

# Fundamental Studies of Liquid Chromatography at the Critical Condition Using Enhanced-Fluidity Liquids

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The improvement in the analysis of telechelic polymer matrixes continues to be a pursuit for many scientists of varying disciplines. This quest for a new technique has led to the continued development of liquid chromatography at the critical condition (LCCC) or liquid chromatography at the critical adsorption point (LC-CAP). LCCC allows for the isolation of one area of the polymer matrix so that other areas of the polymer can be probed with size-exclusion or adsorptive chromatographic modes. Although this technique has been successfully applied to the analysis of telechelic polymers, the practice of LCCC can be difficult. These difficulties include finding and maintaining a solvent system appropriate for the practice of LCCC as well as deterioration of peak shape once the system is operating at the LCCC mode. Because of the specificity of the mobile phase required for the practice of LCCC, the work is routinely practiced by premixing solvents. Previous work with enhanced-fluidity liquid mobile phases demonstrated that these mobile phases removed many of the aforementioned challenges associated with working at the LCCC mode. These mobile phases utilize both pressure and temperature variation in order to maintain the specific solvent strength necessary for the LCCC work. This work studies the coupling and optimization of enhanced-fluidity, EF, liquid mobile phases for LCCC. Several EF-LCCC systems, differing in mobile phase composition, temperature, and pressure, were routinely established, resulting in the effective practice of critical chromatography. The practice of LCCC with on-line mobile phase preparation is demonstrated using commercially available instrumentation. Finally, EF-LCCC is used to analyze triblock and diblock copolymers.

The characterization of a polymer requires that the molecular weight and functionality distribution be determined. Size exclusion chromatography (SEC) techniques have been routinely used to determine the molecular weight distribution of polymer systems.<sup>1–3</sup> Adsorption chromatographic techniques, which are more useful

in elucidating functionality differences, are often complicated by the differences in molecular weight of the polymer analytes. The determination of functionality and functionality distribution becomes even more challenging when the concentration of the functionality is low in comparison to the total polymer system. These complications cause most spectroscopic and adsorption chromatographic techniques to be of little use when determining the functionality distribution of a heteropolymer system.<sup>3</sup>

SEC is dominated by the entropy of transfer,  $\Delta S$ , through porous media, and adsorption chromatographic techniques are dominated by the enthalpic energy of transfer,  $\Delta H$ , of the analyte with the stationary phase.<sup>2,4</sup> The balance of the aforementioned energies of transfer leads to a third chromatographic mode known as liquid chromatography at the critical condition (LCCC or LC-CAP) which results in a Gibb's free energy of transfer,  $\Delta G$ , equal to zero and a distribution coefficient,  $K_d$ , equal to one.<sup>3</sup>

$$\Delta G = -RT \ln K_d = \Delta H - T\Delta S \quad (1)$$

The critical condition for a polymer is achieved when a range of polymers varying only by molecular weight elute under the same chromatographic band.

In a chromatographic system, each component of a heteropolymer system,  $A_xB_y$ , contributes to the total Gibb's free energy of interaction, as described in eq 2.

$$\Delta G = \sum x \Delta G_A + \sum y \Delta G_B \quad (2)$$

For a chromatographic system operating at the critical condition for component A,  $K_{dA} = 1$  regardless of the molecular weight and functionality distribution of component A.<sup>3</sup> The resultant separation, either SEC or adsorption chromatography, will occur on the basis of the heterogeneity of component B of the polymer matrix.

LCCC has found many applications in the characterization of polymer analytes. These applications include the analysis of polymer blends,<sup>5–7</sup> copolymers,<sup>1,8–12</sup> graft copolymers,<sup>13</sup> polymers

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differing in tacticity,<sup>14,15</sup> and end-group.<sup>16</sup> LCCC was utilized with long packed capillaries and off-line MALDI mass spectrometry.<sup>17,18</sup> LCCC has also been used in-series with SEC and nuclear magnetic resonance.<sup>15,19,20</sup> Epoxy resins have been analyzed using LCCC and off-line MALDI-TOF mass spectrometry.<sup>21</sup> Although LCCC resolves difficult polymer systems, the application of the technique is accompanied by challenges.

The most common chromatographic variable manipulated to reach the critical condition is the mobile phase composition. Current drawbacks of LCCC are a sensitivity to slight changes in eluent composition and peak broadening.<sup>22–24</sup> Traditionally, LCCC has been achieved using mobile phases of binary or tertiary liquid mixtures.<sup>11,25</sup> Slight changes in the proportions of these mixtures, (~0.1 mole percent<sup>1,24</sup>) may cause a loss of the critical condition.<sup>22</sup> This sensitivity to eluent composition manifests itself in the fact that most HPLC pumps have an accuracy of 1% v/v.<sup>24</sup> The use of temperature tuning to reach the critical condition has been utilized with liquid mobile phases for the establishment of the critical condition,<sup>12</sup> although the behavior is sometimes unpredictable.<sup>24</sup> Enhanced-fluidity liquid mobile phases were recently used to achieve the critical condition in a consistent and reproducible fashion.<sup>1,17,18</sup>

Enhanced-fluidity liquids are common organic solvents, such as tetrahydrofuran (THF), to which large proportions of a liquified gas, such as CO<sub>2</sub>, are added. The enhanced-fluidity liquid maintains a solvent strength close to that of the organic proportion of the mixture, while the viscosity approaches that of the fluidity modifier.<sup>1,26,27</sup> The high compressibility of enhanced-fluidity liquids provides manipulation of solvent strength by changing the pressure or temperature of the solution, variables that are unavailable with pure liquid mobile phases. The combination of enhanced-fluidity liquids with chromatography results in higher efficiency and faster speed of analysis when compared to their traditional liquid mobile phase counterparts. Improvements in chromatographic performance are documented for SEC,<sup>26,28</sup> chiral HPLC,<sup>29</sup> and normal- and reversed-phase liquid chromatography.<sup>30</sup>

Enhanced-fluidity liquid mobile phases in LCCC were first used to characterize copolymers of poly(styrene-*co*-methyl acrylate) with a mobile phase of THF/CO<sub>2</sub>. Pressure manipulation of the highly compressible enhanced-fluidity liquids was an accurate and reproducible method for reaching the critical condition.<sup>1</sup> Highly efficient LCCC separations of functionalized polymers were also demonstrated when enhanced-fluidity liquid mobile phases were used with very long packed capillary columns. This chromatographic system was then used with off-line MALDI-TOF mass spectrometry for the characterization of poly(propylene glycol) based analytes.<sup>17,18</sup>

The current practice of LCCC is often associated with difficulty in maintaining an appropriate critical mobile phase for a given polymer. With traditional liquid mobile phases, the only effective means of solvent strength modification relies on changing the type or percent ratio of the solvents utilized. Once an appropriate solvent system is established as a critical mobile phase for a polymer, efforts to further improve the LCCC system are rarely attempted because of the potential loss of the critical condition with any perturbation of the system. Previously, an enhanced-fluidity liquid mobile phase of 54 mol % CO<sub>2</sub>/46 mol % THF at 136 atm and ambient temperature was established to be an appropriate mobile phase to effectively reach the critical condition for a molecular weight series of polystyrene standards.<sup>1</sup>

This study expands upon this discovery by further characterizing enhanced-fluidity LCCC. Temperature control was applied along with pressure variation to establish the LCCC mode for polystyrenes. The three variables that effectively alter the solvent strength of an enhanced-fluidity liquid mobile phase—percent composition, temperature and pressure—were manipulated and monitored in an effort to not only find the critical mode for polystyrene but to also find the most effective LCCC system. The density of the mobile phases was documented as a means of monitoring the solvent strength of the enhanced-fluidity liquids. The ability to vary the temperature of the chromatographic system while operating at the critical chromatographic mode allowed for the analysis of the thermodynamic properties of the LCCC system. The overall plate height of each established LCCC system was monitored and the individual contributions to the plate height were calculated.

