High Temperature Size Exclusion Chromatography for High Throughput Analysis

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Introduction

The SEC separation time is essentially determined by the flow rate of the mobile phase with a given column configuration since polymers elute earlier than the total void volume of the column. To reduce the SEC separation time, the ratio of the flow rate to the column void volume needs to be increased, but there is a limitation due to the stability of the packing materials under the increased column pressure and the deterioration in the solute transfer equilibrium Shortening the column length and increasing the column diameter have been the major directions to reconcile the high flow rate with the adverse effects.[1] However, shortening the column length inevitably reduces the number of theoretical plates and a larger column diameter requires the large amount of eluent as well as very high flow rate which sometimes is a burden for an analytical HPLC pump. Recently, Popovici et al. reported on theoretical and practical considerations for the fast SEC analysis of PS samples with five commercial SEC columns at room temperature.[2] They examined the various approaches such as particle size of the packing materials, column length, and the eluent flow rate to change the SEC analysis time. They concluded that a fast separation in SEC is more favorable than suggested by conventional theory, but the trade-off between the analysis time and the resolution has to be made between the column lengths and the flow rate.

The main obstacle achieving both high speed and high resolution in a SEC analysis is the slow mass transfer of the analytes. To increase the mass transfer rate, temperature should be the first parameter to consider. As the temperature increases, the viscosity of the mobile phase decreases and the diffusivity of analytes increases. Furthermore, the low eluent viscosity reduces the column backpressure allowing the use of a high flow rate and a longer column (higher number of plates) without imposing much burden to the solvent delivery pump. In recent years, elevated temperature HPLC has been used increasingly exploiting the benefits of high separation efficiency and high-speed analysis.[3]

While high temperature SEC has been used widely also, its use is mainly limited to the characterization of crystalline polymers, for which high temperature operation above the polymer melting temperature was required to dissolve the polymers. Despite the high temperature operation has many advantages other than increasing polymer solubility as can be seen in the recent development in high temperature HPLC, the merits of the high temperature operation have been hardly exploited in the SEC analysis.

In this study, we made a simple modification to a common SEC apparatus to allow a high temperature operation for the resolution enhancement in the high throughput analysis..

Experimental

For high temperature HPLC operation above the mobile phase boiling point, the back pressure has to be regulated to keep the mobile phase from boiling. The backpressure regulator is usually attached after the detector. In this case, the detector cell should be amenable to high pressure and temperature. 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 common HPLC detectors without any modification. As the backpressure regulator, a narrow-bore stainless steel tubing was used. The backpressure is a function of flow rate and it can be controlled by changing the tubing length and the diameter. For example, a 370 x 0.18 mm tubing provides with a backpressure to increase the boiling point of THF to 135 °C at the mobile phase flow rate of 2.0 mL/min. On the basis of the restrictor backpressure, all SEC experiments were carried out within temperature ranges from ambient temperature to 110 °C. In addition, the restrictor tubing is in contact with a heat exchanger and a small fan is attached to expedite the cooling of the effluent while it passes through the tubing.

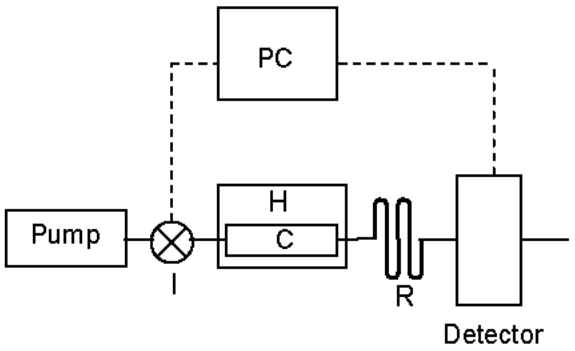

Figure 1. Schematic diagram of high temperature SEC system. I: injector, H: column heater, C: column, R: restrictor, PC: Computer.

Results and discussion

Figure 2 displays the SEC chromatograms of a PS mixture at the room temperature and 110°C. The flow rate was varied and the elution time decreases in proportion to the flow rate. The backpressure of the column, labeled for each chromatogram, increased with the flow rate also. Since the deterioration of the SEC chromatogram is clear at the flow rate higher than 1 mL/min, the flow rate was not increased further. The backpressure at 110°C is much lower than room temperature and the flow rate could be increased further. Opposite to the room temperature experiments, we could not decrease the flow rate at will since we have to maintain a minimum backpressure for THF not to boil. Therefore, the experiments at 110°C were carried out at the flow rate range of 0.9-2.0 mL/min. At the flow rate of 2 mL/min, the SEC separation was completed in 1.5 min without noticeable change in resolution

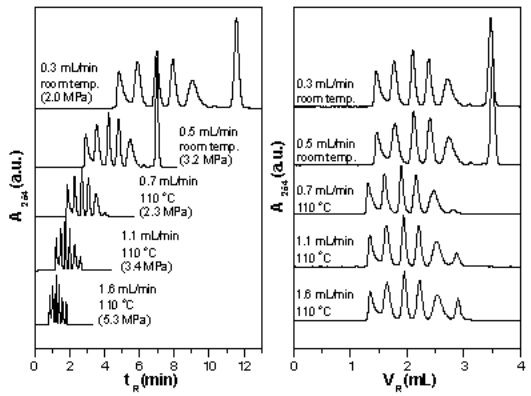

Figure 2. SEC separation of 5 PS standard samples with polypore column (left: t_R plot, right: V_R plot). Column: PL polypore (250 × 4.6 mm, 5 μm particle size), eluent: THF, PS standards: 1530 k, 200 k, 30.9 k, 5.05 k, 690 g/mol

References

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3. C. Zu, D.M. Goodall, S.A.C. Wren *LC-GC Europe* 2004, *17*, 530