirical power equation:

$$PD = 201 \cdot c^{-1.48} \tag{1}$$

where PD is the peak distance (mL) and c is the copolymer composition (molar fraction of styrene). This calibration is applicable to the determination of statistical poly(S/MMA) copolymer composition in a copolymer composition range that is influenced by the mobile phase composition. This application window corresponds to a composition of 0.1–0.5 and 0.2–0.6 molar fraction of styrene for eluent compositions of 74/26 and 70/30% vol. THF/*n*-hexane,[22] respectively.

Based on the aforementioned preliminary experimental results, this article focuses on a comprehensive study of copolymer poly(S/MMA) separation using LC LCA. The first part deals with the evaluation of the suitability of LC LCA methods, with the help of two parameters, the ratio of peak area over peak height and the ratio of the peak area of chromatograms obtained by LC LCA and SEC. In the second part,

the elution mechanism is further studied through off-line fractionation. Three fractions were collected after the separation by the chromatography column and analyzed in terms of molar mass and composition. Composition was determined using the former calibration curve relating peak distance to copolymer molar fraction.

EXPERIMENTAL SECTION

Copolymer Samples

Two series of copolymers were obtained by radical solution polymerization as previously described;[22] the variation in composition and molar mass is illustrated in Figure 1. The first series varies with respect to the styrene/methyl methacrylate ratio in the copolymer (0.08–0.85 molar fraction of styrene), at a constant molar mass (220,000 ± 30,000 g/mol), while the second series possesses molar masses from 20,000 to 250,000 g/mol, at a molar styrene fraction of 0.5 ± 0.02. Molar mass and composition of the copolymers selected herein were determined by light scattering and ^{1}H NMR[22] and are given in Table I.
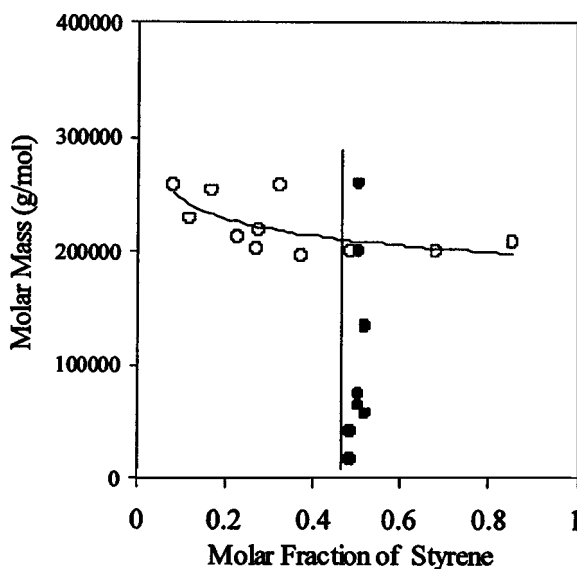
Figure 1. Molar mass as a function of composition for both series of poly-(S/MMA) copolymers (○: various compositions, ●: various molar masses).

Table I. Composition (from ^{1}H NMR) and molar mass (from light scattering) of the copolymers employed

Copolymer designation	Composition (molar fraction of styrene)	M_w (g/mol) 10^3
Poly(S/MMA) 23	0.17 ± 0.01	237
Poly(S/MMA) 24	0.23 ± 0.01	192
Poly(S/MMA) 19	0.28 ± 0.01	261
Poly(S/MMA) 20	0.37 ± 0.01	165
Poly(S/MMA) 3	0.52 ± 0.01	136
Poly(S/MMA) 10	0.85 ± 0.01	244

SEC and *Low* and *High* LC LCA Methods

Tetrahydrofurane (THF) and *n*-hexane, both of HPLC grade, were purchased from SDS (France). Various mobile phases were employed according to the separation mode. The respective THF and *n*-hexane volume contents are given in Table II. *Low* LC LCA corresponds to the lowest *n*-hexane content that permitted a poly(MMA) elution independent of molar mass.

