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Nota applicativa

Enhanced Resolution of Low Molecular Weight Samples Using Advanced Polymer Chromatography (APC) with Multi-Detection

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Abstract

In this application note, two low molecular weight polymers, a polystyrene and a polycaprolactone, were analyzed using the ACQUITY APC and OMNISEC REVEAL in combination to demonstrate the power of comprehensive polymer characterization by multi-detection integrated with the resolution, speed, and efficiency of APC.

Benefits

- Investigate molecular structure, such as branching
- Improve molecular weight accuracy
- Improve lab efficiency – less sample, less time, less solvent

Introduction

Gel permeation/size exclusion chromatography (GPC/SEC) has been and remains the standard analytical technique for analysis of polymer molecular weight. The advent of Waters Advanced Polymer Chromatography (APC) has elevated the technique, greatly improving resolution, data quality, and productivity. Improving on these advantages, Waters has developed low-dispersion detectors that include the ACQUITY UPLC Refractive Index (RI) Detector and the ACQUITY Photodiode Array (PDA) Detector with TaperSlit to complement the ACQUITY UPLC APC System.

Similar improvements have been made to advanced GPC/SEC technologies such as viscometers and light scattering detectors to make them compatible with APC. Malvern Panalytical recently introduced a version of the OMNISEC REVEAL advanced detector unit that through a collaboration with Waters has been optimized for integrated use under APC conditions.

Figure 1. ACQUITY APC System with the OMNISEC REVEAL.

One of the hallmarks of APC is the ability to achieve excellent resolution of low molecular weight samples, while one of the features of OMNISEC REVEAL is the sensitivity of its light scattering detector, especially for low molecular weight samples. In this application note, two low molecular weight polymers, a polystyrene and a polycaprolactone, were analyzed using the ACQUITY APC and OMNISEC REVEAL in combination to demonstrate the power of comprehensive polymer characterization by multi-detection integrated with the resolution, speed, and efficiency of APC.

Experimental

Sample description

One polystyrene sample (A) and one polycaprolactone sample (B) were prepared in THF at a concentration of 9.725 and 3.18 mg/mL, respectively.

LC conditions

LC system:	ACQUITY APC
Detector:	OMNISEC REVEAL with refractive index, light scattering (RALS 90° angle, LALS 7° angle), and viscometer detectors
Vials:	Waters vials with pre-slit septa
Column:	ACQUITY APC XT 4.6 mm × 150 mm A) 45 Å, 125 Å, and 450 Å, B) 45 Å, 45 Å, and 125 Å, in series
Column temp.:	A) 40 °C and B) 35 °C
Sample temp.:	A) 40 °C and B) 35 °C
Injection volume:	A) 20 and B) 50 µL
Flow rate:	A) 0.8 mL/min and B) 0.5 mL/min
Mobile phase:	THF (unstabilized)

Data management

ACQUITY APC operation:	Standalone ACQUITY console software
OMNISEC REVEAL operation:	Data collected and processed using Malvern Panalytical OMNISEC software

For comparison, the polystyrene sample was also analyzed using two 30 cm mixed-bed analytical GPC/SEC columns under the same conditions but with different loadings. The amount of mass introduced to the APC System was 194.5 µg compared to that of the GPC/SEC analysis which was 486 µg per injection.

Results and Discussion

Before assessing the quality of the ACQUITY APC-OMNISEC REVEAL results, it is worth comparing the chromatograms from the two techniques. The overlay of the refractive index samples from both analyses shown in Figure 2 makes the differences in mobile phase and analysis times required visibly obvious. While more than 20 mL of solvent are required for the sample to elute from the pair of mixed-bed analytical GPC columns, the sample eluted from three APC Columns in less than 5 mL. This represents a significant savings both in solvent consumption (in this case, 15 mL) and time per analysis (in this case, ~19 mins). Most importantly, the high resolution provided by the set of APC Columns afforded this sophisticated analysis for various oligomers that were not separated on standard 30 cm analytical GPC/SEC columns.

