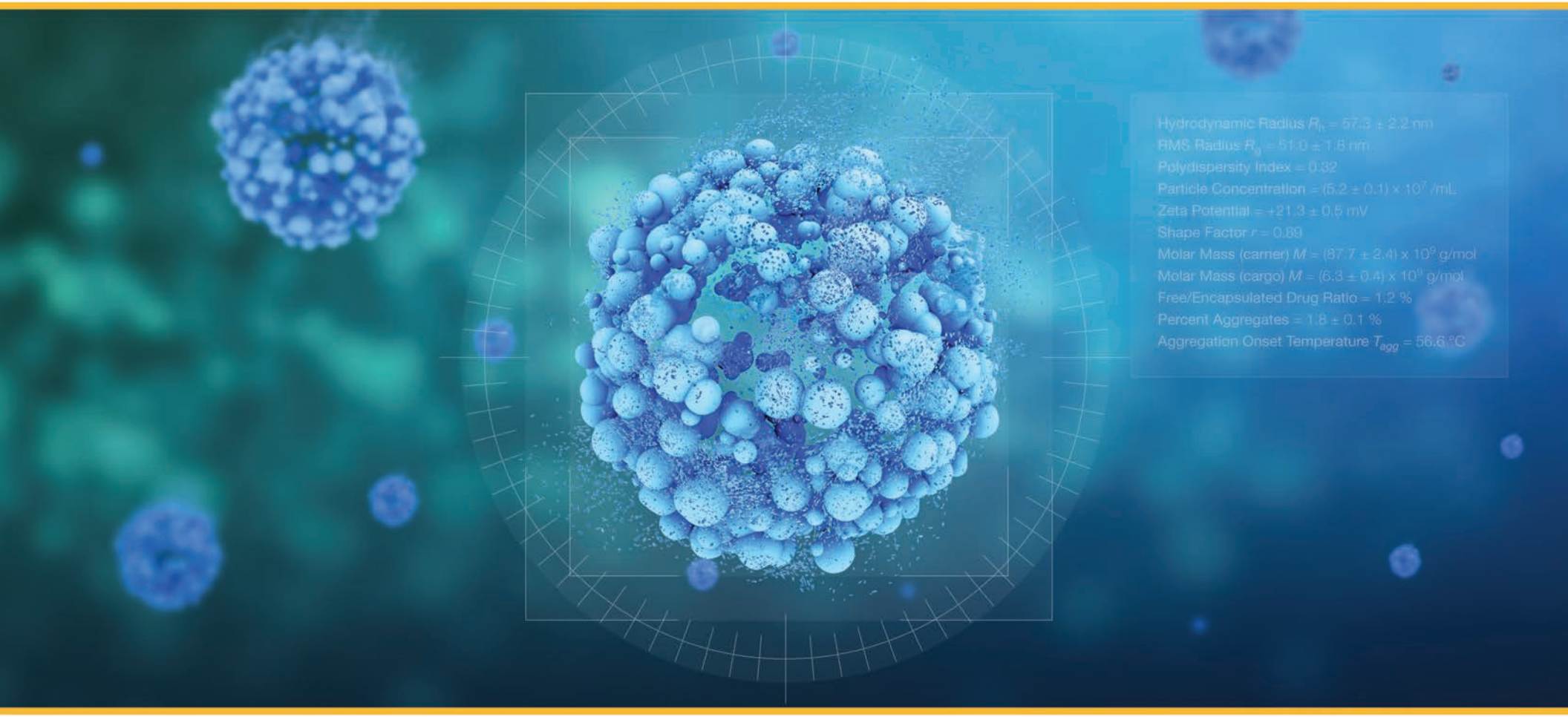
Characterization of Biologics and Complex Drugs



Waters™ | WYATT TECHNOLOGY

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Essential analytical tools

In the course of developing and producing modern therapeutics—including biological and non-biological complex drugs—many analytical challenges arise. Obtaining quantitative results accurately, rapidly and rigorously is key to success in bringing these products to patients.

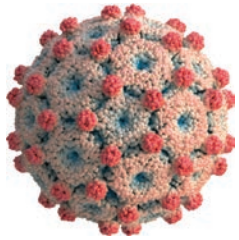
Our mission is to provide you with the finest analytical instruments, and the best technical and scientific support, so you can confidently meet these challenges, day in and day out. Join the thousands of scientists and technology specialists around the globe who know that they can depend on Wyatt Technology, a portfolio of Waters Corporation, to get results.


Did I make what I intended?
Is it a robust product?
Does it do what it is supposed to do?
Does it behave well?

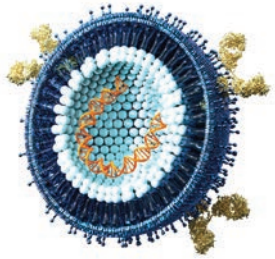
End-to-end Solutions

Advanced characterization for every application

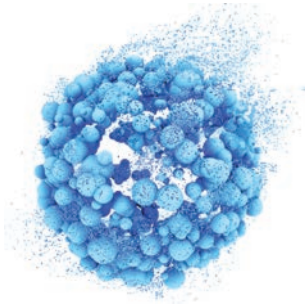
ANTIBODY AND PROTEIN-BASED THERAPY From monoclonal antibodies and therapeutic glycoproteins to fusion proteins, bispecifics, PEGylated proteins and antibody-drug conjugates, multi-angle light scattering (MALS) and dynamic light scattering (DLS) are utilized throughout the discovery, development and production chain to characterize drug molecules, aggregates, fragments, complexes and interactions.



VACCINES MALS, DLS, ELS and FFF are important in the development, production and quality control of vaccines based on viruses and virus-like particles, polysaccharides and protein-polysaccharide conjugates. Light scattering determines size, composition, aggregation, concentration and interactions with serum, beginning with each serotype and on through the final drug product.



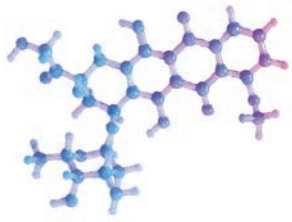
GENE THERAPIES The unique capabilities of light scattering are invaluable in analyzing viruses, exosomes, and synthetic nanoparticles used to deliver RNA and DNA. Light scattering determines the size, concentration, degree of aggregation, shape, zeta potential and relative nucleic acid content of these particles. Platforms include microwell-plate systems for rapid screening, and separation-detection systems combining MALS and DLS modules with chromatographic separation or field-flow fractionation for quantifying critical quality attributes.



NANOMEDICINES Nano-formulations of traditional drug substances, such as lipid nanoparticles, dendrimers, polymer micelles and nano-emulsions, benefit greatly from analysis by light scattering. In addition to size, size distributions and zeta potential, characterization by light scattering reveals shape, concentration and encapsulation efficiency. FFF-MALS is particularly powerful for determining accurate, quantitative distributions of the essential physical attributes of these particles.

PEPTIDES AND NUCLEIC ACIDS Whether homogeneous like insulin and siRNA, or broadly heterogeneous like glatiramer acetate, our instruments are essential in the development and production of therapeutic macromolecules that fall outside of the standard definition of proteins and nanoparticles. Light scattering characterizes molar mass and conformation as well as aggregation, interactions and self-assembly kinetics.

POLYMERS Some of the pharmaceutical polymers that benefit from analysis by light scattering include polymeric excipients used in traditional oral formulations, heparin and hyaluronic acid for direct therapies, and PLGA for drug-delivery nanoparticles and biodegradable devices. Light scattering detectors provide absolute molar mass distributions and also quantify conformation, branching and zeta potential.



SMALL MOLECULES Key applications of our light scattering instrumentation in the field of small-molecule drugs include drug discovery: identification of promiscuous inhibitors and screening for inhibitors of protein-protein interactions during drug discovery, turbidity measurements of drug formulations and process development of nanosolids.

Proteins & mAbs

Light scattering provides a wide range of solutions to the development and production of biotherapeutics based on proteins and mAbs, from quick quality checks of purified solutions through developability and stability assessments, biophysical characterization in solution, analysis of protein-protein interactions and process analytics. Our MALS and DLS instruments are essential throughout the development pipeline.