A CGX 500 silica column (300 mm length, 7 mm ID) with 10 μm particles and an average pore size of 50 nm (Tessek, Praha, Czech Republic) was employed for all experiments. The liquid chromatography system consisted of an L-7100 isocratic pump (Hitachi Instruments, Tokyo, Japan) coupled with a Hitachi L-7400 UV detector operating at a wavelength of 260 nm and a Rheodyne type 7100 injector with an injection loop of 20 μL. Chromatograms were analyzed using D-7000 HPLC system manager interface and software (LaChrom, Merck-Hitachi). Fractions were collected with a L-7650 Merck collector. The standard separation was carried out at 0.5 mL/min flow rate, with a solute concentration of 1.0 mg/mL. All experiments were performed at a controlled temperature of $25 \pm 0.1°$C (Hitachi L-7300 Column oven).

Table II. Eluent composition corresponding to the different methods

| Method	THF (vol. %)	*n*-hexane (vol. %)	Composition application range (molar fraction styrene)
SEC	100	0	0–1.0
Low LC LCA	74	26	0.1–0.5
High LC LCA	70	30	0.2–0.6

RESULTS AND DISCUSSION

In chromatographic methods, whenever a strong adsorption of the macromolecules is observed, such as with LC LCS or LC LCD, irreversible molar mass dependent adsorption of copolymer onto the stationary phase may occur.[17,24] This phenomenon can become a long-term drawback even if macromolecules can be desorbed easily by desorli flushing. In order to evaluate the suitability of the LC LCA methods applied to statistical poly(S/MMA) copolymers, macromolecule recovery and peak broadening were studied in an initial stage.

Suitability of LC LCA Applied to Statistical Poly(S/MMA) Copolymers

Since pure THF corresponds to SEC conditions, the peak area of chromatograms recorded under these conditions represents the total amount of macromolecules injected and will be considered as a reference. The same copolymer solutions were injected by SEC and *low* and *high* LC LCA; the peak area ratios of chromatograms obtained by limiting conditions and SEC are plotted as a function of copolymer composition and molar mass in Figures 2(a) and 2(b). Above a 0.2 molar fraction of styrene (Figure 2(a)), all the points are scattered around unity, indicating that adsorption is not observed regardless of the copolymer composition. The application range of both methods is given in Table II, and the shaded areas correspond to *low* LC LCA and *high* LC LCA, respectively, and overlap in the range of 0.2–0.4. Under the lower limit of the application window, copolymers were eluted according to an LC LCA mechanism.[22] Macromolecules were eluted at the same time as the eluent, and the peak area was completely modified. Furthermore, no influence of molar mass on the peak area is observed in the range from 20,000 to 250,000 g/mol (Figure 2(b)).

