ASTRA V
User's Guide

Version 5.3.4

(M1000 Rev. H)

Contents

1 About ASTRA V

This chapter provides an overview of the ASTRA V software and this manual. It also tells you how to contact Wyatt Technology for support.

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What is ASTRA?

The ASTRA software collects and processes data from dilute macromolecular solutions. It uses this data to calculate the molar mass, radius moments, and other results. In addition, it controls the data acquisition performed by various instruments.

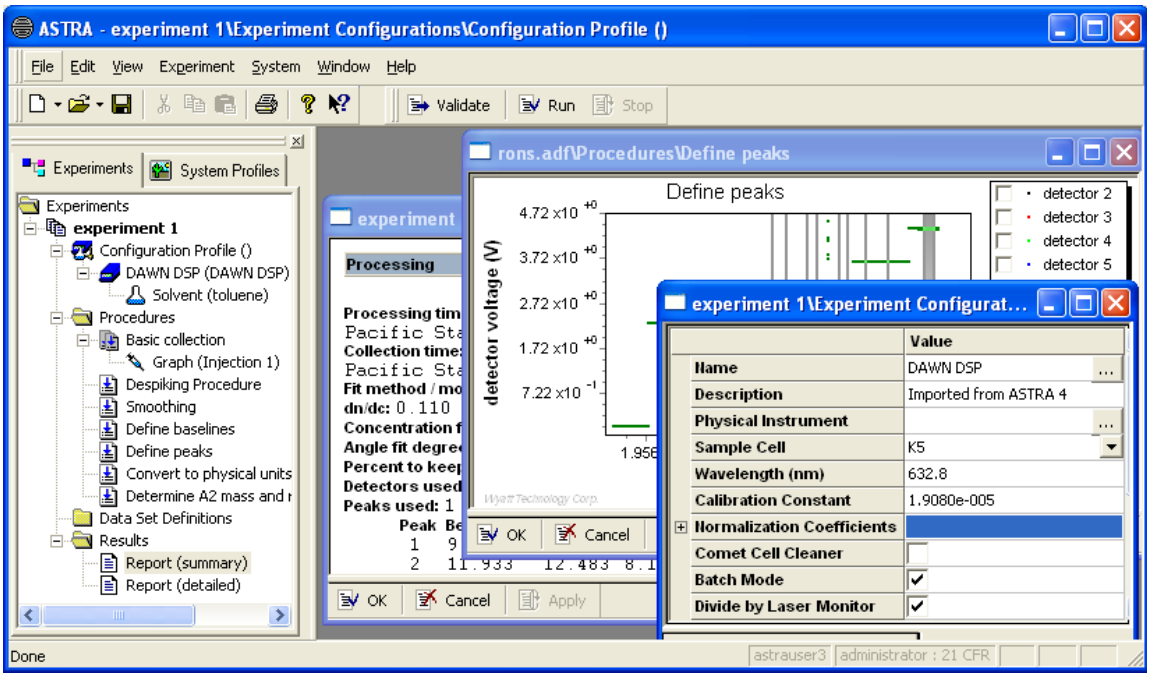

Figure 1-1: ASTRA Environment

How is ASTRA V Different from Earlier Versions

If you created files with ASTRA version 4.70 and higher, this version can import experiments and data from the earlier version.

ASTRA V, rewritten from the ground up, has numerous advantages over previous versions of ASTRA, including the following:

- **Easier to use.** You can get started simply by opening an experiment template, connecting to your physical instruments, and running the experiment. “Run mode” simplifies the ASTRA interface.
- **More powerful.** As you gain more experience with ASTRA, you can begin to create and manage system profiles to extend the experiment templates. “Experiment Builder mode” provides more power.
- **21 CFR Part 11 compliance.** You can store experimental data as required by the FDA, if federal compliance is required in your lab.
- **Less light-scattering centric.** This new version of ASTRA is designed to work with a wide variety of instrument types, without sacrificing functionality for light-scattering data collection. ASTRA V supports all Wyatt Technology instrumentation and analysis methods and adds new functionality.

Using This Manual

This manual describes how to install and configure the ASTRA software for collecting and processing data. It is meant to be used in conjunction with the hardware manual that came with your Wyatt instrument (for example, a DAWN or miniDAWN instrument).

This manual assumes a basic knowledge of Microsoft Windows features and mouse operations.

User Modes

You can use ASTRA in "Run" mode or "Experiment Builder" mode.

Run mode makes it easier to learn to use ASTRA. It may be the mode you prefer even after you are an experienced user. In Run mode, you create experiments using the configuration and procedure templates provided with ASTRA. You can modify configuration and procedure properties, but cannot add or delete instruments or procedures.

Experiment Builder mode allows you to modify the configuration and procedure sequences in a template. The icon to the left identifies portions of this manual that apply only if you turn on Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

In addition, Experiment Builder mode allows you to open multiple procedure windows at once. However, you should be careful with this feature, since changing and applying properties in one window does not generally result in changes to other open procedure windows. To see such changes reflected in other procedure windows, you should close and reopen them.

Operating Tiers

ASTRA V can be purchased with any of the following operating tiers:

- **ASTRA V Basic:** Saves experiments to files. In this manual, the "Basic" icon applies to this mode.
- **ASTRA V with Research Database:** Saves experiments to an experiment database. Does *not* provide 21 CFR Part 11 compliance. In this manual, the "Database" icon applies to this mode.
- **ASTRA V with Security Pack:** Provides 21 CFR Part 11 compliance. This includes saving experiments to an experiment database, user accounts with access levels, and sign off procedures. In this manual, the "Database" and "Security" icons both apply to this mode.

This manual identifies information that is specific to these operating tiers using the following icons:

Table 1-1: Operating Tier Icons

Icon	Description
Basic	Identifies information that applies only to the ASTRA V Basic operating tier.
Database	Identifies information that applies to both of the following operating tiers: ASTRA V with Research Database ASTRA V with Security Pack
Security	Identifies information that applies only to the ASTRA V with Security Pack operating tier.

User Account Levels

Security

As part of the 21 CFR Part 11 compliance of ASTRA V with Security Pack, all users must log in with a unique user id and password. The administrator sets up accounts with one of the following user account levels:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure sequence and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

Security

Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the "Security" icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA V with Security Pack. There are no access restrictions when ASTRA V is used in other operating tiers.

How This Manual is Organized

The first three chapters of this manual provide an overview of ASTRA, explain how to install ASTRA and prepare it for use, and how to get started using ASTRA.

The remaining chapters in this manual correspond to items in the ASTRA environment as shown in Figure 1-2.

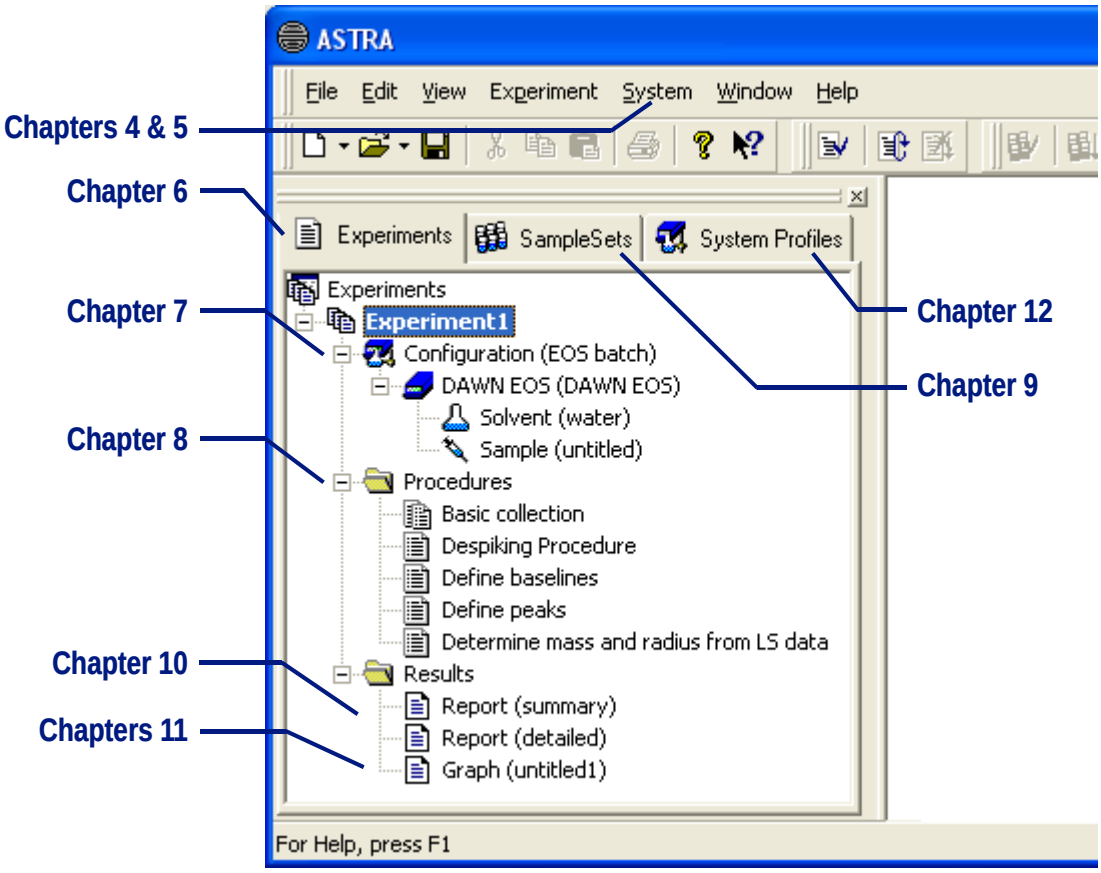

Figure 1-2: Workspace Items and the Chapters that Discuss Them

Manual Conventions

To make it easier to use this manual, we have used the following conventions to distinguish different kinds of information:

- **Menu commands.** This manual indicates menus command to use as follows: **File→Open**. This example indicates that you should open the File menu and select the Open command. You will see this style wherever menu commands are described.
- **Buttons.** In the text you will see instructions to "click" on-screen buttons and to "press" keys on the keyboard.
- **Key combinations.** A plus sign (+) between key names means to press and hold down the first key while you press the second key. For example, "Press ALT+ESC" means to press and hold down the ALT key and press the ESC key, then release both keys.
- **DAWN instrument.** Except where there are details for a particular instrument, when the name will be given, we will refer to the DAWN EOS and miniDAWN instruments simply as the *DAWN*.

- **Molar mass versus Molecular weight.** The IUPAC Definition Committee specifies the term *molar mass* for the sum of the atomic weights of all atoms in a mole of molecules. The term *molecular weight* has the same meaning. You will see *molar mass* used in this manual.

Tip:	See Appendix A, "Menu Quick Reference" for a complete list of keystroke alternatives to the mouse pointer for selecting menu options.

Glossary

The following terms are used in this manual:

- **A2 mass and radius:** Results produced by a traditional Zimm plot analysis.
- **ASTRA:** The data collections, analysis, and lab control software for Wyatt Technology Corporation instruments.
- **batch mode:** Data collection performed in a stand-alone fashion—fluid injected into a cell, not being pumped through plumbing. For example, using the MicroCuvette with the DAWN. Batch measurements are typically on unfractionated samples. Batch mode is also called off-line.
- **configuration:** ASTRA's description of the physical apparatus used to collect data. It is an assembly of profile units describing sample, solvent, and instruments, as well as the connections (both fluid and signal) between them.
- **data set:** The grouping of data produced by a procedure. For example, a typical light scattering measurement might produce a set of data including molar mass and RMS radius.
- **DAWN:** Multi-angle light scattering detectors from Wyatt Technology Corporation. They are used to determine the molar mass, size, and second virial coefficient for macromolecules in solution. Versions include the DAWN EOS and miniDAWN.
- **dn/dc:** The change in a solution's refractive index with a change in the solute concentration. Measured in mL/g.
- **experiment database:** The database in which ASTRA V with Research Database and ASTRA V with Security Pack store experiment information and data.
- **fractionation:** The separation of a polydisperse solution of macromolecules by some physical property of the macromolecules. For example, size exclusion chromatography (SEC) and field flow fractionation (FFF) separate macromolecules based upon size.
- **intrinsic viscosity (IV):** A measure of the capability of a polymer in solution to enhance the viscosity of the solution. Derived using specific viscosity and concentration data.

- **light scattering:** A technique whereby the intensity of scattered light from a macromolecule in solution is measured at multiple angles to determine a molar mass, RMS radius, and second virial coefficient. Also called classical, Rayleigh, or static light scattering.
- **miniDAWN:** A DAWN instrument with three detectors. It is used primarily for characterizing small (less than 50 nm in RMS radius) macromolecules.
- **non-fractionated:** Samples that are typically polydisperse solutions containing a range of macromolecules with different weights, sizes, or conformations.
- **online:** Data collection with solution flowing through the instrument. For example, a pump pushes solvent through an injector, and the resulting solution is passed through a fractionation device and then characterized by instruments downstream. Online measurements are usually for fractionated samples, but can also be for non-fractionated samples.
- **Optilab:** The Optilab instruments (rEX, DSP, and 903) are differential refractometers. These instruments can be used to determine the concentration of a macromolecule in solution, and to measure the *dn/dc* value necessary for determining molar mass in light scattering measurements.
- **physical units:** Units of measurement that have scientific meaning. For example, the DAWN instrument produces voltage signals that must be converted to the physical units of Rayleigh ratio before they can be analyzed to determine mass and radius.
- **procedure:** ASTRA's representation of a process in the collection and analysis of the data. A procedure can be either for collection, data transformation, data analysis, display, instrument configuration, or administrative purposes.
- **profile:** A description of a physical entity in an ASTRA experiment. For example, an instrument, solvent, or sample.
- **QELS (Quasi-Elastic Light Scattering):** This is also known as dynamic light scattering or photon correlation spectroscopy. The WyattQELS instrument measures rapid fluctuations in scattered light intensity to determine the translational diffusion coefficient and hydrodynamic radius for macromolecules in solution.
- **RI:** Refractive index. Used to describe differential refractometer instruments or data from the Optilab rEX, DSP, or 903.
- **system database:** The database in which ASTRA stores templates and profiles. This is separate from the experiment database.
- **ViscoStar:** On-line differential viscometer that measures the intrinsic viscosity and Mark Houwink-Sakurada (MHS) parameters of polymers.

- **system profile:** An ASTRA profile saved in the system database. As such, it is available to all users and can be imported into experiments or sample sets. For example, several common instrument configurations might be saved as system profiles such that they can later be easily used in different experiments.
- **user mode:** You can choose to use ASTRA in "Run" mode or "Experiment Builder" mode. Run mode is easier to use. Certain options are not available in Run mode.
- **workspace:** The portion of the ASTRA interface that shows the Experiments, Sample Sets, and System Profiles tabs and their contents.

Batch Mode vs. Online Mode

The distinction between batch mode and online mode is an important one for all types of instruments.

- **Batch Mode:** In a batch mode experiment, the measurement instrument stands alone, and is not hooked up to a pump. Samples are introduced into the instrument via vials or by injecting slugs of sample that completely fill the sample cell. The concentration of the sample is known, since the researcher has prepared it. Also, the solvent for the sample now needs to be associated with the instrument, since it no longer comes from the pump. Batch mode is also called "non-fractionated".
- **Online Mode:** In an online mode experiment, a measuring instrument such as the Optilab or DAWN EOS is connected to a pump and injector. The solvent flow is controlled by the pump, and the sample is added by the injector. The solution continually flows through the system, and the concentration needs to be measured via an RI or UV instrument. Flow mode is also called "flow" or "fractionated".

Light scattering, ultra-violet, RI, and viscometry instruments can all be run in either batch or flow mode.

Getting More Help

If you have a question about ASTRA, first look in this manual or consult the online help. You can also find late-breaking updates and technical information about your version of ASTRA in the readme file.

Also, be sure to register for and use your Wyatt Technology Support Center account. Go to www.wyatt.com to log in. You'll find FAQs, tutorials, software downloads, newsletters, and ways to order supplies.

If you still cannot find an answer, please contact Wyatt Technology Technical Support.

Contacting Technical Support

Please be prepared to provide the following information when you contact technical support. If you e-mail or fax your question in to us, include *all* of the following information.

- Wyatt Technology instrument serial numbers (located on the back panel).
- ASTRA software version number. The software version number is located on the original distribution CD, or you can view it by selecting **About** from the **Help** menu. The version of ASTRA used to collect the data is included in all reports.
- The type of computer hardware you are using.
- Microsoft Windows version number.
- Exact wording of any messages that appear on your computer screen.
- What you were doing when the problem occurred.
- How you tried to solve the problem.

Contact Information

Website	http://www.wyatt.com
E-Mail Support	astra.support@wyatt.com
FAX Support	(805) 681-0123
Telephone Support	(805) 681-9009
Corporate Headquarters	Wyatt Technology Corporation 6300 Hollister Ave. Santa Barbara, CA 93117 USA

International Support

Outside the USA, you may use one of the contact methods listed here, or you may contact the Wyatt Technology Distributor in the country where you bought your product.

Where to Go from Here

Continue to Chapter 2, “Installing and Setting Up ASTRA” to prepare ASTRA for use.

Be sure to read your hardware manual(s) before attempting to collect data using the software. They contain important safety and operational information.

2 Installing and Setting Up ASTRA

This chapter provides instructions for installing ASTRA on your computer and instructions for preparing it for use.

The ASTRA administrator in your organization should follow the steps in all sections of this chapter to make ASTRA ready for use as described in the remaining chapters.

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System Requirements

In order to use the ASTRA software, you must have the following:

- Microsoft Windows® 2000 (Service Pack 2 or higher), Microsoft Windows XP Professional (32 bit), or Microsoft Windows Vista
- Internet Explorer 5.5 or higher
- At least 150 MB free hard disk space
- At least 512 MB of RAM (1 GB recommended)
- Microsoft Access or Microsoft SQL Server 7 or higher (for ASTRA V with Research Database or ASTRA V with Security Pack only)
- A CD-ROM drive
- Available communication ports as required for data collection. (See the manuals for your instruments for details.)
- A Windows-supported printer and/or plotter
- A Windows-supported mouse

Additionally, adequate experiment storage space is required. Each experiment run with ASTRA will use on average of about 0.5 MB of storage.

Installing the ASTRA Software

This section provides basic instructions for installing ASTRA. You must use the ASTRA installation program to install ASTRA, rather than simply copying the files to your hard disk.

To install ASTRA, do the following:

1. Log in to Windows using an account with Administrator or Power User privileges.
2. Place the ASTRA disk in your CD drive. On most systems, the ASTRA setup procedure will start automatically. (If you downloaded an update, double-click on the downloaded ASTRA_5xxx_Setup.exe file.)

   If the setup procedure does not start automatically, use Windows Explorer or the Run dialog to run setup.exe in the CD's ASTRA folder.
3. Answer the prompts in the setup procedure.

   Choose to perform a full installation so that all the components of ASTRA will be installed. (See "Installing the ISI on Other Computers" on page 5-4 for other types of installations.)

After you install ASTRA, the Windows **Start** menu contains a folder called Wyatt Technology.

Upgrading to a New Version of ASTRA

You can check for newer versions of ASTRA by choosing **Help→Check for Updates** from the ASTRA menus. (You can also use this command to control how often ASTRA checks for updates automatically.)

For recent versions of ASTRA, you can install the new version without deinstalling the old version.

Installing a new version *does not* update the system database, which is where experiment templates and system profiles are stored. This is because you likely want to keep custom experiment templates and system profiles. See "Migrating the System Database" on page 2-3 to update your system database so you have all the latest experiment templates and system profiles.

Note: If you have an older version of ASTRA V, you may be prompted to deinstall the old version of ASTRA before installing the new version. When you deinstall, any files you have created or modified (such as experiment files, your experiment database, and the system database) are not deleted.

To deinstall an old version of ASTRA V, follow these steps:

1. Choose **Start→Control Panel** from the Windows Start menu.
2. Double-click the **Add or Remove Programs** icon.
3. Scroll down in the Add or Remove Programs list to Wyatt Technology ASTRA.
4. Click the **Remove** button.
5. Install the new version of ASTRA as described in the previous section.

   You can choose to install the new version of ASTRA in the same location as the previous version. This allows you to easily continue using the same experiment database and other files.

Migrating the System Database

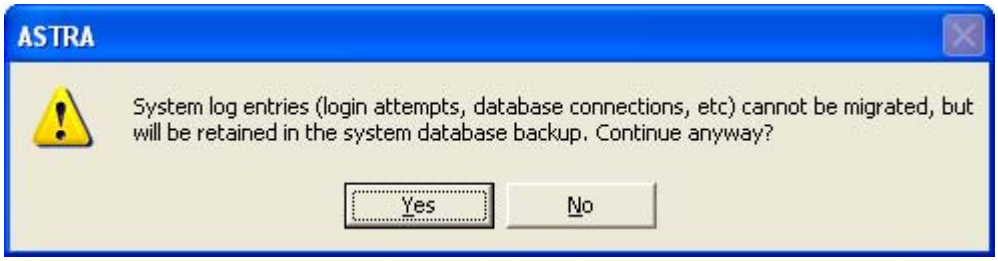
You must use an account with ASTRA Administrator access to follow the steps in this section.

The ASTRA system database stores experiment templates, sample set templates, system profiles, system solvents, and molecular standards. These include items that are provided with ASTRA and items that you customize and save as templates or profiles. All versions of ASTRA use a system database.

The installation does not overwrite an existing system database. To update your system database so you have all the latest experiment templates and system profiles, follow these steps:

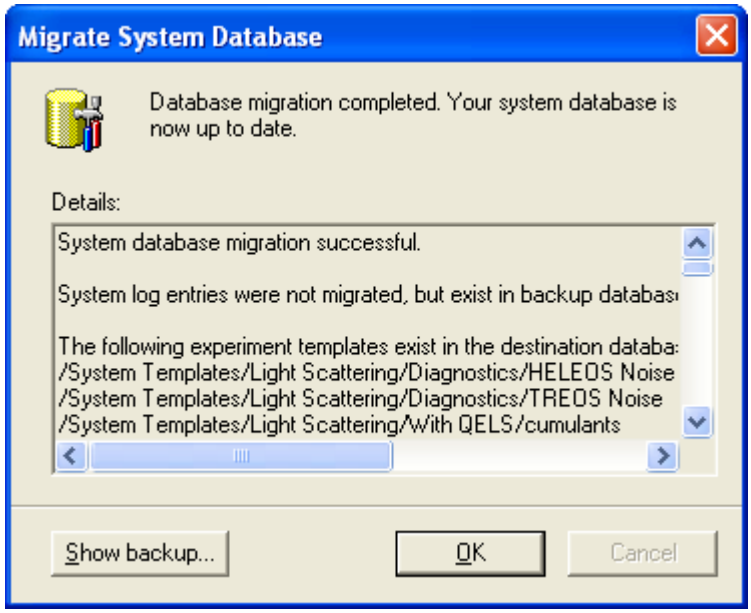
1. Choose **System→Database Administration→Migrate**.
2. You see a message that says the procedure will copy custom templates and profiles to the new database. Click **Migrate** to continue.

3. You see a warning that system log entries (such as login attempts and database connections) are not copied to the new database. To perform the migration, click **Yes**.



Hint: You can use database migration to purge system databases that contain a large number of system log records. Note that the migration *does* copy 21 CFR log information related to templates and profiles it migrates.

4. As your database is migrated, you see progress information. Messages identify any templates or profiles that are not updated because you have customized them.



You can migrate an existing system database created with ASTRA v5.3 or higher.

Whenever you migrate the system database, a backup is created with a filename of ASTRA_System2.bak (or ASTRA_System2_#.bak, where # is a sequence number, if you do multiple migrations). You can click **Show backup** to open a Windows Explorer view of the Database folder of the ASTRA installation, which contains the system database and backup files.

You can rollback a migration by deleting the new ASTRA_System2.mdb file and renaming the backup file to ASTRA_System2.mdb.

Activating Optional ASTRA Features

To activate optional ASTRA features—such as QELS—you use the Feature Activation dialog. In this dialog, you enter keys provided to you by Wyatt Technology Corporation based on your licensing agreement.

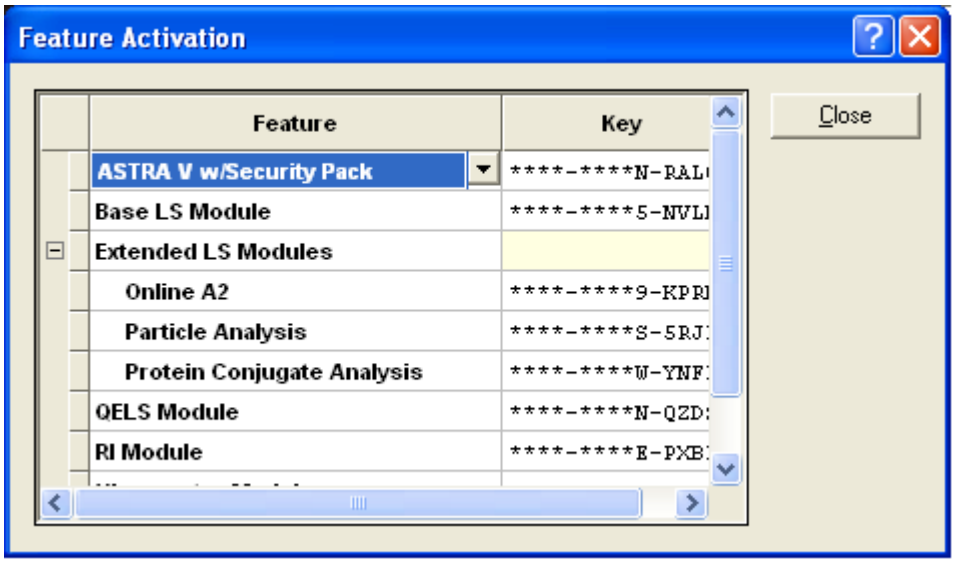


Figure 2-1: Software Activation Dialog

To activate a feature, follow these steps:

1. Double-click the **ASTRA V** icon on your desktop.
2. Choose **System→Feature Activation** to open the dialog above.
3. You can choose the operating tier for ASTRA by selecting either **ASTRA V**, **ASTRA V w/Research Database**, or **ASTRA V w/Security Pack** from the drop-down list.
4. Click in the right column next to a module you want to activate. Options for which no activation key has been entered are blank.
5. Type the registration code exactly as it is printed on your certificate.
6. Click **Close** after you have entered all keys.
7. Close ASTRA.
8. If you selected **ASTRA V w/Security Pack**, go to "Setting Up User Accounts" on page 2-6 for instructions on creating user accounts.
9. Restart ASTRA.

When ASTRA is installed, the entire program and all modules are placed on the computer. You enter a key to unlock optional modules. Certain modules can be used for a 30 day "demo" period. After 30 days have passed, you can purchase an activation key to use these modules. Most users receive an activation key for ASTRA and additional modules when purchasing the software. If you want to add other modules, contact Wyatt Technology Corporation to purchase an activation key for that module.

Setting Up User Accounts

Security

As part of the 21 CFR Part 11 compliance of this software, ASTRA V with Security Pack requires that all users log in with a unique user id and password.

User accounts in ASTRA V with Security Pack are managed as Microsoft Windows user accounts. You create the following four Windows groups, and then assign each user that should be able to access ASTRA to one of the following groups:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure sequence and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

In operating tiers other than ASTRA V with Security Pack, users are not prompted to log in with a user name and password.

Security

Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the "Security" icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA V with Security Pack. There are no access restrictions in other operating tiers.

Setting Up Groups

Security

To create the groups you will use with ASTRA, follow these steps:

1. Log in using a Windows account that has administrator privileges.
2. Right-click on **My Computer** in the Windows Start menu, and select **Manage**. This opens the Computer Management window.
3. In the tree on the left, expand the Local Users and Groups item (which is within the System Tools list).
4. Right-click on **Groups** under Local Users and Groups, and select **New Group**.
5. In the New Group dialog, type the following:
   - "ASTRA Administrator" in the Group name field.
   - "Administers ASTRA accounts and database" in the Description field.
6. Click **Create**.

7. Create the following additional groups in the New Group dialog, and click **Create** after each one. Make sure your use the exact capitalization and spacing shown here.

Group name	Description
ASTRA Researcher	Creates and modifies experiments and profiles.
ASTRA Technician	Runs experiments and saves data.
ASTRA Guest	Read-only access to experiments and results.

8. Click **Close** after you have created all four groups.

Creating Users

Security

You can use existing Windows user accounts or create special accounts for ASTRA access. To create a new user account, follow these steps:

1. In the Computer Management window, right-click on **Users** under Local Users and Groups, and select **New User**.
2. In the New User dialog, type a User name, Full name, Description, and Password as desired.
3. Click **Create**.

Assigning Users to Groups

Security

To assign user accounts to an ASTRA group, follow these steps:

1. In the Computer Management window, right-click on one of the ASTRA groups you added and select **Properties**.
2. Click **Add**.
3. In the "Enter the object names to select" field, type a user name you want to add to this group.
4. Click **OK** in the Select Users dialog.
5. Click **Add** again if you want to add other users to this group.
6. Click OK in the Properties dialog when you have finished adding users to a group.

Note for Networked Accounts

Security

You can use a similar procedure to set ASTRA privileges for networked accounts. Log in to the server used for the active directory. Perform steps similar to those described in the previous sections. However, perform the steps for the active directory instead of the local computer.

Setting up accounts locally or for a networked account determines which domain name users need to type when logging in to ASTRA.

Add groups to either the corporate domain or the local machine, depending on where the user logs in. ASTRA expects to find the Group and User information in the same place. So, for example, you cannot create a local group and add a domain user to this group.

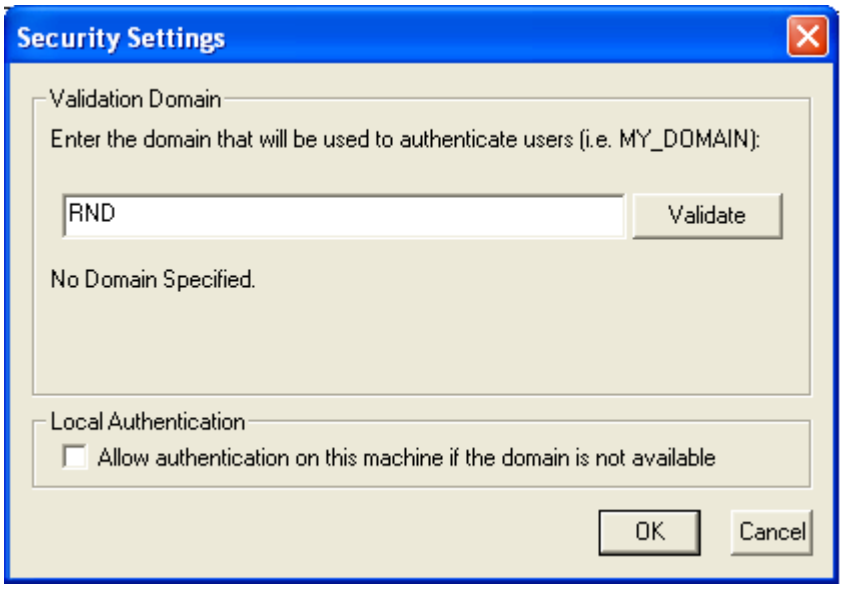
Setting a Validation Domain for Security

Security

You can specify the domain to use for user authentication. This allows you to prevent security problems where a user with a lower security group on the corporate domain could create a local group called "ASTRA Administrator" and use that local group to log into ASTRA.

Follow these steps:

1. Choose **System→Security** to open the following dialog.



2. Type the domain you want to use to authenticate users.
3. Click **Validate** to confirm that the domain name you typed is accessible on the network.
4. If you want users to be able to use ASTRA when the domain is not available, check the Local Authentication box.
5. Click **OK**.

Running ASTRA

To run ASTRA, do one of the following:

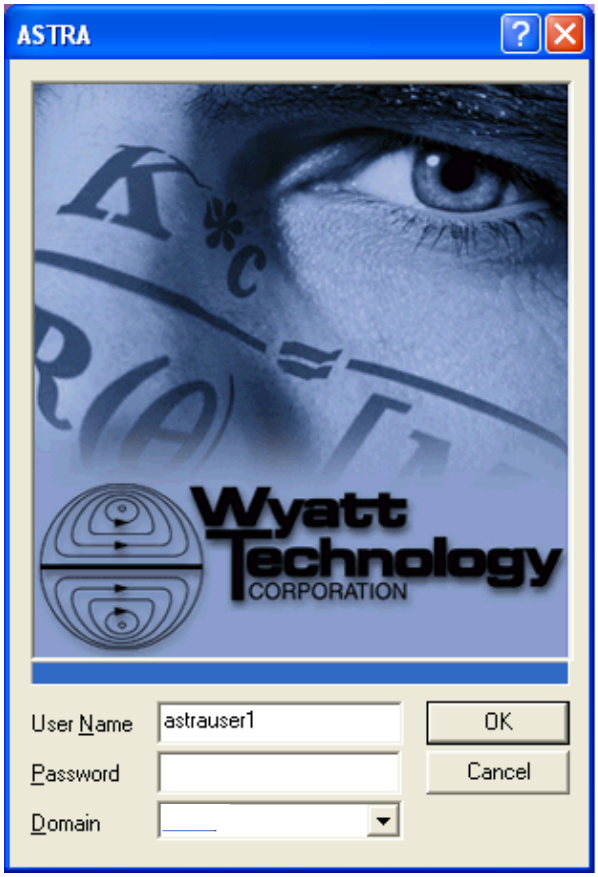
- Double-click the **ASTRA V** icon on your desktop.
- Choose **Programs→Wyatt Technology→ASTRA V** from the Windows Start menu.

It may take a minute or so for ASTRA to open. Avoid closing the initial startup window while waiting.

Security

If you are using ASTRA V with Security Pack, you will be prompted to log in. Use a User Name / Password combination set up as described in "Setting Up User Accounts" on page 2-6.

If ASTRA privilege groups were set up on your local computer, type the name of your local computer for the domain. Otherwise, if the ASTRA privilege groups were added for your networked account, type the domain of your networked account.



Database

By default, ASTRA V with Security Pack and ASTRA V with Research Database store experiments in a Microsoft Access database called ASTRA Experiment. A user with ASTRA Administrator privileges can change to another database by following the steps in "Connecting to a Database" on page 4-3. If you plan to change the database, it is best to do so before you start using ASTRA for experiments.

Accessing and Viewing Hardware

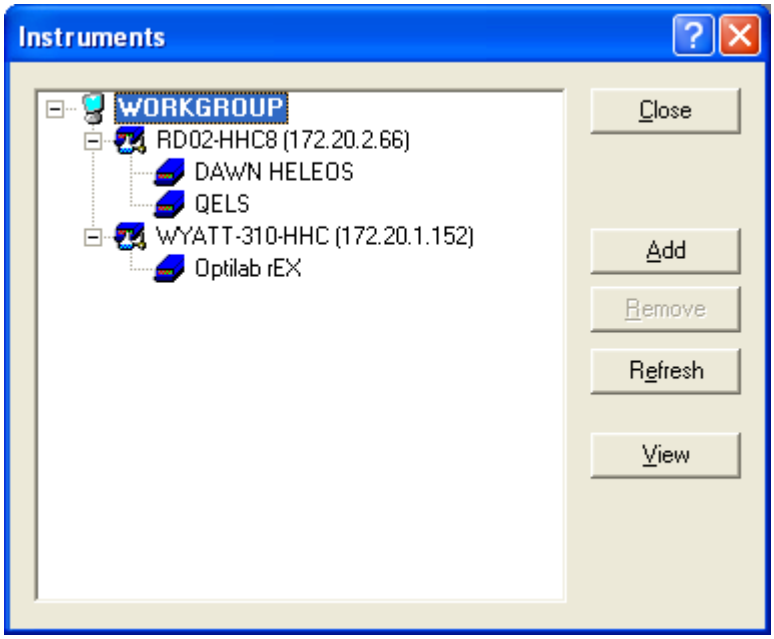
ASTRA's instrument list provides the following capabilities:

- Allows you to add and delete computers or instruments that run an Instrument Server Interface (ISI) to the set available to ASTRA on your computer.
- Shows instruments connected to your network and allows you to launch the Diagnostic Manager for each instrument.

This section gives step-by-step instructions for the initial actions you need to perform to make instruments visible to ASTRA. For more details about the ISI and the Diagnostic Manager, see Chapter 5, "Interfaces to Instruments".

Viewing the Instrument List

To view the list of instruments available to your copy of ASTRA, choose **System→Instruments** from the ASTRA menus. You will see the Instruments dialog.



The list shows the instruments and computers with an ISI installed that your copy of ASTRA knows about. You see instruments that support a direct data connection to ASTRA or instruments that are connected (via USB) to computers you have added to the list.

Note: If you physically connect an instrument to the network after opening this dialog, click **Refresh** to update the list.

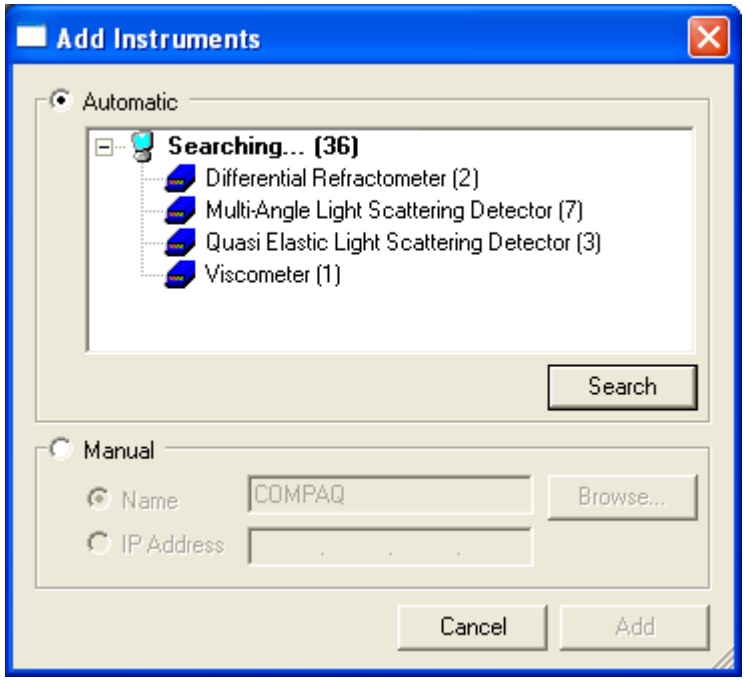
The **View** button opens the Diagnostic Manager described in "Using the Diagnostic Manager" on page 5-5.

Adding an Instrument or Computer to the Instrument List

If you are collecting from a DAWN or WyattQELS instrument connected to your local computer, the first computer you should add to the instrument list is your own local computer. You should also add other computers that have or will have instruments connected to them.

To add an instrument to the instrument list, which you open with **System→Instruments**, follow these steps:

1. In the Instruments dialog, click **Add** to open the Add Instruments dialog. A search for instruments is performed automatically.



2. If the instrument you want to add was found, select that instrument and click **Add**.
3. If your instrument was not found, you can add a computer or instrument by selecting the **Manual** option. Then either type the network name of the computer or instrument, or click the **Browse** button, or select the **IP Address** option and type the numeric IP address. Then click **Add**.

If you click the **Browse** button, expand the network listing so that you can see the instruments and computers on your network.

Instruments and computers that you add to the Instrument dialog are still connected to ASTRA in subsequent ASTRA sessions unless you delete them. Any supported instruments connected to those computers will be available within ASTRA.

See Chapter 5, “Interfaces to Instruments” for more about using the ISI, Instruments, and Diagnostic Manager dialogs.

Once instruments are visible in the Instruments dialog, ASTRA is ready to use for collecting data. Please note that ASTRA can still be used for the analysis of already collected data files without any connection to an instrument.

Removing an Instrument or Computer from the Instrument List

To remove an instrument or computer from the Instrument list (opened with **System→Instruments**), select the name of that resource in the instrument list and click **Remove**.

3 Getting Started

This chapter shows you how to create and run a simple experiment. It assumes that ASTRA has been set up as described in Chapter 2, “Installing and Setting Up ASTRA”.

CONTENTS **PAGE**

Starting ASTRA

To run ASTRA, do one of the following:

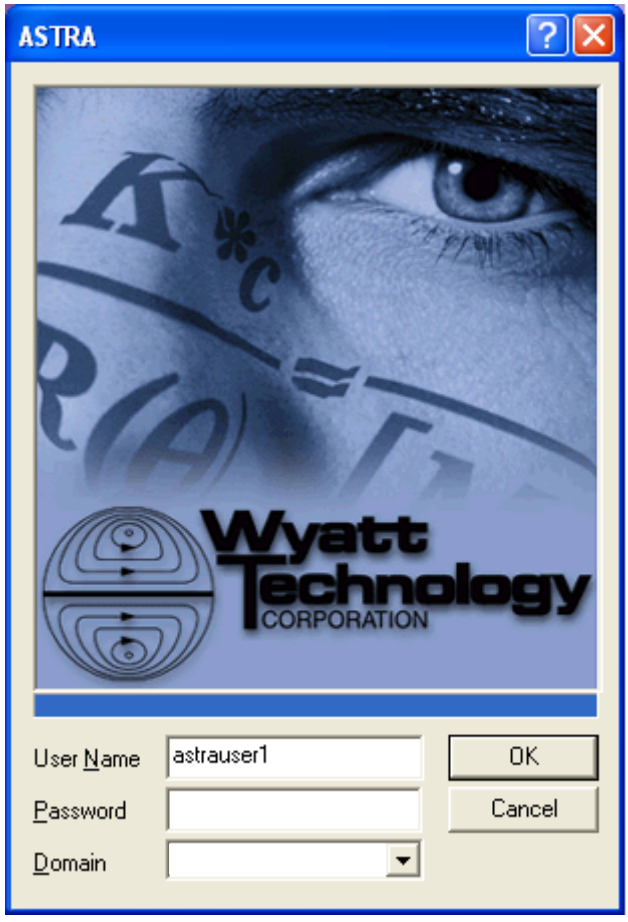
- Double-click the **ASTRA V** icon on your desktop.
- Choose **Programs→Wyatt Technology→ASTRA V** from the Windows Start menu.

It may take a minute or so for ASTRA to open.

Security

If you are using ASTRA V with Security Pack, you will be prompted to log in. Use a User Name / Password combination given to you by the ASTRA administrator. This may be the same as your Windows user name and password.

If ASTRA privilege groups were set up on your local computer, type the name of your local computer for the domain. Otherwise, if the ASTRA privilege groups were added for your networked account, type the domain of your networked account..



The account you use determines the types of actions you can perform within ASTRA. The user levels are as follows:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure sequence and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the "Security" icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA V with Security Pack. There are no access restrictions in other operating tiers.

ASTRA Tutorials on the Support Center

Several tutorials are provided for ASTRA on the Wyatt Technology Support Center. Log in and click the "Tutorials" item. If you don't already have a support center account, you can sign up for one.

Currently, the support center provides the following tutorials. We strongly encourage you to use these tutorials to learn how to use ASTRA.

- **ASTRA V Presentation:** This PowerPoint presentation provides an introduction to ASTRA V and the terminology it uses.
- **ASTRA V Templates Exercise:** This tutorial shows you how to connect to instruments, create experiments from a template, adjust experiment configurations, save experiment and profile templates, and run experiments.
- **ASTRA V SEC-LS Characterization:** This tutorial uses sample experiments that have already been run to show how to use data analysis procedures. You'll need to download and unzip the data files provided. The steps of the tutorial show how to set baselines, normalize the detectors, set peaks, set delay volumes (alignment), set band broadening parameters, assessing whether the flow cell was clean, and viewing molar mass results.
- **ASTRA V Skill Building Exercise:** This tutorial contains a number of brief exercises that ask you to answer questions about the results of various experiments that have already been run. It also contains questions that help you learn to assess the quality of data and troubleshoot detector problems using data. It uses the same experiment and data files as the previous tutorial.

Additional information about various ASTRA features is provided at http://www.wyatt.com/solutions/software/ASTRA.cfm.

Performing a Simple Light Scattering Experiment

In this section, you will use ASTRA to perform a simple batch light scattering experiment. For this experiment, you need to have one of the following instruments connected to either your local computer or one that can be accessed over the network:

- DAWN HELEOS
- DAWN EOS
- DAWN DSP
- DAWN DSP-F
- miniDAWN
- miniDAWN TREOS

For this introductory experiment, it is best to set the instrument up for a simple batch collection. You do not need to connect pumps or other instruments at this time. If you have already set up a flow experiment, you may choose the appropriate "online" template for your setup.

Note: If you don't have a DAWN or miniDAWN light-scattering instrument, you can still follow the steps in this example by selecting an experiment template appropriate to your instrument and setting properties that correspond to the ones described here.

Security

You must use an account with ASTRA Researcher or ASTRA Administrator access to follow the steps in this section.

Checking the Instrument Connection

Your ASTRA administrator has probably already set up connections to computers with instruments you will access with ASTRA. You can confirm this by choosing **System→Instruments** to open the Instruments dialog. This dialog lists instruments connected to computers that can currently be accessed by ASTRA.

If the instrument you want to use for this experiment is not listed, follow the steps in "Accessing and Viewing Hardware" on page 2-10.

Creating an Experiment

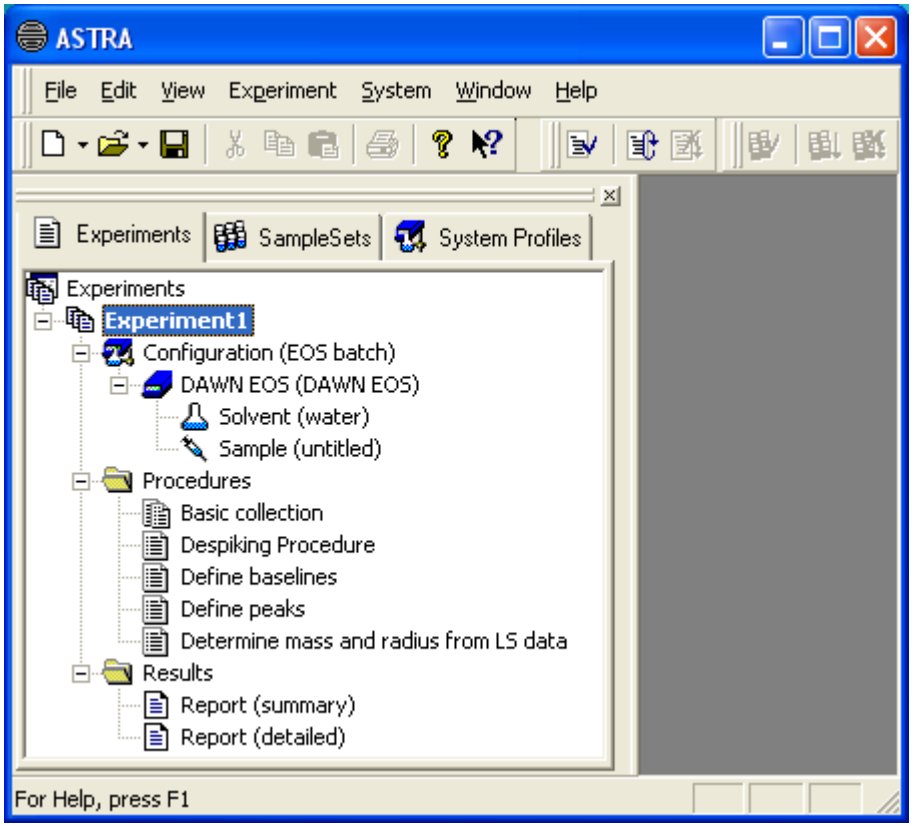
ASTRA provides a large number of experiment templates. Most users will be able to find a template that defines a configuration identical or close to their own experimental setup.

To create an experiment, follow these steps:

1. Choose **File→New→Experiment From Template**. (Ctrl+Alt+T)
2. In the "New from Existing" dialog, open the System Templates folder.

3. Open the “Light Scattering” folder.
4. Select a “batch” template and click **Create**.

   A new experiment called Experiment1 is created based on the template you selected. The Experiments tab in ASTRA shows the parts of the experiment.



You can expand or collapse the nodes in an experiment as desired by clicking on them. Each experiment contains the following categories of items:

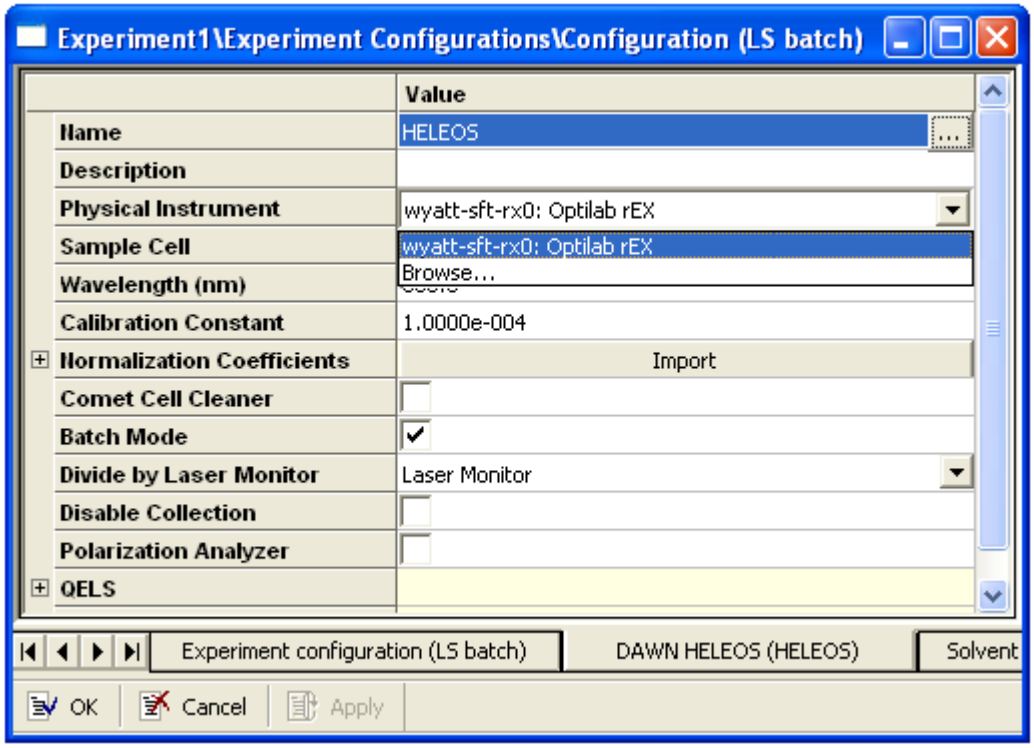
- **Configuration:** The hardware devices and connections used in the experiment. For online (fractionated) experiments, this may include a pump, injector, solvent, sample, DAWN, Optilab, and data connections. For details on all types of items that may be configured and their properties, see Chapter 7, “Configuring Experiments”.
- **Procedures:** The actions to be performed in sequence when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see Chapter 8, “Editing Procedures”.
- **Results:** The reports and graphs to be produced after the experiment procedure has been run. For details, see Chapter 10, “Working With Reports”.

Modifying the Configuration

The experiment template sets most of the properties to values you are likely to use. There are just a few properties you typically need to set.

For the example LS Batch experiment, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties dialog for the configuration. (You can also open this dialog by double-clicking on any part of the Configuration tree in the Experiments tab.)
2. Notice that this dialog has a tab along the bottom for each item in the configuration.
3. Select the tab for the DAWN or miniDAWN instrument. Use the  arrows to the left of the tabs to scroll to the right until you find the tab for the light scattering instrument.



4. In the Physical Instrument row, select an instrument to use for data collection from the drop-down list.
5. Select the tab for the solvent.
6. In the Name row, click the "**...**" button on the far right.
7. In the Copy System Profile dialog, open the System Solvents folder and select the solvent you are using. For example, you may be using toluene. Then click **Copy**. The properties for the solvent you select automatically replace those of the default solvent.
8. Click **Apply** or **OK** at the bottom of the properties dialog.

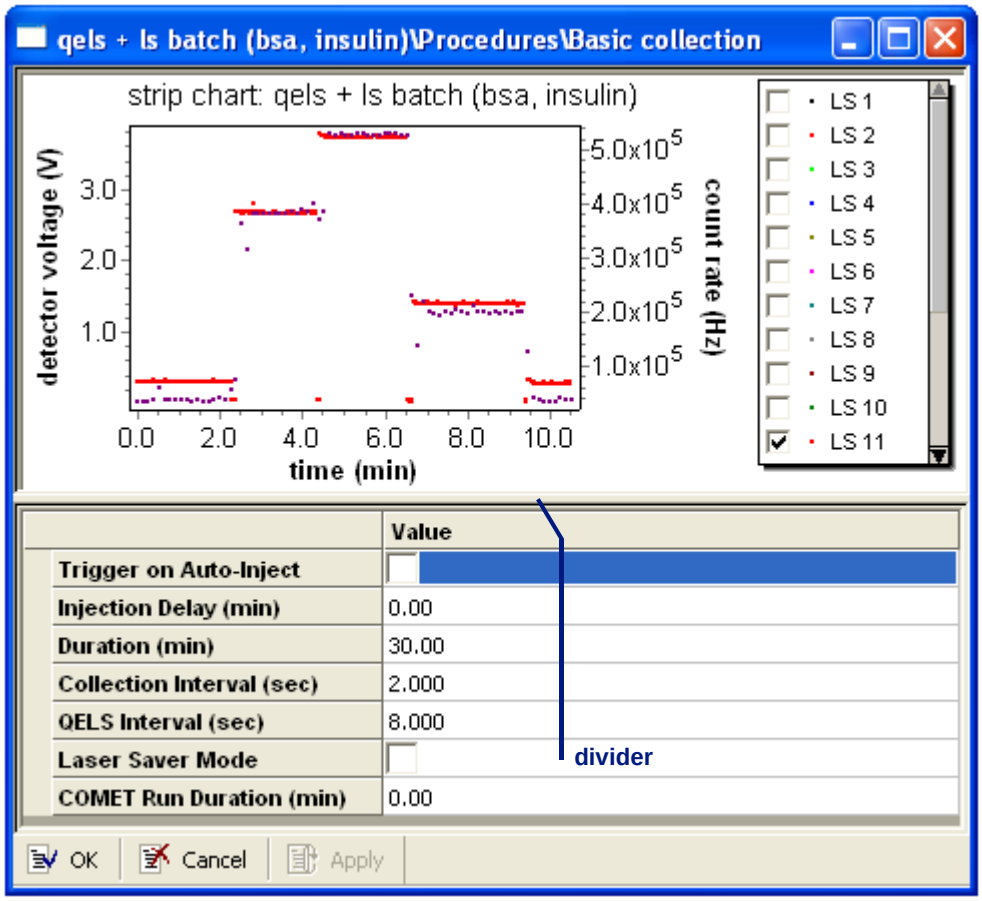
Just by setting two properties you have created an experiment that can be run. The templates provided with ASTRA make it as simple as possible to get to the point where you can run an experiment.

You can set other properties for the experiment, instrument, or solvent if you like. Configurations are described in detail in Chapter 7, “Configuring Experiments”.

Modifying Procedure Settings

At this point, you could run the default experiment. However, to show you more about using ASTRA, we’ll set the duration of the data collection. Follow these steps:

1. In the Procedures node, double-click the Basic collection procedure. This opens the Basic collection dialog.
2. If necessary, resize the dialog to see the Duration property. You can also drag the divider between the graph and the property list to resize the graph.



3. Type a new duration for the collection. For example, since you are simply learning to use ASTRA, you might collect data for only one minute.
4. Click **Apply**.

If you ever want to add procedures to the provided templates, you can use Experiment Builder mode.

Running the Experiment

Experiment procedures prompt you for any information they need in order to run successfully. To run the experiment, follow these steps:

1. Click the **Run** icon in the ASTRA toolbar (Ctrl+Shift+R).

Note: If you are learning to use ASTRA without access to a light-scattering instrument, you can open an experiment with pre-collected data by choosing **File→Open→Experiment** (Ctrl+O) and opening the "Sample Data" folder, then "Practice Experiments", then "batch processing example.vaf". Then skip to step 4.

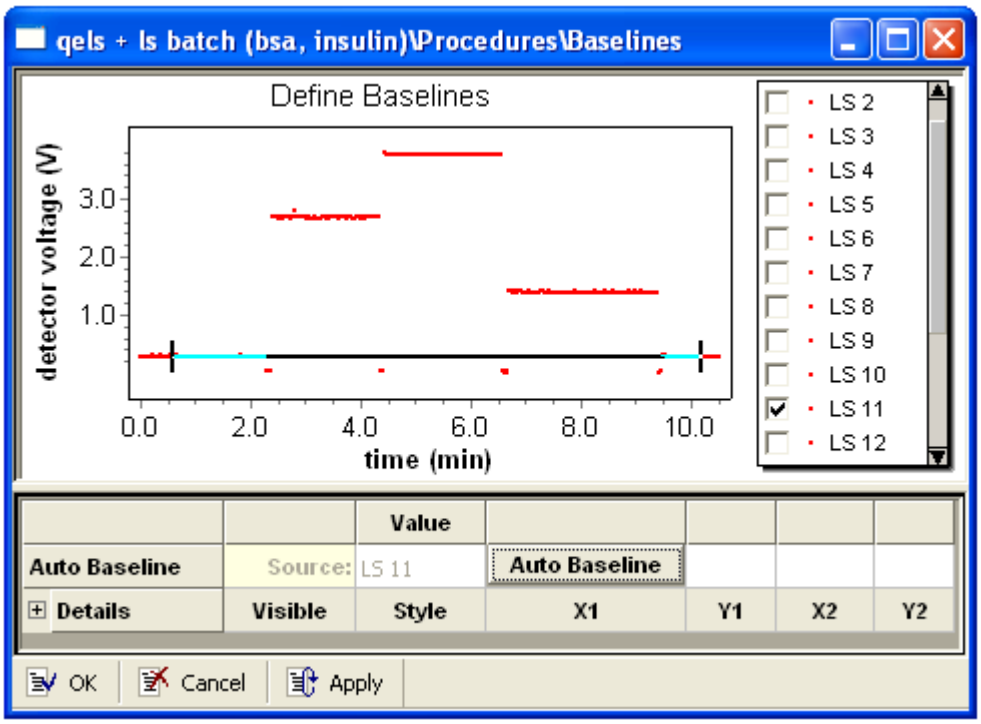
2. Watch the data as it appears in the Basic collection graph. You can enable and disable detector displays in real-time.

Note: While you are collecting data, you can work on setting up other experiments. You cannot modify the experiment that is running.

3. Inject samples and/or start pumps as needed to run the experiment.

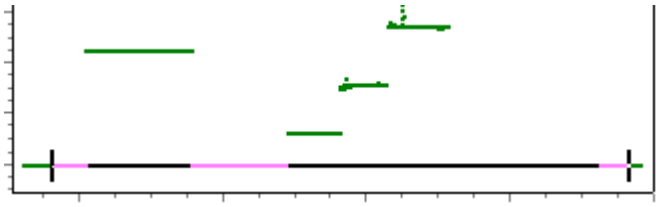
   After data is collected, you see a message that says a baseline needs to be set. Set a baseline by following these steps:

   a. Click **OK** to open the dialog for setting baselines.



   b. If you like, you can select a different detector's data to view.

c. Use your mouse to click on the baseline of the graph at one location and drag to another location on the baseline.



By default, baseline ends snap to the voltage level for a particular time. If you hold down the Shift key, you can then drag the end of a baseline to any location.

d. Scroll down to the bottom of the property list.

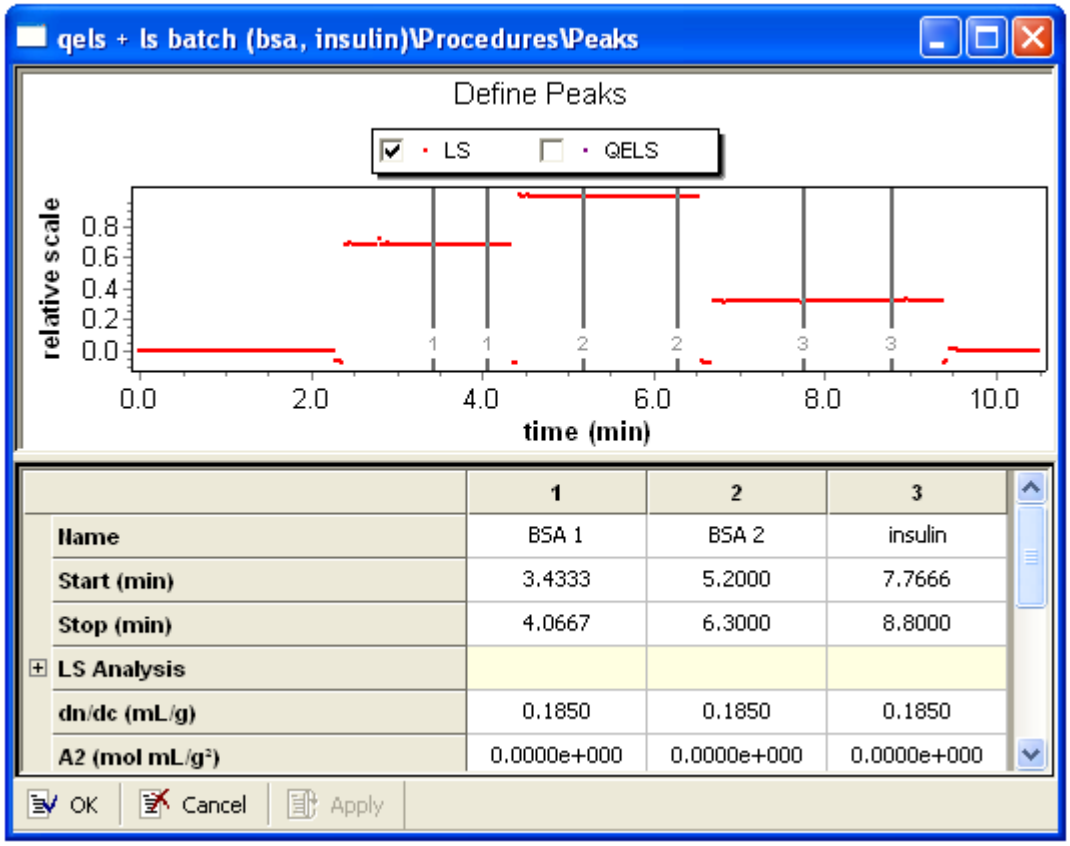
e. In the Auto Baseline row, select the detector for which you set the baseline. (For example, detector 11.)

f. Click **Auto Baseline** to automatically set baselines at the same collection times for all detectors.

g. Click **OK** to continue running the experiment.

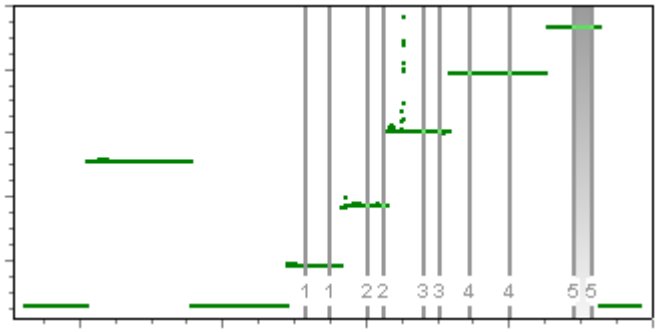
4. You next see a message that says peaks need to be specified. Set peaks by following these steps:

a. Click **OK** to open the dialog for setting peaks.

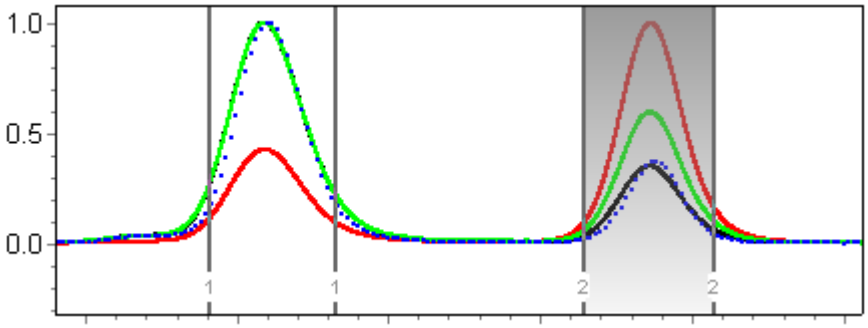


b. Use your mouse to click on one end of a peak range. Then drag to the other end of that peak range.

c. Continue adding peak ranges for the rest of the collected data. A number is shown for each peak that corresponds to the column for that peak below the graph. The selected peak is shaded. For example, these are peaks selected for a batch experiment:



These are examples of peaks selected for an online experiment:



Note: If you want to zoom in on the graph, hold down the Ctrl key and use your mouse drag an outline around the area you want to see. To zoom back out, hold down the Ctrl key and click your right mouse button.

d. Click **OK** to continue running the experiment.

5. The experiment runs to completion, and all the procedure icons in the experiment show that they have been run.

A procedure's state is always indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid sequence location.

For more about running experiments, see Chapter 6, "Creating & Running Experiments".

Viewing Reports

To view a report, simply double-click on it in the experiment tree. You can scroll down to read the results of the data analysis.

For more about setting up and viewing results, see Chapter 10, “Working With Reports”.

Summary

In a few minutes, you’ve created and run an experiment. You have modified the properties of a configurations and set properties such as baselines for a procedure. These are the main types of tasks you will perform when setting up and running your own experiments.

As you become a more advanced user, you may want to learn to perform tasks that are available in Experiment Builder mode. For details, see “User Modes” on page 3-13.

More About the ASTRA Environment

This section explains some general tasks you may perform within ASTRA that were not covered in the sample experiment in the previous section—such as customizing the ASTRA environment and getting help.

For an overview of ASTRA features, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the link to Features.

User Modes

You can use ASTRA in "Run" mode or "Experiment Builder" mode.

Run mode makes it easier to learn to use ASTRA and may be the mode you prefer even after you are an experienced user. In Run mode, you create experiments using the configuration and procedure templates provided with ASTRA. You can modify properties of the configuration and procedures, but cannot add or delete instruments or procedures.

Experiment Builder mode allows you to modify the configuration and procedure sequences in a template. The icon to the left identifies portions of this manual that apply only if you turn on Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**. This mode allows you to open multiple procedure windows at once. However, you should be careful with this feature, since changing and applying properties in one window does not generally result in changes to other open procedure windows. To see such changes reflected in other procedure windows, you should close and reopen them.

User Account Levels

As part of the 21 CFR Part 11 compliance of ASTRA V with Security Pack, all users must to log in with a unique user id and password. The administrator sets up accounts with one of the following user account levels:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure sequence and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

Where necessary, the user level required to perform an action is identified in this manual. Lines above and below the "Security" icon in the left margin (as shown here) highlight such information. Security information is specific to ASTRA V with Security Pack. There are no access restrictions in other operating tiers.

Customizing the Environment

You can drag various parts of the ASTRA window to positions you like better. The command menu bar, the toolbars, and the workspace (with the Experiments and System Profiles tabs) can all be repositioned. ASTRA remembers the positions you chose the next time you start it.

The View menu allows you to hide or redisplay the following parts of the environment. You might want to hide these items to allow you to make a graph display as large as possible on a small computer monitor.

- **View→Standard Toolbar** allows you to hide the standard toolbar. This bar contains icon buttons for creating, opening, and saving experiments, using the clipboard, printing, and getting help.
- **View→Status Bar** allows you to hide the bar at the bottom of the window. This bar shows messages about the experiment status, the current user name, and the access level this user has. It shows graph coordinates when you hold down the Shift key and point your mouse at a graph.
- **View→Workspace** allows you to hide the workspace with its tree view of experiments and system profiles.

  You can choose **System→Preferences→Auto-hide Workspace** to hide the workspace when it is not needed so that you will have more screen space for other dialogs. When the workspace is hidden, you can move your cursor to the edge of the window where the workspace is docked to display the workspace.
- **View→Customize**. The Toolbars tab provides another way to hide various ASTRA toolbars. The Experiment toolbar is the one that contains the Validate, Run, and Stop icons.

You may want to add keystrokes for various menu commands you use frequently. To add a command, follow these steps:

1. Choose **View→Customize** and go to the Keyboard tab.
2. Select a menu in the Category pull-down list.
3. Select a command in the Commands list.
4. Click in the “Press New Shortcut Key” field.
5. Use your keyboard to press a key combination. The key names are shown in the field, and any command to which they are already assigned is shown below.
6. To assign the key combination to the selected command, click **Assign**.

Command Reference

See Appendix A, “Menu Quick Reference” lists of all menu commands, tool bar buttons, and key sequences provided in ASTRA.

Printing

You can print Configuration property dialogs, procedure dialogs, reports, and graphs from ASTRA.

To print, choose **File→Print** (Ctrl+P) and use the Print dialog as you would in other Windows applications.

In addition, you can choose **File→Print Setup** to choose a printer.

Getting Help

The online help for the ASTRA software contains all the information in this manual. Descriptions of properties in the help system are linked to dialogs so that you can quickly learn more about individual properties.

Note:	The online help system does not contain hardware-related information.

To access the online help system, do one of the following:

- Choose **Help→Contents** to open the help system to the beginning topic.
- Choose **Help→Search** to open the full-text search for the help system.
- Choose **Help→Index** to open the index for the help system.
- Press F1 in any dialog to open help about that dialog.
- Click  and then click on a dialog for context-sensitive help.

Exiting from ASTRA

When you have finished working with ASTRA, exit it just as you would any other Windows application. If a file is open, ASTRA closes it. If any changes to an open file haven't been saved, you are prompted to save the changes or cancel the closing of ASTRA.

To close ASTRA, do one of the following:

- Choose **File→Exit**.
- Press ALT+F4.
- Press Alt, F, X.
- Click the X button in the upper-right corner of the ASTRA window.

4 ASTRA Administration

This chapter tells how to administer ASTRA V experiment databases. Such databases are used in ASTRA V with Research Database and in ASTRA V with Security Pack.

CONTENTS **PAGE**

21 CFR Part 11 Support Overview

Security

21 CFR Part 11 contains regulations by the U.S. Food and Drug Administration (FDA) concerning electronic records and electronic signatures. FDA-regulated companies in pharmaceutical, biotechnology, and other industries are under increased scrutiny to comply with 21 CFR Part 11.

Background and Reasons for Compliance

Title 21 of the Code of Federal Regulations includes regulations for food and drugs regulated by the Food and Drug Administration. Part 11 of this title establishes the criteria under which electronic records and signatures can be considered equivalent to paper records and handwritten signatures in processes regulated by the FDA.

FDA-regulated industries must document that proper processes have been followed to insure that products are consistent. Signed documents about various points in the manufacturing processes must be reviewed, securely stored and available for review by the FDA. Reviewing these records was time consuming and required manual searches. 21 CFR Part 11 makes record handling more accurate and efficient for all parties because all of the records stored are digital.

The benefits of becoming 21 CFR Part 11 compliant include the following:

- **Compliance:** This may be a requirement for conducting business. Compliance provides better preparation for FDA inspections.
- **Improved Efficiency:** Electronic records can be searched quickly.
- **Faster Time to Market:** Time delays in approval cycles can be reduced because records can be transferred electronically.
- **Better Quality and Consistency:** Products may be improved and are manufactured in a consistent manner.
- **Improved Research Data:** Compliant electronic records provide better data integration and allow trending information to be better examined.
- **Reduced Cost:** Storage space for hardcopy records is more costly than electronic storage.
- **Reduced Risk:** Compliant electronic records are less vulnerable to signature fraud and misfiling.

For details from the FDA about 21CFR Part 11, see http://www.fda.gov/ora/compliance_ref/part11.

Making use of the 21 CFR Part 11 support in ASTRA V with Security Pack makes your experimental data collection, analysis, and storage compliant with the FDA ruling.

21 CFR Part 11 Support in ASTRA V

Security

ASTRA 21 CFR Part 11 compliance features related to user accounts and logging activities are available only in ASTRA V with Security Pack. The icon to the left identifies information that is specific to ASTRA V with Security Pack.

If you are using these features, you must have an ASTRA administrator to manage 21 CFR Part 11 compliance. That manager will perform the following actions, which are described in this chapter:

- "Connecting to a Database" on page 4-3
- "Managing User Accounts" on page 4-6
- "Using the System and Experiment Logs" on page 4-7
- "Deleting Experiments" on page 4-8

Database

ASTRA features related to the use of an experiment database to store experiments and sample sets are available in both ASTRA V with Security Pack and ASTRA V with Research Database. The icon to the left identifies information that applies to both of these software versions.

For more about 21 CFR Part 11 compliance in ASTRA V including a detailed white paper, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the link to Compliance.

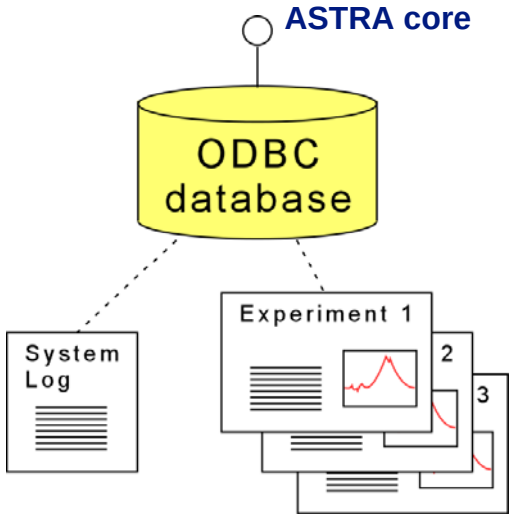
Connecting to a Database

ASTRA V uses two databases:

- **System database:** This database stores experiment templates, sample set templates, system profiles, system solvents, and molecular standards. A system database is used by *all* ASTRA V operating tiers. Each installation of ASTRA V uses its own, local system database. You cannot specify a different database to use as the system database. See "Migrating the System Database" on page 2-3 for information about updating the system database.
- **Experiment database:** This database stores information about experiments, database actions, warnings, and more. This database logs list all activities performed with ASTRA that must be logged for 21 CFR Part 11 compliance. An experiment database is used *only* in ASTRA V with Security Pack and ASTRA V with Research Database. You can specify which experiment database to use.

The rest of this section talks about ASTRA's experiment database, which is sometimes called simply the "database".

ASTRA uses Open DataBase Connectivity (ODBC) to connect to databases. ASTRA has been tested with Microsoft Access and Microsoft SQL Server, version 7 or higher databases.



Database By default, the experiment database is a Microsoft Access database called ASTRA Experiment.

If you have Microsoft SQL Server, Wyatt recommends that you use this for your experiment database. Microsoft Access is a light-weight database that cannot handle the large amounts of data generated by ASTRA; the database operations will become slow as the amount of stored data increases.

Ideally, the experiment database will be a networked database that is backed up regularly by the IT department. Note that adequate database storage is required. If the database is networked, the database server must be accessible to the PC running ASTRA via the network.

If you have no networked Microsoft SQL Server installation available, you can install SQL Server locally. There is a free version of SQL Server called SQL Server 2005 Express Edition that is available from Microsoft.

For more information, go to the Wyatt Support Center website (www.wyatt.com/support) and follow the links to Downloads->Databases. Follow the link to the SQL Server 2005 Express Edition installation and connection instructions. In addition, the “ReadMe Files” directory of your ASTRA installation contains information about database and network issues.

Each experiment run with ASTRA uses an average of about 0.5 MB of storage, so database size should be based on estimated number of experiments to be saved. Also, a database user account and password must be created for the database. The user account must have privileges to create, delete, and modify tables in the database.

Viewing the Current Experiment Database

To see the current experiment database path, follow these steps:

1. Log in to ASTRA using an account with ASTRA Administrator access.
2. Choose **System→Database Administration→Properties**. This opens the Database Properties dialog.

ASTRA V with Security Pack and ASTRA V with Research Database must be connected to an experiment database. Only one database can be used at a time. However, it is possible to connect to different databases for different types of experiments.

Creating a Microsoft Access Experiment Database

Although SQL Server is recommended over Microsoft Access, you can use Microsoft Access for your experiment database. To create a new Microsoft Access experiment database and connect to it with ASTRA, follow these steps:

1. Log in to ASTRA using an account with ASTRA Administrator access.
2. Choose **System→Database Administration→Connect to Database**. This opens the Select Data Source dialog.
3. Connect to an existing ODBC data source or create a new one using this dialog. Remember that only Microsoft Access and SQL Server databases are supported. For example, you might follow these steps:
   - Move to the Machine Data Source tab in the Select Data Source dialog and click **New**.
   - Select **System Data Source** and click **Next**. (Alternately, you could select User Data Source if you only want this account to access this database.)
   - Select **Microsoft Access Driver (*.mdb)** and click **Next**. (Alternately, you could select SQL Server.)
   - Click **Finish**.
   - Type a **Data Source Name**. For example, astra_exp_db.
   - Type a **Description**. For example, ASTRA Experiment Database.
   - Click **Create**.
   - In the New Database dialog, type a database name. For example, astradb.mdb. Also, browse for a location to store your database. For example, c:\Program Files\WTC\Astra V\Database.
   - Click **OK** four times (in the New Database dialog, the success message, the ODBC Microsoft Access Setup dialog, and the Select Data Source dialog).

Managing User Accounts

Security

User accounts in ASTRA V with Security Pack are managed as Microsoft Windows user accounts. You assign each user account that can access ASTRA to one of the following four groups:

- **ASTRA Administrator.** Can change database settings and can create, modify, and delete experiment files. Also has privileges of Researchers, Technicians, and Guests.
- **ASTRA Researcher.** Can create and modify experiment files. Can connect to networked computers and instruments. Also has privileges of Technicians and Guests.
- **ASTRA Technician.** Can run a given experiment procedure sequence and save the resulting data. Also has privileges of Guests.
- **ASTRA Guest.** Has read-only access to experiments and results.

With operating tiers other than ASTRA V with Security Pack, users are not prompted to log in with a user name and password.

Chapter 2, “Installing and Setting Up ASTRA” contains a section on “Setting Up User Accounts” on page 2-6 since that portion of ASTRA administration needed to be done during initial setup. That section provides steps for setting up the groups used by ASTRA, creating user accounts, and assigning users to groups. You can modify and delete user accounts using the same Windows tools.

The status bar at the bottom of the ASTRA window shows the name of the user account that is currently logged in. It also shows the ASTRA group to which that user is assigned.

A user should not be assigned to more than one ASTRA group.

Using the System and Experiment Logs

Database

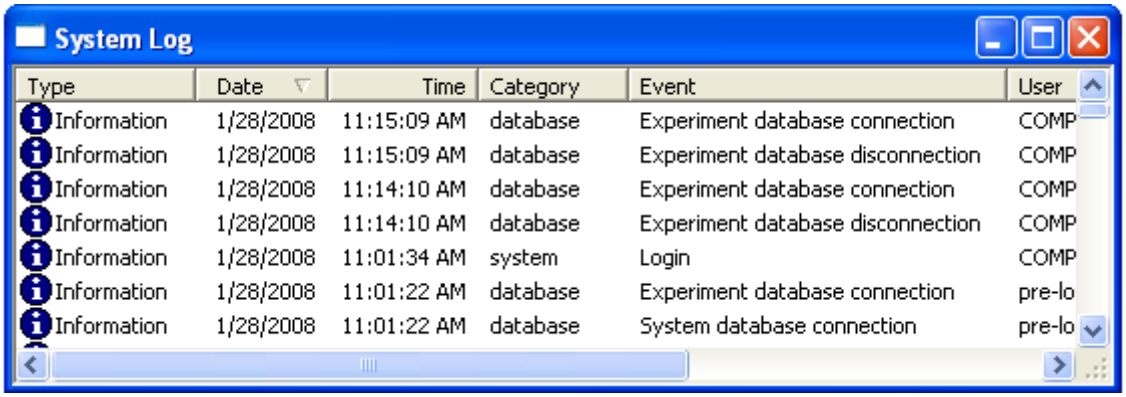
ASTRA V with Security Pack and ASTRA V with Research Database log information about actions performed that relate to experiments. There are two types of logs created by ASTRA:

- **System log:** Shows global actions that include: logging in, logging out, creating, importing, exporting, opening, saving, and deleting an experiment, and signing off on an experiment.
- **Experiment logs:** Shows actions that relate to a particular experiment. These include actions involving procedures, data set definitions, reports, templates, and more. This log shows when the procedures that make up an experiment were run.