Attributes	Solution
Molar Mass	SEC-MALS with DAWN™ (HPLC) or microDAWN™ (UHPLC) for absolute MW of monomers, oligomers, fragments, complexes; ultraDAWN™ for real-time process analytics.
Aggregation	FFF-MALS-DLS with Eclipse™ and DAWN to characterize soluble and insoluble aggregates. DynaPro™ Plate Reader to screen buffers and candidates for colloidal stability.
Conjugation	SEC-MALS-UV-RI with DAWN and Optilab™ to determine the individual MW of protein and glycan, PEG or polysaccharide components in conjugated biotherapeutics.
Stability	HT-DLS/SLS with DynaPro Plate Reader for rate of aggregation under accelerated stability testing, as well as T_{agg}, T_{onset}, A_2, k_D, viscosity of concentrated proteins; DynaPro NanoStar™ and ZetaStar™ for quantifying all of these plus opalescence, manually in cuvettes.
Interactions	CG-MALS with Calypso™ and DAWN to characterize drug-target and protein-excipient interactions, self-association and self-interaction at high protein concentration.

Further information

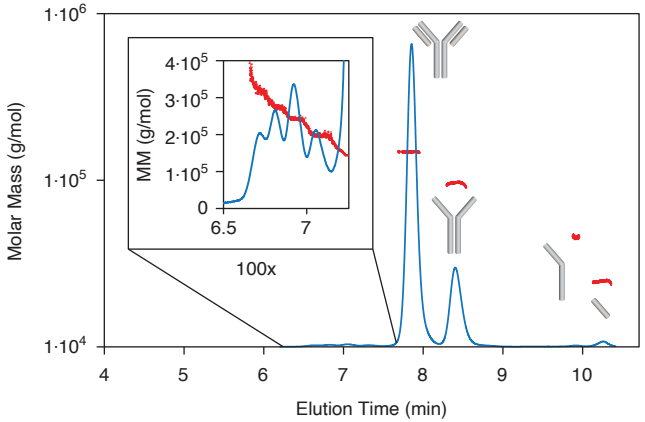
Screening candidates and formulations for aggregation, aggregation propensity and viscosity is ideally carried out in the DynaPro Plate Reader: www.wyatt.com/PlateReader

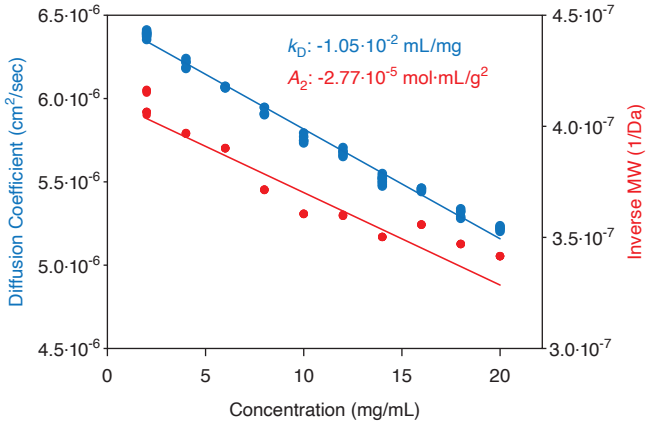
Our customers share their knowledge on the use of SEC-MALS, FFF-MALS, CG-MALS and HT-DLS/SLS to effectively develop mAbs and proteins: www.wyatt.com/Webinars/Biotherapeutics

Characterize aggregates and fragments

Aided by the superb separation power of UHPLC, SEC-MALS identifies and quantifies the peaks observed for a degraded mAb, in terms of molecular weight and total eluting mass. Fragments such as dual and single heavy chains, and small oligomers such as dimers consisting of monomer + fragments, are found.

An orthogonal method, FFF-MALS, utilizes an Eclipse AF4 device with a DAWN to separate and quantify monomers, oligomers and larger aggregates in the range of tens or even hundreds of MDa.





Screen aggregation, colloidal and thermal stability

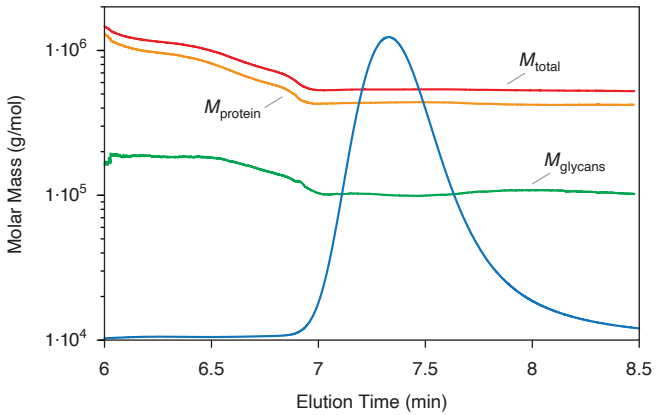
Colloidal stability of a protein based biopharmaceutical is evaluated simultaneously via the second virial coefficient A_2 and diffusion interaction parameter k_D. More positive values indicate higher colloidal stability.

This analysis may be automated in the DynaPro Plate Reader to test multiple candidates for developability, or multiple buffers for formulation. In addition, the instrument rapidly determines the degree of aggregation present, and thermal stability indicators such as aggregation rate and the onset temperature for aggregation.

Quantify conjugated proteins

Characterization of glycosylated or PEGylated proteins, protein DNA complexes and other binary conjugates, to determine the degree of conjugation and heterogeneity, is accomplished with triple detection SEC-UV-MALS-RI. The results of the analysis include the molar mass of the protein and that of the modifier, as well as the total molar mass, at each point along the chromatographic peak, as shown here for a glycoprotein.

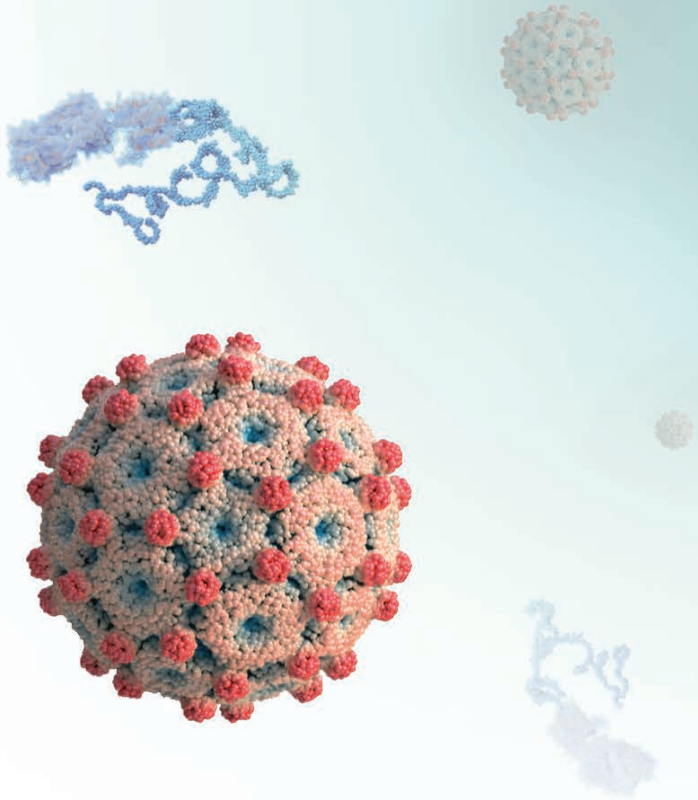
The analysis also calculates the overall extinction coefficient and *dn/dc* of the conjugate, which are essential in other characterization methods. For example, in determining the binding affinity of an antibody to this glycoprotein by CG-MALS, the conjugate's overall *dn/dc* value is needed.



Vaccines

Vaccines tend to be complex products combining multiple serotypes of macromolecules or bionanoparticles, often with adjuvants. Our suite of solutions include field-flow fractionation, which separates such complex samples, along with multiple online detectors to characterize biophysical attributes. Other instruments in the light-scattering toolbox cover deeper research studies as well as formulation and production.

Attributes	Solution
Molar Mass and Size	FFF-MALS-DLS with Eclipse and DAWN to characterize size and molar mass of polysaccharides, viruses, LNPs and VLPs, and quantify aggregates and incomplete capsids; ultraDAWN for LNP, polysaccharide and viral vector process control.
Conjugation	SEC/FFF-MALS-UV-RI with DAWN and Optilab to determine the distributions of molar mass, conjugation ratio and conformation of protein-polysaccharide conjugates or payload and encapsulation efficiency in RNA-bearing LNPs.
Titer	FFF-MALS for determination of fully quantitative virus/VLP size distributions; SLS in a DynaPro Plate Reader, NanoStar or ZetaStar for quick estimate of titer. ultraDAWN for process monitoring.
Binding Affinity	CG-MALS with Calypso and DAWN for characterization of the affinity and absolute stoichiometry of antibody-antigen binding, even when complex, multivalent interactions occur.
Stability	ZetaStar for analyzing zeta potential of viruses and VLPs at physiological ionic strength. DynaPro Plate Reader to assess stability in multiple buffers after stress such as freeze/thaw.