The chromatograms obtained under SEC and limiting conditions are superposed in Figure 3. The maximum of both peaks is aligned to zero. Since experimental retention volumes are different according to both methods, the solvent peak (thin line) is observed only in the case of LC LCA. Figure 3 also reveals an appreciable difference in the peak shape. The ratio of peak area to peak height (S/H) has been utilized as a crude peak broadening indicator. This ratio was calculated from SEC and *low* and *high* LC LCA chromatograms and is plotted as a function of composition as well as molar mass (Figures 4(a) and 4(b)). Considering first the SEC process, the S/H ratio is constant over all the composition range, indicating that this series of copolymer has similar composition and molar mass distributions. In contrast, in the case of the limiting condition processes, the S/H ratio depends strongly on the copolymer composition,
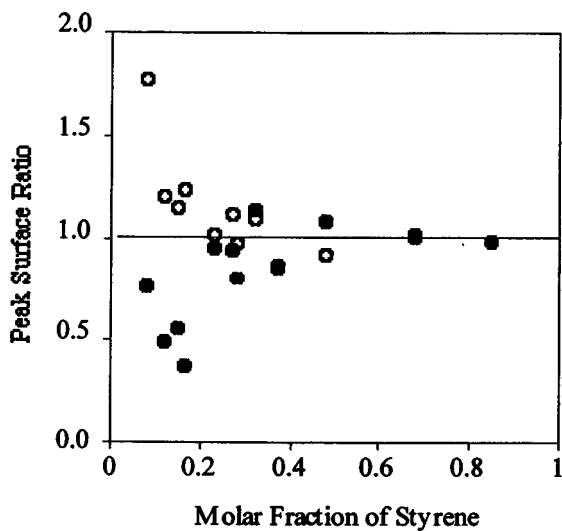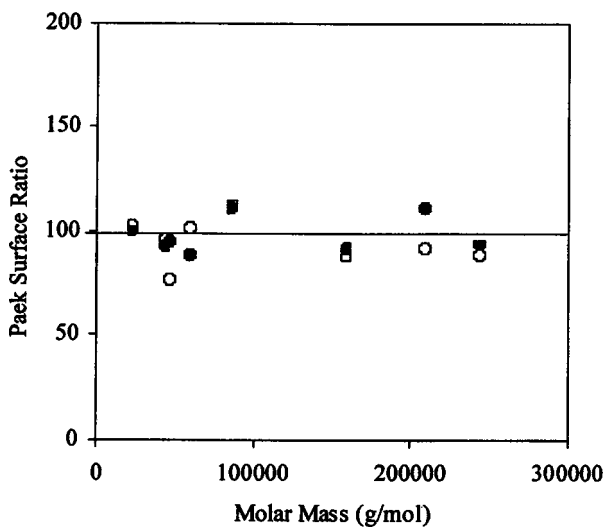
Figure 2. Peak surface ratio between LC LCA and SEC as a function of (a) composition and (b) molar mass. Peak surface ratio (○, ●).

and three domains can be separately described. Therefore, the shaded areas in Figure 4(a) correspond to the application range of the methods: *low* LC LCA (0.1–0.5 molar fraction of styrene) and *high* LC LCA (0.2–0.6 molar fraction of styrene). The elution behavior of the
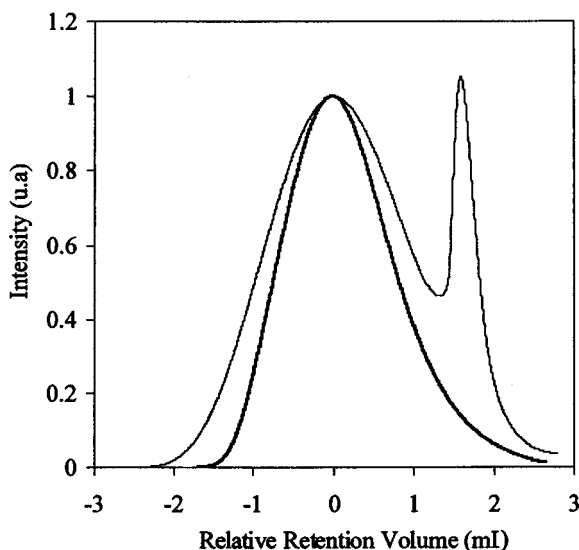
Figure 3. Superposition of SEC (—) and *high* LC LCA chromatograms. In the case of *high* LC LCA, the lines (—) and (—) correspond to the sample and solvent peak respectively. The data were obtained with solution 1, which contains poly(S/MMA) 24. An application range (\\\, ///) for *low* and *high* LC LCA respectively.

macromolecules in the three different zones can explain the variation of the S/H ratio.

1. Below the lower limit of the application range (molar fraction of styrene below 0.1), macromolecules are eluted at the same time as the eluent since the copolymers are mainly composed of MMA units. As a consequence, there is no gradual separation of the solute and macromolecule elution behavior is completely modified, with peaks becoming narrower.
2. In the application range of both *low* and *high* LC LCA methods (0.1–0.6 molar fraction of styrene), the S/H ratio is larger than those calculated by SEC. This indicates a mild peak broadening, as has also been observed by other authors.[17]
3. Above the upper limit of the application range, (i.e., above 0.6 molar fraction of styrene) modification of the peak shape is not observed in comparison to SEC.