As ASTRA Administrator, you should communicate to researchers about the Sign Off procedures they should add to experiments they create. These are likely required by your 21 CFR Part 11 policies and procedures. Sign offs can store information about the user responsible for the experiment, the user who approved the experiment, or the user who reviewed the experiment.

Viewing the System Log

To view the system log, choose **System→Log→Open**.



You can save the system log to a text file by choosing **System→Log→Save As**.

Viewing an Experiment Log

Database

To view the entire experiment database log, choose **System→Database Administration→Log→Open**.

To view the log for the current experiment, right-click any folder in the experiment tree, and choose **Manage→Log**. Or, choose **Experiment→Log→Open** from the menu bar. For information about experiments, see Chapter 6, “Creating & Running Experiments”.

You can save the experiment database log to a text file by choosing **System→Database Administration→Log→Save As**.

Viewing a Sample Set Log

Database

To view a log for a sample set, right-click any item in the sample set tree, and choose **Manage→Log→Open**. Or, choose **SampleSet→Log→Open** from the menu bar. For more about sample sets, see Chapter 9, "Using Sample Sets".

Working with Logs

Database

In any log, the most recent action is shown at the top by default. You can click the column headings to sort the log in other ways, such as by category, event, or user.

You can double-click an entry to view a dialog with more detailed information about that log entry. The buttons in this dialog act as follows:

- **Up Arrow:** Go to the previous entry in the list. Unless you changed the sorting, this is the next entry in time.
- **Down Arrow:** Go to the next entry in the list. Unless you changed the sorting, this is the previous entry in time.
- **Copy Icon:** Copy the text of the event to the clipboard. You can then past the text into another application, such as a word processor.

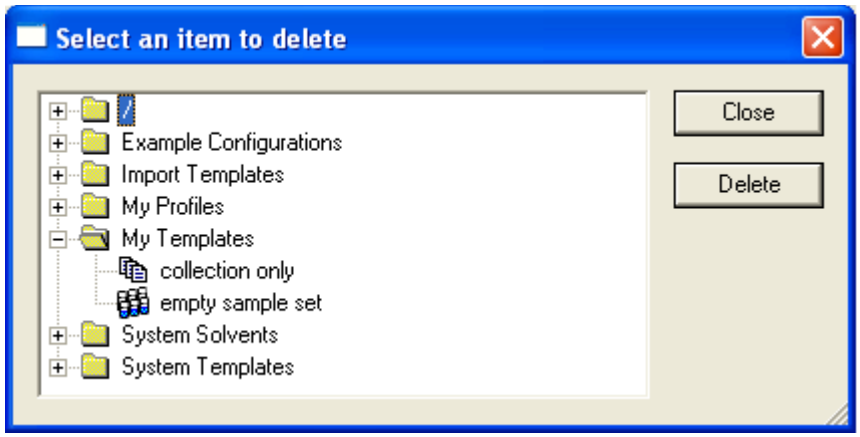
Deleting Experiments

Security

Only an ASTRA Administrator is permitted to delete experiments from the experiment database. You should only delete an experiment if it is permitted by your 21 CFR Part 11 policies and procedures.

To delete an experiment, follow these steps:

1. Choose **System→Database Administration→Delete Items**. This opens the Select an item to delete dialog. Experiments you have saves are in the "/" folder. (This dialog also lists profiles, templates, sample sets, and other items stored in the experiment and system databases.)



2. Highlight the experiment you wish to delete in the list.

3. Click **Delete**.
4. Click **Close** when you have finished deleting experiments.

This command can also be used to delete sample sets, system profiles and experiment templates. For more information, see “Deleting a Sample Set” on page 9-11, “Deleting a Profile” on page 12-7, and “Deleting a Template” on page 6-19.

Performing Database Maintenance

If you are having problems with your experiment database, you can choose **System→Database Administration→Automatic Maintenance** to attempt to correct the problem.

We do not recommend that you use this command unless you are having problems with the experiment database.

5 Interfaces to Instruments

This chapter explains the Instrument Server Interface (ISI), the Diagnostic Manager, and the WCS Client. These can all be accessed through ASTRA. The ISI allows you to connect to, administrate, and acquire data from instruments connected locally or over your network.

CONTENTS **PAGE**

Instrument Server Interface Overview

ASTRA can collect data from various instrument types. More importantly, it can combine this data with analysis procedures. Connections to instruments can be made either locally or through the network.

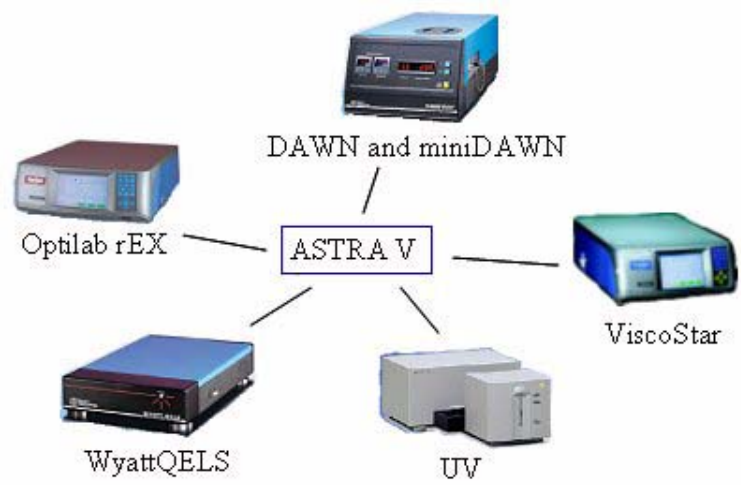


Figure 5-1: ASTRA Instrument Connections

For instructions for connecting to instruments, see "Accessing and Viewing Hardware" on page 2-10.

The Instrument Server Interface (ISI) allows you to access instruments connected locally or across your internal Local Area Network (LAN). That means the instruments you use with ASTRA need not be directly connected to the computer you are using.

Next-generation instruments from Wyatt Technology Corporation—such as the DAWN HELEOS, Optilab rEX, and ViscoStar—can be controlled directly over the network or a local USB connection. They have an integrated ISI that can be accessed directly through the network. You do not need to install an ISI on your local computer to access these instruments.

The following instruments can also be accessed locally or through the network, in much the same way that a printer can be shared on a LAN. For these instruments, you must install the ISI on the computer to which the instrument is connected. See "Installing the ISI on Other Computers" on page 5-4.

- DAWN EOS, DSP, and DSP-F
- miniDAWN
- WyattQELS

Other instruments—including the following—may be connected through the AUX input of another instrument. As a result, the instruments will be shown as part of an experiment configuration.

- Optilab DSP
- Optilab 903

- Generic RI Instrument
- Generic UV Detector
- Generic Viscometer

Figure 5-2 summarizes these types of connections:

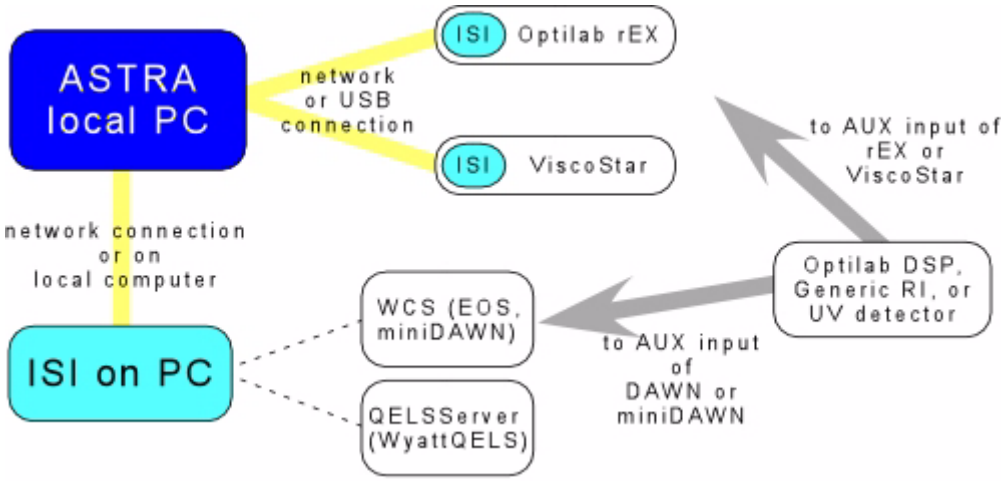


Figure 5-2: Connections Between ASTRA, the ISI, and Instruments

Figure 5-3 shows the ASTRA V with Research Database and ASTRA V with Security Pack architecture in more detail. The circles represent interfaces presented by the ASTRA core, the ISI, and ODBC for use by other parts of the architecture.

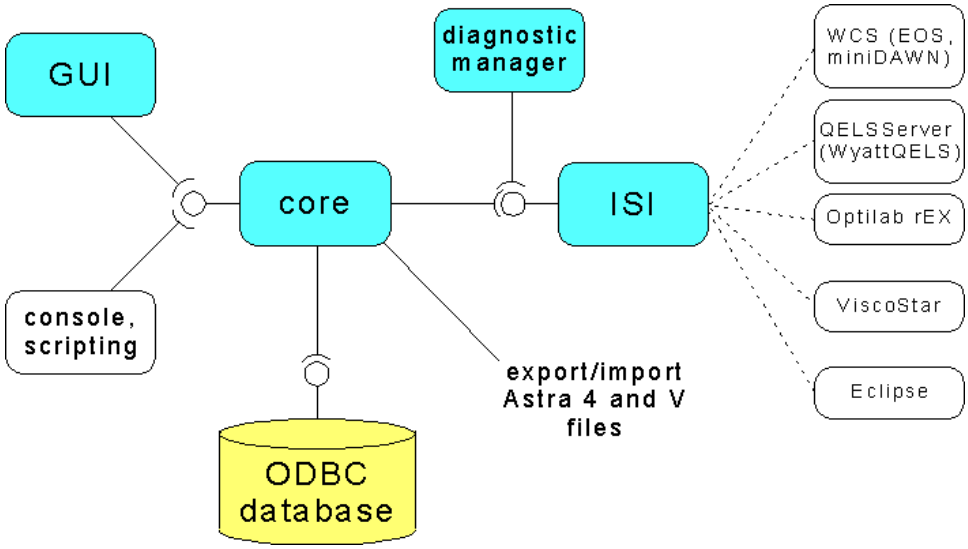


Figure 5-3: ASTRA System Architecture

The core of ASTRA is used by the ASTRA user environment. Console and scripting applications also access the ASTRA core. The ISI presents an interface that is used by both the ASTRA core and the Diagnostic Manager. The ISI, in turn, connects to various instruments.

If you use ASTRA V Basic, the ODBC database shown in Figure 5-3 would be replaced by file access to separate experiment files.

Installing the ISI on Other Computers

When you install the ASTRA software on a computer, the ISI software is automatically installed along with ASTRA.

The ASTRA installation CD allows you to install only the diagnostic manager and the appropriate instrument controllers. If you choose to do this, the ISI is installed without the graphical interface and other components of the ASTRA V software.

Your network administrator may want more technical information about the ISI. The ISI runs as a DCOM server on each machine where it is installed. To successfully connect to an ISI, the computer should allow DCOM connections. This is the standard configuration for Windows 2000 and Windows XP Professional prior to Service Pack 2.

Windows XP Professional SP2 and newer operating systems require manual configuration of the DCOM settings to allow external data connections. For more on DCOM and firewall issues, go to the Wyatt Support Center website (www.wyatt.com/support) and follow the links to **FAQs→Software**.

The "ReadMe Files" directory of your ASTRA installation contains additional information about database and network issues.

The ISI runs a server called the Wyatt Communications Server. In the Processes tab of your Windows Task Manager, this process is called WCS.exe. If ASTRA quits unexpectedly, you may need to end the WCS process through the Task Manager.

Using the Diagnostic Manager

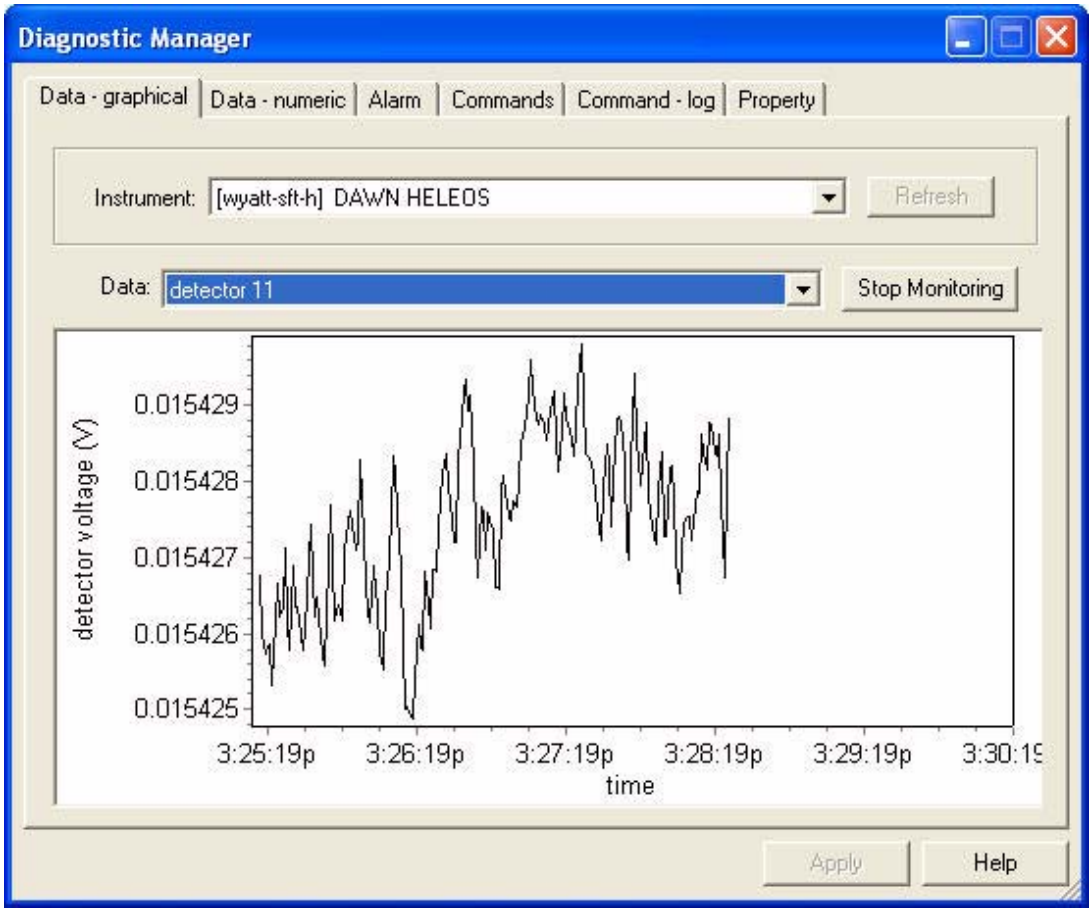
If you choose **System→Instruments**, select an instrument and click **View**, you see the Diagnostic Manager for that instrument. You can also start the Diagnostic Manager from the Windows Start menu by choosing **Programs→Wyatt Technologies→ASTRA→Diagnostic Manager**.

You can use the Diagnostic Manager utility to monitor and control instruments. For example, you can start a collection in your lab, then monitor the progress in your office over the network. Alarms and other state information are reported directly to the Diagnostic Manager, and you can use the Diagnostic Manager to send commands and configuration information to any instrument available within ASTRA.

This manager is not intended for viewing and interpreting data. Instead, it can be used to determine if your instruments are connected and functioning correctly.

Viewing Graphical Data with the Diagnostic Manager

The **Data - graphical** tab of the Diagnostic Manager allows you to view a real-time graph of data received from an instrument. The type of data collected is different for each type of instrument. The following figure shows data collected by a DAWN HELEOS instrument.



To monitor data, follow these steps:

1. Select an instrument to monitor from the Instrument drop-down list.

   If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Refresh** to update the drop-down list.

2. Select the type of Data to monitor. The choices differ depending on the type of instrument. For more about viewing "live data" for light-scattering instruments, see "Viewing and Setting Properties with the Diagnostic Manager" on page 5-10.

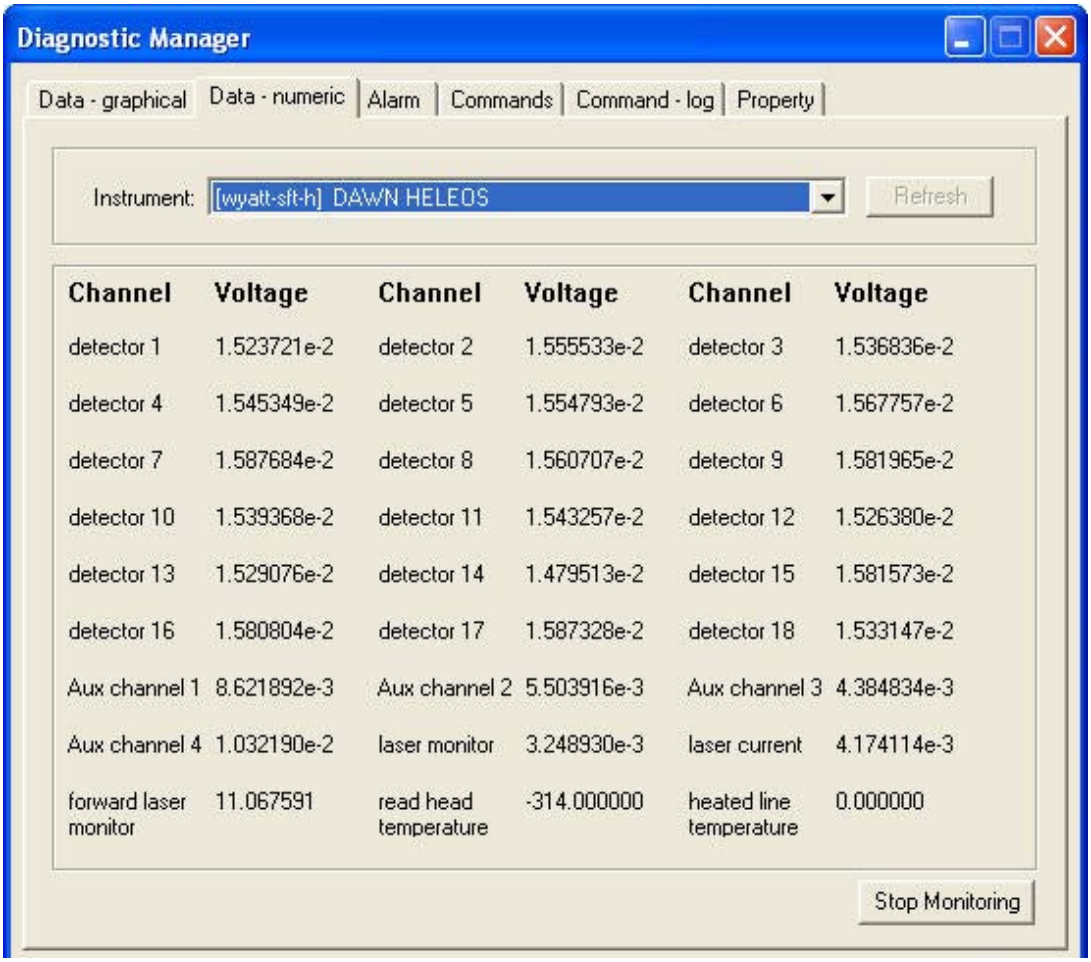
3. Click **Start Monitoring**.

4. To stop the graph, click **Stop Monitoring**.

You can modify the appearance of the graph just as you would in ASTRA. For details, see "Working with Procedure Graphs" on page 8-5.

Viewing Numeric Data with the Diagnostic Manager

The **Data - numeric** tab of the Diagnostic Manager allows you to view real-time numeric data received from an instrument. The type of data collected is different for each type of instrument. The following figure shows data collected by a DAWN HELEOS instrument.



To monitor data, follow these steps:

1. Select an instrument to monitor from the Instrument drop-down list.

   If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Refresh** to update the drop-down list.

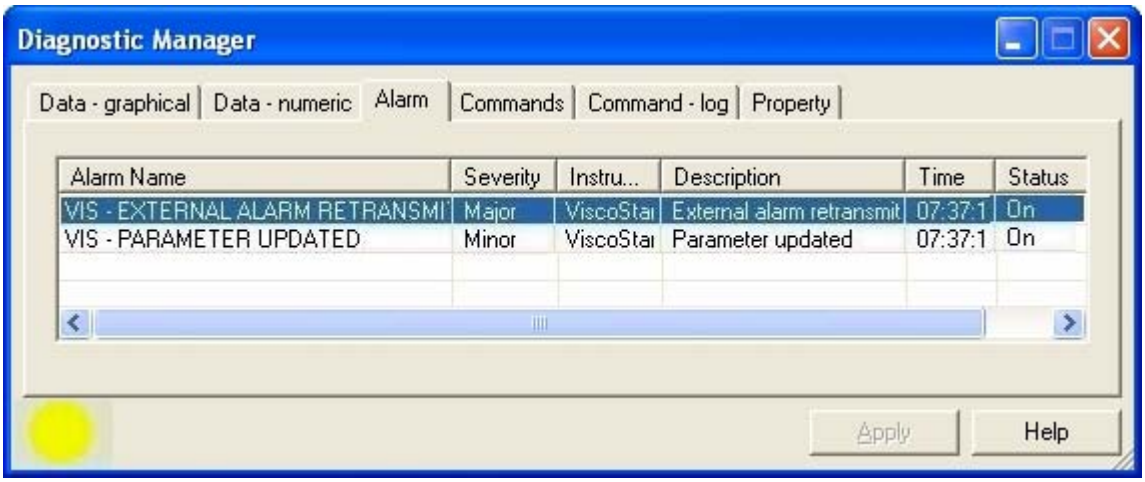
2. Click **Start Monitoring**.

3. To stop the data updates, click **Stop Monitoring**.

Viewing Alarms with the Diagnostic Manager

When an alarm occurs for any instrument available to ASTRA via ISI, a warning signal flashes in all tabs of the Diagnostic Manager. This warning is a blinking circle on the bottom of the Diagnostic Manager.

The **Alarms** tab of the Diagnostic Manager shows alarms sent by all instruments available to ASTRA via ISI. Only alarms sent during the current Diagnostic Manager session are shown.



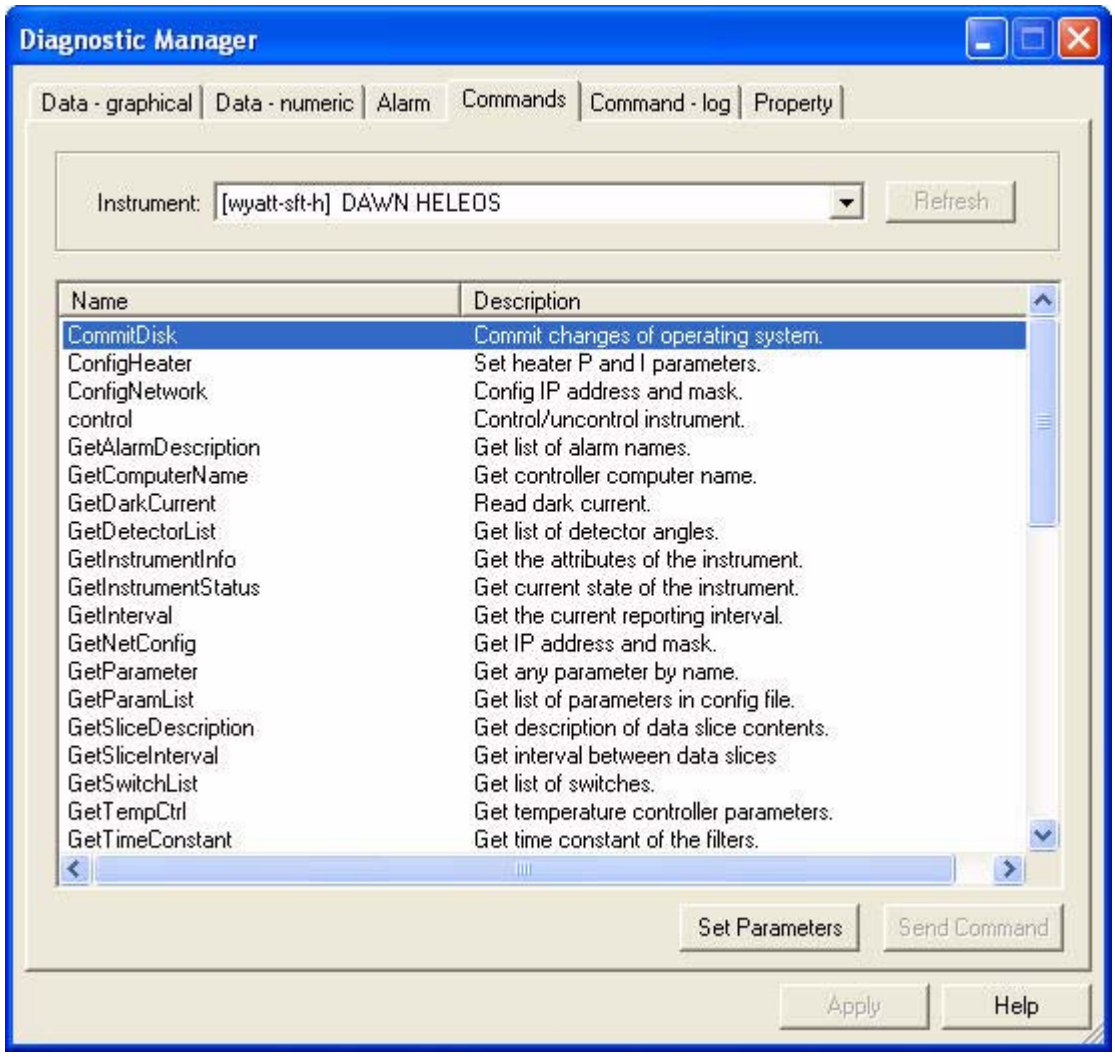
This tab shows the following information about an alarm:

- **Alarm Name:** This column tells what type of alarm occurred.
- **Severity:** The level of alarm severity.
- **Instrument:** Which instrument sent the alarm.
- **Description:** More information about the alarm.
- **Time:** The time the alarm occurred.
- **Status:** Shows whether you have acknowledged an alarm. Acknowledging an alarm hides the blinking circle on the bottom of the Diagnostic Manager window. To acknowledge an alarm, right-click on this column and select "Acknowledged".

You can resize the dialog and its columns to see more text in a column.

Sending Commands with the Diagnostic Manager

The **Commands** tab of the Diagnostic Manager allows you to send commands to instruments. The list of commands you can send is different for each type of instrument. The following figure shows the commands available for the DAWN HELEOS instrument.



To send a command, follow these steps:

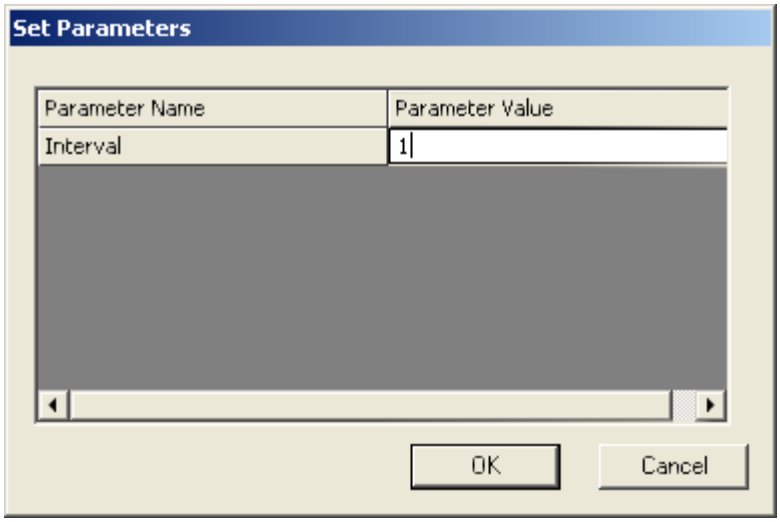
1. Select an instrument from the drop-down Instruments list.

   If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Refresh** to update the drop-down list.

2. Select the command you want to send to the instrument.

3. If the command you selected requires any parameters, the **Send Command** button is disabled until you set those parameters. If this is the case, click **Set Parameters** to open a dialog that asks you for the appropriate parameter values.

For example, if you set the collection interval, you will be asked to provide the new interval in seconds.



Type or select the parameter value and click **OK**.

4. Click **Send Command**.

Viewing the Command Log with the Diagnostic Manager

The **Command - log** tab of the Diagnostic Manager shows commands you have sent to the instrument via the Diagnostic Manager and responses provided by the instrument. Only commands sent during the current Diagnostic Manager session are shown.

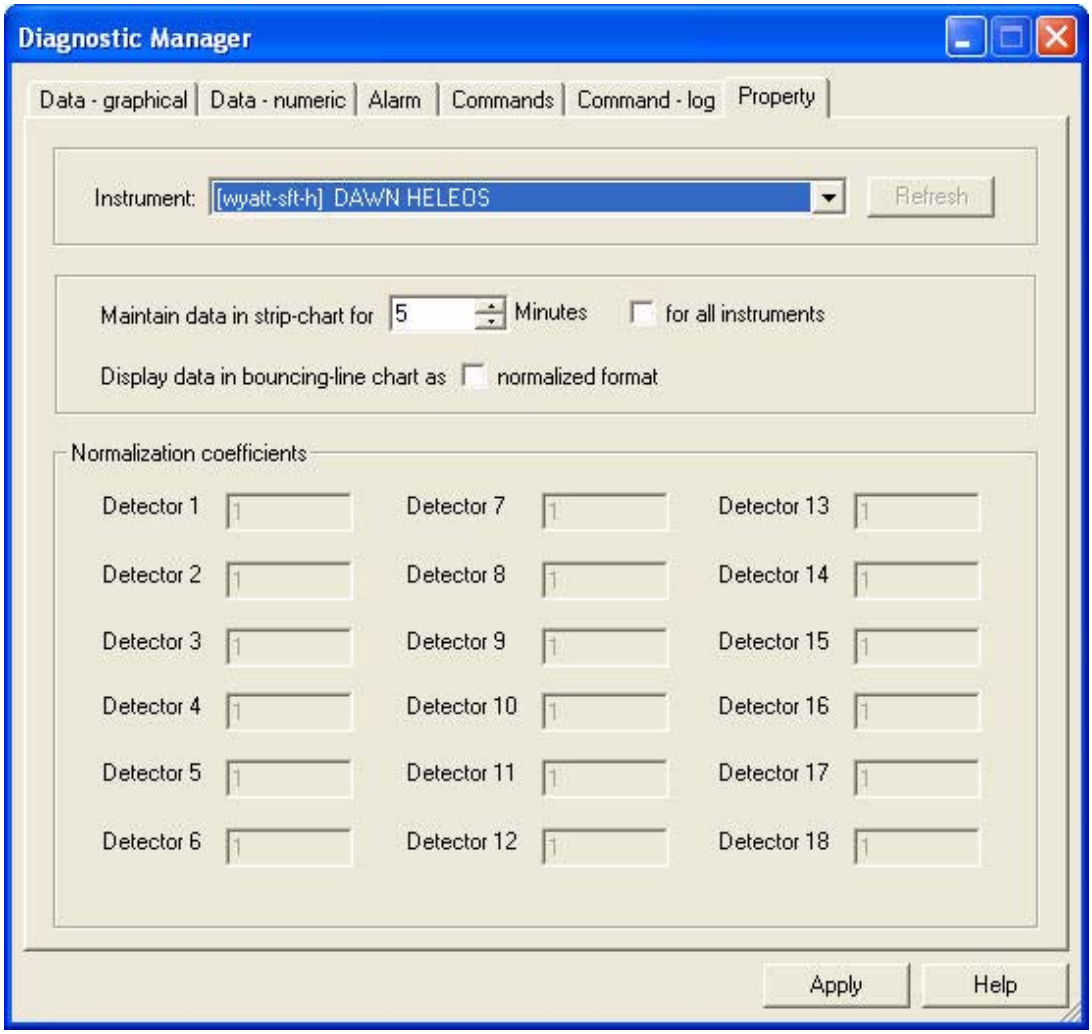
The following figure shows some commands sent to a DAWN HELEOS instrument.

You can drag the borders between column headings to resize the columns.

You can copy data from this log for pasting into other applications.

Viewing and Setting Properties with the Diagnostic Manager

The **Property** tab of the Diagnostic Manager allows you to view and set instrument properties. The list of properties you can set is different for each type of instrument. The following figure shows the properties available for the DAWN HELEOS instrument.



Properties are stored in the Windows registry of the computer to which the instrument is connected. The ISI running on that computer gets the information from the registry.

To set one or more properties, follow these steps:

1. Select an instrument from the drop-down Instruments list.

   If you add computers using ASTRA's Instrument list or you connect additional instruments after opening the Diagnostic Manager, click **Refresh** to update the drop-down list.

2. In the "Maintain data in strip-chart" area, set fields as follows:

   - **Minutes:** Select the number of minutes for which you want data to be displayed in the graphical data page for each strip chart graph.

- **For all instruments:** Put a checkmark in this box if you want the number of minutes to apply to all instruments accessible through the Diagnostic Manager. This setting affects only your view of the data; this setting does not affect users on other computers.
- **Display data in bouncing-line chart as normalized format:** This checkmark is used for Wyatt light-scattering instruments only. If you put a checkmark in this box, the normalization coefficients shown here are applied in the Data - graphical tab (see page 5-5) when "live data" is selected as the data type.

  "Live data" displays the detector voltages as a function of detector number. Applying the normalization constants should make the line essentially flat when looking at the scattering from a solvent. You can use this feature as a diagnostic tool when trying to set the orientation of a scintillation vial on the DAWN. Rotate the vial, view the live data, and try to put the vial in a position such that the live data line is flat.

3. For the remaining fields, change values for any properties you want to modify. The properties shown are different for each instrument type. Refer to the hardware documentation for details.
4. Click **Apply**.

WCS Client Application

For additional diagnostics, you may choose to run the WCS Client, which reports on the activities of the Wyatt Communications Server. This server manages communication between ASTRA and instruments.

You can start the WCS Client from the Windows Start menu by choosing **Programs→Wyatt Technologies→ASTRA→WCS Client**.

Typically, you would use this in cooperation with Wyatt Technical Support.

6 Creating & Running Experiments

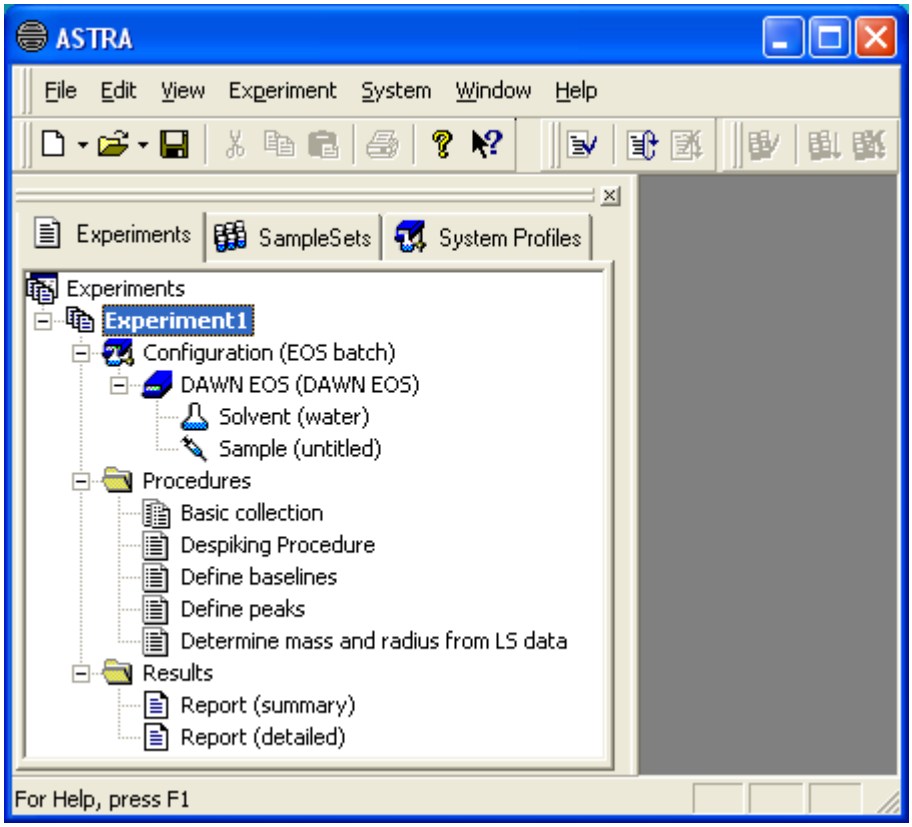
This chapter tells how to work with ASTRA experiments. The details of items contained in an experiment are covered in other chapters. This chapter describes actions you perform with the entire experiment, such as creating a new one, saving it, running it, or exporting it.

CONTENTS **PAGE**

About Experiments

The ASTRA user environment is centered around a structure we call an "experiment," which contains all the information needed to run an experiment and produce results. After you run an experiment, the experiment structure contains the results.

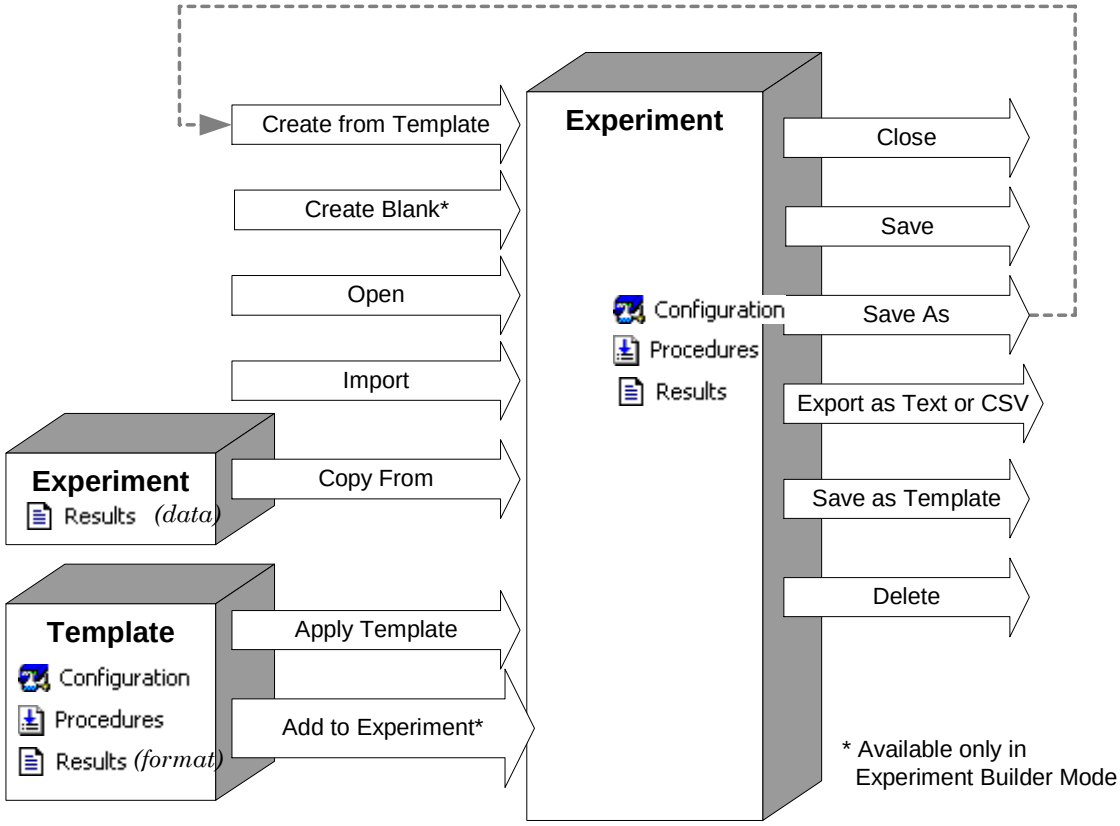
The Experiments tab in ASTRA shows the parts of the experiment.



You can expand or collapse the folders in an experiment as desired. Each experiment contains the following categories of items:

- **Configuration:** The hardware devices and connections used in the experiment. For online (fractionated) experiments, this may include a pump, injector, solvent, sample, DAWN, Optilab, and data connections. For details on all types of items that may be configured and their properties, see Chapter 7, "Configuring Experiments".
- **Procedures:** The actions to be performed in sequence when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures. For details on all types of procedures, see Chapter 8, "Editing Procedures".
- **Results:** The reports and graphs to be produced after the experiment procedure has been run. For details, see Chapter 10, "Working With Reports".

The actions you can perform on an experiment are shown in the following diagram. The arrows that point to the main experiment show ways to open, create, or bring information into an experiment. The arrows that point away from the main experiment show ways to close, save, or export information from an experiment.



The sections in this chapter listed below correspond to the actions shown in the previous diagram. Some commands behave differently depending on whether you are using ASTRA V Basic or a version that uses an experiment database (ASTRA V with Research Database or ASTRA V with Security Pack).

Table 6-1: Actions to Perform on Experiments

Action	Description	See
Create From Default	Make a new experiment based on a template that was saved as the “default template”.	page 6-4
Create From Template	Make a new experiment based on a configuration, procedure, and results template.	page 6-5
Create Blank	Make a new experiment with no default configuration, procedure, or results.	page 6-6
Open	Database: Open an experiment from the experiment database. Basic: Open an experiment from a file. This may include experiments saved with previous versions of Wyatt software.	page 6-7 and page 6-8
Import	Database: Open an experiment saved in a file. This may include experiments saved with previous versions of Wyatt software.	page 6-9

Table 6-1: Actions to Perform on Experiments

Action	Description	See
Export	Save an experiment to a file, or save experiment data to a tab-delimited or comma-separated values file.	page 6-17
Close	Close the current experiment.	page 6-15
Save	Database: Save an experiment to the experiment database. Basic: Save an experiment to a file.	page 6-15 and page 6-16
Save As	Database: Save experiment to the database with different name. Basic: Save an experiment to a file with a different name.	page 6-15 and page 6-16
Export as Text or CSV	Create a text or comma-separated file containing experiment data.	page 6-17
Save As Template	Save the configuration, procedure, and results formats so that they can be used as the basis for future experiments.	page 6-18
Delete	Delete the experiment from the experiment database.	page 6-20
Run	Run the experiment procedure.	page 6-11
Run Indefinitely	Run the experiment procedure ignoring the Duration.	page 6-11
Copy From	Copy results data from one experiment to another.	page 6-21
Apply Template	Create a copy of the experiment. Then apply the analysis procedures and result presentation from the selected template to the copy of the experiment.	page 6-22
Add to Experiment	Add items to the configuration, procedure, or reports. (Experiment Builder mode only.)	page 6-24

Creating New Experiments

The recommended way to create an experiment is from a system template provided with ASTRA. For more customization, you may want to save experiments as templates and use those. Experiment Builders may choose to create experiments from a blank template; however, using a provided template saves time.

Creating Default Experiments

If you have specified a "default" experiment template (see page 6-19), you can choose **File→New→Experiment from Default** to quickly create a new experiment from this template.

You can quickly create and start running an experiment using the default template by choosing **File→Begin Injection** (Ctrl+Shift+V) or clicking the toolbar icon. The experiment is created and begins data collection automatically. See page 6-19 for information on creating default templates.

Creating Experiments from Templates

Templates can be used to create new experiments, or to re-analyze data in a different way. ASTRA comes with over three dozen system templates that allow you to start using ASTRA at its full potential immediately.

Experiment templates set properties to values you are likely to use. There are just a few properties you typically need to modify.

Security You must have at least Technician access to create an experiment from a template. You must have at least Researcher access to modify an experiment that was created from a template.

To create an experiment from a template, follow these steps:

1. Choose **File→New→Experiment From Template**.

Shortcuts: Press Ctrl+Alt+T.
Click the down-arrow next to the icon.

2. In the New from Existing dialog, open the folder that contains the experiment template you want to use. You can choose from the following folders:
   - **System Templates:** These templates are provided with ASTRA for your use. A set of experiment templates is provided for each Wyatt instrument. These templates typically provide a starting point for most experiment types you might perform.
   - **My Templates:** These templates are ones you have saved as described in "Creating a Template" on page 6-18.
   - **Import Templates:** These templates are used when you open an experiment created with the old ASTRA 4 or DNDC 5 software. Creating new experiments from these templates is not recommended.

   If you read about an experiment template you want to use, but don't see it in the New from Existing dialog, see "Migrating the System Database" on page 2-3 to update your system database so you have all the latest experiment templates and system profiles.
3. Select a template and click **Create**.

   A new experiment is created based on the template you selected.

   Templates you use to create experiments are stored in the ASTRA system database. They are not stored in separate files in the installation tree.

Creating Experiments from Scratch

Experiment Builder

You can create and modify blank experiments only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

Security

You must have at least Researcher access to create an experiment from scratch.

You can modify templates and save experiments as templates, so it is unlikely that you will want to work starting from an empty experiment. However, if you want to create an empty experiment, follow this step:

- Choose **File→New→Blank Experiment**.

Shortcuts: Press Ctrl+N.
Click the down-arrow next to the  icon.
Right-click "Experiments" in the workspace and choose **New**.

Opening an Experiment from the Database

Database

You can open and work with any experiment you have saved.

If you are using ASTRA V with Research Database or ASTRA V with Security Pack, experiments are stored in the experiment database, and you open experiments from that database. To open experiments stored in separate files (such as exported experiments or experiments saved with ASTRA 4), see "Importing an Experiment from a File" on page 6-9.

Security

There are no access level restrictions on opening an experiment.

To open an experiment, follow these steps:

1. Choose **File→Open→Experiment**.

Shortcuts:
Press Ctrl+O.
Click the down-arrow next to the  icon.
Right-click "Experiments" in the workspace and choose **Open**.
Open a recently used experiment from the list in the File menu.

2. In the Open dialog, select the experiment you want to import.

   Unless you have created a folder in the database, all the experiments are in the top-level "/" folder.

   As in standard file selection dialogs, you can click the  icon to change the way the list of experiments in the database is viewed. In the detail view, the last data and time the experiment was modified is shown.

3. Select an experiment and click **Open**.

Tips:
You can open multiple experiments by holding down Shift key (for a range) or the Ctrl key (for individual experiments) while selecting experiments.

Opening an Experiment from a File

Basic

You can open and work with any experiment you have saved. If you are using ASTRA V Basic, experiments are stored in separate files with an extension of *.vaf.

To open an experiment, follow these steps:

1. Choose **File→Open→Experiment**.

Shortcuts:
Press Ctrl+O.
Click the down-arrow next to the  icon.
Right-click "Experiments" in the workspace and choose **Open**.
Drag-and-drop an experiment file from Windows Explorer or the desktop to the ASTRA window.
Open a recently used experiment from the list in the File menu.

2. In the Open dialog, navigate to the folder that contains the experiment you want to import.
3. Select a file and click **Open**.

You can open any of the following types of files:

File Extension	Description
*.vaf	Experiment file saved or exported by ASTRA V.
*.vrf	ASTRA V crash recovery file. (See page 6-14.)
*.adf	File saved by ASTRA version 4.70 or higher for a DAWN EOS, DAWN DSP, or DAWN DSP-F. See "Importing ASTRA 4 Files" on page 6-9 for information about fixing problems with these files.
*.mdf	File saved by ASTRA 4 for a miniDAWN.
*.nwf	File saved by DNDC 5.
*.rwf	File saved by RICAL 5.

Imported experiments have a complete set of configuration items, procedures, and results needed to view the experiment.

Importing an Experiment from a File

Basic

This item is disabled in ASTRA V Basic since it is identical to **File→Open→Experiment**.

Database

You can import experiments stored in files. This includes experiments saved with ASTRA 4 and ASTRA V Basic. It also includes experiments exported by ASTRA V.

Security

You must have at least Researcher access to import an experiment.

To import an experiment, follow these steps:

1. Choose **File→Import→Experiment**.

Shortcuts: Press Ctrl+I.
Right-click "Experiments" in the workspace and choose **Import**.
Drag-and-drop an experiment file from Windows Explorer or the desktop to the ASTRA window.

2. In the Import Experiment dialog, navigate to the folder that contains the experiment you want to import.
3. Select a file and click **Open**.

You can open any of the following types of files:

File Extension	Description
*.vaf	File saved or exported by ASTRA V.
*.vsf	ASTRA V sample set file.
*.vrf	ASTRA V crash recovery file. (See page 6-14.)
*.adf	File saved by ASTRA version 4.70 or higher for a DAWN EOS, DAWN DSP, or DAWN DSP-F. See "Importing ASTRA 4 Files" on page 6-9 for information about fixing problems with these files.
*.mdf	File saved by ASTRA 4 for a miniDAWN.
*.nwf	File saved by DNDC 5.
*.rwf	File saved by RICAL 5.

Imported experiments have a complete set of the configuration items, procedures, data set definitions, and results needed to view the experiment.

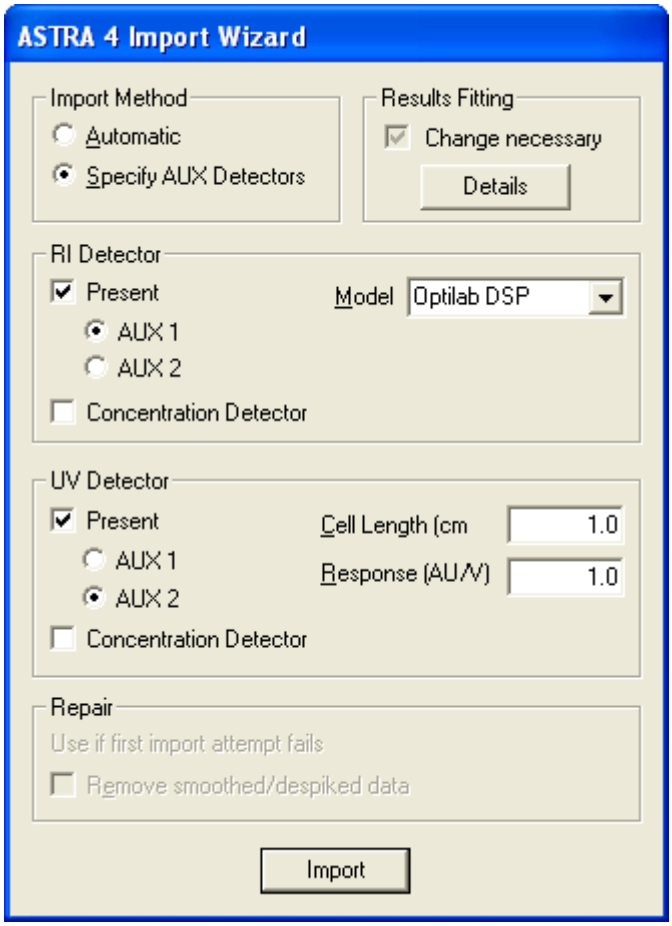
Importing ASTRA 4 Files

ASTRA V can automatically import most ASTRA 4 experiments. However, some issues may occur when importing certain files. For this reason, an Import Wizard allows you to attempt to fix such problems when importing the file.

Typical issues that may cause problems are that in some ASTRA 4 files, the smoothed data is stored, but the original data is not available. Also,

some files may not contain information about how AUX channels were used to receive RI or UV data for concentration calculations. Click the **Details** button for a description of the results fitting changes needed by your experiment.

When you open or import an ASTRA 4 file (*.adf or *.mdf), you see this dialog. The default is to perform an automatic import, and most other options are grayed out.



To import an ASTRA 4 file:

1. If your ASTRA 4 file had a standard configuration (such as, batch, particles, or RI detector alone), click **OK** in the Import Wizard to try an automatic import.
2. If you had a UV detector instead of an RI detector, or in addition to an RI detector, choose the **Specify AUX Detectors** item. Then specify how your RI and/or UV detectors were connected. Note that only one detector should have the **Concentration Detector** box checked. For an RI detector, select the model. For a UV detector, specify the cell length and response value. Then click **OK**.
3. Double-click on a procedure with a graph (such as Peaks) or a results graph. If the graph makes sense, the experiment was imported correctly and you need not repair the file. If the graph looks like a scatter plot (when it should not), close the experiment without saving, and continue to the next steps.
4. Reimport the ASTRA 4 file. Check the box in the **Repair** area to remove smoothed or despiked data. Click **OK**.
5. Again, check to see if the resulting graphs look valid. If they do, the file was imported correctly.
6. If you continue to encounter difficulties importing a file created with ASTRA 4, DNDC, or RICAL, please contact Wyatt Technology Corporation for assistance.

Running an Experiment

Once you have set up an experiment in ASTRA (and the corresponding instruments, connections, solvents, and samples are ready), you can run the experiment.

Security You must have at least Technician access to run experiments.

Validating an Experiment

You can validate an experiment's procedure sequence and configuration by choosing **Experiment→Validate**.

Validation checks the procedure sequence for conflicts. If the experiment collects data, validation also checks that the necessary instruments are connected and available. It checks to make sure the experiment configuration contains a solvent and a sample. In addition, validation checks the collection script. If you use the basic collection procedure, the collection script is built automatically, and validation never finds any problems with the script.

Shortcuts: Press Ctrl+Shift+V.
Right-click any folder in the tree, and choose **Manage→Validate**.

If any procedure in the sequence has a red X on its icon, it is in an invalid location in the experiment sequence or the configuration is missing instruments that produce data needed by the procedure. Modify the sequence as described in "Sequencing Procedures" on page 6-27 or revise the experiment configuration to include the appropriate instruments.

A procedure's state is always indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid sequence location or does not have the necessary data to run.

Starting a Data Collection Run

To start the experiment run, follow these steps:

1. Begin by turning on, warming up, and stabilizing your experimental apparatus. When everything is ready to go, continue with the following steps in ASTRA.
2. Choose **Experiment→Run**.

Shortcuts: Press Ctrl+Shift+R.
Click the Run icon in the experiment toolbar.
Right-click any folder in the experiment tree, and choose **Manage→Run**.

You can alternately choose **Experiment→Run Indefinitely** to run the experiment until you stop it. This command ignores the Duration property for the collection. (The experiment will also stop collecting data if your disk or database runs out of storage space.)

3. For an experiment with a basic collection procedure (as opposed to a custom script), you will be prompted to click **OK** and then inject the sample.
4. The spinning hourglass icon on the experiment node in the workspace shows that the experiment is running. Alternately, the drop-down in the experiment status toolbar shows the current state of the experiment. You see data as it is collected and can use the checkboxes to turn on or off data traces from various sources. Light scattering data is red; RI data is blue; UV data is green, QELS data is magenta, and viscosity data is black.
5. During the experiment, you are prompted for any information a procedure needs in order to run. For example, for light-scattering experiments, you will probably need to set baselines and peaks after the data is collected.

Security

6. If you are using ASTRA V with Security Pack, you will likely be prompted to sign off on an experiment after it runs.

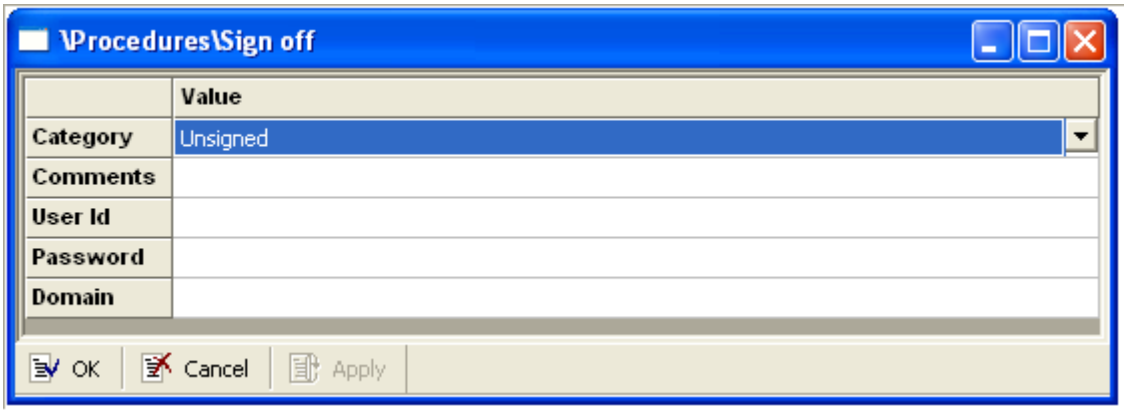
You can quickly create and start running an experiment using the default template by choosing **File→Begin Injection** or clicking the toolbar icon. The experiment is created and begins data collection automatically. See page 6-19 for information on creating default templates.

Signing Off on an Experiment

Security

If you see a message that says some parameters are not set for the Sign off procedure, follow these steps:

1. Click **OK**. You will see the Sign off procedure dialog.



2. In the Sign off dialog, choose a sign off category from the list. The categories are as follows:

Category	Description
Unsigned	This is the initial state for a sign off. You must select another category.
Responsibility	Selecting this category indicates responsibility for performing the experiment according to procedures.
Approval	Selecting this category indicates approval of the experiment.
Review	Selecting this category indicates review of the experiment.

3. In the Comments field, type any additional information required by your standard operating procedures.
4. In the User id, Password, and Domain fields, type the values for your valid ASTRA account, as described in the section on "Starting ASTRA" on page 3-2. Be sure to use uppercase and lowercase correctly in your password. Note that it is not necessary for the person signing off on the experiment to be the same person who logged in initially to begin the ASTRA session.
5. Click **OK**.

Stopping an Experiment

To stop a running experiment, choose **Experiment→Stop**.

Shortcuts: Press Ctrl+Shift+S.
Click the Stop icon in the experiment toolbar.
Right-click any folder in the experiment tree, and choose **Manage→Stop**.

Stopping an experiment with ASTRA stops only the selected experiment from executing. This includes the collection and analysis of data. It does not affect the execution of other experiments in ASTRA, nor does it affect any activity going on outside of ASTRA's control.

See your hardware documentation for information about alarms, emergency stops, and setting up safety interlocks. Alarms may be monitored via the Diagnostic Manager. See "Viewing Alarms with the Diagnostic Manager" on page 5-7 for details.

Re-Running an Experiment for Data Processing

If you modify one or more procedures in an experiment, you can re-run the experiment using the **Run** command. This time, instead of collecting data, only the procedures marked with the not-run icon are performed.

If a Crash Occurs...

While data is being collected, ASTRA stores data in a "crash recovery file" in your My Documents\ASTRA V Recovered Files folder. The filename contains the current date and time and have a file extension of .vrf. For example, 20080324_221518237Experiment.vrf.

When the data collection is complete and data is successfully saved, this file is automatically deleted.

When saving over the network, a problem may occur, for example, if the network connection fails or if you do not have permission to save to a particular location. When this happens, the save fails and the crash recovery file is retained.

Each time ASTRA starts, it checks for crash recovery files in the My Documents\ASTRA V Recovered Files location. If a .vrf file is present, ASTRA opens it and creates a standard ASTRA V .vaf file in the same My Documents location.

Closing an Experiment

You can work with multiple experiments open in ASTRA.

To close an experiment without exiting from ASTRA, follow these steps:

1. In the experiment tree, select an item in the experiment you want to close.
2. Choose **File→Close**.

Shortcuts: Right-click the experiment name in the tree and choose **Close**.
Choose **File→Close All** to close all experiments at once (unless data collection is in progress).

3. If you have made unsaved changes, you are asked whether you want to save them.

Saving an Experiment to the Database

Database

If you use ASTRA V with Research Database or ASTRA V with Security Pack, experiments are saved in the ASTRA database. To save experiments in separate files, see "Exporting an Experiment" on page 6-17.

It is a good idea to save experiments frequently.

Security

You must have at least Technician access to save an experiment.

To save an experiment, follow these steps:

1. Choose **File→Save**.

Shortcuts: Press Ctrl+S.
Click the  icon.
Right-click the experiment name in the tree and choose **Save**.

2. If this is the first time you have saved this experiment, you see the Save As dialog. Otherwise, you are finished saving the file.
3. Type a name for the experiment.
4. If you want to store this experiment in a subfolder, click the  New Folder icon and type a name for the folder. Then open the folder.
5. Make sure the Of Type field shows "Experiments". For information about saving templates, see "Creating a Template" on page 6-18
6. Click **Save**.

To save an experiment with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Shortcuts: Right-click the experiment name in the tree and choose **Save As**.

Saving an Experiment to a File

Basic

It is a good idea to save experiments frequently.

If you are using ASTRA V Basic, experiments are stored in files with an extension of *.vaf.

To save an experiment, follow these steps:

1. Choose **File→Save**.

Shortcuts: Press Ctrl+S.
Click the  icon.
Right-click the experiment name in the tree and choose **Save**.

2. If this is the first time you have saved this experiment, you see the Save As dialog. Otherwise, you are finished saving the file.
3. In the Save As dialog, navigate to the folder you want to contain the file.
4. In the File Name field, type a file name for the experiment. The following characters may not be used in ASTRA file names:

colon	:
question mark	?
quote	“
asterisk	*
forward slash	/
backslash	\
less than	<
greater than	>
pipe	|

5. The Save As Type field shows that the file will be saved with an extension of *.vaf. You can choose an older version of ASTRA V if you like. To save to an ASTRA 4, tab-delimited text, or comma-separated values format, see “Exporting an Experiment” on page 6-17.
6. Click **Save**.

To save an experiment with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Shortcuts: Right-click the experiment name in the tree and choose **Save As**.

Exporting an Experiment

You can export experiment data to separate files. These files can be imported by ASTRA, or you can save experiments in formats that can be imported by spreadsheets.

Basic

If you are using ASTRA V Basic, this command allows you to export experiments as ASTRA 4, tab-delimited, and comma-delimited files. To save an experiment to a different *.vaf file in the current ASTRA format, use the Save As command instead.

Security

You must have at least Researcher access to export an experiment.

To export an experiment, follow these steps:

1. Choose **File→Export→Experiment**.

Shortcuts: Right-click the experiment name in the tree and choose **Export**.

2. In the Export Experiment dialog, navigate to the folder you want to contain the exported file. (Do not export the experiment to a folder that is a read-only folder, such as Sample Data or Analyzed Experiments.)
3. In the File Name field, type a file name for the experiment.
4. In the Save As Type field, select a type. The formats you can export are as follows:

Type	Description
*.vaf	ASTRA V file that can be imported by ASTRA V on this or another computer. You can choose a version of ASTRA for compatibility with earlier versions. (Use Save As instead for ASTRA V Basic.)
*.adf	An ASTRA 4 format for use with earlier versions of ASTRA.
*.txt	Tab-delimited text file for exporting a data set defined by the selected data set definition. This format is easily imported into most spreadsheets.
*.csv	Interpolated comma-delimited text file for exporting a data set defined by the selected data set definition. This format is easily imported into most spreadsheets. Data traces are interpolated so that all measurements are on the same time scale, allowing for easy display in software such as Excel.

5. If you choose a .txt or .csv format, you can also choose a data set definition to export with the experiment. See “Creating Data Set Definitions” on page 11-8 for information about data set definitions.
6. Click **Save**.

If you do not find the organization of the tab-delimited or comma-delimited output useful, try the output described in “Exporting Data” on page 11-17.

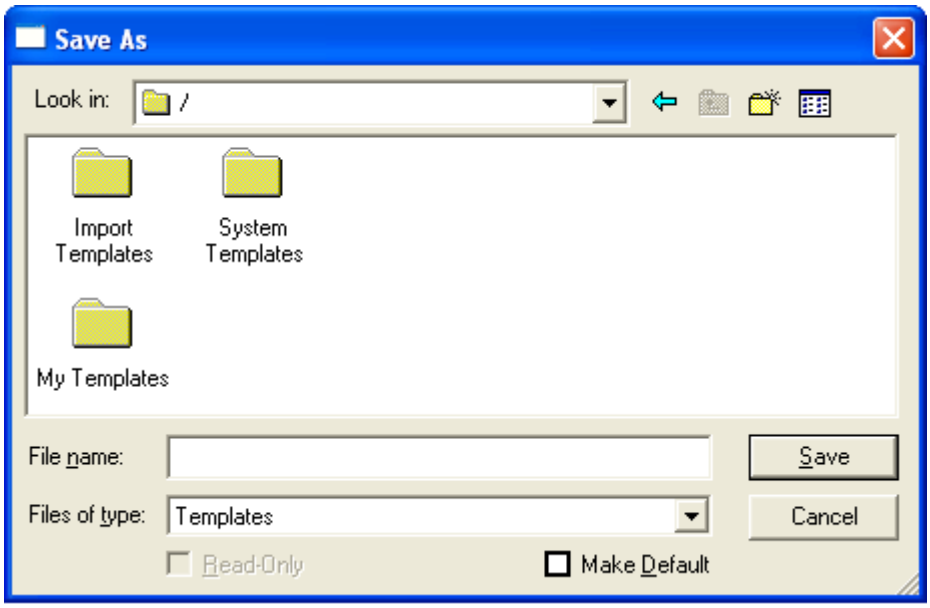
Creating a Template

After you modify an experiment, you may want to save it as a template for other experiments. The saved template includes the configuration, procedure, and result formats.

Security You must have at least Researcher access to save templates.

To save a template, follow these steps:

1. Choose **File→Save As Template**.



2. Navigate to the location in the system database where you want to save this template. The recommended location is the **My Templates** folder or a subfolder you create in My Templates.

   You can create a subfolder by clicking the New Folder icon and typing a folder name. Then open the new folder.

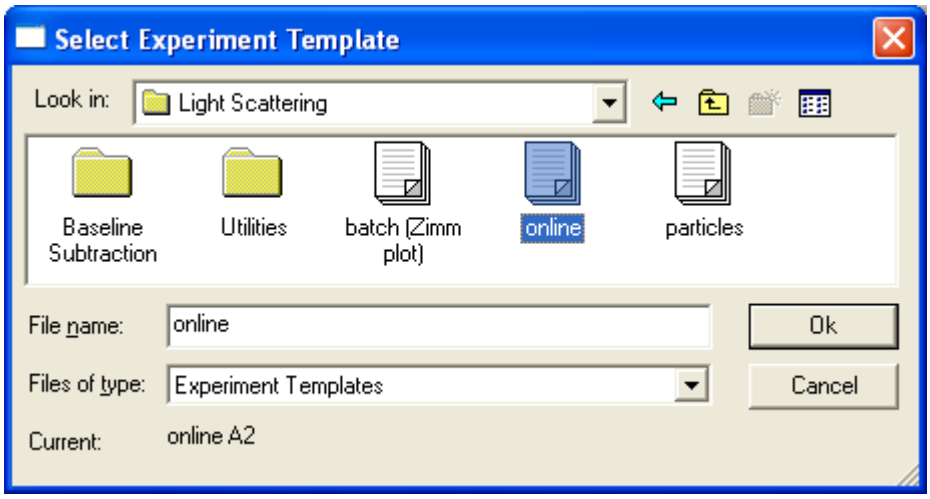
3. Check the **Make Default** box if you want this template to be the default for use with the **File→New→Experiment from Default** command.

4. Click **Save**.

Note: Templates are saved in the system database. This database is separate from the experiment database.

Setting a Default Template

You can specify the default experiment template by choosing **System→Preferences→Set Default Template**. In the Select Experiment Template dialog, choose an existing template from the My Templates folder or the System Templates folder and its subfolders and click **OK**.



The **Current** line shows the currently selected default template.

When you choose **File→New→Experiment from Default**, the template you selected will be used to create a new experiment. The default template is also used when you create a blank sample set as the default template for all samples in the set.

You can also set the default template when saving an experiment template by checking the **Make Default** box.

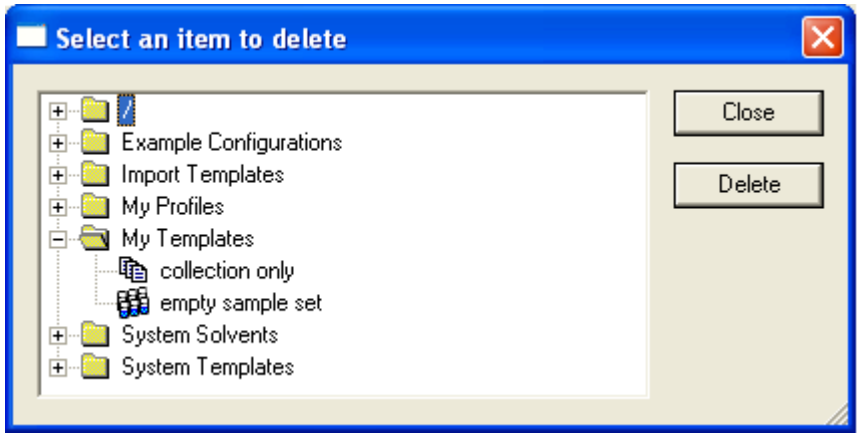
Deleting a Template

Security

You must have at least Researcher access to delete templates.

To delete an experiment template, follow these steps:

1. Choose **System→Database Administration→Delete Items**. This opens the "Select an item to delete" dialog.



2. Find and highlight the template you wish to delete. Templates are generally in the "My Templates" and "System Templates" folders.
3. Click **Delete**.
4. Click **Close** when you have finished deleting templates.

Deleting an Experiment

Security

If you are using ASTRA V with Security Pack, experiments can only be deleted by an ASTRA administrator. See "Deleting Experiments" on page 4-8 for details.

Basic

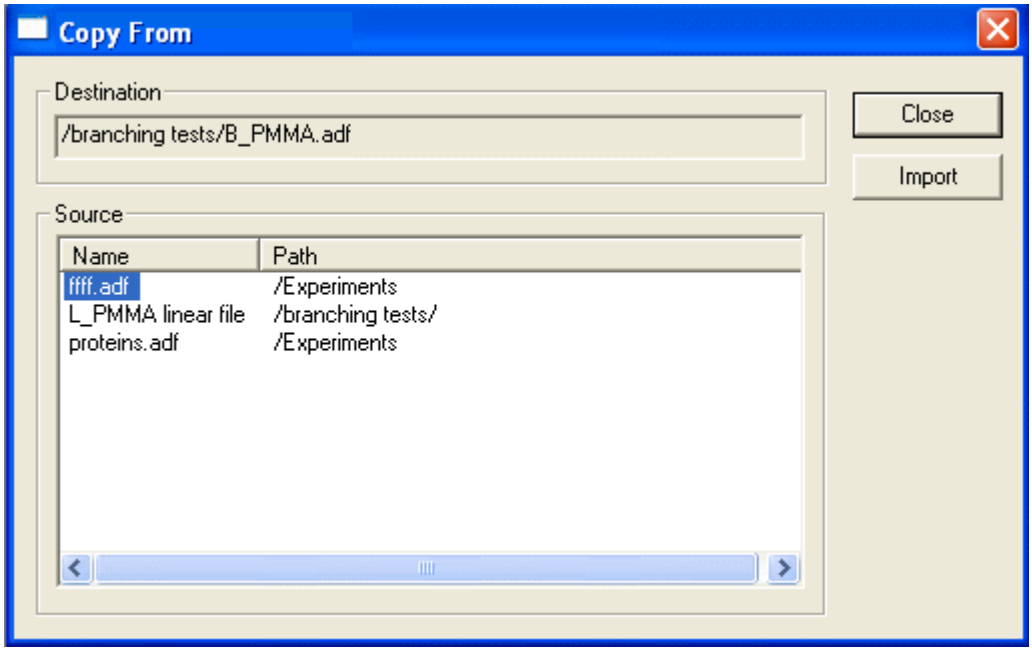
If you are using ASTRA V Basic, any user may delete an experiment by deleting the *.vaf file that contains the experiment.

Copying Data

You can copy data generated for one experiment into another experiment. You might do this to create combined plots of results from several experiments, or to use the data in a procedure such as branching.

To copy data into an experiment, follow these steps:

1. Open both the source and destination experiments. The source experiment contains the data you want to copy. You will copy the data to the destination.
2. Activate the destination experiment by clicking on any part of the experiment in the workspace.
3. Choose **Experiment→Copy From**. The Copy From dialog appears.



4. Select the source experiment you want to use, and click **Import**. A Data Set Definition dialog for the source experiment is shown.
5. Select the data you want to copy into the destination experiment. See “Creating Data Set Definitions” on page 11-8 for information on using this dialog.
6. Click **OK** in the Data Set Definition dialog.
7. Repeat steps 4 through 6 for any additional data you want to copy.
8. When finished, click **Close** in the Copy From dialog.

After you have copied data, you can access it using a standard data set definition in the source experiment. The data set definition allows you to display the data in graphs. In addition, the data is available for procedures such as branching.

Applying a Template

You can apply the procedures and result formats from a template to an experiment you have already run to collect data. This allows you to perform multiple procedure sequences on the same set of raw data. (To apply a template to multiple experiments, see "Applying a Template to Multiple Experiments" on page 6-23.)

For example, after using the "LS batch (Debye plot)" template when collecting data, you might want to apply the "LS batch (Zimm plot)" template to the same data so that you can view the results differently.

Applying a template creates a separate experiment, so you do not lose any of the information in the original experiment. The new experiment has a name that reflects both the original experiment and the template.

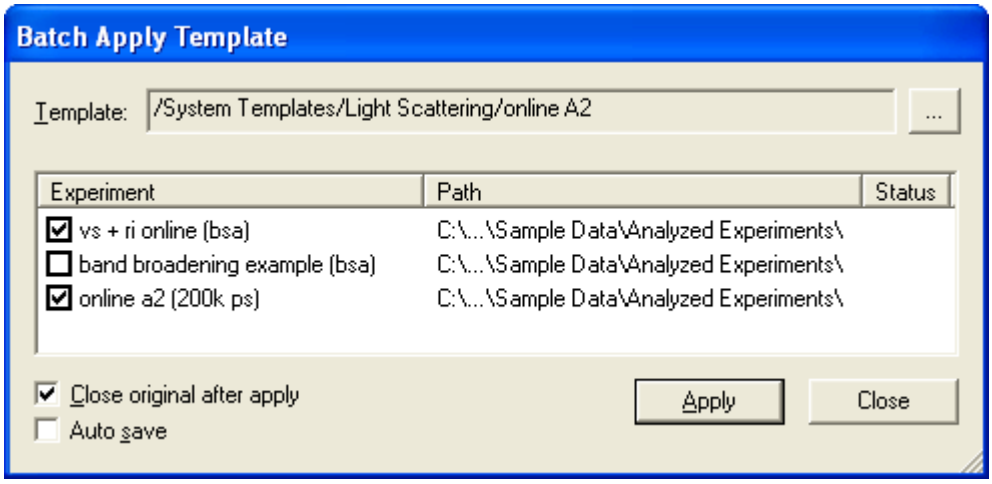
To apply a template, follow these steps:

1. Open the experiment that contains the raw data you want to use.
2. Choose **Experiment→Apply Template**. The New From Existing dialog appears. This is the same dialog you use to create an experiment from a template before data collection.
3. Choose a template to apply to the data. The procedure and result formats in the template will be used. Typically, you would choose a template from the System Templates or My Templates folder.
4. Click **Create**. A new experiment is created and is run automatically. The name of the new experiment combines the names of the template used to create the experiment and the original experiment.
5. After the applied procedure runs, you can view the new results.

Applying a Template to Multiple Experiments

If you want to apply the same experiment template to multiple experiments, follow these steps:

1. Open all the experiments to which you want to apply a new experiment template.
2. Choose **File→Batch Apply Template**.



3. Click "..." and browse for the template you want to apply. The procedure and result formats in the template will be used. Typically, you would choose a template from the System Templates or My Templates folder.
4. Check the boxes next to the open experiments to which you want to apply the selected template.
5. Leave the **Close original after apply** box checked if you want to automatically close the old experiments when new experiments with the template applied are created.
6. Check the **Auto save** box if you want the new experiments that will be created to be saved automatically.
7. Click **Apply**. A new experiment is created for each old experiment and is run automatically. The names of the new experiments combine the names of the template used to create the experiment and the original experiment.

Adding Elements to an Experiment

This chapter provides simple examples for adding elements to the folders in an experiment tree. Other chapters are referenced to provide details about all the various configurations, procedures, data sets, and results that can be added and how to work with these things after adding them to an experiment.

Security

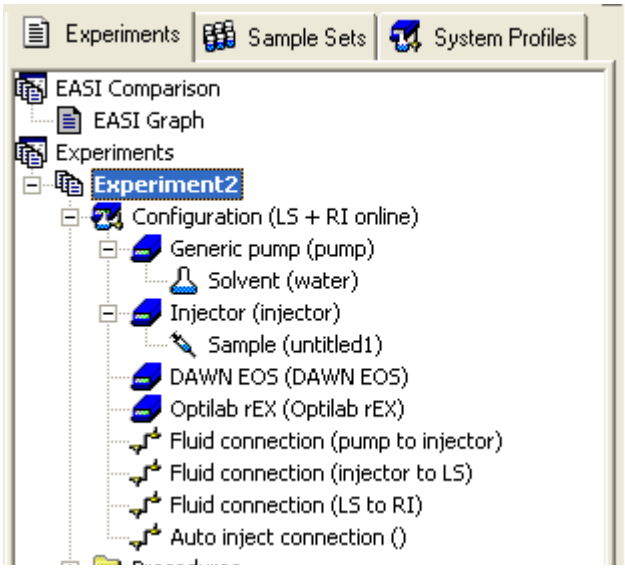
You must have at least Researcher access to add elements to experiments.

Adding to the Configuration

Experiment Builder

You can add items to the configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

The Configuration tree in the Experiments tab shows the hardware configured to be used in the experiment. The templates provided with ASTRA include most instruments and connections you use in a typical experiment. Chapter 7, "Configuring Experiments" contains details about the properties that can be set for each instrument type.

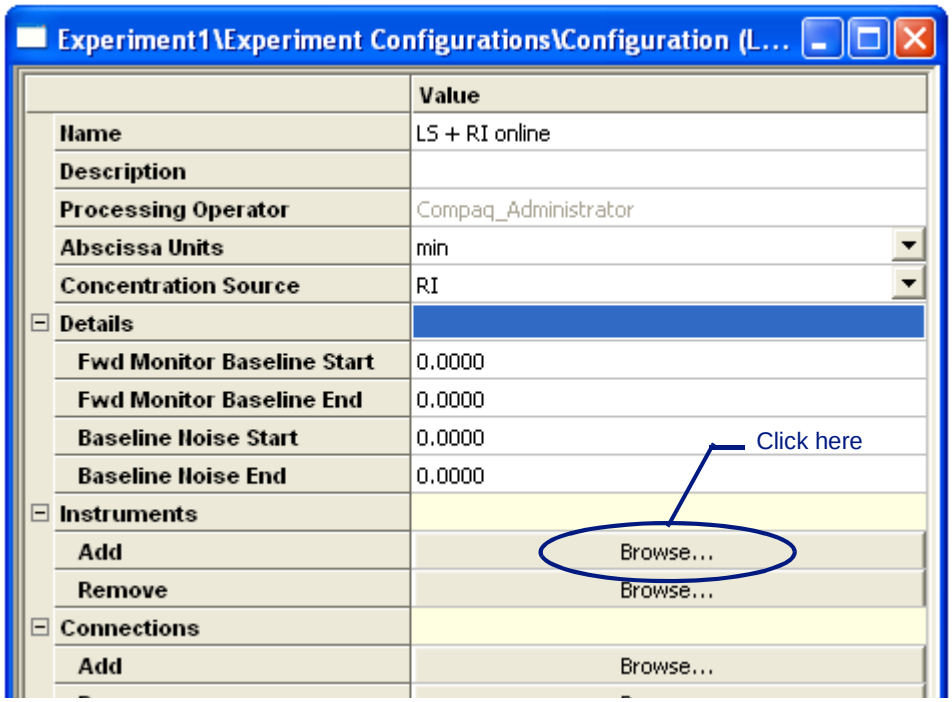


To add instruments and connections, you can specify them in the Experiment Configuration tab of the Experiment Configuration properties dialog. To add an instrument, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties dialog for the experiment configuration, which has a tab for each item in the configuration tree.

