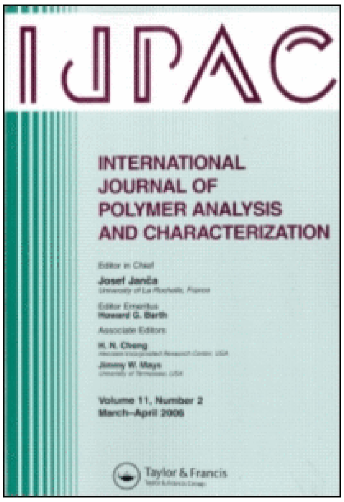

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Determination of the Composition of Statistical Copolymers by Liquid Chromatography Under Limiting Conditions of Adsorption*

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Liquid chromatography under limiting conditions of adsorption (LC LCA) was applied to determine the composition of statistical poly(styrene-methyl methacrylate) (PS/MMA) copolymer standards. Two series of copolymers were prepared and characterized. The first series varies with respect to the styrene/methyl methacrylate ratio in the copolymer, at a constant molar mass (220,000 ± 30,000 g/mol), while the second has molar masses from 20,000 to 250,000 g/mol at a molar styrene fraction of 0.5 ± 0.02. The copolymers were fractionated using size exclusion chromatography (SEC) and LC LCA. In the case of LC LCA, two different ratios of THF to *n*-hexane were employed. Low and high LC LCA were differentiated according to the composition of the binary eluent, the lowest *n*-hexane content corresponding to low LC LCA. The influence of the molar mass on the peak distance, defined as the difference of retention volume between SEC and LC LCA, was negligible in the studied range compared to composition effect. A calibration curve was subsequently established and correlated the peak distance to the copolymer composition. The application range of the calibration curve was found to vary with the percentage of *n*-hexane contained in the eluent and corresponded to 0.1–0.4 and 0.2–0.6 molar fraction of styrene for low and high LC LCA, respectively. Using these calibration curves, the P(S/MMA) copolymer composition distribution calculation was estimated for one sample. Therefore, the limiting condition method can be coupled on-line or off-line with SEC to perform a simultaneous characterization of the compositions and molar mass distributions.

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Keywords: Liquid chromatography; Liquid adsorption chromatography; Critical conditions; Statistical copolymers; Light scattering

INTRODUCTION

The determination of copolymer molar mass, composition and their respective distributions is of a paramount importance in the evaluation and optimization of macromolecular properties. Several analytical methods are available to estimate these parameters independently, though the bivariate distribution, modeled by Stockmayer four decades ago,[1] has still not been reliably quantified, even for simple copolymers.[2]

Size exclusion chromatography (SEC) is known to be a rapid and reliable method for homopolymer characterization. However, as molar mass and composition influence copolymer properties, the retention volume, and hence the elution of macromolecules, depends on both dimensions. During the past thirty years, attempts to overcome this problem have included orthogonal chromatography or cross fractionation,[3,4] multidetector arrangements, as well as coupling liquid chromatography with mass detection, FTIR,[5,6] NMR[7] and mass spectroscopy[8] detectors. An alternative family of methods controlled the balance of entropic and enthalpic influences by mediating the solute/solvent/stationary phase interactions.[9] Liquid adsorption chromatography, therefore, was employed with SEC by Mori to separate statistical poly(styrene/methyl methacrylate) copolymers[10] by composition and subsequently by molar mass. Macrogradient methods have been developed to separate copolymer blends either with continuous[10-12] or stepwise solvent changes[13,14] though they have been shown to lack repeatability.[15] The liquid chromatography under limiting conditions has been also used to characterize block copolymers and polymer blends.[16,17] These methods employ a binary eluent but, contrary to the previous methods, the mobile phase composition is kept constant during the elution time. Besides, macromolecules are dissolved and injected in solution in a good solvent unlike the binary eluent.

In the case of liquid chromatography under limiting conditions of adsorption (LC LCA), the binary eluent strongly promotes the adsorption of macromolecules onto the stationary phase. The mobile phase consists of a mixture of an adsorption promoting liquid, called *adsorli*, and a desorption promoting liquid, referred to as a *desorli*. The *desorli* has higher affinity for the column packing and corresponds to the good solvent for the macromolecules in which polymers are dissolved and injected. As the injection solvent and eluent are different, solute/solvent/stationary phase interactions are complex as previously described.[16-18] The macromolecules move faster than the injection zone. Therefore, when polymers "leave" the injection zone, they encounter the eluent and are retained by adsorption on the column packing until they are reached by the slower moving *desorli*. The macromolecules then desorb and begin to elute again. This process of exclusion, adsorption and desorption occurs until an equilibrium is established where the macromolecules elute on the front (leading edge) of the injection zone.

