Lee, H. C., Lee, H., Lee, W., Chang, T. H. & Roovers, J. Fractionation of cyclic polystyrene from linear precursor by HPLC at the chromatographic critical condition. Macromolecules...

ARTICLE *in* MACROMOLECULES · OCTOBER 2000

Impact Factor: 5.8 · DOI: 10.1021/ma000807b

Fractionation of Cyclic Polystyrene from Linear Precursor by HPLC at the Chromatographic Critical Condition

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Received May 9, 2000
Revised Manuscript Received September 8, 2000

Introduction. The study of the synthesis and physical properties of synthetic cyclic polymers has attracted considerable interest since the discovery that some DNA occurs in a circular form.[1−3] Various physical properties of cyclic polymers have been predicted theoretically[4−8] as well as by computer simulation studies[9−11] and have been examined experimentally.[12−22] Anionic polymerization has been the best method to obtain high molecular weight cyclic polymers with narrow molecular weight distribution. The basic strategy is to form precursor polymers with two carbanion end groups and to have them react intramolecularly, under extreme dilution, with a difunctional electrophile to close the ring.[21−26] However, side reactions produce linear precursor polymers, and intermolecular reactions simultaneously produce dimeric and higher molecular weight linear polymers. Therefore, it is difficult to obtain pure cyclic polymers directly, and fractionation has been necessary in order to obtain cyclic polymers with high purity.

For the fractionation of cyclic polymers from the ring-closure reaction mixture, two methods have been employed most often: fractional precipitation[16,20−22,24−28] and preparative size exclusion chromatography (SEC).[29,30] However, none of the methods have been fully successful in separating the cyclic polymers from the side products. The SEC retention times (hydrodynamic volume of polymer chains) of a cyclic polymer and its linear precursor are not sufficiently different to provide complete resolution of the elution peaks. The ultracentrifugation sedimentation method was reported to be more successful in distinguishing cyclic polymers from the linear precursor by Roovers et al.,[21,26] but it cannot be used easily for preparative purposes. Therefore, it is safe to say that all the synthetic cyclic polymers made so far were contaminated by linear precursors to some extent.

Interaction chromatography (IC) generally refers to the chromatography techniques utilizing the enthalpic interaction between the analytes and the stationary phase while SEC separation is due to the entropic exclusion mechanism. Both, size exclusion and interaction mechanism are operative in the chromatographic fractionation of macromolecules when a porous stationary phase is employed.[31] Often SEC or IC separation involves a mixed mode of two separation mechanisms. If one carefully adjusts the elution conditions (mobile and stationary phase as well as temperature), the chromatographic critical condition can be achieved. At this point the enthalpic interaction effect and entropic exclusion effect are exactly compensated, and polymers elute near the retention time of the injection solvent independent of their molecular weight.[32] Liquid chromatography at the critical condition (LCCC) has been efficiently employed to characterize polymer blends, block copolymers, telechelic polymers, etc.[33−36]

Gorbunov and Skvortsov generalized the Casassa theory of SEC of linear polymers[37] employing the boundary condition that the entropy and enthalpy effects for a polymer chain interacting with a stationary phase are mutually compensated.[38,39] According to their theory, cyclic polymers behave quite differently from linear polymers at the critical condition of linear polymers: the retention of cyclic polymers depends on the size of the polymer chain relative to the pore size while the retention of linear polymers is independent of the molecular weight. The theory predicts the possibility of effective fractionation of linear and cyclic polymers of the same chemical nature and the same molecular weight by LCCC. In this communication we would like to report the successful fractionation of cyclic polystyrenes (PSs) from all linear contaminants by LCCC.

Table 1. Molecular Weights of Cyclic PSs Used

sample code	$M_w \times 10^{-3}$ [a]	sample code	$M_w \times 10^{-3}$ [a]
R17H	(6.9)	R8DC	73.3
R7F	(12.1)	R9E	86.9
R16E	(18)	R18D	161
R1F	23.4	R10DD	198
R4DB	48		

[a] From refs 14 and 16. Values in parentheses are from SEC on the linear precursors.

