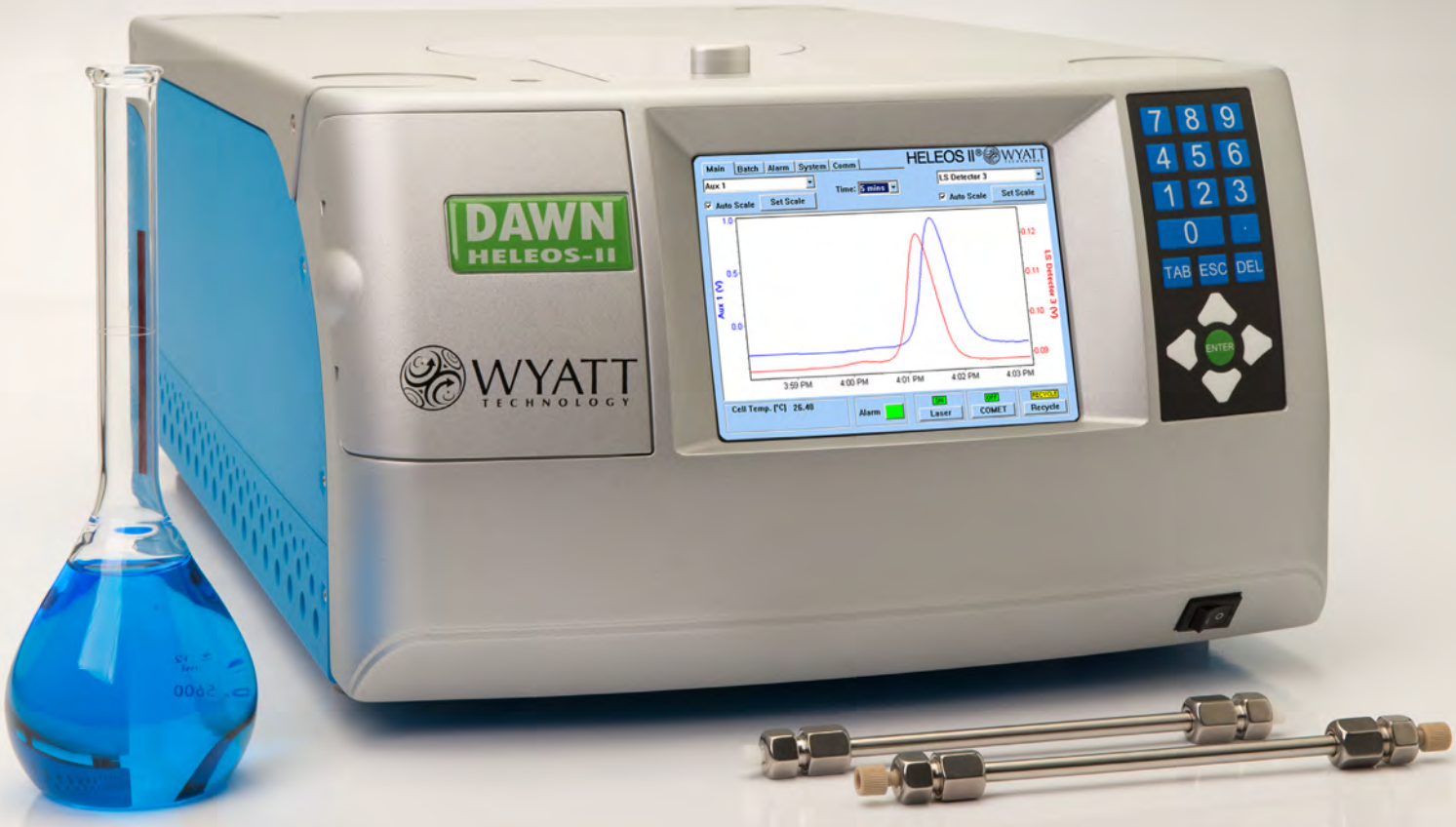
WYATT TECHNOLOGY CORPORATION

DAWN® HELEOS® II

The world's most advanced light scattering instrument for absolute characterization of proteins, polymers, and nanoparticles.



