Characterizing Quality Attributes of GLP-1 Analogs by Light Scattering

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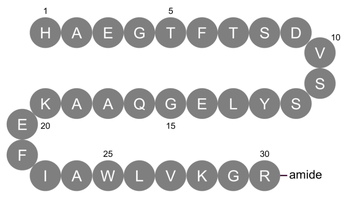
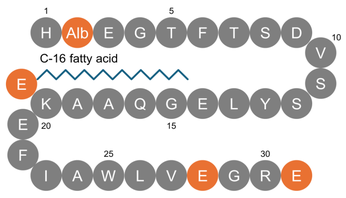
Introduction

Glucagon-like peptide-1 analogs (GLP-1a) have received enormous attention as therapeutic peptides for the treatment of type 2 diabetes and obesity. Accurate characterization of their quality attributes is crucial for ensuring product quality, safety and efficacy. However, analytical challenges remain due to the complexity of peptide structure, propensity for aggregation and fibrillation, purity and impurities, and stability. This poster demonstrates the application of light scattering in identifying and quantifying monomers, oligomers, aggregates of GLP-1a, as well as screening the stability of GLP-1a.

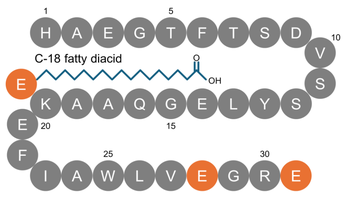
Native GLP-1



Liraglutide



Semaglutide



Wyatt Technology solutions for GLP-1a quality attributes

Multi-Angle Light Scattering

Attributes:
- Absolute molar mass
- Concentration
- Extinction coefficient
- Size and dispersity

Application examples:
- Platform method for routine and in-depth analysis
- Evaluate product consistency, oligomeric identity, and impurities.
- Characterize reversible association/disassociation

Dynamic Light Scattering

Attributes:
- Size, polydispersity
- Colloidal stability metrics such as A_2 and k_D

Application examples:
- Rapid assessment of aggregation
- Storage stability evaluation
- Formulation development
  - pH, ionic strength
  - Excipient

Field-Flow Fractionation

Orthogonal separation to SEC
- Versatile separation technique
- Characterization of aggregates too large for SEC

FFF/SEC-MALS: Routine and in-depth characterization

UPLC™ or HPLC System & UV detector

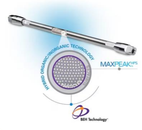
Arc™ Premier LC System

WyattQELS™
Integrated DLS detector

DAWN™
MALS instrument

Optilab™
dRI detector concentration for non-UV/Vis active components

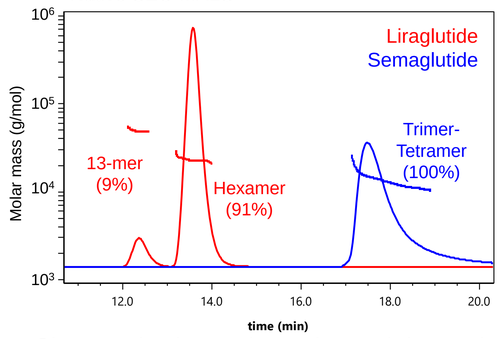
Eclipse™ field flow fractionation system
The ultimate separation system for nanoparticles and macromolecules



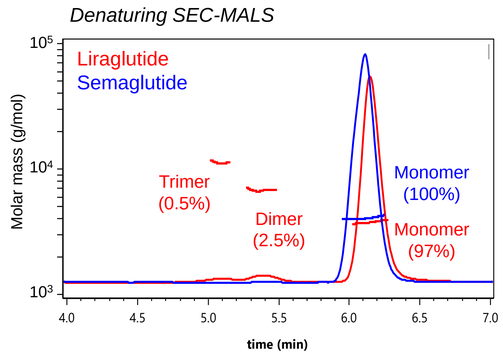
Successful SEC-MALS starts with successful separation.
XBridge™ Premier (HPLC) and ACQUITY™ Premier (UPLC) SEC columns.
Prevent undesired analyte/surface interactions through the power of MaxPeak™ HPS Technology

Platform SEC-MALS for routine analysis- concentration, oligomers identity, aggregation

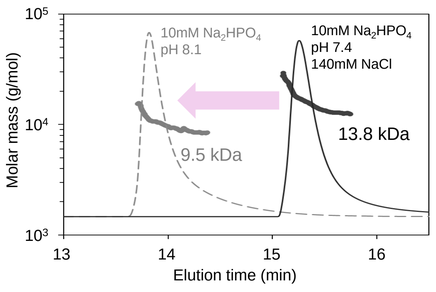
Native SEC-MALS



Denaturing SEC-MALS



Characterization under native and denaturing conditions is standard for peptide-based therapeutics. The multidetector SEC-MALS platform method is simple to implement and compatible with a variety of GLP-1a constructs. A single measurement provides multiple quality attributes from one injection, including concentration, molecular weight, size, polydispersity, and oligomer identity and quantity.



