Aggregate Analyses by Light Scattering - Complementary and Orthogonal

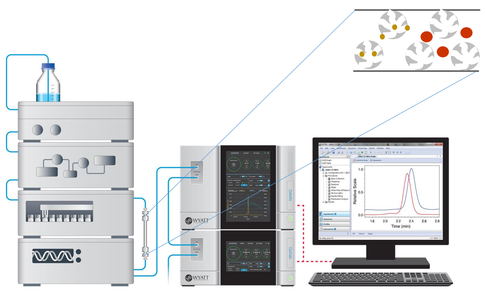
Sigrid Kuebler, Ph.D, Dan Some, Ph.D, and Jeff Ahlgren, Ph.D. — Wyatt Technology

Introduction

Abstract: Biotherapeutic regulatory filings require detailed characterization of degradation pathways including aggregation. Three orthogonal light scattering techniques complement each other to analyze and quantify soluble and sub-micron insoluble aggregates: SEC-MALS, FFF-MALS and CG-MALS. This poster describes the capabilities of each technique and how they work together to provide a comprehensive picture of irreversible and reversible aggregates.

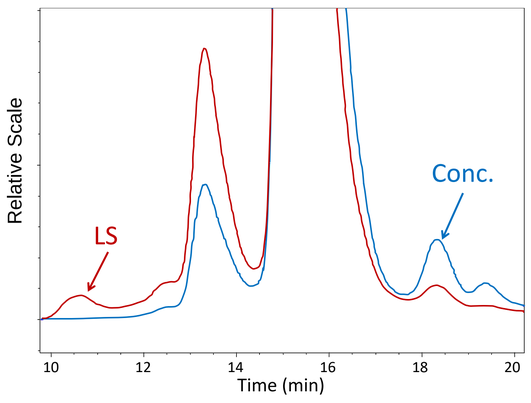
Technique	SEC-MALS	FFF-MALS	CG-MALS
Separation size range	0.5 – 20 nm (column dependent)	1 nm – 1 µm	none
Properties determined	Quantitative distributions of absolute MW and size (R_g, R_h)	Quantitative distributions of absolute MW and size (R_g, R_h)	Average MW and size; A_2(B_{22}), self / hetero-association K_d, co-solute interaction A_{11}
Resolution	Proteins, fragments, small aggregates (column dependent)	Proteins, fragments, soluble and non-soluble aggregates	Stoichiometry and association properties of reversible aggregates and complexes
Separation matrix	Column (high surface area)	Open channel or hollow fiber (low surface area)	none
Change in concentration	Sample dilution	Concentrates samples during focusing step; dilutes after elution	none

SEC-MALS: absolute molar mass of proteins, oligomers and fragments



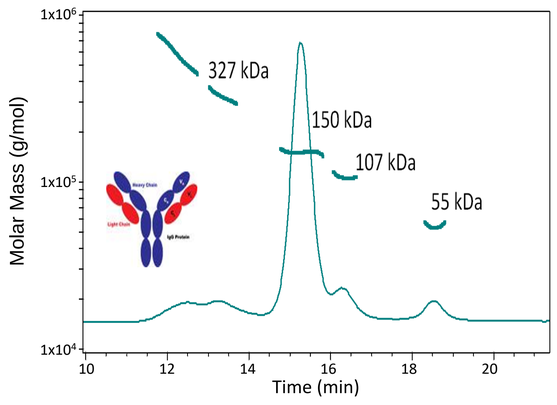
- Any leading HPLC-SEC for separation and UV detection
- High-performance WTC-030S5 SEC column
- DAWN® or miniDAWN® MALS detector
- Optilab® RI detector

Trace Aggregates



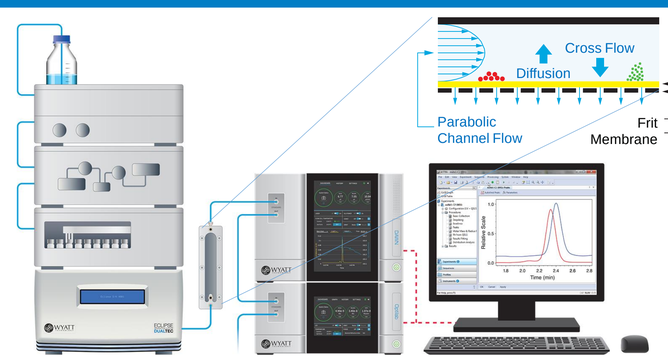
LS is more sensitive to high-MW fractions than concentration detectors

