SEC-MALS Noise Assessment and Cleaning Guide

Summary

An important part of obtaining high quality SEC-MALS data is maintaining a clean system. Contaminants in the system will contribute to a noisy or wavy light scattering baseline thereby decreasing the signal-to-noise ratio and obscuring meaningful signals. This guide will describe how to isolate the source of the noise in the HPLC/MALS system in order to clean and remove contaminants.

Related Technical Notes and References

M1000	ASTRA 6 User's Guide
M3000	miniDAWN TREOS User's Guide
M3200	DAWN HELEOS II User's Guide
M3500	µDAWN User's Guide
TN1007	ASTRA 6 Quick Guide
TN3502	Guidelines for Improving the Quality of SEC-MALS Data in an Aqueous System
TN3503	Troubleshooting a Clog in a SEC-MALS System

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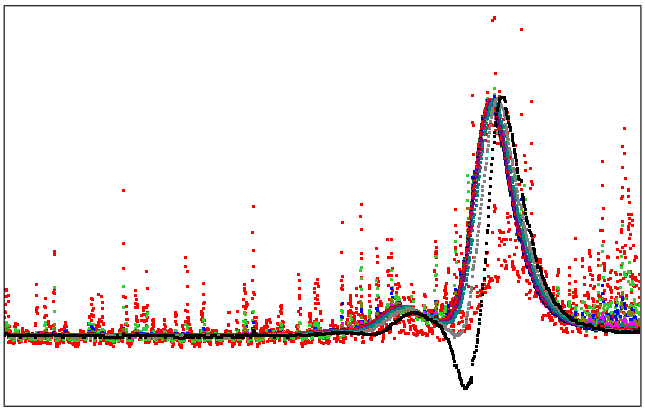

Figure 1: An example of noisy light scattering signals from multiple scattering angles collected from a Bovine Serum Albumin run through a dirty MALS instrument flow cell.

Introduction

Before an SEC-MALS measurement can be started, your chromatography system must be equilibrated. When the baseline is stable, a measurement can be performed. Ideal baseline and noise levels depend on your detector and the system solvent:

HELEOS II (90° detector):

- Noise: < 30 µV (0.5 s collection interval)
- Baseline: Aqueous ~ 0.015 to 0.02 V
- Baseline: THF ~ 0.04 V
- Baseline: Toluene ~ 0.16 V

TREOS (90° detector):

- Noise: < 30 µV (0.5 s collection interval)
- Baseline: Aqueous ~ 0.015 to 0.02 V
- Baseline: THF ~ 0.04 V
- Baseline: Toluene ~ 0.1 V

µDAWN (90° detector):

- Noise: < 60 µV (0.05 s collection interval)
- Baseline: Aqueous ~ 0.015 to 0.025 V
- Baseline: THF ~ 0.04 V
- Baseline: Toluene ~ 0.15 V

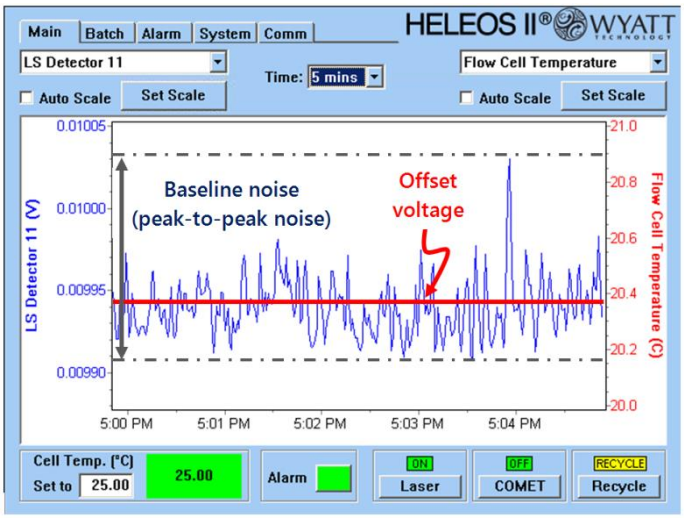

Figure 2. Noise and baseline shown on the front panel of a HELEOS instrument.

