

## High Temperature Size Exclusion Chromatography

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**Abstract:** High temperature size exclusion chromatography (SEC) has been used widely for the characterization of crystalline polymers, for which high temperature operation above the polymer melting temperature is required to dissolve the polymers. However, this high temperature operation has many advantages in SEC separation in addition to merely increasing polymer solubility. At high temperature the eluent viscosity decreases, which in turn decreases the column backpressure and increases the diffusivity of the analytes. Therefore, many reports on the high temperature operation of high performance liquid chromatography (HPLC) have focused on shortening the analysis time and enhancing the resolution. However, the application of high temperature SEC analysis to exploit the merits of high temperature operation is scarce. In this article, therefore, we report on a new apparatus design for high temperature SEC.

**Keywords:** high temperature, size exclusion chromatography, polystyrene.

### Introduction

Size exclusion chromatography (SEC) is the most widely used technique to measure the molecular weight (MW) and the molecular weight distribution (MWD) of polymers. SEC separates the polymer molecules according to the size of polymer chains, a well-defined function of MW for linear homopolymers. SEC has many advantages over other classical fractionation techniques in speed, effort, required amount of sample, etc.<sup>1-5</sup> However, SEC does not exhibit as high resolution as other HPLC techniques mainly due to the slow mass transfer of high MW polymers. Operation at elevated temperature provides with various advantages in this regard. As the temperature increases, viscosity of the mobile phase decreases, and the diffusivity of analytes increases.<sup>6-11</sup>

In recent years, elevated temperature HPLC has been used increasingly exploiting the benefits of high separation efficiency and high throughput analysis.<sup>6,12-14</sup> While high temperature SEC has been used widely also, its use has been restricted to the characterization of crystalline polymers, for which high temperature operation above the polymer melting temperature is required to dissolve the polymers.<sup>15-17</sup> Despite the high temperature operation has many advan-

tages other than increasing polymer solubility as can be seen in the recent development in high temperature HPLC,<sup>18,19</sup> SEC analysis of polymers exploiting the merits of high temperature operation is scarce. Renn and Synovec reported on the reduced analysis time and/or increased separation efficiency for high temperature SEC using a silica based micro-bore column (250 × 1.0 mm ID).<sup>20</sup> However, it was not extended to practical applications. It is likely due to the additional requirements on the apparatus to prevent the eluent from boiling and a detector cell durable at high pressure. In this study, we report a simple way to implement the high temperature operation capability in the SEC analysis.

### Experimental

The SEC apparatus is a typical set-up composed of a solvent delivery pump (Bischoff, 2250), a six-port sample injector (Rheodyne, 7125) equipped with a 50  $\mu$ L injection loop, a refractive index (RI) detector (Shodex, RI-71) as a concentration detector. SEC separations of polystyrene (PS) standards were carried out with two Shodex UT-806M columns (Shodex, 300 × 8.0 mm ID, 30  $\mu$ m particle size) and THF eluent (Samchun, HPLC grade) at a flow rate of 1.0 mL/min. The temperature of the separation column was controlled by column oven (Futechs, AT-4000). A restrictor

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was attached before the detector to control the backpressure.

## Results and Discussion

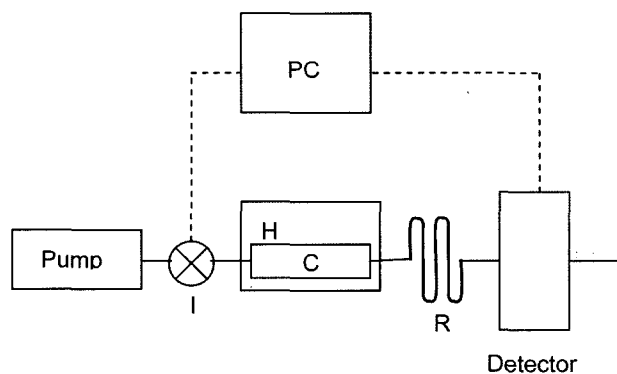
For high temperature HPLC operation above the normal boiling point of the mobile phase, the backpressure has to be regulated to keep the mobile phase from boiling.<sup>18</sup> The backpressure regulator is usually attached after the detector. In this case, the detector cell should endure the high pressure and the cell temperature has to be regulated well. In this study, the backpressure regulator is inserted between the column and the detector so that the detection can be done at atmospheric pressure. The schematic diagram of the apparatus is shown in Figure 1. It allows the use of normal HPLC detectors without modification. A narrow-bore stainless steel tubing was used as the backpressure regulator and the backpressure can be controlled by changing the tubing length. In addition, the effluent temperature can be controlled while it passes through the restrictor tubing.