Prior to application of LC LCA to copolymers, limiting conditions should be first determined using the homopolymers corresponding to the copolymer. Limiting conditions are defined as the binary eluent composition where the retention volume of one of the homopolymers is molar mass independent. In the aim of studying statistical poly(styrene-methyl methacrylate copolymers (PS/MMA), this preliminary step has been previously performed[19] with poly(methyl methacrylate) (PMMA) and polystyrene (PS) homopolymers. At the eluent composition of 74/26%vol of THF/*n*-hexane, PMMA macromolecules were eluted independently of the molar mass, whereas PS were still eluted according to an SEC mode. Moreover, recent LC LCA results have permitted one to define a new parameter, the peak distance, as a convenient metric for copolymer analysis.[20]

In this paper, the separation of statistical P(S/MMA) copolymers using liquid chromatography under limiting of adsorption is discussed. Two series of copolymers were obtained and then characterized in terms of molar mass and composition. The first series has a constant molar mass (220 ± 30 kg/mol) and various compositions (0.08–0.85 molar fraction of styrene), whereas the second exhibits a constant composition (0.5 molar fraction of styrene) and different molar masses (20 to 250 kg/mol). These two families were fractionated using SEC and LC LCA to study separately the effect of copolymer molar mass and composition on the peak distance.

EXPERIMENTAL

Materials

Unless stated otherwise, solvents and reagents were used as received. Styrene (S), 99% purity, methyl methacrylate (MMA), 99% purity, α,α'-azobis-isobutyronitrile (AIBN) and 1-butanethiol (BuSH), 97% purity, were purchased by Fluka (Germany). Tetrahydrofuran (THF) and *n*-hexane, both HPLC grade, (SDS, France) were used without further purification. All other solvents of purum grade were used for copolymer synthesis and other characterization: toluene, deuterated and normal dichloroethane (DCE), carbon tetrachloride (CCl_4), dimethylformamide (DMF) and THF were obtained from SDS.

Polystyrene and poly(methyl methacrylate) standards were purchased from American Polymer Standards Corporation (USA) and exhibit a molar mass range of 9,000–370,000 and of 6,000–350,000 g/mol, respectively.

Copolymer Synthesis

Two series of copolymers, varying either in molar mass or in composition, were prepared by radical solution polymerization in toluene at 60°C. The polymerization conditions are given in Table I. The syntheses were performed in a 0.8-L stainless-steel reactor equipped with a coil heating system and agitated with a mechanic stirrer at 200 RPM. Toluene was first degassed with a nitrogen stream for 1 h at 60°C to remove oxygen. Monomers were then added and total concentration was 4.2 mol/L. The 400 mL solution was then further degassed for 30 min prior to the introduction of AIBN as initiator dissolved in 1 mL of toluene. Reactions were terminated before overall conversion by adding 5 mg of hydroquinone dissolved in acetone and monomer conversion was measured by thermogravimetry (Tab. I).

The initial molar fraction of styrene was varied in order to obtain copolymers with composition ranging from 0.08 to 0.85 molar fraction of styrene with a controlled molar mass of around 200,000 g/mol. The chain-transfer agent (butanethiol) was introduced to produce the second series of copolymer having an identical composition of

TABLE I Summary of polymerization conditions Chain transfer agent concentration (BuSH), initiator concentration (AIBN), initial molar fraction of styrene (f_S), conversion (conv.) and theoretical and experimental copolymer molar fraction of styrene (F_S^{Th} and F_S^{Exp} respectively). All polymerizations were performed at 60°C unless noted by an asterisk in which case the polymerization was carried out at 70°C

Copolymer code	*BuSH (mmol/L)*	*AIBN (mmol/L)*	f_S	*Conv. (%w)*	F_S^{Th}	F_S^{Exp}
P(S/MMA)18	0	0.53	0.46	8.9	0.50	0.50 ± 0.01
P(S/MMA)2	0	1.94	0.47	6.5	0.50	0.50 ± 0.02
P(S/MMA)3	1.0	2.14	0.48	4.5	0.50	0.52 ± 0.02
P(S/MMA)4	2.2	2.20	0.48	4.5	0.50	0.52 ± 0.01
P(S/MMA)6	3.5	2.10	0.47	5.4	0.50	0.50 ± 0.03
P(S/MMA)8*	14.0	14.40	0.47	13.3	0.50	0.48 ± 0.02
P(S/MMA)10	0	2.13	0.90	4.1	0.85	0.84 ± 0.01
P(S/MMA)9	0	2.10	0.72	3.5	0.65	0.68 ± 0.02
P(S/MMA)20	0	2.66	0.30	5.1	0.39	0.37 ± 0.02
P(S/MMA)11	0	2.00	0.28	3.2	0.35	0.32 ± 0.02
P(S/MMA)19	0	2.18	0.20	5.7	0.29	0.28 ± 0.01
P(S/MMA)16	0	2.18	0.20	6.2	0.28	0.27 ± 0.01
P(S/MMA)24	0	2.46	0.14	11.6	0.22	0.22 ± 0.01
P(S/MMA)23	0	2.35	0.09	11.5	0.15	0.16 ± 0.01
P(S/MMA)12	0	1.90	0.08	5.0	0.15	0.15 ± 0.01
P(S/MMA)21	0	2.45	0.06	9.1	0.12	0.12 ± 0.01
P(S/MMA)15	0	2.29	0.04	7.4	0.08	0.08 ± 0.01

0.5 molar fraction and various molar masses. All the copolymers were then purified twice by precipitation in methanol (dissolved in acetone).

