

Live Webinar Q&A Sheet:

Smaller particle and low adsorption columns for fast and efficient characterization of AAV through SEC-MALS Dr. Bala Addepalli

The recorded webinar may be viewed from the <u>Biotherapeutics</u> webinars page. These questions were submitted by live viewers. Additional information on SEC-MALS, DLS, FFF, CG-MALS and RT-MALS may be found on the Wyatt web <u>Library</u> under Webinars, Application Notes, Featured Publications and Bibliography, as well as on the corresponding <u>Product</u> page and <u>Solutions</u> page of our web site.

Please contact <u>info@wyatt.com</u> with any additional questions.

Questions & Answers

GENERAL MALS QUESTIONS

- *Q:* Other than using a MALS detector, is there a way to know if my sample is interacting with the column?
- A: Checking the recovery of analytes, chromatography peak shape (looking for any shoulder or asymmetry), carryover issues, are the good places to start.
- *Q:* Is there an easy rule to follow to avoiding shearing of particles like a relationship between the size, particle size and flow rate?
- A: High flowrates are expected to cause shearing in combination with suboptimal pore size of particles. But BEH450 particles used with GTx columns did not exhibit shearing up to the tested flowrates and its pore size is optimal for AAV monomer and aggregate characterization.
- *Q:* What type of data could I expect if I used a column that wasn't ideal for a MALS detector?
- A: Elevated baseline, particle shedding, High LS noise, very inconsistent and low precision of the measurements of the tested analytes.
- *Q:* How can one be certain of the peak assignment in a chromatogram?
- A: Running appropriate protein standard test mix and known AAV standards can help assign peaks based on the retention time observations.

SEC COLUMN GUIDE (ONLINE HERE)

The Waters | Wyatt[™] columns recommended in our column guide have been selected for their optimal performance with MALS due to their excellent resolution, extended lifetime, and lot-to-lot reproducibility, and minimal particle shedding.

Application	Column Name	DAWN™ Detector type	Molecular weight range	Pore Size (particle 2.5 μΜ)	Column dimension	Column P/N
Proteins	Xbridge [™] Premier Protein SEC Column	DAWN [™] or miniDAWN [™]	10-650 kDa	250 Å	7.8 x 300 mm	<u>186009962</u>
Proteins	Xbridge Premier Protein SEC Column	microDAWN™	10-650 kDa	250 Å	4.6 x 300 mm	<u>186009960</u>
Proteins- Guard	MaxPeak [™] Premier Protein SEC Guard		10-650 kDa	250 Å	4.6 x 30 mm	<u>186009969</u>
Gene Therapeutics	XBridge Premier GTx BEH SEC Column	DAWN or miniDAWN	200-10,000 kDa	450 Å	7.8 x 300 mm	<u>186010587</u>
Gene Therapeutics	XBridge Premier GTx BEH SEC Column	microDAWN	200-10,000 kDa	450 Å	4.6 x 300 mm	<u>186010585</u>
Gene Therapeutics - Guard	Xbridge Premier GTx BEH SEC Guard		200-10,000 kDa	450 Å	4.6 x 30 mm	<u>186010583</u>