Journal of Chromatography A, 1216 (2009) 4606–4610

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Two-dimensional liquid chromatography analysis of synthetic polymers using fast size exclusion chromatography at high column temperature

Kyuhyun Im, Hae-woong Park, Sekyung Lee, Taihyun Chang*

Department of Chemistry and BK 21 School of Molecular Science, Pohang University of Science and Technology, San 31, Hyoja-Dong, Nam-Gu, Pohang 790-784, South Korea

ARTICLE INFO

Article history:
Received 20 January 2009
Received in revised form 14 March 2009
Accepted 25 March 2009
Available online 1 April 2009

Keywords:
2D-LC analysis of synthetic polymer
IC × SEC
Temperature gradient interaction chromatography
High temperature size exclusion chromatography

ABSTRACT

In recent years, two-dimensional liquid chromatography (2D-LC) has been used increasingly for the analysis of synthetic polymers. A 2D-LC analysis provides richer information than a single chromatography analysis at the cost of longer analysis time. The time required for a comprehensive 2D-LC analysis is essentially proportional to the analysis time of the second dimension separation. Many of 2D-LC analyses of synthetic polymers have employed size exclusion chromatography (SEC) for the second-dimension analysis due to the relatively short analysis time in addition to the wide use in the polymer analysis. Nonetheless, short SEC columns are often used for 2D-LC analyses to reduce the separation time, which inevitably deteriorates the resolution. In this study, we demonstrated that high temperature SEC can be employed as an efficient second-LC in the 2D-LC separation of synthetic polymers. By virtue of high temperature operation (low solvent viscosity and high diffusivity of the polymer molecules), a normal length SEC column can be used at high flow rate with little loss in resolution.

1. Introduction

Practically all synthetic polymers are not single molecular species but have multiple distributions in various molecular characteristics such as molecular weight, chain architecture, functionality, composition, etc. Precise analysis of synthetic polymers with multivariate distributions is a difficult task and a single separation method is often not able to provide complete information. A logical strategy to characterize such complex samples is to find liquid chromatography methods which separate exclusively or at least predominantly according to a single molecular characteristic each and to combine them to carry out a multidimensional mapping of the multivariate distribution.

A practical form of multidimensional analysis is two-dimensional liquid chromatography (2D-LC). The 2D-LC analysis is performed through coupling of two LC separations by on-line or off-line. Balke and Patel might be the first to practice a 2D-LC separation of synthetic polymers by combining two size exclusion chromatography (SEC) using different eluents for the separation of copolymers according to molecular weight (MW) and chemical composition [1]. The use of on-line 2D-LC for the characterization of complex synthetic polymer has been increasing in recent years and a variety of combinations of two different LC methods has been utilized; SEC, reversed phase LC (RPLC), normal phase LC (NPLC), liquid chromatography at critical condition (LCCC), etc. [2–28]. In principle, any combination of these LC methods would work as far as the orthogonality between the two LC separations is sufficient. In practice, however, a few additional aspects need to be considered further.

First of all, the mobile phases of the two LC methods have to be compatible. The mobile phase incompatibility often causes break-through (solvent-plug effect) problems and results in poor resolution and/or incomplete sample recovery in the second-dimension (2nd-D) LC separation [4,9,13,21–24,29]. Secondly, the 2nd-D LC separation needs to be executed at a high repeating rate for comprehensive on-line 2D-LC analyses. A comprehensive 2D-LC chromatogram is nothing but a combination of a number of the 2nd-LC chromatograms. Therefore, the 2D-LC analysis time is practically the product of the 2nd-D LC analysis time and the sampling number of the 1st-D LC effluent. Since the number of sampling should be maintained at a sufficient level to avoid serious undersampling, the analysis time of a comprehensive on-line 2D-LC is mainly determined by the speed of the 2nd-D LC separation.

Comprehensive on-line 2D-LC separations of synthetic and natural polymers have been carried out mostly by a combination of interaction chromatography (IC) × SEC, taking advantage of the high resolution of IC in the 1st-D separation and the speed as well as the universality of SEC separation in the 2nd-D. IC separates the solute molecules according to the interaction strength with the stationary phase and shows better sensitivity on chemical nature of the polymers than SEC. In the IC separation, the interaction strength of

* Corresponding author. Tel.: +82 54 279 2109; fax: +82 54 279 3399.
E-mail address: tc@postech.ac.kr (T. Chang).

