[POSTER NOTE]

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Using High Speed/High Resolution Size Exclusion Chromatography Separation of Polymeric Materials with Light Scattering Detection

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OVERVIEW

- **Explore the hyphenation of Waters® ACQUITY® Advanced Polymer Chromatography® (APC™) System with the Malvern OMNISEC Reveal integrated multi-detector module.**
- **Determine the operational conditions of the linked systems and their impact on data quality for the analysis of polymers and proteins.**
- **Assess the additional benefits that Right Angle Light Scattering and Low Angle Light Scattering (RALS/LALS) detection brings to polymer characterization.**

Figure 1. ACQUITY APC SEC Core 1 System with post column flow to the Malvern OMNISEC Reveal.

INTRODUCTION

The polymeric materials market is wide and varied, and producing a competitive product in a timely manner with consistent quality can be a challenge. The ever widening market of polymers can be found in a variety of products: from simple biodegradable shopping bags to complex drug delivery systems. As polymer technology advances, understanding and predicting complex properties of a consistently high quality product drives a desire for more expansive characterization and more flexible analytical instrumentation. This need for complex product knowledge can lead to creative analytical solutions ranging from high speed chromatographic separation to advanced mass spectrometry.[1, 2, 3]

Traditional analytical solutions have often met the need for polymer characterization through coupling liquid chromatography (LC) with detectors such as a refractive index (RI), ultra violet or photo diode array (UV or PDA), evaporative light scattering (ELS) and fluorescence (FLR). Examples of creative analytical solutions are adding a secondary LC system for two dimensional (2D) chromatography or hyphenating the LC system with detectors such as differential pressure viscometry (DPV) and/or light scattering.[4]

With the introduction of high speed/high resolution size exclusion chromatography techniques, the resolution and speed of the separation is maintained from injection to detection with optimized traditional detectors. The novel design of the Waters Advanced Polymer Chromatography (APC) system, including the robust separation column, and low dispersion flow path, is used to yield high resolution and quick mobile phase changes. However, the use of this high speed/high resolution separation technique has seen limited pairing with offline detectors such as light scattering. As the advantages of APC become more widely used in research and industry, the detector options that can maintain the high speed/high resolution of the APC separation are increasing.[5, 6, 7]

In this study, the expansion of the Waters APC approach to the size exclusion separation is presented. The sample is diverted from the ACQUITY APC Core 1 system to a Malvern OMNISEC Reveal integrated multi-detector module for analysis of RI, UV, DPV and RALS/LALS while maintaining the required system dispersion control needed for the APC experiment (Figure 1).[8]

EXPERIMENTAL

Conditions for demonstration measurements

The test conditions are based on using a high speed, high resolution ACQUITY APC SEC Core 1 System coupled to a Malvern OMNISEC Reveal. The two sytems are connected with four thousandths inner diameter tubing and a low pressure poly fitting from the APC PDA outlet to the REVEAL inlet. The first experimental samples evaluated are a mixture of two narrow polystyrene standards dissolved in terahydrofuran. The second experimental sample is a bovine serum albumin standard in aqueous buffer solution. The separations are analyzed using the Malvern OMNISEC Reveal. The data are collected within each system's respective sofware.

SEC conditions

Normal Phase

System:	Waters APC Core 1 System
Eluent:	THF
Flow Rate:	1.0 mL/min
Sample Concentration:	1 mg/mL of each narrow standard (2 mg/mL total)
Injection Volume:	10 µL
Column Temp.:	40 °C
Columns:	Waters 4.6 x 150 mm APC XT 450 Å, 125 Å and 45 Å in series
Conc. Detector:	Waters ACQUITY RI detector @ 40 °C (used only when disconnected from Reveal)
LS and PDV Detector:	Malvern OMNISEC Reveal (settings below)
Coupling Tubing:	0.004 inches ID Stainless Steel tubing

Reverse Phase

System:	Waters APC Core 1 System
Eluent:	Aqueous Buffer
Flow Rate:	1.0 mL/min
Sample Concentration:	1 mg/mL of each narrow standard
Injection Volume:	10 µL
Column Temp.:	25 °C
Columns:	Waters ACQUITY BEH SEC Protein 4.6 x 150 mm 200 Å
Conc. Detector:	Waters ACQUITY RI detector @ 40 °C (used only when disconnected from Reveal)
LS and PDV Detector:	Malvern OMNISEC Reveal (settings below)
Coupling Tubing:	0.004 inches ID Stainless Steel tubing

Malvern Detector Conditions

System:	Malvern OMNISEC Reveal
System Temp:	35 °C

Chromatographic System Control

Waters Empower 3 FR3 Software for instrument control, data acquisition and chromatographic data processing.

