Application Note

High Speed/High Resolution Size Exclusion Chromatography of Low Molecular Weight Polystyrene-Tetrahydrofuran Solutions with In-line Fraction Collection

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This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This application brief demonstrates the ability to separate low molecular weight polymer solutions into well resolved discrete molecular weight fractions through high speed/high resolution size exclusion chromatography, and in-line fraction collection for further analysis.

Benefits

Size exclusion chromatography and fraction collection of polymer solutions are fast and intuitive using Advanced Polymer Chromatography (APC) with Waters Fraction Manager-Analytical (WFM-A).

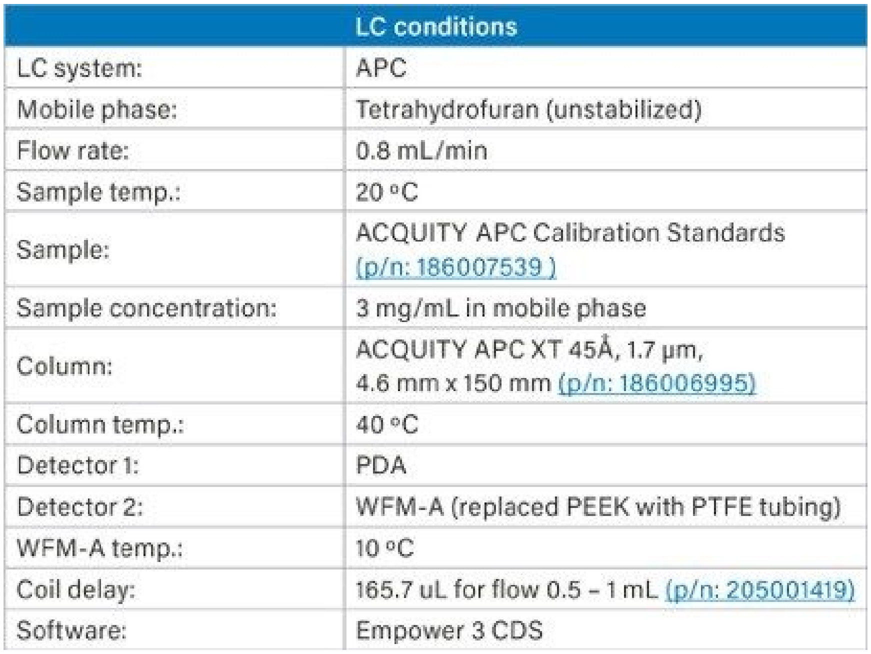
Introduction

Size exclusion chromatography (SEC) has been the standard analysis tool for polymer molecular weight characterization for decades. Polymer molecular weight distribution is used to predict polymer properties of in-process samples and finished products.[1] Collecting individual SEC molecular weight fractions for further analysis using advanced detectors can aid in-process and formulation changes by elucidating polymer chemistry and structure information.

Responding to in-process and product changes with the typical 45 minute SEC analysis time and difficult to program fraction collectors is a challenge. These analysis challenges make the phrase "streamlined workflow" sound optimistic. Size exclusion chromatography and fraction collection of polymer solutions are fast and intuitive using Advanced Polymer Chromatography (APC) with Waters Fraction Manager-Analytical (WFM-A).

In this technology brief, we describe a fast SEC method with simplified fraction collection using Waters Advanced Polymer Chromatography (APC) System[2] with Waters Fraction Manager-Analytical (WFM-A).[3] Providing analysis times of less than 10 minutes with collection of narrow peak fractions make in-process testing more accessible especially if multiple aliquots of the same fraction are required. The resulting time savings can bring a faster resolution to an out of spec product, and the 10 minute assay offers efficient workflows.

Experimental



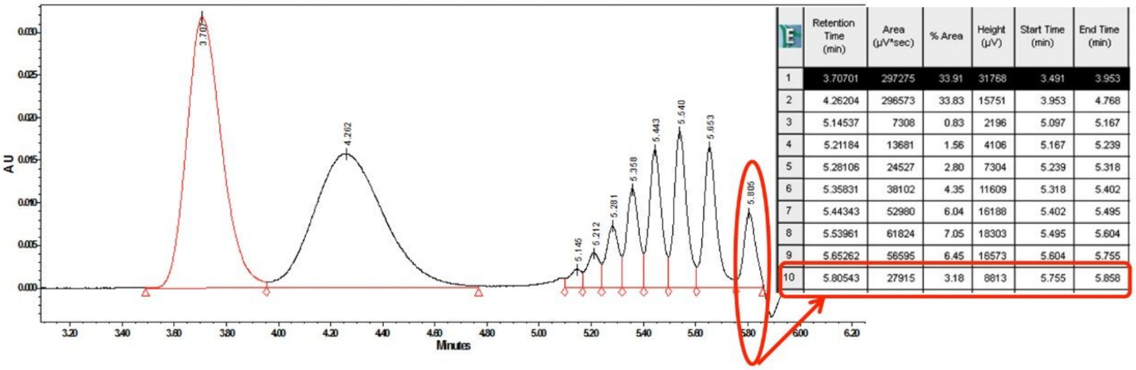
LC conditions	
LC system:	APC
Mobile phase:	Tetrahydrofuran (unstabilized)
Flow rate:	0.8 mL/min
Sample temp.:	20 °C
Sample:	ACQUITY APC Calibration Standards (p/n: 186007539)
Sample concentration:	3 mg/mL in mobile phase
Column:	ACQUITY APC XT 45Å, 1.7 µm, 4.6 mm x 150 mm (p/n: 186006995)
Column temp.:	40 °C
Detector 1:	PDA
Detector 2:	WFM-A (replaced PEEK with PTFE tubing)
WFM-A temp.:	10 °C
Coil delay:	165.7 uL for flow 0.5 – 1 mL (p/n: 205001419)
Software:	Empower 3 CDS

