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Characterization of mixed solutions of hyperbranched and linear polystyrenes by a combination of size-exclusion chromatography and analytical ultracentrifugation

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Abstract

Separation and characterization on mixed solutions of hyperbranched and linear polystyrenes was achieved using size-exclusion chromatography (SEC) as the first dimension and analytical ultracentrifugation (AUC) as the second dimension. The results show that linear and hyperbranched polystyrenes with similar hydrodynamic sizes (one fraction from SEC) can be separated by AUC according to the molar mass, and the separation efficiency decreases with the increasing of the retention volume in SEC. Moreover, the molar masses determined by AUC are consistent with the values measured by SEC-refractive index (RI) and SEC-multi-angle light scattering (MALS) detection. Furthermore, the result shows that the separation efficiency decreases with the increasing of the subchain length of hyperbranched polystyrenes. Our study lays a solid foundation for future studies to separate polymers with different topologies by a combination of SEC and AUC.

KEYWORDS

analytical ultracentrifugation, hyperbranched polymer, size-exclusion chromatography, topology

1 | INTRODUCTION

The separation and characterization of polymers with different molar masses, topologies, or functionalities play an important role in polymer science and technology. In general, size-exclusion chromatography (SEC) is the most widely used tool to characterize polymers and provides the molar mass and molar mass distribution with respect to the hydrodynamic volume of the polymers usually using linear polystyrenes with narrow molar mass distributions as calibration standards.[1–5] However, it is difficult to obtain accurate analysis of complex polymers with different topologies using SEC alone because polymers with the same hydrodynamic volume but different topologies may coelute.[6] No doubt, it is necessary to combine SEC with other methods, such as normal-phase temperature gradient interaction chromatography (NP-TGIC),[7–9] reversed-phase temperature gradient interaction chromatography (RP-TGIC),[10] solvent gradient interaction chromatography (SGIC),[11] and liquid chromatography at critical condition (LCCC)[12] to form a two-dimensional detection system to characterize complex polymers. Many studies have been done to characterize complex polymers in detail using two-dimensional liquid chromatography.[8–17] For example, Radke et al. used a combination of SGIC and SEC to separate and characterize linear and hyperbranched polyester mixtures according to the topology and molar mass.[11] Chang et al. stated that accurate analysis of linear and star polystyrene mixtures cannot be obtained by SEC alone, but a combination of SEC and reversed phase liquid chromatography (RPLC) can solve the problem.[16] Chang et al. also used 2D-LC to identify species in the polystyrene/polybutadiene block copolymer system in detail.[17] However, two-dimensional liquid chromatography also has some drawbacks, such as the "breakthrough" effect, and experimental conditions such as the solvent composition for different systems needs to be adjusted to obtain a good separation.[18]

In addition to liquid chromatography, analytical ultracentrifugation (AUC) is also a good tool for separation of solutes based on the molar mass and the size of the solutes in solutions.[19–35] It has been widely used to characterize polymers and nanoparticles,[19–26] proteins,[27–30] and DNA.[31–35] However, only a few research studies have been done to characterize polymers by a combination of SEC and AUC. Previously, we have used this coupled method to investigate the scaling laws between the sedimentation coefficient and the molar mass of a series of linear poly(2-ethyl-2-oxazoline).[23] In this study, we mainly used this combined method to separate polymers with different topologies, that is, linear and hyperbranched polystyrenes. In the first dimension separation, SEC was used to separate the polymer mixtures according to the hydrodynamic volume, and in the second dimension separation, AUC was used to fractionate and characterize the polymers mainly with respect to the molar mass. As a result, two-dimensional separation maps of polymers with different topologies are obtained. In addition, the effect of the subchain length on the separation efficiency was also investigated.