0021-9673/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2009.03.072

solute with stationary phase is usually controlled during the elution either by changing the eluent composition (solvent gradient elution) [30–32] or by changing the column temperature (temperature gradient elution) [33,34]. Despite the many merits in IC, it is difficult to employ IC for the 2nd-D LC since the gradient elution is difficult to be repeated rapidly. In addition, it is not trivial to establish the eluent compatibility. Usually the solvent strength of an IC eluent is weak to induce adequate interaction strength of solutes with the stationary phase. If the injection solvent (the eluent of the 1st-D LC for a direct on-line coupling) is stronger than the eluent in 2nd-D LC, breakthrough of the sample often occurs [29]. Therefore, the use of IC for the 2nd-D LC is rare unless the isocratic/isothermal elution is possible [21,24] and even in this case, a solvent exchange step is often employed to avoid the sample breakthrough [21,24].

SEC separates polymers according to the hydrodynamic size of the molecules and is most widely used for polymer characterization [35]. In addition, SEC eluents are usually thermodynamically good and chromatographically strong solvents. Therefore, the solvent incompatibility problem is minimized if SEC is used for 2nd-LC, which is the main reason why the IC × SEC combination is most popular in the 2D-LC analysis of polymers [7,11,12,16,18–20,27,28]. Furthermore, SEC can be run fast. The SEC separation time is essentially determined by the flow rate of the mobile phase with a given column configuration since all polymers elute before the total permeation limit (less than the total void volume of the column). Therefore, reduction of the column size and/or increase of flow rate are straightforward ways toward the fast SEC but a trade-off between the analysis time and the resolution is unavoidable [36]. There have been a few efforts to minimize the resolution loss in the fast SEC. Kilz et al. reported on a new design of SEC columns which can reduce the SEC analysis time to about 2 min [37,38]. Recently Chang and coworkers reported on the fast SEC apparatus operated at high column temperature [39,40].

In this study, we would like to demonstrate the utility of the 2D-LC analysis in the analysis of various synthetic polymers. The 2D-LC analysis employs high temperature-SEC (HT-SEC) as the 2nd-D LC, which allows fast separations without much loss in the resolution. This type of 2D-LC separation could be utilized efficiently for the analysis of complex polymers.

2. Experimental

THF, CH_2Cl_2, CH_3CN and 1,4-dioxane are from Samchun Chemical Co. and iso-octane is from J.T. Baker. They were HPLC grade and used as received. The 1st-D IC system consists of a Bischoff HPLC compact pump, a Rheodyne six-port injection valve equipped with a 50 μL loop, and a UV absorption detector (Knauer, K-2501). Temperature of the IC column was controlled by circulating fluid from a programmable bath/circulator (ThermoHaake, C25P) through a column jacket. The 2nd-D SEC apparatus consists of a solvent delivery pump (Bischoff, 2250), a UV absorption detector (TSP, UV2000) operating at a wavelength of 260 nm or 230 nm. Temperature of the SEC column (Polymer Lab., PolyPore; 250 mm × 4.6 mm i.d.) was controlled by a column oven (Futecs, AT-4000). The mobile phase was THF at a flow rate of 1.7 mL/min, which allows an SEC run to be completed in 1.5 min at 110 °C. A narrow-bore tubing was attached between the column and the detector to control the back pressure of the column and to cool the effluent temperature before it reaches the detector. Details of the HT-SEC apparatus were reported previously [39,40]. The two LC systems were interfaced by an electronically controlled 2-position, 10 port switching valve (Alltech, SelecPro) that enables continuous, alternate sampling of the 1st-D IC effluent and injection to the 2nd-D SEC through two sample loops.

