



TOSOH

# A New Paradigm in Light Scattering Technology.

Solution for advanced GPC/SEC detection

## LenS<sub>3</sub> MALS Detector

Accurate

Powerful

EcoSEC GPC

Simple

TSKgel



SECview

High Performance

## LenS<sub>3</sub> MALS-V Dual Detector



Convenience

Experts in Chromatography

TOSOH BIOSCIENCE

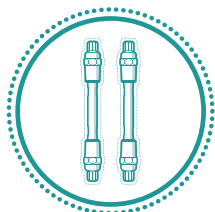
## ***Comprehensive Solution for Advanced Gel Permeation/Size Exclusion Chromatography***

Biopharmaceutical macromolecules such as proteins and antibodies, viral or polymer-based drug delivery vectors, new sustainable polymers and common engineering plastics all require increasingly thorough characterization to develop well-defined and safe products. With over 50 years of experience in chromatography, Tosoh Bioscience provides a complete suite of instruments, columns and software to support your analysis needs.



### **LenS<sup>TM</sup><sub>3</sub> Multi-Angle Light Scattering Detectors**

The LenS<sub>3</sub> MALS detector series offers a revolutionary approach for the measurement of molecular weight (MW) and size distributions of delivery vectors, biomolecules, synthetic polymers, polysaccharides, and biopolymers. Powered by simple, powerful and accurate SECview<sup>TM</sup> software.



### **TSKgel<sup>®</sup> GPC/SEC Columns**

Extensively used in laboratories all over the world, our TSKgel columns are designed for researchers seeking the highest level of performance. Covering the total range of GPC/SEC applications with multiple packing materials, porosities, and dimensions, these columns offer high resolution, excellent reproducibility and long column life.



### **EcoSEC<sup>TM</sup> GPC Systems**

The EcoSEC series of fully automated liquid chromatography systems for gel permeation chromatography is designed for robust polymer analysis. Both solutions, for ambient and for high temperature GPC, combine dual pump solvent systems, sophisticated heating and a highly efficient refractometry detection system to deliver the highest reproducibility.

## **Get Started**

Additional resources are available for helping you implement the LenS<sub>3</sub> Multi-Angle Light Scattering Detector into your laboratory.



### **Web**

Visit [tosohbioscience.com](http://tosohbioscience.com) for videos, product information and ordering.



### **Email**

Our technical service staff is ready to answer questions: [techservice.tbl@tosoh.com](mailto:techservice.tbl@tosoh.com)



### **In Person**

A technical seminar can be arranged on-site or via the web. Request via [seminars@tosoh.com](mailto:seminars@tosoh.com)



## LenS<sub>3</sub> Multi-Angle Light Scattering Detector

### *A Revolutionary Technology for Macromolecular Characterization*

The LenS<sub>3</sub> Multi-Angle Light Scattering Detector offers a revolutionary approach for the measurement of molecular weight (MW) and radius of gyration ( $R_g$ ) of synthetic polymers, polysaccharides, proteins, and biopolymers. LenS<sub>3</sub> is accompanied by SECview Software, offering seamless versatility from data acquisition to processing. Highlighted in *Figure 1* are the features and benefits of this truly innovative detector.

➤ **Figure 1.** Performance and highlights of the LenS<sub>3</sub> Multi-Angle Light Scattering detector

#### Highest Sensitivity

- A unique patent pending optics design
- Green light source: ~2.7x higher scattering intensity

#### Extended $R_g$ Measurement Range

- Extreme low and high measurement angles with high signal-to-noise ratio
- Angular dissymmetry made detectable for smaller molecules
- Patent pending assumption-free calculation method

#### HPLC/UHPLC Compatibility

- Allows usage of semi-micro HPLC columns and narrow-bore UHPLC columns

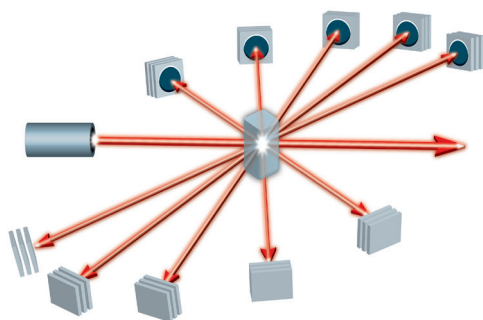
#### Powerful and Intuitive Software

- Easy to learn, use and teach
- MW,  $R_g$ , and much more in a few clicks with no model assumption over the broadest range
- Simple and instantaneous execution and comparison of data processing methods

## Revolutionary Detector Design

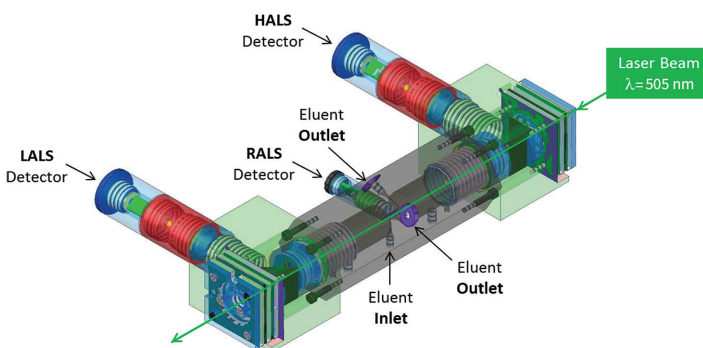
Multi-Angle Light Scattering (MALS) detectors have become a common tool to determine MW and size of macromolecules (Figure 2). The theory is based on the Rayleigh equation, where the intensity of the scattered light,  $R_{\theta}$ , is directly related to molecular weight of macromolecules. Using the traditional Zimm method, one can determine molecular weight, radius of gyration and the second virial coefficient of large scattering molecules on the basis of angular and concentration dependence measurements of the intensity of scattered light from dilute solutions.

➤ Figure 2. Typical MALS detector design



Alternatively, MW can be obtained accurately from a Low Angle Light Scattering (LALS) detector directly without angular extrapolation. The LenS<sub>3</sub> Multi-Angle Light Scattering detector is a revolutionary technology that combines the best of both MALS and LALS detectors. It does not contain a conventional cell and offers an extended flow path that uses 3 angles to provide MALS and LALS analysis, as depicted in Figure 3.

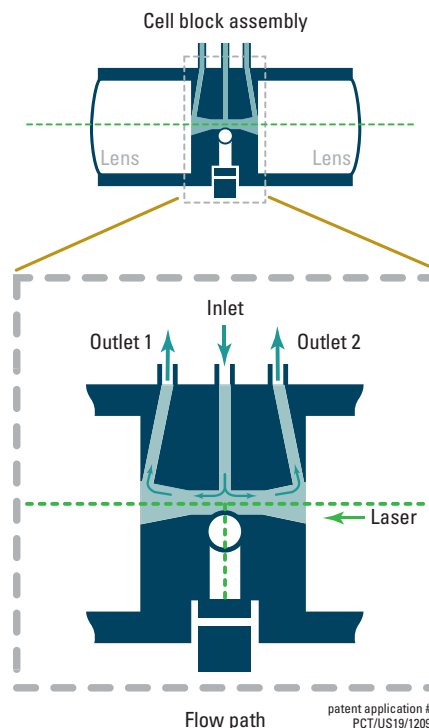
➤ Figure 3. LenS<sub>3</sub> MALS detector design



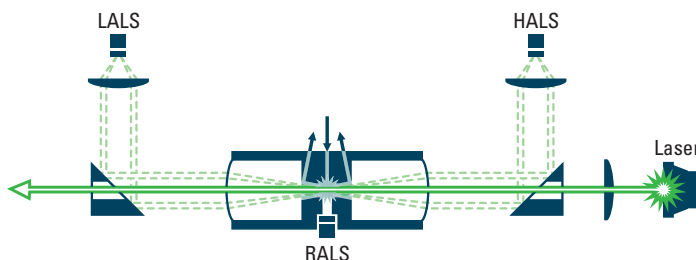
The angles are fixed at 10° (LALS), 90° (RALS) and 170° (HALS), while the inlet flow is split into two at the entrance of the measurement path and exits at two separate outlets.

