How to Use ParSEC

A general purpose Size Exclusion Chromatography Software Package

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Please Read

This is your instruction manual for installing and using your Brookhaven ParSEC chromatography software package. Please read it carefully before making measurements. In *Chapter I: Introduction*, you will find a brief overview of the software and what it can do. *Chapter II: Working with The Program, Two Common Tips* explains how to move around the program. And Chapter *III: Quick Start, A Brief Tour of ParSEC* tells you how to setup the software for your detector configuration, how to setup measurements, collect and analyze data. By reading these three chapters, the reader with some familiarity with SEC will be able to make measurements.

Software undergoes periodic improvements. There are always additions and changes. As these become available, they will be added to the back of this manual as appendices. Please look at the appendices if you cannot find the answer to your questions in the numbered sections of the manual.

Please contact the factory at info@brookhaveninstruments.com if you have questions. Please refer to the version number (click Help) of ParSEC that you are using. If you know when the software was purchased, please supply that information too. Please also indicate from whom the software was purchased. We cannot help you unless you have an authorized version.

Remember the old saying: "When in doubt, read the instruction manual." Sometimes the solution to your problem has already been addressed. You just need to find it. Thanks for purchasing Brookhaven software.

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Chapter I Introduction

ParSEC is a general purpose size exclusion chromatography (SEC) software package. It can be used to collect, record, store, and analyze data from concentration detectors (RI, UV, and ELSD), multi-angle static light scattering (SLS) detectors, dynamic light scattering (DLS) detectors, and bridge viscometers (Visc).

The following detectors are especially well suited for use with ParSEC.

- BI-MwA, Brookhaven's multi-angle SLS instrument for molecular weight distribution (MWD) determination.
- BI-DNDC/DeltaDNDC, Brookhaven's and Bures's differential refractometer, an RI instrument for concentration determination.
- NanoDLS, Brookhaven's DLS instrument for sizing.
- BI-2020/Eta2020, Brookhaven's and Bures's dual detector including an RI and a bridge viscometer for intrinsic viscosity and Mark-Houwink-Sakurada (MHS) parameter determination.

However, Agilent, Waters, Shimadzu, and other manufacturer's detectors can be accommodated too.

Using one of the concentration detectors, and molecular weight standards of the same chemistry as the unknown polymer, ParSEC can be used to calibrate columns and produce a MWD. This is called standard calibration.

Using one of the concentration detectors and a viscometer, as well as many standards, ParSEC can be used to calibrate columns and produce a MWD without the need for the unknown polymer to match the chemistry of any of the standards, provided separation in the columns is by size only. This is called universal calibration.

Using one of the concentration detectors and an SLS detector, ParSEC can be used to produce a MWD without the need for column calibration. In addition, for polymers large enough in size, the radius of gyration can be determined. This is sometimes referred to as absolute molecular weight determination, even though calibration of the SLS and concentration detector is still required.

Using one of the concentration detectors and a DLS detector, ParSEC can be used to determine hydrodynamic radius. If the distribution is broad, then the concentration and size information is combined to produce a size distribution. If the distribution consists of one or more narrow peaks, the size can be used to calculate molecular weight given the Mark-Houwink-Sakurada parameters. This can be especially interesting in the case of ASEC, absolute size exclusion chromatography, a technique with particular application

in the study of protein aggregation. Separation of the monomer from higher order aggregates is not strictly SEC since the separation is done with special columns based on preferential adsorption/desorption rather than size. Nevertheless, ParSEC can be used with this form of chromatography.

Reading the Chapters

In Chapter II, two very useful tips for using the software are discussed, the right and left mouse click within a window to reveal choices. This keeps the interface from being overcrowded and hard to read. **READ THIS CHAPTER next.** Then read Chapter III. If you know chromatography, these two chapters are enough to get you started.

Remember: If what you want to do next is not shown in the top line menu, right click in the current window. If you want to move the baselines, change the integration limits, change axes, or scale axes, left click on the object of interest.

In Chapter III a brief run through of the program in a common configuration is shown. This chapter highlights the overall layout of the program and the normal flow. Many features common to most of the windows are discussed. These are context sensitive. ***This is the chapter to read to quickly learn the program.***

In Chapter IV New Measurements/System Setup is discussed. This is where components of the system are named and setup like the pump, injector, and the various detectors.

In Chapter V New Measurements/Sample Queue is discussed. This is where samples are named and Sample Types are designated (unknown, standards, etc.). It is also where the measurement is initiated when you click “Start Queue”.

In Chapter VI the Database Window is discussed. Finished measurements are automatically stored in the database. Measurements are recalled from the database for reanalysis and comparison with other measurements.

In Chapter VII the Data Collection and Review Windows are discussed. They are related: The Data Collection Window is active when data is being collected. A Review Window appears when recalling data previously saved. After setting the baseline and integration limits, the Elugram Window is obtained simply by checking the appropriate box in the Data Review Window. Integration limits can be further refined in the Elugram view.

In Chapter VIII the Detector Window is discussed. Depending on which detectors are attached and enabled, calculated results such as molecular weight, intrinsic viscosity, radius of gyration and hydrodynamic radius are shown in this window.

In Chapter IX the Distribution Window is discussed. The differential and cumulative molar mass distributions that are obtained from combining concentration and molecular weight from the Detector Window are displayed.

In Chapter X the Results Window and the printout are discussed. This is where you find the molar mass averages, ratios, and radii of gyration. It is also where you find calculated values for the percent sample recovery, dn/dc, and global intrinsic viscosity.

In Chapter XI the Extended Calculations Window is discussed. Here Log-Log plots of data such as Low [η] vs. Log M_w can be used to determine the Mark-Houwink-Sakurada coefficients k and α.

In Chapter XII the Overlay Window is discussed. This is where you can overlay raw data to see how repeatable measurements are even before you calculate anything. Or, you can overlay calculated molecular weight distributions.

Installing Software

1. Find the parsec_vX.XX folder on the directory of the CD, where X.XX is the version number.
2. Double click on the folder to open it.
3. Click on the file ParSECSetup.exe. Follow instructions to install the software.

Alternatively, you may have been sent two zip files: ParSEC_vX.XX_d1.zip and ParSEC_vX.XX_d2.zip. Double click and unzip each file. Find the directory where they were unzipped. Follow step #3 above to install the program.

If successful, you will see “BIC Molecular Weight Software” in the program menu (submenu “Brookhaven Instruments Corp. (Win32).) Alternatively, the default software path is C:\Bicw32\parsec\ParSEC.exe.

Appendices

The appendices are mostly devoted to calculations that many users may wish to ignore. It is here that the various relative molar mass averages and polydispersity indices are defined. Advanced calculations are detailed with several examples from well-studied polymer-solvent systems. The relationship between various detector signals and how calculations are made is also revealed.

In addition, as new changes are made, if they cannot be easily integrated into the main body of the manual, look for their explanation in an appendix.

Chapter II: Working With the Program, Two Common Tips

Here are two useful tips that make it easier to work with the program:

- If what you want to do next in the currently selected window is not shown anywhere on screen, right click in the current window.

- If you want to move baselines or integration limits, select or scale axes, place a cursor, or read out values on the screen, left click (and sometimes drag) on the object of interest: markers, axes, curves, or spin buttons.

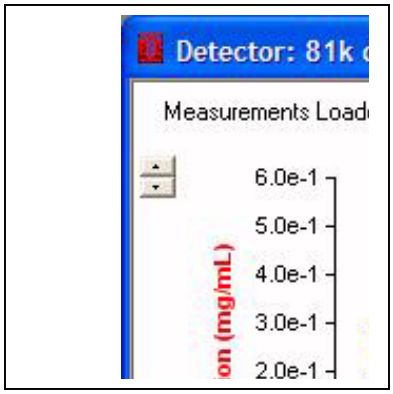
Right Clicking: Revealing choices not shown in the top-line menu

Here is one example of the first tip. In the Detector Window below, it is clear that both the "Concentration" and "M" (molecular weight) axes (the Y axes) are not scaled properly. Though it is harder to tell, neither is the "Elution Volume" (the X axis) scaled properly. You could left click on each axis separately (second tip), and then use the vertical spin buttons to adjust each graph separately. Or, by right clicking the mouse, you bring up a context sensitive sub window with "Autoscale All" as the first choice.

Look at the other choices shown in the popup sub window when you right click. The Graph Settings choice allows you to turn on or off any of the graphs in a particular window. You might, for example, want to show only the molecular weight and concentration plots (as shown here) and not the radius of gyration, intrinsic viscosity or hydrodynamic radius. With the Graphs Settings choice, you can turn these on and off. Or you might have so many Y-axes, it is crowded and you want to see more of the plotted data. You can check the "Collapse Y Axis" box in the Graphs Settings sub window to a single, unlabeled Y-axis.

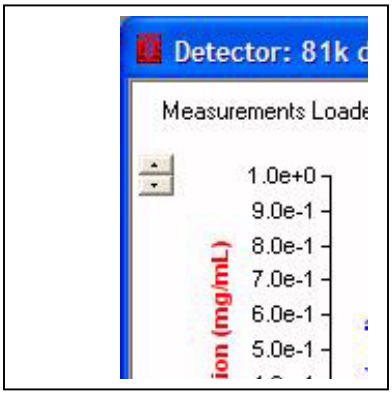
Depending on which window you are in, the Graph Settings choices will change. They are context sensitive. For example, if you are in the Data Collection window (also called Review window when analyzing or reanalyzing data), the Graph Settings allow you to show or not show various signals that are being or have been collected. Collapsing the Y axes for clarity (in some cases) is again a choice as is showing smoothed or unsmoothed data and showing the various signals with and without the inter-detector delays implemented. In the Distribution window, the Graph Settings choices are simpler: Show or not show the cumulative and/or the differential molar mass distribution.

After right clicking, slide the cursor over the "Window" sub window, the one with the filled in black arrowhead pointing to the right. You will find a deeper sub window that allows you to open (if not already) other windows including: Database, Data Collection, Review, Detector, Distribution, Extended Calculations, Results, and Overlay. These choices are available from any window with a graph by right clicking and selecting "Window".



when you are reviewing data attached or signals enabled, in gray rather than black reminder that this feature is rent configuration.

Neither Y axes nor the X axis are scaled properly. Right click and the context sensitive window pops up. Click the top choice, “Autoscale All”.



Now all three axes are scaled properly.

NOTE: When a choice is not possible, such as “Data Collection” without instruments then the choice is shown lettering. This is a not available in the cur-

After right clicking, slide the cursor over “Sample Settings”. Here you can review and change sample parameters, select calculation options, and review calibration constants.

After right clicking, slide the cursor over “Print/Export”. Here you can preview and print results, setup a printer, and export data.

When reviewing data, you can load up to 10 measurements in a pull down scroll box shown in the top of each graph window. Look at the example above. There are 9 measurements loaded. The one currently displayed is the one labeled “81k dxt” with a date and time stamp. This was the user’s way of labeling an 81 kDa Dextran sample. After right clicking, slide the cursor over “Unload From List”. Then click to unload the currently displayed measurement or to empty the entire list.

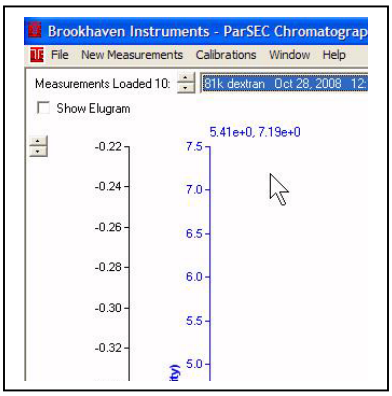
Finally, after right clicking, click “Save” to save any changes you have made.

All this is available with a right click of the mouse in any graphing window. In the “Database” window, a right click brings up a sub window with different choices that will be discussed in detail in Chapter VI.

Here is the point: **IF IN DOUBT, RIGHT CLICK** to reveal choices.

Left Clicking: Moving markers, changing data point readout, scaling

Left clicking of the mouse in a window with a graph allows several choices to be made depending on where the cursor is at the time.



Look at the green bull's-eye target on the blue curve (if you are reading this in color). It is the right-n point/second, this data point corresponds to 992 seconds away from zero (the 1st data point). Now 992 s is 16 minutes and 32 seconds. At a flow rate of 0.5 mL/min, the target should be at 8.267 mL, which it is as read in the box.

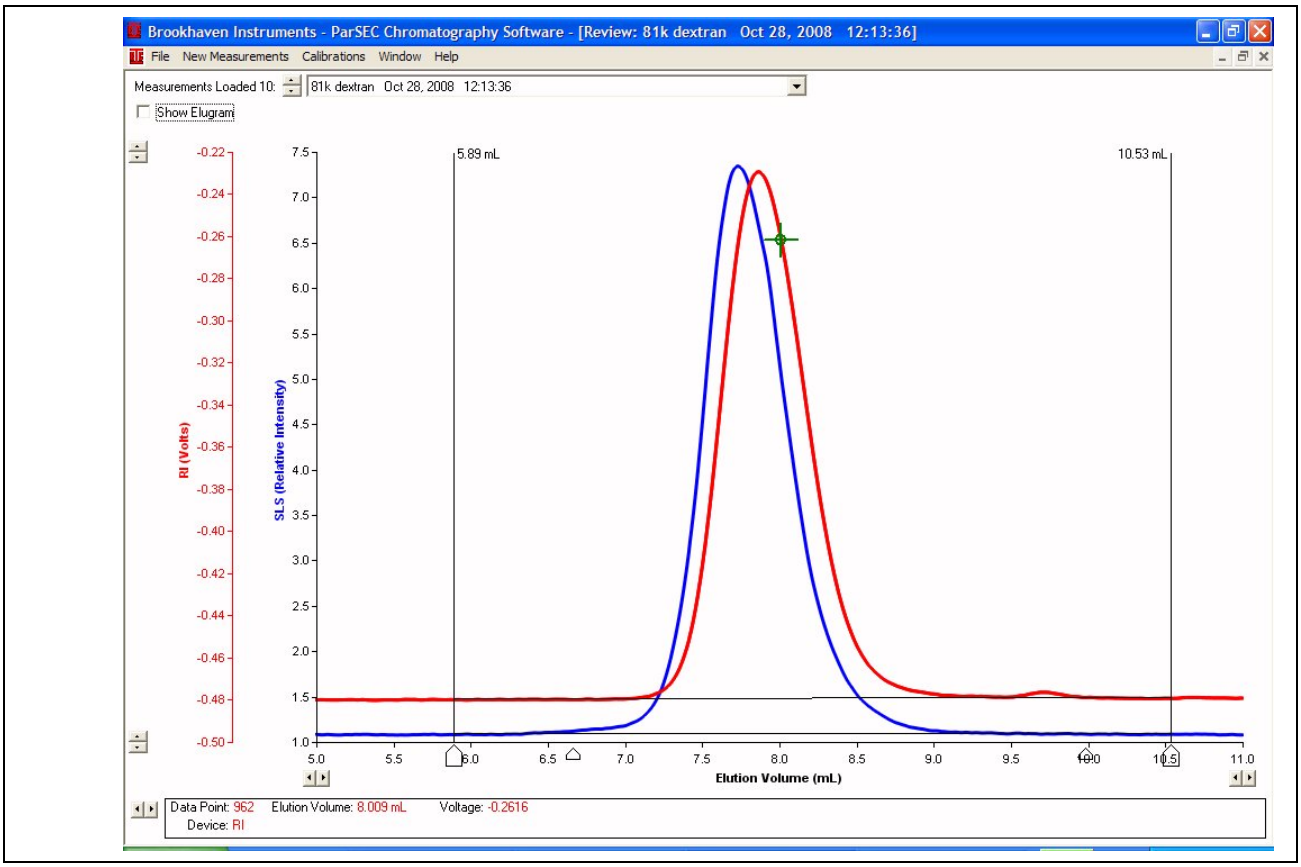
In the same box, the actual axis corresponding to target placement is shown as well as its value. Here it is the static light scattering axis (SLS) and the relative intensity or Y-value is 4.6125. Of the seven angular curves, just the 90° curve is shown for clarity. By clicking the box labeled "Show All Angles", six more SLS curves will appear.

