HIGH SPEED/HIGH RESOLUTION SIZE EXCLUSION CHROMATOGRAPHY OF LOW MOLECULAR WEIGHT POLYSTYRENE -TETRAHYDROFURAN SOLUTIONS WITH FRACTION COLLECTION

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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 SEC separation eluent into individual molecular weight fractions can aid in-process and formulation changes by elucidating polymer chemistry and structure information from advanced analysis techniques.[2]

Responding quickly to in-process and post–process changes has great impact on manufacturing and customer success. With typical 45 minute SEC analysis times and difficult to program fraction collectors, responding rapidly is a challenge. These analytical challenges cause the phrase "streamlined workflow" to sound optimistic. SEC analysis times of less than 10 minutes, with a collection of narrow peak fractions, can make in-process testing accessible: especially if multiple aliquots of the same fraction are required. The time savings can bring a prompt resolution to product not conforming to manufacturing specifications, and the 10 minute assay offers the much desired streamlined workflow.

METHODS

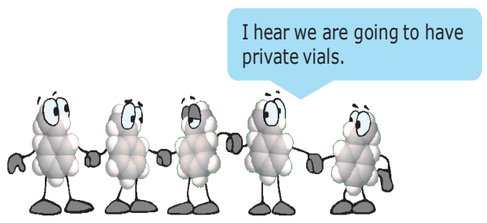


Figure 1.
Advanced Polymer Chromatography (APC) system.[3,4]

Method Conditions:	
LC:	APC
Mobile Phase:	Tetrahydrofuran (unstabilized)
Flow Rate:	0.8 mL/min.
Sample Temp:	20 ºC
Sample:	Low Mwt. Polystyrene Standard (P/N 186007539)
Sample Conc:	3 mg/mL in mobile phase
Columns:	APC XT (4.6 x 150mm) 125A, 2 x 45A
Column Temp:	40 ºC
Detector 1:	PDA
Detector 2:	WFMA (replaced PEEK with PTFE tubing)
WFMA Temp:	10 ºC
Coil Delay:	165.7 uL for flow 0.5 – 1 mL (P/N205001419)
Software:	Empower 3 FR4

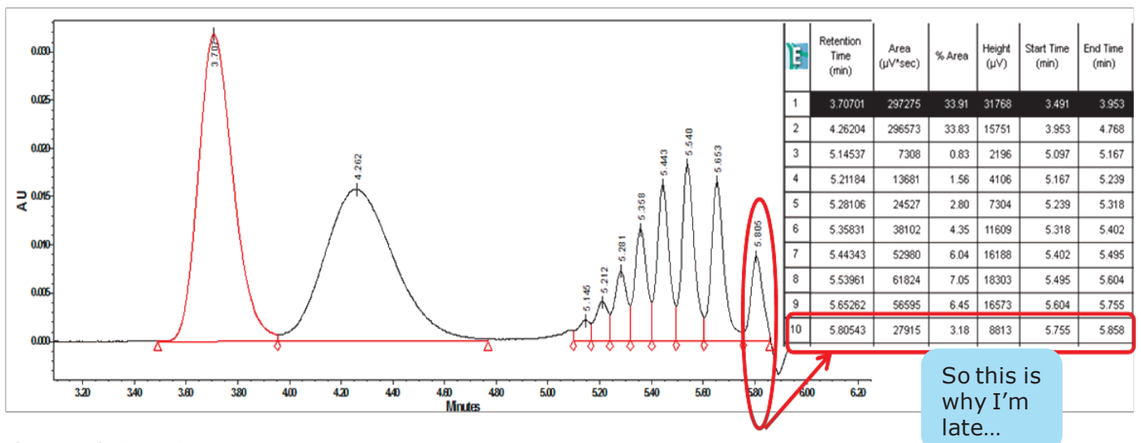
Table 1.
Method conditions for APC separation and WFMA collection.

Figure 2.
Waters fraction manager analytical (WFMA).[5]

The 10 minute SEC and simplified fraction collection is achieved using a single sample, from a calibration kit, containing multiple low molecular weight polystyrenes . 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.

