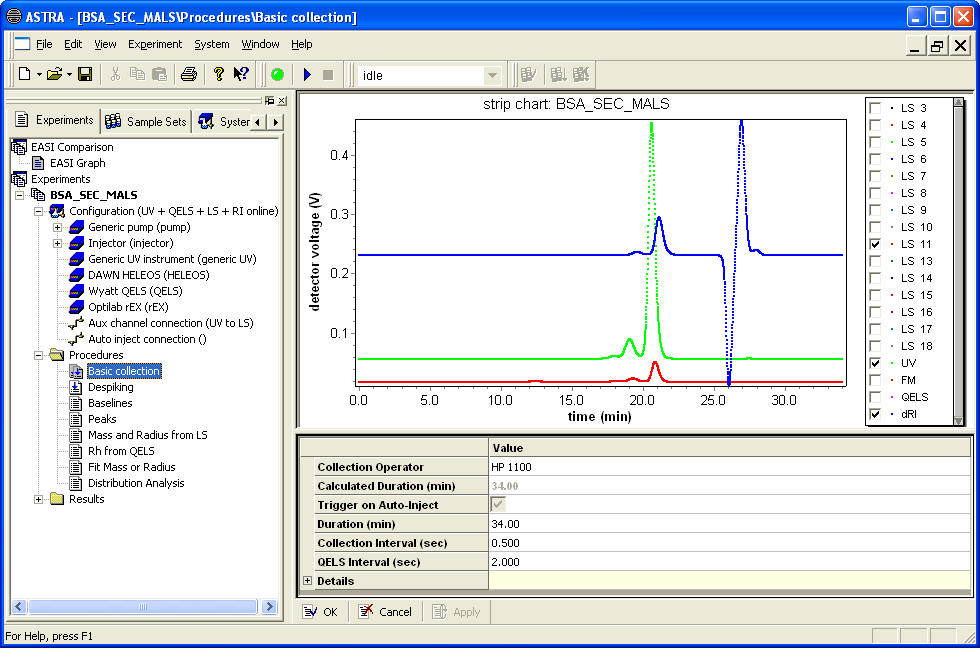
**Computer LAB: SEC-MALS Processing**

### Quick Processing Guide for a validation standard (e.g. BSA or 30 kDa PS) used to determine light scattering parameters for a new HPLC setup (first injection):

**Basic Collection Window after data collection:**



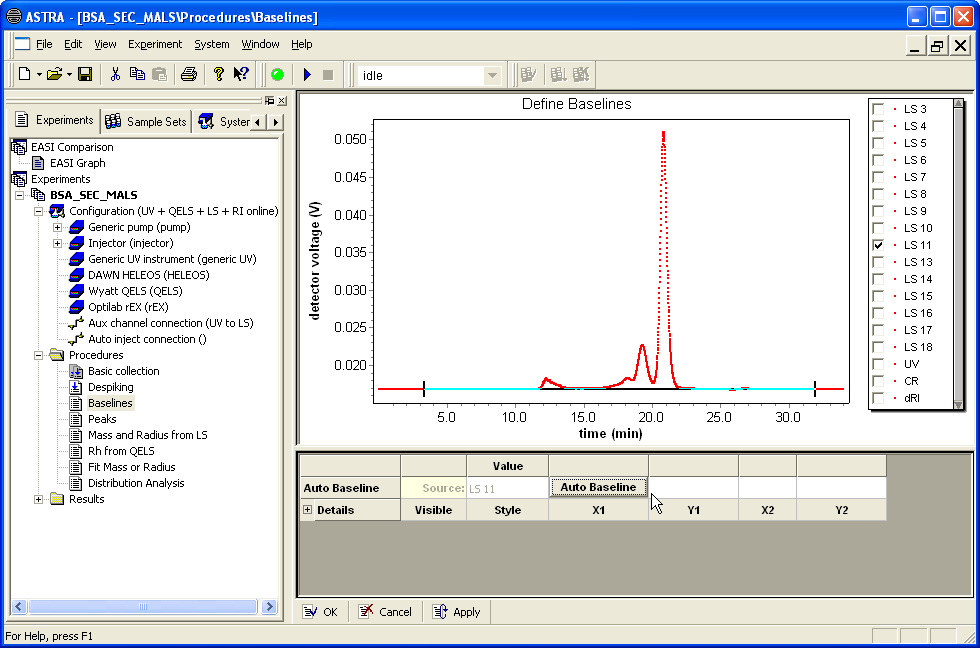
### The processing of a validation standard consists of the following steps:

1. *Setting baselines*
2. *Selecting a peak for Normalization*
3. *Normalization: sample must be an isotropic scatterer (rms radius < 10 nm)*
4. *Alignment (Determine Interdetector Delay): sample must be monodisperse (Mw/Mn < 1.05)*
5. *Band Broadening: sample must be monodisperse*
6. *Adjust peak selection and determine molar mass*

* After performing these processing steps, the experiment can be saved as a **Template** for further use with this particular HPLC setup and mobile phase. Once all these processing steps have been done, you can use this template for all your future data collections.
* This template contains both ***Configuration*** information (Instruments, Normalization, Alignment and Band Broadening) as well as ***Processing*** parameters (if set).
* Refer to the ASTRA manual, tutorials, and ASTRA Processing Workshop for more details.

### 1. Setting Baselines:

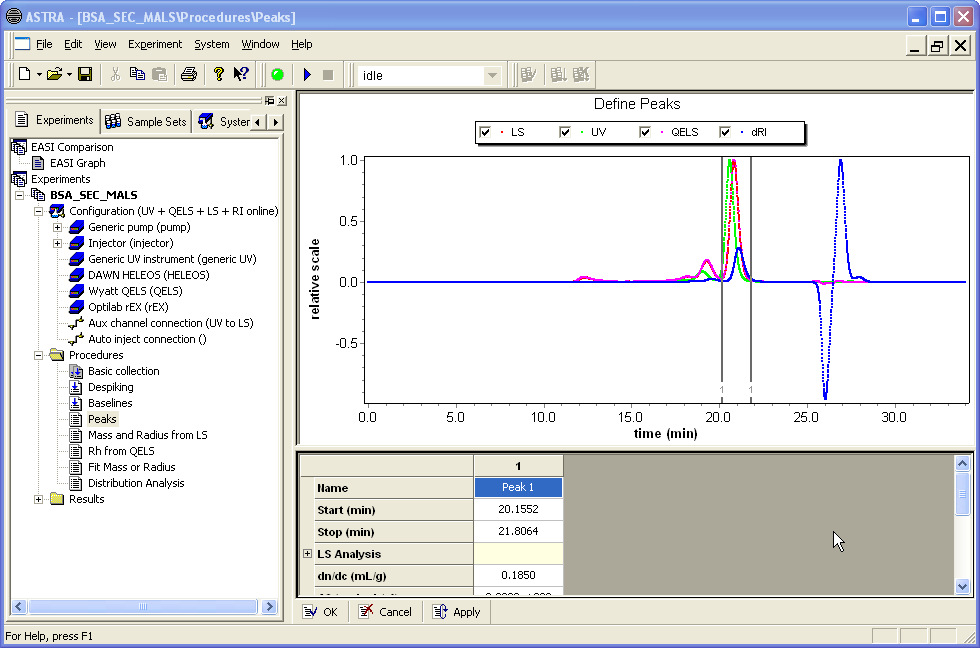
* Click on the **Run** button again to process your data. You will be prompted to set your baselines in the **Define Baselines** window.
* Set baseline for the 90° detector (HELEOS: detector 11, TREOS: detector 2)
* Click on **Autobaseline** to apply settings to all detectors, check baselines for all detectors, in particular for the concentration detectors. Zoom into the graph for better viewing.
* Note: The **Despiking Procedure** will be performed automatically. The default despiking setting is **Normal**. Change to **None** or **Heavy** if desired.



