**LAB: Light Scattering Batch Experiment – Zimm Plots**

### Topics:

* Use of a MALS detector and flow cell for unfractionated samples (batch mode)
* Measure the ***weight average molar mass (Mw)***, the ***z-average root mean square radius (rms radius)***, and the ***second virial coefficient (A2)*** of a Dextran in aqueous solution.

### Goals:

* To become familiar with instrument and ASTRA software set-up
* Collection of batch data
* Optimization of fit parameters for generating a Zimm Plot for the analysis of unfractionated samples.

### What is a batch measurement?

* Measure weight average molar mass, z-average rms radius, and A2 of an ***unfractionated*** sample.

### When to use a batch measurement?

* No SEC method available (e.g. different column and/or different solvent needed)
* The column is suspected to alter the sample (*e.g.* high Mw polymers, adsorption or shearing of the sample on a column)
* To measure A2
* To determine equilibrium constant and kinetics
* To monitor a reaction

### How to perform a batch measurement?

* Measuring LS detector cells: **flow cell**, scintillation vial (HELEOS), microCuvette
* Need to know: dn/dc, concentration (can be also measured with RI or UV in series with LS detector)

### Experimental setup with a flow cell:

* Goal: Stable LS signal (plateau) with known corresponding concentrations
* Different setups:
  + **HPLC pump and a manual injector (500 – 1000 μL)** or
  + **Syringe pump and syringes**
  + Calypso system: three syringe pumps to deliver concentration gradients and online concentration measurement (more on that in a Scatter Session on Day 3)
  + On-line A2 determination (more on that in a Scatter Session on Day 3)
* Key point: Delivery of an air bubble-free and particle-free solution to the flow cell

### Specifics of this lab:

* Goal: Generation of a Zimm plot with at least 5 concentrations
* Use either syringe pump or HPLC pump with DNDC injection system (WISL), flow rate ~0.5 mL/min, exact flow rate not critical
* Collect baseline at start and end of the acquisition: aqueous solvent (100 mM NaNO3, 200 ppm NaN3 as preservative)
* Normalization standard (10 kDa Dextran at ~ 10 mg/mL)
* Five concentrations of sample (0.2– 2.0 mg/mL of high Mw Dextran sample)
* Plumbing of the tubing:
  + Reverse inlet and outlet plumbing to take advantage of the smaller diameter of the inlet tubing to pressurize the flow cell to keep air bubbles dissolved in solution.
* Filters:
  + 0.02 μm (or smallest available pore size) syringe tip filter for solvent
  + 0.2 μm for normalization standard
  + 0.45 μm for high molar mass dextran solutions
* Syringes:
  + For aqueous solvents – rubber tip syringes are okay as well as all polymer or glass
  + For organic solvents – do NOT use syringes containing plungers with a rubber tip as it will dissolve and contaminate your sample. Use all polymer or glass syringes, only!

### Topics:

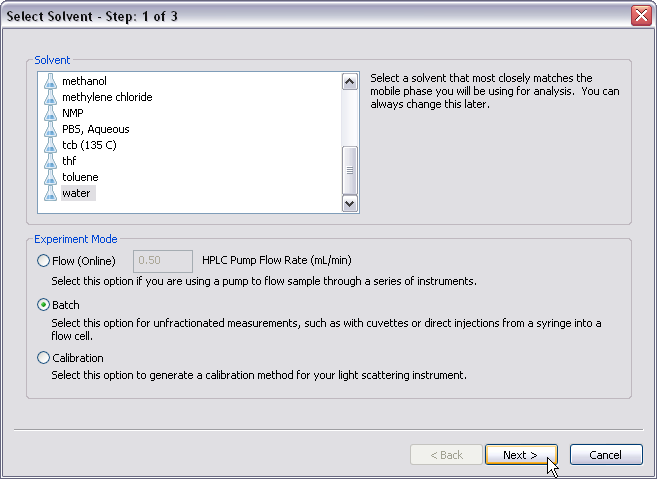
* Setting up a syringe and a syringe pump (or Wyatt DNDC injector kit with an HPLC system) to pass solvent and samples through the DAWN or miniDAWN flow cell.
* Detecting air bubbles in the flow cell visually and by use of the forward laser monitor.
* Assessing the quality of flow cell cleanliness.
* Normalization using an isotropic scatterer (small molar mass polymer).
* Collection of data in ASTRA.
* Processing light scattering data and the generation of a Zimm Plot.
* Evaluation of the Zimm Plot.
* Selecting detector and concentration fit orders
* Deleting concentrations and/or detectors if needed
* Try different polynomial detector fit models (Debye, Zimm and Berry)