   As the chromatographic parameters were kept identical in all injections, peak broadening cannot be attributed to any experimental difference.[25] The peak broadening phenomenon could, however, reveal
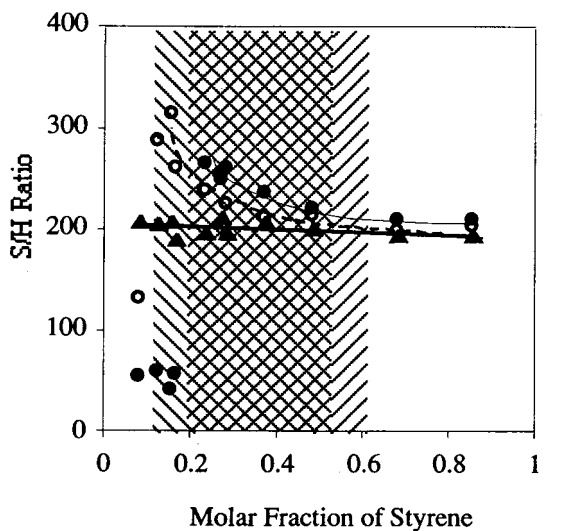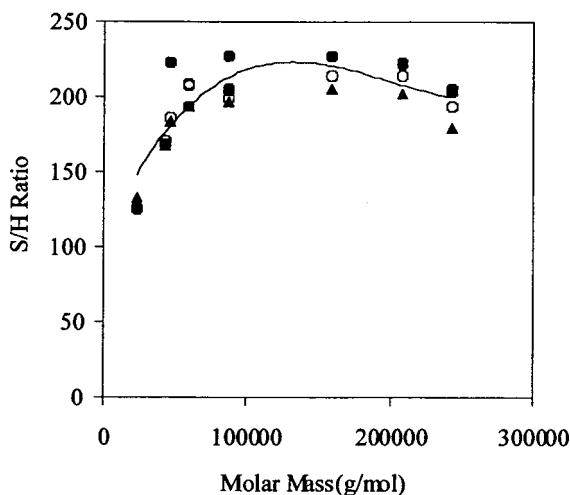
Figure 4. Ratio of the peak surface to the peak height as a function of (a) copolymer composition and (b) copolymer molar mass from chromatograms obtained using SEC (Δ), *low* LC LCA (○), and *high* LC LCA (●).

a modification in the macromolecule separation process between SEC and LC LCA. Adsorption interaction increases with MMA content and the elution process of the macromolecule gradually changes from SEC to LC LCA by decreasing molar fraction of styrene.[22] The increase

of adsorption interactions can explain the peak broadening, as has been previously mentioned[21] in the case of liquid chromatography at the point of exclusion adsorption transition (LC PEAT).
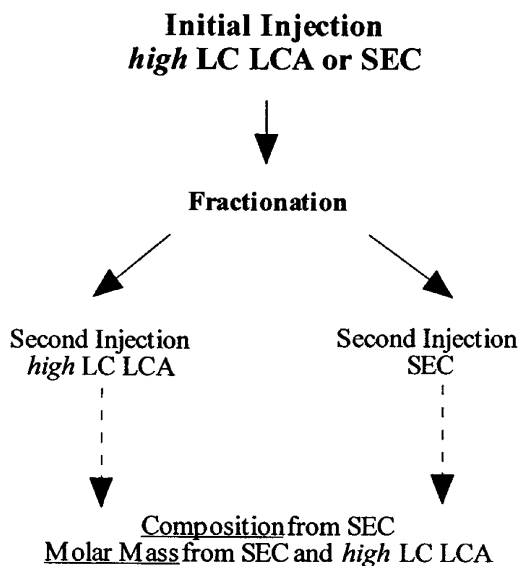
The peak area to height ratio (S/H) depends on the molar mass in a range from 20,000 to 250,000 g/mol, as illustrated in Figure 4(b). This behavior can be attributed to differences in the copolymer synthesis. Contrary to the composition effect, molar mass influence is the same on SEC and the LC LCAs process in the studied range. As the S/H ratio confirms the PD behavior of the copolymer as a function of composition, it can be used to complete the analysis of copolymer elution by LC LCA.

