

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





DAWN[®] The gold standard for absolute molar mass and size

The DAWN 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). It can measure molar masses from hundreds of Daltons to hundreds of millions of Daltons and radii from 10 nm to 500 nm. This capability covers a very broad range of applications from peptides, proteins, ultra-low molecular weight polymers, synthetic and natural polymers, to vesicles, emulsions and nanoparticles. Moreover, it makes absolute measurements, which do not depend on column calibration standards or reference molecules.

The DAWN may be used in several modes

- In continuous-flow detection following chromatographic separation, to characterize distributions of molecular weight and size
- Coupled to an Eclipse[™] Field Flow Fractionation system 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
- Coupled to a Calypso[®] automated compositiongradient system, to assess macromolecular interactions and reaction kinetics as well as average solution properties

Regardless of which mode you choose, the DAWN provides the highest sensitivity, reliability and measurement range of any MALS detector.

Superior technology & 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 from ~15° to 160°.

An exceptionally precise glass-to-metal design sets the stage for measurements of the greatest sensitivity and accuracy, and flow cells that may be replaced on-site without further alignment. The unique flow cell design of the DAWN provides welcome relief from the contamination problems that plague most other light scattering instruments. The DAWN 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 eluent. The DAWN includes an embedded COMET[™] (Cell Operation and Maintenance Enhancing Technology) flow cell cleaning device that virtually eliminates manual cleaning. 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.

Endorsed by the scientific community

No instrument can compare to the DAWN for the characterization of proteins and macromolecules in solution. Our bibliography of thousands of peer-reviewed papers using DAWN and miniDAWN® MALS instruments proves the power of the DAWN to provide the molecular weight and size data that scientists around the world depend on for their daily research. Visit www.wyatt.com/Bibliography to see for yourself.



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

DAWN Advantages

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



New User Interface

The larger responsive display is the starting point of our Smart Services[™] platform and designed to give the user a simple to use, all-in-one system view



Smart Services Platform

A series of user experience enhancements delivering greater ease-of-use and improved workflow

System Ready Monitor

Never waste a run due to incomplete equilibration, excessive mobile-phase noise or sub-optimal detector state. The System Ready Monitor simply does that for you and continuously reports if all systems are optimal right from the front panel. If problems do arise, the System Ready Monitor alerts you by changing from green to yellow to red depending on the severity and provides actionable, real-time guidance on what needs to be done to bring the system back to peak health.

Real-Time Health Indicators

System Ready Monitor indicating that not all is well? For more detailed information on what's holding you back, review the Real-Time Health indicators. Perhaps the Forward Monitor indicator is triggered by a bubble or the Drift indicator by insufficient column equilibration.

Specific indicators can be customized for more or less stringent requirements. For example, the Noise indicator can be set to have a wider acceptable range for aqueous buffers, which typically exhibit more noise than organic solvents.

Collection Mode

Never again worry about a colleague disrupting your experiments. Our dynamic Collection Mode indicator provides a spinning, system status wheel right from the front panel that can't be missed. Want to know whose is operating the instrument? Simply click on the collection icon to determine who is logged in and from what computer they are connected.







WyattQELS - Inline DLS

A unique benefit of Wyatt MALS instruments is their ability to accept an optional embedded dynamic light scattering (DLS) module for inline measurements of hydrodynamic radius. The WyattQELS connects via optical fiber to the DAWN flow cell and collects light scattered from particles in the beam, simultaneously with MALS acquisition.

In order to optimize range and sensitivity for different particle size combinations, the DAWN offers four angular DLS positions—50.5°, 90°, 135° and 150°. These are independent of MALS photodetector positions, and ASTRA will automatically detect the position.

Replaceable Flow Cell

The heart of the DAWN is its flow cell—a highly polished, precision-manufactured glass and metal assembly designed to eliminate stray light for maximum light-scattering sensitivity. Our exclusive design allows the flow cell to be changed without the need for additional laser or optical alignment. This enables a Wyatt field service engineer to perform a flow cell change in the field, minimizing down time and maximum productivity.

CheckPlus™

If concerns about the status of your instrument arise, an expert opinion is just a click away. With CheckPlus, our integrated service application, a complete instrument and system history report can be autogenerated at any time and sent to a Wyatt Service Engineer or Application Scientist for further analysis. This is just another example of how our Smart Services Platform is designed to deliver important customerfacing benefits and simplified use.







MALS 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.



At the low concentrations typical of analytical SEC, the amount of light scattered by a suspension

of molecules is directly proportional to the product of their weight-average molar mass and their concentration. The angular variation of the scattering reveals the molecule's 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 makes no prior assumptions about molecular conformation (in fact, 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 obtained experimentally, from first principles.

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 (RALLS/LALLS), the light from a single stray dust particle can corrupt the data. Low-angle laser light scattering (LALLS) is particularly problematic because dust particles scatter copiously into the low angles.

