Characterization of Linear and Star Polystyrene by Temperature-Gradient Interaction Chromatography with a Light-Scattering Detector

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ABSTRACT: We report the characterization of polystyrene by temperature-gradient interaction chromatography (TGIC) with on-line light-scattering (LS) detection. Since a binary mixture eluent, CH_2Cl_2/CH_3CN (57/43, v/v), was employed in TGIC, a systematic deviation in apparent molecular weight due to preferential sorption was observed. After an appropriate correction, however, correct molecular weights could be obtained. Second virial coefficients obtained from light-scattering measurement showed that the TGIC separation condition is very close to the Θ condition. With the TGIC/LS setup, analysis of an unfractionated six-arm star polystyrene was carried out. The resolution of TGIC was high enough to resolve all the polymer molecules having one to six arms. Comparison of LS detection results with the results obtained by calibration with linear PS standards reveals that elution in TGIC is far more sensitive to molecular weight alone. Thus it provides an excellent route to separating polymer molecules of similar hydrodynamic volume but differing molecular weight.

Introduction

Recently we have introduced the temperature-gradient interaction chromatography (TGIC) technique to characterize the molecular weight distribution of polystyrene (PS) and poly(methyl methacrylate) (PMMA).[1,2] While size exclusion chromatography (SEC) utilizes mainly the entropy change associated with the partition equilibrium to separate macromolecules in terms of their molecular size, interaction chromatography utilizes the enthalpic interaction between solute molecules and the stationary phase. During the separation by TGIC, the partition coefficient of solutes between the stationary and mobile phases is controlled by varying temperature.[3] TGIC provides much higher molecular weight resolution than size exclusion chromatography (SEC). Since the temperature of the column is varied in TGIC during elution, we were concerned that the temperature dependence of refractive index would hamper the use of a light-scattering (LS) or a refractive index (RI) detector which has been widely employed for characterization of polymers. In fact, an RI or LS detector has not been used in solvent gradient HPLC for polymer characterizations due to the background refractive index drift.[4−6] Nevertheless, we have successfully demonstrated that LS and RI detection can be employed, in combination, in the TGIC analysis for determination of the absolute molecular weight distribution of poly(methyl methacrylate) (PMMA).[2] This was possible because the refractive index change with temperature was not so large as that in the solvent gradient method. Therefore, with a minor modification of the apparatus, the background refractive index could be controlled back to a stable value when the eluent reaches the refractive-index-sensitive detectors. Undoubtedly, the use of LS detection greatly enhances the applicability of TGIC in the characterization of a variety of polymers.

In the characterization of PMMA, pure acetonitrile was used as the eluent, which allowed us to use a light-scattering detector without further complications other than temperature control.[2] In most cases of TGIC analysis, however, a binary mixed solvent needs to be adopted as mobile phase for an optimum separation, which makes LS detection even more complicated. For example, in the TGIC analysis of PS, a mixed solvent of acetonitrile/tetrahydrofuran or acetonitrile/methylene chloride was employed.[1,3,7] Polymer chains in a binary mixture of solvent preferentially adsorb one component of the mixture, usually the better solvent of two components, so that the refractive index contrast is not only due to the polymer chain itself but also due to the excess solvent component preferentially adsorbed to the polymer chain.[8,9] A typical light-scattering experiment in such a system does not provide a true molecular weight.[10] The extent of preferential sorption needs to be considered to obtain the true molecular weight. One of the objectives of this study is to determine whether or not light-scattering detection can be employed for the mixed eluent system of TGIC.

The other objective of this study was to characterize branched polymers by TGIC. It is well-known that SEC does not resolve branched polymers as well as linear polymers.[11] SEC does not separate the polymer molecules in terms of their molecular weight but by the size of the polymer chain. The relationship between the molecular weight and the size of a polymer chain for branched polymers is different from that of linear polymers.[12−14] Branching reduces the size of a polymer relative to the size of a linear material of the same molecular weight.[15] For instance, in star polymers with a uniform arm molecular weight, the hydrodynamic volume of star polymers does not change much as the number of arms increases. Therefore it is hard to resolve multiarm star polymers by SEC even when their