Additionally, a green laser ( $\lambda = 505 \text{ nm}$ ) provides approximately 2.7 times higher scattering intensity than a conventional red laser ( $\lambda = 660 \text{ nm}$ ). Greater sensitivity is also provided by the unique design of the light path, as opposed to a conventional flow cell, which allows maximum interaction with solute molecules and a more effective light collection mechanism with lower noise (see Figure 4).

➤ Figure 4. Cell block assembly and flow path of the LenS<sub>3</sub> MALS detector



➤ Figure 5. Optics design of LenS<sub>3</sub> MALS detector



## Revolutionary Calculation Method

The LenS<sub>3</sub> MALS detector's number and the positions of the detector angles enable a unique and unmatched capability to offer users multiple calculation options for MW and R<sub>g</sub>.

### MW determination options:

- *Direct measurement using 10°*
- *Direct measurement using 90°*
- No longer necessary are Zimm plot extrapolations using multi-angle measurement

### R<sub>g</sub> determination options\*:

- *A patent pending method using a novel Angular Dissymmetry Plot (no concentration information necessary)*
- Replaces the historic assumption of total isotropic scattering for lower molecular size

The revolutionary calculation method for R<sub>g</sub> measurement using the angular dissymmetry plot addresses the two major limitations of the Zimm method: enhanced signal to noise allows lower R<sub>g</sub> measurements (R<sub>g</sub> < 10 nm) without requiring solute concentration. This approach uses a proprietary normalization procedure in addition to lower wavelength light source and enhanced optics to accomplish lower size measurements. It requires a minimum of 3 angles with one at the lowest and the other at the highest possible angle positions to provide R<sub>g</sub> data from below 10 nm to over 50 nm.

\* patent application #: PCT/US19/12095: Light Scattering Detectors and Methods for the Same

Whitepaper - Light Scattering for Determination of Molecular Weight and Radius of Gyration [W20116]

Expanding the boundaries of light scattering [TN21107A]



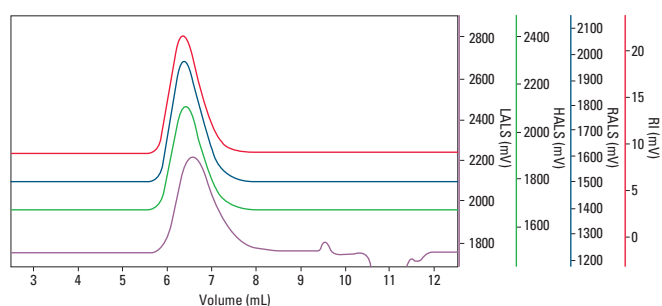
## Accurate Molecular Weight and $R_g$ Determination

### Molecular weight and radius of gyration are measured accurately across the entire distribution with the LenS<sub>3</sub> MALS detector.

#### System & Columns

- EcoSEC® GPC System with RI detector
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × TSKgel® GMH<sub>HR</sub>-H (7.8 mm ID × 30 cm) column

➤ **Figure 6.** LALS (red), RALS (blue), HALS (green) and RI (purple) signals of the NIST SRM706a standard



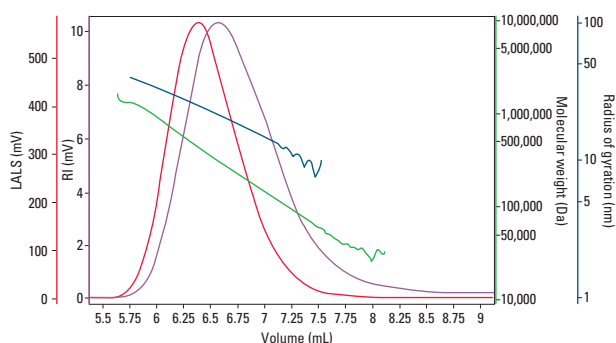
➤ **Table 1.** Measured values of molecular weight and radius of gyration of the NIST SRM706a PS standard by light scattering

Parameter	From LenS <sub>3</sub> MALS	Literature values
$M_n$ (g/mol)	169,030	-
$M_w$ (g/mol)	289,900	285,000 ± 23,000*
$M_z$ (g/mol)	426,780	-
$PDI = M_w/M_n$	1.72	-
$R_{g,z}$ (nm)	27.8	27.8 ± 1*

#### Samples & Conditions

- NIST SRM706a broad polystyrene (PS) standard
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 0.77 mg/mL; injection volume = 20  $\mu$ L

➤ **Figure 7.** Molecular weight (green) and  $R_g$  (blue) distributions overlaid with RI and LALS signals



\* SRM 706; *Polystyrene (Broad Molecular Weight Distribution)*; National Bureau of Standards; U.S. Department of Commerce: Washington, DC (1967); available at <https://www-s.nist.gov/srmors/certificates/706a.pdf> (accessed Mar 2019).

Podzimek, S.; *Light Scattering, Size Exclusion Chromatography and Asymmetric Flow Field Flow Fractionation*; Wiley, 2011; p 233.

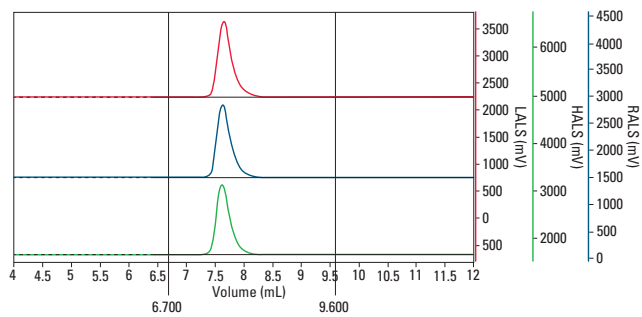
## Radius of Gyration Below 10 nm

**R<sub>g</sub> values of small polymers below 10 nm are reliably measured by light scattering for the first time in history with the LenS<sub>3</sub> MALS detector!**

### System & Columns

- EcoSEC GPC System with RI detector
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × TSKgel GMH<sub>HR</sub>-H (7.8 mm ID × 30 cm) column

➤ **Figure 8.** LALS (red), RALS (blue) and HALS (green) signals with baselines and integration limits for PS 30K standard



➤ **Table 2.** Measured values of radius of gyration, R<sub>g,z</sub>, of PS standards

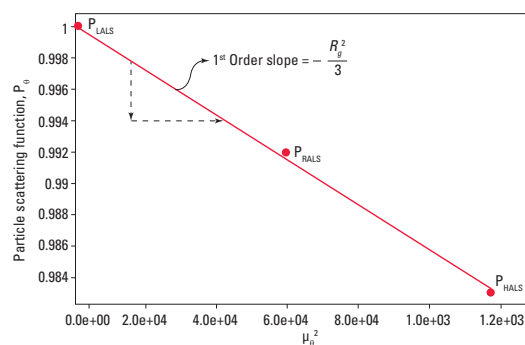
Sample	M <sub>w</sub>	R <sub>g,z</sub>	Literature values*
PS100K	100,400	12.0	13.2
PS40K	40,800	7.6	8.1
PS30K	30,100	6.6	6.8
PS20K	18,600	5.5	5.3
PS10K	10,700	4.6	3.9

\* Literature values calculated using the correlation R<sub>g</sub> = 0.0245·M<sub>w</sub><sup>0.546</sup>

### Samples & Conditions

- Polystyrene (PS) standards below 100K
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 1 - 3 mg/mL; injection volume = 50 μL

