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Temperature gradient interaction chromatography of low molecular weight polystyrene

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Abstract

Separation of low molecular weight polystyrene (PS) by temperature gradient interaction chromatography (TGIC) is reported. As in the separation of high molecular weight PS, temperature is an efficient variable to control the retention of low molecular weight PS in the reversed phase HPLC separation. However, a right choice of the eluent, typically a marginal solvent for the polymeric solute of interest, is crucial for the temperature to play an effective role in the retention control. For an example, PS oligomers were well separated by TGIC under the isocratic elution condition of C18 bonded silica and methanol as the stationary and the mobile phase, respectively. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: Separation of polystyrene oligomers; Temperature gradient interaction chromatography

1. Introduction

Size exclusion chromatography (SEC) has long been used as an efficient tool for the separation of polymers in terms of their molecular weights [1]. However, SEC is not a high resolution tool especially for the separation of low molecular weight polymers. For a high resolution analysis of oligomers, non-exclusion high performance liquid chromatography (HPLC) has been more efficiently employed. The non-exclusion HPLC stands for the HPLC methods utilizing the interaction between the solutes and the stationary phase in contrast to SEC, which will be called henceforth as interaction chromatography (IC). There have been a number of reports on the IC separation of oligomers. The early works till mid 80s were well summarized in the report of Jandera and Rozkošná [2]. An extensive study on the IC separation of polystyrene (PS) oligomers was reported by Lewis et al. [3]. They employed reversed phase HPLC for the analysis of PS oligomers and reported the solvent effect associated with the IC separations [3]. Lochmüller and McGranaghan reported a partial separation of a rather high molecular weight PS oligomers (M_w: 2000) [4] and Shalliker et al. demonstrated a separation of wide molecular weight range of PS from oligomers to high molecular weight by solvent gradient HPLC [5]. Recently Philipsen et al. reported an extensive thermodynamic study on the HPLC separation of oligomeric polystyrene and polyester [6]. There are also many reports on the IC separation of the oligomers other than PS in the literature [7,8]. From these studies, it is well recognized that high resolution separation under isocratic elution condition is possible only for a limited range of molecular weight. As the retention of a polymeric species generally follows the Martin's rule, i.e. $\ln k'$ (capacity factor) is proportional to the degree of polymerization [9], the retention increases steeply with the degree of polymerization that makes it difficult to cover a wide molecular weight range by isocratic elution. In order to extend the molecular weight range, i.e. to control the solute retention during the IC elution, the solvent gradient elution method has been commonly employed.

The retention in IC can be also controlled by changing the temperature instead of changing the solvent composition. The capacity factor, k' is related to the thermodynamic parameters associated with the sorption process of a solute to the stationary phase as follows:

$$\ln K = -\frac{\Delta G^\circ}{RT} = \frac{\Delta S^\circ}{R} - \frac{\Delta H^\circ}{RT} \quad \text{and} \quad k' = K\phi \quad (1)$$

where K is the equilibrium constant of a solute partitioned between the stationary and mobile phase and ϕ is the

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Table 1
PS standards used in this study

Sample code	M_p (g mol^{-1}) (Measured by SEC)	M_w/M_n (Measured by SEC)	Supplied by
1	520	1.14	Polymer Lab.
2	950	1.16	Polymer Lab.
3	1250	1.10	Polymer Lab.
4	1700	1.06	Polymer Lab.
5	3200	1.04	Polymer Lab.
6	7000	1.04	Polymer Lab.
7	11 600	1.03	Polymer Lab.
8	22 000	1.03	Polymer Lab.
9	37 300	1.04	Daelim Inc.
10	68 000	1.03	Home made
11	140 000	1.04	Home made
12	225 000	1.03	Waters
13	402 000	1.02	Waters
14	632 000	1.04	Daelim Inc.
15	1 530 000	1.11	Daelim Inc.

volume ratio of the stationary and mobile phase, V_s/V_m. It is clear from Eq. (1) that the capacity factor is a function of the temperature and the temperature dependence of the capacity factor has been frequently investigated to obtain the thermodynamic parameters associated with the chromatographic separation. However, temperature has not been used widely as a control parameter for the IC retention as the range of the variation is not large due to the solvent boiling as well as freezing (or precipitation of the solutes). In addition, K is weakly dependent on the temperature if ΔH° is not large, which is controlled mainly by the choice of the pair of the mobile and stationary phases.