DAWN HELEOS II

The gold standard for absolute molar mass and size

The DAWN HELEOS II determines the absolute molar masses of proteins, biopolymers, and synthetic polymers, as well as the sizes of vesicles and other sub-micron particles, through multi-angle light scattering (MALS - see sidebar). It can measure molar masses from hundreds of Daltons to hundreds of millions of Daltons and radii from 10 nm to 500 nm, covering peptides and ultra-low-molecular weight polymers through proteins, synthetic and natural polymers, vesicles, emulsions and most nanoparticles. Moreover, it makes *absolute measurements*, which do not depend on standards or semi-empirical "calibration" routines.

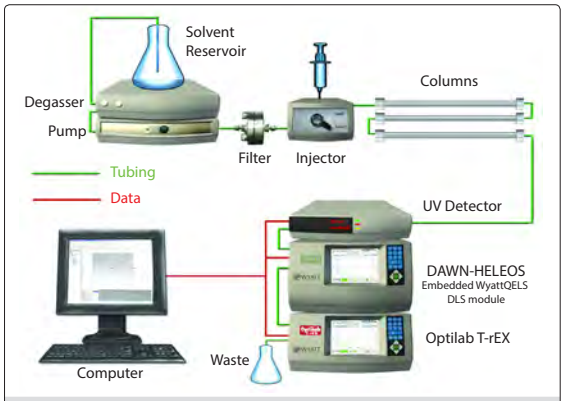
The DAWN may be used in several modes: in continuous-flow detection following chromatographic separation to characterize distributions of molecular weight and size; as a stand-alone unit in a batch or micro-batch mode, to measure average molar masses or sizes; or coupled to an automated composition-gradient system to assess macromolecular interactions and reaction kinetics as well as average solution properties. Regardless of which mode you choose, the HELEOS II provides the highest sensitivity, reliability and measurement range of any MALS detector.

Superior technology and productivity

Eighteen discrete photodetectors are spaced around the DAWN's flow cell in a special geometry, ensuring that measurements are made *simultaneously* over a broad range of scattering angles (typically from 10° – 160°).

An exceptionally rigid glass-to-metal design, combined with innovative vibration isolation, yields an optical bench of the greatest stability, which sets the stage for measurements of the greatest sensitivity and accuracy.

The unique flow cell design of the HELEOS provides welcome relief from the contamination problems that plague most other light scattering instruments. The HELEOS cell isolates the entrance and exit windows from the scattering volume to dramatically suppress stray light. With the help of carefully designed turbulent flow, its windows are continuously washed by the eluant. Coupling the DAWN with an embedded COMET™ (Cell Operation and Maintenance Enhancing Technology) flow cell cleaning system virtually eliminates cleaning; a great advantage when compared with other instruments—some of which can't be cleaned unless they're returned to the manufacturer. Simply press a button or program your data collection to automatically clean the cell after every run. This results in reliable operation with minimum downtime.



The DAWN HELEOS II forms the heart of a modular SEC-MALS system incorporating any analytical HPLC or FPLC instruments. Create the ultimate setup for absolute characterization of proteins, polymers and nanoparticles by adding more Wyatt modules such as an Optilab T-rEX differential refractometer, a ViscoStar differential viscometer, and a WyattQELS embedded DLS detector.

Endorsed by the scientific community

No instrument can compare to the HELEOS II for the characterization of proteins and macromolecules in solution. Our library of *thousands* of peer-reviewed papers using DAWN and miniDAWN™ MALS instruments proves the power of the HELEOS II to provide molecular weight and size data that scientists around the world depend on for their daily research.

Multi-angle means more information...

Multi-angle light scattering (MALS) is the preferred analytical technique for determining molar masses and sizes without making assumptions. And because it is a straightforward, absolute method—a significant advantage over most other analytical techniques—it provides results that are not dependent on calibration standards or measurements that someone else made in another laboratory.

In the limit of low concentration, the amount of light scattered by a suspension of molecules is directly proportional to the product of their weight-average molar mass times their concentration. The angular variation of the scattering (as a function of angle) reveals the molecules' mean square radius. If you want to determine molar masses and sizes over the greatest possible ranges, a MALS instrument is the ideal solution.

Unlike empirical techniques, such as viscometry, MALS requires no assumptions relating the measurements to the results. Furthermore, MALS makes no prior assumptions about molecular conformation (since MALS can **measure** it), nor does it depend upon specific polymer or protein reference standards. All the constants required for determining absolute molar masses are measured experimentally **from first principles**—they do not depend on vague empirical relations.

