

HPLC and UHPLC refractive index detectors with extended measurement range unlike all others





## Optilab<sup>®</sup> Extended-Range Refractometers

dRI Range and Versatility Without Compare

# Universal concentration determination: UHPLC and more

Wyatt Technology's Optilab line of differential refractive index (dRI) detectors provides concentration measurements of proteins, polymers, peptides, oligonucleotides and other macromolecules, regardless of their incorporation of chromophores or fluorophores. All you need is the *dn/dc* value of your molecule in the mobile phase (see sidebar on page 3) and you are ready to determine accurate, reliable concentrations for quantitation as well as light scattering analysis of molar mass and conjugation.

**Optilab** is the workhorse of HPLC-based SEC-MALS, FFF-MALS or SEC-RI. With sensitivity as good or better than the industry's best dRI instruments, an unmatched dynamic range of 12,000,000:1, the added benefits of the Optilab line (outlined below) and compatibility with the most common HPLC equipment, Optilab has been selected for the leading academic, industrial and government labs around the globe.

microOptilab<sup>™</sup>, designed for the narrow peaks of UHPLC-SEC, offers the same benefits as Optilab including best-in-class sensitivity and huge dynamic range. The microOptilab reduces interdetector broadening by 3x and increases the data acquisition rate by 2.5x, making it a perfect companion to the microDAWN<sup>™</sup> UHPLC MALS detector.

**Optilab HC** (high-concentration) is in a class by itself: It measures dRI over a range 7x greater than that of Optilab with only a 2x decrease in sensitivity! Optilab HC, capable of measuring up to 180 mg/mL of protein, is appropriate for semi-prep SEC and highconcentration CG-MALS.

### Optimized for MALS



MALS analysis of molar mass requires three variable inputs: Light scattering intensity (from the MALS detector), concentration and *dn/dc*. Because only dRI detectors can provide two

of the three inputs, they play a special role in MALS measurements. Most importantly, the dRI illumination wavelength must be matched to the MALS detector for optimal results. Broad-band lamps can never provide the same degree of accuracy in MALS analysis.

Optilabs employ a high-power LED operating at the same wavelength as the Wyatt MALS detectors, guaranteeing the perfect match for MALS analysis. Moreover, the large range of an Optilab (even the standard model goes up to 25 mg/mL protein!) means that it can readily measure *dn/dc* when literature values do not suffice.

### Unparalleled parallel detection

Optilab's unique combination of sensitivity and dynamic range are a result of proprietary technology that utilizes an array of 512 parallel detectors, described on page 3.

Optilab's specialized array detection technology imparts another unique capability: Measurement of absolute refractive index (aRI). Since aRI is also necessary for MALS measurements, this ability is quite useful when non-standard solvents serve as the mobile phase, and can even determine the ratio of two solvents in a gradient elution.

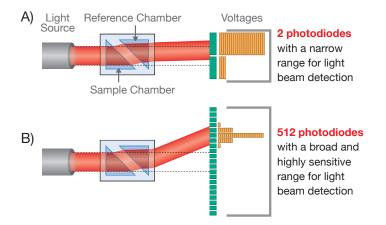
#### Digital diva

Optilab digitizes its signals for transmission to Wyatt's SEC-MALS software package, ASTRA®. Optilab is the only dRI detector that can interact digitally with ASTRA in order to maintain the highest signal quality.

#### Additional unique benefits offered by Optilab:

- Temperature control from 4 °C to 65 °C
- Hard-wired synchronization between online MALS, dRI and the ViscoStar<sup>®</sup> differential viscometer for perfect peak timing

#### Optilab's advanced technology



- A) The principle behind all dRI detection is based on the deflection of light that passes through prismatic sample and reference cells. Just about every dRI detector uses a simple bi-cell, comprising two detectors, to measure that deflection. This arrangement imposes a tradeoff on sensitivity vs. range.
- B) Optilab's 512-detector array means it can achieve maximum sensitivity over a much wider concentration range. In fact, Optilab can reliably quantify a tiny peak at the nanogram level superimposed on a milligram-level peak!

#### Beyond concentration



The refractive index increment, *dn/dc*, is a key parameter specific to an analyte and its solvent. For many macromolecules and solvents, *dn/dc* 

may be found in tables in the literature.

Conveniently, the *dn/dc* of unmodified proteins in aqueous buffer is nearly universal. This means that the concentration of unknown proteins may be measured, facilitating their identification and discriminating between impurities and aggregates of the primary protein.

When a macromolecular conjugate consists of two distinct species, with sufficiently differentiated *dn/dc* and UV extinction coefficients, it is possible to combine UV, dRI and MALS measurements in order to determine the molar mass of each constituent. This capability, supported by Wyatt's ASTRA chromatography software, is widely used to analyze co-polymers, PEGylated proteins, glycoproteins, protein-nucleic acid complexes and surfactant-solubilized membrane proteins.

What if you don't have a literature value for *dn/dc*? Just measure it, using Optilab, carefully-prepared solutions of known concentration and ASTRA! Injections may be made with a syringe pump or an HPLC pump and a Wyatt high-pressure Injection System (WISH). For fully automated *dn/dc* measurements, consider a Calypso<sup>®</sup>.

### New User Interface

The larger responsive display is the starting point of our Smart Services<sup>™</sup> 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.

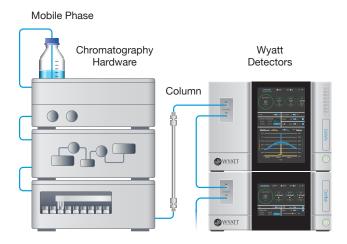
#### **Real-Time Health Indicators**

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.

