

Use of High Speed/High Resolution Size-Based Chromatographic Separation of Polymeric Mixtures with Offline Infrared Detection

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OVERVIEW

- Assess the potential of adding Fourier Transform Infrared (FTIR) analysis to a Size Exclusion Chromatography (SEC) system, to explore operational conditions and their impact on FTIR data generated.
- High speed/high resolution SEC experiments are supported using Waters® ACQUITY® Advanced Polymer Chromatography® (APC™) System, enabling rapid solvent exchange between experiments, and modified FTIR parameters while maintaining chromatographic performance.
- The potential benefits are explored highlighting the spectral identity and effect on chromatographic detector quality.

INTRODUCTION

Advances in polymerization processes have expanded from traditional condensation polymerization and free radical polymerization to include processes such as anionic polymerization, emulsion polymerization, RAFT, ATRP, and others. The development has evolved from simple polymer chains to complex polymers capable of performing multiple functions within a single molecular chain. As these new materials evolve, their control and understanding have come under intense scrutiny, requiring a wide range of analytical technology ranging from chromatographic separation to advanced mass spectrometry.^{1, 2}

Addressing the challenges of material characterization has often been focused on hyphenated detection techniques coupled with separation. This approach utilizes a concentration detector such as a refractive index (RI) detector and/or ultra violet (UV) detection coupled to other analyte specific type detection.³

With the introduction of high speed / high resolution size-based separation techniques, the resolution and speed of the separation is maintained from injection to detection with traditional detector options such as RI and UV detection. This novel approach to the design of the APC separation equipment, including the separation column and entire flow path, are used to yield high resolution. However, the use of this high speed high resolution separation technique has seen limited pairing with offline sample analysis techniques such as infrared spectroscopy.⁴

In this poster note, the expansion of the Waters Advanced Polymer Chromatography (APC) approach to the size-based separation is presented. Utilizing a controlled split flow, the sample is diverted to both a Waters ACQUITY Refractive Index Detector (RI) and an HTX LC -Transform 700 sample collection system (LC-T 700) for Fourier Transform Infrared (FTIR) analysis while maintaining the required system dispersion control needed for the APC experiment.⁵



Figure 1. Waters ACQUITY APC SEC Core 1 System with post column split flow to the ACQUITY RI Detector and the HTX Technologies LC-Transform 700 sample collection system.

EXPERIMENTAL

Conditions for demonstration measurements

The test conditions are based on using a high speed, high resolution ACQUITY APC SEC Core 1 System coupled to an HTX Technologies LC-Transform 700 sample collection system, the LC-T 700. The two detectors are managed by using a post column split flow at a fixed 1:1 ratio, (Figure 1). Samples evaluated are a mixture of two narrow polystyrene standards and a mixture of a narrow polystyrene standard and a narrow polymethylmethacrylate standard dissolved in the chromatographic system mobile phase. All LC-T 700 samples were collected on a germanium disk. The germanium disk was evaluated using the Bruker Tensor II FTIR bench, fitted with a reflection stage produced by PIKE Technologies, specifically designed for the evaluation of the LC-T 700 collection disks.

SEC conditions

System:	Waters APC Core 1
Eluent:	THF
Flow rate:	1.0 mL/min
Sample concentration:	1 mg/mL of each narrow standard (2 mg/mL total)
Injection volume:	10 μ L
Column temp.:	40 $^{\circ}$ C
Columns:	Waters 4.6 x 150 mm APC XT 450 \AA , 125 \AA and 45 \AA in series
Conc. detector:	Waters ACQUITY RI at 40 $^{\circ}$ C
LC-T 700 detector:	Conditions below
Split ratio:	HTX LC-T 700/RI 1:1 using two equal lengths of 0.004 inches ID SS tubing

LC-T Conditions (optimized for APC chromatography)

LC-T 700 system:	HTX LC-Transform 700
Probe temp.:	90 $^{\circ}$ C
Nebulizer gas:	Nitrogen
Gas flow:	3.0 L/min.
Spray nozzle height:	5 mm above the disk surface
Sampling rate:	100 degrees/min.
Collection delay:	2 min.
Sample collection:	310 degrees of rotation

LCT disk FTIR reader conditions

Data collection:	Bruker Opus System with LC-T 4 driver
Resolution:	4 cm^{-1}
Number of scans:	8

Chromatographic system control

Waters Empower[®] 3 FR2 Software was used for instrument control, data acquisition, and chromatographic data processing.

RESULTS AND DISCUSSION

In this study, two sample sets were studied to assess the capability and impact of band spread of the LC-T 700 FTIR. The two samples are chosen to contain 55,000 Dalton (Da) and 31,000 Da polystyrene (PS) and 55,000 Da PS and 31,600 Da polymethylmethacrylate (PMMA). A split flow is used to send a portion of the sample sets to the RI detector, and the resulting APC chromatograms are used to indicate the resolution of the analysis (Figure 2).

The two narrow polymer samples are from a single injection collected on the rotating germanium disk in the LC-T 700 collection device (Figure 3). The two red arrows point to the collection of each narrow polymer distribution corresponding to their chromatographic elution profile.