Performing measurements with an increased baseline voltage or noise decreases the accuracy of the results. Therefore, the root cause of the noise problem should be identified. Noise in the light scattering trace can be caused by particles or contaminants in one or more of these components:

- Light scattering detector (*page 3*)
- Mobile phase (*page 3*)
- HPLC System (*pages 4-5*)
- Upstream detectors (such as a UV detector) (*page 6*)
- Column (*page 7*)

Note: Before you start, contact your HPLC vendor to make sure all components of your HPLC system are compatible with nitric acid and any other solvent used in the cleaning protocols described below. Wyatt's MALS and RI detectors are compatible with 10% nitric acid. ***Do not flush your column with nitric acid.***

Tools and Supplies needed

- Syringe pump
- Pure solvent (e.g. HPLC grade water)
- Alcohol
- 10 mL Syringes
- 0.02 µm syringe tip filters
- Cleaning solvents (see Appendix 1 and recommendations from your HPLC manufacturer)

Procedure

Check the noise in the MALS detector with an offline measurement

1) Take the MALS detector offline:

   a. Disconnect your MALS detector from the flow path. See Figure 3.

   b. Connect a syringe pump to the MALS detector with a 10 mL syringe filled with **pure solvent** and a 0.02 µm syringe tip filter. If your mobile phase is an aqueous buffer, use 18.2 MOhm-cm resistivity water; if your mobile phase is pure organic solvent, use a **new** HPLC grade bottle of that solvent.

   c. Begin flushing at a flow rate between 0.1 mL/min and 0.5 mL/min.

Note: You may substitute the 0.02 µm filter with a 0.1 µm syringe tip filter if the smaller pore size is not available.

2) Collect 5-10 minutes of data in ASTRA and measure baseline noise. You can use the noise method in the **Light Scattering → Diagnostics** folder. Note that the **HELEOS noise**, **TREOS noise,** or **µDAWN noise** methods measure RMS noise. RMS noise (noise standard deviation) can be converted to peak-to-peak noise by multiplying the RMS noise by a factor of 6.6; i.e., (peak-to-peak noise) = 6.6 x (RMS noise).

3) If the peak-to-peak noise is 30 µV or less, the instrument itself is clean; proceed to the next section. Otherwise, flush the MALS detector with 20% alcohol, then 10% nitric acid if needed. See *Appendix 1: General System Cleaning Procedures* for more details.

4) After flushing, check the noise again. If it is acceptable, it is recommended that you continue checking each component of the system using the steps below – the MALS detector may not be the only cause of noise.

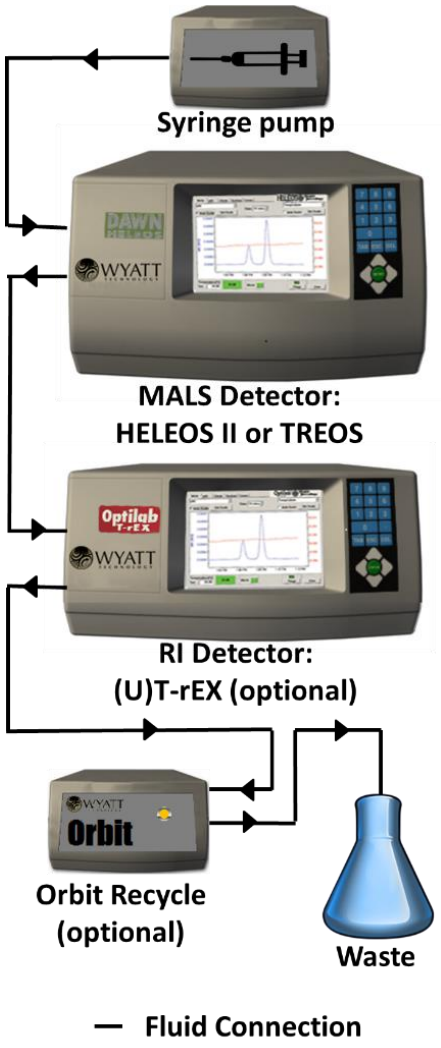

Figure 3. The flow path used to check the noise of the MALS instrument itself.