* Click **OK** to save your settings. This will automatically take you to the **Peaks** Procedure.

### 2. Selecting a peak for Normalization:

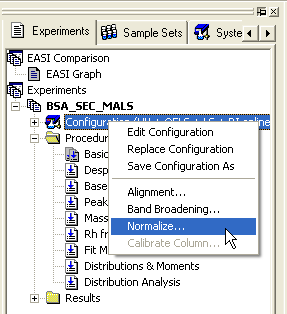
* In the **Peaks** Procedure, select the upper third of the light scattering peak by clicking and dragging across the main peak as shown below (HELEOS: detector 11, TREOS: detector 2)



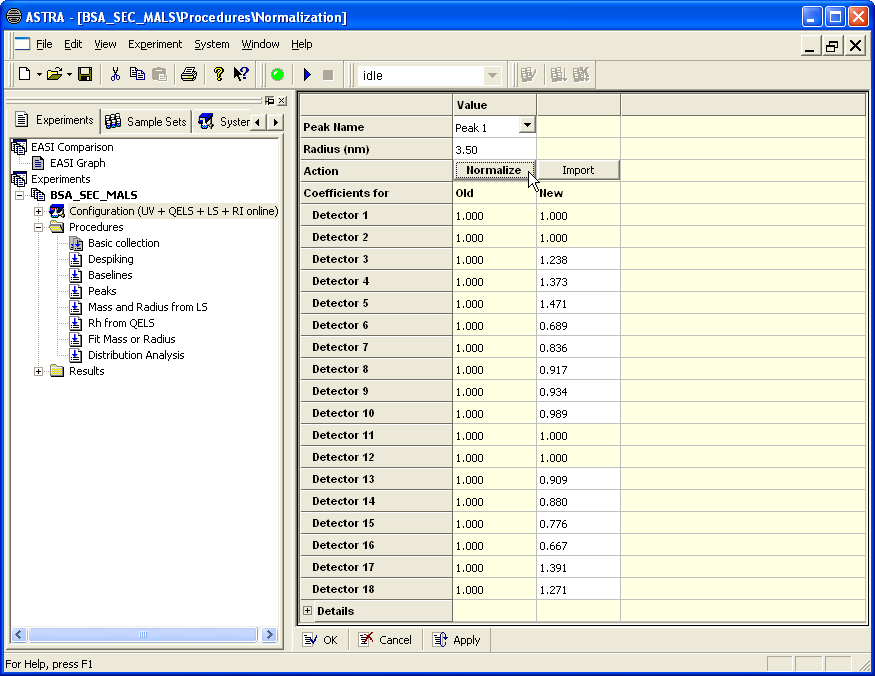
Click **OK**. All procedures have now been run.

### 3. Normalization:

* Right-Click on **Configuration** to select the **Normalization** Procedure:



This will activate the **Normalization** window.

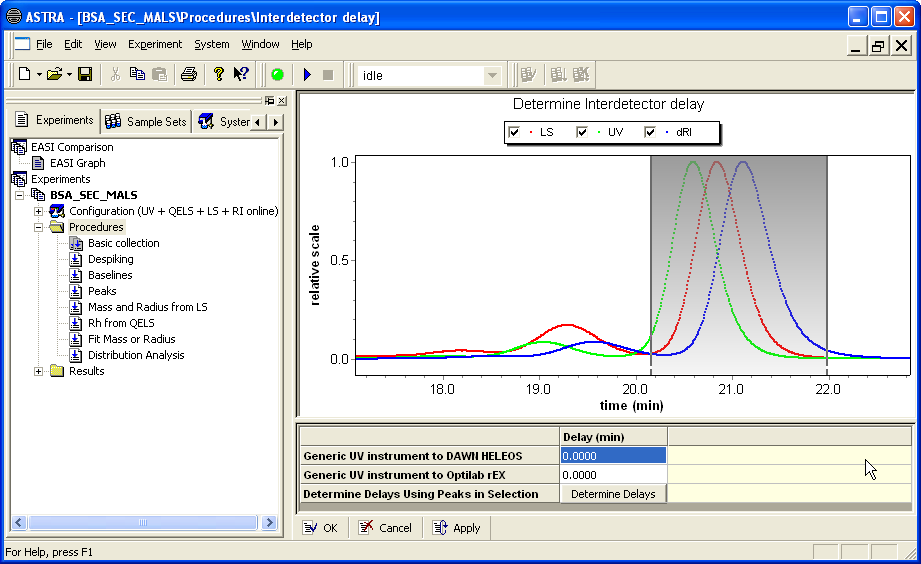


* Select the appropriate **Peak Name** (here: Peak 1) and **Radius** (BSA: 3.5 nm) for your sample. ***Note***: you can estimate the radius for a sample of unknown radius as long as the sample is an isotropic scatterer.
* Click on **Normalize**. All detectors except the 90° detector and the QELS detector should be different from 1.000.
* You can also **Import** Normalization Coefficients from another open ASTRA data file.  
    
  ***HELEOS users only:*** Due to the refraction at the glass/solvent interface, Detector 1 is only available in batch work with a scintillation vial. Detector 2 cannot be used in aqueous buffers, so both detectors have normalization coefficients of 1.0 in this example.  
  Additionally, Detector 12 has been replaced with the QELS optical fiber in this instrument, so the Normalization coefficient is 1.000 for this detector as well.