2 | EXPERIMENTAL SECTION

2.1 | Materials

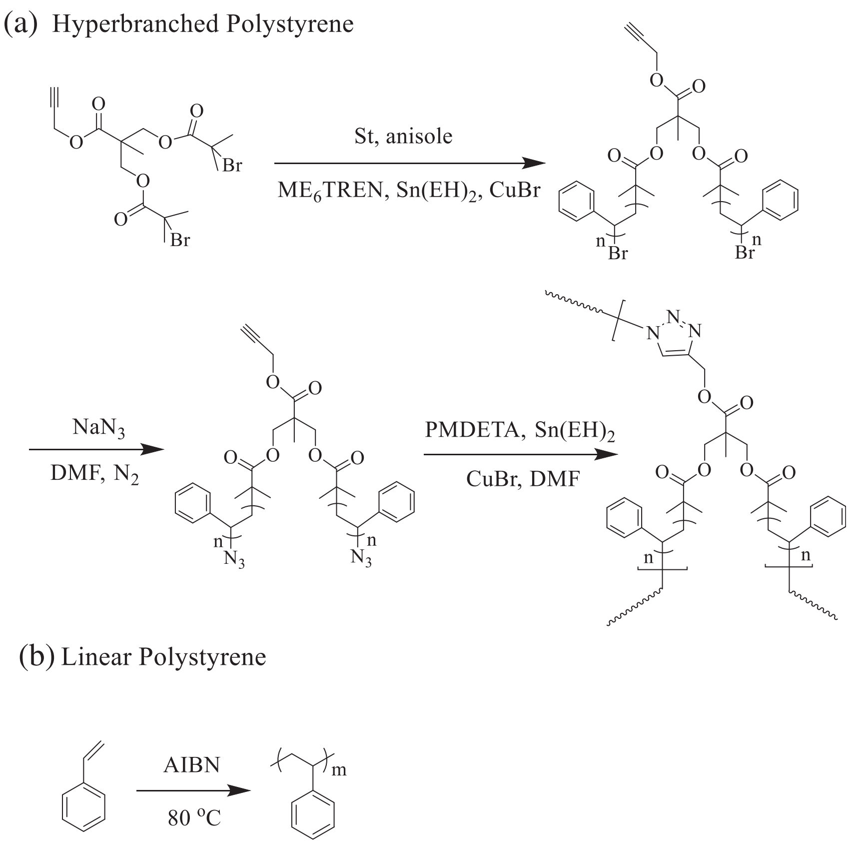
Styrene (St, Sinopharm Chemical Reagent Co., Ltd, 97%) was first passed through a basic alumina column to remove the inhibitor and then distilled under a reduced pressure over calcium hydride (CaH_2). Sodium azide (NaN_3, Sigma-Aldrich, 99%), N,N,N′,N′,N″-pentamethyldiethylenetriamine (PMDETA, Sigma-Aldrich, 99%), tin(II)-2-ethylhexanoate ($Sn(EH)_2$, Aladdin, 95%) were used as received. Dimethylformamide (DMF, Sinopharm Chemical Reagent Co., Ltd, 97%) was purified by distilling under a reduced pressure after dried over anhydrous magnesium sulfate. Tetrahydrofuran (THF, Sinopharm Chemical Reagent Co., Ltd, 97%) and toluene were distilled over sodium. Dichloromethane (DCM) and anisole from Sinopharm Chemical Reagent Co., Ltd were distilled over calcium hydride before use. Tris(2-(dimethylamino)ethyl)amine (Me_6TREN, Aladdin, 98%) was used as received. Copper-(I) bromide (CuBr, Alfa, 98%) was washed with glacial acetic acid to remove soluble oxidized species, filtered, washed with ethanol, and dried under vacuum.

2.2 | Preparation of hyperbranched and linear polystyrenes

Synthetic pathways for hyperbranched and linear polystyrenes are described as follows according to a reported procedure, as shown in Scheme 1.[36–39]

2.3 | Synthesis of polystyrene macromonomers

The initiator propargyl 2,2-bis((2′-bromo-2′-methylpropanoyloxy)methyl) propionate (PBMP) was synthesized according to the literature method.[39] PBMP (0.60 g, 1.28 mmol), St (8.8 ml, 76.9 mmol), Me_6TREN (85 μl, 0.32 mmol), $Sn(EH)_2$ (104 μl, 0.32 mmol), and anisole (8.8 ml) were added to an oven-dried sealing tube with a magnetic stirrer. The tube was degassed by three freeze-pump-thaw cycles, and CuBr (9.8 mg, 68.3 μmol) was added quickly into the tube by a hot long-neck glass funnel when the solution was frozen by liquid nitrogen. After pumping again, the tube was sealed in vacuum and placed into an oil bath thermostated at 90°C for 4 h. The reaction was stopped by liquid nitrogen. After thawing, the polymer solution was diluted in THF. The metal salts were removed by a neutral alumina column, and the crude product was finally precipitated twice in methanol. The polystyrene macromonomer was dried in a vacuum oven for 24 h. Yield: 2.5 g (62.5%), $M_w = 2{,}970$ g/mol (measured by SEC in Figure S1). The polymer is designated as PS-3K, where 3K is the molar mass. Another sample PS-7K was synthesized in the same way by changing the feed ratio of styrene to the initiator and reaction time. These