The germanium disk inserted in the LC-T FTIR accessory allows for the collection of the spectra as a function of the rotational location corresponding to the elution time or volume (Figure 4). The small red dot is the focal point of the data collection. Using the sampling rate of FINE, the spectra is collected at every degree of rotation, or every 0.01 minutes as specifically done in this experiment.



Figure 3
The above image is the germanium collection disk in the HTX LC-T 700. The two spots identified by the red arrows indicate the deposit of the two narrow polystyrene samples collected (top 55000 Da bottom 31000 Da).

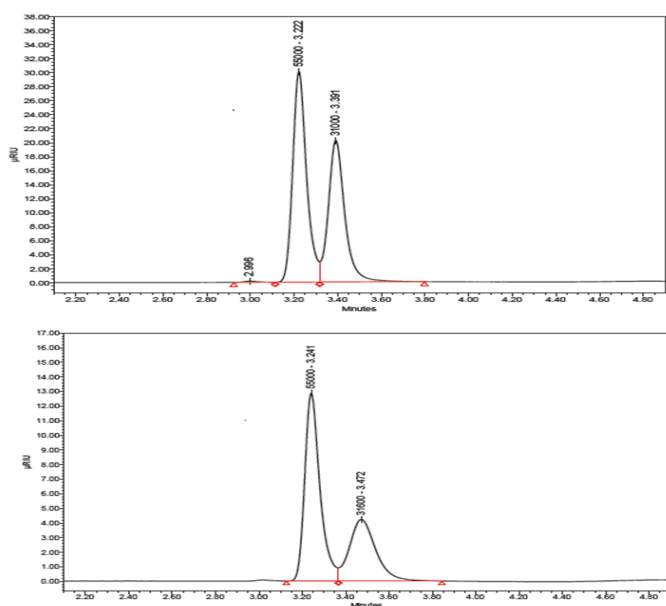


Figure 2. Top chromatogram is a mixture of narrow molar mass PS centered at 55,000 Da and 31,000 Da collected on an RI detector. Bottom chromatogram is a mixture of narrow molar mass PS centered at 55,000 Da and PMMA centered at 31,600 Da collected on an RI detector.

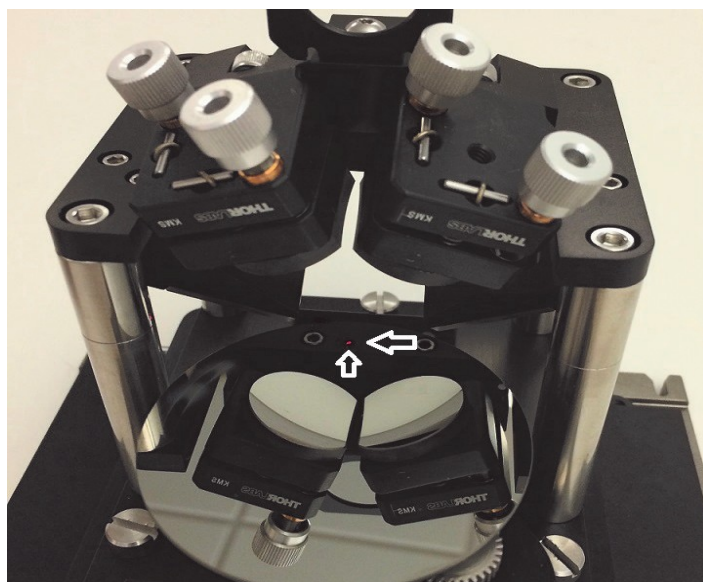


Figure 4. LC-T 700 FTIR Interface. The white arrows pointing to the focused IR collection point (red dot) on the germanium disk.

The typical FTIR spectra collected in Transmission mode is demonstrated below (Figure 5). If the spectra is converted from transmission to absorbance, the local maxima can be selected for polystyrene at 701 cm^{-1} and for polymethylmethacrylate 1726 cm^{-1} . The relative concentration of each narrow polymer standard can be collected as a function of elution volume.

Comparing the signal from the split chromatographic flow, using the refractive index detector (black) and the LC-T 700 collection for FTIR analysis (red), a great match is observed between the detectors for the mixed PS sample (Figure 6-top). The comparison of the two detector modes shows an acceptable $\sim 20\%$ band spread with the FTIR measurement.

Processing the FTIR data for the second sample (Figure 6-bottom), a mixture of $55,000\text{ Da}$ PS and $31,600\text{ Da}$ PMMA, two different frequencies are measured: 701 cm^{-1} for PS (red) and 1726 cm^{-1} for PMMA (green). These plots are overlaid with the signal from the refractive index detector (black). The ability to measure the molar mass distribution for the mixed sample, using the RI detector signal and the distribution of each individual polymer, is based on its spectral identity.