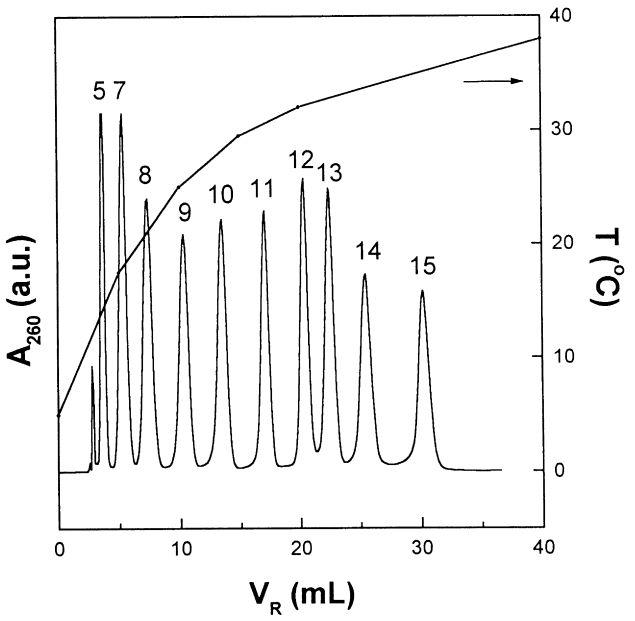

Fig. 1. TGIC chromatogram of ten PS standards whose molecular weights range from 3200 to 1 530 000 (Sample code 5, 7–15 in Table 1). The sharp peak appearing at $V_R = 2.8$ mL is the injection solvent peak. Right ordinate represents the temperature of the circulating fluid in the column jacket. With this temperature program, ten PS standards are nicely separated according to their molecular weights.

To our knowledge, the practical use of temperature gradient in IC for the separation of polymeric species was first reported by Lochmüller and coworkers [10]. They employed temporal as well as spatial gradients of the column temperature in the IC separation of polyethylene oxides. We also found independently that temperature can be used as an efficient tool to control the IC retention of polymeric species and subsequently have reported several applications of temperature gradient interaction chromatography (TGIC) [11–16]. In these studies, however, wide molecular weight range samples, with an emphasis on high molecular weight polymers, were dealt with and the resolution in low molecular weight region appeared not as good as the high molecular weight polymers. We found recently that the resolution in low molecular weight region can be enhanced greatly by lowering the solvent quality of the eluent. With an appropriate choice of the eluent, temperature is a very efficient control variable for the IC separation of oligomers. In this paper we report the result of low molecular weight PS separation by TGIC.

2. Experimental

The experimental setup is practically the same as in our previous report [11]; an HPLC apparatus with single C18 bonded silica column (Alltech, Nucleosil, 100 Å pore, 4.6 mm × 250 mm, 5 μm particle size) and a UV/Vis absorption detector (LDC, Spectromonitor 3000) operated at the wavelength of 260 nm. Polystyrene standards used in this study are listed in Table 1. The mobile phase was either a mixture of CH_2Cl_2/CH_3CN or tetrahydrofuran(THF)/methanol, which were used as received from Aldrich (HPLC grade). The mixture eluents were pre-mixed and delivered by an isocratic pump (LDC, Constametric 3200) at a flow rate of 0.5 mL/min. All the injection samples were dissolved in a small portion of the eluent and injected through a Rheodyne 7125 injector equipped with a 50 μL sample loop. Temperature of the column was controlled in a pre-programmed manner by circulating a fluid from a bath/circulator (Neslab, RTE-111) through a column jacket (Alltech).