Malvern ONMISEC software for OMNISEC Reveal instrument control, data acquisition and chromatographic data processing.

RESULTS AND DISCUSSION

The polymer samples for this study are chosen to define the separation of two polymeric standards. The protein standard is chosen for its common use in biological and pharmaceutical fields. The chromatographic results of the polymers are assessed for RI detector comparison, and the protein is an excellent example of a biological macromolecule for confirmation of detector resolution. The OMNISEC Reveal overlaid detector signals produce a chromatogram of a 65K Dalton (Da) and 30K Da polystyrene (PS) mixture (Figure 2-top). The overlaid detector signals reveal a consistent retention volume throughout the detector flow path providing confidence in the chromatographic resolution. The bottom chromatogram in Figure 2 is generated from exporting the APC RI and Reveal RI data into a spreadsheet and creating a graph. Further data confidence is attained through the comparable instrumental signal analysis in the RI chromatogram.

Figure 3 shows the calculated polystyrene properties of the two standards in graph and tabular form. The RALS/LALS detection, in conjunction with RI, UV and DPV detectors, enable the calculation of the absolute molecular weight, inherent viscosity, and hydrodynamic radius (Figure 4). These polymer properties, specific to each sample, can reveal material characteristics that can be helpful in determining behavior of the polymer in a product matrix or in a solution.[9, 10, 11]

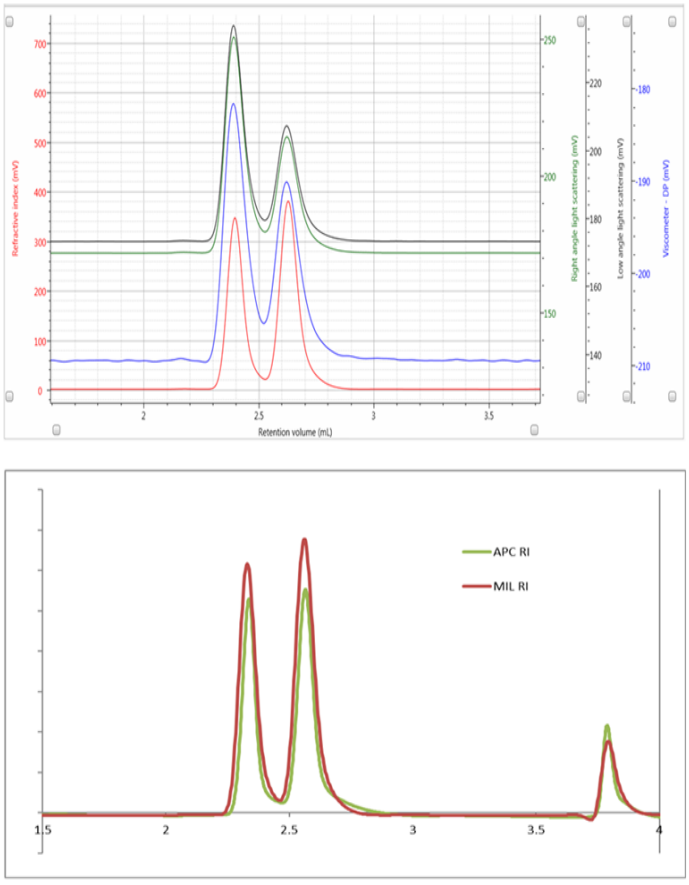


Figure 2. Top chromatogram is a mixture of narrow molar mass PS centered at 65K Da and 30K Da collected on Malvern OMNISEC Reveal RI, DLS, and DPV.
Bottom chromatogram is a mixture of narrow molar mass PS centered at 65K Da and 30K Da collected on a Waters ACQUITY RI detector (green), and a Malvern OMNISEC RI detector (red).

Results by sample and peak

Parameter	Inj. 1 PS30k PS65k 06/09/2016 21:23:19	
	Peak 1	Peak 2
Mn (g/mol)	59,980	28,730
Mw (g/mol)	62,340	29,610
Mz (g/mol)	65,760	30,730
Mw/Mn	1.039	1.031
IVw (dL/g)	0.3288	0.193
Rh(η)w (nm)	6.805	4.464