For accurate and reliable analysis of molecular weight and size, instruments designed for quantitative static light scattering measurements must cover a broad range of scattering angles, hence the term "multi-angle light scattering". This is in contrast to instruments producing scattering measurements at only one or two angles, which are notoriously inaccurate since, with only one or two angles (RALS, LALS), the light from a single stray dust particle can corrupt the data. Low-angle light scattering (LALS) is particularly problematic because dust particles scatter copiously into the low angles.

For more information:

To learn about MALS and its uses in the characterization of macromolecules and nanoparticles, visit these resources:

www.wyatt.com/**Theory** - theory overview of multi-angle (static) and dynamic light scattering

www.wyatt.com/**SEC-MALS** - introduction to SEC-MALS applications and instrumentation

www.wyatt.com/**FFF-MALS** and www.wyatt.com/**CG-MALS** - advanced applications of MALS technology for nanoparticle characterization and biomolecular interactions

www.wyatt.com/**HELEOS** - additional DAWN HELEOS II features

HELEOS II Advantages

- Compatible with *any* HPLC system.
- Sensitive operation is achieved by means of a flow cell design that eliminates virtually all stray light.
- Measures nanoparticles from 10 nm to 1 µm and boosts sensitivity for small molecules down to 200 g/mol, thanks to 18 optimally-placed scattering angles.
- Constant, highly stable laser output is maintained by the integrated laser monitor.

The DAWN HELEOS II may be positioned downstream of all types of HPLC, FPLC, or GPC systems for analysis of molar mass and size distributions.

- Sensitive even in the presence of strong scattering from high concentrations or large particles, thanks to 24-bit A/D conversion.
- Corrects for sample absorption at the laser wavelength via the Forward Laser Monitor.

Optional modules:

- Integrated COMET ultrasonic device automates *in situ* cell cleaning.
- Flow-to-batch conversion kit permits use of a 10 µL cuvette or a 10 mL scintillation vial.
- IR laser and fluorescence blocking filters enable characterization of fluorescent samples.

Optics & Electronics

First to incorporate DSP electronics

The HELEOS' engineering elegance is reflected in its integrative approach to electro-optical and mechano-optical design. The HELEOS has no mirrors, prisms, or moving parts that could become misaligned, damaged, or contaminated—elements of particular concern when any measurements are made. Developed through years of research and analysis by the world's leaders in light scattering instrument design, the kinematically-engineered flow cell, manifolds, read head, and laser mounts are unified on a single optical bench.

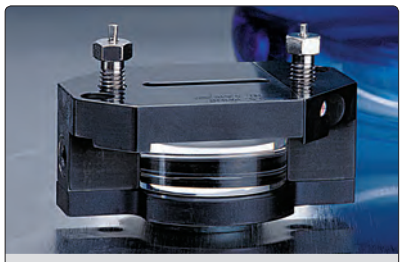
Wyatt Technology pioneered the coaxial flow cell, which lies at the heart of the HELEOS. Its revolutionary design allows it to be cleaned and replaced without any instrument realignment. The flow cell *minimizes* sample volume and stray light but allows multi-angle measurements to be performed simultaneously over the widest range of useful angles. The nitrogen purge option makes the DAWN HELEOS II robust even in dusty or humid environments or when operated at low temperatures (below the dew point).

The DAWN's temperature control capabilities are just as impressive. Our 0.05°C temperature regulation generates reproducible and stable baselines. For work between -15°C and +150°C (or room temperature to +210°C with the high-temperature model), this feature allows new classes of experiments to be performed with great simplicity. Whether you're studying protein folding at 4°C or polyolefins at 135°C, the temperature control options of the HELEOS II give you incomparable versatility.

Decades ago, we pioneered the use of solid-state hybrid photodiode detectors in our classical light scattering instruments, and ever since have maintained the highest level of custom electro-optic components. Each analog signal (one for each angle) is processed by its own dedicated Digital Signal Processing (DSP) integrated circuit with 24-bit digital conversion for extremely high resolution. You get the highest resolution data over the *entire* measurement range.

Four auxiliary analog inputs (with their own DSP circuits) are included for interfacing to third-party external detectors, such as DRI, UV, PDA, etc. so you can leverage investments in third-party instruments. We also measure the transmitted light with a forward monitor photodetector—a valuable capability for studying samples that *absorb*, as well as scatter, light.