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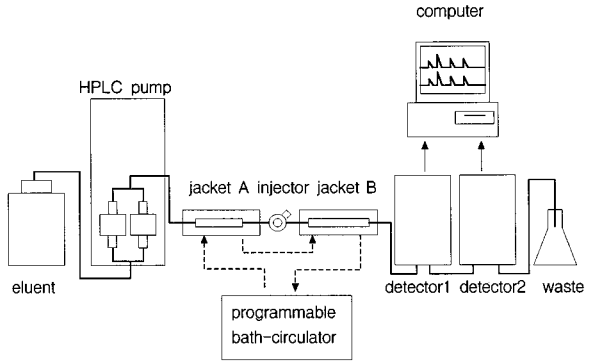

Figure 1. Schematic diagram of TGIC apparatus. Jacket A holds an empty HPLC column to precontrol the temperature of the eluent while jacket B holds a separation column.

molecular weights are far enough apart to be resolved in the case of linear polymers. Unlike SEC, TGIC utilizes the interaction between the polymer segment and the stationary phase; thus, it should be possible to separate branched polymers with greater sensitivity to molecular weight. In this paper we report the results of star PS characterization by TGIC coupled with light-scattering detection.

Experimental Section

A schematic of the TGIC apparatus is shown in Figure 1. It is practically the same as that reported previously except that we added temperature-controlling parts before and after the separation column.[3] Before the injector (Rheodyne 7125), eluent was passed through an empty 2.1-mm-diameter HPLC column whose temperature was controlled in a water jacket. The water-jacket temperature was maintained at the same temperature of the water jacket containing the separation HPLC column. This temperature control of eluent before it reached the separation column improves the resolution by reducing the temperature gradient across the cross section of the column. While the resolution was clearly improved for a 4.5-mm-diameter HPLC column, there was no noticeable change for a 2.1-mm-diameter column. It appears that 2.1-mm columns are thin enough for the single-column jacket to control the uniform temperature of the separation column (at least at the flow rates employed).

After the separation column, the eluent first passes through a UV/vis detector in order to equilibrate the temperature close to room temperature before it reaches a temperature-sensitive detector such as an RI or LS detector. As elaborated later, this moderate effort of temperature control works very well in eliminating baseline drift in the RI or LS chromatograms. Other than the column-temperature-controlling part, a typical isocratic HPLC apparatus equipped with a C18 bonded silica column (Alltech, Nucleosil, 100 pore, 250 mm × 2.1 mm, 5-μm particle size) was used. The mobile phase was a mixture of CH_2Cl_2/CH_3CN premixed in the ratio 57/43 by volume. Both solvents were HPLC grade and used as received from Aldrich. The flow rate was 0.1 mL/min.

Ten PS samples of narrow molecular weight distribution (M_w and M_n are, respectively, the weight-average and number-average molecular weights) were used in this study: 56 700 (M_w/M_n = 1.06), 87 100 (M_w/M_n = 1.06), 152 000 (M_w/M_n = 1.04), 233 000 (M_w/M_n = 1.04), 366 000 (Waters, M_w/M_n = 1.03), 444 000 (M_w/M_n = 1.05), 630 000 (M_w/M_n = 1.04), 726 000 (Tosoh, M_w/M_n = 1.05), 1 090 000 (Tosoh, M_w/M_n = 1.08), 1 530 000 (M_w/M_n = 1.11). 7 PS samples were gifts from Daelim Industrial Co. Other PSs were obtained from Waters and Tosoh Co. The weight-average molecular weights and the M_w/M_n values were determined in this laboratory by SEC with a light-scattering detector (LDC Analytical, KMX-6). Injection samples were made in the same solvent as the mobile phase at a concentration from 1.5 to 3.5 mg/mL, depending on the molecular weight of the polymers to obtain optimum light-scattering intensity. Samples were injected through a 7125 Rheodyne injector with a 20-μL sample loop. Chromatograms were recorded with a UV/vis absorption detector (LDC Analytical, Spectromonitor 3200) at the wavelength of 260 nm, a low-angle laser-light-scattering (LALLS) detector (LDC Analytical, KMX-6), and a refractive index (RI) detector (Waters, R401). The specific refractive index increment (dn/dc) of PS in the mixed solvent was measured to be 0.213 mL/g by a differential refractometer (LDC Analytical, KMX-16) at 25 °C. The obtained chromatograms were analyzed with PCLALLS (LDC Analytical) software. The molecular weight calculation method used with on-line LALLS detection was reported elsewhere in detail.[16,17]