Determination of Copolymer Molar Mass

Copolymer molar mass was measured by static light scattering (Dawn DSP multiangle laser photometer equipped with a HeNe laser operating at 632.8 nm, Wyatt Technologies SA, Santa Barbara, USA). Specific refractive index increment (*dn*/*dc*) values were measured using an Optilab DSP interferometric refractometer (Wyatt) at a wavelength of 632.8 nm. All solvents and solutions were filtered using 0.22 and 0.45-μm pore-size Teflon filters, respectively (Bioblock, France). Triplicate measurements of molar mass and *dn*/*dc* were obtained in four different solvents (toluene, THF, DMF and DCE) and made it possible to calculate the absolute weight-average molar mass of each copolymer according to light scattering theory for copolymers.[21] The values obtained are summarized in Table II.

TABLE II Molar mass characterization of copolymers Weight-average molar mass of P(S/MMA) copolymers determined by LS (M_w^{LS}) and SEC (M_w^{SEC}), the dispersity index (DI) and the relative difference between both methods (ΔM_w)

Copolymer code	M_w^{LS} *g/mol*	M_w^{SEC} *g/mol*	*DI*	ΔM_w
P(S/MMA)18	237,000	261,000	1.36	−0.15
P(S/MMA)2	223,000	201,100	1.56	0.09
P(S/MMA)3	136,000	135,600	1.61	0.01
P(S/MMA)4	69,800	75,400	1.59	−0.07
P(S/MMA)6	46,700	57,900	1.55	−0.19
P(S/MMA)8	25,000	18,600	1.28	0.35
P(S/MMA)10	244,000	210,000	1.50	0.11
P(S/MMA)9	236,000	200,900	1.52	0.17
P(S/MMA)20	161,500	219,500	1.40	0.26
P(S/MMA)11	221,000	258,800	1.61	0.15
P(S/MMA)19	261,000	197,200	1.44	0.32
P(S/MMA)24	192,000	214,500	1.40	−0.10
P(S/MMA)23	237,000	254,000	1.38	−0.07
P(S/MMA)12	201,000	296,000	1.60	−0.32
P(S/MMA)21	219,000	230,000	1.43	−0.05
P(S/MMA)15	262,000	258,000	1.49	−0.02
Average value*	220,000 ± 30,000	230,000 ± 32,000	1.48 ± 0.08	

* Average value of molar mass and dispersity index are calculated only with the copolymer series varying in composition.

Determination of Copolymer Composition

The theoretical composition can be estimated using the instantaneous copolymer composition Eq. (1):

$$F_S^{Th} = \frac{r_S f_S^2 + f_S f_{MMA}}{r_S f_S^2 + 2 f_S f_{MMA} + r_{MMA} f_{MMA}^2} \quad (1)$$

where: f_S, f_{MMA} are the monomer molar fractions, r_S and r_{MMA} are the reactivity ratios of S and MMA, respectively and $r_S = 0.52$, $r_{MMA} = 0.47$ at 60°C.[22]

Copolymer composition was experimentally determined by ^1H NMR with a Brücker AC 200 Spectrometer (Germany). Copolymers were dissolved in a mixture of deuterated DCE and CCl_4 at a ratio of 1 : 2. The chemical shifts of P(S/MMA) are shown in Table III.

TABLE III ^{1}H NMR chemical shifts of the P(S/MMA) copolymer

Peak	*Chemical shift (ppm)*	*Chemical groups*	*Monomer*
A	7.1	C_6H_5—	styrene
D	2–1.2	—CH_2—CH—	
C	3.6–2.1	—O—CH_3	
B	0.3–1.2	—CH_3—	MMA
D	2–1.2	—CH—C—	

The peak surfaces, S_A and S_B, correspond to S and MMA monomer, respectively and were used to calculate the copolymer molar fraction of styrene F_S according to:

$$F_S^{Exp} = \frac{S_A/5}{S_A/5 + S_B/3} \qquad (2)$$

SEC, Low and High LCLCA

In SEC, pure THF was employed as the eluent and THF (*desorli*) and *n*-hexane (*adsorli*) were used in low and high LC LCA. The ratios of THF and *n*-hexane were calculated by weight and subsequently expressed as volume percentages neglecting the volume change. The eluent composition in low LC LCA and high LC LCA for PMMA was 74/26 THF/*n*-hexane %vol and 70/30 %vol, respectively.

A CGX 500 silica column with 10-μm particles and a pore size of 50 nm (Tessek, Praha, Czech Republic) was employed as the stationary phase for all the experiments. The liquid chromatograph consisted of an L-7100 isocratic pump (Hitachi Instruments, Tokyo, Japan) coupled with an Hitachi L-7400 UV detector and a Rheodyne type 7100 injector (Merck) with an injection loop of 20 μL. Operating wavelengths of 260 nm and 235 nm were used in the case of copolymer, PS and PMMA, respectively. Chromatograms were analyzed using D-7000 HPLC system manager interface and Merck-Hitachi software. The standard separation involved 0.5 mL/min flow rate and a solute concentration of 1.0 mg/mL. All the experiments were performed at a controlled temperature of 25 ± 0.1°C in a Hitachi L-7300 Column oven.

