Biophysical
Instrumentation
Facility

SEC MALS OPERATION PROTOCOL (09/04/23)

Pre-run requirements:

- User must provide some sample QC, ideally in the form of a UV trace from a standard SEC experiment.
- User must either use standard running buffer (PBS or 25 mM Hepes pH 7.5, 150 mM NaCl), or must provide the composition of preferred buffer for their experiment. Buffers with a pH above 7.5. are not compatible with the set of Wyatt silica columns due to column shedding at high pH. Glycerol containing buffers should not be used. Users will need a minimum of 500 mL buffer, but best to have them bring 1 L.
- Check the MQ H2O bottle on the instrument for contamination (Shouldn't be a problem, but did happen once).
- Check the waste bottle, empty if necessary.
- Turn the HPLC lamp on. The lamp needs 10-15 mins to warm-up, but the pre-sample steps take much longer than this.
- Turn on the MALS and dRI instruments.
- Place a full vial of running buffer in the wash position (pos. 99) of the autosampler.
- Prior to injection, samples should be at a minimum centrifuged for 10 minutes at max speed in a microcentrifuge, or filtered to 0.2 uM.

System Equilibration:

- Place the A line of the HPLC into freshly filtered buffer, seal the bottle with the cap to prevent dust/particulate from entering the buffer.
- Open the purge valve on the HPLC, and program the HPLC to flow 100% A at 5 mL/min using either the HPLC control software or the "Gameboy" controller. Adjust the maximum flow gradient on the control software to 100 ml/min^2 to allow the flow to ramp-up faster (sometimes the instrument/software goes back to the default value of 0.1). Allow the buffer to flow to waste for 6 minutes.
- Set the flow to 0
- Once flow has stopped, reset the flow gradient to 0.1 ml/min^2, then close the purge valve
- Adjust the flow rate to 0.5 mL/min using either the HPLC control software or the "Gameboy" controller.
- On the MALS, activate the COMET (on-line sonicator). On the dRI, open the purge valve to allow buffer to enter the reference cell. Run the COMET for 10 mins.
- Monitor the noise, wander, and drift values on both the MALS and DRI. Once green, the system is ready for the initial BSA run. At a minimum this will take 1 hour, maybe longer depending on the buffer composition and state of the column/instrument. Once everything has stabilized,

close the purge valve on the dRI. Run a minimum of 2 column volumes (~40 mL), but optimal data quality is achieved with 5+ CVs of equilibration.

- If noise, wander and drift values do not improve after 8-10 CVs, there is likely contamination in the instrument/column, and both will need to be cleaned (See cleaning protocol document).

BSA Standard run:

- Prepare a standard of BSA at 2 mg/mL (I usually make 5 mL at a time for easier weighing) in running buffer. Filter to 0.2 uM.
- Place a low-volume disposable insert into a vial, and carefully pipette 75 uL of BSA solution into the insert. Cap the vial, and place into the autosampler.
- Open the ASTRA software, create a new sequence. Fill in the BSA sample name, vial position, concentration, set injection volume to 50 uL, and select the default method for the wyatt columns + guard (40 min method, 0.5 mL/min).
- Press run. You will be prompted to save the sequence before the sequence actually runs, make sure to stay by the instrument to do this or else nothing will actually run.
- After the BSA standard has run, open the results in the Astra software and proceed with data analysis. For the BSA standard, ake sure to align, normalize, and run band broadening in the configuration tab. This configuration will be used adjust data for all samples running in the same buffer on the same day.

  Right click configuration, select save configuration as, save this configuration with the column and date.

Sample run:

- Place a low-volume disposable insert into a vial, and carefully pipette 75 uL of sample into the insert. Cap the vial, and place into the autosampler.
- Open the ASTRA software, navigate to the sequence tab, right click and add a sample. Fill in the sample name, vial position, concentration, set injection volume to 50 uL, and select the default method for the wyatt columns + guard (40 min method, 0.5 mL/min). Repeat for multiple injections, assuming the samples are stable at RT
- Press run. The instrument keeps track of what has been run in a sequence, so don't worry about samples that have already been run. You will be prompted to save the sequence after every modification, its totally fine to overwrite the same sequence file each time for one set of experiments as the output data is saved separately.
- After the sample run has finished, open the results in the Astra software, right click the configuration tab, select replace configuration, and load the BSA configuration saved earlier.
- Proceed to data analysis.

Data analysis:

BSA Control:

Follow along with software prompts to analyze data.

- First, set baselines for all LS detectors, UV, and dRI. For good quality data, auto baseline detection will work well.
- Next, select peaks for analysis. Be selective for the area around the apex of any apparent peaks.
- Follow below for the BSA control run:
  - <u>Alignment (Aligns detector signals for UV, MALS, dRI)</u>
    - Right click configuration, click alignment.
    - Highlight a distinct peak, click align signals (upper left).
    - Click OK to apply settings (bottom left).
  - <u>Band Broadening (Adjust for sample diffusion through tubing)</u>
    - Right click configuration, click band broadening.
    - Reset the values in the terms table at the bottom.
    - Select apex of peak, click perform fit (Upper left).
    - Click OK to apply settings.
  - <u>Normalization (Normalize detector signal)</u>
    - Right click configuration, click Normalization.
    - Choose a distinct peak, click normalize.
    - Click OK to apply settings.

<u>Samples:</u>

Follow along with software prompts to analyze data.

- First, set baselines for all LS detectors, UV, and dRI. For good quality data, auto baseline detection will work well.
- Next, select peaks for analysis. Be selective for the area around the apex of any apparent peaks.
- Right click the configuration tab, click replace configuration, and load the configuration file saved for the BSA sample generated earlier.
- Examine results in both EASI graph and EASI table.
- Save data file.
- Export graphs
  - Right-click any graph.
  - Click edit then export.
  - Choose desired export parameters. Graphs and table can be output direct as PDF using the print to PDF option, or each parameter can be output as CSV, allowing for custom plotting in other software.

Post-run:

The HPLC will not stop flow after a sequence is run!! Do not leave the system after a sequence finishes.

- Set the flow gradient to 0.1 ml/min^2, then set the flow rate to 0.
- Once flow has stopped completely, transfer the A line back into water. Cap the bottle to prevent dust.
- Open the purge valve on the HPLC, and program the HPLC to flow 100% A at 5 mL/min using either the HPLC control software or the "Gameboy" controller. Adjust the maximum flow

gradient on the control software to 100 ml/min^2 to allow the flow to ramp-up faster (sometimes the instrument/software goes back to the default value of 0.1). Allow the water to flow to waste for 6 minutes.

- Set the flow to 0
- Once flow has stopped, reset the flow gradient to 0.1 ml/min^2, then close the purge valve
- Adjust the flow rate to 0.5 mL/min using either the HPLC control software or the "Gameboy" controller.
- On the MALS, activate the COMET (on-line sonicator). On the dRI, open the purge valve to allow buffer to enter the reference cell. Run the COMET for 60 mins (On the MALS touchscreen, click system then set the COMET run to 60 min).
- Equilibrate in water for a minimum of 2 CVs (40 mL)
- Set flow rate to 0
- Empty waste

For long term storage, Columns should be stored in 20% ethanol at 4°, or in a buffer containing 0.02% NaN$_3$.