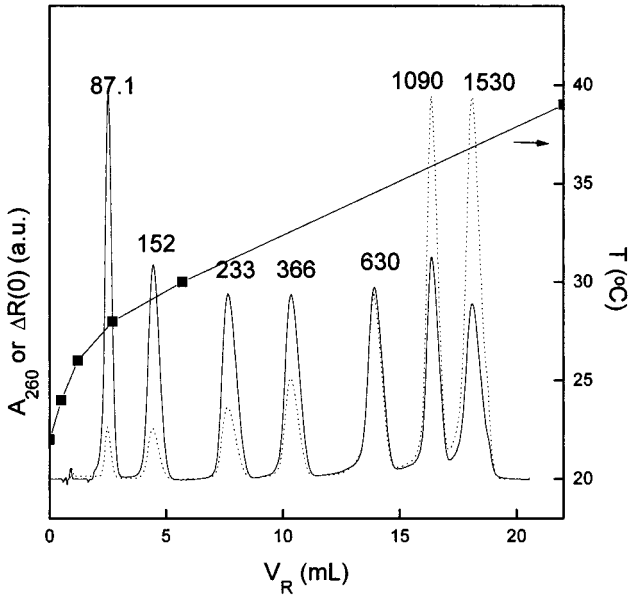

Figure 2. TGIC chromatograms of seven PS samples recorded with a UV/vis detector (solid line) and an LALLS detector (dotted line). Column temperature was changed in a stepwise manner during the elution, as shown in the plot. Molecular weight ($\times 10^3$) is shown on top of each peak.

For the SEC analysis, the same HPLC apparatus used with TGIC was employed except for the following. Four SEC columns (Polymer Lab, 2 × Mixed C, 1 × Mixed D, 1 × Mixed E) were used, and the column temperature was maintained at 40 by use of a column oven (Fiatron, CH-460). The eluent was tetrahydrofuran (THF), and the flow rate was 1.0 mL/min. An injection sample of PS star was made in THF at the concentration of 1 mg/mL, and the injection volume was 100 μL. The analysis scheme was similar to that of TGIC/LALLS analysis.[16,17]

Results and Discussion

On-Line LS Detection in Mixed Eluent. In Figure 2 the TGIC chromatograms of seven PS standards recorded with a UV/vis detector and an LALLS detector are displayed. The temperature of the column was raised by a series of five linear ramps from 22 to 39 °C, as shown in the right ordinate. The baselines of both chromatograms are very stable, indicating that the temperature variation during the elution causes little refractive index drift when eluent reaches the detectors. Complete separation of seven standard PSs is achieved, and the relative intensities of the signals from the UV/vis and LALLS detectors reflect the molecular weight differences of eluted samples. The numbers on each peak position are the weight-average molecular weights determined by SEC/LALLS.

Figure 3 shows TGIC chromatograms of 366 000 PS (sample 5 in Table 1) recorded by UV/vis and LALLS detectors. The temperature of the column was raised

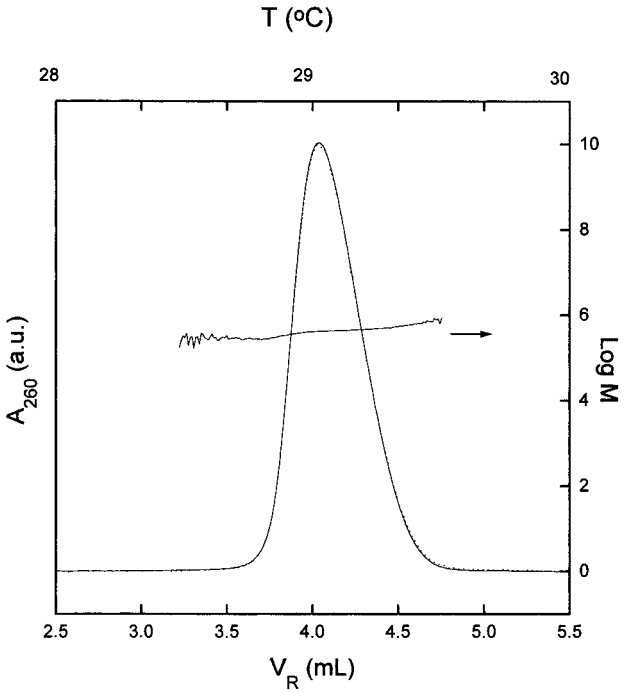

Figure 3. TGIC chromatogram of a 366 000 PS sample recorded with a UV/vis detector (solid line) and an LALLS detector (dotted line). Since the molecular weight distribution is narrow, both chromatograms are nearly overlapped. Temperature was programmed as shown in the top abscissa, and the calibration curve (log M vs V_R) is also shown in the plot.

