

## Use of High Speed/High Resolution Size-Based Chromatographic Separation of Surfactants and Oligomeric Materials with Single Quadrupole Mass Spectrometry

Michael O'Leary, Jennifer Gough, Tanya Tollifson  
Waters Corporation, Milford, MA USA

### OVERVIEW

- Assess the potential of adding mass spectrometry (MS) to a Size Exclusion Chromatography system (SEC), explore operational conditions and their impact on MS data generated.
- High speed/high resolution SEC experiments are supported using the Waters® ACQUITY® Advanced Polymer Chromatography® (APC™) System, enabling rapid solvent exchange between experiments, modified MS parameters, and ionization modes while maintaining chromatographic performance.
- The potential benefits and limitations of different ionization modes are explored highlighting the molar mass coverage and data complexity from the operating conditions.



Figure 1. Waters ACQUITY APC System with post column split flow to the ACQUITY RI and ACQUITY SQ Detector 2 Mass Spectrometer.

### INTRODUCTION

Recent developments in polymerization processes have utilized a wide array of strategies. 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 size exclusion chromatography (SEC) separation. One approach, the so called triple detector approach, involves the use of a concentration detector such as a refractive index (RI) detector, a viscosity detector, and a multi angle light scattering detector to provide additional information from the polymer analysis. Other schemes have employed other detectors such as infrared and even mass spectrometry (MS) coupled to the SEC experiment .

Exploring the many variables for optimum operation of the SEC-MS experiment for individual sample types has proven to be difficult due to the relatively long test cycle time and even longer equilibration time required for typical SEC experiments. With the introduction of the Waters Advanced Polymer Chromatography (APC) System's size-based separation technique, a novel approach is used to yield a high speed and high resolution 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 MS detection due to the need to control material ionization and solvent matrix effects. In this study, the expansion of the APC approach to the size-based separation is presented. A single system control platform is evaluated to pair the chromatographic system to the MS detector system. This enables a high throughput and high resolution evaluation of size-based separation of polymeric material while allowing for controlled ionization without interfering with the chromatographic separation.<sup>1</sup>

## EXPERIMENTAL

### Conditions for demonstration measurements

The test conditions are based on using a high speed, high resolution ACQUITY APC SEC System<sup>1</sup> coupled to a Waters SQ Detector 2 single quadrupole mass spectrometer. The two detectors are managed by using a post column split flow at a fixed 2:1 ratio, shown in Figure 1). Samples evaluated are PEG and Tergitol surfactants dissolved in the chromatographic system mobile phase.

### SEC conditions

System:	ACQUITY APC System
Eluent (PEG):	20/80 Methanol/water 0.05% formic acid
Eluent (Tergitol):	95/5 Methanol/water
Flow rate:	0.6 mL/min
Injection volume:	10 $\mu$ L
Column temp.:	40 $^{\circ}$ C
Columns:	2 Waters 4.6x150 mm BEH SEC 125 $\text{\AA}$
Conc. detector:	ACQUITY RI Detector @40 $^{\circ}$ C (split between RI & mass spec)
Split ratio:	Refractive Index / mass spectrometer 2:1

### MS conditions

MS system:	SQ Detector 2
Probe type:	ESI & IonSABRE™ II APCI
Ionization mode:	ESI <sup>+</sup>
Capillary voltage:	3.0 kV
Desolvation temp.:	500 $^{\circ}$ C ;
Source temp.:	150 $^{\circ}$ C
Cone voltage:	10 V
Sampling rate:	5 Hz
MS scan range:	80 to 2000 <i>m/z</i>
Ionization mode:	APCI <sup>+</sup>
Corona Current:	1-4 $\mu$ A (4 $\mu$ A for increased signal)
Desolvation temp.:	500 $^{\circ}$ C
Source temp.:	150 $^{\circ}$ C
Cone voltage:	5-50 V (5 V optimum for low charge)
Sampling rate:	5 Hz
MS scan range:	80 to 2000 <i>m/z</i>

## RESULTS AND DISCUSSION

In this study, two modes of ionization were evaluated. Each ionization mode was assessed relative to their impact on the interpretation of the polymer composition and structure. Using a high speed and high resolution SEC separation, each mode can be quickly optimized to assess the results from each ionization mode.