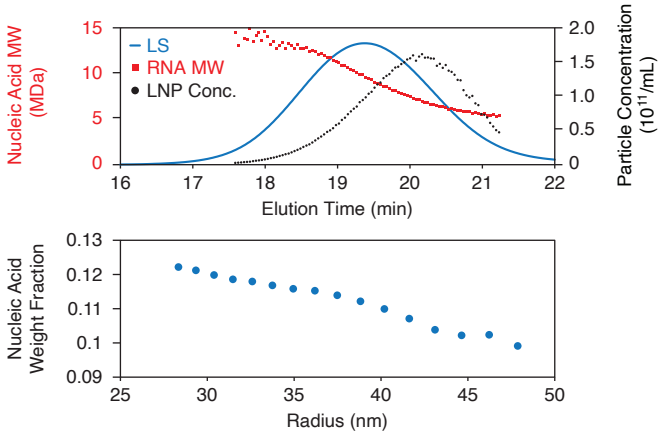
Further information

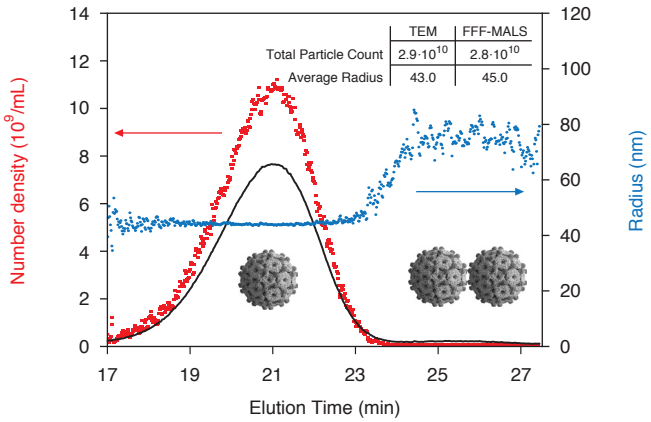
View application notes, white papers and webinars on the use of light scattering and field-flow fractionation in vaccine development, production and quality control: www.wyatt.com/Vaccines

Download application notes and white papers describing the characterization of vaccines and other biotherapeutics with light scattering: www.wyatt.com/AppNotes/Biotherapeutics

LNP-RNA analysis

Though SEC is also used, FFF is the tool of choice for separating lipid nanoparticles (LNP) encapsulating nucleic acids. The data from online detectors – MALS (possibly with embedded DLS), UV and dRI – characterize and quantify not only average size, polydispersity, particle concentration, morphology and stability, but also encapsulation efficiency and the size-based LNP payload distribution in terms of MW, weight fraction and number of DNA or RNA molecules per particle.





Product validation

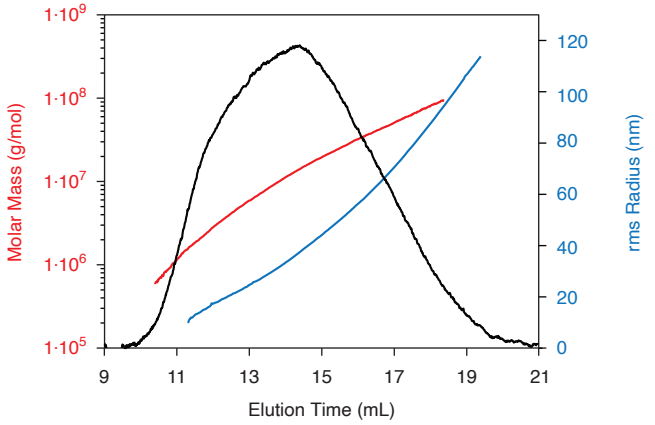
In these influenza virus subpopulations, both size and total particle concentration are quantified in detail by FFF-MALS. Comparison with particle size and counting by TEM shows discrepancies of just 2% in virus concentration and 5% in size. A small dimer peak is also identified.

Alongside FFF-MALS, the DynaPro Plate Reader is highly productive for rapid screening of processing conditions and formulations. In addition to sizing dozens to hundreds of virus samples under standard conditions and/or elevated temperatures, it can also determine viral particle concentration.

Protein-polysaccharide conjugates

FFF-MALS is the preferred means of characterizing protein-polysaccharide conjugate vaccines that extend above tens or hundreds of MDa in MW. AF4 creates no shear-induced degradation while fractionating the entire range; MALS determines molar mass and size simultaneously.

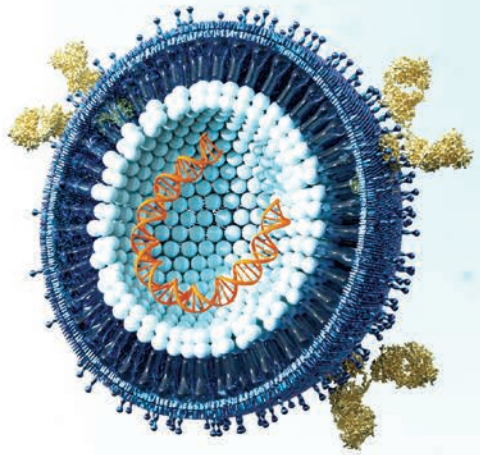
RT-MALS with ultraDAWN is a cost-effective solution for process development, monitoring and control of polysaccharide homogenization processes.



Gene Therapies

Biophysical characterization of small viral vectors, such as AAVs, is performed with size-exclusion or ion-exchange chromatography coupled to light-scattering, UV and RI detectors. Field-flow fractionation separates larger vectors such as lentivirus, exosomes or lipid nanoparticles. Gene therapies and nanoparticles may be quickly tested for size, aggregation, charge and concentration by our dynamic and electrophoretic light scattering instrument lines.

Attributes	Solution
Size, Shape	Automated, in-plate DLS with the DynaPro Plate Reader for sizing. SEC-MALS-DLS or FFF-MALS-DLS with Eclipse and DAWN for detailed size and shape distributions. ultraDAWN for process monitoring.
Empty:Full Ratio and Payload	SEC/IEX/FFF-MALS-UV-RI for analysis of relative genetic content vs. capsid mass (AAV) or lipid mass (LNP), even if empty and full gene-delivery vectors are not separated. ultraDAWN for AAV purification.
Aggregation	SEC/FFF-MALS for quantifying percent aggregate. ultraDAWN for estimating aggregate content in real time during production and purification processes.
Concentration/ Titer	SEC/FFF-MALS separates by size, then determines the particle concentration for accurate titer by size. NanoStar, ZetaStar or DynaPro Plate Reader for rapid, low-volume quantitation of particle concentration. ultraDAWN for process monitoring.
Zeta Potential and Stability	DynaPro Plate Reader, NanoStar or ZetaStar for formulation screening and accelerated stability testing. ZetaStar to measure size and zeta potential simultaneously.



Further information

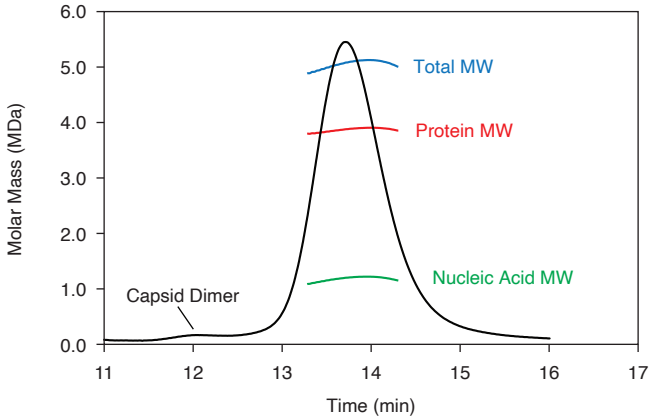
Download application notes on characterizing AAVs or exosomes, and the brochures for the instruments used in these analyses: www.wyatt.com/GeneTherapies

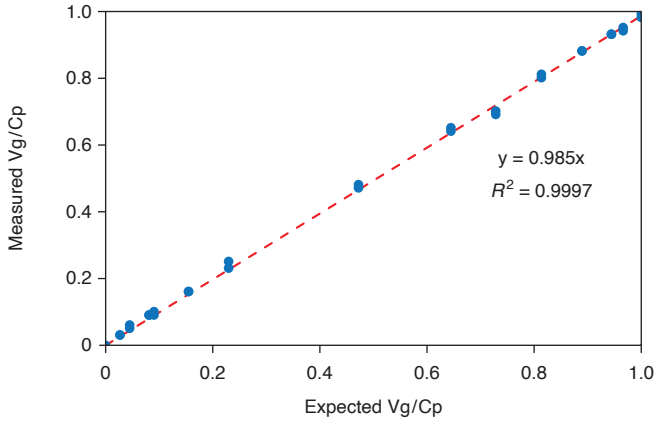
Discover the premier light-scattering instrument for characterizing viral and non-viral vectors. MALS determines size, concentration, aggregation and payload: www.wyatt.com/DAWN

DNA content

ASTRA™ software's AAV analysis module utilizes SEC-MALS-UV-RI data to determine nucleic acid and proteinaceous content of the viral vector, calculating the molecular weight of each component, at each point along the peak. Since the full genome is 2.4 MDa while the average DNA molecular weight is 1.2 MDa, these viruses are partially filled.