➤ **Figure 9.** Angular dissymmetry plot for PS 30K



where,

$$\mu_\theta = \frac{4\pi n_p \sin\left(\frac{\theta}{2}\right)}{\lambda}$$

n<sub>p</sub> = refractive index of mobile phase

λ = wavelength of laser

$$P_\theta = N_\theta \cdot \frac{I_\theta}{I_0}$$

N<sub>θ</sub> = normalization factor for angle θ

I<sub>θ</sub> = scattering intensity for angle θ

I<sub>0</sub> = scattering intensity for angle 0

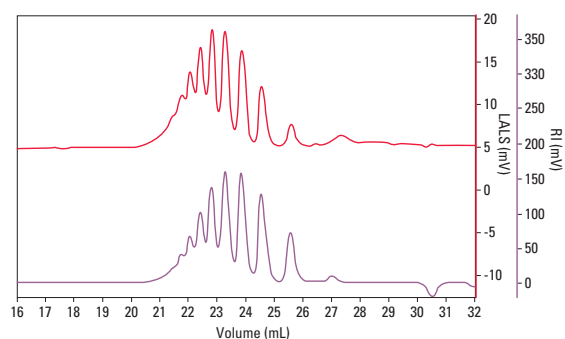
## Ultra Low Molecular Weight Oligomers by LALS

### The Low Angle Light Scattering detector is capable of measuring molecular weight down to 200 Da!

#### System & Columns

- EcoSEC GPC System with RI detector
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × G3000H<sub>XL</sub>, 1 × G2500H<sub>XL</sub> and 1 × G2000H<sub>XL</sub> (all 7.8 mm ID × 30 cm) columns

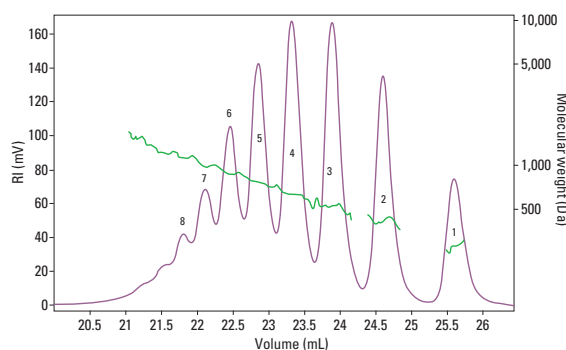
➤ **Figure 10.** LALS (red) and RI (purple) signals of the A-500 PS standard



#### Samples & Conditions

- Tosoh A-500 polystyrene (PS) standard
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Concentration = 8.54 mg/mL; injection volume = 50 µL

➤ **Figure 11.** Molecular weight distribution (green) overlaid with RI signal



➤ **Table 3.** Theoretical molecular weights of the oligomers present in A-500 and  $M_p$  values obtained from LALS (using an average  $dn/dc$  of 0.170)

Peak #	Theoretical MW	$M_p$ from LALS
1	266	255
2	370	377
3	474	503
4	578	618
5	682	725
6	786	849
7	890	985
8	994	1105



## Superior Sensitivity

# The LenS<sub>3</sub> Multi-Angle Light Scattering detector achieves repeatable molecular weight measurement down to 15 ng of PS 100K in THF!

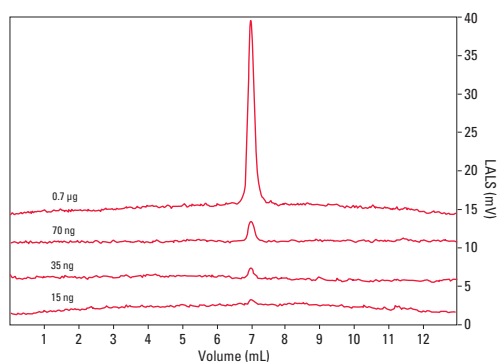
### System & Columns

- EcoSEC GPC System with RI detector
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × TSKgel GMH<sub>HR</sub>-H (7.8 mm ID × 30 cm) column

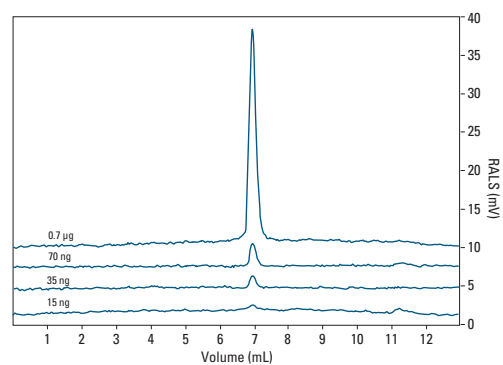
### Samples & Conditions

- Tosoh 100K polystyrene (PS) standard F-10
- THF at 1.0 mL/min; T = 35 °C (pumps, column and RI)
- Decreasing injected mass

➤ **Figure 12.** LALS signals for decreasing injected mass of PS 100K standard F-10



➤ **Figure 13.** RALS signals for decreasing injected mass of PS 100K standard F-10



➤ **Table 4.** Molecular weights by LALS and RALS for decreasing injected mass of PS 100K standard F-10

Injected mass	MW by LALS	MW by RALS
0.7 µg	102,110	101,570
70 ng	101,520	100,960
35 ng	101,150	100,870
15 ng – inj. 1	-	102,180
15 ng – inj. 2	-	100,980
15 ng – inj. 3	-	101,390
15 ng – inj. 4	-	99,800
15 ng – inj. 5	-	100,760
15 ng – Average	-	101,020 ± 780 (0.77%)

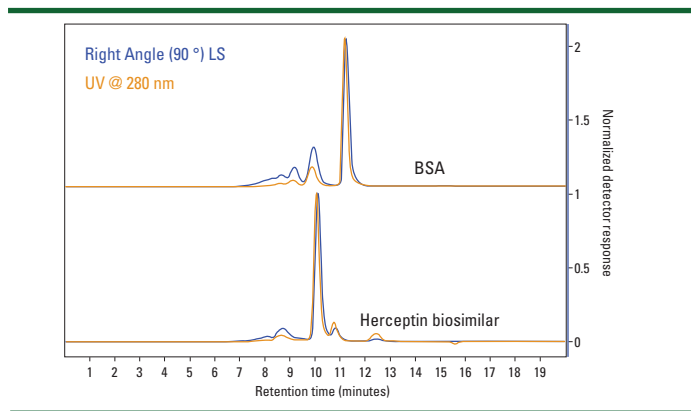
## Protein Aggregates and Fragments Identification and Quantitation

### Oligomers and fragments of BSA and monoclonal antibodies are easily detected and identified with the LenS<sub>3</sub> MALS detector.

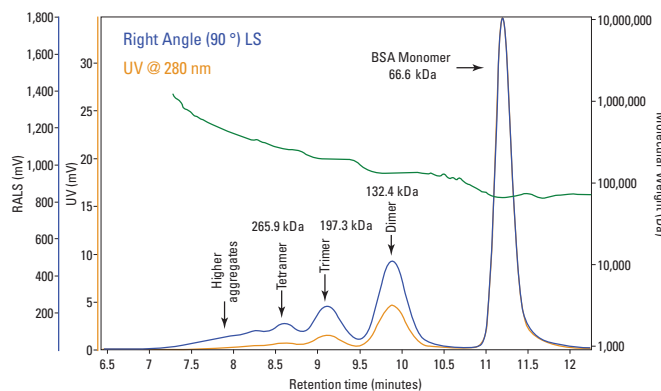
#### System & Columns

- Thermo Fisher Dionex Ultimate® 3000 UHPLC system with UV detector @ 280 nm
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW3000 (4.6 mm ID × 30 cm) column

➤ **Figure 14.** Raw chromatograms of BSA and Herceptin biosimilar samples, with light scattering and UV signals



➤ **Figure 15A.** Molecular weight (green) distribution curve and values of BSA oligomers overlaid with UV (orange) and RALS (blue) signals