### Notes:

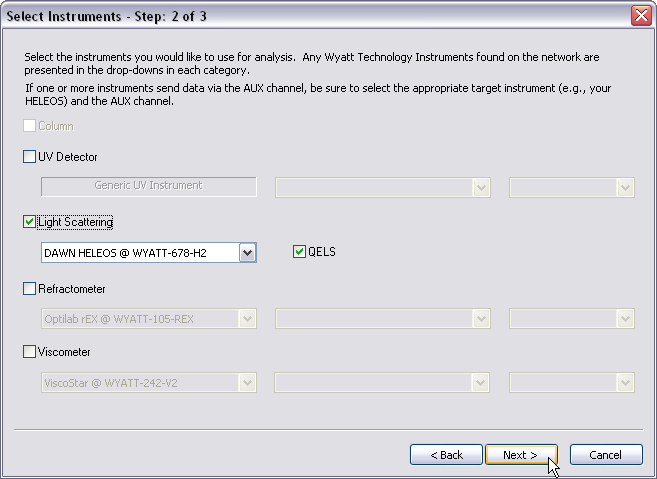
* A list with part numbers of supplies and equipment used in this session can be found in **Section 14** of your LSU course binder.
* Example ASTRA data files for both HELEOS and TREOS are located on your USB memory drive in: **LSU Training Binder\04 – Light Scattering Batch Lab.**
* Instructions on how to perform Zimm plots using ASTRA 5 software are located on your USB memory drive in: **LSU Training Binder\04 – Light Scattering Batch Lab\04 - LS Batch Lab Zimm Plot 2011 - ASTRA 5.**

**ASTRA 6 Data Collection:**

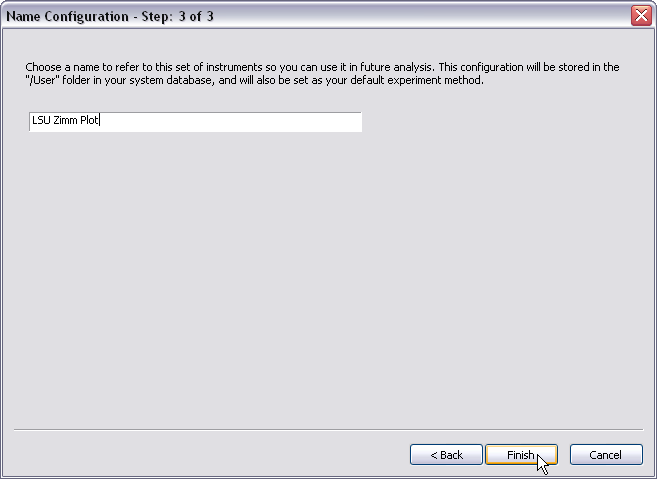
***Open*** ASTRA 6 (current version) and select **System** → **Configuration Wizard**. In the **Select** **Solvent** Dialog, select your **Solvent** (water) and set the **Experiment Mode** to **Batch**.



Click on **Next** to go to the **Select Instrument** Dialog. Select your Light Scattering Instrument (e.g. Heleos or Treos). If your instrument has a QELS unit, the QELS checkbox will be automatically checked.



Click on **Next** to go the **Name Configuration** Dialog. Choose a name for this configuration, e.g. LSU Zimm Plot. Click on **Finish**. You have now created a Method that you can run.



In ASTRA, go to **File** → **New** → **Experiment from Default**. This will open a new file called **Experiment 1** using the **Configuration** that you’ve just created. We will now need to make a few changes to our Configuration and Procedures before we can start data collection.