A small amount of dimer was also identified. The module also determines percent aggregate in addition to capsid concentration (particles/mL), and the Cp/Vg ratio.





Process validation

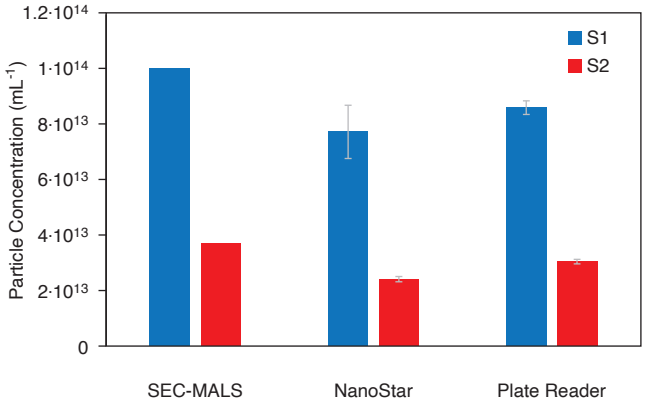
Validation tests, carried out by mixing pre-determined proportions of known empty and full viral vectors, show that ASTRA's proprietary SEC-MALS method for determining the relative loading of AAVs with single-stranded DNA is accurate, precise and repeatable.

The simplicity of the method and its inherent validatability make it especially useful for quality control and lot release purposes. For PAT purposes, in-process monitoring of particle size is accomplished by real-time MALS with the ultraDAWN.

Quality control and process screening

A few microliters of sample and a few seconds of measuring time suffice to determine particle size and concentration in the NanoStar or DynaPro Plate Reader, making it easy to test multiple serotypes S1, S2,…, process parameters or storage buffers.

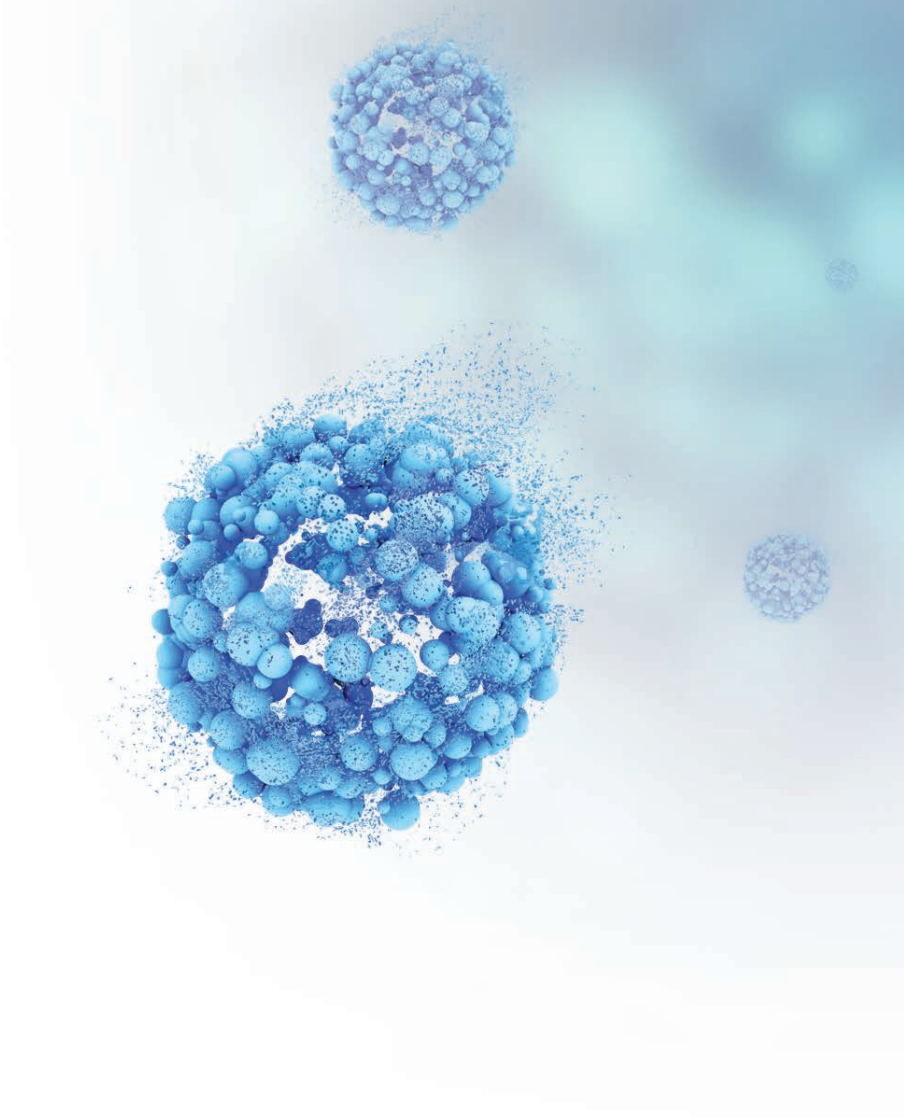
Results for AAVs compare well with an orthogonal and fully analytical method, SEC-MALS. For larger gene vectors such as lentivirus or lipid nanoparticles, the comparable analytical method is FFF-MALS, which separates, quantifies and characterizes particles up to 1000 nm.



Nanomedicines

Light scattering and field-flow fractionation provide characterization capabilities essential in developing novel nanoparticle drug delivery systems (nanoDDS), whether the carrier vehicle is a liposome, dendrimer, polymer micelle, emulsion or some other nanoparticle. Light scattering can be applied to monitor production processes as well as product development, process development and quality control.

Attributes	Solution
Size, Shape, Size Distribution	Automated, in-plate DLS with the DynaPro Plate Reader for sizing. FFF-MALS-DLS with Eclipse and DAWN for detailed size and shape distributions. ultraDAWN for process monitoring.
Drug Loading	SEC/FFF-MALS-UV-RI for spectroscopic analysis of drug content vs. size (fluorescence may be used instead of UV absorbance). SEC/FFF-MALS-DLS for structural evaluation of relative drug content and location.
Encapsulation Efficiency	SEC/FFF-MALS separates free drug from nano-delivery vehicles, then quantifies both substances. Payload may also be quantified.
Concentration/ Titer	SEC/FFF-MALS separates by size, then determines the particle concentration. NanoStar, ZetaStar or DynaPro Plate Reader for quickly estimating concentration over many formulations or conditions. ultraDAWN for process monitoring.
Zeta Potential and Stability	DynaPro Plate Reader, NanoStar or ZetaStar for aggregation and accelerated stability testing. ZetaStar to measure size and zeta potential simultaneously.