3. Results and discussion

Fig. 1 shows a typical TGIC chromatogram of ten PS standards whose molecular weight ranges from 3200 to 1 530 000. Each peak is labeled with the corresponding sample code listed in Table 1. This TGIC chromatogram was obtained by isocratic elution with 57/43 (v/v) mixture of CH_2Cl_2/CH_3CN while the column temperature was varied from 5 to 38°C as shown in the figure. All ten PSs are well

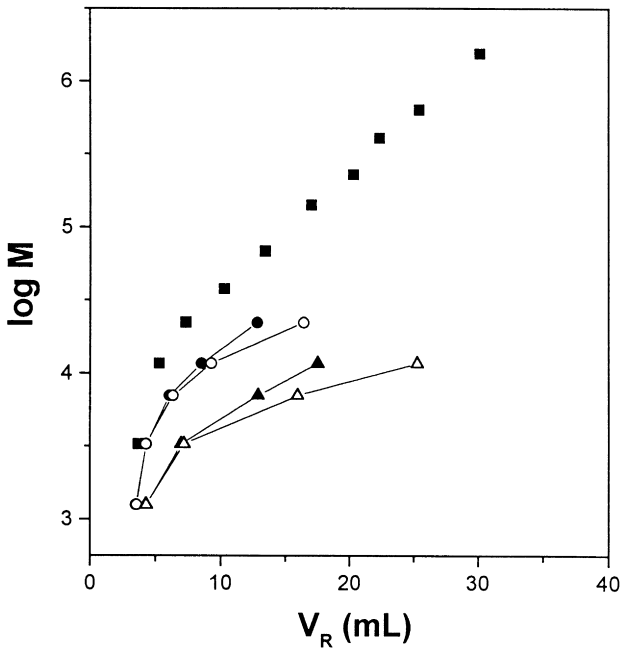

Fig. 2. Calibration curves, log M vs. retention volume (V_R), of chromatograms obtained in this study; ■: Fig. 1, ●: Fig. 3(a), ○: Fig. 3(b), ●: Fig. 3(c), △: Fig. 3(d). Refer to the text for the details.

separated according to their molecular weight sequences and the sharp peak appearing around $V_R = 2.8$ mL is the injection solvent peak. Molecular weight calibration curve, log M vs. retention volume (V_R), can be constructed from the peak positions of the chromatogram as shown in Fig. 2 (filled squares). The calibration curve shows a good linear relationship over the wide molecular weight range higher than 10 000, but curves down steeply at the lower molecular weight region. It indicates that the resolution of low molecular weight PS is not as good as the high molecular weight ones. Unlike SEC, in TGIC the retention of the polymeric solutes can be controlled with relative ease by changing the temperature gradient program [11,13]. In order to improve the resolution of TGIC at the low molecular region, we examined the effect of changing the temperature program and the resulting chromatograms are displayed in Fig. 3. Fig. 3(a) and (b) show two TGIC chromatograms of five low molecular weight PS standards (Sample code 3, 5–8) using the two different temperature programs as shown in the plots; the column temperature is initially kept at 0°C till the injection solvent peak starts to elute out ($t_R = 5$ min), then the temperature is raised linearly with time at two different rates, 1.0°C/min in (a) and 0.5°C/min in (b). Eluent composition is $CH_2Cl_2/CH_3CN = 57/43$ (v/v), the identical composition with which the chromatogram shown in Fig. 1 is obtained. The retention of PS 8 ($M_p = 22\,000$) is clearly increased in (b) relative to (a), but it is not clear for the lower molecular weight ones. In order to compare the retention more clearly, the calibration curve of two chromatographic runs are plotted in Fig. 2, where filled and open circles correspond to the chromatograms of Fig. 3(a) and (b), respectively. Comparing the retentions at three different temperature programs including that of Fig. 1, it is evident that the retention of the higher molecular weight species is more strongly affected as we change the temperature program. It is a natural consequence of the thermodynamics associated with the chromatographic separation. According to the Martin's rule, ΔG° is proportional to the degree of polymerization, thus the temperature dependence of the capacity factor has to be larger for higher molecular weight samples according to Eq. (1). If we want to spread the retention of the low molecular weight PS further, i.e. to make the retention become more sensitive to the temperature change, we need to make the ΔG° larger for the sorption process, which can be done easily by making the eluent condition poorer for PS. For this purpose, we employed 50/50 (v/v) mixture of CH_2Cl_2/CH_3CN instead of the 57/43 mixture used previously. The solvent quality of the 50/50 mixture of CH_2Cl_2/CH_3CN is so poor that PS of molecular weight over 100 000 does not dissolve completely at low temperature. Measured cloud temperature of PS ($M_w = 100\,700$) in this mixture was about 11°C. Under this eluent condition, TGIC chromatograms using the same temperature programs used in Fig. 3(a) and (b) are displayed in Fig. 3(c) and (d), respectively. In this chromatogram four low molecular PS samples (sample code 3, 5–7) were used. As displayed in Fig. 2, the calibration curves clearly show a larger shift in the retention of the low molecular weight samples.

