Sensitive Polymer Analysis using Critical Point Chromatography and ELSD

Application Note

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Introduction

Liquid chromatography under critical conditions (LCCC), or critical point chromatography, is a technique used to investigate very small differences between the chemical structures of polymers. These differences could arise through the use of co-monomers or through the introduction of end-group functionality. Traditional interactive chromatographic techniques are often insensitive to small changes in structure and critical point chromatography has become the method of choice for these analyses.

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LCCC relies on carrying out isocratic chromatography at the so-called critical point for the polymer under investigation. In liquid chromatography of polymers, the samples are introduced into an eluent flowing through a column packed with porous media. Any retention of the polymer on the column media results from a reduction in the free energy of the polymer in solution, which can be described by:

$$\Delta G = \Delta H - T \Delta S$$

where Δ G is the change in Gibbs' free energy, Δ H is the change in enthalpy and Δ S is the change in entropy. Adsorption of the polymer by a reversed/normal-phase mechanism will result in a negative Δ H and, therefore, a negative Δ G, whereas if a size exclusion mechanism occurs then Δ S will be positive and again Δ G will be negative. Separation of any components of the polymer occurs if the reduction in Δ G differs between the individual components. For many polymers, controlling the choice of chromatographic eluent and column determines whether or not the retention mechanism is primarily or exclusively adsorption or size exclusion. Critical point conditions are reached for a given polymer/solvent combination when Δ H and Δ S are balanced and there is no change in Δ G during the analysis. Under these conditions, all components of a polymer with the same chemical composition will elute at the total permeation limit of the column, regardless of the molecular weight. At the critical point, the polymer is said to be 'chromatographically invisible', as no separation is obtained. For the majority of polymers, the critical point can be determined by making the appropriate selection of column, temperature and eluent. Critical point chromatography is very useful for obtaining compositional information for polymers with differing end groups or those containing co-monomers. If critical conditions are applied for one component of a sample, that component becomes chromatographically invisible and any separation observed is controlled by other components.

Critical point chromatography is carried out under isocratic conditions and, therefore, can be performed on a standard LC system composed of a pump, injection valve, a reversed/normal-phase HPLC column and concentration detector, without the need for complex equipment. LCCC is well illustrated using a PLRP-S column and the Agilent ELSD for the analysis of modified polyethylene glycol (PEG) and poly(styrene-b-methyl methacrylate) (PMMA).

Instrumentation

Column:	PLRP-S 100Å 5 µm, 150 x 4.6 mm (p/n PL1111-3500)
Detection:	Agilent ELSD

Materials and Reagents

Analysis of PEG
Eluent: 49% Acetonitrile in Water
Analysis of PMMA
Eluent: Polystyrene - 47% ACN in THF;
Polymethylmethacrylate - 17% ACN, 17% Water, 66% THF

Conditions

Flow Rate:	1.0 mL/min
Injection Volume:	20 µL

Results and Discussion

LCCC analysis of end-group modified polyethylene glycol

Critical point chromatography was used to analyze a PEG that had been modified with amine end groups (Figure 1). Critical point conditions for PEG were established by analyzing a series of PEG narrow standards of different molecular weights using different isocratic combinations of acetonitrile and water.

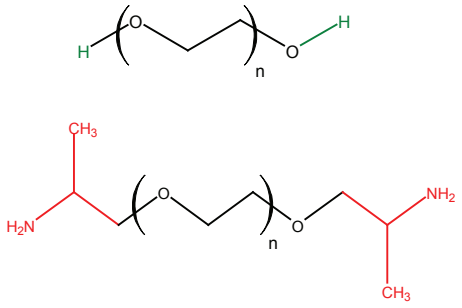

Figure 1. The structure of the original and the modified PEG materials.

Figure 2 shows chromatograms of the standards in SEC and reversed-phase mode, and at the critical point where elution is independent of molecular weight.

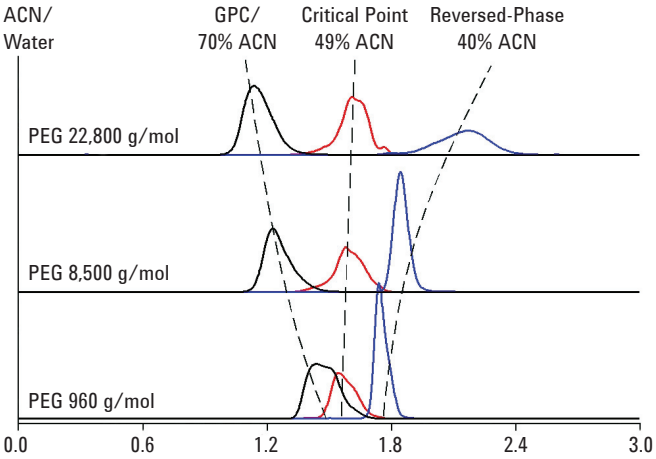

Figure 2. Analysis of PEG in SEC and reversed-phase to reveal the critical point.

