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HPLC and MALDI-TOF MS Analysis of Highly Branched Polystyrene: Resolution Enhancement by Branching

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Temperature gradient interaction chromatography (TGIC) and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were applied for the characterization of highly branched polystyrenes (PS) prepared by linking living polystyryl anions using 4-chlorodimethylsilylstyrene. Reversed-phase (RP)-TGIC showed an unexpectedly high resolution according to the number of branches despite significant overlap of the molecular weight as confirmed by MALDI-TOF MS. The enhancement of the resolution is ascribed to the contribution of the nonpolar groups in the branched PS: the dimethylsilyl groups in the branching unit as well as the *sec*-butyl initiator groups. As the number of branches increases, the number of nonpolar groups increases, which in turn increases the RP-TGIC retention synergistically with increasing molecular weight. In contrast, a poorer resolution was found in normal-phase-TGIC, in which the nonpolar groups reduce the retention. The resolution in RP-TGIC appears superior to that of liquid chromatography at the chromatographic critical condition (LCCC) of PS. It is seemingly due to the synergistic contribution of the incremental PS molecular weight to the functionality in the branched PS in RP-TGIC while only the functionality contributes to the separation in LCCC. This type of resolution enhancement could be utilized efficiently for the analysis of highly branched polymers such as dendrimers or hyperbranched polymers.

Rigorous analysis of branched polymers is a difficult task. Separation of polymers according to their molecular weight has been carried out most widely by size exclusion chromatography (SEC).[1,2] SEC separates the polymers in terms of their apparent hydrodynamic chain size, and it has been most successful in the analysis of linear homopolymers in which a relatively simple correlation exists between the molecular weight and the hydrodynamic size. For branched polymers, however, the resolution of SEC is not as good as linear polymers since the hydrodynamic chain size does not increase with molecular weight as much as in the case of linear polymers. For more rigorous characterization of model branched polymers, interaction chromatography (IC) has been proven to provide a much higher resolution.[3−6] In IC, the retention is less sensitive to the chain architecture and is mainly determined by molecular weight. For model branched polymers with branches of the "same" chain length (e.g., star-shaped polymers with branches prepared by anionic polymerization), we previously could resolve the polymer species of different numbers of branches by IC with a far better resolution than SEC.[4,5] However, practically all the synthetic polymers have finite molecular weight distributions, which deteriorates the resolution of the star-shaped polymers with different numbers of branches as the number of branches increases.

With increasing interest in highly branched polymers such as dendrimers and hyperbranched polymers in recent years,[7−9] high-resolution analysis of highly branched polymers becomes more important. Recent advances of the ionization method in mass spectrometry (MS) such as the matrix-assisted laser desorption/ionization (MALDI) method have made it possible to rigorously analyze relatively high molecular weight polymers.[10−13] However, the MALDI method still cannot fully cope with polymers of wide

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Table 1. Molecular Characteristics of PS Precursor and Branched PS

samples	CDMSS/*sec*-BuLi (molar ratio)	M_w (M_n) (kg/mol)[a]	M_w (M_n) (kg/mol)[b]
precursor			2.3 (2.2)
BS45	0.45	6.6 (4.8)	5.4 (3.9)
BS87	0.87	22.7 (15.0)	13.5 (9.0)

[a] Determined by light scattering detection. [b] Determined by calibration with PS standards.

molecular weight distribution, and it is desirable to hyphenate a proper separation method with the mass detection method. In this paper, we report an unexpectedly high enhancement in liquid chromatography resolution for a highly branched polymer due to the functionality increasing proportionally with the number of branches, which was confirmed by MALDI-time-of-flight (TOF) MS.