Experimental Section. Nine cyclic PS samples in the molecular weight range from 5000 to 200 000 were used. The polymerization and fractionation scheme as well as characterization results were reported previously.[16,26] The molecular weight characterization results are summarized in Table 1. A typical isocratic HPLC apparatus was used with different columns for SEC and LCCC. The SEC system included two cross-linked PS gel columns (Polymer Lab., mixed C). For LCCC system, a single C18 bonded silica column (Nucleosil C18AB, 100 Å pore, 250 × 4.6 mm) was used. In LCCC experiments, the column was put in a jacket connected with a bath/circulator so that the column temperature was kept constant at 43 °C. The mobile phase was a mixture of CH_2Cl_2/CH_3CN (57/43, v/v), and the flow rate was 0.5 mL/min. In the SEC experiments, the column temperature was kept at 40 °C in a column oven, and the eluent was THF at the flow of 1.0 mL/min.

Results and Discussion. In Figure 1 are displayed the SEC chromatograms of nine cyclic PSs of different molecular weight and their linear precursors. The ring closure of two living chain ends causes the reduction in the hydrodynamic volume of the polymer chains so that cyclic PS elutes at a longer retention time than its linear precursor. However, the difference of the hydrodynamic

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10.1021/ma000807b CCC: $19.00
Published on Web 10/14/2000

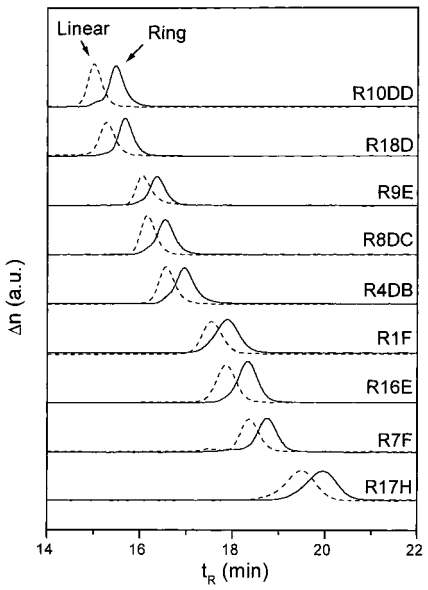

Figure 1. SEC chromatograms of cyclic PS (solid line) and corresponding linear precursor (dashed line). Linear PS precursors are eluted earlier than cyclic PSs, but the elution peaks are partially overlapping. Some elution peaks of cyclic PSs show small shoulders at lower retention, indicating the contamination of the linear precursors.

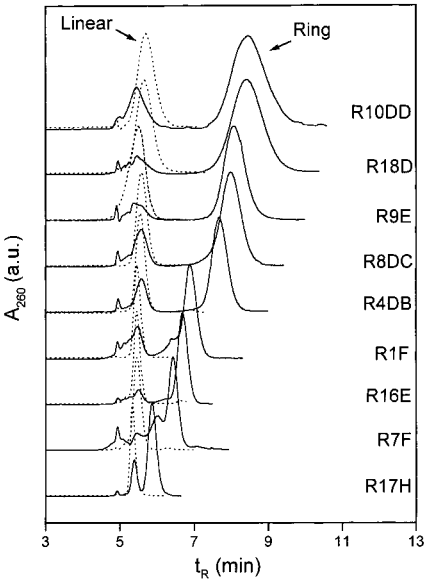

Figure 2. LCCC chromatograms of cyclic PSs and corresponding linear precursors at the chromatographic critical condition of linear PS. Linear precursors (dotted line) elute at about the same retention time (5.4 min) independent of the molecular weight while the retention of cyclic polymer increases with molecular weight. The small peaks appearing near $t_R = 5$ min are the injection solvent peaks. The elution peak of cyclic polymers is completely separated from the linear precursors down to the baseline. The chromatograms of cyclic PS (solid line) show that most of the samples contain contaminants mostly the linear precursors.

volume between linear and cyclic PS is not sufficiently large to provide their complete resolution. Although a visible amount of linear PS precursors appears in some chromatograms of cyclic PS as shoulders, it is difficult to quantify the linear contamination of the cyclic polymers.

