

Standard Operating Procedure GPC/SEC

Ryan Mulvenna, February 2014

Description of Process

This SOP is dedicated to the proper, safe handling and use of the Gel Permeation Chromatography/Size Exclusion Chromatography (GPC/SEC) in FRNY 2182. It relies on differences on the hydrodynamic diameter of a polymer sample to determine its' molecular weight (M_n) and dispersity (\bar{D}) versus calibration standards.

Required Training

Purdue School of Chemical Engineering Safety Orientation
P.O.W.E.R. Lab safety orientation (including fume hood induction)

Engineering and Ventilation Controls

The pressure rated GPC (130 bar max.) self contained GPC features Agilent Software designed for automation and error detection.

Personal Protective Equipment (while using the GPC)

EYE PROTECTION: Safety glasses or goggles.

PROTECTIVE CLOTHING: Nitrile gloves, lab coat.

ENGINEERING CONTROL: Self contained unit.

GPC Sample Preparation

1. To make a sample, dissolve 10 mg of ***dried*** polymer into 1 mL of ***inhibitor free tetrahydrofuran (THF)***.
2. Inject the dissolved sample into a GPC vial ***through a 20 μ m PTFE filter*** (kept in the first drawer directly underneath the GPC). Seal with a GPC vial cap, and place in the automated sample holder, noting its numbered position.

GPC Sample Testing

1. Ensure that there is at least 100 mL per sample to be analyzed + 400 mL total in the THF solvent bottle (The front bottle) and that there is enough space for the eluted solvent for the waste bottle (The back bottle).
2. Ensure that you are on the Instrument Control (online) software: Method and Run Control Tab (Appendix 1). *Right-click Access Sequence Context Menu*, and select *Sequence Parameters*, and then enter the folder name and operator name you wish the data to be placed in.
3. Ensure that you are on the Instrument Control (online) software: Method and Run Control Tab (Appendix 1). *Right-click Access Sequence Context Menu*, and select *Sequence Table*. Enter the numbered position of the sample(s) in the vial column, type the sample name into the sample name and data file columns, use "AGB_METHOD_27JAN2012" for every sample in the Method Name Column, and set the Inj/Vial column equal to 1. Add sample rows by clicking the Insert button as necessary. Click OK once complete.
4. Ensure that you are on the Instrument Control (online) software: Method and Run Control Tab (Appendix 1). Make sure that all five control windows are displayed in the green. If this is not the case, click the corresponding I/O button on the screen to reset the device.
 - 4a. Sometimes, the RID detector may not be active and be displayed in yellow. This usually means that the RID reference window needs to be purged. To do this, *Right-click* the RID control window and click *Control*. In the popup window, select *Purge Reference Cell, On For*, and enter the time for *30 minutes*, then click *OK*. After the reference cell has been purged, the GPC should be ready for use.
5. When ready with the loaded samples, click the *Sequence* button in the Method and Run Control Tab to run the experiments (Appendix 1).

GPC Sample Analysis

1. Ensure that you are on the Instrument Control (online) software, and click on the Data Analysis tab. (Appendix 1).
- 2a. To export a trace, click *File, Export File, CSV File*. On the popup window, select "Signal" as the data source and click "Browse" to change the name and its save location. Click OK and confirm all further actions.

- 2b. To analyze the sample, once in the Data Analysis tab, go to *File, Load Signal* and select the sample GPC trace you wish to load (Appendix 2).
3. Go to *GPC, Calculate GPC Results* to open the Agilent GPC-Addon for GPC peak analysis (Appendix 3).
4. To load a sample, click on the folder icon in the top left corner of the screen and select your sample (RID.ch file in the sample name subdirectory). This will open the data trace, with the name being displayed in the top left corner.
5. While the data trace plot is selected, ensure that the proper calibration file is selected. If not, click on *Calibration Load, Load* to correctly load the correct calibration sample.
6. Click and drag the red triangles in the data trace window to border the elution peaks on both sides.
7. Click on *Window, Cascade* and then *Window, Mass Distribution* and *Window, Elutogram* to obtain the M_n and \bar{D} .
8. To obtain an overlay of multiple peaks, Highlight the elutogram window, click on *Overlay, Delete All Curves* and confirm. With the desired elutogram window still highlighted, click *Overlay, Include Curve* and confirm. Repeat steps 2b-8 to load more overlay plots. On the last overlay after clicking on *Include Curve* and confirm, click *Overlay, Overlay* to see the combined traces of your samples.