If you did display all the angles, you can cycle the target through them by left clicking on the left-right horizontal arrows in that same horizontal line. At each angle, the value is shown in the box as is the angle (replacing the 90°).

Instead of clicking on the curve, you can move the target to the left or right by left clicking on the left-right horizontal arrows to the left of the word "Data Point" in the box.

So far the target and data point read out is stuck on the SLS axis. To read out any other axis, in this case the RI axis, left click on that Y-axis. The target will jump vertically and come to rest on the red (if you are seeing this in color) curve. The data point and elution volume correspond to that for the RI X-axis, and the Y-axis value will be labeled "RI (volts)". For this particular case, it will be approximately -0.35 volts. For a more exact result, just click on the RI axis and then read the value in the box.

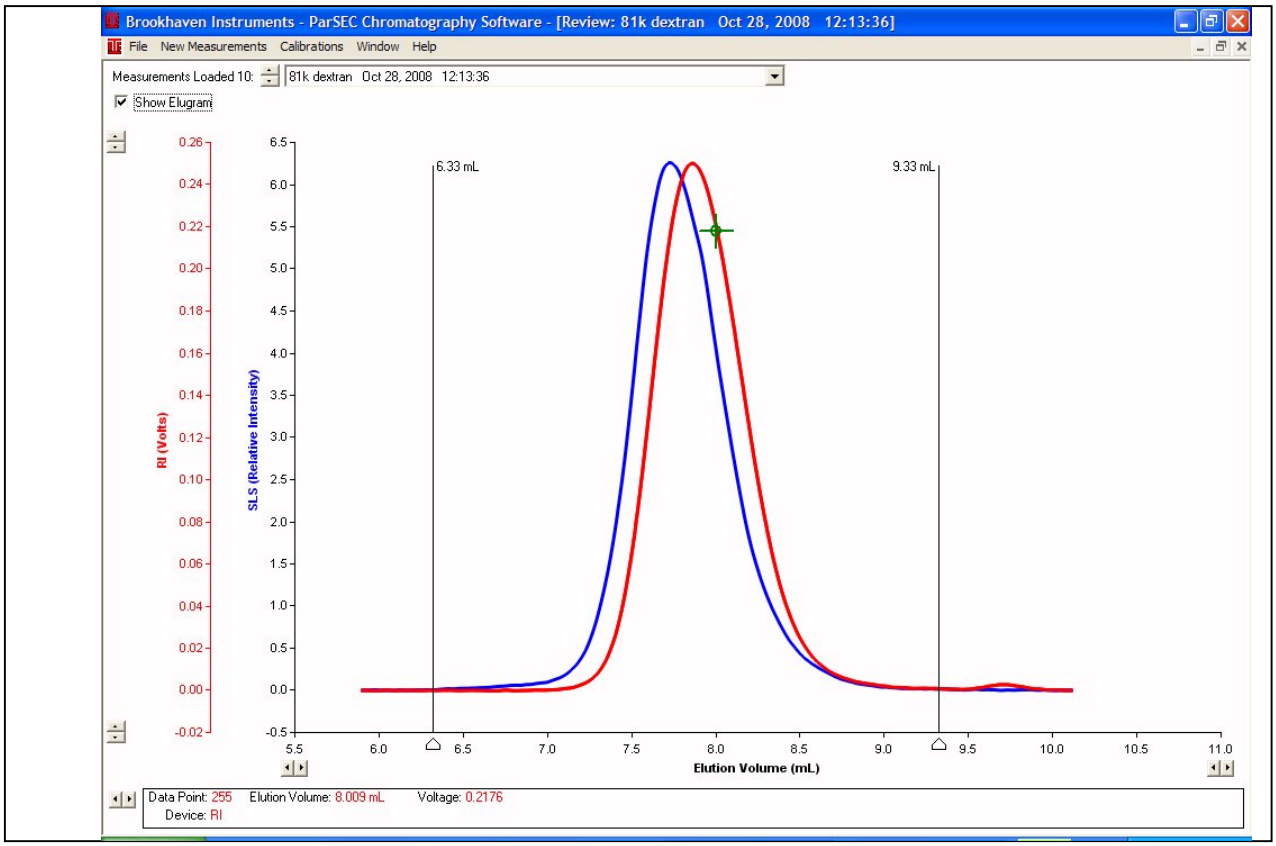
The target position is not the same as a general cursor position on the plot. A target position corresponds to a point on one of the plots displayed. The cursor position is any x,y pair on the plot, wherever the cursor is located. Look at the cursor near the top of the SLS axis. The position is given as 5.41 mL (elution volume) and 7.19 (relative intensity) for the SLS curve because that is the curve currently selected. If you have color readout, you would see that the SLS axis is in blue, as is the curve itself, as are the cursor coordinates (5.41, 7.19) and as are the readouts in the data box. When you left click on the RI axis (in red), the green target will jump to the red curve, the cursor coordinates will be in red, and so will the readouts in the data box. It is color-coordinated on screen.



The large, baseline markers, here shown at 5.89 and 10.53 mL are moved by left clicking and dragging each one separately. The vertical lines and values disappear when you move the cursor away from the marker. To guide the eye when setting the baseline, horizontal lines appear. And they disappear when you move the cursor away from the markers.

Setting the baseline is the first step in limiting the calculation. And if the curves are sitting on a rising or falling baseline, then the value of the baseline between the marker positions is subtracted from each of the signals to compensate for this type of linear drift. RI detectors are very sensitive and show such thermal drifts.

The two smaller markers, always inside or coincident with the larger, baseline markers are the integration limits for calculations. They can be set in the Review window (see top line to reveal what type of window you are dealing with) or, by left clicking in the upper left box labeled “Show Elugram”, the following window appears:



In this picture, the cursor is sitting on one of the integration limits and that is why you see the vertical markers and the limits labeled at 6.33 mL and 9.33 mL. The baseline was set wider, as it normally should be, and all subsequent calculations will be carried out between these two integration limits. In the Elugram view, the baseline markers disappear and the window allows for somewhat easier setting of the integration limits. [NOTE: The inter-detector delay has been shifted for clarity, not for accuracy.]

The X and Y axes can be scaled individually. To scale the X-axis, left click the left-right horizontal arrows at either end of the axis. These allow you to stretch or compress either end of the scale while leaving the other end fixed.

To scale the selected Y-axis, left click the up-down vertical arrows at either end of the far left Y-axis. These allow you to stretch or compress either end of the selected scale while leaving the other end fixed.

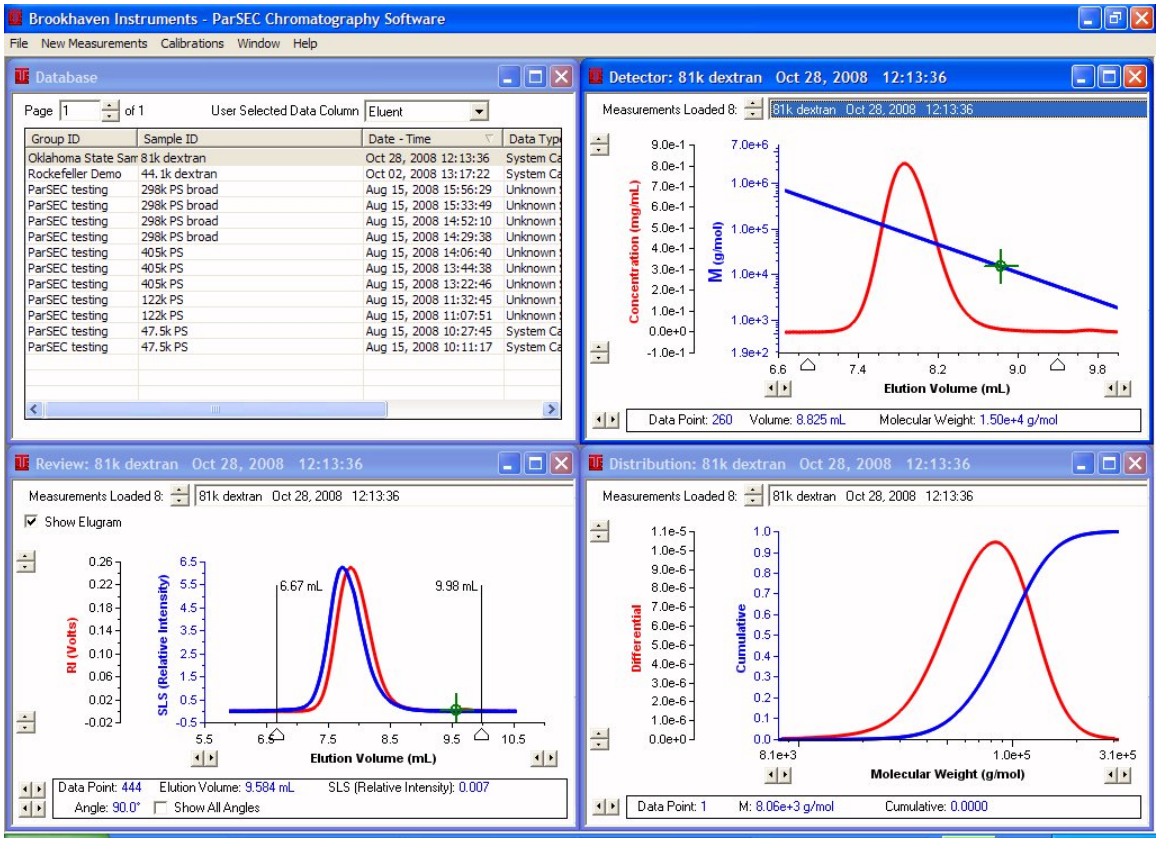
Remember: The selected Y-axis is the one you last clicked on. It is the one whose data is shown in the box and labeled. It is the one the target is attached to.

Here is the point: **LEFT CLICK** to drag markers, scale axes individually, set the target marker on the selected curve, and move through the data points in the data box.

Chapter III: Quick Start, a Brief Tour of ParSEC

A Common Set of Windows

Below is a view of four common windows. It is suggested that you open the program—even if it is not connected to any detectors—and run through some of the features



described here to get a feel for the program.

When you run the program for the first time, if you have not been assigned an Operator ID, select one of your own choosing. If you only see the top menu choices, and no open windows, follow these instructions for creating a common 4-window configuration:

- ❖ Click File/Database. The Database Window will open.
- ❖ Click on one of the default files sent with the software or any other file previously run and saved. The Review Window will open.
- ❖ Then right click in the window and move the cursor over to “Window”.
- ❖ From the list that appears, click on “Detector”. The Detector Window will open.

- Right click again, move the cursor over to “Window” again, and click on “Distribution”. The Distribution Window will open.

- If either the Detector or Distribution windows are empty, right click and click on “Autoscale All”. Now there should be curves in all three graphing windows.

- Last, in the top menu, click “Window” followed by “Smart Tile”.

The upper left window is the Database Window. When measurements are finished in the “Data Collection” Window (see section III of this chapter), they are automatically added to this window. From this window, one can select previous measurements for review and analysis. It is possible to filter and sort data files in this window for easier viewing and comparisons. Right clicking in this window allows you to load (ASCII files), delete, search, archive (as binary file), reload archive files (binary files), or export (as ASCII file) one or more selected files.

The lower left window is the Review/Elugram Window used to display raw data from saved data files. To add a data file to the window, double click on it. Or, click once to highlight it, right click, and then click “Load”. Up to 10 measurements can be loaded into this window at one time. In this example, eight of the possible 10 have been loaded. The name of the one displayed is shown. Use the scroll buttons to change which of the eight are displayed. To unload the current file, or to unload all of them, right click in this window and select “Unload Current” or “Unload All” from the “Unload From List” selection.

The Review Window has two faces. In the regular Review Window view (unchecked), the baseline markers (larger) can be set (drag and drop). A baseline is determined and subtracted from all signals yielding the excess signal values (solvent subtracted values). In the Elugram Window view (checked), the one shown above, the baseline markers disappear and it is the integration limit markers (smaller) that can be set. All subsequent calculations are limited by the upper and lower integration limits.

In the example shown here, the shift between the red (RI detector) and blue (BI-MwA detector) curves indicates the inter-detector delay has not been enabled. To enable, uncheck “Show Elugram”, right click, click “Graph Settings”, and check the “Interdetector Delay” box. Return to the Elugram Window view.

