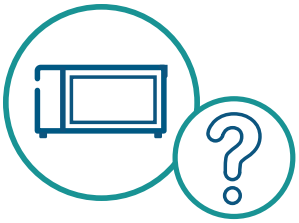

MALS Application-related FAQs

What types of samples can be analyzed using SEC-MALS?

SEC-MALS is usually implemented in the analysis of synthetic polymers (e.g. linear and branched polymers), biopolymers and polysaccharides, proteins and other biomolecules, and drug delivery systems including Adeno-Associated Viruses (AAVs), lipid nanoparticles (LNPs), and other virus-like particles.

What does MALS measure that SEC alone cannot?

MALS measures absolute molecular weight directly independent of column calibration standards. SEC alone provides only relative molar mass based on elution volume and assumptions about molecular conformation, hence if the properties of calibration standards are not matching the analyzed samples the molecular weight determination might not be correctly determined.

Does MALS require column calibration standards?

No. SEC-MALS does not require calibration standards for molecular weight determination. Columns are used only for separation, not for MW calibration.

Can MALS be used without SEC (batch mode)?

Yes. MALS can be used in batch mode if the sample is monodisperse and well-defined, though SEC-MALS is preferred for polydisperse or aggregated samples.

When should I use SEC-MALS instead of calibration-based SEC?

We recommend implementing SEC-MALS when no suitable calibration standards exist, when the sample has complex architecture (branched, star, conjugated), when there is a need for true molar mass, not relative values, and when information on structural or compositional changes are needed.

Why does SEC-MALS MW not match SEC-calibrated MW?

Because SEC calibration assumes similar hydrodynamic behavior to the standards. SEC-MALS reports true molecular weight regardless of shape or chemistry.

What is dn/dc and why is it so important?

dn/dc is the refractive index increment that converts RI signal to mass concentration. The squared dn/dc value is a part of MW determination equations from MALS, meaning even small errors significantly impact molecular weight results.

Can I use a literature dn/dc value?

Yes, but only if solvent composition, wavelength, and temperature closely match your experiment. For copolymers, conjugates, or non-aqueous solvents, we strongly recommend measuring dn/dc with an instrument set-up and method used in the sample analysis. The dn/dc can be determined by measuring the refractive index response as a function of precisely known, monodisperse sample concentration that span at least one order of magnitude.

When should I measure dn/dc experimentally instead of using a default value?

You should measure dn/dc when working with new chemistries, copolymers, conjugated molecules (such as polymer-drug conjugates, or antibody-drug conjugates), non-aqueous solvents and regulatory-relevant data.

Why can different dn/dc inputs lead to different molecular weight results for the same sample?

Different dn/dc inputs lead to different molecular weight (MW) results because dn/dc is in the core equations that convert detector signals into absolute molar mass. Dn/dc is a key input parameter needed to back-calculate a MW for any observed concentration response measured by RI response and light scattering signal intensities measured by MALS.

Regardless of chromatographic performance, varying the dn/dc input will lead to significantly varied MW values for the same set of measured MALS signals.

Why can the same sample have different valid dn/dc values?

Dn/dc is not a universal constant for a polymer or protein. It depends on solvent composition (salt, buffer, cosolvents (DMSO, DMF, MeOH, etc.), wavelength of refractive index detector, temperature, and chemical composition (copolymer, conjugates,..). Therefore, one sample can have multiple dn/dc values, but only one is correct for the exact experimental conditions.

Can UV replace RI as a concentration detector?

Yes, if the extinction coefficient (dA/dc) is known and constant at the selected absorbance wavelength used for concentration determination; however, RI remains more universal because it does not require a chromophore.

What are the best practices for storing my MALS detector?

It is strongly recommended to store MALS in 20% methanol for long term storage. Always ensure miscibility when exchanging between mobile phases to prevent solvent clogs in the detector plumbing. Moreover, avoid stopping the flow with any mobile phase containing salts to avoid crystallization and damaging of MALS flow cell.

Why do I need multiple angles in MALS?

Multiple angles allow separation of molecular weight and size (Rg) effects. For MW calculations, it is possible to directly measure MW by measuring the scattering intensity at one angle close enough to the scattering beam; however, for size determination of Rg, multiple angles are needed for determining angular dependence.

Why are some low-angle data sometimes excluded from the data analysis?

Low-angle detectors are more sensitive to dust, large aggregates, and baseline noise (low angles in a traditional MALS detector are close to the incident laser beam). Excluding low-angle detectors from scattering models is common practice in traditional MALS setups that rely on data fitting for MW and R_g; however, this increases the uncertainty of the resultant MW and R_g outputs.

What are Mn, Mw, and Mz?

Mn (number-average molecular weight) is the average molecular weight calculated by giving equal weight to each molecule, regardless of its size. It reflects the number distribution of molecules in the sample using the number of molecules. M_i signifies the molecular weight for a given molecule i, and ci signifies the number of molecules associated with (N_i) for a given measurement multiplied by M_i.

$$Mn = \Sigma(ci) / \Sigma(ci / Mi)$$

Mw (weight-average molecular weight) is the average molecular weight calculated by giving more weight to heavier molecules, according to their mass contribution. It is therefore more sensitive to higher-molecular-weight species than Mn.

$$Mw = \Sigma(ci \cdot Mi) / \Sigma(ci)$$

Mz (z-average molecular weight) is a higher-order average that gives an even stronger emphasis to the highest-molecular-weight species in the distribution. It is particularly sensitive to very large molecules or aggregates.

$$Mz = \Sigma(ci \cdot Mi^2) / \Sigma(ci \cdot Mi)$$

Does SEC separation quality affect MALS measurements?

Yes. Poor separation can lead to overlapping species, incorrect concentration assignment, and biased MW values, especially for Mn

Why does Mw remain stable while Mn changes when separation worsens?

Mw is intensity-weighted and dominated by higher-mass species, while Mn is sensitive to low-mass tails and peak slicing, making it more sensitive to chromatographic quality. Additionally, this Mn change between two different chromatographic separations can lead to significant change in the sample polydispersity values.

Why do aggregates yield strong MALS signals?

Scattering intensity increases with molecular weight. Trace amounts of aggregates can disproportionally contribute to LS signal relative to its monomeric components and significantly skew average MW results.

Why don't detector intensities match the relative abundance observed with my MALS signals?

RI or UV responses depend solely on the sample concentration, while MALS signal is dependent on both sample concentration and MW. This can result in any number of disparities when directly comparing LS responses to concentration. High MW species yield significant scattering signals at low abundances, while low MW species yield weak scattering responses at high abundances.

How do I ensure accuracy for low-signal samples?

To improve accuracy we recommend increasing injected mass (within column limits), improving dn/dc accuracy (manual determination under the chromatographic conditions instead of using literature values), using RI rather than UV if possible, minimize baseline noise and temperature fluctuations.

How do band broadening and noise correction settings affect MALS data analysis?

Noise correction reduces the influence of baseline noise on detector signals, improving data stability. Band broadening correction compensates for peak dispersion between detectors caused by system volume and tubing. When these corrections are disabled, molecular weight and distribution results may be less accurate or inconsistent.

Why can poor chromatographic separation or non-monodisperse standards affect MALS results?

MALS assumes that all molecules contributing to a signal slice are well separated chromatographically. Co-elution of species (e.g. monomer with aggregates) leads to averaged or distorted molecular weight results. Non-monodisperse standards are not suitable for MALS calibration because the software cannot assign a single, well-defined molecular weight to the peak.

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