Figure 3 shows a chromatogram of the amine-modified PEG material, before and after neutralization of the amine functionality with hydrochloric acid.

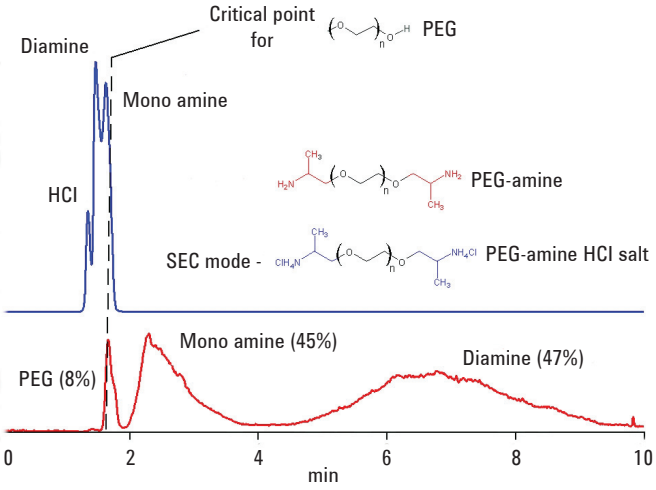

Figure 3. Amine modified PEG before and after neutralization with HCL.

Before the addition of the acid, one peak was observed at total permeation (corresponding to unmodified PEG) and two peaks were observed eluting in interactive mode (after total permeation of the column). The two peaks eluting in interactive mode were assigned as the mono and di-amine end-group modified PEGs. Based on the peak areas, the ratio of components assigned as 8% PEG, 45% mono-amine and 47% di-amine. Addition of the hydrochloric acid changed the elution to SEC mode (elution before the PEG peak), indicating the sensitivity of the chromatography at critical conditions to sample chemistry.

LCCC Analysis of Poly(styrene-b-methyl methacrylate)

Critical point chromatography was used to analyze a sample of poly(styrene-b-methyl methacrylate) block copolymer, whose structure is shown in Figure 4.

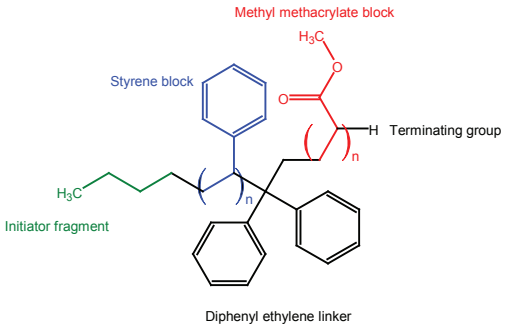

Figure 4. Structure of poly(styrene-b-methyl methacrylate).

Critical conditions were established for both polystyrene and polymethyl methacrylate by running narrow standards of varying molecular weight using different isocratic mixtures of solvents.

Figure 5 shows critical point diagrams for the polystyrene and polymethyl methacrylate standards.

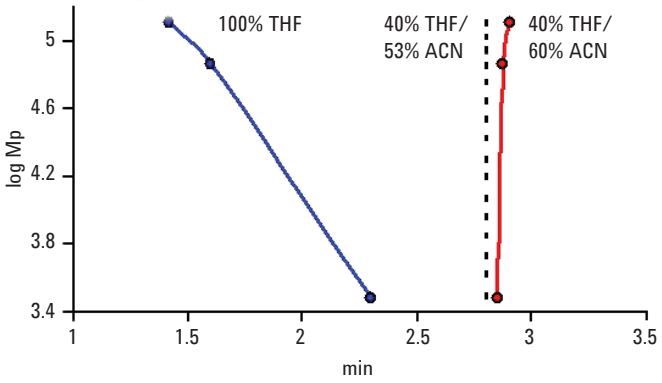

Figure 5a. Critical point diagrams for PS.

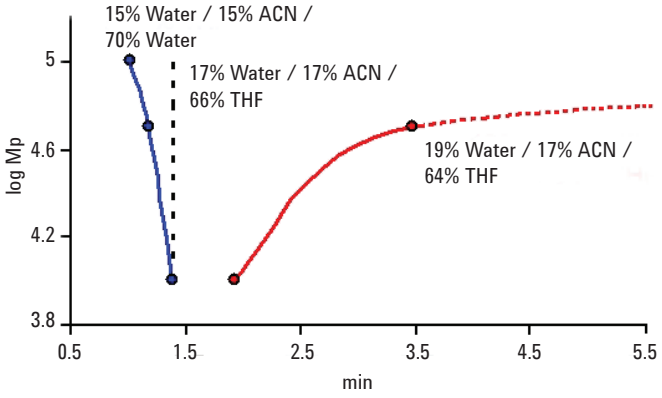

Figure 5b. Critical point diagrams for PMMA.

Under the critical conditions for PMMA, chromatography of PS resulted in elution based on adsorption mode.