Table 1. Molecular Weight Characterization of PS standards by LALLS

	M_w ($\times 10^3$)			
sample	SEC/ LALLS	TGIC/ LALLS	batch LALLS	corrected[c]
1	56.7	62.2 (9.7%)[a]	61.5/2.98[b]	56.7
2	87.1	95.5 (9.6%)[a]		87.1
3	152	163 (7.5%)[a]		149
4	233	254 (8.8%)[a]	249/−0.757[b]	232
5	366	401 (9.6%)[a]		366
6	444	475 (7.1%)[a]		433
7	630	692 (9.8%)[a]		631
8	726	784 (8.0%)[a]	790/−10.2[b]	715

[a] Percent deviation from the value of SEC/LALLS. [b] Second virial coefficient ($\times 10^{-6}$ mol mL/g^2). [c] TCIC/LALLS results corrected for preferential sorption.

slowly at the rate of 0.067 °C/min from 28 to 30 °C. The low rate of temperature change expands the elution peak to allow a more accurate analysis. In addition, it shows that we can control the retention of a polymer easily by changing the temperature programming, which can expedite this type of analysis. When the elution is started at 28 °C, the retention volume (V_R) of the peak is greatly reduced (4 mL versus 10 mL) in comparison with that of the elution peak of the same polymer in Figure 2. Therefore precise experiments on eight PSs were carried out individually with optimized temperature programming for each polymer sample. Figure 3 also shows a plot of molecular weight (log M) versus V_R which was obtained from the analysis of both UV/vis and LALLS chromatograms. The calibration curve is nearly flat, indicating that the molecular weight distribution of the polymer sample is very narrow. Due to the narrow distribution of the PS sample, the elution peaks recorded with the UV/vis and LALLS detectors nearly overlap each other.

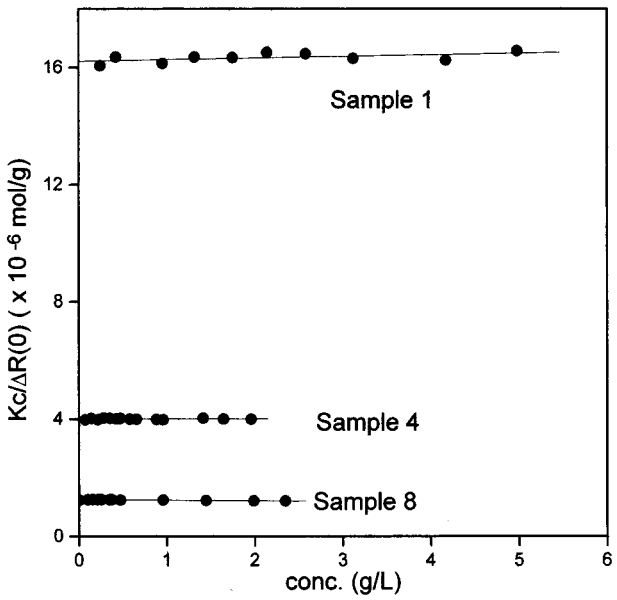

Figure 4. Batch LALLS result of samples 1, 4, and 8 in Table 1. Their apparent molecular weights are in good agreement with the on-line SEC/LALLS results as listed in Table 1. Also the second virial coefficients are practically 0, showing that the eluent is nearly a Θ solvent for PS.

