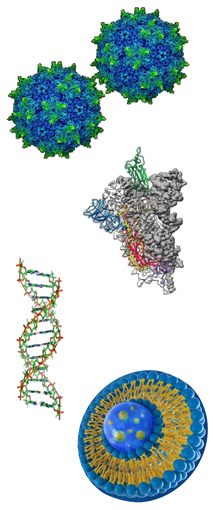
Advances in Downstream PAT for Biologics, Vaccines and Gene Vectors

Daniel Some – Waters | Wyatt Technology
Contact: dsome@wyatt.com

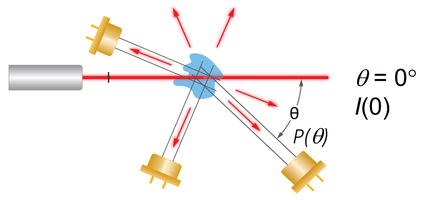
Abstract

Multi-angle light scattering (MALS) is a versatile technology most commonly found in analytical laboratories for characterizing biophysical properties such as protein or polynucleotide molar mass, size and conformation; size and concentration of virions; and the genomic payload of viral and non-viral gene vectors.

Real-time multi-angle light scattering (RT-MALS) is a novel use of MALS which brings many of those capabilities to preparative systems and process development laboratories. RT-MALS determines key product attributes of biologics, in-line or on-line with downstream and fill-finish processes, and can be used to monitor aggregation, distinguish product from impurities, identify process endpoints and more.

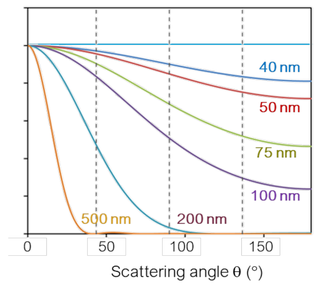


How MALS works: scattered intensity vs. angle



1. **MW**: the *average intensity* of scattered light, extrapolated to 0°, with concentration (UV) yields the **weight-average molar mass**:

$$I_{\mathrm{LS}}(0) \propto M \times c$$



2. **Size:** The *angular variation* of scattered light yields the **z-average rms radius** R_g (or other dimension).

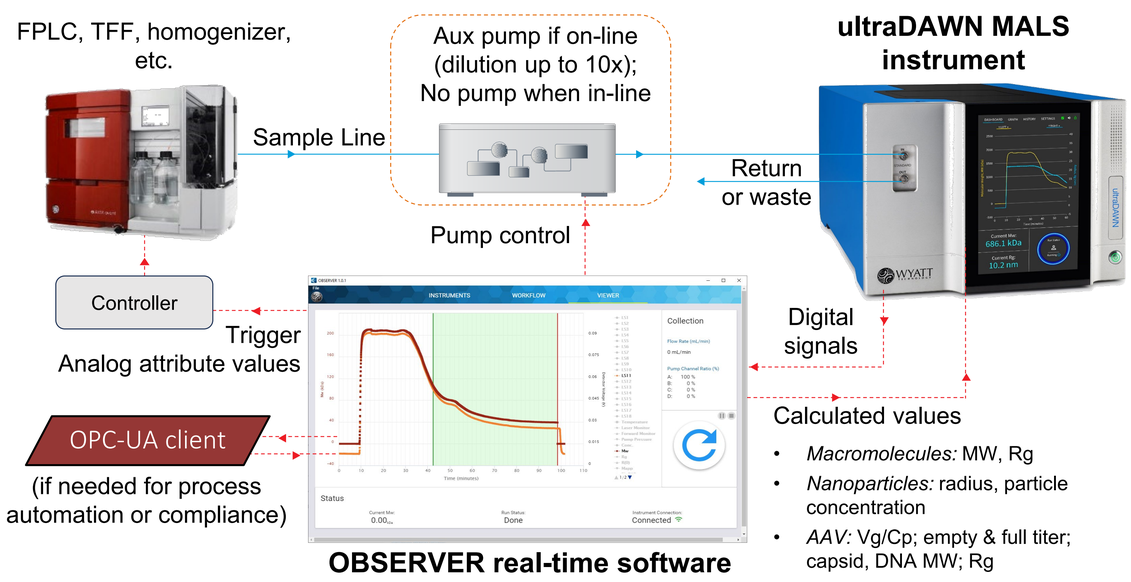
3. **Particle concentration**: the intensity extrapolated to 0° and particle volume (from the size), yield the **particle concentration *N***:

$$I_{particle}(0°) \propto N \times V^2_{\mathrm{particle}}$$

4. **Payload & titer:** The intensity and 2 UV signals (260 nm & 280 nm) yield capsid and DNA MW, Vg/Cp, empty and full titer.