SCHEME 1 Schematic diagram of the synthesis of hyperbranched and linear polystyrenes

samples were also analyzed by SEC (Figure S1), and the ^{1}H NMR spectra of the samples are present in Figure S2.

2.4 | Azidation substitution reaction

PS-3K (2.40 g, 0.80 mmol) and NaN_3 (0.52 g, 8.0 mmol) were placed in a round-bottomed flask and bubbled with nitrogen gas for 10 min. Then, 24 ml of DMF was injected into the closed system with a syringe. After degassing by nitrogen for another half an hour, the mixture was covered from light and stirred for another 24 h. The mixture was stirred for 2 min after the addition of 4.8 ml of DCM, and centrifuged at 4,000 rpm for 10 min. After removing the salts, the supernatant was precipitated in methanol once. After being dried in a vacuum oven for 24 h, the polystyrene macromonomer with one alkyne functional group and two azide functional groups was collected (yield ~ 1.59 g, PS-3K-N_3). PS-7K was substituted with azide groups by changing the feed ratio of NaN_3 to the polymer by the similar method. The characteristic absorption peak of azide group at 2,100 cm^{-1} is observed, as shown in Figure S5, indicating the successful substitution of azide groups. It can be clearly observed that after the azide reaction, the signal of the methine proton at the chain end changed from 4.30–4.50 ppm to 3.80–4.10 ppm and the original peak completely disappeared (Figure S2), indicating that the substitution was complete.[37]

2.5 | Preparation of hyperbranched polystyrene via “click” reaction

PS-3K-N_3 (1.50 g, 0.50 mmol), PMDETA (21 μl, 0.10 mmol), $Sn(EH)_2$ (65 μl, 0.20 mmol), and DMF (3.75 ml) were added into a dry sealing tube with a magnetic stirrer. After three freezing-pumping-thawing cycles, CuBr (14.8 mg, 0.10 mmol) was quickly added into the frozen system, and the tube was sealed under vacuum. The reaction system was stirred at room temperature for 24 h. The polymer solution was diluted in THF and passed through a neutral alumina column to remove the metal salts. The hyperbranched polystyrene was obtained after precipitating in methanol once and drying in a vacuum oven for 24 h (yield ~ 1.42 g, HB-PS-3K, ^{1}H NMR in Figure S3, HB-PS-7K, ^{1}H NMR in Figure S4). Following the same procedure, another hyperbranched samples (HB-PS-7K) was synthesized from the macromonomer PS-7K-N_3. The increase in the molar mass (Figure S1) and the attenuation of the azide absorption peak (Figure S5) demonstrate the success of the click reaction. Figure S6 shows the retention volume dependence of the degree of polymerization of the macromonomer of the two hyperbranched polystyrenes. In addition, the average degree of polymerization can be calculated as 6 using the ratio of the absorbances of the azide groups and benzyl groups (A_{2107}/A_{3026}) before and after the click chemistry reaction.[40]

2.6 | Synthesis of linear polystyrene

St (2.2 ml, 19.2 mmol), azobisisobutyronitrile (AIBN) (10.2 mg, 62.1 μmol), and toluene (7 ml) were added into a dry tube. After three freezing-pumping-thawing cycles, the tube was sealed under vacuum. The polymer solution was diluted in THF and precipitated twice in methanol. The linear polystyrene was dried in a vacuum oven for 24 h. Another two PSs with different molar masses were synthesized by changing the feeding ratio of styrene to the initiator and reaction time. These three linear PSs were dissolved in THF, and then precipitated in methanol to obtain a linear PS sample with a wide distribution. Finally, the linear polystyrene was dried in a vacuum oven overnight. The weight-average molar mass (M_w) and polydispersity index are 35,850 g/mol and 2.6, respectively.