Shortcuts: Double-click any part of the configuration tree in the Experiments tab. Right-click any part of the experiment tree and choose **Manage→Configuration→Edit**.

2. In the Experiment Configuration tab, click the **Browse** button in the row to add instruments.



3. In the Add Instrument dialog, find the instrument you want to add to the experiment. For example, you might navigate to the /Profile/Instrument/UV Instrument folder to select a profile of a UV instrument.

   Profiles of many instruments are provided with ASTRA, and you can create additional profiles as described in Chapter 12, "Working with System Profiles".

4. Select the instrument profile you want to add and click **Open**. The instrument is added to your experiment configuration and you can edit its properties by double-clicking it in the configuration tree.

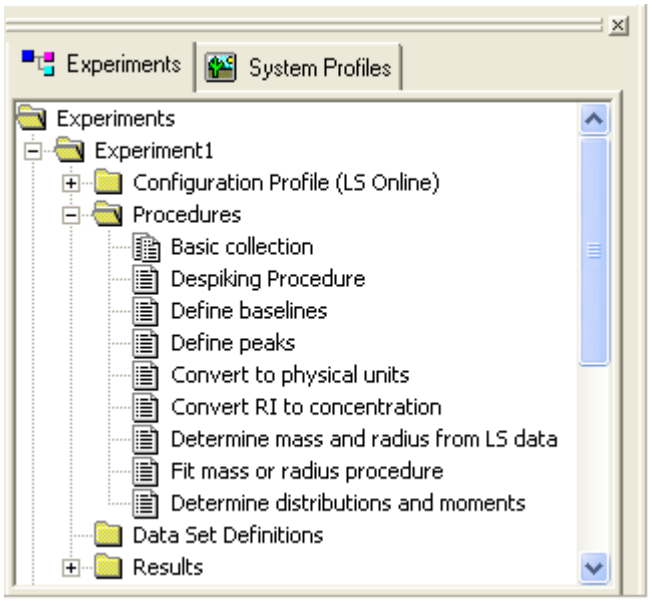
5. To add a connection, follow the same steps but click the **Browse** button in the row to add connections.

Importing an experiment configuration (with **Experiment→Experiment Configuration→Replace Configuration**) replaces the entire experiment configuration (all instruments and connections) with a different experiment configuration. Adding an instrument or connection adds only that item without replacing or removing other items.

Adding Procedures

Experiment Builder You can add procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

The Procedures folder in the experiment tree shows actions ASTRA performs in sequence when you run the experiment. For details on all types of procedures, see Chapter 8, "Editing Procedures".

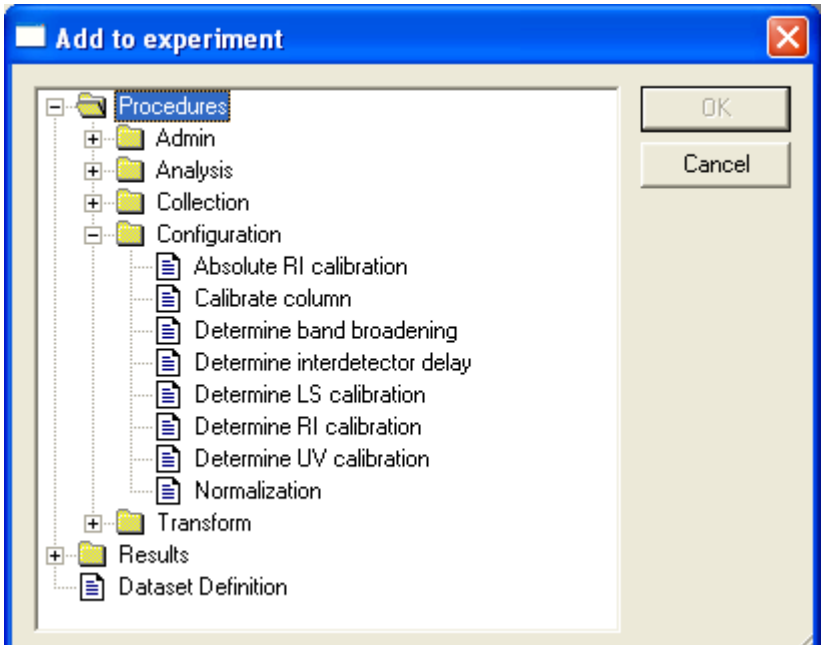


To add a procedure to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results folders of the experiment.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.

2. Open a folder under Procedures and select the procedure you want to add. See Chapter 8, "Editing Procedures" for descriptions of all procedures.



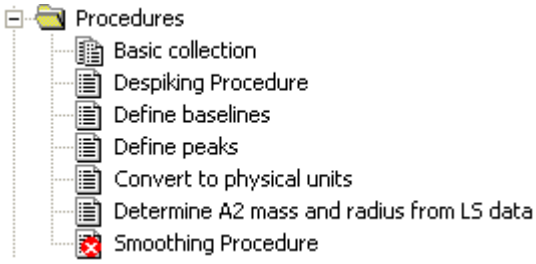
3. Click **OK**.

Sequencing Procedures

Experiment Builder

You can change the sequence of the procedure only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

If a procedure is in an invalid location in the experiment sequence, it will have a red X on its icon.



To correct the problem, drag the procedure to a location in the sequence where its icon and the icons that follow it have no red X.

Chapter 8, "Editing Procedures" has details on the correct location in the sequence for each procedure, and the data required for each procedure to be run.

Note that the standard experiment templates already contain the necessary procedures for collecting and analyzing the data in the correct sequence.

Adding Data Set Definitions

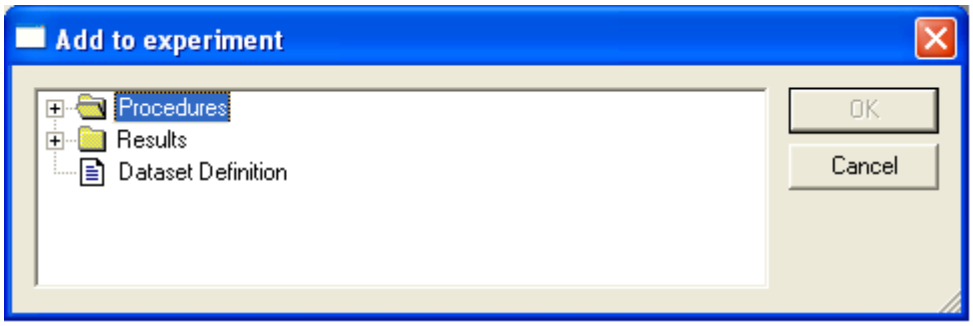
Experiment Builder You can create data set definitions only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. You can see the Data Set Definitions folder in the workspace only if you opened the experiment while in Experiment Builder Mode.

Data Set Definitions make additional sets of data available for use by reports. For details about creating and using data set definitions, see Chapter 11, "Working With Graphs".

To add a data set definition to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.



2. Select Data Set Definition and click **OK**. The data set definition is added to the Data Set Definitions folder of the experiment tree. (Some older experiments may not have a Data Set Definitions folder.)
3. See Chapter 11, "Working With Graphs" for details about specifying the data in the set.

Adding Reports and Graphs

Experiment Builder You can add reports only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. In Run mode, you can add graphs by choosing **Experiment→Graph→Add Custom Plot** or **Experiment→Graph→Add Parametric Plot**.

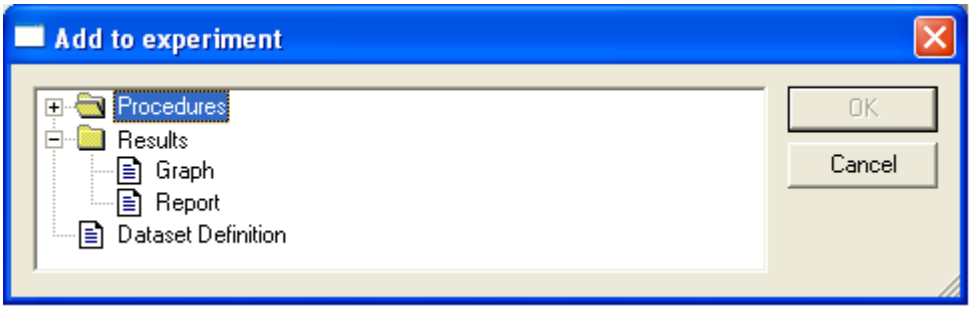
The results of an experiment are available through the reports and graphs you add to an experiment. For details about customizing reports and graphs, see Chapter 10, "Working With Reports".

To add a report or graph to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.

2. Open the Results folder and select Graph or Report.



3. Click **OK**. The graph or report is added to the Results folder of the experiment tree.
4. See Chapter 10, "Working With Reports" for details about reports and Chapter 11, "Working With Graphs" for details about graphs.

7 Configuring Experiments

This chapter explains how to configure your experiments in ASTRA to reflect the instruments, connections, solvents, and samples you use to collect and process data. This is accomplished using ASTRA V configurations and system profiles.

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About Configurations and Profiles

The configuration of an experiment reflects not only the physical apparatus used to collect the data, but also elements such as the solvent and sample. In describing the configuration, ASTRA V breaks up the different parts of the experiment into logical units called *profiles*.

The types of profiles that are available are shown in Figure 7-1. Each instrument has a profile that contains parameters specific to that instrument. In addition, connections between instruments are represented by profiles. Finally, elements used in the apparatus, such as the solvent and sample, are represented by profiles as well.

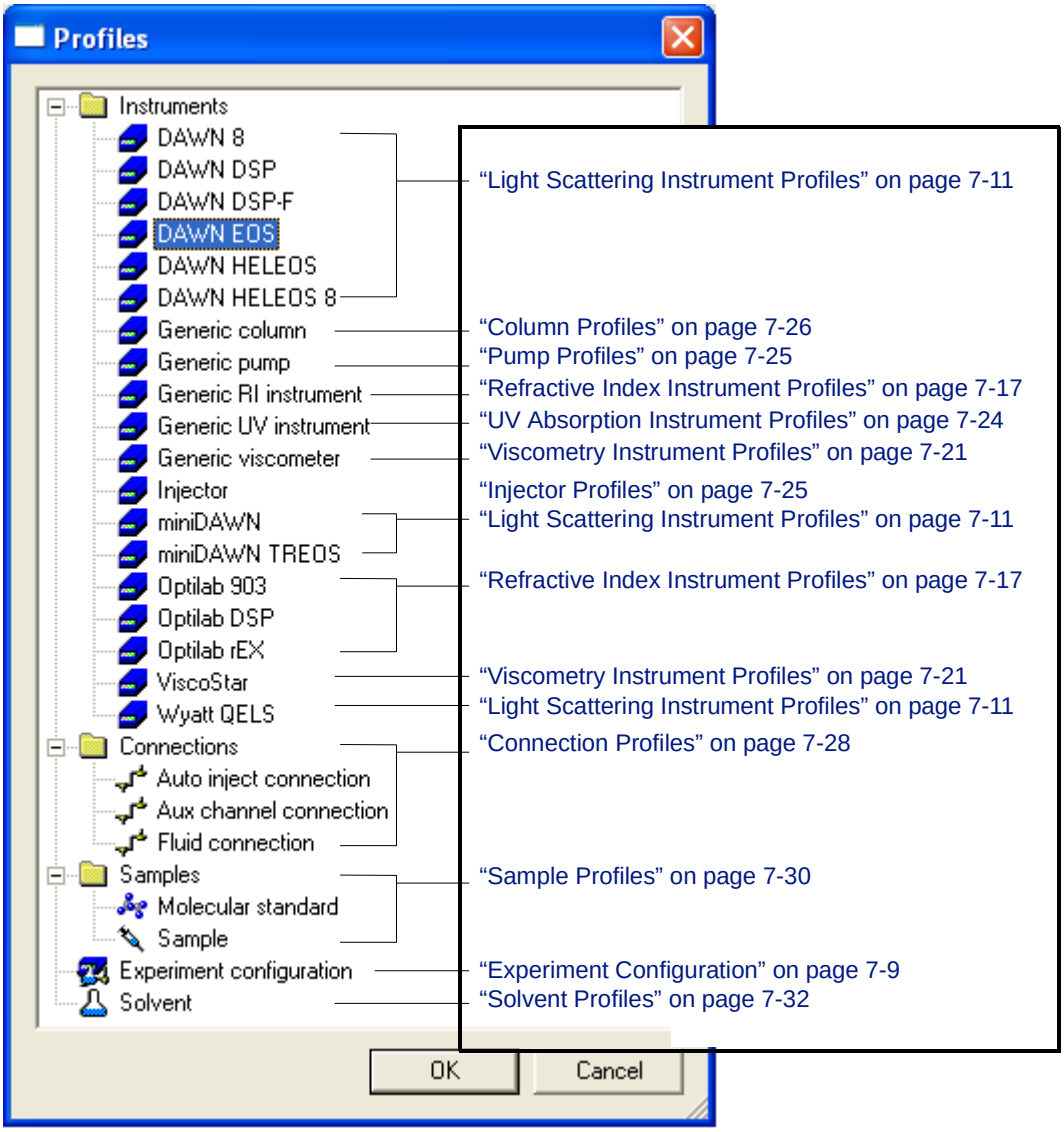


Figure 7-1: Profile types available in ASTRA V

In an experiment, the set of profiles describe how an experiment is set up. This is called the *configuration*. You can also create profiles that are stored outside of experiments and can be copied into experiments as needed; these are called *system profiles*.

This chapter focuses on using profiles in configurations. The experiment templates provided with ASTRA contain commonly used configurations. However, as you gain more experience using ASTRA, you may want to use system profiles in conjunction with your experiments. See Chapter 12, "Working with System Profiles" when you are ready to learn more about using system profiles.

In Run mode, you use the configuration items provided in the templates. While you can modify the properties of items, you cannot add items to or remove items from a configuration.

Experiment Builder

You can add items to a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties dialog, you need to close and reopen it after enabling Experiment Builder mode.

Security

You must have at least Researcher access to work with configurations and system profiles. If you are a Technician or Guest, you have read-only access to profiles.

This chapter describes the types of profiles contained in configurations and how their properties can be modified. Each profile type has a property list similar to that shown in Figure 7-2.

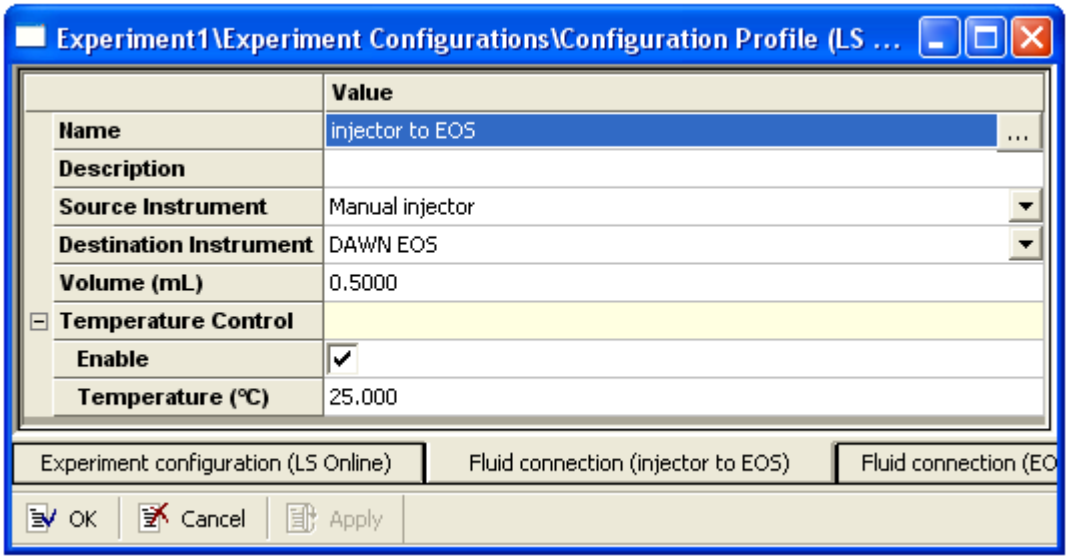


Figure 7-2: Typical property dialog (fluid connection example)

Configuration Example

The Configuration tree in the Experiment tab of the workspace shows both the hardware configured to be used in the experiment, as well as additional elements such as the solvent and sample. The experiment templates provided with ASTRA V include most common configurations. A configuration for an online light-scattering experiment apparatus is shown in Figure 7-3. The same experiment is shown in the schematic of Figure 7-4. Comparing these two figures highlights the logical structure of the configuration and its constituent profile elements in the workspace.

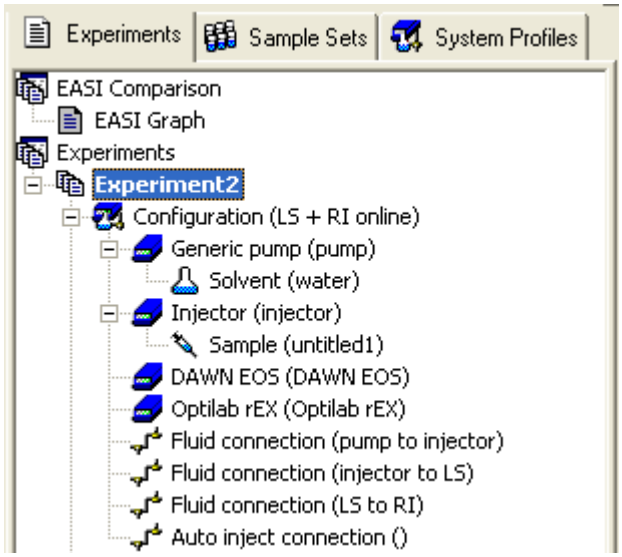


Figure 7-3: Configuration for an LS Online Experiment

The hardware setup for the configuration in Figure 7-3 would be organized similar to Figure 7-4:

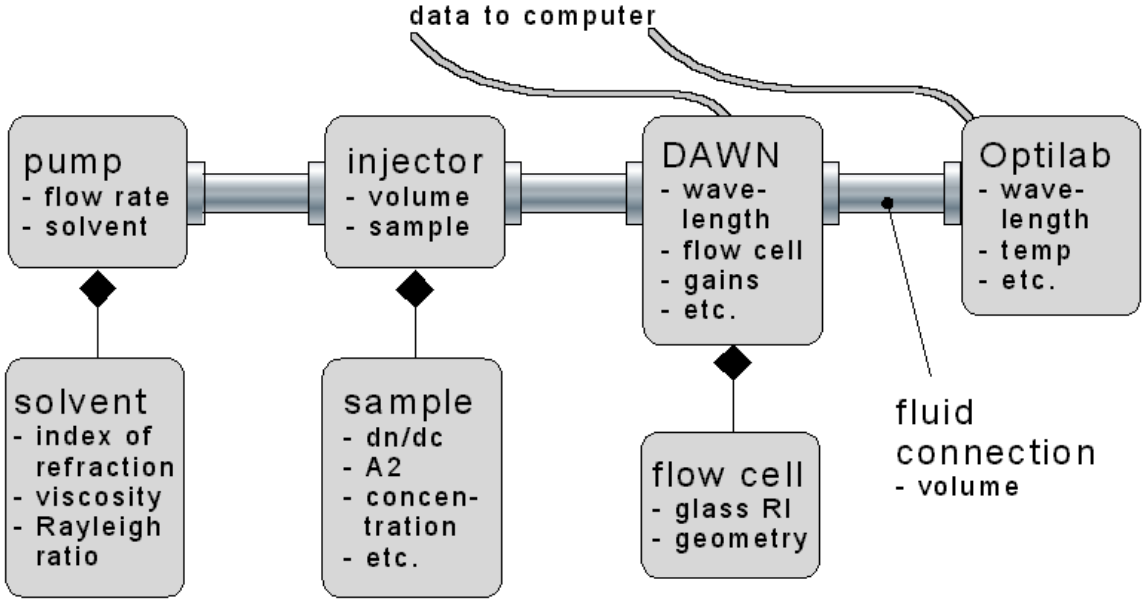


Figure 7-4: Hardware Connections for LS Online Experiment Apparatus

Using Configurations

Each experiment has a Configuration item that contains descriptions of all of the physical components used in the experiment.

The actions you can perform on a configuration are simple. The complexity comes from the large number of physical components that can be used and the large number of properties some of these components have.

Security

You must have at least Researcher access to work with configurations and system profiles. If you are a Technician or Guest, you have read-only access to profiles.

Editing a Configuration

To set properties of a configuration component, follow these steps:

1. Double-click on a component in the Configuration node of the Experiment tab in the workspace. This opens the properties dialog and selects the tab for that component. See Figure 7-2 for an example.

Shortcuts: Choose **Experiment→Configuration→Edit**.

2. Set properties by typing, selecting from a list, or checking a box.

   You can expand or hide lists of related properties if there is a + or - sign next to a property.

3. Alternately, you may click the browse button ("...") to the right of the Name property and locate a system profile to use to replace the existing property values for this item.

4. You can move to other tabs to view or set properties for other items.

Shortcuts: Double-click on an item in the Configuration tree to move to its tab.
Move to a tab using the  tab arrows.

5. Click **Apply** or **OK** to make the changes.

The remaining sections of this chapter contain details about the properties you can set in the various tabs.

Adding Instruments and Connections

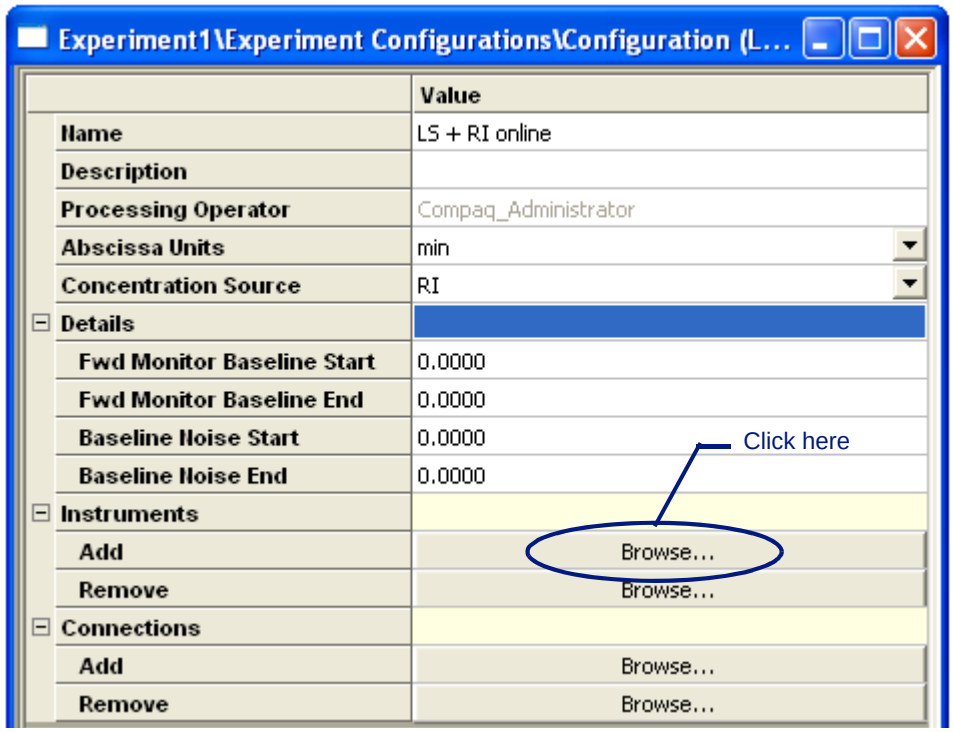
Experiment Builder

You can add items to a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties dialog, you need to close and reopen it after enabling Experiment Builder mode.

Note: No pre-defined instrument profiles are provided in the ASTRA system database. In order to have instruments available to select in the Add Instrument dialog as described in the following procedure, you need to first save instrument profiles in your system database as described in "Creating Profiles" on page 12-3.

To add instruments and connections, you can specify them in the Experiment configuration tab of the Configuration properties dialog. To add an instrument, follow these steps:

1. Choose **Experiment→Configuration→Edit**. This opens the properties dialog for the configuration, which has a tab for each item in the configuration tree. Alternately, double-click the Configuration node in the Experiment tab of the workspace.
2. In the Experiment configuration tab, click the **Browse** button in the row to add instruments.



3. In the Add Instrument dialog, find the instrument you want to add to the experiment. This must be an instrument profile you have saved as described in "Creating Profiles" on page 12-3. For example, you might navigate to the "My Profiles" folder to select a profile you have created.
4. Select the instrument profile you want to add and click **Open**. The instrument is added to your configuration and you can edit its properties by double-clicking it in the configuration tree.
5. To add a connection, follow the same steps but click the **Browse** button in the row to add connections.

Removing Instruments and Connections

Experiment Builder

You can remove items from a configuration only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties dialog, close and reopen it after enabling Experiment Builder mode.

1. Choose **Experiment→Configuration→Edit**. This opens the properties dialog for the configuration, which has a tab for each item in the configuration tree.
2. In the Experiment configuration tab, click the **Browse** button in the row to remove instruments or the row to remove connections.
3. In the Remove Instrument Profile or Remove Connection Profile dialog, check the box next to the item you want to remove and click **OK**.

Replacing an Experiment Configuration or Item

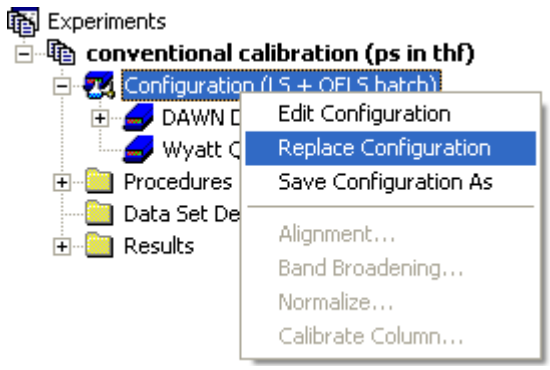
It is possible to replace an *entire configuration* with an experiment configuration stored as a system profile. A number of experiment configurations are provided with ASTRA. You can also save your own experiment configurations as described in "Creating Profiles" on page 12-3. For example, you may have a standard experiment configuration you want to use in many different experiments.

You can also replace a *single instrument or other item* with a saved system profile. No pre-defined instrument profiles are provided with ASTRA. In order to have instruments available, you need to save instrument profiles in your system database as described in "Creating Profiles" on page 12-3.

See Chapter 12, "Working with System Profiles" for more about system profiles.

To import a *complete configuration*, follow these steps:

1. Choose **Experiment→Configuration→Replace Configuration**. (Or, right-click on the "Configuration" node in the experiment, and choose **Replace Configuration**.) You see the Select Profile dialog.



2. Browse the system database for a configuration to import. In addition to any experiment configurations you have saved, ASTRA provides a number of configurations in the "Example Configurations" folder. These are organized by the experiment type and instruments involved.

3. When you find a profile, select it and click **Open**. The experiment configuration you selected replaces the existing one.

To replace an *individual item* in the configuration with another item of the same type, follow these steps:

1. Right-click on a node in the configuration and choose the **Replace** command for that item from the pop-up menu.
2. Browse the database for an item to import. You can only select from items of the corresponding type. That is, you can replace a sample with a sample, a solvent with a solvent, and so on for connections and instruments. If you are replacing an instrument, you can select any type of instrument.

Note: No pre-defined instrument profiles are provided with ASTRA. In order to have instruments available, you need to first save instrument profiles in your system database as described in "Creating Profiles" on page 12-3.

3. When you find a profile, select it and click **Open**. The item you selected replaces the existing item.

If you later edit properties of items you imported, there is no effect on the system profile from which it was imported. Likewise, modifying a system profile does not affect experiments that imported that system profile.

Exporting a System Profile

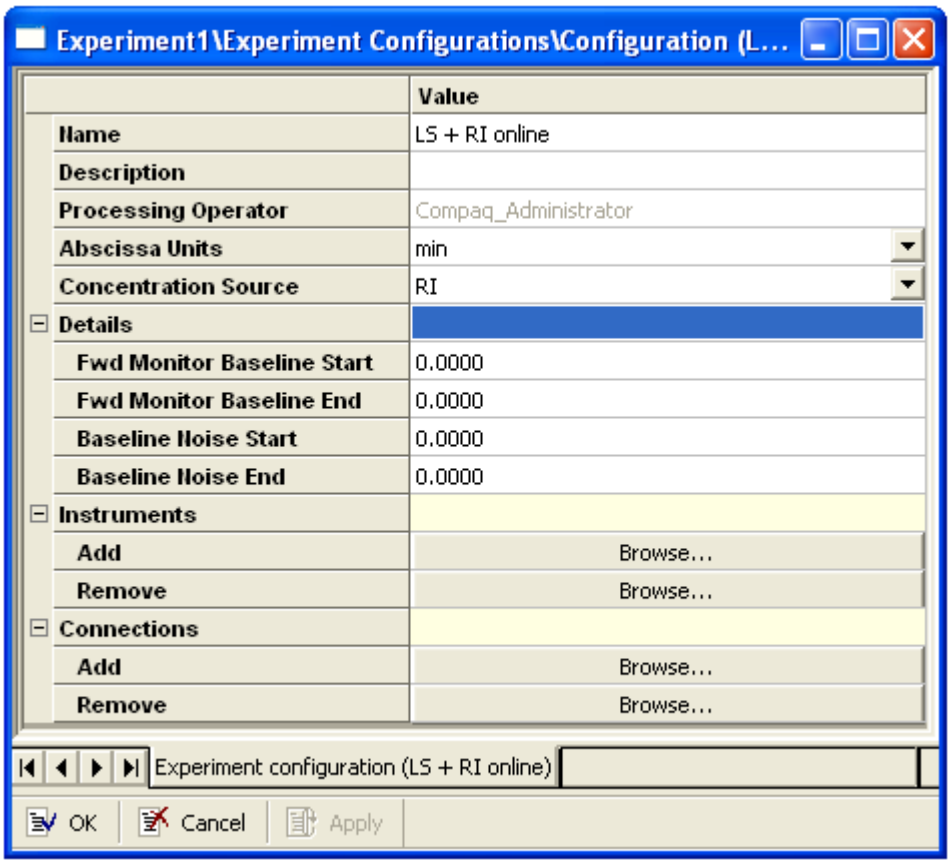
One way to create a system profile is to export items from an experiment. To do this, follow these steps:

1. If you have more than one experiment open, make sure the one you want to export from is selected in the experiment tree of the workspace.
2. Select the item in the configuration you want to export. (Any items nested at a lower level will be exported along with the item you select. For example, in the following figure, exporting the injector creates a system profile that contains the injector and the sample. If you export the configuration item, the entire configuration is saved as a system profile.)
3. Choose **Experiment→Configuration→Save Configuration As**. Or right-click on an item and choose the **Save As** item from its right-click menu.
4. In the Save As dialog, choose the folder where you want to save the system profile. Then type a name for the profile you are creating, and click **OK**.

See Chapter 12, "Working with System Profiles" for more about using a profile you export.

Experiment Configuration

An *experiment configuration* groups together all the profile components used in a particular experiment. For information about creating a new experiment and the associated configuration, see “Creating New Experiments” on page 6-4.



You can set the following properties for an experiment configuration:

Table 7-1: Experiment Configuration Properties

Field	Description
Name	Name of the experiment configuration. Make this name brief enough to be easily selected from your list of experiment configurations.
Description	Description of the experiment configuration, which typically contains more information than the Name.
Processing Operator	The current user. This changes each time you load the experiment. It is the source for the Processing Operator field in reports. In ASTRA V Basic, you can edit this field to show the name you want listed in a report. See “Operator Names in Reports” on page 10-2 for details.
Abscissa Units	The x axis units for display. The default units are milliliters for an online (flow) experiment and minutes for a batch experiment. Also available are milliseconds, seconds, and hours. This setting affects the units for a number of fields in the experiment procedures.

Table 7-1: Experiment Configuration Properties

Field	Description
Concentration Source	Select the source of concentration data you want to use in this experiment. The list shows concentration sources, such as RI and UV, currently in your experiment configuration (if any).
Details->Fwd Monitor Baseline Start / End	If you selected “Forward Monitor” in the Divide by Laser Monitor field of the configuration for your light scattering instrument (see page 7-11), you should specify the start and end points for a region that corresponds to pure solvent. This pure solvent region acts as a baseline for the forward laser monitor signal. If you do not specify a region, the average of the forward laser monitor signals for the first 10% of the collected data is assumed to be the default "pure solvent" range for calculating the average forward monitor signal.
Details->Baseline Noise Region Start / End	Specify the start and end points for a region to be used for the baseline noise computation. If you do not specify a region, the first and last 10% of the run are used to assess baseline noise. These fields allow you to override the default if there are artifacts in these regions.
Add Instruments	Click **Browse** to select an instrument profile to add to the experiment. This property and the ones that follow are available only in Experiment Builder Mode.
Remove Instruments	Click **Browse** to select an instrument profile to remove from the experiment.
Add Connections	Click **Browse** to select a connection profile to add to the experiment.
Remove Connections	Click **Browse** to select a connection profile to remove from the experiment.

Experiment Builder Buttons to add and remove instruments and connections are visible only in Experiment Builder mode, which you enable by choosing **System→Preferences→Experiment Builder Mode**. If you have already opened the Configuration properties dialog, close and reopen it after enabling Experiment Builder mode.

Light Scattering Instrument Profiles

An *instrument* is any hardware device used in an experiment. Light scattering instruments measure the molar mass, RMS radius, and second virial coefficient of a sample via Rayleigh scattering.

DAWN HELEOS and DAWN HELEOS 8 Profiles

The DAWN HELEOS is the default light scattering instrument in most light scattering experiment templates. You can set the following properties for a DAWN HELEOS or DAWN HELEOS 8 instrument:

Table 7-2: DAWN HELEOS Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." on the far right, and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose "Browse..." to open the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-10.)
Sample Cell	Select the type of sample cell used during data collection. The options are: K5, F2, Scintillation Vial, MicroCuvette, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value (1/(V cm)). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See "LS Calibration" on page 8-19 for a way to determine this value.
Normalization Coefficients>1-18 (1-8 for HELEOS 8)	Type the normalization coefficients for the detectors or use the normalization procedure (see page 8-32) to set these values. Detector 11 always has a normalization coefficient of 1. (This is Detector 5 on a HELEOS 8.) Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the **Import** button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the DAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.

Table 7-2: DAWN HELEOS Properties

Field	Description
Divide by Laser Monitor	Select the laser monitor option you want to use. The options are “Laser Monitor”, “Forward Monitor”, and “none”. -- “Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. -- “Forward Monitor”: The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- “none”: No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. No additional wiring is required for the DAWN HELEOS or DAWN HELEOS 8 to collect forward laser monitor data.
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the DAWN collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the DAWN hardware manual for details.
QELS>Option	Check this box if the DAWN instrument has a detector replaced with a QELS fiber.
QELS>Replaced Detector	If QELS is enabled, type the number of the detector replaced for the QELS fiber.
Band Broadening>Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

DAWN EOS, DAWN DSP, DAWN DSP-F, and DAWN 8 Profiles

The properties that may be defined for a DAWN EOS, DAWN DSP, DAWN DSP-F, and DAWN 8 are identical.

You can set the following properties for a DAWN instrument:

Table 7-3: DAWN Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click “...” on the far right, and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose “Browse...” to open the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-10.)
Sample Cell	Type of sample cell used during data collection. The options are: K5, F2, Scintillation Vial, MicroCuvette, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)

Table 7-3: DAWN Instrument Properties

Field	Description
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value (1/(V cm)). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See “LS Calibration” on page 8-19 for a way to determine this value.
Normalization Coefficients>1-18 (1-8 for DAWN 8)	Type the normalization coefficients for the detectors or use the normalization procedure (see page 8-32) to set these values. Detector 11 always has a normalization coefficient of 1. (This is Detector 5 on a DAWN 8.) Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the **Import** button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the DAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-8.
Divide by Laser Monitor	Select the laser monitor option you want to use. The options are “Laser Monitor”, “Forward Monitor”, and “none”. -- “Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. -- “Forward Monitor”: The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- “none”: No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. To use the Forward Monitor, you must route the instrument’s forward laser monitor signal through one of the AUX inputs on the instrument.
Fwd Monitor Aux Channel	If you selected “Forward Monitor” in the field above, specify which AUX input on the instrument receives the forward laser monitor signal.
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the DAWN collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the DAWN hardware manual for details. (Not available for the DAWN 8.)
QELS>Option	Check this box if the DAWN instrument has a detector replaced with a QELS fiber.
QELS>Replaced Detector	If QELS is enabled, type the number of the detector replaced for the QELS fiber.
Band Broadening>Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

Table 7-3: DAWN Instrument Properties

Field	Description
Temperature Control> Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).
Temperature Control> Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Temp Controlled Line> Enable	Check this box if the plumbing line in the instrument is set to maintain a specified temperature (heated or cooled).
Temp Controlled Line> Temperature	If the line is temperature controlled, specify the temperature to which it is set. Use °C.
Detector Amp Gains>1-18 (1-8 for DAWN 8)	Select the detectors gains to use for each laser detector. The options are 1, 21, and 101.
Auxiliary Channel Gains> 1-2	Select the auxiliary channel gains for channels 1 and 2. The options are 1, 10, 100, and 1000.

miniDAWN and miniDAWN TREOS Profiles

You can set the following properties for a miniDAWN or miniDAWN TREOS instrument:

Table 7-4: miniDAWN and miniDAWN TREOS Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose "Browse..." to open the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-10.)
Sample Cell	Type of sample cell used during data collection. The options for the miniDAWN are: K5, F2, and MicroCuvette. The options for the miniDAWN TREOS are: K5, F2, Scintillation Vial, MicroCuvette, and Magic glass.
Wavelength	The wavelength of the laser that produces scattered light from the sample cell and its contents. (nm)
Calibration Constant	Type the Instrument Specific Calibration Constant (ISCC) value (1/(V cm)). Light scattering instruments use the ISCC in the computation of the Configuration Specific Calibration Constant (CSCC). See "LS Calibration" on page 8-19 for a way to determine this value.
Normalization Coefficients (1-3)	Type the normalization coefficients for the detectors or use the normalization procedure to set these values. Detector 2 always has a normalization coefficient of 1. Normalization is the process by which each detector signal is related to the 90° detector signal and the Instrument Specific Calibration Constant. Click the **Import** button to import normalization coefficients from an open experiment.
Comet Cell Cleaner	Check this box if a COMET cell cleaner is to be used with the miniDAWN instrument. Please see the COMET hardware manual for more information about the COMET cell cleaner.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.

Table 7-4: miniDAWN and miniDAWN TREOS Instrument Properties

Field	Description
Divide by Laser Monitor	Select the laser monitor option you want to use. The options are “Laser Monitor”, “Forward Monitor”, and “none”. -- “Laser Monitor”: The light scattering signals are divided by the laser monitor, which corrects for fluctuations in the laser intensity due to power fluctuations. -- “Forward Monitor”: The light scattering signals are divided by the forward laser monitor, which corrects for both laser intensity fluctuations and absorbance by the sample. -- “none”: No correction is performed for laser intensity fluctuations or sample absorbance. Use this option only if signal levels are so low that digital noise from the laser monitor signal can contaminate data. To use the Forward Monitor with the miniDAWN (but not the miniDAWN TREOS), you must route the instrument’s forward laser monitor signal through one of the AUX inputs on the instrument. No additional wiring is required for the miniDAWN TREOS to collect forward laser monitor data.
Fwd Monitor Aux Channel	If you selected “Forward Monitor” in the field above, specify which AUX input on the instrument receives the forward laser monitor signal. (Used for the miniDAWN only.)
Disable Collection	Check this box to disable data collection for this instrument. For example, if the light scattering instrument has the QELS option, it is possible to disable the collection and collect QELS data alone.
Polarization Analyzer	Check this box if the polarization option is currently installed on the instrument. See the hardware manual for details. (Available for the miniDAWN TREOS only.)
Temperature Control> Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled). (Available for the miniDAWN only.)
Temperature Control> Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C. (Available for the miniDAWN only.)
Band Broadening>Enabled	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.
Auxiliary Channel Gains> 1-2	Select the auxiliary channel gains for channels 1 and 2. The options are 1, 10, 100, and 1000. (Available for the miniDAWN only.)

WyattQELS Profiles

A WyattQELS device is a Quasi-Elastic Light Scattering device. You can set the following properties for a WyattQELS instrument:

Table 7-5: WyattQELS Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.

Table 7-5: WyattQELS Instrument Properties

Field	Description
Physical Instrument	Choose an instrument from the drop-down list. If your instrument is not listed, choose “Browse...” to open the Instruments dialog. (See “Accessing and Viewing Hardware” on page 2-10.)
Use QELS Temperature Probe	Check this box if the instrument is set to use a temperature probe signal during collection. Uncheck this box if you have a temperature-controlled DAWN or miniDAWN. If this box is unchecked, the temperature is taken from the temperature set for the DAWN or miniDAWN instrument.
Model	The instrument model. Options are: Wyatt QELS, Flex 99, and Flex 99 ADN.

Refractive Index Instrument Profiles

A *refractive index instrument* measures the differential refractive index (dRI) of a solution in order to calculate the concentration of the sample. In order to calculate the concentration from the differential refractive index, it is necessary to know the dn/dc value for the sample.

Optilab rEX Profiles

You can set the following properties for a Optilab rEX instrument:

Table 7-6: Optilab rEX Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Click "..." and select from the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-10.)
Wavelength	The wavelength of the light used in the instrument. (nm)
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See "Band Broadening" on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See "Band Broadening" on page 8-14 for an explanation of the mixing term. The units are in microliters.

For Optilab rEX users, there are several utility templates in the **System Templates->RI Measurement->Optilab rEX Specific** folder. These experiment templates include "Purge On", "Purge Off", and "Zero dRI". We recommend that you purge the Optilab rEX when not running samples; the "Purge On" template is a convenient way to automate this as part of a sample set. In addition, Optilab rEX templates for absolute RI calibration and RI calibration from a peak are included in this folder.

The purge valves on Optilab rEX instruments are automatically closed at the start of data collection. The exception to this is when absolute RI analysis is conducted, where the Optilab rEX purge valve must be left open.

Optilab DSP Profiles

You do not select a Physical Instrument for the Optilab DSP because ASTRA V does not support a direct data connection to this instrument. When using the Optilab DSP, it is necessary to add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the Optilab DSP signal. See “AUX Connection Profiles” on page 7-29 for details.

You can set the following properties for a Optilab DSP instrument:

Table 7-7: Optilab DSP Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell	Type of sample cell used during data collection. The options are: P2, P2L, P10, P100, P10L, P20, P12, and ENGRCELL.
Temperature	If this instrument is temperature controlled, specify the temperature to which it is set.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

Optilab 903 Profiles

You do not select a Physical Instrument for the Optilab 903 because ASTRA V does not support a direct data connection to this instrument. When using the Optilab 903, it is necessary to add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the Optilab 903 signal. See “AUX Connection Profiles” on page 7-29 for details.

You can set the following properties for a Optilab 903 instrument:

Table 7-8: Optilab 903 Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell	Type of sample cell used during data collection. The options are: P2, P2L, P10, P100, P10L, P20, P12, and ENGRCELL.
Scale	The scale corresponds to the scale selected on the Optilab 903 instrument. Possible values are 2, 5, 10, 20, 50, and 100.
Offset	The offset voltage is determined during the Optilab 903 setup procedure. Please see the Optilab 903 hardware manual for instructions on determining the offset.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See "Band Broadening" on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See "Band Broadening" on page 8-14 for an explanation of the mixing term. The units are in microliters.

Generic RI Instrument Profiles

You can create a Generic RI Instrument profile for any third-party refractive index instrument for which data is collected through the AUX input of another instrument.

You do not select a Physical Instrument for a Generic RI Instrument profile because ASTRA V does not support a direct data connection to such instruments. Instead, add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the signal. See "AUX Connection Profiles" on page 7-29 for details.

You can set the following properties for a generic RI instrument:

Table 7-9: Generic RI Instrument Properties

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Temperature Control> Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).

Table 7-9: Generic RI Instrument Properties

Field	Description
Temperature Control> Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

Viscometry Instrument Profiles

A *viscometer* measures the specific viscometry of a solution.

Wyatt's ViscoStar measures specific viscosity. When combined with concentration data from an RI or UV concentration detector, specific viscosity can be used to calculate intrinsic viscosity. Intrinsic viscosity, in turn, combined with data from light scattering measurements, can be used to derive the hydrodynamic radius (r_h) and molecular shape information.

ViscoStar Profiles

You can set the following properties for a ViscoStar instrument:

Table 7-10: ViscoStar Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Physical Instrument	Click "..." and select from the Instruments dialog. (See "Accessing and Viewing Hardware" on page 2-10.)
Dilution Factor	If you are using a UV detector plumbed before the ViscoStar in the flow sequence, use the default dilution factor of 1.00. If you are using an RI detector (or any other instrument) plumbed after the ViscoStar, see "Measuring the Dilution Factor" on page 7-22 to determine the value to enter here.
Capillary Volume	The internal capillary volume of the ViscoStar instrument. This value is used to correct for certain types of mixing effects. This does not include the volume of the adjustable reservoir. You can compute the internal capillary volume using the provided experiment template.
Specific Viscosity Mode	If you are not using a concentration detector with the ViscoStar, check the Specific Viscosity Mode box. Intrinsic viscosity can only be calculated using both specific viscosity and concentration data.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See "Band Broadening" on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See "Band Broadening" on page 8-14 for an explanation of the mixing term. The units are in microliters.

The purge valves on ViscoStar instruments are automatically closed at the start of data collection.

Measuring the Dilution Factor

If an RI detector (or other instrument) is plumbed after the ViscoStar in the flow sequence, the sample exiting the ViscoStar is diluted by approximately a factor of 2. Therefore the RI detector does not measure the same concentrations that flowed through the LS and ViscoStar instruments. To correct for this, you should measure the dilution factor experimentally.

To measure the dilution factor, use a sample that is known to elute 100%. The detailed report shows the resulting Dilution Factor, which you can enter in the ViscoStar or Generic Viscometer profile.

You should check the dilution factor occasionally, since it will change over time as samples that coat the tubing slowly build up.

To learn more, see the "Measuring the System Dilution Factor" section in the ViscoStar User's Guide.

Generic Viscometer Profiles

You can create a Generic Viscometer profile for any high-temperature third-party viscometer for which data is collected through the AUX input of another instrument.

To create a generic viscometer profile, choose **File->New->System Profile** and select Generic Viscometer and click OK. Name your viscometer profile. Then, you can double-click the viscometer profile in the System Profiles tab to set its properties.

You can set the following properties for a generic viscometer:

Table 7-11: Generic Viscometer Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Dilution Factor	If you are using a UV detector plumbed before the ViscoStar in the flow sequence, use the default dilution factor of 1.00. If you are using an RI detector (or any other instrument) plumbed after the ViscoStar, see "Measuring the Dilution Factor" on page 7-22 to determine the value to enter here.
AUX input mode	Specify the data provided by the AUX input from the viscometer. The options are "differential and inlet pressure" (default), "specific viscosity", and "differential pressure alone". The Waters viscometer can be set to provide either of the first two types of data; other viscometers may provide differential data pressure alone.
Fixed IP (psi)	If you chose "differential pressure alone", specify the fixed inlet pressure for the viscometer in psi.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see "Batch Mode vs. Online Mode" on page 1-8.

Table 7-11: Generic Viscometer Profile Fields

Field	Description
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

UV Absorption Instrument Profiles

A *UV absorption instrument* measures the absorbance of a sample in the ultra-violet region of the spectrum. The absorbance can be converted to a concentration if the cell length of the UV absorption instrument is known and if the UV extinction coefficient for the sample is known.

Generic UV Detector Profiles

You can create a Generic UV Instrument profile for any third-party UV instrument for which data is collected through the AUX input of another instrument.

You do not select a Physical Instrument for a Generic UV Instrument profile because ASTRA V does not support a direct data connection to such instruments. Instead, add an AUX connection to the experiment configuration to indicate which AUX channel and instrument are to be used to read the signal. See “AUX Connection Profiles” on page 7-29 for details.

You can set the following properties for a generic UV instrument:

Table 7-12: Generic UV Instrument Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click “...” and select a profile to use.
Description	Description of the instrument, which typically contains more information than the Name.
Wavelength	The wavelength of the light used in the instrument. (nm)
Cell Length	The length of the sample cell in cm.
UV Response Factor	The conversion factor from absorbance units (AU) to volts for the UV aux output. Please see the hardware manual for the UV detector to determine this value.
Temperature Control> Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).
Temperature Control> Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.
Batch Mode	Check this box if the instrument is to be used in batch mode. Checking this box associates a single sample and solvent configuration with the instrument configuration. For a description of the difference between batch mode and flow mode, see “Batch Mode vs. Online Mode” on page 1-8.
Band Broadening>Enable	Check this box to enable band broadening. This box should be checked only if valid instrumental and mixing terms are entered for the band broadening parameters. These parameters are usually determined by running the Band Broadening procedure (see page 8-14). If band broadening has been enabled, you can disable it using this check box.
Band Broadening> Instrumental Term	See “Band Broadening” on page 8-14 for an explanation of the instrumental term. The units are in microliters.
Band Broadening> Mixing Term	See “Band Broadening” on page 8-14 for an explanation of the mixing term. The units are in microliters.

Injector Profiles

An *injector* consists of an injection loop that injects the sample into the flowing solvent or mobile phase stream from the pump. ASTRA supports both manual and autoinjectors, each of which may provide an auto-inject signal from which data collection can be triggered.

You can set the following properties for an injector:

Table 7-13: Injector Profile Fields

Field	Description
Name	Name of the instrument. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the injector, which typically contains more information than the Name.
Injected Volume (mL)	The volume of the solution injected in milliliters. This is the same as the sample loop volume.

An injector configuration always has a sample configuration associated with it in a profile. See "Sample Profiles" on page 7-30.

If the injector provides an auto-inject signal, you should also have an autoinjector connection in your configuration as described in "Autoinjector Connection Profiles" on page 7-29.

Pump Profiles

Pumps move the mobile phase or solvent through the experimental apparatus. Therefore, a pump has a solvent profile associated with it. When setting up a pump profile, you select an available solvent profile to associate with the pump.

ASTRA supports profiles for generic third-party pumps. You can set the following properties for a generic pump:

Table 7-14: Generic Pump Profile Fields

Field	Description
Name	Name of the pump. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the pump, which typically contains more information than the Name.
Flow Rate	The rate at which the pump runs in mL/min.

A pump configuration always has a solvent configuration associated with it in a profile. See "Solvent Profiles" on page 7-32.

Column Profiles

Columns are used in size exclusion chromatography (SEC) to fractionate a mixture of polymer sizes.

In ASTRA, both conventional and universal column calibration can be performed. In conventional calibration, the analyzed polymer is the same as the polymer used for calibration. In universal calibration, the polymers may be different. Universal calibration requires either a viscometer (and concentration detector) or known values for the Mark-Houwink-Sakurada K and α coefficients. The dn/dc value is required for universal calibration (as it is necessary for intrinsic viscosity calculations), but not for conventional calibration.

ASTRA lets you store a profile for a generic column that contains coefficients obtained from column calibration experiments (see page 8-37). You can set the following properties for a generic column:

Table 7-15: Generic Column Profile Fields

Field	Description
Name	Name of the column. If you have already created a system profile for this instrument, click "..." and select a profile to use.
Description	Description of the column, which typically contains more information than the Name.
Plate Count	The column manufacturer provides the initial plate count as documentation, but this value changes over time. You may enter the current plate count here as documentation when you perform an experiment. The plate count is sometimes called "Efficiency". It quantifies the separating efficiency of the column in terms of the "number of theoretical plates (N)". The specific calculation varies by column manufacturer, but generally measures how well the column is packed and its kinetic performance. In general, higher plate counts indicate more efficient columns. More efficient columns yield narrower peaks than less efficient ones.
Asymmetry Factor	The column manufacturer provides the initial asymmetry factor as documentation, but this value changes over time. You may enter the current asymmetry factor here as documentation when you perform an experiment. The asymmetry factor describes the shape of peaks generated by the column. The distance between the elution volume at the peak apex (V_a) and the front of the chromatogram at 10% of the peak apex (V_f) is divided by the distance between V_a and the backside of the chromatogram at 10% of the peak apex (V_b). A value greater than one indicates a "tailing" peak, in which the bulk of material elutes after the apex. Likewise, a value less than one indicates a "leading" peak in which the bulk of the material elutes prior to the apex.
Resolution	The column manufacturer provides the initial resolution as documentation, but this value changes over time. You may enter the current resolution here as documentation when you perform an experiment. The resolution quantifies the ability of a column to separate different species. This is typically measured for a column by injecting two different species into the column, and then measuring the distance between the peaks and the peak widths. The relation is $R_s = 2(V_2 - V_1)/(W_2 + W_1)$, where V is the elution volume for each species and W is the width of each peak at the baseline.

Table 7-15: Generic Column Profile Fields

Field	Description
Calibration Technique	The type of column calibration performed. The options are: none, Conventional, Universal with Viscometer Data, and Universal without Viscometer Data.
Flow Marker	The elution volume of the flow marker, which is used when combining peak data from multiple experiments. If zero, the flow marker correction is not used.
Mark-Houwink-Sakurada K	The known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This is only used if you select the Universal without Viscometer Data calibration technique. In this case, the equation used is: $[\eta] = K M^{a}$
Mark-Houwink-Sakurada a	The known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This is only used if you select the Universal without Viscometer Data calibration technique.
Conventional Calibration Function	Expanding this row shows the A_i coefficients of the following linear regression equation, where M is the polymer molar mass and V is the elution volume: $\log(M) = A_0 + A_1 V + A_2 V^2 + A_3 V^3 + \ldots$ Note that a column profile can contain results both for a conventional and universal calibration.
Universal Calibration Function	Expanding this row shows the A_i coefficients of the following linear regression equation, where M is the polymer molar mass, η is the intrinsic viscosity, and V is the elution volume: $\log(M[\eta]) = A_0 + A_1 V + A_2 V^2 + A_3 V^3 + \ldots$ Note that a column profile can contain results both for a conventional and universal calibration.

Connection Profiles

A *connection* is an interface between two instruments. There are three types of connections:

- A *fluid connection* represents a piece of physical tubing that routes the solution from one instrument to the next.
- An *AUX connection* represents a physical wire from the AUX output of one instrument to the AUX input of another instrument.
- An *auto-inject connection* represents a physical connection between the auto-inject output of an injector and the auto-inject input of an instrument.

A *connection profile* stores information about a connection between two specific instruments. Connection profiles are referenced by configurations. Connections must be specified in an experiment that uses more than one type of instrument. (The WyattQELS instrument is an exception, since it is associated with a DAWN or miniDAWN instrument.)

See Figure 7-3 in "Configuration Example" on page 7-4 for a diagram that shows the typical connections in an online light scattering experiment.

Fluid Connection Profiles

A *fluid connection profile* describes a plumbing (tubing) connection through which solvent or a solution flows between instruments.

Experiment Builder Fluid connections are hidden in Run mode. To see them you must enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

You can set the following properties for a fluid connection:

Table 7-16: Fluid Connection Properties

Field	Description
Name	Name of the connection. If you have already created a system profile for this connection, click "..." and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Instrument	Select the type of source instrument. The drop-down list shows the instrument profile types that are available for a connection.
Destination Instrument	Select the type of destination instrument. The drop-down list shows the instrument profile types that are available for a connection.
Volume (mL)	The fluid volume displacement that is a result of the plumbing (tubing) between instruments. This can be set manually or determined via the alignment procedure. Typically, the volume only needs to be set for fluid connections between instruments that collect data. (mL)
Temperature Control> Enable	Check this box if the instrument is set to maintain a specified temperature (heated or cooled).
Temperature Control> Temperature	If this instrument is temperature controlled, specify the temperature to which it is set. Use °C.

AUX Connection Profiles

An *AUX connection profile* describes a connection from the analog output of the source instrument to the analog input of the destination instrument.

If there is no AUX connection and the instrument is capable of collecting data and communicating data to the PC, the instrument-to-PC connection is implied and does not require a profile.

You can set the following properties for an AUX connection:

Table 7-17: AUX Connection Properties

Field	Description
Name	Name of the connection. If you have already created a system profile for this connection, click "..." and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Instrument	Select the type of instrument that sends analog data over this connection. The drop-down list shows the instrument profile types that are available for a connection.
Destination Instrument	Select the type of instrument that receives analog data over this connection. The drop-down list shows the instrument profile types that are available for a connection.
AUX Channel	The input AUX channel number on the destination instrument.
Calibration Constant	Constant value by which the AUX signal should be scaled. This constant can be set manually or determined through one of the calibration procedures. The default value is 1.0. See "Differential RI Calibration" on page 8-21 and "UV Calibration" on page 8-31.

Autoinjector Connection Profiles

An *Autoinjector connection profile* describes a physical connection between the auto-inject output of an injector and the auto-inject input of an instrument.

You can set the following properties for an Autoinjector connection:

Table 7-18: Autoinjector Connection Properties

Field	Description
Name	Name of the connection. If you have already created a system profile for this connection, click "..." and select a profile to use.
Description	Description of the connection, which typically contains more information than the Name.
Source Instrument	Select the type of instrument that sends the auto-inject signal over this connection. The drop-down list shows the instrument profile types that are available for a connection.
Destination Instrument	Select the type of instrument that receives the auto-inject signal over this connection. The drop-down list shows the instrument profile types that are available for a connection.

Sample Profiles

A *sample* is the substance being tested. It is dissolved in the solvent, forming a solution. The solution is placed in or flows through a sample cell. A sample may be injected or may be a molecular standard used as a reference standard.

A *sample profile* stores information about samples to be used in experiments. Sample profiles are used by injector profiles and by instruments configured to run in batch (that is, standalone) mode.

Sample Profiles

A *sample* profile describes a sample for which you are determining properties.

You can set the following properties for a sample:

Table 7-19: Sample Properties

Field	Description
Name	Name of the sample. If you have already created a system profile for this sample, click "..." and select a profile to use.
Description	Description of the sample, which typically contains more information than the Name.
dn/dc	dn/dc value associated with the sample in mL/g. The dn/dc value is used when the sample concentration is to be determined using a refractive index instrument. The value entered for the profile is used as a default value when peaks are set for the data.
A2	Second viral coefficient (A^2) value associated with the sample in mol mL/g^2. The value set here is used as a default value for peaks set in the experiment.
UV Extinction Coefficient	The extinction coefficient in mL/(g cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument. The value entered here is used as a default value when peaks are set for the data.
Concentration	The concentration of the sample in g/mL.

An injector configuration always has a sample configuration associated with it in a profile. See "Injector Profiles" on page 7-25.

An autoinjector configuration always has a configuration for a sample associated with it in a profile. The properties tab for samples has a table with a row of the properties in Table 7-19 for each sample well. See "Autoinjector Connection Profiles" on page 7-29.

Molecular Standard Profiles

A *molecular standard* profile describes a commonly used sample—such as BSA monomer—that has well-known properties. Such profiles are used as reference standards for processes such as normalization with a light scattering instrument.

Molecular standard profiles are associated with a peak in the data. The values set for the molecular standard profile will be used in the peak.

You can set the following properties for a molecular standard:

Table 7-20: Molecular Standard Properties

Field	Description
Name	Name of the standard. If you have already created a system profile for this sample, click "..." and select a profile to use.
Description	Description of the standard, which typically contains more information than the Name.
Reference Wavelength	The wavelength at which the dn/dc or UV extinction value is accurate. (nm)
dn/dc	dn/dc value associated with the sample. (mL/g)
A2	Second viral coefficient (A^2) value associated with the sample. (mol mL/g^2)
Molar Mass	Molar mass value associated with the sample. (g/mol)
Intrinsic Viscosity	A measure of the capability of a polymer in solution to enhance the viscosity of the solution. Derived using specific viscosity and concentration data.
Radius>Type	Type of radius specified. May be RMS, Hydrodynamic, or Geometric.
Radius>Value	Radius value associated with the sample. (nm)
UV Extinction Coefficient	The extinction coefficient in mL/(g cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument.

Solvent Profiles

A *solvent* is a substance in which another substance is dissolved, forming a solution. The solution is placed in or flows through a sample cell.

A *solvent profile* stores information about solvents used in experiments, such as toluene. Profiles for common solvents (water, toluene, THF, etc.) are supplied with ASTRA. You cannot change the values in existing solvent profiles. You can build and modify custom profiles for any other solvents you use.

You can set the following properties for a custom solvent:

Table 7-21: Solvent Properties

Field	Description
Name	Name of solvent profile. Typically, this is the name of the chemical. You can click "..." to choose from a list of common solvents in the System Solvents folder or solvent configurations you have saved as system profiles.
Description	Description of the solvent, which may show more information than the Name.
Refractive Index	Displays the computed refractive index of the solvent at the wavelength used in the experiment. This property is shown only if this profile is part of an experiment configuration (as opposed to when you are editing a system profile).
Viscosity	Displays the computed viscosity of the solvent at the temperature used in the experiment. This property is shown only if this profile is part of an experiment configuration.
Rayleigh Ratio	Displays the computed Rayleigh ratio of the solvent at the wavelength used in the experiment. This property is shown only if this profile is part of an experiment configuration.
Refractive Index Model	The model used to specify the refractive index. May be **Fixed** or **Polynomial**. Set the model type before setting the parameters required for that model. • If **Fixed**, specify the Reference Refractive Index. • If **Polynomial**, specify the Refractive Index Model Parameters. These are used to compute the Refractive Index.
Refractive Index Model> Reference Refractive Index	Specify the refractive index of the solvent at the wavelength and temperature you will use. This property is required for both Fixed and Polynomial models.
Refractive Index Model> Parameters 2-5	If the Refractive Index model is **Polynomial**, set Parameters 2-5 using the following polynomial model, which is used to compute the Refractive Index. $$n(L) = P_1 + \frac{P_2}{L^2} + \frac{P_3}{L^4} + \frac{P_4}{L^6} - P_5 P_6$$ • P_1 is the reference refractive index. • P_2 through P_5 are solvent-specific constants. • L is the wavelength in micrometers (µm) of the laser in the light scattering instrument. This is taken from the light scattering instrument profile as part of the configuration, so it does not need to be specified here.
Refractive Index Model> Reference Temp.	If the Refractive Index model is **Polynomial**, set the Reference temperature (P_6 in the previous equation) (°C) for which this fit is valid.
Rayleigh Ratio Model	The model used to specify the Rayleigh ratio of the solvent. May be **Fixed** or **Corrected Lambda^4**. Set the model type before setting the parameters required for that model.

Table 7-21: Solvent Properties

Field	Description
Rayleigh Ratio Model> Reference Rayleigh Ratio	If the Rayleigh Ratio Model is **Fixed**, specify the Rayleigh ratio of the solvent in 1 / (cm) at the wavelength and temperature you will use. If the Rayleigh Ratio Model is **Corrected Lambda^4**, specify the Rayleigh ratio of the solvent at the reference wavelength in 1 / (cm).
Rayleigh Ratio Model> Reference Wavelength	If the Rayleigh Ratio Model is **Corrected Lambda^4**, set the reference wavelength in µm. R(θ) is the calculated solvent Rayleigh ratio using this formula: $$R(\theta) = P_1 \times \left(\frac{P_2}{L}\right)^4 \times \left(\frac{n_L}{n_{L_0}}\right)^2 \times \left(\frac{(n_L - 1)}{(n_{L_0} - 1)}\right)^2$$ • P_1 is the Reference Rayleigh Ratio of the solvent • P_2 is the Reference Wavelength in µm • L is the wavelength in µm of the laser in the light scattering instrument. This is taken from the light scattering instrument profile as part of the configuration, so it does not need to be specified here. • n_L is the refractive index of the solvent at the wavelength of the laser in the light scattering instrument. This is calculated using the formula specified for the Polynomial Refractive Index Model. • n_{L0} is the refractive index of the solvent at P_2 (the reference wavelength). This is calculated using the formula specified for the Polynomial Refractive Index Model.
Viscosity Model	The model used to specify the viscosity of the solvent. May be **Fixed**, **Linear**, or **Exponential**. Set the model type before setting the parameters required for that model.
Viscosity Model> Reference Viscosity	If the Viscosity Model is **Fixed**, specify the viscosity of the solvent in P (Poise) at the temperature you will use. If the Viscosity Model is **Linear** or **Exponential**, specify the viscosity of the solvent in P (Poise) at the reference temperature.
Viscosity Model> Model Parameter 2	If the Viscosity Model is **Linear**, set this parameters using the following model, where n(T) is the viscosity as a function of temperature. $$n(T) = P_1 + P_2(T - P_3)$$ • P_1 is the viscosity in P at the reference temperature. • P_2 is linear temperature dependence of the viscosity (P/°C). • P_3 is the reference temperature for the model in °C. • T is the temperature as determined by the appropriate device's temperature probe in °C. If the Viscosity Model is **Exponential**, set this parameters using the following model, where n(T) is the viscosity as a function of temperature. $$n(T) = P_1 \exp(-P_2(T - P_3))$$ • P_1 is the viscosity in P at the reference temperature • P_2 is the exponential temperature dependence of the viscosity in 1 / °C. • P_3 is the reference temperature for the model in °C. • T is the temperature as determined by the appropriate device's temperature probe in °C.
Viscosity Model> Reference Temp.	If the Refractive Index model is **Linear** or **Exponential**, set the Reference temperature (P_3 in the previous equations) (°C) for which the reference viscosity is valid.

8 Editing Procedures

This chapter explains how to set up your experiment in ASTRA V to collect and analyze data. This is done using ASTRA V procedures.

CONTENTS **PAGE**

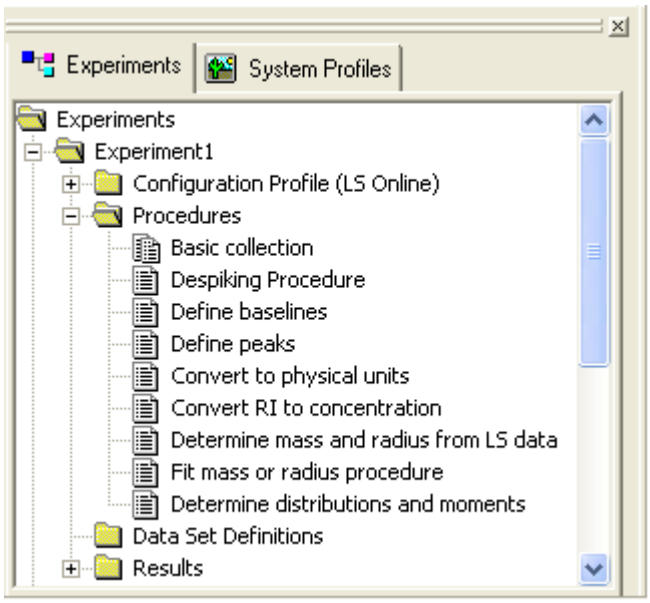
About Procedures

Data collection and analysis in an experiment are broken down into a logical sequence of units called *procedures* in ASTRA V. The procedures are performed in sequence when the experiment is run. There are configuration, collection, transformation, analysis, and administrative procedures.

Security

You must have at least Researcher access to add procedures, and at least Technician access to modify existing procedures. If you are a Guest, you have read-only access to procedures.

The Procedures node in the experiment tree shows actions ASTRA performs in sequence when you run the experiment.

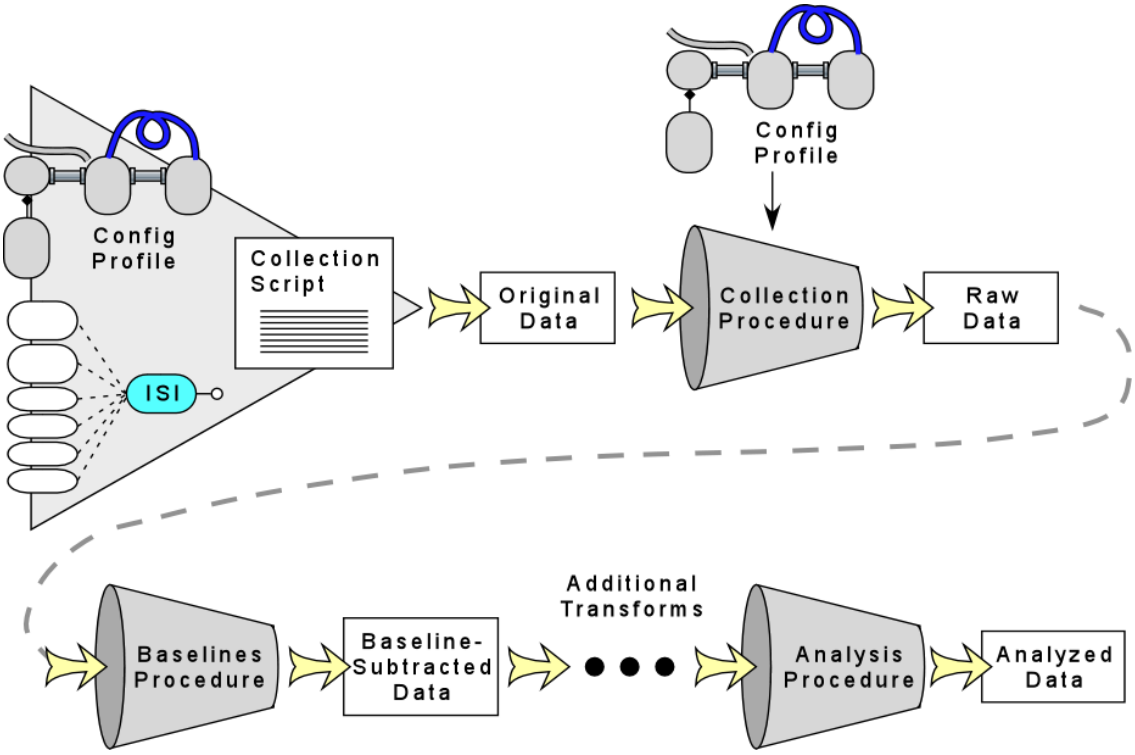


A procedure's status is indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid sequence location or does not have the necessary data to run.

About Data Processing in ASTRA

When ASTRA runs a procedure, the data is modified in the sequence specified by the set of procedures in the experiment. The following figure shows a typical sequence.



Initially, a collection procedure is responsible for gathering data through the ISI from instruments specified in the configuration. The *original data* created by the collection procedure is then forever kept with the experiment in an unmodified state. The same procedure then performs preprocessing on this original data based on the configuration to create what is called the *raw data*. For example, the original data contains the AUX channel traces from an instrument, but the raw data uses the experiment configuration to route that data to the appropriate instrument specified by the AUX connection.

After a transformation procedure runs—such as setting baselines—the data used by subsequent procedures has the transform applied. A number of transformations can be applied in sequence to the data.

After an analysis procedure runs, the experiment also contains *analyzed data* that can be displayed in reports.

Working With Procedures

Most users will not need to add, remove, or resequence procedure items. The templates provided with ASTRA V contain procedures for most common experiments.

Security

You must have at least Researcher access to add procedures, and at least Technician access to modify existing procedures. If you are a Guest, you have read-only access to procedures.

Editing Procedure Settings

To set properties of a procedure, follow these steps:

1. Double-click on a procedure in the experiment. This opens the dialog for that procedure.

   The dialog shows different types of information depending on the type of procedure. Some procedures have a graph and properties; some have only properties; some have a message that says the procedure has no user configurable parameters.

2. Set properties by typing, selecting from a list, checking a box, or clicking a "..." browse button.

   Rows shaded in yellow are read-only. You cannot change the value.

   You can expand or hide lists of related properties if there is a + or - sign next to a property name.

3. Click **Apply** or **OK** to make the changes.

In Run mode, you cannot open a dialog for a procedure unless that procedure has already been run (has the  run icon) or the procedure is the first one in the sequence that needs to be run.

In Run mode, you can open only one procedure at a time. When you open a procedure dialog, any other procedure dialog you have opened closes automatically. This prevents you from relying on information that may no longer be true due to changes in the settings for other procedures.

Certain procedures are hidden in Run mode if you do not need to interact with them. All procedures are shown in Experiment Builder mode.

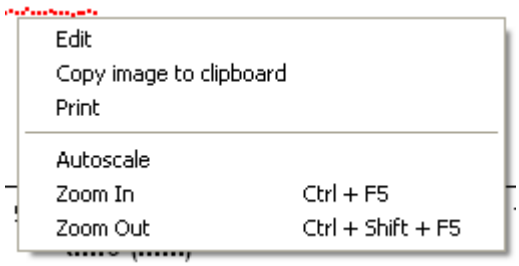
Experiment Builder

You can open any set of procedure dialogs if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**. Remember that changes to the settings for one procedure affect other procedures. The data shown for later procedures may be incorrect if you have changed earlier procedure settings.

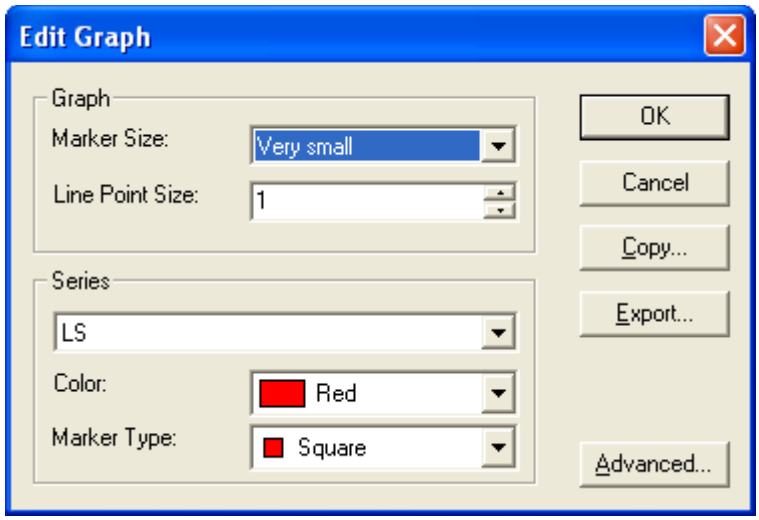
Working with Procedure Graphs

A number of procedure dialogs contain graphs. You can manipulate these graphs in the following ways:

- **Select Detectors or Data:** If there is a list of detectors or data sources above or to the right of the graph, you can use checkmarks to indicate the data you want to view. Some graphs can display multiple data sets in different colors (for example, collection). Others display only one data set at a time (for example, defining baselines).
- **Zoom In:** Hold down the Ctrl key and your left mouse button. Drag a rectangle around the data you want to view larger. Or, press Ctrl+F5 to zoom in. Press F5 to open the Scale Graph dialog.



- **Zoom Out:** Hold down the Ctrl key and click your right mouse button. Each click undoes one zoom in action. Alternately, you can press Ctrl+Shift+F5 to zoom out one level.
- **Edit:** You can modify a variety of aspects of a graph's styles by double-clicking a graph and using the Edit Graph dialog.



This dialog has the following fields:

Table 8-1: Edit Graph Fields

Field	Description
Marker Size	Choose the marker size you want to use. The options are: very small, small, medium, and large. Use Marker Size for lines made up of individual data points; use Line Point Size for fitted curves.
Line Point Size	Set the width of the line when the line is a fitted curve. Use Marker Size if the line is made up of individual data points.
Series	Choose the data series for which you want to change the color or marker type.

Table 8-1: Edit Graph Fields

Field	Description
Color	Select the line or marker color you want to use in the graph. Changing this property changes the line color for all chromatogram traces.
Marker Type	Select the marker type you want to use in the graph. The default is square.

You see the effects of your changes as you make them without closing this dialog. Click **OK** to save your changes. Graph customizations—such as line weight, color, marker style, and title changes—are not saved when you close procedure dialogs that contain graphs.

If you click **Copy**, the graph is in your "clipboard" and you can paste the graph into another program.

If you click **Export**, you can choose to save the graph in one of the following formats:

- data saved as Microsoft Excel file (.xls)
- data saved as comma-delimited text file (.csv)
- data saved as tab-delimited text file (.txt)
- data saved as tagged XML file (.xml)
- image saved as JPEG file (.jpg)

If you click **Advanced**, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

For more about modifying graph displays, see "Viewing and Modifying Graphs" on page 11-13.

When to Modify Procedures

Some procedures prompt you to perform some action, such as marking baselines, when the experiment runs. Other procedures have default values for all their properties.

For procedures other than data collection, you can easily modify the properties after the initial experiment run. Procedures not affected by your changes still show the  run icon. Procedures that need to be re-run show the  not run icon.

Then, you can re-run the experiment using the **Run** command. This time, instead of collecting data, only the procedures marked with the not run icon are performed.

Advanced Procedure Editing

The following subsections discuss advanced ways of managing procedures in an experiment.

Most users will not need to add, remove, or resequence procedure items. The templates provided with ASTRA V contain procedures for most common experiments. You may contact Wyatt Technical Support if you are having difficulty creating an experiment template for your setup.

Experiment Builder If you want to add, remove, or resequence procedures as described in this section, enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

Security You must have at least Researcher access to add, delete, or sequence procedures.

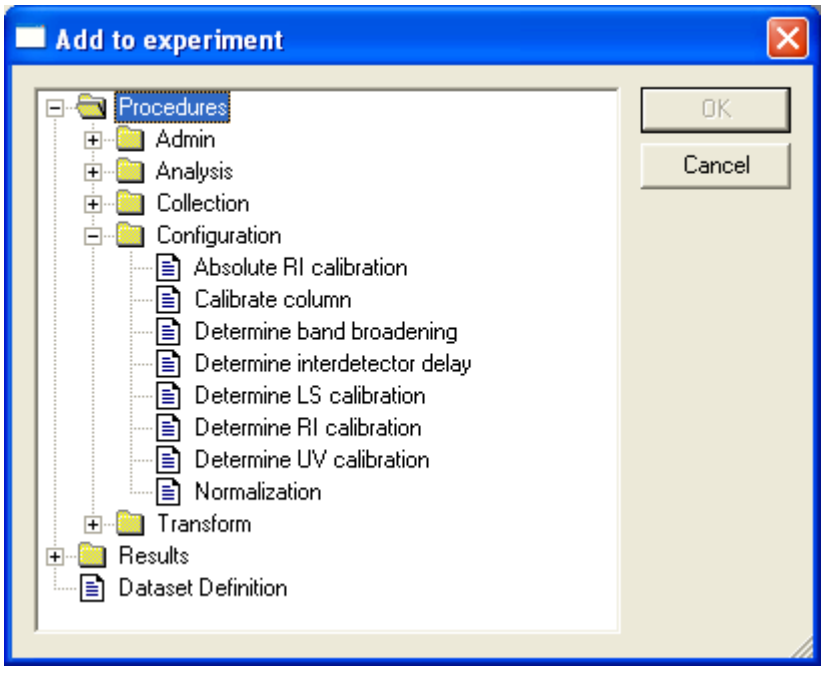
Adding Procedures

Experiment Builder You can add procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

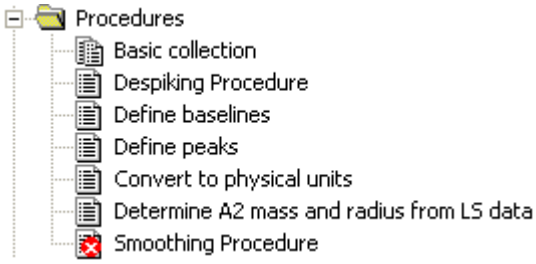
To add a procedure to an experiment, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.