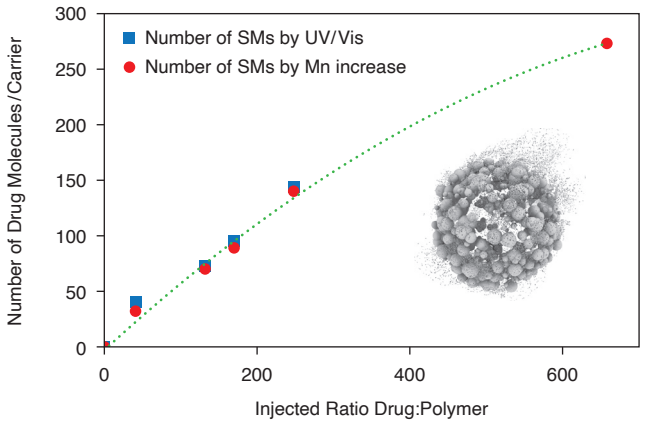
Further information

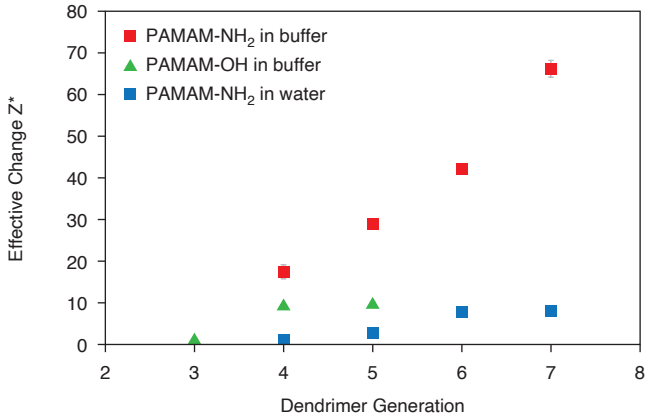
Learn how ultraDAWN is used to monitor and control liposome production processes in order to maintain accurate size specifications: www.wyatt.com/RT-MALS

Learn how light scattering instruments characterize the properties, attributes and stability of therapeutic / diagnostic nanoparticles: www.wyatt.com/Webinars/Nanoparticles

Encapsulation efficiency

AF4-MALS separates small drug molecules (SM) from delivery nanoparticles (NP) to determine the free:bound drug ratio and the amount of drug incorporated into the NPs, along with detailed NP size distributions and concentration. Here, two orthogonal, online methods were used to evaluate encapsulation in a polymersome: 1) quantification of free drug by UV/Vis; and 2) molar mass of the NP + incorporated SM by MALS and dRI. The results are shown to agree closely. Zeta potential of the polymersomes can be measured by the ZetaStar. (Figure reproduced with permission of A. Lederer, Leibniz Institute for Polymer Research.)





Enhancing bioavailability

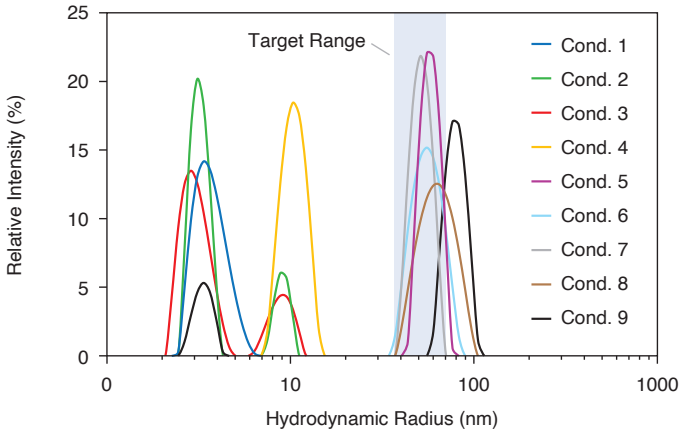
The surface charge Z^* of a drug nanocarrier must have the right sign and magnitude for optimal bioavailability and delivery to the desired organ. ZetaStar measures nanoparticle charge and zeta potential, even for samples at very high ionic strength and when the particle scatters relatively little light, such as the dendrimers shown here dissolved in a high-ionic strength buffer containing 500 mM acetic acid + 500 mM NaO_3. Analyses were automated with an autosampler.

When automation is not required, the NanoStar or the ZetaStar are ideal for further characterizing size, stability and aggregation by DLS and SLS.

Formulation and process development

The DynaPro Plate Reader increases productivity during liposomal formulation development, automatically quantifying size, aggregation and concentration over a large number of conditions. Here the instrument identifies only one formulation, Cond. 7, producing a size distribution entirely within the desired window of 40-70 nm.

Temperature-ramp or isothermal stability studies may be carried out directly in the instrument. Freeze-thaw and other stresses may be applied in the industry-standard plates used by this instrument. Measurements of zeta potential can also be automated, by combining the ZetaStar with an autosampler and pump.



Peptides & Nucleic Acids

Even small peptides of a few hundred g/mol can be analyzed by light scattering to determine molar mass, but SEC-MALS is especially powerful in measuring distributions of heterogeneous peptides, free RNA and protein-DNA complexes. High-throughput screening for stability, self-association and inhibition of protein-protein interactions is effected by light scattering in well plates.

Attributes	Solution
Molar Mass	SEC-MALS with DAWN and Optilab for molar mass: homogeneity and distributions. ultraDAWN to monitor downstream processes.
Conformation	SEC-MALS-DLS with DAWN and WyattQELS™ to characterize size and conformation.
Stability	Dynamic and static light scattering in the DynaPro Plate Reader, NanoStar or ZetaStar to test degradation under stressed conditions such as elevated temperature or freeze-thaw.
Self-association	CG-MALS with Calypso and DAWN to quantify size, kinetics and affinity of oligomerization. DynaPro Plate Reader, NanoStar or ZetaStar to screen self-interaction under multiple conditions.
Complexation	SEC-MALS to determine the absolute stoichiometry and size of protein-nucleic acid complexes; CG-MALS for analysis of binding affinity.

Further information

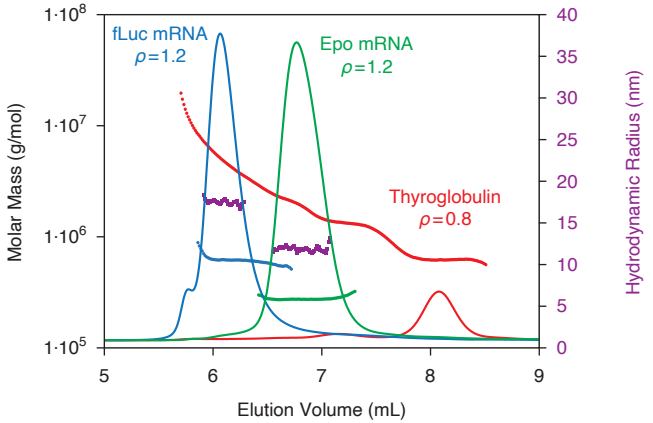
Application notes contributed by our customers include SEC-MALS method development for mRNA analysis: www.wyatt.com/AppNotes/Biotherapeutics

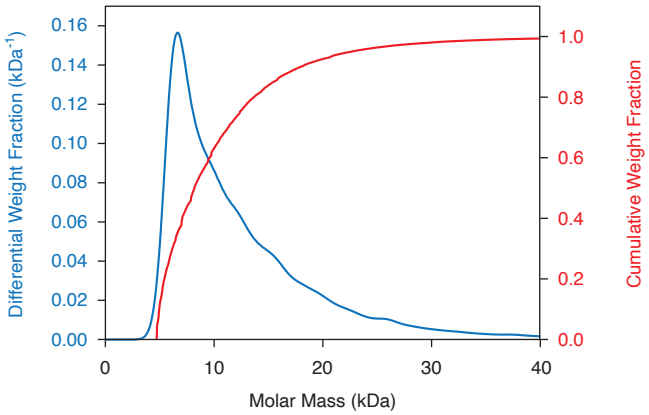
View open-access webinars on the Wyatt website to learn how heterogeneous peptides are characterized by SEC-MALS: www.wyatt.com/webinars/Biotherapeutics

Biophysical attributes

Entirely unstructured, mRNA elutes much earlier than a globular protein of the same MW such as thyroglobulin. This conformational difference is reflected in the shape factor, $\rho = R_g : R_h$, determined by simultaneous MALS and DLS measurements.

SEC-MALS does not rely on column calibration with globular standards, and provides the correct MW regardless of elution volume. In addition, it distinguishes single- and double-stranded nucleic acids which have similar diffusion coefficients and elute at about the same volume in SEC.





Heterogeneous peptides

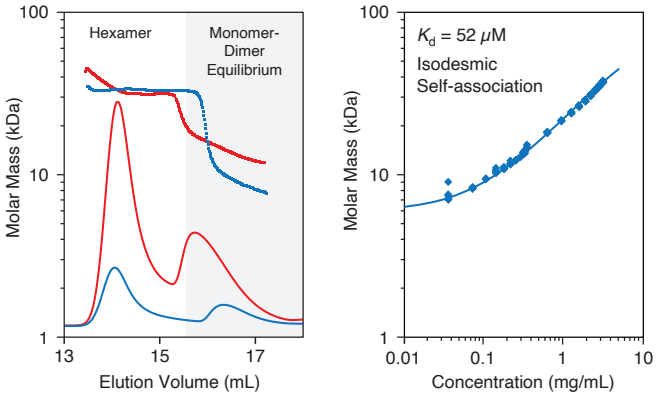
Glatiramer acetate is formed by reduction of high-molecular-weight precursors. SEC-MALS determines the complete molar mass distribution as well as the moments (M_n, M_w, M_z) and polydispersity ratio (M_w/M_n) for characterization and quality control.

