

Temperature Controllable HPLC Column for Preparative Fractionation of Polymers

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Abstract: An HPLC column with a self-contained temperature control device was constructed for preparative temperature programmed interaction chromatography. Two Peltier plates were attached to a large bore column (120×22 mm i.d.) and the column temperature was controlled by PID mode feed back control. At a flow rate of 1.5 mL/min, the column temperature could be increased and decreased at a rate as high as 50 °C/min and 10 °C/min, respectively, which is much faster than using a column jacket and bath/circulator. The rapid heating and cooling rates allows a high repetition rate of chromatographic fractionation. The performance of the temperature controllable column was demonstrated successfully by the fractionation of homo-polymer precursors from diblock copolymers.

Keywords: polymer fractionation, HPLC column, temperature control, Peltier device, temperature gradient interaction chromatography.

Introduction

Practically all synthetic polymers are not a single molecular species but have distributions in various molecular characteristics such as molecular weight, chain architecture, microstructure, composition, etc. Therefore, fractionation of polymers is an indispensable process in polymer research to characterize the polymer samples as well as to obtain the samples with a narrow distribution in molecular characteristics for detailed studies of various physical properties dependent on the molecular characteristic.

Polymer fractionation has been carried out for long by fractional precipitation or fractional dissolution methods.¹ The development of size exclusion chromatography (SEC) has brought about a great progress in polymer fractionation and nowadays most of analytical fractionations are performed by SEC.^{2,3} SEC can fractionate polymer samples with much higher resolution in shorter time than classical methods. However, a preparative scale SEC fractionation is still not commonly used due to the low sample loading capacity of SEC and overloading causes serious band broadening. Also its sensitivity to polymer chain size (hydrodynamic volume) sometimes limits the utility of SEC.

In recent years, interaction chromatography (IC) has been applied to polymer fractionation.⁴⁻⁶ In contrast to SEC, IC is sensitive to both chemical nature and molecular weight of

polymers. Therefore IC can be used to fractionate polymers according to various molecular characteristics, which is not possible with SEC. For example, IC can separate copolymers according to their chemical composition, branched polymers according to their molecular weight, stereoregular polymers according to their tacticity, block copolymers according to the length of individual block, etc.⁷

Unlike SEC, IC fractionation of synthetic polymers (ensembles of wide distribution in various molecular characteristics such as molecular weight, composition, microstructure and chain architecture) commonly requires control of the solute-stationary phase interaction strength during the elution to elute such complex polymers in a reasonable time span. The most commonly employed method to vary the interaction strength is to change the eluent composition during the elution (solvent gradient elution).⁸ Although the solvent gradient IC fractionation of synthetic polymers works well for the fractionation according to the chemical heterogeneity, the shortcomings of the method are in the difficulty to use many useful detection methods for polymer characterizations such as differential refractometry, light scattering, and viscometry due to the background signal drift. The background signal drift makes the quantitative analysis difficult, if not impossible. The other method used to control the interaction strength is the column temperature variation.^{7,9}

Chromatographic separation is a consecutive equilibrium process of the solutes between the stationary and the mobile phase during the elution. Since the equilibrium constant is a

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function of temperature, the chromatographic retention is affected by temperature and temperature can be used an active experimental variable to control the chromatographic retention.^{10,11} While the solvent gradient elution has been widely employed to control the solute retention in IC separation, however, the temperature variation has not attracted good attention. It is mainly due to the weak effect of temperature change on LC retention relative to the solvent composition change. In recent years, however, column temperature programming in LC has been used increasingly.¹² In particular, temperature gradient interaction chromatography (TGIC) has demonstrated its merits over SEC in the analysis of polymer systems by virtue of its high resolution (lower band broadening)^{13,14} and sensitivity on molecular weight (not chain size) and chemical nature.¹⁵⁻²³ Furthermore, IC shows much larger sample loading capacity than SEC.⁹

