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

Author: Michael O'Leary¹, Jennifer Gough¹, Damian Morrison^{1,} Alain Creissen² Affiliation: 1; Waters Corporation, Milford, MA USA 2; HTX Technologies LLC, Chapel Hill, NC, USA

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 has come under intense scrutiny utilizing a wide range of analytical technology ranging from chromatographic separation to advanced mass spectrometry.

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.

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

In this study 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 for** Fourier Transform InfraRed (FTIR) analysis (LCT 700) while maintaining the required system dispersion control needed for the APC experiment.

THE INTERNATIONAL SYMPOSIUM ON GPC/SEC AND RELATED TECHNIQUES ptember 26th - 29th 2016 Amsterdam, Netherlands gpcevent.com

METHODS

Conditions for demonstration measurements:

The test conditions are based on using a high speed, high resolution ACQUITY APC SEC system¹ coupled to an HTX Technologies LC-Transform 700 sample collection system, the LCT 700. The two detectors are managed by using a post column split flow at a fixed 1:1 ratio, (see figure 1). Samples evaluated are a mixture of two polystyrene standards and a mixture of a narrow narrow polystyrene standard and a narrow polymethylmethacrylate standard dissolved in the chromatographic system mobile phase. All LCT samples were collected on a Germanium disk. The Germanium disk was evaluated using Bruker Tensor II FTIR bench fitted with a reflection stage produced by PIKE Technologies specifically designed for the evaluation of the LCT collection disks.

SEC Conditions

•	System	Waters APC Core 1 System
•	Eluent:	THF
•	Flow rate:	1.0 mL/min
•	Sample Concentration	1 mg/ml of each narrow standard
		(2 mg/ml total)
•	Injection volume:	10 μL
•	Column Temp.:	40 ° C
•	Columns	Waters 4.6X150 mm APC XT 450 Å,
		125 Å and 45 Å in series
•	Conc. Detector	Waters ACQUITY RI detector @40 \degree C
•	LCT detector	Conditions below
•	Split ratio	HTX LCT Detector/RI 1:1 using two
	•	equal lengths of 0.004" i.d. SS tubing

LCT Conditions (optimized for the APC chromatography)

_CT system:	HTX LC-T ransform700
Probe Temperature:	90 °C
Nebulizer gas	Nitrogen
Gas flow	3.0 liters/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

Bruker Opus System with LCT 4 driver
Fine (1 degree of rotation/ spectra)
4 cm-1
8

Chromatographic System Control

Waters Empower 3 FR2 Software for instrument control, data acquisition and chromatographic data processing from the RI detector.

Figure 1. Waters ACQUITY Core 1 APC system with post column split flow to ACQUITY RID and LCT 700 sample collector

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

RESULTS 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 2.80 3.00 3.20

Figure 2. Top chromatogram is a mixture narrow molar mass polystyrene centered at 55,000 Da and 31,000 Da collected on a RI detector. Bottom chromatogram is a mixture narrow mo*lar mass polystyrene centered at 55,000 Da and polymethyl* methacrylate centered at 31,600 Da collected on a RI detector.

Figure 3. The above image is the germanium collection disk in the HTX LCT 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 4. LCT – FTIR Interface. The white arrows pointing to the focused IR collection point (red dot) on the germanium disk.



DISCUSSION

In this study two sample sets are studied to assess the capability and impact of band spread of the LCT FTIR. The two samples are chosen to contain 55,000 Dalton (Da) and 31,000 Da polystyrene (PS) and 55,000 Da polystyrene and 31,600 Da polymethylmethacrylate (PMMA). The APC chromatograms using a potion of the split flow supplying the refractive index detection of the two sample sets are shown in figure 2 indicating the resolution of the analysis.

In figure 3 we see the two narrow polymer samples from a single injection collected on the rotating germanium disk in the LCT 700 collection device. The two red arrows point to the collection of each narrow polymer distribution corresponding to their chromatographic elution profile.

Figure 4 shows the germanium disk inserted in the LCT FTIR accessory allowing for the collection of the spectra as a function of the rotational location corresponding to the elution time or volume. The small red dot is the focal point of the data collection. Using the sampling rate, FINE, we can collect spectra every degree of rotation or in this experiment every 0.01 minutes a spectra is collected.

Figure 5 shows the typical FTIR spectra collected in Transmission mode. If we convert the spectra from transmission to absorbance we can select the local maxima 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 LCT 700 collection for FTIR analysis (red) we can see the great match is observed between the detectors for the mixed polystyrene sample (figure 6-top). The comparison of the two detector modes shows an acceptable ~20% band spread with the FTIR measurement.

Processing the FTIR data for the second sample, a mixture of 55,000 Da PS and 31,600 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) - bottom of figure 6. Here we can see the ability to measure the molar mass distribution for the mixed sample using the RI detector signal and the distribution of each individual polymer based on its spectral identity.



Waters THE SCIENCE OF WHAT'S POSSIBLE.

Figure 5 Top image is an infrared Transmission spectra of polystyrene. The bottom image is an infrared Transmission spectra of polymethylmethacrylate. Based on the spectra the transition band at 701 cm⁻¹ is selected for polystyrene and the band at 1726 cm⁻¹ is selected for polymethylmethacrylate.



Figure 6. Top image is an overlaid plot of the injection of 2 narrow polystyrene samples showing the intensity of the infrared spectra at 701 cm⁻¹ against the elution time of the separation (red) and the refractive index detector signal as a function of elution time (black).

Bottom image is an overlaid plot of the injection of a narrow polystyrene and a narrow polymethylmethacrylate sample showing the intensity of the infrared spectra at 701 cm⁻¹ (PS) against the elution time of the separation (red), the intensity of the infrared spectra at 1726 cm⁻¹ (PMMA) against the elution time of the separation (green) and the refractive index detector signal as a function of elution time (black).

CONCLUSION

- Coupling the APC, high resolution / high speed SEC system to the LCT 700 is easily managed with the selection of the correct split ratio, tubing diameter and sample / scanning rates.
- The impact of the LCT 700 on chromatographic band spread is minimal, approximately 20% increase in band spread.
- In the example studied, the benefit of the additional FTIR data to the SEC experiment is clear.
- The high speed chromatography offered by the APC is a perfect match to the experimental conditions available with the LCT 700 system enabling high resolution FTIR data collection.