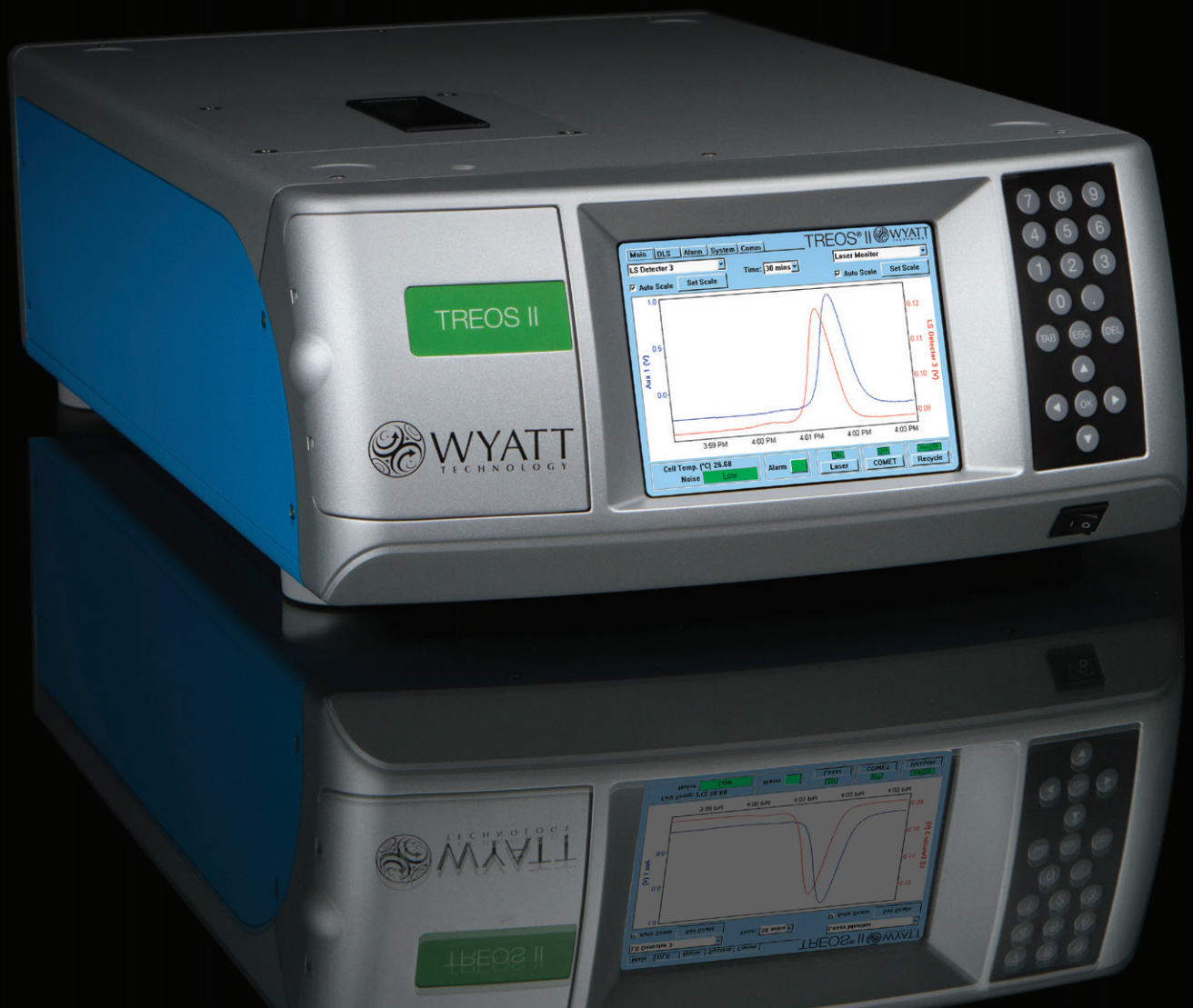
WYATT TECHNOLOGY CORPORATION

miniDAWN® TREOS® II

Multi-angle light scattering for absolute characterization of proteins, polymers, and nanoparticles by SEC-MALS



miniDAWN TREOS II

Multi-Angle Light Scattering (MALS) Detector

Determine absolute molar mass and size in solution

The miniDAWN TREOS II offers the level of performance and capability you have come to expect from our award-winning instruments. Determine the absolute molar masses and sizes of macromolecules and nanoparticles in solution from hundreds—to millions of Daltons—without the need for column calibration standards.