Despite the successful control of the retention itself, however, the resolution of the low molecular weight samples does not appear to be improved significantly in the four chromatograms in Fig. 3. The four low molecular weight PS samples are not completely resolved down to the baseline even in Fig. 3(d). There are two possible causes for the incomplete separation of four PS samples; either the limited resolving power of TGIC or the inherently overlapped distribution of low molecular weight PS samples. In order to test the resolution of TGIC at the low molecular weight range, we tried to fractionate PS 5 under the elution condition of Fig. 3(d). In this experiment, two drops (about 30 μL) of TGIC elution fractions at two different V_R of 6.3 and 8.3 mL were collected, then injected together for a separate TGIC run at the same temperature program. Fig. 4 shows the two TGIC chromatograms of PS 5 (dotted line) and the two fractions (solid line). Two peaks of the fractionated samples are somewhat broadened from the original cut but still much narrower than that of unfractionated PS 5. The molecular weight distribution of each peaks in Fig. 4 were calculated by use of the calibration obtained from the peak positions of the PS samples in Fig. 3(d). The weight average molecular weight of PS 5 and two fractions are determined as 3330, 2430, and 4120, respectively. And the M_w/M_n of these are 1.08, 1.001, and 1.001. Therefore, it is clear that the overlap of the elution peaks in Fig. 3 is due to the overlap of the molecular weight distribution of the PS samples.

As shown in the inset of the Fig. 3(d), PS 3 ($M_p = 1250$)

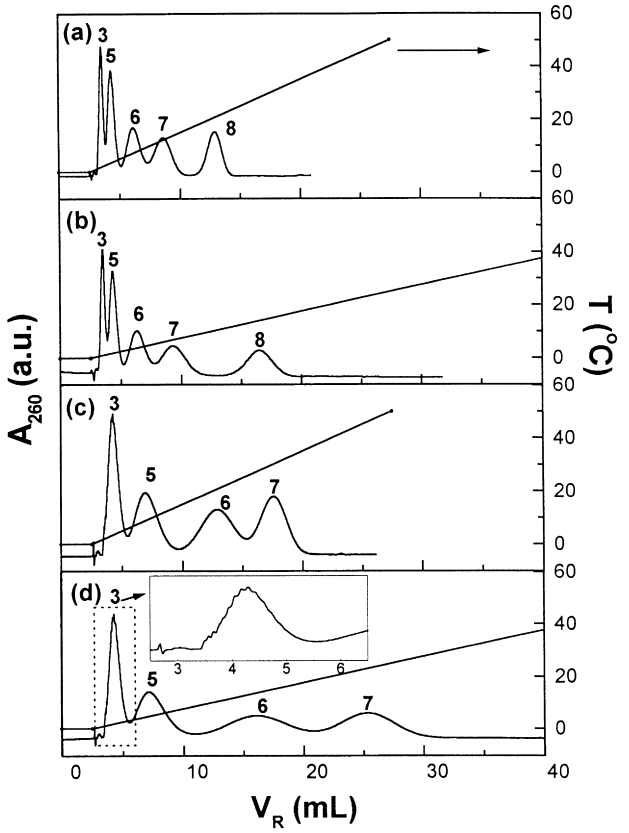

Fig. 3. TGIC chromatograms of low molecular weight PS standards (Sample code 3, 5–8) with two different temperature programs and with two different mobile phase compositions. Column temperature was raised linearly with time after the injection solvent peak starts to elute out (t_R = 5 min) as shown in the plots; (a) and (c) 1°C/min, (b) and (d) 0.5°C/min. Mobile phase compositions are CH_2Cl_2/CH_3CN = 57/43 (v/v) for (a) and (b), 50/50 (v/v) for (c) and (d), respectively. In the inset of (d) a magnified plot of the PS 3 (M_p = 1250) is displayed where the partial resolution of each oligomeric species is observed.