Any remaining aggregates would be filtered by the column and not be quantified by SEC-MALS. Such particles are quickly identified by DLS using the NanoStar or ZetaStar. Extended characterization of aggregates is possible with an Eclipse/DAWN FFF-MALS system.

Self-association

The presence of insulin self-association is indicated by SEC-MALS (left), where—alongside the presence of a robust hexamer peak—dynamic equilibrium is evident in the monomer-dimer region. The shift in the equilibrium for different injected concentrations is the hallmark of reversible interactions.

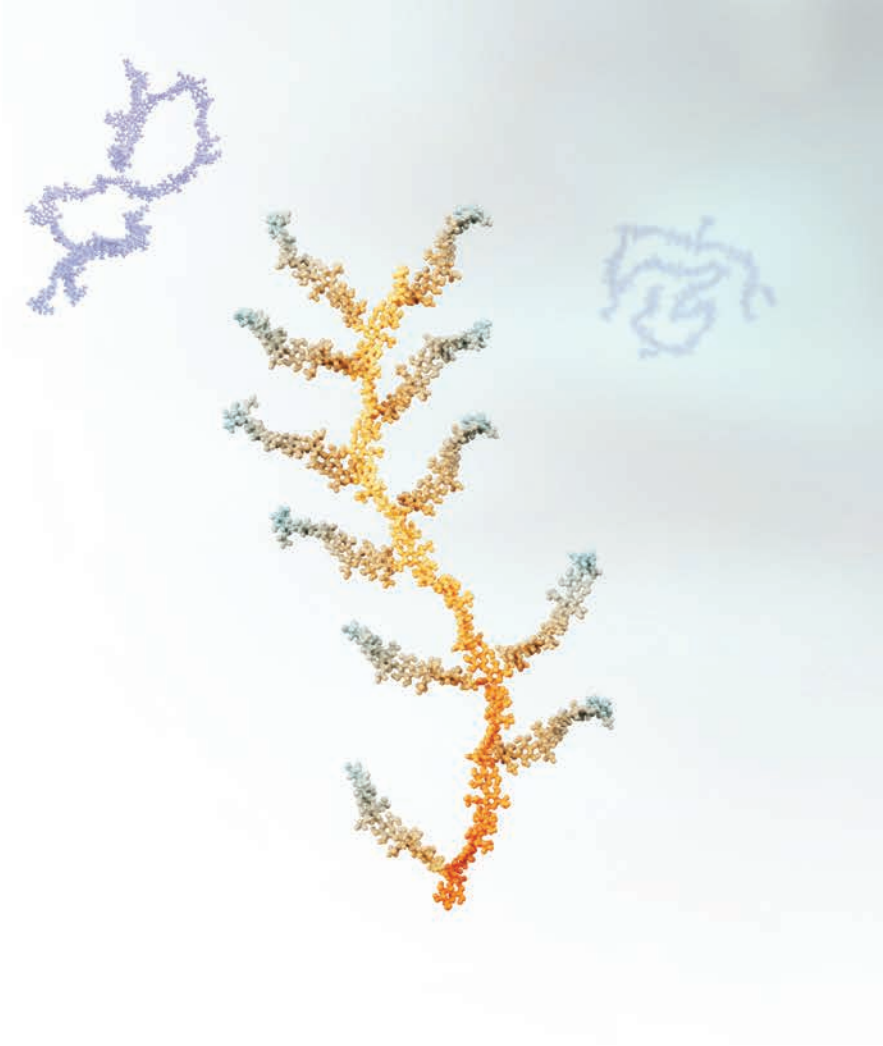
The equilibrium dissociation constant of self-association, K_d, is quantified by CG-MALS (right). Analysis performed by the CALYPSO™ software takes into account how the weight-average molar mass changes with concentration.



Polymers

Polymer characterization by analytical size-exclusion chromatography with column calibration relies on the assumption that the analyte has the same conformation, density and column interactions as the reference molecules. MALS analysis is independent of a polymer's column-elution properties and the need for compatible standards; it provides absolute molar mass, size, conformation and branching ratio.

Attributes	Solution
Molar Mass Distributions and Moments	SEC-MALS with DAWN and Optilab for absolute molar mass, M_w, M_n, M_z and polydispersity. FFF-MALS with Eclipse to fractionate polymers that are incompatible with SEC separations. ultraDAWN to monitor polymerization/depolymerization processes/conjugation.
Conformation and Branching	SEC-MALS-IV with DAWN and ViscoStar™ to determine size distributions, molecular conformation such as compact (branched), random coil or stiff, and branching ratio.
Copolymer Composition	SEC-MALS-UV-RI to analyze the molar mass of copolymer components, if at least one exhibits UV absorption.
Solubility	Dynamic light scattering with NanoStar or ZetaStar and quartz cuvette to test solubility in organic solvents.



Further information

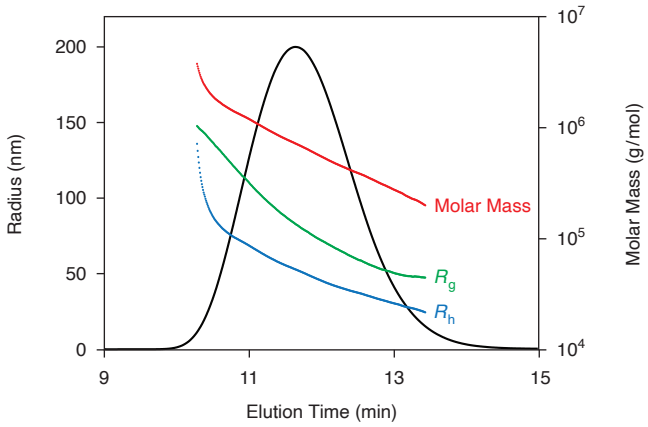
The combination of MALS, differential viscometry and refractive index measurements provides comprehensive characterization of all bio/pharmaceutical polymers: www.wyatt.com/Biopolymers

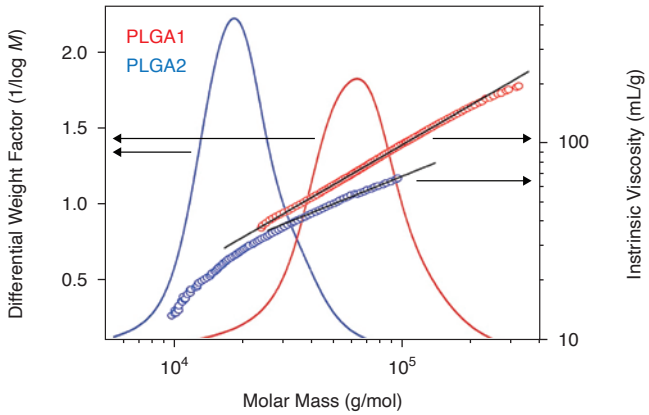
DAWN is the ultimate multi-angle light scattering detector for polymer characterization, offering maximum sensitivity and range: www.wyatt.com/DAWN

Absolute molar mass and size

With SEC-MALS-IV, hyaluronic acid can be fully characterized in terms of molar mass, rms radius and hydrodynamic radius, at each elution volume. MW moments and polydispersity are then calculated.

The high-molecular-weight fraction of this material is at the upper limit of fractionation by SEC. Samples containing extended HMW content are better fractionated and characterized by AF4-MALS, which can analyze molar masses in the hundreds of millions.





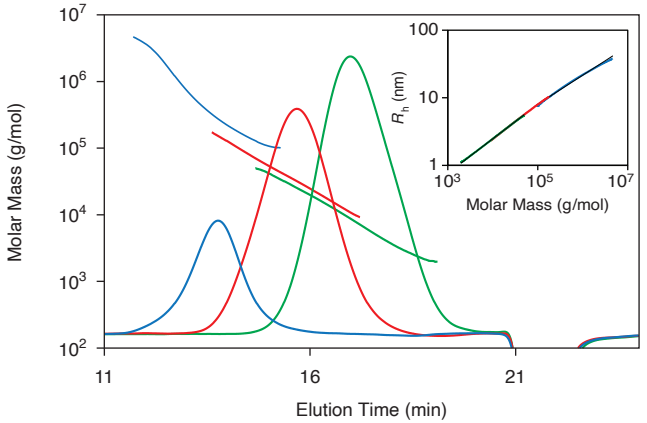
Compare distributions and conformations

PLGA conformation depends on branching, the ratio of lactic to glycolic acid and the overall molar mass distribution. Branching and conformation are determined from the plot of intrinsic viscosity, determined by ViscoStar, against molar mass, determined by DAWN.