The high resolution and large sample loading capacity render IC more suitable than SEC for a large scale, high resolution fractionation of complex polymers. We have already demonstrated the utility of IC for the small prep-scale fractionation of cyclic polymers and block copolymers.^{24,25} In most of the TGIC separations, the column was put in a column jacket and the column temperature was controlled by circulating fluid from a bath/circulator, which allows precise control of the column temperature.⁹ The elution time is controlled by column temperature and the rate of temperature change dictates the analysis time. In particular, the cooling rate after a TGIC run (commonly a heating process) determines how fast the analysis can be repeated. The heating and cooling rate of the commercial bath/circulator are limited due to the large thermal mass and especially the slow cooling rate.²⁶

In this paper, we report on the construction of an HPLC column with self-contained temperature control device to improve the speed of column temperature control. The temperature control was done by Peltier element plates directly attached to the column. Peltier devices, known as thermoelectric modules, are solid-state devices that function as heat pump. By changing the polarity of the applied voltage, it can either heat or cool the system. The performance of the temperature controllable column was tested for the fractionation of homo-polymer precursors from diblock copolymers.

Experimental

Materials. Polystyrene-*block*-polyisoprene (PS-*b*-PI) (30 k-18 k) was synthesized by living anionic polymerization.^{27,28} Polystyrene-*b*-polybutadiene (PS-*b*-PB) (28k-14k) was purchased from Polymer Source Inc. (Dorval, Canada).

SEC Analysis. For 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 multi-angle laser light scattering detector (MALLS, Wyatt, mini-DAWN) and a refractive index detector

(Wyatt, Optilab DSP). Eluent was THF (Samchun, HPLC grade) at a flow rate of 0.8 mL/min. Polymer samples were dissolved in THF at ~1.0 mg/mL and the injection volume was 100 μ L.

IC Analysis and Fractionation. TGIC apparatus is a typical LC instrument composed of a solvent delivery pump (Bischoff, 2250), a six-port sample injector (Rheodyne, 7125) equipped with a 100 μ L injection loop, a UV absorption detector (TSP, UV100) as a concentration detector. For analytical TGIC analyses, a C18 bonded silica column (Kromasil C18, 5 μ m, 100 Å pore, 150×4.6 mm I.D.) was used. The mobile phase was a CH₂Cl₂/CH₃CN mixture (75/25, v/v, Samchun, HPLC grade) at a flow rate of 0.5 mL/min. For the prep-scale separation, a home made temperature controllable column was used as described later in detail. The column was packed by C18 silica particles (RS Tech, 5 μ m, 100 Å pore) using a slurry packer (Alltech) at 6,000 psi. Mobile phase was the same as in the analytical TGIC analysis at a flow rate of 1.5 mL/min.

Results and Discussion

Figure 1 illustrates the temperature controllable column developed in this study. Two Peltier plates (40 × 40 mm) were attached to the opposite sides of a large bore column (120 × 22 mm i.d.), which was custom-made by steel. Unlike the commercial column, the outer cross section was square-shaped to make a good contact between the flat Peltier plates and the column surface. Other exposed walls of the column were thermally insulated to reduce the heat loss. On the opposite side of each Peltier plate is attached a heat sink and a fan to equilibrate the outer surface temperature of the Peltier plate with room temperature as quickly as possible. A small thermocouple is attached to the outer surface of the column to monitor the column temperature. The column temperature is feed-back controlled in the PID mode by changing the DC voltage applied to the Peltier element. By changing the polarity of the DC voltage, Peltier device can either heat or cool the column.

The efficiency of the column temperature control was examined by monitoring the temperature of the column sur-

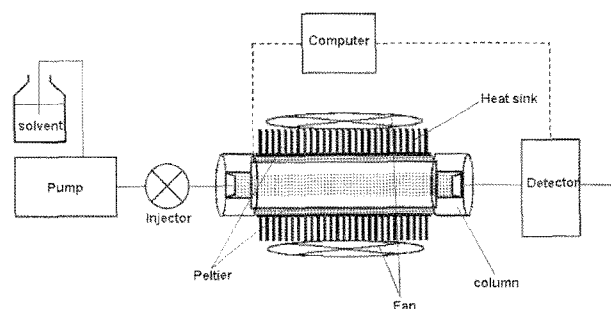


Figure 1. Schematic of high speed temperature controllable column.