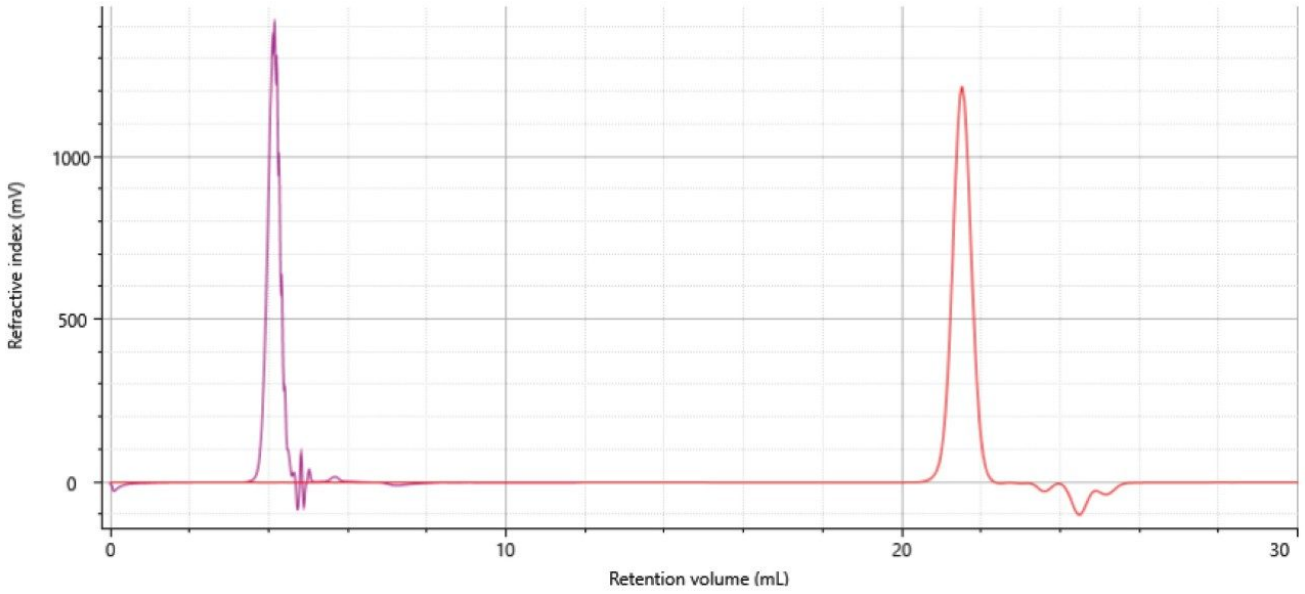

Figure 2. Overlay of refractive index signals of the low molecular weight polystyrene sample analyzed using three ACQUITY APC Columns (purple) and two analytical GPC/SEC columns (red).

Closer examination of the chromatograms obtained on the integrated ACQUITY APC-OMNISEC REVEAL system indicate that in addition to much lower retention volume and retention time, excellent signal strength was acquired using all three of the detectors (RI, RALS, and viscometer), as shown in Figure 3. This combination of data allows for the calculation of absolute molecular weight, intrinsic viscosity, hydrodynamic radius, and for structural comparisons to be made using a Mark-Houwink plot.

Figure 3 shows the triple detector chromatogram of the polystyrene sample measured on the integrated ACQUITY APC-OMNISEC REVEAL system. Plotted across the top of the chromatograms is the molecular weight of the sample. As expected for a synthetic polymer sample, the molecular weight steadily decreases from the larger, earlier eluting material across the sample's distribution to the smaller, later eluting oligomers.