Based on the slope of the conformation plot, PLGA1 is found to have a linear conformation with higher molar mass than PLGA2. PLGA2 exhibits the onset of branching (lower slope) at approximately 20 kg/mol.

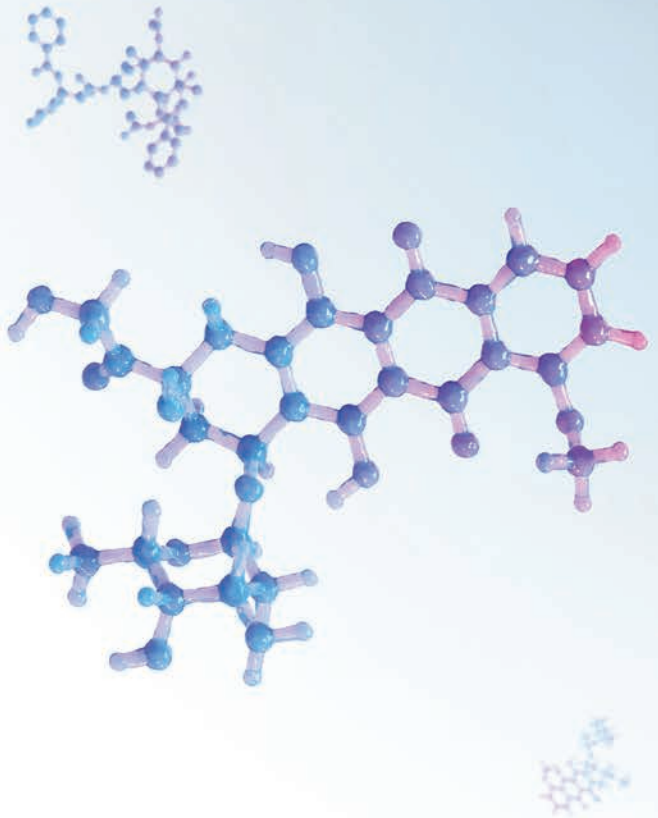
Absolute characterization

High-MW dextrans may elute differently in GPC—even though they have the same conformation and chemistry—as a result of different column loading or aging. Even though the molar mass values obtained by MALS differ at the same elution volume for these three dextrans (nominally 10, 40 and 500 kDa), the conformation plots (inset) determined by MALS plus intrinsic viscosity measurements prove that they are identical in conformation.



Small Molecules

While the primary uses of MALS, DLS and ELS for small-molecule drug development include characterization of target proteins, polymer excipients and nanoparticle delivery vehicles, there are several applications specific to the small-molecule drugs and excipients. The DynaPro Plate Reader, in particular, is highly beneficial for screening aspects of drug properties including aggregation and functionality.



Attributes	Solution
Size and Aggregation	Automated, in-plate DLS/SLS with the DynaPro Plate Reader for sizing and aggregation. NanoStar when automation is not needed.
Inhibition and Interactions	DynaPro Plate Reader to perform *in vitro* screening of protein-protein interaction inhibition when both proteins are soluble. CG-MALS with Calypso to quantify dissociation kinetics and binding affinity.
Free/Bound Drug Ratio	FFF-MALS separates free drug from nano-delivery vehicles, then quantifies both substances. Appropriate for small-molecule drug compounds as well as peptides, proteins or oligonucleotides.
Quality	Dynamic light scattering with DynaPro Plate Reader, NanoStar or ZetaStar to check for cross-linked nanoparticles in soluble excipients such as mannose. NanoStar or ZetaStar to perform turbidity measurements of drug formulations.

Further information

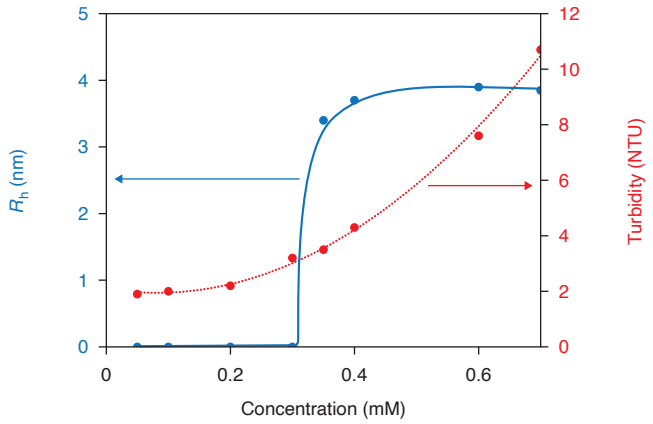
The DynaPro Plate Reader screens size and molar mass in standard microwell plates to assess aggregation, solubility and particle concentration: www.wyatt.com/DynaPro

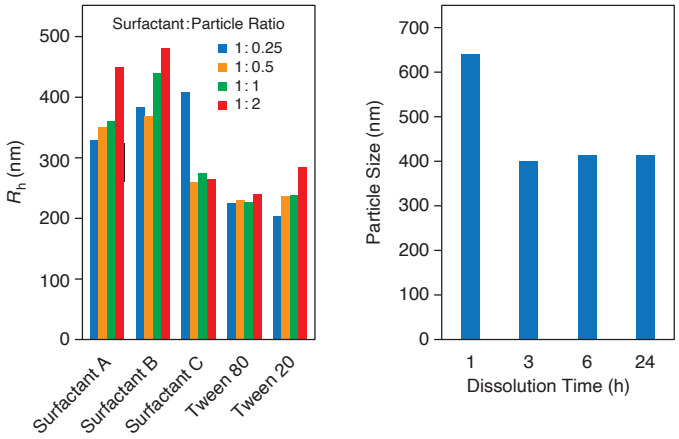
Wyatt team members and customers share their knowledge in both instrument-specific and application-specific presentations: www.wyatt.com/webinars

Excipient behavior

Excipients are key to effective and bioavailable APIs. In addition to polymer molar mass and degradation, light scattering—both static and dynamic—characterizes surfactant micelles for size, CMC and CMT, with and without API present.

If only a few APIs or solvents need to be tested, then the cuvette-based NanoStar or ZetaStar is sufficient. The DynaPro Plate Reader is ideal for testing a large range of solvents and excipients, if they are compatible with plastic well plates. Both instruments are equipped for static and dynamic light scattering measurements.





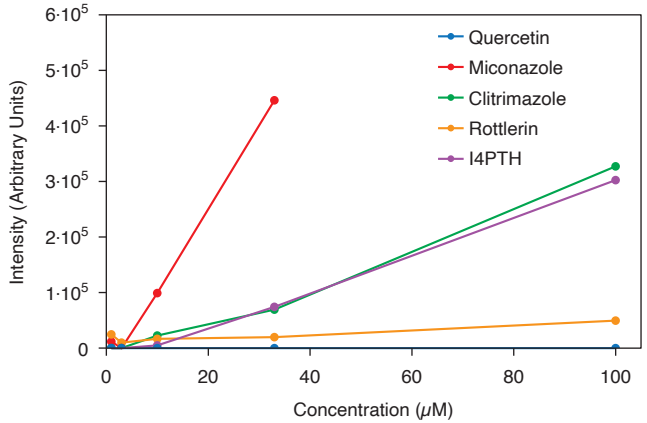
Nanomilling process optimization

Multiple conditions such as those used in acoustic milling may be tested in the DynaPro Plate Reader, in order to identify the ideal recipe for creating stable nanosolids with enhanced bioavailability.

For a continuous production process, the ultraDAWN may be more appropriate since it determines particle size in real time.

Promiscuous inhibitors

Promiscuous inhibitors are aggregated molecules that bind non-specifically to target proteins. The DynaPro Plate Reader is used to check screening ‘hits’ for such aggregation. Here we see how the intensity varies with concentration; two of the analytes maintain very low scattering intensity at the highest concentration, indicating that they do not aggregate at all in this solvent.