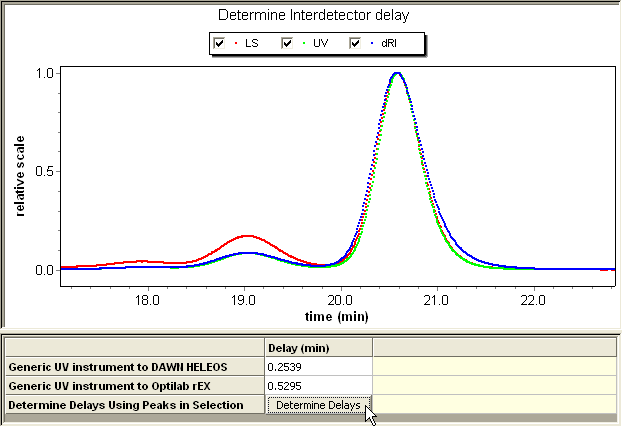
Click **OK** to close the **Normalization** window.

### 4. Alignment:

* Right-click on **Configuration** and select **Alignment**. This will open the **Determine Interdetector Delay** window.
* Zoom into the BSA monomer peak and select a peak region as shown.



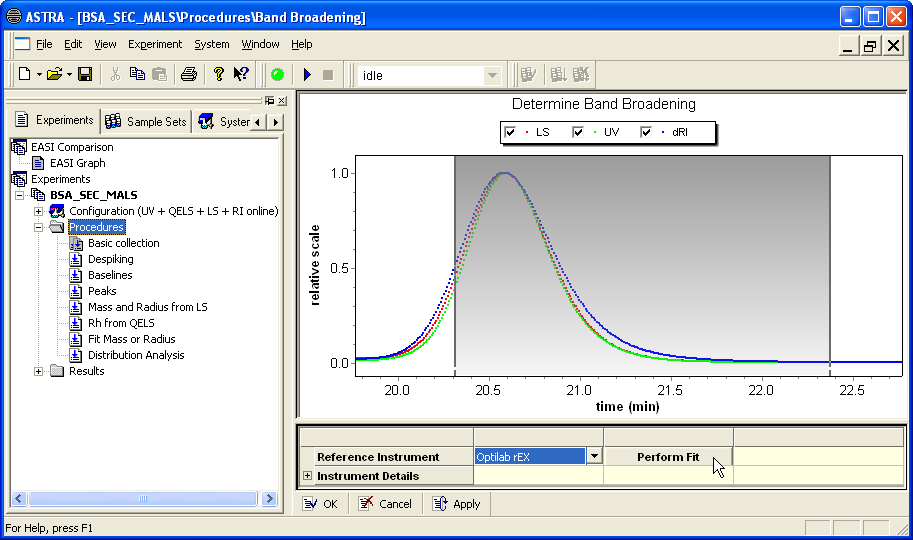
* Click on **Determine Delays**. The **Delays** will be different from zero and the properly aligned detectors will look like this:



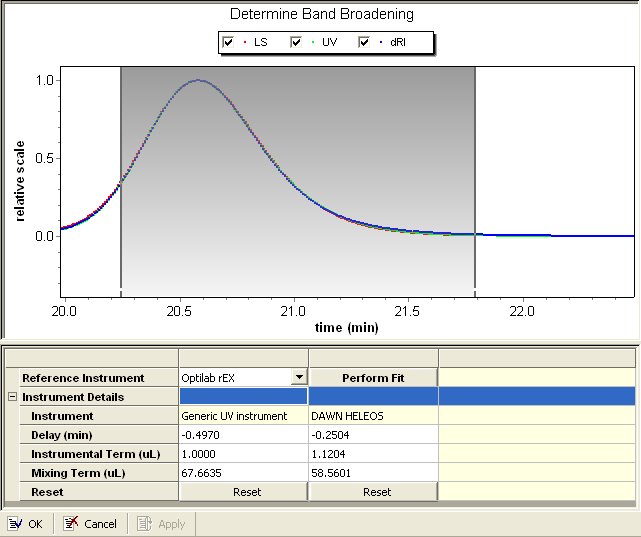
* Peak broadening is particularly visible for the dRI detector. This will be corrected in the **Band Broadening procedure** below. Click **OK** to close the **Determine Interdetector Delay** window.

### 5. Band Broadening:

* Right-Click on **Configuration** and select **Band Broadening**. This will open the **Determine Band Broadening** Procedure.
* Select the limits of the BSA monomer peak as shown (you want to set the left peak limit at ca. half height of the peak.



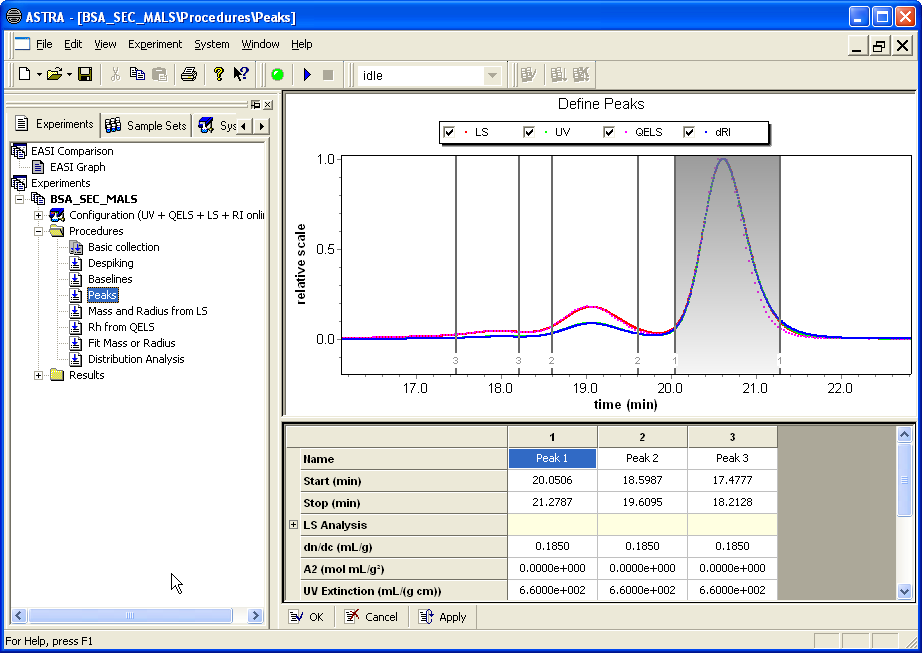
* The Reference Instrument will be automatically selected. Click on **Perform Fit.**
* The properly broadened peak should now look like this:



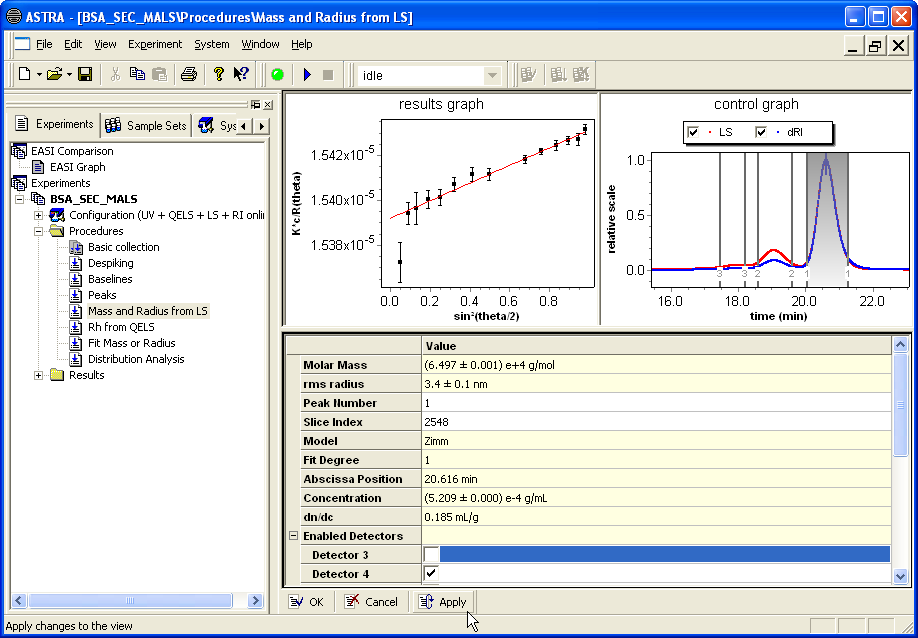
* In **Instrument Details**, you can check that the **Instrumental Terms** should be close to 1.0 and the **Mixing Terms** for a UV or LS detector will be approximately 60.
* Click **OK** to save your settings and close the **Determine Band Broadening** window.

### 6. Adjust Peak Selection and determine molar mass:

* Double-click on the **Peaks** procedure in the workspace and adjust your peak range settings:

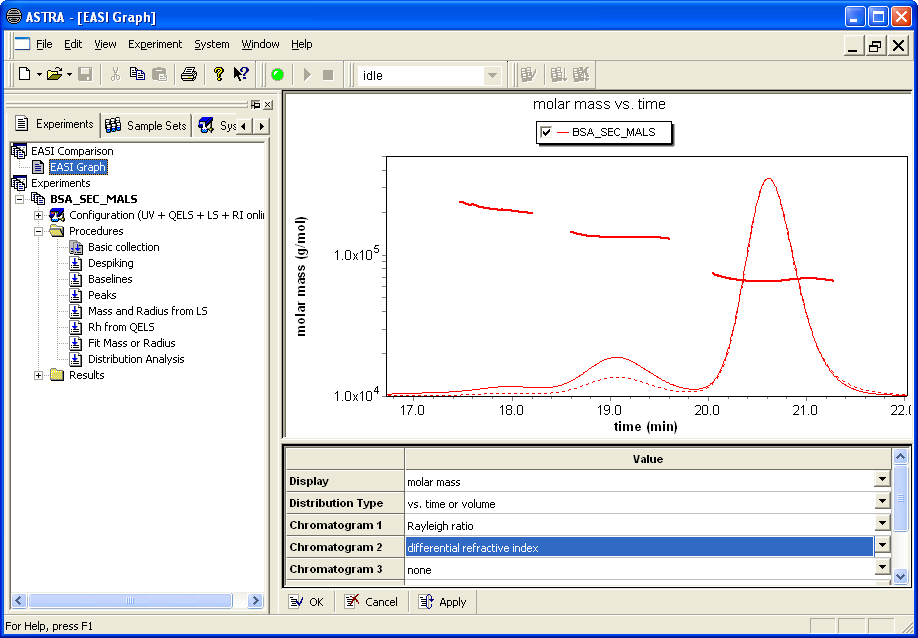


* Click **OK** to close the **Define Peaks** window
* Open the **Mass and Radius from LS** procedure. All data points in the **Results Graph** (Debye Plot) should form a straight line (within the error bars). Some of the low and high angle detectors might need to get disabled to achieve this (see e.g. detectors 3 and 4 below).
* The molar mass and the radius are displayed for each slice of the selected peak in the chromatogram.