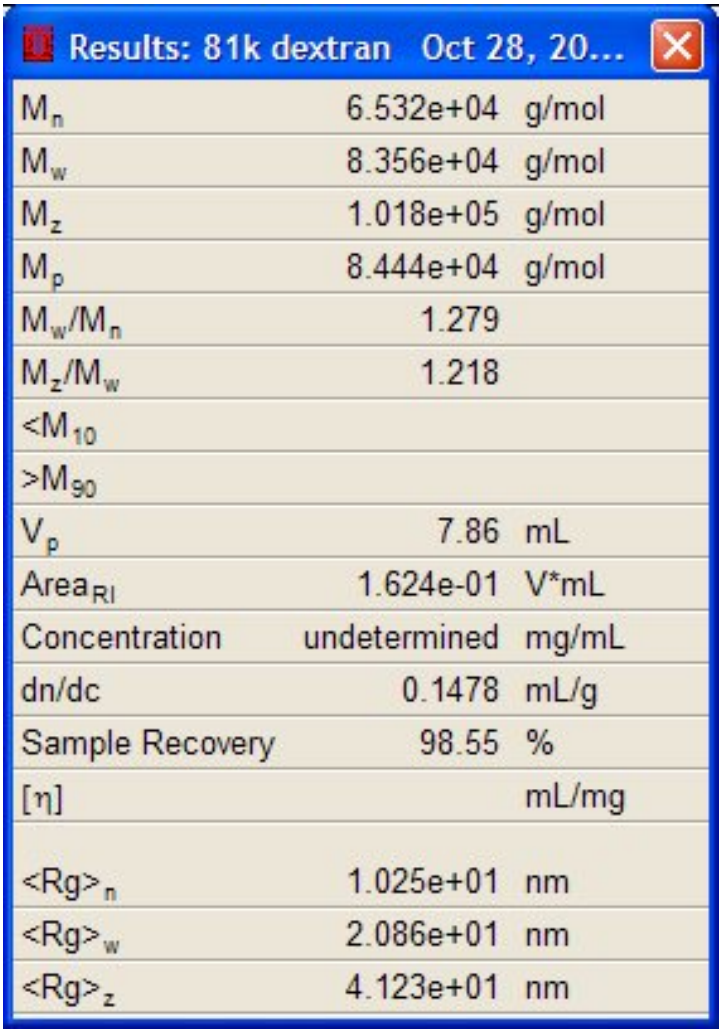
Interactive Windows

The windows are interactive. If you enable or change the inter-detector delays, the baseline markers or integration limits, the results in the Detector and Distribution Windows change accordingly. This is also true for the Results Window and any other calculations derived from changing parameters (such as markers). It is automatic. You do not have to force a recalculation.

The upper right window is the Detector Window. Raw signals are turned into calculated results for concentration, molecular weight, radius of gyration (not shown), intrinsic and specific viscosity (not shown) and hydrodynamic diameter (also not shown) vs. elution volume with the baseline subtracted and the calculations performed only between the selected integration limits. To turn on (show) or turn off (not show) a particular plot, right click, select “Graph Settings” and check or uncheck boxes corresponding to particular results. You can also change integration limits in this window.

The lower right window is the Distribution Window. In this window, the differential and cumulative (integral) molecular weight distributions (MWD’s) are displayed. Strictly speaking, these are relative molar mass distributions, but both terminologies are in common use. If you want to turn off either distribution, right click, select “Graph Settings” and check or uncheck the “Differential” or “Cumulative” boxes.

Can’t wait to see results? From any of the graphing windows, right click, select “Window”, and click on “Results”. The Results Window will look like this:



M_n	6.532e+04	g/mol
M_w	8.356e+04	g/mol
M_z	1.018e+05	g/mol
M_p	8.444e+04	g/mol
M_w/M_n	1.279	
M_z/M_w	1.218	
$<M_{10}$		
$>M_{90}$		
V_p	7.86	mL
$Area_{RI}$	1.624e-01	V*mL
Concentration	undetermined	mg/mL
dn/dc	0.1478	mL/g
Sample Recovery	98.55	%
[η]		mL/mg
$<Rg>_n$	1.025e+01	nm
$<Rg>_w$	2.086e+01	nm
$<Rg>_z$	4.123e+01	nm

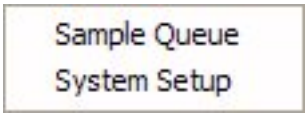
Can't wait to see printed results? From any of the graphing windows, right click, select "Print/Export", and click on "Print".

A List of Things to Do To Get Started

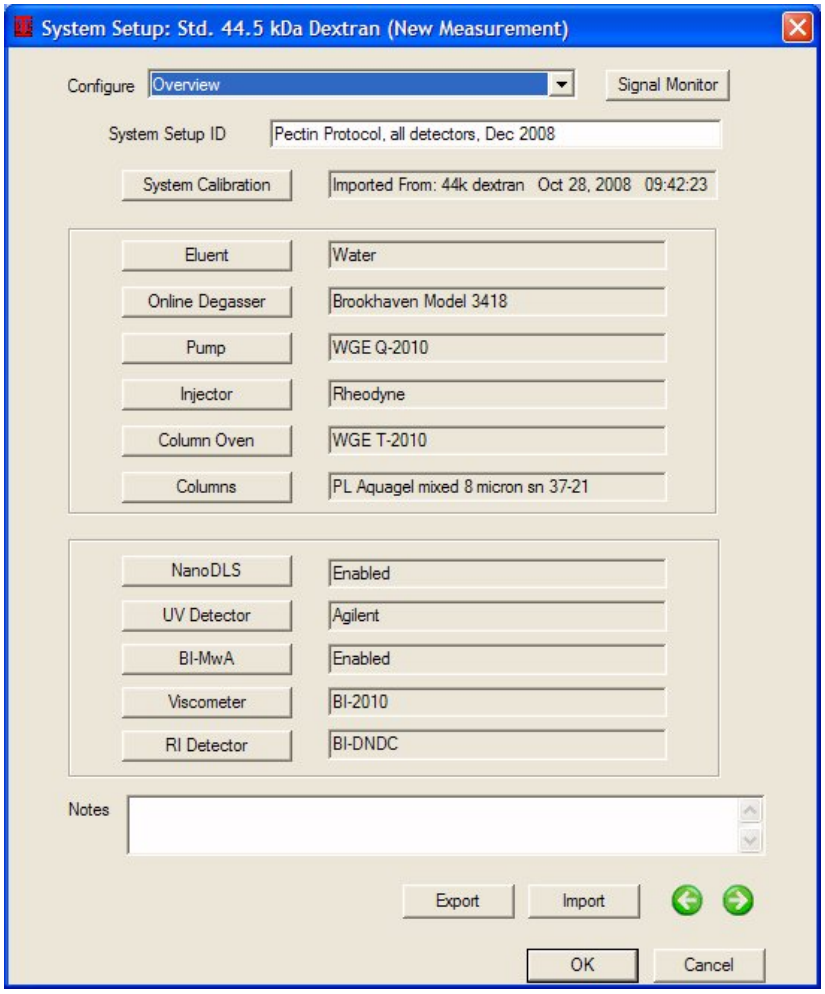
In this section, it is assumed you have not yet configured or setup the system. Then this is the first step:

I. System Setup

In the top menu, click "New Measurements". There are two choices:



Click "System Setup". Here is what you see for a previously configured system: The Overview.



There are several interesting points about this Overview Window.

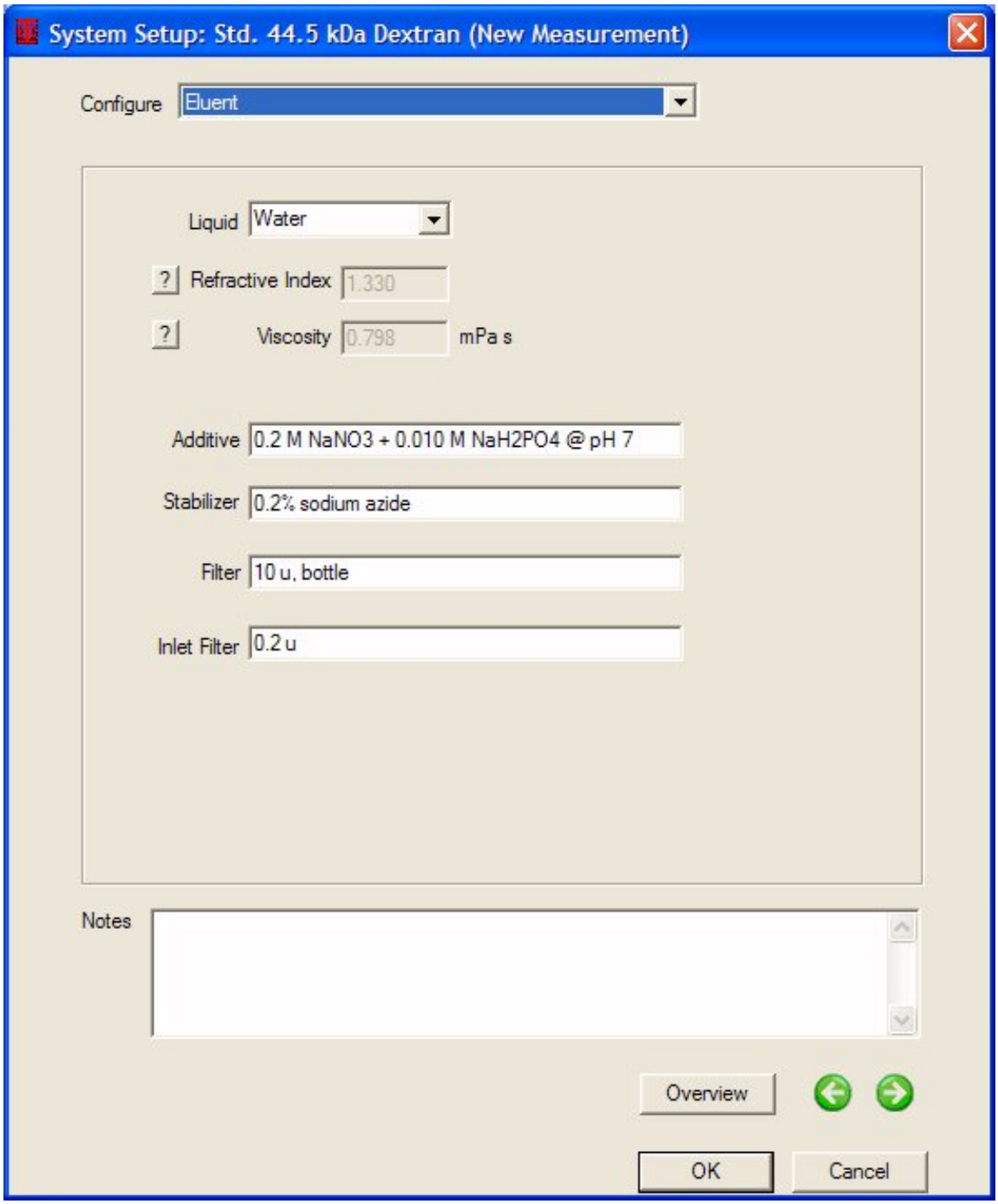
(a) The full setup, as defined by entries in the many choices listed, will be applied to new measurements, in particular one that is already in the Sample Queue labeled “44.5 kDa Dextran (New Measurement)”. NOTE: You can reach this “Overview” when you review previous measurements. In that case, the information is for the reviewed data file, not for a new measurement. Always look at the title bar of this window to determine which setup you are dealing with: Currently loaded or New Measurements.

(b) The full setup was saved (click “Export” at the bottom of the screen) and reloaded (click “Import” at the bottom of the screen). When saved, the “System Setup ID” was given as “Pectin Protocol, all detectors, Dec 2008”. These system setup files are given the extension .bisys. They are found in C:\Bicw32\parsec\SystemSetups. System setups are saved along with any notes you may have entered as reminders. Re-loading a system setup, with minor changes, will save you lots of time.

(c) You can move between the various setup windows in three ways: click the command buttons; use the left-right green arrows at the bottom of the screen; or click the down arrow in the top line labeled “Config-ure”. Then select the window you want to work in. One of these three methods will be the most convenient depending on where you are in the screen, but all work the same.

(d) “System Calibration” contains inter-detector delays, calibration con-stants (called calibration factors in some parts of the world), and nor-malization constants if you have the BI-MwA light scattering detector. Since we are assuming you are setting up the configuration for the first time, there is no need to examine this window further. It will either be empty or contain a default set of values.

Click “Eluent”. Here is what you see for this particular system configuration:



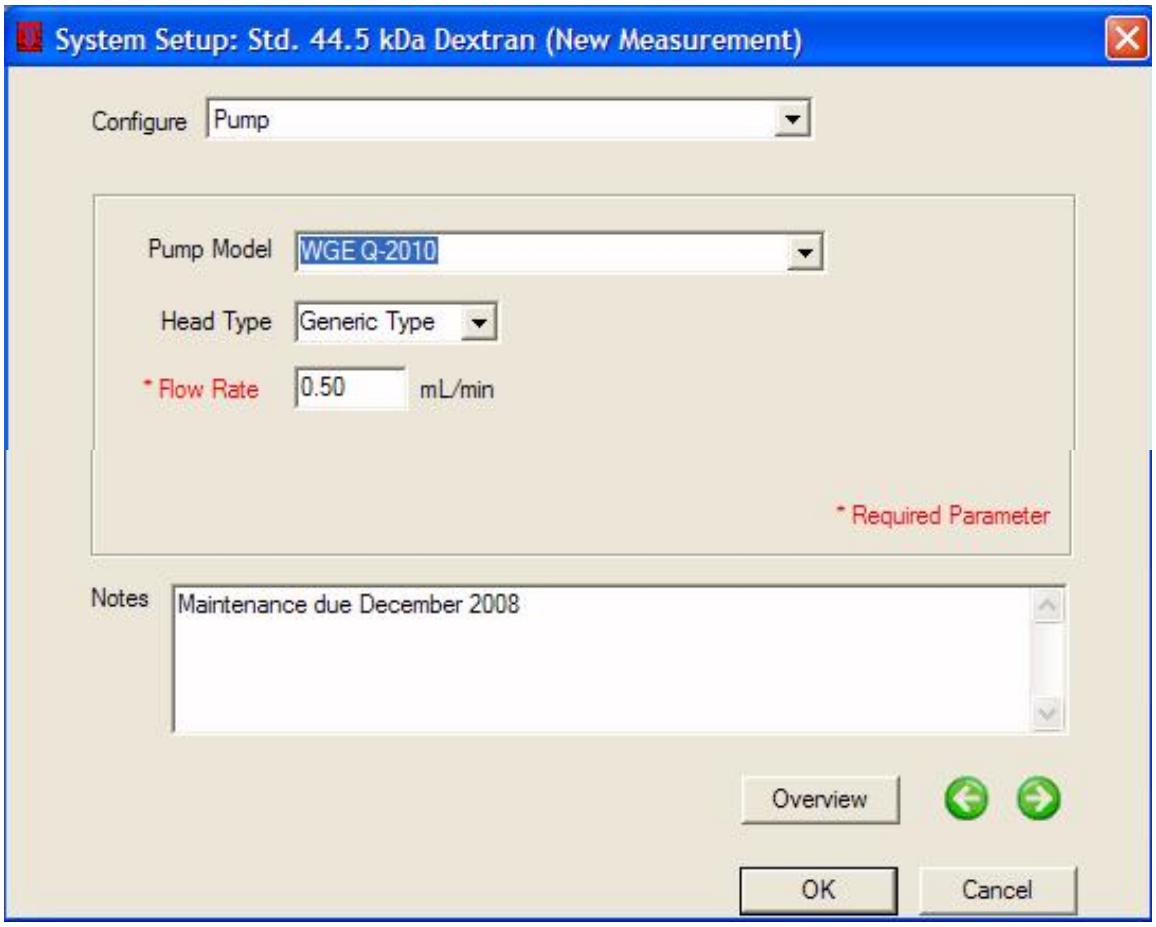
A list of common liquids is located in the “Liquids” combination box. Or, select “Unspecified” and type in a name. If the liquid is from the list, and the temperature was set elsewhere, then the refractive index and viscosity

are known and displayed. If an unspecified liquid is input, these grayed out values become available for editing. Enter values for the refractive index and viscosity of the liquid at the temperature you have set for the BI-MwA and the NanoDLS, if it too is enabled.