EXPERIMENTAL SECTION

Preparation of Branched Polystyrenes. Branched polystyrenes (PS) were prepared by coupling polystyryl anion with 4-chlorodimethylsilylstyrene (CDMSS).[14] CDMSS was prepared by following the literature procedure.[15] The preparation of PS was performed in a drybox filled with argon gas, and all solvents used were HPLC grade. Styrene, cyclohexane, and tetrahydrofuran (THF) were distilled with proper drying agents prior to transferring into a drybox. They were further purified by passing through a column filled with activated neutral alumina. Polystyryl anion was prepared by adding 0.38 mL of *sec*-butyllithium (*sec*-BuLi, 1.3 M in cyclohexane) to a solution of styrene (1.5 mL) in cyclohexane (15 mL) and THF (40.6 μL). The solution was stirred for 30 min at 60 °C. Two portions of 5 mL of solution were transferred into separate flasks. PS precursor was prepared by quenching the polystyryl anion solution with methanol, and the branching reactions were carried out by adding 12.5 (BS45) and 25 mg (BS87) of CDMSS to each flask, respectively. Molar ratios of CDMSS to the polystyryl anion (*sec*-BuLi) were 0.45 and 0.87, respectively. The branching reactions were allowed to proceed for 30 min with stirring at 60 °C before termination with methanol.

SEC Analysis. For the SEC analysis, two mixed-bed columns (Polymer Lab. Mixed C, 300 × 8.0 mm i.d.) were used at a column temperature of 40 °C. SEC chromatograms were recorded with a multiangle laser light scattering detector (Wyatt, mini-DAWN) and a refractive index detector (Wyatt, Optilab DSP) using THF (Duksan) mobile phase at a flow rate of 0.8 mL/min. Polymer samples for the SEC analysis were dissolved in THF at an appropriate concentration (~1.0 mg/mL), and the injection volume was 100 μL. The characterization results of mother branched PS are listed in Table 1.

HPLC Analysis. For the reversed-phase-temperature gradient interaction chromatography (RP-TGIC) analysis, a C18 bonded silica column (Luna C18, Phenomenex, 5-μm particle size, 100-Å pore, 250 × 4.6 mm) was used. The mobile phase was a CH_2Cl_2/CH_3CN mixture (55/45, v/v, Duksan) at a flow rate of 0.5 mL/min. For liquid chromatography analysis at the chromatographic critical condition (LCCC), the same column as in RP-TGIC was used. Mobile phase was the same as in the RP-TGIC analysis. The critical condition was established at 50.5 °C.

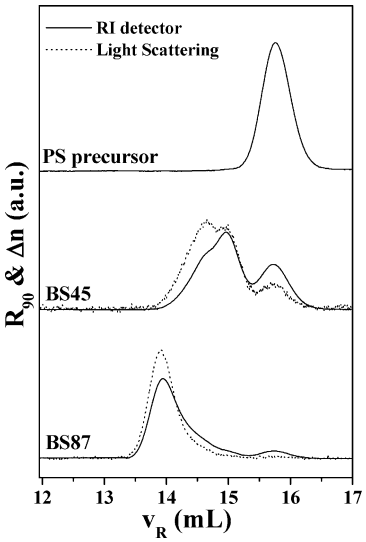

Figure 1. SEC chromatograms of PS precursor and two branched PS made from different molar ratios of CDMSS to *sec*-BuLi (BS45, 0.45; BS87, 0.87). Separation condition: two mixed-bed columns (Polymer Lab, PL mixed C, 300 × 8.0 mm), THF eluent at column temperature of 40 °C.

For the normal-phase (NP)-TGIC analysis, a silica column (Alltech, 5-μm particle size, 50-Å pore, 250 × 4.6 mm) and a mixture (58/42, v/v) of isooctane (Mallinckrodt) and THF (Duksan) were used (0.5 mL/min). The samples were prepared by dissolving the polymers in a small volume of the corresponding eluents. The temperature of the column was controlled by circulating fluid from a programmable bath/circulator (Neslab, RTE-111) through a homemade column jacket. The chromatogram was recorded with a UV absorption detector (TSP, UV 100) operating at a wavelength of 260 nm.