The backpressure under a restrictor tubing of  $380 \times 0.18$  mm ID was measured at different flow rates. The boiling point of THF was estimated from the Clausius-Clapeyron equation at six different mobile phase flow rates.<sup>21</sup>

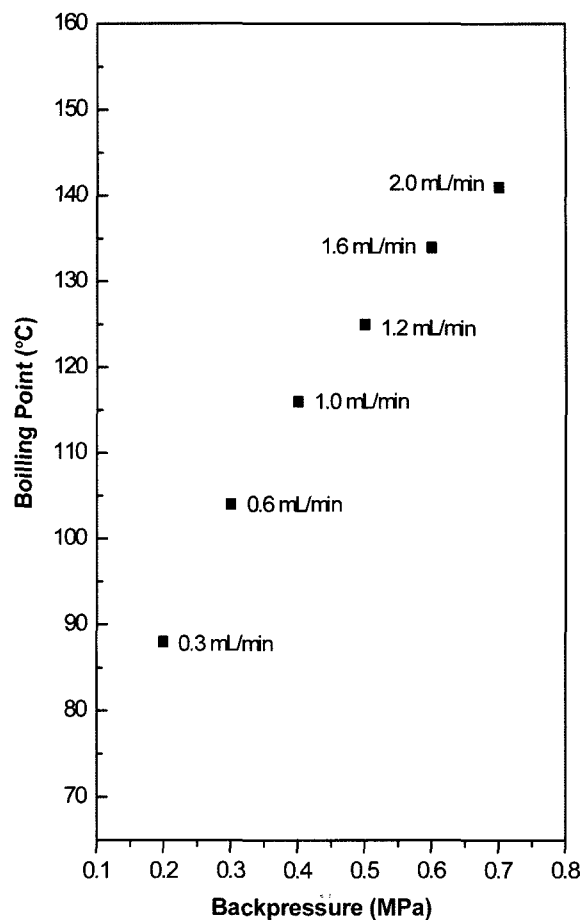
$$\ln \frac{p}{p_o} = \frac{\Delta H_{vap}^o (T - T_o)}{RT_o T} \quad (1)$$

where  $p_o$  is the atmospheric pressure,  $\Delta H_{vap}^o$  is the standard enthalpy of vaporization,  $R$  is the gas constant, and  $T_o$  is the normal boiling point. As displayed in Figure 2, the backpressure increases with the flow rate, which in turn increases the boiling point of THF. For example, at a flow rate of 1.0 mL/min, the backpressure from the restrictor is 0.4 MPa, at which the boiling point of THF is 116 °C.

To examine the column temperature effect on the SEC resolution, SEC experiments were carried out with PS standard samples. Figure 3 displays the SEC chromatograms of two sets of PS standard mixtures recorded with a refractive