Note the *information* boxes with the question mark (?) icons. Whenever you see one of these icons, click for more information and guidance if you need it.

The remainder of the window is devoted to additional information, if you have it, on how the eluent was treated. When you save the entire configuration, this information is available for later review.

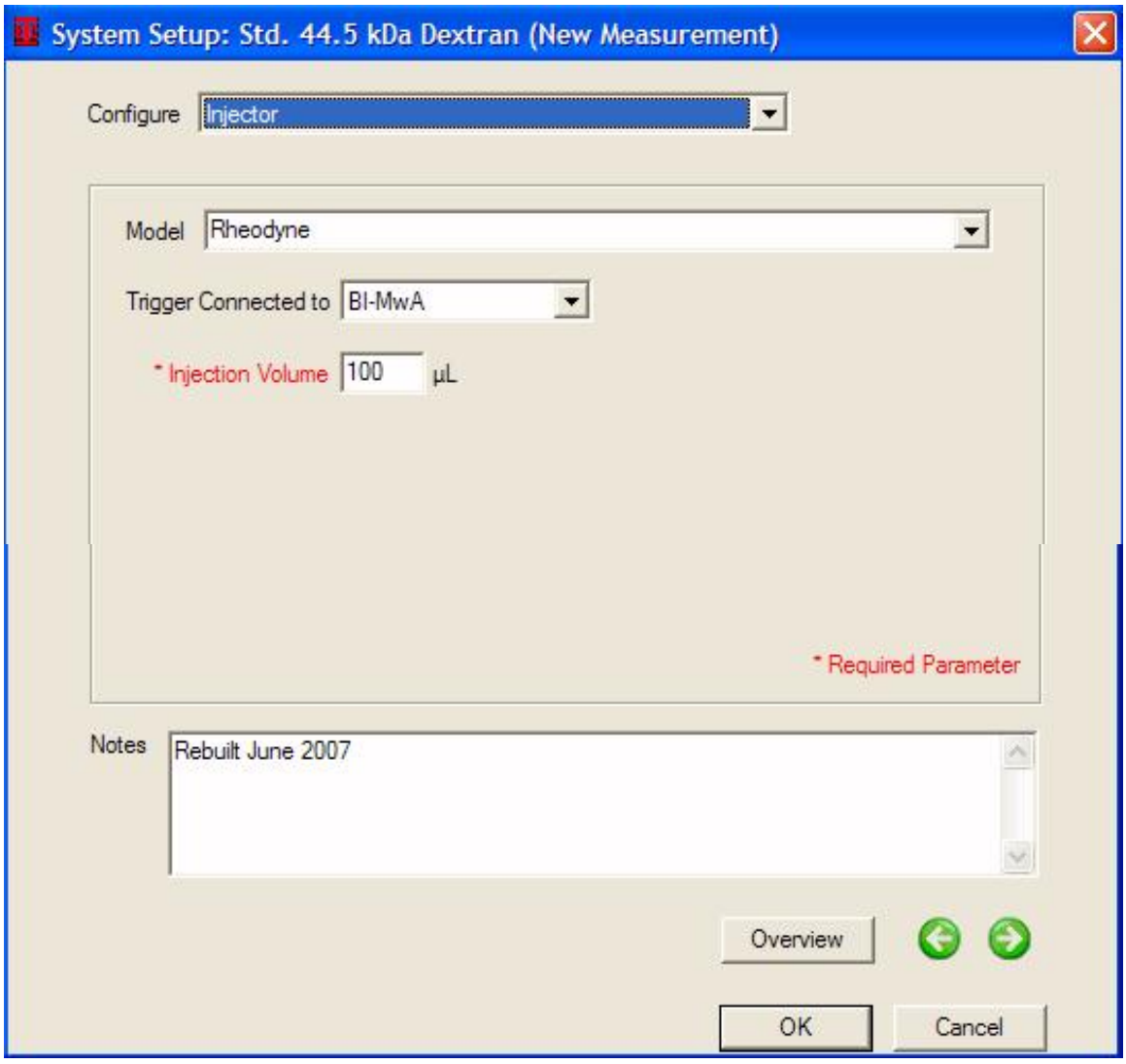
Click "Pump". Here is what you see for this particular system configuration:



A list of common pump models is located in the "Pump" combination box. Or, select "Unspecified" and type in a name. There is also a choice for micro, analytical or undefined head type for future reference. The most important input value is the flow rate. It is required. NOTE: When any parameter

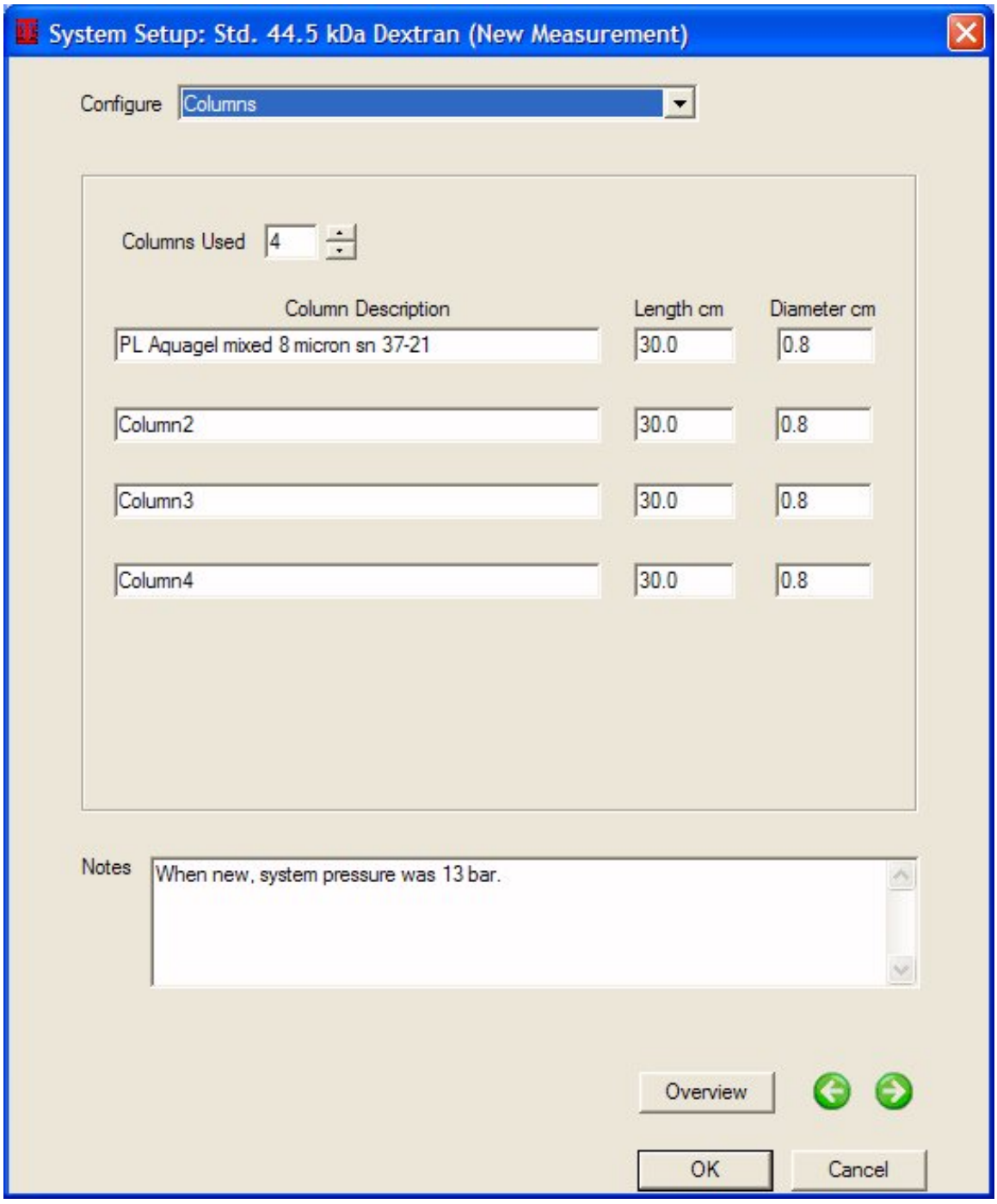
is required, it is flagged with a red label and an asterisk. The Notes box has an important piece of information about when maintenance is due. Pumps due require periodic maintenance and this is a good place for the alert.

Click "Injector". Here is what you see for this particular system configuration:



A list of common injector models is located in the "Injector" combination box. Or, select "Unspecified" and type in a name. Auto-samplers can also be specified in this page. The most important input value is the injection volume. It is required. NOTE: When any parameter is required, it is flagged with a red label and an asterisk. The Notes box has an important piece of information about when the injector was last rebuilt. Injectors do require periodic maintenance, though not as often as the pumps, and this is a good place for the alert.

Click “Columns”. Here is what you see for this particular system configuration:

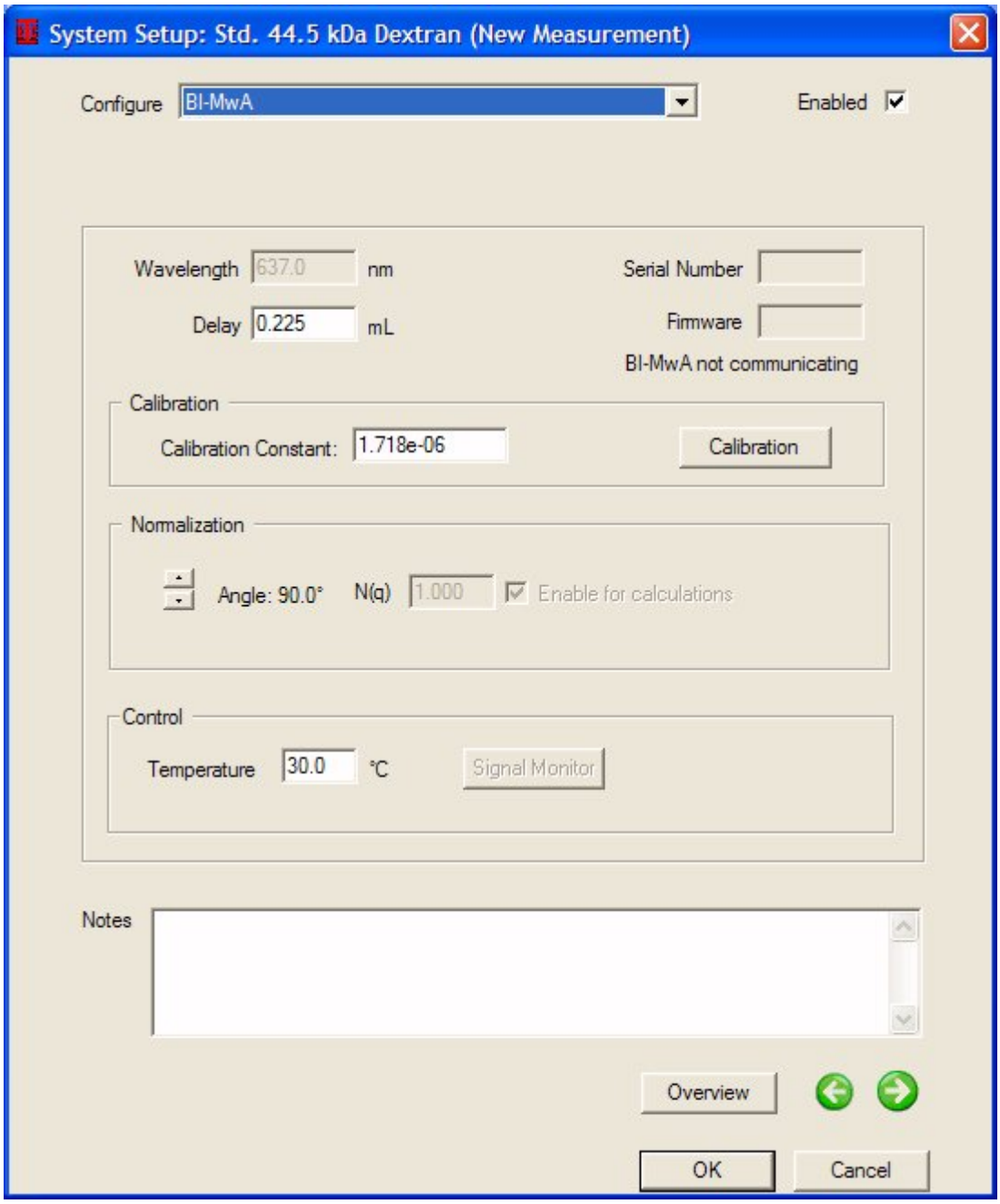


From one to four columns can be described here, including their length and diameter. Good lab practice for any type of chromatography requires that the system pressure is monitored over time. As it rises, it suggests pre-

column filters or columns themselves are becoming plugged. The notes section here is an excellent place to note the pressure for future reference.

Just two of the many detectors will be discussed here: The BI-MwA static light scattering detector, for so-called absolute molecular weight determination; and the BI-DNDC differential refractometer, an RI device for concentration determination.

Click "BI-MwA". Here is what you see for this particular system configuration:



If a detector is to be used in a particular configuration, you must check the "Enabled" box in the upper right of the screen as shown here.

The BI-MwA's wavelength, Serial Number and Firmware version are displayed but grayed out. For purposes of writing the manual, this particular BI-MwA was not attached to a computer and so it is not communicating. For this reason, the Serial Number and Firmware version are not shown.

The inter-detector delay, here 0.225 mL, is determined when calibrating the system. To test the effects of a different value, you can edit it here manually.