MALDI-TOF MS. For the MALDI-TOF MS experiments, a Bruker Reflex III mass spectrometer was used. The spectrometer is equipped with a nitrogen laser (λ = 337 nm), a pulsed ion extraction, and a reflector. Polymer solutions were prepared in THF at a concentration of 5 mg/mL. The matrix, 1,8-dihydroxy-9(10*H*)-anthracenone (dithranol, Aldrich, 97%), was dissolved in THF at a concentration of 20 mg/mL. A 5-μL aliquot of the polymer solution was mixed with 50 μL of the matrix solution and 1.5 μL of a silver trifluoroacetate (Aldrich, 98%) solution (1 mg/mL) in THF, respectively. A 0.5-μL portion of the final solution was deposited onto a sample target plate and allowed to dry in air at room temperature.

RESULTS AND DISCUSSION

Figure 1 displays the SEC chromatograms of the PS precursor and the two branched PS, BS45 and BS87. The number-average molecular weight of the linear PS precursor eluting at $V_R \approx 15.8$ mL was determined as 2.2 kg/mol by calibration with standard PS samples. The two branched PS show multiple peaks representing the leftover precursor PS and high molecular weight polymers

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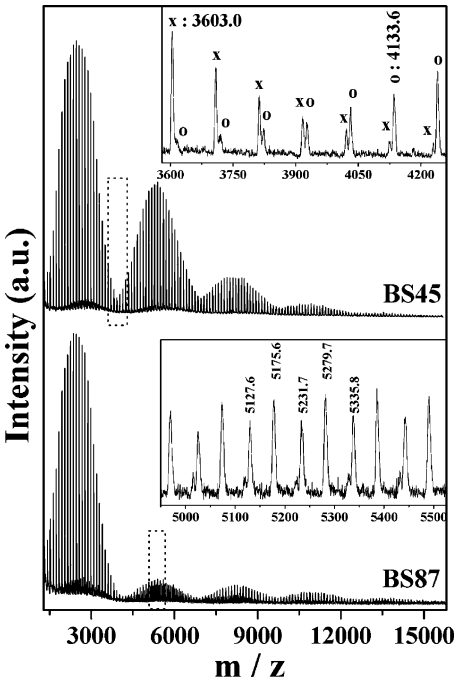


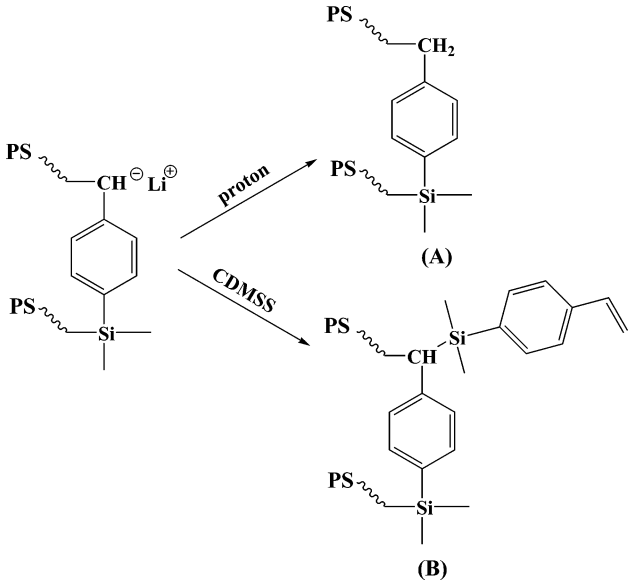
Figure 2. MALDI-TOF MS spectra of BS45 and BS87. In the insets, magnified spectra of the boxed regions are shown. Matrix, dithranol; salt, silver trifluoroacetate.

branched by CDMSS. The major elution peaks of the two branched PS show multimodal structures due to the species of small number of branch chains, but the resolution is not high enough to identify individual polymer species. This is an expected resolution in an SEC separation of highly branched polymers.[5,14] Therefore, it is not possible for SEC analysis to provide more detailed information beyond the average branch numbers from the molecular weights determined by light scattering detection.

