Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Using High Speed/High Resolution Size Exclusion Chromatography Separation of Polymeric Materials with Dynamic and Multi-Angle Light Scattering Detection

Jennifer Gough[1], Robert Birdsall[1], Damian Morrison[1], John Champagne[2] , Kyle Rogers[2]
[1]Waters Corporation, Milford, MA USA, [2]Wyatt Technology Corporation, 6330 Hollister Ave.; Santa Barbara, CA USA

OVERVIEW

- **Explore more detection with the Waters® ACQUITY® Advanced Polymer Chromatography® (APC™) System and the Wyatt µDAWN™ multi-angle and quasi-elastic light scattering (MALS/QELS).**

- **Bring the high resolution separation capabilities of APC to light scattering analysis.**

- **Experience the simplicity of the APC/ µDAWN hyphenation and the benefits that are brought to polymer characterization.**

INTRODUCTION

Polymeric materials are an integral part of everyday tasks, and there is great complexity behind the research and manufacturing of these high quality and competitive products. From the simple task of carrying food in biodegradable shopping bags to battling diseases with intricate drug delivery systems, these everyday tasks are possible through an exacting polymer process. As technologies advance, there is a drive to understand and predict material properties through more expansive characterization and flexible analytical instrumentation. Creative analytical solutions, ranging from high speed chromatographic separation to advanced mass spectrometry, have become the answer for complex product knowledge.[1, 2, 3]

Traditional analytical solutions have often been the answer for polymer characterization. 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) have met traditional needs. Examples of creative analytical solutions include adding a secondary LC system for two dimensional (2D) chromatography or hyphenating the LC system with detectors such as dynamic light scattering (DLS) and multi-angle light scattering (MALS).[4]

LC separations with resolution and speed have not always been synonymous. Yet, with the innovative design of the Waters Advanced Polymer Chromatography (APC) system, including the robust separation column and low dispersion flow path, the system yields high resolution, short analysis times, and quick mobile phase changes. This high speed/high resolution separation technique works best with low dispersion detectors, availability and diversity of which continue to increase as the advantages of APC become more widely used in research and industry.[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 system to a Wyatt µDAWN with MALS/QELS and RI detector while maintaining the required system dispersion control needed for the APC experiment (Figure 1).[8]

Figure 1.
ACQUITY APC System with post column flow to the µDAWN.

EXPERIMENTAL

Conditions for demonstration measurements

The test conditions are based on using a high speed, high resolution ACQUITY APC SEC System coupled to Wyatt µDAWN and Optilab UT-rEX detectors. The two sytems are connected with seven thousanths inner diameter tubing and a low pressure poly fitting from the APC PDA outlet to the µDAWN inlet. The first experimental samples evaluated are a mixture of narrow polystyrene (PS) standards and of a 200K dalton PS standard dissolved in terahydrofuran (THF). The second experimental samples are a bovine serum albumin (BSA) standard and a protein standard mixture in aqueous buffer solution. The separations are analyzed using the Wyatt µDAWN and RI. Each system's respective sofware is used for their respective instrument control, data collection and data processing.

SEC conditions

Organic Solvent Method

System:	Waters APC System
Eluent:	THF
Flow Rate:	0.7 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 µDAWN)
LS and RI Detector:	Wyatt µDAWN and Optilab UT-rEX (settings below)
Coupling Tubing:	0.007 inches ID Stainless Steel tubing

Aqueous Method

System:	Waters APC System
Eluent:	Aqueous Buffer: 150mM NaCl, 20mM Phosphate, pH=6.5
Flow Rate:	0.6 mL/min
Sample Concentration:	1 mg/mL of each narrow standard
Injection Volume:	2 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 µDAWN)
LS and RI Detector:	Wyatt µDAWN and Optilab UT-rEX (settings below)
Coupling Tubing:	0.007 inches ID Stainless Steel tubing

Wyatt Detector Conditions

LS Detector:	Wyatt µDAWN
LS Temp:	Ambient
RI Detector:	Optilab UT-rEX (25°C)