Eight standard PSs were examined in detail, as listed in Table 1. The M_w values of these samples are summarized in the TGIC/LALLS column of Table 1. They show a systematic deviation from the M_w's obtained from SEC/LALLS analysis, which are believed to be the correct values. The percent error shown in parentheses, which stands for the deviation from the value of the SEC/LALLS measurement, is approximately constant, approximately 9%, independent of molecular weight. This systematic deviation was thought to be caused primarily by preferential sorption, but the temperature effect would contribute in part, since different molecular weight polymers are eluted at different column temperatures. For the TGIC separation of PS in this study, the higher the molecular weight, the higher the column temperature required for optimum separation. Therefore it is possible that the background refractive index difference due to the temperature difference contributed to the observed systematic deviation. In order to clarify the issue, a batch LALLS experiment was carried out for three PS samples (1, 4, and 8) in the identical mixed solvent system used in TGIC experiments. The experiment was carried out at ambient temperature (22 ± 0.5 °C), and the 6−7° annulus was used to collect the scattered light. Figure 4 shows the plots of $Kc/\Delta R(0)$ versus c, where K, c, and $\Delta R(0)$ are the instrument constant, the polymer concentration and the excess Rayleigh factor at the angle 0, respectively. From the intercept at $c = 0$ and the slope, the weight-average molecular weight and the second virial coefficient were obtained and are listed in Table 1. The molecular weights obtained from batch LALLS are in excellent agreement with the results of TGIC/LALLS, which clearly indicates that the systematic deviation from the results of SEC/LALLS is entirely, within experimental precision, due to preferential sorption in the mixed solvent system. In Table 1 it appears that preferential sorption does not depend on polymer molecular weight, although it is generally accepted that the preferential sorption coefficient decreases with increasing molecular weight.[8] It is likely in this system

that preferential sorption is not strong and the molecular weight range of PS samples is not wide enough to reveal a clear molecular weight dependence. Therefore we can calculate the correct molecular weights by multiplying by the same correction factor for all samples. In practice we used the internal standard method to obtain the molecular weight easily if preferential sorption is independent of molecular weight.[18]

$$\frac{Kc_{\mathrm{std}}}{\Delta R(0)_{\mathrm{std}}} = \frac{1}{M_{\mathrm{w\ std}}} \tag{1}$$

$$\frac{Kc_{\mathrm{sam}}}{\Delta R(0)_{\mathrm{sam}}} = \frac{1}{M_{\mathrm{w\ sam}}} \tag{2}$$

where the subscripts std and sam stand for standard and sample, respectively. Since $\Delta R(0)$ is proportional to the area of the elution peak in the LALLS chromatogram, eqs 1 and 2 can be reduced as follows.

$$M_{\mathrm{w\ sam}} = M_{\mathrm{w\ std}} \cdot \frac{\Delta R(0)_{\mathrm{sam}}}{\Delta R(0)_{\mathrm{std}}} \cdot \frac{c_{\mathrm{std}}}{c_{\mathrm{sam}}} = M_{\mathrm{w\ std}} \cdot \frac{\mathrm{area}_{\mathrm{sam}}}{\mathrm{area}_{\mathrm{std}}} \cdot \frac{c_{\mathrm{std}}}{c_{\mathrm{sam}}} \tag{3}$$

If the preferential sorption coefficient is independent of molecular weight, the contribution of the preferential sorption to the scattered intensity is canceled out in eq 3 and we can get a correct molecular weight of the samples. This method has a number of advantages. For example, the dn/dc of the sample as well as the loop volume of the injector does not need to be measured independently. The results of corrected M_w in Table 1 are calculated with eq 3 with respect to sample 5 (M_w 366 000) as the "standard" sample. The molecular weights determined by this method are in excellent agreement with the values obtained by SEC/LALLS analysis.

Figure 4 also reveals that the second virial coefficients (A_2) of PS in the mixture of CH_2Cl_2/CH_3CN are nearly 0 and that the eluent is close to a Θ solvent. In the previous study for the characterization of PMMA, the mobile phase of CH_3CN was also a Θ solvent for the polymer. Although further systematic studies are called for in order to understand the detailed separation mechanism, inferring from these two examples, it seems that this poor, near Θ, solvent condition is required for a successful TGIC separation.