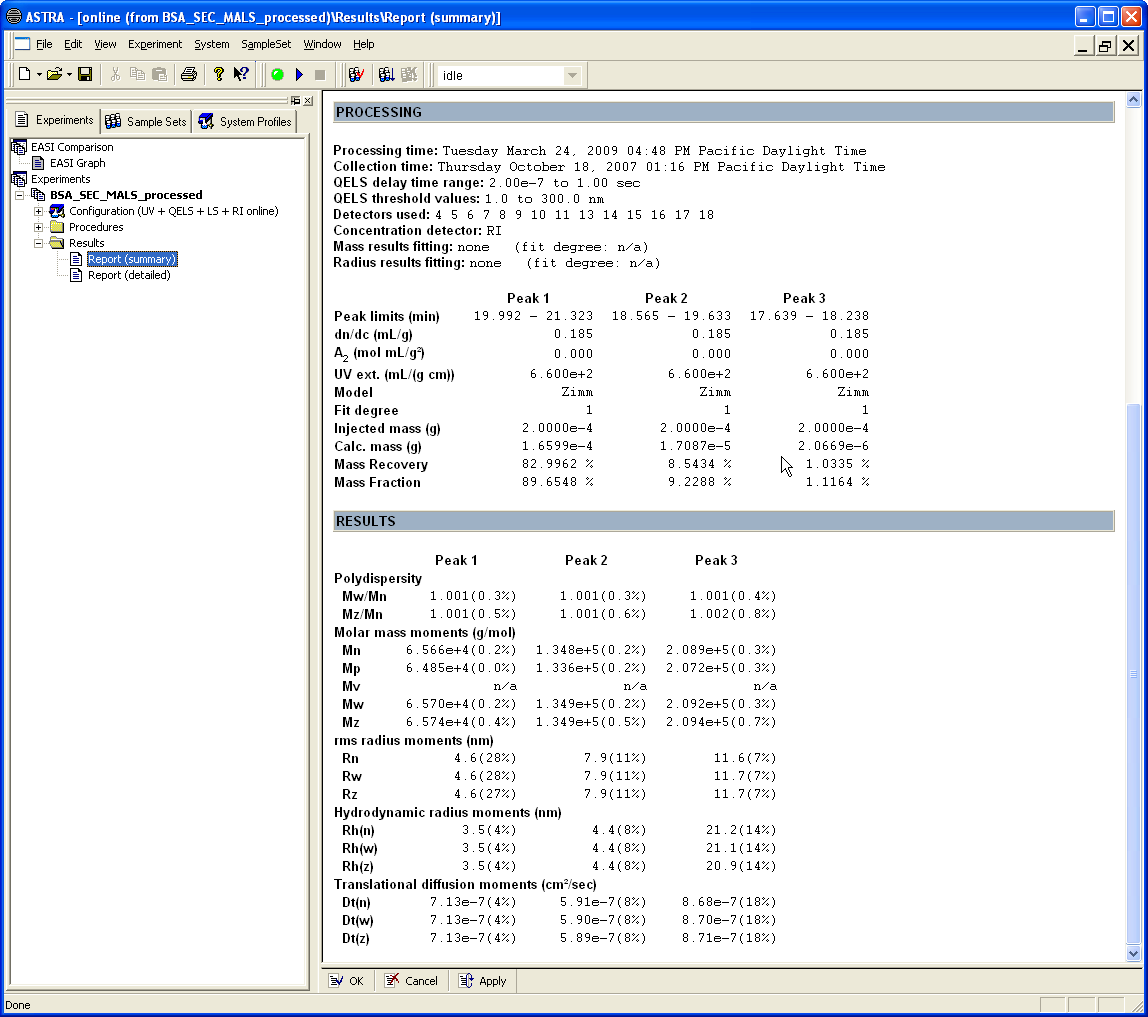


The molar mass can be displayed either graphically or numerically as shown on the next page.

**Graphical display in EASI Graph:**

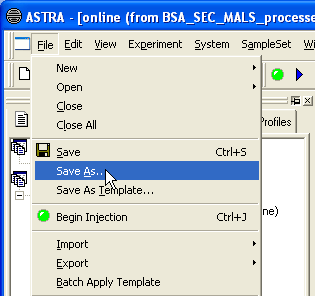


**Numerical display as Report in the Results folder:**



### Running and processing data once a first injection sample has been run and processed (see above)

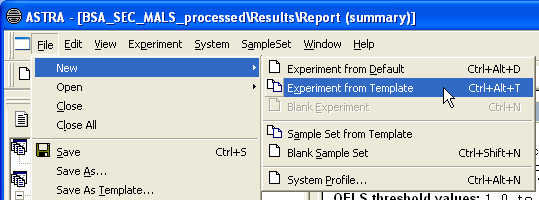
* 1. Save the data file that you have processed above (e.g. My\_First\_BSA\_Injection.vaf)



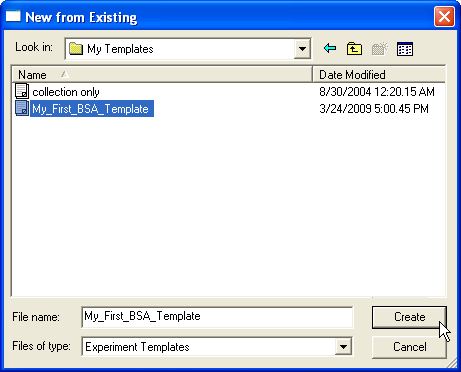
* 1. Also save this file as a template in the My Templates Folder (e.g. My\_First\_BSA\_Template)

|  |  |
| --- | --- |
|  |  |

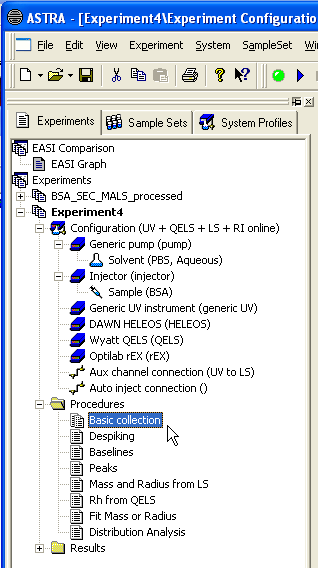
* 1. For the next experiment, select File → New → Experiment from Template



* 1. Select your template (My\_First\_BSA\_Template)in the My Templates and click Create:

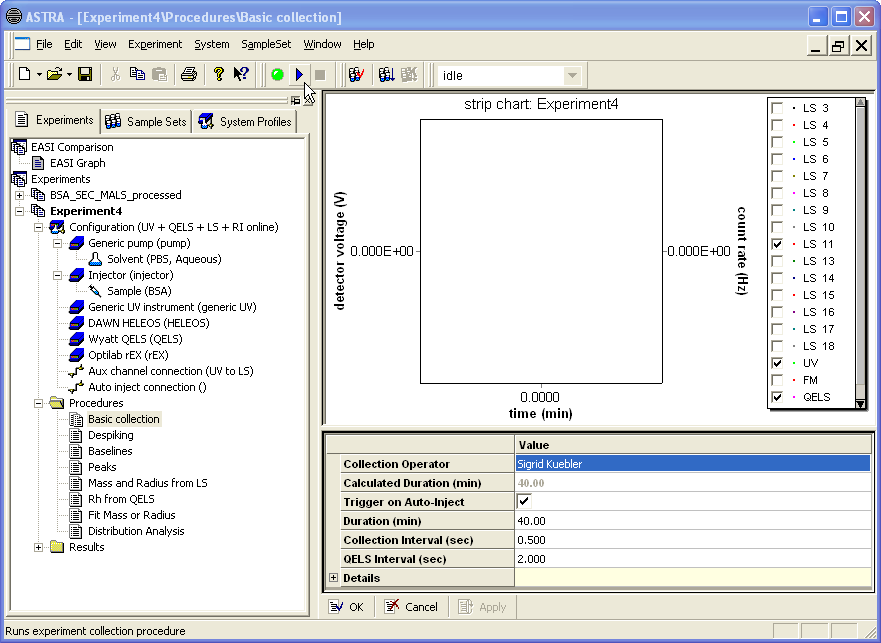


* 1. This will open up a new Experiment in the ASTRA workspace:



**Note that all Configuration parameters, such as your pump, Injector and physical instruments, as well as Normalization Coefficients, Interdetector Delay Volumes and Band Broadening are already set correctly in this template for your current HPLC system setup.**

* 1. Set Duration and Collection Interval for your new experiment and click on the “Run” icon to start data collection. You may want to adjust parameters, such as dn/dc or UV extinction coefficients as well (can be also done post-acquisition).



* 1. After data collection is completed, you may have to click the “Run” icon again to process your data.

***You will now only need to:***

* + - set or adjust baselines,
    - select peak limits and adjust parameters, e.g. dn/dc, UV extinction coefficient, ..
    - and view your results!