Once collected, the data are preprocessed by a high-speed embedded computer to eliminate noise spikes arising from dust or other contaminants. The noise rejection algorithm provides the greatest possible noise rejection *without* distorting the real data. Typically, other light scattering devices use long time-constant analog filters resulting in peak distortion due to their integration of noise generated by dust and debris. Since the analog-to-digital conversion is performed on-board the HELEOS, low light scattering signals are not prone to environmental "noise" or pick-up, which can cause erroneous data conversions. The HELEOS' digital output signals are transmitted from the instrument to a PC via Ethernet for network operation or a USB interface when standalone operation is desired. The highly reliable digital data transfer protocol includes a time stamp and checksum with each packet, insuring that data are neither lost nor corrupted.



The integrated axial flow cell assembly insures highly reproducible measurements at all angles simultaneously.

How It Works...

The DAWN HELEOS II utilizes a fixed, multi-angle detector array, which measures data from all angles simultaneously. Complete multi-angle measurements are made in less time than it takes a mechanically-driven device to make a measurement at a single angle.

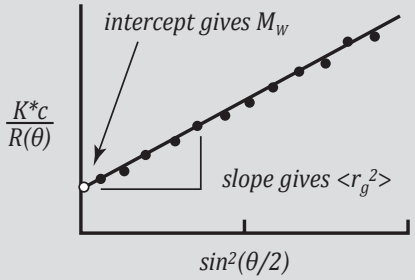
In the limit of very small concentrations, as is the usual case for liquid chromatography, the fundamental relationship linking the intensity of scattered light, the scattering angle, and the molecular properties is simply:

$$\frac{K^*c}{R(\theta)} = \frac{1}{[\, M_W P(\theta)\,]} + 2A_2c + O(c^2)$$

where:

- $R(\theta)$ is the excess Rayleigh ratio, proportional to the intensity of light scattered by the sample at each angle θ,
- c is the sample concentration (g/L),
- M_W is the weight-average molar mass (grams per mole),
- A_2 is the second virial coefficient (a measure of intermolecular interactions),
- K^* is an optical parameter that depends on laser wavelength, solvent refractive index and the sample's refractive increment dn/dc.

The function $P(\theta)$ describes the scattered light's angular dependence, a function of the molecule's size and internal structure. This measurement is absolute and does not require any **a priori** knowledge of molecular conformation or branching. A plot of $K^*c/R(\theta)$ vs. $sin^2\,(\theta/2)$ in the limit of the very low concentrations characteristic of chromatography experiments yields a curve whose intercept at $\theta = 0$ yields M_W and whose initial slope is proportional to the molecule's mean square radius $\langle r_g^2 \rangle$.



Many scattering angles in a MALS detector does not guarantee the best measurements. Optimal flow cell design, smart optical engineering and sophisticated data processing algorithms all combine to reduce stray light and other noise sources, while maximizing useful signals.

Wyatt's decades of experience in MALS technology ensure the most sensitive and robust instrumentation.

Optional embedded modules include the COMET ultrasonic flow cell cleaning system and WyattQELS™ DLS detector.

ASTRA® Software

Absolute molecular weights & sizes by SEC-MALS

ASTRA, the most powerful software package for collecting and analyzing light-scattering data associated with proteins, macromolecules, and nanoparticles, comes with each HELEOS instrument. ASTRA is used equally well for batch (unfractionated) or chromatographically-separated samples.

By coupling a HELEOS II to your favorite chromatograph, ASTRA determines the distributions of absolute molar masses and sizes of fractionated samples in an intuitive way that permits simultaneous collection and processing of data files.

ASTRA includes specialized routines for the following:

- Absolute molar mass, size, and second virial coefficients
- Dynamic light scattering analysis of molecular/nanoparticle size
- Differential refractive index (dRI) and UV acquisition to measure sample concentrations and extinction coefficients
- Off-line or online *dn/dc* determinations
- Nanoparticle number densities
- Differential viscometry measurements to determine intrinsic viscosities, polymer conformation and branching ratio

Independent means of verification

For data collected from separated samples, ASTRA will:

- Calculate polydispersity values
- Produce the molar mass and rms radius for each elution slice
- Determine protein mass and fraction for protein conjugates
- Extend analyses far beyond "Triple Detection", where MALS, RI, UV, QELS, and viscometer data may be collected simultaneously
- Correct for inter-detector band broadening
- Calculate oligomeric (e.g. aggregate) fraction distributions
- Process the chromatographic data without reference to standards, quasi-empirical calibration techniques, or a priori assumptions about molecular structure/conformation
- Calculate the precision of each result
- Comply with 21 CFR Part 11 regulations

Solid science

With ASTRA, every quantity required to make an unequivocal molar mass determination has its own independent means of verification—from the *dn/dc* determination, to the concentration, to the absolute intensity of light scattered at each angle. ASTRA even reports the uncertainties of the measurements, so you can get an instant feel for the quality of your data.