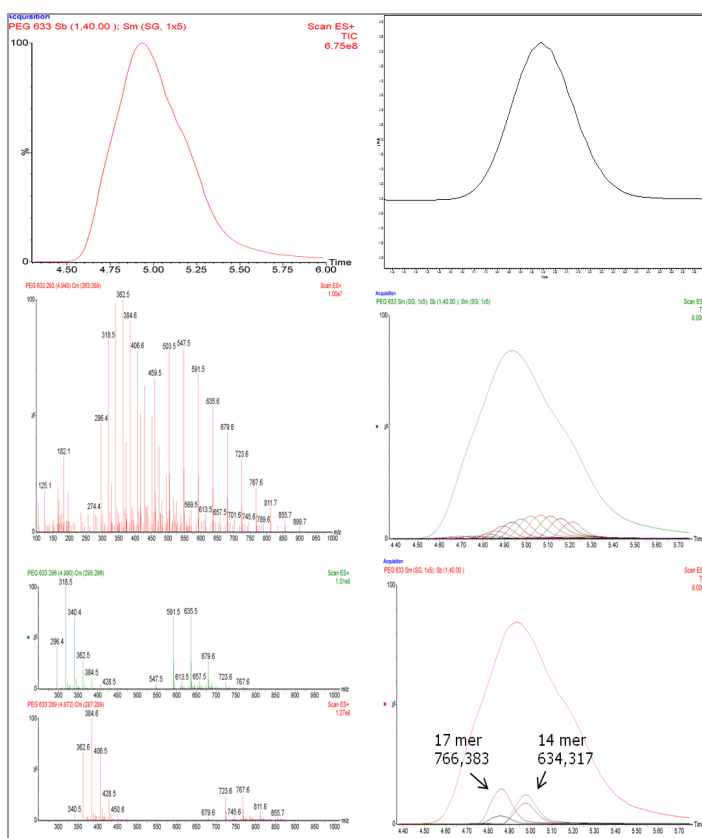


Figure 2. The top left image shows the Total Ion Chromatogram (TIC) using ESI conditions of a '633' Da PEG standard; and the top right the corresponding RI signal from the same injection.

The mid left spectra is the ESI collected across the entire size distribution showing the mass of each molecule separated by 44 Da (each EO unit or mer), as well as a second lower mass series separated by 22 due to the mass of each mer divided by the double charge ( $m/z$ ).

The mid right chromatogram shows the TIC for the sample and the Extracted Ion Chromatogram (XIC) for the different chain lengths present in the 'narrow standard'.

The bottom left spectral set shows the spectra of two chromatographic (size) slices showing the impact of the charge 1 and charge 2 state of the sample.

The bottom right overlaid chromatogram set shows the distribution of the sample and that of the 17 and 14 mer chain length. The overlapping peaks are due to the two charge states of the molecule.

In Figure 2, we see the chromatographic profile of the polyethylene glycol (PEG) sample is available from the total ion chromatogram (TIC) using Electro Spray Ionization (ESI) showing good comparison with the more conventional RI signal for the polymer distribution. Further, we can examine the mass spectra of the sample, and see the sample is made up of several different polymer chains. Additional investigation showed that for each chain, there can be at least two ions relating to the mass ( $m$ ) and its charge ( $z$ ). Showing many  $m/z$  values for multiple charges can make interpretation difficult as shown for the two examples of the 17 and 14-mer length chains.

In Figure 3, two longer chain polymer samples were evaluated. Here the situation becomes more complicated as the degree of charging increases with increasing polymer length when using ESI. Further, as the polymer chain length increases, the signal for the single charged chains all but disappear as the multiple charging species dominate.