***Notes:***

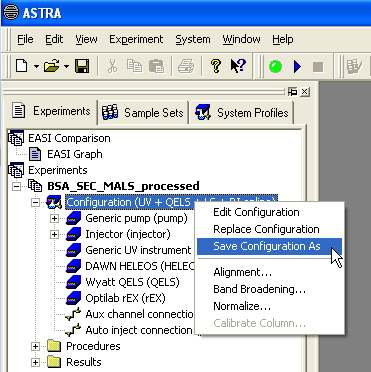
* If you template contains processing information such as baselines and peak settings, the data file will be automatically processed. If you do not want processing parameters in your templates, delete those and then save this data file as a *template*.
* If you change your HPLC setup (e.g. tubing between detectors, add detectors) or change your mobile phase, you may need to renormalize or determine Interdetector delay volumes and Band Broadening Parameters. Please refer to Section 6 of your LSU course binder for more information.

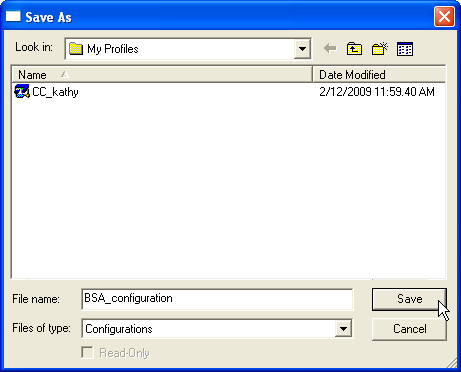
### Applying Normalization Coefficients, Interdetector Delay and Band Broadening parameters after a data file has already been run (Replacing Configurations)

***Notes:***

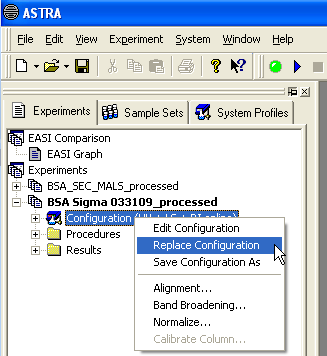
* On existing data, *Templates* only apply to *Procedures*, not settings such as Normalization Coefficients, Interdetector Delay, Band Broadening parameters, Calibration constants, solvents, etc. which are part of the *Configuration* of an Experiment.
* To change these *Configuration* parameters, the *Configuration* of the existing data file will need to be *replaced*.

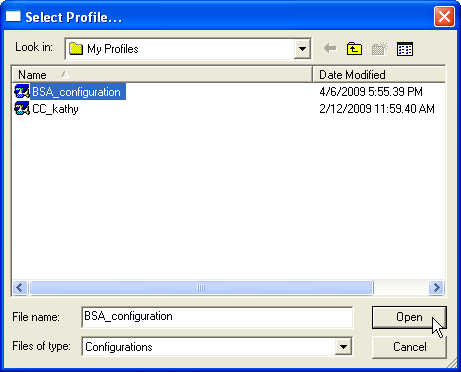
1. Save the configuration of a data file that has the desired settings in the My Profiles folder (e.g. “BSA\_configuration”):





2. Open the data file that you want to apply this Profile to and choose Replace Configuration with the desired Profile (“BSA\_configuration”):





|  |  |
| --- | --- |
|  | ***The data file  “BSA Sigma 033109\_processed”  will now contain the desired Configuration parameters, such as Normalization Coefficients, Interdetector delay volumes, Band Broadening parameters, etc.*** |

ASTRA Calculations Workshop

**Objectives**:

1. To learn how ASTRA computes molar masses, radii, and computed masses and the role that constants and processing parameters play in those results and their uncertainties.

2. To learn more advanced processing procedures such as assessing quality of the data using the overlay plot, selection of fit formalism and polynomial order, on-line dn/dc determinations, and results fitting.

3. Introduction to EASI graph - distribution plots and custom plots.

**BSA\_SEC\_MALS.vaf** (or similar BSA injection**):**

found in LSU memory key, ASTRA example files or collected during LSU lab.

1. Set baselines, peaks, and alignment; normalize, calculate band-broadening parameters. These procedures are covered in detail in the corresponding HTML self-guided tutorial SEC-LS Characterization.chm.
2. Students learn that alignment, band broadening, and normalization coefficients need not be measured for every sample run.
3. Review baseline noise, effect of changing the collection interval, and the role of De-spiking. ASTRA chooses the lowest data point in a collection interval, so increasing the collection interval is one way to de-spike the data.
4. Dissect the Debye plot in “Determine mass and radius from LS data” window.
5. Quick review of slope-intercept results from Debye plot.
6. Demonstrate moving through the chromatogram for slice-by-slice results.
7. Show how to disable individual detectors.
8. Discuss known sources of uncertainty: baseline noise in first & last 10% of collection + a fit line least squares regression through LS data.
9. Discuss the use of LS Model and LS fit degree, changing those values in the Peaks window. Explain the fundamental difference in capabilities of miniDAWN vs. DAWN.
10. Show calculation for concentration: [ RI(V) \* RIcc ÷ dn/dc ] or simply (RIU ÷ dn/dc) for rEX.
11. Work through detailed report, line by line. Discuss “Calculated Mass” and the role of flow rate in this calculation. On-line dn/dc uses injected mass, and this topic will be covered in a later section.

**Mark-Houwink Plot (PS706).vaf:** Poly(styrene) Processed - found in ASTRA example files.

1. Show EASI graph distribution plots - discuss.

2. Show “Fit mass or radius procedure.” Explain and discuss uses.

3. Demonstrate right-click on EASI graph to export .jpg and Excel files.

**Note for QELS & DynaPro users:**

Custom aqueous solvent viscosity calculator “SEDNTERP” freeware is available at www.jphilo.mailway.com

Download & install SDTR0601.exe. A copy of the program is available on your USB memory key in: **\Other Resources.**

|  |
| --- |
| **ASTRA 5.3.4 Detailed Report for BSA\_SEC\_MALS\_processed** |

**Experiment name:** \\Wyatt-data\Users\skuebler\LSU\ASTRA data files for LSU presentations\ BSA\_SEC\_MALS\_processed

**Sample:**

**Description:** BSA  
**Concentration:** 2.000e-3 g/mL  
**Injected volume:** 0.100 mL

**Processing Operator:** Sigrid Kuebler

**Collection Operator:** HP 1100

**Collection Astra Version:** 5.3.4.4

|  |  |  |
| --- | --- | --- |
| TFN1B94 |  |  |

|  |  |
| --- | --- |
| TFN1B97 | TFN1B9A |

|  |  |
| --- | --- |
| TFN1B9D | TFN1BA0 |

|  |  |  |
| --- | --- | --- |
| TFN1BA3 |  |  |

|  |
| --- |
| **CONFIGURATION** |

**Light scattering instrument:** DAWN HELEOS

**Cell type:** K5  
**Laser wavelength:** 658.0 nm  
**Calibration constant:** 2.7156e-4 1/(V cm)  
**Replaced detector:** 12  
**Temperature control:** n/a  
**Temperature:** n/a  
**Band broadening correction:** yes   (Instrumental: 1.000 uL   Mixing: 59.031 uL)  
**Collection interval:** 0.500 sec