Fractionation of Statistical Copolymers

Classical cross-fractionation has been commonly applied for the determination of two copolymer properties and generally implies a first separation by SEC and a subsequent separation by another method, such as gradient HPLC, for example.[26] SEC separates by molecular size, which is influenced by molar mass and composition. In this study, the first step consists in a separation using LC LCA followed by SEC and LC LCA separations, since the only way to experimentally deconvolute the molar mass and composition distribution is by having the first separation according to only a single parameter.

Experimentally, two peaks are observed in the chromatograms (data not shown): the first, between 5 and 9 mL, corresponds to the sample peak and the second, 9–10 mL, is attributed to the solvent. Three fractions, having a volume between 0.5 and 1.5 mL, were collected at the exit of the column during the time corresponding to the sample peak (5–9 mL). The composition and molar mass are determined on the three fractions by reinjecting them in the column using either SEC or LC LCA methods, as illustrated by Scheme 1. For each fraction, the molar mass was calculated from a universal calibration curve based on poly(S) and the SEC retention volume. The composition resulted from the peak distance (PD), defined above.

Figure 5 shows an initial injection performed by *high* LC LCA and second injection by SEC, and Figure 6 shows the same initial injection followed by a *high* LC LCA separation. The peak distance of each fraction is then calculated by difference of retention volume obtained by *high* LC LCA and SEC. As the copolymer solution is diluted approximately 80 times after the first injection, the initial solution concentration was increased from 1 to 5 mg/mL so that the signal of the second injection could be more precisely detected. The decrease of the concentration by a factor of 80 does not affect the value of peak distance. The former

Initial Injection
high **LC LCA or SEC**

$\downarrow$

Fractionation

Second Injection   **Second Injection**
high LC LCA   SEC

Composition from SEC
Molar Mass from SEC and *high* LC LCA

Scheme 1. Off-line fractionation procedure for both SEC and *high* LC LCA methods.

calibration curve determined previously at higher concentration of copolymer (1 mg/mL) can be used without limitation.

The three fractions were collected after separation of copolymer solutions having different composition distributions. The molar mass and composition of the various copolymers are provided in Table I. Solution 1 contains only poly(S/MMA)24 and is considered as a narrow composition distribution. Solution 2 is a blend of three different copolymers and has the same average composition of 0.23 ± 0.06 molar fraction of styrene, though it exhibits a larger composition distribution than solution 1. Solution 3 corresponds to a bimodal composition distribution and consists of a blend of two copolymers having very different compositions: 0.17 and 0.85 respectively for poly(S/MMA)23 and poly(S/MMA)10. The three different solutions were fractionated according to Scheme 1. Both SEC (open symbols) and *high* LC LCA (filled symbols) were used as a first injection. For the three subsequent fractions, molar mass and composition are calculated according to their respective calibration curve. Molar mass is then plotted as a function of composition in Figure 7 and will be herein discussed in detail.