3 | EQUIPMENT

3.1 | Size exclusion chromatography

The weight-average molar mass (M_w), number-average molar mass (M_n), and the polydispersity index (PDI = M_w/M_n) of the samples were determined by a Waters 1515 SEC instrument equipped with three Waters Styragel columns (HR2, HR4, and HR6) and connected with a multi-angle light scattering detector (MALS, Wyatt WEA-02) and a refractive index detector (RI, Wyatt WREX-02) at 35°C. The injection volume was 50 μl. A refractive index increment (dn/dc) value of 0.185 ml/g was used to calculate the M_w of PS in THF.[41] THF was used as the eluent at a flow of 1.0 ml/min and the instrument was calibrated by a series of polystyrene standards.

3.2 | Analytical ultracentrifugation (AUC)

Sedimentation velocity (SV) experiments were carried out in a Proteomelab XL-A analytical ultracentrifuge (Beckman Coulter Instruments) at 20.0°C at a wavelength of 260 nm using 12-mm double-sector cells. The rotational speed was 40,000 rpm or 58,000 rpm, which is dependent on the molar mass of polymers. Note that centerpieces made from aluminum were used in this study because THF was used as the organic solvent to dissolve PS polymers. In each experiment, 200 data sets describing the time- and radial-dependence of the polymer concentration during the sedimentation and diffusion processes were obtained and analyzed by SEDFIT using $c(s, f/f_0)$ model.[42,43] With a combination of the Svedberg equation and the Stokes–Einstein equation:

$$D = \frac{k_B T}{6\pi\eta R_h} \tag{1}$$

$$M = \frac{s \cdot RT}{D(1-\bar{\upsilon}\rho_s)} \tag{2}$$

$$s = \frac{M \cdot (1-\bar{\upsilon}\rho_s)}{6\pi\eta R_0 N_A (f/f_0)} \tag{3}$$

We can obtain the information of molar mass M, hydrodynamic radius R_h, frictional ratio f/f_0, and sedimentation coefficient s, where the N_A, k_B, T, ρ_s, $\bar{\upsilon}$, η, and R_0 refer to Avogadro's number, the Boltzmann constant, absolute temperature, solvent density, partial specific volume of the solute, solvent viscosity and the radius of a spherical particle that has the same mass and density as the solute, respectively. Herein, the value of the partial specific volume $\bar{\upsilon}$ of polystyrene in THF was 0.923 ml/g according to the value reported by Schubert et al.[20]

4 | RESULTS AND DISCUSSION

It is well known that SEC has been employed widely to fractionate and characterize polymers according to the hydrodynamic volume of polymers. Thus, the characterization of polymer mixtures with different topologies cannot be achieved by SEC alone, which requires other methods such as TGIC,[7–10] SGIC,[11] and LCCC.[12] In addition, AUC is a powerful technique to separate polymers based on the molar mass and the size.[42] Therefore, in this study, a combination of SEC and AUC has been used to separate a mixture of linear PS and hyperbranched PS. Hyperbranched polystyrenes with two different subchain lengths (HB-PS-3K and HB-PS-7K) were first synthesized from AB_2-type polystyrene macromonomers by a combination of atom transfer radical polymerization (ATRP) and "click" chemistry.[44,45] The linear polystyrene was synthesized by conventional radical polymerization using AIBN as the initiator. The detailed characterization data of the macromonomer, the hyperbranched polystyrene and linear polystyrene are summarized in Table 1.

Figure 1 shows the SEC curves of linear polystyrene (L-PS), hyperbranched polystyrene (HB-PS-3K), and a mixture of HB-PS-3K and L-PS (Mixture-3K) with a weight ratio of 5:1. The ratio of the mixture is to ensure that each component in the mixture can be detected effectively in AUC experiments. Each fraction with a volume of 0.5 ml from 21.25 min and a total of 15 fractions were collected, as shown in Figure 1, which is similar to the method used by Radke et al. for the separation of polyesters with different degrees of branching.[11] Since the SEC separation is governed by the hydrodynamic volume of polymers, each fraction should contain hyperbranched and linear polystyrenes with similar hydrodynamic volume but different molar masses due to the different topologies. The same mixed solution was fractionated three or four times by SEC to ensure sufficient amount of samples for the second-dimensional separation by AUC. Each fraction was further diluted or concentrated to make sure that the absorbance value at a wavelength of 260 nm is in the range of 0.2–1.2 to meet the requirements of AUC measurements.

