Communications to the Editor

Characterization of Binary Polymer Mixtures by Simultaneous Size Exclusion Chromatography and Interaction Chromatography

Hee Cheong Lee and Taihyun Chang*

Department of Chemistry, Pohang University of Science and Technology, Pohang, 790-784, Korea

Received April 9, 1996
Revised Manuscript Received July 12, 1996

In the analysis of macromolecules, both synthetic and biological, high performance liquid chromatography (HPLC) has many advantages over the classical techniques such as selective precipitation from dilute solution,[1] selective extraction,[2] and selective turbidmetric titration[3] in terms of time, effort, required amount of samples, and so on. The chromatographic methods applied for the analysis of macromolecules can be largely divided in terms of separation mechanism into size exclusion chromatography (SEC) and interaction chromatography (IC). SEC is a simple and universal method in the analysis of macromolecules which employs the separation mechanism based on the partition equilibrium of solute molecules between the same solvent located in the mobile phase and in the pores of the packing material, the stationary phase.[4] Therefore it utilizes mainly the entropic effect in the partition equilibrium to separate the macromolecules in terms of their molecular size. As a result, the higher molecular weight macromolecules are eluted first and the sample solvent molecules are usually eluted at the end of the chromatogram.

On the other hand, IC separates the solute molecules by mainly enthalpic interaction with the stationary phase such as adsorption or partition. Therefore IC has been widely used to separate copolymers in terms of their chemical composition.[5,6] However, IC can separate the macromolecules by their molecular weight since the interaction also depends on the molecular weight of the polymer chain.[7] Since retention volume typically increases exponentially with molecular weight, a solvent gradient elution is commonly adopted for the optimum resolution.

In recent years, the practical use of polymer mixture has increased rapidly, and rapid characterization of their constituents becomes more and more important. Although SEC is the most universal method for polymer characterization, the application of conventional SEC to polymer mixtures is only possible if the hydrodynamic volumes of each component in a mixture are fairly different. There have been several approaches to get around of this problem. For examples, Lee et al. isolated the contribution from individual components of a binary polymer mixture by use of multiple detectors.[8] Balke and Patel fractionated each component of a polymer mixture by "orthogonal chromatography" by use of IC and SEC in sequence.[9] Pasch[10] and Hunkeler et al.[11] reported the application of "SEC under critical conditions" for the analysis of binary polymer mixtures. More recently, Berek and co-workers successfully employed the "eluent switching" method to separate polymer mixtures.[12,13] In this paper we report an elegant method, temperature gradient HPLC, which employs an isocratic mobile phase and one type of column for the simultaneous characterization of binary polymer mixtures in single elution.

Table 1. PMMA and PS Standards Employed in This Study

code	polymer	M_w ($\times 10^3$)	M_w/M_n[a]	manufacturer[b]
1	PMMA	1500	1.11	APSC
2	PMMA	501	1.09	APSC
3	PMMA	77.5	1.06	APSC
4	PMMA	8.5	1.14	PC
5	PMMA	2.0	1.10	PC
a	PS	1.7	1.06	PL
b	PS	5.1	1.05	PL
c	PS	11.6	1.03	PL
d	PS	22.0	1.03	PL
e	PS	37.3	1.04	DIC
f	PS	68.0	1.03	PL
g	PS	114	1.05	DIC
h	PS	208	1.05	PC
i	PS	502	1.04	PL
j	PS	1090	1.08	TC
k	PS	2890	1.09	TC

[a] Provided by the manufacturers. [b] APSC, American Polymer Standards Corp.; PC, Pressure Chemical; PL, Polymer Labs.; DIC, Daelim Industrial Co.; TC, Tosoh Co.