2. Open a folder under Procedures and select a procedure to add.
3. Click **OK**. The procedure is added to the end of the experiment.
4. The procedure is likely to have a red X on its icon when it is placed at the end of the experiment. The red X means the procedure is in an invalid location in the sequence (or it requires data from an instrument that is not in the configuration).



   A procedure's state is always indicated by its icon, as follows. (Collection procedures have a special two-page icon for all states.)

	Procedure has not been run since the procedure was last modified.
	Procedure has been run successfully.
	Procedure is currently running.
	Procedure is in an invalid sequence location or does not have the necessary data to run.

5. Drag the procedure to a position in the sequence where its icon changes to show it is in a valid location.

Deleting Procedures

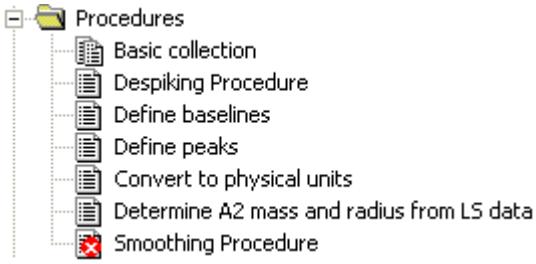
Experiment Builder You can delete procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

To delete a procedure from an experiment, select the procedure and press the **Delete** key.

Sequencing Procedures

Experiment Builder You can resequence procedure items only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

If a procedure is in an invalid location in the experiment sequence, it will have a red X on its icon.



To correct the problem, drag the procedure to a location in the sequence where its icon and the icons that follow it have no red X. See the section about the specific procedure in this chapter for information about its valid positions in an experiment.

Validating a Procedure Sequence

To check the procedure and configuration, choose **Experiment→Validate**.

Shortcuts: Right-click an experiment tree folder, and choose **Manage→Validate**.

In addition to checking for a correct procedure sequence, validation also tests to make sure instruments in the configuration are connected and available when you are getting ready to collect data. It also validates collection scripts for experiment builders. If you use the basic collection procedure, the collection script is built automatically, and validation never finds any problems with the script.

If any procedure in the sequence has a red X on its icon, it is in an invalid location in the experiment sequence or it requires data from an instrument that is not in the configuration. Modify the sequence as described in "Sequencing Procedures" on page 8-8 or revise the experiment configuration to include the appropriate instruments.

Collection Procedures

Your experiment will typically contain a collection procedure as the first procedure in the experiment sequence. The following types of collection procedures are available:

- "Basic Collection" on page 8-10
- "Script Collection" on page 8-12

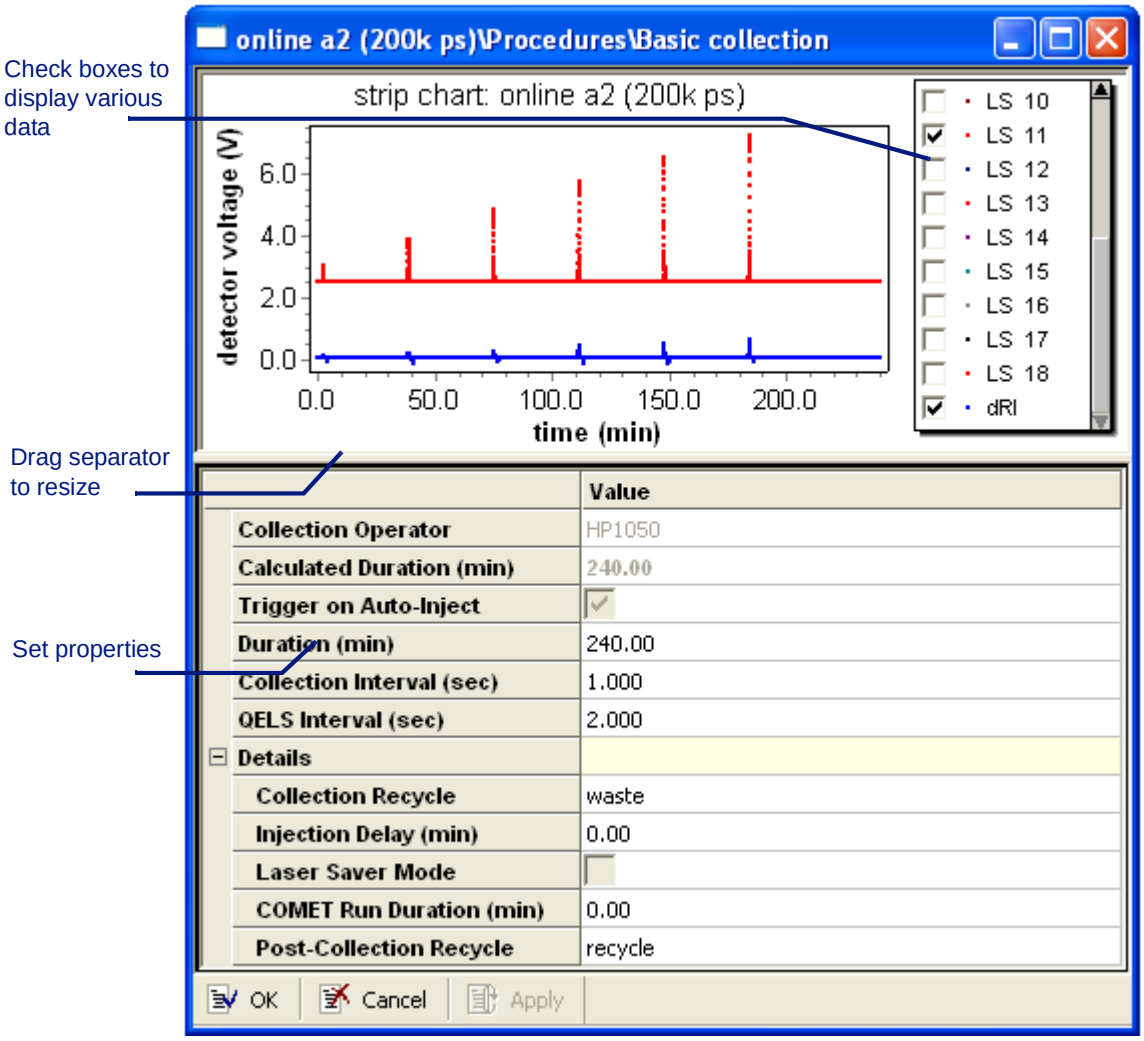
Basic Collection

The Basic collection procedure collects data from the instruments specified in the Experiment Configuration. You can set properties for this procedure before running the experiment. When you run the experiment, this procedure runs automatically, without prompting for parameters. You can use this dialog to view data as it is being collected.

If multiple instruments are collecting data, you can choose to view any set of signals in the checkboxes to the right of the graph. You may need to scroll to see some checkboxes. Signals other that light scattering (LS) are shown at the end of the list. This may include the forward laser monitor (FM) and refractive index. Light scattering data is shown in red; RI data is blue; UV data is green, QELS data is magenta, and viscosity data is black.

The x-axis of the graph uses units specified by the Abscissa Units property of the experiment configuration (see page 7-9). By default, the units are milliliters for an online experiment and minutes for a batch experiment.

The y-axis of the graph uses raw data units. For light-scattering experiments, this is in volts. The data is converted to other units by a later transformation procedure.



The properties you can set are as follows:

Table 8-2: Basic Collection Properties

Field	Description
Collection Operator	The user at the time data collection is started. See “Operator Names in Reports” on page 10-2 for details.
Calculated Duration	Shows the total duration of the collection based on the Duration, Injection Delay, and COMET Run Duration.
Trigger on Auto-Inject	Check this box if an auto-inject signal will signal the start of data collection.
Duration	The time or fluid volume for which data is to be collected. The units are determined by the Abscissa Units property of the experiment configuration.
Collection Interval (sec)	How often the light-scattering or other instrument should collect data. The default is every 0.25 seconds. This interval may be set to a multiple of 0.125 seconds. This interval is used to set the collection interval for Wyatt LS instruments, ViscoStars and/or the Optilab rEX.
QELS Interval (sec)	How often the QELS instrument (if there is one) should collect data. The default is every 2 seconds. This interval may be set to a multiple of 1 second. The maximum interval is 1 hour.

Table 8-2: Basic Collection Properties

Field	Description
Details->Collection Recycle	Controls how the Recycle valve is during the collection. This may be set to “waste” or “recycle”. The default is “waste”
Details->Injection Delay	The delay in time or fluid volume between injection and the start of data collection. The units are determined by the Abscissa Units property of the experiment configuration. The default is zero.
Details->Laser Saver Mode	Turn the light-scattering instrument’s laser off after collection is finished. If you will not collect more data for at least an hour or so after finishing this collection, it is best to turn off the laser. However, you want to be sure not to cycle the laser frequently, since this will shorten the life-span of the laser. If you are using this collection as part of a sample set, do not check the Laser Saver Mode box. Instead, use one of the System Templates->Light Scattering->Utilities->turn laser off templates as the last experiment template in the sample set.
Details->COMET Run Duration (min)	After data collection, run the COMET cell cleaner for a specified duration. You should also check the Comet Cell Cleaner box in the configuration dialog for the light-scattering instrument. See the COMET hardware manual for more information about the COMET cell cleaner.
Details->Recycle Valve Post-Collection	Controls how the Recycle valve is set at the end of the collection. This may be set to “waste” or “recycle”. During a collection, the Recycle valve is always set to “waste”.

You can change the Duration while an experiment is running. However, you cannot change the collection interval or auto-inject signals after collection has started.

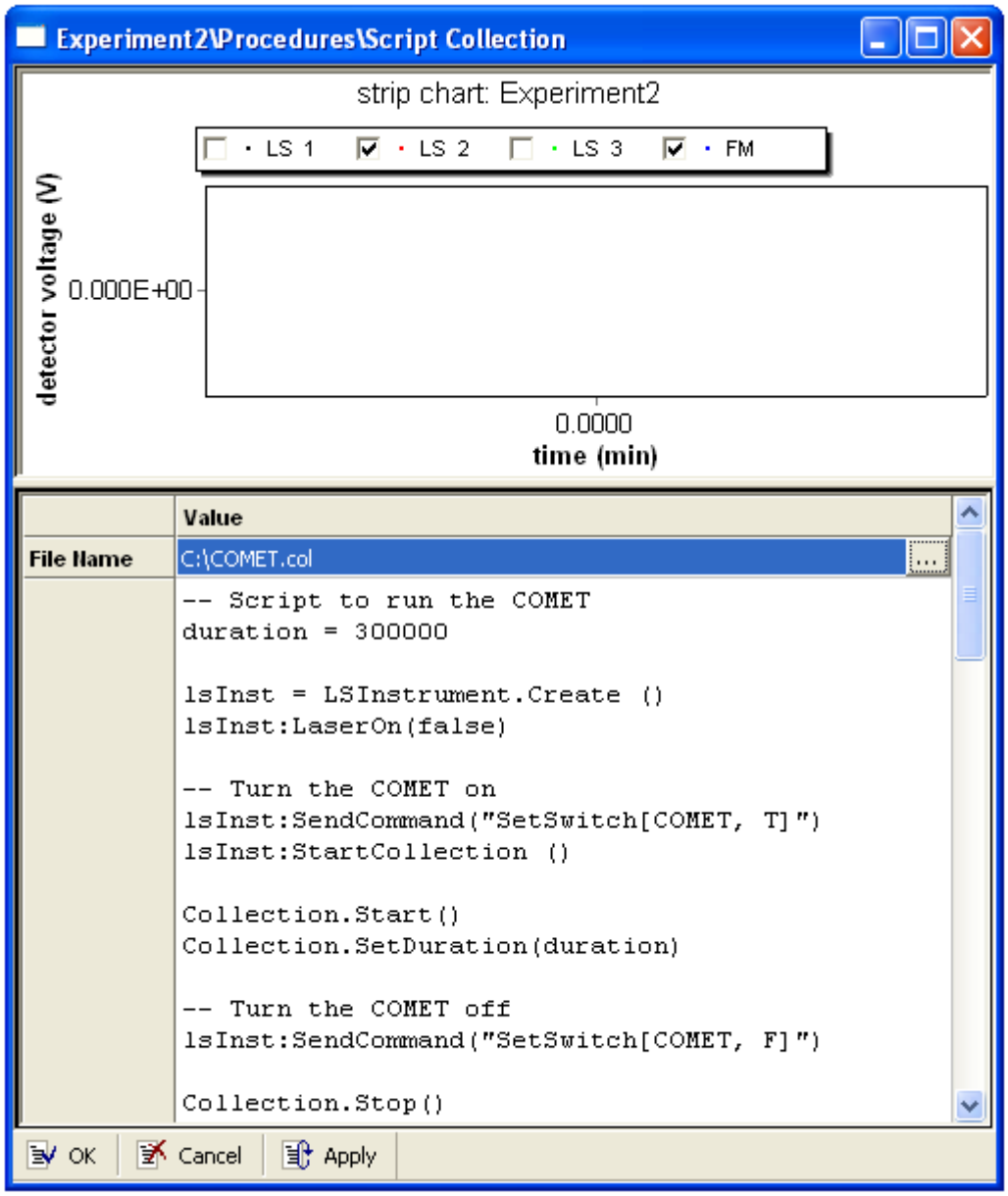
The “Basic Collection” procedure automatically closes all purge valves, switches recycle valves to waste (by default), and deactivates the COMET feature prior to data collection. The exception to the closing of purge valves is when absolute RI analysis is conducted, the Optilab rEX purge valve is left open as required.

Script Collection

You can customize your data collection by using a script collection. To learn more about writing collection scripts, and the scripting language, see Appendix C, “Data Collection with Scripts”. Several simple collection scripts are included with ASTRA V, and are used, for example, in the experiment templates for light scattering calibration and in utility templates for turning off the later and for using the COMET cell cleaner.

The graph in the Script collection procedure dialog behaves the same as the graph in the Basic collection procedure dialog.

To select a script for the procedure, click the "..." button for the File Name property. In the Open dialog, select the script you want to use and click **OK**. Collection scripts typically have a file extension of *.col. You can type a script directly in the Script property row, but using a separate text editor is recommended.



Configuration Procedures

You may need to calibrate your instruments or measure various aspects of their behavior. These procedures may be used in separate calibration experiments, or integrated into other experiments.

The following configuration procedure types are available:

Band Broadening

This procedure allows you to correct for the effects of interdetector band broadening between instruments in an online experiment. Such broadening can distort the peak shape between instruments, resulting in incorrect results for analysis methods that require comparing the signal of two different instruments. The band broadening procedure allows you to correct for the broadening.

To do so, collect data from a narrow, monodisperse sample for use in determining the band broadening parameters.

For an example experiment that determines band broadening, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "band broadening example (BSA).vaf experiment in the Sample Data->Analyzed Experiments folder.

When to Determine Band Broadening

This procedure only needs to be performed once when you connect the instruments or change the tubing between the instruments. The band broadening remains the same until you change the length of tubing between the instruments or change the instrument sequence.

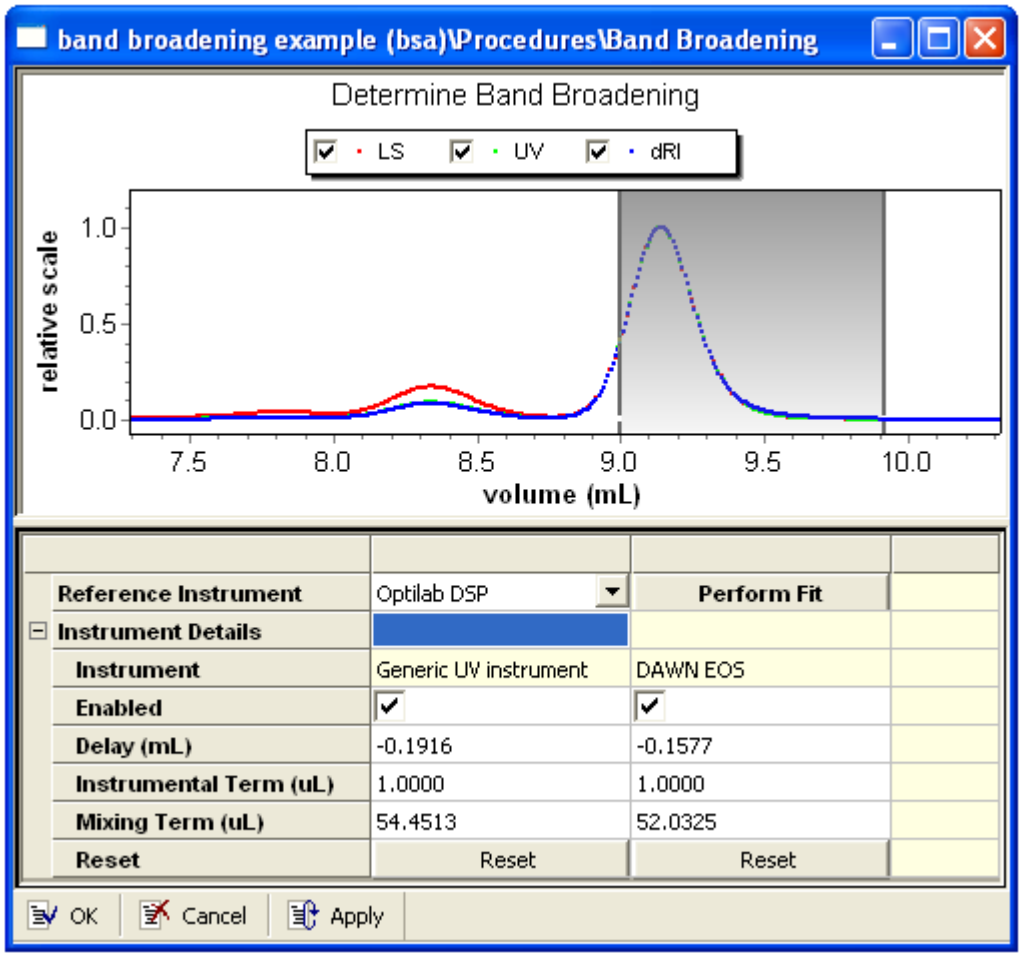
For more about how and why you need to correct for band broadening, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Band Broadening correction.

How to Determine Band Broadening

To calculate appropriate band broadening terms, follow these steps:

1. Open the Band Broadening procedure view. If an experiment has already been run, you can open this view by choosing **Experiment→Configuration→Band Broadening**.

   The procedure view looks similar to the following:



2. For the **Reference Instrument** property, select the instrument that has the broadest signal. Typically, this is the last instrument in the flow path. However, if there is a viscometer in the flow path, choose the viscometer as the Reference Instrument. The inherent instrumental broadening in the viscometer is usually greater than the broadening resulting from flow between instruments.
3. Drag a peak range on the graph to create a range marker to be used to determine the band broadening parameters. It is important that you select a monodisperse peak. Be careful to select a region of the peak that is free from contamination by other eluting species. Typically, the range marker should be set from a position about halfway up the leading edge of the peak to a point just past the peak where all detector signals have returned to the baseline.
4. Click **Perform Fit** to calculate the terms and update the graph.

5. Examine the fit between the two traces and the values for the Instrumental Term and the Mixing Term. The expected values vary depending on whether there is a viscometer in the instrument series and whether despiking or smoothing has been performed.
   - **No Viscometer and No Despiking or Smoothing:** The value of the instrumental term should be small (1 µl).
   - **No Viscometer and Despiking or Smoothing Performed:** The value of the instrumental term will generally be larger than 1 µl but smaller then the mixing term.
   - **Viscometer Used:** The instrumental term will generally be comparable to or larger than the mixing term. Despiking and smoothing should not make much difference if there is a viscometer.
6. If the resulting instrumental term is significantly larger than expected and the match between the peaks is not good, you should repeat the fit. To do this, click **Reset**, then enter seed values for the instrumental and mixing terms.
   - **No Viscometer Used:** Use seed values of 1 µl for the instrumental term and 40 µl for the mixing term.
   - **Viscometer Used:** Use seed values of 20 µl for the instrumental term and 20 µl for the mixing term.
7. Click **Perform Fit** again.
8. If repeated attempts to obtain a good fit fail, either verify that the baselines are set correctly or choose a different range for the fit.
9. When the fit between the two traces looks good, click **OK** or **Apply** to re-run the experiment with the band broadening correction.

This procedure has the following properties:

Table 8-3: Band Broadening Properties

Field	Description
Reference Instrument	The instrument with the largest degree of broadening in the instrument series. This is typically the last instrument in the series, unless a viscometer is present, in which case the viscometer should be chosen as the reference instrument.
Perform Fit	Click **Perform Fit** when you are ready to use the peak marker to calculate the band broadening terms. The graph shows the corrected data.
Instrument Details> Instrument	The instrument trace to broaden. This is set automatically when you choose a reference instrument.
Instrument Details> Enabled	Check this box to enable band broadening for specific instruments.
Instrument Details> Delay (mL)	The interdetector volume (or time) between the reference instrument and the instrument to broaden. (The units are determined by the Abscissa Units field in Experiment Configuration.) You can set the initial seed value here or through the alignment procedure. The band broadening procedure recalculates the interdetector volume when determining the band broadening parameters. The resulting interdetector volume is generally different from that obtained from the alignment procedure.
Instrument Details> Instrumental Term	A term that defines the degree of broadening due to instrumental effects—that is, not due to mixing—in the band broadening calculation.

Table 8-3: Band Broadening Properties

Field	Description
Instrument Details> Mixing Term	A term that defines the degree of broadening due to mixing in the band broadening calculation.
Instrument Details> Reset	Click **Reset** to change the instrumental and mixing terms back to zero and update the graph.

Experiment Builder This procedure is hidden in the Experiment tree in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure in the tree, choose **System→Preferences→Experiment Builder Mode**.

Interdetector Delay (Alignment)

If an online experiment has already been run, you can open the Interdetector Delay procedure view by choosing **Experiment→Configuration→Alignment**.

ASTRA uses the volume delay between different instruments to correlate their measurements. Determine the delay volumes by collecting data on a monodisperse sample, then aligning the resulting peak for each instrument collecting data.

Once delay volumes between instruments have been determined, ASTRA subtracts them from each instrument to correct for the time it takes fluid to propagate between instruments.

When to Determine the Delay

This procedure only needs to be performed once when you connect the instruments or change the tubing between the instruments. The volume delay will remain the same until you change the length of tubing between the instruments or change the instrument sequence.

How to Determine the Delay

To determine the delay volumes, follow these steps:

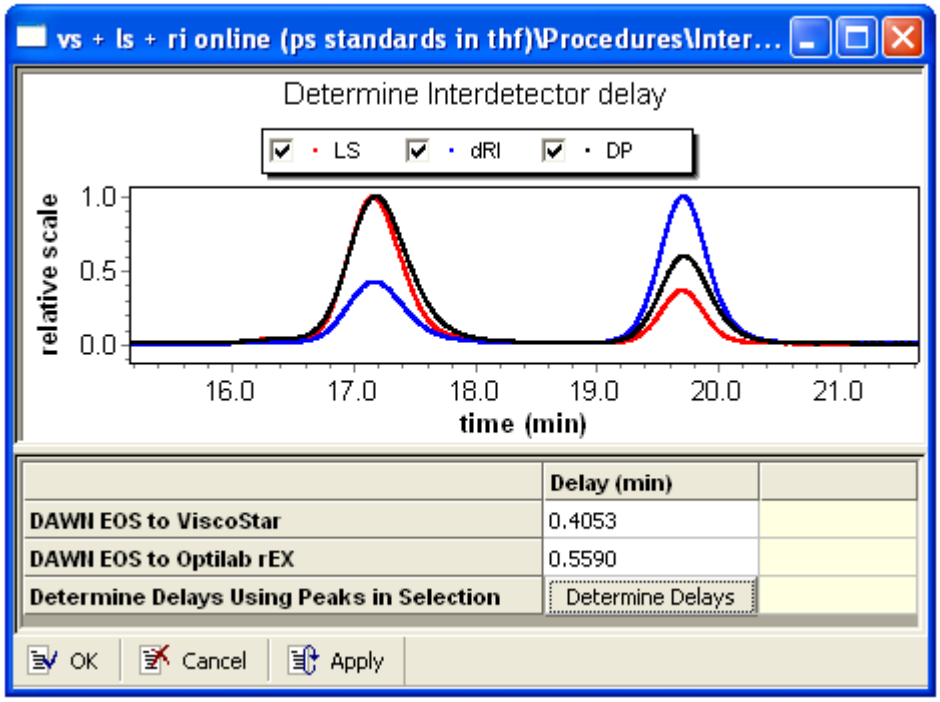
1. Prepare a monodisperse sample for data collection.

   A monodisperse sample is necessary for determining the volume delay between the DAWN and other instruments. It should have a polydispersity of less than 1.05. A suitable sample for alignment may be a narrow polystyrene, polyethylene oxide or a non-aggregated protein.

   Broad standards will *not* have peaks overlaid even when the system is properly aligned. This is perfectly normal and due to the different characteristics of molar mass detectors and concentration detectors. (Use the “Band Broadening” on page 8-14 procedure to correct for this.)

2. Create an experiment that includes the Interdetector Delay procedure. For best results, set the collection interval to 0.125 second.

3. The dialog for this procedure looks as follows.



The relative heights of the peak for the traces are auto scaled to match each other.

4. Click the **Determine Delays** button. The delays between instruments are automatically calculated. This procedure stores the interdetector delays in the properties of the fluid connections in the experiment configuration.

5. Alternately, you can type delay values directly in the property field. The graph shows the peaks corrected for the delay values currently entered.

This procedure has the following properties:

Table 8-4: Interdetector Delay Properties

Field	Description
Instrument relation	A separate row is shown for each fluid connection between the light scattering instrument and other instruments.
Delay	The time or fluid volume between the two instruments. The units are determined by the Abscissa Units property of the experiment configuration.
Determine Delays	Click this button to automatically calculate the delays between instruments.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

LS Calibration

A DAWN instrument needs to be calibrated to enable ASTRA to convert its signals to Rayleigh ratios. You must determine its calibration constant before using ASTRA to calculate absolute molar masses.

Calibration should be performed in batch mode; that is, before connecting the DAWN to a fractionation system.

This section describes the behavior of the separate procedure that performs calibration. In practice, this procedure is not used in isolation. Instead, you create an experiment using the calibration template for your instrument. That experiment contains a number of procedure items that run in sequence to turn the laser on and off, set peaks, and more.

When to Calibrate

Wyatt Technology calibrates each DAWN during manufacture and includes the calibration constant on the Quality Control report shipped with the instrument. However, you should calibrate the DAWN in your own lab and compare the value you obtain with the value on the QC report to verify that no internal damage occurred during shipment.

A DAWN should be recalibrated for any change that may affect the value of the scattering signal at the 90° detector. Calibrate if you:

- Disassemble the flow cell.
- Change the 90° detector photo diode.
- Change the laser gain.
- Change the jumper setting on the amplifier PCB for the 90° detector. If you do this, make sure you change the corresponding detector gain in the DAWN profile.
- Have not calibrated in the last 30 days. The passage of time may affect the signal.
- Realign the laser beam. (older DAWN DSP models only)

See “Measured Quantities and Calibration” on page D-4 for a discussion of calibration theory.

How to Calibrate

To perform a calibration experiment, follow these steps:

1. Set up your equipment for a batch (non-flow) experiment.
2. Create a new calibration experiment by choosing **File→New→Experiment From Template**.
3. In the New from Existing dialog, open the “System Templates” folder, then “Light Scattering”, then “Calibration”, and then the experiment for your light-scattering instrument.
4. Click **Create**.
5. Double-click the light-scattering instrument in the configuration.

6. In the properties dialog, select the appropriate physical instrument, sample cell, and wavelength.
7. Select the Solvent tab or double-click the Solvent in the configuration.
8. Click the "..." button for the Name property and open the System Solvents folder. Then select your solvent from the list of solvent profiles and click **Copy**.

   You should calibrate the instrument using a pure solvent with a well characterized Rayleigh ratio. We recommend calibrating with HPLC-grade toluene for the following reasons:

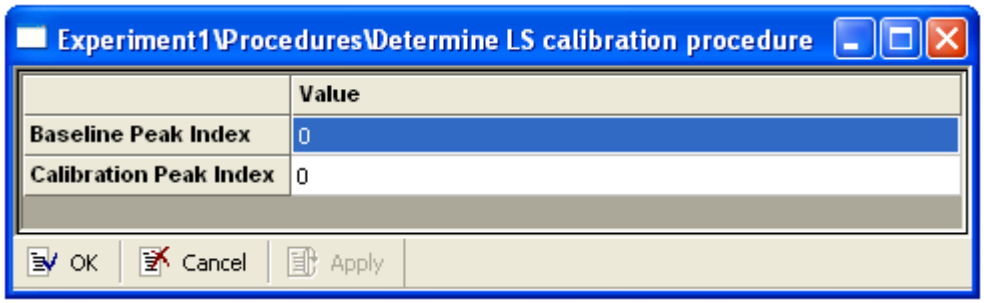
   - It has a high and accurately determined Rayleigh ratio.
   - It is generally a dust-free solvent.
   - Its refractive index is very similar to that of the flow cell windows.
9. Inject the solvent into the flow cell using a syringe pump. The solvent must be pure and free of particulates; we recommend that you use a 0.02 µm syringe filter attached to the syringe.
10. Wait until solvent is flowing through the cell and the front panel display for the 90° detector (detector 11 on most DAWNs or detector 5 on a DAWN 8) is stable. The variation in the signal should be 5 mV or less.
11. Choose **Experiment→Run** to begin running the calibration experiment. The calibration constant is calculated and written to the DAWN instrument configuration property dialog and the final calibration report.
12. Use the calibration constant in other experiment configurations in either of the following ways:
    - Type the calibration constant in the property dialog for the DAWN instrument in other experiments.
    - Export the calibrated DAWN profile to the system profiles, then import the profile whenever you create a new experiment. See Chapter 12, "Working with System Profiles" for details.

The accuracy of this constant may be improved by repeating the measurement a few times and averaging the results.

DAWN DSP and DAWN DSP-F: ASTRA prompts the user to turn the laser on and off at the appropriate times.

Setting Calibration Properties

The values for this procedure are set in the calibration template. Typically, you will not need to modify them. You can double-click on the LS calibration procedure to open its property dialog:



This procedure has the following properties:

Table 8-5: LS Calibration Properties

Field	Description
Baseline Peak Index	Number of the peak marker in the Peaks dialog that marks data collected with the laser off. The calibration template contains a pre-set baseline peak.
Calibration Peak Index	Number of the peak marker in the Peaks dialog that marks data collected with the laser on. The calibration template contains a pre-set calibration peak.

Differential RI Calibration

Most materials, when dissolved in a solvent, change the refractive index of the solution by an amount proportional to their concentration. This proportionality factor is called dn/dc, a factor that is usually independent of molar mass especially for molar masses greater than roughly 10,000 g/mol. The dRI detector's output is proportional to the change in refractive index, which in turn is equal to the product of the concentration and dn/dc.

For “analog” dRI instruments that send signals to the AUX input of another instrument, the proportionality factor relating detector output voltage to n is called the calibration constant. This constant is inversely proportional to dRI detector sensitivity. It is the number required by ASTRA to convert the voltage output of the dRI detector into changes in refractive index units when reading the dRI instrument signal through the AUX input of another instrument.

The Wyatt Optilab rEX is intended to communicate digitally with ASTRA in refractive index units. As such the Optilab rEX dRI calibration constant is an internal constant stored on-board the Optilab rEX itself.

When to Calibrate

dRI instruments manufactured by Wyatt Technology, including the Optilab rEX and the Optilab DSP, come pre-calibrated.

Other dRI instrument manufacturers might or might not supply an approximate dRI calibration constant. ASTRA needs an accurate dRI calibration constant since any error in the dRI calibration constant is directly proportional to the error in molar mass. The calibration constant can vary 10-15% from the manufacturer's approximate value, so calibration of any third-party dRI instrument is strongly recommend prior to use.

In all cases dRI measurement performance should be checked regularly (annually) against a standard with a known *dn/dc* value. Changing solvents does not affect the dRI calibration constant. If the calculated mass for a known sample changes over time, consider calibrating the dRI as part of your troubleshooting effort.

Note: For use with certain chromatography systems and the ASTRA V software, dRI calibration may be performed by injecting a suitable standard sample and verifying that the injected and calculated masses are equal. This option is often much more efficient than the off-line approach described below, particularly with organic mobile phases. Contact Wyatt Technical Support and review the section “RI Calibration from Peak” on page 8-75 for more information.

Off-line dRI calibration may be performed using any substance with a well-characterized *dn/dc* value. In all cases the recommended standard is *anhydrous* sodium chloride dissolved in pure de-ionized water. This solution has a *dn/dc* of 0.172 mL/g at a wavelength of 690 nm (0.181 mL/g at 488 nm, 0.174 mL/g at 633 nm or 658 nm, and 0.170 ml/g at 900 nm).

Appropriate concentrations should be prepared using pure distilled water and *clean* glassware. The lowest and highest recommended concentrations vary by instrument.

- For the Optilab rEX: ~0.1 mg/mL and ~5mg/mL.
- For all other dRI instruments: ~0.1mg/mL to 1.2mg/mL.

Use six or more concentrations prepared within 1% or better accuracy to increase the precision of the final determination. Wyatt Technology provides validated, pre-mixed NaCl solutions in the ideal concentration range for calibration of any dRI instrument (Wyatt Technology part number P8400, NaCl Solutions Kit.) NaCl concentrations are as follows: 0.0 (blank), 0.1, 0.5, 1.0, 1.2, 2.0, 3.0, 4.0, and 5.0 mg/mL in nanopure water.

dRI instruments can differentiate between solvent that has been saturated with ambient gases and those that have been degassed. It is possible to detect concentration differences in the 1 ppm range. Consequently, standard samples and solvent blanks must be prepared from the same solvent stock. After preparing the standard solutions, fill two or three extra containers with water from the same solvent flask to use for “blank” analyses.

Samples should be kept well-sealed to avoid evaporation. Some solvents (such as water) keep fairly well for a number of months, but concentration changes may occur over time due to evaporation or contamination.

NaCl solutions may be infused into the dRI instrument directly via syringe pump or with an HPLC pump and an injector as described below.

HPLC Pump with Injector

This arrangement is shown in Figure 8-1. Use 0.01 in. ID tubing between the injector and the Optilab rEX, and 0.02 in. ID tubing elsewhere. A flow rate of 0.5–1.0 mL/minute is ideal. Use a large sample loop, 0.5–1.0 mL to ensure that each injection produces a clear plateau and not a peak as it passes through the Optilab rEX.

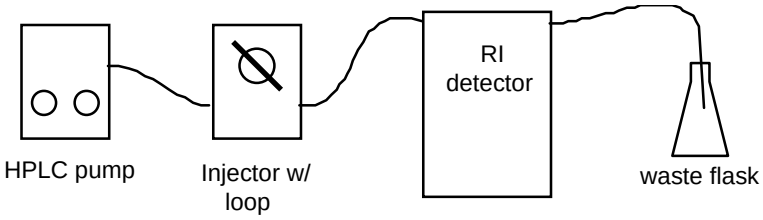


Figure 8-1: Setting up an HPLC pump and injector for calibration

The objective is to inject *known* concentrations into the detector. If a peak with a rounded top and no clear plateau is obtained, the concentration at the top of the peak will be unknown; flat-topped plateaus indicate that the cell has been fully flushed with sample solution.

A syringe pump fitted with a large syringe may be used instead of the HPLC pump.

Syringe Pump Infusion

Another option is to connect the Optilab rEX as shown in Figure 8-2, using a syringe pump. Use 0.02 in. ID tubing for both connections. A recommended flow rate is 0.1–0.2 mL/minute. The syringe must be rinsed and dried thoroughly or replaced between samples to avoid contamination of one concentration by the next sample. When the syringe is disconnected to change samples, the pressure change and injected air may cause an unstable baseline for several minutes.

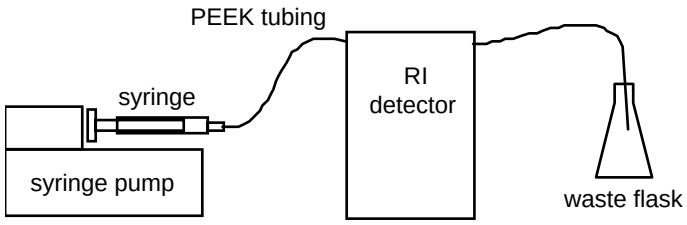


Figure 8-2: Setting up a syringe pump for calibration

Collecting Data

The dRI instrument's sample and reference cells should be flushed with high purity water (first solvent to be analyzed) for several minutes before making measurements. Confirm that the temperature is stable, and activate the instrument's purge feature while flowing pure water for several minutes. Deactivate the purge, flow with water for several more minutes, then zero the instrument.

Collect data using ASTRA V software as follows:

1. Start the ASTRA V software and select **File->New->Experiment from Template**. In the dialog box that opens, select the **System Templates** folder, then the **RI Measurement** folder, and finally open the **RI calibration** template.
2. In the experiment, expand the **Configuration** node and verify that the proper experimental parameters have been entered. Update the configuration if necessary.
3. Expand the **Procedures** node of the template. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. A typical Duration for the dRI calibration experiment is 20 minutes or more. Close the dialog by clicking the **OK** button, and run the experiment.
4. Introduce pure solvent (blank) into the reference cell, making sure the solvent flows through the system at a constant rate.

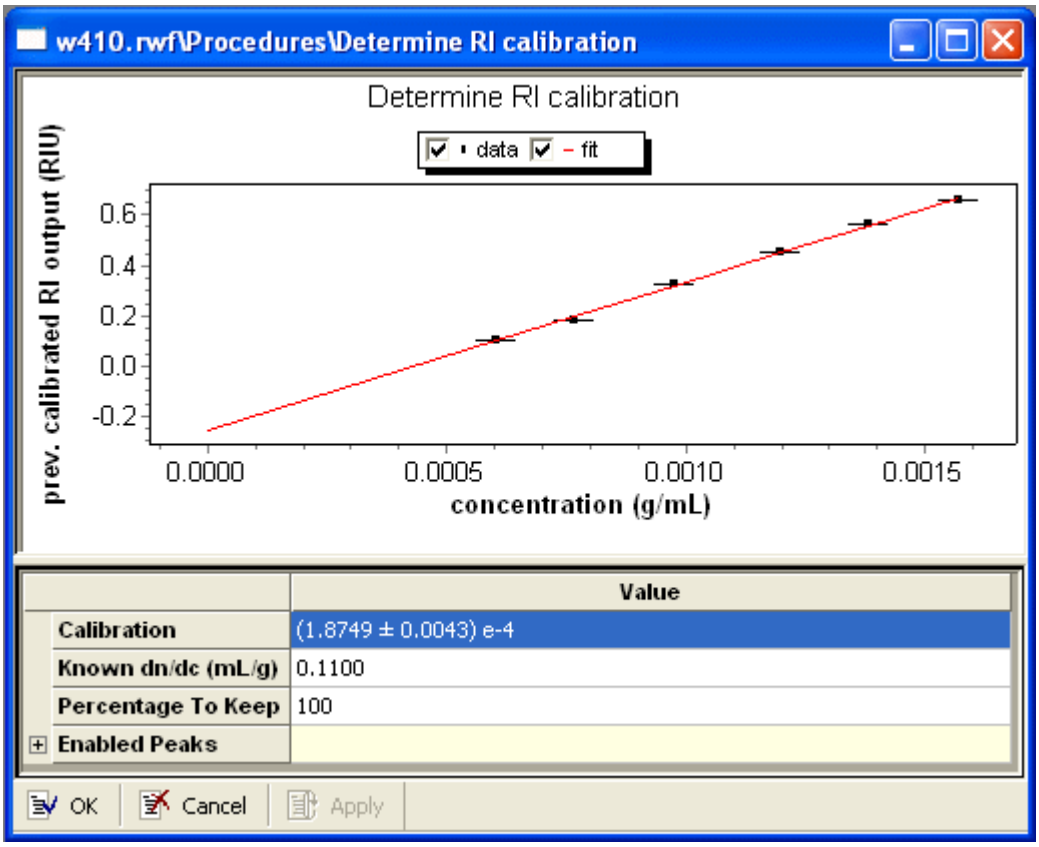
   dRI detectors do not react favorably to sudden changes in flow as the detector drift would overwhelm the signal. Thus, it is important to maintain a nearly constant flow through the instrument while measuring the solutions.
5. While ASTRA V collects data, begin to introduce the series of prepared standards into the sample cell of the detector. Each standard solution is introduced one at a time, *beginning with the lowest concentration*.
6. Wait for the signal to stabilize (allow signal to reach a plateau), which may take several minutes, while the previous solution is completely rinsed out of the cell. After all the standards have been injected, re-inject a pure solvent sample (blank) to re-establish the baseline.
7. Once a good baseline signal is acquired, stop the ASTRA V data collection.

Data Processing

After the data collection has ended, follow these steps:

1. ASTRA V may produce a message box informing you that baselines need to be set. Click **OK**, and the software will expand the Procedures section of the experiment and open the **Baselines** window. Otherwise click the **Run** button to proceed.

2. In the **Define Baselines** window, define the baseline using the solvent blanks that where injected before and after the standard solutions. Click and drag from one solvent plateau to the other, such that a baseline is drawn under the sample solution plateaus. Click **OK** to close the baselines window.
3. In the **Define Peaks** window, define a peak for each of the flat plateau regions associated with each injected standard. Click and drag over the maximum flat region for each sample injection. Do not set peak regions for the "blank" injections.
4. As peak regions are defined, a table beneath the Define peaks graph shows a new column associated with each respective peak. In these columns, under the Refractive index node, enter the known concentration for each standard in the row titled Concentration (g/mL). Once you have assigned all the peaks their respective concentrations, click on the OK button at the bottom to close the pane.
5. Double-click the **RI Calibration** procedure to open its property dialog:



This procedure has the following properties:

Table 8-6: RI Calibration Properties

Field	Description
Calibration	The resulting calibration constant.
Known dn/dc	The known dn/dc value for the calibration standard used.

Table 8-6: RI Calibration Properties

Field	Description
Percentage to Keep	The percent of the marked peak data to use for calibration. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks	This list shows the peaks used in the fit to determine the calibration constant. Checking or unchecking a peak adds or removes it from the fit to determine the calibration constant.

6. Enter the known dn/dc into the row titled Known dn/dc (mL/g) and click **Apply**. The value in the **Calibration** row should update once this known value is entered.
7. Expand the **Results** node of the experiment and double-click the **Report (summary)** line. The report displays the new Calibration constant.

Optilab rEX dRI Calibration Results

For the Optilab rEX, the reported "Calibration Constant" is actually a correction factor. A value of 1.0000 would indicate that the Optilab rEX's internal dRI calibration constant has remained unchanged. If the value is 1.0120, the Optilab rEX dRI calibration constant has increased by a factor of 1.2%.

1. Multiply the old dRI calibration constant by the correction factor to determine the new dRI calibration constant. The old dRI calibration constant is viewable in the **Constants** window on the Optilab rEX front panel.

   The calculation is:

   (old dRI calibration constant) x (ASTRA V correction factor) = new dRI calibration constant

   For example:

   3.3828e-3 x 1.012 = 3.4234e-3

Note: You must calculate the new dRI calibration constant manually, using the old dRI calibration constant and the correction factor provided by ASTRA.

2. Tab to the **System** screen on the Optilab rEX front panel. Open the **Constants** window, and enter the new dRI calibration constant. Tab to the **Apply** button, and press Enter.

Optilab DSP or Third-Party Instrument dRI Calibration Results

Enter the reported dRI calibration constant for "analog" instruments in the "calibration constant" field of the AUX connection profile for the RI detector. This value is not set in the AUX connection profile automatically.

Absolute RI Calibration

You can calibrate the Optilab rEX aRI measurement as described in this section. This procedure is also summarized in the *Optilab rEX User Guide* section titled "Instrument Calibration for aRI."

We recommend that you verify and/or calibrate the absolute Refractive Index (aRI) measurement before operating the instrument in aRI mode for the first time, and it is recommended that you check these values for accuracy on a periodic basis.

General Information

Optilab rEX aRI measurement performance should be checked against one or more standards. For a complete aRI calibration, at least 3 pure solvents with known aRI values (which are specific for the operating wavelength of the Optilab rEX) should be used. See Table 8-7 for recommendations. It is imperative that each solvent infused into the Optilab rEX must be miscible with the solvent that it replaces.

The Optilab rEX aRI is factory-calibrated using the following four solvents in the following order:

1. High Purity Water (NANOpure water with a final 0.2 micron filter)
2. Methanol (HPLC-Grade Fisher A452-4)
3. Tetrahydrofuran (GPC-Grade w/BHT Burdick & Jackson Cat. 341-4)
4. Toluene HPLC Grade (HPLC-Grade Burdick & Jackson Cat. 347-4)

Table 8-7: Known aRI Values for Various Solvents at Various Wavelengths

Solvent	685nm	658nm	633nm	532nm	514nm	488nm
Water	1.3303	1.3309	1.3316	1.3347	1.3354	1.3364
Methanol	1.3242	1.3247	1.3253	1.3282	1.3289	1.3300
Tetrahydrofuran	1.4015	1.4022	1.4029	1.4069	1.4079	1.4094
Toluene	1.4882	1.4896	1.4910	1.4995	1.5017	1.5053

Note: It is imperative that each solvent be miscible with the solvent it replaces, anytime the Optilab rEX is flushed with a new solvent.

Collecting Data for Optilab rEX aRI Calibration

1. The Optilab rEX should be purged (flow liquid with the Purge on) with high-purity water for several minutes before beginning the aRI calibration procedure. Confirm that the temperature is stable at 25°C, and operate the Optilab rEX with the Purge ON for all data collection (the Purge indicator will be yellow on the **Main** tab).
2. On the Optilab rEX front panel, navigate to the **System** tab, and open the **Constants** dialog. Record the values listed for dRI calibration constant, dRI offset, aRI calibration constant, and aRI offset.

3. Collect data using the ASTRA V software. As of the time of publication of this user's guide, the ASTRA collection procedure is as follows (refer to the current *ASTRA V User's Guide* for more information):
   a. Start the ASTRA V software and select **File->New->Experiment from Template**.
   b. In the dialog that opens, select the **System Templates** folder, then the **RI Measurement** folder, then the **Optilab rEX Specific** folder, and finally the **absolute RI calibration** template.
   c. Expand the **Configuration** node and verify that the proper experimental parameters have been entered.
   d. Expand the **Procedures** node of the template. Click on the **Basic collection** procedure and enter appropriate values for each of the fields. Close the pane by clicking the **OK** button.
   e. Run the experiment.

Introduce Solvents

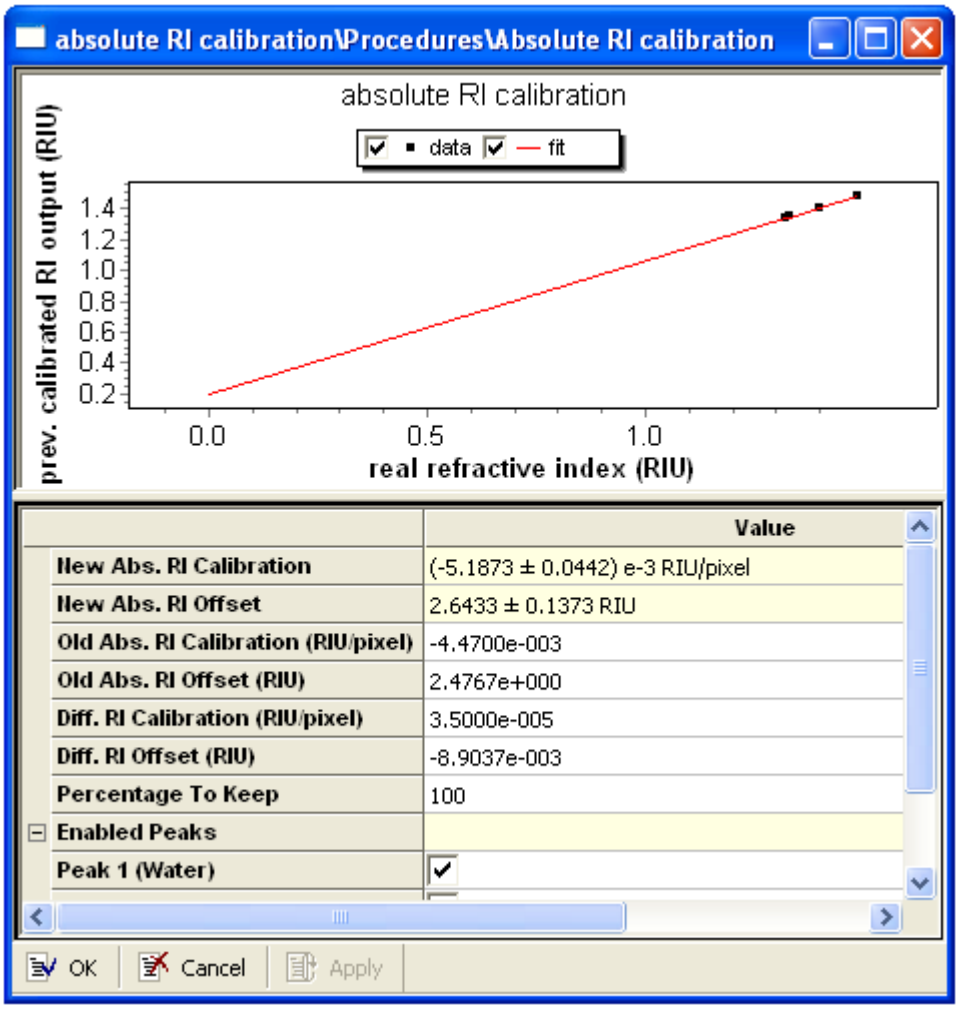
1. Flow the pure solvents directly into the Optilab rEX at a constant flow rate, using a syringe pump or an HPLC pump set in the flow rate range of 0.5mL/min to 1.0mL/min.
2. After flowing roughly 4-5 mL of solvent, toggle the purge valve to **PURGE OFF** for 15 seconds, and then toggle it back to **PURGE ON** for 15 seconds. Repeat this cycle for 2-3 minutes. Cycling the purge valve in this manner creates just enough agitation in the flow path to displace air bubbles and thoroughly remove the previous solvent.
3. On the Optilab rEX **System** tab, go to the **LED** button and press Enter. Adjust the **Percent max power** setting so that the **Light intensity** is close to, but not above, 7.8 Volts. Close the LED Intensity dialog and return to either the **Main** or **aRI** tab.
4. Complete the solvent introduction process by leaving the purge valve in the **PURGE ON** state for roughly one minute, and then turn the syringe pump off. After three minutes of stopped flow the Optilab rEX is stable. During this time ASTRA V will record the stable, flat no-flow region. Note the time of this no-flow period, which will be referenced during data processing.
5. Repeat this solvent introduction procedure (beginning with step 1 of this list) for each remaining solvent.

ASTRA Calculations

Follow these steps in ASTRA V:

1. After data collection, open the **Procedures** section of the experiment, and click **Define peaks**. On the graph, identify the flat no-flow regions associated with each solvent. Define a peak for each solvent by selecting a plateau of approximately 30 seconds located near the end of the flat region.

2. Beneath the graph a table is shown with a column for each defined solvent. Expand the **Refractive index** node. A row will now be visible titled **Real**. In each solvent's column, enter the known aRI value. Make sure that it is the correct aRI value for the Optilab rEX's operating wavelength. Table 8-7 lists aRI values for several solvents at various wavelengths. Click the **OK** button to close the window.
3. Click on the **Absolute RI Calibration** procedure to open its settings pane. Enter the **dRI calibration constant, dRI offset, old aRI calibration constant** and **old aRI offset**. These are the numbers recorded in Step 2 on page 8-27. Click **OK** to close the window.



4. Click the Run experiment icon on the icon toolbar at the top of the window. The template completes its data processing.
5. Expand the **Results** node and double click the **Report** line. The report will display the new aRI calibration constant and new aRI offset. Save the ASTRA V file. You may also want to print the report.

6. Go to the **System** tab on the Optilab rEX, tab to the **Constants** button and press Enter. Enter the new values into the Optilab rEX in the Constants dialog. Tab to the **Apply** button and press Enter. Tab to the **Close** button and press Enter. The Optilab rEX aRI is now recalibrated.
7. Measure and record the aRI value for pure ethanol, which should be near 1.35 after the correct constants are in place and after purging the rEX with ethanol as described in the section "Introduce Solvents" on page 8-28.

Setting Absolute RI Calibration Properties

This procedure has the following properties:

Table 8-8: Absolute RI Calibration Properties

Field	Description
New Abs. RI Calibration	The resulting absolute RI calibration constant. These are measured in refractive index units (RIU) per pixel on the photodiode.
New Abs. RI Offset	The resulting absolute RI offset in RIU.
Old Abs. RI Calibration	The previously used absolute RI calibration constant from the Optilab rEX.
Old Abs. RI Offset	The previously used absolute RI offset from the Optilab rEX.
Diff. RI Calibration	The differential RI calibration constant from which the absolute value was determined (from the Optilab rEX).
Diff. RI Offset	The differential RI offset from which the absolute value was determined (from the Optilab rEX).
Percentage to Keep	The percent of the marked peak data to use for calibration. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks	This list shows the peaks used in the fit to determine the calibration constant. Checking or unchecking a peak adds or removes it from the fit to determine the calibration constant.

The graph shows a fit to the absolute RI values using the previous absolute RI calibration data and the measured refractive index data.

For an example experiment that determines the absolute RI calibration, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "absolute RI calibration.vaf" experiment in the Sample Data->Analyzed Experiments folder.

You can place this procedure with other analysis procedures and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine the dn/dc or RI calibration in a procedure, only the first one is valid.

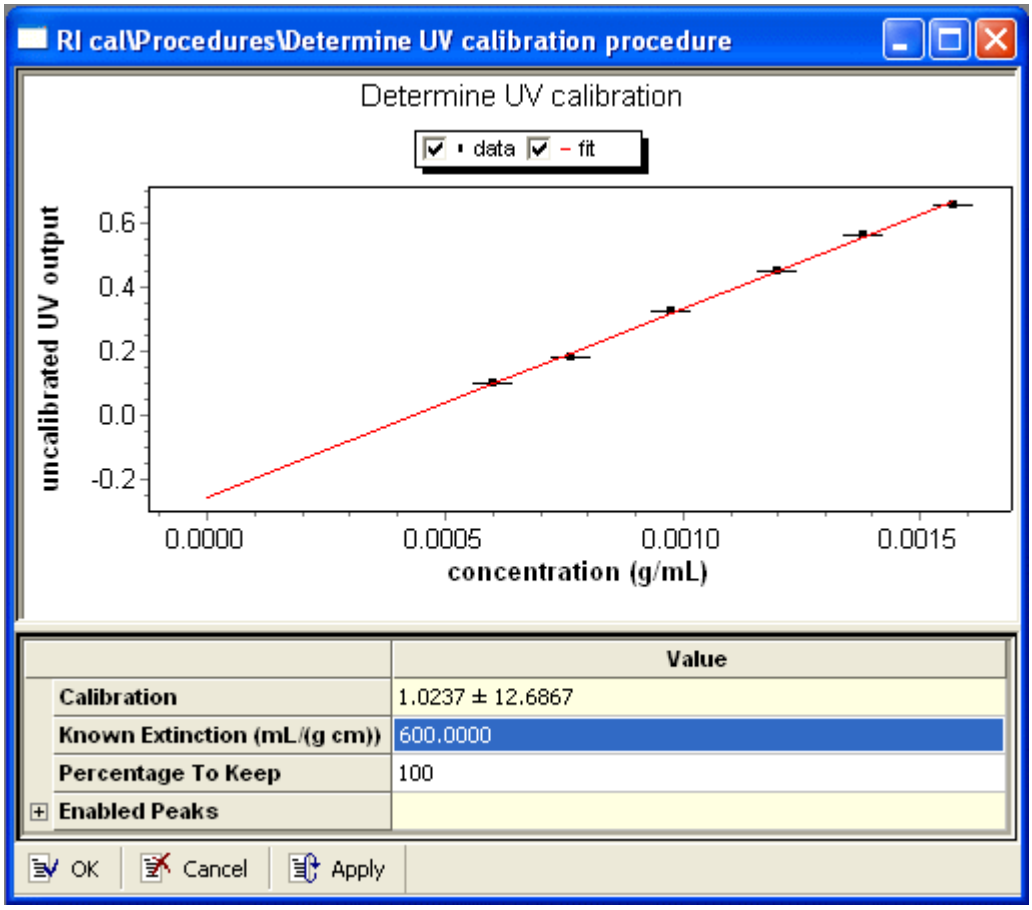
UV Calibration

The UV response factor supplied by the instrument manufacturer is generally accurate, and the calibration constant for the UV AUX connection can be set to 1. If, however, you suspect that the specified response factor is incorrect, you can determine the UV calibration directly.

The procedure for determining the UV calibration constant is very similar to that for determining the RI calibration constant. The main difference is that the Known Extinction (in mL/(g cm)) of the sample is used instead of the known *dn/dc* of the sample.

The UV calibration constant varies with the extinction coefficient of the particular sample. See *Wyatt Technical Note #15* for more instructions on using a UV detector.

Double-click on the UV calibration procedure to open its property dialog:



This procedure has the following properties:

Table 8-9: UV Calibration Properties

Field	Description
Calibration	The resulting UV calibration constant.
Known Extinction	The known extinction value for the calibration standard used.

Table 8-9: UV Calibration Properties

Field	Description
Percentage to Keep	The percent of the marked peak data to use for calibration. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Enabled Peaks	This list shows the enabled peaks.

Normalization

If an experiment has already been run, you can open the Normalization procedure view by choosing **Experiment→Configuration→Normalize**.

Normalization is the process by which the various detectors' signals are related to the 90° detector signal and the instrument calibration constant. By definition, the 90° detector always has a normalization coefficient of 1. (This is detector 5 on a DAWN 8 and detector 11 on all other DAWNs.) Good normalization is an important component in achieving good results from a DAWN.

When to Normalize

The first time you use ASTRA after installing your DAWN or miniDAWN, you need to normalize the detectors. Thereafter you will need to normalize only under certain conditions:

- Whenever you collect data from a sample whose solvent is different from that used for the previous normalization.
- For aqueous solvents, whenever the solvent offsets you just collected are better than those used for computing normalization coefficients earlier. (Better means lower voltages.)
- Whenever you reinstall the DAWN flow cell.
- Whenever you alter the laser alignment. (Laser alignment is necessary only for DAWN DSP and DSP-F instruments.)

See "Normalization" on page D-8 for details on how normalization coefficients are used in calculations.

Selecting a Normalization Standard

The normalization standard you use should have a low molar mass and a low polydispersity. It should be highly concentrated and use the same solvent you plan to use for your experiments. The issues related to these requirements are discussed in the following list:

- **Isotropic Scattering:** The important concept to understand about normalization is that a very small molecule scatters light *isotropically*, meaning with equal intensity in all directions. So if we inject a very small molecule into the DAWN flow cell, we might expect to measure equal voltages at all detectors. This is not the case for several reasons:
  - Different detectors are collimated differently to improve performance and thus do not "see" equal lengths of the flow cell bore.
  - Refractive index effects come into play and change the light intensity and scattering angles.

- Individual detectors vary somewhat in sensitivity.

We overcome these conditions by injecting a very small molecule and computing factors to force the light intensity to be equal for all detectors. As long as we inject a molecule whose size is too small to be measured accurately by the DAWN (smaller than 10 nm), the exact size does not matter.

A good isotropic standard is a sample molecule with a diameter of less than about 1/20th of the wavelength of the incident light, which is the case for random coil molecules with a molar mass below 50,000, and also for most proteins. Perform the normalization at the flow rate and in the solvent you intend to use to run samples.

- **Low Polydispersity:** It is inappropriate to normalize with a broad distribution (polydispersity greater than 1.2) or unknown sample. Always use a relatively narrow distribution (polydispersity less than 1.1), low molar mass sample for normalization.

  It is usually appropriate to use the same sample for normalization that you used to determine the delay volumes between the DAWN and other instruments. If there are absolutely no narrow standards available in the solvent you are using, you may be able to normalize properly by setting the peak region to include only the central part. Nevertheless, use a narrow standard if one is available.

- **Same Solvent as Samples:** Due to the changes in scattering angle with solvent refractive index, the normalization needs to be performed in the same solvent as the samples you want to analyze. For chromatography, we recommend one of the following:
  - A 30,000 g/mol narrow polystyrene in toluene or THF, having an RMS radius of about 5 nm.
  - A 20,000–30,000 g/mol polysaccharide such as pullulan or dextran in water, or a PEO, also having an RMS radius of about 5 nm.
  - A monomer protein such as BSA in water, having an RMS radius of 3 nm.

- **High Concentration:** The standard you inject for normalization is at a higher concentration than normal. This is to improve the signal-to-noise ratio of the measurement. Aim for a ratio of at least 100:1 for the normalization peak.

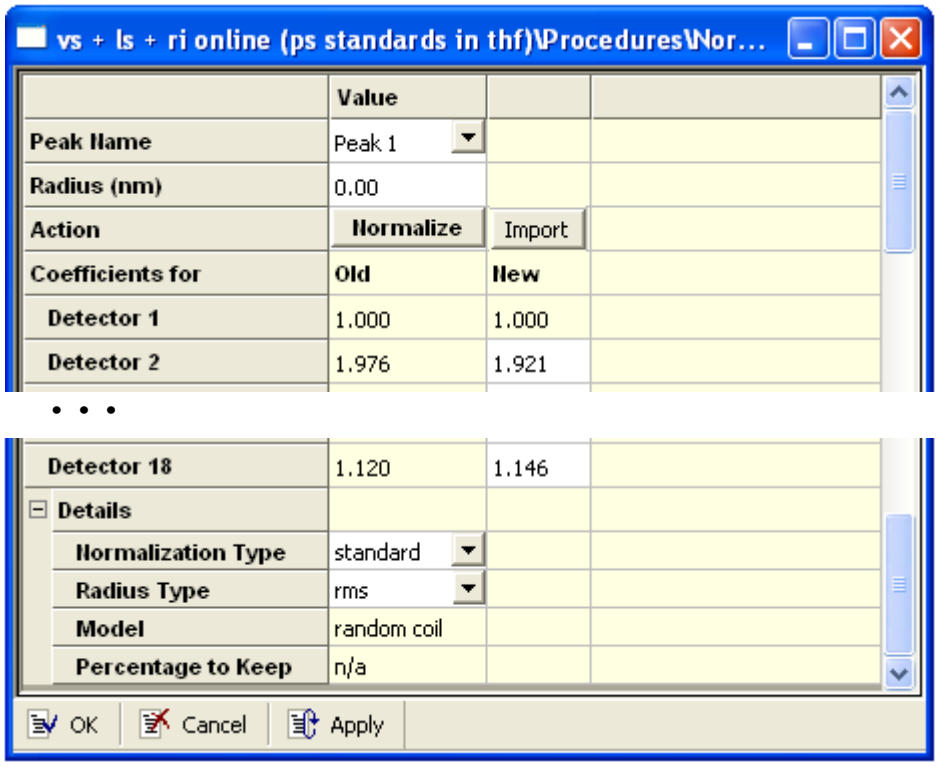
- **Batch Mode Issues:** For batch measurements, you do not have the advantage of molar mass separation as you do in chromatography. Any aggregates in your sample will not be separated and may cause normalization errors. Therefore, we recommend higher concentrations of non-aggregating lower molar mass standards for normalization in batch mode. A 10–15 mg/mL solution of 4000 g/mol polystyrene in toluene or THF, or 5000 g/mol dextran, pullulan or PEO in water, works well. All of these have RMS radii of about 2 nm.

Running a Normalization Experiment

The sample and solvent you use for normalization are important. Follow these steps to normalize:

1. Choose a normalization standard as described earlier in this section.
2. Create a new experiment from the template appropriate for your type of experiment.
3. Run the experiment and set baselines and peaks as described for those procedures. Use a narrow peak for normalization.

   For online experiments, set a peak symmetrically over a monomer peak (exclude any multimer peaks). For batch mode, set a peak region over the plateau corresponding to your normalization standard.
4. Perform the normalization by choosing **Experiment→Configuration→Normalize**. You see the property view for the procedure.



5. In the property view, specify the peak and radius for the sample. If you are using a Mie or sphere model for the sample, specify that the radius is a geometric radius.
6. If this is a batch experiment, specify the fraction of data to keep for the normalization.
7. Click the **Normalization** button.
8. The newly calculated normalization coefficients are displayed next to the previous coefficients. If you wish to use the new normalization coefficients, click **OK** or **Apply**. Otherwise, click **Cancel**.

9. You can view the calculated normalization coefficients in the Experiment Configuration dialog for the DAWN or miniDAWN.

Importing Normalization Coefficients

Instead of performing a normalization, you can import normalization coefficients from another experiment by clicking the **Import** button. The source experiment must contain normalization coefficients and must be open before you click the **Import** button.

Setting Normalization Properties

You can set properties for this procedure before or during the experiment. Double-click on the Normalization procedure to open its property dialog:

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

This procedure has the following properties:

Table 8-10: Normalization Properties

Field	Description
Peak Number	Type the number of the peak that corresponds to your normalization standard.
Radius	The radius of the normalization standard in nm.
Action	Click the **Normalization** button in this row when you are ready.
Coefficients for Detectors	The Old column shows previous normalization coefficients for each detector. The New column shows the computed normalization coefficients.
Normalization Type	The type of normalization to use. Options are standard and area. • "Standard" normalization uses the Rayleigh Ratio peak apex as the basis for normalizing. In effect, it divides the peak apex for each detector angle by the peak apex value for the 90-degree detector. • "Area" integrates Rayleigh Ratios over the entire peak. The Rayleigh Ratio peak areas for each detector angle are divided by the area for the 90-degree detector to yield the normalization coefficient. This method provides better performance than "standard" normalization.
Radius Type	The type of radius specified. Options are RMS, geometric, and hydrodynamic. If you are using a Mie or sphere model for the sample, specify that the radius is a geometric radius.
Model	Displays the fitting model being used.
Percentage to Keep	If this is a batch experiment, specifies the fraction of data to keep for performing the normalization. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.

Checking the Normalization Coefficients

After normalizing the detectors, you need to make sure that the coefficients you obtained are accurate. You can use the Debye plot ("About Debye Plots" on page 8-61) to do this.

If you use the Debye plot, inject a sample with a radius around 20 nm; a linear polymer with a molar mass about 200,000 g/mol is suitable. Set baselines and mark the peak, then use the Mass and Radius from LS Data

procedure (page 8-59) to display the Debye plot using the Debye model and a Fit Degree of 1. It is a good idea to step through several data slices at the top of the peak (use the +/- keys) to get a feeling for the random noise in the data. If one detector is consistently off the fitted line (above or below) its normalization coefficient needs to be redetermined.

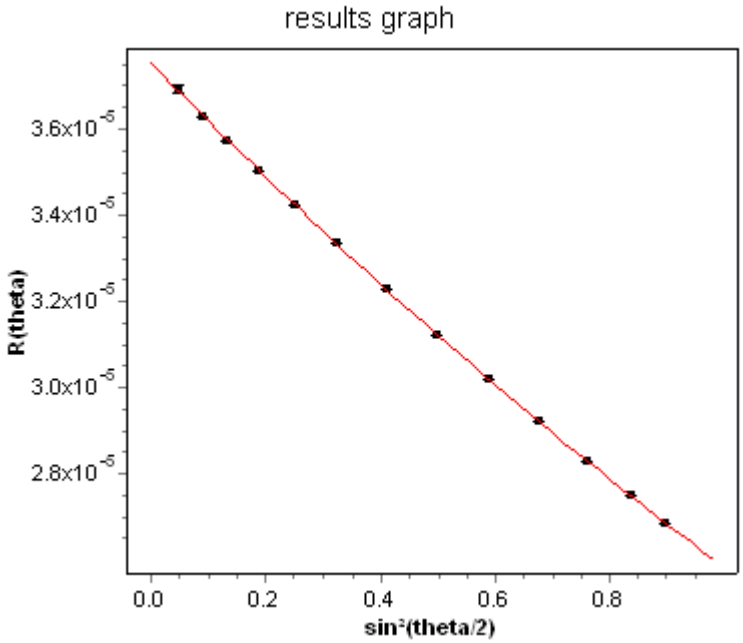


Figure 8-3: Debye plot showing accurate normalization coefficients for all angles

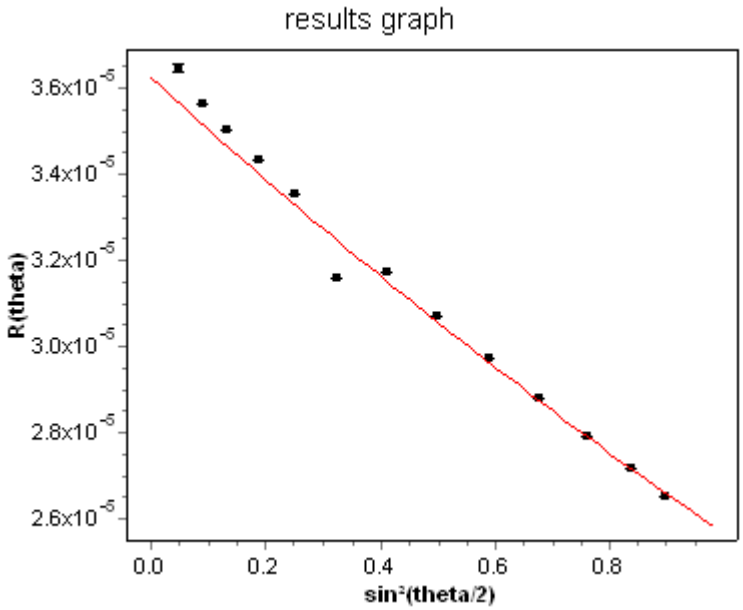


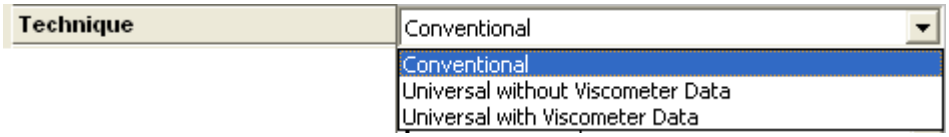
Figure 8-4: Debye plot showing incorrect normalization coefficient for detector 9

Calibrate Column

ASTRA provides this procedure for developing a column profile. Such profiles can be used to compare the absolute molar masses derived from light-scattering results to the relative molar masses derived from conventional size-exclusion chromatography. Such comparisons can illustrate possible errors generated by relative molar mass measurements and may be useful for characterization of branching.

This procedure determines the calibration constants stored in the generic column profile (see page 7-26).

Two main types of column calibration are available. “Conventional calibration” and “universal calibration.” For conventional calibration, the analyzed polymer must be the same as the polymer used for calibration. For universal calibration, the polymers may be different.



The calculation for universal calibration requires either intrinsic viscosity data or known Mark-Houwink-Sakurada K and a coefficients for the polymers used for calibration and the polymer to be analyzed. The dn/dc value is required for universal calibration (as it is necessary for intrinsic viscosity calculations), but not for conventional calibration.

You can see example experiments that perform column calibration by choosing **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database) and opening the conventional calibration or universal calibration .vaf file in Sample Data-> Analyzed Experiments. For an experiment template choose **File→New→Experiment From Template**, and open the “universal calibration” template in the System Templates->Viscometry folder.

For more, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Conventional and Universal Column Calibration.

When to Calibrate

This procedure only needs to be performed when a new column is used, or if you think the column has aged to a point that its behavior needs to be reassessed.

Analysis of a sample using universal calibration takes place in two logical phases. First, the column profile must be determined by measuring the behavior of a set of known molecular standards when passed through the column. Once this "determine column calibration" phase is complete, the unknown sample can be analyzed in a separate experiment.

In an experiment, this procedure must come after the Baselines and Peaks procedures. If multiple detectors are used, this procedure must also come after the Interdetector Delays and Band Broadening procedures.

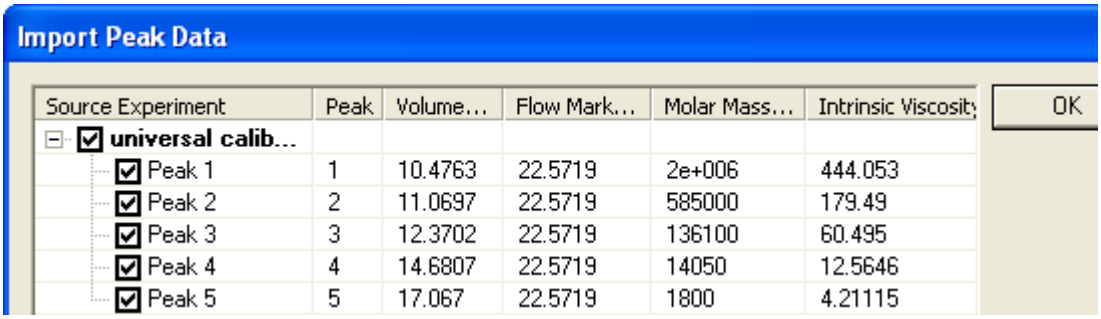
Running a Column Calibration

To calibrate a column, follow these steps:

1. Set up the equipment for an online experiment with your SEC column.
2. Choose **File→New→Experiment From Template** to open a new experiment. For conventional calibration, the template is in the System Templates->RI Measurement folder. For universal calibration, the template is in the System Templates->Viscometry folder.
3. Run the experiment using a set of known molecular standards.

   If you are performing a universal calibration without viscosity data, use a standard that is available in several known molar masses and for which the Mark-Houwink-Sakurada K and a coefficients are known (for example, polystyrene).
4. For each experiment, define peaks:
   - Enter masses or select predefined molecular standards for each peak in the Peak view.
   - For universal calibration, supply intrinsic viscosity information in the Extended Parameters if viscometry data is not present in the experiment.
5. Choose **Experiment→Configuration→Calibrate Column**. Examine the column calibration data fit. For the "Universal Calibration with Viscosity Data" technique, an additional column shows the intrinsic viscosity of the molecular standard.
   - You can specify a flow marker (peak) to use in the calibration. Select between positive and negative peaks to search for the peak maximum or minimum. If you select the same flow marker for each experiment, it is used when combining data to yield a more accurate curve.
   - You can view individual peak entries by pressing the "+" sign next to the Peak label.
6. Save the completed experiment.
7. Repeat the previous steps for any additional mixtures of known molecular standards as many times as necessary to cover the full column range. Use the flow marker you selected in each mixture.
8. Open all column calibration experiments you saved for this column.

9. In the Calibrate Column dialog, click the **Import Peak Data** button. You will see a dialog that lists other open experiments that contain column calibration data. Check the boxes next to any peaks you want to import. The grid shows the peak number, elution volume, flow marker, molar mass, and intrinsic viscosity for each peak. Then click **OK**.



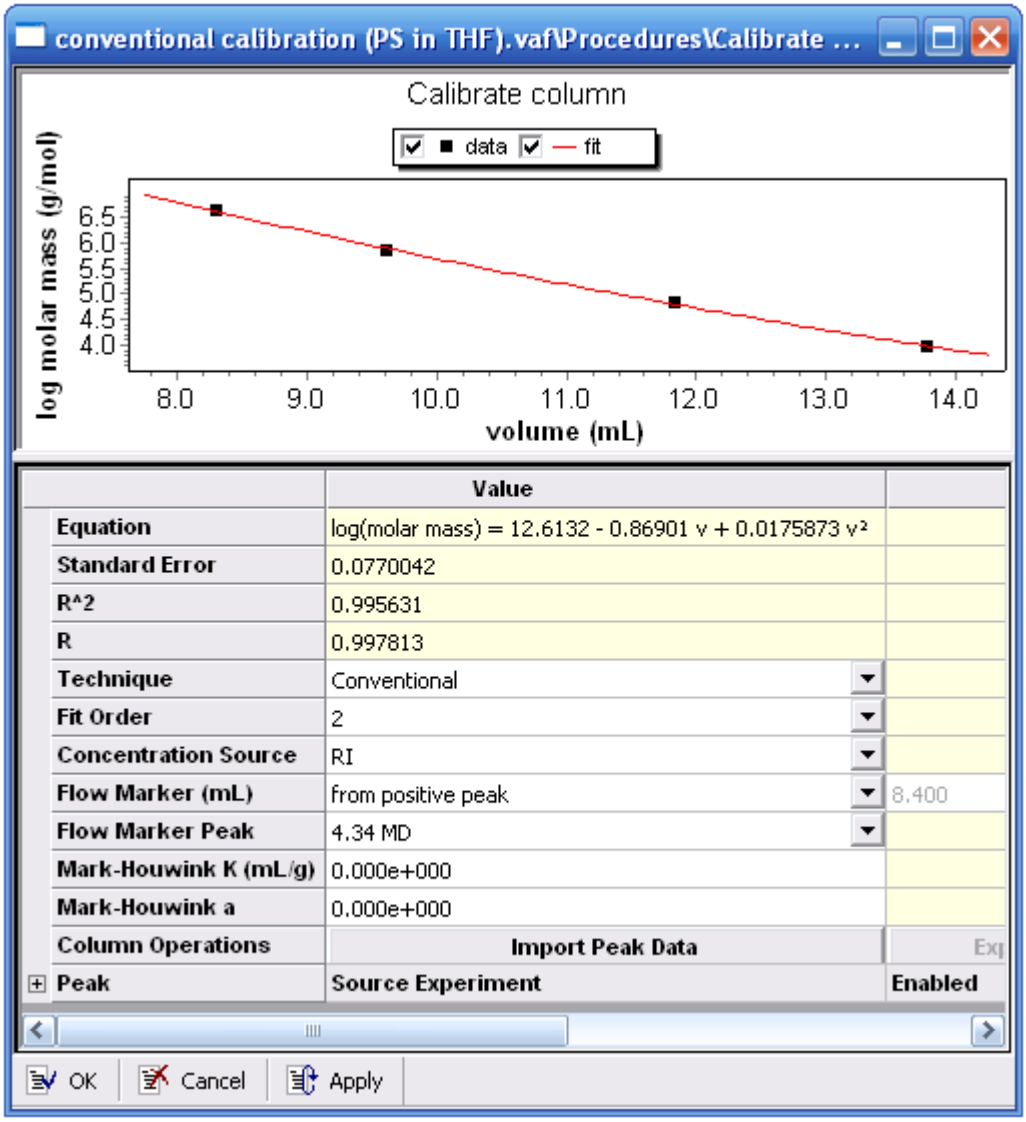
10. Confirm that the peaks have been added to the list and adjust the curve fit order. Then, click **Apply** to store the imported peaks.
11. Click **Export** in the Calibrate Column dialog to open the Save Calibrated Column Profile dialog. (You can click **Export** only after you have clicked **Apply**.)
12. Select a folder for your column profile, and type a name for the column. Click **Save** to store the profile. In future experiments, you can import this profile in the column profile (see page 7-26).

Setting Column Calibration Properties

Experiment Builder

This procedure is hidden in the Experiment tree in Run mode. However, you can still open it in Run mode by choosing **Experiment→Configuration→Calibrate Column**.

You can set properties for this procedure after you run the experiment collection. Double-click on the Calibrate column procedure to open its property dialog:



The graph shows a plot of the data and the linear regression. The red fit line provides visual feedback as to the quality of the fit.

This procedure has the following properties:

Table 8-11: Column Calibration Properties

Field	Description
Equation	Shows the resulting equation from the linear regression. The coefficients are the ones that will be stored in the Column profile.
Standard Error	Also known as the residual standard deviation, this shows the standard deviation of the observed data from the fit values. If the fit degree equals the number of degrees of freedom, this is zero (the fit is the same as the observed values).