Different elution fractions from SEC were further measured by the AUC method. In ultracentrifugation experiments, the sedimentation coefficient of one solute is proportional to buoyant molar mass and inversely proportional to the hydrodynamic radius (R_h), as shown in Equation (3), so that in principle the hyperbranched and linear polystyrenes in each fraction should have different sedimentation coefficients due to the similar hydrodynamic radius but different molar masses and shapes. Experimental sedimentation profiles curves and fitting data for Mixture-3K-fraction4 (the fourth fraction of the mixture of HB-PS-3K and L-PS) are shown in Figure 2a. From left to right, the polymers sediment to the bottom driven by the centrifugal field as time increases. At the beginning of the experiment, the solutes were dispersed evenly in the sample cell. At the end of the experiment, all the polymer chains sediment toward the bottom of the cell under the centrifugal force. In addition, Figure 2b shows the residual values at different radial positions with the best-fit root-mean-square derivation of 0.006, which is slightly higher than the typical system noise of 0.005.[30]

In general, the $c(s)$ model in SEDFIT software developed by Schuck is the most commonly used model for analyzing AUC data to obtain the sedimentation coefficient distribution of the solutes in solutions.[42,43] The $c(s)$ distribution is obtained by accurate solutions of Lamm equation using the maximum entropy regularization method.[42] In $c(s)$ model by assuming the same of the frictional ratios of different solutes, the number of the species in solutions can be identified, however, the accurate molecular molar masses of the solutes with different frictional ratios cannot be obtained. Later, a two-dimensional size-and-shape distribution model $c(s, f/f_0)$[43] was further developed to solve this problem by calculating s and f/f_0 simultaneously with no assumptions of scale laws, and this model has been applied to the mixtures varied in s, M, and frictional ratio,[43] such as proteins[43] and nanoparticles.[22] In this study, we mainly used $c(s, f/f_0)$ model to investigate the difference in molar masses and fractional ratios between linear and hyperbranched polymers.

Figure 3a shows the size-and-shape distribution of Mixture-3K-fraction4 using $c(s, f/f_0)$ model implemented in the Sedfit software.[43] The result clearly shows that two species with the sedimentation coefficients of 3.71 S

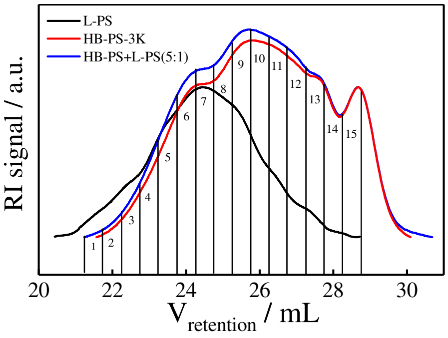


FIGURE 1 Overlay of SEC chromatograms of linear polystyrene (L-PS, black line), hyperbranched polystyrene (HB-PS-3K, red line) and the mixture of HB-PS-3K to L-PS with a weight ratio of 5: 1 (blue line). Stationary phase: three Waters Styragel columns (HR2, HR4, and HR6), mobile phase: THF, flow rate: 1 ml/min, detection: Refractive index detector. The vertical lines indicate the fraction limits [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Molar mass characteristics of polystyrene macromonomer, hyperbranched polystyrene, and linear polystyrene

		^{1}H NMR	SEC		
Samples		M_n (g/mol)	M_n (g/mol)	M_w (g/mol)	M_w/M_n
Macromonomer	PS-3K	2,990	2,700	2,970	1.10
	PS-7K	7,840	6,420	6,900	1.07
Hyperbranched PS	HB-PS-3K	—	7,390	24,450	3.30
	HB-PS-7K	—	17,090	53,120	3.10
Linear PS	L-PS	—	14,070	35,850	2.60

and 5.38 S exist in the system. The corresponding frictional ratios (f/f_0) of these two species are 1.89 and 1.69, respectively, which is defined as the ratio of the friction coefficient (f) experienced by the solute during the sedimentation to the theoretical friction coefficient (f_0) of the ideal sphere with the same molar mass. The linear and hyperbranched polystyrenes in each fraction should have similar hydrodynamic volume and hyperbranched polystyrene should have a larger sedimentation coefficient due to its more compact structure.[11] Moreover, the f/f_0 of hyperbranched sample is smaller than that of linear polystyrene, indicating the more symmetric structure of hyperbranched samples.[46]