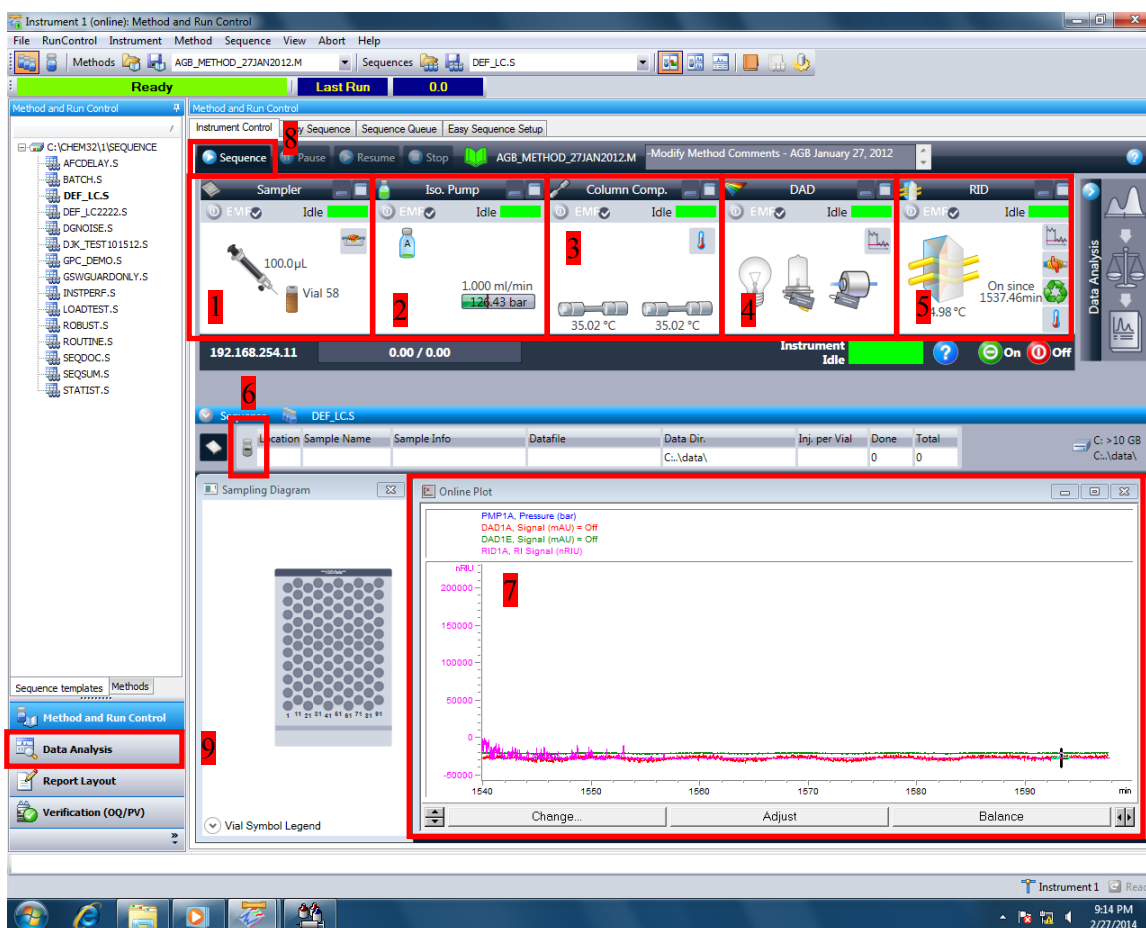
GPC Sample Analysis

Once every six months, the GPC needs to be calibrated with polystyrene GPC samples to ensure its continued accuracy form drift.