A commercially available supercritical fluid chromatography (SFC) instrument operating with enhanced-fluidity liquid mobile phases was utilized for the first time for the practice of LCCC. This type of commercial instrument features on-line mixing and pressure control of an enhanced-fluidity liquid mobile phase. An SFC instrument is the only type of commercially available chromatographic system that can practically couple LCCC with on-line mobile phase preparation, allowing for time-saving and reproducible practice of LCCC. Pressure and temperature was varied while working at the critical chromatographic mode, and the efficiency was monitored. Finally, the utility of LCCC with enhanced-fluidity liquid mobile phases will be demonstrated with the analysis of diblock and triblock polymer systems of *block*-copoly(styrene/poly(ethylene-*co*-butylene)) and *block*-copoly(sty-

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Table 1. Size Exclusion Calibration Curve Data of Polystyrene Standards

| temp<br>K | slope<br>log $M_w$ /mL <sup>a</sup> | pore vol<br>mL <sup>b</sup> | interstitial vol<br>mL |
|-----------|-------------------------------------|-----------------------------|------------------------|
| 313.15    | -2.53 ± 0.03                        | 0.69 ± 0.02                 | 0.86 ± 0.01            |
| 323.15    | -2.60 ± 0.04                        | 0.67 ± 0.01                 | 0.86 ± 0.01            |
| 333.15    | -2.71 ± 0.02                        | 0.67 ± 0.01                 | 0.86 ± 0.01            |
| 343.15    | -2.96 ± 0.13                        | 0.67 ± 0.02                 | 0.82 ± 0.02            |
| 353.15    | -2.71 ± 0.05                        | 0.65 ± 0.01                 | 0.82 ± 0.01            |

<sup>a</sup> The slope of the calibration curve was calculated using a linear fit at a 95% confidence interval using SigmaPlot software (version 4). <sup>b</sup> The pore volume was calculated by subtracting the retention volume of a totally permeating PS standard ( $M_w = 590$ ) from the retention volume of a totally excluded PS standard ( $M_w = 2\,316\,000$ ).

Table 2. Comparison of Instrument Parameters

| temp<br>K | plate height<br>cm × 10 <sup>-3</sup> | $\Delta P^a$ commercial<br>atm | $\Delta P^a$ home-built<br>atm |
|-----------|---------------------------------------|--------------------------------|--------------------------------|
| 313.15    | 7.8 ± 0.6                             | 39.4                           | 35.3                           |
| 323.15    | 8.1 ± 0.6                             | 38.5                           | 33.6                           |
| 333.15    | 7.7 ± 0.2                             | 36.5                           | 31.6                           |
| 343.15    | 7.0 ± 0.4                             | 35.7                           | 28.3                           |
| 353.15    | 6.7 ± 0.6                             | 34.0                           | 29.6                           |
| 363.15    | 5.6 ± 0.7                             | 30.6                           | 27.5                           |

<sup>a</sup> Standard deviation in the pressure measurements were ≤ 1.02 atm.

rene/poly(ethylene-*co*-butylene)/styrene), respectively. These samples were analyzed at the critical condition for polystyrene with two mobile phase systems differing in composition and operational temperature and pressure.

## EXPERIMENTAL CONDITIONS

**Materials.** Polystyrene samples ( $M_w = 590$ ,  $M_w/M_n = 1.07$ ;  $M_w = 5120$ ,  $M_w/M_n = 1.08$ ;  $M_w = 12\,600$ ,  $M_w/M_n = 1.08$ ;  $M_w = 18\,700$ ,  $M_w/M_n = 1.03$ ;  $M_w = 29\,300$ ,  $M_w/M_n = 1.01$ ;  $M_w = 44\,100$ ,  $M_w/M_n = 1.07$ ;  $M_w = 114\,200$ ,  $M_w/M_n = 1.04$ ;  $M_w = 2\,316\,000$ ,  $M_w/M_n = 1.02$ ) were purchased from Scientific Polymer Products, Inc. (Ontario, NY). Polystyrene samples ( $M_w = 13\,104$ ,  $M_w/M_n = 1.04$ ;  $M_w = 382\,100$ ,  $M_w/M_n = 1.16$ ) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Polyethylene samples ( $M_w = 15\,520$ ,  $M_w/M_n = 1.08$ ;  $M_w = 108\,000$ ,  $M_w/M_n = 1.32$ ;  $M_w = 155\,000$ ,  $M_w/M_n = 3.66$ ) were purchased from Scientific Polymer Products, Inc. (Ontario, NY). Dicarboxy terminated polystyrene ( $M_w \approx 50\,000$ ) was purchased from Scientific Polymer Products, Inc. (Ontario, NY). Triblock copolymer samples of poly(styrene/poly(butylene-*co*-ethylene)/styrene) were supplied by Shell Chemical Co. (Houston, TX). Properties of the triblock polymers are given in Table 3. Tetrahydrofuran (THF) (stabilized, 99.9% pure) was purchased from Mallinckrodt Specialty Chemicals Co. (Paris, KY) and distilled over sodium metal and benzophenone to remove water. An isomeric mixture of xylene (100% pure) was provided and used as received from Ashland Chemical Co. (Columbus, OH). Supercritical fluid chromatography (SFC)/supercritical fluid extraction (SFE) grade CO<sub>2</sub> (99.99995% pure) was used as received from Praxair (Allentown, PA). Polymer samples, 1–3 mg/mL, were prepared in the liquid portion of the enhanced-fluidity mobile phase.

Table 3. Description of Copolymer Samples

| sample <sup>a</sup> | structure <sup>b</sup> | % PS/% PB- <i>co</i> -PE <sup>c</sup> | $M_w^d$<br>KDa      |
|---------------------|------------------------|---------------------------------------|---------------------|
| G1650               | PS-PE-PB-PS            | 29/71                                 | 83                  |
| G1652               | PS-PE-PB-PS            | 29/71                                 | 77                  |
| G1657               | PS-PE-PB-PS            | 13/87                                 | 103 (shoulder ~ 76) |
| G1726               | PS-PE-PB               | 30/70                                 | 77, 40              |

<sup>a</sup> Kraton sample notation from Shell Chemical Co. (Belpre, OH). <sup>b</sup> Polystyrene (PS), polyethylene (PE), polybutylene (PB). See Figure 6 for structure. <sup>c</sup> Weight average ratio. <sup>d</sup> Molecular weights of the total copolymer system were established by size exclusion chromatography relative to PS (experimental conditions are described in Figure 7A). Standard deviation in molecular weight values were ≤ 1.4 KDa. (Product literature details the molecular weight of the copolymers from highest to lowest molecular weight as: G1650, G1657, G1652, and G1726 with G1650 and G1657 as "medium" and G1652 and G1726 as "low").

