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MALS analysis of FPLC-separated proteins

Your Challenge

- You need to know not only if but also what elutes from size-exlusion separations on an FPLC.
- Knowledge of molecular weight (MW) helps you characterize your eluted molecules.

Our Solution

LenS3 Multi-Angle Light-Scattering Detector

- Highly sensitive & reproducible MALS measurements.

What was done?

- Three monoclonal antibodies & two BSA samples were analyzed by SEC-MALS using a LenS3 MALS detector.

What was the result?

- MW of mAb and BSA samples was reliably determined by LenS3, also for low concentration impurities

Know what comes off your column after FPLC size exclusion separation by determining the molecular weight of eluting sample components with the LenS3 MALS detector.

Your Benefit

Know instead of guess: MW analysis of the eluate of FPLC separations with LenS3.

Analysis of proteins and mAbs

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https://www.separations.eu.tosohbioscience.com/products/chromatography-instruments-and-accessories/stand-alone-detectors

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Application Note

Characterization of Biomacromolecules by FPLC coupled with the LenS3 MALS Detector

Size exclusion chromatography (SEC), specifically gel filtration chromatography (GFC), is an important tool for the separation of biomaterials and biopharmaceutical products such as monoclonal antibodies (mAbs). With the increasing need to acquire an in-depth understanding of physical properties, the addition of a multi-angle light scattering (MALS) detector to the common chromatography system has attracted great attention.

ÄKTA pure® is a commonly utilized FPLC system for the purification of proteins, peptides, and nucleic acids. This application note demonstrates the compatibility of the ÄKTA pure with the LenS3 MALS detector. The coupling provides the user with the capability not only to qualitatively detect each fraction that is separated by the column connected to ÄKTA system, but also to quantitatively determine the absolute molecular weight and the size of each species, including the targeted molecules, impurities, aggregates, and fractions.

Material and Methods

Instrumentation:	ÄKTA pure 25 in-line with UV detector (@ 280 nm) and LenS3 MALS detector
Column:	Agarose-based size exclusion column for 10-600 kDa proteins, 10 mm ID x 30 cm L
Mobile phase:	100 mmol/L sodium phosphate, 100 mmol/L sodium sulfate, 0.01% sodium azide, pH 6.8
Flow rate:	0.60 mL/min
Temperature:	ambient
Injection vol.:	35 µL

Results and Discussion

Monoclonal antibodies (mAbs)

Samples & Conditions

• NIST mAb (0.48 mg/mL); Trastuzumab (0.79 mg/mL, Herceptin® biosimilar); Adalimumab (0.45 mg/mL, Humira® biosimilar)

• dn/dc = 0.187 mL/g; dA/dc (NIST mAb) = 1.42 mL/g; dA/dc (Trastuzumab) = 1.48; dA/dc (Adalimumab) = 1.4

Figure 1. Molecular weight distribution overlaid with UV signals of NIST mAb (red), Trastuzumab (blue), and Adalimumab (green).

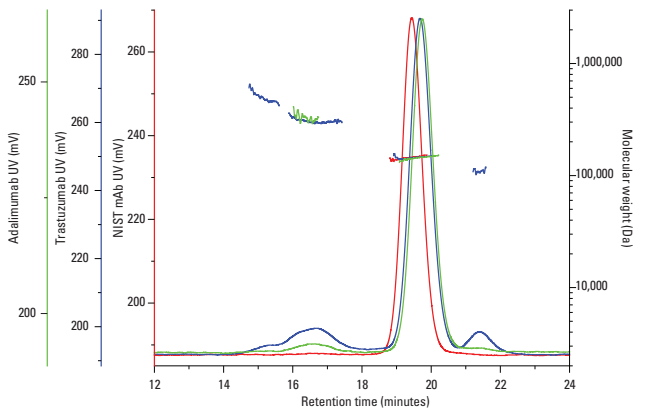


Table 1. Molecular weight characterization of mAbs by LenS3.

Peak	Fragment	Monomer	Dimer	Trimer
NIST mAb	N/A	147,198	N/A	N/A
Trastuzumab	116,081 (1.40%)	147,118 (79.62%)	306,051 (12.40%)	487,714 (0.64%)
Adalimumab	97,930 (2.72%)	146,059 (96.53%)	329,747 (0.75%)	N/A

Conclusion

• The calculated molecular weights of fragments, monomer, dimer and trimer from different mAbs show that these molecular weights are very similar to the reported molecular weight from literature. Results demonstrated that LenS3 can determine mAb molecular weights accurately.