$$\frac{Vg}{Cp} = \frac{M_{DNA\ (MALS)}}{M_{DNA\ (full\ genome)}}$$

RT-MALS: ultraDAWN™ *instrument* and OBSERVER™ *software*



- *Macromolecules:* MW, Rg
- *Nanoparticles:* radius, particle concentration
- *AAV:* Vg/Cp; empty & full titer; capsid, DNA MW; Rg

Case study: Flow-through protein purification

Flow-through HIC remove mAb aggregates

During the run the column saturates and eventually aggregates break through

UV does not differentiate monomers, aggregates

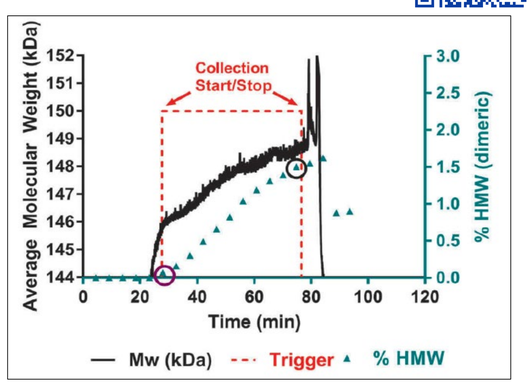
Goals:

- Identify optimal time to cut pool for maximum yield & specified quality
- Calculate %HMW in final pool

RT-MALS solution:

- %HMW (assuming monomer-dimer mixture) is calculated from weight-average molar mass.
- Trigger goes ON when monomers are found (MW > 146 kDa) and OFF when reaching 1.5% dimer.
- RT-MALS optimizes pool regardless of variations in input load and content, buffer variations or column aging, and calculates %HMW in the pool.

Read the paper:

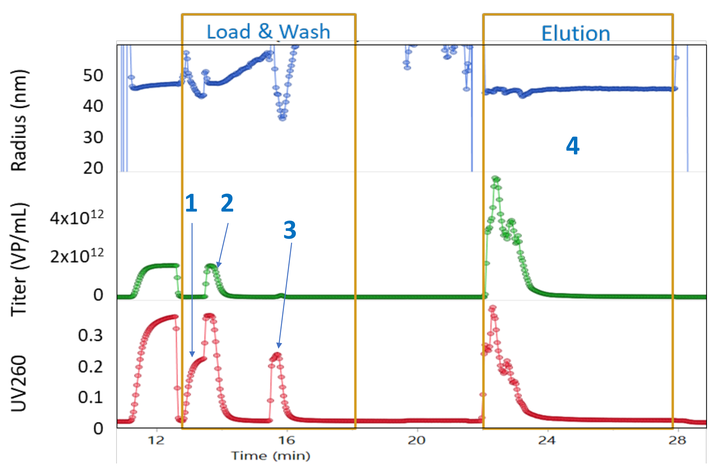


Patel et al., *mAbs* **10**(7): 945-950 (2018)

$$\%HMW = \frac{c_d}{c_{tot}} = \frac{M_{w,m-d}}{M_m} - 1$$

c_d: dimer concentration
c_{tot}: total protein concentration
M_m: monomer molar mass
$M_{w,m\text{-}d}$: total solution weight-average molar mass

Case study: Adenovirus purification



1. **Load:** High UV + no MALS indicates impurities washing though
2. **Load:** High UV + High MALS indicates viruses breaking through
3. **Wash:** High UV + no MALS indicates impurities washing off