Lederer et al.[47] used SEC to fractionate linear and hyperbranched polyesters separately and further injected different fractions into SEC-RI-MALLS system to obtain the accurate molar mass of linear and hyperbranched samples. They found that the molar mass of the hyperbranched polyesters at the same retention volume was higher than that of linear samples. Radke et al.[11] studied the retention time of the branched and linear polyesters in a given SEC fraction on the gradient liquid chromatography and they found that branched species have the higher retention time. They explained that branched polymers have higher molar mass than linear components due to their compact structure, which leads to stronger adsorption strength and higher retention time. In this study, the molar mass of hyperbranched polystyrene is also higher than that of the linear ones with the same retention volume. So, it is clear that the species with higher sedimentation coefficient belong to hyperbranched polystyrenes due to the higher molar mass and lower f/f_0, as indicated in Equation (3). Note that the extinction coefficients of linear and hyperbranched polystyrenes are basically the same (Figure S7), so the magnitude of $c(s, f/f_0)$ can be easily obtained from the absorbance of the two species. There are two peaks in the integrated $c(s, *)$ distribution from $c(s, f/f_0)$, as shown in Figure 3b, which belong to the linear and hyperbranched polystyrenes.

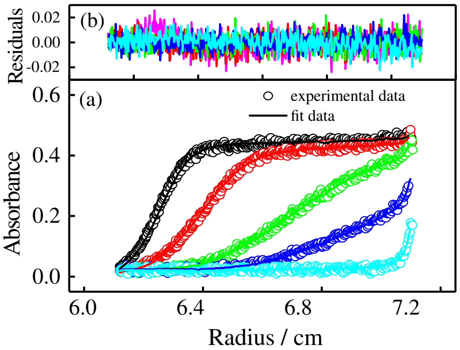


FIGURE 2 Sedimentation velocity analysis of Mixture-3K-fraction4 at 40,000 rpm and 20°C. (a) The collected data (discrete points) and fit data (solid line); (b) fit residuals [Color figure can be viewed at wileyonlinelibrary.com]

After proving that a combination of AUC and SEC can be used to separate linear and hyperbranched polystyrenes in one fraction, we then used AUC to measure all the fractions of the mixture of linear polystyrene and hyperbranched polystyrene with a macromonomer molar mass of 3,000 g/mol (HB-PS-3K) from SEC. Figure 4 shows a two-dimensional contour plot of the mixture of the linear polystyrene and the hyperbranched polystyrene (HB-PS-3K). The retention volume in the first dimension (SEC) and the sedimentation coefficient in the second dimension (AUC) are plotted as the abscissa and the ordinate, respectively. As mentioned above, the species with higher sedimentation coefficient can be assigned to the hyperbranched polystyrene due to its higher molar mass than linear PS. The result also shows that the difference of sedimentation coefficients between the two species in each fraction increases with decreasing the retention volume, which is similar to the findings by Radke et al.[11] In their study, Radke et al. used a two-dimensional liquid chromatography to separate linear and branched polyesters and they found that the