RESULTS AND DISCUSSION

Copolymer Molar Mass and Composition

The statistical P(S/MMA) copolymers obtained as described in the experimental part were characterized in terms of composition and molar mass. Radical solution polymerization provided copolymers possessing both molar mass and composition distributions.[1] In order to avoid an over broadened shift during polymerization, the reactions were terminated at monomer conversions between 4–12 wt% (Tab. I).

The copolymer composition was determined experimentally by ^{1}H NMR using the surface of specific peak for each monomer (Tab. III). The obtained values are in very good agreement with the calculated compositions based on the recipes employed and known reactivity ratios (Tab. I).

Weight-average molar masses were determined by LS (M_w^{LS}) using experimental results of *dn*/*dc* measured for each copolymer. As shown in Figure 1, the refractive index increment is proportional to the copolymer composition as could be expected.[21] Moreover, the extrapolated values at 0 or 1 molar fraction of styrene are in good agreement with *dn*/*dc* of PMMA and PS, respectively, determined by other authors.[23] Weight-average molar masses were also calculated based on SEC chromatograms (M_w^{SEC}) performed in pure THF, using a calibration curve with polystyrene standards (Tab. III). The relative difference (ΔM_w) between both methods is given by the ratio

$$\Delta M_w = \frac{M_w^{\mathrm{LS}} - M_w^{\mathrm{SEC}}}{M_w^{\mathrm{SEC}}} \tag{3}$$

Table II reveals that molar mass difference between light scattering and SEC averages 13% (range of 1 and 35%). The influence of the multivariate distribution on elution behavior of copolymers could be an explanation of why chromatographic and photometric data need not agree. Furthermore, the dispersity index (DI) measured by SEC is approximately 1.5. This is an acceptable value to employ the copolymers as broad liquid chromatography standards.

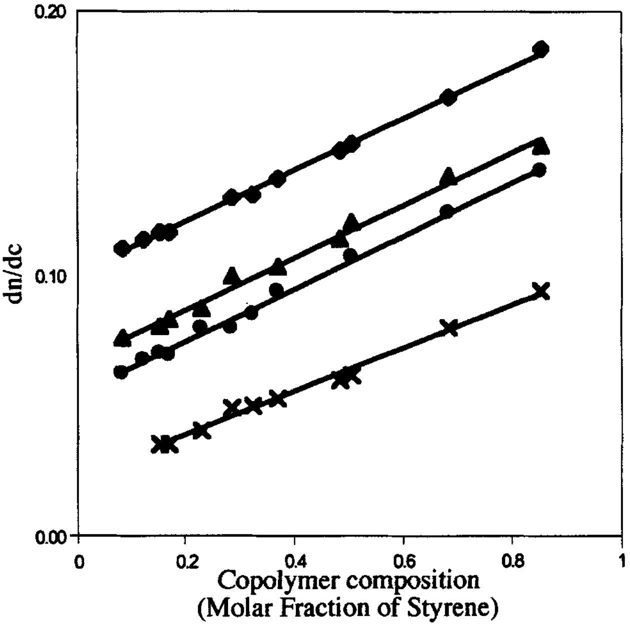

FIGURE 1 Specific refractive index increment (dn/dc) as a function of the copolymer composition (f_S) in various solvents. ◆ THF, $dn/dc = 0.0967f_S + 0.1022$, $R^2 = 0.998$; ▲ DMF, $dn/dc = 0.0983f_S + 0.0684$, $R^2 = 0.983$; ● DCE, $dn/dc = 0.1002f_S + 0.0555$, $R^2 = 0.993$; × Toluene, $dn/dc = 0.0824f_S + 0.0232$, $R^2 = 0.991$.

Application Range of Limiting Conditions of Adsorption

The limiting conditions previously found[19] have been reproduced here as shown on the plot of retention volume of PMMA standards *versus* molar mass (Fig. 2). The sensitivity of LC LCA towards eluent composition has been subsequently evaluated by investigating another eluent composition containing an additional level of *adsorli*. The calibration curves performed at the new eluent composition of 70/30% vol THF/*n*-hexane is plotted in Figure 2. In the molar mass range within 6,000 to 350,000 g/mol, a vertical line is obtained indicating that limiting conditions of adsorption are obtained. The eluent composition of 70/30% vol THF/*n*-hexane will be referred to as high LC LCA to distinguish it from low LC LCA which is defined as the minimum level of *adsorli* (26% vol *n*-hexane) required to obtain a retention independent of the molar mass. A slight shift is observed between low