Figure 2 shows the MALDI-TOF mass spectra of BS45 and BS87. The average molecular weight of the precursor PS (the first peak envelope of the two spectra) is in agreement with the SEC analysis result. For the two branched PS, we can easily identify the progressive appearance of the peak envelopes representing the polymer species with multiple branches. Their molecular weights increase as integral multiples of the PS precursors, as expected. The insets show expanded mass spectra of the dotted box regions of the mass spectra. For BS45, the expanded spectrum shows the overlap region of the peak envelops corresponding to the species containing one (×) and two (O) PS precursor chains. The low molecular weight side envelope (×) represents the precursor PS terminated with hydrogen, and the molecular weight corresponds to 104.15*n* (*n* styrene units) + 57.12 (one *sec*-butyl initiator) + 107.87 (Ag^+ ion) + 1.01 (one terminal H). For example, with $n = 33$, the peak molar mass of the structure was found to be 3603.0, as compared to the calculated mass of 3602.9. In the high molecular weight side envelope (O), the polymer should contain one branching unit and two PS precursor chains (Scheme 1(A)). The molecular weight corresponds to 104.15*n* (*n* styrene units) + 57.12 × 2 (two *sec*-butyl initiators) + 107.87 (Ag^+ ion) + 161.30 (one branching unit) + 1.01 (one terminal H). For example, with $n = 36$, the molar mass was found to be 4133.6, as compared to the calculated mass of 4133.8.

On the other hand, BS87 shows a more complicated pattern. It also contains significant amount of +56 (in fact, +160 for the same degree of polymerization of styrene) mass peaks relative to the peaks found in BS45. The molar mass is consistent with the molecular structure terminated with CDMSS (+161.30 (dimethylsilylstyrene) − 1.01 (one hydrogen) ≈ 160) by the termination reaction of polymer anions with a chlorosilyl group instead of a proton (Scheme 1B). It is reasonable to observe more CDMSS-terminated polymers when a larger equivalent of CDMSS is added. According to the MALDI-TOF mass analysis as elaborated later, the content of CDMSS-terminated species reaches maximum at four-branched species, which remains to be elucidated in connection with the reaction kinetics of the system. Except for the difference in the terminal group, it is clear that BS45 and BS87 consist of the identical series of different branched PS but with different relative abundance.

Scheme 1. Proton-Terminated (A) and CDMSS-End-Capped (B) Two-Branch PS



We can also note two features in the mass spectrum: (1) The overlap of peak envelop becomes more significant as the number of branches increases, which is natural due to the finite molecular weight distribution of the precursor PS. The full width at half-maximum (fwhm) of the precursor PS is ∼1100 amu, which amounts to ∼40% of the average molecular weight. (2) The intensity of the peaks decreases rapidly as the number of branches increases. This is due to the mass discrimination phenomenon in the MALDI-TOF mass spectrometry, which allows a precise analysis only for the polymers with narrow molecular weight and composition distribution.[16,17] Therefore, although much more detailed information is available from the MALDI-TOF mass spectra than SEC chromatogram, the information is limited due to the difficulty in MALDI process to cope with the wide molecular weight distribution of the branched PS.

Figure 3 displays RP-TGIC chromatogram of the branched PS together with several PS standards (linear chain). The first sharp peak appearing at $t_R \approx 6$ min is the injection solvent peak, and we can observe a series of the peaks representing the branched PS of increasing numbers of branches. It is surprising to see that

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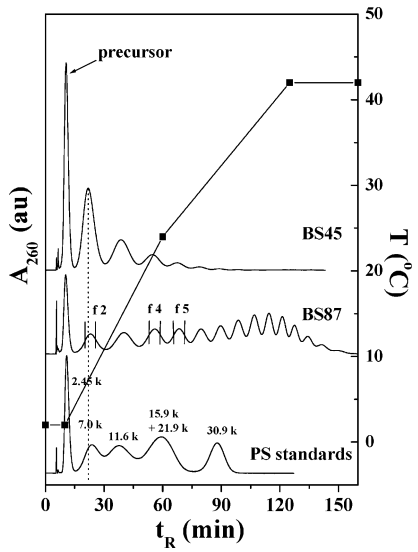

Figure 3. RP-TGIC separation of branched PS and six linear PS standards. Column, C18 bonded silica, 100 Å, 250 × 4.6 mm; eluent, CH_2Cl_2/CH_3CN (55/45, v/v); flow rate, 0.5 mL/min. Temperature program is shown in the plot.