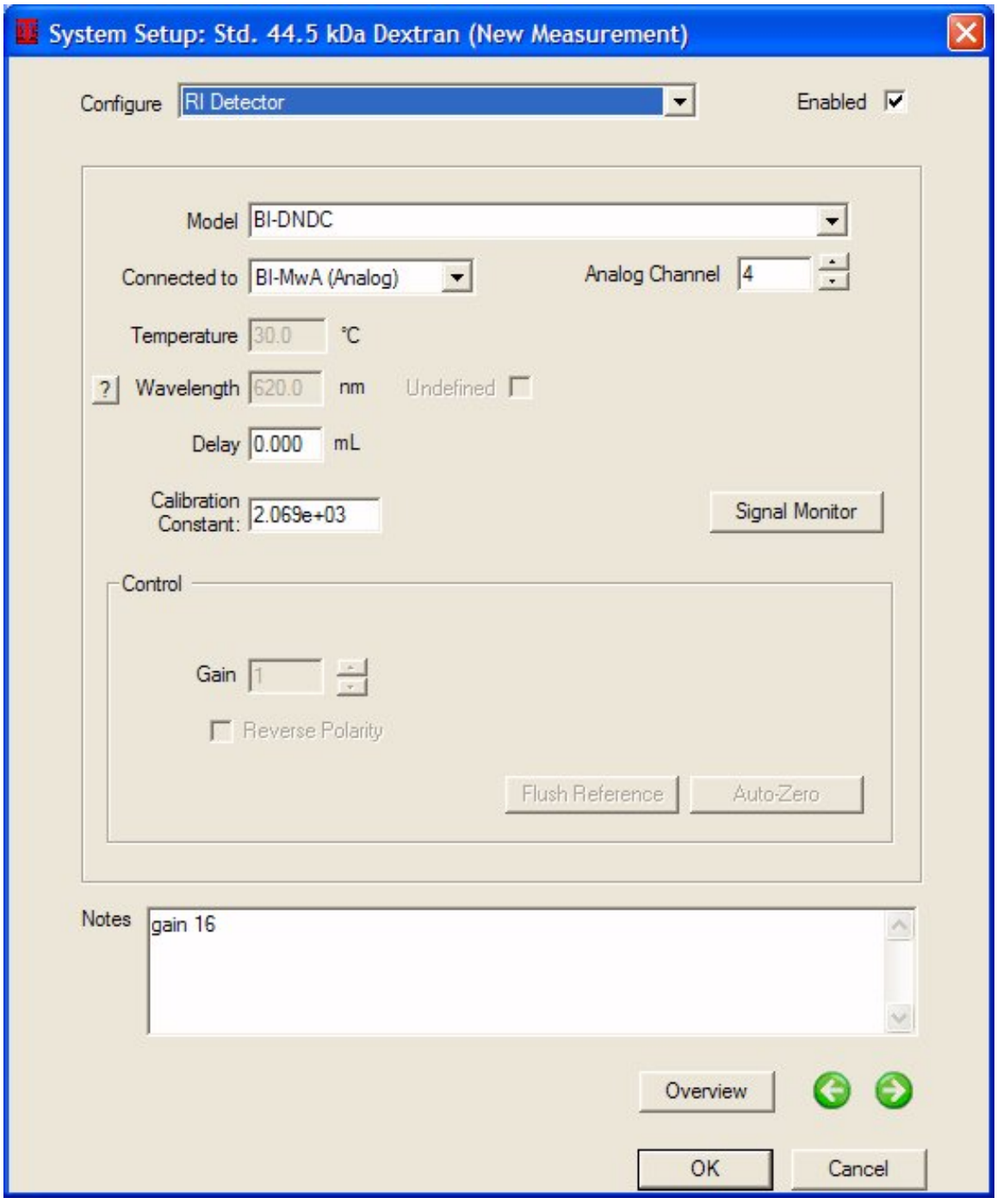
The calibration constant, here 1.718e-6, was determined when calibrating the system using a polymer standard. However, if you click "Calibration" in this window, you will have an opportunity to calibrate the BI-MwA using a pure liquid of known Rayleigh Ratio such as toluene. Both types of calibration are acceptable. Using a pure liquid is more accurate but not as convenient as using a standard polymer to calibrate multiple detectors, to determine inter-detector delays, and to normalize the BI-MwA multi-angle detector.

Normalization constants for six of the seven BI-MwA angles (by definition the constant for 90° scattering is unity) are obtained using a Rayleigh scatterer such as a polymer of sufficiently low size. These constants are used to normalize angular differences arising mostly from the different fibers and include angular effects ($\sin\theta$, the so-called volume correction). It may come to pass that one angle is not performing well and by un-checking the "Enable for calculations" box, its value is not used in any calculations.

Cell temperature inside the BI-MwA is set if the option has been ordered. Up to 80 °C may be set. There is no cooling; only heating.

By clicking on "Signal Monitor", a graph is displayed showing voltage or normalized signal intensity in arbitrary units, whichever is appropriate for that particular detector, vs. time. Do this whenever you "enable" a new detector. In this way you can, before setting up the entire system, before entering information into the Sample Queue, determine if the newly enabled detector is properly attached to the system and if the signal is reasonable. In the case of the BI-MwA, you can view one angle at a time or all angles together.

Click "RI Detector". Here is what you see for this particular system configuration:



Notice that the "Enabled" box is checked in the upper right of the screen indicating this detector is being used.

The “Model” pull-down box allows a choice of common RI detectors, here the BI-DNDC.

The “Connected to” pull-down box allows a choice, here the BI-MwA analog inputs. This means that the analog output of the RI Detector is connected to one of the analog-to-digital inputs accessible from the rear of the BI-MwA. The “Analog Channel” scroll box indicates that it was channel 4. There are 27 channels.

In addition to the BI-MwA analog inputs, one can also connect detectors to the analog inputs of the NanoDLS and the WGE ETA 2010 differential viscometer. A digital connection is also allowed.

Note the ***information*** boxes with the question mark (?) icons. Whenever you see one of these icons, click for more information and guidance if you require it.

If the RI detector does not use a narrow-band wavelength source, check the “Undefined” box. This will force concentration calculations based on slice-to-area ratios under the voltage vs. elution volume curve generated by the RI detector. It will also force the user to input a value of the differential refractive index, dn/dc, at or close to that of the source used in the static light scattering device, the BI-MwA. Such a value is required to accurately calculate several, but not all, MWD statistics.

The inter-detector delay, here 0.000 mL, indicates the BI-DNDC is the first detector following the columns.

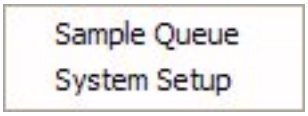
The calibration constant, here 2.069e+3, was determined when calibrating the system using a polymer standard, the same standard that can be used to calibrate the BI-MwA and determine its normalization constants.

Useful Tip

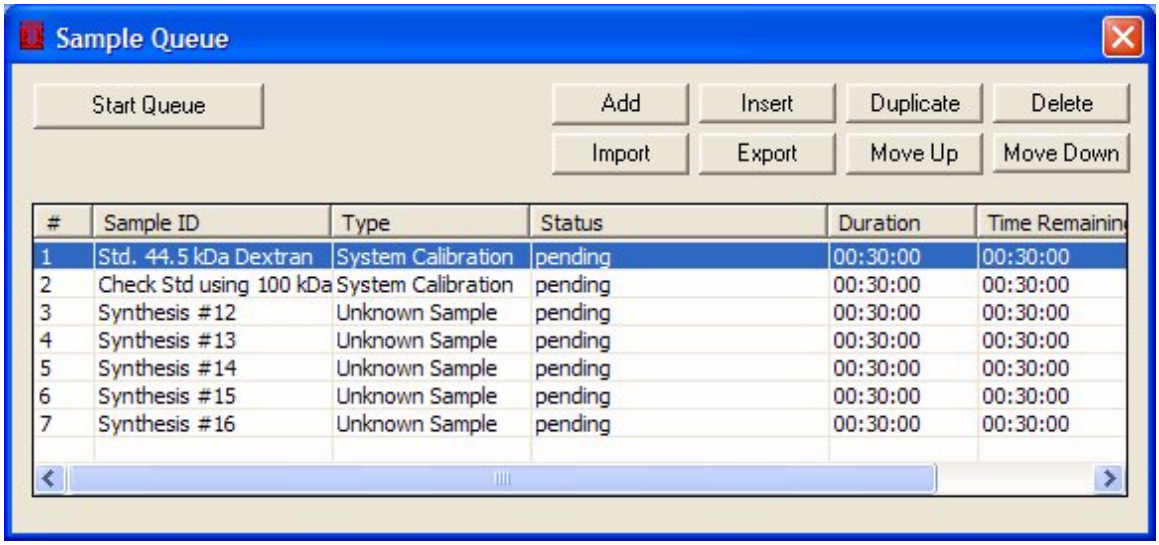
Remember to “Export” the configuration after making any changes or additions. This saves it under a name of your choosing. The configuration can be used again by clicking “Import”. It is easier to import a similar configuration and make a few changes than to start over with a large number of entries. The “Export” and “Import” command buttons are located at the bottom of the New Measurements/System Setup/Overview window.

II. Sample Queue.

In the top menu, click “New Measurements”. There are two choices:



Click “Sample Queue”. Here is what you see for a previously configured system:



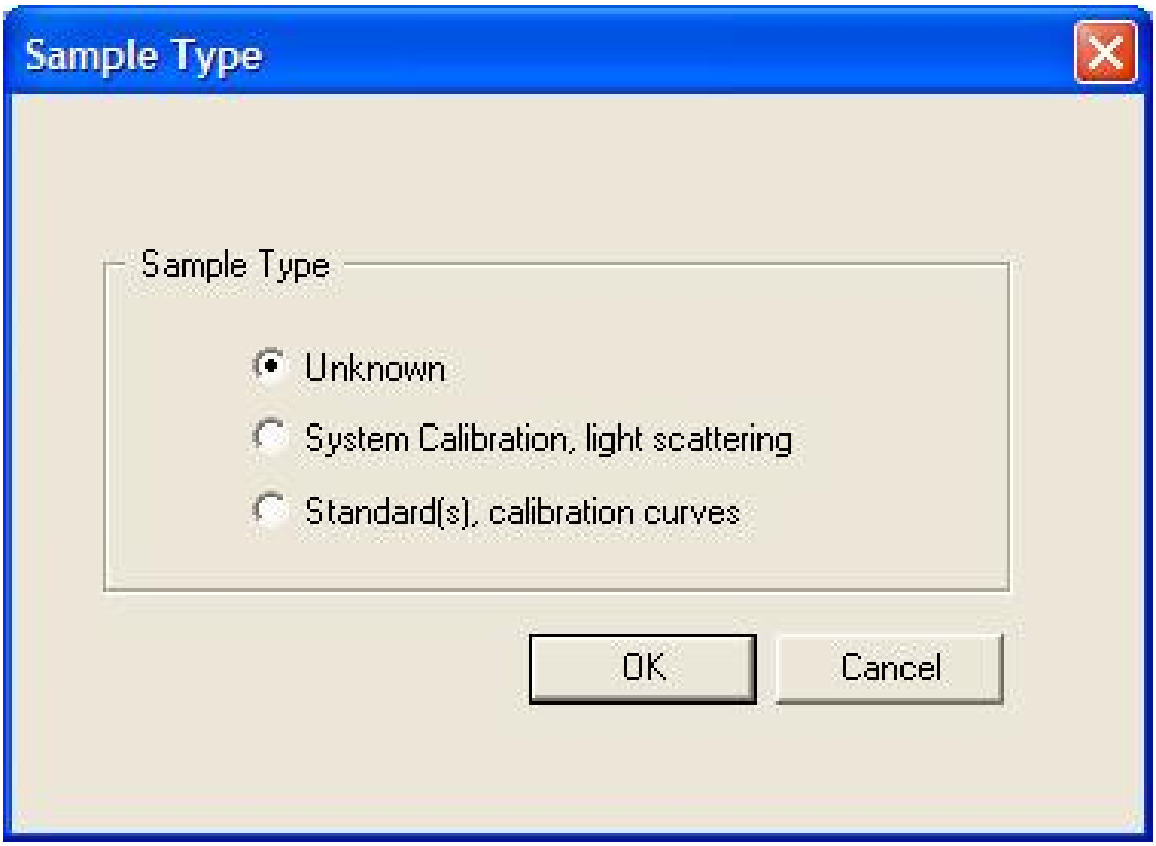
Even if you are making just one run, you must “Add” it to the Sample Queue. Measurements proceed until stopped manually or until the last one in the Sample Queue is completed and stored in the Review Window.

To assist in building up a Queue and arranging it the way you want, several of the other command buttons are of use. Click on an entry and you can move it up or down one step at a time with the commands “Move Up” and “Move Down”, or you can “Delete” it entirely. You may wish to run a sample more than once under the same conditions. In this case, click “Duplicate”. Duplication followed by minor changes to parameters such as Sample ID, concentration, etc. is also effective in setting up new entries. To make such changes, double click on the entry that was duplicated. Duplicates are always added at the bottom of the queue.

You may wish to “Insert” a new entry between two others. Click on the entry above the insertion point and proceed to fill in the answers to the same questions when adding a new entry. See below.

It takes time to enter a large number of runs in a queue. And if you intend on running the same or similar queues in the future, make sure to "Export" and name the queue. It will end with the extension .bisq. It can then be imported using the "Import" command button.

When you click "Add" or when you "Insert", here is what you will see:

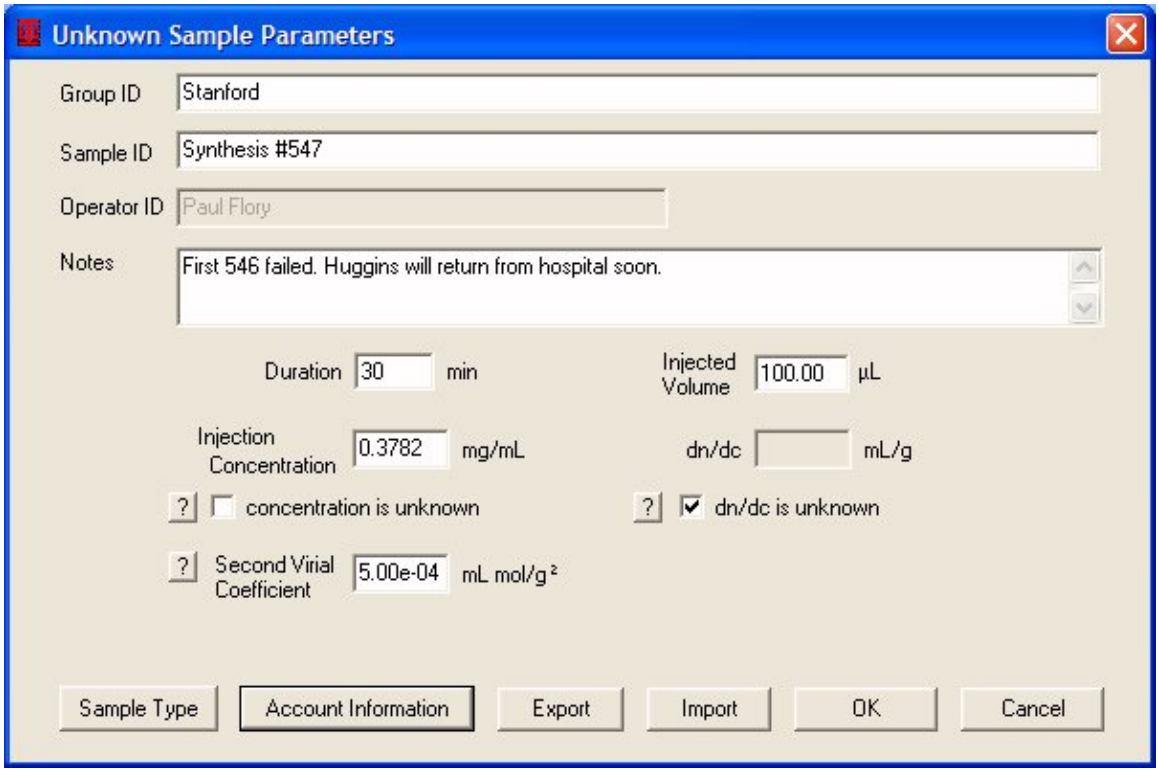


There are three sample types, each with its separate informational window:

- Unknowns, what you measure most often;
- A known, single standard used for calibration and normalization of the static light scattering device, other detectors, and determination of inter-detector delays;
- And known single or mixtures of narrow standards used in standard and universal (viscometer) calibration.