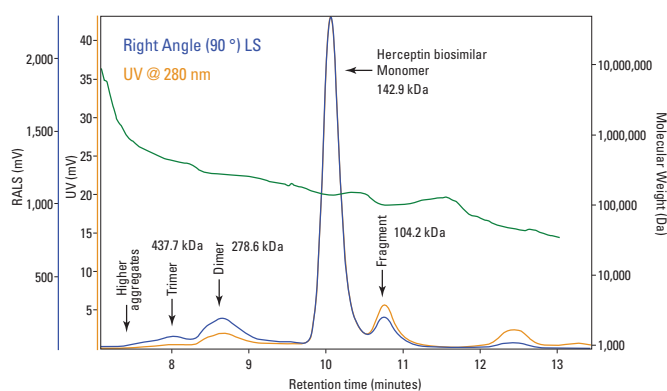


Peak	MW (kDa)	Area (%)
Monomer	66.6	74.1
Dimer	132.4	16.7
Trimer	197.3	5.4
Tetramer	265.9	2.1
Aggregates	Up to 1,000+	1.7

#### Samples & Conditions

- Bovine Serum Albumin (BSA) and Herceptin® biosimilar
- 100 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8 + 100 mmol/L Na<sub>2</sub>SO<sub>4</sub>
- Flow rate = 0.25 mL/min
- Concentration:  
BSA = 3.58 mg/mL, injection volume = 10 μL  
mAb = 2.75 mg/mL, injection volume = 7 μL

➤ **Figure 15B.** Molecular weight (green) distribution curve and values of Herceptin biosimilar overlaid with UV (orange) and RALS (blue) signals



Peak	MW (kDa)	Area (%)
Fragment	104.2	11.8
Monomer	142.9	69.1
Dimer	278.6	8.3
Trimer	437.7	1.3
Aggregates	Up to 5,000+	0.3

## Superior Sensitivity for mAb

Superior sensitivity for mAbs can be demonstrated down to 2 ng of sample loading.

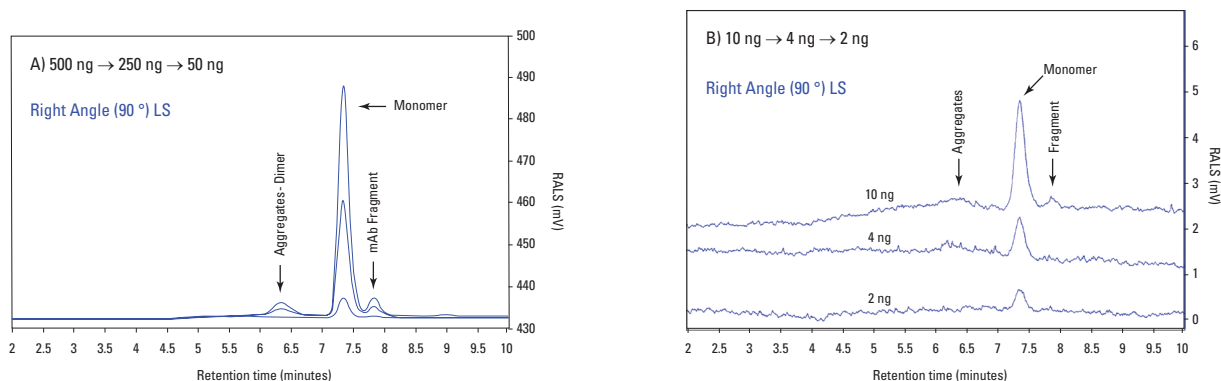
### System & Columns

- Thermo Fisher Dionex Ultimate 3000 UHPLC system with UV detector @ 280 nm
- LenS<sub>3</sub> Multi-Angle Light Scattering detector
- 1 × TSKgel UP-SW3000 (4.6 mm ID × 30 cm) column

### Samples & Conditions

- Herceptin biosimilar
- 100 mmol/L NaH<sub>2</sub>PO<sub>4</sub>, pH 6.8 + 100 mmol/L Na<sub>2</sub>SO<sub>4</sub>
- Flow rate = 0.35 mL/min
- Injected mass from 500 ng down to 2 ng

➤ **Figure 16A and 16B.** Light scattering signal (RALS) of Herceptin biosimilar monomer, fragment and aggregates with decreasing injected mass



## Analysis of Unpurified and Purified Oligonucleotides

# Rapid and accurate molecular weight profiling of small oligonucleotides is now possible!

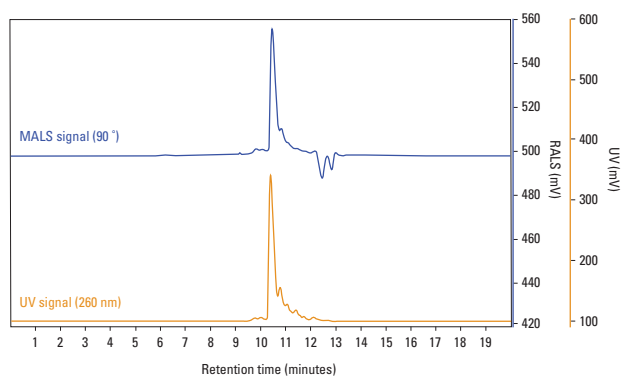
### System & Columns

- Thermo Fisher Dionex Ultimate 3000 UHPLC system with UV detector @ 260 nm
- LenS3 Multi-Angle Light Scattering detector
- 1 x TSKgel UP-SW2000 (4.6 mm ID x 30 cm) column

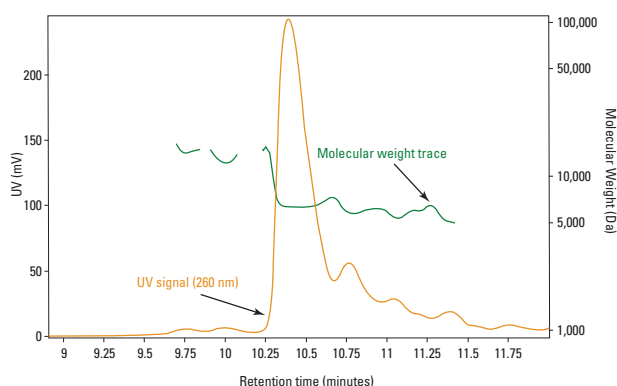
### Samples & Conditions

- 20-bases custom oligonucleotide with MW=6,141 Da
- 0.5 mol/L NaCl + 0.1 mol/L EDTA + 0.1 mol/L Na<sub>2</sub>SO<sub>4</sub> + 0.05% NaN<sub>3</sub> in 0.1 mol/L phosphate buffer, pH 7.52
- Flow rate = 0.30 mL/min
- Injection volume = 10 µL
- Concentration:  
Purified sample = 0.3 mg/mL  
Unpurified sample = 1 mg/mL

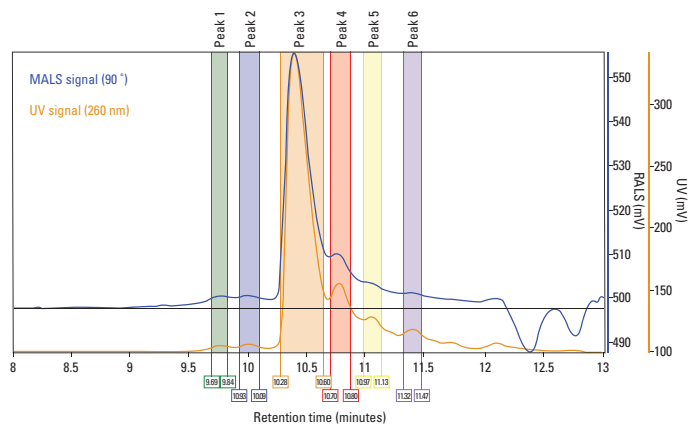
➤ **Figure 17.** RALS (blue) and UV (orange) signals of the unpurified 20-mer