**Instrumentation.** *In-House-Built Chromatographic System.* The in-house chromatographic system included an ISCO 260D syringe pump from ISCO, Inc. (Lincoln, NE); a Valco W-series high-pressure injection valve with an injection volume of 200 nL from Valco Instruments (Houston, TX); and two 250 × 2.0 mm i.d. Betasil silica (200-Å pore size, 5-μm particle size) columns in series from Keystone Scientific, Inc. (Bellefonte, PA). The silica column was chosen because the pore structure will not vary as a result of temperature variations. Column temperature was controlled with a Varian 3700 capillary gas chromatographic oven with temperature control of ± 0.2 K from Varian (Palo Alto, CA). The accuracy of the temperature of this oven was calibrated with a mercury thermometer. The column and connecting tubing in front of the injector were held at the experimental temperature for at least 0.5 h before data collection. Detectors used included a Spectroflow 757 UV-vis absorbance from Kratos Analytical Instruments (Ramsey, NJ) at a wavelength of 254 nm with a 250-μm-i.d. homemade fused-silica view cell. The view cell was made by applying a low flame to remove the polyimide coating from the fused-silica capillary (Polymicro Technologies, Inc., Phoenix, AZ). A 30-μm-i.d. fused-silica capillary of appropriate length was attached to the outlet of the detector to control the flow rate and function as a restrictor. A Varex MKIII ELSD evaporative light scattering detector with an SFC nebulizer from Alltech, (Deerfield, IL) was also used. The ELSD detector was placed in-line with the column via an appropriate length of 50-μm-i.d. fused-silica capillary, which also functioned as a flow restrictor. Nitrogen gas (Praxair, Allentown, PA) was used as a nebulizer gas at a flow rate of 2.5 L/min, and the drift tube temperature was maintained at 363 K (unless otherwise noted). Data acquisition was achieved with an IBM Thinkpad using EZChrom software (version 6.7) from Scientific Software, Inc. (San Ramon, CA).

*Commercial Chromatographic System.* The commercial chromatographic system utilized a Gilson SF3 supercritical fluid chromatograph (Gilson, Inc., Middletown, WI). The system consisted of a model 308 pump with a 10 SC thermostated pump head that was used to pump liquified CO<sub>2</sub>. The pump head cooling coil was chilled with a mixture of ice and water that was continuously circulated using a Techne Tempunit Thermoregulator (model TU-16D from Techne, Inc., Princeton, NJ) with an accuracy of ± 0.2 K. A second pump (model 306 with a 5 SC pump head) was used to deliver THF to the chromatographic system. A binary mixture of THF/CO<sub>2</sub> was mixed in a dynamic mixer



(model 811C) using a 1.5-mL mixing chamber. Injections of 2  $\mu$ L were made using an external injection loop injector (model 7725i) from Rheodyne L. P. (Rohnert Park, CA). A variable wavelength UV detector (model 151) with a high-pressure flow cell (6- $\mu$ L cell volume) was used to monitor the effluent at a wavelength of 254 nm. The column outlet pressure was maintained with a model 821 pressure regulator. Data acquisition was achieved with Unipoint System control software (version 1.80) from Gilson, Inc. (Middletown, WI).

**Density Apparatus.** Density measurements were made using a DMA 45 digital density meter (Anton Parr K. G.; Graz, Austria) that was connected to a DMA-512 measuring cell. The density meter has an accuracy of  $1 \times 10^{-4}$  g/cm<sup>3</sup>. An ISCO 260D syringe pump (ISCO, Inc., Lincoln, NE) delivered the enhanced-fluidity liquid mixtures to the density meter and maintained the pressure. The tubing between the pump and the density meter was coiled and placed in a waterbath that had temperature control of  $\pm 0.1$  K (Techne TC-16U from Techne, Inc., Princeton, NJ) to maintain a constant temperature before density measurements were recorded. The temperature-regulated water was also circulated through the heating mantle of the density cell of the DMA-512 to maintain a constant temperature. The whole apparatus was wrapped in a fiberglass insulation blanket (Carborundum Co., Niagara Falls, NY), and the system was equilibrated at each temperature for 0.5 h before measurements were recorded.

**Procedure.** Mobile phase mixtures for the home-built system were prepared by placing the appropriate amount of THF or xylene into a syringe pump then adding a specific amount of CO<sub>2</sub> from another syringe pump. The pressure of the CO<sub>2</sub> was carefully controlled so that the density was known, allowing for the transfer of an accurate volume of CO<sub>2</sub>. Mixing of the enhanced-fluidity liquid mobile phase was achieved by alternating the volume of the syringe pump for  $\sim 5$  min and then allowing the liquid to equilibrate for a minimum of 12 h prior to use. Batch preparation of the enhanced-fluidity mobile phases allows for an accuracy of 0.004 mL.

Pressure control of the home-built chromatographic system was achieved by adjusting the inlet pressure,  $P_i$ , at the head of the column with the pump used for solvent delivery and was monitored with the pressure transducer at the pump head. The pressure drop of the system was monitored by placing a pressure transducer with an accuracy of  $\pm 1$  atm (model 204, Setra Systems, Inc.; Acton, MA) between the column outlet and the detector. The flow rate was maintained by adjusting the length of the fused-silica restrictor placed at the column outlet. The commercial instrument utilized a pressure regulator at the column outlet,  $P_o$ , for pressure control of the system. The Gilson outlet pressure transducer was calibrated with the Setra pressure transducer described above. The column inlet pressure transducer was also calibrated with the Setra pressure transducer.

Chromatographic peaks were analyzed using PeakFit software (version 4) from Jandel Scientific (San Rafael, CA). Calibration curve data were analyzed using a linear regression curve with a 95% confidence using SigmaPlot software (version 4) from SPSS, Inc. (Chicago, IL). The polystyrene with  $M_w$  of 2316 KDa was used to determine the interstitial volume of the system. Polystyrene with  $M_w$  of 590 Da was exposed to the most pore volume for this chromatographic system.<sup>26</sup>

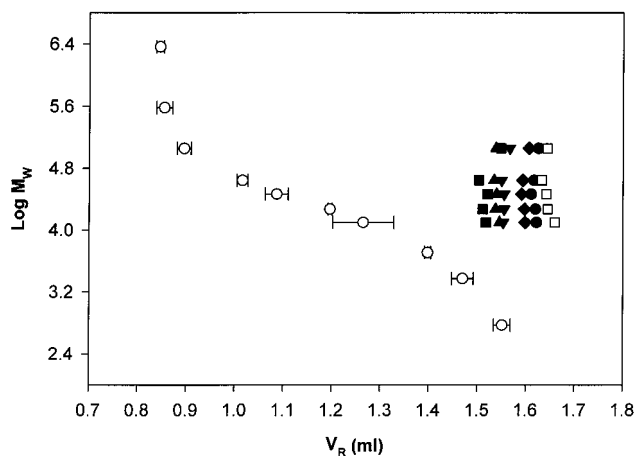


Figure 1. Calibration curves of PS standards at the critical condition versus temperature using the home-built system, 54% CO<sub>2</sub>/46% THF (unless otherwise noted), 0.36 mL/min flow rate, UV detection, and a bare silica stationary phase: (○) size exclusion mode with 100% THF, 313 K, 0.15 mL/min; (□) 313 K, 240 atm; (●) 323 K, 252 atm; (◆) 333 K, 263 atm; (▼) 343 K, 280 atm; (▲) 353 K, 336 atm; (■) 363 K, 349 atm. (Column head pressure,  $P_i$ , is listed; error bars are smaller than plot symbols.)