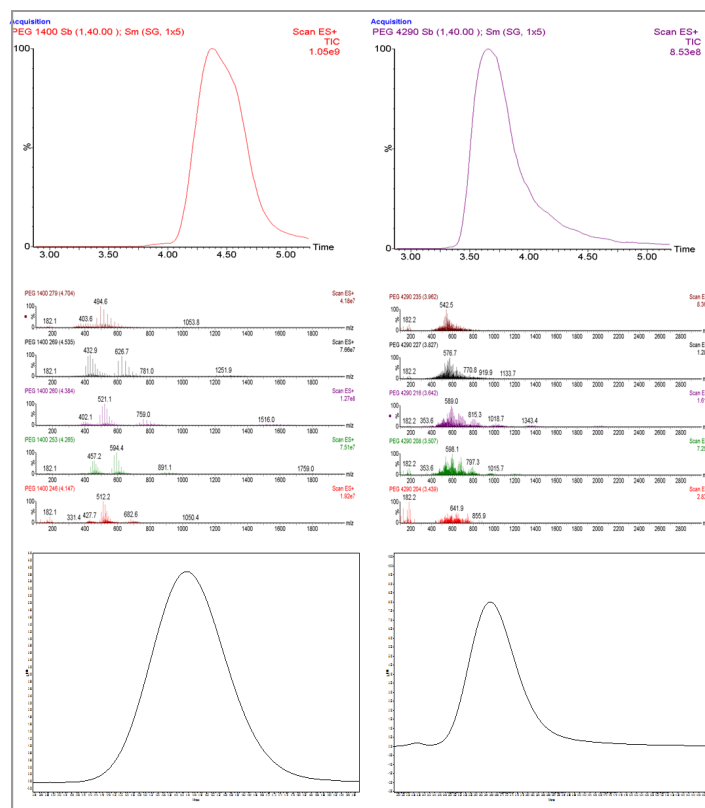


Figure 3. Top left image is the total ion chromatograms (TIC) using ESI conditions of a '1400' Da PEG standard; the mid left is the individual mass spectra as a function of retention time (size) showing +1, +2, +3 and +4 with increasing charging with chain length; and the bottom left is the corresponding RI chromatographic trace for the polymer sample.

Top right image is the total ion chromatograms (TIC) using ESI conditions of a '4000' Da PEG standard; the mid right is the individual mass spectra as a function of retention time (size) showing +2, +3, +4, +5, +6 and +7 with increasing charging with chain length (note, +7 dominating); and the bottom right is the corresponding RI chromatographic trace for the polymer.

In Figure 4, we see the effects using Atmospheric Pressure Chemical Ionization (APCI). Here we find the parent chain length will dominate the spectra at any point in the chromatogram as the charge or z will be 1. The ionization mode is shown to be optimized to control any in-source fragmentation by decreasing the cone voltage applied to the source resulting in a dominant signal for each protonated molecule under investigation.

In Figure 5, the APCI mode shows the same charge control for a longer chain PEG sample polymer with single charges dominating, and the resulting *m/z* being equal to the mass of the polymer chain.

In Figure 6, the APCI mode is used to evaluate a copolymer sample, Tergitol ( $\text{CH}_3(\text{CH}_2)_{14}(\text{C}_2\text{H}_4\text{O})_5\text{H}$ ). Quickly the data set becomes more complicated due to the subtle structural changes in length of both the alkyl and ethylene oxide (EO) in the sample—in some cases with nearly identical size. The distribution of each chain molecule can be assessed using the mass of each chain in its single charge state showing unique composition.

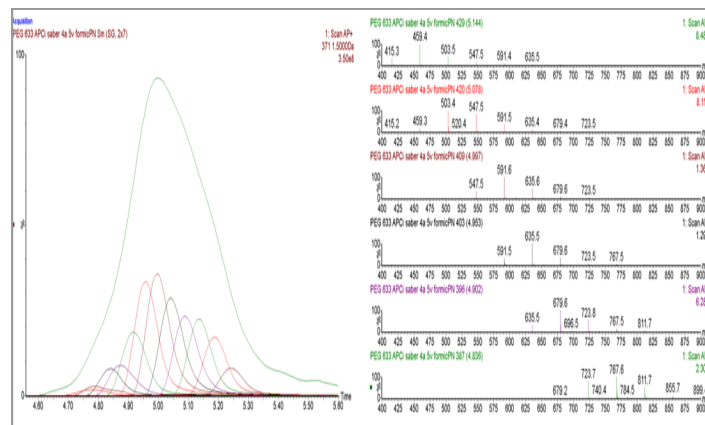


Figure 5. Left image TIC and XIC of '633' PEG using APCI. On the right is the mass spectra of the components of PEG 633.