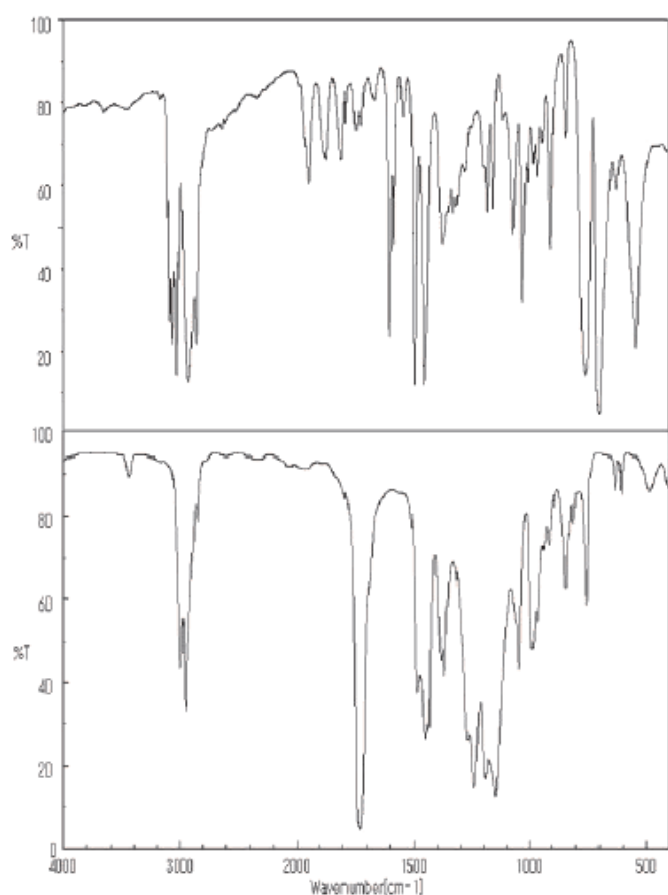


Figure 5

Top image is an IR Transmission spectra of PS. The bottom image is an IR Transmission spectra of PMMA. Based on the spectra, the transition band at 701 cm^{-1} is selected for PS and the band at 1726 cm^{-1} is selected for PMMA.

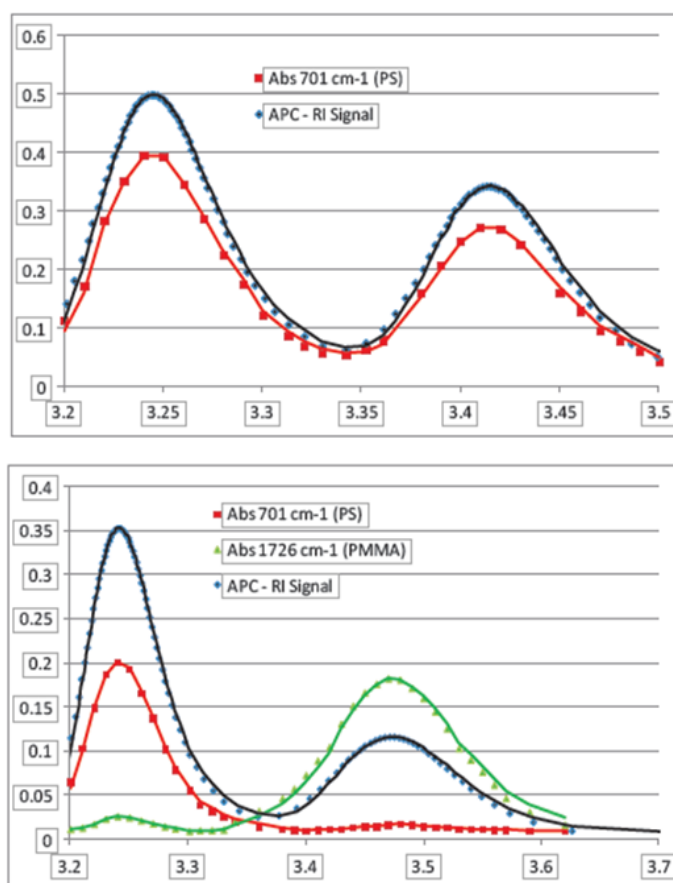


Figure 6

Top image is an overlaid plot of the injection of two narrow PS samples, showing the intensity of the IR spectra at 701 cm^{-1} against the elution time of the separation (red) and the RI detector signal as a function of elution time (black).

Bottom image is an overlaid plot of the injection of a narrow PS and a narrow PMMA sample showing the intensity of the PS IR spectra at 701 cm^{-1} against the elution time of the separation (red), the intensity of the PMMA infrared spectra at 1726 cm^{-1} against the elution time of the separation (green) and the RI detector signal as a function of elution time (black).

CONCLUSIONS

- Coupling the APC, high resolution / high speed SEC Core 1 System to the LC-T 700 is easily managed with the selection of the correct split ratio, tubing diameter, and sample scanning rates.
- The impact of the LC-T 700 on chromatographic band spread is minimal: approximately 20% increase in band spread.
- In the example studied, adding unique polymer-specific FTIR spectral identity to the SEC experiment offers the benefit of identification and qualification of individual polymers in a polymer mixture.
- The high speed chromatography offered by the ACQUITY APC System is a perfect match to the experimental conditions available with the LC-T 700 system enabling high resolution FTIR data

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