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Rapid molecular weight analysis of polymers by temperature gradient interaction chromatography

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Abstract

Temperature gradient interaction chromatography (TGIC) has been established as a high-resolution technique for the characterization of synthetic polymers. So far, most of the TGIC investigations focused on the high-resolution analysis and little effort has been made on the reduction of the analysis time. In this study, we examined the effect of the column heating rate, the eluent flow rate, and the column length on the TGIC analysis time. We found that the heating rate is the most important experimental parameter to control the TGIC retention time. With a C18 silica column (50 mm × 4.6 mm I.D.), a set of PS standards of wide molecular weight range (5–648 kg/mol) could be separated within 4 min at a heating rate of 8 °C/min.

Keywords: Temperature gradient interaction chromatography (TGIC); Fast separation; Polystyrene

1. Introduction

Temperature programming has seen increasing use recently as an experimental parameter in liquid chromatography (LC) [1,2]. The temperature effect on liquid chromatographic retention is evident from the following formula of the retention factor, k

$$k \equiv \frac{V_R - V_m}{V_m} = K\frac{V_s}{V_m} = \exp\left(-\frac{\Delta G^\circ}{RT}\right)\frac{V_s}{V_m} \quad (1)$$

or

$$\ln k = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \ln\phi, \qquad \phi \equiv \frac{V_s}{V_m} \quad (2)$$

where V_R, V_m, and V_s stand for the retention volume, the mobile phase volume, and the stationary phase volume in the LC column, respectively. The distribution constant, K is defined as the ratio of the solute concentrations distributed in stationary phase to the mobile phase and is related to the standard Gibbs free energy change (ΔG°) associated with the transfer of solute molecules from the mobile to the stationary phase.

The above formulae indicate that the solute retention is affected by temperature if the solute transfer involves an enthalpy change (Eq. (2)). In the size exclusion chromatography (SEC), the most widely employed method in the polymer characterization, the distribution of polymer solutes between the pore and the interstitial space is mainly governed by the conformational entropy change of polymer chains and the retention is usually insensitive to the column temperature [3,4]. On the other hand, in the interaction chromatography (IC), the enthalpic interaction between the solutes and the stationary phase plays a major role for the solute distribution and the solute retention changes with temperature [5].

For some years, we have employed column temperature programming to control the retention of polymer solutes. The temperature gradient interaction chromatography (TGIC) has been successful in many applications such as high-resolution analysis of the molecular weight distribution [6–10], functionality [9,11], polymer mixtures [3,9], branched polymers [12–18], block copolymers [15,16,19–21], etc. In these TGIC

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separation studies, we did not monitor the temperature inside the column but the bath temperature of the fluid circulating through the column jacket since it is not trivial to monitor the temperature inside the column during the experiment and the precise monitoring of the column temperature was not necessary in practice as far as the chromatographic retention is reproducible with a given temperature program of the circulating fluid. Also we have not made a serious effort to shorten the separation time since our primary interest has been in the high-precision analysis. While it is obvious that a rapid change of the column temperature can reduce the separation time, there were concerns on the adverse effects due to the possible temperature lag between the circulating fluid and inside the column in addition to the limitation in the heating rate of a bath/circulator. Therefore, the TGIC analysis time has remained comparable to that of conventional size exclusion chromatography.

A few years ago, Bruheim et al. reported on the rapid separation (11 min) of polystyrene standards (2–400 kg/mol) by using a packed capillary column (0.32 mm I.D.) to reduce the heat capacity of the column [22]. For the separation, they raised the column oven temperature up to 150 °C with the temperature gradient as steep as 40 °C/min. Despite the successful reduction in the analysis time, the quality of the chromatogram was not as good as the ones obtained with a slow temperature change. Furthermore, it seems unnecessary to raise the temperature to such an extreme level since most of the TGIC analyses have been performed in a moderate temperature range. Therefore, there might have been a large deviation between the oven temperature and the eluent temperature in the column. In this study, we carried out a more systematic study to reduce the TGIC separation time. We monitored the eluent temperature directly in off-line and increased the heating rate up to 8 °C/min while maintaining the thermal equilibrium between the circulating fluid and the eluent.