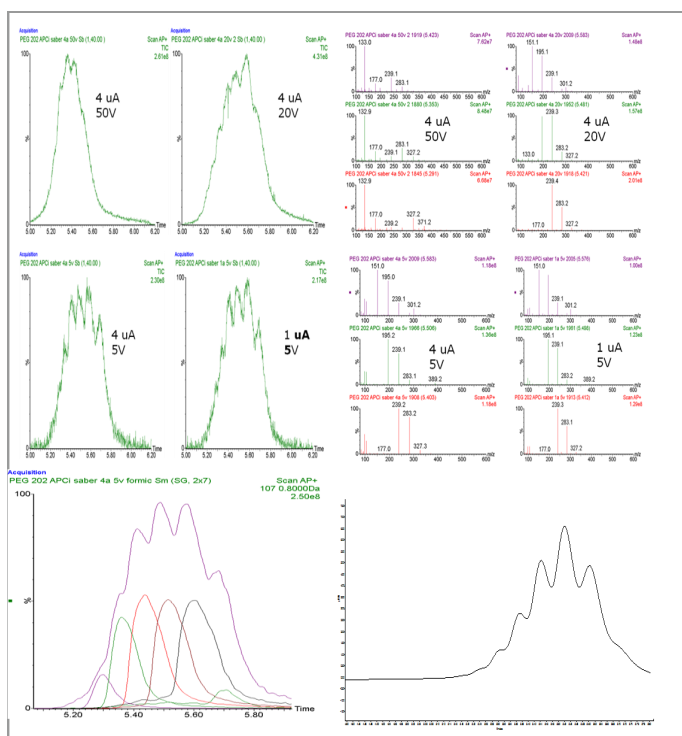


Figure 4. Top left set of images are the TIC of PEG '202' standard collected using Atmospheric Chemical Ionization (APCI) with varied corona current and cone voltage settings. Top right are spectra collected from each ionization condition extracted at the elution time corresponding to the signal maxima.

Bottom left image is the overlay of the smoothed TIC data of the PEG standard and the XIC spectra of the individual components in the mixture using the optimized cone voltage (5V) and corona current (4uA) settings. The bottom right image is the corresponding RI chromatographic trace for the polymer sample.

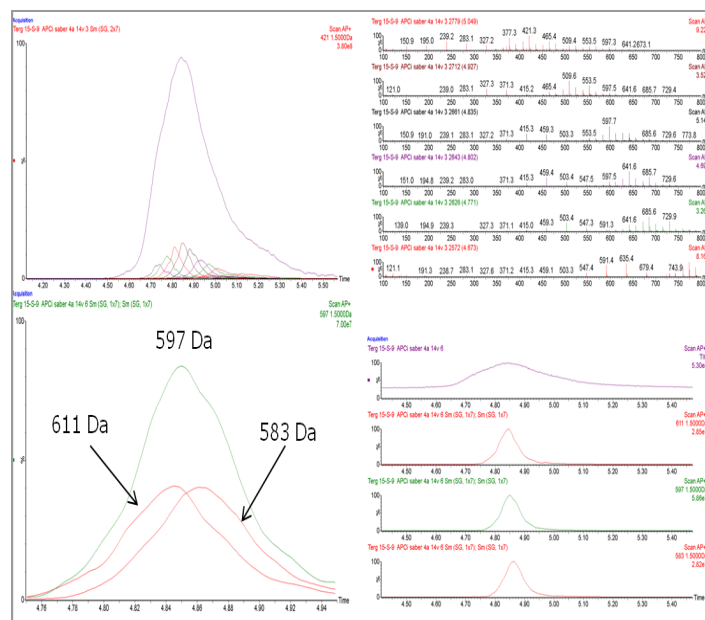


Figure 6. Top left image TIC and XIC of Tergitol ( $\text{CH}_3(\text{CH}_2)_{14}(\text{C}_2\text{H}_4\text{O})_5\text{H}$ ) using APCI. Top right are spectra collected from each ionization condition extracted at the elution time corresponding to the signal maxima.

Bottom left are the XIC of fixed EO content and varied  $\text{CH}_2$  length. Bottom right XIC relative to the full sample distribution.

## CONCLUSIONS

- Using a single quadrupole instrument shows a potential for coupling MS to APC for rapid polymer analysis and MS optimization.
- Use of ESI yields rich data sets for interrogation, but caution must be taken when evaluating the data due to charging effects with increasing chain length with increasing charge dominating the signal.
- Use of APCI allows for simplifying the mass spectral signal allowing for easier data interpretation.

## References

1. ACS Fall Meeting, Boston, MA, Polymer Division Paper ID 2253501 August 2015.
2. Waters ACQUITY APC Product Ordering Guide, November 2014.
3. Rapid Polymer Analysis with Atmospheric Solids Analysis Probe/ Mass Spectrometry (ASAP/MS), Waters Technology Brief no [720003840en](#).
4. Tergitol™ Manufactured by The Dow Chemical Co., Midland MI Supplied by Spectrum Chemicals New Brunswick, NJ, USA.

Waters, ACQUITY, Advanced Polymer Chromatography, and The Science of What's Possible are registered trademarks of Waters Corporation. APC and IonSABRE are trademarks of Waters Corporation. All other trademarks are the property of their respective owners.