TGIC Separation of Star PS. Figure 5 shows TGIC chromatograms of an unfractionated six-arm star PS recorded with a UV/vis detector (solid line) as well as a LALLS detector (dotted line). The star polymer was prepared by linking living PS chains with 1,2-bis-(trichlorosilyl)ethane as described previously.[19] Prior to the linking reaction, about five units of isoprene are incorporated onto each living PS chain to reduce steric hindrance during the linking reaction.[19] A part of the PS arm was taken out before the linking reaction to measure the molecular weight of an individual PS arm. The molecular weight of the PS arm was determined separately by SEC/LALLS as 80 000. From the chromatogram it is evident that all the elution peaks of the linking reaction products, from one- to six-arm star PSs, can be identified. Four-, five-, and six-arm stars are not fully resolved, and five-arm stars appears as a shoulder of the main peak, six-arm star PS. The numbers above each peak are the molecular weights of the respective peak position determined by two different methods. The top numbers were obtained from a calibration curve made by linear PS standards (M_{CAL}) while the bottom ones in bold type were from LALLS (M_{LS}). Assuming the selective sorption is independent of chain architecture, the LALLS molecular weights are corrected for the selective sorption by the same procedure as described earlier. Except for the case of the six-arm star, the molecular weights determined by two different methods are fairly close to the molecular weight of the corresponding PS stars, that is the molecular weight of an arm multiplied by the number of arms. For the six-arm star, M_{CAL} shows the largest deviation from the expected value, 434 000, while the LALLS detection gives us 492 000, which is close to the expected value from the molecular weight of the arm.

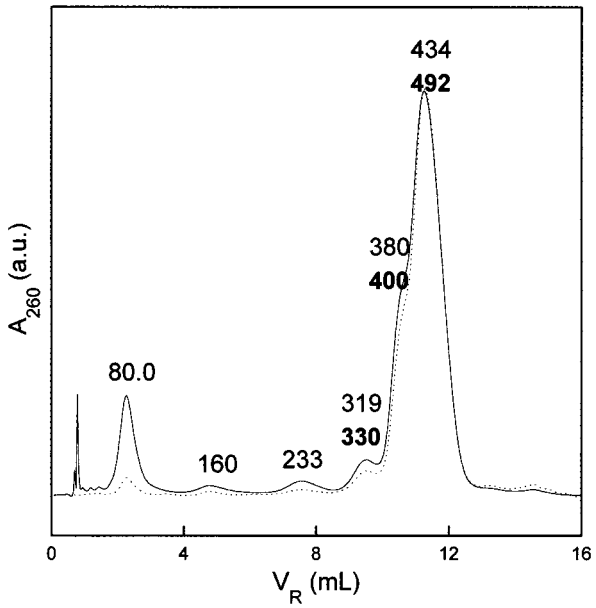

Figure 5. TGIC chromatograms of a six-arm star PS recorded with a UV/vis detector (solid line) and an LALLS detector (dotted line). All six species having one to six arms are resolved. The M_{CAL} of the peak position is marked on top of each peak. For four- to six-arm stars, M_{LS} was also measured and marked with bold type under the M_{CAL}. They are matched well except for six-arm star PS.

For comparison, the SEC chromatogram of the same star PS sample is displayed in Figure 6. The numbers at the top of each peak have the same meaning as in Figure 5. In this chromatogram, we could locate the two-arm star (V_R = 22.7 mL) and perhaps the three-arm star as a small shoulder of the main peak, but the star PSs of more than three arms are not resolved at all. In addition, while M_{CAL} and M_{LS} of the single-arm PS are in good agreement, the molecular weight of star PSs deviates significantly from the true molecular weight of the star PSs as the number of arms increases. The main peak, apparently an unresolved mixture of four-, five-, and six-arm stars, shows a peak M_{CAL} of 347 000 while M_{LS} is 457 000. This poor resolution of SEC for branched polymers mainly arises from the separation mechanism of SEC, which separates the polymeric solutes according to their sizes in solution.

These results indicate that the retention behavior in TGIC is far less sensitive to the molecular architecture than SEC, thus providing higher resolution as well as M_{CAL} values which are closer to the correct molecular weight for branched polymers. This is a reasonable result considering that the separation mechanism of