## RESULTS AND DISCUSSION

**Pressure and Temperature Variation.** The enhanced-fluidity liquid mobile phase established for LCCC of polystyrene with a Jordi gel divinylbenzene column was 54 mol % CO<sub>2</sub>/46 mol % THF at 136 atm and 298 K.<sup>1</sup> In this study, this bulk mobile phase composition was also found to be effective for reaching the critical condition of polystyrene with a stationary phase of bare silica.

Because the solvent strength of an enhanced-fluidity liquid can be tuned by changing the pressure and the temperature of a specific mobile phase composition, it seemed likely that combinations of different pressures and temperatures could be used to achieve the critical condition for the same mobile phase composition. To test this possibility for a constant mobile phase composition of 54 mol % CO<sub>2</sub>/46 mol % THF, the temperature of the column was varied from 313 to 363 K in 10 K intervals. For each temperature, the pressure was varied until the critical condition was found for polystyrene standards with  $M_w$  that varied from 12.6 to 114.2 KDa. Figure 1 shows the resultant calibration curves for the critical conditions achieved at the different pressure and temperature combinations. As the temperature of the LCCC system was raised through a temperature range of 313 to 363 K, it was necessary to raise the inlet pressure of the system from 240 to 349 atm in order to maintain the critical condition.

The pressure and temperature compensation observed when approaching the critical condition for the polymer standards is consistent with the need to maintain a constant solvent strength to achieve the critical condition. Next, the density of each of the six mobile phase conditions was measured to determine if the solvent strength necessary to reach the critical condition corresponded to a specific bulk density. A densitometer was placed in-line between the pump and the injector of the chromatographic system. Figure 2 shows that the density did remain approximately constant over the critical conditions studied.

One other notable feature observed when these six critical conditions were determined is that the retention volume for the critical-mode calibration curves shifted to lower values as the

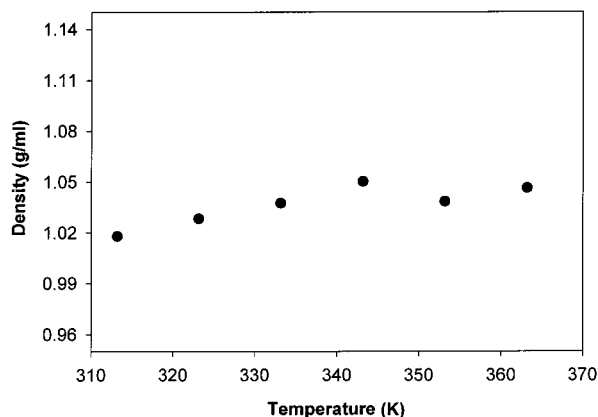


Figure 2. Density of the mobile phase, 54% CO<sub>2</sub>/46% THF, versus temperature at the same conditions as Figure 1. (Error bars are smaller than the plot symbols.)

temperature of the system was increased (see Figure 1). To better understand why the measured retention volume might be shifting with temperature, a series of size-exclusion calibration curves of the same polymers were collected over a similar temperature range using the same silica stationary phase with a mobile phase of pure THF. An exclusion-mode calibration curve was not determined for the polystyrenes at 363 K, because the higher-molecular-weight polymers did not elute from the column under this condition. The SEC calibration curves, differing only in operational temperature, experienced a slight shift in retention (see Table 1). Ideally, when operating purely in the exclusion mode, the same calibration curve shape should be maintained, regardless of the operating temperature. Any variation in the SEC curves with temperature may be due to several variables: a change in the dimensions of the stationary phase, a change in the viscosity of the mobile phase, a change in the dimensions of the polymer analyte or a manifestation of a nonideal exclusion mode mechanism.<sup>4,31</sup> The use of a silica stationary phase eliminates the possibility for a change in the dimensions of the packing material with temperature, although the apparent pore volume that the polymer experiences within the chromatographic system may be altered with temperature as a result of slight changes in the viscosity of the mobile phase (the inlet pressure of the system varied by ~20 atm or about one-half the pressure drop of the system).

Table 1 shows that the apparent pore volume under SEC conditions was the same, except for the highest temperature studied (353 K), at which a slight shift to a smaller apparent pore volume was noted. Both the molecular weight range of the polystyrene polymer standards and the temperature range evaluated were not extremely high, so a variation in the polymer dimensions with temperature change was not expected.<sup>32</sup> In addition, the slope of the calibration curve increased as the temperature of the chromatographic system was raised from 313 to 353 K (see Table 1).

The enthalpy of transfer,  $\Delta H$ , for adsorptive interactions is typically exothermic, which would cause the retention volume to

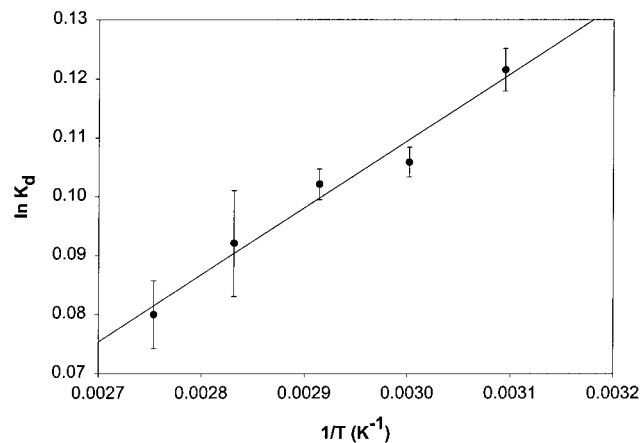


Figure 3. Linear regression of  $\ln K_d$  versus reciprocal temperature at the critical condition for the PS standard with a  $M_w$  of 29 300 and a polydispersity of 1.01.

decrease as the temperature was increased. Therefore, the small shift in retention volume under SEC conditions shows that the SEC conditions are not completely ideal in that entropic interactions should be exclusively controlling the separation. However, the extent of enthalpic interactions has obviously increased at the critical condition, and this most likely caused the greater shift in the retention time.

Using the pore volume and dead volume determined under SEC conditions for the studied temperature range, the distribution coefficients of the polystyrenes were calculated at the critical conditions. Over the temperature range explored, the flow rate was rigorously maintained, as well as the density (as noted in Figure 2), indicating that the shift in retention volume was thermodynamically driven. The variation in retention volume at the critical condition with temperature change was reflected in changes in the distribution coefficient,  $K_d$ , as described in eq 3.

$$\ln K_d = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} + \ln F \quad (3)$$

A phase ratio ( $F$ ) of 0.92 was obtained using information supplied by the manufacturer of the columns (pore volume of 0.9 mL/g and packing density of 0.4 g/mL).<sup>33</sup> Because these values were approximate, the error in  $\Delta S$  could not be calculated, but it may be as high as 30%. Figure 3 shows a plot of  $\ln K_d$  versus  $1/T$ , which allowed for the calculation of  $\Delta H$  from the slope and  $\Delta S$  from the intercept. The enthalpy at the critical conditions was  $-226.1 \pm 22.1$  cal/mol, and the entropy was found to be equal to  $-0.3$  cal/mol K. An approximate balance between the entropically driven exclusion mode and the enthalpically driven adsorption mode was achieved.

**Band Dispersion at the Critical Condition for a Functionalized Polymer.** The most effective critical mobile phase from the six established for this chromatographic system was selected by monitoring the plate height for a functionalized polystyrene sample (dicarboxy-terminated polystyrene) examined at the critical mode for polystyrene. Figure 4A shows the change in plate height of the dicarboxy-terminated polystyrene at the critical condition

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(33) Technical information supplied by Keystone Scientific, I., Bellefonte, PA. 2001.

