WYATT TECHNOLOGY
6300 Hollister Avenue
Santa Barbara, CA 93117
Tel: +1 (805) 681-9009
Fax: +1 (805) 681-0123
Web: www.wyatt.com

Live Webinar Q&A Sheet:

Vaccines, Well Characterized? Light Scattering Solutions for Biophysical Characterization in Vaccine R&D

The recorded webinar may be viewed from the Biotherapeutics webinars page. These questions were submitted by live viewers. Additional information on MALS, DLS, and FFF may be found on the Wyatt web Library under webinars, application notes, featured publications and bibliography, as well as on the corresponding Product page and Theory page of our web site.

Please contact info@wyatt.com with any additional questions.

Applications

Q: *Can you perform protein conjugate analysis for a peptide-polysaccharide conjugate?*

A: Yes, as long as the weight fraction of the total peptide content is more than 3-5% of the conjugate.

Q: *Can you do three component conjugate, like lipid-carbohydrate-protein?*

A: Generally, protein conjugate analysis can only measure the composition of two-component conjugates. However, if two of the three components have similar *dn*/*dc* values and UV extinction coefficients, like in the case of lipid-carbohydrate-protein conjugates, then we could combine lipid and carbohydrate as one component. By applying protein conjugate analysis, we can then determine the molar masses from the protein as well as from the combined lipid and carbohydrate.

Q: *Are there any excipient limitations for DLS?*

A: Only when the product of size and concentration of the excipient are comparable to that of the sample of interest, then excipient will compromise the

DLS measurement. One such example is the presence of micelles in membrane protein solution.

Q: *What temperature range can be screened with the DLS Plate Reader?*
A: From 4 to 85 °C. If the aggregation temperature is higher than 85°C, the aggregation rate (i.e., the onset time of radius vs. time plot) at an elevated temperature may be used to indicate the formulation stability instead of the use of aggregation temperature.

Q: *What are the possibilities of using light scattering technology for analyzing toxoid and anti-toxin flocculation (an alternative method to Ramon flocculation method)? Is this possible to use CG-MALS tech for flocculation analysis purpose?*

A: MALS measures molar mass of macromolecules in solution or size of uniform nanoparticle suspension. The precipitation from the flocculation method will clog the tubing used in CG-MALS, making it unsuitable.

Q: *For future applications, can you count the number of Ebola virus particles in a blood sample?*

A: Light scattering is not specific enough to discriminate Ebola virus particles from other particles of similar size (including other types of viruses) that might be present in the blood. Additionally, Ebola viral loads of interest tend to be quite small – tens or hundreds per mL – and MALS would not be able to count this small number.

Q: *Can you elaborate on how MALS gives the number of particles present? What MALS data values are used?*

A: The MALS angular variation yields the particle's R_g. Assuming the refractive indices of the particle and solvent are known, combining these with the size allows calculation of scattered intensity per particle (into each angle). Then we divide the measured total scattered intensity by the scattering intensity calculated for a single particle to estimate the number of particles.

Q: *Can CG-MALS be used to estimate the homogeneity of Antigens? Like not all the Ag sample is folded or functional?*

A: CG-MALS does not separate species, so we cannot quantify the homogeneity in terms of amount of aggregate or fragment, like we can with SEC-MALS. However, by fitting the CG-MALS data, we can solve for an "incompetent

fraction" which is not available for binding, such as an unfolded or nonfunctional portion of the antigen.

Q: *How do you remove light scattering contribution from the UV signal so we can do conjugate analysis with the three detector system?*

A: UV extinction is a combination of absorbance and loss due to scattering. Since scattering increases with the sixth power of the radius while absorbance varies with the third power (the molecular volume), the larger the molecule or particle, the more light it scatters relative to the amount it absorbs. If the scattering loss can be determined, then the extinction can be corrected to determine just the absorption component.

Scattering varies inversely as the fourth power of the wavelength for most macromolecules. A viable approach to correct for scattering at 280 nm is to simultaneously collect UV signal at a longer wavelength (say λ_L, typically around 350 nm) where the protein does not absorb, so the extinction signal at that wavelength is only produced by light scattered from the molecule. Then the scattering portion at 280 nm is estimated by multiplying the signal at wavelength λ_L times the factor of $(\lambda_L/280)^4$. This is subtracted from the total the UV extinction signal at 280 nm to determine just the absorption signal. Currently, this feature is not incorporated in ASTRA software for protein conjugate analysis, but will be implemented in the near future.

Q: *What is the minimum value of Rg where reporting this value by MALS is accurate?*

A: 10 nm.

Q: *How much sample do you need for performing MALS?*

A: While the recommended typical sample load for SEC-MALS of proteins or polysaccharides is ~ 0.1 mL at 1 mg/mL, excellent results may be obtained with much less. Please refer to the formulas on Slide 25 for minimum concentration at the flow cell and minimum SEC load.

Q: *I struggle with getting good enough resolution in SEC-MALS for my nanoparticles. Data you have presented looks better-- do you have suggestions for columns or buffer conditions?*

A: For nanoparticles, I would suggest FFF for fractionation. FFF offers a much wider range of separation, the ability to tune flow conditions to optimize separation at specific size ranges, compatibility with a wider range of aqueous solvents, and far less surface area for nanoparticles to stick to.

Q: *In DLS, measuring vesicles, we get an intensity % value. Can we convert it to mass %?*

A: Yes, with known size (measured by DLS) and specific volume (assumed via a model relating conformation to molar mass).

Q: *Is it possible to use LS in the study of fungi?*

A: Not the intact fungi as LS measures sizes from 0.3 nm to several μm. However, the polysaccharides and polymers exacted from the fungi can be characterized by LS.

Q: *Vesicle quantification: is there an automated method to perform it (ASTRA VI...?)*

A: Similarly to the use of FFF-MALS for liposome quantification as presented in the webinar, vesicles may be sized and their number density estimated.