Chromatographic System Control

Waters Empower 3 FR3 Software and Wyatt ASTRA Software

Using High Speed/High Resolution Size Exclusion Chromatography Separation of Polymeric Materials with Dynamic and Multi-Angle Light Scattering Detection

2

RESULTS AND DISCUSSION

The polymer samples for this study are chosen to define the separation of a polymer standard mixture and a single narrow polymer standard. The protein standards are chosen for their common use in biological and pharmaceutical fields. Comparison of the RI detector signals of the mixed polymer standards are used to assess RI resolution and analysis quality throughout the analyte flow path. The single protein is an excellent example of a biological macromolecule for confirmation of detector resolution, and the protein standard mixture adds confidence to instrumental analysis flexibility from organic solvent to aqueous chromatography within an eight hour day.

Below in Figure 2 is a chromatogram of a PS narrow standard mixture of four molecular weights. The overlaid RI analysis reveals a consistent retention time for the APC and Optilab detectors. The RI data is generated from the Wyatt ASTRA data. Data confidence is increased through the comparable instrumental signal analysis in the RI chromatogram.

Figure 3 shows the 200K Dalton (Da) PS correlation function and control curve from the µDAWN, which provides the hydrodynamic radius value. The MALS/QELS detection, in conjunction with RI detector, enable many calculations such as the absolute molecular weight, calculated mass, and mass fraction (Table 1). The constant RI detector response is indicative of peak/sample homogeneity (Figure 4). These polymer properties reveal material characteristics that can be helpful in determining behavior of the polymer in a product matrix or solution.[9-11]
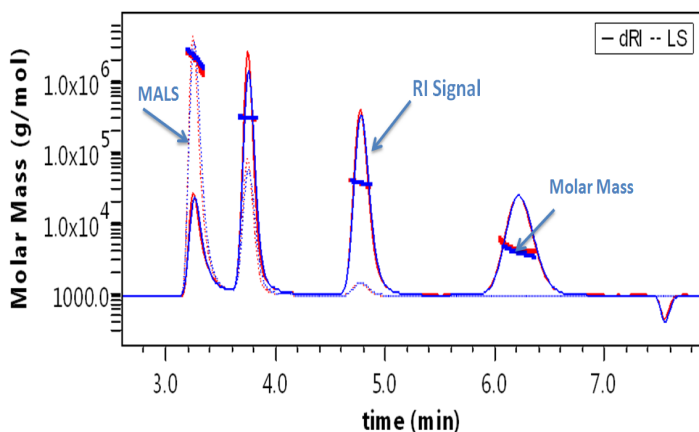

Figure 2.
APC RI signal in blue, and Optilab RI signal in red of PS standard mix.
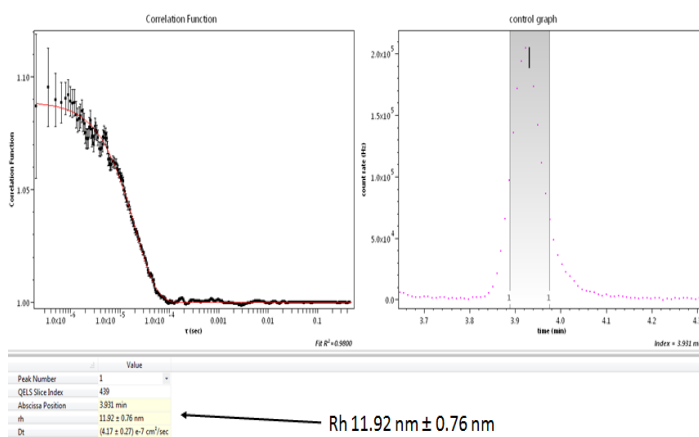
Table 1.
Molecular weight customizable table of 200K Da PS.

Figure 3.
QELS correlation and control graphs: 200K Da PS.