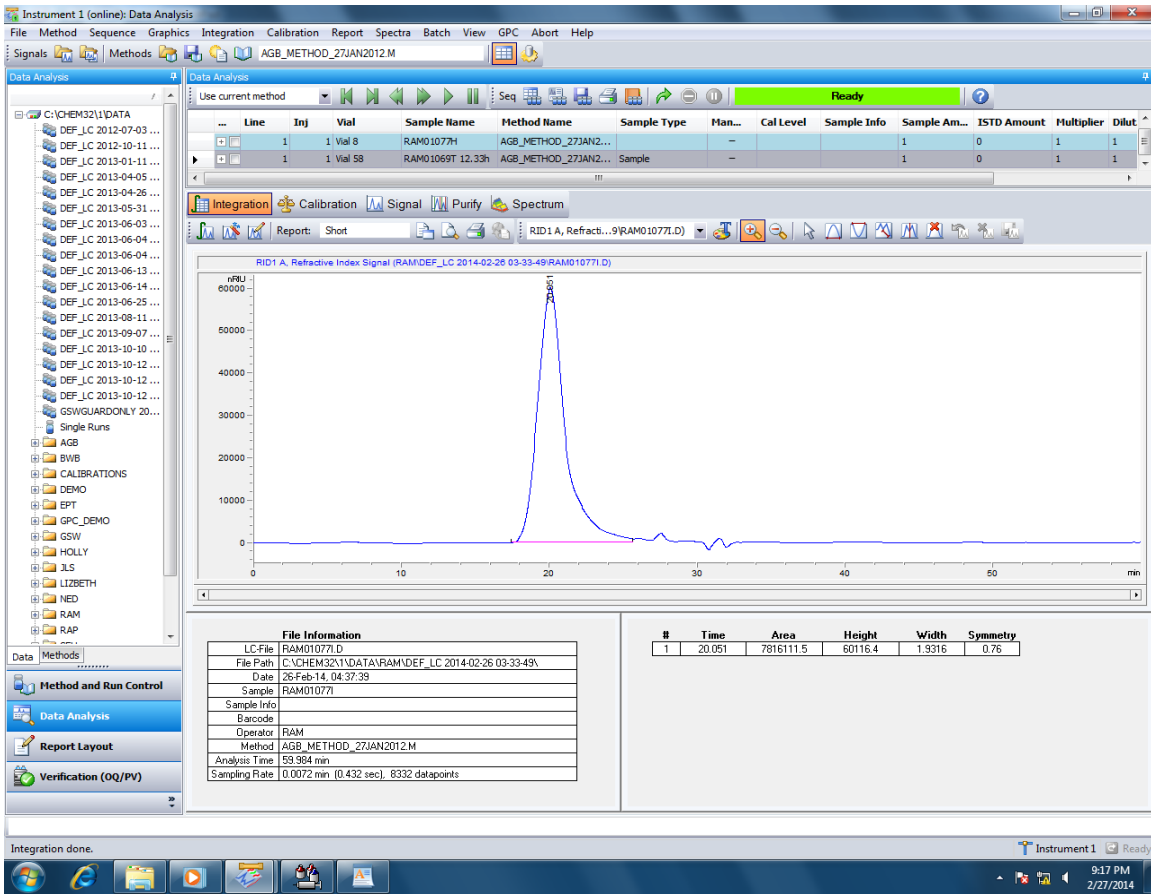
1. Prepare the Agilent EasiCal calibration standards by dissolving both polymer tipped samples separately in a vial with 1 mL of **inhibitor free THF**.
2. Run the samples as defined in the "GPC Sample Testing" section of this SOP.
3. Switch to the Agilent GPC-Addon for GPC peak analysis window for one of the calibration standards (Appendix 3).
4. Click on the Calibration Window on the bottom left of the screen to maximize.

5. While the Calibration Window is highlighted, click on *File, New* to load a new empty calibration file. In the top of the window, change the "Fit" drop down menu value from "none" to "Polynom 7".
6. Click and drag the red triangles in the data trace window of one of the calibration files to border the elution peaks of one of the standard samples.
7. Right click the x-axis of the data trace window and select *Find Maximum*. If this option is not available, or if an error occurs, manually click and drag a box around the peak to maximize zoom to manually read the peak maximum.
8. Enter the value into the "Volume" column. Enter the molecular weight of the material into the "Molar Mass" column and set "Stat. Weight" equal to 1. Enter the file name of the GPC plot file that you extracted this peak into "Sample Name".
9. Repeat steps 6-8 for the other peaks in the calibration plot (molecular weight goes from high-to-low with increasing elution volume). Remember to load the other calibration sample run and enter its data into the calibration file.
10. Once the calibration file has been populated, highlight its' screen and click *File, Save*. Save it in the "Calibrations" folder with the name "*YearMonthDay_RI*" of the calibration.