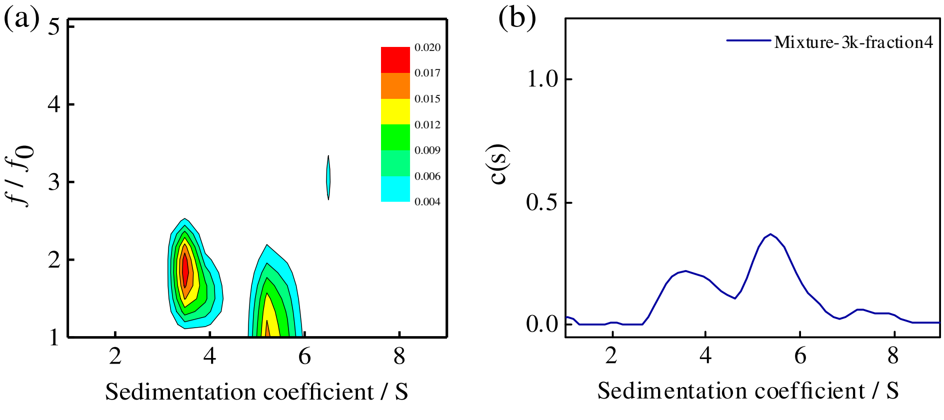


FIGURE 3 Sedimentation velocity analysis of the sample Mixture-3K-fraction4 sedimenting at a rotor of speed of 40,000 rpm at 20°C: (a) the two-dimensional distribution $c(s, f/f_0)$ shown in 2D contours and (b) the corresponding integrated $c(s, *)$ distribution [Color figure can be viewed at wileyonlinelibrary.com]

separation efficiency in the lower molar mass region decreases due to the decreasing in the number of branch points. In current study, the differences in molar mass and shape between linear PS and hyperbranched PS decrease with decreasing the number of branch points, so it is difficult to separate in the low molar mass region. Figure 4 clearly shows that the separation is efficient when the retention volume is smaller than a critical retention volume (V_c) ~ 24.5 ml.

Note that the traditional two-dimensional liquid chromatography can be used to separate polymers and can give information about the molar masses of each component in the polymer mixture using different detectors. Herein, during the fractionation of the mixture of linear polystyrene and hyperbranched polystyrene by AUC, information about the molar mass and the shape can also be provided using $c(s, f/f_0)$ model implemented in the Sedfit software. Schuck et al. showed that the molar masses of the proteins fitted by the $c(s, f/f_0)$ model are closer to the theoretical molar masses.[43] In our study, the linear polystyrene was fractionated individually and then each fraction was injected in SEC to acquire molar mass of linear polystyrene in each fraction using a refractive index detector. Figure 5a illustrates the comparison of molar masses of linear polystyrene using the RI detector and AUC method. The result clearly shows that the values of molar mass are consistent with each other for five fractions, confirming that AUC can separate and characterize the molar masses of linear polystyrene at the same time. In addition, the sedimentation coefficient of linear polystyrene scales with the molar mass obtained by AUC with a scaling index of 0.46 (data not shown), which is slightly smaller than the value 0.48 reported by McCormick and Pavlov et al. in different solvents, indicating that the linear polystyrene adopts a random coil conformation in THF.[48–50]

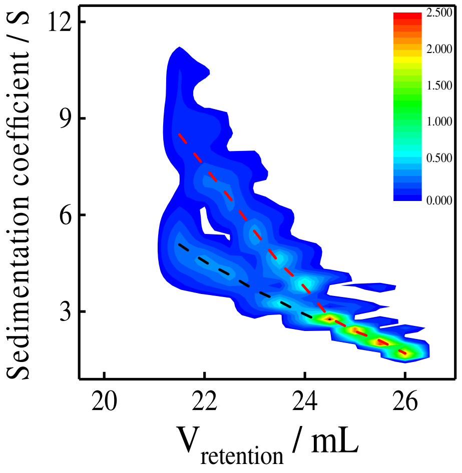


FIGURE 4 SEC × AUC separation of a mixture of linear polystyrene and hyperbranched polystyrene with a macromonomer molar mass of 3,000 g/mol (HB-PS-3K). The lines are to guide the eyes [Color figure can be viewed at wileyonlinelibrary.com]

Similarly, molar mass of hyperbranched polystyrene component can also be obtained by AUC and compared with the values measured by SEC-MALLS instead of SEC-RI due to the different topologies between the hyperbranched PS and linear PS standards for SEC, as shown in Figure 5b. The result shows that for hyperbranched polystyrene, the values are also close to each other. After obtaining the accurate molar masses of hyperbranched and linear polystyrenes ($M_{\text{w, h-PS}}$ and $M_{\text{w, l-PS}}$), the ratio of $M_{\text{w, h-PS}}$ to $M_{\text{w, l-PS}}$ ($r = M_{\text{w, h-PS}}/M_{\text{w, l-PS}}$) at the critical retention volume (Figure 4) can be calculated as ~1.2, that is, for two polymer samples with different molar masses and similar hydrodynamic radius, the separation by AUC will be efficient if the ratio r is higher than 1.2.