2. Experimental

The temperature gradient interaction chromatography (TGIC) apparatus is a typical isocratic reversed-phase HPLC system equipped with a C18 bonded silica column (Nucleosil C18, 100 Å pore, 3 μm particle size, 50 mm × 4.6 mm I.D.). A long piece of large bore tube (440 cm long, 0.03 in. I.D.) is installed between the pump (TSP, Spectra series P100) and the injector (Rheodyne 7125) to pre-equilibrate the temperature of the eluent before it reaches the column. The preheating tubing was wound to a 1 in. diameter coil to fit into a column jacket. Temperature of the column and the preheating tube was controlled by circulating fluid from a bath/circulator (THERMO Haake, PII C25P) through the jackets, which encase the column and the coiled preheating tube. To increase the heating rate, an external immersion heater (1 kW) was put into the bath. Eluent was a mixture of CH_2Cl_2 and CH_3CN (HPLC grade, Duksan) at a composition of 57/43 (v/v). The concentration of injection samples was 0.3 mg/mL and the injection volume was 5 μL. The chromatograms were recorded by an UV–vis detector (Spectra series UV100) operated at 260 nm. Eight polystyrene (PS) standards of wide molecular weight range (5.1–1800 kg/mol) were used in this study. Their molecular characteristics are listed in Table 1.

Table 1
Molecular characteristics of the polystyrenes

PS samples	M_w (M_w/M_w)[a]
5.1k	5 050 (1.05)
15.3k	15 300 (1.03)
30.9k	30 900 (1.03)
55.2k	55 200 (1.07)
98.9k	98 900 (1.06)
205k	205 000 (1.03)
632k	631 600 (1.04)
1800k	1 800 000 (1.11)

[a] Determined by SEC according to the calibration with polystyrene standards.

3. Results and discussion

Fig. 1 displays the temperature of the eluent before and after the column. A thin thermocouple was inserted through a short 0.03 in. bore tube (thicker than the 0.007 in. bore LC tubing) attached to the respective temperature monitoring position of the eluent flow. We monitored the eluent temperature at two different heating rates, 2 and 8 °C/min. Before this study, the typical heating rate in TGIC measurements was 2 °C/min or lower mainly due to the limitation in the heating capacity of the bath/circulator. The heating rate was increased by aid of external heating from an immersion heater. As shown in the figure, the temperature of the eluent after passing through the preheating tube follows the temperature

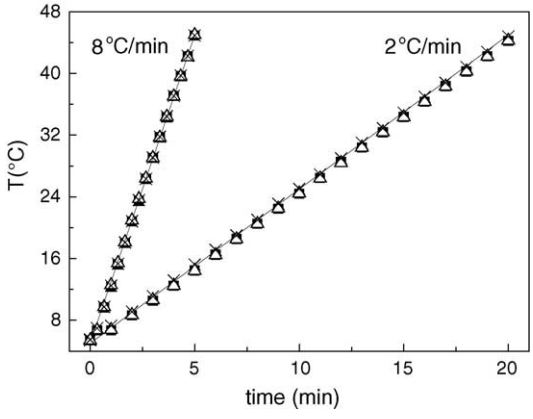

Fig. 1. The eluent temperature monitored before and after the separation column (Nucleosil C18, 100 Å pore, 3 μm particle, 50 mm × 4.6 mm I.D.). Solid lines are bath temperatures programmed at heating rates of 2 and 8 °C/min. Different symbols represent the measured temperatures. (×) After passing through the preheating tubing; (■) after the column with preheating and (△) after the column without preheating. Eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min.

of the circulating bath near perfectly. It ensures that the eluent temperature is equilibrated with the bath temperature by the time it reaches the injector, which is connected to the column inlet. The coiled preheating tubing has an internal volume close to 2 mL. Therefore the residence time of the eluent in the tube is 2 min at a flow rate of 1 mL/min, which is evidently long enough for the eluent to follow the temperature ramp as fast as 8 °C/min. We might be able to reduce the volume of the preheating tubing further, but it was not pursued due to the lack of significant merit.

The eluent temperature at the column outlet was also monitored with and without the preheating tubing. As shown in Fig. 1, the eluent temperatures at the two configurations are hardly distinguishable over the temperature range of 5–45 °C. The residence time of the eluent in the column (50 mm × 4.6 mm I.D.) is 0.65 min at a flow rate of 1 mL/min. During this period the temperature was raised more than 5 °C at a heating rate of 8 °C/min. In the absence of the preheating tube, the eluent entering the column is at the ambient temperature (∼27 °C). Therefore, in this case, the eluent temperature had to change as much as 22 °C while it passes through the column. The results displayed in Fig. 1 clearly demonstrate that the heat transfer across the column wall and the packing materials is fast enough to allow reasonable thermal equilibrium by the time when the eluent exits from the column even without preheating the eluent.