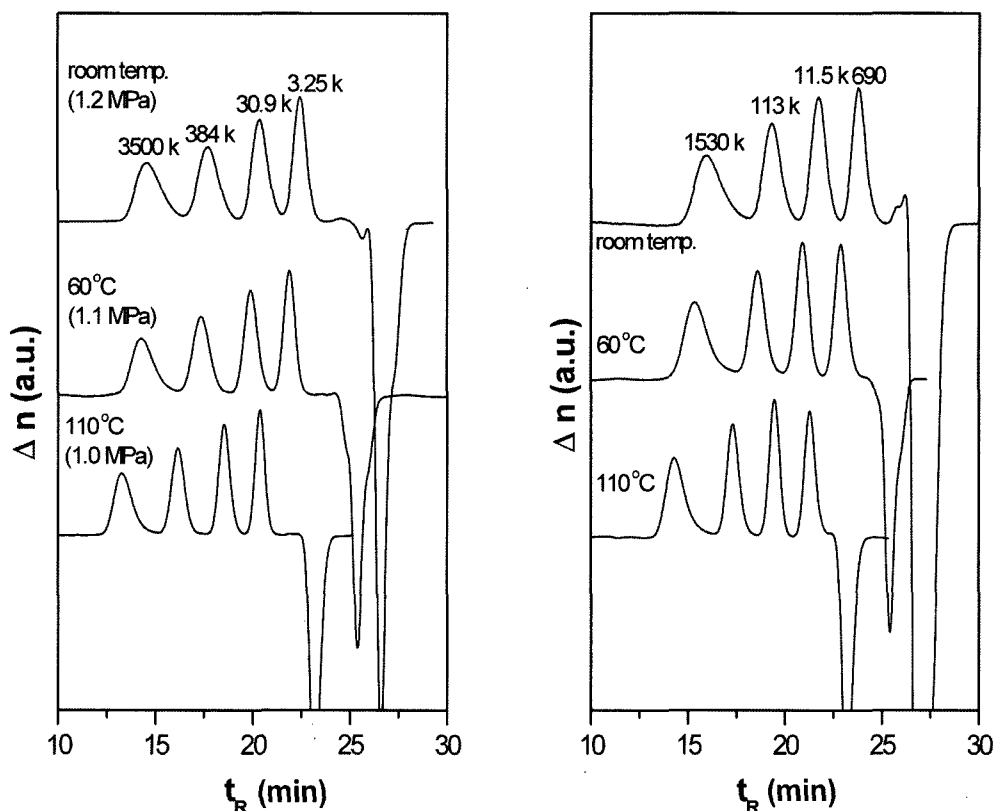
**Figure 1.** Schematic diagram of high temperature HPLC system. I: injector, H: column heater, C: column, R: restrictor.



**Figure 2.** The estimated boiling point of THF under the backpressure of a narrow-bore tubing ( $380 \times 0.18$  mm ID). The flow rates are labeled for each data point.

index detector at three different column temperatures. The flow rate was kept constant at 1.0 mL/min. The backpressure was measured at the solvent delivery pump and it is the backpressure exerted by the columns and the restrictor tubing. The pressure at the outlet of the column should be the same as the one measured with restrictor tubing only. According to the data shown in Figure 2, the pressure is sufficiently high to keep THF from boiling at 110 °C. In this SEC experiment, a refractive index detector was employed. A refractive index detector is more universal, but more sensitive to the temperature fluctuation than a UV/vis absorption detector. If the effluent is not sufficiently cooled down when it reaches the detector, it may cause a signal drift in the refractive index detection. As shown in Figure 3, the baseline is very stable indicating the temperature of the effluents and the detector cell are well equilibrated. This is another merit of this restrictor design.

Comparing the SEC chromatograms obtained at RT, 60 and 110 °C, first we can notice the shift of the peak position. The injection solvent peak (the negative peak eluting last)



**Figure 3.** SEC chromatograms of two sets of PS standard mixtures recorded with a RI detector at three different column temperatures. PS standards: 3500, 1530, 384, 113, 30.9, 11.5, 3.25, 0.69 kg/mol. Column: two Shodex UT-806M columns (300×8.0 mm ID, 30 μm particle size), eluent: THF at a flow rate of 1.0 mL/min.

shifts significantly to a shorter elution time. This is likely due to the thermal expansion of the eluent as well as the packing materials resulting in the reduction of the total void volume in the column. The total void volume of the column decreases from 26.9 mL at room temperature to 23.2 mL at 110°C. Figure 4 displays the calibration curves constructed from the elution peak positions of the eight PS standards at three different temperatures. The shift of the elution peak position occurs for all PS samples of different MW indicating that the pore volume is also reduced due to the thermal expansion of the eluent and the packing materials. The magnitude of the peak shift gets relatively smaller for the higher MW samples and the calibration curve becomes steeper as the column temperature increases.