➤ **Figure 18.** Molecular weight distribution (green) of the unpurified 20-mer



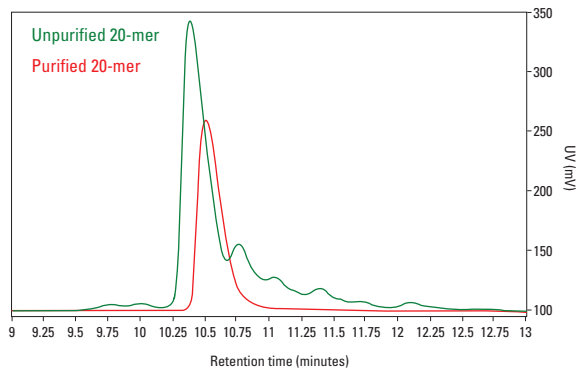
➤ **Figure 19.** Peak analysis of the unpurified 20-mer



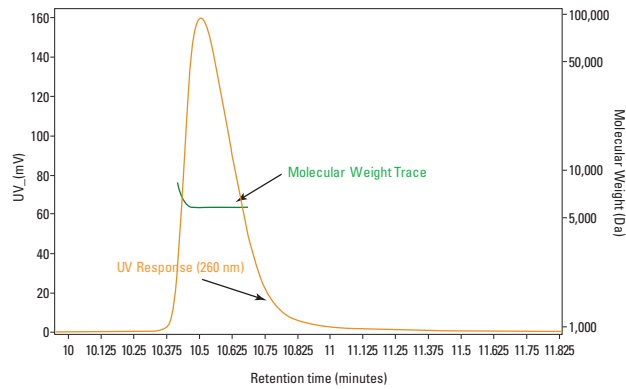
➤ **Table 5.** Retention time and molecular weight of each peak (triplicate injection)

Peak	Retention time (min)	% RSD	MW (Da)	% RSD
1	9.774	0.1%	13,599	2.1%
2	10.012	0.0%	11,550	1.9%
3	10.398	0.1%	6,398	0.7%
4	10.776	0.1%	5,751	1.5%
5	11.053	0.1%	5,177	2.3%
6	11.422	0.2%	4,446	5.5%

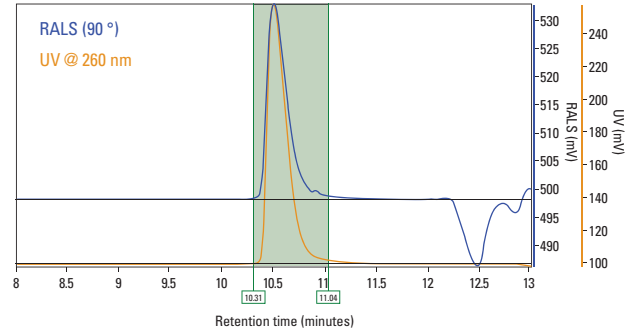
➤ **Figure 20.** Overlay of the unpurified (green) and purified (red) 20-mer UV chromatograms



➤ **Figure 21.** Molecular weight distribution (green) of the purified 20-mer



➤ **Figure 22.** Peak analysis of the purified 20-mer



➤ **Table 6.** Retention time and molecular weight of the purified 20-mer (triplicate injection)

Injection	Retention time (min)	MW (Da)
1	10.431	6,066
2	10.443	6,023
3	10.445	6,038
Average	10.440	6,042
<b>%RSD</b>	<b>0.1%</b>	<b>0.3%</b>





## Optional Viscometer for LenS<sub>3</sub> – LenS<sub>3</sub> MALS-V Dual Detector

### *A Unique and Flexible Solution for Triple Detection GPC/SEC of Polymers*

The LenS<sub>3</sub> MALS detector can be equipped with an optional integrated viscometer. The LenS<sub>3</sub> MALS-V dual detector thus offers a simple and compact solution for triple detection measurements when combined with a concentration detector (RI or UV). Just like with light scattering data, the SECview software includes seamless viscometry data acquisition and processing to get the most out of advanced detection GPC/SEC analyses. The features and benefits of this state-of-the-art triple detection solution are highlighted in Figure 23.

➤ **Figure 23.** Performance and highlights of the LenS<sub>3</sub> MALS-V dual detector

#### **Integrated MALS & Viscometry Dual Detector**

- 2-in-1 detector module with small footprint
- Patent-pending\* internal flow path with minimized dead volume and band broadening

#### **High-performance and versatile Triple Detection solution**

- High-sensitivity dual detector
- Broad MW and size measurement range ( $R_g$  and  $R_h$ ) for all types of polymers
- Compatible with any GPC/SEC system

#### **Easy-to-use and advanced Triple Detection GPC/SEC software**

- Seamless execution of triple detection, universal, and conventional calculations
- Easy conformation, shape and branching analysis

### *A perfect complement to MALS for polymer analysis*

The intrinsic viscosity (IV) of polymers reflects how dense and how flexible polymer chains are in dilute solutions. The lower the IV, the more compact the polymer is while rigid polymer chains show a higher IV than flexible random coils.

When combined with light scattering measurements, the MW and IV distributions provide a wealth of structural information about polymers:

- conformation (random coil, sphere, rod)
- density of the backbone
- presence of branching (number of branches, branching frequency)
- hydrodynamic radius ( $R_h$ )
- Mark-Houwink parameters

The viscometer also allows the determination of MW by universal column calibration for comparison with legacy methods or for special applications to polymers with unfavorable optical properties (e.g. weak scatterers, light absorption and fluorescence).

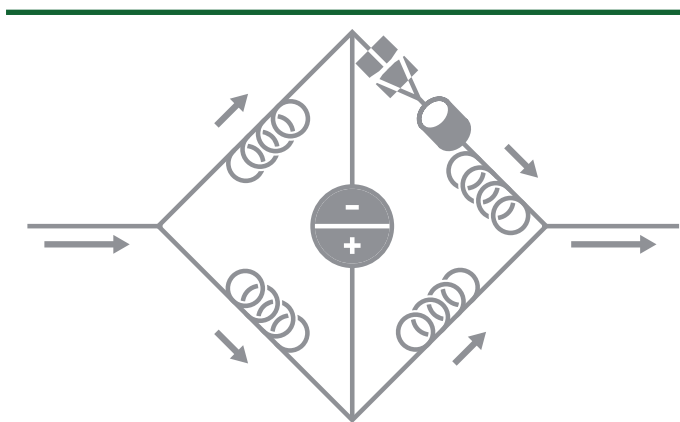
## Unique Dual Detector Flow Path

While the use of advanced light scattering and viscometry detectors enhances conventional GPC/SEC analyses, forming an optimal setup raises some non-trivial technical challenges. Minimizing inter-detector volumes and the resulting band broadening effect is certainly the most challenging issue that multi-detection systems design must address.

Typical multi-detection setups place the light scattering detector first in series with the viscometer, thus generating extra dead volume from the connection between the two detectors.

The LenS<sub>3</sub> MALS-V dual detector incorporates the light scattering cell chamber directly into the 4-capillary bridge of the differential viscometer. (Figure 24). Before entering the delay volume, the sample will first flow through the MALS flow cell, which has a very low resistance. The capillary in this branch of the viscometer is adjusted to ensure the bridge is correctly balanced. This fully integrated design allows the two measurements (light scattering and viscometry) to occur almost simultaneously, which minimizes dead volume and peak broadening greatly.