Table 1. Instrument conditions.

Results and Discussion

In this example, we demonstrate the 10 minute SEC assay and simplified WFM-A fraction collection workflow using a single vial of low molecular weight polystyrenes from a calibration kit. The first procedural step is an SEC analysis for determination of sample peak retention times using the parameters in Table 1.

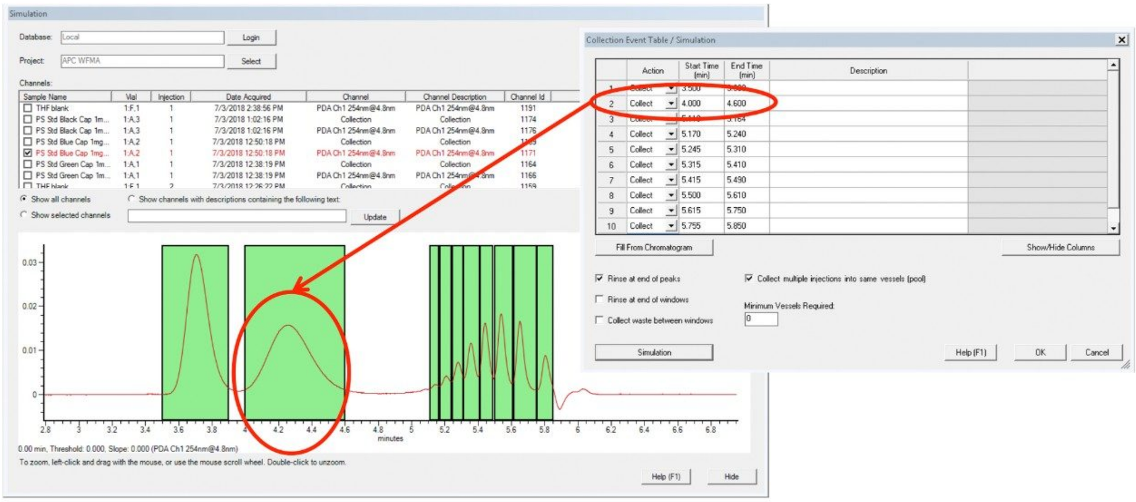
The second step is applying the peak retention times to the fraction collection timing. The peak retention times are calculated using the Empower CDS Software for processing SEC molecular weight, as shown in Figure 1.



	Retention Time (min)	Area (µV*sec)	% Area	Height (µV)	Start Time (min)	End Time (min)
1	3.70701	297275	33.91	31768	3.491	3.953
2	4.26204	296573	33.83	15751	3.953	4.768
3	5.14537	7308	0.83	2196	5.097	5.167
4	5.21184	13681	1.56	4106	5.167	5.239
5	5.28106	24527	2.80	7304	5.239	5.318
6	5.35831	38102	4.35	11609	5.318	5.402
7	5.44343	52980	6.04	16188	5.402	5.495
8	5.53961	61824	7.05	18303	5.495	5.604
9	5.65262	56595	6.45	16573	5.604	5.755
10	5.80543	27915	3.18	8813	5.755	5.858

Figure 1. APC analysis of low molecular weight polystyrenes.

The peak elution times from the first SEC analysis (Figure 1) are entered into the WFM-A collection table, and the WFM-A Software provides a simulation of the collection (Figure 2). If the simulation meets the experimental needs, the next SEC analysis is collected. The analysis is repeated until the desired volume is achieved in each fraction vial.