Furthermore, we used this two-dimensional method to study the effect of the macromonomer molar mass on the separation efficiency. Figure 6 shows two-dimensional chromatogram for the mixtures containing linear polystyrene and hyperbranched polystyrenes with macromonomer molar masses of 7,000 g/mol (HB-PS-7K). Figure 6 shows similar results as Figure 4, that is, two species with different sedimentation coefficients can be observed and the separation efficiency decreases with the

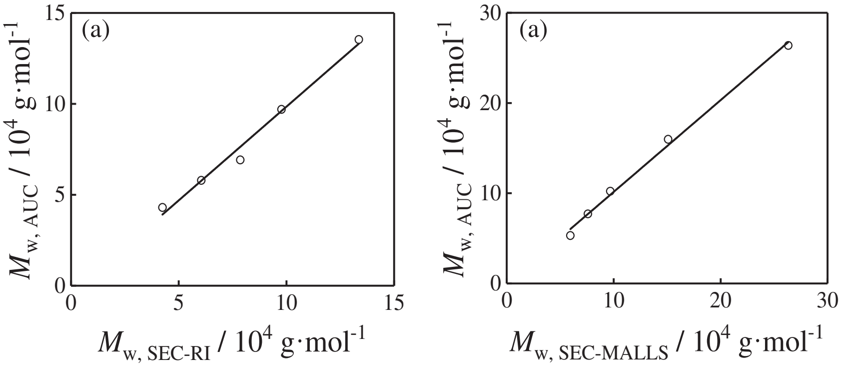


FIGURE 5 Comparison of the molar masses of (a) linear components and (b) hyperbranched portion in the mixture measured by SEC-RI or SEC-MALLS (*x*-axis) and AUC (*y*-axis)

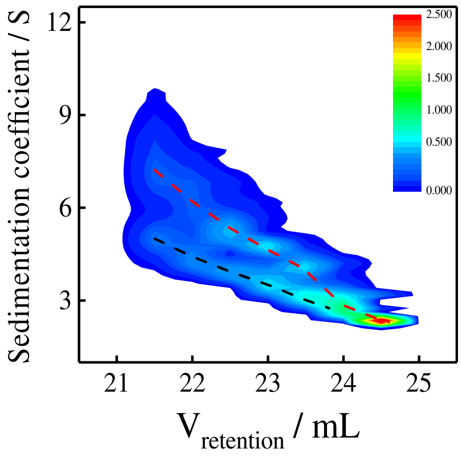


FIGURE 6 SEC × AUC separation of a mixture of linear polystyrene and hyperbranched polystyrene with a macromonomer molar mass of 7,000 g/mol (HB-PS-7K) [Color figure can be viewed at wileyonlinelibrary.com]

decreasing of the molar mass of polymers. It is clear that the sedimentation coefficient difference between the linear polystyrene and hyperbranched polystyrene (HB-PS-3K) (Figure 4) in one fraction with the same retention volume is larger than that for HB-PS-7K. For example, the sedimentation coefficient difference at the retention volume of 21.75 ml for HB-PS-3K is 4.09 S, which is larger than 2.59 S for HB-PS-7K. The reason is that the degree of branching is lower for hyperbranched polystyrene with longer subchain length, which results in the smaller difference of the molar mass between linear PS and hyperbranched PS in one fraction with the same retention volume.[51] Note that, Radke et al. stated that the separation efficiency was worse for the longer arm than for the shorter one and they separated the linear and star shaped-structures for arm molar masses up to 42,000 g/mol through improving the SEC resolution.[8,9]

5 | CONCLUSIONS

We have demonstrated that a combination of SEC and AUC can be used to separate and characterize mixed solutions of hyperbranched and linear polystyrenes. The results show that the separation efficiency decreases with the decreasing of the molar mass of the polymer and the increasing of the retention volume, and the mixture cannot be separated at high retention volume. The separation efficiency also decreases with the increasing of the subchain length of the hyperbranched polystyrene. Moreover, this method can obtain the accurate molar mass of the individual component in one fraction eluted from the SEC column and avoid the problem of solvent incompatibility that often occurs in conventional 2D liquid chromatography. Our study provides the framework for future studies to separate polymers with different topologies by a combination of SEC and AUC.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

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