A typical isocratic HPLC apparatus was used for the experiments. Three C18 bonded silica columns having different pore sizes (Alltech, Nucleosil, 100, 500, 1000Å pore size each, 250 × 4.5 mm) were connected in series to enhance the resolution of the size exclusion region. The particle size of the column packing materials was 5−7 μm. Eleven polystyrene (PS) standards and five poly(methyl methacrylate) (PMMA) standards were employed in this study and are listed in Table 1. The columns were put in a jacket connected to a bath/circulator so that the column temperatures was controlled in a pre-programmed manner. The mobile phase was a mixture of CH_2Cl_2/CH_3CN at the composition of 57/43 (v/v) and the flow rate was 0.5 mL/min. A mixture of 11 PS and five PMMA standards was made at the concentration of about 1 mg/mL for each polymer with the eluting solvent and the injection volume was 50 μL. The wavelength of the UV/vis detector was set at 235 nm.

In Figure 1 are displayed the isothermal IC chromatograms of the PS and PMMA mixture at six different temperatures. The mobile phase is a reasonably good solvent system for PMMA, and the interaction between the PMMA chain and the hydrophobic C18 bonded silica surface should not be strong, so the PMMA standards are found to be separated mainly by the size exclusion mechanism and eluted before the solvent peak.[4] The solvent peak, labeled as "S" in the figure, appears at near the retention volume (V_R) of 9 mL at 0 °C, the peak position of which changes slightly with

* Author to whom correspondence should be addressed.

S0024-9297(96)00536-0 CCC: $12.00

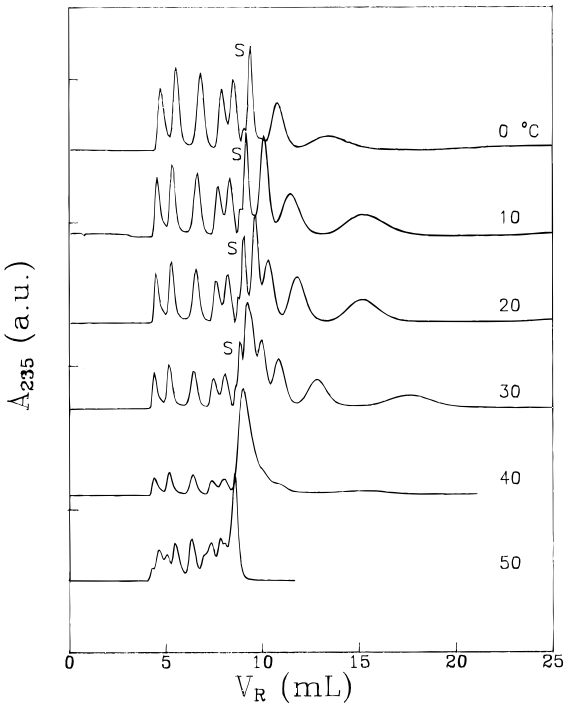

Figure 1. Isothermal HPLC chromatograms of the 11 PS and five PMMA standards mixture at six different temperatures. Three serially connected C18 bonded silica columns are used, and the mobile phase is a mixture of CH_2Cl_2/CH_3CN(57/43 (v/v)). The first five peaks that eluted before the solvent peak (labeled as "S") are PMMA standards for which peak positions change little with temperature. On the other hand, PS standards are eluted after the solvent peak and their retention volume strongly depends on the column temperature. Refer to the text for details.

temperature. The slight change is believed to be due to the thermal expansion of the system. Since PMMA standards are separated by a size exclusion mechanism, higher molecular weight polymers are eluted at lower V_R. Also, we can notice that the elution peak positions of PMMA standards do not change much with the temperature either. This is because the size exclusion mechanism is an entropic effect which does not depend strongly on temperature. Although the peak intensity appears to become smaller as the temperature is increased, it is an artifact from the intensity normalization process. The chromatograms are normalized for the most intense peak in each chromatogram, and the peak appearing near the solvent peak becomes quite intense at the high temperature, as explained later.