Characterize:

- Proteins, small peptides and oligonucleotides
- Synthetic and natural polymers
- Nanoparticles, virus-like particles and vesicles

Superior technology and productivity

Outstanding sensitivity Use as little as 25 ng injected sample (100 kDa polystyrene in THF; varies for other samples and solvents).

Field serviceable The TREOS II is engineered with self-contained modules that are easily replaced.

System-ready monitor Noise levels are monitored in real-time. An all-green status indicator lets you know when your system is ready for data collection.

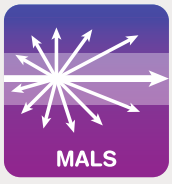
Invest for the future Upgrade to a UHPLC-compatible low-volume MALS instrument by simply replacing the optical module, right in your lab.

DLS module The WyattQELS™ module fits inside the TREOS II to provide you with on-line dynamic light scattering for determining radii down to 0.5 nm.

Self-cleaning All light scattering cells are subject to contamination by particles. Press a button to activate the embedded COMET™ ultrasonic module. For maximum reliability and uptime, program the TREOS II to automatically clean the cell after every run.

Knowledge without assumptions

Multi-Angle Light Scattering



Using first principles, MALS is a well-established technique in the field of macromolecule and nanoparticle characterization. By eliminating common assumptions that are necessary in size exclusion column calibration, you can be more precise and confident in your results.

Not all light scattering instruments are designed the same. The miniDAWN TREOS II incorporates three angles of detection for greater sensitivity and repeatability.

- Determine with absolute confidence molar masses and sizes without the need for column calibration or reference standards
- Identify column interactions, aggregation or other non-ideal characteristics that chromatography alone cannot determine
- Light scattering instruments with only one or two angles (LALLS, RALLS) are notoriously susceptible to poor data quality due to dust particles—but not the TREOS II

TREOS II Advantages

- Highest sensitivity in class: 25 ng of 100 kDa polystyrene in THF
- Superior MW determination for small molecules down to 200 g/mol
- Compatible with any HPLC, upgradeable to UHPLC
- Wide measurement range for particles 10 to 150 nm in radius
- Corrects for absorbing samples via the Forward Laser Monitor
- System-ready monitor simplifies data collection
- Field serviceable
- 21 CFR Part 11 compliant software

Start with standard HPLC, then upgrade to a micro-flow cell for use with UHPLC.

The miniDAWN TREOS II gives you access to both worlds.

Optional modules

- UHPLC-compatible module with micro-flow cell
- WyattQELS dynamic light scattering detector
- Integrated COMET ultrasonic device automates *in situ* cell cleaning

Molar mass in a single click? Absolutely!

Load Sample → Hit ‘Run’ → Get Results

Quick Setup:

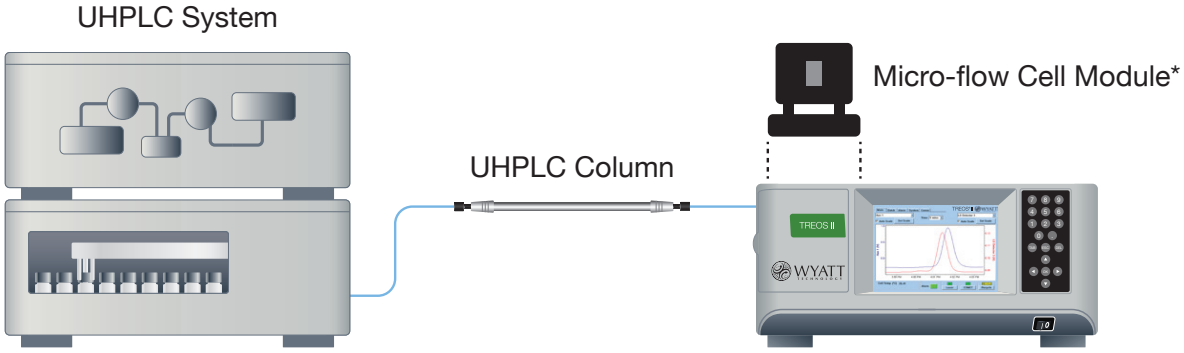
The TREOS II is controlled by ASTRA®, the leading software for SEC-MALS. ASTRA’s Method Wizard lets you set up a default method optimized for your sample type in three easy steps:

1. Select experiment type
2. Input parameters
3. Hit Run

ASTRA will:

- Synchronize data collection with your HPLC
- Autoset parameters to determine MW and R_g
- Generate custom reports and graphs
- Prepare for the next run

Upgrading to UHPLC? No problem!



TREOS II

Micro-flow Cell Module:

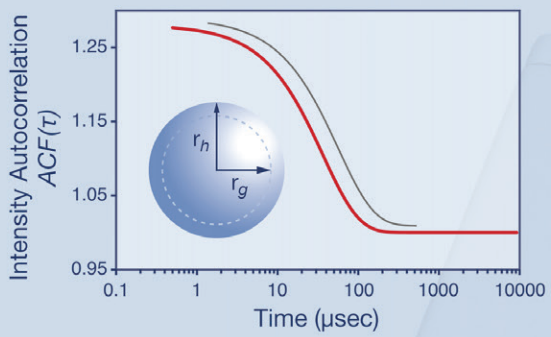
Whenever you are ready for UHPLC, simply upgrade the TREOS II with a drop-in micro-flow cell module—and you’ll have a µDAWN®.

**Sold separately*

UHPLC Provides:

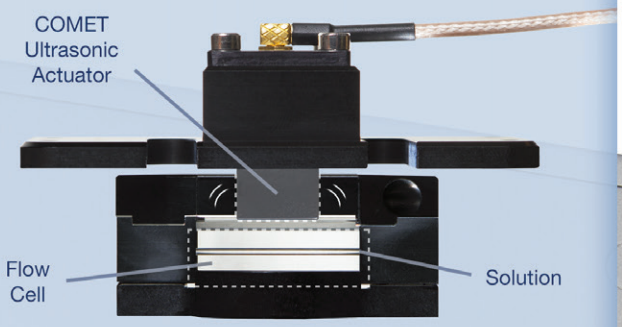
- Higher throughput
- Higher resolution
- Better sensitivity
- Lower sample consumption