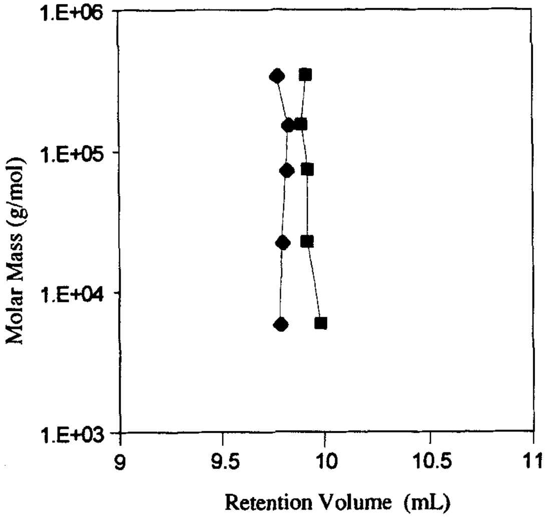

FIGURE 2 Calibration curve for narrow PMMA standards in mixed eluents using a silica based sorbent. (74/26% vol THF/*n*-hexane: low LC LCA (◆) and 70/30% vol THF/*n*-hexane: high LC LCA (■)).

and high LC LCA which can be attributed to an enhancement of PMMA and stationary phase interactions, enthalpic effect increasing with *n*-hexane content. The retention volume is also independent of the molar mass over a given range of eluent composition. Low and high LC LCA conditions can be applied without extreme molar mass limitations and mobile phase sensitivity, which plague competing methods such as critical conditions.[24] Furthermore, LC LCA use of an isocratic pump greatly improves repeatability, in contrast with the macrogradient method.[25]

Peak Distance Suitability

Both of the aforementioned series of copolymer were fractionated according to the various elution modes: SEC, low and high LC LCA. Figures 3a and 3b show the plot of retention volume *versus* copolymer molar mass and composition. The retention volume depends strongly on copolymer molar mass as illustrated by Figure 3a with a nearly constant shift toward higher values by increasing *n*-hexane content in the eluent. On the contrary, the behavior of the retention volume

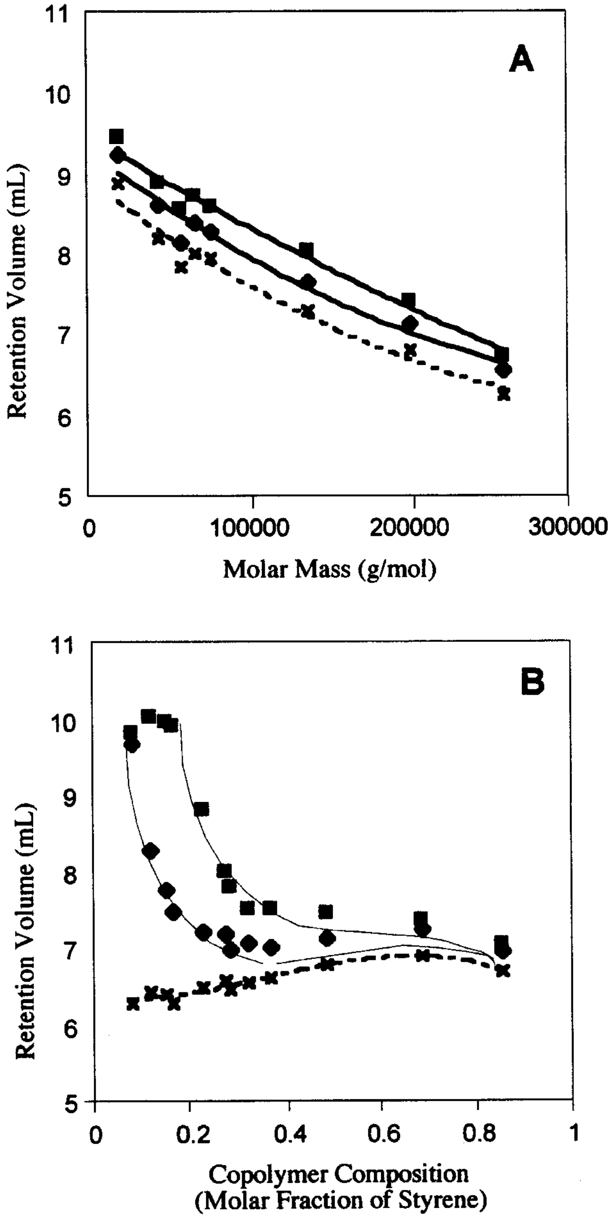

FIGURE 3 Retention volume (mL) obtained by SEC (×), low LC LCA (◆) and high LC LCA (■). (A) retention volume *versus* copolymer molar mass; (B) retention volume *versus* copolymer composition.