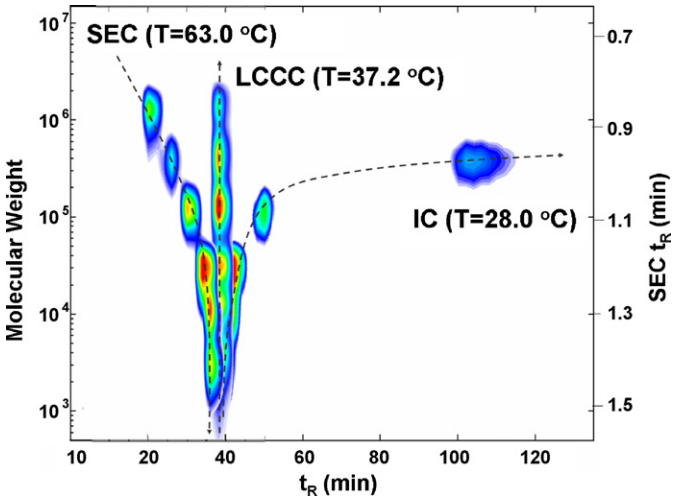

Fig. 1. 2D-LC chromatograms recorded by a UV absorption detector (260 nm) showing polymer molecular weight (SEC) vs. 1st-D LC retention time at different column temperatures; SEC (T = 63.0 °C), LCCC (T = 37.2 °C), IC (T = 28.0 °C). PS standards: 3250, 10k, 30.9k, 113k, 384k, 1800k. 1st-D RP-TGIC (abscissa): C18 bonded silica column (Nucleosil, 7 μm, 500 Å, 150 mm × 4.6 mm, i.d.); CH_2Cl_2/CH_3CN = 57/43 (v/v) eluent at a flow rate of 0.05 mL/min. 2nd-D SEC (ordinate): Polymer Lab, PolyPore; 250 mm × 4.6 mm i.d., 5 μm; THF eluent at a flow rate of 1.7 mL/min; column temperature: 110 °C.

3. Results and discussion

The familiar fan-shaped diagram showing the transition from SEC mode to IC mode via LCCC upon the change of column temperature or mobile phase composition can be constructed by on-line 2D-LC separation. Fig. 1 is the overlay of three 2D-LC chromatograms of 6 different MW polystyrene (PS) standard samples; SEC × SEC, LCCC × SEC and IC × SEC. For the three chromatographic separations, the separation condition of the 1st-D LC was identical except for the column temperature. A C18 silica column (Nucleosil, 7 μm, 500 Å, 150 mm × 4.6 mm, i.d.) and CH_2Cl_2/CH_3CN mixture (57/43, v/v) were used as the stationary and mobile phase, respectively. At the column temperature of 63.0 °C, the PS samples are separated by SEC mode eluting high MW sample first while they are separated by IC mode at 28.0 °C eluting high MW samples later. At 28.0 °C, the highest MW (1800k) PS sample is retained too strongly to elute. At 37.2 °C, all the samples of different MW elute at the same elution volume, indicating critical conditions. The effluent of the 1st-D separation is subjected to the 2nd-D SEC separation operated at 110 °C. The SEC analysis can be repeated every 1.6 min. Each 2D-LC chromatogram is an overlay of ~90 SEC chromatograms.

Fig. 2 displays an NPLC × SEC 2D-LC chromatogram recorded by a UV absorption detector (260 nm) of 3 different MW PS-H and PS-OH (2k, 10k, 113k) pairs. Each pair of PS-H and PS-OH was prepared from the same anionic polymerization batch. After the polymerization of styrene monomer was completed, a half of the polystyryl anions was terminated with isopropanol to yield normal H-terminated PS (PS-H) while the other half was reacted with ethylene oxide to yield PS-CH_2CH_2OH (PS-OH) [41–43]. Therefore, PS-H and PS-OH in each pair are the same polymers except for the end group. The 2D-LC separation carried out according to the functionality in the 1st-D NPLC and according to the MW in the subsequent 2nd-D SEC separation. As shown in Fig. 2, a mixture of 3 different MW pairs of PS-H and PS-OH yields well-resolved 6 peaks. The isocratic and isothermal 1st-D NPLC separation is done near the LCCC condition of PS for the separation according to the functionality. However, the separation condition is slightly off from the LCCC condition and the chromatogram shows a weak MW dependence along the ordinate. Nonetheless, the 1st-D NPLC separation demonstrates