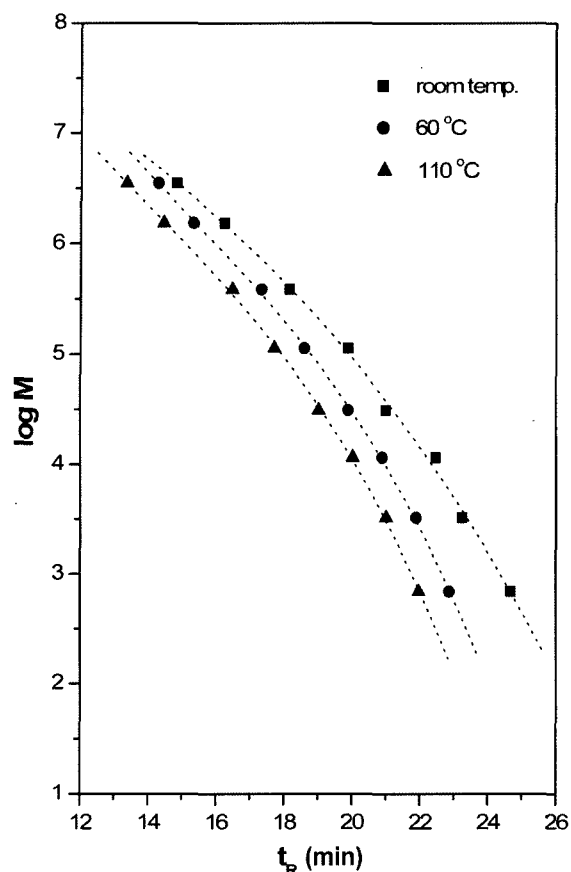
The steeper calibration curve corresponds to the lower resolution in general since a large slope ( $\Delta M/\Delta t_R$ ) means small elution time difference for a given MW difference. However, the width of the elution peak in Figure 3 decreases significantly as the column temperature increases. To estimate the resolution change quantitatively, the resolution factor was calculated from the following equation.<sup>22</sup>

$$R_S = \frac{2 \times \Delta Z}{W_A + W_B} \quad (2)$$

where  $\Delta Z$  is the distance between the two adjacent elution peaks,  $W_A$  and  $W_B$  are the peak widths of the adjacent peaks at the baseline. The higher the  $R_S$  value is, the higher the resolution is. This type of analysis is rarely used for the SEC analysis of polymers which is not a single chemical species but have MW distribution. However, it is a simple and valid method to compare the relative resolution of the same set of PS standards at different separation conditions. The result summarized in Table I shows that the resolution factor increases as the temperature increases for entire MW range. Despite the distance of the adjacent peaks ( $\Delta Z$ ) decreases as the temperature increases (a steeper calibration curve), the peak widths are reduced more to improve the resolution. The reduction of the peak width is the results of the facilitated mass transfer of the polymer chains.

In summary, high temperature operation improves the SEC separation efficiency a great deal and a common SEC apparatus can be modified for the high temperature run simply by addition of a column oven and a restrictor tubing.

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**Figure 4.** Calibration curves constructed from the elution peaks of eight PS standards at three different column temperatures.

**Table I. The SEC Resolution Factor of PS Samples at Three Different Temperatures**

Codes between Two Samples*	Resolution Factor ( $R_s$ )		
	Room Temp.	60 °C	110 °C
(1)	1.28	1.44	1.66
(2)	1.35	1.50	1.76
(3)	1.31	1.38	1.62
(4)	1.27	1.50	1.73
(5)	1.24	1.46	1.64
(6)	1.19	1.36	1.49

\*3500-(1)-384-(2)-30.9-(3)-3.25, 1530-(4)-113-(5)-11.5-(6)-0.69 kg/mol.

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