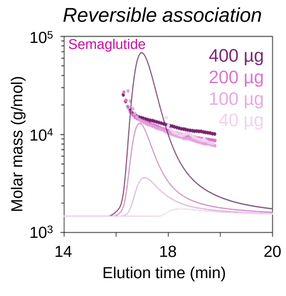
Ensure product consistency and identity

GLP-1a's oligomerization is sensitive to pH and ionic strength, so precise analytical methods are essential, especially since UV alone can lead to inaccurate identity determination. MALS clearly shows that the oligomeric state of semaglutide changes from a tri-/tetramer mixture to dimers after buffer exchange.

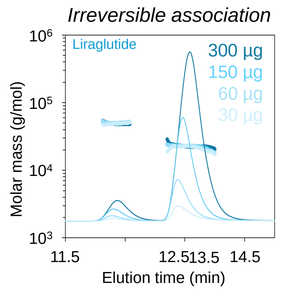
Reversible association/dissociation

The multidetector SEC-MALS technique can help distinguish reversible from irreversible associations by directly measuring the molar mass distribution. It clarifies any ambiguity from UV detection alone, as MALS does not rely on elution time to determine molar mass.

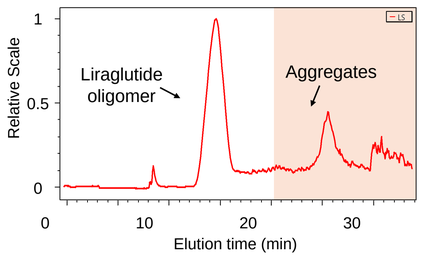
Reversible association



Irreversible association



FFF Fractogram, smaller particles elutes first



FFF -- Characterization of aggregates too large for SEC

Given the importance of aggregation, regulatory agencies require orthogonal data and assessment. FFF is a widely used separation technique recognized by the FDA that fills the characterization gap. FFF flexible separation method can separate particles that are too large for SEC analysis.

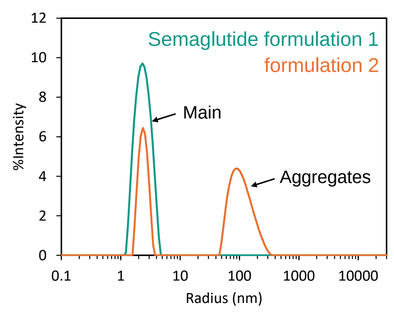
DLS and SLS: Rapid formulation and stability screening

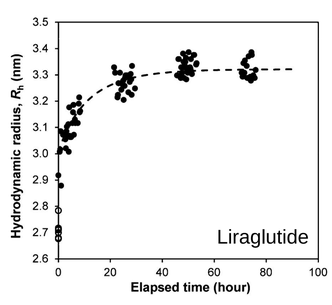
DynaPro™ NanoStar™ instrument

- ✓ Traditional DLS, with SLS for size, molar mass and particle concentration
- ✓ Rapid, low volume measurements in quartz or disposable cuvettes

DynaPro Plate Reader

- ✓ High-throughput, automated DLS/SLS
- ✓ Screen size distribution and aggregation in standard plates





Fast and sensitive assessment of aggregation

DLS provides quick, non-invasive analysis of sample stability and aggregation. It provide size and polydispersity in less than 1 minute with only a few microliters of sample.

Storage stability characterization

Isothermal size measurement provides insight into storage stability. An increase in size indicates an unstable construct or an unoptimized buffer condition.

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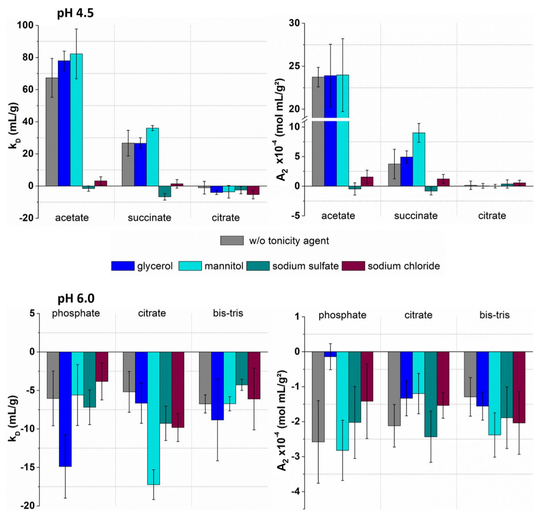
Liraglutide pH and excipient screening



Ranking formulation stability with industry-standard microwell plates

Non-specific interactions are crucial for optimizing biotherapeutic candidates and formulations. DLS assesses these interactions and colloidal stability via the diffusion interaction parameter (k_D), while SLS quantifies the second virial coefficient (A_2). Together, k_D and A_2 offer complementary insights into solute interactions, aiding in formulation ranking and selection.

The DynaPro Plate Reader is compatible with industry-standard microwell plates and excels at high-throughput formulation screening.

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Conclusions

- ✓ DLS/SLS provides rapid screening of aggregates and impurities throughout entire GLP-1a product life cycle.
- ✓ SEC-MALS platform denaturing and non-denaturing analytical methods provides in-depth characterization of identify and quantification of monomer, reversible oligomer, and irreversible aggregates for GLP-1a.
- ✓ FFF-MALS provides orthogonal characterization of aggregation and extend characterization of particles too large for SEC.

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