End-to-end support

Advanced characterization at each step

Development of efficacious and robust pharmaceuticals, whether biologics or non-biological complex drugs, is a long and difficult process. Our suite of analytical technologies, based on light scattering and related techniques, can assist you at each stage. Our uniquely versatile instrumentation for biophysical screening, characterization and process analytics is essential from candidate discovery and selection through optimization, purification, formulation, manufacturing and quality control. Below are just a select set of phase-specific applications – contact us to learn how we can help meet your challenges.

Discovery & Optimization

Candidate Selection

- Protein quality control
- Developability assessment

Affinity Maturation

- Drug-target binding studies
- Optimization of crystallization buffers

Early Stage Formulation

Formulation Screening

- Aggregation and aggregation propensity
- Nanomedicine encapsulation efficiency

Stability Studies

- Accelerated stability
- Quantify stress-induced degradants

Late Stage Formulation

Formulation Screening

- Colloidal, thermal and chemical stability
- Viscosity and opalescence of concentrated mAbs

Formulation Studies

- Protein-protein and protein-excipient interactions
- Characterize drug product

Early Process Development

Process Development

- Inline monitoring to optimize pools
- Quantify aggregates and fragments in fractions

Process Characterization

- Characterize drug substance
- Analyze process-induced degradation

Late Process Development

Process Development

- Inline and at-line monitoring to optimize cutoff

Process Characterization

- Determine physical titer and empty:full ratio of gene vectors
- Comparability assessment between lots, processes

Manufacturing & QC

Regulatory Filing

- Biophysical characterization

Process Analytics

- Real-time monitoring of molar mass and size

Quality Control

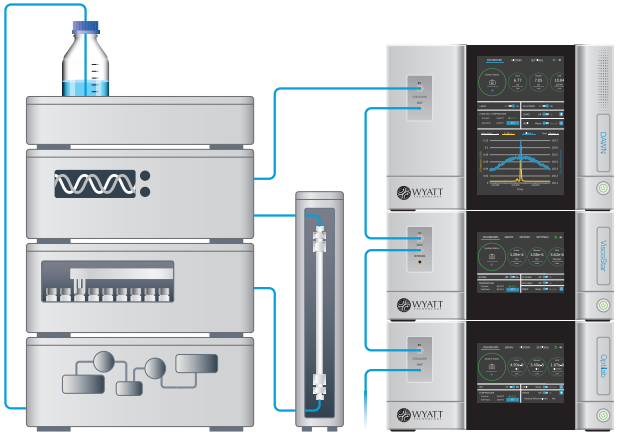
- Protein-polysaccharide molar mass distributions

Characterization Toolkit

SEC-MALS Multi-angle light scattering with size-exclusion chromatography

In SEC-MALS, the SEC column is only used to separate molecules by size. Molecular weight, size, concentration, conjugate composition and conformation are all determined directly by the online MALS, DLS, UV and differential refractive index (dRI) detectors. There is no need for column calibration standards.

A SEC-MALS system consists of standard HP-SEC instrumentation plus a DAWN MALS detector, an Optilab dRI detector and ASTRA software. A WyattQELS dynamic light scattering module may be embedded in the MALS instrument to measure sizes below 10 nm in radius. For analysis of the conformation of polymers below about 1 MDa, a ViscoStar differential viscometer is added. The low-volume microDAWN, microOptilab™ and microViscoStar™ SEC-MALS instruments are optimized for the narrow peaks of UHP-SEC.



DLS & ELS Dynamic and electrophoretic light scattering

DLS determines particle sizes R_h from < 1 nm to > 1 µm. Size distributions may be measured even without fractionation, though at low resolution, and nanoparticle concentrations or viral titers calculated. Analysis of R_h vs. temperature and concentration yields stability indicators such as aggregation temperature T_{agg} and the diffusion interaction parameter k_D. DLS instruments can also measure static light scattering (SLS) to determine molar mass, second virial coefficient A_2 and turbidity.

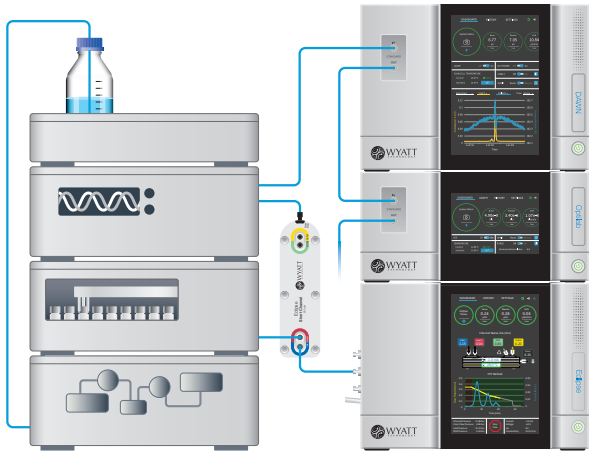
The DynaPro NanoStar and ZetaStar measure DLS and SLS in 2 µL quartz cuvettes or 4 µL disposable cuvettes. The DynaPro Plate Reader measures DLS and SLS in standard 96, 384 or 1536 microwell plates. The ZetaStar also measures in a flow cell. These instruments all use DYNAMICS™ software for control, data acquisition and analysis. The NanoStar and ZetaStar may also be operated via the intuitive DYNAMICS Touch™ on-board app.

ELS determines a particle's zeta potential to assess colloidal stability, isoelectric point in buffer, N/P ratio, etc. The ZetaStar measures DLS and ELS simultaneously, permitting full automation with an autosampler. Its flow cell can be pressurized to suppress bubbles, formed by electrolysis in high-salt solutions, which interfere with sensitive ELS measurements.

FFF-MALS Multi-angle light scattering with field-flow fractionation

In FFF-MALS, size-based separation of macromolecules and particles from 1 to 1000 nm is accomplished with an Eclipse system implementing asymmetric-flow field-flow fractionation (AF4). The analytes are characterized with downstream detectors that include MALS, DLS, UV, dRI, fluorescence and/or differential viscometer, providing similar biophysical properties as SEC-MALS, but over a wider size range.

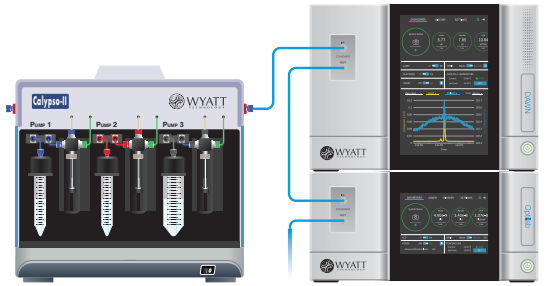
FFF has additional advantages over SEC including no shear, small surface area and tunable separation power. The Eclipse system uses industry-standard HPLC pumps, autosamplers and fraction collectors for guaranteed reliability and minimal sample consumption. VISION™ software controls the HPLC and Eclipse, and coordinates data acquisition by ASTRA.



CG-MALS Composition-gradient multi-angle light scattering

Composition-gradient MALS characterizes biomolecular interactions by preparing and measuring a series of compositions or concentrations. The variation in the solution's weight-average molar mass with composition is analyzed to quantify binding affinity and absolute stoichiometry (i.e., the number of each type of molecule present in the complex). In the case of non-specific interactions, self- and cross-virial coefficients are determined.

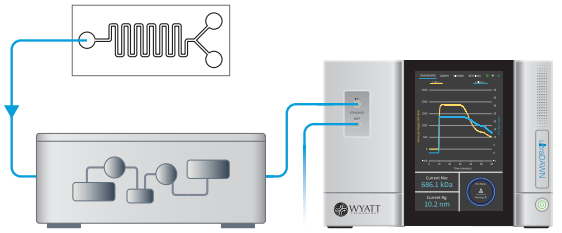
A CG-MALS system includes a Calypso composition-gradient system, a DAWN or miniDAWN™ MALS detector, an Optilab or generic UV concentration detector, and the CALYPSO™ software.



RT-MALS Real-time process analytics with multi-angle light scattering

Product attributes such as molar mass, size, Vg/Cp and nanoparticle concentration are evaluated by RT-MALS for advanced process monitoring and control. Molar mass measurements signal end points for polymerization/depolymerization or protein purification, while size monitoring of nanomedicines such as lipid nanoparticles or viral vectors is invaluable during purification, diafiltration and fill-finish.

An RT-MALS system adds an ultraDAWN instrument directly inline with the process, or online via a low-volume continuous feed. OBSERVER™ software calculates product attributes up to five times per second and sets triggers to start and stop a reaction or collect pools. It can control the process directly, or feed data and triggers via OPC-UA to process control software for GMP operation.



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