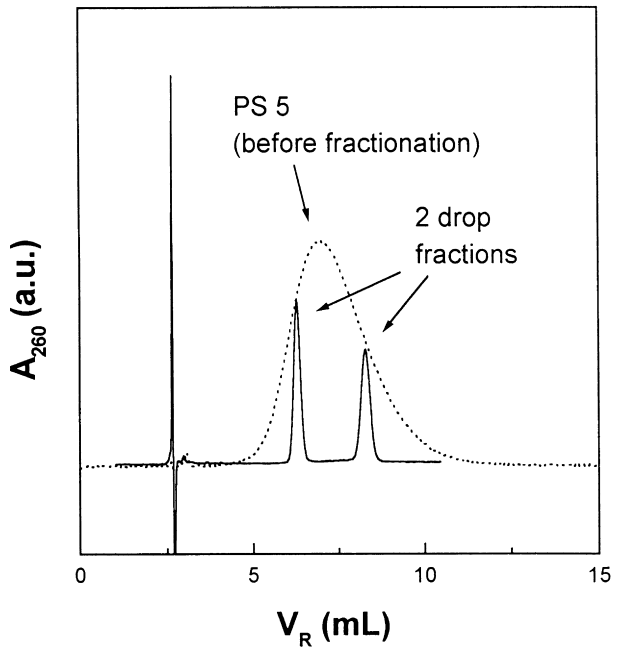

Fig. 4. TGIC Chromatogram of PS 5 (M_p = 3200) (dotted line) and two drop fractions (about 30 μL) collected at V_R of 6.3 mL and 8.3 mL (solid line). TGIC separation condition is the same as in Fig. 3(d). M_w/M_n (M_w) values of the three samples are determined as 1.08 (3330), 1.001 (2430), and 1.001 (4120), respectively.

shows a fine splitting of the peak that comes from the partial separation of each PS oligomeric species. In order to improve the resolution of the oligomers, it was necessary to lower the solvent quality of the eluent further. For example, we can improve the resolution at this range by increasing CH_3CN content in the mixed eluent. In that case, however, isomers of different tacticity of each PS oligomer species are separated to generate a numerous peaks, which in turn makes the peak identification difficult [3,17]. The reason why such an isomeric separation takes place in a few specific solvent conditions is not fully understood. In order to avoid the complication, we employed methanol as a poor solvent for the TGIC separation of PS oligomers, which is known not to involve such a complication [3]. A fine resolution TGIC chromatogram of a mixture of PS 1 (M_p = 520) and PS 4 (M_p = 1700) is displayed in Fig. 5 in which 5/95 (v/v) mixture of THF/methanol is used as the eluent. THF, a good solvent for PS, was used in order to adjust the solvent quality. Column temperature was varied from −5 to 95°C at the ramping speed of 0.77°C/min. We

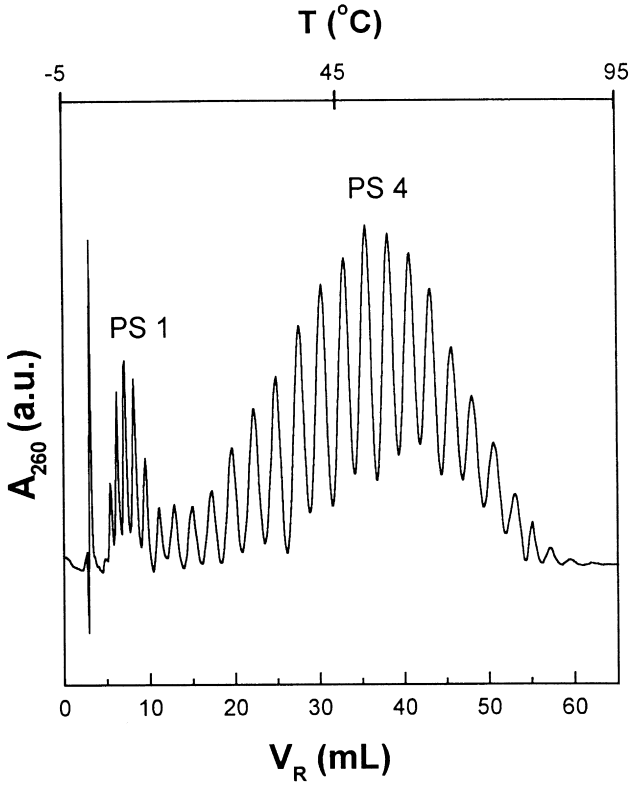

Fig. 5. TGIC chromatogram for mixture of PS 1 (M_p = 520) and PS 4 (M_p = 1700). For the resolution enhancement at the lower molecular weight region, 95/5 (v/v) methanol/THF was used as the eluent. Column temperature was varied from −5 to 95°C at the ramping speed of 0.77°C/min.