We will examine each in turn as the choices also determine how slice concentrations are calculated and that has a great effect on the final answers.

(a) If you filled in the radio button for “Unknown” and then clicked “OK”, this is what you see:



The Group ID is like a folder into which various data files are kept. It helps to organize the database. Duration defaults to 30 minutes. It can be different for individual runs in the same queue. The “Injected Volume” will be copied from the Injector Window in the System Setup; however, it can be changed here and it too can be different for individual runs in the same queue.

Unless you have checked “concentration is unknown”, you must fill in the “Injection Concentration” in mg/mL; otherwise, you cannot leave the window except through cancelation. If the concentration is unknown, check the box and read the information accessible by clicking the associated question mark icon. There you will learn that slice concentrations can be calculated even without knowing the injected concentration.

The value of dn/dc is used in two ways: If you have an RI device, and its wavelength is close to that of the light scattering device, then dn/dc is used to determine both slice concentrations and in the light scattering equations to determine M_w. If you don’t know dn/dc, it can be calculated from RI data (not UV data) and then used in the light scattering equation, but this only works well if the two wavelengths are close (as they are with the BI-DNDC and BI-MwA). However, if you are using an old RI device, one with “white” light or a wavelength far from the visible, say 700 nm or greater,

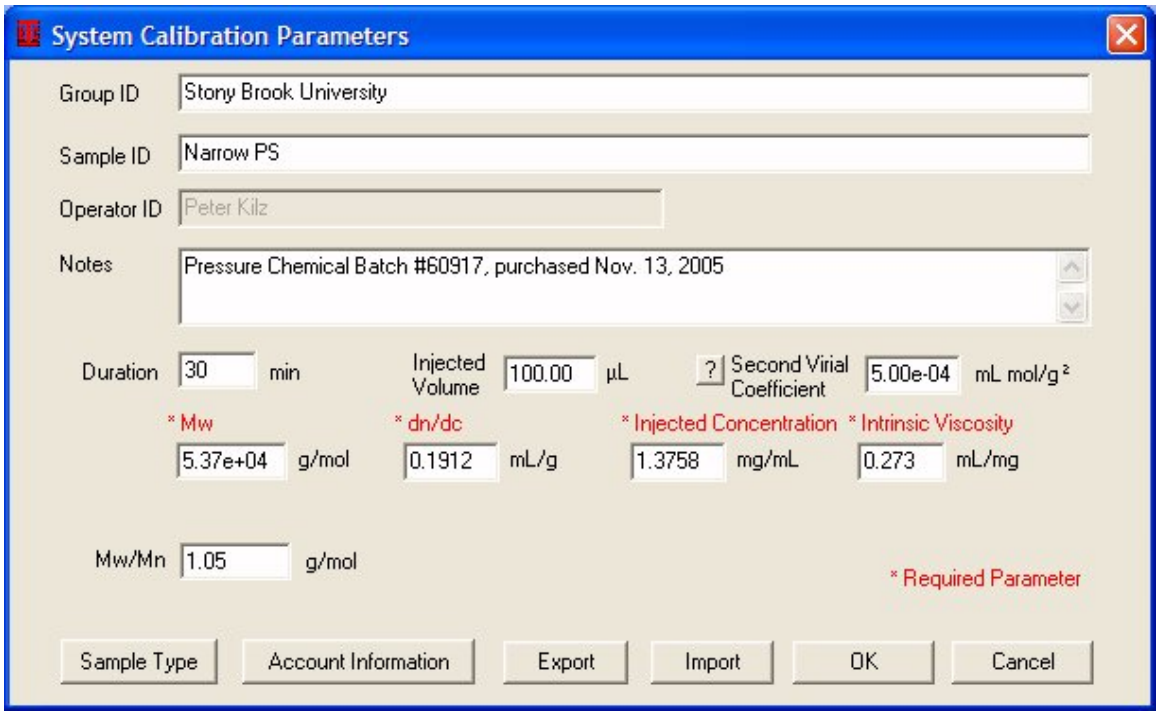
you will need to enter dn/dc corresponding to the wavelength in the light scattering device.

Click on the question mark icon labeled “Second Virial Coefficient” for a discussion of when this value is important. Generally, it is important for higher concentrations and molecular weights, say greater than a few mg/mL and 1 Mg/mol. If in doubt, compare results using zero and the default value. If the change is substantial, consult the literature for a better estimate of this coefficient.

As before, if you expect to measure similar samples often, click the “Export” command button and give the saved file a name. The extension is .bsp1. You can “Import” such files and save time in subsequent runs.

Whereas the “Notes” field is useful in describing the sample and any preparation, the “Account Information” window is used for keeping notes on any third party request for the measurement.

(b) If you filled in the radio button for “System Calibration, light scattering” and then clicked “OK”, this is what you see:

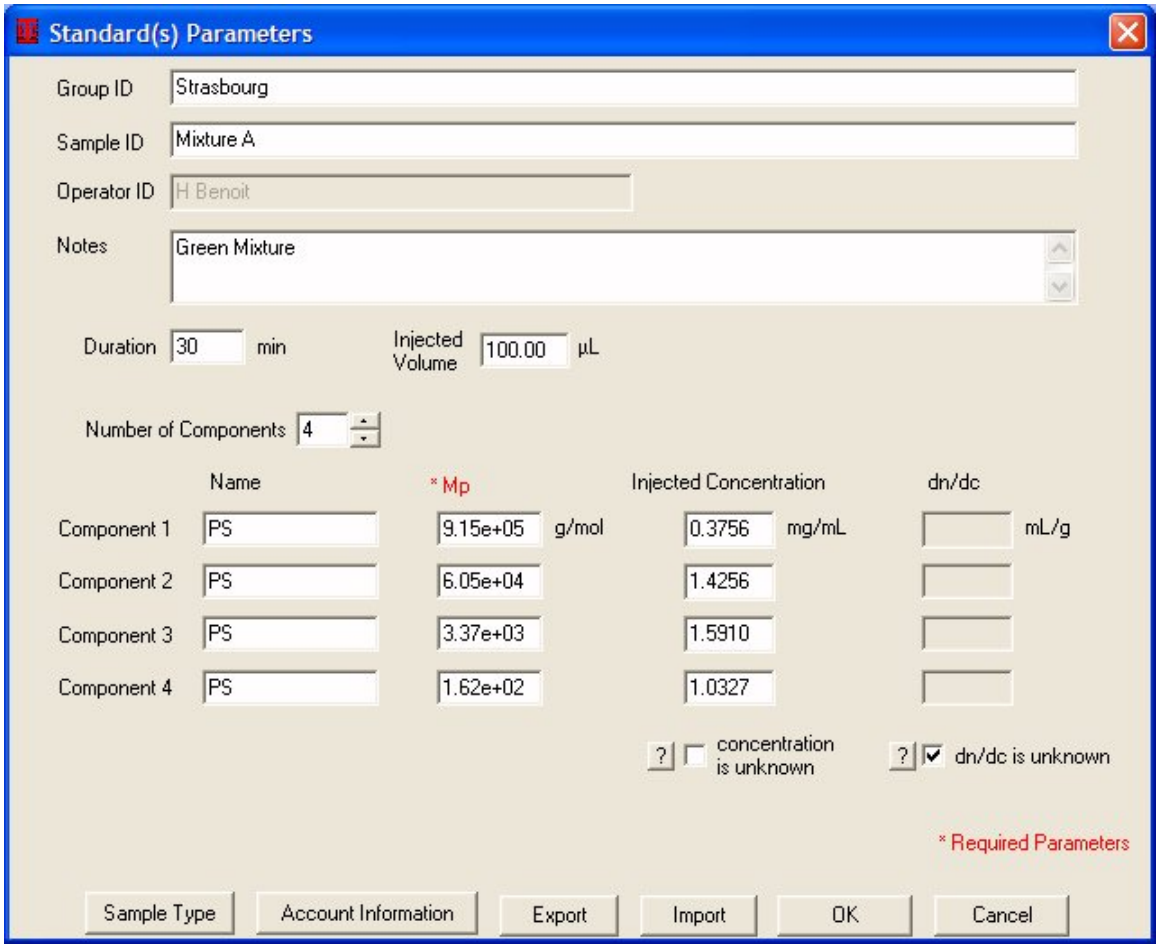


Choose this sample type when you want to use a standard polymer with known properties to calibrate all the detectors, including the light scattering device, normalize angular intensity data, and determine the inter-detector delays.

The window will change depending on what detectors you have enabled in the System Setup. If you have enabled a viscometer, then the Intrinsic Viscosity becomes a required parameter (labeled in red and with an asterisk).

If the standard used shows the ratio of the weight- and number-average molecular weights, then fill it in. Sometimes that value is useful in a fine adjustment to the inter-detector delay, especially for broader standards like Dextran with M_w/M_n around 1.35.

(c) If you filled in the radio button for "Standard(s), calibration curves" and then clicked "OK", this is what you see:

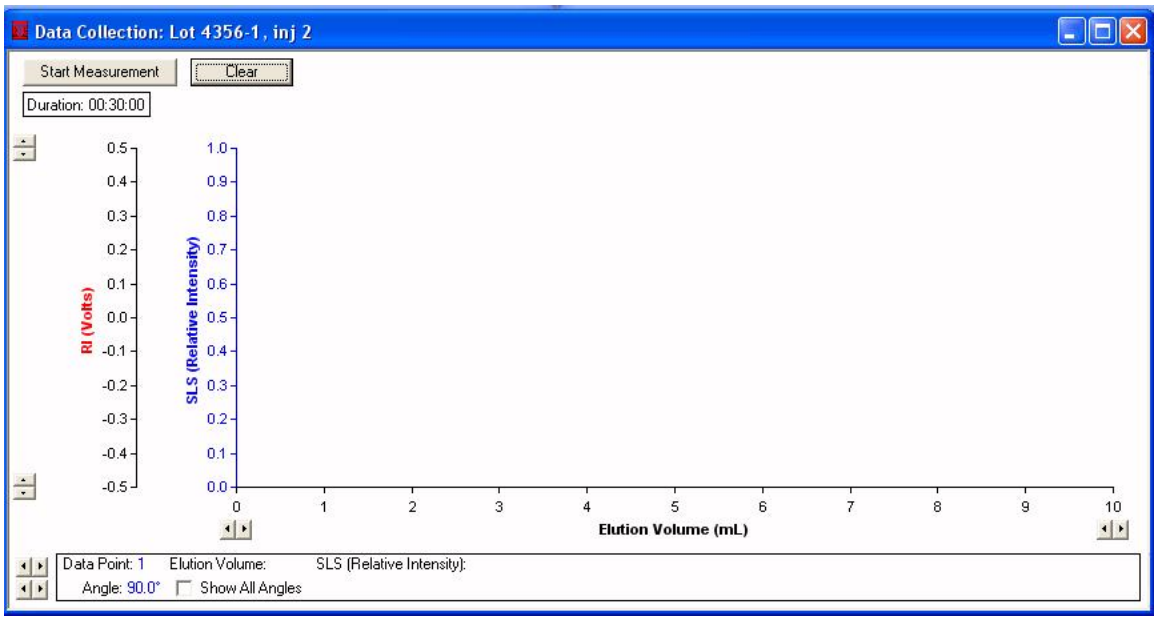


You can select from one to four standards. Commonly, standards come in a mixture. The required parameter is the molecular weight at the peak, M_p, determined from SEC/GPC, for it is the elution volume corresponding to this given peak's molar mass that will be used to construct calibration curves (Ln M_p vs. V_e), not M_w.

II. Making Measurements.

Here we assume you have configured the system (New Measurements/System Setup) that properly identifies the detectors you want enabled; you have verified using the "Signal Monitors" that each detector is registering properly; and you have set up a "Sample Queue" with one or more samples. In the beginning, such samples will be standards either for one or the other type of calibration, or validation standards.

After entering the last sample in the queue, click "Start Queue". You will see the Data Collection Window that looks like this:

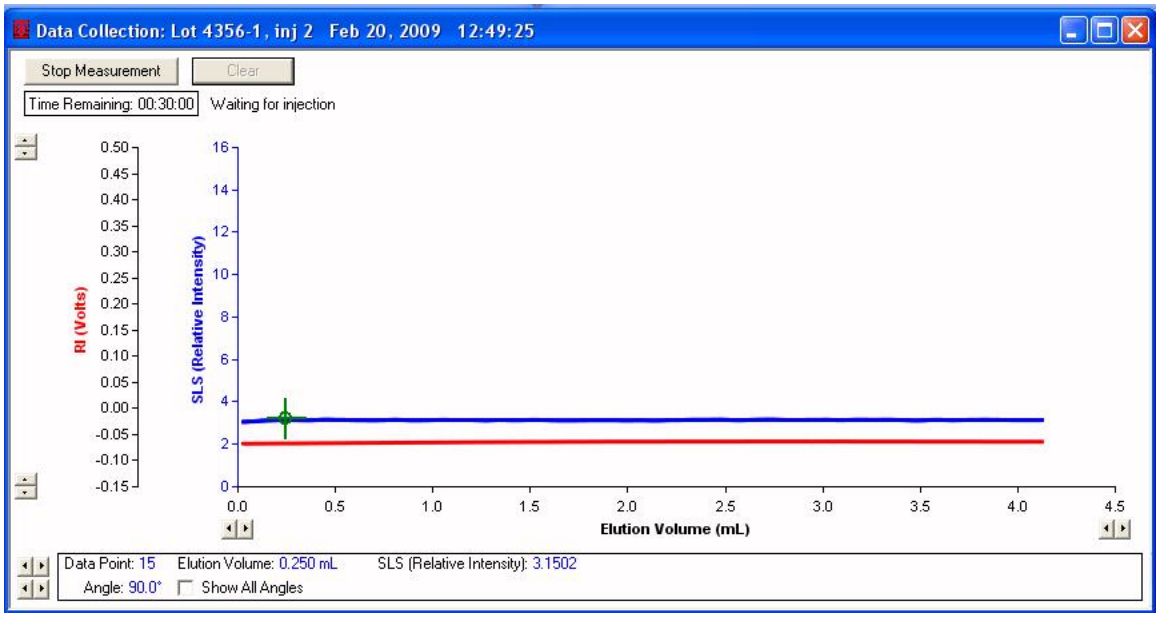


The Y-axes for two detectors are displayed: RI concentration detector in red and static light scattering detector in blue. There may be more. To turn them on, right click (see discussion in Chapter II). Click on "Graph Settings" and mark or unmark the check boxes as desired.