|  |  |  |  |
| --- | --- | --- | --- |
| **Detector** | **Scattering angle** | **Gain** | **Normalization coefficient** |
| 1 | n/a | n/a | 1.000 |
| 2 | n/a | n/a | 1.000 |
| 3 | 14.4° | n/a | 1.230 |
| 4 | 25.9° | n/a | 1.371 |
| 5 | 34.8° | n/a | 1.471 |
| 6 | 42.8° | n/a | 0.689 |
| 7 | 51.5° | n/a | 0.835 |
| 8 | 60.0° | n/a | 0.917 |
| 9 | 69.3° | n/a | 0.933 |
| 10 | 79.7° | n/a | 0.989 |
| 11 | 90.0° | n/a | 1.000 |
| 12 | n/a | n/a | 1.000 |
| 13 | 110.7° | n/a | 0.909 |
| 14 | 121.2° | n/a | 0.880 |
| 15 | 132.2° | n/a | 0.776 |
| 16 | 142.5° | n/a | 0.668 |
| 17 | 152.5° | n/a | 1.391 |
| 18 | 163.3° | n/a | 1.271 |

|  |  |
| --- | --- |
| **Aux channel** | **Gain** |

**RI Instrument:** Optilab rEX

**Cell type:** n/a  
**Wavelength:** 658.0 nm  
**Band broadening correction:** n/a   (Instrumental: n/a   Mixing: n/a)

**UV Instrument:** Generic UV instrument

**Wavelength:** 280.0 nm  
**Cell length:** 1.000 cm  
**UV response factor:** 1.000 AU/V  
**Band broadening correction:** yes   (Instrumental: 1.000 uL   Mixing: 67.445 uL)

**QELS instrument:**

**Model:** Wyatt QELS  
**Use temperature probe:** yes  
**Collection interval:** 2.0 sec

**Solvent:** water

**Refractive index:** 1.331  
**Viscosity:** 8.9450e-3 g/(cm sec) (valid if QELS temperature not used)

**Flow rate:** 0.500 mL/min

**Fluid Connections:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Source Instrument** | **-** | **Destination Instrument** | **Delay Volume** |
| Generic pump | - | Injector | 0.000 mL |
| Injector | - | Generic UV instrument | 0.000 mL |
| Generic UV instrument | - | DAWN HELEOS | 0.124 mL |
| DAWN HELEOS | - | Optilab rEX | 0.125 mL |

**Aux Connections:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Source Instrument** | **-** | **Destination Instrument** | **Aux Channel** | **Calibration Constant** |
| Generic UV instrument | - | DAWN HELEOS | 2 | 1.0000 |

|  |
| --- |
| **PROCESSING** |

**Processing time:** Tuesday September 02, 2008 10:03 AM Pacific Daylight Time

**Collection time:** Thursday October 18, 2007 01:16 PM Pacific Daylight Time

**QELS delay time range:** 2.00e-7 to 1.00 sec

**QELS threshold values:** 1.0 to 10.0 nm

**Detectors used:** 4 5 6 7 8 9 10 11 13 14 15 16 17 18

**Concentration detector:** RI

**Mass results fitting:** none   (fit degree: n/a)

**Radius results fitting:** none   (fit degree: n/a)

**Baselines:**

|  |  |  |
| --- | --- | --- |
| **Series** | **Endpoints** | **Type** |
| detector 3 | (1.159, 0.037) -- (33.066, 0.037) | manual x, auto y |
| detector 4 | (1.159, 0.023) -- (33.066, 0.023) | manual x, auto y |
| detector 5 | (1.159, 0.016) -- (33.066, 0.016) | manual x, auto y |
| detector 6 | (1.159, 0.019) -- (33.066, 0.019) | manual x, auto y |
| detector 7 | (1.159, 0.018) -- (33.066, 0.018) | manual x, auto y |
| detector 8 | (1.159, 0.018) -- (33.066, 0.018) | manual x, auto y |
| detector 9 | (1.159, 0.017) -- (33.066, 0.017) | manual x, auto y |
| detector 10 | (1.159, 0.017) -- (33.066, 0.017) | manual x, auto y |
| detector 11 | (1.159, 0.017) -- (33.066, 0.017) | manual x, auto y |
| detector 13 | (1.159, 0.017) -- (33.066, 0.017) | manual x, auto y |
| detector 14 | (1.159, 0.018) -- (33.066, 0.018) | manual x, auto y |
| detector 15 | (1.159, 0.018) -- (33.066, 0.018) | manual x, auto y |
| detector 16 | (1.159, 0.020) -- (33.066, 0.020) | manual x, auto y |
| detector 17 | (1.159, 0.017) -- (33.066, 0.017) | manual x, auto y |
| detector 18 | (1.159, 0.020) -- (33.066, 0.020) | manual x, auto y |
| raw UV absorbance data | (1.159, 0.055) -- (33.066, 0.055) | manual x, auto y |
| count rate | (1.159, 87370.792) -- (33.066, 87814.375) | manual x, auto y |
| differential refractive index data | (1.159, -0.000) -- (33.066, -0.000) | manual x, auto y |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Peak 1** | **Peak 2** | **Peak 3** |
| **Peak limits (min)** | 19.992 - 21.517 | 18.565 - 19.633 | 17.639 - 18.238 |
| **dn/dc (mL/g)** | 0.185 | 0.185 | 0.185 |
| **A2 (mol mL/g²)** | 0.000 | 0.000 | 0.000 |
| **UV ext. (mL/(g cm))** | 6.600e+2 | 6.600e+2 | 6.600e+2 |
| **Model** | Zimm | Zimm | Zimm |
| **Fit degree** | 1 | 1 | 1 |
| **Injected mass (g)** | 2.0000e-4 | 2.0000e-4 | 2.0000e-4 |
| **Calc. mass (g)** | 1.6902e-4 | 1.7087e-5 | 2.0669e-6 |
|  |  |  |  |