WyattQELS module

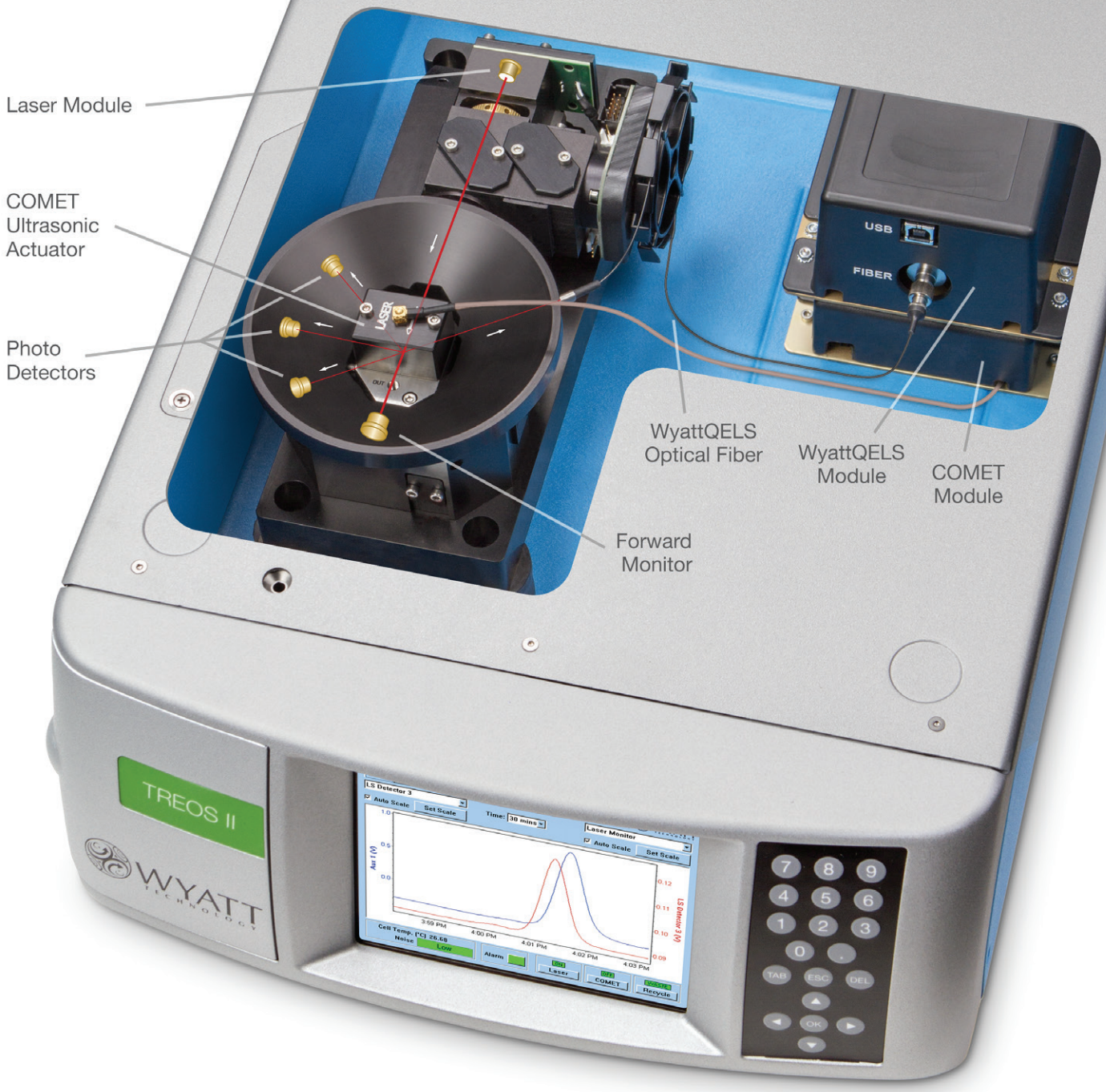


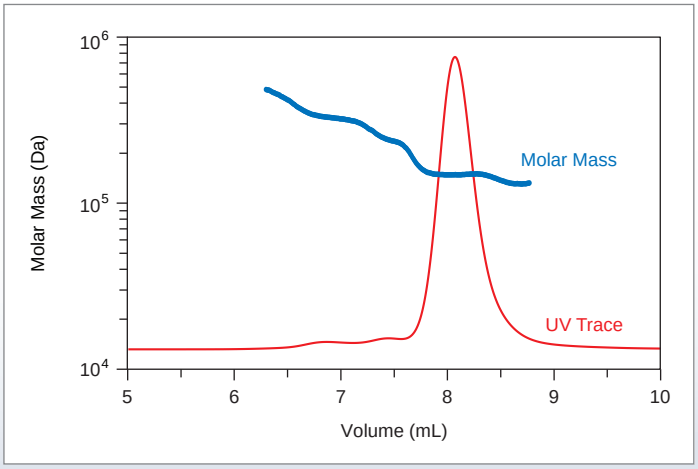
Measure the hydrodynamic radius of your sample with the WyattQELS dynamic light scattering module