Data courtesy of Janssen Vaccines & Prevention

4. **Elution:**

- **UV:** indicates "something" is eluting, does not differentiate virus from impurities
- **MALS:** confirms monomeric AdV by constant radius
- **MALS:** Integrates titer peak to determine pooled titer
- **Validation:** Good agreement of size and titer with offline reference data. RT-MALS saves ~ 2 weeks turn-around time for offline analytics

	Size (nm)	Titer (1/mL)
RT-MALS	R_{geom}: 45.7	7.31 x 10^{11}
Reference off-line method	R_h: 48.6	6.07 x 10^{11}

Case study: Viral vector ultrafiltration

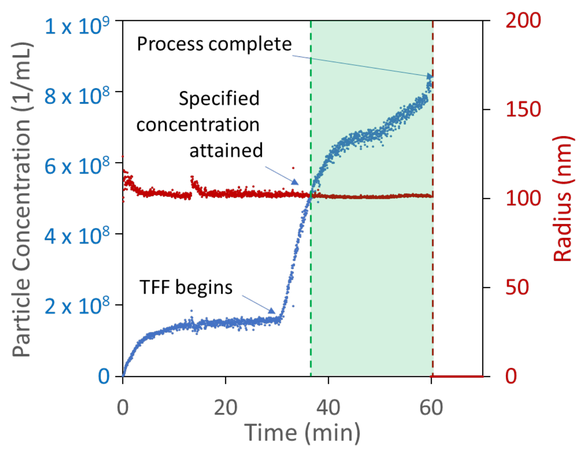
Ultrafiltration by TFF to increase concentration of drug substance

Goals:

- Signal final concentration end point
- Flag possible degradation
- Identify membrane fouling

RT-MALS solution:

- OBSERVER monitors trigger condition: titer > 5 x 10^8 mL^{-1}
- Response time ~ 12 seconds, does not delay processing
- Monitor average virion size to ensure no degradation
- RT-MALS can also monitor the permeate to identify membrane fouling



Case study: AAV enrichment

Ion-exchange chromatography removes empty capsids and other impurities

Read the application note:

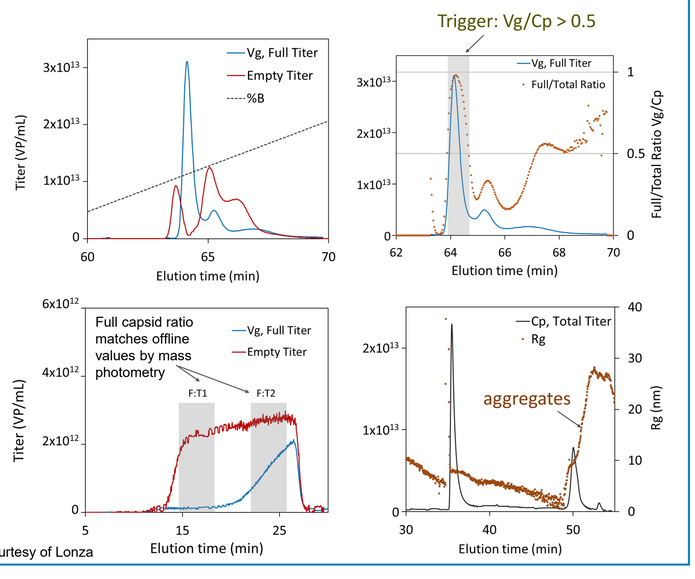
UV signal does not provide quantitative Vg/Cp or titer, or identify aggregates.

Goals:

- Optimize column loading and collect enriched vectors by Vg/Cp in real time;
- Identify aggregates, calculate titer

RT-MALS solution:

- *Linear gradient* (top): OBSERVER trigger set to collect Vg/Cp > 0.5
- OBSERVER calculates final full and empty titers, Vg/Cp in pool
- *Step gradient* (bottom): In Column Load, confirm discard of empty AAVs
- Strip peak identified as aggregates by size R_g



Data courtesy of Lonza

Conclusions

For more info:

- RT-MALS - with ultraDAWN and OBSERVER - directly monitors relevant CQAs and other product attributes in downstream processing of biologics like mAbs and viral vectors
- In process development, RT-MALS enhances process understanding and accelerates time to market by cutting down on offline analytics
- In production, RT-MALS increases productivity, yield and quality while adding flexibility for transferring across scales and sites.