Table 8-11: Column Calibration Properties

Field	Description
R^2	Shows the adjusted R squared value from the fit. This quantity can be used to gauge the quality of a linear fit. The closer this is to one, the better the fit. If the fit degree equals the number of degrees of freedom, this is set to zero to indicate that statistical interpretation of the results is not possible.
R	Shows the square root of the adjusted R squared value. The closer this is to one, the better the fit. If the fit degree equals the number of degrees of freedom, this is set to zero to indicate that statistical interpretation of the results is not possible.
Technique	The type of column calibration to perform. The options are Conventional, Universal with Viscometer Data, and Universal without Viscometer Data. For conventional calibration, the analyzed polymer must be the same as the polymer used for calibration. For universal calibration, the polymers may be different. Universal calibration requires either viscometer data or known values for the Mark-Houwink-Sakurada K and a coefficients.
Fit Order	You can choose linear regression up to 7th order. If the fit order exceeds the number of degrees of freedom, the graph and equation portions of the display indicate that no fit is possible.
Concentration Source	If multiple concentration instruments are present, select the one to use in determining the column calibration. You can use this field to switch between multiple concentration sources when deciding which peak to use. The setting here does not affect the setting in the “Experiment Configuration” on page 7-9.
Flow Marker (mL)	Use the drop-down menu to select whether or not a flow marker is to be set, and how. Drop-down options are as follows: - None - no flow marker is to be used. The flow marker value is set to zero. - Enter value - enter the flow marker value in mL in the cell to the right of the drop-down. - From positive peak - the flow marker will be determined from the apex of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down. - From negative peak - the flow marker will be determined from the lowest point of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down.
Flow Marker Peak	Select the peak for the flow marker if you used one.
Mark-Houwink-Sakurada K	The known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique is selected.
Mark-Houwink-Sakurada a	The known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique is selected.
Peak	Expanding this row shows the peaks in the experiment. The enabled checkbox determines which peaks to include or exclude from the fit. The molar mass used for each peak comes from the Peaks dialog. You can enter Intrinsic Viscosity for each peak here in the Calibrate Column dialog.

Transformation Procedures

The transformation procedures allow you to mark portions of the collected data or to convert the collected data in some way.

The following transformation procedure types are available:

Despiking

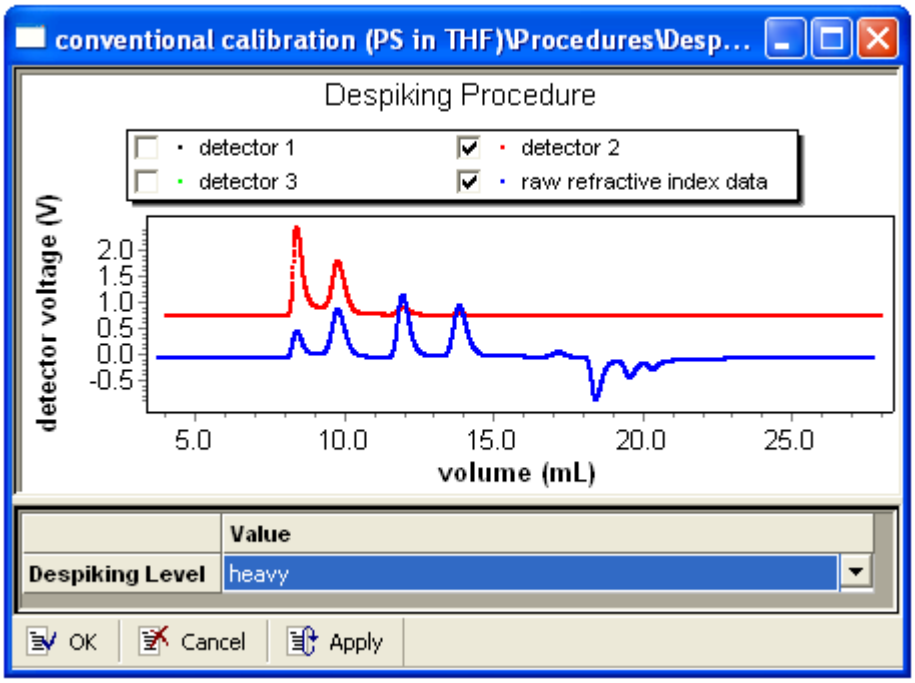
The Despiking procedure removes spurious noise spikes from the collected data. Such spikes are likely caused by dust in the solvent.

This procedure may be placed at any point in the experiment sequence before the analysis procedures that determine the final results. This procedure runs automatically without prompting for a value.

You can set the property for this procedure before running the experiment, or you can modify it after running the experiment and re-run the experiment to see the effects of changing the setting.

If your light scattering data is noisy, you may want to run one of the experiment templates provided with ASTRA for diagnostic purposes. For an experiment template choose **File→New→Experiment From Template** to open a template in the System Templates->Light Scattering ->Diagnostics folder. The "LS noise" template characterizes baseline detector noise.

Double-click on the procedure to open its property dialog:



If data has already been collected for this experiment, the graph shows the data with the currently selected despiking level applied.

The property you can set is as follows:

Table 8-12: Despiking Properties

Field	Description
Despiking Level	Choose the degree of despiking. The options are none, normal, or heavy. The default is normal.

Smoothing

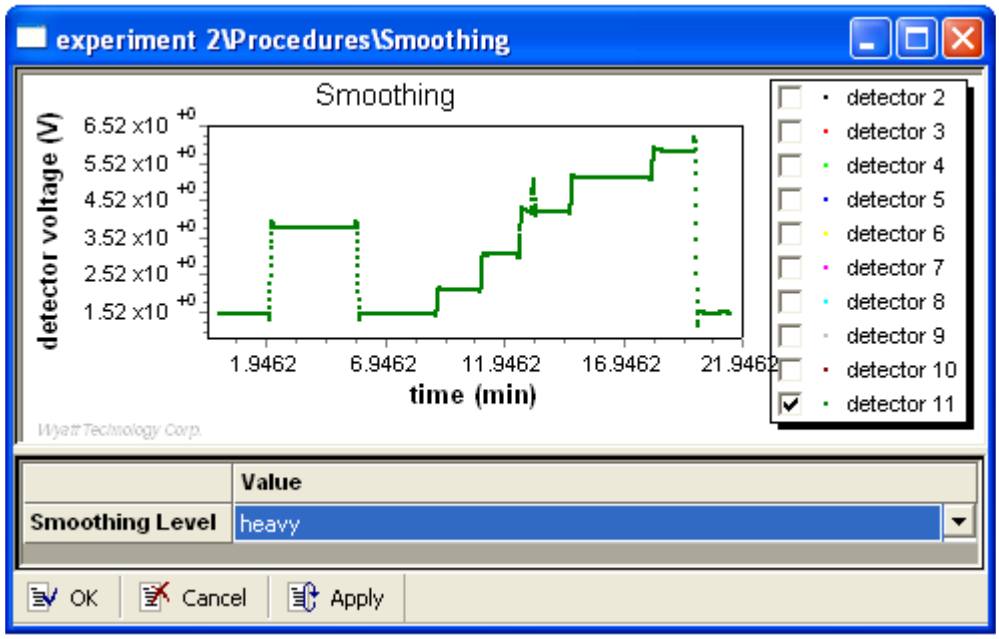
The Smoothing procedure smooths noisy data. Smoothing can be useful in certain circumstances, but in general it is better to remove the source of the noise, such as particulates in the mobile phase and pump pulsations, rather than to smooth the data.

ASTRA smooths data using the Savitsky-Golay technique of least-squares smoothing. Use this procedure with care—the height of very sharp peaks may be reduced somewhat by the smoothing process.

This procedure may be placed at any point in the experiment sequence before the analysis procedures that determine the final results. This procedure runs automatically without prompting for a value.

You can set the property for this procedure before running the experiment, or you can modify it after running the experiment and re-run the experiment to see the effects of changing the setting.

Double-click on the procedure to open its property dialog:



If data has already been collected for this experiment, the graph shows the data with the currently selected smoothing level applied.

After you change the smoothing level, you should check the baselines and peaks to make sure no changes are needed because of the smoothing.

Smoothing always acts on the raw data. You cannot increase smoothing by re-smoothing.

The property you can set is as follows:

Table 8-13: Smoothing Properties

Field	Description
Smoothing Level	Choose the degree of smoothing. The options are none, normal, or heavy. The default is none.

Note: Smoothing improves the appearance of the displayed data, but not the precision. To increase the precision of the calculated MM and radius you need to increase the signal to noise of the system by reducing the baseline noise and/or increasing the signal from the polymer.

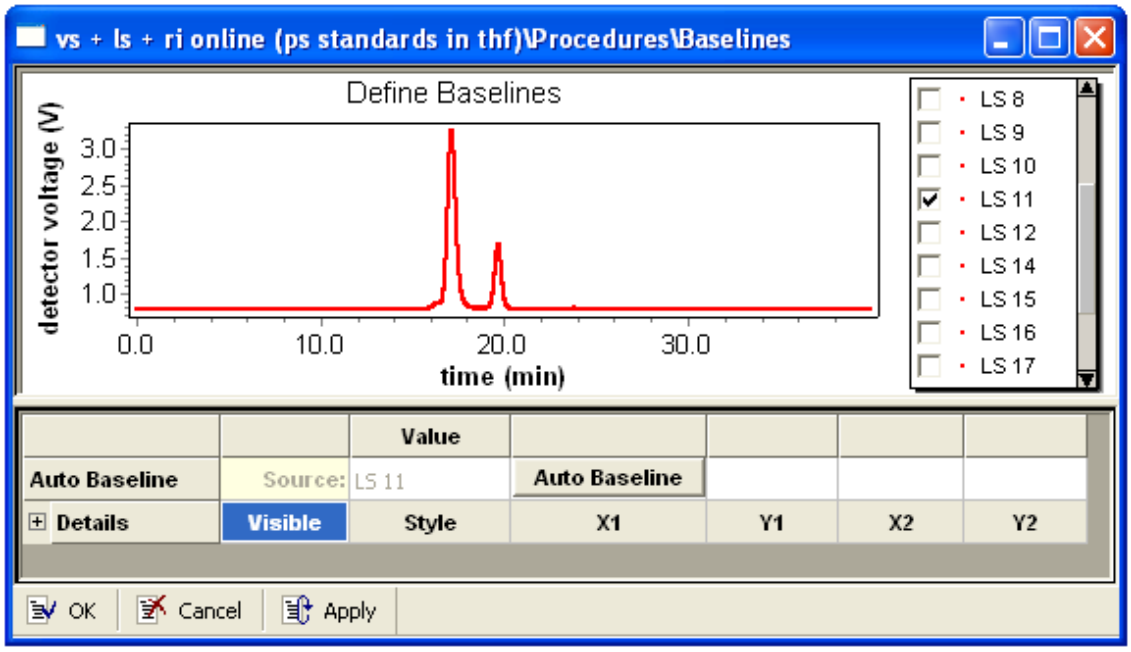
Baselines

Setting a baseline enables ASTRA to subtract the base signal from the collected data. For light-scattering experiments, the baseline level includes the photodiode dark offset and the solvent scattering.

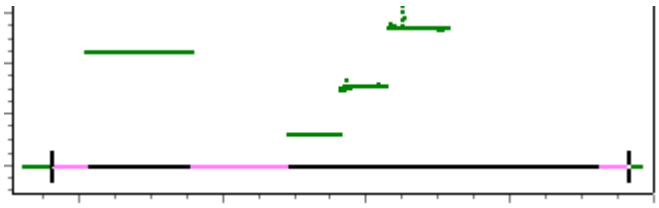
This procedure is normally placed after any despiking or smoothing you want to perform and before conversion or analysis procedures.

When this procedure runs, you see a message that says a baseline needs to be set. Set a baseline by following these steps:

1. Click **OK** to open the dialog for setting baselines.



2. In the list to the right of the graph, select the detector for which you set the baseline. (For example, detector 11.) To check the quality of your data, select different detectors from the detector list and examine the peaks.
3. Click on the graph to add a baseline to the collected data. (You can press **Delete** to remove a baseline.)
4. Use your mouse to drag the baseline ends to appropriate locations.



Set the baseline ends far enough from the peak (where the baseline is flat) so they do not interfere with the signal.

By default, baseline ends snap to the voltage level for a particular time. If you hold down the Shift key, you can drag the end of a baseline to any location. This may be useful if the collection was interrupted before the signal returned to the original baseline.

5. Click **Auto Baseline** to automatically set baselines at the same collection times for all detectors.
6. Check the automatic baseline settings by examining the baseline for each light scattering detector in turn. If necessary, you can modify the baseline for an individual detector.
7. If you are using other detectors (RI, UV, or viscometer), you should set baselines for them independently. Other detectors are affected quite differently by chromatographic details such as injection peak, pump fluctuations, and baseline stability.
8. Click **OK** to continue running the experiment.

You can see the details of the baselines selected for each detector by expanding the Details list.

You can clear all baseline settings by deleting the baseline for the source detector and then clicking the **Auto Baseline** button.

When you position a baseline, the properties set for each detector are as follows:

Table 8-14: Baseline Properties

Field	Description
Auto Baseline	Click this button to automatically set baselines based on the selected detector.
Visible	This box is checked if the data from this detector is shown in the graph.
Style	"None" indicated no baseline is set. "Snap-Y" indicates the Y value of the endpoint is calculated based on the Y value of the surrounding data points. "Manual" indicates that the X and Y endpoints are manually specified and are not taken from the Y value of the endpoint data.
X1	Shows the x-axis coordinate of the left end of the baseline for this detector.
Y1	Shows the y-axis coordinate of the left end of the baseline for this detector.
X2	Shows the x-axis coordinate of the right end of the baseline for this detector.
Y2	Shows the y-axis coordinate of the right end of the baseline for this detector.

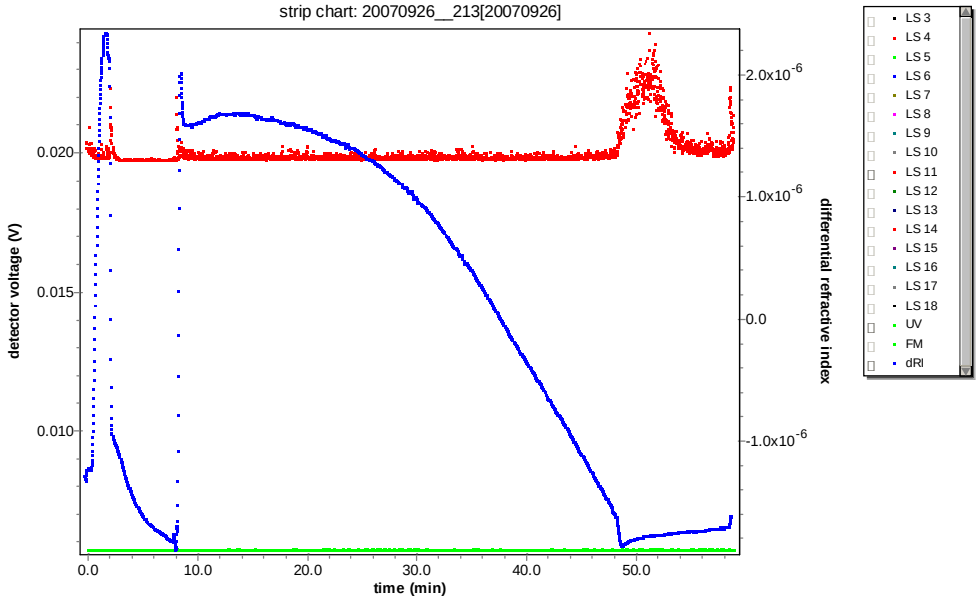
If your light scattering baseline drifts, you may want to run one of the experiment templates provided with ASTRA for diagnostic purposes. For an experiment template choose **File→New→Experiment From Template** to open a template in the System Templates->Light Scattering ->Diagnostics folder. The "LS noise" template reports baseline detector noise and drift.

Blank Baseline Subtraction

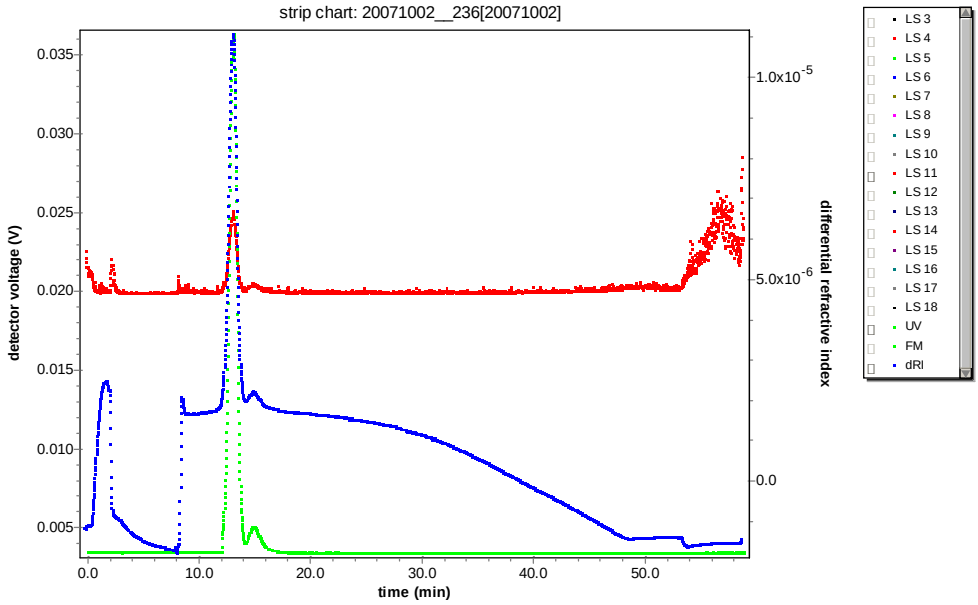
While standard baseline subtraction is useful for combining instrument data during chromatography runs, there are many cases where predetermined changes in flow rate, temperature, and other effects can cause instrument baselines to drift such that the standard linear baseline subtraction feature cannot correct for the problem.

When the baseline changes are caused by a repeatable set of conditions, such as Eclipse instrument flow adjustments, a set of "blank" data can be collected that will model these effects. By subtracting this "blank" run, we can generate well-behaved result data.

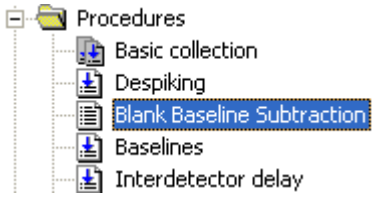
1. Collect sample "blank" data for the experiment run by running an injection without any sample. ASTRA collects data about changes in baseline conditions caused by the collection environment. This collection should have the same characteristics—duration, solvent, instruments, etc.—as the sample runs, and should be subject to the same conditions—temperature control, use of Eclipse cross-flow levels, etc. For example, this blank run shows a fluctuating dRI baseline (blue):



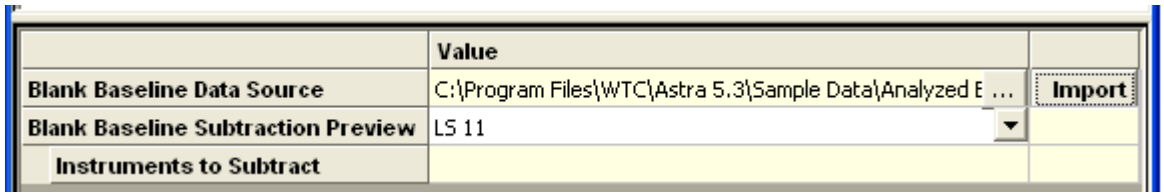
2. Run a data collection for the samples.



3. Keep both experiments open in ASTRA.
4. Right-click on the "sample" experiment, and select the **Manage->Add To Experiment** menu option:
5. Open the **Transform** category, and add the **Blank Baseline Subtraction** procedure to the experiment.
6. Drag this added procedure to position it right after the Despiking procedure.

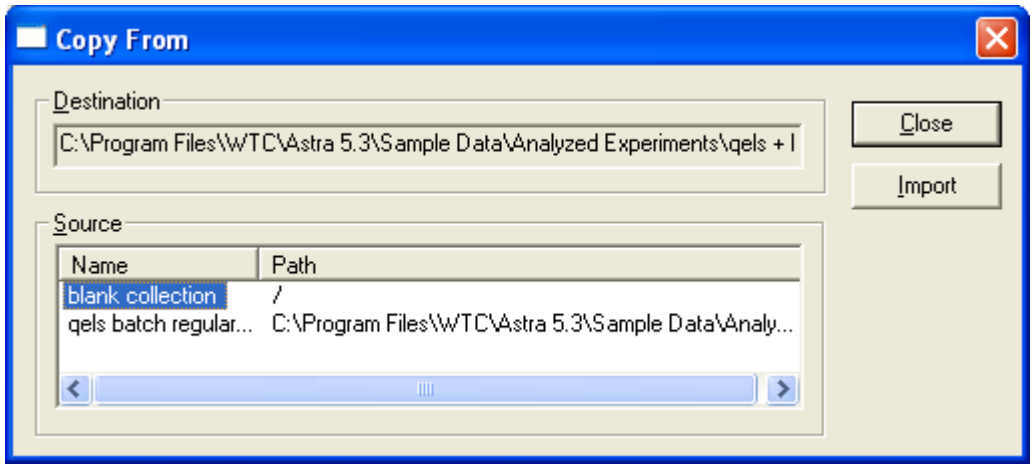


7. Open the "Blank Baseline Subtraction" procedure.

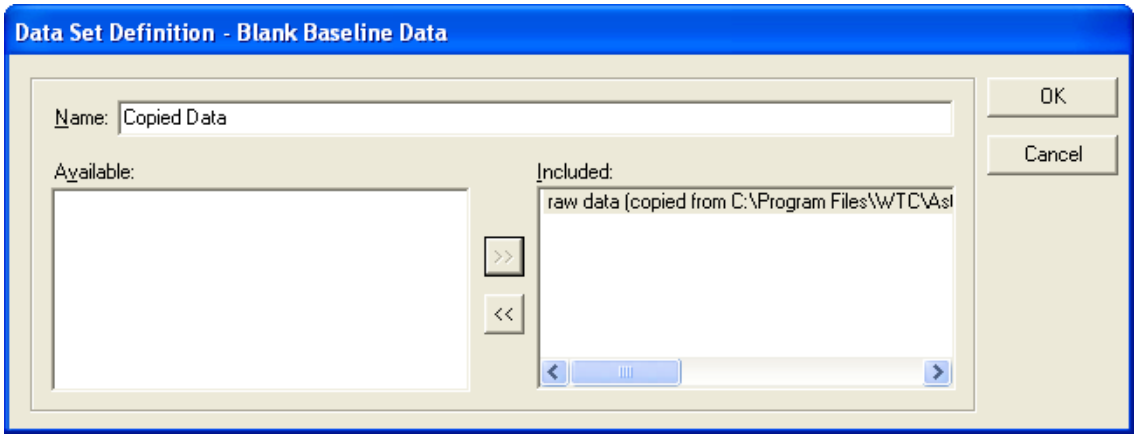


8. Click the **Import** button on the right end of the first row to open the Copy From dialog.

9. In the Source list, select the “blank” experiment and click **Import**..

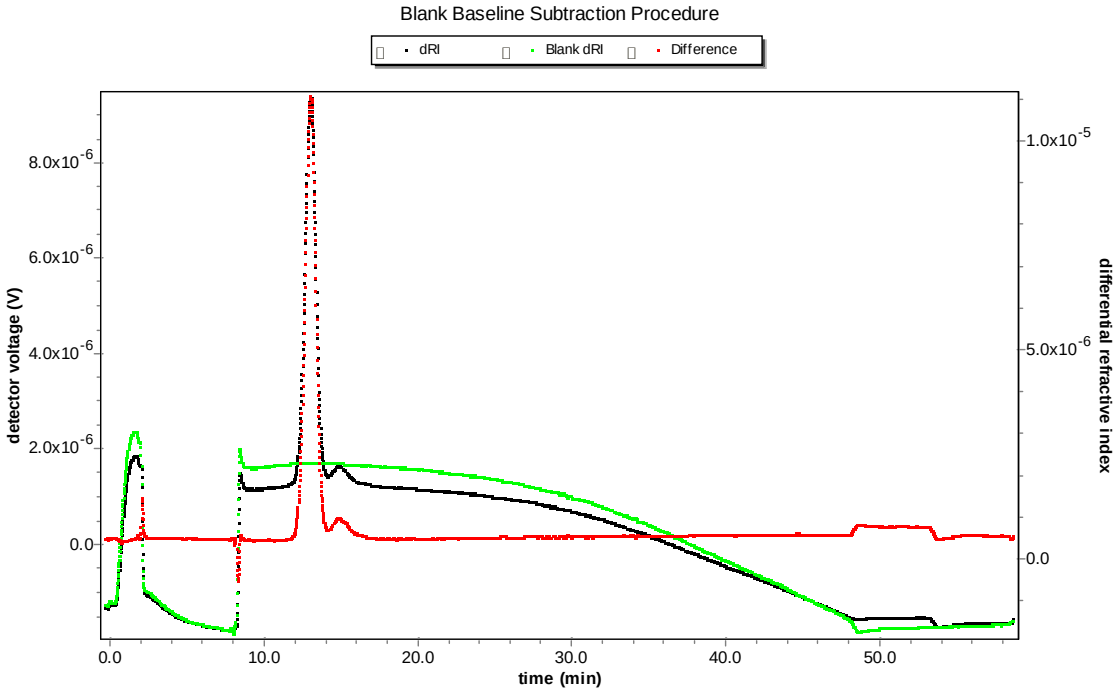


10. In the **Blank Baseline Subtraction** procedure, click the Browse ("…") button for the **Blank Baseline Data Source**. Choose one of the **Available** data sets and move it to the **Included** list. Typically, a good choice is “raw data, despiked”. Then click **OK**.



11. In the **Instrument to Subtract** list, choose which instruments should have their baseline subtracted.

12. You can then use the **Blank Baseline Subtraction Preview** to see how the blank subtraction affects the signal:



13. You can fine-tune the baseline subtraction by using the standard Baselines procedure to specify the zero-point for the signal.

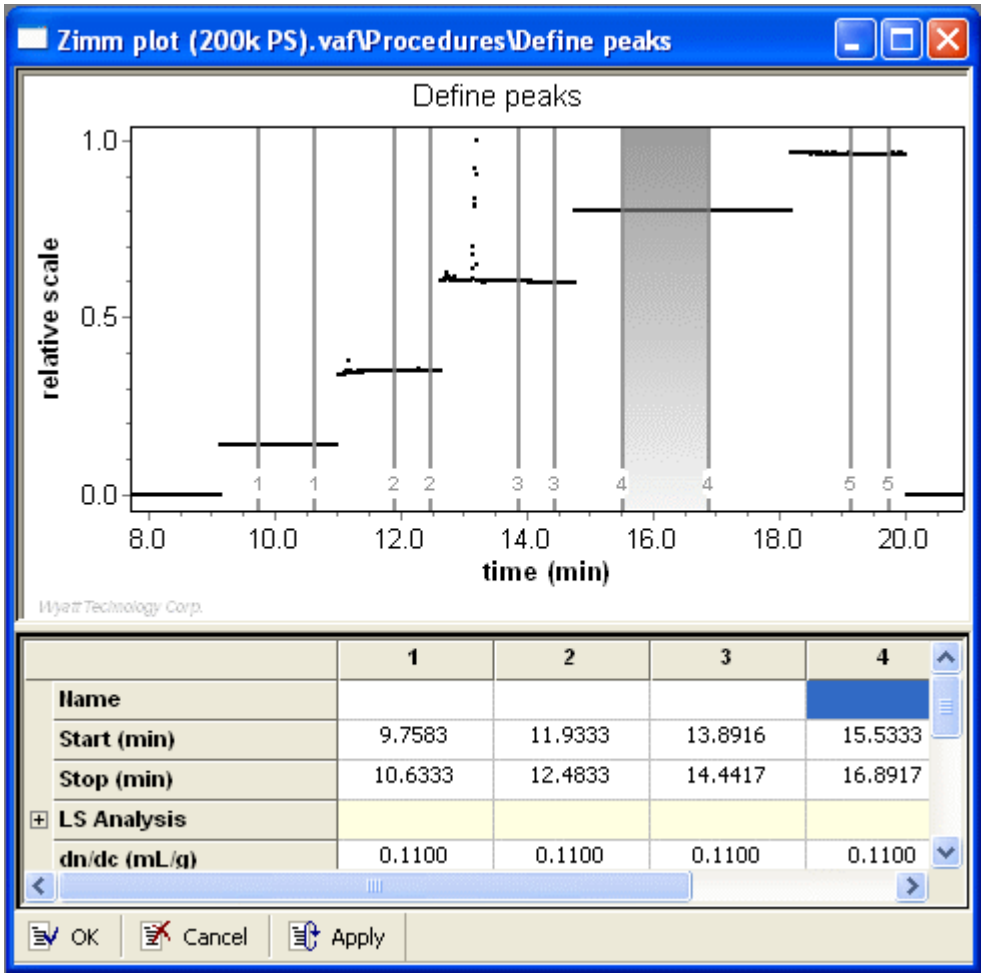
A comparison of the blank-subtracted and raw data files shows that in this case the unsubtracted sample underestimates the molar mass.

Peaks

After collecting data on your sample and setting baselines, you need to define the peak regions. This is done by marking the beginning and end point of every peak you want to include in processing.

When this procedure runs, you see a message that says peaks need to be specified. Set peaks by following these steps:

1. Click **OK** to open the dialog for setting peaks.

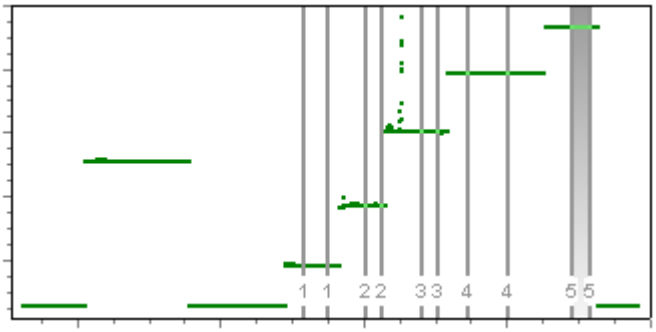


2. You can check additional detector boxes to view multiple sets of data. The colors for data shown use the following default colors: light scattering data is red, refractive index data is green, UV data is blue, viscosity data is gray, and QELS data is magenta. Multiple detector angles use colors assigned by the graphing system.
3. Click on the graph to add a peak range to the collected data.
4. Use your mouse to drag the ends of the range to appropriate locations for the leftmost peak you want to analyze.

Note: For online experiments, we recommend setting peak endpoints so that the signal-to-noise ratio for both the light scattering and concentration detectors is greater than or equal to 2. This may necessitate excluding an aggregate peak (for which there may be a strong light scattering signal but no RI signal) or a low molar mass tail of a broad distribution sample (for which the light scattering signal will be small even though the RI signal is strong).

5. Continue adding peak ranges for the rest of the collected data.

   A number is shown for each peak that corresponds to the column for that peak below the graph. Peaks are numbered in the order you create them, not necessarily from left to right. The selected peak is shaded.



Note: If you want to zoom in on the graph, hold down the Ctrl key and use your mouse drag an outline around the area you want to see. To zoom back out, hold down the Ctrl key and click your right mouse button.

6. If you selected "Forward Monitor" in the Divide by Laser Monitor field or the configuration for your light scattering instrument (see page 7-11), you should create a "peak" for pure solvent and specify the number of this peak in the "Experiment Configuration" on page 7-9. This pure solvent peak acts as a baseline for the forward laser monitor signal. If you do not specify such a peak, the average of the first ten percent of the forward laser monitor signal range is used as a baseline.
7. After setting the peaks, enter the relevant information for each peak as needed in the property list. Depending on the type of analysis to be performed, different properties need to be specified. See Table 8-15 to

determine which properties you need to specify (the procedure lists for some properties may not be complete). The list contains the following fields for each peak:

Table 8-15: Peak Properties

Field	Description
Name	A name you can give to the peak for use in reports.
Start	The x-axis starting point for the peak. If you set peaks using the graph, the Start and Stop values are set automatically. Alternately, you can type values in these fields. The units are determined by the Abscissa Units property of the experiment configuration.
Stop	The x-axis ending point for the peak. The units are determined by the Abscissa Units property of the experiment configuration.
LS Analysis>Model	Set this parameter when you perform any of the following analysis procedures: Radius from LS Data Mass and Radius from LS Data Number from LS Data Protein Conjugate Analysis The available models are: Zimm (the default), Debye, Berry, random coil, sphere, Mie, coated sphere, and rod. See “Choosing a Fit Model” on page 8-55 for details.
LS Analysis>Fit Degree	If you selected Zimm, Debye, or Berry as the fit model, you must specify the fit degree here. The fit degree default is 1. The range is 0 to 5 for the DAWN, and 0 or 1 for the miniDAWN. See “Choosing a Fit Model” on page 8-55 for details.
dn/dc	The dn/dc value for this peak. Set this parameter if you are performing any of the following analysis procedures: Mass and Radius from LS Data Differential RI Calibration UV Extinction from RI Protein Conjugate Analysis If you are performing a Protein Conjugate Analysis, this parameter corresponds to the dn/dc value for the protein.
A2	The second viral coefficient for this peak. Set this parameter if you are performing any of the following analysis procedures: Mass and Radius from LS Data Protein Conjugate Analysis **Caution:** If you enter too large a second virial coefficient the molar mass may become negative or artificially large, depending on the method used. See Appendix D, “Light Scattering Theory”.
UV Extinction	The UV extinction for this peak. Set this parameter if you are using a UV detector for concentration and are performing any of the following analysis procedures: Mass and Radius from LS Data Protein Conjugate Analysis If you are performing a Protein Conjugate Analysis, this parameter corresponds to the extinction value for the protein.

Table 8-15: Peak Properties

Field	Description
Refractive Index> Real	The real index of refraction for the peak. Set this parameter if you are performing any of the following analysis procedures: Mass and Radius from LS Data (using Mie or coated sphere) Radius from LS Data (using Mie or coated sphere) Number from LS Data If you are using the coated sphere model, this parameter corresponds to the real refractive index of the core of the coated sphere.
Refractive Index> Imaginary	The imaginary index of refraction. Set this parameter for the same analysis procedures listed for the Real Refractive Index.
Concentration	The concentration of the sample for this peak. Set this parameter if you are performing any of the following analysis procedures: A2 Mass and Radius from LS Data Dn/dc from RI Data Extinction from UV Data Mass and Radius from LS Data (batch mode with no concentration detector)
Injected Mass	The mass of the sample injected in grams. If you do not enter a value in this field and you have provided all the necessary parameters (Concentration and sample volume), ASTRA computes the Injected Mass. Alternately, you can specify the value here. Procedures that use this injected mass value account for viscometer dilution factor effects if the concentration detector is downstream from a viscometer. Set this parameter if you are performing any of the following analysis procedures: Dn/dc from Peak UV Extinction from Peak
Eta Analysis> Model	Specify the model to use for intrinsic viscosity calculations for this peak. The model may be Huggins, Kraemer, or Solomon-Gatesman. The default is Huggins. ASTRA has historically used the Huggins relation with a "Huggins Constant" of zero. For more about ETA analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Intrinsic Viscosity Models.
Eta Analysis> Huggins Constant (k')	If you select the Huggins model, you can specify a Huggins constant here to be used in the calculation. The default is zero.
Eta Analysis> Kraemer Constant (k'')	If you select the Kraemer model, you can specify a Kraemer constant here to be used in the calculation. The default is zero.
Molecular Standard	If you wish to specify a molecular standard for the peak, select the system profile for that standard from the drop down list. The values of the molecular standard parameters are then used for the peak. See Chapter 12, "Working with System Profiles" for more about creating molecular standard system profiles.
Extended Parameters> Modifier dn/dc	If you are using the Protein Conjugate Analysis procedure, specify the dn/dc value in mL/g fro the modifier.
Extended Parameters> Modifier UV Extinction	If you are using the Protein Conjugate Analysis procedure, specify the extinction coefficient in mL/(g cm) for the modifier.
Extended Parameters> Coating Thickness	If you are using the coated sphere LS model, specify the coating thickness in nm.
Extended Parameters> Coating Real RI	If you are using the coated sphere LS model, specify the real RI of the coating.
Extended Parameters> Coating Imag RI	If you are using the coated sphere LS model, specify an RI value for the coating. This RI value should be corrected for absorption.
Extended Parameters> Rod Radius	For Rod LS model calculations, ASTRA assumes that the thickness of a rod-shaped particle is insignificant (0.0 nm) compared to its length. If the thickness is significant, enter its thickness or approximate thickness in nm.

Table 8-15: Peak Properties

Field	Description
Extended Parameters> Molar Mass (g/mol)	If you are calibrating a column (or using molecular standards with known molar masses), enter the known molar mass of the molecular standard for this peak.
Extended Parameters> Intrinsic Viscosity (ml/g)	If you are performing a universal column calibration (with viscosity data), enter the known intrinsic viscosity of the molecular standard for this peak.
Extended Parameters> Mark-Houwink-Sakurada K	If you are performing viscosity-based calculations, enter the “K” fit parameter for the Mark-Houwink-Sakurada analysis for this peak.
Extended Parameters> Mark-Houwink-Sakurada a	If you are performing viscosity-based calculations, enter the “a” fit parameter for the Mark-Houwink-Sakurada analysis for this peak.

8. Click **OK** to continue running the experiment.

Choosing a Fit Model

The light-scattering fit models are as follows:

- **Zimm model:** uses the K*c/R(θ) formalism. The Zimm model should be used for molecules that have RMS radii smaller than 50 nm and that do not conform to another standard model such as random coil or sphere. The Zimm model has the advantage over the Debye model in that it often requires a lower fit degree for the same size molecule. For large (>50 nm) molecules, the Zimm model often produces a negative molar mass and should not be used.
- **Debye model:** Uses the R(θ)/K*c formalism. It gives better results over a wider range of molar mass, including the very large (greater than ~10^6 Daltons or ~100 nm RMS radius). But you may need to delete high angle detectors to improve the fit of the extrapolation since the curvature can be very large.
- **Berry model:** Uses the sqrt(K*c/R(θ)) formalism. It can be useful, in combination with deleting high angle data, when analyzing molecules with RMS radii greater than 50 nm.
- **Random coil model:** Uses the formula for a theoretical random coil molecule rather than a polynomial to fit the angular light scattering data.
- **Sphere model:** Uses the analytical formula for a sphere rather than a polynomial to fit the angular light scattering data. Use this model only with known spherical samples, such as lattices. Note that if the spheres are aggregated, this model may not fit since the aggregated particles may be of any shape.
- **Rod model:** Uses the analytical formula for a rod rather than a polynomial to fit the angular light scattering data. It is necessary to specify the rod radius when using this model.
- **Coated sphere model:** Uses the analytical formula for a coated sphere rather than a polynomial to fit the angular light scattering data. It is necessary to specify the coating thickness and core and coating refractive indices when using this model.

- **Mie model:** Uses the Mie analysis for a sphere rather than a polynomial to fit the angular light scattering data. It is necessary to specify index of refraction when using this model. If you are using the Lorenz-Mie theory (as this model does), the particle need not satisfy the criteria for Rayleigh-Debye Gans scattering. As a result, this is the most general method for analyzing spheres of any size.

See the "Determination of Molar Mass and Sizes" on page D-11 for a discussion of the fit models.

Broaden

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. It applies the terms calculated by the "Band Broadening" procedure. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

For an example experiment that corrects for band broadening, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "band broadening example (BSA).vaf experiment in the Sample Data->Analyzed Experiments folder.

Convert to Physical Units

This procedure is only visible in Experiment Builder mode. It converts instrument signals to physical units, if necessary. For example, light scattering values in volts are converted to Rayleigh ratios.

You may place this procedure in a location after the collection procedure and before the analysis procedures. Any procedures that follow this one will display detector data in physical units rather than voltages.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

Convert to Concentration

This procedure converts the refractive indexes measured by an RI instrument or UV absorbance data to concentrations. It is only visible in Experiment Builder mode. The Experiment Configuration (see page 7-9) contains a Concentration Source field that allows you to choose between RI and UV data if both are available.

You may place this procedure in a location after the collection procedure and before the analysis procedures.

If the *dn/dc* value is specified for a peak region, any procedures that follow this one display RI data as concentrations for each peak region. If the UV extinction coefficient is specified for the peak region, any procedures that follow this one will display UV data as concentrations for each peak.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

Convert Specific to Intrinsic Viscosity

This procedure converts the specific viscosity measured by a viscometer such as the ViscoStar to intrinsic viscosity using concentration data. See the *ViscoStar User's Guide* for details about the calculation that is performed.

You may place this procedure after the peaks are defined and before the analysis procedures. You must also place the "Convert to Physical Units" procedure before this procedure.

If both RI and UV concentration data were collected for this experiment, use the procedure "Convert to Concentration" on page 8-56 to specify which set of data to use for concentration calculations.

There are no properties to set for this procedure. It runs without prompting for any values. The procedure "Peaks" on page 8-51 lets you select a model to use for intrinsic viscosity calculations and any constants required by the selected model. The model may be Huggins, Kraemer, or Solomon-Gatesman.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

For more about viscometry data collection and analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Viscometry.

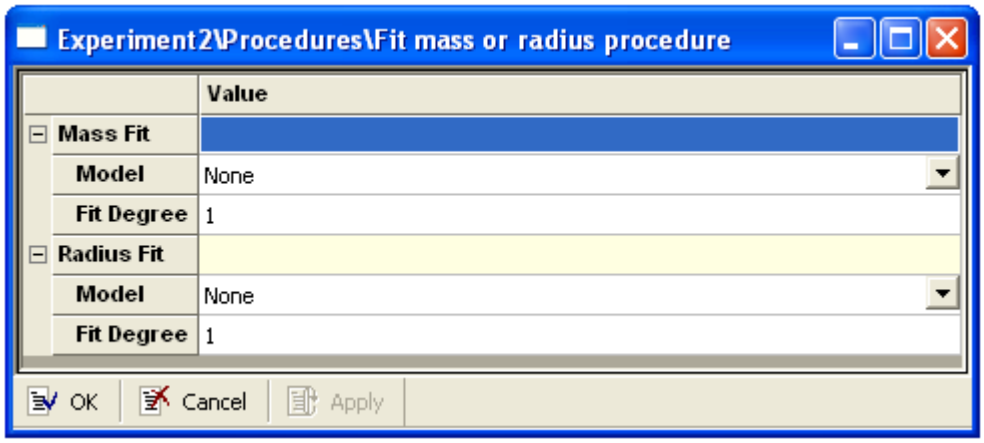
Fit Mass or Radius

You can use a curve fitting model to fit the mass and radius results.

You can set the properties for this procedure before running the experiment, or you can modify them after running the experiment and re-run the experiment to see the effects of changing the settings.

If you set a model and fit degree for the radius, the fit line is shown in graphs of RMS radius, including EASI graphs.

To set fit properties, open the dialog for the procedure.



The properties for this procedure are as follows:

Table 8-16: Fit Mass or Radius Properties

Field	Description
Mass Fit>Model	The model to use to fit the mass. Options are none (the default), polynomial, and exponential.
Mass Fit>Degree	The fit degree for the mass. The default is 1. The allowed range is 0 to 5. This field is disabled if the fit type is "None," which means no fit will be performed.
Radius Fit>Model	The model to use to fit the radius. Options are none (the default), polynomial, and exponential.
Radius Fit>Degree	The fit degree for the radius. The default is 1. The allowed range is 0 to 5. This field is disabled if the fit type is "None," which means no fit will be performed.

Fit the mass or radius data if you with to obtain more accurate peak moments and distribution results for mass and radius ranges that have significant scatter due to lower signal to noise ratios.

After choosing a fit, you must view the appropriate molar mass or radius vs. volume graph to visually determine whether the fit is acceptable or not. If the fit is not good, ASTRA reports results that are meaningless. You should try both polynomial and exponential fitting before deciding which one to use.

Analysis Procedures

The analysis procedures calculate various results using the data.

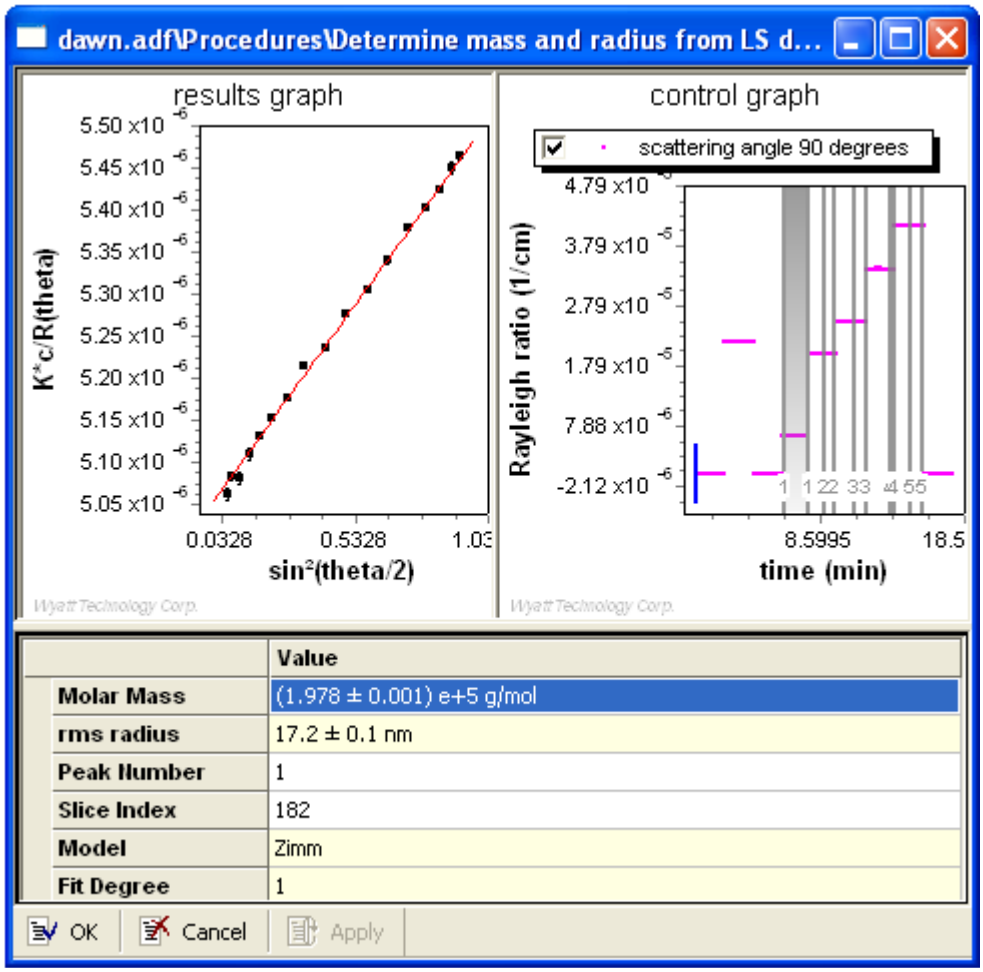
The following analysis procedure types are available:

Mass and Radius from LS Data

This procedure calculates the molar mass and RMS radius of the sample. Both light scattering and concentration data are required. For an online experiment, either an RI or UV detector provides the concentration data. For a batch measurement, the concentration can be specified for the peak ranges.

For more about light scattering analysis of molar mass, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Molar mass via light scattering.

The procedure has the following dialog:



The left graph shows the results in a Debye graph. See “About Debye Plots” on page 8-61.

The right graph shows the baseline and peaks for the selected detector.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the mass. If you place multiple methods that determine mass in a procedure, only the first one will be valid.

The properties for this procedure are as follows:

Table 8-17: Mass and Radius Properties

Field	Description
Molar Mass	Shows the calculated molar mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Peak Number	Type the number of the peak for which molar mass and RMS radius should be calculated.
Slice Index	Displays the selected slice number. alternately, you can enter the slice number for which to view results.

Table 8-17: Mass and Radius Properties

Field	Description
Model	This field shows the light-scattering model selected for this peak in the Peaks dialog. This field is display only.
Fit Degree	This field shows the fit degree selected for this peak in the Peaks dialog. This field is display only. This information is valid only if the Zimm, Debye, or Berry model has been selected for the peak.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Concentration	This field shows the concentration of this peak. This field is display only.
dn/dc	Shows the dn/dc value for this peak. This field is display only.
Enabled Detectors> 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.

About Debye Plots

The following procedure views show a Debye plot:

- “Mass and Radius from LS Data” on page 8-59
- “Number from LS Data” on page 8-69
- “Radius from LS Data” on page 8-71

Debye plots let you view the light scattering data for each slice of the peak and see the weighted least-squares fit to the data. This plot is a good place to check the appropriateness of the polynomial used for fitting. It also allows you to check visually the normalization coefficients for the DAWN.

When viewing a Debye plot, there are two separate graphs. On the right is the control graph. On the control graph, use the mouse to select a peak and slice for which the Debye plot on the left graph is to be viewed. the selected peak is highlighted, and a cursor shows the selected slice. You can use the arrow keys to scroll through the various slices.

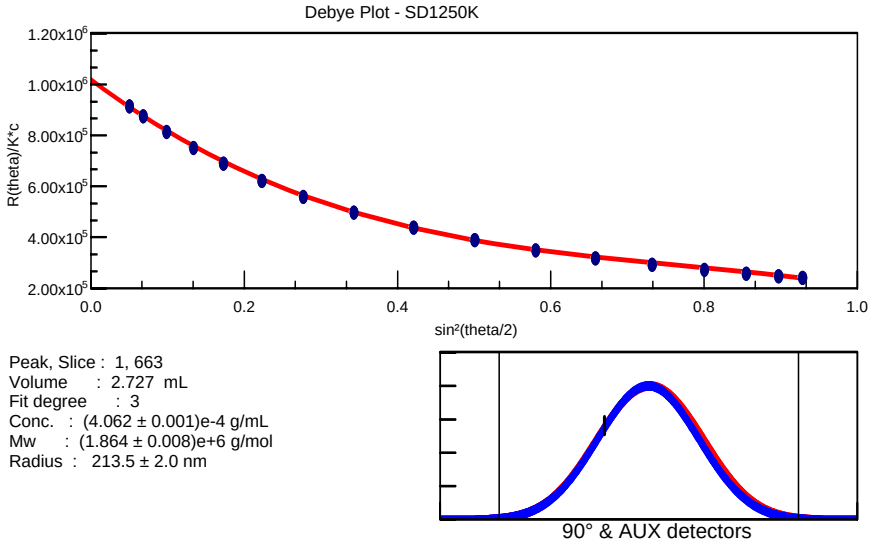


Figure 8-5: Debye plot with Fit degree of 3

Fitting the Light Scattering Data

Random coil, sphere, coated sphere, rod, and Mie models do not require a fit degree. Hence, the Debye plot can be used to assess the efficacy of the fit model, and flag noisy detectors or a poor normalization.

For the Debye, Zimm, and Berry models, the angular data is fit to a polynomial expansion. Hence it is necessary to specify a fit degree for these models. Fit degree can be set form 0 to 5.

When using the Debye, Zimm, or Berry model and determining the fit degree, it is often sufficient to choose a fit degree that gives the smallest error. As an example, compare the result in Figure 8-5 with that of Figure 8-6. The error in MM has increased. The only difference is the Fit Degree chosen for the calculation. In this example, using a Fit Degree of 2 increases the error in the data and gives biased low results. It is also obvious in Figure 8-6 that a fit degree of 2 is inappropriate due to the systematic deviations of the data with respect to the fit.

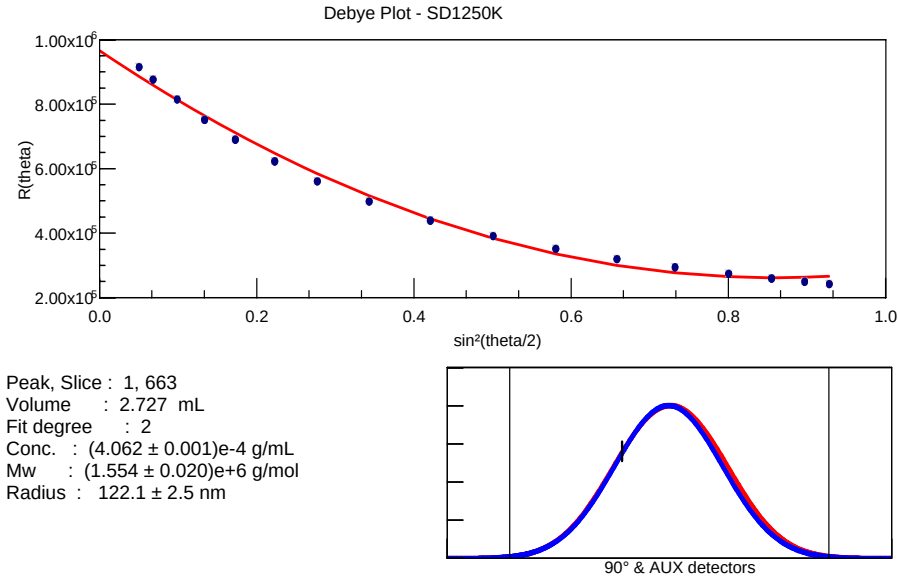


Figure 8-6: Debye plot with Fit degree of 2

Sometimes minimizing the error in the data is not a sufficient criterion. In fact, several polynomials may give very similar errors. This typically happens when you use the Debye model and have a lot of low angular curvature in the data. You must then visibly make sure that the low angle data is fit well, otherwise the fitted molar mass and radius will be incorrect.

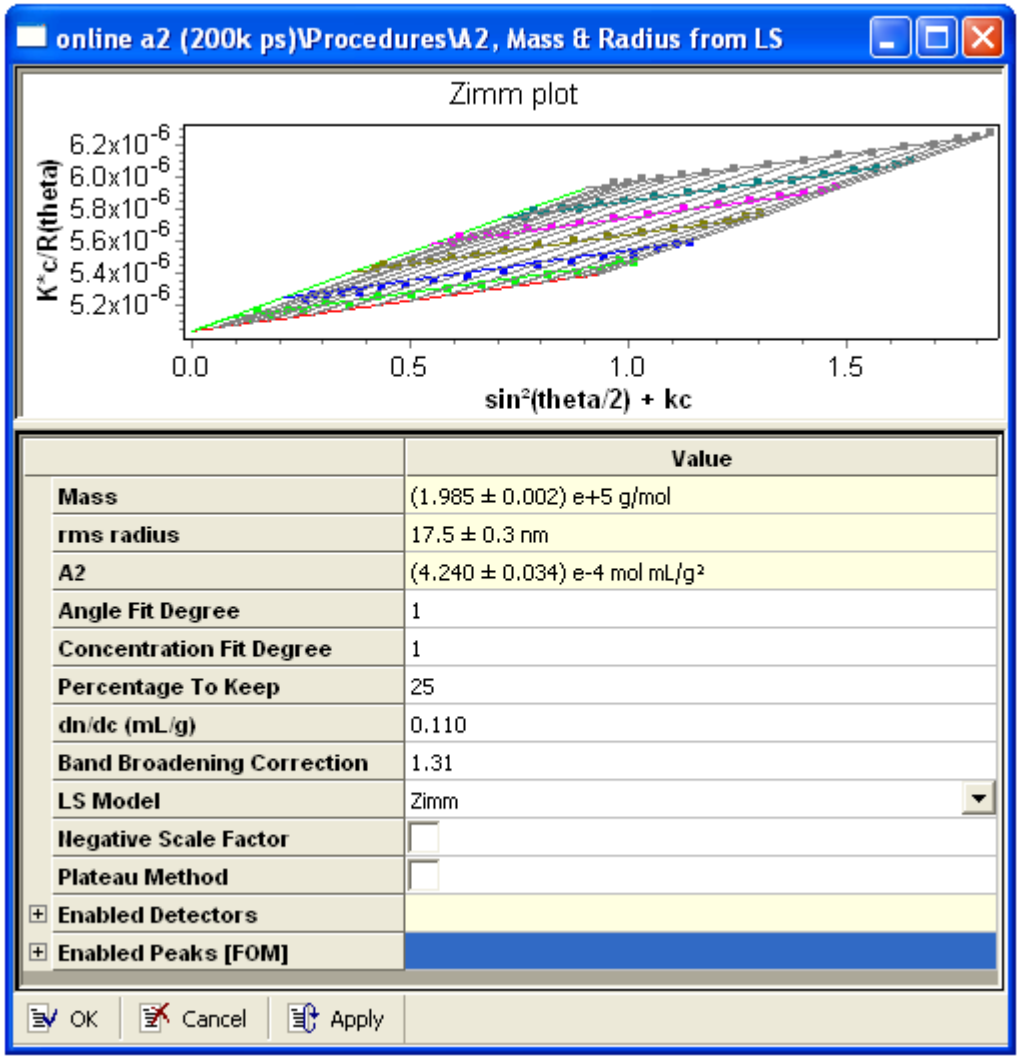
There are cases when no model accurately fits the angular curvature in the Debye plot. Typically, this is due not to a failure in the fit model, but to incomplete fractionation; the polydispersity in the sample cannot be reproduced by a model that assumes a monodisperse sample.

A2 Mass and Radius from LS Data

This procedure calculates the second virial coefficient (A_2), mass, and RMS radius of the sample based on light scattering data as a function of angle and concentration. For A_2 and A_3 calculation, see “Online A2” on page 8-65.

For more about determining the second virial coefficient, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Second virial coefficient.

The procedure has the following dialog:



By default, the graph shows the results in a Zimm plot style, although ASTRA V does not use a traditional Zimm plot analysis. Instead, a global fitting algorithm is used to present all concentration and angular data together. In the presentation, the grid represents the best fit results from the global fit. The quality of the fit can be assessed by seeing how the measured data points lie with respect to the best fit grid. The data points for each peak are shown in a different color.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the mass. If you place multiple methods that determine mass in a procedure, only the first one will be valid.

Previously, A_2 measurements could only be done in batch (plateau) mode. ASTRA now supports A_2 measurement in online mode through use of the Plateau calculation method. Use the "online A2" experiment template in the System Templates->Light Scattering folder for online A_2 measurement. A completed "online A2" experiment is also available for importing.

The properties for this procedure are as follows:

Table 8-18: A2 Mass and Radius Properties

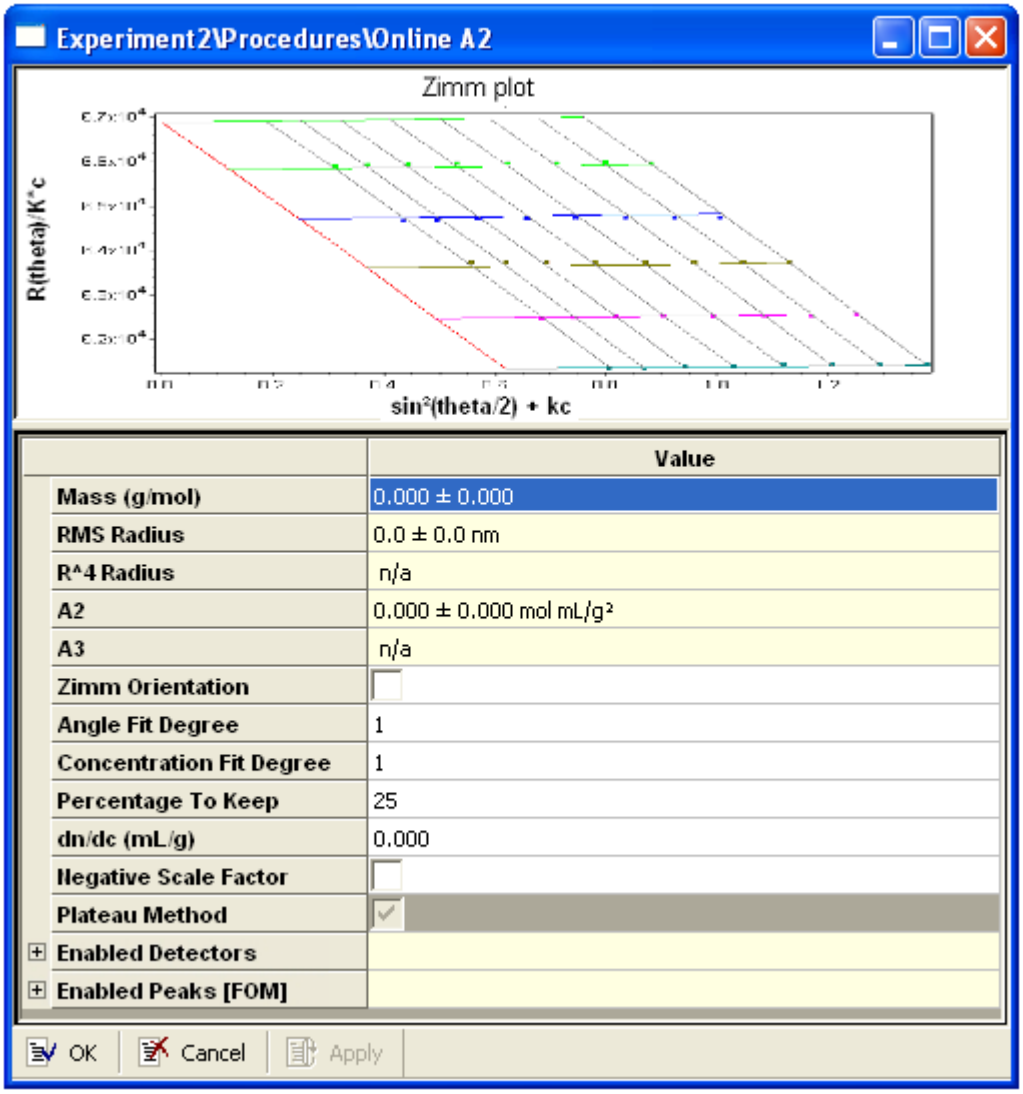
Field	Description
Mass	Shows the calculated mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
A2	Shows the calculated second virial coefficient. This field is display only.
Angle Fit Degree	The angular fit degree. May range from 0 to 5.
Concentration Fit Degree	The concentration fit degree. May range from 0 to 5.
Percentage to Keep	Type the percentage of each peak to use for calculating the A2 mass and radius. The default is 25%. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
dn/dc (mL/g)	Specify the dn/dc value for the sample. If the dn/dc value is zero, the default is taken from the sample in the configuration.
Band Broadening Correction	If you are doing online Zimm plots, specify the band broadening correction factor. The corrupting effects of band broadening boil down to a single multiplicative correction factor. You can typically measure this by performing the Plateau method for a well-understood standard. Once this factor is measured, you can use this value on all subsequent analyses to correct for the effects of band broadening.
LS Model	Choose the calculation model to use. The units for the y-axis in the graph change as appropriate for the model you select. The options are Zimm (the default), Debye, Berry, Random Coil, and Sphere.
Negative Scale Factor	Put a checkmark in this box to use a negative scale factor for the Zimm plot.
Plateau Method	Put a checkmark in this box if this is a batch experiment (or an online experiment with plateaus rather than peaks).
Enabled Detectors> 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.
Enabled Peaks [FOM]> 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [FOM] value shown for each peak is a "Figure of Merit," which is a unitless value that reflects the ability to measure A2 accurately. If the FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A2 accurately.

Online A2

This procedure calculates the second and third virial coefficients (A_2 and A_3, respectively), mass, and RMS radius of the sample based on light scattering data as a function of angle and concentration.

For more about determining the second virial coefficient, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Second virial coefficient.

The procedure has the following dialog:



By default, the graph shows the results in a Zimm plot style, although ASTRA V does not use a traditional Zimm plot analysis. Instead, a global fitting algorithm is used to present all concentration and angular data together. In the presentation, the grid represents the best fit results from the global fit. The quality of the fit can be assessed by seeing how the measured data points lie with respect to the best fit grid.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the mass. If you place multiple methods that determine mass in a procedure, only the first one will be valid.

Previously, A_2 measurements could only be done in batch (plateau) mode. ASTRA now supports A_2 measurement in online mode. Use the "online A2" experiment template in the System Templates->Light Scattering folder for online A_2 measurement. A completed "online A2" experiment is also available for importing.

The properties for this procedure are as follows:

Table 8-19: Online A2 Properties

Field	Description
Mass	Shows the calculated mass. This field is display only.
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
R^4 Radius	Shows the calculated R^4 radius. This is a physical quantity derived from the Zimm series expansion. It is similar to the RMS or geometric radius, except that it is the "fourth-root-mean-fourth-power." This field is display only.
A2	Shows the calculated second virial coefficient. This field is display only.
A3	Shows the calculated third virial coefficient. This field is display only.
Zimm Orientation	Toggle the display between the Zimm and Debye plotting formalisms.
Angle Fit Degree	The angular fit degree. May range from 0 to 2.
Concentration Fit Degree	The concentration fit degree. May range from 0 to 2.
Percentage to Keep	When performing a batch analysis, type the percentage of each peak to use for calculating the A2 mass and radius. The default is 25%. If the plateau is flat (not drifting) in the peak range, using the default value is recommended. For online analysis, this field is unused.
dn/dc (mL/g)	Specify the dn/dc value for the sample. If the dn/dc value is zero, the default is taken from the sample in the configuration.
Negative Scale Factor	Put a checkmark in this box to use a negative scale factor for the Zimm plot.
Plateau Method	Put a checkmark in this box if this is a batch experiment (or an online experiment with plateaus rather than peaks).
Enabled Detectors> 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.
Enabled Peaks [FOM]> 1 to n	You can omit peaks from the plot by removing the checkmark next to the peak number. The [FOM] value shown for each peak is a "Figure of Merit," which is a unitless value that reflects the ability to measure A2 accurately. If the FOM for a peak is less than 1 or slightly greater than 1, then the peak will help measure A2 accurately.

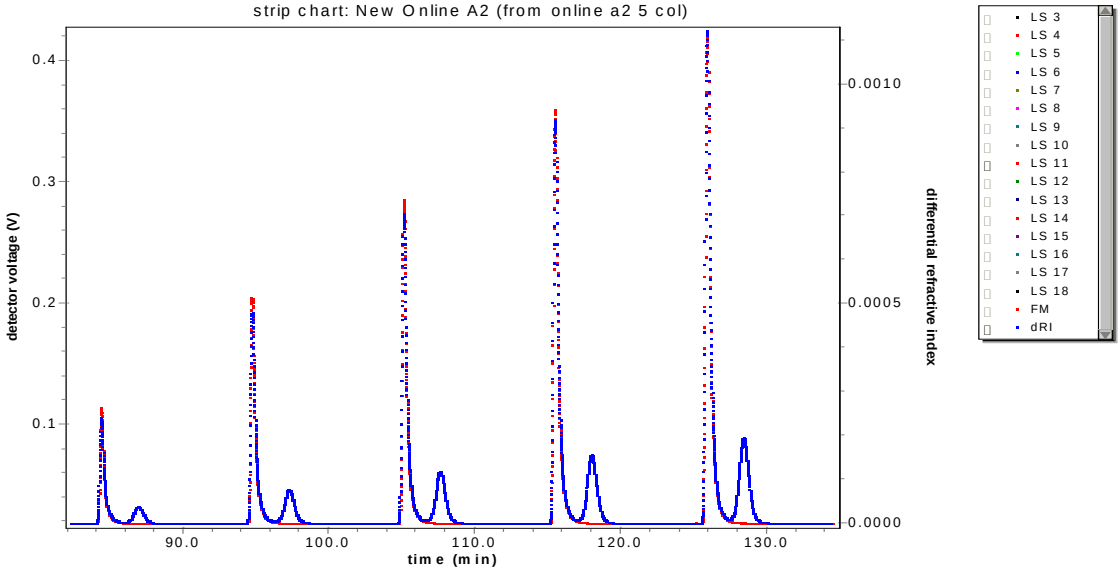
The Online A2 model is based on a series expansion of the Zimm model in concentration. It is best suited for small molecules for which the Berry model is not applicable. You cannot specify the light scattering fit model for this analysis.

Determining A2 Using the Online A2 Procedure

The Online A2 procedure extends the analysis to include the third virial coefficient (A_3) and removes the need for a band broadening correction.

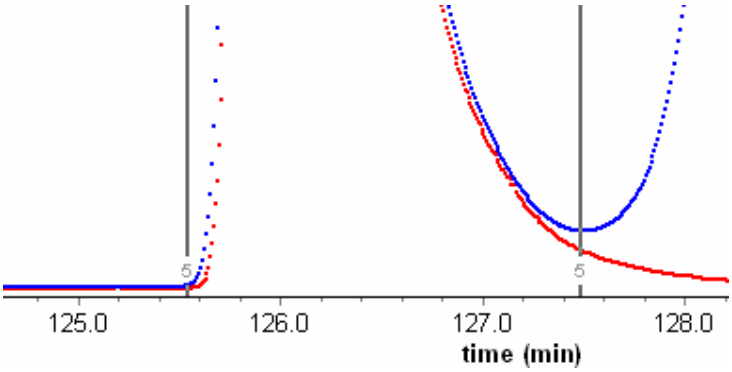
To analyzing a sample for A_2, follow these steps:

1. Create a new experiment using the System Templates->Light Scattering folder->online A2 template.
2. Adjust the configuration to reflect your light scattering and concentration detectors (the default is a HELEOS and an Optilab rEX)
3. Run a data collection consisting of a series of injections of varying concentrations. The following example shows five samples. These injections should be collected during the same analysis run.



4. Set baselines as you would normally.
5. Select peak ranges for each of the peaks. It is important to make the peaks widths, and thus the analyzed volumes, as similar as possible.
   - Since the higher concentration sample peaks may be wider, you may wish to use this as the width to set all peaks.
   - Typically, you should start the leading edge of the peak right before the signal begins to rise from the baseline.
   - Likewise, you should choose the trailing edge of the peak to correspond to either the baseline or the inflection point if the signal rises towards a secondary peak.

For example, the following peak range was selected for Peak 5 starting just before the peak rises from the baseline and ending at the point where the concentration signal passes through zero slope to start the second peak:



Note that this corresponds to a peak width of 127.48 - 125.55 = 1.93 min. You should keep the other peaks to as close to the same width as possible while still capturing the leading and trailing edges of the peak.

6. The "Online A2" procedure is now ready for processing. When you open the procedure, be sure the *dn/dc* value is set to the correct value for the sample being analyzed. If you have previously set a *dn/dc* value in the sample profile (in the experiment configuration) the number should already be filled in.

7. By adjusting the fit degree (from 0-2 for **Angle Fit Degree** and **Concentration Fit Degree**) you can change the types of results produced. The following table shows which results will be listed as "n/a" depending on your fit degree settings:

Table 8-20: Online A2 Results for Various Fit Degrees

Angle Fit Degree (Sin2(T/2))	Concentration Fit Degree	Results Excluded
0		R^4 Radius, RMS Radius
1		R^4 Radius
2		None
	0	A2, A3
	1	A3
	2	None

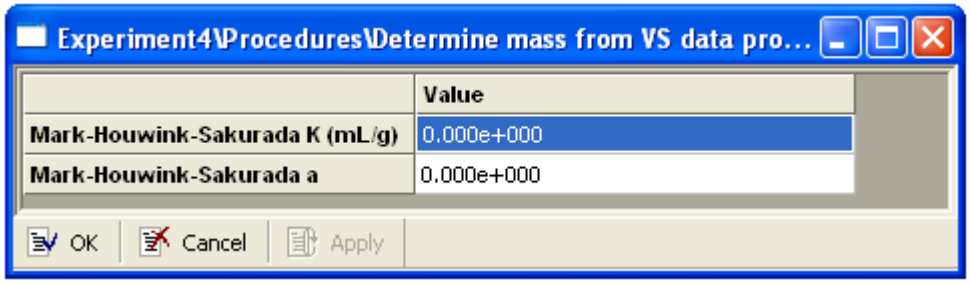
Mass from VS Data

This procedure calculates the molar mass using viscosity data It uses a Mark-Houwink-Sakurada analysis to calculate the intrinsic viscosity. See the "Operating Principles and Theory" appendix of the *ViscoStar User's Guide* for details.

You can use the Mark-Houwink-Sakurada procedure on page 8-83 to determine the appropriate Mark-Houwink-Sakurada parameter values for your polymer, solvent, and temperature combination. Then, you can use those values in this procedure to determine the mass.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the mass. If you place multiple methods that determine mass in a procedure, only the first one will be valid.

The procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-21: Mass from VS Data Properties

Field	Description
Mark-Houwink-Sakurada K	The "K" fit parameter for the Mark-Houwink-Sakurada analysis.
Mark-Houwink-Sakurada a	The "a" fit parameter for the Mark-Houwink-Sakurada analysis.

For more about viscometry data collection and analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Viscometry.

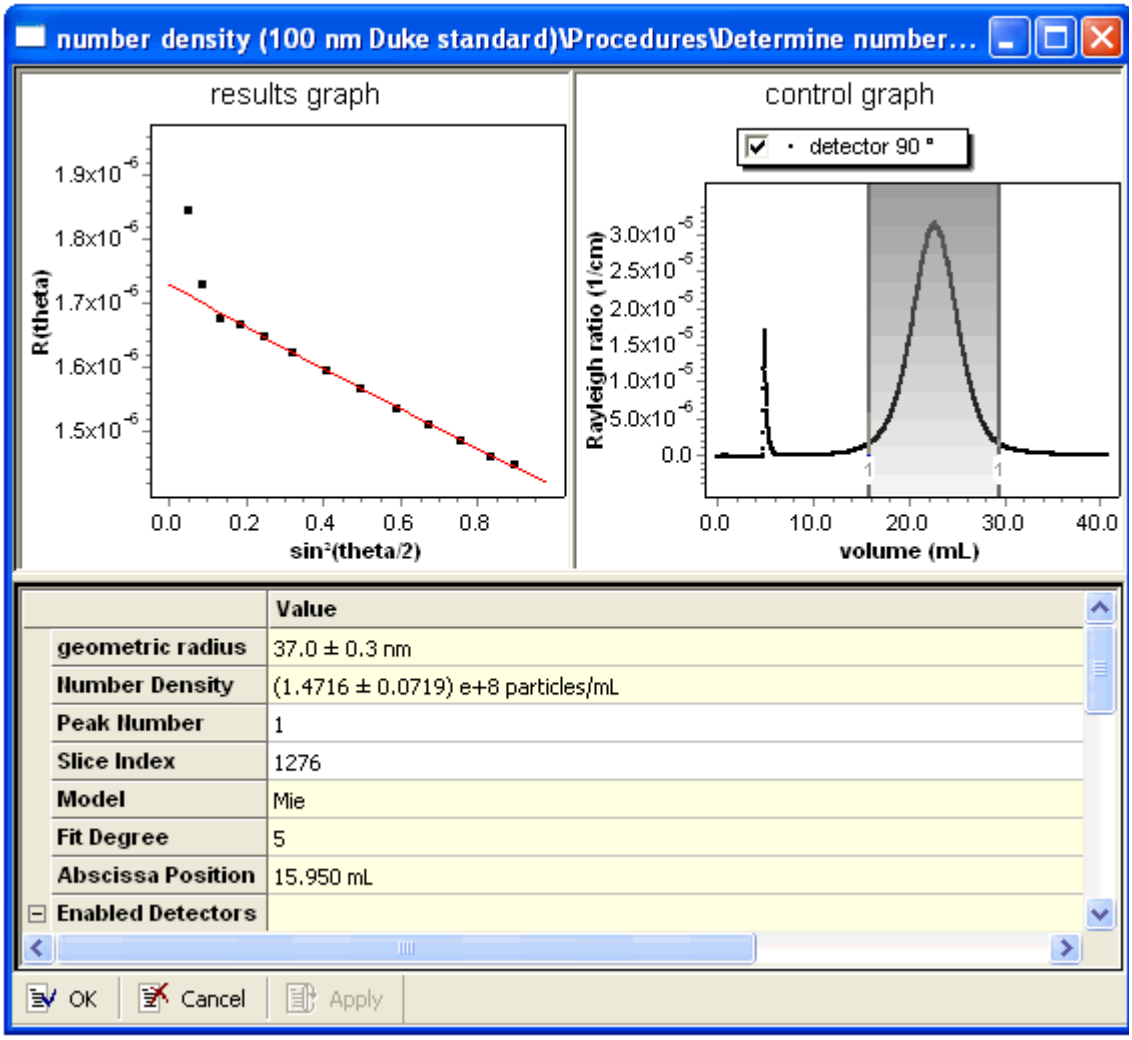
Number from LS Data

This procedure calculates the radius and the number of particles per mL (density) in the sample. It is necessary to specify the refractive index of the sample in the Peaks procedure to determine the number density. This procedure is normally used with online (fractionated) experiments.

For more about determining and using number density, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Particles and number density.

You can place this procedure with other analysis procedures, and after all the transformation procedures.

The procedure has the following dialog:



The graph display is a standard Debye plot.

The properties for this procedure are as follows:

Table 8-22: Number from LS Data Properties

Field	Description
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Number Density	Shows the number density in particles per mL. This field is display only.
Peak Number	Displays the number of the peak for the Debye plot.
Slice Index	Displays the index for the slice displayed in the Debye plot. Alternately, you can type a slice index here.
Model	This field shows the light-scattering model selected for this peak in the Peaks dialog. This field is display only.
Fit Degree	This field shows the fit degree selected for this peak in the Peaks dialog. It is valid only if the Zimm, Debye, or Berry model is selected. This field is display only.

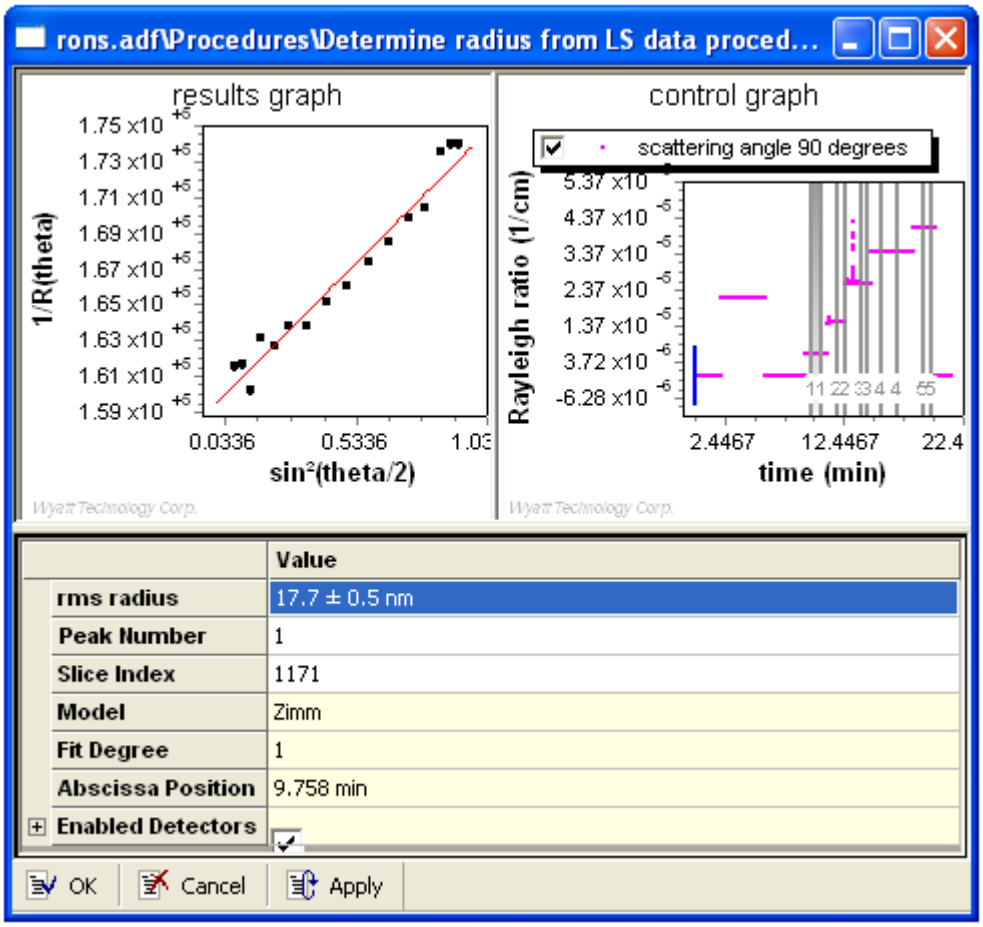
Table 8-22: Number from LS Data Properties

Field	Description
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Enabled Detectors> 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark..