COMET module



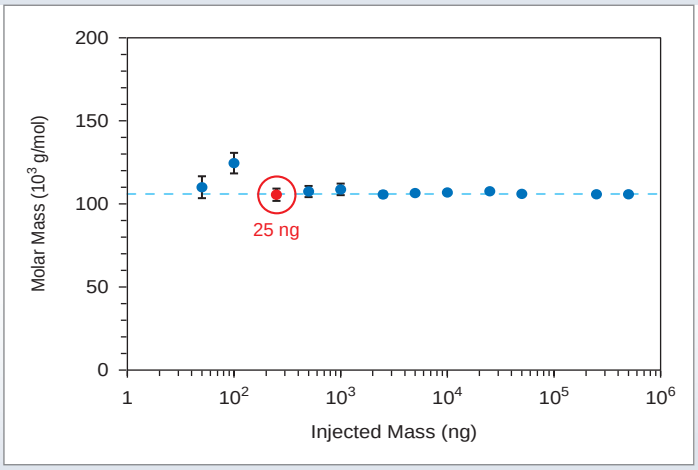
The proprietary COMET module uses ultrasonic vibrations to clean the flow cell of particles





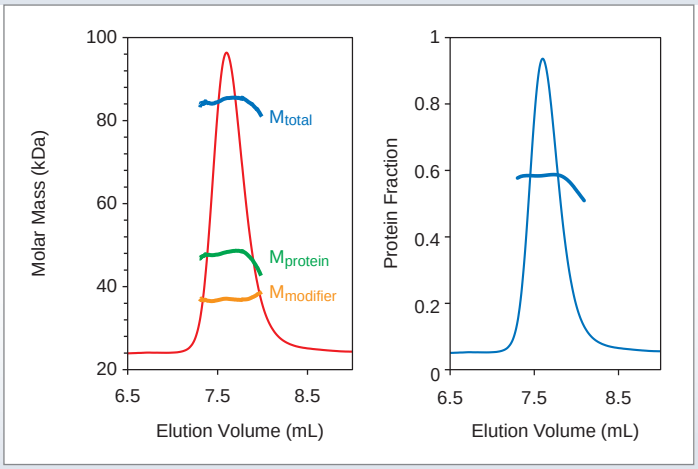
Absolute Molar Mass Analysis

The TREOS II is a perfect MALS detector for size-exclusion chromatography (SEC) in order to determine absolute molar masses and sizes of proteins (IgG and oligomers shown here as an example) or polymers eluting from the SEC column. Its superior solvent compatibility and minimal maintenance requirement make it a robust tool for SEC-MALS. Furthermore, it can be augmented with an online DLS option and/or the ViscoStar® III viscometer.



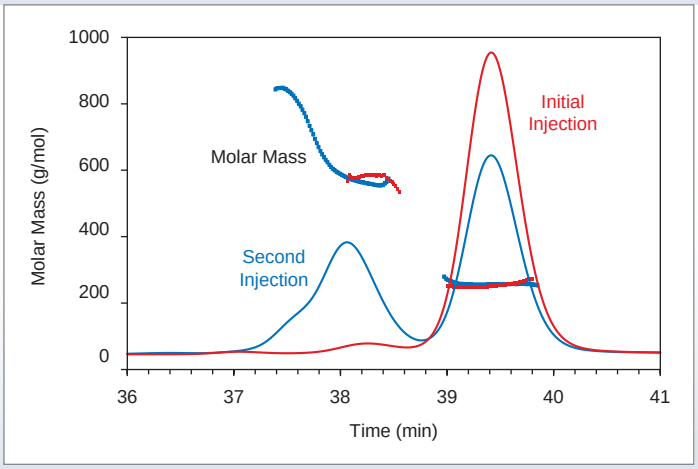
Sensitivity, Precision and Accuracy

The TREOS II provides sensitive, precise, and accurate measurements of the molar masses (MM) of polymers as demonstrated by the graph of MM vs. Injected Mass from a 1.05 x 10^5 g/mol polystyrene. The results were obtained from a 7.8 x 300 mm SEC column using THF as the mobile phase. Each data point is the average from three runs. The graph shows that accurate and precise MM is readily achieved with an injected mass of 25 ng and above.