On the other hand, the retention volume of PS standards, being eluted after the solvent peak (S), strongly depends on the column temperature. At 0 °C only four standard PS's are eluted in the retention volume range shown in the plot. Also, the elution sequence is reversed, so lower molecular weight polymers are eluted first. This is because PS's are separated mainly by interaction mechanism. As the temperature is raised, the retention volumes are progressively reduced so that all 11 PS standards shown are eluted in the chromatogram at 40 °C while most of the peaks are merged near the solvent peak position. This is a rather well-known phenomenon known as the "critical condition", where the size exclusion effect and interaction effect cancel out each other.[10,11,14] At the critical condition a polymer is eluted at the same V_R independent of molecular weight, which makes the peak near the retention volume of 9 mL become very intense. At 50 °C, PS standards are also eluted at the size exclusion

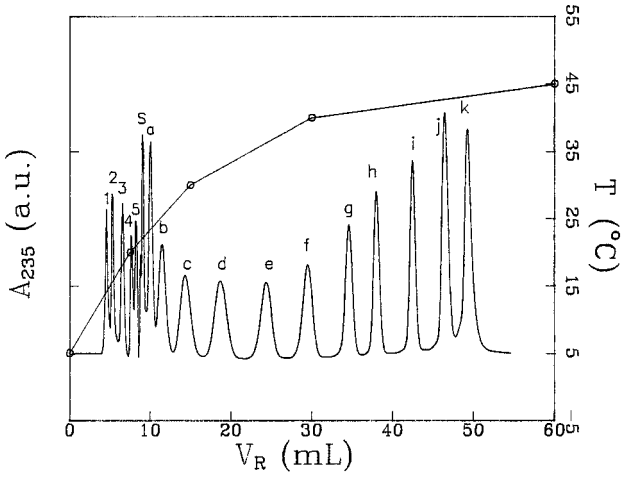

Figure 2. Temperature gradient HPLC analysis of the set of 11 PS and five PMMA standards listed in Table 1. All the elution peaks are labeled with the same code as in Table 1. The temperature of the circulating fluid is programmed to change by five segmented linear ramps from 5 to 45 °C as shown in the figure.

region, so both polymers are eluted before the solvent and the elution peaks of both polymers are overlapped each other.

Therefore it is clear that two polymer species are separated by different chromatographic separation mechanism simultaneously. Taking advantage of the temperature effect in IC, temperature gradient HPLC analysis of the same set of PS and PMMA standards was carried out, and the chromatogram is shown in Figure 2. In the figure all the elution peaks are labeled with the same code as in Table 1. The temperature of the column, more accurately, the circulating fluid, is programmed to change by four segmented linear ramps from 5 to 45 °C in order to optimize the separation in the IC region. The near perfect separation of 16 polymer standards is achieved by single isocratic elution. The resolution of IC is so great that we can achieve a baseline separation for PS standards for which the average molecular weights differ only by factor of 2 or less. As reported previously, a single C18 bonded silica column is good enough to provide a comparable resolution for PS in the IC region.[15] We employed three columns in this study in order to enhance the resolution in the SEC region.

In summary, we demonstrated that PS/PMMA mixture can be separated with a good resolution in terms of their chemical nature and molecular weight simultaneously. Our approach shares the same concept as in the critical condition LC[10,11,14] in the sense of using both size exclusion and interaction mechanism while we use both separation mechanisms simultaneously to characterize both polymer components in a single elution. We do not think that the solubility effect plays a role for the IC separation of PS's, since all the PS standards are completely soluble at the given separation condition. The cloud point of the highest molecular weight PS is found to be about 0 °C in the eluent mixture. Therefore, we are convinced that the main separation mechanism for PS is the enthalpic interaction. When we employed bare silica columns and a selectively good solvent system for PS, we found that the separation mechanism could be reversed for PS/PMMA mixture, although it needs further refinement to achieve a comparable resolution to that found in this study. A detailed study on the separation mechanism will be reported later.

Acknowledgment. This work is supported in part by grants from KOSEF (95-0502-07-01-3). We thank Mr. Wonmok Lee for his help. We also appreciate the helpful comments of Prof. Dusan Berek of the Slovak Academy of Sciences.

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MA960536Y