Fig. 2 shows the TGIC chromatograms of seven polystyrene (PS) standards of different molecular weights

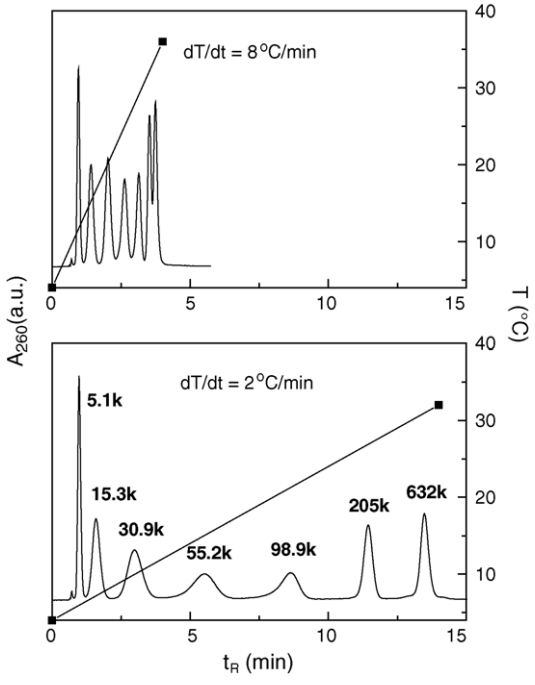

Fig. 2. TGIC chromatograms of seven PS standards at two different heating rates, 8 °C/min (top) and 2 °C/min (bottom). The molecular weights of the PS standards are labeled for the corresponding elution peak. Column: Nucleosil C18, 3 μm, 100 Å, 50 mm × 4.6 mm I.D.; eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min.

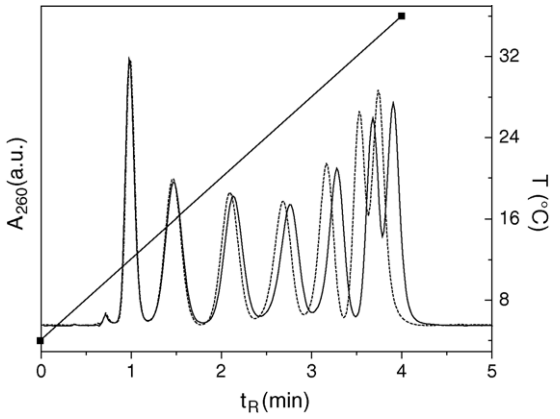

Fig. 3. Preheating effect on TGIC chromatogram at a heating rate of 8 °C/min. Solid line is the chromatogram obtained with preheating and the dashed line is without preheating. Ambient temperature was 27 °C. Column: Nucleosil C18, 3 μm, 100 Å, 50 mm × 4.6 mm I.D.; eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min.

ranging from 5.1 to 632 kg/mol. They are obtained by use of two different linear temperature ramps at 2 °C/min (bottom) and 8 °C/min (top) with preheating. The fast heating rate reduces the separation time of the PS standards greatly and all the PS standards elute within 4 min. Therefore it confirms to us that the reduction of TGIC analysis time can be achieved without relying on extreme temperature gradient if a good temperature equilibration can be achieved. However, the resolution at a heating rate of 8 °C/min is inferior to that of 2 °C/min, in particular for the high molecular weight species.

The effect of eluent preheating on the TGIC separation is displayed in Fig. 3, in which two TGIC chromatograms obtained with and without preheating are compared. Despite the exit temperature appears to follow the bath temperature well with or without preheating as shown in Fig. 1, the retention time of the PS standards is shortened without preheating and the effect is more conspicuous for high molecular weight polymers. The reduction in the retention time without preheating must be the result of the different (ambient) temperature of the eluent entering the column, which is much higher than the column temperature at the initial stage of the separation. The effect is larger for the high molecular weight PS, whose retention is more sensitively affected by temperature since ΔH° in Eq. (2) is proportional to the degree of polymerization according to the Martin's rule [23]. It is interesting to note that the resolution with and without preheating appears not much different. However, we employed the preheating tube for all the experiments since it ensures the temperature equilibrium between of the stationary and the mobile phase from the column inlet.