Note that you can start a data collection immediately and change all parameters post acquisition, but it’s just better practice to set everything up correctly before starting a run.

### Configuration:

* Expand the “+” sign next to **Configuration** and double-click on **DAWN HELEOS** (or **miniDAWN TREOS**).
* Change the **Sample Cell** to the correct type. We are going to use the flow cell for our Zimm plot experiment today, so the type will be **Fused Silica** (for newer instruments) or **K5** (for older instruments). You may also use the **Microcuvette** or **Scintillation vial** for this type of experiment.
* Enter the **Calibration Constant** for your instrument (from the Certificate of Performance or your most recent calibration with toluene). Note: The calibration will need to be performed with the correct **Sample Cell**, e.g. when you use the Microcuvette, you will also need to calibrate the Microcuvette.
* If you have a HELEOS with QELS, check that the **Replaced Detector** is correct (Default is 12).
* Click on **OK** to save your settings and close the DAWN HELEOS tab.
* Click on the “+” sign next to DAWN HELEOS and double-click on **Sample**. Give your sample a **Name** (e.g. *Dextran*) and change **dn/dc** to the correct value, e.g. **0.138 mL/g** for dextran. Click on **OK** to save your settings and close the Sample tab. We’ve now set all the correct configuration parameters for our Zimm plot experiment.

### Procedures

* Note that the Procedures in our Experiment do not include the Zimm plot analysis (they are actually a Debye Plot – more about that tomorrow). To perform the Zimm plot analysis, we will need to apply a different *Method*.
* In the File Menu, go to **Experiment** → **Apply Method** or right-click on your Experiment in the Workspace and select **Apply Method**. This will open the **New from Existing** Dialog. In the Dialog, navigate to **System** → **Methods** → **Light scattering** → **batch (Zimm plot)**. This will create a new experiment that is called **batch (Zimm plot) (from Experiment 1)**. This new experiment contains all necessary procedures, i.e. *Basic collection*, *Despiking*, *Baselines*, *Peaks*, and *A2, Molar Mass &Radius from LS*.
* In the **Basic collection** Procedure, set the **Duration** to **30 min**. Leave the **Collection Interval** at **2 sec** and the **QELS Interval** at **5 sec** (the QELS Interval parameter is irrelevant if you do not have a QELS or don’t want to collect QELS data). Leave the **Trigger on Auto-Inject** box unchecked.
* Click on **Apply** to save your settings (**Apply** will leave the basic collection window open).

### Data Collection

* Click on the Run Icon (the green arrow on the menu bar) or select Processing → Run to start the experiment.
* Collect 5 minutes of Baseline using filtered solvent
* Collect 3-5 minutes 10 kDa Dextran (for Normalization)
* Collect 3-5 minutes of each concentration of the unknown Dextran in solvent (0.2 – 2.0 mg/mL)
* Finish with original baseline solvent for 3-5 min to complete the baseline.

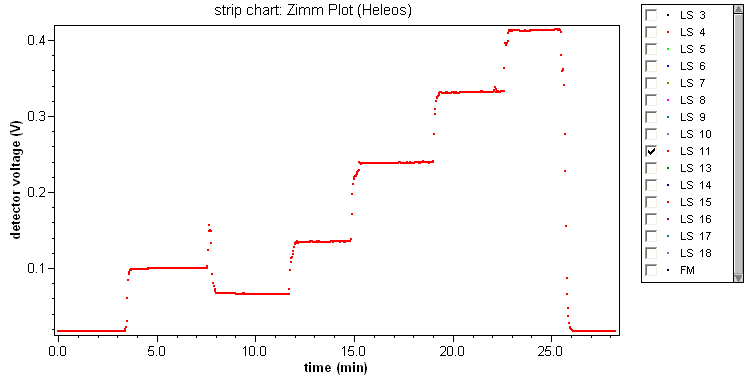
**Important: Syringe-tip filters are required for batch mode light scattering operation!**

**Notes:**

* In ASTRA 6, the data collection can be paused and resumed (this was not possible in ASTRA 5).
* You can always extend the acquisition time by using the **Run Indefinitely** option if you find yourself running short of time (**Experiment \ Run Indefinitely**). Use **Stop Collection** to end the run when the data collection is complete.