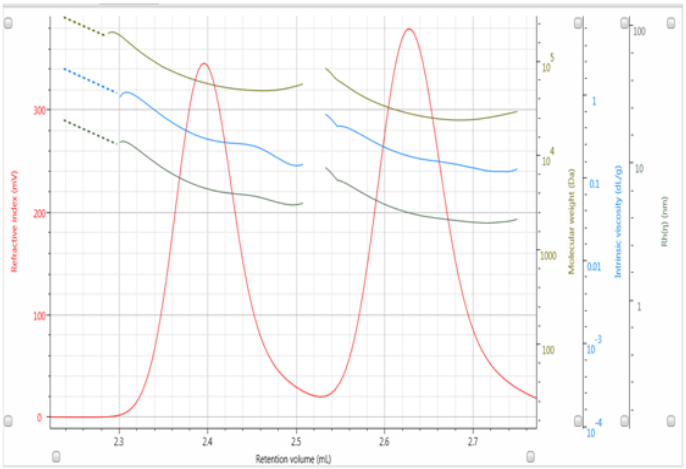


Figure 3. The top table shows the Malvern OMNISEC Reveal calculations for the 65K Da and 30K Da PS derived from the RI, DLS, and DPV.
The bottom image is a chromatogram from the Malvern OMNISEC Reveal of the PS mixture, molecular weight, intrinsic viscosity, and hydrodynamic radius.

The advantages of gaining further polymeric characterization with the OMNISEC Reveal can also be applied to the polymer-like characteristics of proteins. The BSA analysis is demonstrated below in the chromatographic overlay of each detector, bringing confirmation to the resolution maintained throughout the analyte flow path (Figure 5). The molecular weight, and hydrodynamic radius of the protein analysis in Figure 6, and tabulated in Figure 4, can be used to predict the behavior of the analyte in solution.[10, 11]

Results by sample and peak.				
Parameter	Inj. 3 BSA 08/09/2016 11:53:17			
	Peak 1	Peak 2	Peak 3	Peak 4
Mw (g/mol)	370,800	206,000	132,900	66,770
Mw/Mn	1.109	1.004	1.001	1.011

Results by sample and peak.		
Parameter	Inj. 3 BSA 09/09/2016 11:34:14	
	Peak 1	Peak 2
Rh(η)w (nm)	4.94	3.694

Figure 4. The table is the Malvern OMNISEC Reveal calculations for the BSA molecular weight and hydrodynamic radius derived from the RI, LS, and IV.

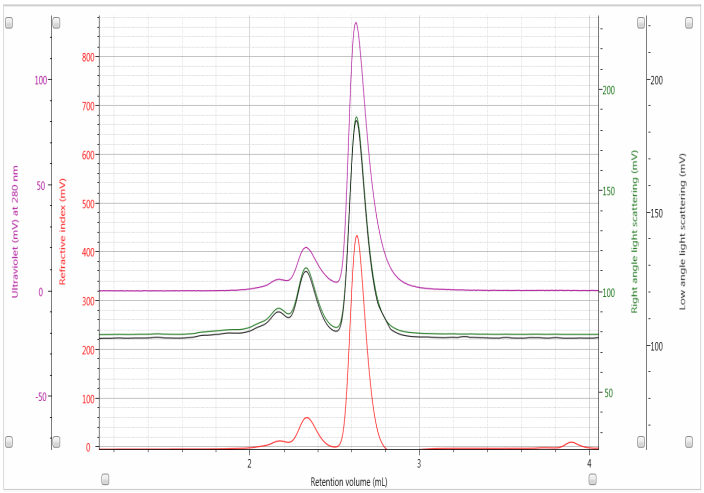
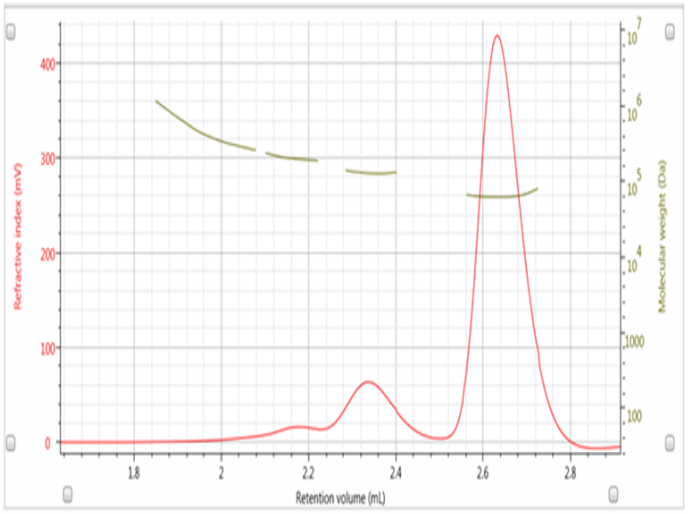


Figure 5. The overlaid chromatograms from the Malvern OMNISEC Reveal of the BSA refractive index, RALS/LALS, and DPV.