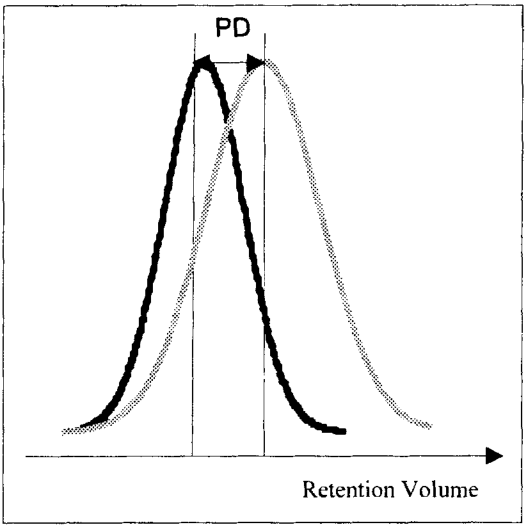

FIGURE 4 Schematic of the method employed in the peak distance calculation (— SEC; — LC LCA).

versus copolymer composition is completely different between SEC and both LC LCA methods and it is further analyzed with the help of the Peak Distance. Indeed, the peak distance method was first pointed out as a convenient parameter to study copolymers by limiting conditions of solubility.[20] The peak distance (PD) is defined as the difference between LC LCA and SEC retention volume at the maximum of the elution peak:

$$\mathrm{PD} = V_R(\mathrm{LC\ LCA}) - V_R(\mathrm{SEC}) \qquad (4)$$

The distance between the two retention volumes is schematized in Figure 4 and exemplified with some copolymers (Tab. IV). The peak distance will be employed hereinafter, as a parameter for the characterization of statistical copolymers using LC LCA.

Molar Mass and Composition Influences on Peak Distance

The most important problem raised by copolymer analyses *via* SEC is the separation of both molar mass and composition effects on the

TABLE IV Illustration of peak distance calculation Retention volumes obtained by SEC ($V_{R,\mathrm{SEC}}$), low LC LCA ($V_{R,\mathrm{LC\ LCA}}$) and the peak distance of some copolymers

Copolymer code	V_{SEC} *(mL)*	$V_{LC\ LCA}$ *(mL)*	*PD (mL)*
P(S/MMA)2	6.80	7.15	0.35
P(S/MMA)4	7.85	8.17	0.32
P(S/MMA)24	6.51	7.24	0.74

elution process. The influence of copolymer molar mass and composition on peak distance is subsequently discussed.

From Figure 5, it appears that using low LC LCA, PD remains constant around an average value of 0.35 ± 0.03 mL in the investigated range of molar mass. In the case of high LC LCA, as the *adsorli* (*n*-hexane) content is more important than in low LC LCA, adsorption/exclusion effects are even more significant. As a result, PD is more important and corresponds to 0.65 ± 0.09 mL. With increasing interactions between the stationary phase and the solute, the retention volume is increased and leads to higher PD values. Moreover, contrary

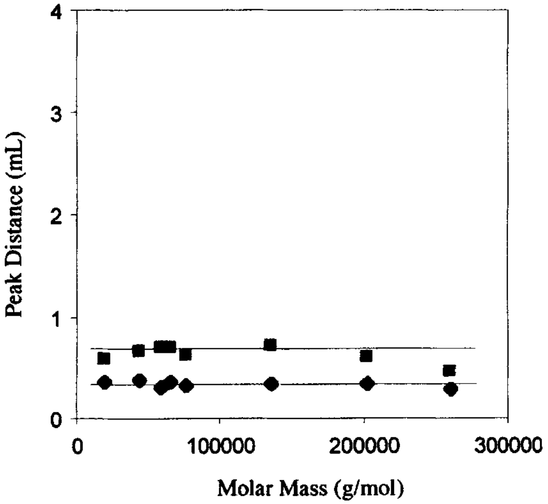

FIGURE 5 Peak distance as a function of molar mass obtained for low and high LC LCA for copolymer having a constant molar fraction of styrene of 0.5. (◆ low LC LCA; ■ high LC LCA).

to low LC LCA, a slight maximum in the curve PD as a function of molar mass is observed in high LC LCA. Nevertheless, this slight nonlinearity of PD is dwarfed by the dependence of PD on copolymer composition, as is described in the following.

From Figure 6, peak distance is seen to depend strongly on the copolymer composition within a certain range. Using low LC LCA, PD varies from 3.5 to 0.35 mL when the molar fraction of styrene increases from 0.1 to 0.4. This window of application is shifted toward higher styrene content, between 0.2 and 0.6 molar fraction in the case of high LC LCA conditions. However, in both cases, the change in PD as a function of copolymer composition (3 mL) is one order of magnitude larger than the molar mass effect (0.3 mL) observed in Figure 5. At a copolymer composition of 0.5 molar fraction, which is within the application range in the case of high LC LCA separation mode, the influence of molar mass on PD is negligible compared to the composition effect. Therefore, it can be considered that the peak distance depends primarily on the copolymer composition. This result is of paramount importance since it will permit us to apply low or high LC LCA to decouple copolymer composition and molar mass influence on copolymer elution.

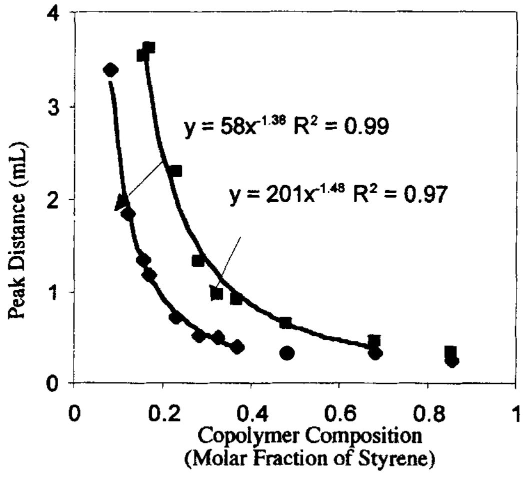

FIGURE 6 Peak distance as a function of composition obtained for low and high LC LCA. (◆ low LC LCA; ■ high LC LCA).