With the HELEOS II and ASTRA, pump speed fluctuations no longer play a role in determining molar masses: regardless of when the sample elutes, ASTRA will calculate its absolute molar mass at each elution volume. Changing your columns, your HPLC tubing connections, even your flow rate won't affect the HELEOS' performance at all.

ASTRA's analytical algorithms are based on rock-solid science developed at Wyatt Technology and are found in no other software package. For example, proprietary global fitting algorithms ensure that no extrapolation is needed in computing molar mass, size, or second virial coefficients. Branching ratios are computed *directly*.

Versatile reporting

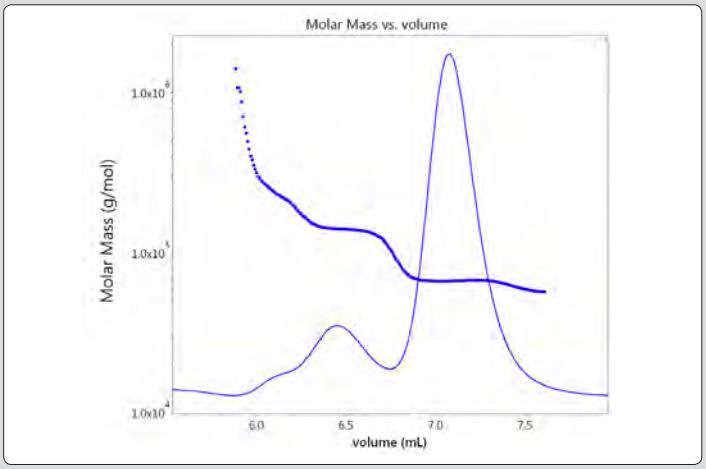
The results of these calculations are displayed in the following *graphical displays:*

- Molar Mass and Molecular Size vs. Volume
- Differential Molar Mass and Molecular Size Distributions
- Cumulative Molar Mass and Molecular Size Distributions
- Long chain branching calculations and graphics
- Conformation plots of log (M_W) vs. log (r_g)
- Three-dimensional plots displaying each light scattering detector's response as a function of elution volume
- Custom plots of any measured or calculated quantity on abscissa and ordinate axes

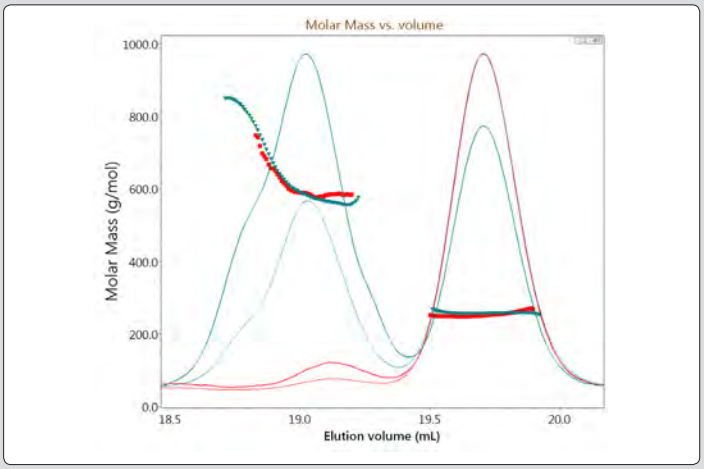
ASTRA's graphs use the following, easily exportable formats: metafile, bitmap, JPEG, PNG, GIF, PCX, PDF, even Postscript for the greatest possible compatibility with your reporting require-ments. In addition, you can create Bar, Pie, Line, and Scatter plots from the data with just a few clicks of the mouse. You have complete control over tick marks, scaling, line weight and widths, patterns, fills, etc. ASTRA has industrial strength graphing built right in.