Check the noise of mobile phase with an offline measurement (aqueous only)

1) If the peak-to-peak noise determined in step 2 of the "*Check the noise in the MALS detector with an offline measurement*" section was 30 µV or less in pure solvent, use the syringe pump to flush your instrument with your mobile phase that was used during collections in which high noise levels were observed.

2) Collect 5-10 minutes of data in ASTRA and measure baseline noise.

3) If the peak-to-peak noise is 30 µV or less, the mobile phase is clean; proceed to the next section. Otherwise, replace mobile phase and see if the noise improves.

Check the noise of pump and inline filter flowing directly into the MALS

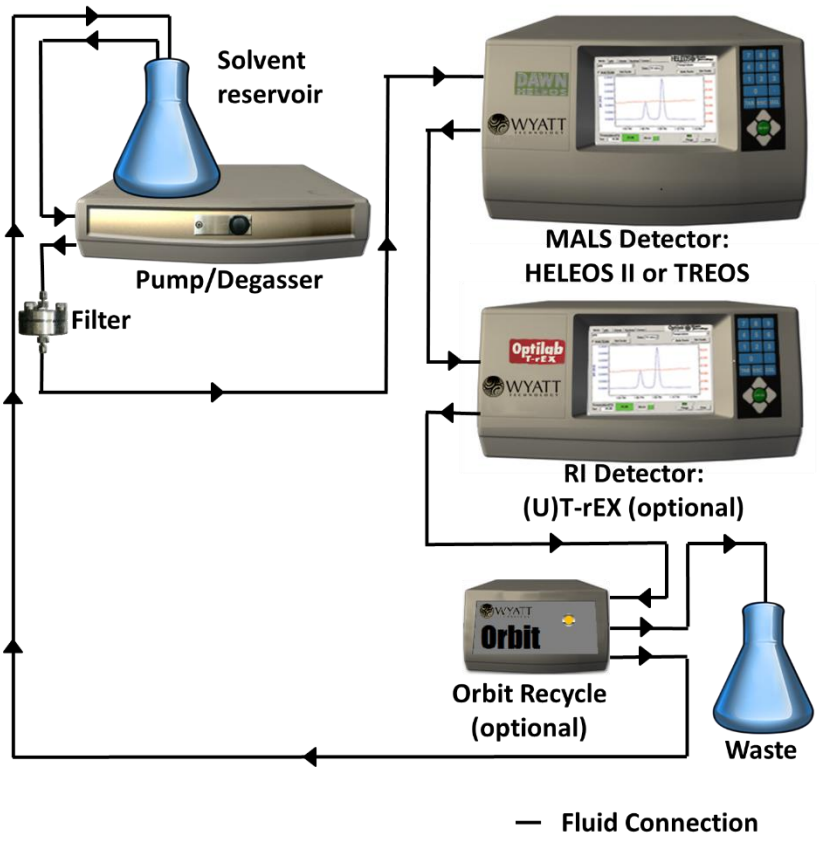

Figure 4. The flow path used for checking the noise of the pump, degasser, and inline filter.

Note: Avoid introducing air into the MALS detector. Make sure that the tubing used is filled with liquid. It is recommended to flush a couple of drops out of the tubing when connecting to the MALS detector.

1) Connect the HPLC pump to the MALS detector bypassing all components between the pump/degasser and the MALS detector (except the inline filter). See Figure 4.
2) Set the flow rate to 0.5-0.8 mL/min. It will take 5-10 min for the initial noise to decrease. Record 5-10 minutes of data using your standard online ASTRA method. Uncheck the "Trigger on Auto-Inject" checkbox in the Basic Collection window and hit the Run button.
3) If the peak-to-peak noise is 30 µV or less, the pump and inline filter are clean; proceed to the section below. If it is higher, check the pump frit and the inline filter.
    - The inline filter membrane should be replaced monthly.
    - Check the titanium frit as well. You can sonicate the titanium frit for cleaning or replace it (Upchurch part number: A-342-02).
    - If none of the above improves the noise level, clean the HPLC pump according to the vendor instructions.
4) After cleaning, check the noise again. If it is acceptable, it is recommended that you continue checking each component of the system using the steps below – the pump may not be the only cause of noise.