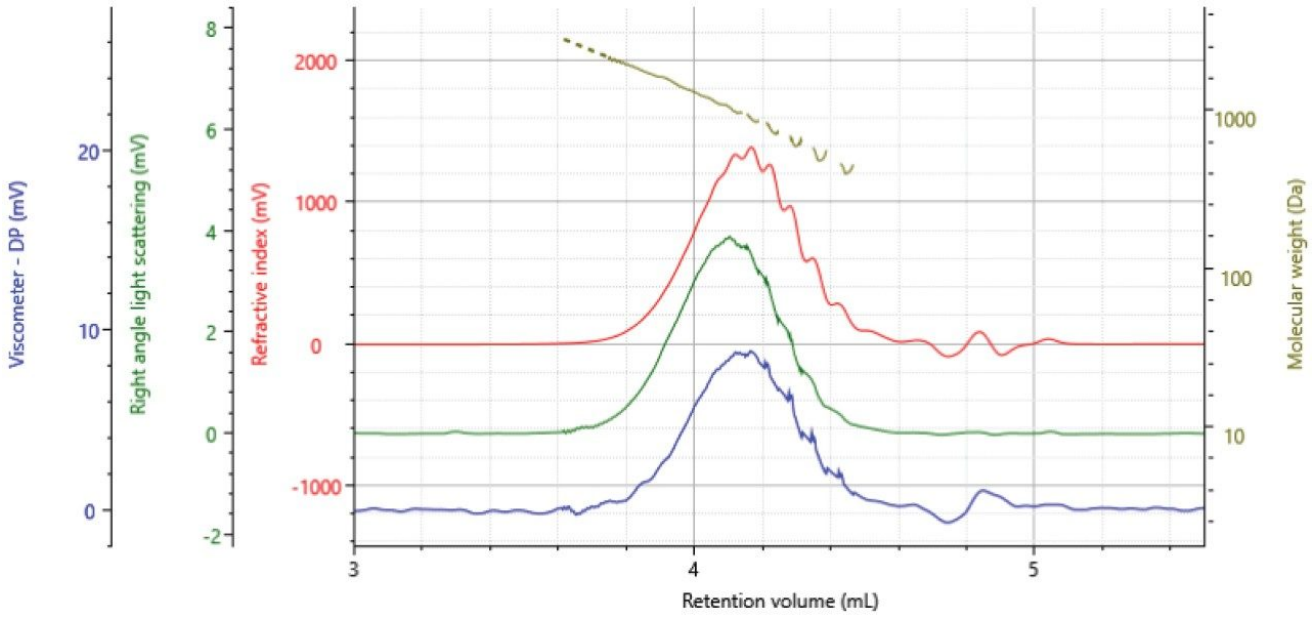

Figure 3. Triple detector chromatogram of the low molecular weight polystyrene sample; refractive index (red), right angle light scattering (green), viscometer (blue), and molecular weight (gold).

On the low molecular weight side of the sample's peak, individual oligomers showed some resolution, enough to identify several peaks with their own set of integration limits. The breaks in the molecular weight plot represent the location of those limits, which signify five individual oligomers in addition to the main portion of the distribution. The molecular characterization data for these six peaks is summarized in Table 1.

	Peak 1		Peak 2		Peak 3		Peak 4		Peak 5		Peak 6	
	Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD	Mean	% RSD
Mn (g/mol)	1,214	1.1	883.6	1.1	765.6	1.1	655.5	1.8	539.4	2.4	444.8	1.8
Mw (g/mol)	1,260	1.3	885.1	1.2	768	1.3	659.3	2.0	544	2.7	451.8	2.0
Mw/Mn	1.038	0.2	1.002	0.1	1.003	0.1	1.006	0.2	1.008	0.3	1.016	1.0
IVw (dL/g)	0.03361	2.8	0.03012	2.2	0.02946	1.0	0.02931	1.3	0.02907	1.6	0.02988	4.9
Rh(η)w (nm)	0.8724	1.2	0.7504	1.1	0.7103	0.6	0.6737	1.1	0.6298	1.4	0.5967	2.2
Frac. of sample (%)	49.99	2.3	14.89	3.1	13.95	0.3	11.14	3.9	6.473	6.8	3.554	3.9
Likely oligomer	9+		8		7		6		5		4	

Table 1. Molecular characterization data from three injections of the low molecular weight polystyrene sample.

The molecular weights of the six fractions designated by the integration limits (calculated using a dn/dc value of

0.185 mL/g for polystyrene in THF) range from 1260 Da down to 452 Da. Based on the molecular weight of styrene (104 Da), these fractions represent the oligomers starting with a degree of polymerization of four (n = 4) up to nine or more (n = 9+). The intrinsic viscosity and hydrodynamic radius values for each fraction were calculated as well, all with excellent precision over the three injections. The weight fraction of each oligomer is listed, with the n = 9+ fraction accounting for half of the sample and the n = 4 fraction representing only 3.56% of the sample. This demonstrates complete characterization of multiple low molecular weight species within a larger sample distribution using minimal sample, solvent, and time.

For the analysis of the polycaprolactone sample the column set was altered with the goal of improving resolution of the oligomers. The ACQUITY APC Column with 450Å pores (p/n: 186007010) was therefore replaced with a duplicate of the column with 45 Å (p/n: 186006995) pores to create a column set consisting of three 150 mm APC columns: 45, 45, and 125 Å (p/n: 186007000) in series. The amount of polycaprolactone introduced to the system was 159 µg, which, like the polystyrene sample, is less than the 300 to 500 µg typically used in GPC/SEC analyses for the same low molecular weight sample.

Similar to the polystyrene sample shown in Figure 3, the molecular weight plot of the polycaprolactone sample descends consistently throughout the chromatogram and is broken up into segments representing the identifiable oligomers. In addition to the main peak, five oligomeric species were selected. The molecular characterization data for these six peaks is summarized in Table 2. Again, for comparison, it was not possible to separate the various oligomers of polycaprolactone on the standard 30 cm analytical GPC/SEC columns.