37° C for 6 months



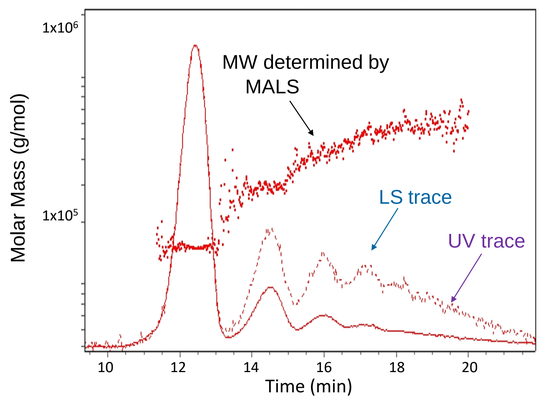
MALS + RI determines absolute molar masses of known and unknown proteins

FFF-MALS: quantify soluble and insoluble aggregates



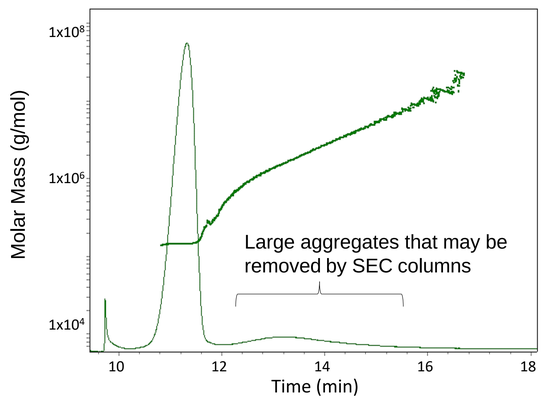
- Eclipse® FFF + HPLC for separation and UV detection
- DAWN® or miniDAWN® MALS detector
- Optilab® RI detector

Resolve and analyze oligomers…



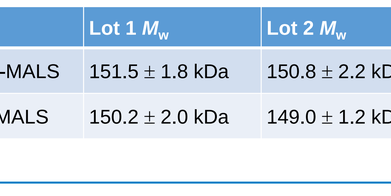
Eclipse® FFF is a recognized orthogonal technique to SEC for quantifying oligomers

…and all sizes of aggregates



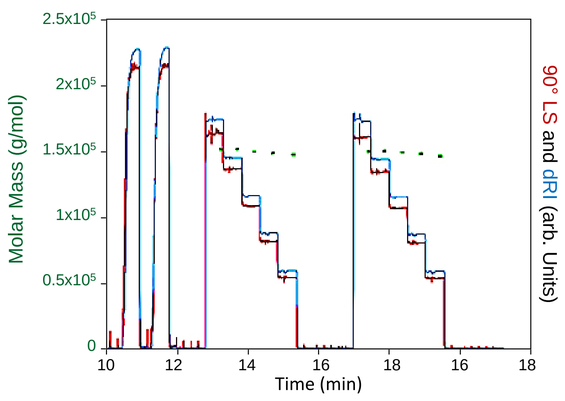
Large aggregates that may be removed or disrupted by SEC columns remain intact and are characterized in FFF

CG-MALS: validate overall M_W and protein-protein interactions



- Calypso® composition-gradient system
- DAWN® or miniDAWN® MALS detector
- Optilab® RI detector

Lot-to-lot comparability and SEC-MALS validation



	Lot 1 M_w	Lot 2 M_w
SEC-MALS	151.5 ± 1.8 kDa	150.8 ± 2.2 kDa
CG-MALS	150.2 ± 2.0 kDa	149.0 ± 1.2 kDa

Automated CG-MALS measurements:

- Validate overall SEC-MALS or FFF-MALS M_w to determine if aggregates have been filtered out or disrupted.
- Identify reversible aggregates at actual formulation concentrations
- Compare lots in terms of weight-average molar mass and protein-protein interactions (A_2 / B_{22}).
- Highlight differences in A_2 that indicate changes in surface residues (e.g. deamidation) or higher-order structural shifts.

Conclusions

Orthogonal and complementary

- Mutually orthogonal biophysical techniques for characterizing molar mass, size and aggregates
- Comparison of results from these complementary techniques identifies aggregate properties and behavior.

SEC-MALS with a DAWN provides:

- Quantitative distributions of absolute molar mass and size
- Identification of fragments and foreign proteins as well as oligomers
- Characterization of conjugates such as PEGylated or glycosylated proteins

FFF-MALS with an Eclipse and DAWN provides:

- Orthogonal approach to SEC-MALS for characterizing biopharmaceuticals.
- A wider range of separation than SEC with no shear, less dilution and little surface interactions
- Characterization of analytes too large for SEC: protein-polysaccharide conjugate vaccines, lipoproteins, liposomes, viruses and virus-like particles.

CG-MALS with a Calypso and DAWN provides:

- Indication of aggregate loss in SEC or FFF
- Measures of protein-protein interactions including self-association and co-solute interactions
- A quick, simple way to compare lots