Check the noise of the autosampler

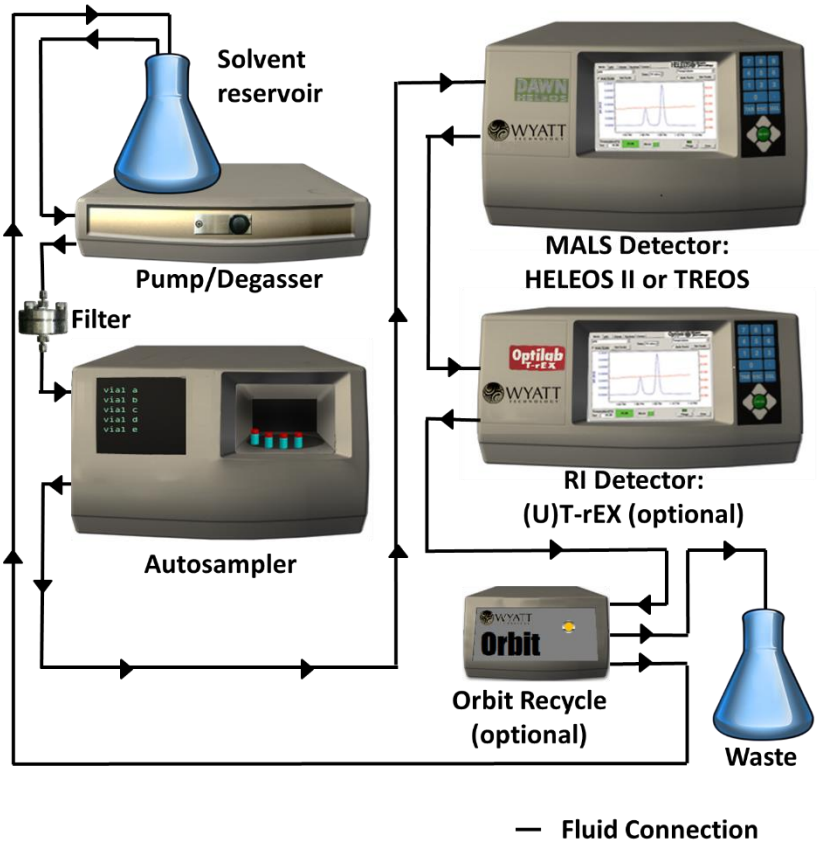

Figure 5. The flow path used for checking the noise of the autosampler.

1) Add the autosampler into the flow path. See Figure 5.
2) Set the flow rate to 0.5-0.8 mL/min. It may take 5-10 min for the initial noise to come down. Record two ASTRA collections using your standard online ASTRA method. Uncheck the "Trigger on Auto-Inject" checkbox in the Basic Collection window and hit the Run button.
2) Check the noise with the injection valve in bypass mode.
3) Check the noise with the injection valve in mainpass (flushing through the loop). If the noise increases significantly when the system is in mainpass mode, the autosampler loop is dirty and needs to be cleaned.
4) To clean the loop, switch the system to 20% alcohol and make 5-10 injections every 5 minutes. Change back to your mobile phase and check the new noise level. It should be less than 30 µV. If the noise is acceptable it is recommended that you continue checking each component of the system using the steps below – the autosampler may not be the only cause of noise.