In Figure 2, LCCC chromatograms of both cyclic PSs (solid line) and their linear precursors (dotted line) at the critical condition of linear PS are displayed. We note

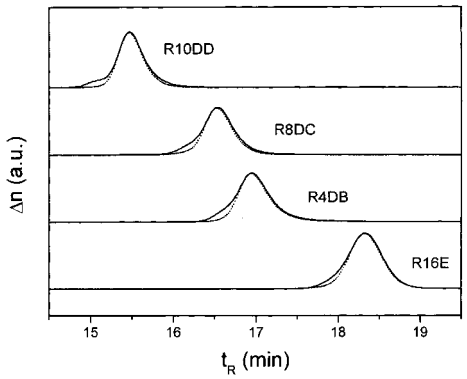

Figure 3. SEC chromatograms of four cyclic PSs fractionated by LCCC (dotted line) in comparison with the cyclic PSs before the fractionation (solid line), which are identical chromatograms shown in Figure 1.

that linear PSs elute at nearly the same retention time around 5.4 min independent of molecular weight. This is the elution behavior of the linear polymer chains at the chromatographic critical condition. From the chromatograms of cyclic PSs, it is clear that all the cyclic PSs purified by fractional precipitation method contain contaminants, mostly the linear polymers eluted at the retention time of the linear precursors. The small peaks appearing near $t_R = 5$ min are the injection solvent peaks. We also find other subsidiary peaks for R1F and R7F appearing between the linear polymer peak and the main ring peaks, which have not been identified yet. We can also note that the cyclic peak broadens with molecular weight, which is a behavior expected in the interaction chromatographic separation operated in the isocratic mode.[40]

On the other hand, at the same elution conditions, the retention time of cyclic PSs increases with molecular weight and the cyclic PSs are completely separated from their linear precursors. The general trend of the cyclic PSs' retention with respect to the molecular weight is in agreement with the theoretical prediction by Gorbunov and Skvortsov.[26,39] A more detailed investigation including the pore size dependence will be reported in the future. We collected the main elution peaks of LCCC of four cyclic PS samples and subjected them to a new SEC analysis. The SEC chromatograms of the four cyclic PSs fractionated by LCCC are shown in Figure 3 as dotted lines in comparison with the cyclic PSs before the fractionation (solid line), which clearly demonstrate the elimination of the linear precursor contaminants.

It is worth mentioning that Pasch et al. applied LCCC for the fractionation of cyclic PS earlier.[41] They reported that the retention of cyclic PSs was longer than the linear precursors, which is consistent with the theoretical prediction.[26,39] However, they found that the retention time of cyclic PSs was also independent of molecular weight; i.e., both linear and cyclic PSs appear simultaneously at the chromatographic critical condition, which is in conflict with the theoretical prediction. We suspect that the relatively low molecular weight range of the PS samples (1860−25 000), relatively large contribution of the functional groups involved in the ring closure reaction, and many side reaction products could be responsible for the discrepancy.

In conclusion, we have successfully fractionated cyclic PSs from their linear precursors by LCCC at the critical condition of linear PSs, and the general trend of the retention behavior of the cyclic polymers is in agreement

with the theoretical prediction. Since LCCC can be practiced on a preparative scale, this would be the best available method to obtain pure cyclic polymers.

Acknowledgment. This work was supported in part by the Korea Research Foundation (BK21 project). We thank Dr. A. A. Gorbunov for helpful discussions.

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MA000807B