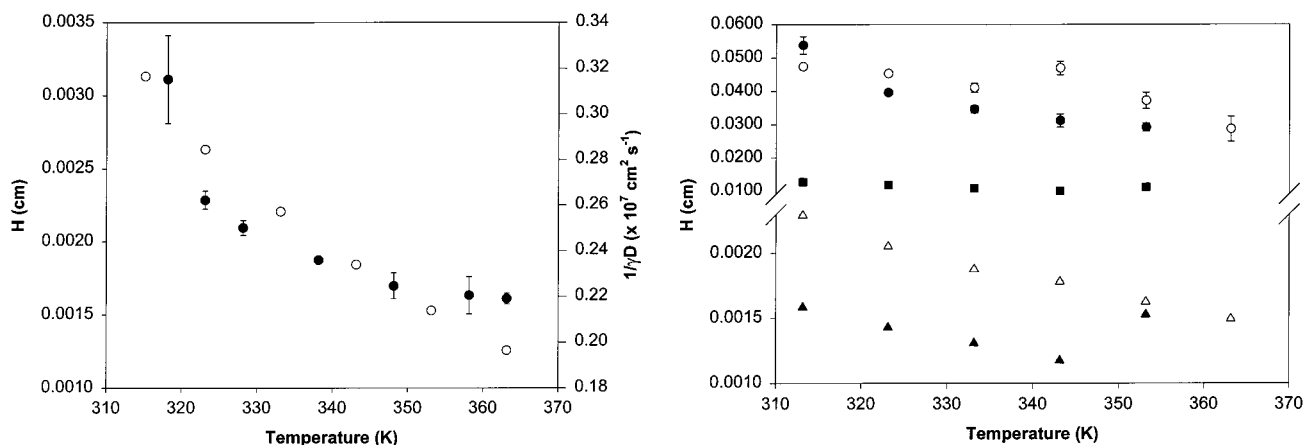


Figure 4. (A) Variation of diffusion coefficient of PS standard with temperature and variation of plate height of dicarboxy-terminated polystyrene at the critical condition for polystyrene with temperature: (●) plate height, (○) reciprocal of the diffusion coefficient times the obstruction factor. (B) Plate height contributions versus temperature for PS standard ( $M_w = 29\,300$ ). Open symbols are LCCC mode, closed symbols are SEC mode. (●) total plate height, (○) total plate height, (■) polydispersity, (▲) stagnant mobile phase, and (△) stagnant mobile phase. (Error bars are smaller than plot symbols.)

for polystyrene for each temperature studied. This plot demonstrates that the chromatographic efficiency doubled from  $\sim 15\,000$ – $30\,000$  plates over the temperature range explored, with the highest efficiency observed at the higher temperatures.

Equation 4 describes the individual contributions to the band dispersion for a system operating at the exclusion mode.<sup>4,31,34</sup>

$$H = A + \frac{B}{u} + \frac{K_d}{1.7(1 + K_d)^2} \left[ \frac{d_p^2 u}{\gamma_m D_m} \right] + \frac{L \ln p}{D^2 V_r^2} \quad (4)$$

$A$  is the band dispersion contribution due to the multiple flow paths in the packed bed.  $B/u$  describes the dispersion due to longitudinal diffusion. The third term describes the band dispersion due to the flow profile and also band dispersion in the stagnant mobile phase contained in the pores. The fourth term describes the band dispersion due to the polydispersity of the polymer.  $K_d$  is the partition coefficient,  $d_p$  is the pore diameter (cm),  $\gamma_m$  is the obstruction factor,  $u$  is the linear velocity ( $\text{cm s}^{-1}$ ),  $D_m$  is the mobile phase diffusion coefficient ( $\text{cm}^2 \text{ s}^{-1}$ ),  $L$  is column length (cm),  $p$  is the polydispersity of the polymer,  $D$  is the slope of the calibration curve (plot of  $\ln$  molecular weight versus retention volume), and  $V_r$  is the retention volume of the polymer (mL).

At the critical condition, the second and fourth terms of eq 4 should not contribute to band dispersion. Therefore, the band dispersion should vary approximately inversely with the diffusion coefficient of the polymer. Figure 4A shows that as the temperature of the system was raised from 313 to 363 K, the reciprocal of the mobile phase diffusion coefficient times the obstruction factor of a similar size polymer (molecular weight of 13 104) drops by 50%, and this agrees with the approximate 50% decrease in the plate height over the same temperature range.<sup>34</sup>

**Size Exclusion and Critical Condition Band Dispersion Comparison.** To better understand the band dispersion changes that happen when moving from size exclusion conditions to the

critical mode using enhanced-fluidity liquid mobile phases, the band dispersion of a polystyrene standard with a molecular weight of 29 300 and a polydispersity of 1.01 was studied for both modes of chromatography.

First, the individual components of eq 4 were calculated from the experimental band dispersion data collected using 100% THF as the mobile phase over the temperature range of 313–353 K at a reduced velocity of 2.0. The second term in eq 4, due to longitudinal diffusion, was calculated to be  $\leq 5.8 \times 10^{-6}$  cm and did not contribute greatly to the plate height. The third and fourth terms, due to mass transport in the stagnant mobile phase in the pores and the polydispersity of the analyte, respectively, are shown in Figure 4B. Under SEC conditions, the plate height due to the diffusion in the stagnant mobile phase and the polydispersity of the polymer sample contribute significantly to the total plate height of the chromatographic system. Also as expected, the plate height due to diffusion in the stagnant mobile phase was lower at the higher operating temperatures as a result of an improvement in the mobile phase diffusion coefficient. Broadening due to the polydispersity of the polymer did not vary significantly with temperature change. By subtracting the calculated values for the third and fourth terms of eq 4, the approximate contribution due to multiple flow paths in the packed columns,  $A$ , could be determined. Clearly from the data in Figure 4B, under SEC conditions, multiple flow paths contributed significantly to the total band dispersion.

Next, the individual components for band dispersion while operating at the LCCC mode, using the 54 mol %  $\text{CO}_2$ /46 mol % THF and the appropriate pressures over the temperature range of 313–363 K, were determined for a reduced velocity of 2.6. The plate height contribution due to the polydispersity of the polymer was essentially zero as a result of the vertical slope of the calibration curve. The band dispersion at the critical condition due to the mass transport within the pores (Figure 4B) was a significant fraction of the total measured band dispersion. At the CC mode, slight adsorptive interactions between the polymer and the stationary phase occur. This contribution would cause the  $K_d$  to increase and, therefore, increase the third component in eq 4.

(34) Yuan, H. Ph.D. Dissertation, The Ohio State University, Columbus, OH, 1997.

The EF-LCCC demonstrated two benefits: first, the efficiency of the chromatographic system was maintained as the polymer standards moved from the exclusion to the critical mode, and second, the efficiency of the functionalized polymer analytes was high. Previous studies observed that when moving from SEC to CC conditions, (while maintaining a constant flow rate) the plate height of the polymer standards increased by as much as 50%.<sup>22,24</sup> Herein, the flow rate at the CC mode was matched to approximately the same reduced velocity ( $\mu d_p/D_m$ ) as that for the SEC mobile phase conditions.<sup>34</sup> This study demonstrated that the overall plate height of the polymer standards operating at the CC mode was equal to (or only slightly higher than) that experienced for SEC. This maintenance of efficiency when working from the exclusion mode to the critical mode was the result of the benefits gained when utilizing mobile phases with higher diffusion coefficients. The critical chromatography was further improved with increased operating temperature, which provided a highly effective LCCC system.