Application Range of PD for Copolymer Composition Determination

As the sorbent is the same in SEC, low, or high LC LCA, in terms of porosity and surface chemistry, the difference between the separation modes can be attributed to the modification of the chemical properties of the eluent. In contrast to the purely entropic SEC, adsorption interactions, in the presence of *n*-hexane (*i.e.*, low or high LC LCA), are enhanced since *n*-hexane plays a role of an adsorption promoting agent (*adsorli*).[16, 17] PS homopolymer elution follows a SEC process as previously shown[17] as long as the eluent contains less than 64% vol of *n*-hexane. At an eluent composition of 26 or 30% vol of *adsorli*, styrene units are not sensitive to *n*-hexane and do not participate to enthalpic interactions. Adsorption occurs mainly between MMA units and the silica particles. As a consequence, the macromolecules are eluted according to different elution processes, depending on their chemical composition. The calibration curve of PD as a function of copolymer composition (Fig. 6) can be interpreted as having three regions. Considering first the PD calculated using SEC and low LC LCA data, the three ranges are defined as follows:

(1) Copolymers with a composition under 0.1 molar fraction of styrene are eluted with a constant retention volume identical to that of the solvent. The PD is constant in this range implying a lower limit to the copolymer analysis (Fig. 6). In such cases, adsorption interactions between solute and stationary phase are maximized and the copolymers are eluted under a LC LCA process independent of the composition and molar mass. Consequently, the peak distance method cannot be applied in this range to determine copolymer composition.

(2) By increasing the styrene content, the adsorption interactions between MMA and silica particles are drastically reduced and even disappear as the copolymer composition reaches approximately a molar fraction of styrene of 0.4–0.5. Macromolecules are eluted according to a process moving gradually from LC LCA towards "pure" SEC. The application window between 0.1–0.4 molar fraction corresponds to a *transition mode*. As a consequence, the difference between both retention volumes obtained by low LC LCA and SEC, is no longer constant and the peak distance can be

related to the copolymer composition. *This region is suitable for simultaneous copolymer composition and molar mass characterization.*

(3) Above the upper limit of the application range, *i.e.*, 0.4–0.5 molar fraction, the macromolecules are eluted according to a SEC mode and the shift of retention volume obtained using low LC LCA compared to SEC values is constant. As a consequence, the PD is obviously constant and is no longer a suitable parameter to estimate copolymer composition.

In the case of the high LC LCA separation mode, as *n*-hexane content is higher than in low LC LCA, the aforementioned adsorption effect plays a more significant role in the separation process. Therefore, as the MMA units of macromolecules have a more important influence on the elution process, the application range is shifted to lower MMA content: from 0.1–0.4 to 0.2–0.6 molar fraction of styrene.

Overall, the statistical copolymer elution, as well as the peak distance, can be modified using limiting conditions of adsorption by adjusting the eluent composition. In the range where LC LCA and SEC mode are competing, the plot of PD *versus* composition can be precisely fitted with power equations (Fig. 6). The calibration curves obtained using both low and high LC LCA allow one to determine the copolymer composition. Moreover, from this kind of separation, it will be possible to calculate the composition distribution as will be demonstrated in the following section of this paper.

Composition and Composition Distribution Calculation

By employing the calibration curves obtained in Figure 6, the composition distribution can be calculated from the chromatogram data obtained by either SEC and low LC LCA, or SEC and high LC LCA. The calculation is exemplified with sample P(S/MMA)16, which has not been used to draw the calibration curve. The SEC and LC LCA normalized peaks are plotted in Figure 7. The peak distance is then calculated as a function of retention volume, when the normalized intensity obtained by LC LCA is equal to the normalized intensity determined by SEC (Fig. 8). The composition can be finally deduced

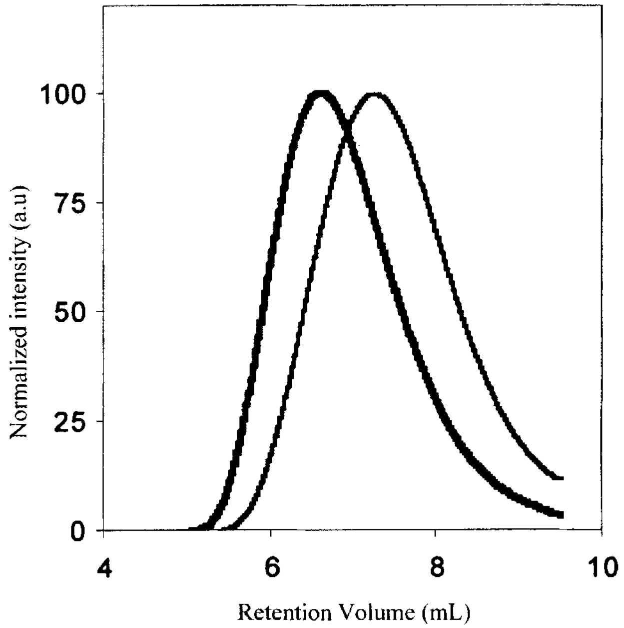

FIGURE 7 SEC and low LC LCA normalized chromatograms for sample P(S/MMA)16. (— SEC; ······ LC LCA). Polymer characterization is summarized in Table I.