Remember: If in doubt, right click to bring up choices appropriate for this window and also for motoring around the program.

Notice that the "Duration" is displayed but nothing is happening. To begin, click "Start Measurement".

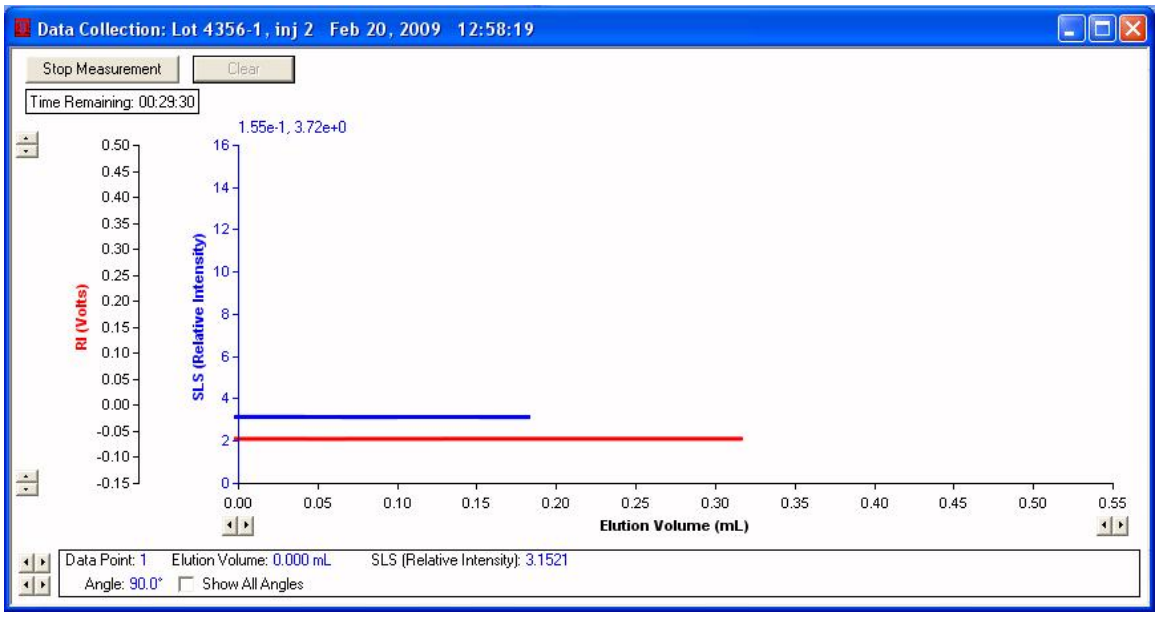
This is what you see next, well after about 4 minutes (flow rate 1 mL/min):



Signals are being displayed but not recorded. This allows you another opportunity to look for any gross instability in the system. If you see any, you can “Stop Measurement” or “Stop Queue”, depending on what you want to accomplish.

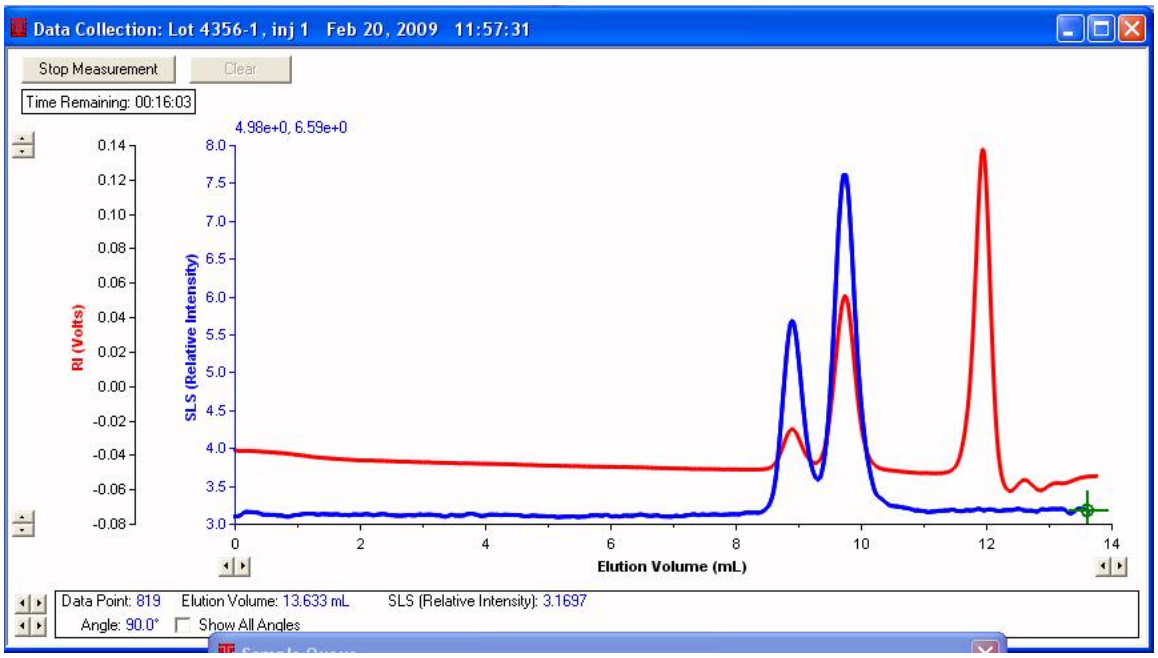
“Time Remaining” is still the original duration of 30 minutes and now the system is “Waiting for injection”.

Here is what you see 30 seconds after the injection trigger is sensed:



The x-axis has reset, starting with zero elution volume. There are 29 minutes and 30 seconds remaining. The inter-detector delay has not been enabled, since the RI detector signal is ahead of the static light scattering signal. This difference is the inter-detector delay. It means the RI device is after the columns and before the light scattering device. If the inter-detector delay had been set, the red and blue lines would be of equal length.

Here is what you see in the “Data Collection” window a little less than half way through the original 30 minute duration:



Here the inter-detector delay was enabled and this explains the nice overlap of the two peaks in blue and red. The far peak in red, along with the ripples following it, the one without any corresponding light scattering signal in blue, are the so-called solvent or system peaks. They arise from the difference in refractive index of the solvent (the smallest molecule in the injection solution) and the eluent. Sometimes the solvent peak is negative. It depends on the sign of the difference in the refractive index. In any case, solvent and eluent molecules have molecular weights that are too small for a light scattering device to sense, but highly sensitive RI detectors can easily sense the difference.

At this point, one can click “Stop Measurement”. When we do, the measurement is saved in the database and is then ready for analysis in the Review Window.

The high sensitivity of the RI detector is often accompanied by a slow drift as shown for the red signal above. In the Review Window, by setting the baseline markers, any drift in any of the signals is accounted for. Click “Show Elugram” to remove the effect of drifting baselines. Then set the smaller integration limit markers to highlight just the region around the peaks where you want to calculate. Now results are available in the Detector, the Molecular Weight Distribution, and any other windows.

An observant person will notice that the last slide corresponds to Injection 1 at 11:57 am; whereas, the slide before that one corresponds to Injection 2 at 12:58 pm. Do not be confused. The slides were not chosen for absolute continuity but only to demonstrate what to look for.

Appendix A: Definitions of Average Molar Masses

Let n_i equal the number of chains each of molar mass M_i. The number-average molar mass is defined as:

$$M_n = \overline{M}_n = \langle M_n \rangle = \frac{\sum_i n_i M_i}{\sum_i n_i}.$$

The mass-average molar mass, M_w, more commonly called the weight-average molar mass is defined as:

$$M_w = \overline{M}_w = \langle M_w \rangle = \frac{\sum_i n_i M_i^2}{\sum_i n_i M_i}.$$

The Z-average molar mass is defined as:

$$M_z = \overline{M}_z = \langle M_z \rangle = \frac{\sum_i n_i M_i^3}{\sum_i n_i M_i^2}.$$

For a monodisperse distribution, $M_n = M_w = M_z$. For a polydisperse distribution, the following relationship holds: $M_n < M_w < M_z$.

A polydispersity index PDI that is always >1 can be defined as either

$$PDI_{w/n} = \frac{M_w}{M_n} = \frac{\sum_i n_i \sum_i n_i M_i^2}{\left(\sum_i n_i M_i\right)^2},$$

or as

$$PDI_{z/w} = \frac{M_z}{M_w} = \frac{\sum_i n_i M_i \sum_i n_i M_i^3}{\left(\sum_i n_i M_i^2\right)^2}.$$

Appendix A: Definitions of Average Molar Masses

If the distribution is thought of as continuous, where M is now a continuous variable, and f(M) is the differential distribution by number, that is f(M)dM is proportional to the number of polymer chains with molar masses between M and M+dM, then the above discrete definitions are replaced by these integral definitions:

$$M_n = \frac{\int M\, f(M)dM}{\int f(M)dM},$$

$$M_w = \frac{\int M^2 f(M)dM}{\int M\, f(M)dM},$$

$$M_z = \frac{\int M^3 f(M)dM}{\int M^2 f(M)dM},$$

$$PDI_{w/n} = \frac{M_w}{M_n} = \frac{\int f(M)dM \int M^2 f(M)dM}{\left(\int M\, f(M)dM\right)^2},$$

$$PDI_{z/w} = \frac{M_z}{M_w} = \frac{\int M\, f(M)dM \int M^3 f(M)dM}{\left(\int M^2 f(M)dM\right)^2}.$$

Real polymer distributions are discrete, of course, and these integral definitions are used in theoretical calculations once a suitable, continuous function f(M) is defined. Given that f(M) is the differential distribution by number, then M·f(M) is the differential distribution by mass.

Experimentally one does not count the number of molecules. Instead, the concentration c_i is determined. By redefining n_i to be the number of chains per unit volume each with molar mass M_i, then $c_i = n_i \cdot M_i$. Now the original summations can be replaced by the following ones:

$$M_n = \frac{\sum_i c_i}{\sum_i c_i / M_i},$$

$$M_w = \frac{\sum_i c_i M_i}{\sum_i c_i},$$

Appendix A: Definitions of Average Molar Masses

$$M_z = \frac{\sum_i c_i M_i^2}{\sum_i c_i M_i},$$

$$PDI_{w/n} = \frac{M_w}{M_n} = \frac{\sum_i c_i / M_i \sum_i c_i M_i}{\left(\sum_i c_i\right)^2},$$

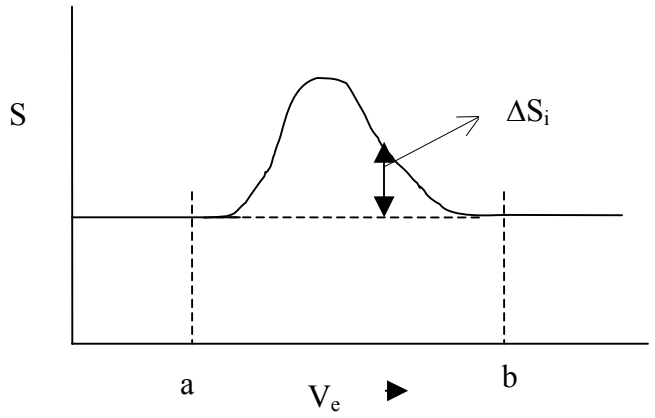
$$PDI_{z/w} = \frac{M_z}{M_w} = \frac{\sum_i c_i \sum_i c_i M_i^2}{\left(\sum_i c_i M_i\right)^2}.$$

These definitions show that a set of $\{c_i, M_i\}$ obtained experimentally can be used to calculate the important MWD statistics. The c_i are obtained from the concentration detector. The M_i are obtained from the SLS detector without the need to calibrate the columns, or the M_i are obtained by standard or universal calibration. In the latter case, a viscosity detector is required.

Appendix B: Basic MWD Calculation Methods

Concentration Signals, S, vs. Elution Volume, V_e

A typical concentration detector signal is displayed below. It could represent the signal in volts from the RI or UV device. The baseline is placed and extended under the peak. Then integration limits, *a* and *b*, are determined.



We define the i^{th} slice as a vertical cut at the i^{th} elution volume $(V_e)_i$. Assuming the data collecting rate is once per second, then the i^{th} slice occurs at the i^{th} second after injection defines the start of the measurement. For example, a 30 minute run consists of 1800 slices. Remember: From the slice concentration c_i and the corresponding M_i values, one can determine all the important statistics for the MWD. Note that data are collected discretely; however, it is convenient to represent the curve as continuous.

Excess quantities are defined as the difference between the signal and the baseline (extended under the curve). It is these excess quantities that are related to concentration, molecular weight, and intrinsic viscosity. The excess signal at $(V_e)_i$ is indicated in the plot as ΔS_i.

For an RI device, the excess signal is proportional to the product of three factors:

$$\Delta S_i = f_{RI} \times dn/dc \times c_i . \qquad \text{Eq. 1}$$

Where f_{RI} is the instrument calibration factor, a constant independent of polymer or solvent or column; dn/dc is the specific refractive index, also known as the differential refractive index, and it is a function of polymer, solvent, wavelength, and temperature; and c_i is the concentration of the i^{th} slice.

Rearranging the equation yields one method for obtaining the slice concentrations:

$$c_i = \frac{\Delta S_i}{f_{RI} \times dn/dc}. \qquad \text{Eq. 2}$$

Obviously, when using this method, both the instrument calibration factor and the differential refractive index must be known.

There is another way that doesn't require either of these factors. First, determine the area under the curve, A_{RI}, by integration:

$$A_{RI} = \int_a^b \Delta S \cdot dV_e \,. \qquad \text{Eq. 3}$$

Next, substitute to get this expression:

$$A_{RI} = \int_a^b \Delta S \cdot dV_e = f_{RI} \times dn/dc \times \int_a^b c \cdot dV_e = f_{RI} \times dn/dc \times m_{inj}\,. \qquad \text{Eq. 4}$$

This last integral yields a mass, the injected mass, m_{inj}, provided that all the polymer elutes from the column. This assumes there is no retention. This assumption is inherent in the idea of successful column chromatography. In fact, by comparing injected mass with that calculated from the area under the curve, one can check retention was insignificant.