### Data Processing

***Basic Collection Window after data collection:***



Baseline

unknown dextran

10 kDa dextran for normalization

***Baselines:***

After the data collection has been stopped, a message will appear that informs you that no baselines have been defined. Click on **OK** to go to the baselines procedure.

* In the **Define Baselines** window, left-mouse click and drag to define the baseline for the 90° detector (LS 11 for the Heleos, LS 2 for the TREOS).
* Click on **Set All** to define baselines for all other detectors. Note: **Autofind Baselines** will generally not work for batch experiments like Zimm plots.
* Check the other detector baselines to verify correct baseline settings.
* Click on **OK** to save your settings and close the procedure. Then click on **OK** in the message dialog that informs you that the file does not have any peaks defined. This will automatically advance to the **Peaks** procedure.

***Define Peaks***

* Left-click and drag on the 10 kDa Dextran plateau to select a flat peak region for normalization
* Click on **OK** to save your settings and close the window.
* **Notes:** 
  + We’ve only set the 10 kDa Dextran peak for Normalization for now. After Normalization we will go back and define all other peaks.
  + Once baselines and peaks have been set, data processing is complete as indicated by the blue vertical arrows on the page icons next to the procedures.

***Normalization***

* To open the Normalization procedure, right-click on **Configuration** and select **Normalize**, or go to **Experiment** → **Configuration** → **Normalize**.
* Select **Peak Name** (**Peak1**) and leave the **Radius** at **3 nm**.
* Click on the **Normalize** button. This will set the normalization coefficients for all detectors, which will now be different from 1.000. Normalization coefficient for the 90° detector will be 1.000 (since it’s the reference). Normalization coefficients for the QELS replaced detector and low angle detectors that are not visible in your sample cell (e.g. detector 1 or also 2 if you have a K5 cell) will also remain at 1.000.
* Click **OK** to save your settings and close the Normalization window. We will now set the peaks for our unknown dextran sample

***Define Peaks***

* Delete peak 1 (only necessary for normalization, not for Zimm plot) by highlighting the peak on the graph or table and hit the “Delete” button on your keyboard.
* Define peaks for concentrations C1-C5 (see below) and enter the concentrations for each plateau, e.g.:

C1 = 0.2 mg/mL

C2 = 0.5 mg/mL

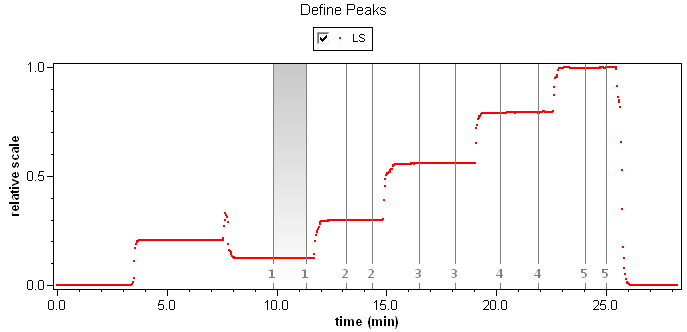
C3 = 1.0 mg/mL

C4 = 1.5 mg/mL

C5 = 2.0 mg/mL

* To zoom in and out of the graph:  
  *Ctrl + left mouse click* and define a region to *zoom in*

*Ctrl + right mouse click* to *zoom out* (stepwise)