Figure 4.
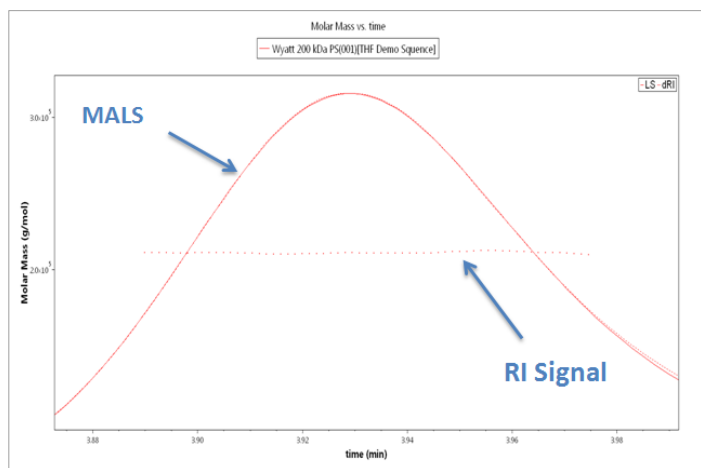RI is constant across 200K Da PS LS peak: consistent signal across peak.

Using High Speed/High Resolution Size Exclusion Chromatography Separation of Polymeric Materials with Dynamic and Multi-Angle Light Scattering Detection

3

The advantages gained by polymer characterization with the μDAWN and Optilab can also be applied to the polymer-like characteristics of proteins. The BSA analysis is demonstrated below in the table and in the chromatogram, bringing confirmation to the resolution through the exhibited monomer, dimer and trimer (Table 2 & Figure 5). The hydrodynamic radius of the BSA in Figure 6 can be used to predict the behavior of the analyte in solution. The protein standard mixture, seen in Figure 7, demonstrates the separation and detection capability of the combined systems and their flexibility to operate with an organic solvent method (normal phase) or an aqueous method (reverse phase).[10, 11]

The red horizontal lines near each peak in the chromatograms below represent the molar mass distribution, and these can have linear, convex or concave shape. In this study, the BSA and protein mix exhibited some non-linear behavior in their molar mass distribution. This curvature is related to detector response of an analyte. A constant detector response is indicative of a homogenous sample that is baseline resolved from neighboring peaks in the chromatogram. Deviation from a constant detector response or non-linear response can be a result of increased peak heterogeneity or non-resolved peaks.[12]

Experiments: All | Peaks: All | Scalars: Custom | Abscissa: min

	Peak 1			Peak 2			Peak 3		
	Mw (kDa)	Calculated mass (μg)	Mass fraction (%)	Mw (kDa)	Calculated mass (μg)	Mass fraction (%)	Mw (kDa)	Calculated mass (μg)	Mass fraction (%)
BSA(002)[Aqueous Demo Sequence 3]	64.794 (±0.430%)	1.25	87.4	148.986 (±5.442%)	0.16	11.4	296.629 (±12.702%)	0.02	1.2
Average	64.794	1.25	87.4	148.986	0.16	11.4	296.629	0.02	1.2
Standard deviation	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
% Standard deviation	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Minimum	64.794	1.25	87.4	148.986	0.16	11.4	296.629	0.02	1.2
Maximum	64.794	1.25	87.4	148.986	0.16	11.4	296.629	0.02	1.2