DISCUSSION & RESULTS

The peak retention times are calculated using the Empower software for processing SEC molecular weight (Graph 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

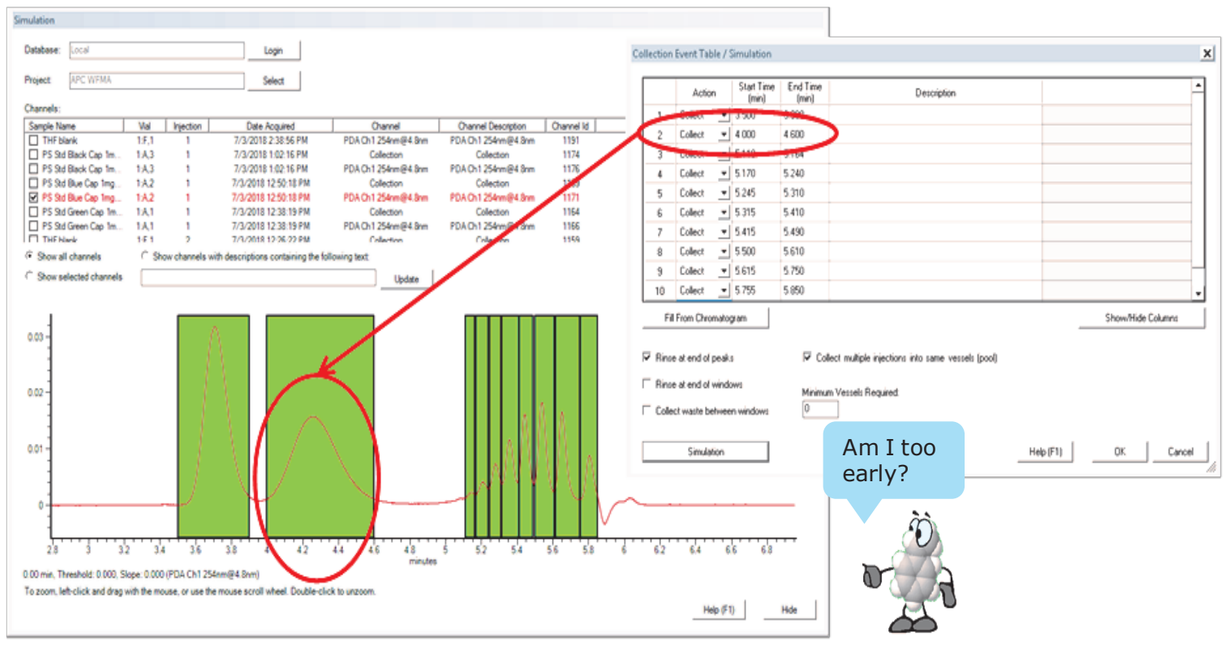
Graph 1 & Table 2.
APC integration of polystyrene standards & table of integration values using Empower 3 software.

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 in Graph 2 and the maximum vial capacity of 2mL. This calculation can be done automatically using the custom calculation feature in Empower software.