from PD by using the calibration curve, determined previously under the same conditions and can be plotted as a function of normalized intensity (Fig. 9). From this transformed chromatogram, the composition distribution can be defined in the same manner as the weight and number molar mass averages are commonly calculated. The number- and the weight-average compositions of P(S/MMA)16 are respectively equal to 0.265 and 0.269 molar fraction of styrene. The average composition determined by the peak distance method is identical to the average value obtained by ^{1}H NMR (Tab. I: 0.27 mol fraction of styrene). Nevertheless, the dispersity index of the experimental composition distribution corresponds to 1.01. This value is very low even if the copolymerization was carried out at the azeotropic point and the reaction stopped at 6.2% of conversion. As the fitting curve is not linear, it cannot be excluded that the real composition distribution may be slightly underestimated.

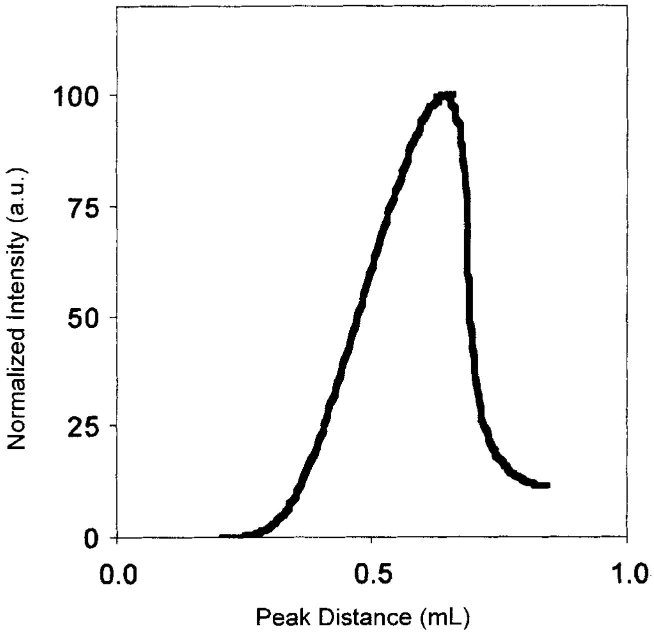

FIGURE 8 Normalized intensity as a function of peak distance for sample P(S/MMA)16. Polymer characterization is summarized in Table I.

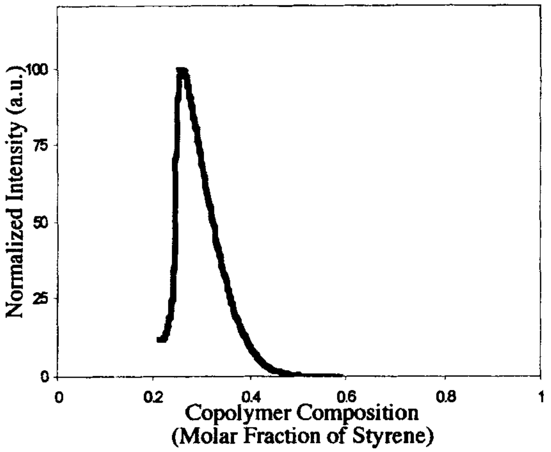

FIGURE 9 The copolymer composition distribution for sample P(S/MMA)16.

CONCLUSIONS

Limiting conditions of adsorption can be applied for statistical P(S/MMA) copolymers in a range of eluent composition between 26 and 30% vol in *n*-hexane. A calibration curve involving the peak distance method, defined as the difference of retention volume between SEC and LC LCA separation modes, leads to the calculation of the composition of statistical P(S/MMA) copolymers between 0.1 and 0.6 molar fraction of styrene independent of their molar mass. This is valid over the range between 20,000 and 250,000 g/mol. LC LCA is an excellent complement for size exclusion chromatography and allows one to realize, in an isocratic chromatographic system, the decoupling of composition and molar mass.[26] Furthermore, as liquid chromatography process gives a gradual separation of the macromolecules, the composition distribution can also be determined as is discussed in a latter study.[27]

In the aim of elucidating the mechanism of macromolecules elution, the fractions obtained under various conditions are still under study. Off-line experiments are being carried out in a first step prior to testing an on-line coupled system, as already has been done by some other authors with different systems.[28, 29] By adding a SEC column in series with the first LC LCA system, both composition and molecular size of copolymer will be quantified simultaneously.[27] Further improvements will be focused on enlarging the application window of LC LCA separation. This will include improvement to column sensitivity by utilizing smaller particle size, multiple columns or substituting *n*-hexane by a weaker *adsorli.*

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