Fig. 4 shows a TGIC chromatogram employing a nonlinear temperature program in an attempt to improve the resolution. We can see that the resolution in high molecular weight region is substantially improved and even 1800 kg/mol PS is fully resolved in 6 min. This resolution improvement costs the separation time to some extent. It takes about 5 min to separate the PS standards up to 632 kg/mol while it takes

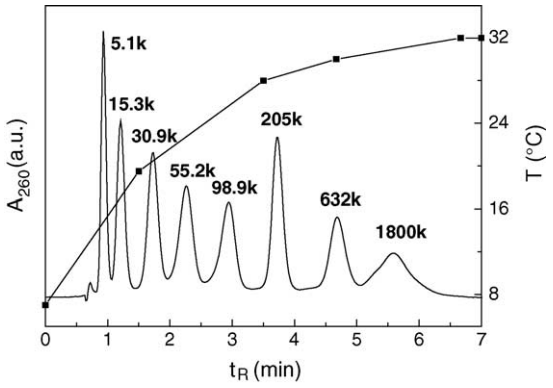

Fig. 4. TGIC chromatogram of 8 PS standards with a non-linear temperature program. Temperature program is shown in the plot. Column: Nucleosil C18, 3 μm, 100 Å, 50 mm × 4.6 mm I.D.; eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min.

4 min to separate the same set of seven PS standards under the linear heating ramp at 8 °C/min as shown in Figs. 2 and 3.

We have also examined the column length dependence of the polymer solute retention as shown in Fig. 5. All the columns employed have 4.6 mm internal diameter and are packed with an identical packing materials (Nucleosil C18, 100 Å pore, 3 μm particle size). The retention time of the PS increases with the column length in general, but the most conspicuous change with the column length arises from the void volume difference among the different length columns. All the PS samples elute in the IC separation mode after the injection solvent peak. The lowest molecular weight PS eluting near the injection solvent peak reflects the elution delay due to the column void volume. The retention times of the PS standards at different column length are summarized in Table 2.

It is notable that the retention time range from the lowest to highest molecular weight PS elution is not in the order of the column length. The 250 mm long column exhibits the shortest

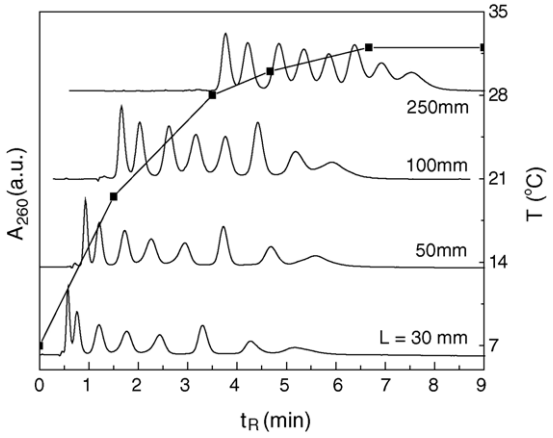

Fig. 5. TGIC chromatograms obtained with different length columns at a temperature program shown in the plot. Column: Nucleosil C18, 3 μm, 100 Å, 4.6 mm I.D.; eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min.

Table 2
TGIC retention time (min) of PS standards at different column length[a]

MW (kg/mol)	Column length (mm)			
	30	50	100	250
Solvent (t_o)	0.40	0.65	1.19	3.08
5.1	0.58	0.93	1.66	3.77
15.3	0.75	1.21	2.03	4.21
30.9	1.21	1.73	2.62	4.84
55.2	1.77	2.26	3.17	5.35
98.9	2.44	2.94	3.76	5.86
205	3.31	3.72	4.42	6.39
632	4.27	4.68	5.18	6.93
1800	5.17	5.59	5.93	7.53

[a] TGIC separation condition is the same as shown in Fig. 5.

retention time range of 3.76 min (7.53 min for 1800 kg/mol and 3.77 min for 5.1 kg/mol sample) since most of the solute migration along the column takes place at high temperature over a small temperature range. At low temperature, the high molecular weight PS strongly interacts with the stationary phase and moves very slowly. For example, it would take 5.17 min for the highest molecular weight PS (1800 kg/mol) to travel 30 mm distance (t_R in the 30 mm long column) in the 250 mm long column. At that time, the temperature of the column already reaches higher than the critical temperature at this separation condition (30.3 °C). The separation mechanism switched to mainly the size exclusion mechanism above the critical temperature and the 1800 k PS quickly migrates the remaining distance of 220 mm in 250 mm long column in 2.36 min ($t_{R,250\,mm} - t_{R,30\,mm}$), which is faster than the speed of the eluent ($t_{o,250\,mm} - t_{o,30\,mm}$ = 2.68 min).