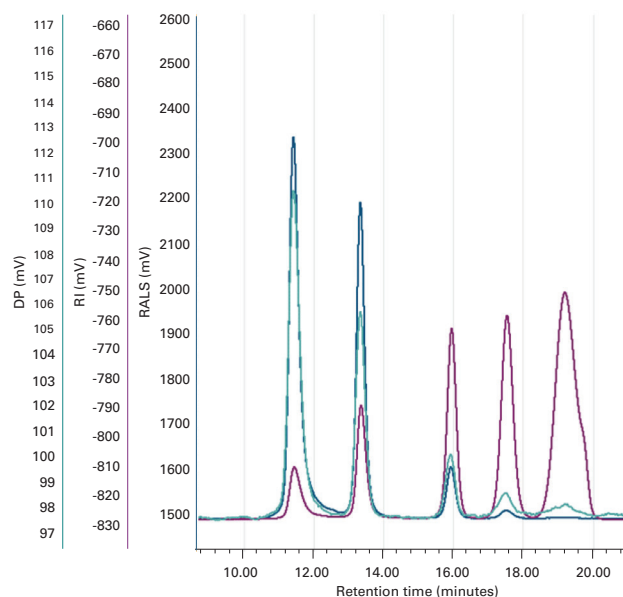
➤ Figure 24. Flow path diagram of the LenS<sub>3</sub> MALS-V dual detector



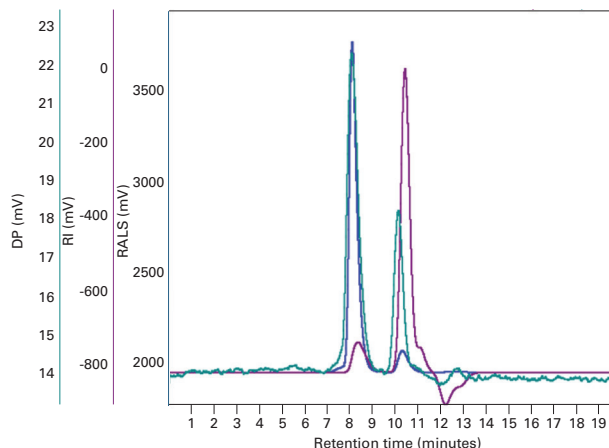
## Triple detection with high resolution and high sensitivity

This unique flow path preserves the quality of the separation obtained from high resolution GPC/SEC columns, even when using semi-micro columns, while maximizing the sensitivity of the viscometer, as shown in figures 25 a) and b), Figure 26 and Table 7.

➤ Figure 25a. Triple chromatogram (RI, LS and DP) of polystyrene standards 2.89 MDa (F-288), 430k Da (F-40), 41k Da (F-4), 5k Da (A5000) and 500 Da (A500) obtained using 2x TSKgel GMH<sub>HR</sub>-M columns (7.8 mm ID x 30 cm L) with EcoSEC Elite GPC system and LenS<sub>3</sub> MALS-V dual detector at 1.0 mL/min, 40° C.

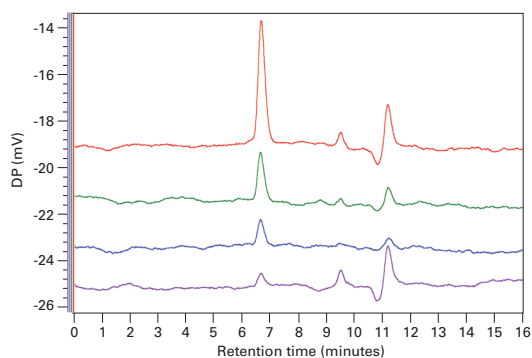


➤ Figure 25b. Triple chromatogram (RI, LS and DP) of polystyrene standards 200 kDa (F-20) and 2500 Da (A2500) obtained using 1x TSKgel SuperHM-H semi-micro column (6 mm ID x 15 cm L) with EcoSEC Elite GPC system and LenS<sub>3</sub> MALS-V dual detector at 0.3 mL/min, 40° C.





➤ **Figure 26.** DP signal for decreasing injected mass of PS 100K standard F-10, using EcoSEC Elite GPC system with LenS<sub>3</sub> MALS-V dual detector and 1× TSKgel GMH<sub>HR</sub>-M columns (7.8 mm ID × 30 cm L) at 1.0 mL/min, 40° C.



➤ **Table 7.** Intrinsic viscosity obtained for decreasing injected mass of PS 100K standard F-10

Injected mass	Intrinsic viscosity (dL/g)
5 µg	0.449
2 µg	0.449
1 µg	0.451
0.5 µg	0.447



## Molecular weight, size and intrinsic viscosity distributions

The full and accurate picture of polymers is obtained rapidly by Triple Detection GPC/SEC with the LenS<sub>3</sub> MALS-V dual detector and the SECview software.

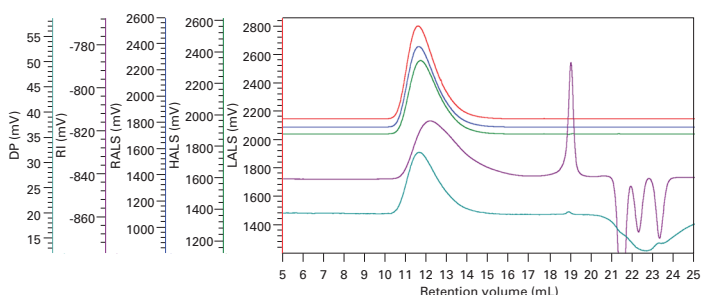
### System & Columns

- EcoSEC® Elite GPC system with RI detector
- LenS<sub>3</sub> MALS-V dual detector
- 2x TSKgel® GHH<sub>HR</sub>-M (7.8 mm ID x 30 cm) column

### System & Columns

- Broad distribution polystyrene (PS) sample
- THF at 1.0 mL/min, T=40°C (pumps, columns and RI)
- Concentration = 0.65 mg/mL; Injection Volume = 50 µL

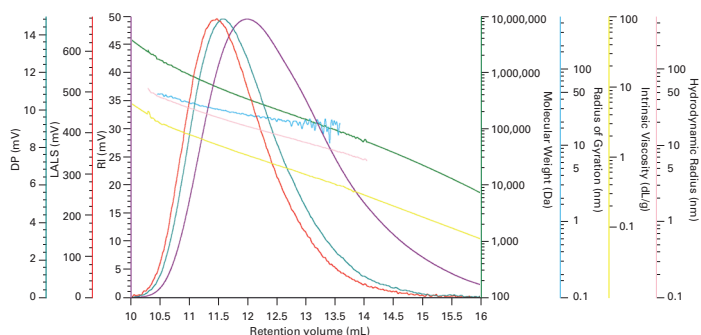
➤ **Figure 27.** Triple chromatogram of the broad polystyrene sample with LALS (red), RALS (blue), HALS (green), RI (purple) and viscometer DP (teal) signals.



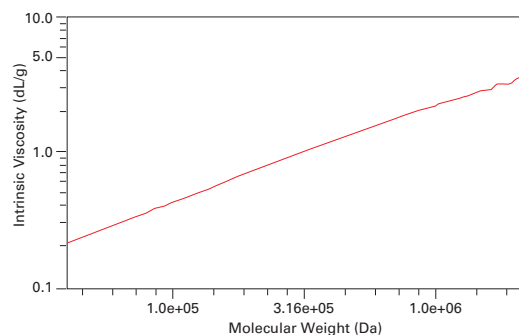
➤ **Table 8.** Measured values of molecular weight, radius of gyration, hydrodynamic radius and intrinsic viscosity of the broad polystyrene sample by triple detection GPC/SEC

Result	Triple Detection
M <sub>n</sub> (Da)	109,010
M <sub>w</sub> (Da)	298,320
PDI	2.74
R <sub>g</sub> (nm)	29.2
R <sub>h</sub> (nm)	16.1
IV (dL/g)	0.88

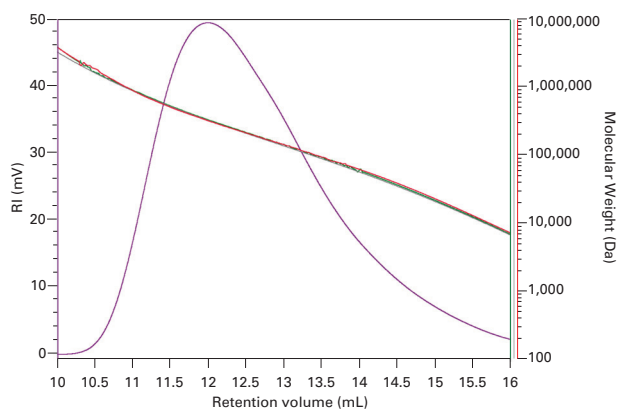
➤ **Figure 28.** Molecular weight (green), R<sub>g</sub> (blue), R<sub>h</sub> (pink) and intrinsic viscosity (yellow) distributions overlaid with RI, LALS and DP signals.