The separation of the copolymer solutions through an SEC process, i.e., initial injection using SEC, will be considered first. The plot of molar mass versus composition corresponds almost to a vertical line for the three solutions, and the value of the calculated composition corresponds
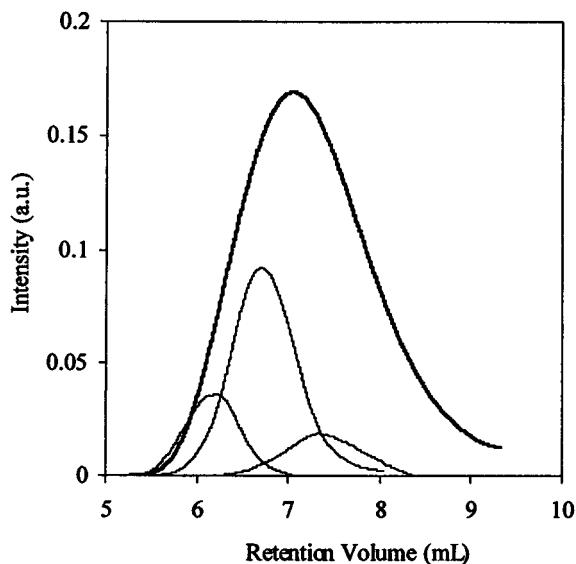
Figure 5. Fractionation of solution 3, containing poly(S/MMA) 10 and poly(S/MMA) 23. The initial injection was performed using *high* LC LCA, and the three fractions were subsequently injected in an SEC. Note that the peak intensity of the initial injection and the fractions are not on the same scale and are expressed in arbitrary units (a.u.).

to the average composition of the mixed sample. The polymer chains contained in the three fractions differ only in molar mass. As a consequence, the drift due to composition effect, during an SEC process, is also very weak. This is particularly pointed in the case of solution 3, where the composition of the fractions, 0.35 molar fraction of styrene, is close to the arithmetical average of the copolymer composition values. In contrast, in the first fraction the copolymers collected possess the highest molar masses. Molar masses are significantly different in the three fractions, indicating that the separation mechanism is primarily dependent on the molar mass.

The two-dimensional plots, molar mass versus composition, obtained using *high* LC LCA (filled symbols) and SEC (open symbols) as initial injections can be then compared for the three different solutions. Theoretically, an infinite slope would indicate a separation according to molar mass and a slope equal to zero should represent a separation according only to composition. Solution 1 (triangle symbols) exhibits a narrow composition distribution, and a slight decrease of the slope, from 6.4×10^6 to 3.0×10^6, is observed between both types of initial injection, SEC and *high* LC LCA, respectively. Since both straight lines are very
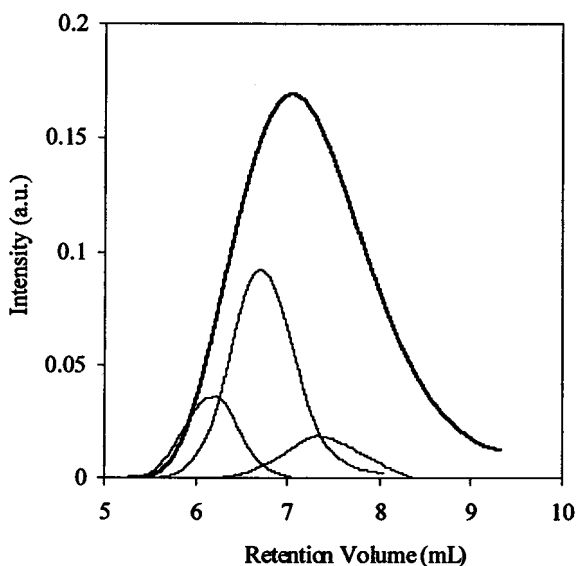
Figure 6. Fractionation of solution 3, containing poly(S/MMA) 10 and poly(S/MMA) 23. The initial injection was performed using high LC LCA, and the three fractions were subsequently injected using *high* LC LCA. Note that the peak intensity of the initial injection and the fractions are not on the same scale and are expressed in arbitrary units (a.u.).

close in terms of molar mass and composition, it seems that the difference between both elution methods (SEC or *high* LC LCA) is slight. Enlarging the composition distribution of the copolymer (solution 2, square symbols), the difference between the slope of molar mass versus composition for both types of initial injection, *high* LC LCA and SEC, is enhanced, 7.0×10^6 and 1.9×10^6, respectively. The average compositions are slightly different in the three fractions obtained by *high* LC LCA compared to SEC, indicating that separation is more influenced by composition. The enthalpic separation mechanism (LC LCA) in the first stage is more sensitive to the composition of statistical copolymer than the entropic elution process (SEC).