Database: Local

Project: APC WFMA

Channels:

Sample Name	Vial	Injection	Date Acquired	Channel	Channel Description	Channel Id
☐ THF blank	1:F,1	1	7/3/2018 2:38:56 PM	PDA Ch1 254nm@4.8nm	PDA Ch1 254nm@4.8nm	1191
☐ PS Std Black Cap 1m...	1:A,3	1	7/3/2018 1:02:16 PM	Collection	Collection	1174
☐ PS Std Black Cap 1m...	1:A,3	1	7/3/2018 1:02:16 PM	PDA Ch1 254nm@4.8nm	PDA Ch1 254nm@4.8nm	1176
☐ PS Std Blue Cap 1mg...	1:A,2	1	7/3/2018 12:50:18 PM	Collection	Collection	[illegible]
☑ PS Std Blue Cap 1mg...	1:A,2	1	7/3/2018 12:50:18 PM	PDA Ch1 254nm@4.8nm	PDA Ch1 254nm@4.8nm	1171
☐ PS Std Green Cap 1m...	1:A,1	1	7/3/2018 12:38:19 PM	Collection	Collection	1164
☐ PS Std Green Cap 1m...	1:A,1	1	7/3/2018 12:38:19 PM	PDA Ch1 254nm@4.8nm	PDA Ch1 254nm@4.8nm	1166
☐ THF blank	1:F,1	2	7/3/2018 12:26:22 PM	Collection	Collection	1159

● Show all channels
○ Show channels with descriptions containing the following text:
○ Show selected channels

0.00 min, Threshold: 0.000, Slope: 0.000 (PDA Ch1 254nm@4.8nm)

To zoom, left-click and drag with the mouse, or use the mouse scroll wheel. Double-click to unzoom.

	Action	Start Time (min)	End Time (min)	Description
1	Collect	3.500	[illegible]	
2	Collect	4.000	4.600	
3	Collect	[illegible]	5.164	
4	Collect	5.170	5.240	
5	Collect	5.245	5.310	
6	Collect	5.315	5.410	
7	Collect	5.415	5.490	
8	Collect	5.500	5.610	
9	Collect	5.615	5.750	
10	Collect	5.755	5.850	

☑ Rinse at end of peaks
☐ Rinse at end of windows
☐ Collect waste between windows
☑ Collect multiple injections into same vessels (pool)
Minimum Vessels Required: 0

Figure 2. Simulation of WFM-A fractions.

For this experiment, the separation was collected three times in the same vials. The fraction collection maximum volume (Equation 1) was based on the greatest peak retention time (Figure 2) and the maximum vial capacity of

2 mL.

Equation 1. [(4.6-4.0 min) 1 mL/min.]3 = 1.8 mL.

The WFM-A vials were moved to the sample manager, and the SEC analysis was repeated for confirmation of the molecular weight separation (Figure 3). Note the lowest molecular weight peaks are not baseline resolved. The fractions of non-resolved peaks would not be expected to result in a purely single peak molecular weight, and this expectation is evident in the overlapping fractions of 994 Da to 266 Da shown in Figure 3. If further precise fractions were needed, each vial could undergo its own SEC analysis and fraction collection.

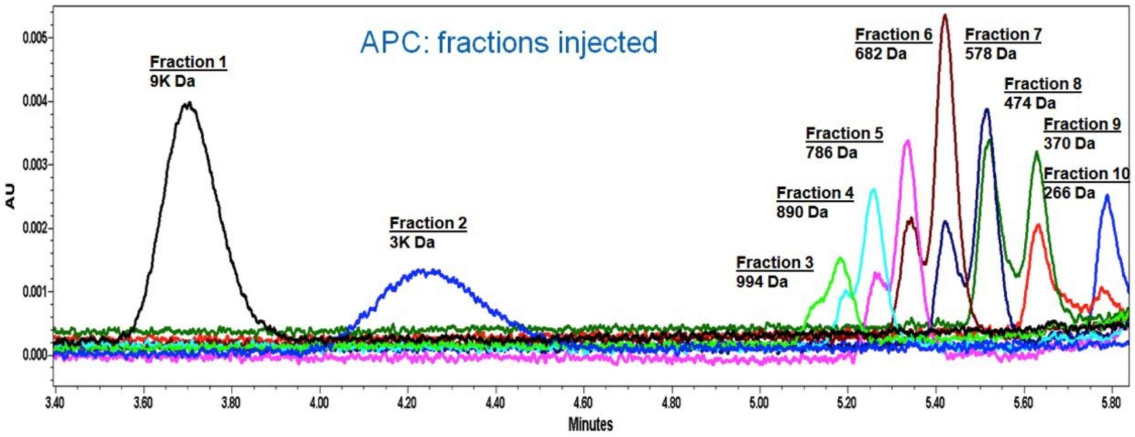


Figure 3. APC analysis of individual fraction vials.

Conclusion

SEC analysis and fraction collection of narrow polystyrene standards is completed quickly and precisely using the APC System in-line with the WFM-A.

References

1. L. Ślusarski *et al.* Importance of the Surface Layer for Polymer Materials. Solid State Phenomena, 94:. 305-308, 2003.
2. Waters APC System online
3. Waters WFM-A, online

Featured Products

ACQUITY Advanced Polymer Chromatography System <https://www.waters.com/134724426>

Waters Fraction Manager - Analytical <https://www.waters.com/134782878>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

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