Chromatograms of two polystyrene narrow standards eluting under polymethyl methacrylate critical conditions are shown in Figure 6.

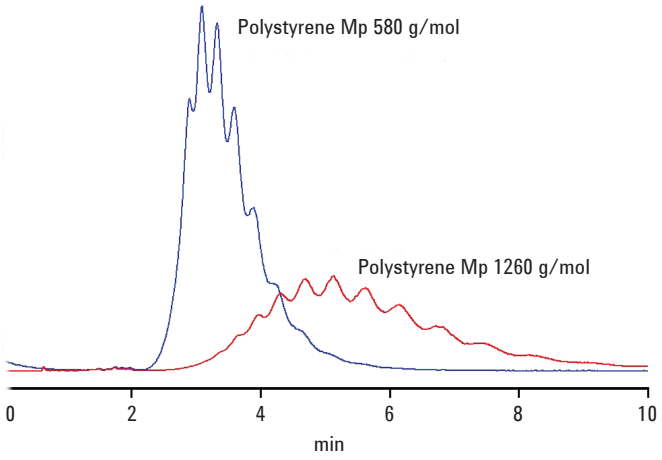

Figure 6. Chromatograms of polystyrene standards under polymethyl methacrylate critical conditions.

Under the critical conditions for PS, PMMA resulted in elution based on SEC.

Figure 7 shows chromatograms of a series of polymethyl methacrylate narrow standards and a SEC calibration curve obtained under the polystyrene critical conditions.

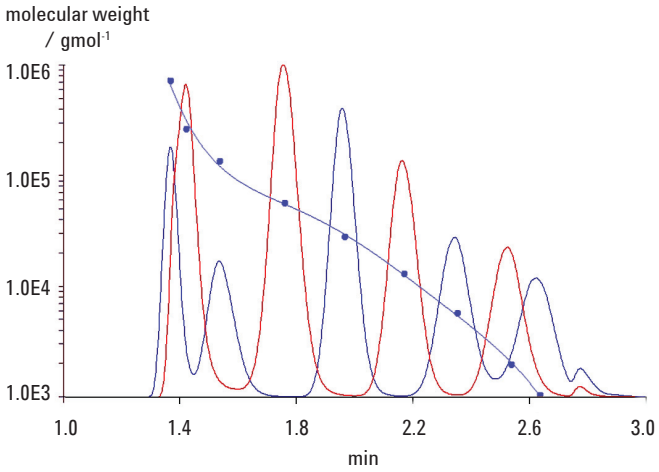

Figure 7. PMMA standards and SEC calibration curve run under PS critical conditions.

The poly(styrene-b-methyl methacrylate) sample was analyzed under the critical conditions for polystyrene. Using these conditions, the elution is controlled purely by the molecular weight of the methyl methacrylate block of copolymer, the polystyrene block is 'chromatographically invisible'.

Figure 8 shows the molecular weight distributions of the polystyrene block by conventional GPC using polystyrene standards before introduction of the methyl methacrylate and growth of the PMMA block, and the PMMA block under critical conditions for polystyrene using PMMA standards.

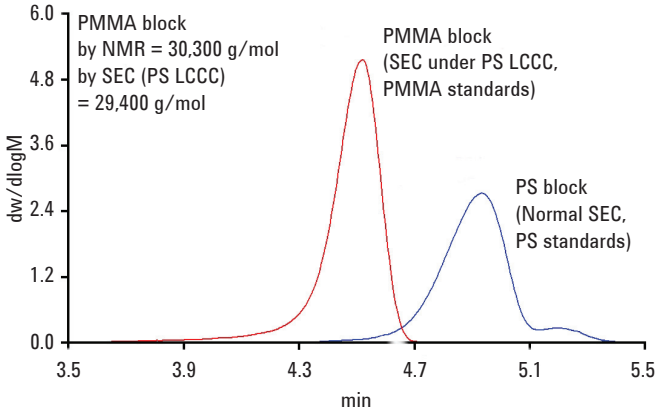

Figure 8. Molecular weight distributions of the styrene block before reaction with PMMA, and the PMMA block under critical conditions for polystyrene.

Using these results, the molecular weight of the total polymer and the comparative block lengths could be determined as approximately 71% polystyrene, 29% polymethyl methacrylate. The results showed good agreement with the results from NMR experiments.

Conclusion

Critical point chromatography is a powerful tool for analyzing small chemical changes in the structure of polymers, such as the inclusion of co-monomers to a polymer backbone or a change in end-groups.

In many cases, traditional chromatography techniques are not sufficiently sensitive to show these changes, and critical point chromatography offers a cheaper and easier route to structural analysis compared to other techniques that have been used in the past, such as NMR.

However, critical point chromatography is extremely sensitive to the chemistry of the sample and column and so a specific methodology must be developed for each application.

PLRP-S columns and the Agilent ELSD is an ideal combination for these challenging applications.

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