Once operating at the critical condition for PS, the band dispersion for the functionalized polymer (dicarboxy terminated polystyrene) was markedly less than that of a comparable molecular-weight polystyrene standard operating at both the SEC and CC conditions (compare band dispersion data in 4A to that in 4B).

The utility of enhanced-fluidity liquid mobile phases with LCCC was realized with the use of the variables of temperature and pressure, in addition to the bulk mobile phase composition. This is a vast improvement over those LCCC systems utilizing "traditional" liquid mobile phase systems in which there was little or no improvement in either the achievement or maintenance of the critical condition. The lack of control over these systems leaves no variables available for the exploration into more efficient critical chromatographic systems.<sup>22,24</sup>

It is clear that the use of enhanced-fluidity liquid mobile phases allows for the most comprehensive control of the solvent system for the practice of LCCC. The practice of LCCC with enhanced-fluidity liquids not only was found to be routine, but also allowed for the operation of the technique with an efficient system. The ability to vary temperature while maintaining the critical condition allowed for the study of the thermodynamics and band dispersion contributions.

**Commercial Instrument.** To further explore the utility of coupling enhanced-fluidity liquids with LCCC, a commercial SFC instrument was used. The commercial instrument was evaluated using the same column, mobile phase, flow-rate, and temperature range that was evaluated using the home-built system. Figure 5 shows that the critical condition was achieved for a molecular weight series of polystyrene standards with pressure and temperature tuning of the commercial SFC instrument. The commercial instrument showed similar trends in the shift of retention volume of each critical condition, as compared to the home-built system.

Three differences between the operation of the home-built system and the commercial system were noted. The overall operating pressure of the commercial instrument was lower than that of the home-built instrument. The amount of pressure change necessary to reestablish the critical condition as the temperature was increased was also much lower than that for the home-built

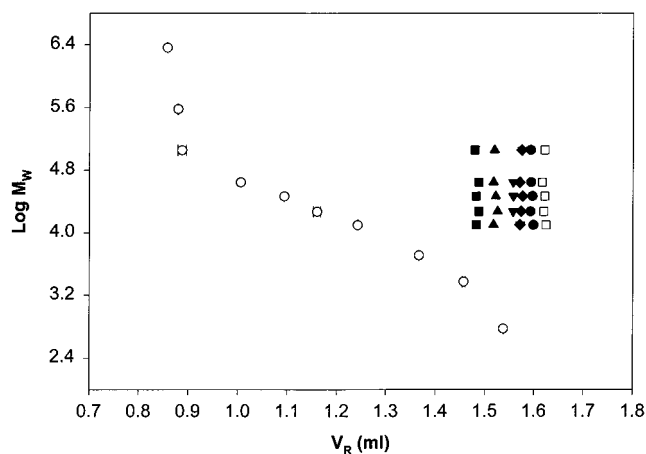


Figure 5. Calibration curves of PS standards at the critical condition versus temperature using the commercial system, 54% CO<sub>2</sub>/46% THF (unless otherwise noted), 0.36 mL/min, UV detection, and a bare silica stationary phase: (○) size exclusion mode with 100% THF, 313 K; (□) 313 K, 166 atm; (●) 323 K, 166 atm; (◆) 333 K, 170 atm; (▼) 343 K, 170 atm; (▲) 353 K, 172 atm; and (■) 363 K, 176 atm. (Column outlet pressure,  $P_o$ , is listed; error bars are smaller than plot symbols.)

system. The reproducibility of the retention volume was improved over that of the home-built system, and the overall efficiency of the commercial instrument was lower than the corresponding chromatographic conditions operating with the home-built instrument.

The first difference appears to be the result of a small error in the mobile phase composition. It was previously noted that two enhanced-fluidity liquids differing by  $\sim 0.3$  mol % have approximately the same solvent strength when the mobile phase containing less carbon dioxide was operating at an inlet pressure that was  $\sim 100$  atm lower.<sup>35</sup> It was further noted that the fluid containing 0.3 mol % more THF required an inlet pressure adjustment of 67 atm in order to span chromatographic modes from exclusion to critical to adsorption. The mobile phase containing less THF required an inlet pressure adjustment of over 102 atm in order to achieve the same chromatography. This is consistent with the results obtained with the commercial instrument. Both the overall inlet pressure ( $\sim 139$  atm less than the home-built instrument) and the approximately minor amount of pressure change necessary for the maintenance of the critical condition over the temperature range explored appears to correspond to a mobile phase that is  $\sim 0.5$  mol % higher in THF content than the mobile phase utilized with the home-built system. This error in mobile phase composition was most likely the result of error in mixture proportions generated by the commercial system. This slight increase in THF content of the enhanced-fluidity liquid was also seen in the slight increase in pressure drop across the columns when compared to similar chromatographic conditions with the home-built system (Table 2).

The improved reproducibility of retention volume is illustrated by the smaller error bars in Figure 5 for the commercial instrument when compared to the similar calibration curve (Figure 1) collected with the home-built system (although both systems showed high reproducibility of retention volume when operating

(35) Souvignet, I. Ph.D. Dissertation, The Ohio State University, Columbus, OH, 1996.



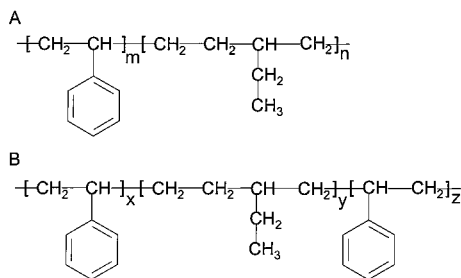


Figure 6. Structure of the diblock copolymer (A) and triblock copolymer (B) samples.

at the critical condition). This improvement was due to the commercial instrument's greater accuracy of flow-rate control as a result of independent manipulation of the pressure tuning and flow rate.

The final operational difference between the two systems was the overall efficiency of each system. Table 2 details the plate height of the system when examining dicarboxy-terminated polystyrene operating at the LCCC mode for polystyrene (58.66 vol % THF/41.34 vol % CO<sub>2</sub> operating at a flow rate of 0.36 mL/min over an outlet pressure range of 166–176 atm at a temperature range of 313–363 K). The plate height of the commercial instrument was approximately 30% greater than the plate height for this analyte operating at similar conditions with the home-built chromatographic system. The lower efficiency of the commercial instrument was due to the higher amount of extracolumn volume in comparison to the home-built system.

The beneficial feature of the commercial SFC instrument was the on-line mixing feature of the enhanced-fluidity liquid mobile phase and the accurate control of the flow rate. An SFC instrument is the only type of chromatographic system available for the practical use of on-line mobile phase mixing with LCCC. This type of system saves time by alleviating the need for premixed mobile phases and allows for easy changes in the bulk properties of the solvent system without long preparation and equilibration times. As demonstrated, once the bulk solvent system of the enhanced-fluidity liquid mobile phase was chosen, the appropriate solvent strength necessary for the practice of LCCC was found and maintained in a routine and reproducible fashion by fine-tuning the mobile phase with pressure and temperature modification. In this case, a lower operating pressure than the home-built system allowed for the consistent practice of LCCC. This is not only a time-saving practice but it overcomes the requirement of premixing a mobile phase system to 0.1 mole percent accuracy, which is the case for LCCC attempted with traditional liquid mobile phases. If the commercial instrument were configured with less extracolumn void volume, it would be a good candidate for the practical application of LCCC with careful mobile phase selection and maintenance.