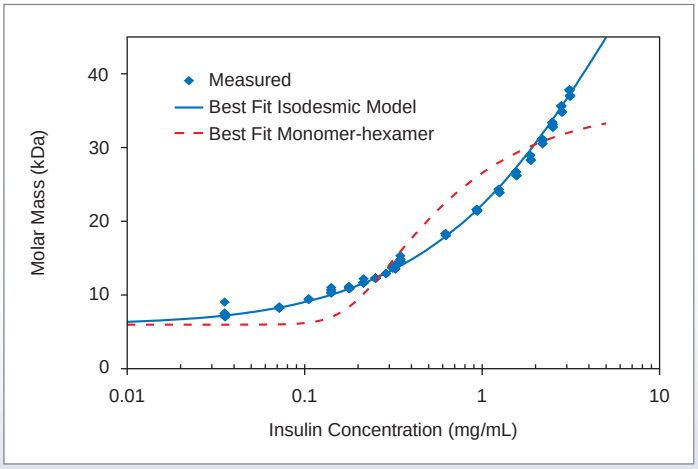
Protein Conjugates and Block Copolymers

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



Small Polymers and Peptides

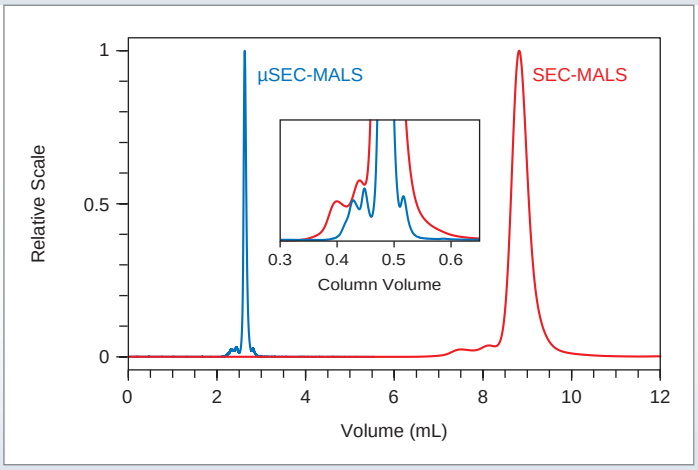
Methylene diphenyl 4,4'-diisocyanate (MDI) has a molar mass of 250 Da and will readily form oligomers in THF. The superior sensitivity of the TREOS II accurately characterizes low molar mass molecules like MDI and their behavior, without reference to standards or column calibration of any kind. Over time, the sample aggregated and shows more oligomers (blue curve) compared to the initial injection (red curve).



Reversible Self-Association

In conjunction with a Calypso composition gradient system, the TREOS II can analyze protein self-association. The Calypso® creates a series of dilutions, and its software analyzes the concentration dependence of the molar mass to determine K_d and stoichiometry.

Here we see the self-association of insulin vs. concentration. A monomer-hexamer association model clearly does not fit the data. However, an isodesmic self-association model proves an excellent fit, indicating K_d = 52 µM.



From HPLC to UHPLC

Unlike any other instrument on the market, the TREOS II can be initially configured for an HPLC setup and later upgraded to a UHPLC system. The results from these two configurations of the same instrument are presented here by the red trace (SEC-MALS) and blue trace (µSEC-MALS), illustrating the versatility of the TREOS II to be upgraded to a µDAWN detector in your lab.

How Multi-Angle Light Scattering Works

In the limit of very small concentrations, as is the usual case for size exclusion or gel permeation chromatography (SEC or GPC), the fundamental relationships linking the intensity of scattered light, the scattering angle, and the molecular properties are simply:

$$M_W = R(0)/K^*c$$

$$P(\theta) = R(\theta)/K^*M_W c$$

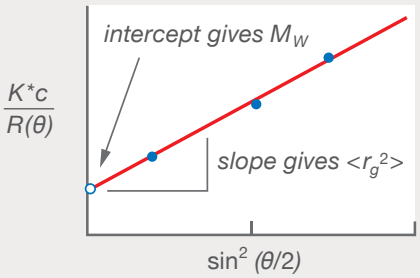
where:

- M_W is the weight-average molar mass (grams per mole),
- $R(\theta)$ is the measured excess Rayleigh ratio, proportional to the intensity of light scattered by the sample at each angle θ, and $R(0)$ is the value of $R(\theta)$ at $\theta=0$,
- c is the sample concentration (g/L),
- 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.