Check the noise of the UV detector (if applicable)

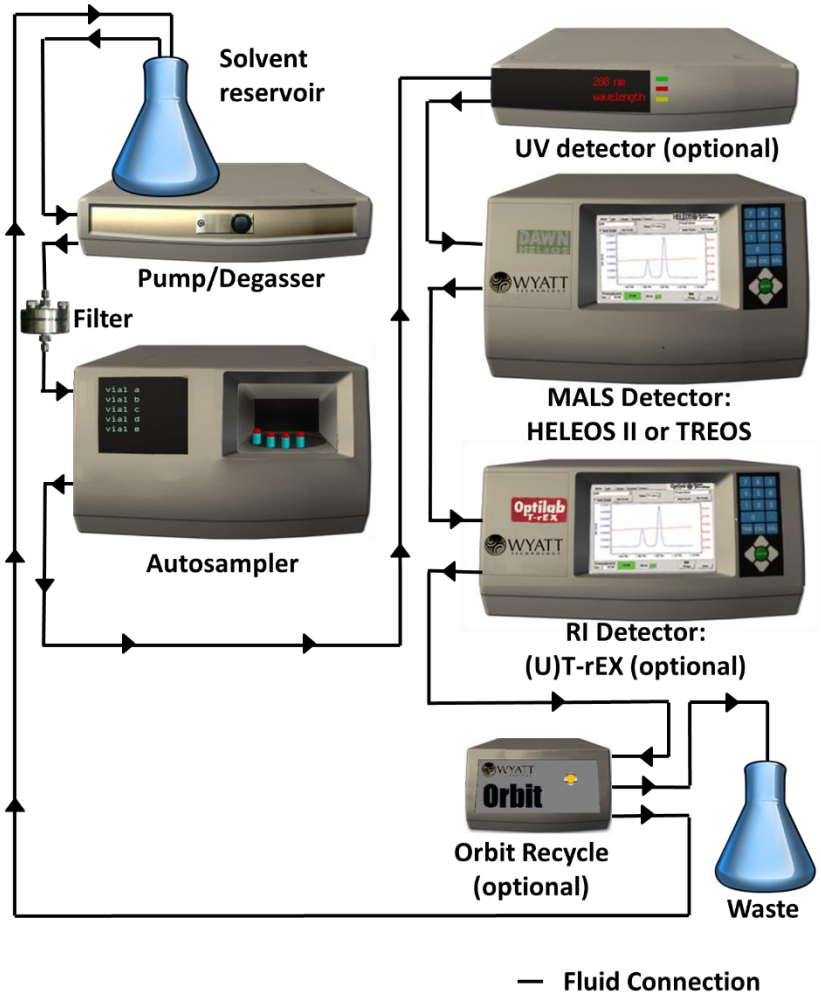

Figure 6. Flow path used for checking the noise of the UV detector.

3) Add the UV detector into the flow path.

4) Set the flow rate to 0.5-0.8 mL/min. It may take 5-10 min for the initial noise to decrease. Record data using your standard online ASTRA method. Uncheck the "Trigger on Auto-Inject" checkbox in the Basic Collection window and hit the Run button.

5) If the peak-to-peak noise is 30 µV or less, the UV detector is clean. Otherwise, clean the UV detector according to vendor instructions and check the noise level again. You may need to replace the UV detector's flow cell.

6) Before testing the last source of noise (the column), proceed to the next section to prepare the system for attaching the column.

Flush the entire system (except for the columns)

1) After completing all cleaning procedures according to manufacturer recommendations, flush the HPLC/MALS system from the last cleaning solvent into your mobile phase through a series of miscible solvents (e.g. methanol to water to buffer).
2) If you ran a nitric acid wash, check the pH of the mobile phase after it passes through the detectors; it should no longer acidic from the nitric acid wash. Also change the filter membrane of the inline filter if present.
3) Record the noise in ASTRA. Uncheck the "Trigger on Auto-Inject" checkbox in the Basic Collection window and hit the Run button.
4) If the peak-to-peak noise is 30 µV or less, proceed to the next section. If the noise is still high, consider changing the tubing.