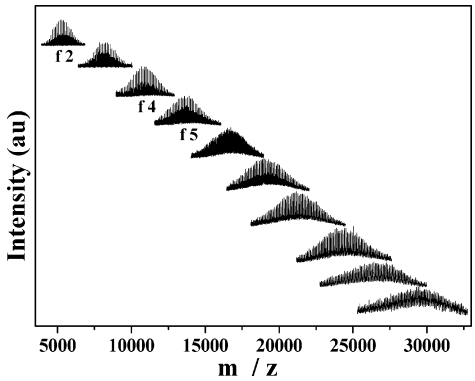

Figure 4. MALDI-TOF MS spectra of 10 RP-TGIC fractions of BS87 from 2 to 11 branches.

RP-TGIC resolves branched PS up to 14−15 branch species clearly. Considering that the fwhm of the precursor PS amounts to ∼40% of the average molecular weight, the molecular weight difference of the branched species alone cannot account for such an excellent resolution of the highly branched PS. For example, the molecular weight difference between the polymer with 10 branches and 11 branches is only ∼10% on average, and they would not be resolved by molecular weight difference alone. To confirm the speculation, RP-TGIC elution peaks of BS87 were fractionated near the peak maximum positions (indicated with small vertical bars for a few peaks in the chromatogram) and subjected to MALDI-TOF MS analysis. Figure 4 displays the resulting MALDI-TOF mass spectra.

As expected, the average molecular weight increases as integral multiples of that of PS precursor and the molecular weight distribution of the branched species overlap each other significantly as the number of the branches increases. Considering that only narrow fractions were collected near the elution peak maximum to avoid the overlap region of the adjacent elution peaks, the real overlap in molecular weight must be more serious between the species of the adjacent branching numbers. This confirms to us that the high resolution in RP-TGIC separation is not due to the molecular weight only. In addition, we ran RP-TGIC for a few PS standards of molecular weight similar to branched PS in the same condition. As also shown in the bottom of Figure 3, the resolution for the PS standards is much poorer than the branched PS. Also we can note that the RP-TGIC retention of linear PS standards is smaller than the branched PS of similar molecular weight except for the first peak, which is the linear PS precursor. This clearly indicates that the high resolution in the RP-TGIC separation of the branched PS must have been assisted by extra chemical moieties in them, which made the RP-TGIC retention of the branched PS increase over linear PS.

The only aliens in the branched PS to affect the RP-TGIC retention relative to linear PS standard are dimethylsilyl branching units and *sec*-butyl initiator moieties. The number of these two groups increases with the number of branches. The nonpolar groups must have increased the RP-TGIC retention synergistically with increasing molecular weight of branched PS.[18,19] Convincing evidence of the contribution of the dimethylsilyl group can be found from the comparison of the RP-TGIC retentions between BS45 and BS87. As shown in the MALDI-TOF mass spectra in Figure 2, BS87 contains CDMSS-terminated species while BS45 does not. The extra dimethylsilyl terminal group in BS87 clearly shifts the RP-TGIC elution peak position of BS87. A vertical dotted bar is drawn in Figure 3 to aid the visual comparison. Such a retention shift is conspicuous for the species with lower branch numbers. It is consistent with the observation that the relative content of CDMSS-terminated species is highest at four branched species. Other evidence of the resolution enhancement due to the nonpolar groups in RP-TGIC can be found from the NP-TGIC separation of the branched PS. We reported previously that NP-TGIC could separate linear PS according to the molecular weight with a resolution comparable to RP-TGIC.[18] However, for this branched PS, the NP-TGIC exhibits a much poorer resolution for BS87 than linear PS standards as shown in Figure 5. The retention increase with molecular weight must have been largely compensated by the increasing number of the nonopolar groups in the branched PS contributing to the retention in a manner opposite to the case of RP-TGIC.