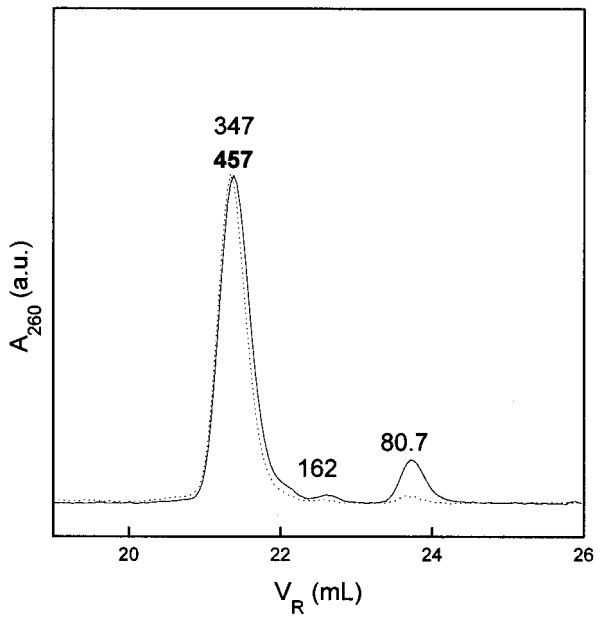

Figure 6. SEC chromatograms of a six-arm star PS recorded with a UV/vis detector (solid line) and an LALLS detector (dotted line). Four- to six-arm star PSs are not resolved at all while one- and two-arm species are resolved. The M_{CAL} of the peak position is marked on top of each peak. For the main peak, unresolved four- to six-arm stars, M_{LS} was also measured and marked with bold type under the M_{CAL}.

TGIC is the interaction between polymer segments and the stationary phase. Although high branching should impede the interaction due to higher segmental density near the branching point(s), TGIC is undoubtedly more sensitive to the number of segments than SEC.

In summary, we showed that light-scattering detection can be employed in a mixed, isocratic, chromatographic analysis when proper care is taken to account for selective sorption. To our knowledge, this is the first successful demonstration of the use of a light-scattering detector in mixed solvent HPLC. Employment of a light-scattering detector for TGIC makes the technique much more powerful, since the molecular weight distribution can be obtained without relying on the calibration with standard polymers. In addition, TGIC separates branched polymers far better than SEC due to its separation mechanism: interaction chromatography.

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References and Notes

(1) Lee, H. C.; Chang, T. *Polymer* **1996**, *37*, 5747.
(2) Lee, W.; Lee, H. C.; Chang, T.; Kim, S. B. *Macromolecules* **1998**, *31*, 344.
(3) Lee, H. C.; Lee, W.; Chang, T. *Korea Polym. J.* **1996**, *4*, 160.
(4) Quarry, M. A.; Stadalius, M. A.; Mourey, T. H.; Snyder, L. Y. *J. Chromatogr.* **1986**, *358*, 1.
(5) Shalliker, R. A.; Kavanagh, P. E.; Russell, I. M. *J. Chromatogr.* **1991**, *543*, 157.
(6) Lochmüller, C. H.; Jiang, C.; Elomaa, M. J. *J. Chromatogr. Sci.* **1995**, *33*, 561.
(7) Lee, H. C.; Chang, T. *Macromolecules* **1996**, *29*, 7294.
(8) Kratochvil, P. *Classical Light Scattering from Polymer Solutions*; Elsevier: New York, 1987.
(9) Casassa, E. F.; Eisenberg, H. *Adv. Protein Chem.* **1964**, *19*, 287.
(10) Stockmayer, W. H. *J. Chem. Phys.* **1950**, *18*, 58.
(11) Barth, H. G., Mays, J. W., Eds. *Modern Methods of Polymer Characterization*; John Wiley & Sons: New York, 1991.
(12) Stockmayer, W. H.; Fixman, M. *Ann. N.Y. Acad. Sci.* **1953**, *57*, 334.
(13) Zimm, B. H.; Stockmayer, W. H. *J. Polym. Sci.* **1959**, *37*, 19.
(14) Casper, R.; Biskup, V.; Lange, H.; Phol, U. *Makromol. Chem.* **1976**, *117*, 1111.
(15) Zimm, B. H.; Stockmayer, W. H. *J. Chem. Phys.* **1949**, *17*, 1301.
(16) Lee, H. C.; Ree, M.; Chang, T. *Polymer* **1995**, *36*, 2215.
(17) Lee, H. C.; Chang, T. *Bull. Korean Chem. Soc.* **1996**, *17*, 648.
(18) Lee, H. C.; Chang, T. *Bull. Korean Chem. Soc.* **1995**, *16*, 640.
(19) Frater, D. J.; Mays, J. M.; Jackson, C.; Sioula, C.; Efstradiadis, V.; Hadjichristidis, N. *J. Polym. Sci., Polym. Phys. Ed.* **1997**, *35*, 587.

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