Dividing Eq. 1 by Eq. 2, and rearranging, one finds: $c_i = \frac{\Delta S_i \cdot m_{inj}}{A_{RI}}$ Eq. 5

Thus, the slice concentration can be calculated without calibrating the RI device and without knowing the value of dn/dc.

Both Eq. 2 and Eq. 5 have their uses depending on how much is known about the properties of the polymer solution and the RI instrument. For example, the traditional standard calibration using an RI device was often carried out using a wavelength in the infrared or even with a white light source (multiple wavelengths spanning the visible). Eq. 5 can still be used in these circumstances but not Eq. 2. Even if the source has a single wavelength in the infrared, and dn/dc were known, it is very likely that the same value could not be used with an SLS instrument using a visible wavelength. The values of dn/dc differ sufficiently such that the error could be significant.

Remember that injected mass is simply the injected concentration, C_{inj}, times the loop volume, V_L:

$$c_i = \frac{\Delta S_i \cdot C_{inj} \cdot V_L}{A_{RI}}\,. \qquad \text{Eq. 6}$$

Loop volumes, typically in the range of 50 to 250 μL for polymer characterization (much smaller for HPLC and small molecule characterization) can have errors as large as

± 10%. Depending on how the detectors are calibrated, this error may contribute significantly, or it may cancel out.

For a UV-Vis detector, the absorbance, A, is defined as Log (I_o/I_t), where I_o is the incident intensity (the baseline value) and I_t is the transmitted intensity at the same elution volume. It follows from Beer's law that:

$$A_i = \varepsilon \times b \times c_i \,. \qquad \text{Eq. 7}$$

Where ε is the absorptivity (wavelength dependent); b is the path length in the cell; and c_i is the i^{th} slice concentration as before. Actually, Beer's law is normally written

$$I_t = I_o \cdot e^{-\varepsilon bc} \quad .$$

Since the exponent is always positive, it follows that the transmitted light is always smaller than the incident light. Thus, $Ln(I_t) - Ln(I_o) = - \varepsilon$ x b x c. The difference in natural logarithms is analogous to ΔS. The signal is inverted to show a positive peak and this removes the minus sign. And one may think of the definition for absorbance as another way of removing the minus sign. Since Ln(X) = 2.303 x Log(X), the constant Ln 10 = 2.303 is, if you will forgive the expression, *absorbed* into the constants used when normalizing such measurements.

Now Eq. 7 and Eq. 1 are formerly similar. Just as we can calibrate an RI device to determine f_{RI} , given dn/dc, we can calibrate a UV-Vis detector to determine ε, given path length b. However, whereas dn/dc is known for many polymer/solvent pairs that may be used to calibrate an RI device, it is less convenient to use a standard with a known absorptivity. Instead, by integrating Eq. 7 over the peak, equations analogous to Eq. 5 and 6 are obtained as follows:

$$c_i = \frac{A_i \cdot m_{inj}}{A_{UV}}, \qquad \text{Eq. 8}$$

and

$$c_i = \frac{A_i \cdot C_{inj} \cdot V_L}{A_{UV}} \,. \qquad \text{Eq. 9}$$

Where A_{UV} is the area under the absorbance curve and is formally given by analogy to Eq. 3 as:

$$A_{UV} = \int_a^b A \cdot dV_e \quad . \qquad \text{Eq. 10}$$

Concentration Signals, S, vs. Elution Volume, V_e

THIS IS A DRAFT and so this section is not yet finished.

Appendix C: Extended Calculations

R_g – R_H and Geometric Dimension Relationships

For spheres, $(R_g)^2 = 3/5\ R^2$, where R is the geometric radius of the sphere and R_g, the radius of gyration, is determined from static light scattering, SLS. For a spherical polymer[1], biomacromolecule (many globular proteins are close enough), dendrimers, or nanoparticles with small molecules and ions attached to the surface, the geometric radius is replaced by the hydrodynamic radius, R_H. Using dynamic light scattering, DLS, one can determine R_H directly, assuming the correlation function is analyzed for center-of-mass diffusion (translational diffusion).

Thus, for globular shapes, $R_H/R_g = 1/\sqrt{(3/5)} = 1.291$.

For a random coil polymer in a theta solvent at the theta temperature, $R_H/R_g = 0.665$ and in a good solvent, $R_H/R_g = 0.640$.[2] If you have to remember these numbers, use 0.65 or 2/3 for both cases.

For a long, thin, rod-like macromolecule (prolate ellipsoids with Length/Diameter, L/b, aspect ratios > 10), $(R_g)^2 = L^2/12$. The diameter of the rod plays no part in R_g if the aspect ratio is large enough.

For rod-like macromolecules, including those short enough where the diameter matters,

$$R_H / R_g = \frac{3^{1/2}}{Ln(L/b) - \gamma}.$$

Where, $\gamma = 0.3$ and accounts for end effects of the rod. (See reference 2.) The value of R_H/R_g for a rod is 0.640 when L/b = 20. This value is close to that for random coils. But for smaller L/b, the ratio is larger: 0.865 when the ratio is 10 and 1.323 when the ratio is 5.

R_g – M Relationships

In general, for a given shape, as the molecular weight increases, the size must increase too. How fast it increases depends on the shape of the molecule and the polymer-solvent interactions. In general, $R_g = k\ M^{\nu}$

In a so-called “good” solvent, the monomers of a linear, random-coil, homopolymer are surrounded by solvent in a dilute solution. Polymer-solvent contacts are favored over polymer-polymer or solvent-solvent interactions. The extension of the polymer is

greatest. In this case, $R_g \sim M^{3/5}$ is a result first given by Flory.[3] The second virial coefficient, A_2, is positive.

An experimental example is polystyrene in toluene or benzene at room temperature from 200 kg/mol to 50 Mg/mol:[4]

$R_g(\text{nm}) = 0.0123 \times (\text{M in g/mol})^{0.593}$.

Such results are obtained by plotting Log R_g vs. Log M for either a series of very narrowly distributed polymers, each measurement typically made in batch mode, or using chromatography on a broad sample ($M_w/M_n > 2$). In either case, it is highly recommended to use at least two full decades in molar mass; otherwise, such Log-Log plots are notorious for yielding the wrong answer. In this particular case, the range of molar masses was 250:1 or 2.5 decades.

The pre- and exponential factors agree quite well with those given by renormalization theory, 0.01234 and 0.5936 (see reference 2). And the exponent is very close to 3/5 from Flory's simpler theory. Using this formula to <u>estimate</u> R_g for polystyrene in other good solvents yields reasonable approximations. Goods solvents dissolve the polymer readily.

However, the pre-exponential factor and exponent will differ for another random coil, linear homopolymer. Values in the range of 0.55 to 0.65 are common for the exponent.

In a so-called "theta" solvent, at the theta temperature, various effects cancel and the size is proportional to the square root of the number of monomers. That is $R_g \sim M^{1/2}$. The second virial coefficient, A_2, is zero. Thus, the solution behaves as if it were ideal until third order effects (A_3Mc^2) become important. Each polymer/solvent pair has, in principle, a theta temperature. However, it may be outside the range where the polymer/solvent can form a solution.

An experimental example is polystyrene in cyclohexane at 35.4 °C from 800 kg/mol to 50 Mg/mol (see reference 2):

$R_g(\text{nm}) = 0.02675 \times (\text{M in g/mol})^{0.5040}$.

As the solvent becomes "poor", the polymer-polymer and solvent-solvent interactions become stronger than the polymer-solvent interactions. The second virial coefficient, A_2, is less positive, even somewhat negative. The polymer begins to fold up, driving towards a globular shape. For a sphere, the hydrodynamic volume, $V_H \sim (R_H)^3 \sim (R_g)^3$. The volume is proportional to the mass. Therefore, it follows that $R_g \sim M^{1/3}$.

It is hard to find such cases starting with a synthetic, random coil polymer, for when A_2 is negative and polymer-polymer interactions are strong, they are also strong

between chains. Aggregation occurs. Thus, the molecular weight and R_g determined are of the aggregates.

For long, thin rods, $R_g \sim L \sim M$, since adding monomer increases the length and molecular weight linearly. In this idealized case, the exponent for a rod is 1.

It is clear that the exponent in a Log R_g vs. Log M plot carries shape information. If it is close to 1, the shape is rod-like, if it is close to 0.6 the shape is that of a random coil in a good solvent, if it is 0.5 the shape is that of a random coil under theta conditions, and as the exponent approaches 1/3 the shape approaches that of a spherical particle.

R_H – M Relationships

In the first section, it was shown that for a given shape there is a fixed relationship between R_g and R_H. It follows that if DLS is used to determine R_H, then the exponent in R_H-M relationships also indicates something about the shape.

For example, DLS measurements on 14 globular proteins, ranging from lysozyme (14.7 kg/mol) to thyroglobulin (670 kg/mol) at room temperature gave this result:[5]

Globular Proteins: $R_H(\text{nm}) = 0.0309 \times (\text{M in g/mol})^{0.428}$.

Clearly, many of the globular proteins are slightly elongated since the exponent is not 1/3. Given a direct measurement of R_H using DLS, this equation can predict the molecular weight of a globular protein to within 10%. Not bad if the goal is to determine whether or not the protein has aggregated.

How about other biomacromolecules? Here are average results in water at room temperature as given in the Brookhaven Instrument's BI-PSDW software manual:

Starburst Dendrimers: $R_H(\text{nm}) = 0.0860_5 \times (\text{M in g/mol})^{0.30}$

Branched Polysaccharides: $R_H(\text{nm}) = 0.0367 \times (\text{M in g/mol})^{0.44}$

Linear Polysaccharides: $R_H(\text{nm}) = 0.0151 \times (\text{M in g/mol})^{0.55}$.

The exponent for dendrimers confirms that these polymers made from trifunctional monomers are sphere-like. The branched polysaccharides are as dense as a globular protein given the similarity of the exponents. And linear polysaccharides in water behave as many linear homopolymers in a "good" solvent do.

R_H – D Relationships

The relationship between hydrodynamic radius and translational diffusion coefficient for center-of-mass motion is given by the Stokes-Einstein equation:

$$D_T = k_B T / 6\pi\eta R_H \ .$$

Where D_T is the translational diffusion coefficient, T is temperature in Kelvin; k_B is Boltzmann's constant (1.3806 E-23 $J \cdot K^{-1}$); and η is the bulk viscosity of the liquid (0.8904 mPa·s for water at 298.15 °K). The previous four R_H – M relationships were in fact calculated from these four $D_T = k_D \cdot M^{\beta}$ relationships (all at 25 °C, pure water):

Globular Proteins: $D_T(cm^2 \cdot s^{-1}) = 7.89E\text{-}5 \times (M \text{ in g/mol})^{-0.428}$

Starburst Dendrimers: $D_T(cm^2 \cdot s^{-1}) = 2.86E\text{-}5 \times (M \text{ in g/mol})^{-0.30}$

Branched Polysaccharides: $D_T(cm^2 \cdot s^{-1}) = 6.71E\text{-}5 \times (M \text{ in g/mol})^{-0.44}$

Linear Polysaccharides: $D_T(cm^2 \cdot s^{-1}) = 1.63E\text{-}4 \times (M \text{ in g/mol})^{-0.55}$

A relationship such as $D_T = k_D \cdot M^{\beta}$ is known as a Mark-Houwink-Sakurada (MHS) equation with the pair of constants, $\{k_D, \beta\}$, known as the MHS constants. A more familiar set of MHS constants is described in the next section. All such MHS equations are empirical. [The SLS equation relating excess scattered intensity to molecular rate is not empirical. It is more fundamental. Thus the name "absolute" attached to molecular weight determination using SLS even though the instrument itself has to be calibrated.]

This relationship can be used to calculate a molecular weight (not M_n or M_w, but M_D) from DLS measurements of D_T provided the model is adequate to describe the experimental situation.

$[\eta]$ – M Relationships

The most famous MHS equation relates intrinsic viscosity, $[\eta]$, to molecular weight, M, as follows:

$$[\eta] = k_\eta \cdot M^{\alpha} \ .$$

Where the pair of constants, $\{k_\eta, \alpha\}$, also MHS constants, depend on the solvent, the solute (macromolecule), and the temperature. The constants α are related to shape with val-

ues >1 for rods, 0.7 to 0.8 for random coils in good solvents, and 0.5 in theta solvents. This relationship can be used to calculate a molecular weight, called the viscosity-average molar mass, M_v, from intrinsic viscosity measurements of [η] provided the model is adequate to describe the experimental conditions.

While this equation was used extensively before SEC/GPC became commonplace, a more common place to find the use of intrinsic viscosity is this empirical equation that is ultimately derived from the Einstein equation for spheres:

$$[\eta] \cdot M = \Phi \cdot V_H \, .$$

Where V_H is the hydrodynamic volume and Φ is a constant equal to about 2.8 x 10^{23} for all flexible chains.[6] If it were a universal constant, then the product of intrinsic viscosity and molecular weight would be the same for all polymers with the same hydrodynamic volume. Since hydrodynamic volume is responsible for pure size exclusion chromatography, it follows that setting up a curve of [η]·M vs. V_R, where V_R is retention volume in an SEC experiment, should lead to different polymers of different molecular weights falling on the same curve. Then a measurement of the intrinsic viscosity of an unknown would lead to its molecular weight if it too followed the "universal" curve.

Many nonpolar flexible chain polymers and copolymers in nonpolar liquids do indeed, over limited molecular weight ranges, follow such a curve. And, so, this empirical equation provides a method of calibrating columns if standards that match the unknown in structure and chemistry are not available for standard calibration.

But the equation is not universal. Several polymers, especially ones in water and polar or charged macromolecules with retention mechanisms other than size, do not follow the universal curve. The constant Φ is suppose to be independent of structure and shape. But it isn't for enough polymers that "universal" calibration is not, after all, quite so universal.

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[1] Polymers are sometimes limited to synthetic polymers. And macromolecules include both polymers and biomacromolecules such as proteins. Throughout this appendix, the words polymer and macromolecule are interchangeable.

[2] I. Teraoka, *Polymer Solutions: An Introduction to Physical Properties*, Table 3.1, page 187, John Wiley & Sons, New York (2002).

[3] P.J. Flory, *Principles of Polymer Chemistry*, Cornell University Press, Ithaca, New York (1953).

[4] J. de Cloizeaux, G. Jannink, *Polymers in Solution : Their Modelling and Structure*, Oxford University Press: Clarendon (1990).

[5] K. Mattison, private communication of DLS measurements made with a Brookhaven BI-200SM multiangle SLS/DLS goniometer circa 1995.

[6] Paul C. Hiemenz and Timothy P. Lodge, *Polymer Chemistry, Second Editon*, CRC Press, Boca Raton, Florida (2007).