• LenS3 provides an accurate and absolute MW calculation that is independent of retention time shift, caused e.g. by column use history.

Bovine Serum Albumin (BSA)

Samples & Conditions

- BSA from Sigma Aldrich (purity 98% by agarose gel electrophoresis)
- Concentration = 4.68 mg/mL
- dn/dc = 0.185 mL/g; dA/dc = 0.667 mL/g

Figure 2. LALS (red), RALS (blue), and UV (yellow) signals of the BSA, and its molecular weight distribution (green).

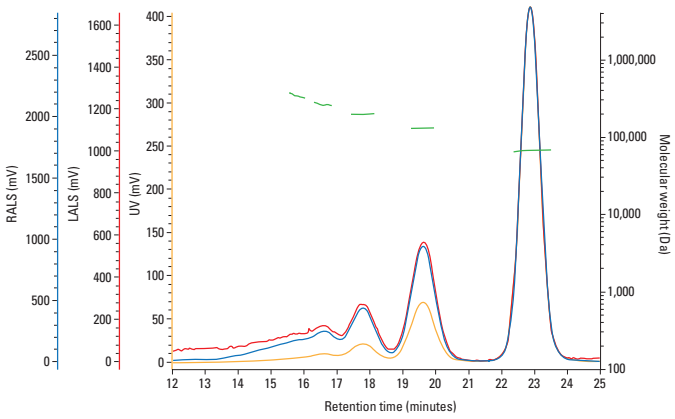


Table 2. Molecular weight characterization of BSA by LenS3.

Peak	Assignment	Retention time (min)	MW (Da)	%
1	Monomer	22.890	66,753	74.39
2	Dimer	19.625	130,473	15.56
3	Trimer	17.786	197,473	4.94
4	Tetramer	16.611	263, 962	3.53
5	Pentamer	16.056	338,954	1.14

Table 2. Reproducibility of molecular weight characterization of BSA by LenS3.

Injection #	Monomer	Dimer	Trimer	Tetramer	Pentamer
1	66,753	130,473	197,473	263,962	338,954
2	66,902	130,207	196,442	261,635	339,866
3	66,979	130,927	196,226	265,314	341,306
Average	66,878	130,536	196,714	264,637	340,042
%RSD	0.172	0.279	0.339	0.706	0.349

Conclusion

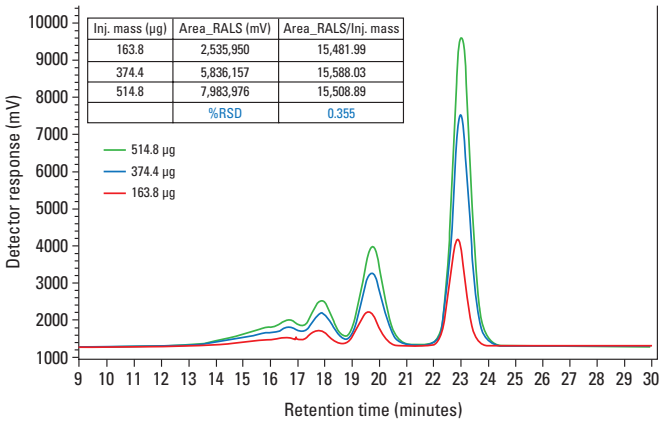
- LenS3 has great sensitivity to detect and calculate not only the protein monomer but also the aggregates even at trace amounts (e.g. pentamer with 1.14 % which corresponds to 1.86 µg.)!
- The sensitivity and robust performance of LenS3 enable achieving a %RSD <1% even for the pentamer.

Detection Linearity

Samples & Conditions

- BSA from Sigma Aldrich (purity 98% by agarose gel electrophoresis) with various injection amounts.

Figure 3. RALS signal overlay of BSA with various injection loads.



Conclusion

- LenS3 provides an exceptional detection linearity to different sample loading amounts (below 0.5% deviation).

Summary

Coupled with an ÄKTA pure FPLC system, the LenS3 MALS detector provides:

- Unprecedent sensitivity for characterization of protein monomer and aggregates.
- High reproducibility and precision.
- Accurate and reliable molecular weight calculation (independent of the retention time).
- Exceptional linearity to different injection loading amounts.

This complimenting use of the LenS3 MALS detector with an ÄKTA pure FPLC system has not been approved or sponsored by Cytiva. Tosoh Bioscience does not have a relationship with Cytiva.