➤ **Figure 29.** Mark-Houwink plot of the broad polystyrene sample obtained by triple detection GPC/SEC



➤ **Figure 30.** Overlay of molecular weight distributions obtained by light scattering (green), conventional calibration (grey) and universal calibration (red) with RI signal.



➤ **Table 9.** Measured values of molecular weight moments by conventional calibration, universal calibration and light scattering

Result	Conventional calibration	Universal Calibration	Light Scattering
M <sub>n</sub> (Da)	105,460	110,630	109,010
M <sub>w</sub> (Da)	291,800	292,370	298,320
M <sub>z</sub> (Da)	507,960	515,590	519,200
PDI	2.77	2.64	2.74

## Characterization of Dextrans

# Triple Detection GPC/SEC is now easy-to-use for the complete analysis of polysaccharides with structural differences.

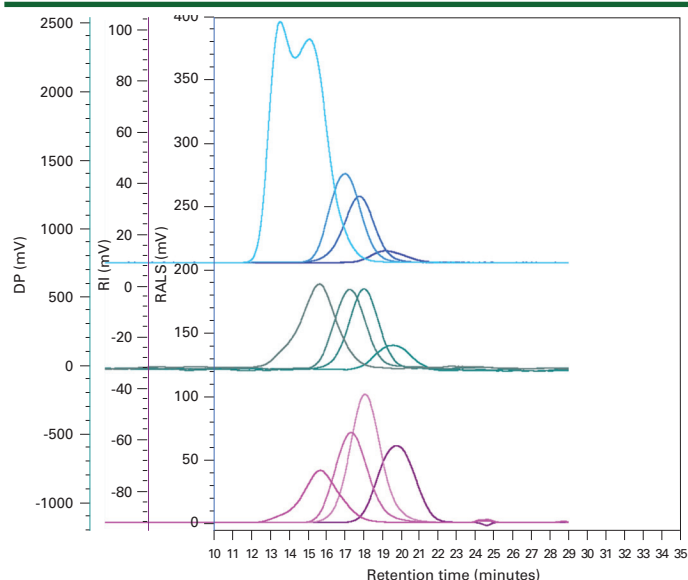
### Samples & Conditions

- EcoSEC Elite GPC system with RI detector
- LenS<sub>3</sub> MALS-V dual detector
- 2x TSKgel GMPW<sub>XL</sub> (7.8 mm ID x 30 cm) column

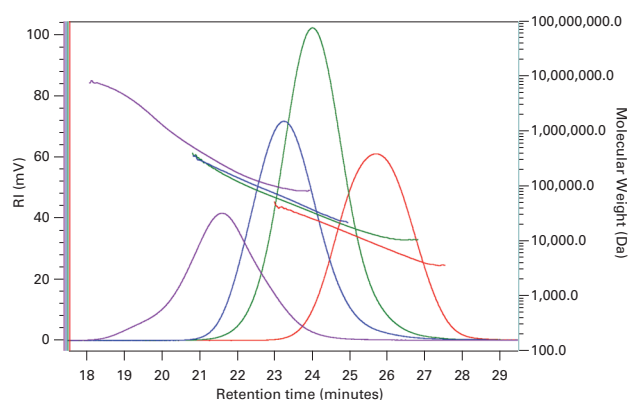
### System & Columns

- Dextran samples 10K, 40K, 70K and 320K
- NaNO<sub>3</sub> 0.1 mol/L at 0.7 mL/min, T=40° C (pumps, columns and RI)
- Concentration = 1-4 mg/mL; Injection Volume = 50 μL

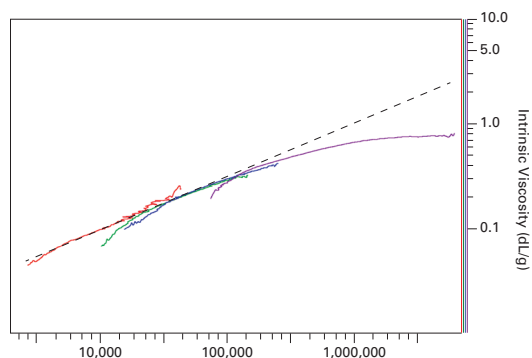
➤ **Figure 31.** Triple chromatograms of the dextran samples with RALS (blue), RI (purple) and viscometer DP (teal) signals



➤ **Figure 32.** Molecular weight distributions overlaid with RI signals of the dextran 10K (red), 40K (green), 70K (blue) and 320K (purple) samples.



➤ **Figure 33.** Overlay of the Mark-Houwink plots of the dextran 10K (red), 40K (green), 70K (blue), 320K (purple) samples and linear reference (black), showing increasing density with increasing MW due to the presence of branching.

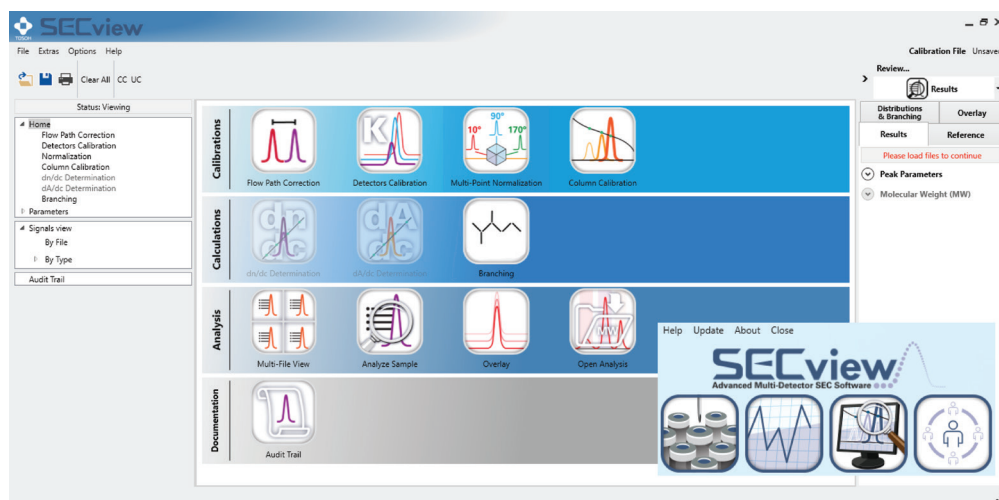


➤ **Table 10.** Measured values of molecular weight, radius of gyration, hydrodynamic radius and intrinsic viscosity of dextran samples by triple detection GPC/SEC

Results	Dextran 10K	Dextran 40K	Dextran 70K	Dextran 320K
M <sub>n</sub> (g/mol)	7,680	28,550	54,950	212,390
M <sub>w</sub> (g/mol)	10,150	37,200	71,510	503,290
PDI	1.32	1.30	1.30	2.37
R <sub>g</sub> (nm)	-	9.4	10.6	23.3
R <sub>h</sub> (nm)	2.5	4.7	6.4	15.4
IV (dL/g)	0.09	0.175	0.24	0.46