Radius from LS Data

This procedure calculates the radius of the sample based on the light scattering signal alone. This procedure is normally used with online (fractionated) experiments.

The procedure has the following dialog:



You can place this procedure with other analysis procedures, and after all the transformation procedures.

The graph display is a standard Debye plot. The properties for this procedure are as follows:

Table 8-23: Radius from LS Data Properties

Field	Description
Radius (geometric or RMS)	Shows the calculated radius. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Peak Number	Displays the number of the peak for the Debye plot.
Slice Index	Displays the index for the slice displayed in the Debye plot. Alternately, you can type a slice index here.
Model	This field shows the light-scattering model selected for this peak in the Peaks dialog. This field is display only.
Fit Degree	This field shows the fit degree selected for this peak in the Peaks dialog. It is valid only if the Zimm, Debye, or Berry model is selected. This field is display only.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Enabled Detectors> 1-18	This list has a checkmark next to detectors whose data is used in the calculation. You can disable individual detectors by removing the checkmark.

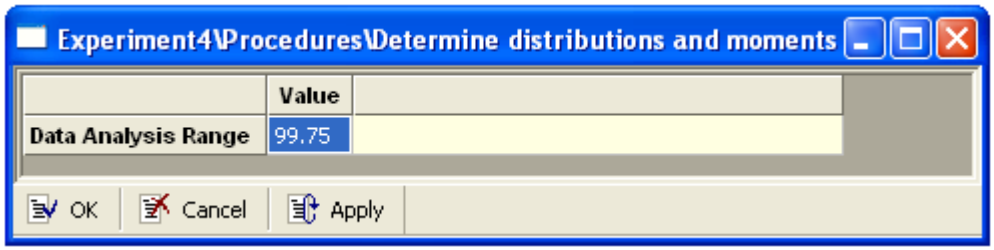
Distributions and Moments

This procedure calculates the cumulative and differential molar mass and radius distributions, as well as the moments (mass × length^2) and averages for various quantities for each peak. This procedure is normally used with online (fractionated) experiments. It is used by the "Distribution Analysis" on page 8-85.

You can place this procedure with other analysis procedures, and after all the transformation procedures.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. To see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

The procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-24: Distributions and Moments Properties

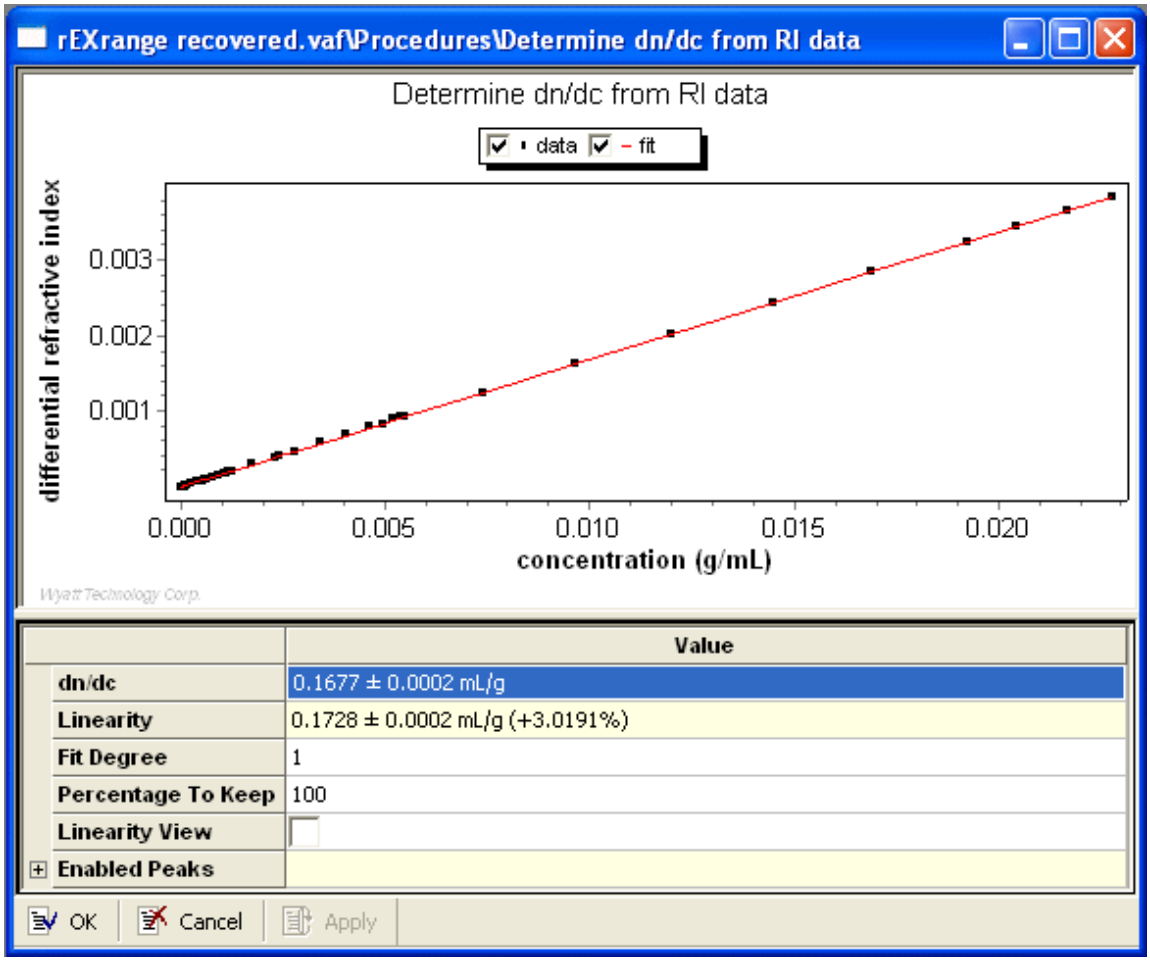
Field	Description
Data Analysis Range	Fine-tunes the amount of data used in the distribution and moments calculation. For large amounts of data, the calculations can take a long time to perform; consequently, the range is set to 99.75 by default. If you wish, you can set it to 100% to use the full range of data in the analysis.

For more about distributions, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Distributions.

Dn/dc from RI Data

This procedure calculates the dn/dc of the sample and the linearity of the result using data from an RI detector.

The procedure has the following dialog:



The graph shows a fit to the differential refractive index and concentration data.

For an example experiment that determines dn/dc from RI data, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "dndc measurement.vaf experiment in the Sample Data->Analyzed Experiments folder.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

The properties for this procedure are as follows:

Table 8-25: dn/dc from RI Data Properties

Field	Description
dn/dc	Shows the calculated dn/dc. This field is display only.
Linearity	Shows the linearity of the dn/dc result. This field is display only.
Fit Degree	Type a fit degree to use for fitting the data. The value may be either 1 or 2.
Percentage to Keep	Type the percentage of the peaks to use for calculations. By default, 100% is used. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Linearity View	Put a checkmark in this box to view the linearity plot.
Enabled Peaks> 1 to n	You can omit peaks you marked from the plot by removing the checkmark next to the peak number.

For more about determining dn/dc, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->dn/dc and UV extinction determination.

Dn/dc from Peak

This procedure calculates the dn/dc using the peak data from a batch mode run. You can use data from a batch mode RI run to calculate either the calibration constant (if dn/dc is known) or dn/dc (if the calibration constant is known). See the manual for your RI instrument for details.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

This procedure uses the injected mass value specified for the peak. The procedure adjusts for viscometer dilution factor effects if the concentration detector is downstream from a viscometer.

RI Calibration from Peak

This procedure calculates the refractive index calibration using the peak data from a batch mode run. You can use data from a batch mode RI run to calculate either the calibration constant (if dn/dc is known) or dn/dc (if the calibration constant is known). See the manual for your RI instrument for details.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the dn/dc or RI calibration. If you place multiple methods that determine dn/dc or RI calibration in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

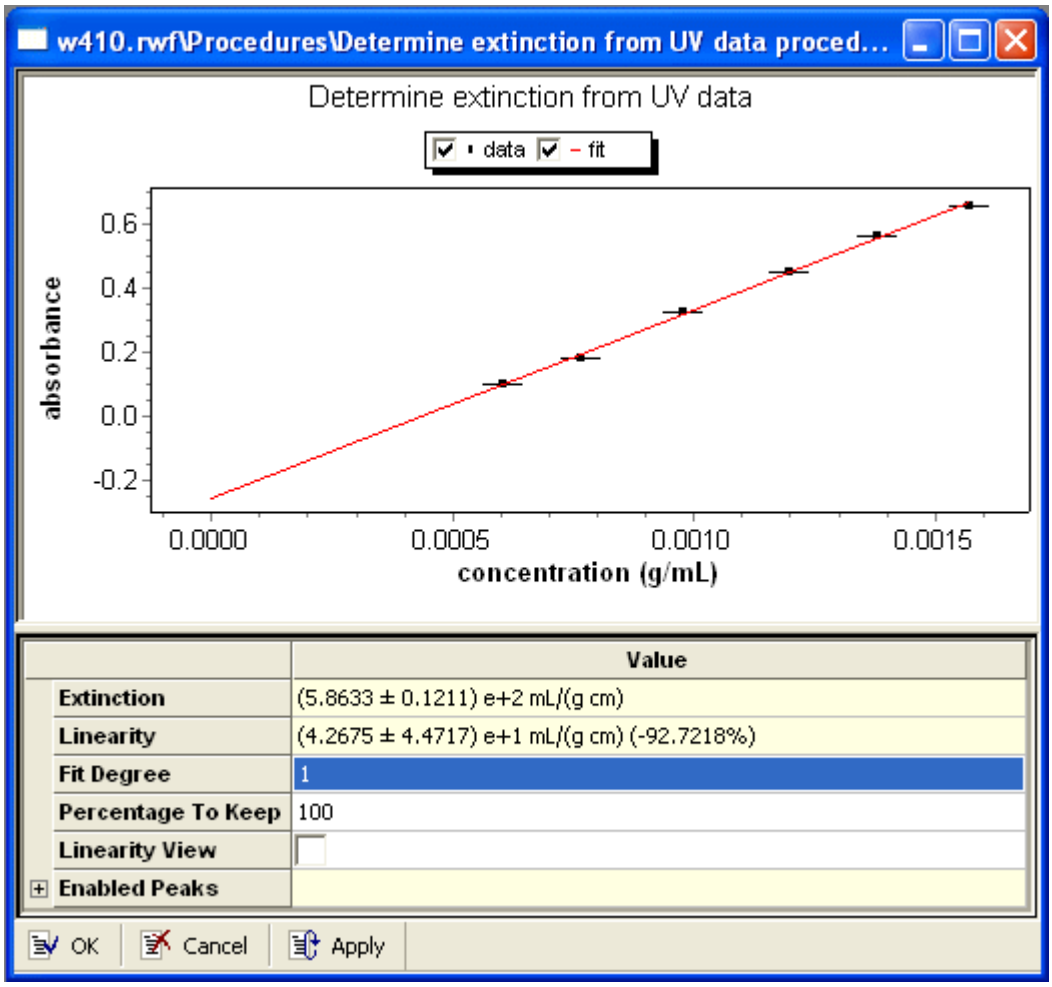
Extinction from UV Data

This procedure calculates the extinction value of the sample and the linearity of the result using data from a UV detector.

For more about determining UV extinction, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->dn/dc and UV extinction determination.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the UV extinction. If you place multiple methods that determine UV extinction in a procedure, only the first one will be valid.

The procedure has the following dialog:



The graph shows a fit to the UV absorbance and concentration data.

The properties for this procedure are as follows:

Table 8-26: Extinction from UV Data Properties

Field	Description
Extinction	Shows the calculated extinction. This field is display only.
Linearity	Shows the linearity of the extinction result. This field is display only.
Fit Degree	Type a fit degree to use for fitting the data. The value may be either 1 or 2.
Percentage to Keep	Type the percentage of the peaks to use for calculations. By default, 100% is used. If the plateau is flat (not drifting) in the peak range, using the default value is recommended.
Linearity View	Put a checkmark in this box to view the linearity plot.
Enabled Peaks> 1 to n	You can omit peaks you marked from the plot by removing the checkmark next to the peak number.

UV Extinction from Peak

This procedure calculates the ultra-violet extinction using peak data.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the UV extinction. If you place multiple methods that determine UV extinction in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

This procedure uses the injected mass value specified for the peak. The procedure adjusts for viscometer dilution factor effects if the concentration detector is downstream from a viscometer.

UV Extinction from RI

This procedure calculates the ultra-violet extinction using the RI data.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines the UV extinction. If you place multiple methods that determine UV extinction in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

For more about determining UV extinction, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->dn/dc and UV extinction determination.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

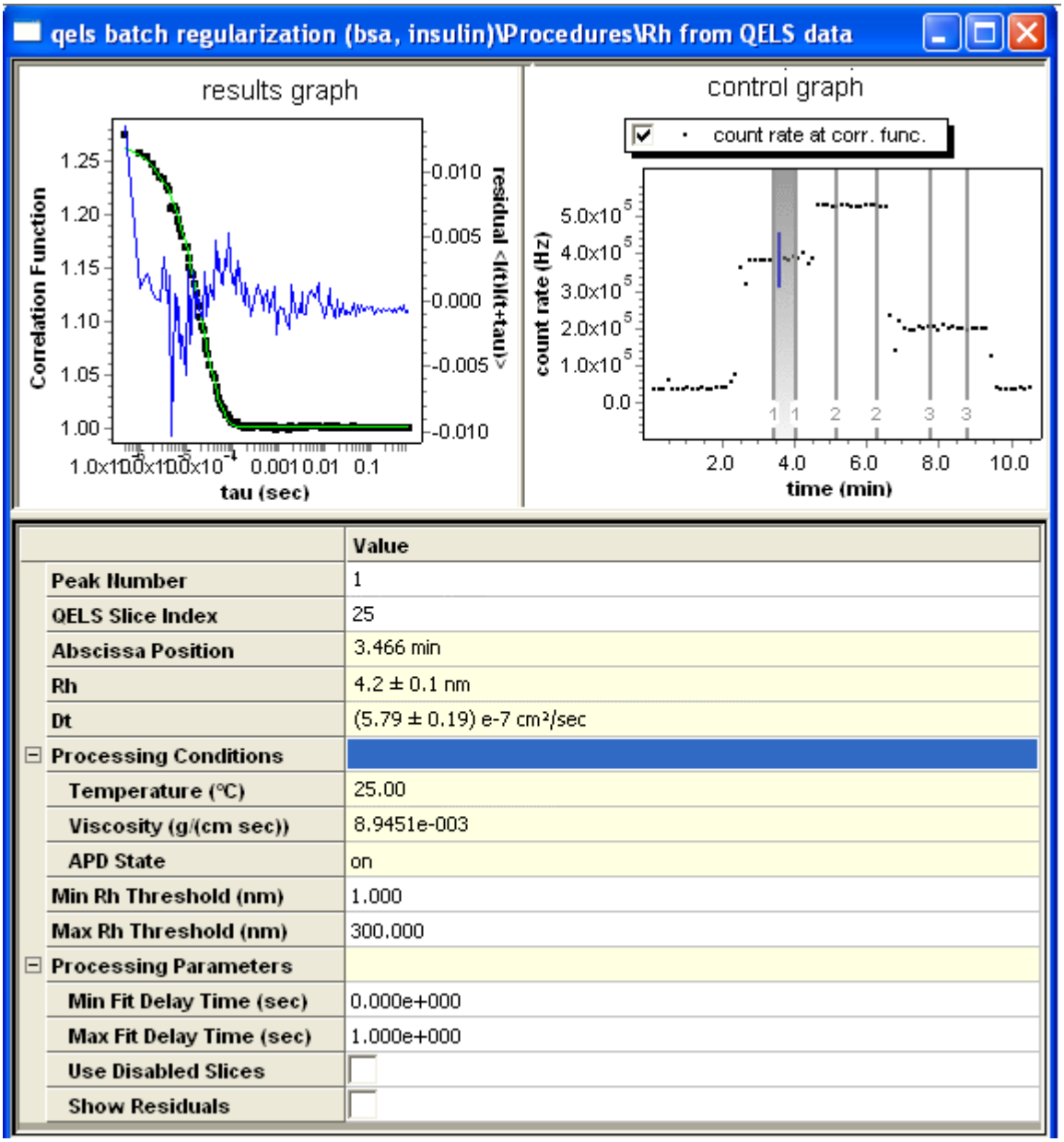
Rh from QELS Data

This procedure calculates the translational diffusion (Dt) and hydrodynamic radius (Rh) using QELS data. You can use this procedure if your experiment configuration uses the Wyatt QELS option in online mode. If you use QELS in batch mode, see “Regularization” on page 8-90.

For more about analyzing online QELS data, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Online QELS.

For an example experiment that determines Rh from QELS data, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "band broadening example (BSA).vaf" experiment in the Sample Data->Analyzed Experiments folder. For an experiment template choose **File→New→Experiment From Template** to open a template in the System Templates->Light Scattering->With QELS folder.

The procedure has the following dialog:



The graph is used to show the fit to the QELS correlation function. Use the graph on the right to select a peak and slice. The left graph shows the correlation function and fit results for the peak and slice you select. The quality of the fit can be determined from the left graph.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines Rh. If you place multiple methods that determine Rh in a procedure, only the first one will be valid.

Data points excluded from the calculations by the max and min properties are shown in red. QELS data collection and analysis tolerates gaps that may occur in QELS data collection due to instrument problems.

The properties for this procedure are as follows:

Table 8-27: Rh from QELS Data Properties

Field	Description
Peak Number	The peak number of the displayed correlation function.
QELS Slice Index	Displays the slice number for the correlation function. You may type a slice number here.
Abscissa Position	This field shows the position on the x-axis for the peak and slice selected. This field is display only.
Rh	Shows the calculated hydrodynamic radius (Rh) value. This field is display only.
Dt	Shows the calculated translational diffusion (Dt) value. This field is display only.
Processing Conditions	
->Temperature	Shows the temperature at which this slice was collected. This field is display only.
->Viscosity	Shows the viscosity at this slice index.
->APD State	Shows the state of the avalanche photo diode (APD) in the WyattQELS detector. If the QELS count rate exceeded the APD protection threshold, it is automatically turned off. This field displays the state of the APD for the correlation function. If the APD was off, the correlation function is probably not valid, and is by default excluded from the analysis. This field is display only.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis
Processing Parameters	
->Min Fit Delay Time	Data with a lower delay time than the value you type is not used in the fit to the correlation function.
->Max Fit Delay Time	Data with a higher delay time than the value you type is not used in the fit to the correlation function.
->Use Disabled Slices	Check this box if you want to include disabled slices (that is, slices for which the APD was turned off) in the calculation.
->Show Residuals	Check this box if you want to show residuals from the fit to the QELS correlation function.
->ACF Display Binning	Adjust the number of auto-correlation function bins to display. By default, data is shown as displayed by the correlator board. If you choose “heavy” a larger number of points is shown, which smooths the signal.

Rh from VS Data

This procedure calculates the hydrodynamic radius (Rh) using the viscosity data. You can use this procedure if your experiment configuration includes a viscometer.

You can place this procedure with other analysis procedures, and after all the transformation procedures. A procedure sequence can contain only one procedure that determines Rh. If you place multiple methods that determine Rh in a procedure, only the first one will be valid.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

For more about viscometry data collection and analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Viscometry.

Branching

This procedure calculates the branching ratio between a linear and branched polymer sample. This analysis is based on the fact that at a constant molar mass the molecular size decreases with increasing degree of branching.

Branching can be characterized by either the radius-based branching ratio (g), which is defined as the ratio of the mean square radius of a branched and linear molecule, or the intrinsic viscosity-based branching ratio (g'), which is defined as the ratio of the intrinsic viscosities of a branched and linear molecule. In both cases, the linear and branched molecules must have the same molar mass. The two types of branching ratios are related by a term called the “drainage parameter” (e).

Linear Models

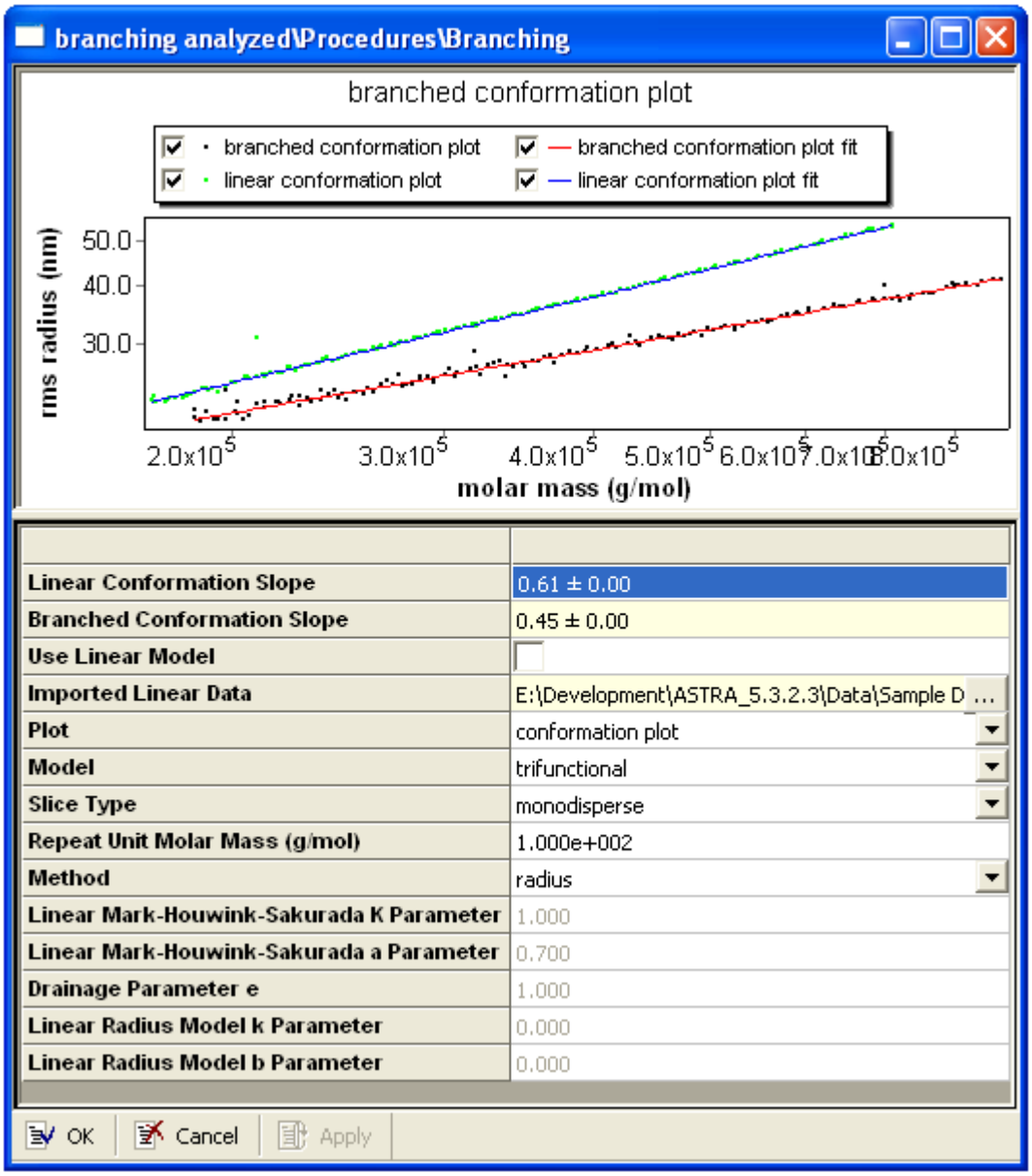
Branching analysis requires the comparison of a branched sample to a linear reference of the same molar mass. The linear reference data can be obtained by performing a collection for a sample with the requisite molar mass, and then importing the data into the branching procedure. However, in many cases, it is sufficient to use an ideal linear model for comparison.

A linear model is used when the “Use Linear Model” check box is enabled. Depending on the type of analysis and instruments in the experiment, you can use a model based on radius data or intrinsic viscosity. The models may be based on measurements made in earlier experiments where a confirmation or Mark-Houwink-Sakurada plot were created, or from published values.

- **Radius Model:** The radius model is specified using a slope (k) and offset (b) for a conformation plot of RMS radius as a function of molar mass.
- **Viscosity Model:** The viscosity model is specified using the Mark-Houwink-Sakurada K and a parameters.

For an example experiment that determines Rh from QELS data, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "branching analyzed.vaf" experiment in the Sample Data->Practice Experiments->Branching folder.

The procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-28: Branching Properties

Field	Description
Linear Conformation Slope	The slope of the linear conformation plot fit line. This is calculated from the graph, and is not settable.
Branched Conformation Slope	The slope of the branched conformation plot fit line. This is calculated from the graph, and is not settable.
Use Linear Model	Select this check box to use a linear model (rather than imported data) to perform the analysis.
Imported Linear Data	Select the data to use for the linear sample. Choose **Experiment→Copy From** in the menus to copy the linear data into the experiment. Then, click "**...**" to select the copied data for the branching procedure. The data you select is used if the "Use Linear Model" box is unchecked.
Plot	Select the type of plot to view.
Model	Select either trifunctional, tetrafunctional, star, or comb branching.
Slice Type	Select monodisperse or polydisperse. Note that the star and comb branching models require a monodisperse slice type.
Repeat Unit Molar Mass	Specify the repeat unit molar mass for long chain branching in g / mol.
Method	Select radius, mass, or viscosity as the method. If you select mass, you must also specify the Linear Mark-Houwink-Sakurada "a" and Drainage parameters. If the current experiment does not have a light scattering detector, the "radius" option is not available. Likewise, if a viscometer is not present, the "viscosity" option is not available.
Linear Mark-Houwink-Sakurada K Parameter	Specify this parameter if you chose the viscosity method and checked the "Use Linear Model" box.
Linear Mark-Houwink-Sakurada a Parameter	Specify this parameter if you chose the mass or viscosity method and checked the "Use Linear Model" box.
Drainage Parameter e	Specify this parameter if you chose the mass method.
Linear Radius Model k Parameter	Specify this parameter if you chose the radius or mass method and checked the "Use Linear Model" box.
Linear Radius Model b Parameter	Specify this parameter if you chose the radius or mass method and checked the "Use Linear Model" box.

For more about branching analysis, see "Branching Calculations" on page D-19 and go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis-> Branching calculations.

Parametric Plot

The parametric plot procedure generates a new data set for two different types of x-y data that share the same x-axis. For example, you can use this procedure to create a plot of RMS radius vs. molar mass.

The properties for this procedure are as follows:

Table 8-29: Parametric Plot Properties

Field	Description
X Data	Choose the data that will be used for the x-axis of the parametric plot.
Y Data	Choose the first data set that will be used for the y-axis of the parametric plot.
Y Data2	Optionally choose a second data set to be used for the y-axis of the parametric plot.

See “Creating Data Set Definitions” on page 11-8 for information about the dialog that appears when you click the “...” button for either the X Data or the Y Data property.

Peak Areas

This procedure calculates the area under the defined peaks. If you use the peak_areas.html report template, your report will contain the values calculated by this procedure. These values are peak area,% peak area, and retention time.

You can place this procedure after the Peaks procedure.

There are no properties to set for this procedure. It runs without prompting for any values.

Experiment Builder This procedure is hidden in Run mode. It is performed automatically as part of certain procedures. If you want to see this procedure, enable Experiment Builder mode by choosing **System→Preferences→ Experiment Builder Mode**.

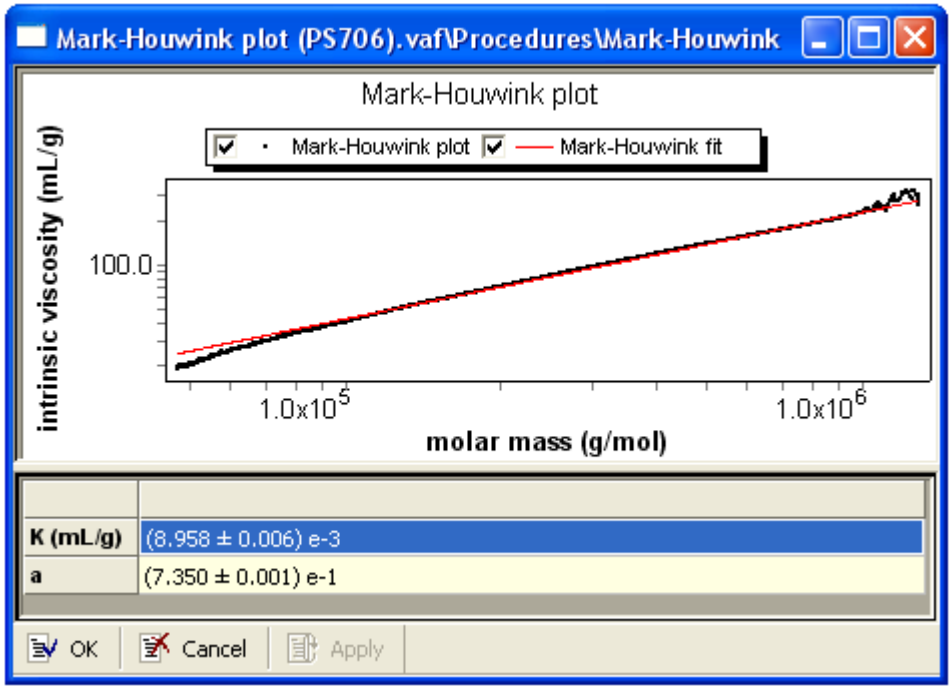
Mark-Houwink-Sakurada

This procedure calculates the Mark-Houwink-Sakurada K and a fit parameters using viscosity data. If you use one of the Mark-Houwink-Sakurada report templates, your report will contain the values calculated by this procedure.

For an example experiment that uses the Mark-Houwink-Sakurada procedure, choose **File→Open→Experiment** (or **File→Import→ Experiment** if you are using ASTRA V with Research Database) and open the “Mark-Houwink-Sakurada plot (PS706).vaf” experiment in the Sample Data->Analyzed Experiments folder. For an experiment template choose **File→New→Experiment From Template** to open the “online” template in the System Templates->Viscometry->With Light Scattering folder.

You can place this procedure at the end of the procedure sequence. This procedure can be used in the same experiment as "Distribution Analysis" and other analysis procedures.

The procedure has the following dialog:



The diagram shows the fit for the Mark-Houwink-Sakurada plot. The procedure selects K and a parameters to make the red line as close to straight as possible. The values of the K and a parameters vary depending on the polymer, solvent, and temperature.

The properties for this procedure are as follows:

Table 8-30: Mark-Houwink-Sakurada Properties

Field	Description
K	The resulting "K" fit parameter for the Mark-Houwink-Sakurada analysis.
a	The resulting "a" fit parameter for the Mark-Houwink-Sakurada analysis.

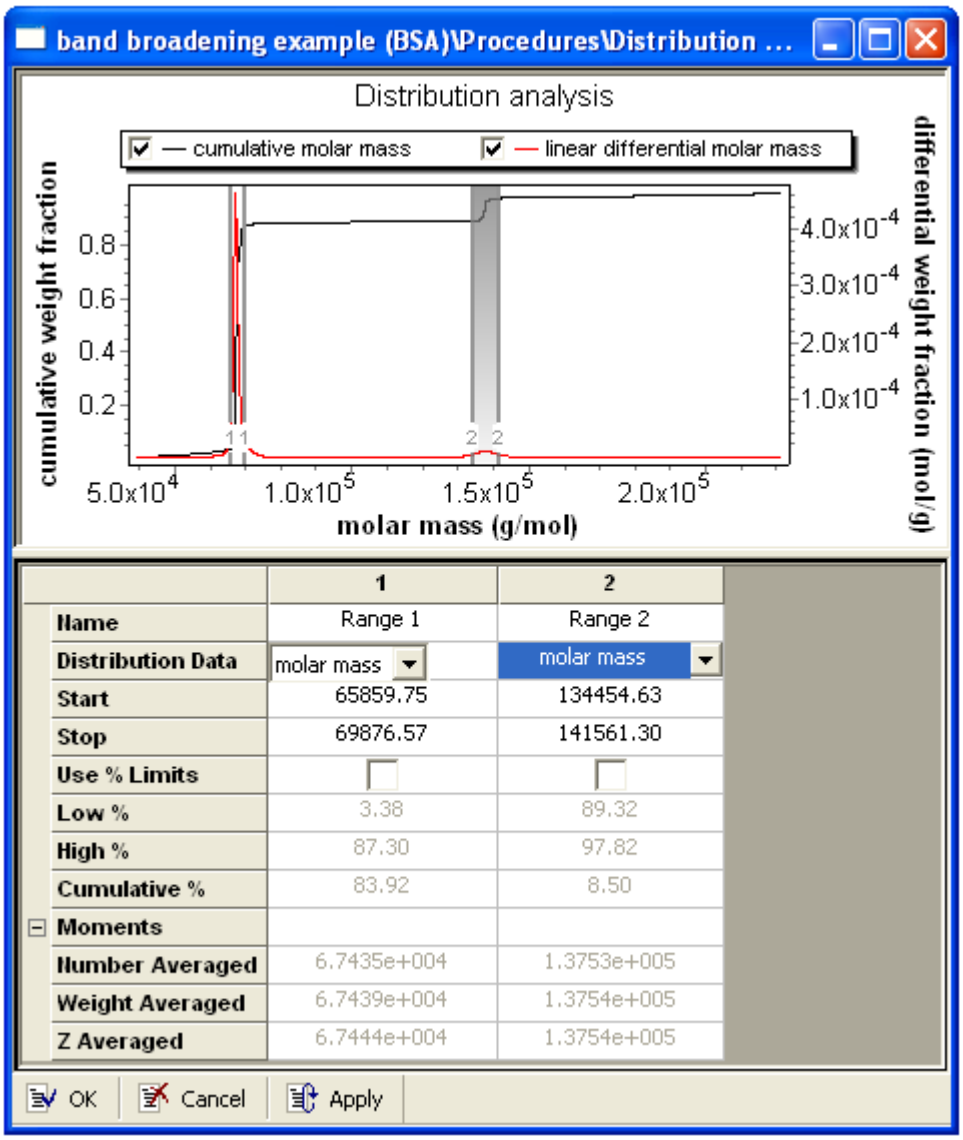
For more about viscometry data collection and analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Viscometry.

Distribution Analysis

This procedure analyzes the distribution of the geometric radius or other selected data item within the sample for an online experiment.

You can place this procedure at the end of the procedure sequence.

The procedure has the following dialog:



The graph shows the linear and cumulative distribution of the geometric radius or other selected data. Click and drag on the graph to select a range such as a peak. A separate column is created in the properties area for each range you create.

For an example experiment that performs a distribution analysis, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "band broadening example" experiment in the Sample Data->Analyzed Experiments folder.

The properties for this procedure are as follows:

Table 8-31: Distribution Analysis Properties

Field	Description
Name	Name of the range for this column. The default names are Range 1, Range 2, etc. You can change these as desired.
Distribution Data	The type of data to plot on the x-axis for distribution analysis. Options vary depending on the data in the experiment. Examples are molar mass, RMS radius, hydrodynamic radius, and translational diffusion.
Start	The starting point on the x-axis of the range.
Stop	The ending point on the x-axis of the range.
Use % Limits	Check this box if you want to specify specific percentages in the cumulative number fraction trace for the beginning and end of the range.
Low %	If you check the Use % Limits box, specify the cumulative number fraction at which you would like to begin the range. If you do not check the box, this field shows the cumulative number fraction at the start of the range you created with your mouse.
High %	If you check the Use % Limits box, specify the cumulative number fraction at which you would like to end the range. If you do not check the box, this field shows the cumulative number fraction at the start of the range you created with your mouse.
Cumulative %	This property shows the difference between the High % and Low %, which is the percent of the sample that falls within this range.
Moments-> Number Averaged	This field reports the number-averaged value of the moment for the selected range.
Moments-> Weight Averaged	This field reports the weight-averaged value of the moment for the selected range.
Moments-> Z Averaged	This field reports the Z-averaged value of the moment for the selected range.

For more about distribution analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis-> Distributions.

Copolymer Analysis

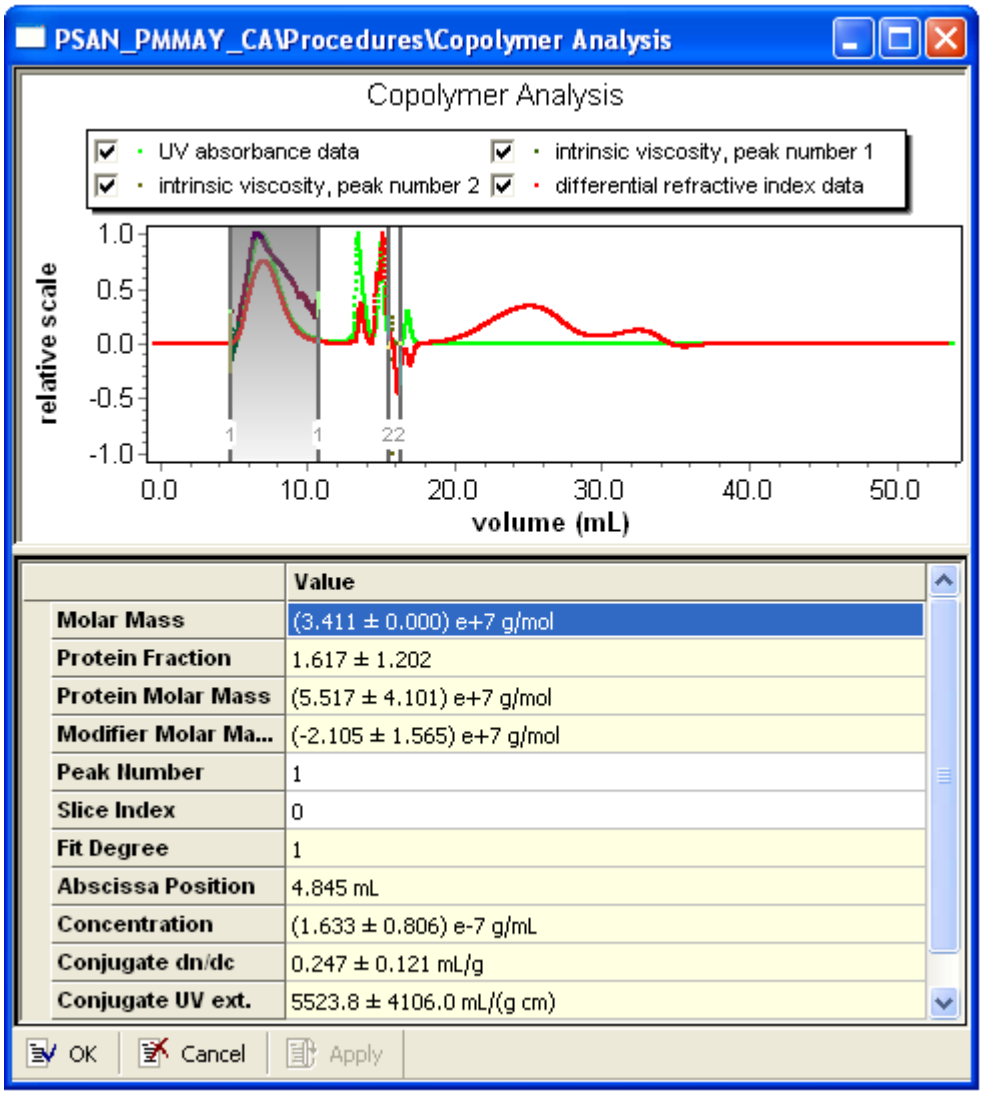
Like the “Protein Conjugate Analysis” on page 8-88, this analysis technique allows you to differentiate between two polymers with the same molecular size. This analysis procedure requires use of a viscometer in conjunction with a UV and RI detector.

The Copolymer Analysis dialog shows the total mass and protein fraction on a slice by slice basis. ASTRA calculates the size of the complex, mass of the complex, and masses of the constituents, and the uncertainties for these values.

This procedure should be placed in the sequence with the analysis procedures, but before the fit mass and radius procedure if it occurs.

For an experiment template that performs a Copolymer Analysis, choose **File→New→Experiment from Template**, and open the “Copolymer Analysis” experiment in the System Templates->Viscometry folder.

This procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-32: Copolymer Analysis Properties

Field	Description
Molar Mass	Shows the total molar mass for the currently selected peak.
Protein Fraction	Shows the protein fraction for the currently selected peak.
Protein Molar Mass	Shows the molar mass of the protein indicated by the selected peak.
Modifier Molar Mass	Shows the molar mass of the protein modifier indicated by the selected peak.
Peak Number	Click on the peak for which you want to view values.
Slice Index	Shows the current slice index, which is indicated by a vertical blue line in the graph.
Fit Degree	Shows the fit degree selected for this peak in the Peaks procedure.
Abscissa Position	The value on the x-axis for the selected peak and slice.
Concentration	The concentration at the selected slice.
Conjugate dn/dc	The dn/dc at the selected slice.
Conjugate UV ext.	The UV extinction at the selected slice.

Protein Conjugate Analysis

This procedure analyzes protein conjugates, which are an important class of copolymers. This analysis requires the use of a light scattering detector in conjunction with both a UV and RI detector.

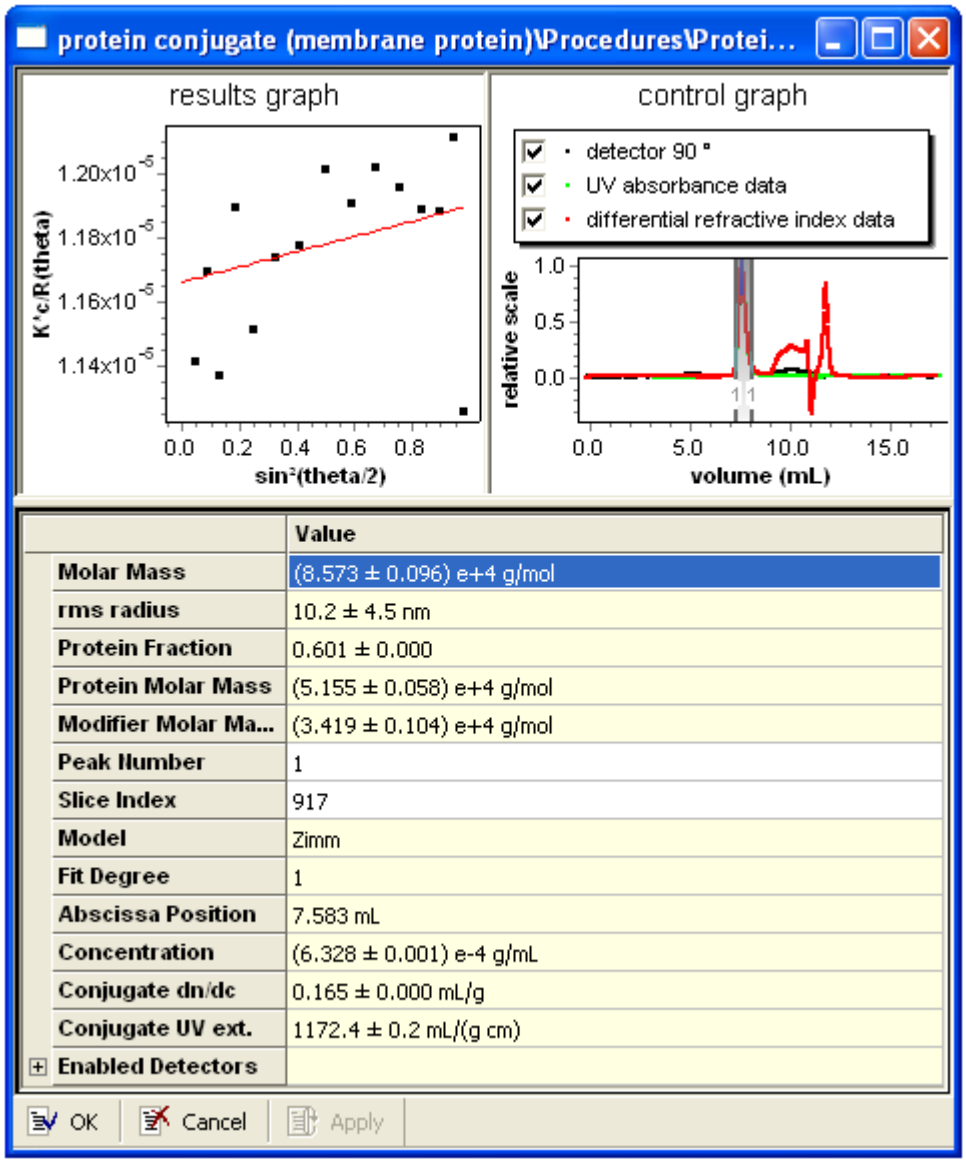
The Protein Conjugate Analysis dialog allows you to see the total mass and protein fraction on a slice by slice basis. ASTRA calculates the size of the complex, mass of the complex, and masses of the constituents, and the uncertainties for these values.

For an example experiment that performs a protein conjugate analysis, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "protein conjugate" experiment in the Sample Data->Analyzed Experiments folder. For an experiment template choose **File→New→Experiment From Template** to open the "protein conjugate" template in the System Templates->Light Scattering folder or the "protein conjugate" template in the System Templates->Light Scattering->With QELS folder.

For more about protein conjugate analysis, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Protein conjugates and copolymers.

This procedure should be placed in the sequence with the analysis procedures, but before the fit mass and radius procedure if it occurs.

The procedure has the following dialog:



The left graph shows a Zimm plot of the results. The right graph shows collection data and peaks.

The properties for this procedure are as follows:

Table 8-33: Protein Conjugate Analysis Properties

Field	Description
Molar Mass	Shows the total molar mass for the currently selected peak.
Radius	Shows the calculated radius for the currently selected peak. The type of radius (RMS or geometric) displayed depends upon the LS fit model specified for the peak. This field is display only.
Protein Fraction	Shows the protein fraction for the currently selected peak.
Protein Molar Mass	The molar mass of the protein indicated by the selected peak.
Modifier Molar Mass	The molar mass of the protein modifier indicated by the selected peak.
Peak Number	Click on the peak for which you want to view values.

Table 8-33: Protein Conjugate Analysis Properties

Field	Description
Slice Index	Shows the current slice index, which is indicated by a vertical blue line in the graph.
Model	Shows the model selected for this peak in the Peaks procedure.
Fit Degree	Shows the fit degree selected for this peak in the Peaks procedure.
Abscissa Position	The value on the x-axis for the selected peak and slice.
Concentration	The concentration as the selected slice.
Conjugate dn/dc	The dn/dc at the selected slice.
Conjugate UV ext.	The UV extinction at the selected slice.
Enabled Detectors	The detectors to enable for this analysis.

Regularization

This procedure regularizes the results of a QELS batch experiment using the DYNALS regularization algorithm from ALANGO. See "Regularization" on page F-6.

The Regularization procedure supports the reporting the results that were previously available in the separate QELSBatch program. This procedure now reports the mean, peak, and standard deviation of the reported values (hydrodynamic radius and translational diffusion).

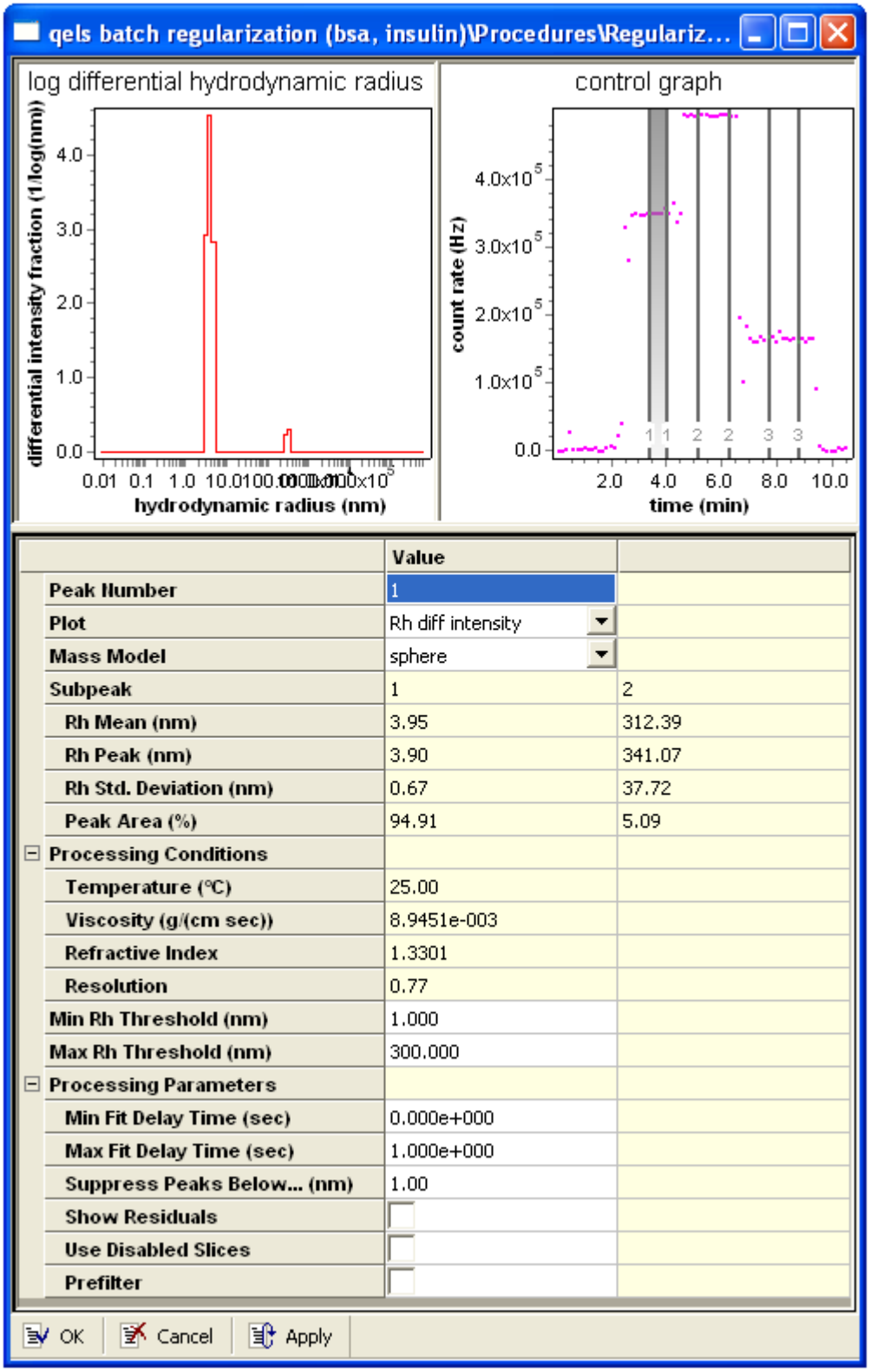
The regularization procedure provides a way to analyze a batch sample (a vial of some mixture of substances). Rather than setting up a chromatography system to separate the components using a column or membrane system, you can use the QELS data to identify the various Rh values of the mixture in the vial.

For an example experiment that performs regularization, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the "QELS batch regularization" experiment in the Sample Data->Analyzed Experiments folder. For an experiment template choose **File→New→Experiment From Template** to open the "regularization" template in the System Templates->Light Scattering->With QELS folder.

For more about analyzing batch QELS data, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->QELS batch.

You can place this procedure at the end of a QELS batch experiment procedure.

This procedure has the following dialog:



The left graph shows various plots depending on the Plot property. The right graph shows the defined peaks for the batch experiment.

The properties for this procedure are as follows:

Table 8-34: Regularization Properties

Field	Description
Peak Number	Click on a peak in the graph or type a number here to select a peak to regularize.
Plot	Select the type of plot you want to view in the left graph. The options are “correlation function”, “Rh diff intensity”, “Rh diff weight”, “Dt diff intensity”, “Rh cumulative intensity”, “Rh cumulative weight”, and “Dt cumulative intensity”.
Mass Model	Select the mass model you want to use in the computation. Options are “sphere” and “random coil”.
Subpeak	Summary of individual components identified by ASTRA in each analytical peak. You cannot modify these values.
->Rh Mean	The mean hydrodynamic radius of the subpeak.
->Rh Peak	The hydrodynamic radius at the peak of the subpeak.
->Rh Std. Deviation	The standard deviation of the peak hydrodynamic radius from the mean.
->Peak Area	The area under the subpeak as a percentage of the entire peak.
Processing Conditions	Conditions (per peak) during the regularization analysis. You cannot modify these values.
->Temperature	Shows the temperature at which the data was collected.
->Viscosity	Shows the viscosity of the solvent. This value comes from the solvent profile (page 7-32).
->Refractive Index	Refractive index of the sample for the given peak.
->Resolution	A value that represents the optimal smoothing of the distribution given the noise level of the correlation function. It varies from 0 (for very noisy data) to 1 (for data with very good signal-to-noise. In general, the lower the resolution value, the more uncertain the actual widths and structure of the final distribution.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis. The Min and Max Rh Thresholds are applied after all other analysis, to allow you to discard results that fall outside a desired range. For example, you could discard results greater than 300 nm). If the “Prefilter” box is not checked, the Min/Max Rh Thresholds are ignored.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis.
Processing Parameters	Various values that govern the processing of the data. Users do not frequently need to change these values.
->Min Fit Delay Time	Type the minimum number of seconds for the fit delay. The default is 0 seconds.
->Max Fit Delay Time	Type the maximum number of seconds for the fit delay. The default is 1 second.
->Suppress Peaks Below	Type a size in nanometers below which peaks should be omitted from the regularization.
->Show Residuals	Check this box if you want the correlation function graph to show residuals. The default it to omit residuals.

Table 8-34: Regularization Properties

Field	Description
->Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photo diode (APD) is triggered. So, any measurement where the APD was triggered is excluded from analysis unless you specifically check the “Use Disabled Slices” check box. See “Rh from QELS Data” on page 8-77 for more about the avalanche photo diode (APD).
->Prefilter	If the "Prefilter" box is checked, data points in the correlation function view that fall outside the minimum or maximum delay times (the “Min Fit Delay Time” and “Max Fit Delay Time” fields) are discarded from the fit. You can see the fit line stop short of these points, and the points themselves change color to red. The rest of the points are still included in the analysis. If this box is unchecked, data points outside the range are used in the fit.

For an example of the results you can view with the regularization procedure, open the “QELS batch regularization (BSA, insulin)” sample file, and open its Regularization procedure. This procedure allows you to see the characteristics of three mixtures (the three peaks in the right graph).

- **Correlation function plot.** This graph gives an idea of how well the data fits the average correlation function over the peak. Peaks 1 and 2 match pretty well, while peak 3 shows some variation. Generally, the more species of different sizes you have, the worse the correlation function matches the data.
- **Rh diff intensity plot.** This graph gives an idea of the number of each type of species by showing the intensity fraction as a function of hydrodynamic radius. For peak 1, there seems to be a lot of particles with a radius of 4 nm and a much smaller number with a 300 nm size. Peak 2 shows (again) a high number of ~4 nm particles and a smaller number of ~50 nm particles. The larger particles are on the right side of the graph.
- **Rh diff weight plot.** This graph shows the distribution of Rh by weight fraction. In peaks 1 and 2 the larger particles don’t even register on this view.
- **Dt diff intensity plot.** This graph shows the intensity fraction as a function of translational diffusion. That is, the particles that move the most are toward the right-side of the graph, while the slower particles are toward the left side.
- **Rh cumulative intensity plot.** This graph gives an idea of the overall intensity produced by constituents below a specific size. For peak 1, most of the intensity seems to be delivered by particles in the range of 4 nm through 300 nm.
- **Rh cumulative weight plot.** This graph shows the contribution to weight fraction for different sizes.
- **Dt cumulative intensity plot.** This graph shows the cumulative intensity as a function of translational diffusion. Again, this graph shows that the bulk of the intensity is contributed by fast-moving (smaller) particles.

Mass from Column Calibration

This procedure calculates the mass of a sample based on the elution volume of a peak through a column. You must have determined the response of the column to a series of known standards prior to using this procedure. See “Calibrate Column” on page 8-37 for details.

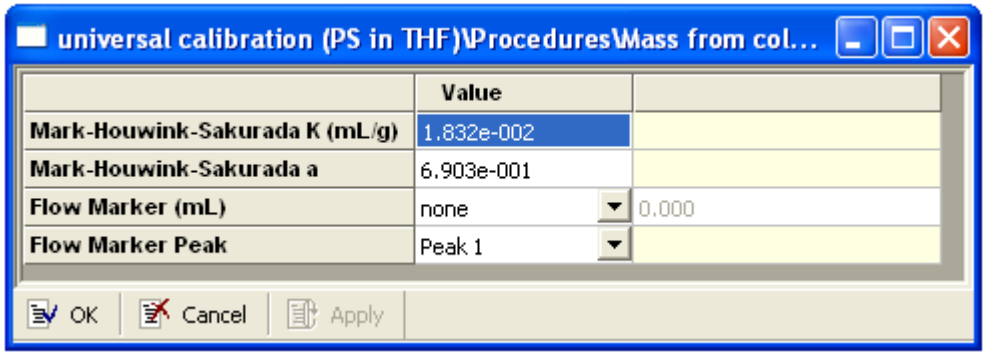
Two main types of column calibration are available. “Conventional calibration” and “universal calibration.” For conventional calibration, the analyzed polymer must be the same as the polymer used for calibration. For universal calibration, the polymers may be different.

For example experiments that perform this procedure, choose **File→Open→Experiment** (or **File→Import→Experiment** if you are using ASTRA V with Research Database), and open the “universal calibration” or “conventional calibration” experiment in the Sample Data->Analyzed Experiments folder. For an experiment template choose **File→New→Experiment From Template**, and open the “universal calibration” or “determine column calibration” template in the System Templates->Viscometry folder.

For more, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Data Analysis->Conventional and Universal Column Calibration.

You can place this procedure with other analysis procedures, and after all transformation procedures. A procedure sequence can contain only one procedure that determines the mass from column calibration. If you place multiple methods that determine mass in an experiment, only the first one will be valid. Your experiment may or may not contain the procedure “Calibrate Column” on page 8-37. If your experiment does not contain the Calibrate Column procedure, you should import the results of a column calibration using “Column Profiles” on page 7-26.

This procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-35: Mass from Column Calibration Properties

Field	Description
Mark-Houwink-Sakurada K	Type the known Mark-Houwink-Sakurada K parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique was used.
Mark-Houwink-Sakurada a	Type the known Mark-Houwink-Sakurada a parameter of the polymer used for calibration. This is only used if the Universal without Viscometer Data calibration technique is selected.
Flow Marker	Use the drop-down menu to select whether or not a flow marker is set and how. Drop-down options are as follows: - None - no flow marker is to be used. The flow marker value is set to zero. - Enter value - enter the flow marker value in mL in the cell to the right of the drop-down. - From positive peak - the flow marker will be determined from the apex of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down. - From negative peak - the flow marker will be determined from the lowest point of the peak selected in the Flow Marker Peak drop down. The resulting value is displayed in mL to the right of Flow Marker drop down.
Flow Marker Peak	Select the peak for the flow marker if you used one.

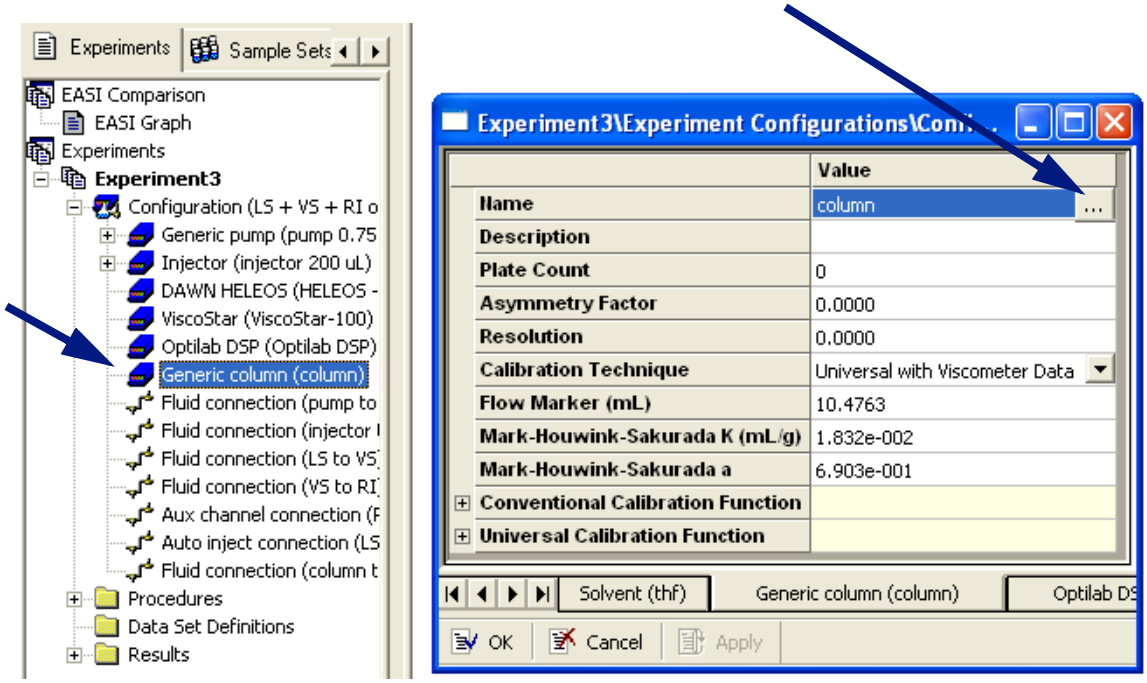
The error in the molar mass is estimated from the standard error of the calibration fit. If the fit degree is equal to the degrees of freedom, a default lower limit uncertainty of 0.001 is used in place of the standard error.

Using a Column Profile to Determine Mass

To use a column calibration to measure the characteristics of an unknown sample, follow these steps:

1. Choose **File→New→Experiment From Template**, and open the "universal calibration" or "determine column calibration" template in the System Templates->Viscometry folder.
2. Expand the configuration, and double-click on the "Generic Column" item.
3. In the Calibration Technique field, select the type of calibration you performed on this column.

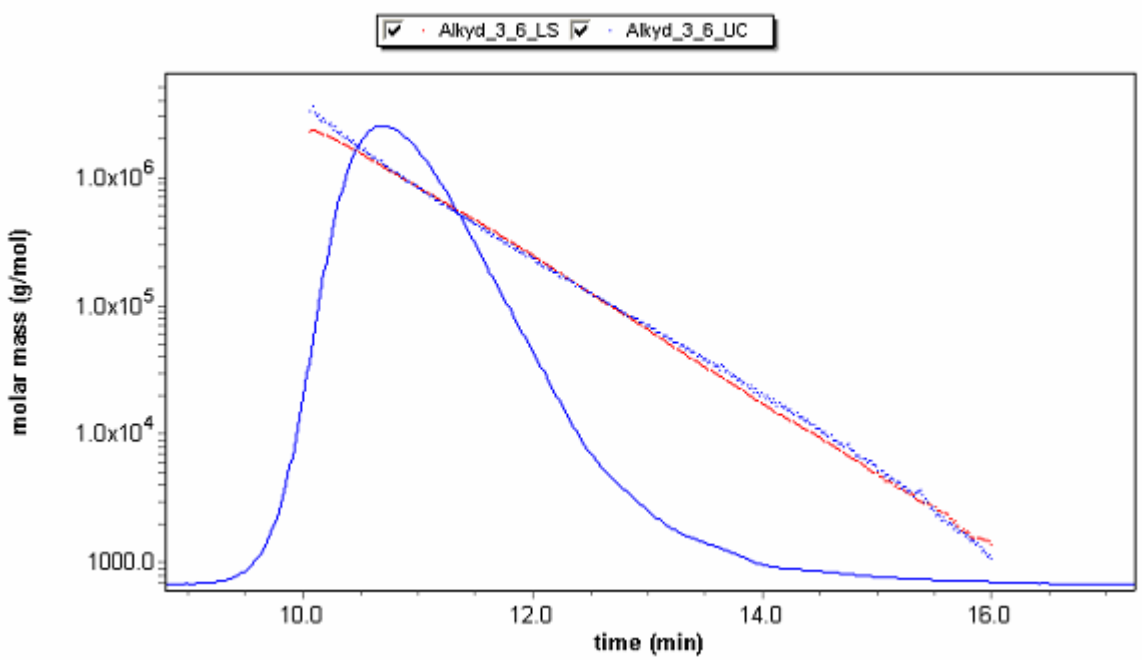
4. Press the "…" button next to the Column name, and select a column calibration you performed earlier.



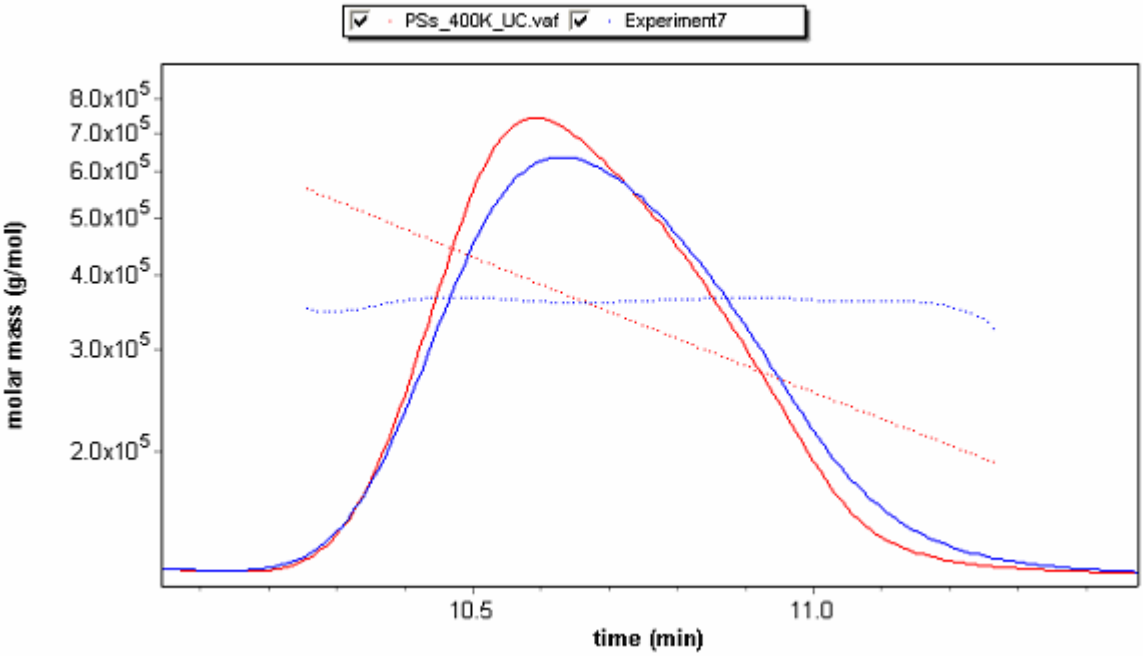
The column profile is updated with the coefficients from the known sample run.

5. Select the particular instruments used at your location, configure the interdetector delay, and make any other experiment configuration changes you normally make.
6. Choose **Experiment→Configuration→Save Configuration As** to save this experiment as a template for later use.
7. Now use this experiment to collect data on an unknown sample, or apply this template to already-collected data that was collected with the column and configuration used in the template. The results are calculated using the calibrated column values.
8. In the "Mass from column calibration" procedure, choose a Flow Marker type and Flow Marker Peak (if any).

9. Finally, you can evaluate the molar mass of the unknown sample. In the following image, the mass characteristics are very close to the light scattering values:



As a counter-example, the following plot illustrates the false polydispersity displayed by universal calibration:



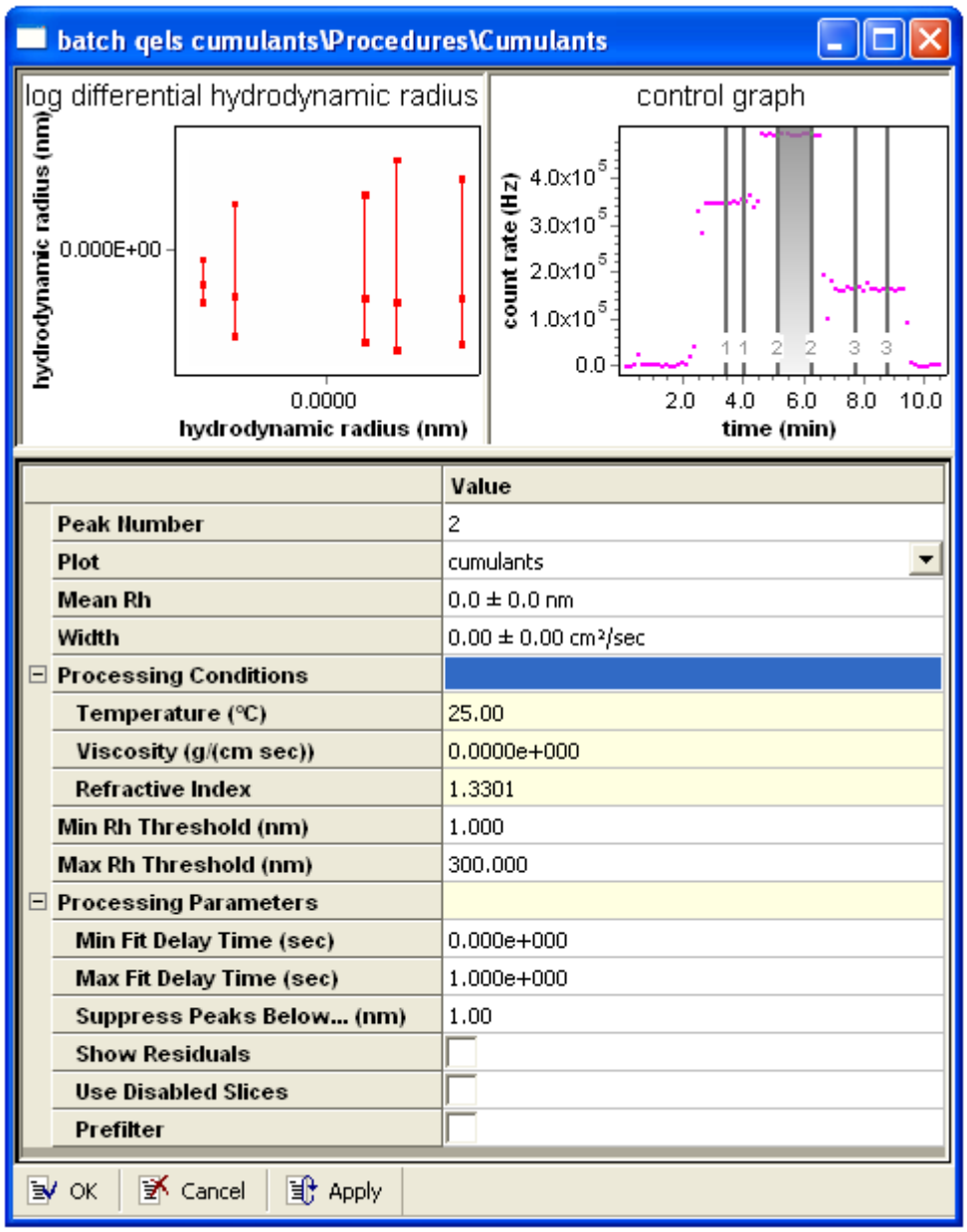
Cumulants

Cumulants analysis is a strategy for extracting information about the underlying size distribution of a polydisperse sample from the correlation function. In a nutshell, the cumulants method involves fitting the correlation function not to a single decay time, but to a Gaussian distribution of decay times. This method retrieves the mean and variance for the distribution. “Cumulants” on page F-2 provides details on cumulants analysis.

Cumulants analysis is used with QELS data. Thus, an experiment configuration must include a light-scattering instrument and Wyatt QELS.

You can place this procedure with other analysis procedures and after all transformation procedures. A sequence cannot contain both the Cumulants procedure and the procedure for “Regularization” on page 8-90.

This procedure has the following dialog:



The properties for this procedure are as follows:

Table 8-36: Cumulants Properties

Field	Description
Peak Number	Click on a peak in the graph or type a number here to select the peak for the cumulants calculation.
Plot	Choose whether to show the correlation function or the cumulants plot in the left graph. The cumulants plot shows the log differential hydrodynamic radius.
Mean Rh	Shows the mean hydrodynamic radius (Rh) for the peak. You can override the calculated value by typing a known value.
Width	Shows the width in cm^2/sec.
Processing Conditions	Conditions (per peak) during the regularization analysis. You cannot modify these values.
->Temperature	Shows the temperature at which the data was collected.
->Viscosity	Shows the viscosity of the solvent. This value comes from the solvent profile (page 7-32).
->Refractive Index	Refractive index of the sample for the given peak.
Min Rh Threshold	Fitted Rh values with a lower radius than the value you type are not used in the analysis. The default is 1 nm. The Min and Max Rh Thresholds are applied after all other analysis, to allow you to discard results that fall outside a desired range. For example, you could discard results greater than 300 nm). If the “Prefilter” box is not checked, the Min/Max Rh Thresholds are ignored.
Max Rh Threshold	Fitted Rh values with a higher radius than the value you type are not used in the analysis. The default is 300 nm.
Processing Parameters	
->Min Fit Delay Time	Data with a lower delay time than the value you type is not used in the fit to the correlation function. The default is 0.
->Max Fit Delay Time	Data with a higher delay time than the value you type is not used in the fit to the correlation function. The default is 1.
->Suppress Peaks Below	Type a size in nanometers below which peaks should be omitted from the analysis.
->Show Residuals	Check this box if you want the correlation function graph to show residuals. The default it to omit residuals.
->Use Disabled Slices	ASTRA normally discards the entire slice if the avalanche photo diode (APD) is triggered. So, any measurement where the APD was triggered is excluded from analysis unless you specifically check the “Use Disabled Slices” check box. See “Rh from QELS Data” on page 8-77 for more about the avalanche photo diode (APD).
->Prefilter	If the "Prefilter" box is checked, data points in the correlation function view that fall outside the minimum or maximum delay times (the “Min Fit Delay Time” and “Max Fit Delay Time” fields) are discarded from the fit. You can see the fit line stop short of these points, and the points themselves change color to red. The rest of the points are still included in the analysis. If this box is unchecked, data points outside the range are used in the fit.

In the cumulants plot, the average hydrodynamic radius and the distribution values at one standard deviation are presented. This creates an “error bar” appearance for the graph, but here the error bars indicate the width of the fitted distribution. They are asymmetric because the hydrodynamic radius is inversely proportional to the symmetric decay time distribution

Administration Procedures

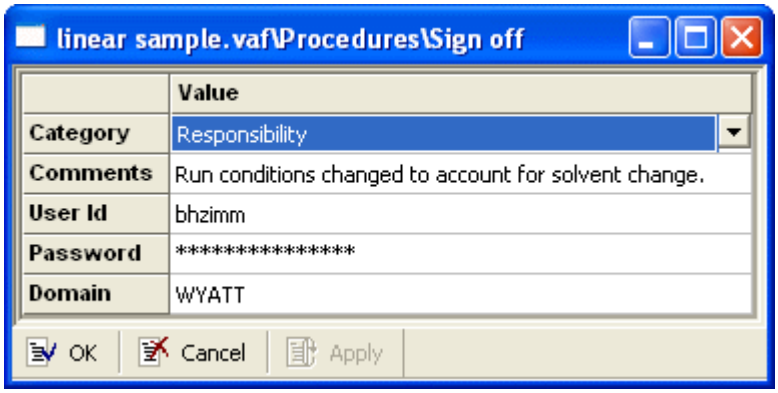
Administration procedures add an experiment log entry for the audit trail.

Sign Off

Security

In order to comply with 21 CFR Part 11, your operating procedures may require that one or more electronic sign offs be associated with each experiment. A Sign off procedure may be located at any position in the experiment sequence specified by your policies and procedures.

The user who runs the experiment is prompted to set parameters for the Sign off procedure. The sign off dialog looks similar to the following:



The properties to enter are as follows:

Table 8-37: Sign Off Properties

Field	Description
Category	The type of sign off. Options are Responsibility, Approval, and Review.
Comments	Any comments about this experiment.
User Id	A valid ASTRA user ID. This need not be the same user ID used to log into this ASTRA session or this Microsoft Windows session.
Password	The password for the specified user ID. This is case-sensitive.
Domain	The domain for the specified user ID.

Electronic signatures can be executed for any data collected in ASTRA. The electronic signature is saved in the experiment log associated with the data, and shows up in the report associated with the experiment.

Multiple electronic signatures can be executed for an experiment. In the reported results, ASTRA flags any electronic signatures that were made before the last modification. Failed electronic signature attempts are recorded in the system log with an alarm status for immediate notification of system administrators.

9 Using Sample Sets

This chapter explains how to configure and run sample sets in ASTRA V.

CONTENTS **PAGE**

About Sample Sets

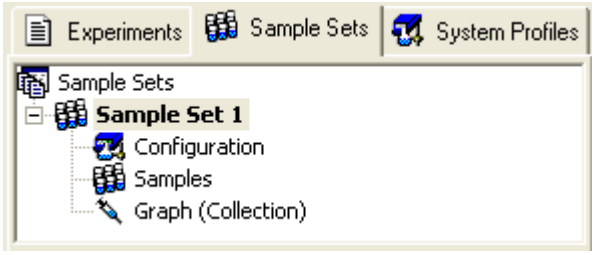
ASTRA sample sets provide an easy way to manage experiment sequences with multiple samples. A sample set can be configured with a default experiment template, such that all samples are collected in the same fashion. Or, a different experiment template can be specified for each sample, making it possible to collect different types of data for each sample. For example, you might do a QELS collection on two out of ten runs.

A sample set is used to collect data from a sequence of injections, usually from an autosampler. The sample set creates a new experiment from a predefined template for each injection, and then runs the experiment to collect and analyze the data. Therefore, a sample set can create many experiments.

Security

You must have at least Researcher access to create sample sets. If you are a Technician, you can run sample sets. If you are a Guest, you have read-only access to sample sets.

ASTRA V has a Sample Sets tab in the workspace that allows you to create, edit, and run sample sets.



The procedures run for a particular sample are determined by referencing an experiment template for each sample. The samples may reference the same or different experiment templates.

Creating New Sample Sets

You can create sample sets starting from a blank configuration or from a template you have created. Blank sample sets are easy to work with. If you often perform experiments with the same set of samples, using a template can save time in setting up the sample set.

Security You must have at least Researcher access to create sample sets.

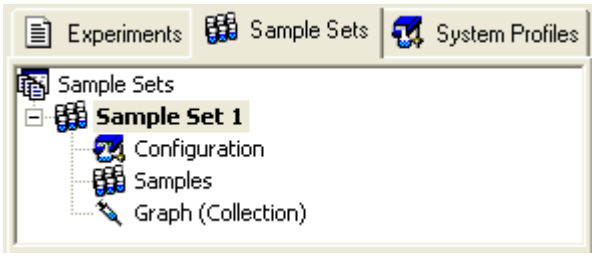
Creating Blank Sample Sets

Create an empty sample set by following this step:

1. Choose **File→New→Blank Sample Set**.

Shortcuts: Press Ctrl+Shift+N.
Click the down-arrow next to the icon.

You see a sample set configuration in the Sample Sets tab of the workspace.