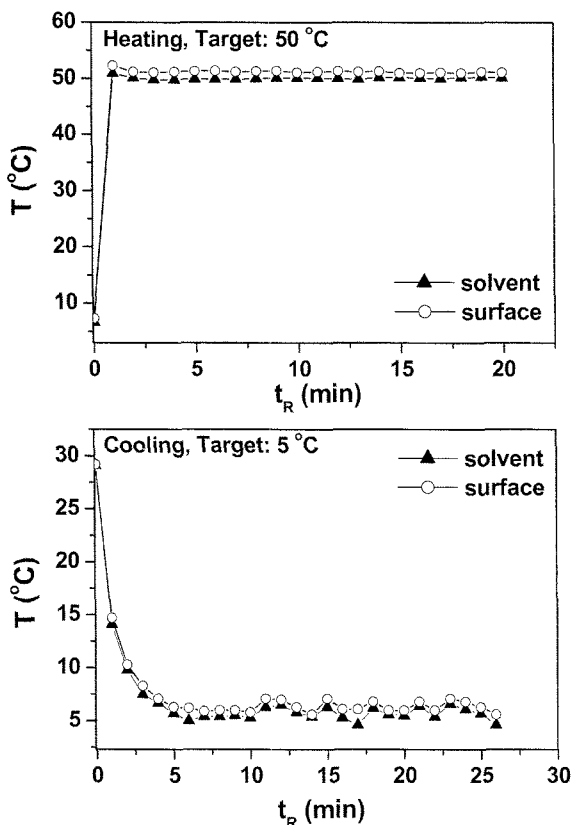


Figure 2. Comparison of the column surface temperature (surface) and the eluent temperature at the column outlet (solvent) for the temperature controllable column in the heating (top) and cooling (bottom) process. Eluent: CH₂Cl₂/CH₃CN mixture (75/25, v/v) at a flow rate of 1.5 mL/min. Column: (C18 silica, 5 μm, 100 Å pore, 120 × 22 mm i.d.).

face and the eluent temperature at the column outlet using small thermocouple probes and the results are displayed in Figure 2. The column surface temperature is slightly higher (about 1 °C) than the eluent temperature at the column outlet for both high (50 °C) and low temperature (5 °C). However, the deviation is consistent and would not cause much trouble in the column performance for the preparative purpose. The temperature stability at low temperature is not as good as at high temperature and small temperature fluctuation is found in the cooling curve at low temperature. Despite the slightly imperfect temperature control, it can be used efficiently for the fractionation of block copolymers as detailed later. Most important merit of the temperature controllable column is in its fast heating and cooling rate, which can expedite the repeated fractionation. The maximum heating and cooling rates are found as 50 and 10 °C/min, respectively and the system can be ready for the next run in a few minute.

Block copolymers are often prepared by anionic polymerization by sequential feeding of different monomers.²⁹⁻³¹

Such prepared diblock copolymers may contain homo-polymer precursor of the first fed monomer to some extent due to the inadvertent termination when the second monomer is added. It is known to be serious when the second monomer is acrylates.^{20,32} If the composition of the first monomer is high, it is not even easy to detect the presence of the homo-polymer precursor by SEC since the SEC retention times of the block copolymer and the homo-polymer precursor are not much different. In IC separation, however, they can be fractionated easily by setting the separation condition in such a way that homo-polymer precursor is in SEC elution condition while block copolymer is in IC separation condition.^{20,32-35} In such a separation condition, homo-polymer precursor elutes out readily before the elution of the injection solvent while block copolymer is adsorbed at the column and does not elute. The adsorbed polymer can be harvested later by changing the elution condition, for example changing the column temperature or solvent composition.^{20,36,37} If the column temperature can be changed