#### CheckPlus™

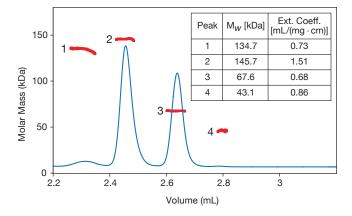
If concerns about the status of your instrument arise, an expert opinion is just a click away. With CheckPlus, our integrated service application, a complete instrument and system history report can be autogenerated at any time and sent to a Wyatt Service Engineer or Application Scientist for further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







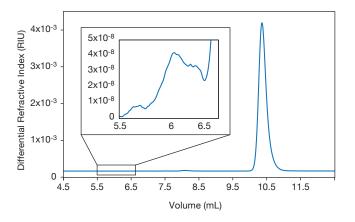
#### Absolute molar mass with MALS



Absolute molar mass is determined from light scattering intensity and concentration, without reference to column calibration or assumptions regarding conformation and non-ideal column interactions. Optilab is perfectly matched to Wyatt Technology's MALS detectors, making dRI measurements at the same wavelength as the MALS laser.

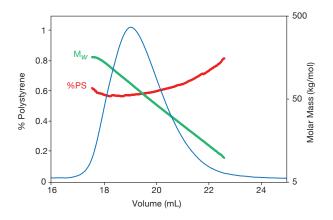
In this example, the molecular weight and extinction coefficient of unidentified proteins may be determined unequivocally using Optilab for concentration measurement, since nearly all proteins have the same *dn/dc* value.

#### Unmatched range and sensitivity



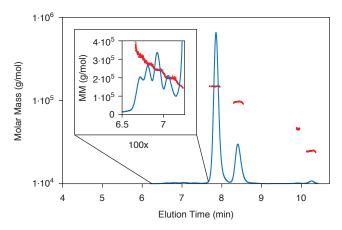
While Optilab and microOptilab offer the same sensitivity as the top industry dRI detectors for HPLC and UHPLC, no other can match its dynamic range. This chromatogram of galactose shows just a portion of the full range. The impurity shown in the inset, validated across five replicates with perfect repeatability, has been magnified by >100,000x relative to the primary sugar peak.

## Analyze copolymer's molar mass and composition



The triple-detector combination of MALS, dRI and UV is essential in characterizing conjugated complexes such as glycoproteins or copolymers for which no reliable column calibration standards exist. Here poly(styrene-co-acrylic acid) is analyzed to determine both total molar mass and percent polystyrene across the entire peak.

#### Aggregates and fragments



The power of UHPLC for separating aggregates and fragments combines with MALS to unequivocally identify small quantities of impurities in an IgG sample. Each of the aggregate peaks shown in the 100x inset represent a fraction of one percent of the monomer total mass yet is well-quantified by MALS.

# Specifications

-	Optilab	Optilab HC	microOptilab	
Differential Refractive Inde	x			
Range (RIU)	-0.0047 to +0.0047	-0.0026 to +0.034	-0.0047 to +0.0047	
Peak-to-peak Noise (RIU)*	± 7.5 x 10 <sup>-10</sup>	± 1.5 x 10 <sup>-9</sup>	± 1.5 x 10 <sup>-9</sup>	
Response Time				
(User Selectable)	0.1 to 5 seconds	0.1 to 5 seconds	0.04 to 5 seconds	
Absolute Refractive Index				
Range (RIU)	1.2 to 1.8	1.2 to 1.8	1.2 to 1.8	
Sensitivity (RIU)	± 0.002	± 0.002	± 0.002	
Fluidics				
Band Broadening **	<12 µL	<12 µL	< 4 µL	
Fill Volume	51.4 µL	51.4 µL	13.7 µL	
(inlet tubing + flow cell)				
Outlet Tubing	370 µL	370 μL	228 µL	
Pressure				
Back pressure created at	<22 psi (1.5 bar)	<22 psi (1.5 bar)	<320 psi (22 bar)	
inlet port (@ 0.5 mL/min				
H <sub>2</sub> O, 20 °C)				
Maximum back pressure	30 psi (2 bar)	30 psi (2 bar)	30 psi (2 bar)	
allowed at outlet port				
Sample Temperature				
Control Range	4 °C to 65 °C	4 °C to 65 °C	4 °C to 65 °C	
Regulation	± 0.005 °C	± 0.005 °C	± 0.005 °C	
Light Source	658 nm $\pm$ 20 nm standard; other wavelengths available			
Safety Sensors	Vapor and liquid (leak)			
Electronics				
Digital Output	22+ bits @ 10 Hz (Optilab) or 25 Hz (microOptilab)			
Analog Output	$\pm$ 10 V or $\pm$ 1 V output, 18 bits resolution			
Computer Interface	Ethernet			
Front Panel Display	162.5 mm, 16-bit, hig	162.5 mm, 16-bit, high resolution touch screen displays		
	signal graphs, instrument settings and diagnostics			
Auxiliary I/O				
Inputs	Analog In (16 bits, $\pm$ 10 V A/D), Zero dRI, Purge,			
	Recycle In, Autoinject In, Alarm In			
Outputs	Autoinject Retransmit, Alarm Out, Recycle Out (to switch			
	Orbit™ between waste and recycle)			
Dimensions	58 cm (L) x 36 cm (V	58 cm (L) x 36 cm (W) x 18 cm (H)		

\*Short term peak-to-peak noise is measured per ASTM-E1303-95(2000) using a 2 second collection interval (4 second time constant) for Optilab or a 0.5 sec collection interval (1-sec time constant) for the microOptilab, and temperature set to 25 °C (both).

\*\*Defined as broadening of the peak, measured over full width at half max, flow rate = 0.5 mL/min for Optilab and Optilab HC and 0.3 mL/min for microOptilab.

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

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

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#### 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.

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

#### Learn more at www.wyatt.com