Check the noise of the columns

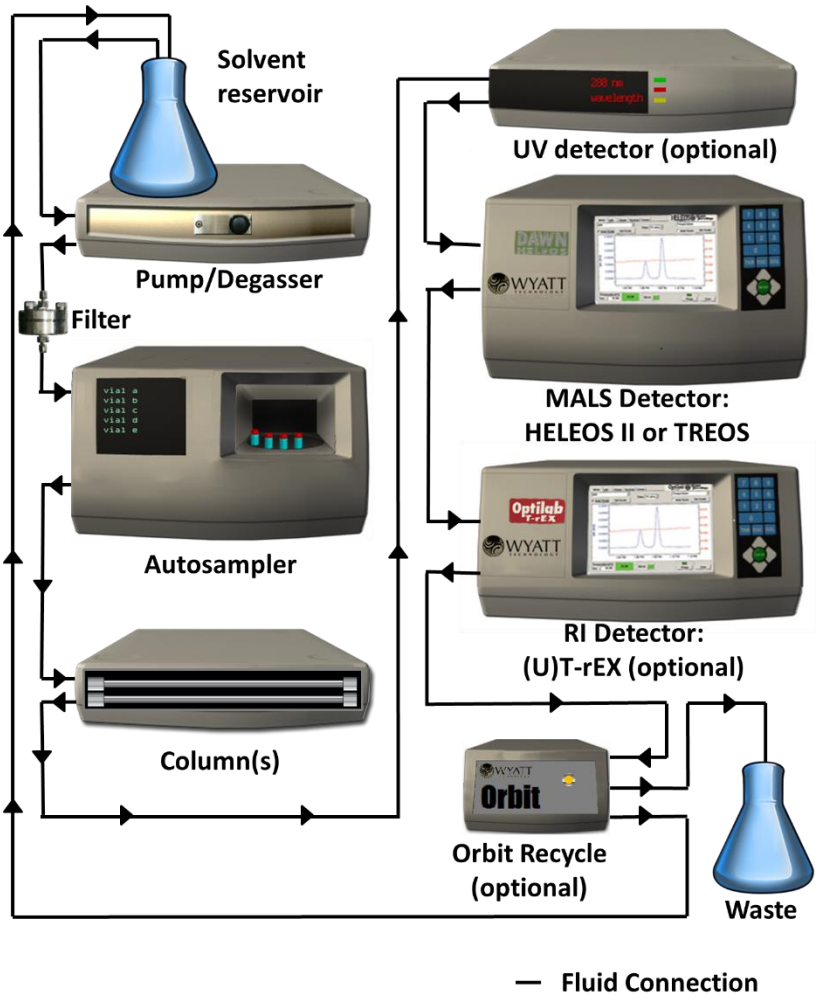

Figure 7. Flow path used to check the noise of the columns.

1) Disconnect the MALS detector from the flow path prior to connecting the column for its initial equilibration.
2) Decrease the flow rate of your system to 0.1 ml/min. Add the column to the flow path and increase the flow rate slowly at a rate of 0.1 ml/min^2.
3) Once you've reached your standard system flow rate, let the column equilibrate per the manufacturer's instructions.
4) Once the column is equilibrated, reconnect the MALS detector to the flow path. Regular downstream HPLC detectors can often be connected at the chromatography flow rate since they do not add significant back pressure for typical flow rates and solvents. UHPLC detectors should not be connected at the regular flow rate as they add significant back pressure; instead, they should be connected at zero or near zero flow rate (follow your column manufacturer's recommendations). Estimated back pressures in water at typical flow rates are listed in Table 1 for HPLC detectors and in Table 2 for UHPLC detectors.

Table 1. Estimated back pressure for HPLC detectors in water at a flow rate of 1 mL/min.

Instrument	[psi]	[bar]
DAWN HELEOS	~51	~ 3.5
miniDAWN TREOS	~44	~ 3
Optilab rEX/TrEX	~ 29	~ 2
ViscoStar	~ 58	~ 4

Table 2. Estimated back pressure for UHPLC detectors in water at a flow rate of 0.5 mL/min.