$R(0)$ cannot be measured directly since the non-scattered laser beam propagates in this direction and overwhelms scattered photons. Therefore $R(0)$ is determined *most accurately* by extrapolating the measured $R(\theta)$ values to $\theta=0$.

The miniDAWN TREOS II utilizes a fixed, multi-angle detector array, which measures data from all three scattering angles simultaneously. This makes multi-angle data collection and analysis a snap. The measurement is absolute and does not require any *a priori* knowledge of molecular conformation or branching in order to calculate molar mass.

Molecular size may be determined from a traditional plot of $K^*c/R(\theta)$ vs. $\sin^2(\theta/2)$, which yields a curve whose initial slope is proportional to the molecule's mean square radius $\langle r_g^2 \rangle$.



At concentrations above those typical of SEC/GPC, intermolecular interactions modify the light scattering equation:

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

A_2 is the second virial coefficient, a measure of intermolecular interactions. A_2, which may be determined from the concentration dependence of $R(\theta)$, is used to study intermolecular interactions and to obtain improved accuracy of M_W analyses.

Specifications

Measurements	
Molar Mass Range	200 Da to 10 MDa*
Molecular Size Range (r_g)	≈ 10 nm to 50 nm, up to 150 nm with shape-specific models
Molecular Size Range (r_h)	In flow mode, 0.5 nm – 40 nm†; batch mode 0.5 nm - 1 µm (requires WyattQELS DLS module or DynaPro® NanoStar® + fiber optic connection)
Sensitivity	25 ng of 100 kD polystyrene in THF*
Fluidics	
Mobile Phase Compatibility	All-solvent compatible (aqueous and organic); Wetted materials are 316 stainless steel, fused silica and Kalrez
Optics	
Detectors	
MALS Detectors	High-gain, high dynamic range photodiodes at 3 detection angles
Auxiliary Detectors	Laser monitor for stabilization feedback; forward transmission monitor to correct signals for absorbing samples and to assess data quality
Dynamic Range	> 16,000,000:1
DLS Detector (optional)	WyattQELS dynamic light scattering module installs directly inside the TREOS II chassis. Alternatively, the optical fiber pickup of the DynaPro NanoStar cuvette-based DLS instrument may be installed in the TREOS II.
Laser Properties	
Laser Wavelength	658 nm ± 4 nm
Laser Power Control	Programmable 10% - 100%
Flow Cells	Fused Silica, optimal for solvent refractive index less than 1.50
Temperature Options	Ambient only
Electronics	
Analog Inputs	2 differential analog inputs with 24 bit resolution; Input range -10 V to +10 V
Analog Outputs	1 analog output 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 Hz to 0.0001 Hz
Front Panel Display	162.5 mm, 16-bit, high resolution touch screen displays signal graphs, instrument settings and diagnostics
Dimensions	
Size	60 cm (L) x 36 cm (W) x 17 cm (H)
Weight	14.25 kg

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

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

† Assuming a flow rate of 0.3 mL/min.

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

With installations in *more* than 65 countries, *more* than 12,000 refereed journal publications citing its instruments, and over 25 PhD scientists, Wyatt Technology is the **world's leading manufacturer of instruments and software** for absolute macromolecular and nanoparticle characterization. Our dedication to providing customers with comprehensive training and personal support make us the gold standard in this field.

For more information

www.wyatt.com/Theory
Theory overview of static and dynamic light scattering

www.wyatt.com/SEC-MALS
Introduction to SEC-MALS, applications and instruments

www.wyatt.com/TREOS
Additional TREOS II features

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