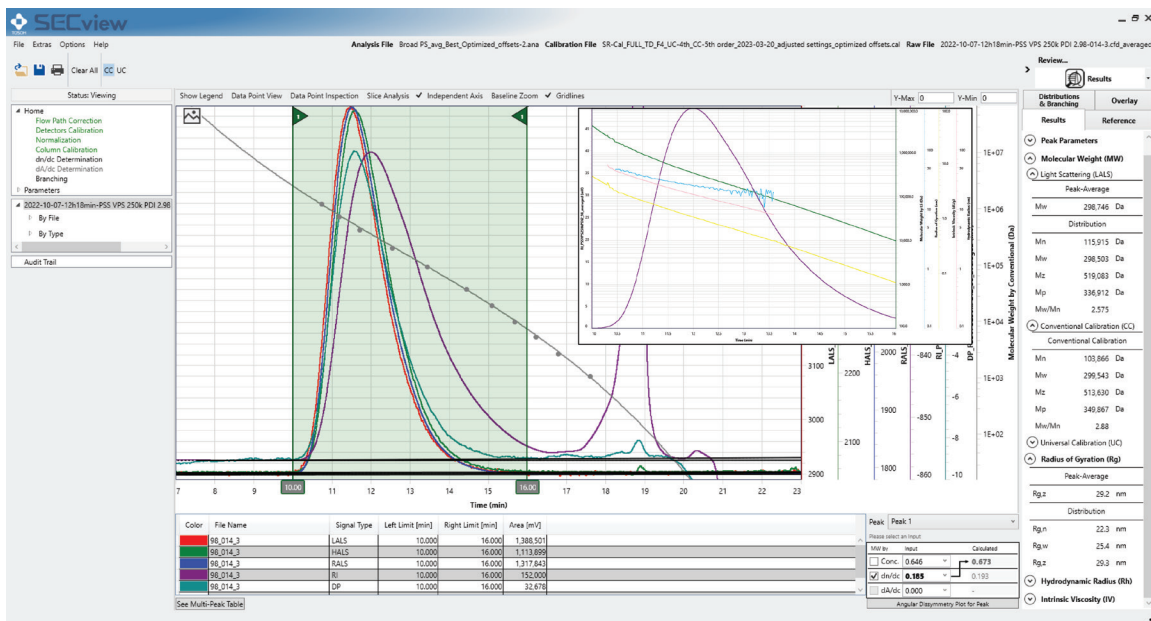
# Introducing SECview: the most user-friendly multi-detector GPC/SEC software

Designed from the ground up by the industry's top experts, SECview provides the most intuitive platform, performing basic to the most advanced multi-detector GPC/SEC analysis. Totally focused around enhancing the user-interface experience, SECview is a welcoming fresh perspective that streamlines the complex calculations required by the advanced detectors. This allows users to swiftly obtain results and complete the analysis without lengthy and cumbersome steps.



## SECview is a total and complete GPC/SEC solution!

Whether the goal is to obtain Absolute Molecular Weight and Radius of Gyration data from the LenS3 MALS detector or to perform Conventional Column Calibration (CCC) for routine GPC/SEC analysis, the new software platform can easily accommodate all data processing techniques. In fact, SECview is the only GPC/SEC data processing module today that performs analysis of sample-of-interest using multiple calibration methods SIMULTANEOUSLY!



← Data from MALS

← Data from CCC

← Data from MALS

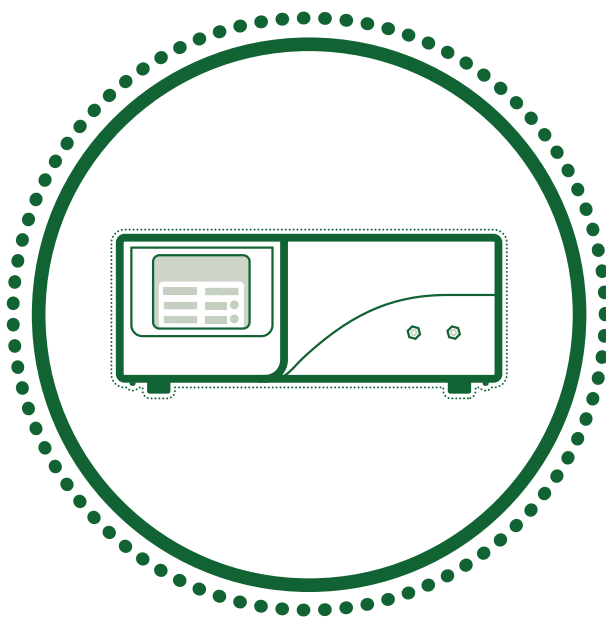
SECview is 21-CFR-Part 11 compliant and provides the necessary tools for audit trails, system validation and multi-user access for users operating in the FDA-regulated environment.

### SECview Features

- Simultaneous multi-method execution and analysis
- Multi-point  $dn/dc$  and UV extinction coefficient determination
- Automatic peak detection for conventional column calibration method
- Multi-peak selection and “independent” data processing
- Adaptable multiple-injection overlay platform
- Advanced peak band broadening and inter-detector volume correction algorithms
- Direct access to the raw data signals while offering powerful de-spiking and smoothing options
- Access to data point cursors on chromatograms and derived graphs
- Easy export of raw data to ASCII files and graph/chromatogram to picture file

### SECview Parameters

- Absolute molecular weight by low angle ( $10^\circ$ ) and right angle ( $90^\circ$ ) light scattering
- Molecular weight in peak average and distribution (*PDI*) forms
- Radius of gyration by 3 angle MALS using the patent pending angular dissymmetry plot
- Concentration, injection-mass recovery,  $dn/dc$ , and UV extinction coefficient



## Specifications

Light scattering	
Number of measurement angles	3
Position of the measurement angles	LALS (10°) RALS (90°) HALS (170°)
Cell geometry	proprietary conical flow path (single inlet, dual outlets)
Laser source type	diode
Laser power	20 mW +/- 5
Laser wavelength	505 nm
Laser temperature control	yes
Wetted material	teflon, PEEK, glass, stainless steel
Maximum flow rate	2 mL/min
Inlet position	front or side
Baseline noise (in THF @ 1 mL/min)	< 1 mV
Baseline drift (in THF @ 1 mL/min)	< 1 mV / 30 min
MW range	< 200 to 10 <sup>7</sup> Da
R <sub>g</sub> range	< 2 nm to > 50 nm (in progress)
Viscometer (optional)	
Measurement principle	4-capillary differential bridge
Dynamic range	5 kPa
Linearity	0.1% full scale
Baseline noise (in THF @ 1 mL/min)	< 0.2 Pa
Baseline drift (in THF @ 1 mL/min)	< 0.25 Pa / 60 min
Volume	23 µL per capillary
Maximum flow rate	4 mL/min in THF; 2 mL/min in water
Maximum pressure	200 kPa
Maximum detector backpressure at outlet	50 kPa
Wetted material	Teflon, stainless steel, PEEK
Delay volume	Small, Medium or Large
General	
A/D board channels / resolution	8 channels / 24 bits
Acquisition rate	10 Hz
Dynamic range	+/- 10 V
Analog inputs	RI, UV and start signal
Connection to PC	ethernet
Dimensions	36.5 (W) × 48.5 (D) × 13 (H) cm = 14.4" × 19.1" × 5.1"
Weight	16 kg = 35 lbs for LenS <sub>3</sub> MALS; 17 kg = 37 lbs for LenS <sub>3</sub> MALS-V
Intellectual property	PCT/US19/12090: Light Scattering Detectors and Sample Cells for the Same PCT/US19/12095: Light Scattering Detectors and Methods for the Same PCT/US2023/025136: Detector for Viscosity and Light Scattering and Methods for the Same

## Ordering Information

Part #	Description
0040000	LenS <sub>3</sub> Multi-Angle Light Scattering Detector
0040001	UHPLC Conversion Service Kit for LenS <sub>3</sub> MALS Detector
0040200	LenS <sub>3</sub> MALS-V Multi-Angle Light Scattering Detector

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