It should be also pointed out that the resolution of the TGIC separation depends on the temperature program and the resolutions observed for the columns of different length in Fig. 5 certainly do not represent the optimized situation for each column. The longer the column is, the better resolution should be expected if an optimized temperature program were applied. However, the long column costs a penalty of the larger void volume. Under the given temperature program, the 50 mm length column appears to allow the best separation of the PS standards in both resolution and analysis speed among the four different length column. The resolution of low molecular weight PS is poorer in the 30 mm long column than the 50 mm long column without gaining much in the analysis speed. If we adjust the temperature program for the 30 mm long column, we may be able to improve the resolution at the low molecular weight samples. But it requires additional separation time and we have not pursued it further.

Another separation parameter we have examined is the flow rate as shown in Fig. 6. As easily expected, the retention time of the PS standards decreases as the flow rate increases. However, the extent of the retention time decrement is not proportional to the flow rate under a given temperature program. We plotted the retention time of each peak relative to the flow rate in Fig. 7 for easy comparison. The flow

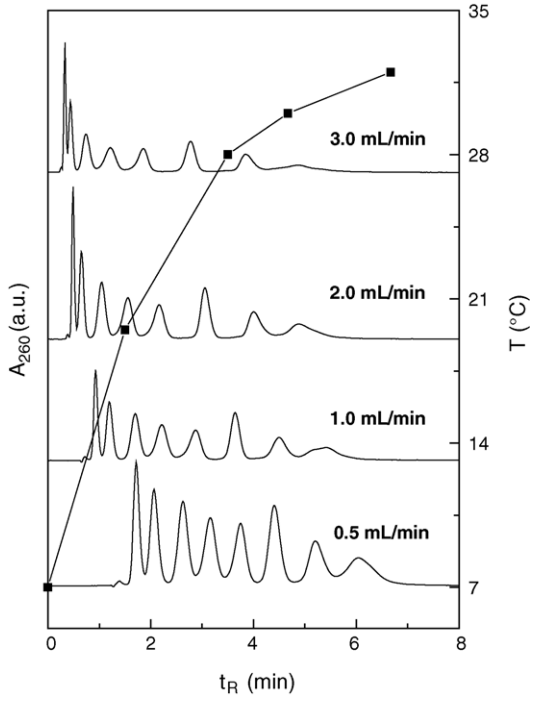

Fig. 6. TGIC chromatograms obtained at different flow rates. Column: Nucleosil C18, 3 μm, 100 Å, 50 mm × 4.6 mm I.D.; eluent: CH_2Cl_2/CH_3CN = 57/43 (v/v); flow rate: 1 mL/min. Temperature program is shown in the plot.

rate effect diminishes as the flow rate increases but the total analysis time changes no more than 1 min over the flow rate variation from 0.5 to 3 mL/min. In particular, the retentions of the high molecular weight PS level off at high flow rates, which clearly indicates the dominating temperature effect on the TGIC retention.

In summary, we have examined the effect of the heating rate, the column length, and the eluent flow rate on TGIC retention of PS standards. We found that the fast heating rate was able to reduce the TGIC separation time of polymer samples greatly without much deterioration of the resolution. Employment of a shorter column and increasing the flow rate also help reduce the analysis time further, but the effect is not as large as the heating rate change. The major effect of shortening the column length comes from the reduction of the column void volume and the column temperature mainly controls the solute retention in the column. The column length affects the resolution, but the effect is significant only for low MW polymers. The high MW polymers having a strong interaction with the stationary phase move very slowly in the column at low temperature and start to move fast along the column after the column temperature reaches a certain level, at which the retention mechanism is switched to the SEC mechanism. The flow rate effect is least significant among the experimental parameters we have examined. However, the three experimental conditions should be considered together to optimize the TGCI separation condition.

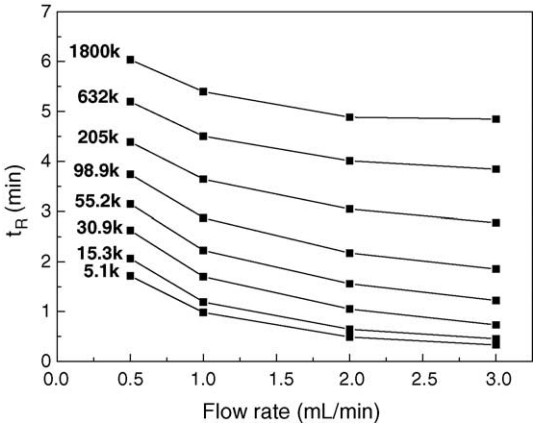

Fig. 7. TGIC retention time vs. eluent flow rate for each PS standards. The retention time represents the peak maximum position in Fig. 6. The retention time decreases with the flow rate increase, but levels off at high flow rate.

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