Solution Supreme

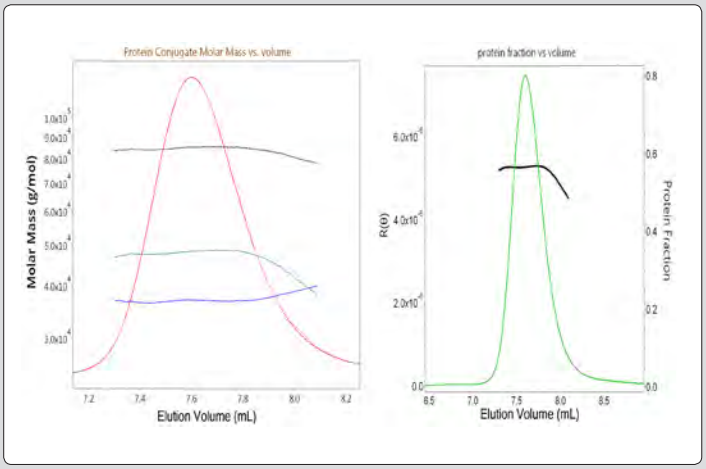
ASTRA provides a complete solution to analytical light scattering. With a full suite of capabilities comprising instrument control, sequence programming, multi-instrument data collection, analysis modules, result visualization and reporting, you can find everything you need for productive and robust characterization of proteins, polymers, nanoparticles and more.



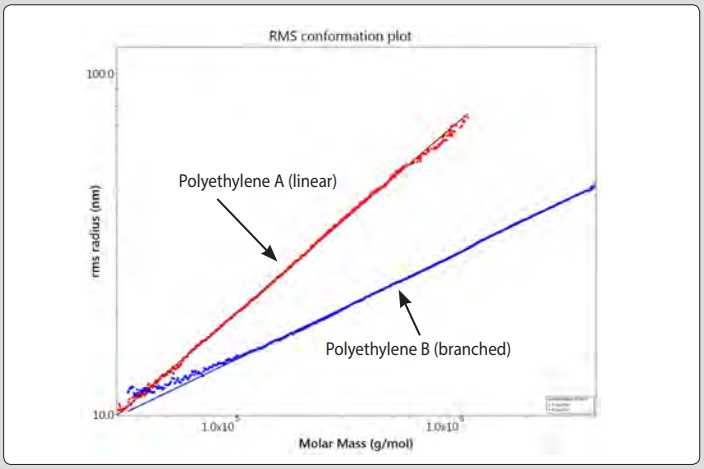
ASTRA's Band Broadening Correction accounts for interdetector dispersion, mathematically adjusting peaks so that each data 'slice' provides matched signals from each detector in the chromatographic elution series. This algorithm is responsible for the uniform molecular weights across the BSA monomer, dimer and trimer peaks plus additional oligomers eluting from the SEC column.



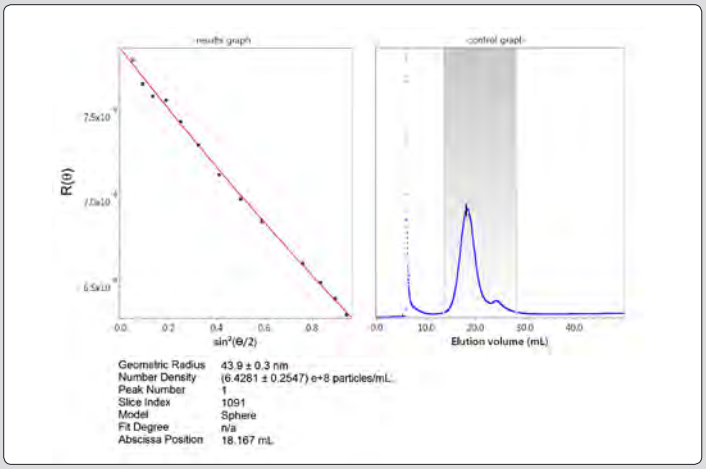
Methylene diphenyl 4,4'-diisocyanate (MDI) has a molar mass of 250 Da and will readily form oligomers in THF. The superior sensitivity of the HELEOS is essential in characterizing molecules like MDI that have such low molar masses.