**Applications.** The coupling of enhanced-fluidity liquids with LCCC was utilized for the analysis of polymers of *block*-copoly(styrene/poly(butylene-*co*-ethylene)) and *block*-copoly(styrene/poly(butylene-*co*-ethylene)/styrene) that are detailed in Figure 6. The block copolymer samples described in Table 3 consisted of different weight percentages of poly(butylene-*co*-ethylene) (PE-*co*-PB) and polystyrene (PS). These samples were examined using two LCCC mobile phase compositions (46 mol % CO<sub>2</sub>/54 mol % THF and 55 mol % xylene/45 mol % CO<sub>2</sub>). Figure 7A details the

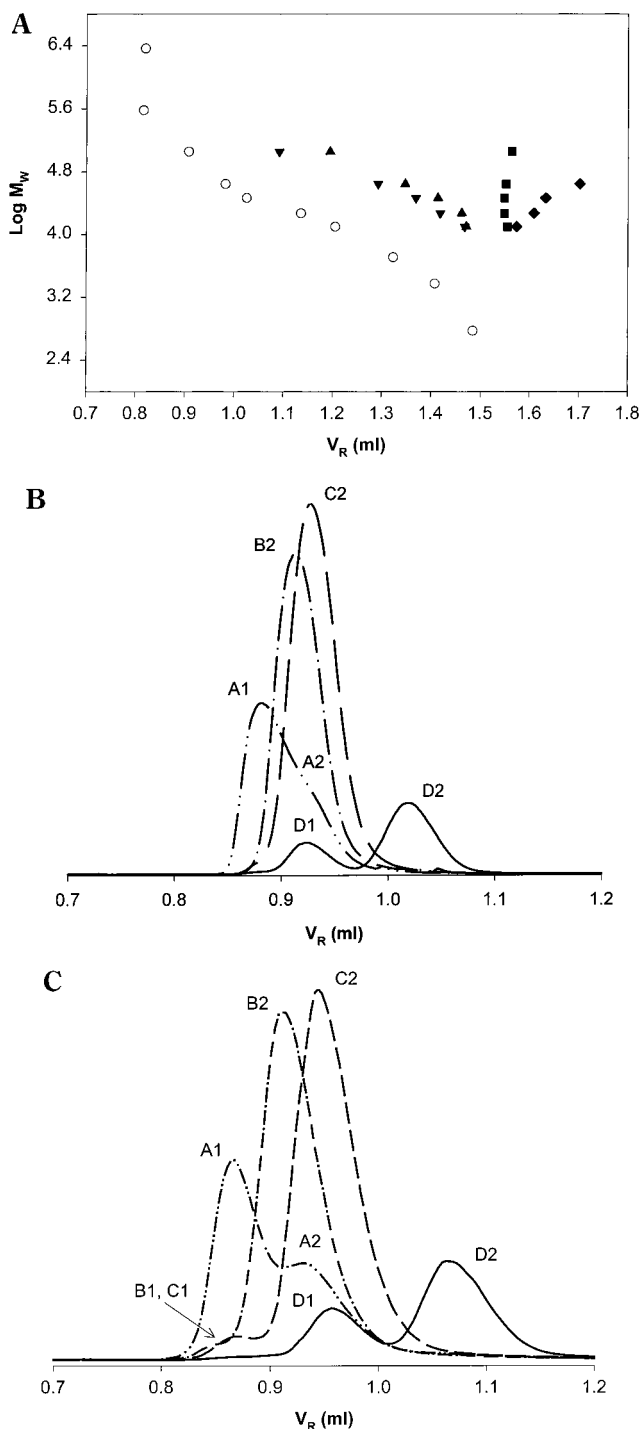


Figure 7. (A) Calibration curves of PS standards with pressure change, 54% CO<sub>2</sub>/46% THF (unless otherwise noted) at 343 K, and 0.36 mL/min using ELSD detection and a bare silica stationary phase: (○) 100% THF, (▼) 442 atm, (▲) 374 atm, (■) 288 atm, and (◆) 255 atm. (Column inlet pressure, P<sub>i</sub>, is listed; error bars are smaller than plot symbols.) (B) Chromatograms of copolymers at the SEC mode corresponding to the calibration curve (○) detailed in panel A. (---) G1657, (- · -) G1650, (- -) G1652, and (—) G 1726. (C) Chromatograms of copolymers at the LCCC mode corresponding to the calibration curve (■) detailed in panel A. (---) G1657, (- · -) G1650, (- -) G1652, and (—) G 1726.

utility of pressure change of the enhanced-fluidity liquid mobile phase in order to achieve the three modes of chromatography with one bulk mobile phase composition (46 mol % CO<sub>2</sub>/54 mol



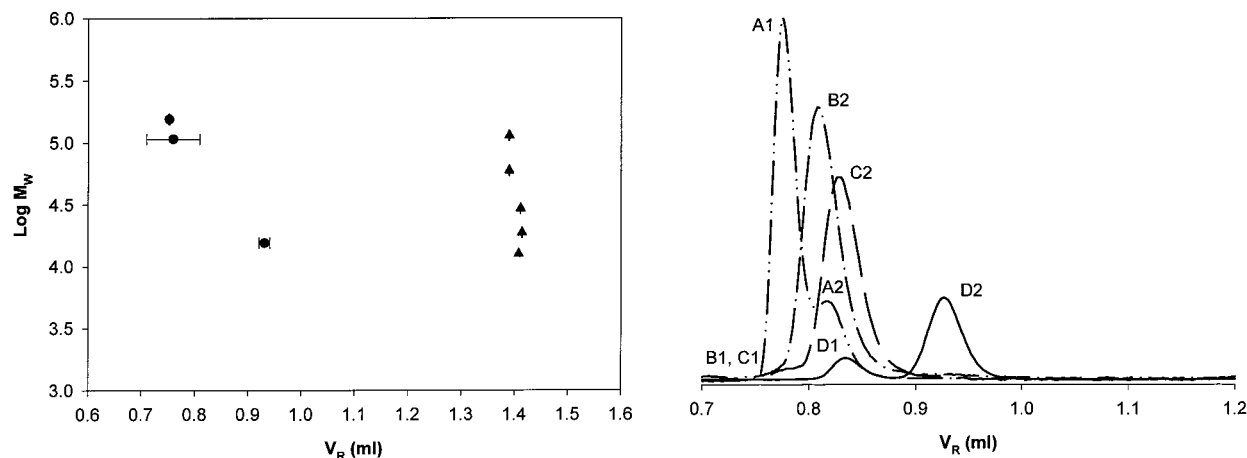


Figure 8. (A) Calibration curves of PS and PE standards at the critical condition of PS; 45% CO<sub>2</sub>/55% xylene, 413 K columns,  $P_i$  of 435 atm, 0.36 mL/min flow-rate, ELSD detection with drift tube temperature of 393 K, and a bare silica stationary phase. PE, (●) standards are in size exclusion mode and PS, (▲) standards are in the critical mode. (B) Chromatograms of the copolymers at the conditions in panel A: (---) G1657, (---) G1650, (---) G1652, and (—) G1726.

% THF at 343 K) for PS standards. At an inlet pressure >288 atm, an exclusion mode was established. At a pressure of 288 atm, the critical mode was achieved, and at pressures lower than 288 atm, adsorption mode chromatography was established.