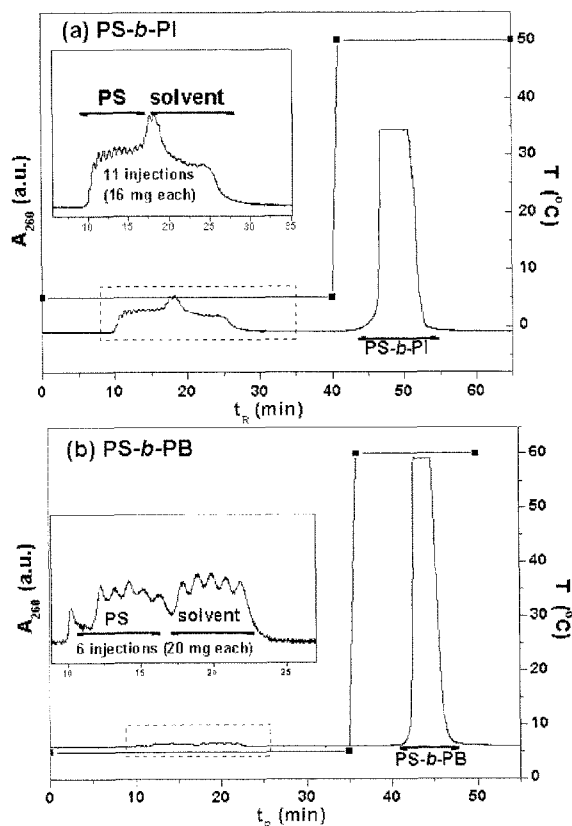


Figure 3. Preparative IC profiles with multiple injections showing the separation of PS precursors from as-synthesized (a) PS-*b*-PI and (b) PS-*b*-PB diblock copolymers. In the inset, the enlarged portion of the dotted box (the elution peaks of homo-PS) is shown. Column temperature is also plotted in the figure. Eluent: CH₂Cl₂/CH₃CN mixture (75/25, v/v) at a flow rate of 1.5 mL/min. Column: (C18 silica, 5 μm, 100 Å pore, 120 × 22 mm i.d.). Injection volume: 100 μL at a concentration of (a) 160 mg/mL, (b) 200 mg/mL.

rapidly, temperature gradient elution is preferred to solvent gradient elution since the solvent change and equilibration of an LC column is a time consuming process. The temperature controllable column developed in this study is suitable for the purification process since the column temperature can be changed rapidly and the requirement of temperature precision is not strict. The temperature controllable column was tested for the purification of PS-*b*-PI and PS-*b*-PB diblock copolymers from their homo-polymer precursor.

Figure 3 displays the chromatograms of preparative IC fractionations of (a) PS-*b*-PI and (b) PS-*b*-PB diblock copolymers. For the separation, the home-made temperature controllable column was used and the block copolymer samples were repeatedly injected. In this reversed phase IC separation, diene polymers (PI and PB) interact with the stationary phase (octadecyl chains coated on the silica particles) more strongly than PS. Therefore, the separation condition was selected in such a way that PS homo-precursors elute in the SEC separation regime (elution before the injection solvent peak) while the block copolymers containing a diene polymer block are fully adsorbed at the stationary phase at the initial separation condition (low temperature). The homo-PS precursors elute before the injection solvent peak for every injection and a complex multiple peak of homo-PS

and injection solvent appears at $10 \text{ min} < \text{retention time } (t_R) < 30 \text{ min}$.²⁰ After the homo-PS elution is completed, the column temperature is increased to elute the trapped block copolymers. The accumulated block copolymer concentration is high and the detector signal is saturated. The injection sample concentration was 160 mg/mL and the injection volume was $100 \mu\text{L}$. Through the multiple injection, $\sim 200 \text{ mg}$ quantity of homo-polymer free block copolymers could be obtained within an hour. By virtue of the rapid cooling rate of the column, such a separation can be repeated without much delay and a gram quantity of the purified block copolymers can be obtained in a few hours.

To verify the purity of thus fractionated block copolymers, as-synthesized block copolymers and fractionated block copolymers were compared by analytical SEC and TGIC. Figures 4 and 5 display the TGIC and SEC chromatograms of the block copolymers before and after the PS homo-polymer precursor removal from the as-synthesized PS-*b*-PI and PS-*b*-PB, respectively. Both diblock copolymers contain about 4 wt% of homo-PS. As shown in the inset of Figure 4(a) the presence of homo-PS only causes a slight tailing in the SEC chromatogram which is not easy to be recognized with SEC analysis alone. SEC separates polymer molecules according to their hydrodynamic volume and SEC cannot resolve byproducts that have similar hydrodynamic volume to the major products. But the difference in the SEC chro-