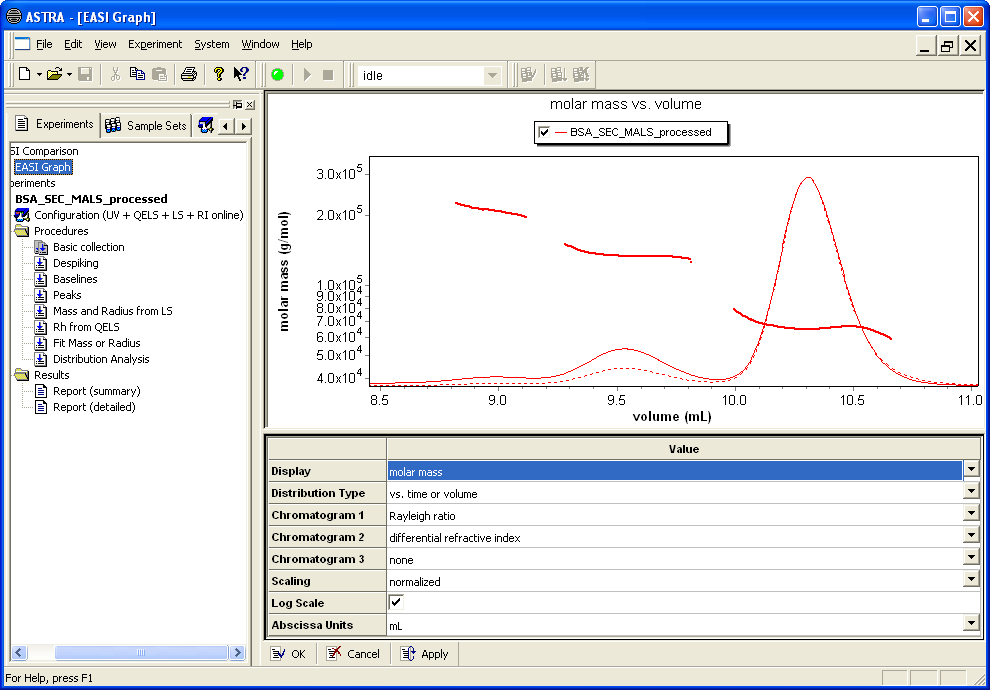
|  |
| --- |
| **RESULTS** |

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Peak 1** | **Peak 2** | **Peak 3** |
| **Polydispersity** | | |  |
| **Mw/Mn** | 1.001(0.3%) | 1.001(0.3%) | 1.001(0.4%) |
| **Mz/Mn** | 1.002(0.5%) | 1.001(0.6%) | 1.002(0.8%) |
| **Molar mass moments (g/mol)** | | |  |
| **Mn** | 6.541e+4(0.2%) | 1.348e+5(0.2%) | 2.089e+5(0.3%) |
| **Mp** | 6.485e+4(0.0%) | 1.336e+5(0.2%) | 2.072e+5(0.3%) |
| **Mw** | 6.549e+4(0.2%) | 1.349e+5(0.2%) | 2.092e+5(0.3%) |
| **Mz** | 6.557e+4(0.5%) | 1.349e+5(0.5%) | 2.094e+5(0.7%) |
| **M(avg)** | 6.500e+4(0.0%) | 1.349e+5(0.0%) | 2.086e+5(0.0%) |
| **rms radius moments (nm)** | | |  |
| **Rn** | 4.9(31%) | 7.9(11%) | 11.6(7%) |
| **Rw** | 4.8(30%) | 7.9(11%) | 11.7(7%) |
| **Rz** | 4.8(29%) | 7.9(11%) | 11.7(7%) |
| **R(avg)** | 3.8(1%) | 7.8(1.0%) | 11.6(0.9%) |
| **Hydrodynamic radius moments (nm)** | | |  |
| **Rh(n)** | 3.5(4%) | 4.2(7%) | 2.4(19%) |
| **Rh(w)** | 3.5(4%) | 4.2(7%) | 2.4(19%) |
| **Rh(z)** | 3.5(4%) | 4.2(7%) | 2.4(19%) |
| **Rh(avg)** | 3.4(0.7%) | 4.0(1%) | 2.1(5%) |
| **Translational diffusion moments (cm²/sec)** | | |  |
| **Dt(n)** | 7.18e-7(4%) | 6.01e-7(8%) | 1.08e-6(18%) |
| **Dt(w)** | 7.17e-7(4%) | 6.00e-7(8%) | 1.08e-6(18%) |
| **Dt(z)** | 7.17e-7(4%) | 5.99e-7(8%) | 1.08e-6(18%) |
| **Dt(avg)** | 6.99e-7(0.7%) | 5.54e-7(1%) | 1.02e-6(5%) |

**Conformation plot slope:** 0.85±0.01 log(nm)/log(g/mol)

**Distribution Analysis Results:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Name** | **Type** | **Start - End** | **Limits (%)** | **Cumulative %** | **Moments** |
| Range 1 | molar mass | 50744.8 - 105113.1 g/mol | 0.3 - 89.8 | 89.5 | Mn=6.546e+004, Mw=6.554e+004, Mz=6.560e+004 g/mol |
| Range 2 | molar mass | 108379.1 - 169279.3 g/mol | 89.8 - 98.9 | 9.1 | Mn=1.348e+005, Mw=1.349e+005, Mz=1.349e+005 g/mol |
| Range 3 | molar mass | 171392.5 - 214042.3 g/mol | 98.9 - 100.0 | 1.1 | Mn=2.061e+005, Mw=2.063e+005, Mz=2.064e+005 g/mol |



**ASTRA FAQs**

**What are the “illegal” characters Astra prohibits from file names?**

The following characters are not allowed as part of file names:

The colon:                     :  
Question Mark:              ?  
Quote:                          "  
Asterisk:                       \*  
Forward Slash:              /  
Back slash:                   \  
Less than:                     <  
Greater than:                 >  
Pipe:                             |

These are reserved by the operating system to indicate path information, disk/device, or are ‘wild card’ characters used for pattern matching of file names.  The standard file browser dialogs will reject these characters, as will the fields containing components of file names (such as the ‘name’ portion of the sample set grid).

**Where does Astra get the names for the “Collection” and “Processing” operator entries in the reports?**

**In ‘basic’ mode:**

* The “Processing Operator” is determined at the time the report is generated, and is retrieved from Windows as the “currently logged in user”.  So on the “Aqueous-1” machine, this will show up as “Aqueous-1”.
* The “Collection Operator” is determined at the time the “StartCollection” message is generated, and is again retrieved from Windows as the “currently logged in user”.   This is then embedded in the collected data, and retained forever after.

**In 21CFR mode:**

* The “Processing Operator” is determined at the time the report is generated, and is the login information for the user currently logged into Astra.  It ignores the Windows “currently logged in user”.
* The “Collection Operator” is determined at the time the “StartCollection” message is generated, and is again based on the login information for the user currently logged into Astra.  It ignores the Windows “currently logged in user”.   This is then embedded in the collected data, and retained forever after.