The separation of polymers according to functionality difference has been done most widely by LCCC.[20,21] At the critical condition of a homopolymeric species, the repeating units of the polymer chains become "invisible" and the polymers elute at the same retention volume independent of their molecular weights. If the polymers contain different functional groups, the retention is controlled by functionality only. There are many successful examples on the LCCC application for the individual block analysis for block copolymers[20−23] and functionality analysis.[20,21,24,25] We also applied LCCC for this highly branched PS. Figure 6 displays

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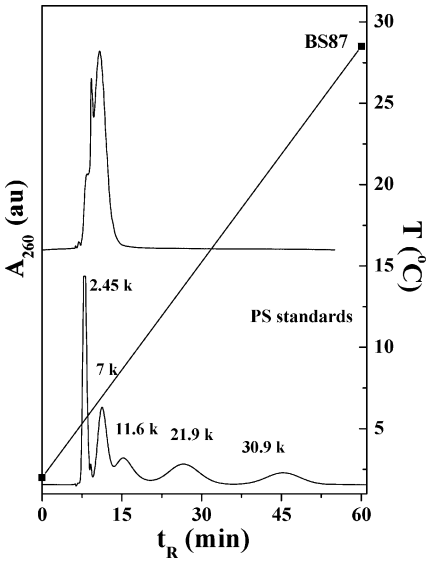

Figure 5. NP-TGIC chromatograms of BS87 and five linear PS standards. Column, bare silica, 50 Å, 250 × 4.6 mm; eluent, isooctane/THF (58/42, v/v); flow rate, 0.5 mL/min. Temperature program is shown in the plot.

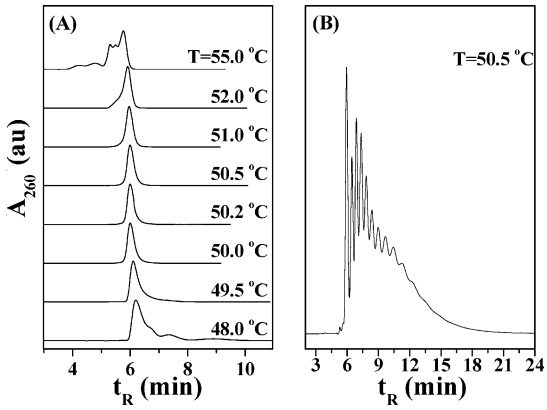

Figure 6. (A) RPLC chromatograms of six PS standards (M_w: 2.5, 7.0, 15.6, 30.9, 62, 113 kg/mol) to establish the critical condition. (B) LCCC chromatogram of BS87 at the critical condition of PS: Column, C18 bonded silica, 100 Å, 250 × 4.6 mm; eluent, CH_2Cl_2/CH_3CN (55/45, v/v); flow rate, 0.5 mL/min.

the LCCC chromatogram of BS87. The critical condition was established at the RP-TGIC condition at the column temperature of 50.5 °C as shown in Figure 6A. The LCCC chromatogram (B) could resolve the branched PS reasonably well. The separation of LCCC is due to the increasing number of functionalities as the branching number increases. However, the apparent resolution is not as elevated as RP-TGIC when compared to Figure 3. Therefore, it clearly supports our conclusion that the unexpectedly high resolution in RP-TGIC separation is ascribed to the synergistic contribution of increasing molecular weight as well as the increasing number of nonpolar moieties as the branching number increases.

In summary, we have shown that the highly branched PS system can be characterized in detail by combination of RP-TGIC and MALDI-TOF MS. The enhancement of resolution by the structural characteristics of the branched PS would open an improved way to characterize highly branched polymer systems such as dendrimers or hyperbranched polymers since they usually carry functional groups and the number of functional groups often varies with molecular weight in a systematic manner. If a good separation condition can be established to enhance the resolution by functional groups in a synergistic manner with molecular weight (e.g., normal phase for polar groups and reversed phase for nonpolar groups), it would be possible to elucidate the structure of highly branched polymers in more detail.

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