In the case of solution 3 (circle symbols), containing two copolymers with very different compositions (0.17 and 0.85 molar fraction of styrene), the composition of the three fractions obtained after SEC separation corresponds to the average value of the blend and is almost identical within the three fractions, as previously mentioned. In contrast, in the case of *high* LC LCA, the compositions determined in the three fractions are close to that of poly(S/MMA)10 (molar fraction: 0.85 of styrene). Indeed, poly(S/MMA)23, having a molar fraction of styrene
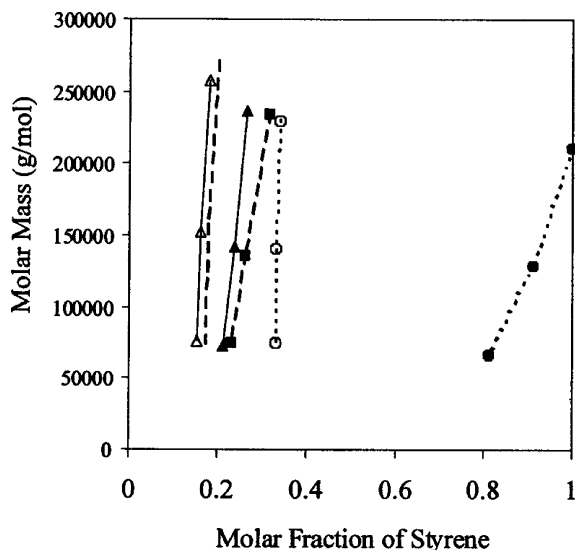
Figure 7. Molar mass versus composition for solutions 1, 2, and 3 (open symbols: initial injection in SEC, filled symbols: initial injection in *high* LC LCA). Solution 1: poly(S/MMA) 24, Δ; Solution 2: poly(S/MMA) 23 + poly(S/MMA) 19 + poly(S/MMA) 24, □; Solution 3: poly(S/MMA) 23 + poly(S/MMA) 10, ○.

of 0.17, is outside of the application range of *high* LC LCA, which has a lower limit of 0.2 molar fraction of styrene. Regarding the elution process, it appears that poly(S/MMA)10 and poly(S/MMA)23 chains are collected respectively in the sample peak and solvent peak. Moreover, Figure 7 shows that the three fractions obtained using *high* LC LCA present molar mass values almost identical to those obtained with SEC. As a consequence, we can conclude that macromolecules contained in solution 3 were separated according to their composition by using *high* LC LCA system, as was the goal at the outset of this research.