How Multi-Angle Light Scattering works

The DAWN utilizes a fixed, multi-angle detector array, which measures data from all angles simultaneously.

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:

$$\frac{K^*c}{R(\theta)} = \frac{1}{M_{\rm w}P(\theta)}$$

where:

- *R*(θ) 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 (g/mol)
- 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



With some extra math, MALS can analyze macromolecules beyond the low-concentration limit.

Insulin oligomerization



Depending on the formulation, insulin may be primarily monomeric or hexameric. SEC-MALS shows that the primary peaks are quite monodisperse despite their non-Gaussian shape. In this example of a primarily hexameric formulation (blue), MALS highlights the presence of monomer-dimer equilibrium, demonstrated by the varying molar mass across the second peak which changes behavior with concentration (not shown). The monomeric formulation (red) exhibits a series of low-abundance aggregates ranging from dimer to hexamer.

Inherently disordered proteins



Molar mass, as determined by MALS, vs. elution volume of kinase fragment (red), bovine serum albumin (BSA, blue) and alcohol dehydrogenase (ADH, green). The fragment, which is quite monodisperse, elutes early relative to its molar mass due to a partially-disordered conformation. Molar masses deduced from the elution volumes of kinase fragment and ADH are shown to be misleading when compared with absolute molar masses from SEC-MALS.

Protein complexes and conformations



Pure interleukin 4 trap (IL4-trap) elutes earlier than the IL4 : IL4-trap complex, despite its lower molecular weight. MALS MW analysis (small red symbols) indicates the expected MW values. Online DLS $R_{\rm h}$ data (open blue symbols) show the reason for the late elution: IL4 stabilizes the trap to form a compact IL4 : IL4-trap complex.

Exosome sizing



Separated by Eclipse field-flow fractionation, serum components including proteins, lipoproteins and exosomes span a size range from a few nanometers to over one hundred nanometers in radius. A WyattQELS dynamic light scattering module embedded in the DAWN measures the smaller sizes while the DAWN's MALS functionality measures the larger sizes with higher sensitivity.



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 DAWN is essential in characterizing molecules like MDI that have such low molar masses.

Protein conjugate and copolymer analysis



ASTRA's Protein Conjugate algorithm makes use of data from MALS, UV and RI detectors to characterize conjugated proteins and copolymers. This analysis determines the molecular weights of the protein, modifier and complete conjugate as well as average extinction coefficient and *dn/dc*.

Biopolymer degradation: Distributions



Sodium alginate, a common food additive, is degraded under the irradiation that was applied to sterilize the food product. Here the dramatic decrease of molecular weight across the entire size range is shown.

Biopolymer degradation: Conformation



Conformation plots of sodium alginate before and after irradiation indicate that, in addition to reducing the molecular weight, the radiation also causes the molecules to change from a globular to extended conformation. The combined phenomena point to the breaking of internal cross-links.

Specifications

Measurements	
Molar Mass Range	200 Da to 1 GDa*
Molecular Size Range (R_{q})	pprox 10 to 500 nm, up to 1000 nm with shape-specific models
Molecular Size Range (R_h)	In flow mode, 0.5 nm to 300 nm [†] ; batch mode 0.5 nm to 1 μm (requires WyattQELS DLS module or DynaPro [®] NanoStar [®] + fiber optic connection).
Sensitivity	10 ng of 100 kDa polystyrene in THF or 200 ng of BSA in PBS, assuming standard 7.8 mm x 300 mm SEC column
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.
Dynamic Range	3,300,000:1
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 ± 4 nm: 785 nm optional for use with fluorescing samples
Laser Power Control	Programmable 10% to 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, Recycle In/Out (operates Orbit™ recycle valve)
Computer Interface	Ethernet
Front Panel Display	162.5 mm, 16-bit, high resolution touch screen displays signal graphs, instrument settings and diagnostics
Dimensions	58 cm (L) x 36 cm (W) x 26 cm (H)

Left to Right Geofrey K. Wyatt, Chief Executive Officer Dr. Philip J. Wyatt, Chairman of the Board Clifford D. Wyatt, President

For more than 35 years, we've operated as one of the very few remaining family-owned businesses in the analytical instrument industry. With installations in more than 65 countries, over 15,000 refereed journal publications citing our instruments and more than 25 PhD scientists on staff, we take great pride in the worldwide recognition that Wyatt Technology has received as a leading manufacturer of instruments and software for absolute macromolecular and nanoparticle characterization. Our dedication to providing customers with comprehensive training and personal support has made us the gold standard in this field.

The DAWN is one of many tools in Wyatt's Light Scattering Toolkit for Essential Macromolecular and Nanoparticle Characterization.

Learn more at www.wyatt.com

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

[†] Configuration and flow-rate dependent.

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.

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