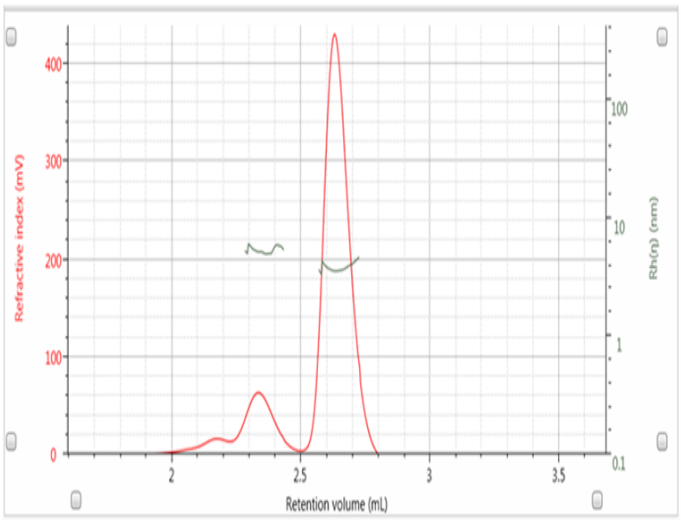


Figure 6. The images are chromatograms from the Malvern OMNISEC Reveal of the BSA refractive index, molecular weight, and hydrodynamic radius.

CONCLUSIONS

- Coupling the APC, high resolution / high speed SEC Core 1 system to the OMNISEC Reveal is easily managed with the selection of the correct tubing diameter.
- The impact of the OMNISEC Reveal on chromatographic band spread is minimized with reduced inter-detector tubing volume and placement of the Reveal RI at the beginning of the OMNISEC detector path.
- In the example studied, the benefit of adding the integrated multi-detector module with RI, RALS/ LALS, PVD, and UV to the SEC experiment offers the ability to calculate absolute molecular weight, intrinsic viscosity and hydrodynamic radius. These calculations can predict polymer behaviors in solutions/product matrices and bring confidence to sample quality through extended quantification.[9-11]
- The high speed chromatography offered by the APC is matched well to the experimental conditions available with the OMNISEC Reveal enabling greater characterization for the complex properties of polymers and proteins.

References

1. Xian, Jun Loh; *Polymers for Personal Care Products and Cosmetics,* Royal Society of Chemistry, Cambridge, U.K., 2016.
2. Srivastava, Apurva, *et al.* (2016), "Polymers in Drug Delivery". *Journal of Biosciences and Medicines* 4: 69 -84.
3. Montaudo, M. S., Puglisi, C., Samperi, F. and Montaudo, G. (1998), "Application of size exclusion chromatography matrix-assisted laser desorption/ ionization time-of-flight to the determination of molecular masses in polydisperse polymers". *Rapid Commun. Mass Spectrom.,* 12: 519–528.
4. Provder, Theodore; Urban, Marek W.; Barth, Howard G.; *Hyphenated Techniques in Polymer Characterization,* American Chemical Society, Washington, DC 1994.
5. "ACQUITY Advanced Polymer Chromatography System", Waters Corporation, 9 November 2016, http://www.waters.com/webassets/cms/library/docs/720004570en.pdf
6. "OMNISEC Reveal", Malvern Instruments Ltd., 9 November 2016, http://www.malvern.com/Assets/MRK2179.pdf
7. "ACQUITY APC Columns: Increased Speed and Resolution for Aqueous and Organic Polymer Separations", Waters Corporation, 9 November 2016, http://www.waters.com/webassets/cms/library/docs/720004580en.pdf
8. "International Symposium on GPC/SEC and Related Topics", Amsterdam, N.L., 26 September 2016
9. Van Krevelen, D.W.; Te Nijehuis, K.; *Properties of Polymers.* 4th Ed., Elsevier Science, Amsterdam, N.L., 2009.
10. Lu, Y., An, L., Wang, Z. (2013),"Intrinsic Viscosity of Polymers: General Theory Based on a Partially Permeable Sphere Model", *Macromolecules,* Issue 14, Volume 46, 5731-5740.
11. Pan, S., Ahirwal, D., Nguyen, D.A., Sridhar, T., Sunthar, P., Prakash, J.R. (2014) "Viscosity Radius of Polymers in Dilute Solutions: Universal Behavior from DNA Rheology and Brownian Dynamics Simulations", *Macromolecules,* Issue 21, Volume 47, 7548-7560.