If you have defined a default experiment template (see page 6-18 and page 6-19), that template is the default for all samples in the new blank sample set.

Creating a Sample Set from a Template

To create an experiment from a template you have created, follow these steps:

1. Choose **File→New→Sample Set From Template**.

Shortcuts: Click the down-arrow next to the icon.

2. In the New from Existing dialog, open the folder that contains the sample set template you want to use. You can choose from the following folders:
   - **My Templates:** These templates are ones you have saved as described in "Saving Sample Sets as Templates" on page 9-11.
3. Select a template and click **Create**.

   A new sample set is created based on the template you selected.

Opening a Sample Set

You can open and work with any sample set you have saved. If you are using ASTRA V with Research Database or ASTRA V with Security Pack, experiments are stored in the experiment database, and you open experiments from that database. If you are using ASTRA V Basic, sample sets are stored in separate files with an extension of *.vsf.

To open an experiment, follow these steps:

1. Choose **File→Open→Sample Set**.

Shortcuts: Press Ctrl+Shift+O.
Click the down-arrow next to the  icon.

2. In the Open dialog, navigate to the folder that contains the sample set you want to open.
3. Select a file and click **Open**.

Importing an ASTRA Sample Set

Security

You must have at least Researcher access to import sample sets.

You can import ASTRA sample sets saved with ASTRA V and ASTRA 4. ASTRA V sample sets have a file extension of *.vsf. ASTRA 4 sample sets have a file extension of *.ss.

Basic

This item is disabled in ASTRA V Basic since it is identical to **File→Open→Sample Set**.

To import an ASTRA sample set, follow these steps:

1. Choose **File→Import→Sample Set**.

Shortcuts: Press Ctrl+Shift+I.

2. In the Import dialog, navigate to the folder that contains the sample set you want to import.
3. In the Files of type field, select the type of sample set file you want to import.
4. Select a file and click **Open**. The sample set is shown in your Sample Sets tab with the filename you imported.
5. To save the sample set in your experiment database, choose **File→Save**.

Importing an Empower Sample Set

Security | You must have at least Researcher access to import sample sets.

ASTRA can connect directly to the database for the Waters Empower™ chromatography software to read in Empower sample sets. So, you can set up your chromatography run in Empower, and then import the data into ASTRA for light scattering and related data analysis.

To import an Empower data set, follow these steps:

1. Create the sample set definition in Empower.
2. Choose **File→Import→Empower Sample Set**.
3. Log in to the Empower database using your Empower user ID and password.

4. Find and select the sample set you want to import from the import dialog and click **Open**. The sample set is shown in your Sample Sets tab with the name of the sample set you imported.

   ASTRA converts the Empower sample set to a full-featured ASTRA sample set. Sample names and identifying information are all brought over from Empower.

5. To save the sample set in your experiment database, choose **File→Save**.

For more about using ASTRA V with Empower, go to http://www.wyatt.com/solutions/software/ASTRA.cfm and follow the links to Connectivity->Interoperability with Waters Empower Software.

Editing a Sample Set

To edit a sample set, you use the property dialog for the sample set.

To use this dialog, follow these steps:

1. Choose **Sample Set→Edit**. This opens the properties dialog for the sample set. You will see the Sample Set property dialog, which contains the “Configuration”, “Samples“, and “Collection” tabs.

Shortcuts: Double-click “Configuration”, “Samples“, or “Collection” in the sample set tree in the Sample Sets tab.

2. You can set properties by typing, selecting from a list, or checking a box. Use the + or - next to a property to expand or hide lists of related properties.
3. You can move to other tabs in the dialog to view or set properties.
4. Click **Apply** or **OK** to make the changes.

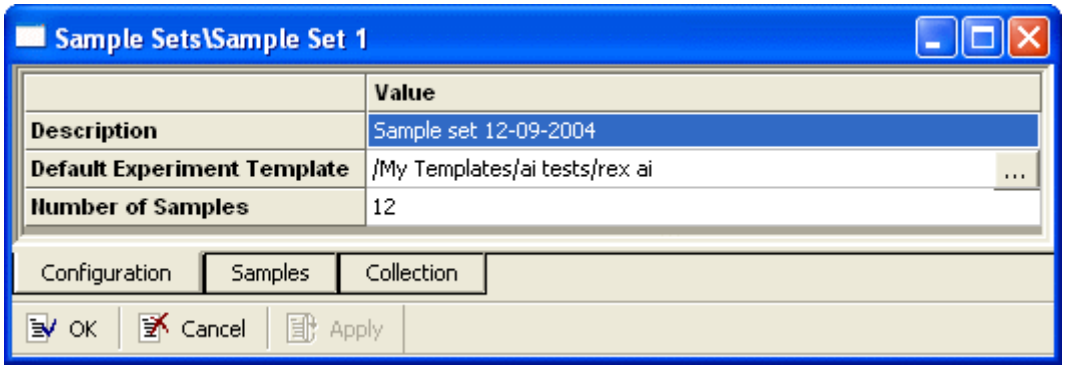
The remaining sections of this chapter contain details about the properties you can set in the various tabs.

The Sample Set property dialog has tabs for the following items:

- **Configuration:** Sets global properties for the sample set configuration.
- **Samples:** Sets properties for samples in each well of the sample tray. Also specifies the experiment template to be used for each sample.
- **Collection:** Shows collection data as samples are being run.

Configuration Tab

The Configuration tab of the Sample Set property dialog looks like this.



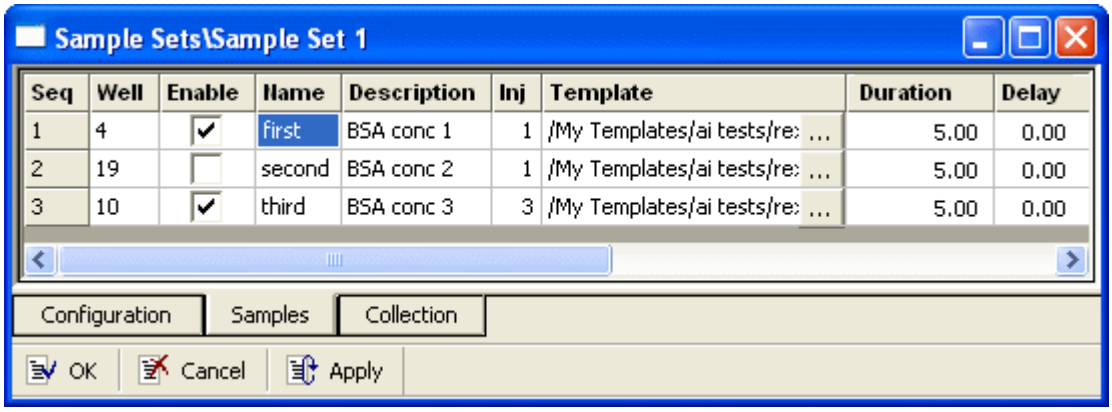
You can set the following properties for a sample set configuration:

Table 9-1: Experiment Configuration Properties

Field	Description
Description	Description of the sample set configuration.
Default Experiment Template	The experiment template to assign initially to all the samples. The template can be overridden on a sample-by-sample basis in the Samples tab. For information on choosing experiment templates, see “Creating Experiments from Templates” on page 6-5. For information on creating experiment templates, see “Creating a Template” on page 6-18.
Number of Samples	The number of samples in the sample set. This can be changed in the Samples view after the initial configuration.

Samples Tab

The Samples tab of the Sample Set property dialog looks like this.



While a sample set is being run, you can change the values for a sample that has not yet been run. Once a particular sample has been run, its fields become noneditable.

If you right-click on a sample row, the pop-up menu allows you to add, delete, and reorder the samples before they are run. These operations are all available even while the sample set is being run. Note that you must select the **Apply** button to apply any changes you make to the sample set while it is running.

You can set the following properties for a sample:

Table 9-2: Sample Properties

Field	Description
Seq	The sequence number in the sample set. This field is non-editable.
Well	The number of the injection well in the sample tray. This is for informational purposes only. You need not use this field.
Enable	If this box is checked, the sample is enabled for the sample set run.
Name	Name of the file to be generated by the sample set. The sample set name is appended to this file name. If multiple injections are requested for a sample, the injection number is also appended to the name. If no name is specified, ASTRA generates a unique name for the generated file.
Description	Description of the sample, which typically contains more information than the Name.
Inj	The number of injections for the sample.
Template	The experiment template to use as the source for instrument configuration, procedure, and result formatting information for this sample. The default experiment template is set in the Configuration tab, but you can override it on a sample-by-sample basis here. If the experiment template itself is modified after you choose it here but before the sample set is run, the modified version of the experiment template is used. For information on choosing experiment templates, see “Creating Experiments from Templates” on page 6-5. For information on creating experiment templates, see “Creating a Template” on page 6-18.

Table 9-2: Sample Properties

Field	Description
Duration	The time or fluid volume for which data is to be collected. The units are determined by the Abscissa Units property of the experiment configuration.
Delay	The delay in time or fluid volume between injection and the start of data collection. The units are determined by the Abscissa Units property of the experiment configuration. The default is zero.
dn/dc	dn/dc value associated with the sample in mL/g. The dn/dc value is used when the sample concentration is to be determined using a refractive index instrument. The value entered for the profile is used as a default value when peaks are set for the data.
A2	Second viral coefficient (A^2) value associated with the sample, measured in mol mL/g^2. The value set here is used as a default value for peaks set in the experiment.
UV Ext	The UV extinction coefficient in mL/(g cm). The extinction coefficient is used when the concentration of the sample is to be determined using a UV absorption instrument. The value entered here is used as a default value when peaks are set for the data.
Conc	The concentration of the sample in g/mL.
Vol	The injection volume of the sample in mL.

In order to avoid cycling the laser frequently, which will shorten its life-span, you should make sure the Laser Saver Mode box is not checked in the Basic Collection configuration of the templates you use for the samples. Instead, you can add one more sample to the sample set and use one of the System Templates->Light Scattering->Utilities->turn laser off templates as the last experiment template in the sample set.

Seq	Well	Enable	Name	Description	Inj	Template
1	0	✔			1	/My Templates/collection only
2	0	✔			1	/My Templates/collection only
3	0	✔			1	/My Templates/collection only
4	0	✔			1	/My Templates/collection only
5	0	✔			1	/My Templates/collection only
6	0	✔			1	/My Templates/collection only
7	0	✔			1	/My Templates/collection only
8	0	✔			1	/System Templates/Light Scattering/Utilities/turn laser off

The order of precedence for values such as dn/dc, A2, UV extinction, concentration, and so on is as follows:

1. Peak values defined in the specified experiments are used if they exist.
2. Values from the Samples tab are used next.
3. The sample profile is used if no other values are set.

Collection Tab

The Collection tab of the Sample Set property dialog allows you to view data as it is collected during a sample set run.

While a sample set is running, the collection data is displayed for the current sample. If an autoinject signal is expected to trigger the injection, this view displays a message indicating the sate of the collection.

Running Sample Sets

Sample sets act as sets of experiments that can be run as a group. Rather than running each separate experiment from the Experiments tab, you run the collection from the Sample Sets tab.

Validating a Sample Set

You can validate an entire sample set by choosing **Sample Set→Validate**.

Validation checks for conflicts in the procedure sequences for all samples in the sample set. If an experiment collects data, validation also checks that the necessary instruments are connected and available. In addition, validation checks the collection scripts. If your templates use the Basic Collection procedure, the collection script is built automatically, and validation never finds any problems with the script.

Shortcuts: Click the Validate Sample Set icon  in the experiment toolbar.

Procedures are not shown in the Sample Sets tab. You can view them by creating new experiments from templates as described in "Creating Experiments from Templates" on page 6-5. For information about modifying the procedure sequence, see "Sequencing Procedures" on page 6-27.

Running a Sample Set

To start the sample set run, follow these steps:

1. Begin by turning on, warming up, and stabilizing your experimental apparatus. When everything is ready to go, continue with the following steps in ASTRA.
2. Choose **Sample Set→Run**.

Shortcuts: Click the Run Sample Set icon  in the experiment toolbar.

3. During a sample set run, the live data can be viewed in the Collection tab, and the state of the sample set execution can be monitored in the Samples tab.

After a sample has been run, its row in the Samples tab is shown with a blue background.

After the full sample set has been run, a message says "Sample set run complete."

Stopping a Sample Set

To stop a running sample set, choose **Sample Set→Stop**.

Shortcuts: Click the Stop Sample Set icon  in the experiment toolbar.

A message says "Sample set run manually stopped."

Stopping a sample set with ASTRA stops only the collection and analysis of data. It does not affect any activity going on outside of ASTRA's control.

See your hardware documentation for information about alarms, emergency stops, and setting up safety interlocks. Alarms may be monitored via the Diagnostic Manager. See "Viewing Alarms with the Diagnostic Manager" on page 5-7 for details.

Viewing a Sample Set Log

Database

To view a log for a sample set, choose **Sample Set→Log->Open**. Double-click on a line to see more details. You can choose **Sample Set→Log->Save As** to save the log to a file.

Saving Sample Sets

To save a Sample Set, follow these steps:

1. Choose **File→Save**.

Shortcuts: Press Ctrl+S.
Click the  icon.

2. If this is the first time you have saved this sample set, you see the Save As dialog. Otherwise, you are finished saving the file.
3. In the Save As dialog, navigate to the folder you want to contain the file.
4. In the File Name field, type a name for the sample set.

Basic

The Save As Type field shows that the file will be saved with an extension of *.vsf if you are using ASTRA V Basic.

Database

If you are using ASTRA V with Research Database or ASTRA V with Security Pack, the sample set is saved in the experiment database.

5. Click **Save**.

To save a sample set with a different name or location, choose **File→Save As** and follow steps 3 through 6 above.

Saving Sample Sets as Templates

A sample set can be saved as a template by selecting "Templates" in the Of Type field in the Save As dialog. Once you have configured a sample set, save it as a template before running it. You can then create new sample sets from the template without building a new one from scratch each time.

Exporting Sample Sets

You can export a sample set from the experiment database to a file with an extension of *.vsf. This file is a binary file that can only be imported by ASTRA V.

To export a sample set, follow these steps:

1. Select the sample set you want to export in your Sample Set tab.
2. Choose **File→Export→Sample Set**.
3. Select the directory where you want to save the file, and type a file-name for the sample set.
4. Click **Save** to create the file.

Deleting a Sample Set

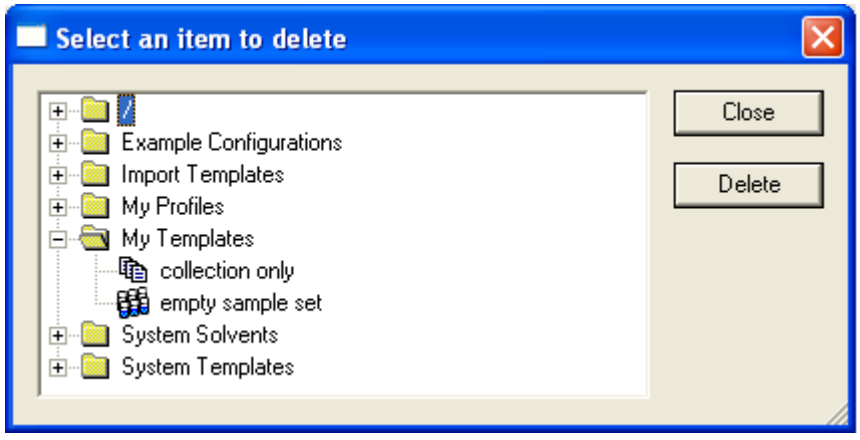
Basic

If you are using ASTRA V Basic, you may delete a sample set by deleting the *.vsf file that contains the sample set.

Database

If you are using ASTRA V with Research Database or ASTRA V with Security Pack, delete a sample set by following these steps:

1. Choose **System→Database Administration→Delete Items**. This opens the Select an item to delete dialog.



2. Highlight the sample set you wish to delete in the list.
3. Click **Delete**.
4. Click **Close** when you have finished deleting sample sets.

Security

If you are using ASTRA V with Research Database or ASTRA V with Security Pack, you must have Administrator access to delete a sample set.

You can delete sample set templates by choosing **System→Database Administration→Delete Items** and using the dialog to delete sample set templates you no longer need.

10 Working With Reports

This chapter explains how to create, customize, and print reports with ASTRA.

CONTENTS **PAGE**

About Reports

The templates provided with ASTRA V include reports that show the results computed for the experiment. Most templates include a summary report and a detailed report. Typically, these reports will need little or no modification. You can simply view and print the reports produced when you run an experiment.

To view a report, double-click on its name in the Results list of the experiment tree in the workspace.

Operator Names in Reports

Basic

If you are using ASTRA V Basic, the operator names shown in reports are assigned as follows:

- **Processing Operator:** This is determined at the time the report is generated. It is the Microsoft Windows "currently logged in user". So on the "Aqueous-1" machine, this will show up as "Aqueous-1".
- **Collection Operator:** This is determined at the time data collection is started. It is also the "currently logged in user" obtained from Microsoft Windows. This username is stored with the collected data, so that it is retained even if processing and report generation is performed by another user.

Database

If you use ASTRA V with Research Database or ASTRA V with Security Pack, the operator names shown in reports are assigned as follows:

- **Processing Operator:** This is determined at the time the report is generated. It is the username for the user currently logged into ASTRA. The Microsoft Windows "currently logged in user" is ignored.
- **Collection Operator:** This is determined at the time data collection is started. It is also the username for the user currently logged into ASTRA. The Microsoft Windows "currently logged in user" is ignored. This username is stored with the collected data, so that it is retained even if processing and report generation is performed by another user.

Changing a Report Format

Experiment Builder You can choose a different report format only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

When you open a report while in Experiment Builder mode, you see the following properties for the report. You do not see this property list if you open the report while in Run mode.

Table 10-1: Report Properties

Field	Description
Graphs>	Check the box or boxes for graphs you want to include in the report.
Template> Description	You can change the name of the report to be shown in the experiment tree list by modifying this name.
Template>File	Click the "..." button, then locate the XML file you want to use. A large number of reports are provided with ASTRA in the Reports folder. (For example, C:\Program Files\WTC\ASTRA 5\Reports.)

Customizing Report Formats

ASTRA provides about 50 report templates, so you are likely to find templates that apply to your experiments. However, you may also need to modify a template to suit your needs.

The report templates are provided as XML files, which are similar to HTML files. However, you should not edit these files with a web page editor, because they use special <wtc_field> tags to identify data to be shown in the report. Many web page editors would remove or modify these non-standard tags.

If you are comfortable editing web pages using a text editor, you can modify the format of the provided report templates as desired. For example, you might want to add your company logo or department information.

The report formats provided with ASTRA V are located in the Reports folder under the ASTRA installation folder. This is usually similar to C:\Program Files\WTC\ASTRA 5\Reports.

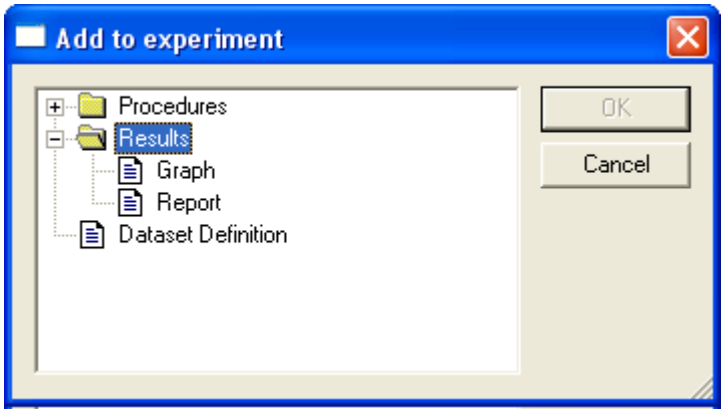
Adding a Report

Experiment Builder You can add reports only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

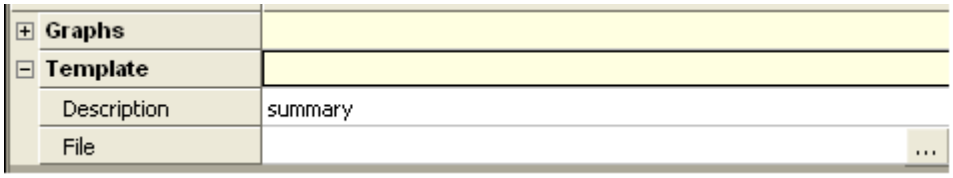
To create a new report, follow these steps:

1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.

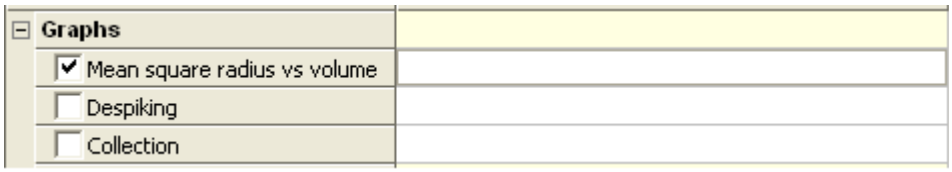


2. Close the Procedures nodes, and select Report (in the Results folder). Then click OK.
3. Double-click the "Report (untitled1)" item that was added to the Results node in the experiment tree.
4. In the property list for the report, expand the Template list and type a Description to appear at the top of the report.



5. For the Template File, click the "..." button.
6. Select a report file to use as a template for the report. A number of summary and detailed templates are provided as XML (web page) files in the Reports folder under the ASTRA installation folder. (To customize reports, see "Customizing Report Formats" on page 10-3.)

7. If you want to add any graphs to your report, expand the Graphs list and check the boxes for any graphs you want to include. The report displays graphs in the sequence you check the boxes. The list and format of the graphs comes from the graphs in the Results section of your experiment and any graphs in the procedures in your experiment. See "Creating Custom Plots" on page 11-10 for information on adding a graph to your experiment.



   Note that you cannot add EASI graphs to reports because they are aggregates of multiple experiments, and cannot be part of a particular experiment's report.

8. Click **OK**.
9. Choose **Experiment→Run** to run or re-run the experiment. After the procedure runs successfully, the report and other results are generated.

Applying Report Formats

You can apply report and graph formats (along with procedures) from a template to an experiment you have already run to collect data. For example, after using the "LS batch (Debye plot)" template when collecting data, you might want to apply the "LS batch (Zimm plot)" template to the same data so that you can view the results differently.

Applying a template creates a separate experiment, so you do not lose any of the information in the original experiment.

To apply a template, follow these steps:

1. Open an experiment containing raw (source) data you want to use.
2. Choose **Experiment→Apply Template**. The New From Existing dialog appears. This is the same dialog you use to create an experiment from a template before data collection.
3. Choose a template to apply to the data. Typically, you would choose a template from the System Templates or My Templates folder.

   The procedures and result formats (reports and graphs) in the template are used in place of those in your source experiment. The source data and the source experiment configuration are not changed.
4. Click **Create**. A new experiment is created.
5. Select the new experiment and click the Run icon ▶ in the toolbar.
6. After the applied procedure runs, you can view the new results.

Printing a Report

To print a report, do one of the following:

- Choose **File→Print**.
- Select the report window, right-click and select **Print**.

Tip:	To copy a report to the Windows clipboard, highlight text, right-click, and choose **Copy**. Then, move to another application and paste.

To change the printer setup, do the following:

1. Choose **File→Print Setup**.
2. Make any changes in the Print Setup dialog and click **OK**.

To preview the report layout, do the following:

1. Choose **File→Print Preview**.
2. You can use the Print Preview window to change the print setup or to print the report.

Exporting a Report

To export data from a report, we recommend that you use the procedure described in “Exporting Data” on page 11-17 to export data from a graph. The **Experiment→Report→Export** command exports the experiment data, rather than the report data.

11 Working With Graphs

This chapter describes how to create and use EASI graphs, data set definitions (DSDs), and DSD-based graphs.

CONTENTS **PAGE**

About ASTRA Graphs

You can use the following ways to create graphs using ASTRA:

- **Procedure Graphs.** Many procedure dialogs contain graphs that you can print. See "Working with Procedure Graphs" on page 8-5 for information about these graphs.
- **EASI Graphs.** These graphs are flexible and easy to create. You make selections and see the resulting graph in the same dialog. EASI graphs are not saved with the experiment. See "Using EASI Graphs" on page 11-3 for details.
- **Custom Plots.** These graphs are more powerful (and more complicated to create). They use a data set definition to identify the data to plot. You can use custom plots to graph multiple types of data against any x-axis values you choose. See "Creating Data Set Definitions" on page 11-8 and "Creating Custom Plots" on page 11-10 for details.
- **Parametric Plots.** The parametric plot procedure generates a data set for two different types of x-y data that share the same x-axis. For example, you can use this procedure to create a plot of RMS radius vs. molar mass. See "Parametric Plot" on page 8-83.
- **Surface Plots.** You can create a 3D surface plot of detector data. See "Creating Surface Plots" on page 11-12

For all types of graphs, see the following sections:

- "Viewing and Modifying Graphs" on page 11-13 for information about how to zoom in and out and change the look of the graph.
- "Printing Graphs" on page 11-15 for information about printing any type of graph.
- "Exporting Graphs" on page 11-16 for information about saving graphs to image files.

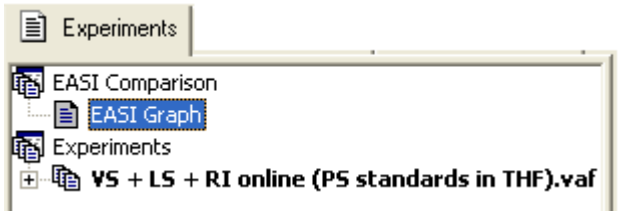
Using EASI Graphs

EASI graphs are intended to be used to quickly visualize results. They are not saved as part of an experiment. You can only have one EASI graph.

To begin creating an EASI Graph, do one of the following:

- Choose **Experiment→EASI Graph**.
- Right-click an experiment tree folder, and choose **Manage→EASI Graph** from the right-click menu.

The EASI Graph window opens, and the Experiments tab lists an EASI Graph item that you can use to reopen this window during the current session.

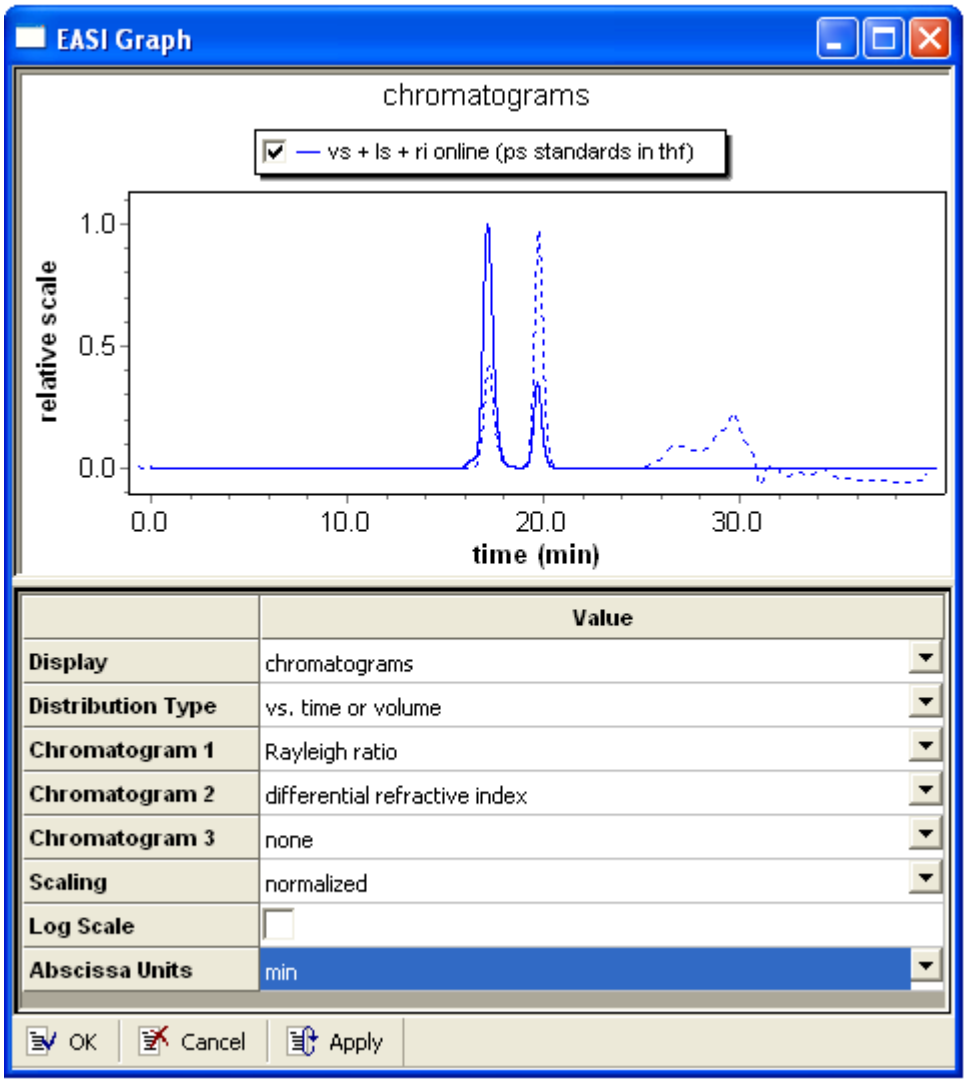


Although you cannot save it, you can print an EASI graph by right-clicking on the graph and choosing **Print** from the pop-up menu.

To customize an EASI Graph, follow these steps:

1. Change any properties in the EASI Graph dialog.
2. Click **Apply** to see the effects of your changes. A message is shown if the experiment does not contain the data for the selected graph type.

The EASI Graph dialog looks similar to this:



The EASI Graph has the following properties:

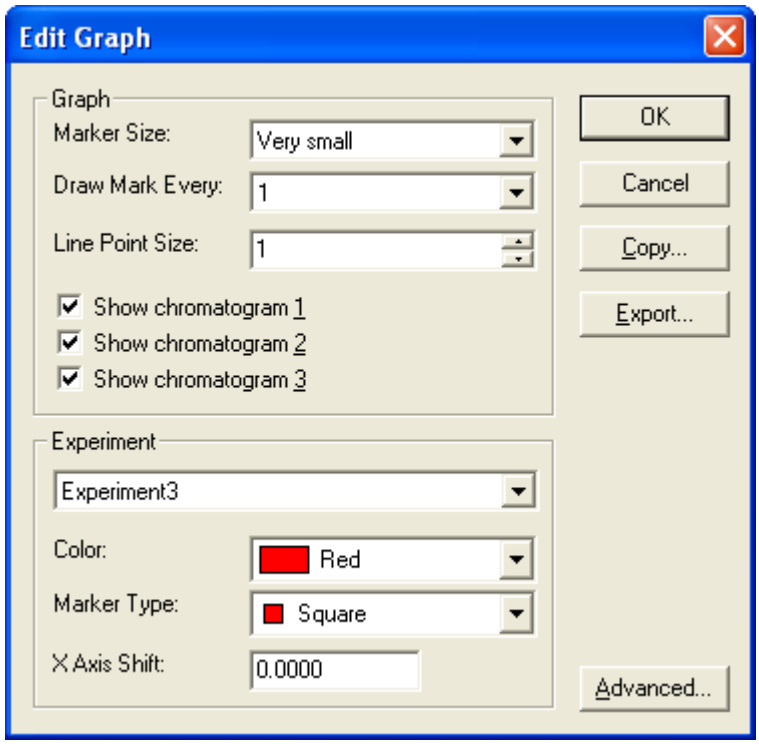
Table 11-1: EASI Graph Properties

Field	Description
Display	Choose the type of display you want. This will be the main data set plotted. (The thicker solid line.) The options are: chromatograms, concentration, molar mass, RMS radius, Rh, RMS conformation plot, Rh conformation plot, intrinsic viscosity, Mark-Houwink-Sakurada plot, geometric radius, mean-square radius, translational diffusion, branching ratio (g and g'), branch units per molecule, long-chain branching, protein conjugate, and instrument voltages.
Distribution Type	Choose the item you want to plot the display against. The options are: vs. time or volume, cumulative weight fraction, differential weight fraction, cumulative number fraction, and differential number fraction. If you choose "vs. time or volume", that will be the x-axis of the plot. If you choose one of the fractional options, your choice will be the y-axis of the plot.
Chromatogram 1	Choose a data set you want to plot along with the main display data. A thinner solid line is used for this data set. The options are: none, Rayleigh ratio, differential refractive index, UV absorbance, QELS count rate, and specific viscosity.

Table 11-1: EASI Graph Properties

Field	Description
Chromatogram 2	If you want to plot a third set of data, choose an item here and check the Show Chromatogram 2 property. A dashed line is used for this data set. The options are the same as for Chromatogram 1.
Chromatogram 3	If you want to plot a fourth set of data, choose an item here and check the Show Chromatogram 3 property. The options are the same as for Chromatogram 1.
Scaling	Choose whether to use normalized or relative scaling. "Normalized" means all data of a particular type (e.g., RI data) is scaled against the largest value of that type across all experiments. "Relative" means values in each experiment are scaled from 0 to 1, allowing all traces to have the same magnitude regardless of the actual values compared between experiments.
Log Scale	Check this box if you want a log scale used for the y-axis of the graph. The axis that log scaling applies to depends on the type of plot.
Abscissa Units	Choose the x-axis scale to use for a Distribution Type of time or volume. The options are min (minutes), mL (milliliters), h (hours), sec (seconds), or msec (milliseconds).

To further customize the appearance of an EASI graph, right-click on the graph and choose **Edit** from the pop-up menu. This opens the Edit Graph dialog, which looks like this:



This dialog has the following fields:

Table 11-2: Edit Graph Fields

Field	Description
Marker Size	Choose the marker size you want to use. The options are: very small, small, medium, and large. Use Marker Size for lines made up of individual data points; use Line Point Size for fitted curves.
Draw Marker Every	Choose how often you want markers to appear on the line. The options are every 1, 2, 3, 5, 10, 20, 30, or 50 data points.
Line Point Size	Set the width of the line when the line is a fitted curve. Use Marker Size if the line is made up of individual data points.
Show Chromatogram 1	Check this box to show a line for the first Chromatogram data set. The default is on.
Show Chromatogram 2	Check this box to show a line for the second Chromatogram data set. The default is off.
Show Chromatogram 3	Check this box to show a line for the third Chromatogram data set. The default is off.
Experiment	Choose the experiment for which you want to graph data.
Color	Select the line or marker color you want to use in the graph. Changing this property changes the line color for all chromatogram traces.
Marker Type	Select the marker type you want to use in the graph. The default is square.
X Axis Shift	Type a numeric value by which the x-axis values should be shifted. The number will be added to the existing times or volumes. You can use a negative value, for example, if 15 mL of solvent were run through the system initially, you might use an X Axis Shift of -15.

You can see the effects of your changes as you make them without closing this dialog.

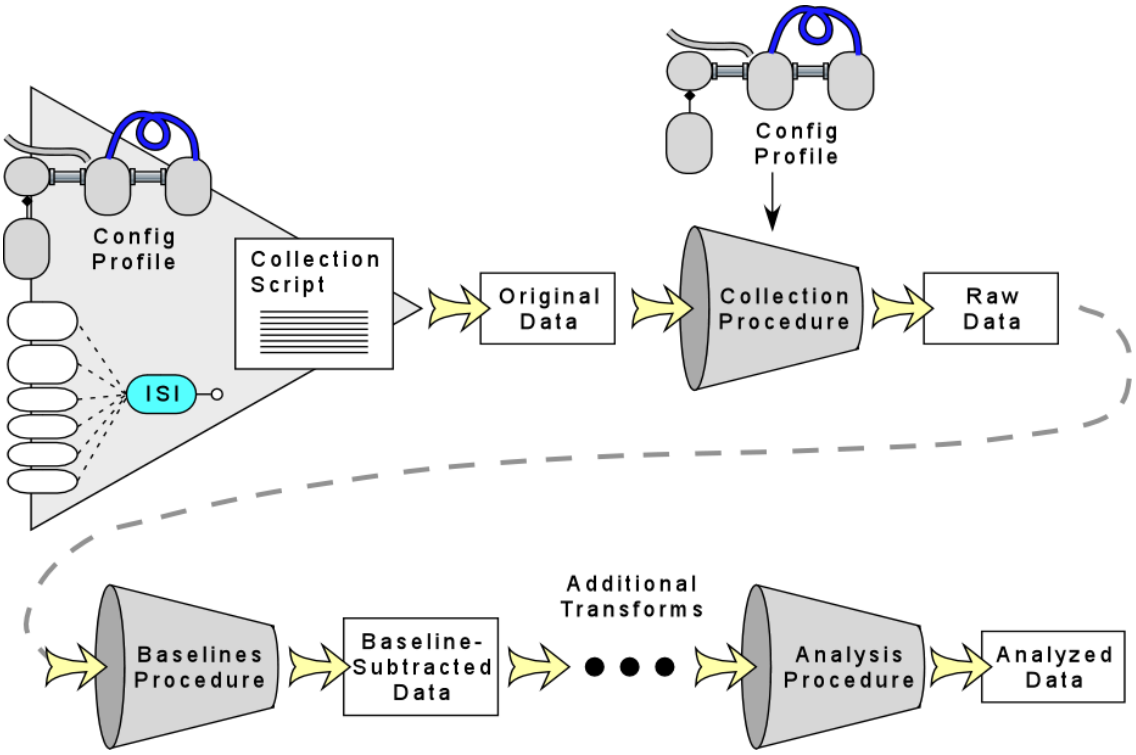
If you click **Advanced**, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

Using Custom Plots and Data Set Definitions

Data set definitions are used to create graphs. When you create and use data set definitions, you have access to more data sets stored within an experiment and have more control over which data is plotted.

Data Collection and Storage

ASTRA experiments store a set of data for each procedure in the experiment. Each set represents the data after the procedure ran. The following figure shows some of the typical data sets.



The data sets include the following:

- **Original data:** In addition to raw detector voltages, this data set typically includes the laser monitor signal and any auxiliary input data.
- **Raw data:** This is the data gathered by the data collection procedure. For a light-scattering experiment, this is the detector voltages. The raw data is kept with the experiment, so that data can be reprocessed if you modify the procedure and can be viewed in reports if desired.
- **Raw data after each transform:** This data has the results of despiking, smoothing, baselines, peaks, and other transformations. A separate data set for each transformation is stored in the experiment.
- **Data after conversion:** This data has the results of conversions, such as from detector voltages to Rayleigh ratios.
- **Analyzed data:** This data has been processed to arrive at results such as molar mass, RMS radius, or other values.

Creating Data Set Definitions

Experiment Builder

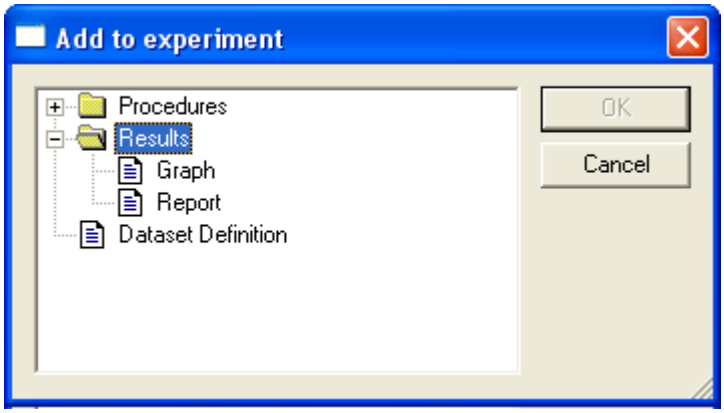
Data set definitions are visible in the experiment tree of the workspace and can be created only if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**.

You can create a data set definition before or after you run an experiment.

To create a new data set definition, follow these steps:

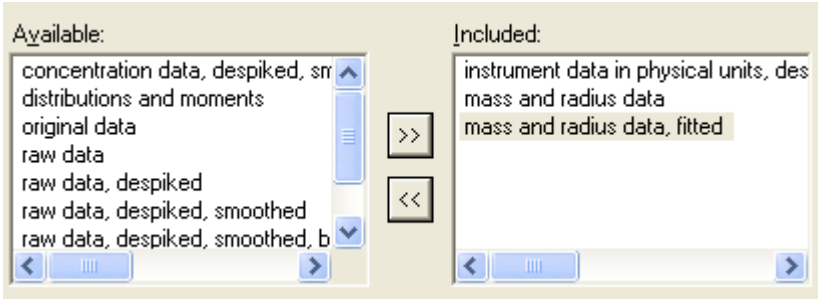
1. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results nodes of the experiment.

Shortcuts: Press Ctrl+Shift+P.
Right-click any folder in the experiment tree, and choose **Manage→Add To Experiment**.

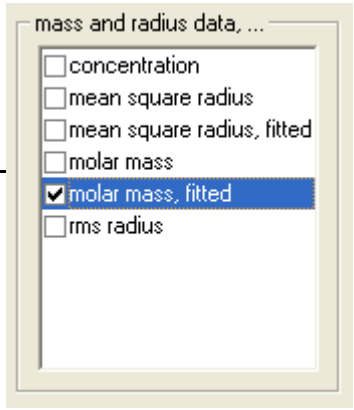


2. Close or scroll past the Procedures node, select Data Set Definition, and click OK.
3. Double-click the "untitled" item that is added to the Data Set Definitions folder in the experiment tree.
4. In the Data Set Definition dialog, type a name for this data set. Typically the name should describe what you want to graph. For example, "molar mass vs. volume".
5. In the Available list, set one or more data set stages you want to graph data from. Each of these stages represents the data after an individual procedure runs. See "Data Collection and Storage" on page 11-7.

6. Click the >> button to move the selected items to the Included list.

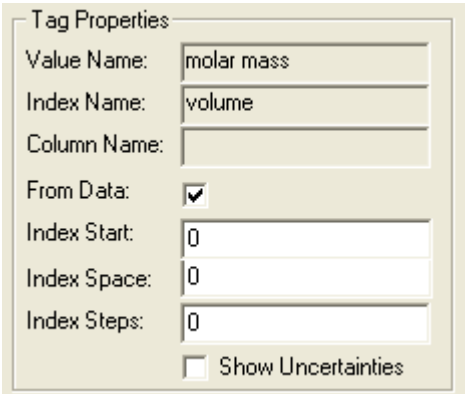


7. Select an item you have moved to the Included list. In the list of data in that stage, place checkmarks next to the data you want to graph.



Note: Be aware that all the data items selected must be graphable against the same x-axis (index) values. For example, both molar mass and concentration can be graphed against volume (if that was the abscissa unit selected in the experiment configuration). However, the "fit of RMS radius vs. molar mass" can only be graphed against an x-axis of the molar mass.

8. When you select an item in the checkmark list, the Tag Properties fields change to describe aspects of that data. Some fields are modifiable for some items. Some fields vary depending on the item selected.



9. Modify values as needed for the data items you selected. All the changes you make for various items will be saved when you later click **OK**.

The properties in the list are as follows:

Table 11-3: Data Set Definition Properties

Field	Description
Value Name	The name of the selected item. (The y-axis values.)
Index Name	The index against which the item can be plotted. (The x-axis values.)
Column Name	Some data set items have column names that describe the type of data.
From Data	If you have selected a function tag, this box specifies whether the function is calculated from the x-axis data values. If not, you need to specify the index start and increment in the following fields.
Index Use	This property is not yet implemented.

Table 11-3: Data Set Definition Properties

Field	Description
Index Value	This property is not yet implemented.
Column Use	If you are using a matrix tag, you can specify how the columns are to be used. Select which column values to use. The options are: DSD_USE_ALL: Use all column values. DSD_CONTROL_VARIABLE: This option is not yet implemented. DSD_AT_VALUE: Use only one column with the value specified below.
Column Value	If you are using a matrix tag and are using only one column, this is the value of the column to use. For example, 90 degrees for the right-angle detector.
Index Start	If you are using a function tag and are not calculating the function from data, specify the starting index.
Index Space	If you are using a function tag and are not calculating the function from data, specify the range the x-axis should span.
Index Steps	If you are using a function tag and are not calculating the function from data, specify the total number of index points.
Show Uncertainties	Check this box if you want the graph to contain uncertainty error bars for this item.

10. Put a checkmark in the **Iterate Experiment Data Over Injection** box if there are multiple injections in the experiment that are to be displayed all at once.
11. If you checked the box to iterate, select the items to iterate.
12. Click **OK** to save your changes.

Using Data From Multiple Experiments

If you want to graph data from multiple experiments, you can copy data from one experiment to another. The copied data is shown in the "Available" list in the Data Set Definition dialog. See "Copying Data" on page 6-21 for details.

Other Uses for Data Set Definitions

When you export an experiment, you can choose to export the data matching a data set definition as a tab-delimited or comma-delimited text file. For details, see "Saving an Experiment to a File" on page 6-16 or "Exporting an Experiment" on page 6-17.

Creating Custom Plots

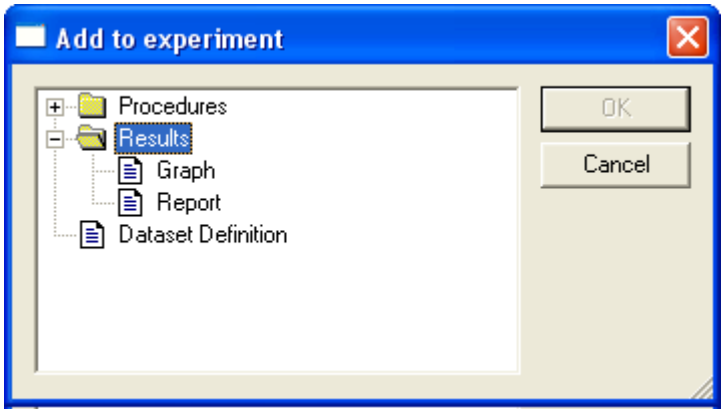
In Run mode, you can add custom graphs by choosing **Experiment→Graph→Add Custom Plot** from the menus. This opens the Data for Custom Plot dialog, and you can specify the data to display. Both the data set definition and results graph are created.

Experiment Builder You can also add graphs as follows if you enable Experiment Builder mode by choosing **System→Preferences→Experiment Builder Mode**:

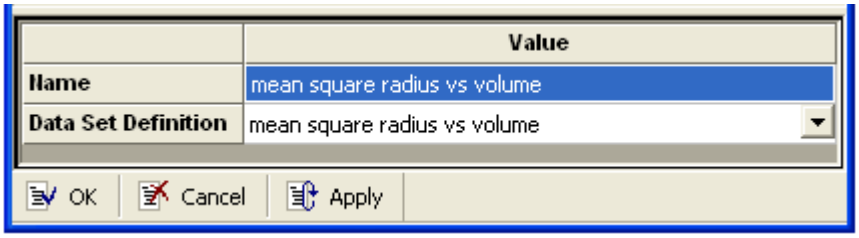
1. Create a data set definition for the data to be graphed as described in "Creating Data Set Definitions" on page 11-8.

2. Choose **Experiment→Add to Experiment**. This opens the Add to Experiment dialog, which allows you to add items to the Procedures, Data Set Definitions, and Results folders of the experiment.

Shortcuts: Press Ctrl+Shift+P.



3. Select Graph (in the Results folder) and click OK.
4. Double-click the "Graph (untitled1)" item that was added to the Results folder in the experiment tree.
5. In the property list for the graph, type a Name to appear at the top of the graph.
6. For the Data Set Definition, select a data set definition you have already created.

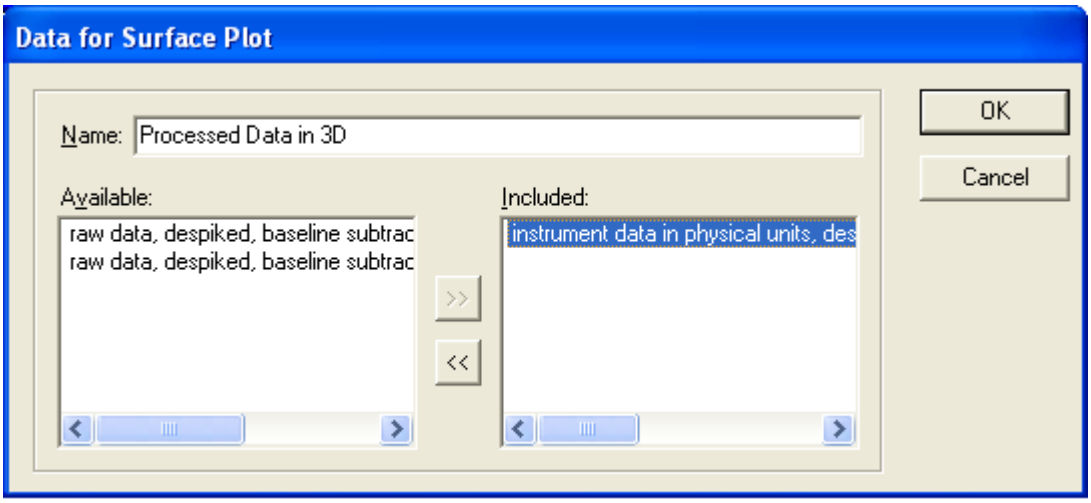


7. Click **OK**.
8. Choose **Experiment→Run** to run or re-run the experiment. After the procedure runs successfully, the graphs and reports are generated.

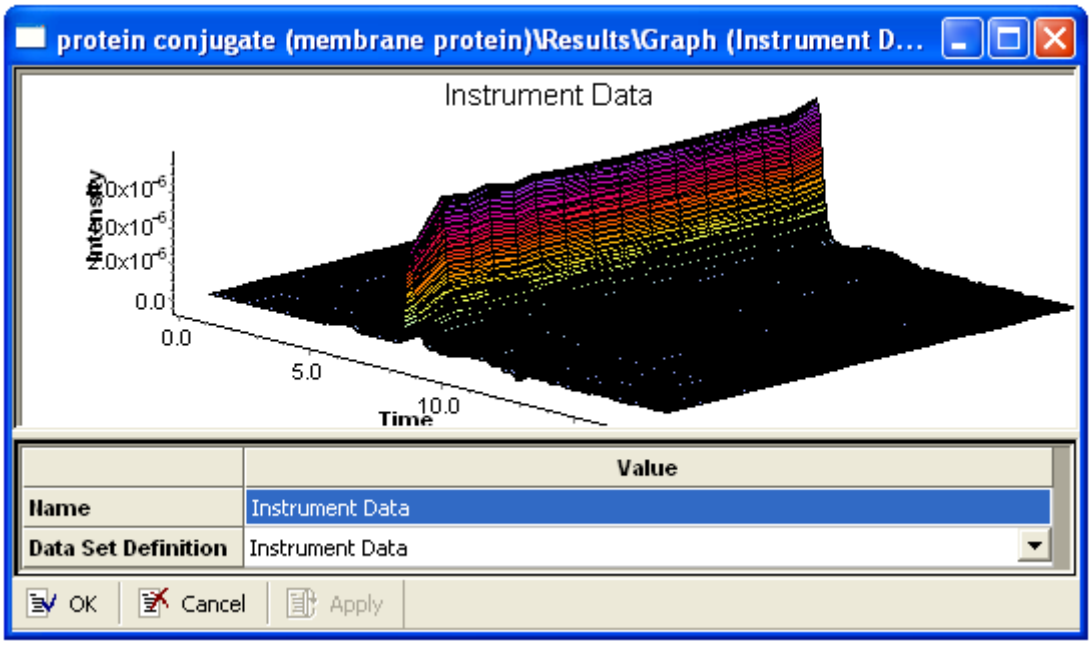
Creating Surface Plots

You can create a 3D surface plot of detector data. To create a 3D surface plot, follow these steps:

1. Choose **Experiment→Graph→Add Surface Plot** from the menus. This opens the Data for Surface Plot dialog.



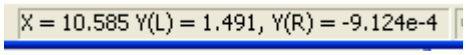
2. Type a name for your plot.
3. Select one of the types of available data and click the **>>** button. You can scroll the list to the right to read the full description of each data set. The sets listed have data for multiple detectors. Only data sets that can be plotted in three dimensions are listed here.
4. Click **OK**. Both a data set definition and the 3D graph are created.
5. Double-click on the new graph listed in the Results folder for this experiment in your workspace. It may take some time to load graphs of large amounts of data.



Viewing and Modifying Graphs

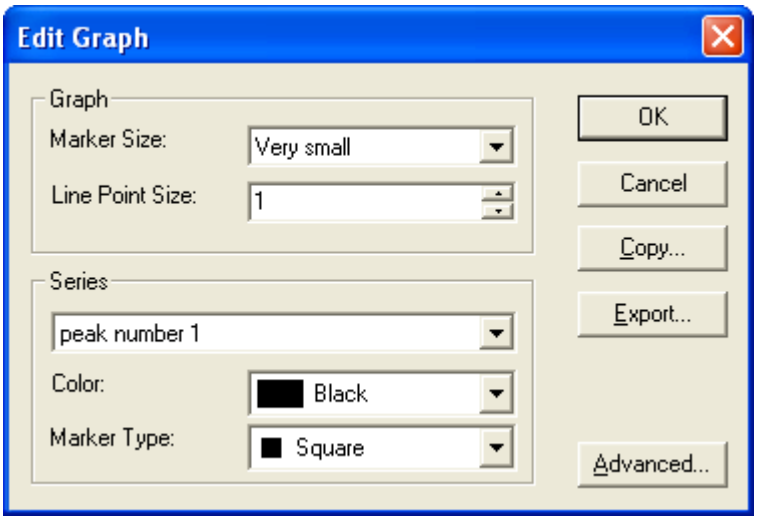
The graphs you generate can be manipulated in a number of ways.

You can see the coordinates for your current mouse location in a graph by holding down the Shift key. The coordinates are shown in the status bar. If the Y axis has more than one scale, the left axis is shown as Y(L) and the right axis is shown as Y(R).



Customizing Line Colors and Widths

To change the line colors and widths in a graph, either double-click the graph or right-click and choose **Edit**. This opens the Edit Graph dialog.



The **Marker Size** and **Line Point Size** fields let you set the width of the line. Some lines are made up of individual data points and some are drawn as a line. Use the field that applies to your graph.

To change the color of a data set, choose a data **Series**. Then, in the **Color** field select a defined color or "Custom" to choose other colors.

You can see the effects of your changes as you make them without closing this dialog.

If you click **Advanced**, you have much more control over the graph display is provided than is described in this manual. For help on settings in the Advanced dialog, move to a field and press F1.

Graph customizations—such as line weight, color, marker style, and title changes—are saved for EASI graphs, custom plots, parametric plots, and surface plots. However, such graph customizations are not saved when you close procedure dialogs that contain graphs.

See page 11-6 for details about the EASI Graph version of the Edit Graph dialog.

Zooming In and Out Graphs

- **Zooming in:** Hold down the Ctrl key and your left mouse button. Drag a rectangle around the data you want to view larger. Then release the mouse button. Alternately, you can press Ctrl+F5 to zoom in one level.
- **Zooming out:** Hold down the Ctrl key and click your right mouse button. Each click undoes one zoom in action. Alternately, you can press Ctrl+Shift+F5 to zoom out one level.

Repositioning Graphs

When you hold down both the Shift key and the right mouse button, you can drag the data to view a different portion of the graph. This is useful when you have zoomed in and want to scroll around the graph.

Press Ctrl+right mouse button to restore the graph to its original position.

Axis Settings

To control the values shown on each axis, follow these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.
2. In the **Chart** tab, choose the **Axis** tab. (The editing dialog has multiple levels of tabs.)
3. In the **Axis** tab, you can select an axis and then change many aspects of how that axis is displayed. For example, click **Change** under Minimum or Maximum to change the range of values for that axis. Also, this tab has a checkbox to turn logarithmic scaling on and off.
4. If you drag the Edit Graph dialog to the side, you can see the effects of changes in your graph as you make them.
5. Click **Close** when you are finished changing the display.

Other tabs of interest in the advanced Edit Graph dialog are as follows:

- **Chart→Axis→Title:** Modify axis title display.
- **Chart→Axis→Labels:** Modify axis label formats and font.
- **Chart→Axis→Ticks:** Modify major ticks on selected axis.
- **Chart→Axis→Minor:** Modify minor ticks on selected axis.
- **Chart→Titles:** Modify graph title text, location, and format.
- **Chart→Legend:** Modify graph legend location and format.

For help on settings in the advanced Edit Graph dialog, move to a field and press F1.

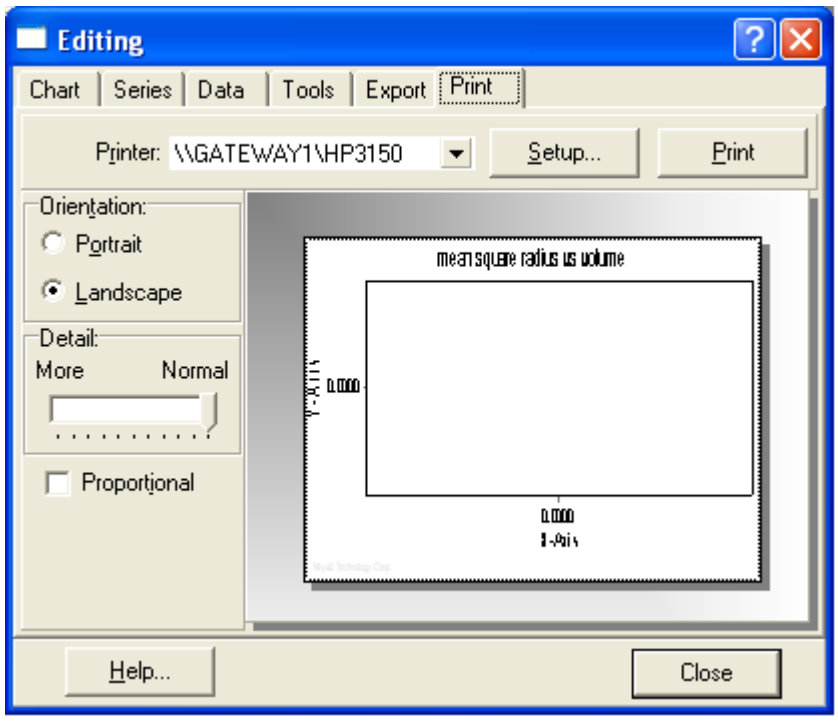
Printing Graphs

To print a graph, do either of the following:

- Right-click on the graph and choose **Print**.
- Choose **File→Print** from the menus.

To print a graph with more control over the output, follow these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.
2. Choose the **Print** tab from the top row of tabs.



3. In the Printer list, choose the printer you want to send the graph to. Click **Setup** if you want to adjust printing properties.
4. Choose an orientation of Portrait or Landscape. The default is Landscape, which is appropriate for most graphs.
5. Use the Detail slider to adjust the quality of the output and number of major tick marks on the axes.
6. Put a checkmark in the Proportional box if you want the graph to have a height and width proportional to the current graph display. After you check this box, you can drag the dotted lines on the preview to resize the graph on the page.
7. When you are ready to print, click **Print**.

Copying Graphs to the Clipboard

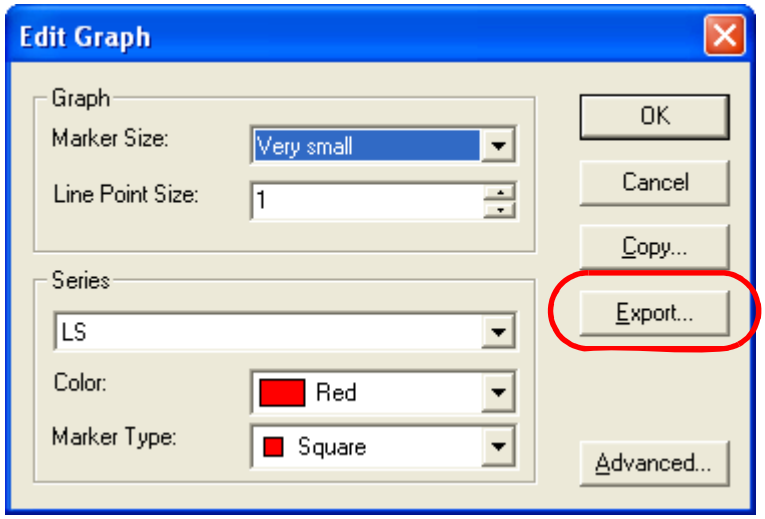
To copy a graph to the clipboard for pasting into another applications, press Ctrl+C while in the dialog that contains the graph. Alternately, right-click on the graph and choose **Edit** from the pop-up menu. Then, click the **Copy** button in the Edit Graph dialog.

Exporting Graphs

You can export graphs as pictures or data for use in other applications. You do this with the dialog you see when you double-click on a graph. The **Experiment→Graph→Export** command exports experiment data, rather than graph data.

Exporting Pictures

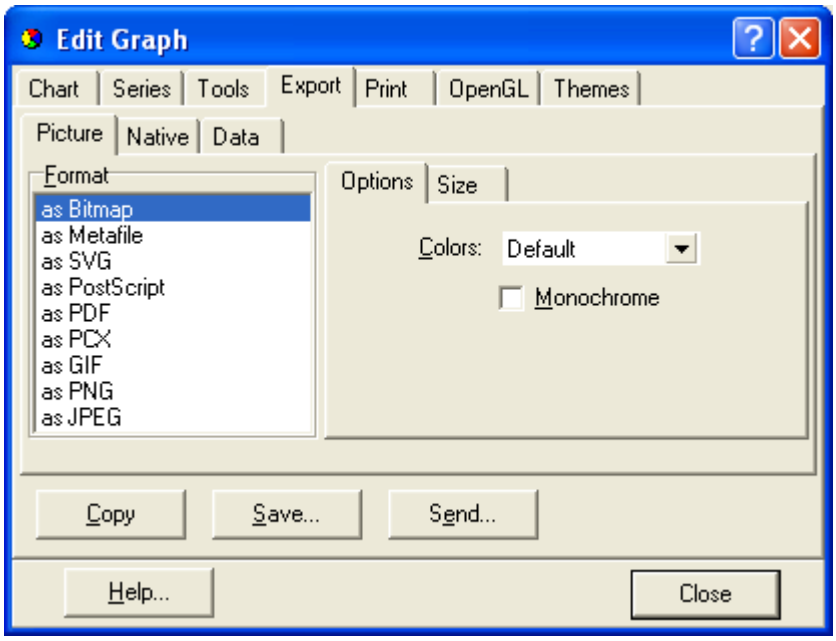
To quickly export a graph image, double-click on a graph to open the Edit Graph dialog. Click the **Export** button and choose the JPEG output file type. See "Working with Procedure Graphs" on page 8-5 for details.



To export a graph to a graphics file with more control over the output, follow these steps:

1. Double-click on a graph. In the Edit Graph dialog, click **Advanced**.
2. Choose the **Export** tab from the top row of tabs.

3. Choose the **Picture** tab from the second row of tabs.



4. Select a file format to export. Metafile is a vector-based Windows Metafile (.wmf) used by applications such as Microsoft Word. SVG is also a vector-based format. Bitmap, GIF, JPEG, PNG, and PCX are all pixel-based image formats with different types of compression. PDF is the Adobe Acrobat format. PostScript is an output format used by many printers. Since encapsulated PostScript is created, some applications can import graphics in this format.
5. The **Options** and **Size** tabs offer different settings depending on the format you select.
6. Once you have set the format and options, click one of these buttons:
   - **Copy:** Store the graphic on the clipboard in this format for pasting into another application.
   - **Save:** Send the graphic to a file of this type. You are prompted for the file name and location.
   - **Send:** Send the graphic to an application such as Microsoft Outlook. This is typically used to email the graphic.

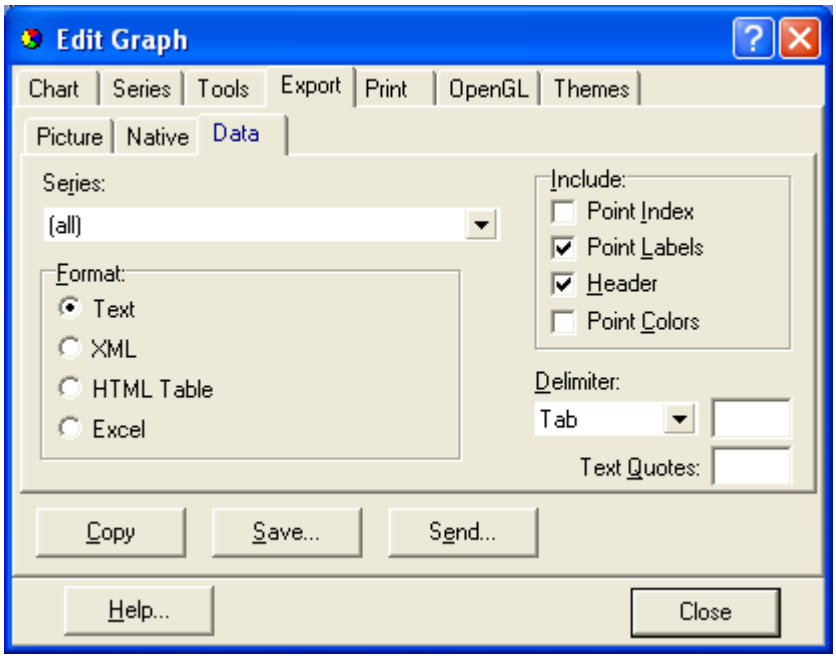
Exporting Data

To quickly export graph data, double-click on a graph to open the Edit Graph dialog. Click the **Export** button and choose a data output file type. See “Working with Procedure Graphs” on page 8-5 for details.

To export a graph to a data file that you can use with a spreadsheet with more control over the output, follow these steps:

1. Double-click on a graph to open the Edit Graph dialog. Then click **Advanced**.
2. Choose the **Export** tab from the top row of tabs.

3. Choose the **Data** tab from the second row of tabs.



4. Select the output format you want: text, XML, HTML table, or Microsoft Excel. If you select Text, you can select a Delimiter to separate the fields. Delimiters are commonly used if you will be importing the data into a spreadsheet or database. You can also specify a quote character (usually blank, ‘, or “) to use around text in the output.
5. Select any other information you want to include in the data file, such as headers.
6. Once you have set the format and other options, click one of the following buttons:
   - **Copy:** Store the data on the clipboard in this format for pasting into another application.
   - **Save:** Send the data to a file of this type. You are prompted for the file name and location. The default file extension matches the format you selected in the Edit Graph window.
   - **Send:** Send the data to an application such as Microsoft Outlook. This is typically used to email the data.

If you do not find the organization of data output useful, try the output described in “Exporting an Experiment” on page 6-17.

12 Working with System Profiles

This chapter explains how to create and use system profiles in ASTRA V.

CONTENTS **PAGE**

About Profiles

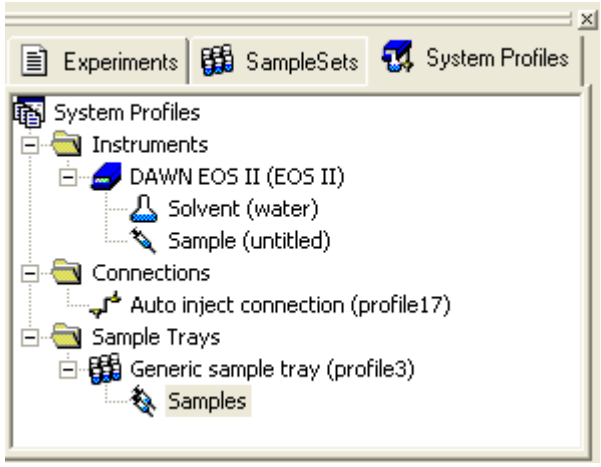
In an experiment, a set of instrument and connection profiles describes how an experiment is set up. This is called the *configuration*. You can also create profiles that are stored outside of experiments and can be copied into experiments as needed; these are called *system profiles*.

The experiment templates provided with ASTRA contain commonly used configurations. However, if your instrument setup differs from the default templates, you may find that you need to modify new experiments in the same way each time you create one.

System profiles allow you to save time by creating reusable blocks of information about your instruments and experiment setup. You can copy this information into new experiments. (Another way to save time is to save experiments as templates as described in "Creating a Template" on page 6-18.)

This chapter focuses on system profiles. See Chapter 7, "Configuring Experiments" for information about configurations and for reference information about the properties of all the available profile items that make up configurations and system profiles.

You can work with system profiles in the System Profiles tab of the workspace.



Creating Profiles

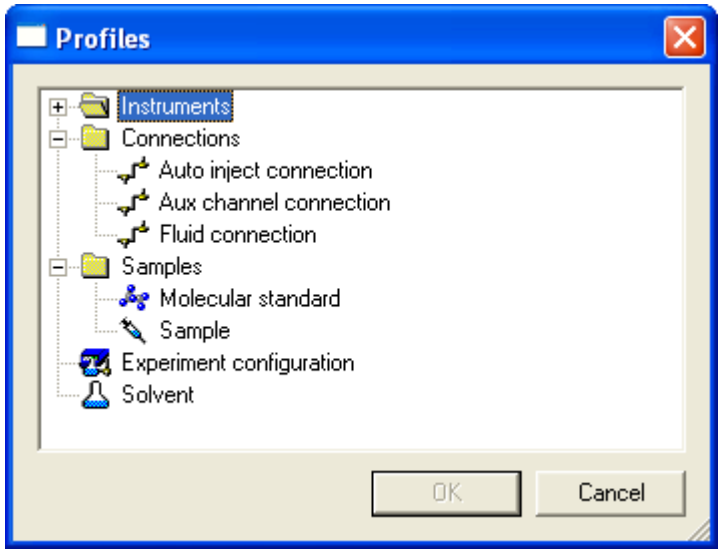
There are several ways to create system profiles. These profiles are stored in the system database, rather than in separate files. Experiments use copies of system profiles, but modifying the portion of an experiment configuration that came from a profile does not affect the system profile itself.

Creating a New Profile

To create a profile with settings used in your experiments using the default profiles as a starting point, follow these steps:

1. Choose **File→New→System Profile**. You will see the Profiles dialog.

Shortcuts: Press Ctrl+Alt+N.
Click the down-arrow next to the icon.
Right-click on any profile item in the workspace, and choose **New**.



2. In the Profiles dialog, select the type of profile you want to create.
3. Click **OK**.
4. In the Save As dialog, type a name for the profile. You can also select a folder in the system database to contain this profile.
5. Double-click the item you created in the System Profiles tab of the workspace to open its property dialog.

   If you created a profile type that has multiple components, there will be a tab for each component. For example, a light scattering instrument in batch mode has a tab for a solvent and a sample. This information is stored with and imported with the instrument profile.
6. Edit the properties as needed. See Chapter 7, "Configuring Experiments" for details about the properties of all profile types.
7. Click **OK** or **Apply** to save your changes.

Exporting a System Profile

You can create a system profile by saving it from an experiment. To do this, follow these steps:

1. If you have more than one experiment open, make sure the one you want to export from is selected in the experiment tree of the workspace.
2. Select the item in the configuration you want to export. (Any items nested at a lower level will be exported along with the item you select. For example, in the following figure, exporting the injector creates a system profile that contains the injector and the sample. If you export the configuration item, the entire configuration is saved as a system profile.)
3. Choose **Experiment→Configuration→Save Configuration As**. Or right-click on an item and choose the **Save As** item from its right-click menu.
4. In the Save As dialog, choose the folder where you want to save the system profile. Then type a name for the profile you are creating, and click **OK**.

Note:	No pre-defined instrument profiles are provided with ASTRA. In order to have instruments available, you need to first save instrument profiles in your system database.

Modifying Profiles

The system profiles are contained in the system database. To work with a system profile you have already created or one of the profiles provided with ASTRA V, you first open the profile in the System Profiles tab of the workspace. Then you can view, edit, or rename the profile.

Opening a Profile

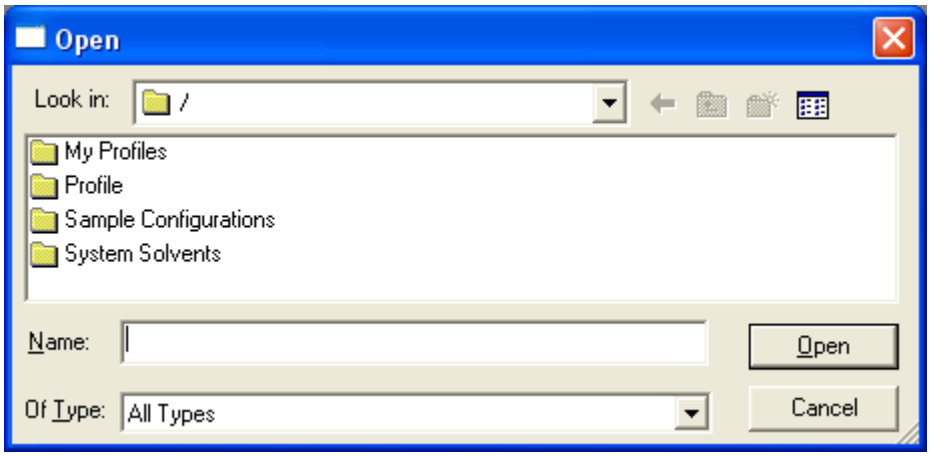
If you just created a system profile, it is open and listed in the System Profiles tab of the workspace. If the profile is not listed, you need to open it before you can modify it.

To open a system profile, follow these steps:

1. Select **File→Open→System Profile**.

Shortcuts: Press Ctrl+Alt+O.
Click the down-arrow next to the  icon.
Right-click on any profile item in the workspace, and choose **Open**.

2. In the Open dialog, locate the profile you want to open.



Profiles may be stored in any of the following folders:

- **My Profiles:** A handy place to save profiles you create.
- **Example Configurations:** A set of complete configurations.
- **System Solvents:** A collection of solvent profiles.

3. You can select the type of profile you want to find in the Of Type list.

   As in standard file selection dialogs, you can click the  icon to change the view of the list of experiments in the database. In the detail view, the last data and time the experiment was modified is shown.

4. Click **Open**. You see the profile listed in the System Profiles tab of the workspace.

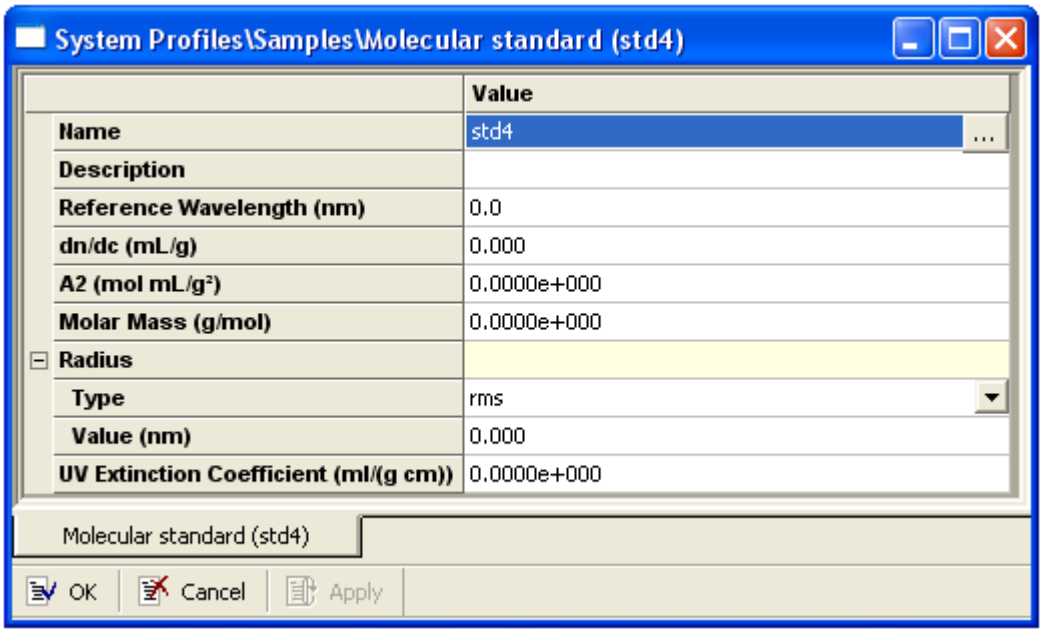
Editing a Profile

To modify a system profile that you have opened, follow these steps:

1. Double-click the item you created in the System Profiles tab of the workspace to open its property dialog.

   If you created a profile type that has multiple components, there will be a tab for each component. For example, a light scattering instrument in batch mode has a tab for a solvent and a sample. This information is stored with and imported with the instrument profile.

2. Edit the properties as needed. See Chapter 7, "Configuring Experiments" for details about the properties of all profile types.



3. Click **OK** or **Apply** to save your changes.

Information from a system profile is copied when it is used in an experiment. After being copied, there is no link between the system profile and the experiment. So, editing a system profile has no effect on experiments to which the system profile was previously copied. In addition, modifying portions of an experiment configuration that came from a system profile has no effect on the original system profile.

Saving a Profile

Changes to profiles are automatically saved to the system database when you click **OK** or **Apply** in their property dialog. If you attempt to close the dialog without saving, you are asked whether to save the changes.

Duplicating a Profile with Save As

To save a profile with another name, follow these steps

1. Select a profile in the System Profiles tab of the workspace.
2. Choose **File→Save As**.

Shortcuts: Right-click on any profile in the workspace, and choose **Save As**.

3. In the Save As dialog, select the system database location where you want to save the new profile.
4. Type a name for the new profile.
5. Click **Save**.

You can use Save As and then delete the original system profile to rename a system profile.

Closing a Profile

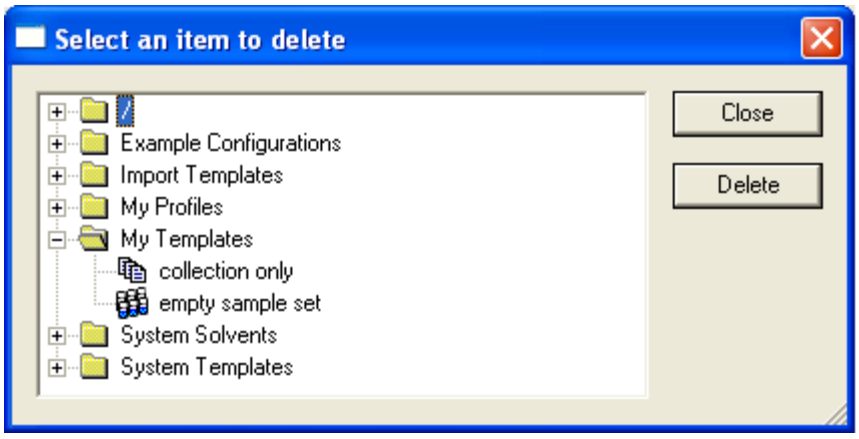
To close a profile, select the profile you want to close and choose **File→Close**. The profile is still available for use in the system database; it is simply not shown in the System Profiles tab.

Shortcuts: Right-click on any profile in the workspace, and choose **Close**.

Deleting a Profile

To delete an existing profile, follow these steps:

1. Select **System→Database Administration→Delete Items**.
2. In the Select an item to delete dialog, select a profile to delete. Profiles are usually in the "My Profiles" folder.



3. Click **Delete**.
4. You are asked if you are sure you want to delete the selected profile. Click **Yes** if you are sure.
5. Click **Close** when you are finished deleting profiles.

Using Profiles

The benefit of creating system profiles is that you can use them to save time when configuring your experiments.

If you later edit properties of items you import or copy from a system profile, there is no effect on the system profile from which it was obtained.

For more about using profiles, see "Using Configurations" on page 7-5.

Adding an Item to a Configuration

You might want to add a component to an experiment. For example, you may have added a UV instrument to an experiment.

To add an item to an experiment configuration using a system profile, follow these steps:

1. If you have more than one experiment open, make sure the one you wish to modify is selected in the experiment tree of the workspace.
2. Choose **Experiment→Configuration→Replace Configuration**. You see the Select Profile dialog.
3. In the Of Type field, select the type of item you are looking for: Connections, or Instruments.
4. Browse the system database for a profile to import.
5. When you find the profile, select it and click **Open**.

