TOSOH

Performance Data

Increased Sensitivity with LenS3 MALS Detector

Introduction

The necessity to better understand the biophysical and chemical characteristics of macromolecules such as monoclonal antibodies (mAbs) has led to the use of more advanced analytical techniques at every level of the development process. Size exclusion chromatography (SEC) is one such method that separates analytes within a sample solution based on their hydrodynamic radii to provide size and purity of a biotherapeutic monomer. It can be further expanded through the use of multi-angle light scattering (MALS) for the determination of molecular weight (MW) of monomers, aggregates and fragments.

Through improvements in both U/HPLC instrumentation and chromatography columns, both resolution and sensitivity have increased with analytical SEC. This increase in sensitivity has allowed users to inject less material for quantitation and analysis by UV, although these amounts are not always suitable for traditional MALS detectors.

The LenS3 multi-angle light scattering detector introduces a novel fluidic and optical design that dramatically increases the sensitivity of the light scattering signal by increasing the signal to noise (S/N) ratio. This provides a significant improvement in the limit of quantification (LOQ) and limit of detection (LOD), allowing molecules of interest to be analyzed at the optimal range of the analytical column as well as providing the ability to monitor aggregates and fragments of low relative abundance. The reduction of noise due to the new cell block assembly adds the useability of clear signals even at an extreme low angle (10°) and extreme high angle (170°). Molecular weight can now be accurately determined directly without extrapolation by use of a true low angle light scattering signal (LALS) for all sample sizes, or right angle light scattering signal (RALS) for small sized molecules, both with extensively higher sensitivity than a conventional MALS detector.

Objective

To demonstrate the capabilities and compare the improved sensitivity of the LenS3 MALS detector to an existing competitive system.

Material and Methods

Chromatographic conditions for all injections

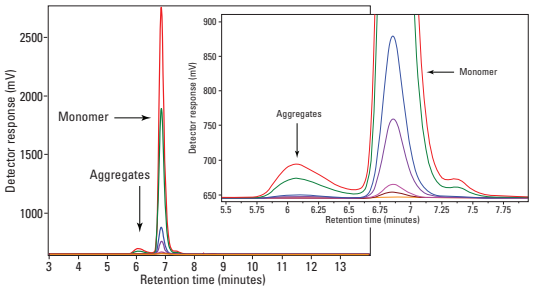
Column:	TSKgel® UP-SW2000, 2 µm, 4.6 mm ID × 30 cm
Instrument:	Thermo Scientific Vanquish™ UHPLC
Mobile phase:	PBS, pH 6.7 (refractive index 1. 331)
Flow rate:	0.35 mL/min
Temperature:	ambient
Detection:	UV @ 280 nm; MALS (LenS3 and competitive MALS detector)
Sample:	Rituximab; MW ~150,000 Da (dA/dc = 1.42 mL/g; dn/dc = 0.185 mL/g)
Load:	see tables 1 and 2

The LenS3 and a competitive MALS detector were coupled to a Thermo Scientific Vanquish UHPLC, with UV acting as the concentration detector. Monoclonal antibody with an expected molecular weight of ~150,000 Da was prepared at different concentrations and injected at 10 µL (as indicated on the table in *Figure 1*) onto a TSKgel UP-SW2000 SEC column.

Results and Discussion

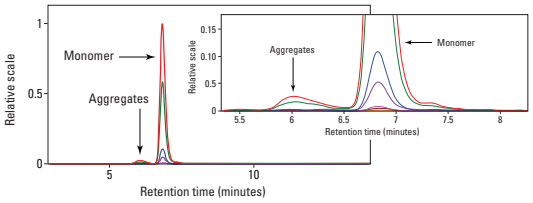
An overlay of the right angle light scattering signals (RALS) used to calculate MW and S/N for both detectors is shown in *Figure 1*. Signal to noise and molecular weight were recorded and compared between the two detectors using identical chromatographic conditions.

Figure 1. Overlay of RALS signals for LenS3 and competitive MALS detector



LenS3 MALS Detector

#	Concentration (mg/mL)	Load (µg)	SN Monomer	Injection volume (10 µL) Monomer -MW	Injection volume (10 µL) Aggregate -MW
1	1	10	8,000	150,373	296,538
2	0.5	5	5,000	149,641	291,396
3	0.1	1	800	151,003	301,290
4	0.05	0.5	400	150,675	309,150
5	0.01	0.1	80	150,240	-
6	0.005	0.05	40	152,160	-
7	0.001	0.01	10	143,072	-



Competitive MALS Detector

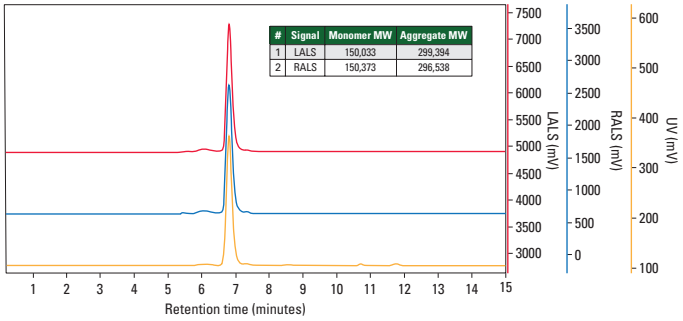
#	Concentration (mg/mL)	Load (µg)	SN Monomer	Injection volume (10 µL) Monomer -MW	Injection volume (10 µL) Aggregate -MW
1	1	10	530	149,800	317,200
2	0.5	5	310	150,400	298,300
3	0.1	1	60	150,600	169,600
4	0.05	0.5	20	152,100	1,194,000
5	0.01	0.1	5	121,900	-
6	0.005	0.05	2	701,500	-
7	0.001	0.01	-	-	-

Competitive separations may not be representative of all applications.

The LenS3 MALS detector yielded a 15 fold increase in sensitivity at 10 µg mass on column using high resolution analytical SEC. This superior S/N ratio allows for molecular weight determination of the monomer and aggregate even at very low concentrations, with LOD as low as 10 ng. LOD of the LenS3 is 5 fold lower than the competitive detector, allowing detection of very low levels of high molecular weight (HMW) and low molecular weight (LMW) impurities which are immunogenic to the human body when injected.

Data quality of the results produced by LenS3 includes a clear LALS signal *(Figure 2)* which is used here to determine a molecular weight of 150,033 Da for the monomer and 299,394 Da for the aggregate, closely matching the results produced by RALS.

Figure 2. LenS3 overlay of LALS, RALS and UV of 1 mg/mL mAb material injected at 10 µL (10 µg load)



Conclusion

Pushing the limits of both quantification and detection, the LenS3 MALS detector is the first choice when analyzing monomer, aggregates and fragments of macromolecules for reliable MW determinations across different amounts of injection. The same detector can provide high sensitivity and can be coupled to a wide range of instruments including HPLC, U/HPLC and FPLC, making it a suitable detector at every step of the development process. Not only is the LenS3 a fit for HPLC and UHPLC methods, but also for applications where sample is limited, concentration is low or for the monitoring of low levels of analyte.