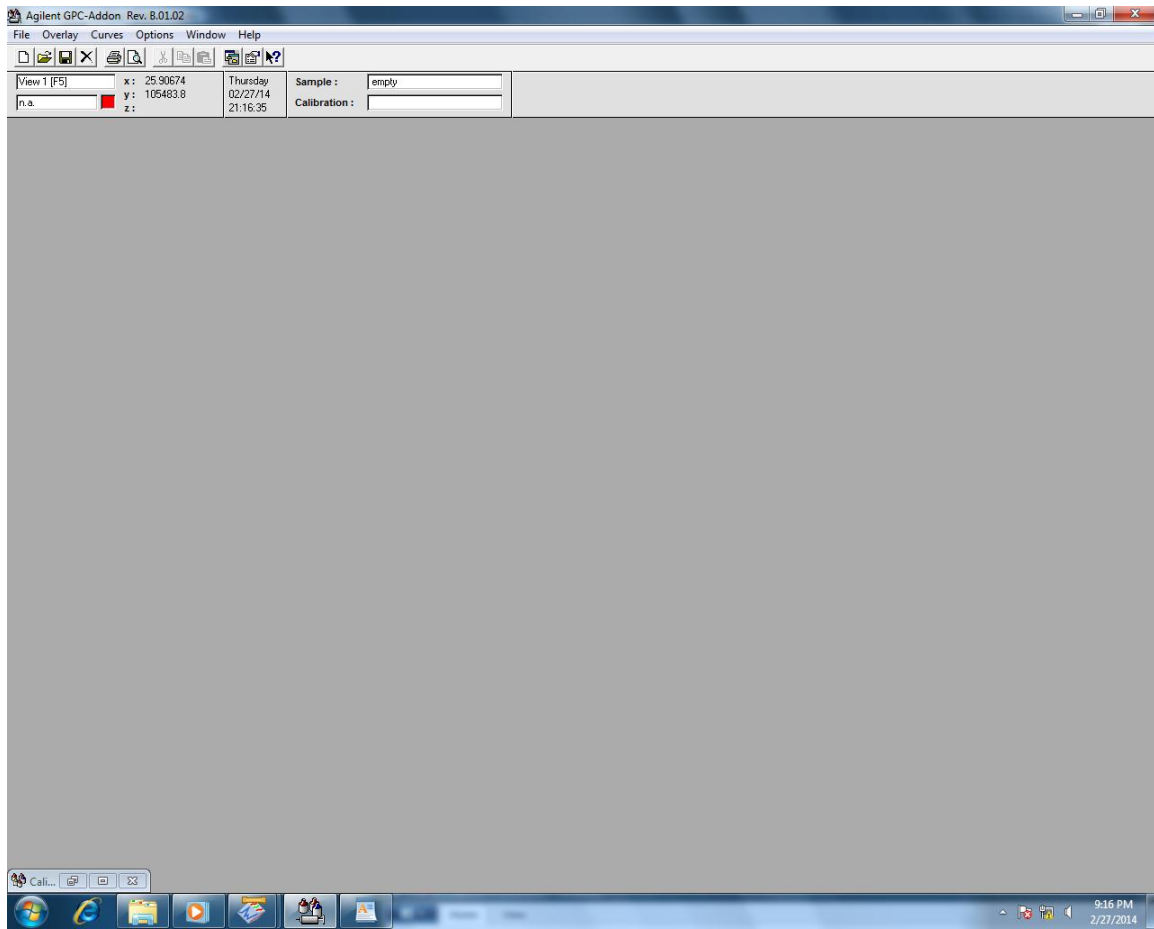
Appendices



Appendix 1: Instrument Control (online) software: Method and Run Control Tab. (1) Sample control, (2) Pump Control, (3) Column Temperature Control, (4) Diode Array/Detector Control, (5) Refractive Index (RI) Control, (6) Access Sequence Context Menu, (7) Online Plot, (8) Sequence, (9) Data Analysis.



Appendix 2: Instrument Control (online) software: Data Analysis Tab.



Appendix 3: Agilent GPC-Addon for GPC peak analysis.