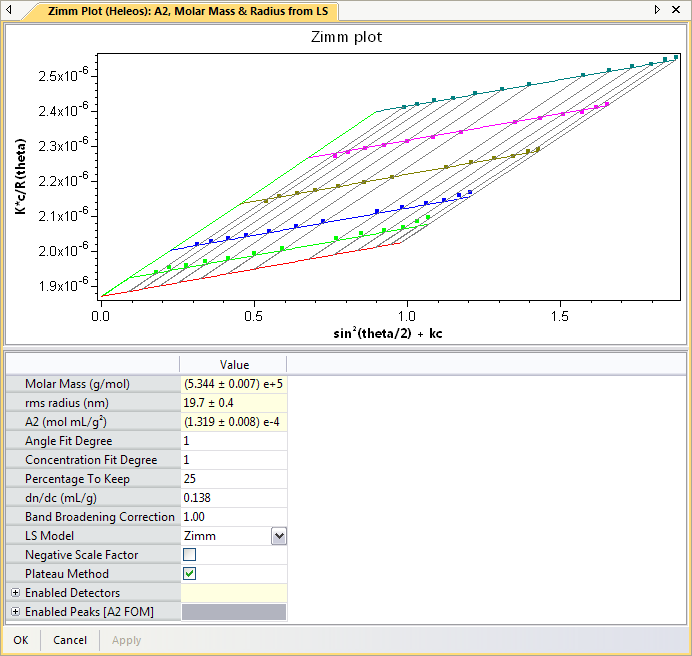


**Important: Select peak regions across the flat section of each individual plateau!**

***And then…***

***A2, Molar Mass and Radius from LS data***

* Double-click on the procedure to open – voila!



**rms radius:**

From slope of angular dependence

**Molar mass:**

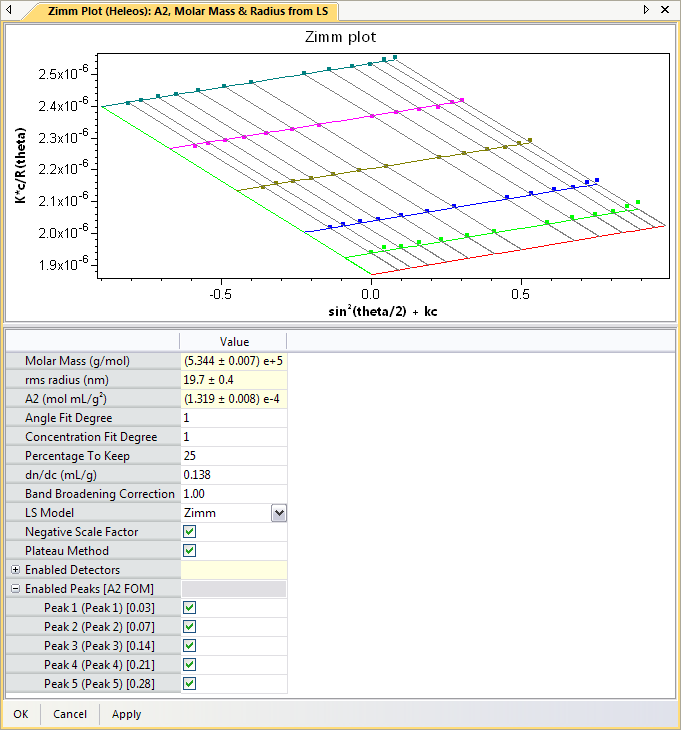
From extrapolation to zero angle and zero concentration (intercept)

**A2:**

From slope of concentration dependence

**A few hints:**

* Make sure that **Plateau method** is checked and the value for *dn/dc* has been entered;
* Delete concentrations and detectors (by expanding the “+” sign of **Enabled Detectors** and **Enabled Peaks** to uncheck selected detectors and concentrations), if necessary;
* Generally, a ***Negative Scale Factor*** **k** (checked as shown on the next page) makes the data easier to view. It does not change the results!



**Molar mass:**

From extrapolation to zero angle and zero concentration (intercept)

**rms radius:**

From slope of angular dependence

**A2:**

From slope of concentration dependence

***Figure of Merit (FOM)***

* The *Figure of Merit* indicates the fraction of the scattered light that originating from the *2A2Mc* term in the light scattering equation. A FOM of 0.05 means that 5% of the signal is due to the contribution of the *2A2Mc* term.
* If the FOM is very low (e.g. 0.05 or less) for the highest concentration measured, the determination of A2 will not be very precise. However, molar mass and rms radius (if greater than 10 nm) can still be determined precisely.
* If the FOM is very high (in the order of 1.0) higher order terms (the third virial coefficient) of the LS equation need to be included in the calculations.