SEC-MALS: CMI Guide to Working with Custom Solvents

Measure Solvent aRI dn/dc in custom solvent Edit SEC-MALS parameters

Introduction

MALS calculations are dependent on a good estimate both the refractive index (aRI) of the solvent and the refractive index increment (dn/dc) for the protein sample in that solvent.

Most proteins have a dn/dc near to 0.185 in PBS or water. The absolute refractive index (aRI) of PBS (and of water) is 1.33. However, some custom solvents have a refractive index that is different enough from 1.33, to affect the dn/dc of protein in the custom solvent.

Measure Solvent Refractive Index

The absolute refractive index of a solvent can be measured using the Optilab TrEx. You should measure this for every buffer. If it varies significantly from 1.33 RUI, then you may need to make a custom solvent profile and adjust the dn/dc for your protein.

1. Equilibrate your column and the flow path with running buffer (typically overnight)
    - Confirm the Optilab TrEx is in the PURGE ON position (flow through the reference flow cell)
2. Turn on the Optilab TrEx LED
3. Go to the aRI tab and record the absolute refractive index (in RUI) of your custom solvent

Estimate Refractive Index Increment (dn/dc) in a Custom Solvent

The refractive index increment of any protein in any given solvent can be measured directly. However, this requires large quantities of protein of known concentration. See the CMI Guide to Protein Conjugate Analysis for instruction on measuring dn/dc directly. However, a reasonable estimate for the dn/dc of proteins in a custom solvent (solvent B) can be calculated using this formula:

$$(dn/dc)_{solventB} \cong (dn/dc)_{solventA} - v_p(n_{solventB} - n_{solventA})$$

v_p = partial specifc volume (~ 1)
$(dn/dc)_{solventB}$ = refractive index increment of sample in solvent B
$n_{solventB}$ = absolulte refractive index of solvent B

Example:

$(dn/dc)_{PBS}$ = 0.185 ml/g
n_{PBS} = 1.330
$n_{solventB}$ = 1.350 (eg. 10% glycerol)
$\rightarrow (dn/dc)_{solventB} \cong$ 0.165 ml/g

Edit your SEC-MALS parameters

If the refractive index of your solvent differs from 1.33 by 0.02 RUI or more, you should consider making a custom solvent profile and adjusting the dn/dc for protein.

1. Open Astra Software
2. Create a Custom Solvent Profile
    a. Open an existing solvent profile: File → Open → Profile.
    b. Navigate to the UserMethods Folder
    c. Open an existing custom solvent profile (eg. 10percent_glycerol_solvent_profile)
3. Change the refractive index model
    a. Expand the Refractive Index Model (by clicking the + next to model)
    b. Change the model to Fixed (if not already set)
    c. Enter the value of your solvent's absolute refractive index for Reference Refractive Index
    d. Use Save As to save your custom solvent profile
4. Set the Solvent Profile
    a. In the SEC-MALS experiment, expand HPLC Device (HPLC Pump)
    b. Double-Click on Solvent
    c. Use the menu by Name to find your custom solvent profile
    d. Click Ok to save the solvent profile to the experiment
5. Set the adjusted Protein dn/dc
    a. In the SEC-MALS experiment, expand HPLC Device (HPLC Sampler)
    b. Double-Click on Sample
    c. Adjust the dn/dc for your protein sample
    d. Click Ok to save to the experiment
    e. If you have already called peaks, either change the parameters in the peaks or delete existing peak calls and call new peaks to use the modified parameters.

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Contact cmi@hms.harvard.edu with questions.

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