Table 2.
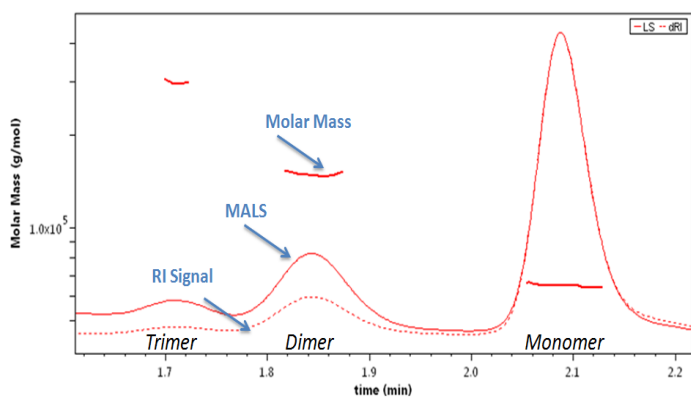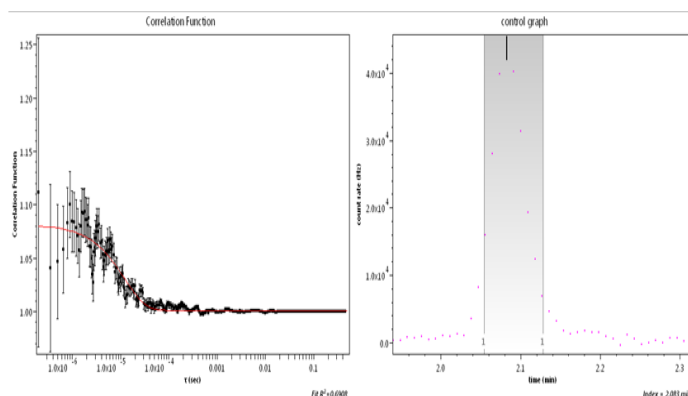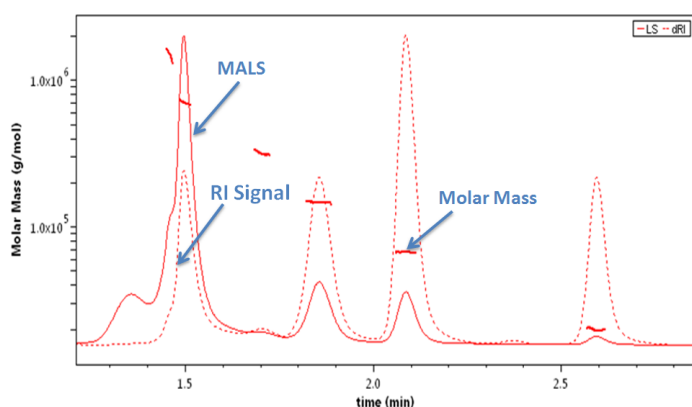The table is the Wyatt ASTRA calculations for the BSA molecular weight of peaks 1, 2 and 3.

Figure 5.
LS and RI chromatogram of BSA: horizontal lines

Figure 6.
QELS correlation and control graphs: BSA.

Figure 7.
LS and RI chromatogram of protein standard mix: horizontal lines denote molar mass distribution.

Using High Speed/High Resolution Size Exclusion Chromatography Separation of Polymeric Materials
with Dynamic and Multi-Angle Light Scattering Detection

4

CONCLUSIONS

- Coupling the APC, high resolution / high speed SEC system to the Wyatt µDAWN and RI detectors is easily managed with the selection of the correct tubing diameter.

- The impact of the Wyatt detectors on chromatographic band spread is minimized with their small inter-detector dispersion design capable of interfacing with Waters ACQUITY ultra high performance systems.

- In the example studied, the benefit of adding the MALS/QELS and RI to the SEC experiment offers the ability to calculate absolute molecular weight 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 µDAWN 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. Kenrick, Sophia; Berges, Aym; "Absolute Molar Mass in UHPLC via the µDawn and UT-rEX", Wyatt Technology Corporation, 18 November 2016, http://www.wyatt.com/files/literature/app-notes/sec-mals-proteins/absolute-molar-mass-uhplc.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. "Online MALS-QELS (Quasi-Elastic Light Scattering)", Wyatt Technology Corporation, 18 November 2016, http://www.wyatt.com/files/literature/app-notes/wyattqels-hydrodyn.pdf

9. "International Symposium on GPC/SEC and Related Topics", Amsterdam, N.L., 26 September 2016, http://www.cvent.com/events/international-symposium-on-gpc-sec-and-related-techniques/agenda-ca6d750bd4a34d429f5d0373eb4148fa.aspx

10. Van Krevelen, D.W.; Te Nijehuis, K.; *Properties of Polymers.* 4th Ed., Elsevier Science, Amsterdam, N.L., 2009.

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.

12. Slotbloom, D.J., Duurkens, R.H., Olieman, K., Erkens, G.B. (2008), "Static Light Scattering to Characterize Membrane Proteins in Detergent Solution", *Methods,* Issue 46, 73-82.