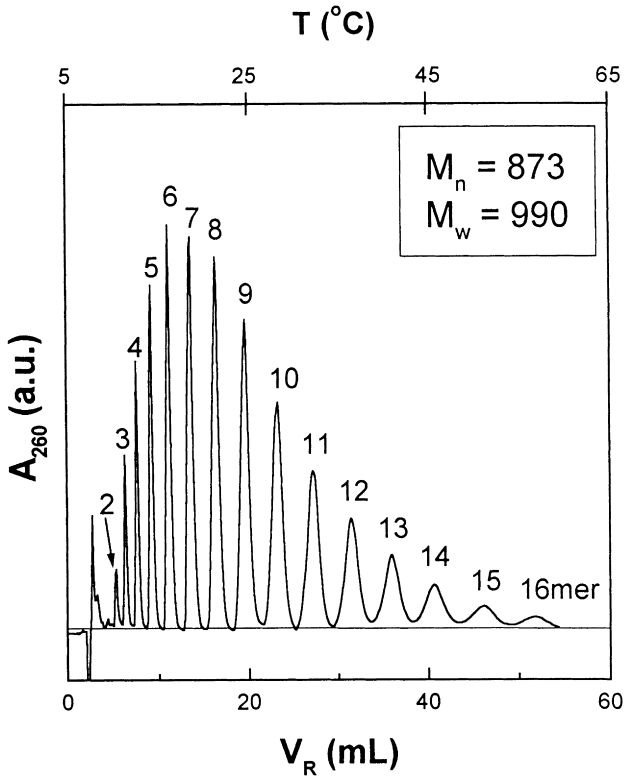

Fig. 6. TGIC Chromatogram for PS 2 ($M_p = 950$). Pure methanol was used as the eluent. Column temperature was varied from 5 to 70°C at the linear ramping speed of 0.5°C/min. Individual oligomeric peaks are well separated down to the baseline and the absolute molecular weight distribution was calculated by integrating each peaks. M_n and M_w obtained are 873 and 990, respectively.

can identify all the oligomeric species present in two polymers, which unambiguously confirms that the molecular weight distribution of two polymers overlaps. The importance of the choice of an eluent for the optimum resolution of oligomeric species is more clearly demonstrated in Fig. 6 where a TGIC chromatogram of PS 2 ($M_p = 950$) is displayed. We used pure methanol as the eluent to obtain this chromatogram, which is well known as a non-solvent for PS [18]. As even the low molecular weight PS is not completely soluble in pure methanol, THF was mixed with CH_3CN to 50 vol% in the injection sample solution to dissolve the PS sample completely. The resolution in the low molecular weight region is greatly enhanced so that we can fully resolve every oligomeric species from monomer to 16 mer. From the chromatogram we can easily measure the relative abundance of each oligomer assuming the absorptivity is independent of the degree of polymerization and calculate the absolute number and weight average molecular weight as 873 and 990, respectively. The importance of the right choice of the solvent in IC separation is not new but has been recognized and studied long to predict an optimum eluent system to enhance the resolution of HPLC. One of the most popular methods to predict the solvent effect in the HPLC separation is the solvent strength concept which is similar to the concept of solvent quality or solubility parameter widely used in polymer science [19]. Although the solvent strength concept provides a useful guideline for the IC separation of small molecules, it often could not predict the retention behavior of polymers well [20]. It obviously requires a further study on the polymeric solute–stationary phase interaction associated with the separation mechanism of the interaction chromatography.

In summary, we examined the role of temperature and eluent composition in the IC separation of low molecular weight PS. Retention of PS can be controlled by changing temperature as well as eluent composition. It was found to be an efficient method to use temperature as a fine-control parameter and to use the eluent composition as a coarse control variable of the retention to optimize the resolution over a relatively narrow molecular weight range. It appears that a poor solvent condition is required for an efficient separation by TGIC. This is likely because the interaction between the PS and the reversed phase stationary phase, long hydrocarbon chain is inherently weak.

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