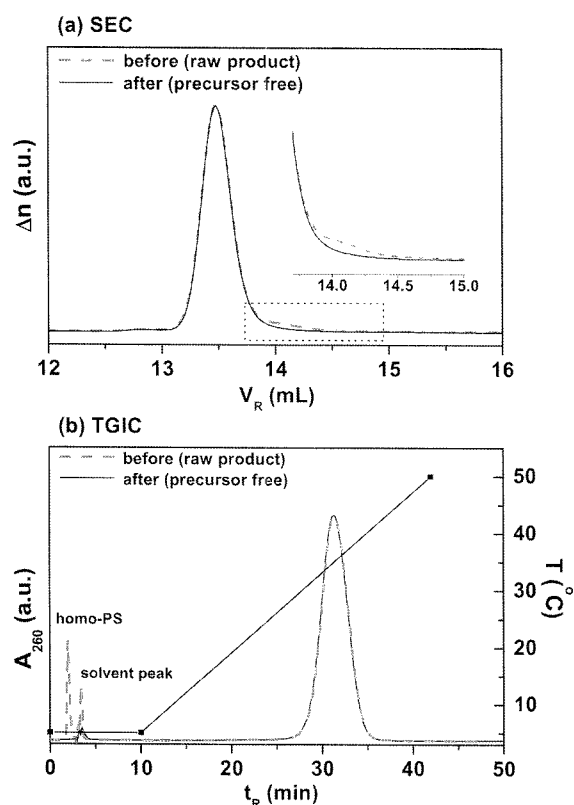


Figure 4. (a) SEC and (b) analytical TGIC profiles of PS-*b*-PI diblock copolymers before and after the homo-PS removal. In the inset, the portion of the dotted box in (a) is enlarged. Column temperature is shown in the plot of the TGIC chromatogram.

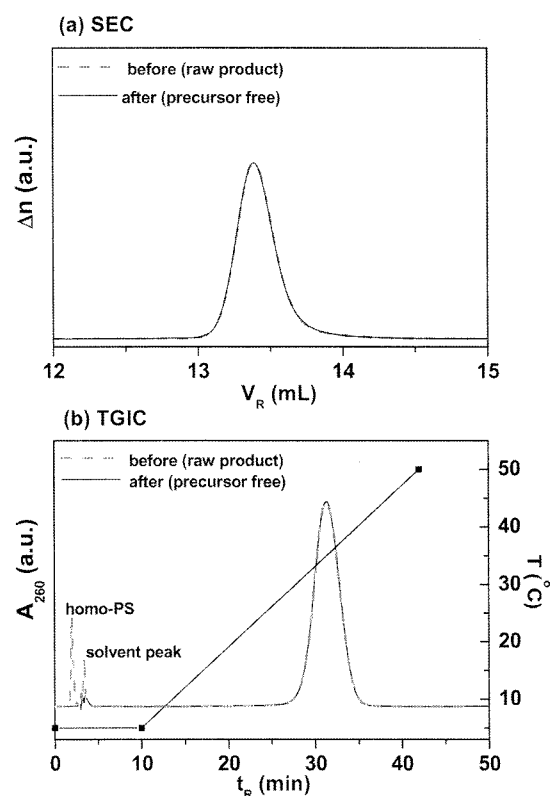


Figure 5. (a) SEC and (b) analytical TGIC profiles of PS-*b*-PB diblock copolymers before and after the homopolymer PS removal.

matogram between the as-synthesized and PS-precursor removed samples clearly shows the removal of homo-PS precursor. It is even more difficult to see the difference by SEC for PS-*b*-PB in which the PS composition is higher (Figure 5(a)). On the other hand, in the TGIC analysis in Figures 4(b) and 5(b), homo-PS elutes ($t_R \sim 2$ min) before the solvent peak ($t_R \sim 4$ min) and well-separated from the block copolymer peak eluting at $t_R \sim 30$ min. After the homo-PS removal, the homo-PS precursor peak disappears completely.

In summary, we constructed a prep-scale HPLC column integrated with a temperature control device. The heating and cooling rate of the column is much higher than the conventional temperature control method for TGIC. We have demonstrated that this temperature controllable column worked well for the large scale fractionation of block copolymers from the prematurely terminated homo-polymer precursors. A single run with multiple injections was able to purify a few hundred mg quantity of block copolymers and it can be repeated rapidly thanks to the high heating/cooling rate of the column.

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