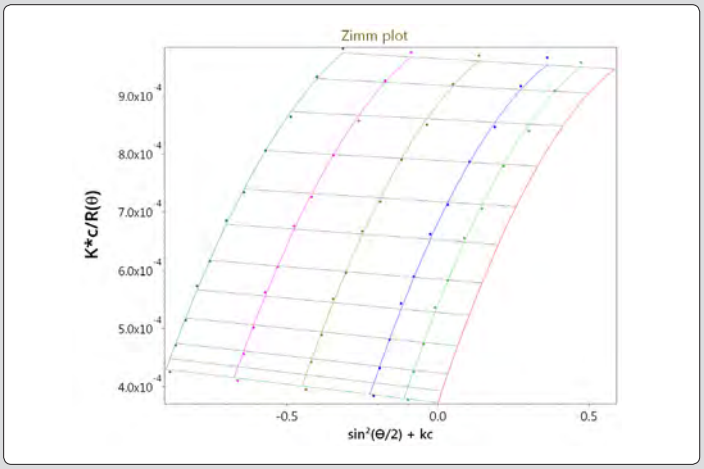
The ASTRA workspace has rich features for characterizing proteins using the Protein Conjugate Analysis algorithims with simultaneous signals from UV, RI, and MALS. The characterization includes molecular weight, extinction coefficient, stoichiometry, and composition analysis.



ASTRA's rms radius vs. molar mass plot reveals branching without column calibration or reference standards. Here, the branching of Polyethylene B is apparent by its significantly smaller slope when it is contrasted with linear Polyethylene A.



ASTRA calculates the number density of each data slice and the total number of particles of an eluted peak from the MALS data. In this example, both the radius and the number density are displayed for one data slice of the selected peak from an FFF run.



ASTRA can also be used for "batch" sample analysis when the molecules of interest are too large for conventional column chromatography. In this example, a high molar mass starch was characterized using batch measurement capabilities and applying the global fitting routines of ASTRA.

Specifications

Measurements	
Molar Mass Range	200 Da to 1 GDa*
Molecular Size Range (r_g)	≈ 10 to 500 nm, up to 1000 nm with shape-specific models
Molecular Size Range (r_h)	In flow mode, 0.5 nm – 300 nm†; batch mode 0.5 nm - 1 µm (requires WyattQELS DLS module or DynaPro® NanoStar® + fiber optic connection).
Sensitivity	0.4 µg/mL BSA; 0.2 µg BSA typical HPLC loading*
Fluidics	
Mobile Phase Compatibility	All-solvent compatible (aqueous and organic). Wetted materials are 316 stainless steel, fused silica or F2 glass, and Kalrez.
Optics	
Detectors	
MALS Detectors	High-gain, high dynamic range photodiodes at 18 detection angles.
Auxiliary Detectors	Laser monitor for stabilization feedback; forward transmission monitor to correct signals for absorbing samples and to assess data quality.
A/D Resolution	24 bit (detector dynamic range > 16,000,000)
DLS Detector (optional)	WyattQELS dynamic light scattering module installs directly inside the DAWN chassis. Alternatively, the optical fiber pickup of the DynaPro NanoStar cuvette-based DLS instrument may be installed in the DAWN.
Laser Properties	
Laser Wavelength	658 nm; 785 nm optional for use with fluorescing samples
Laser Power Control	Programmable 10% - 100%
Flow Cells	Fused Silica, optimal for solvent refractive index less than 1.50; F2, optimal for solvent refractive index above 1.50
Temperature Options	• Ambient • Heated/Cooled (HC) -15°C to +150°C • Ultra-High-Temperature (UHT) ambient to +210°C
Electronics	
Analog Inputs	4 differential analog inputs with 24 bit resolution. Input range -10 V to +10 V
Analog Outputs	2 analog outputs from user selectable measurements channels -10 V to +10 V
Other Inputs/Outputs	Alarm in, Alarm out/retransmit, Auto-Inject in, Auto inject contact closure retransmit
Computer Interface	Ethernet
Transmission Rate	Software selectable from 36.6 to 0.0001 Hz
Front Panel Display	162.5 mm, 16-bit, high resolution touch screen displays signal graphs, instrument settings and diagnostics
Dimensions	60 cm (L) x 36 cm (W) x 23 cm (H)

* depending on *dn/dc*, the sample concentration, and chromatography conditions, this is typical.

† assuming a flow rate of 0.3 mL/min and DLS detection at 160°.

Wyatt Technology is committed to continual improvement. Specifications subject to change without notice.

WARRANTY: All Wyatt instruments are guaranteed against manufacturing defects for 1 year.

With installations in *more* than 65 countries, *more* than 11,000 refereed journal publications citing its instruments, and 18+ PhD scientists on site, Wyatt Technology is the **world's leading manufacturer of instruments** for absolute macromolecular characterization. It is the only company in the world focused exclusively on such systems, their design, and their applications.

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