

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

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GOAL

Demonstrate 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.

BACKGROUND

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).

Table 1. Instrument conditions.

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

In this technology brief, we describe a fast SEC method with simplified fraction collection using Waters™ Advanced Polymer Chromatography™ (APC) System² 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.

THE SOLUTION

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.

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.

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.

$$\text{Equation 1. } [(4.6-4.0 \text{ min}) 1 \text{ mL/min.}]3 = 1.8 \text{ mL.}$$

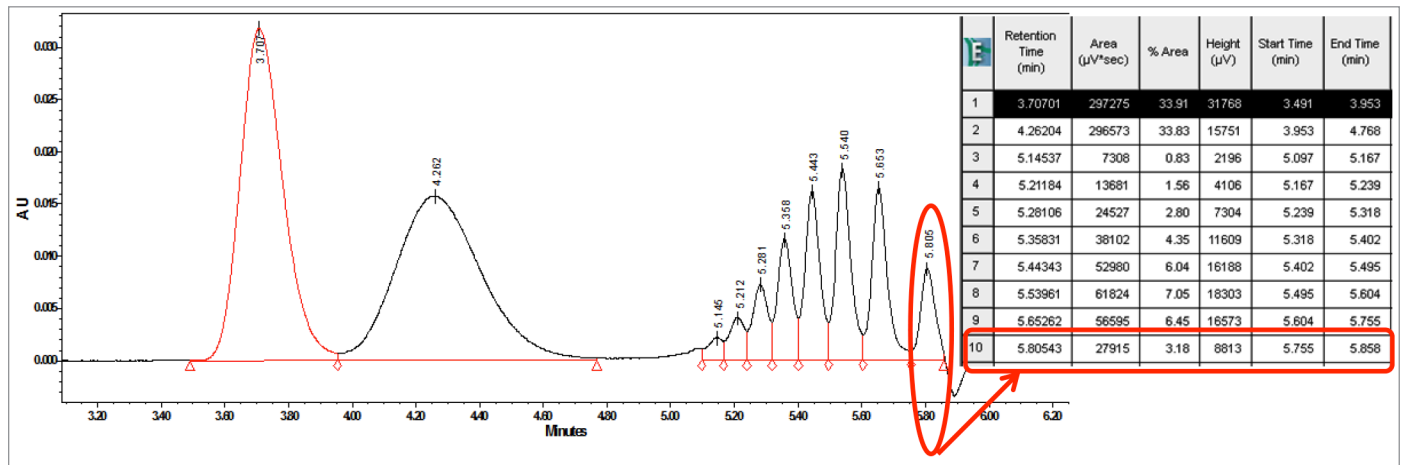


Figure 1. APC analysis of low molecular weight polystyrenes.