Figure 7B shows the chromatograms of the copolymer samples at the exclusion mode for PS standards with a mobile phase of 100% THF, 343 K, and a reduced velocity of 2.0. Table 3 details the molecular weight assignment of these samples by establishing a linear regression to the PS calibration curve detailed in Figure 7A. At these conditions, sample G1726 eluted under two chromatographic bands, and samples G1650 and G1652 were eluted under one chromatographic band in the expected elution order. Sample G1657 was the earliest eluting copolymer and appeared to elute under one chromatographic band (band A1) with a slight shoulder at the tail end of the band (band A2). The literature provided by the manufacturer predicts an exclusion mode elution order as follows: G1650, G1657, and G1652, then G1726. Actual values for molecular weight were not provided, but samples G1650 and G1657 are categorized as similar in molecular weight (medium) and samples G1652 and G1726 are similarly categorized (low).<sup>36</sup> On the basis of the product information, sample G1657 should have a retention volume that lies between samples G1650 and G1652, indicating that the slight shoulder of this chromatographic band represents the actual sample. This information also suggests that the first elution band of sample G1726 corresponds to the copolymer (the elution band at the high retention volume is suspected to be a reaction precursor). It is noted that samples G1657, G1652, and G1726 were essentially unresolved at these conditions. The application of LCCC offered more information about these copolymer samples.

To further classify these copolymer samples, analysis at the critical mode for the PS standards at the same temperature (343 K) and a similar reduced velocity (2.6) was established. When operating at the critical condition for the PS standards, the copolymers eluted in the size-exclusion mode. Figure 7C shows that the elution bands for the copolymers shifted, and greater resolution between the samples was achieved. At the LCCC

conditions, the large elution band for sample G1657 was shifted to a lower retention volume, and the shoulder (band A2) was resolved and shifted to a higher retention volume. Samples G1650 and G1652 were also shifted to higher retention volumes, and a small shoulder was resolved (B1 and C1, respectively). Both bands B1 and C1 coeluted with band A1. The two bands for sample G1726 (D1 and D2) were shifted to higher retention volumes. While operating at the critical condition for the PS standards, it became apparent that the three triblock copolymers (G1650, G1652, and G1657) are blended with a high-molecular-weight byproduct. This byproduct behaves chromatographically differently from the copolymer samples. Further information was gained by analyzing the molecular weight information of the PB-*co*-PE block of the polymer samples.

The copolymer samples were analyzed with a critical mobile phase of 55 mol % xylene/45 mol % CO<sub>2</sub> and a temperature of 413 K. This was established so that PE standards could be examined and used to establish molecular weight data for the PE-*co*-PB block of the copolymer samples. Figure 8A shows that at a pressure of 435 atm, this chromatographic system was operating at the critical condition for the PS standards and the size-exclusion mode for the PE standards. Figure 8B shows the chromatograms of the copolymer samples. At these conditions, resolution of two bands for sample G1657 and a front-end shoulder for samples G1650 and G1652 were apparent.

The establishment of an SEC calibration curve while operating the PS standards at the critical mode allowed for the assignment of molecular weight information for the copolymer samples without the influence of the PS block. Table 4 lists the molecular weight data calculated for the PB-*co*-PE block of the copolymer samples against the PE standards. As expected, samples G1650 and G1657 were found to have a similar molecular weight. Samples G1652 and G1726 were also found to have a similar molecular weight that was lower than the aforementioned samples. This information is consistent with the information provided in the product literature.

According to LCCC theory, the newly established molecular weight information should be lower than the total copolymer

(36) Technical information provided by Shell Chemical Co., Belpre, OH, 1997.

Table 4. Molecular Weight of PB-co-PE Block of Copolymers at LCCC Conditions

| sample | band | $M_w^a$ PB-co-PE (KDa) |
|--------|------|------------------------|
| G1657  | A1   | 109                    |
| G1657  | A2   | 71                     |
| G1650  | B1   | 109                    |
| G1650  | B2   | 75                     |
| G1652  | C1   | 109                    |
| G1652  | C2   | 58                     |
| G1726  | D1   | 54                     |
| G1726  | D2   | 16                     |

<sup>a</sup> Molecular weights were established by size exclusion chromatography relative to PE (experimental conditions are described in Figure 8A). Standard deviation in molecular weight values were  $\leq 2.4$  KDa.

molecular weight assignment by a factor equal to the weight percentage of the block made chromatographically "invisible." The PS block of samples G1650 and G1652 corresponds to 29 wt % of the total copolymer sample. Samples G1726 and G1657 contain 30 and 13 wt % PS, respectively. The assumption was made that the total molecular weight values (listed in Table 3) were valid (despite the lack of resolution of the samples). On the basis of this assumption, the molecular weight value established for the PB-co-PE block of the diblock sample (G1726) was precisely 30% less than the total molecular weight assignment as expected. The triblock samples were not shifted to as high a retention volume as expected. Sample G1657, containing only 13 wt % PS, showed a molecular weight shift of 9%, corresponding to an error of 7%. Sample G1652, containing 30 wt % PS, showed a molecular weight shift of 25%, corresponding to an error of 6%. Sample G1650, the highest molecular weight sample, showed a molecular weight shift of only 10 wt %, corresponding to an error of 20%. These errors may be due to a lack of accuracy in assigning the molecular weight of the total copolymer samples because of the low resolution of these samples at the SEC mode and the lack of more appropriate calibration standards. Lee, et al. observed that the greater the molecular weight difference between two blocks of a copolymer system, the more the molecular weight assignment of the resolved block (while the other block was operating at the critical condition) was in error.<sup>12</sup> This might be an explanation for the results observed, although this cannot be confirmed because of a lack of standards for these "real world" polymer samples. A more

comprehensive study may include the establishment and subsequent analysis of these samples at the critical condition for the PE-co-PB block.

The utilization of LCCC with enhanced-fluidity liquids allowed for the comprehensive analysis of the triblock and diblock samples studied. The ability to use liquefied carbon dioxide as a nonsolvent for these polymer samples allowed for the routine establishment of two critical mobile phase systems for PS with pressure tuning of the solvent. Improved resolution with LCCC revealed the presence of a byproduct blended with the samples. The establishment of a xylene-containing mobile phase at a high temperature allowed for the size exclusion elution of PE standards while PS was made chromatographically "invisible." This allowed for the establishment of molecular weight data for the polymer block experiencing resolution (the PE-co-PB block of the copolymer samples). The copolymers with similar molecular weight ranges were established, which was not the case with the SEC analysis.

## CONCLUSION

This study demonstrated the ease of use of LCCC with enhanced-fluidity liquids. With these liquids, critical chromatographic conditions are readily maintainable with pressure and temperature control. This can increase the number of polymers that are available for LCCC analysis by offering new solvent systems for polymers that were impossible to analyze in the past. Not only is the achievement of the critical condition routine with enhanced-fluidity liquids but an efficient LCCC system for a given polymer analyte can be readily found, further increasing the utility of the technique. The ability to operate the EF-LCCC system over a range of temperatures offers flexibility in the pursuit of applications, such as lower solubility samples and thermodynamic studies. This type of control over the system for critical chromatography is one that had not been previously demonstrated. EF-LCCC studies with a commercial SFC instrument proved to be routine, allowing for the application of the technique in a routine and time-efficient manner. The analysis of both triblock and diblock copolymer samples was successful using two EF-LCCC systems.

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