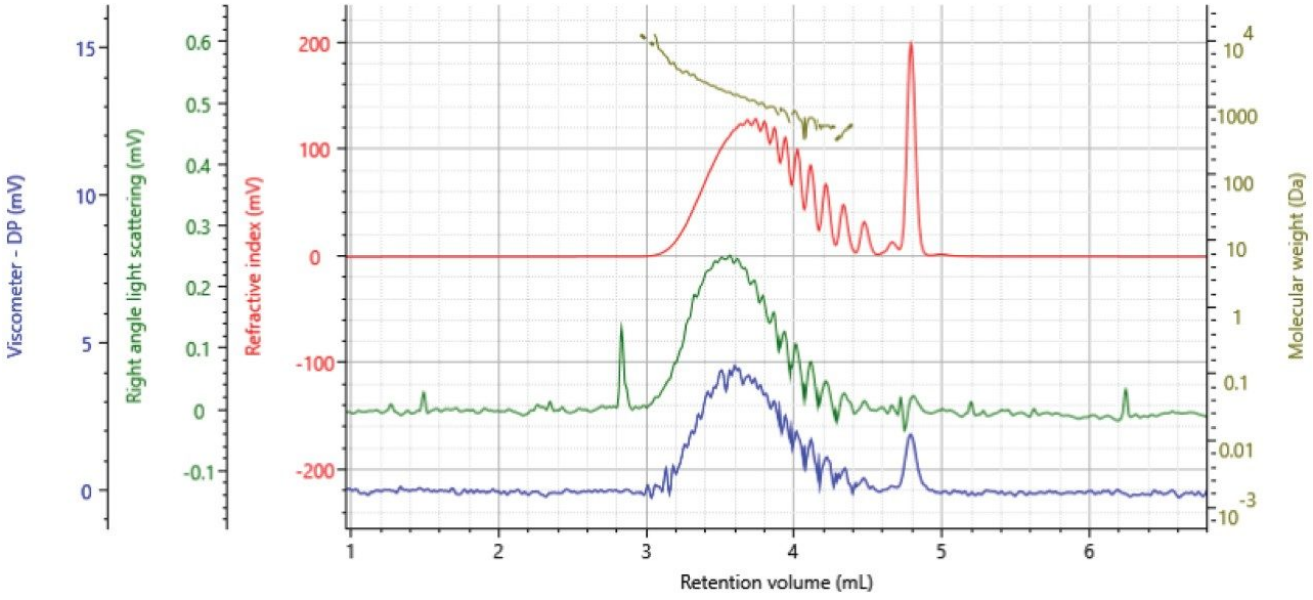

Figure 4. Triple detector chromatogram of the low molecular weight polycaprolactone sample; refractive index (red), right angle light scattering (green), viscometer (blue), and molecular weight (gold).

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6
Mn (g/mol)	1,479	829.6	733.7	622.8	532.6	431.1
Mw (g/mol)	1,711	848.4	757.8	639.3	533.1	439.9
Mw/Mn	1.157	1.023	1.033	1.026	1.001	1.02
IVw (dL/g)	0.06008	0.04014	0.03765	0.03559	0.03208	0.0284
Rh(η)w (nm)	1.158	0.812	0.7642	0.7072	0.6467	0.5781
Frac. of sample (%)	69.9	7.697	7.488	6.182	5.092	3.636
Likely oligomer	**9+**	**8**	**7**	**6**	**5**	**4**

Table 2. Molecular characterization data from the low molecular weight polycaprolactone sample.

As can be seen in Table 2, the molecular weights of the six fractions of the polycaprolactone sample (calculated using a dn/dc value of 0.072 mL/g for polycaprolactone in THF) range from 1711 Da down to 440 Da. The molecular weight of caprolactone (114 Da) is similar to that of styrene, and as such, similar fractions were

observed in the sample; the oligomers starting with a degree of polymerization of four (n = 4) up to nine or more (n = 9+). While the IV values of the polycaprolactone fractions were similar to the analogous polystyrene slices, the hydrodynamic radius values of the polycaprolactones were larger than those of the polystyrene, with the largest difference in the n = 9+ segment. While this fraction represented about 50% of the polystyrene sample, it comprised about 70% of the polycaprolactone sample.

Conclusion

The economy and efficiency of the Waters ACQUITY APC System combined with the advanced OMNISEC REVEAL GPC/SEC detectors facilitated the complete characterization of low molecular weight polystyrene and polycaprolactone samples. The individual oligomers of both samples could be observed and characterized separately due to the resolution of the ACQUITY APC XT Columns and the sensitivity of the OMNISEC REVEAL detectors – with standard 30 cm analytical GPC/SEC columns it is not possible to resolve these oligomers at all. The impact of reduced solvent usage and time savings to obtain comprehensive characterization of samples using the ACQUITY APC-OMNISEC REVEAL system presents obvious benefits to both researchers and manufacturers working with polymeric materials.

Acknowledgements

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