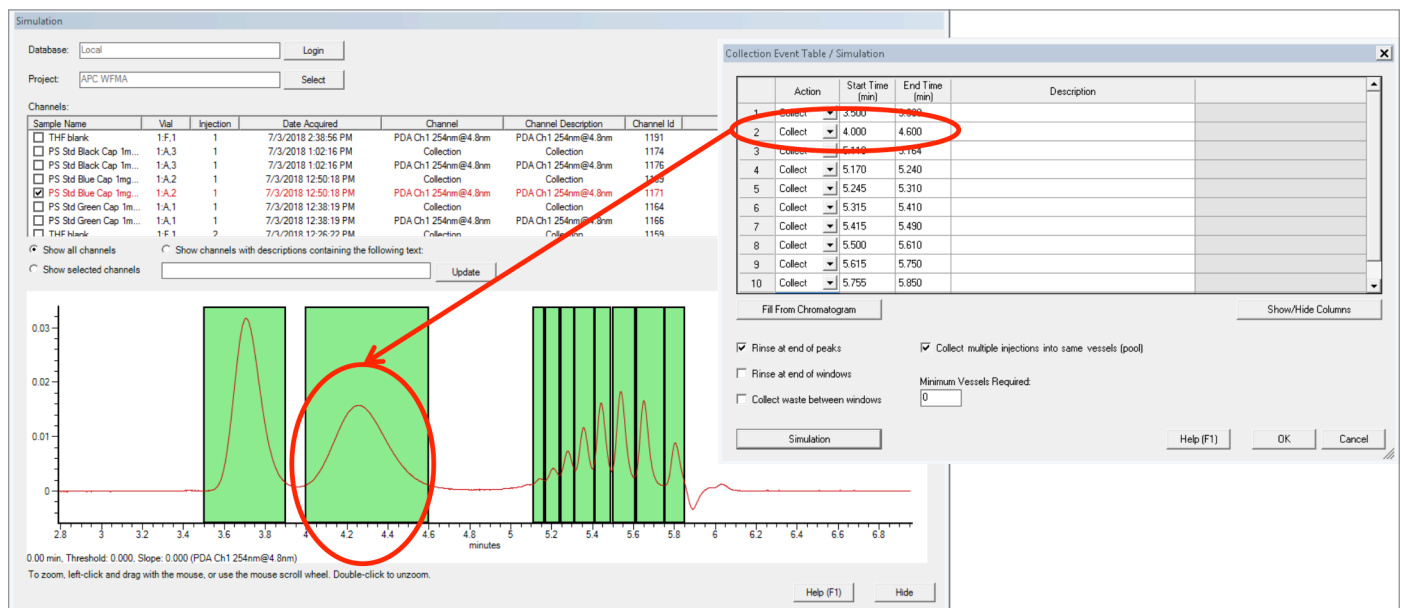


Figure 2. Simulation of WFM-A fractions.

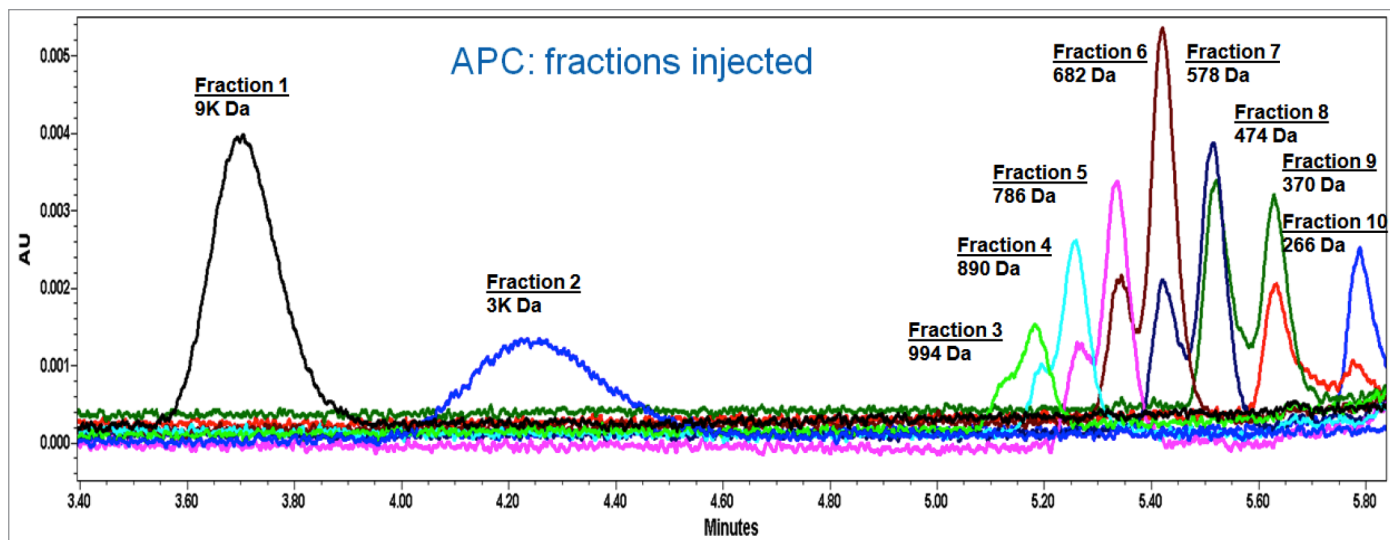


Figure 3. APC analysis of individual fraction vials.

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.

SUMMARY

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](#)

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