Instrument	[psi]	[bar]
µDAWN	~ 116	~ 8
UT-rEX	~ 232	~ 16

5) After reconnecting the MALS detector, continue flushing the column. Once the initial noise of the column has decreased after connecting the column to the MALS detector, purge the RI detector for at least 30 minutes.
6) Record an ASTRA collection (5-10 min) for your setup and note the noise. It should be 50 µV or lower. Up to 100 µV noise can be tolerated for sample injections with a high signal-to-noise ratio, e.g. high molecular weight polymers or particles.
7) If the noise is still high, consider reconditioning or cleaning the column per manufacturer's instructions. If cleaning is not successful it may be necessary to replace the column.

Appendix 1: General System Cleaning Procedures

If your system is in an aqueous mobile phase:

Flush all of the affected HPLC components with the following solvents in this order:

1) 50 mL water
2) 100 mL or more of a mixture of IPA or other alcohol and water (1:1)
3) 50 mL water
4) 100 mL or more of 10% nitric acid
5) 100 mL water

Note: If cleaning solutions such as Tergazyme or Hellmanex are compatible with the HPLC system according to the manufacturer, then an additional cleaning step can be performed using these solutions as they can be effective at removing proteins.

If your system is in an organic mobile phase:

If you wish to clean your system with nitric acid, make sure that either your mobile phase is compatible with water or you use 100 mL or more of an intermediate solvent (100% alcohol) before flushing into water.

A flushing sequence for organic solvents may look like this:

1) 100 mL or more of 100% alcohol
2) 50 mL water,
3) 100 mL or more of 10% nitric acid
4) 100 mL water
5) 100 mL or more of 100% alcohol
6) Organic mobile phase

Notes: Make sure to change the inline filter membrane after flushing the HPLC system with nitric acid before reconnecting your column and running experiments.

If in-situ cleaning of the MALS detector as described above does not improve the cleanliness of the flow cell, disassembling the flow cell and cleaning must be performed as described in the *Service and Maintenance* section of the instrument's User's Guide. A detailed description of the cell cleaning procedure is also provided in your LSU binder and a detailed video for cell cleaning can be found in the Wyatt support center at www.wyatt.com/support (navigate to the Tutorials section).

Appendix 2: General Recommendations

- Always ramp the flow rate of the column gradually at a rate of 0.1 mL/min^2, both when increasing and decreasing the flow rate. Do not open or close the purge valve on the HPLC while flowing with the column attached; instead, slowly ramp down the flow rate before opening or closing the purge valve.
- Clean out the fan filters on the back of the MALS and Optilab instruments every three months. An ambient MALS instruments should run at a temperature ~ 5 °C above room temperature if sufficient space for ventilation around the instrument is provided.
- Check the mobile phase daily with a laser pointer for particulates. Alternatively, you can use a batch DLS instrument to detect particulates.
- Switch off the MALS instrument laser (on the front panel when not in use), or use the Laser Saver Mode in ASTRA (basic collection procedure) which accomplishes the same thing after running an acquisition. Install the latest firmware for HELEOS II, TREOS, and µDAWN instruments. Switch off the MALS instrument completely if you are not using for more than three days. It takes about 30 min to warm up the instrument electronics. The laser warmup itself is completed within a few seconds.
- Consider a column cleaning or regeneration step of your column according to the manufacturer's recommendation. Disconnect the MALS instrument when performing column cleaning.
- If you need to open the top cover of the MALS or Optilab instruments and reach inside, make certain that you ground yourself electrostatically. Disposable wrist straps are available in the MALS instrument hardware kits.
- When installing new loops or UV flow cells, pre-flush with alcohol (100% or 20% according to the manufacturer's specification) before installing.
- When not using the HPLC system, flush into 20% alcohol and keep running at 0.1 mL/min.
- Purchase a syringe pump for the lab for offline work (a basic model will do since the exact flow rate delivery is not critical).
- Perform maintenance on your HPLC system at regular intervals (according to your HPLC manufacturer's recommendations).

Note: Please see *TN3502 – Guidelines for Improving the Quality of SEC-MALS Data in an Aqueous System* for more tips on performing successful SEC-MALS experiments.