The composition influence on the *high* LC LCA elution remains quite far from an ideal elution process based on composition, which would be identified by a horizontal line in a representation of molar mass versus composition. Nevertheless, the copolymer composition effect is enhanced in *high* LC LCA compared to SEC. In the case of SEC, the composition of the three fractions is identical and corresponds to the average composition value of the solution, independent of the distribution. This is not the case for *high* LC LCA. Most particularly, for macromolecules possessing very different chemical compositions, macromolecule separation is dramatically influenced by their composition. Moreover, the solution having a bimodal distribution shows that during elution through *high* LC LCA, the composition effect comes *in addition to* the molar mass
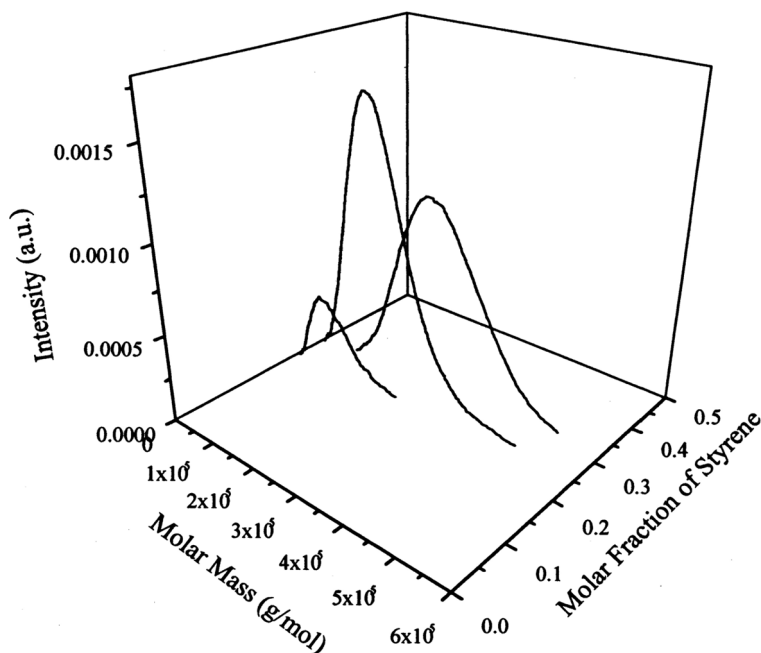
Figure 8. Three-dimensional plot of the molar mass versus composition of a co-polymer blend consisting of a poly(S/MMA) 23, poly(S/MMA) 19, and poly(S/MMA) 24 solution after fractionation with the initial injection in *high* LC LCA.

effect. Indeed, on one hand, due to the influence of composition, polymer chains having high styrene content (above 0.8 molar mass) are collected in the sample peak and are completely separated from polymer chains having low styrene content. On the other hand, since the three fractions of the sample peak present different molar masses, elution of the solute was also performed according to molar mass.

Simultaneous Molar Mass and Composition Distribution

Figure 8 provides another illustration of the decoupling of composition and molar mass effect during LC LCA elution. As described by Scheme 1, the two off-line combinations of two liquid chromatographic systems were used, LC LCA followed by SEC and LC LCA followed by LC LCA. Figure 8 shows that the three analyzed fractions (solution 2) exhibit different compositions and molar masses. That is, a continuous molar mass distribution is decoupled from a discrete composition distribution. Specifically, using the off-line combination of LC LCA followed by SEC,

it was also possible to determine the molar mass distribution as a function of composition. By multiplying the number of collected fractions, or performing on-line coupled LC LCA-SEC, it would be possible to draw a more detailed 3-D representation of the sample. To the authors' knowledge, Figure 8 represents the first evidence of decoupling of composition and molar mass distribution for statistical copolymers. Additionally, using *high* LC LCA, the separation of two copolymers having different compositions was possible. This could lead to other applications of LC LCA.

CONCLUSIONS

This work completes the study of the LC LCA mechanism[27] and shows that adsorption of statistical copolymers onto the stationary phase during elution was not observed using either *low* or *high* LC LCA. The peak shape was also evaluated through the ratio of peak area to peak height. This crude parameter was shown to be sufficiently significant to elucidate a difference in the elution mechanisms between SEC and limiting conditions. Moreover, it corroborates the limit of the application range of *low* and *high* LC LCA methods already presented.[22] LC LCA can be employed to determine copolymer composition using the calibration curve of PD versus molar fraction at various eluent compositions.

Overall, LC LCA-SEC off-line association shows that LC LCA can be considered a new tool to analyze composition and composition distribution as a function of molar mass. This could be studied in more detail by multiplying fractions (on-line injections) in order to give a more reliable three-dimensional description of the copolymer. The application of LC LCA to various polymer systems coupled with SEC canprovide an isocratic bivariate distribution. The authors believe that the power of the method complements its previously demonstrated versatility.[20] Other applications of LC LCA such as the separation of copolymer blends having different compositions or structures and the control of copolymer purity could be envisaged.

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