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

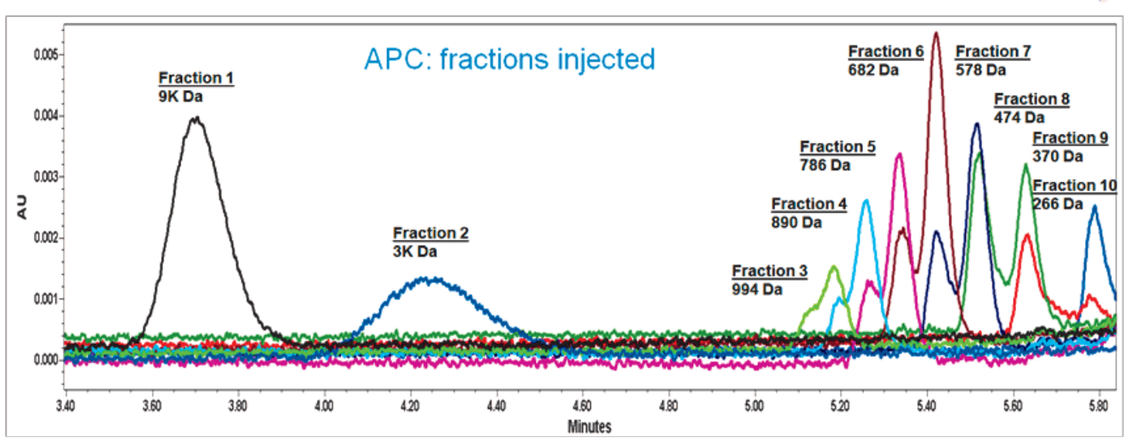
The WFMA vials were moved to the sample manager, and the SEC analysis was repeated for confirmation of the molecular weight separation (Graph 3 & 4). Note the lowest molecular weight peaks are not baseline resolved. The fractions of non-resolved peaks would not be expected to result in a purely single peak molecular weight, and this expectation is evident in the overlapping fractions of 994 Daltons to 266 Daltons. If further precise fractions were needed, each vial could undergo its own SEC analysis and fraction collection.

The peak elution times from the first SEC analysis (Table 2) are entered into the WFMA collection table (Table 3), and the WFMA software provides a simulation of the collection (Graph 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.

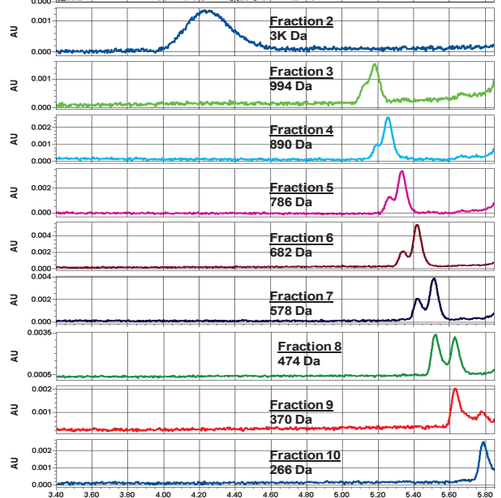


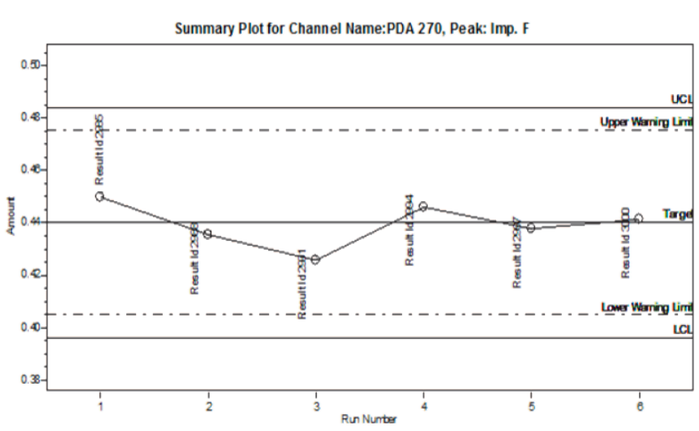
Graph 2 & Table 3.
WFMA fraction collection simulation from event table retention times using Empower 3 software.

Each collected fraction is now ready for further characterization of chemistry or structure by nuclear magnetic resonance (NMR), Fournier transform infrared (FTIR), etc.. Also, each of the fraction peak areas or retention times can be used for tracking the values in a control chart as a tool for process control (Graph 5).



Graph 3 & 4.
APC chromatograms displayed in overlay and stack plots of individual PS fraction vials.





Graph 5.
Example of a control chart created using Empower 3 software.

CONCLUSIONS

- **APC analysis and WFMA fraction collection is completed quickly and precisely.**
- **Empower software enables intuitive and seamless methods for SEC and fraction collection.**
- **High speed/ high resolution separation and fraction collection can enable process control and further sample elucidation.**

References

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