The item is added to your experiment. (If you want to import an entire configuration, see "Replacing an Entire Configuration" on page 12-9.)

Note: No pre-defined instrument profiles are provided with ASTRA. In order to have instruments available, you need to first save instrument profiles in your system database as described in "Creating Profiles" on page 12-3.

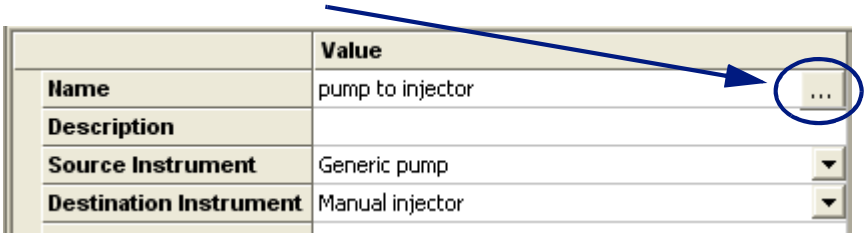
Replacing a Single Configuration Item

You can copy all the properties set for a system profile into the property dialog for an item in an experiment configuration. This has the effect of replacing the item in the experiment with a system profile

To copy from a system profile to an experiment, follow these steps:

1. Double-click on an item in Configuration tree in the Experiments tab of the workspace to open its property dialog.

2. Click the browse button ("...") to the right of the Name property.



3. In the Copy System Profile dialog, find the profile you want to copy from. The Of Type field is automatically set to match only the type of item you are editing.
4. Click **Copy**. The values of properties in the system profile are copied to the experiment.

Note: No pre-defined instrument profiles are provided with ASTRA. In order to have instruments available, you need to first save instrument profiles in your system database as described in "Creating Profiles" on page 12-3.

Replacing an Entire Configuration

You can replace the entire configuration with an experiment configuration that has been saved as a system profile. For example, you might have a standard experiment configuration that you want to use in many different experiments. You can import that configuration into an existing experiment.

To import a completely new configuration, follow these steps:

1. If you have more than one experiment open, make sure the one you wish to modify is selected in the experiment tree of the workspace.
2. Choose **Experiment→Configuration→Replace Configuration**. You see the Select Profile dialog.
3. In the Of Type field, select Experiment Configurations.
4. Browse the system database for a profile to import. A number of configurations are provided with ASTRA V in the Example Configurations folder.
5. When you find a profile, select it and click **Open**.
6. If you select the profile of an instrument or connection, the item is added to your experiment. If you select the profile of an entire configuration, that configuration replaces the existing one.

For more information about replacing a configuration or part of a configuration with a system profile, see "Replacing an Experiment Configuration or Item" on page 7-7.

A Menu Quick Reference

This chapter contains a quick reference for ASTRA V menu commands and keyboard shortcuts.

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Modes and User Levels

The following tables list the commands in the ASTRA V menus. The Description column includes a link to more information about the command. The Modes column identifies when the command is available as follows:

- **Builder:** This command is inactive unless you enable **System→Preferences→Experiment Builder Mode**, which is only available to researchers and administrators.
- **G:** Guest user level
- **T:** Technician user level
- **R:** Researcher user level
- **A:** Administrator user level
- **DB and Security:** This command exists only when using ASTRA V with Research Database or ASTRA V with Security Pack.

  If a command is not limited to "DB and Security", it is always available in ASTRA V Basic, even if user levels are listed. The user levels apply only to ASTRA V with Research Database and ASTRA V with Security Pack.

File Menu

The File menu contains the following commands:

Command	Keyboard Shortcut	Description	Modes
File→New			
→Experiment from Default		Create an experiment from the default template. See page 6-4.	T, R, A
→Experiment from Template	Ctrl+Alt+T	Create an experiment from a template. See page 6-5.	T, R, A
→Blank Experiment	Ctrl+N	Create an empty skeleton of an experiment. See page 6-6.	Builder: R, A
→Sample Set from Template		Create a sample set from a template. See page 9-3.	T, R, A
→Blank Sample Set	Ctrl+Shift+N	Create an empty skeleton of a sample set. See page 9-3.	R, A
→System Profile	Ctrl+Alt+N	Create a system profile. See page 12-3.	R, A
File→Open			
→Experiment	Ctrl+O	Open an existing experiment. See page 6-7 and page 6-8.	All
→Sample Set	Ctrl+Shift+O	Open an existing sample set. See page 9-4.	All
→System Profile	Ctrl+Alt+O	Open an existing system profile. See page 12-5.	All

Command	Keyboard Shortcut	Description	Modes
File→Close		Close the active item. See page 6-15 and page 12-7.	All
File→Close All		Close all items in the current tab. See page 6-15.	All
File→Save	Ctrl+S	Save the selection. See page 6-15 and page 6-16.	T, R, A
File→Save As		Save the selection with a new name. See page 6-15 and page 6-16.	T, R, A
File→Save As Template		Save the experiment as a template. See page 6-18.	T, R, A
File→Begin Injection	Ctrl+Shift+V	Create an experiment from the default template and start running it. See page 6-4.	T, R, A
File→Import			
→Experiment		Import an experiment from a file. See page 6-9.	DB and Security: R, A
→Sample Set		Import a sample set from an ASTRA 4 or ASTRA 5 file. See page 9-4.	DB and Security: R, A
→Empower Sample Set		Import a sample set created with Waters Empower. See page 9-5.	R, A
File→Export			
→Experiment		Save the selected experiment to a separate file. See page 6-17.	R, A
→Sample Set		Export a sample set from the database to a file. See page 9-11.	R, A
File→Batch Apply Template		Apply experiment template to multiple experiments. See page 6-23.	T, R, A
File→Print	Ctrl+P	Print the currently active view. See page 3-15.	All
File→Print Preview		Preview the currently active report. See page page 10-6.	All
File→Print Setup		Set up page formatting for printing. See page 3-15.	All
File→Recent Experiments		Open a recently used experiment. See page 6-7 and page 6-8.	All
File→Exit		Close all windows and exit from ASTRA V. See page 3-15.	All

Edit Menu

The Edit menu contains the following commands:

Command	Keyboard Shortcut	Description	
Edit→Undo	Ctrl+Alt+Backspace	Undo the previous action. This command is currently disabled.	All
Edit→Cut	Ctrl+Shift+NumDel	Cut the currently selected item and place it on the clipboard. This command is available only as appropriate.	All
Edit→Copy	Ctrl+C	Copy the currently selected item to the clipboard. This command is available only as appropriate.	All
Edit→Paste	Ctrl+V	Paste the contents of the clipboard to the current loacation. This command is available only as appropriate.	All
Edit→Delete		Delete the currently selected item. This command is available only as appropriate.	All
Edit→Select All	Ctrl+A	Select everything in the active view. This command is available only as appropriate.	All

View Menu

The View menu contains the following commands:

Command	Keyboard Shortcut	Description	
View→Standard Toolbar		Hide and show OK, Apply, Cancel toolbar at bottom of views. See page 3-14.	All
View→Status Bar		Hide and show status bar at bottom of main window. See page 3-14.	All
View→Workspace		Hide and show workspace with Experiment tree, Sample sets tree, and System Profile tree. See page 3-14.	All
View→Customize		Open dialog to customize toolbars and keyboard shortcuts. See page 3-14.	All

Experiment Menu

The Experiment menu contains the following commands:

Command	Keyboard Shortcut	Description	
Experiment→Configuration			
→Edit		Open the property dialog for configuration of the currently selected experiment. See page 7-5.	All (view-only as Guest)
→Replace Configuration		Import a configuration item or entire configuration from a system profile. See page 7-7 and page 12-4.	R, A
→Save Configuration As		Export a configuration item or an entire configuration to a system profile. See page 7-8 and page 12-3.	R, A
→Alignment		Opens procedure dialog for determining the interdetector delay. See page 8-17.	R, A
→Band Broadening		Opens procedure dialog for correcting effects of fluid mixing between instruments. See page 8-14.	R, A
→Normalize		Opens procedure dialog for relating detector signals to the 90 degree detector signal and the instrument calibration constant. See page 8-32.	R, A
→Calibrate Column		Perform a column calibration for a SEC column. See page 8-37.	R, A
Experiment→Add To Experiment	Ctrl+Shift+P	Add a procedure, report, graph, or data set definition to an experiment. See page 8-7, page 10-4, and page 11-10.	Builder: R, A
Experiment→Copy From		Copy data from one experiment to the current experiment. See page 6-21.	R, A
Experiment→Apply Template		Apply procedures and results to the current experiment, creating a new experiment. See page 6-22.	T, R, A
Experiment→Validate		Validate the experiment procedure sequence and instrument availability. See page 6-11.	T, R, A
Experiment→Run	Ctrl+Shift+R	Start the experiment run. See page 6-11.	T, R, A
Experiment→Run Indefinitely		Run the experiment data collection until manually stopped. See page 6-12.	T, R, A
Experiment→Stop	Ctrl+Shift+S	Halt the experiment. See page 6-13.	T, R, A
Experiment→EASI Graph		Add a customizable graph to the results. See page 11-3.	All
Experiment→Report			
→Export		Export a report to a text file or a comma-separated values file. See page 10-6.	R, A
→Page Setup		Set up page display for printing reports. This command is not yet implemented.	--
→Properties		View the properties of the report. This command is not yet implemented.	--

Command	Keyboard Shortcut	Description	
Experiment→Graph			
→Add Custom Plot		Add a custom plot to the results. See page 11-10.	R, A
→Add Parametric Plot		Add a parametric plot to the results. See page 8-83.	R, A
→Add Surface Plot		Add a surface plot to the results. See page 11-12.	R, A
→Auto Scale		Auto scale the current graph. This command is not yet implemented.	R, A
→Scale		Change graph scale manually. This command is not yet implemented.	R, A
→Export		Export a graph to a text file or a comma-separated values file. See page 11-16.	R, A
→Page Setup		Set up page display for printing graphs. This command is not yet implemented.	--
→Properties		View the properties of a graph. This command is not yet implemented.	--
Experiment→Sign Off		Allow user to sign off experiment for 21 CFR Part 11 compliance. See page 6-12.	T, R, A
Experiment→Log→Open		Display experiment log. See page 4-7.	All
Experiment→Log→Save As		Save experiment log to a file. See page 4-7.	All

Sample Set Menu

The Sample Set menu contains the following commands:

Command	Keyboard Shortcut	Description	
Sample Set→Edit		Open the property dialog for configuration of the currently selected sample set. See page 9-5.	All (view-only as Guest)
Sample Set→Validate		Validate the sample set procedure sequence and instrument availability. See page 9-9.	T, R, A
Sample Set→Run		Start to run the sample set. See page 9-9.	T, R, All
Sample Set→Stop		Halt the active sample set. See page 9-10.	T, R, A
Sample Set→Log→ Open		Display sample set log. See page 9-10.	All
Sample Set→Log→ Save As		Save sample set log to a file. See page 9-10.	All

System Menu

The System menu contains the following commands:

Command	Keyboard Shortcut	Description	
System→Instruments		View list of connected instruments. See page 2-10.	All
System→Database Administration			
→Connect to Database		Connect to a different experiment database. See page 4-3.	DB and Security
→Delete Items		Delete experiments, sample sets, profiles, or templates from database. See page 4-8, page 6-19, page 9-11, page 12-7.	DB and Security: R, A
→Migrate		Update system database with latest ASTRA templates and profiles. See page 2-3.	DB and Security: A
→Automatic Maintenance		Perform database maintenance. See page 4-9.	DB and Security: All
→Log→Open		View experiment database log. See page 4-7.	DB and Security: All
→Log→Save As		Save experiment database log to a file. See page 4-7.	DB and Security: All
→Properties		View properties of experiment database. See page 4-3.	DB and Security
System→Feature Activation		Activate features by providing activation key. See page 2-5.	DB and Security: A
System→Preferences			
→Experiment Builder Mode		Set to Experiment Builder mode. See page 1-3.	DB and Security: R, A
→Auto-hide Workspace		Set the workspace to hide when not in use. See page 3-14.	All
→Set Default Template		Set the default template to use when creating a new experiment. See page 6-19.	T, R, A
System→Security		Specify a domain to use for user authentication. See page 2-8.	DB and Security: A
System→System Log→Open		View system database log. See page 4-7.	All
System→System Log→Save As		Save system database log to a file. See page 4-7.	All

Window Menu

The Window menu contains the following commands:

Command	Keyboard Shortcut	Description	
Window→Close	Ctrl+F4	Close current view.	All
Window→Close All		Close all views.	All
Window→Next	Ctrl+Tab	Move next view to front.	All
Window→Previous	Ctrl+Shift+Tab	Move previous view to front.	All
Window→Cascade		Arrange open views in cascading fashion.	All
Window→Tile Horizontal		Arrange open views in column (wide views).	All
Window→Tile Vertical		Arrange open views in row (tall views).	All
Window→Windows		Open a list of windows to select from.	All

Help Menu

The Help menu contains the following commands:

Command	Keyboard Shortcut	Description	
Help→Contents	F1	Open help table of contents. See page 3-15.	All
Help→Search		Open search tab of online help.	All
Help→Index		Open index tab of online help.	All
Help→Wyatt Online		Open Wyatt Support Center website.	All
Help→Check for Updates		Look for newer versions of ASTRA available for download. See page 2-3.	All
Help→About ASTRA		Open version and copyright information about ASTRA V.	All

B System Templates

This appendix provides an overview of the ASTRA V System Templates

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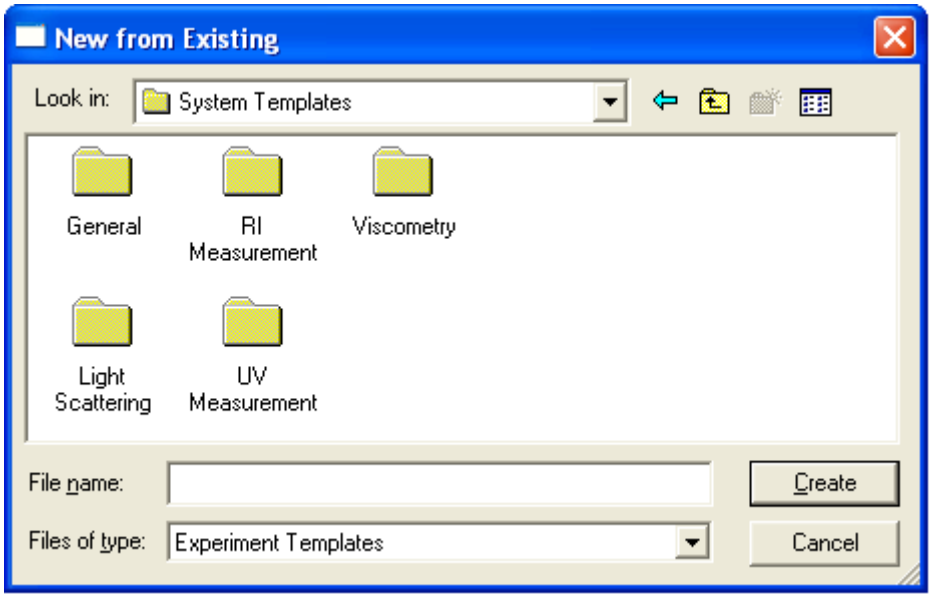
Overview

This appendix provides an overview of the ASTRA V system templates. These templates are stored in five different folders: General, RI Measurement, Viscometer, Light Scattering, UV Measurement. This appendix is organized according to the folders that contain the templates.



Some templates are only usable with a feature activation keys.

Some templates need to be applied to an already existing experiment and some are used to run a new experiment. This information is provided along with the name of each template, using these abbreviations:

- CT = Templates used to collect new data.
- AT = Templates that are generally applied to an already run experiment to analyze data in an additional way.

Hint: If you read about an experiment template you want to use, but don't see it in the New from Existing dialog, see "Migrating the System Database" on page 2-3 to update your system database so you have all the latest experiment templates and system profiles.

General

The following templates are provided in the General folder.

Concentration Determination (AT)

After you select the peaks zones and enter the dn/dc value for each peak, the relative concentration and calculated mass is displayed in the report for each peak.

RESULTS			
	Peak 1	**Peak 2**	**Peak 3**
concentration moments (g/mL)			
c(avg)	1.06e-4(0.0%)	9.12e-6(0.0%)	2.10e-6(0.0%)
calculated mass (g)			
m	6.3631e-5	6.8543e-6	9.0903e-7

Peak Areas (AT)

After you select the peaks zones, the following data is displayed in the report. The peak area units depend on the units you have chosen in the configuration for the abscissa unit.

RESULTS			
	Peak 1	**Peak 2**	**Peak 3**
RI Instrument (RIU min)			
Peak Area	2.943e-5	3.170e-6	4.204e-7
UV Instrument (absorbance min)			
Peak Area	1.034e-1	1.084e-2	1.212e-3
Viscometer (specific viscosity min)			
Peak Area	n/a	n/a	n/a
DAWN or miniDAWN (Rayleigh ratio min)			
Detector			
1	2.147e-1	4.485e-2	8.013e-3
2	2.139e-1	4.375e-2	7.568e-3
3	2.137e-1	4.356e-2	7.470e-3

UV Extinction from RI Peak (AT)

First select the peak of interest. You need to know and enter the dn/dc value of the molecule and the calibration constants of the UV and RI. The report shows the UV extinction coefficient in ml/(g.cm) calculated from the refractometer signal.

If you know the dn/dc of the molecule of interest, it is better to determine the epsilon using this template and not the 100% mass recovery template where the 100% mass recovery is hardly verified.

The advantage of using this template is that there is no effect based on the flow rate, the recovery, etc. That is, you don't have to make assumptions for these parameters.

RESULTS

Calculated UV extinction based upon concentration from known dn/dc and RI detector.

	Peak #1
UV ext. (mL/(g cm))	6.467e+2

Light Scattering

The following templates and folders are provided in the Light Scattering folder.

Baseline Subtraction

The following template is provided in the Light Scattering->Baseline Subtraction folder.

Online (CT)

Use this template to run an online light scattering experiment to measure the baseline for later baseline subtraction.

Calibration (CT)

To calibrate a Wyatt Light scattering instrument, you need to create a new experiment (**File->New->Experiment from Template**) and choose the template corresponding to your light scattering detector.

The calibration is done with a batch injection of pure and filtered toluene.

Globally the method used in the template first collects data for 30 seconds with the laser on and then does a second measurement with the laser off (dark voltage). The intensity is measured at the 90° angle. ASTRA V analyzes the difference between both signals to convert the volt signal into meaningful units. The resulting calibration constant is shown in the report.

Results

Calculated calibration constant: `1.0000e-4 1/(V cm)`

Templates are available for the following instruments:

- Dawn 8
- Dawn DSP
- Dawn DSP-F
- Dawn EOS

- HELEOS
- HELEOS 8
- miniDAWN
- TREOS

Utilities (CT)

When you run a single experiment or sample set, the Comet templates allow you to program the Comet cell cleaner for a certain amount of time (shown in the name of the template). Note that in these templates the laser is automatically turned off.

- HELEOS Comet 5min
- HELEOS Comet 10min
- HELEOS Comet 2hour
- TREOS Comet 5min
- TREOS Comet 10min
- TREOS Comet 2hour

Laser off templates can only be used with a sample set. In the United States, the recommendation is not to turn the laser off if it's for less then 3 weeks.

Before using a "Laser off" template, you need to customize it. This means to perform the physical instrument connection and save the resulting experiment as a template before running a sample set.

- Turn EOS laser off
- Turn HELEOS laser off
- Turn miniDawn laser off
- Turn TREOS laser off

The "Orbit on" template places the Orbit device in Recycle mode for the TREOS and HELEOS.

Diagnostics

The following templates are provided in the Light Scattering->Diagnostics folder.

Detector Overlay (AT)

This template allows you to visualize an overlay of the light scattering detectors. It is a very useful tool for trouble shooting:

- You can check the photodiodes' normalization.
- You can check for laser misalignment. All the detector signals should look the same for a monodisperse sample.
- You can check the for a dirty cell. If the cell is dirty, the peaks are not the same shape for all detectors.

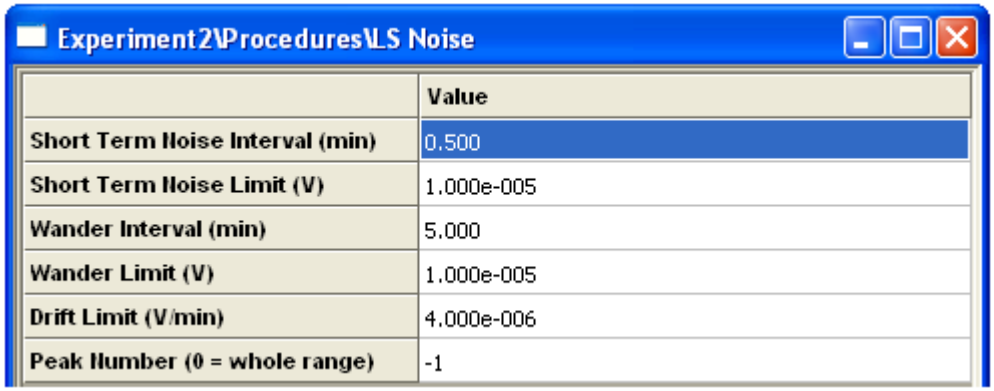
View 2 (Overlay Test) (CT)

This is similar to the Detector Overlay template.

Noise (AT)

- HELEOS Noise
- TREOS Noise
- EOS Noise
- miniDawn Noise

The noise templates analyze the noise level of the light scattering detectors. To do so, you must measure a stable baseline. Some criteria are already entered in the template in the “LS Noise” procedure.



The report indicates whether the measured noise levels are within specification.

With QELS

Batch (CT)

Use this template to do batch QELS measurements. Inject the sample directly into the light scattering detector.

The Rh from QELS graph is displayed in the “Rh from QELS” procedure.

Online (CT)

Use this template to do online QELS measurements. Run the sample through the chromatography system and detectors.

The Rh from QELS graph is displayed in the “Rh from QELS” procedure.

Regularization (AT)

Apply this template to perform a regularization regression, which permits the calculation of the size distribution of a sample.

For example, if you have a bad separation and several entities leave the column at the same time, regularization reveals this co-elution because there will be several peaks with the regularization.

Cumulants (AT)

Similarly to the Regularization analysis, applying this template calculates the size distribution of the sample. This template fits the correlation function data to a cumulant distribution.

Particles (AT)

In particles mode, it is possible to measure the size (radius) and number density of a sample using just a light scattering detector without any concentration detector. Note that you won't be able to measure the molar mass using this template.

In addition, this template computes Rh using QELS data.

Protein Conjugate (CT)

This template permits measurement of Rh from QELS data as well as RMS radius and molar mass using Protein Conjugate analysis.

Batch (Debye plot) (CT)

Use this template to do batch injection of a sample of known concentration (only one concentration).

The Debye plot is displayed together with the molar mass and radius.

Batch (Zimm plot) (CT)

Light scattering can be used to measure the second virial coefficient (A2) of a macromolecule. ASTRA supports the analysis of rapid injections of small volumes of a sample. This proprietary online analysis can retrieve A2 using a fraction of the sample needed for traditional measurements.

ASTRA uses a proprietary global fitting method to upgrade the Zimm plot to a more robust, modern analysis. The global fitting method removes all extrapolations from the Zimm plot, helps identify inconsistent data sets, and provides more precise and robust results for A2. As with all analysis procedures in ASTRA, the global fit view offers immediate visual confirmation of fit quality using a visual representation of the Zimm plot. You can change parameters in the grid view and view fit results immediately.

It is recommended that you inject six concentrations of the sample.

For this template you need to manually enter the concentration of each injected sample.

Branching (AT)

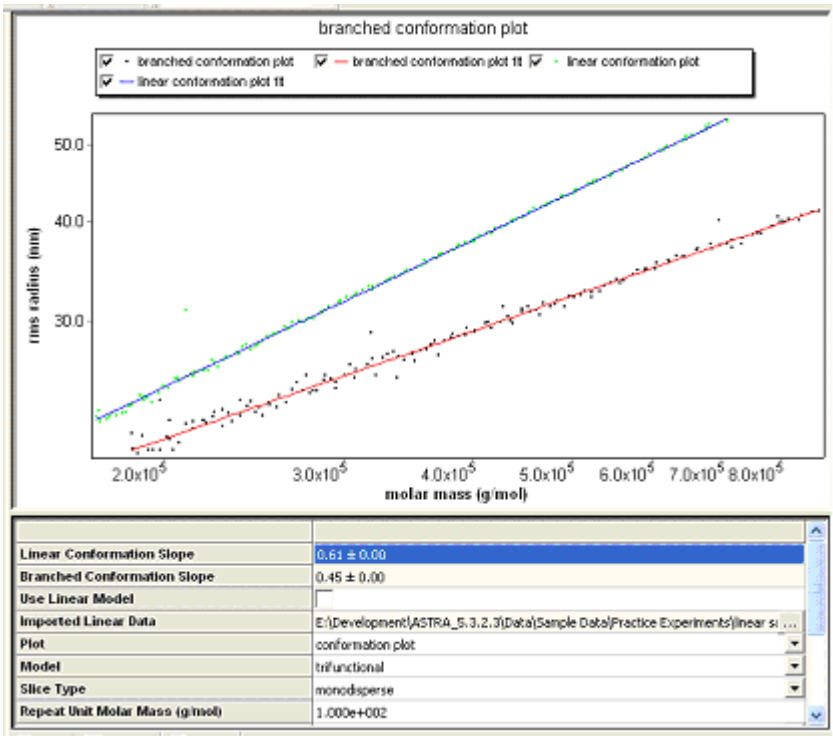
The branching characteristics of a polymer can only be determined if MALLS (Multi-Angle Laser Light Scattering) data of a linear example of the polymer exists.

Branching ratio, g (Branching parameter)

$$g = \left(\frac{R_{br}^2}{R_{lin}^2} \right)_M$$

R = root mean square radius (radius of gyration)

The “Branching” procedure shows the branched conformation plot and the slope of the linear and branched polymer.



The branching ratio is displayed in the report.

Online (CT)

Use this template to run basic online light scattering experiments.

With this template, the sample is injected online, using a concentration detector to measure the actual concentration (and mass) for each peak.

The RI or UV signal is used to determine the concentration and the light scattering for the intensity. With this template, the concentration doesn’t need to be entered manually.

The results graph shows the mean square radius vs. volume.

Online (Zimm Plot) (CT)

This template is similar to the Online template except that the data is displayed in a Zimm plot using physical units.

Online A2 (CT)

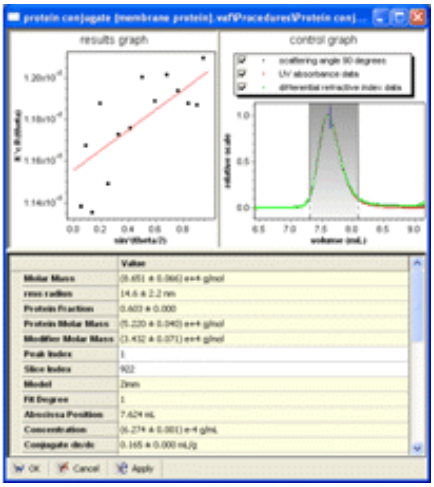
This template is similar to the Online template. In addition to mass and radius, the A2 value is computed and a Zimm plot is shown. This is similar to the Zimm batch analysis, except that it is performed in an online mode.

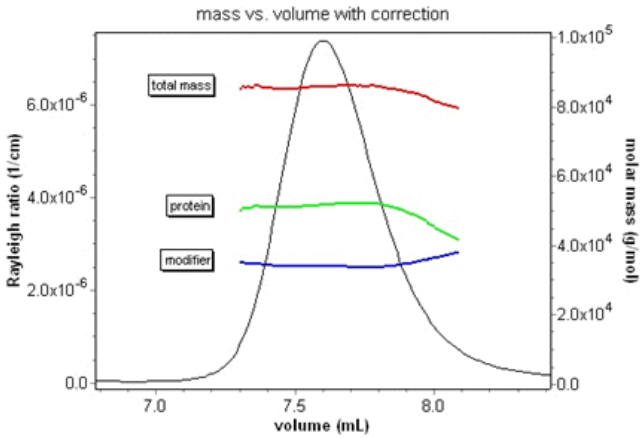
Protein Conjugate (AT)

This template allows you to determine the molar mass, size, and relative polymer fractions of a copolymer using light scattering. All that is required are two additional detectors that have differing sensitivities to the constituent polymers. Traditionally, light scattering has been used in conjunction with an RI and UV detector for this purpose.

An important class of copolymers are protein conjugates. For example, researchers often need to determine the fraction of protein in glycosylated and pegylated proteins, as well as membrane protein-detergent complexes. ASTRA has native support for protein conjugate and copolymer analysis using a light scattering detector in conjunction with a UV and RI detector.

You need to enter the dn/dc and UV extinction values for the protein and modifier. Total mass and protein fraction on a slice-by-slice basis are displayed. ASTRA calculates the size of the complex, mass of the complex, and masses of the constituents, displaying them with rigorous uncertainties. In addition, the concentration and calculated dn/dc values are displayed for that elution volume.





Particles (AT)

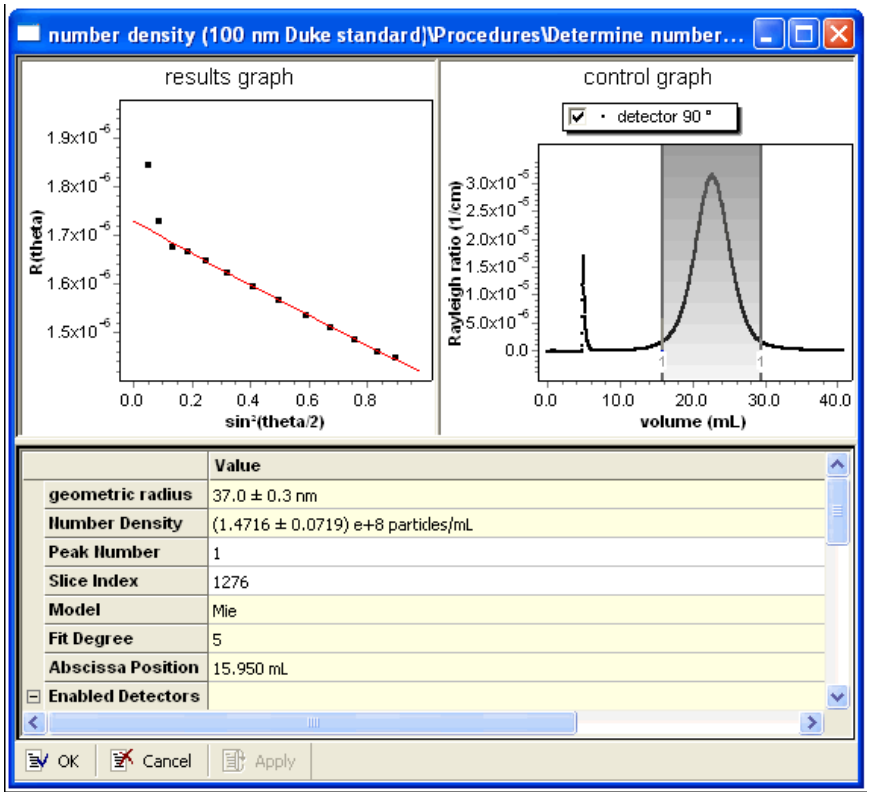
In particles mode, it is possible to measure the size (radius) and number density of a sample using just a light scattering detector without any concentration detector. Note that you won't be able to measure the molar mass using this template.

Number Density (AT)

This template provides a procedure to calculate number density, which means how many particles you have in your sample. Particle measurements are especially suited for use when a light scattering instrument is

coupled to a fractionation technique such as Field Flow Fractionation (FFF) or Capillary Hydrodynamic Fractionation (CHDF), but concentration is not measured.

This procedure calculates the radius and the number of particles per mL (density) in the sample. You must specify the refractive index of the sample in the Define Peaks procedure to determine the number density. This template is normally used with online (fractionated) experiments.



Remarks:

- Model: This field shows (display only) the light-scattering model selected for this peak in the Define Peaks dialog.
- Fit Degree: This field shows (display only) the fit degree selected for the peak.

RI Measurement

The following templates and folders are provided in the RI Measurement folder.

100% Mass Recovery Methods

The following templates are provided in the RI Measurement->100% Mass Recovery Methods folder.

dn/dc from Peak (AT)

Keep in mind that this template works on the hypothesis that all the mass injected is retrieved under the peak. This may not be a valid assumption for certain types of samples.

For each measurement slice of the peak, the refractive index is known due to the refractometer. You then need to enter the amount of injected sample in grams and the flow rate. The software can calculate the dn/dc value since it is proportional to these parameters.

RI Calibration from Peak (CT)

This template is used to calibrate any refractometer (Wyatt or generic) EXCEPT for the Optilab rEX, which uses a special template. Note that the main difference is in the experiment configuration. For this template there is a pump, injector, light scattering instrument, and refractometer.

To perform the calibration, you need to enter the precise injected mass and to know the dn/dc of the solvent. The calculation of the calibration constant is based on finding the same calculated mass.

Optilab rEX Specific

The following templates are provided in the RI Measurement->Optilab rEX Specific folder.

Absolute RI Calibration (CT)

The template is specifically for calibrating the Optilab rEX for absolute refractive index measurements. Absolute measurements means that there is no reference to a standard. So for these analyses, the purge valve must be ON.

Inject at least three different solvents with known (and different) refractive indexes (e.g., toluene, THF, and water).

RI Calibration from Peak (AT)

This template is only used to calibrate the Optilab rEX. For this template there is a pump, injector, and an Optilab rEX. No other instruments are necessary.

To do the calibration, you need to enter the precise injected mass and to know the dn/dc of the molecule in the specific solvent. The calculation is based on the assumption of 100% mass recovery, and the aim is to find the calibration constant needed to have the calculated mass equal to the injected mass.

Zero dRI (CT)

This template sets the dRI calibration constant value to zero.

Orbit On (CT)

This template places the Orbit device in Recycle mode for the Optilab rEX.

Purge On (CT)

We recommend that you purge the Optilab rEX when not running samples; the "Purge On" template is a convenient way to automate this as part of a sample set.

Purge Off (CT)

You can use this template in combination with the "Purge On" template in a sample set to close the purge valves on an Optilab rEX instrument.

The purge valves on Optilab rEX instruments are automatically closed at the start of data collection. The exception to this is when absolute RI analysis is conducted, where the Optilab rEX purge valve must be left open.

Diagnostics

The following template is provided in the RI Measurement->Diagnostics folder.

Grimace (AT)

The Grimace template shows an overlay (to display it use an EASI graph). Molar masses are plotted.

The template allows you to determine if there is secondary band broadening. If there is secondary band broadening, for a monodisperse sample, the flat line will become a curve (that is, a "grimace" shape).

Conventional Calibration (CT)

Use this template for configurations that contain only a refractometer.

Inject several monodisperse samples with well-known molar masses. Then do a calibration curve; this will allow you to compare the elution volume for your sample.

If two molecules have the same molar masses but different radii, there is an error in the calculation.

RI Calibration (CT)

Use this template only with batch measurements. The experiment configuration contains only an Optilab rEX.

Inject at least three concentration of sodium chloride, which has a well-known dn/dc. The slope allows ASTRA to determine the calibration constant.

Determine CC Column Profile (CT)

Analysis of a sample using a universal or conventional column calibration takes place in two distinct phases. First, the response of a column to a set of standards with known molecular weights must be measured. Once this "determine column calibration" phase is complete, the unknown sample can be analyzed.

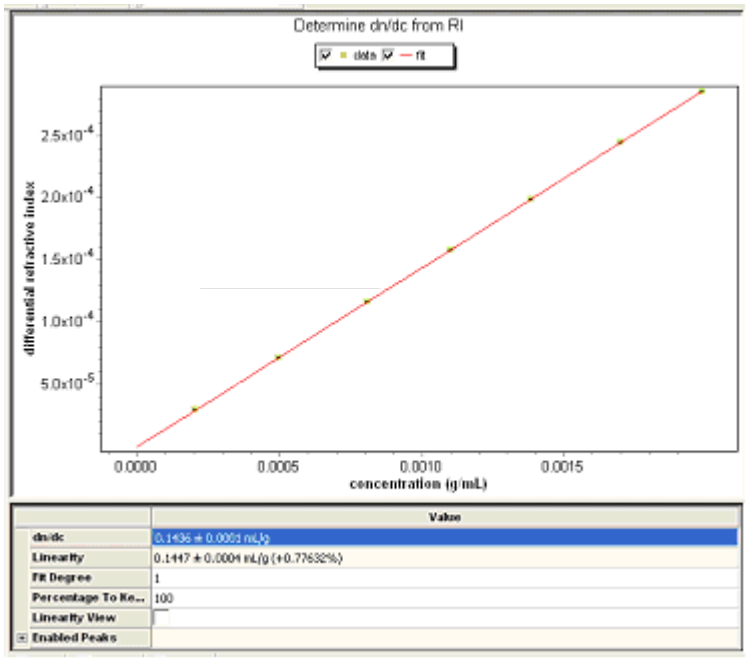
The "determine column profile" template allows you to set up the column profile for Conventional Calibration.

Batch (Determine dn/dc) (CT)

The determination of dn/dc for a specific sample in a solvent is done by manually injecting several concentrations of the sample. Additionally, the solvent used to dissolve the sample should also be injected before and after the samples.

When you have run the experiment, define the peaks and the concentration of each sample. These concentrations have to be very precise.

In the "dn/dc from peak" procedure, the dn/dc value, errors, and graph are displayed.



Copolymer Analysis (AT)

This template is for experiments without a light scattering instrument.

To use this template, a conventional column calibration profile is required. The molar mass is determined from the elution time and not from light scattering data.

For example, you might use this template to determine the quantity of monomer A and monomer B.

RI Peak Areas (AT)

When this template is applied to an experiment, only the refractometer peak area for each selected peak is shown in the report.

RESULTS			
	Peak 1	**Peak 2**	**Peak 3**
RI Instrument (RIU min)			
Peak Area	2.943e-5	3.170e-6	4.204e-7

UV Measurement

The following folders is provided in the UV Measurement folder.

100% Mass Recovery Methods

The following template is provided in the UV Measurement->100% Mass Recovery Methods folder.

UV extinction from peak (AT)

The UV extinction coefficient is calculated under the hypothesis that 100% of the injected mass is recovered. To perform the calculation, ASTRA needs to know the flow rate and the injected mass.

The value is shown in the Report in ml/(g.cm).

RESULTS

Calculated UV extinction assuming 100% mass recovery.

	Peak 1
UV ext. (mL/(g cm))	5.645e-1

Viscometry

The following templates and folders are provided in the Viscometry folder.

Calibration

The following template is provided in the Viscometry->Calibration folder.

Determine Universal Column Profile (CT)

Analysis of a sample using universal or conventional column calibration takes place in two distinct phases. First, the response of a column to a set of standards with known molecular weights must be measured. Once this "determine universal column profile" phase is complete, the unknown sample can be analyzed.

This template allows you to set up the column profile for Universal Calibration.

With Light Scattering

The following templates are provided in the Viscometry->With Light Scattering folder.

Branching (VS+LS) (AT)

This template lets you determine the branching ratio using either viscosity or light scattering data. It does not require conventional or universal calibration, as it measures the mass using the light scattering signal.

Online (CT)

This is the standard online template for use when you have a light scattering detector and a viscometer.

Diagnostics

The following template is provided in the Viscometry->Diagnostics folder.

VS Noise (CT)

This template measures the noise of the viscometer and analyzes its level. To do so, a stable baseline needs to be measured. Some criteria are already provided in the template, they can be found in the "Viscometer noise" procedure. The report indicates whether the measured noise levels are within specification.

	Value
Short Term Noise Interval (min)	0.500
DP Short Term Noise Limit (psi)	2.200e-005
IP Short Term Noise Limit (psi)	1.000e-002
Wander Interval (min)	5.000
DP Wander Limit (psi)	1.000e-004
IP Wander Limit (psi)	5.000e-002
DP Drift Limit (psi/min)	6.000e-006
IP Drift Limit (psi/min)	1.670e-003
Peak Number (0=whole range)	-1

Utilities

The “Orbit on” template places the Orbit device in Recycle mode for the ViscoStar.

Copolymer Analysis (AT)

This template is for use with viscometer data. It is the equivalent of the Protein Conjugate template, but for viscometry.

Online (CT)

This is the standard online template for use when you have only a viscometer and no light scattering instrument.

Branching from VS Data (AT)

This template is the equivalent of the branching template found in the Light Scattering folder. However, this one measures branching using viscometer data.

To use this template you must first do a column calibration and set a column profile.

Mass from VS Data (AT)

This template calculates the molar mass distribution all along the chromatogram based on the viscometer data.

Universal Calibration (CT)

To use this template, you must have a saved column profile.

In the Generic Column of the experiment configuration, select the corresponding column profile by clicking the “...” button.

C Data Collection with Scripts

This appendix describes the scripting language you can use for script-based data collection.

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Introduction

ASTRA V embeds a powerful, general-purpose programming language called Lua in its data collection system. Scripts can be written using the normal syntax and features present in the core Lua language version 5.1.3. For details about Lua, see http://www.lua.org.

You use scripts with the procedure described in "Script Collection" on page 8-12. When you run a script, any syntax errors are reported in a message.

The following sections provide an overview of the features available for creating custom collection scripts.

Note: Writing scripts is an advanced feature, almost never needed for typical data collection tasks. For users who want to construct novel collections involving significant interaction with the instrument settings, valve position, laser power levels, and so forth, it can provide a powerful means of interacting with your Wyatt instruments.

Scripts allow you to issue commands to instruments to prepare a collection, collect data for a specific period of time, and so forth. This provides a powerful mechanism for customizing data collection. Internally, scripts form the foundation of the Basic Collection procedure, which is simply a graphical interface to the features of the script collection interpreter.

Collection

The overall collection is controlled by issuing commands in the Collection namespace. These commands are used to interact with the user, and to control the collection status.

Commands are issued using the following syntax:

```
Collection.[command]
```

where [command] is one of the following commands:

Command	Effect
PromptUser ("text")	Pop up a message to the user that must be responded to (via the "OK" button) for the collection to proceed.
Start ()	Tell ASTRA to begin listening for data from instruments.
SetDuration ([milliseconds])	Wait for the specified number of milliseconds (while collecting data).
SetInjectToCollectDelay ([milliseconds])	Set the number of milliseconds ASTRA will wait after receiving an auto-injection signal before proceeding.
Stop ()	Tell ASTRA to stop listening for data.
WaitForMessage ([instrument], "[message]")	Hold the collection until the string [message] is received by [instrument]. The [instrument] is an instrument name, retrieved using one of the “Create” commands described in the next section. The only [message] currently available is ISI_INSTRUMENT_AUTOINJECT, which indicates that an autoinject signal has been received by the specified instrument.

Interacting with Instruments

To issue commands to an instrument, you must first obtain an instrument reference that links the specific physical instrument in the experiment configuration to the instructions in the script. This is done using one of the following commands:

Command	Effect
LSInstrument.Create ()	Get a reference to the experiment configuration's static light scattering instrument.
QELSInstrument.Create ()	Get a reference to the experiment configuration's dynamic light scattering instrument.
RIInstrument.Create ()	Get a reference to the experiment configuration's refractive index instrument.
VIInstrument.Create ()	Get a reference to the experiment configuration's viscometer.

Once you have obtained a reference to one or more instruments, you can issue commands to the instrument. Instruments respond to a general set of messages, as well as some instrument-specific commands.

Common Instrument Commands

All instruments understand the following messages. Commands are issued using the following syntax:

```
[instrument].[command]
```

where [instrument] is the instrument reference obtained by the "Create" method defined in “Interacting with Instruments” on page C-3.

For example, the following commands turn the recycle valve of a light scattering instrument to the “waste” position:

```
lsInst = LSInstrument.Create ()
# Turn the recycle valve off
lsInst.SendCommand("SetSwitch[Recycle, F]")
```

The complete set of instrument commands is shown in the following table:

Command	Effect
GetInstrumentLabel ()	Get a string version of the instrument's name. Useful for generating messages to the user.
Enabled ()	Return true or false, indicating whether the “Disable Collection” flag in the instrument configuration is enabled.
SetCollectionInterval ([seconds])	Set the instrument data collection interval in to the specified number of seconds.
SendCommand ([command])	Tell the instrument to perform a particular command. [command] is an instrument command string (described elsewhere).
StartCollection ()	Tell the instrument to begin transmitting data to ASTRA. This provides finer control over when instruments begin transmitting collection data.

Static Light Scattering Instrument Commands

All static light scattering instruments understand the following messages.

Commands are issued using the following syntax:

```
[instrument].[command]
```

where [instrument] is the instrument reference obtained by the "Create" method defined in “Interacting with Instruments” on page C-3.

Command	Effect
LaserOn ([state])	Set the laser status to on (true) or off (false). [state] is either true or false.
RunCOMET ([state])	Sets the COMET to on (true) or off (false). [state] is either true or false. This command has no effect on instruments without a COMET device.
DitherOn ([state])	Sets the laser dithering status to on (true) or off (false). [state] is either true or false.

Dynamic Light Scattering, Refractometer, and Viscometer Commands

There are currently no Dynamic Light Scattering Instrument, Refractometer, or Viscometer-specific instrument commands.

Examples

The following examples show some useful collection scripts. You can learn about additional features of the Lua programming language by visiting the website at http://www.lua.org, or by reading the book *Programming in Lua*, second edition, by Roberto Ierusalimschy.

The following script collects data for three auto-injections:

```
lsInst = LSInstrument.Create ()
lsInst:LaserOn(true)
lsInst:SetCollectionInterval(0.5)
lsInst:StartCollection ()

for i=1,3 do
   Collection.WaitForMessage(lsInst,
     "ISI_INSTRUMENT_AUTOINJECT")
   if i == 1 then  -- start collection on 1st iteration
      Collection.Start()
  end
  Collection.SetDuration(30000)
end

Collection.Stop()
```

The following script collects data for a light scattering instrument calibration. It prompts the user to press **Enter** to continue once the instrument is ready.

```
lsInst = LSInstrument.Create ()
lsInst:LaserOn(true)
lsInst:SetCollectionInterval(0.125)
lsInst:StartCollection ()
Collection.PromptUser("Press Enter to Start Calibration.")
Collection.Start()

-- Run 30 seconds with laser
Collection.SetDuration(30000)

-- Run 30 more seconds without laser ...
lsInst:LaserOn(false)
Collection.SetDuration(30000)
lsInst:LaserOn(true)
Collection.Stop()
```

The following script turns COMET features on and off as needed during the collection (see the **bold** portions in the example).

```
lsInst = LSInstrument.Create ()
lsInst:LaserOn(true)
lsInst:SetCollectionInterval(0.125)
lsInst:SendCommand("SetSwitch[COMET, T]")
lsInst:LaserOn(true)
lsInst:StartCollection ()
Collection.Start()
Collection.SetDuration(600000)
lsInst:SendCommand("SetSwitch[COMET, F]")
Collection.Stop()
```

Sample experiments with collection scripts for running the COMET cell cleaner and turning lasers on and off are provided in the **System Templates->Light Scattering->Utilities** folder of the system database.

For Optilab rEX users, there are several experiment templates in the **System Templates->RI Measurement->Optilab rEX Specific** folder. These templates have scripts for “Purge On”, “Purge Off”, and “Zero dRI”.

You may contact Wyatt Technical Support if you have a specific need to create additional collection scripts.

D Light Scattering Theory

This appendix reviews the application of the basic theory of laser light scattering to the determination of molar mass and root mean square radii. The text covers basic quantities, the relation to measurements, calibration and normalization, and determination of molar masses, sizes and distributions.

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Introduction

Although gel permeation chromatography (GPC) provides good separation of molecules by their hydrodynamic radius, traditionally it has been necessary to calibrate GPC columns using standard samples of known molar mass in order to determine the molar mass of an unknown sample. Unfortunately, appropriate standards having the same composition and conformation as the unknown specimen are often not available.

If the value of dn/dc (differential refractive index increment) or the total mass of eluting solute is known, light scattering measurements can provide an absolute measurement of molar mass when used in series with a concentration-sensitive detector such as a refractive index (RI) detector. Light scattering can provide a continuous measurement of molar mass if sample concentrations are high enough to provide adequate signals.

In essence, light scattering measurements automatically provide a column calibration curve for every sample, obviating time-consuming, conformation-dependent calibration procedures. When techniques such as reverse phase chromatography are used, separation is not based on molecular size, and it is often difficult or impossible to calibrate with known standards. Also, the situation is more complicated for certain types of heterogeneous co-polymers for which dn/dc is a function of the molar mass.

Basic Quantities

The symbol used to describe the angle-dependent light scattering is R_θ called the Rayleigh ratio. It is defined as:

(Equation 1)

$$R_\theta = \frac{I_\theta r^2}{I_0 V}$$

where I_θ is the scattered intensity, I_0 is the intensity of the incident beam, V is the volume of the scattering medium, and r is the distance between the scattering volume and the detector. Eq. 1 implies that the dimensions of R_θ are (length)$^{-1}$.

Note: The DAWN instruments are provided with *vertically* polarized light sources; this fact is assumed in Eq. 1 and all derivations to follow.

When studying a solution of solvent plus solute, it is convenient to use R_θ to represent the *excess* scattering of the solution compared to that of the solvent alone:

(Equation 2)

$$R_\theta = \frac{\left(I_\theta - I_{\theta,solvent}\right) r^2}{I_0 V}$$

where $I_{\theta,solvent}$ is the scattered intensity of the solvent. For a pure solvent we use Eq. 1; for a solution we use Eq. 2. The philosophy behind Eq. 2 is simple: we are merely describing the scattering after subtracting the "baseline" of pure solvent. In this case R_θ is often called the *excess* Rayleigh ratio of the solute.

As we shall see below, knowledge of R_θ at a number of different angles leads directly to the weight average molar mass and mean square size of the solute molecules, making R_θ the most important measured quantity in light scattering.

Measured Quantities and Calibration

How do we measure R_θ? If we try to use Eq. 1 or Eq. 2 directly, we run into trouble immediately. First, the quantities we can measure directly are detector voltages, not light intensities. Because the measured voltages are proportional to light intensities, we must calibrate our detectors. This calibration is not difficult and will be explained shortly.

A more serious problem is that due to the geometry of the sample cell in the DAWN or miniDAWN, each detector "sees" a different scattering volume V and subtends a different solid angle with respect to the scattering volume. Refractive index differences among various solvents and sample cells exacerbate this problem. Calculating V for each detector is thus extremely complex and prone to inaccuracies.

The solution is to use the proportionality between R_θ and I_θ in Eq. 1 to derive a calibration factor which gives the correct value of R_θ for a known scatterer. Fortunately, some common solvents have been thoroughly studied, and their Rayleigh ratios are well known, allowing us to use the GPC solvent itself as the calibration standard. Using pure solvent as the scattering standard makes the calibration completely independent of any polymer sample.

Let us first consider scattering at 90°. We absorb not only the detector sensitivity but also all the geometrical volume and solid angle factors into a single Configuration Specific Calibration Constant, called A_{CSCC}.[1] By using the proportionality between detector voltage and light intensity, Eq. 1 may be written as:

(Equation 3)

$$R_{90} = A_{CSCC}\left(\frac{V_{90} - V_{90,dark}}{V_{laser} - V_{laser,dark}}\right)$$

where V_{90} and $V_{90,dark}$ are the 90° detector signal voltage and dark offset voltage, respectively, and V_{laser} and $V_{laser,dark}$ are the laser monitor signal and dark offset, respectively. Dark offsets are obtained with the laser turned off. Note that dividing by the laser monitor signal compensates for any changes in laser intensity due to power supply fluctuations, temperature drift, laser aging, etc.

As an example, suppose we calibrate with toluene. Pure filtered toluene has a Rayleigh ratio of 1.406 x 10^{-5} cm^{-1} at a wavelength of 632.8 nm.[2] Suppose that using our DAWN or miniDAWN sample cell we observe a 90° scattering signal of about 1 V. The laser monitor signal is factory-set to be near 5 V, and the dark offsets are much smaller than this, so Eq. 3 implies

1. A_{CSCC} is not displayed in the software.
2. W. Kaye and J.B. McDaniel, *Applied Optics*, vol. 13, No. 8, 1974, pp. 1934–1937.

$A_{CSCC} \approx 7.0 \times 10^{-5}$ cm^{-1}. Of course, this is just an example, and the measured constant may be quite different depending on the instrument and conditions (laser wavelength, etc.).

Toluene provides a large scattering signal; in fact, toluene has the highest Rayleigh ratio of any of the common solvents, and is thus highly desirable for use as a calibrator. Many other solvents can, theoretically, be used for calibration of the DAWN or miniDAWN but we do not recommend them.

The astute reader will point out that since we know the Rayleigh ratio for toluene, and since the scattering from toluene is relatively large, we ought to be able to calibrate with toluene, measure our samples in water, and still obtain correct results. The complication lies in the geometrical factors which describe the volume of scatterer seen by the 90° detector as well as the solid angle it subtends. These factors depend on the refractive index of both the solvent and the glass of which the sample cell is made (see the DAWN or miniDAWN Hardware Manual).

Thus the "constant" A_{CSCC} is really dependent on the solvent type and cell type. That is why we call it a configuration specific calibration constant. To allow users to calibrate with one solvent and/or cell and make measurements with another, we must have an "instrument" constant that is truly independent of these changing factors and is instead only a function of the particular instrument and the sample cell geometry.

This instrument constant A_{inst} is related to A_{CSCC} by Eq. 4:

(Equation 4)
$$A_{\mathrm{CSCC}} = A_{\mathrm{inst}}\left(\mathrm{Reflection\ Correction}\right)\left(\mathrm{Geometry\ Correction}\right)$$

First, the "Reflection Correction" will be considered. The reflection correction represents the reflective losses at each interface in the sample cell. For example, the incident laser beam loses intensity at the air-glass interface of the sample cell, and the glass-solvent interface as well. Similarly, the scattered light that is to be detected also suffers from reflective losses at the solvent-glass and glass-air interfaces as it leaves the sample cell. If the solvent is changed, or a different cell is used, these reflective losses will change, and hence it is necessary to correct for them if A_{inst} is to be independent of solvent and cell glass.

The reflection correction can be considered in terms of the transmitted intensity between media 1 and 2 with indices of refraction n_1 and n_2, respectively. Transmitted intensity is given by the Fresnel equations as:

(Equation 5)
$$T_{12} = \frac{4n_1n_2}{\left(n_1 + n_2\right)^2}$$

If g represents the flow cell glass, s represents the solvent, and a represents air, then the reflection correction can be written as

(Equation 6)
$$\mathrm{ReflectionCorrection} = \frac{1}{T_{sg}^{2}T_{ga}^{Nunc}}$$

where $Nunc$ is the number of uncoated glass-air interfaces the incident and scattered light have to traverse. It is assumed that the reflective losses at a coated, i.e., antireflection coated, interface are negligible.

The "Geometry Correction" for a sample cell is not so straightforward to determine as the reflection correction. There are examples of analytical expressions derived for simple cell geometres[1,2], but there are no simple analytical expressions for more complicated geometries such as that of the K5 and F2 flow cells. In addition, these analytical expressions are valid for instances where the source of scattered light is either a point source or a completely illuminated volume, neither of which hold for the instance of scattered light being collected from a line source like a collimated laser beam. Therefore, the geometry correction has been calculated for each sample cell using computer ray-tracing simulations based upon the exact geometry of the sample cell, laser beam, and detection optics for the 90 degree detector in the DAWN and miniDAWN.

For the K5 and F2 flow cell, the resulting geometry correction goes as nsng, i.e., the index of refraction of the solvent times the index of refraction of the glass, respectively. The complete expression taking into account the reflection and geometry corrections is therefore:

(Equation 7)

$$A_{\mathrm{CSCC}} = A_{\mathrm{inst}} \frac{n_s n_g}{T_{sg}^2 T_{ga}}$$

for the standard flow cell with an antireflection coated entrance window and uncoated exit surface, and the transmission terms are calculated using Eq. 5.

For the scintillation vial, both the geometry and solvent-glass reflection corrections were folded into the ray tracing calculations, so only the factor for the reflection correction due to the two uncoated glass-air interfaces of the scintillation vial are in the final expression. The resulting complete formula for the scintillation vial is:

(Equation 8)

$$A_{\mathrm{CSCC}} = A_{\mathrm{inst}} \frac{n_s^{1.797}}{T_{ga}^2}$$

For the MicroCuvette, both the geometry and solvent-glass reflection corrections were folded into the ray tracing calculations. Both the entrance and exit windows for the MicroCuvette are anti-reflection coated, so there are no explicit reflection correction terms in the final equation. The resulting complete formula for the MicroCuvette is

(Equation 9)

$$A_{\mathrm{CSCC}} = A_{\mathrm{inst}} n_s^{1.983}$$

1. C.I. Carr, Jr. and B.H. Zimm, "Absolute Intensity of Light Scattering from Pure Liquids and Solutions", *J. Chem. Phys.* vol. 18, p. 1616 (1950).
2. J.J. Hermans and S. Levinson, "Some Geometric Factors in Light-Scattering Apparatus", *J. Opt. Soc. Am.*, vol. 41, p. 460 (1951).

When you perform a calibration, ASTRA calculates a configuration-specific constant from Eq. 3, but you never see this number. It is immediately converted to the instrument constant A_{inst} via Eq. 7, Eq. 8, or Eq. 9 depending on the cell type. The A_{inst} value is reported as the "Calibration Constant" and is the value entered in the DAWN or miniDAWN profile.

If at some later time you change A_{inst} in the instrument profile, ASTRA will recalculate A_{CSCC}. ASTRA also recalculates A_{CSCC} if the solvent or cell type is changed. This process may sound complicated, but it enables you to calibrate with one solvent and make measurements with another, and the software efficiently handles all the details.

Note: Since changing the sample cell may require a realignment of the laser, we recommend you always recalibrate afterwards.

The calibration measurements should be made with *great care* as the accuracy of all other measurements depends upon them. As long as the system is left undisturbed it is not necessary to recalibrate, but we advise making occasional checks using a standard polymer, as photodiode sensitivity may change with age. The calibration should be performed with HPLC-grade toluene filtered through the smallest available filter (0.02 μm) immediately before making the measurement using the ASTRA program. The cleanliness of the cell is vital for this purpose. Be sure to leave the DAWN or miniDAWN instrument and the laser switched on for one hour before making any measurements.

Normalization

At this point we have calibrated the 90° detector in an absolute sense: the calibration is totally independent of any sample we might wish to study. In other words, we can measure R_θ accurately for any solvent or sample, assuming it gives a large enough signal. Furthermore, the calibration can be traced directly to the scattering from pure, well-understood solvents.

So far we have ignored all angles except 90°. Each detector has its own geometrical factors and angular sensitivity to measured light intensity. Furthermore, these effects vary from solvent (and sample) to solvent. We would like to quantify this effect so that we can correct for it. If not, we will mistake solvent and geometric readhead effects for characteristics of our sample, resulting in poor data.

Therefore, we use a set of *normalization coefficients* N_θ to relate each detector to the 90° detector. These coefficients must be determined using the *same flow rate* (same pressure) and the *same solvent* that are used for the GPC measurement, since the refractive index of the solvent changes the scattering angles and the geometrical factors for each detector.

We must use a sample that is an isotropic scatterer (one which scatters equally in all directions), so that we can be sure that the variations measured are due to detector geometry and not some interaction of the sample with the light. The normalization coefficient for the 90° detector is assigned a value of 1.0, while the other detectors are adjusted by varying amounts to yield uniform results.

These coefficients must be determined using the same flow rate (same pressure) and the same solvent that will be used for the chromatography measurement, since the refractive index of the solvent changes the scattering angles for each detector.

The process of normalization is quite simple. We assume that the 90° detector has already been calibrated as described above. To normalize, we introduce an isotropic scatterer (*i.e.*, R_θ is independent of θ) and compute a set of coefficients so that each detector gives the same R_θ as the 90° detector when its signal is multiplied by its coefficient. Expressed algebraically we have:

(Equation 10)

$$R_\theta = N_\theta A_{CSCC}\left(\frac{V_\theta - V_{\theta,\,dark}}{V_{laser} - V_{laser,\,dark}}\right)$$

For Eq. 3 and Eq. 10 to agree when $\theta = 90°$, N_{90} must be exactly unity.

Thus Eq. 10 gives us a way to calculate Rayleigh ratios at any detector angle. We recommend normalizing with a low molar mass sample which has a radius less than about 10 nm (molecules this small scatter nearly isotropically). For polystyrene in toluene or THF this corresponds to a molar mass of less than about 50,000 g/mol.

In practice we need not measure the various detector dark offsets $V_{90,dark}$ in Eq. 10. This is because the instrument is typically used to study samples in solution, not solvents by themselves. Thus we are interested in the *excess* Rayleigh ratio of the eluting sample, compared with the baseline of solvent alone. We therefore use an alternative form of Eq. 10:

(Equation 11)

$$R_\theta = N_\theta A_{CSCC}\left(\frac{V_\theta - V_{\theta,\,baseline}}{V_{laser} - V_{laser,\,dark}}\right)$$

where R_θ is taken to be the excess Rayleigh ratio, and $V_{\theta,baseline}$ is the detector voltage far away from the sample peak. The quantity $V_{\theta,baseline}$ is due to the scattering from pure solvent [*cf.* Eq. 2] and the diode dark offset. Eq. 11 is the one used in ASTRA.

Implementation

ASTRA provides two normalization techniques: "Standard" normalization, which uses the normalization calculation used since ASTRA 4, and "Area" normalization, which uses a new method based on integration over the peak.

Standard Normalization

In practice, $V_\theta - V_{\theta,baseline}$ is not determined from a single data slice, but from the result of following the steps below:

1. Select a sample peak to use for normalization.
2. Using the data points for the center half of the peak (that is, the half of the peak centered on the peak apex), fit them to a 6th order polynomial of the form:

(Equation 12)

$$y = a + bx + cx^2 + dx^3 + ex^4 + fx^5 + gx^6$$

   Note that there must be at least seven points in the "center half" of the selected peak for the normalization calculation to run.
3. The apex of the fit curve, y in Eq. 12, provides $V_\theta - V_{\theta,baseline}$. The maximum y is found iteratively by plugging into Eq. 12 the x value for each slice used in the fit, and selecting the largest resulting y. This method is used due to the relatively small number of points typically involved, and to preclude the chance of finding local maxima.
4. Repeat steps 2 and 3 first for the 90° degree detector, then for each light scattering detector for which a normalization coefficient is to be calculated.
5. Set any negative y values to 1.0.
6. Finally, divide the results of Eq. 12 for all detectors by the result of Eq. 12 for the 90° detector. This yields the desired normalization coefficients.

Area Normalization

Better normalization results were found when the results were calculated from the integration of the Rayleigh Ratio peak as follows, rather than just using the peak apex:

1. Select a sample peak to use for normalization.
2. Integrate the Rayleigh Ratios over the entire peak.
3. Repeat the integration for each light scattering detector.
4. Any negative results are set to 1.0.
5. Finally, set the normalization coefficients for all detectors equal to the result of the integration for the current detector divided by the result for the 90º detector. This forces the 90º detector to equal 1.0.

Determination of Molar Mass and Sizes

We begin by reviewing briefly the relationship between the data collected by the DAWN system and the molecular parameters derived from them. We start with Eq. 13 from Zimm [*J. Chem. Phys.* **16**, 1093–1099 (1948)]:

(Equation 13)

$$\frac{R_\theta}{K^* c} = MP(\theta) - 2A_2 cM^2 P^2(\theta)$$

where

- c is the mass concentration of the solute molecules in the solvent (g/mL)
- M is the weight average molar mass (g/mol)
- A_2 is the second virial coefficient (mol mL / g^2)
- $K^* = 4\pi^2 n_0^2 (dn/dc)^2 \lambda_0^{-4} N_A^{-1}$ is an optical constant where n_0 is the refractive index of the solvent at the incident radiation (vacuum) wavelength, λ_0 is the incident radiation (vacuum) wavelength, expressed in nanometers, N_A is Avogadro's number, equal to 6.022 x 10^{23} mol^{-1}, and dn/dc is the differential refractive index increment of the solvent-solute solution with respect to a change in solute concentration, expressed in mL/g (this factor must be measured independently using a dRI detector).
- $P(\theta)$ is the theoretically-derived form factor, approximately equal to $1 - 2\mu^2 \langle r^2 \rangle / 3! + \cdots$, where $\mu = (4\pi/\lambda)\sin(\theta/2)$, and $\langle r^2 \rangle$ is the mean square radius. $P(\theta)$ is a function of the molecules' z-average size, shape, and structure.
- R_θ is the excess Rayleigh ratio (cm^{-1})

Eq. 13 is the basis of the calculations in ASTRA as well as of the Zimm plot technique, which is often implemented in a batch sample mode. Eq. 13 assumes vertically polarized incident light and is valid to order c^2. The task is now to determine, for each slice, the molar mass and mean square radius.

It is possible to solve Eq. 13 in a variety of ways, leading to a number of different fit methods. We shall consider the Debye, Zimm, Berry and Random Coil methods.

Debye Fit Method

First, construct a Debye plot, that is, a plot of $R_\theta / K^* c$ vs. $\sin^2(\theta/2)$. Second, fit a polynomial in $\sin^2(\theta/2)$ to the data, and thereby obtain the intercept at zero angle, $R_0 / K^* c$, as well as the *slope* at zero angle, $m_0 \equiv d\left[R_\theta / K^* c\right] / d\left[\sin^2(\theta/2)\right]_{\theta \to 0}$. Note that as θ approaches zero, the form factor $P(\theta)$ approaches unity. Therefore, Eq. 13 becomes:

(Equation 14)

$$\frac{R_{\theta \to 0}}{K^* c} = \frac{R_0}{K^* c} = M - 2A_2 c M^2$$

If $A_2 = 0$, then

(Equation 15)

$$M = \frac{R_0}{K^* c}$$

Otherwise, solving Eq. 14 for M yields:

(Equation 16)

$$M = \frac{2\left(1 - \sqrt{1 - 8A_2 c\left(\frac{R_0}{K^* c}\right)}\right)}{8A_2 c}$$

Care must be exercised in solving Eq. 14. If A_2 or c is too large, there will be no real solution, which means that a higher order formulation of Eq. 13 is required. In addition, only one of the two solutions of Eq. 14 is physically reasonable. Also, the standard solution to the quadratic equation is susceptible to round-off error as $A_2 \to 0$. Therefore, we use an alternate form of Eq. 16, obtained by simple algebra:

(Equation 17)

$$M = \frac{2\left(\frac{R_0}{K^* c}\right)}{1 + \sqrt{1 - 8A_2 c\left(\frac{R_0}{K^* c}\right)}}$$

Eq. 17 is not susceptible to round-off error. Note that the expression in Eq. 17 reduces to $R_0 / K^* c$ in the case $A_2 = 0$, as it must.

To find the mean square radius $\langle r^2 \rangle$ for the slice, substitute the first two terms in the expansion for $P(\theta)$ into Eq. 13 to obtain:

(Equation 18)

$$\left\langle r^2 \right\rangle = \frac{-3 m_0 \lambda^2}{16\pi^2 M (1 - 4A_2 Mc)}$$

Zimm Fit Method

To perform calculations with the Zimm fit method, which is a fit to K^*c / R_θ vs. $\sin^2(\theta/2)$, we need to expand the reciprocal of Eq. 13 to first order in c:

(Equation 19)

$$\frac{K^*c}{R_\theta} = \frac{1}{MP(\theta)} + 2A_2c$$

The appropriate results in this case are

(Equation 20)

$$M = \left(\frac{K^*c}{R_0} - 2A_2c\right)^{-1}$$

and

(Equation 21)

$$\langle r^2 \rangle = \frac{3m_0\lambda^2 M}{16\pi^2}$$

where $m_0 \equiv d\left[K^*c / R_\theta\right] / d\left[\sin^2(\theta/2)\right]_{\theta\to 0}$

Berry Fit Method

To perform calculations with the Berry method, which is a fit to $\sqrt{K^*c / R(\theta)}$ vs. $\sin^2(\theta/2)$, we must expand the square root of the reciprocal of Eq. 13 to first order in c:

(Equation 22)

$$\sqrt{\frac{K^*c}{R_\theta}} = \frac{1}{\sqrt{MP(\theta)}} + A_2c\sqrt{MP(\theta)}$$

In this case the results are:

(Equation 23)

$$M = \frac{4}{\left(\sqrt{K^*c / R_\theta} + \sqrt{K^*c / R_\theta - 4A_2c}\right)^2}$$

and

(Equation 24)

$$\langle r^2 \rangle = \frac{3\lambda^2 m_0}{8\pi^2\sqrt{M}(1/M - A_2c)}$$

where

$$m_0 \equiv \frac{d\left[\sqrt{K^*c / R_\theta}\right]}{d\left[\sin^2(\theta/2)\right]_{\theta\to 0}}$$

Random Coil Fit Method

To derive the Random Coil fit method, we go back to Eq. 13, but instead of fitting a polynomial to $R_\theta / K^* c$ vs.$\sin^2(\theta/2)$ as in the Debye method, we insert into Eq. 13 the theoretical form factor $P(\theta)$ for random coils, which was first derived by Debye [P. Debye, *J. Phys. Coll. Chem.* **51**, 18 (1947)]:

(Equation 25)

$$P(\theta) = \frac{2}{u^2}\left(e^{-u} - 1 + u\right)$$

where $u = (4\pi / \lambda)^2 \left\langle r^2 \right\rangle \sin^2(\theta / 2)$. Since $P(\theta)$ is a nonlinear function of its parameter $\langle r^2 \rangle$, we use an iterative nonlinear least squares fit. Unlike the other fit methods, the Random Coil method assumes the polymers are approximately random coils. This can be an advantage for large random coil molecules because it allows the fit to proceed with fewer parameters than would otherwise be required in a simple polynomial fit, and the result can be lower estimated errors.

You can select which calculation type—Zimm, Berry, Debye, or random coil—you wish to employ in the Peaks procedure (page 8-51). If you own a miniDAWN, the calculation type must be either Zimm or Random Coil.

The result of these calculations is that for each slice i we have the molar mass M_i and the mean square radius $\langle r^2 \rangle_i$. Technically, the molar mass is weight-averaged and the mean square radius is z-averaged. Assuming good chromatographic separation, these quantities can be used together with the concentration c_i (measured with a concentration-sensitive detector) to find the molar mass and radius moments, as described next.

Model Fit Method

To derive the fit method for the various "modeled" form factors (sphere, coated sphere, rod), we fit the Zimm equation to $R_\theta / K^* c$ vs. $\sin(\theta/2)$, as in the Debye method, we insert into Eq. 13 the theoretical form factor $P(\theta)$ for the desired model. Form factor models have been derived for spheres, coated spheres, and rods and are covered in the text by van de Hulst[1]. Note that the sphere and coated sphere models yield geometric radius, while the rod model produces a length.

Sphere

(Equation 26)

$$P(\theta) = \frac{3}{u^3}\left(\sin u - u\cos u\right)$$

where $u = (4\pi / \lambda) r \sin(\theta/2)$.

Rods

(Equation 27)

$$P(\theta) = \int_0^{2u} \frac{\sin t}{t}\, dt - \frac{\sin^2 u}{u^2}$$

where $u = (2\pi / \lambda)\, L \sin(\theta/2)$, and L is the rod length (L >> rod radius).

1. *Light Scattering by Small Particles*, Wiley, New York [1957]

Mie Fit Method

In the Lorenz-Mie Fit method, Maxwell's equations for electromagnetic radiation are solved under the assumption of spherical particles. The Mie solution does not require the particle to satisfy the Raleigh-Gans-Debye criteria, and is therefore the most general method for analyzing spheres of any size.

To derive the fit method for various "modeled" form factors (sphere, coated sphere, rod), we fit the Lorenz-Mie equation to R_θ / K^*c vs. $\sin(\theta/2)$. The extended Lorenz-Mie exact calculation for a coated sphere (single layer) is given in the text by Bohren and Huffman based on the paper of A. L. Aden and M. Kerker[1].

Note that the Mie fit yields a geometric radius.

Molar Mass and RMS Radius Moments

ASTRA calculates the following molar mass and RMS (root mean square) radius moments for each peak. All summations are taken over one peak.

Number-average molar mass:

(Equation 28)

$$M_n = \frac{\sum c_i}{\sum \frac{c_i}{M_i}}$$

Weight-average molar mass:

(Equation 29)

$$M_w = \frac{\sum \left(c_i M_i \right)}{\sum c_i}$$

z-average molar mass:

(Equation 30)

$$M_z = \frac{\sum \left(c_i M_i^{\,2} \right)}{\sum \left(c_i M_i \right)}$$

Number-average mean square radius:

(Equation 31)

$$\left\langle r^2 \right\rangle_n = \frac{\sum \left(\frac{c_i}{M_i} \left\langle r^2 \right\rangle_i \right)}{\sum \frac{c_i}{M_i}}$$

1. *J. Appl. Phys.* 22, 1242-1246 [1951]

Weight-average mean square radius:

(Equation 32)

$$\left\langle r^2 \right\rangle_w = \frac{\sum\left(c_i \left\langle r^2 \right\rangle_i\right)}{\sum c_i}$$

z-average mean square radius:

(Equation 33)

$$\left\langle r^2 \right\rangle_z = \frac{\sum\left(c_i M_i \left\langle r^2 \right\rangle_i\right)}{\sum\left(c_i M_i\right)}$$

The quantities c_i, M_i, and $\langle r^2 \rangle_i$ in these equations are respectively the mass concentration, molar mass (g/mol), and mean square radius of the i^{th} slice. The often-quoted RMS radii are simply the square roots of the appropriate mean square radii. ASTRA also calculates two polydispersity values: M_w / M_n and M_z / M_n.

The uncertainty-weighted average molar mass (M_{avg}) is calculated as follows:

(Equation 34)

$$M_{avg} = \left(\frac{\sum_i M_i \sigma_{M_i}^2}{\sum_i \sigma_{M_i}^2} \right)$$

σ_{M_i} is the uncertainty in the molar mass measurement. The error in this calculation is defined as follows:

(Equation 35)

$$\sigma_{M_{avg}} = \frac{1}{\sum_i \sigma_{M_i}^2}$$

Uncertainties in Calculated Quantities

ASTRA calculates uncertainties for all reported quantities. By analyzing the baseline data at the beginning and end of the chromatogram, ASTRA determines the statistical fluctuation in each detector's output, including all photodiodes and the AUX signals.

Each detector is weighted based on the fluctuations (noise) seen in the first and last 10% of the data points, up to 100 data points. Whichever end is least noisy is used to calculate the weighting factor. (For batch mode calculations, data points within each plateau are used to calculate the detector weighting factors for each concentration.)

The error bars in the Debye plot do not represent this weighting factor directly. The Debye plot involves performing an nth order polynomial fit $R_\theta / K^* c$ to (for the Debye Fit Method), $K^* c / R_\theta$ (for the Zimm Fit Method), $\sqrt{K^* c / R(\theta)}$ (for the Berry Fit Method), or $P(\theta)$ (for the Random Coil Fit Method). The error bar calculation therefore involves the weighting factor, the normalized R(theta) value as well as a concentration uncertainty factor and the Chi-squared value returned from the fit. If the normalization is off for some detectors, then the Chi-squared value from the fit tends to increase, causing all error bars to grow. Hence, changes to the normalization coefficients will affect the error bars shown in the Debye plot, as well as the uncertainties in the overall peak results.

The different errors combine according to the usual rules for propagation of errors to yield a standard deviation (depending on calculation method) for each slice. These in turn allow calculation of uncertainties in the molar mass and size for each slice, and hence uncertainties in the calculated molar mass and size averages.

Remember, these uncertainties are statistical only, and do not include any of the many possible systematic errors that may be present. Examples are errors in dn/dc, the DAWN calibration constant, the AUX calibration constants, and the normalization coefficients.

Use the reported uncertainties as a measure of the statistical consistency of the data, never as an absolute limit on the error in your results.

Out of Range Values

Occasionally, electrical noise or a very low concentration or light scattering signal may cause the calculated molecular weight at a particular slice to be a negative number. For low molecular weights, often the mean square radius at a particular slice will be negative due to random noise in the Debye plot for that slice. Also, noise may cause both the calculated molecular weight and the mean square radius to have uncertainties larger than the values themselves. In these cases, special considerations are called for.

When calculating molecular weight averages, ASTRA first checks the calculated molecular weight values of all slices to be included in the calculations to find out if any of them are negative. ASTRA then removes slices that have negative values before calculating the averages.

When calculating mean square radius averages ASTRA includes values from all slices in the summation. If the sum of the mean square radii is positive, ASTRA will calculate the root mean square averages. If it is negative, the resulting root mean square averages will be set to zero.

In addition to the above, if any of the slices to be included in the averages have uncertainties larger than the values themselves, ASTRA will exclude them from the averages.

When plotting data in the Distribution Plots, ASTRA removes any slices that have negative values.

Differential Distribution Calculations

ASTRA V uses an adaptive binning technique for determining the differential distributions. It works both with the direct results, and with data that has been fit with results fitting.

Branching Calculations

ASTRA performs a number of sophisticated branching calculations. These are described below.

Branching Ratio: Radius Method

The branching ratio g_M is formally defined[1] as:

(Equation 36)

$$g_M = \left(\frac{\langle r^2 \rangle_{br}}{\langle r^2 \rangle_{lin}} \right)_M$$

where $<r^2>_{br}$ and $<r^2>_{lin}$ are the mean square radii of branched and linear (unbranched) polymer samples to be compared. Note that the ratio is taken at the same molar mass, *not* at the same volume. In general, for a given molar mass, the branched polymer will have a smaller radius, so g_M will lie between 0 and 1.

ASTRA calculates g_M this way: If no results fitting method has been selected, ASTRA uses the raw RMS Radius vs. MM data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from MM vs. Volume and RMS Radius vs. Volume directly in the branching calculations. For a number of points (300 points per decade of molar mass), Eq. 36 is applied.

In order to obtain useful branching information, the two files (linear and branched) should overlap as much as possible in molar mass. The branching ratio g_M can only be calculated in this region of overlap, since only in this region can radii be found at the same molar mass.

To use this method, select the Radius method in the Branching properties view. See “Branching” on page 8-80”.

Branching Ratio: Mass Method

If the molecular radii are too small to be calculated accurately, then we must use another method. Assuming the Flory-Fox equation is valid,[2] it can be shown that:

(Equation 37)

$$g_M = \left(\frac{M_{lin}}{M_{br}} \right)_V^{\frac{(a+1)}{e}}$$

where M_{lin} and M_{br} are the molar masses of a linear and branched polymer, respectively, a is the Mark-Houwink-Sakurada parameter for the linear polymer, and e is the drainage parameter, ranging from 0.5 for a non-draining polymer to 1.0 for a free-draining polymer to 1.5 for a Flory-

1. B.H. Zimm and W.H. Stockmayer, *J. Chem. Phys.*, **17**, 1301 (1949).
2. L.P. Yu and J.E. Rollings, *J. Appl. Polym. Sci.*, **33**, 1909–1921 (1987).

Fox polymer.[1] A value of 0.5–1.0 seems most used in the literature. The effect of the choice of e on the results can be seen in the figure below, which shows g_M for various values of e using a Mark-Houwink-Sakurada parameter a of 0.7, typical for a random coil. Notice that the ratio in Eq. 37 is taken at constant elution volume V.

If no results fitting method has been selected, ASTRA uses the raw MM vs. Volume data for both the linear and branched files. If a results fitting method has been selected, ASTRA uses the fitted data from MM vs. Volume in the branching calculations. For each slice of the branched file, ASTRA obtains the linear molar mass from the slice in the linear file having the elution volume closest to that of the branched slice.

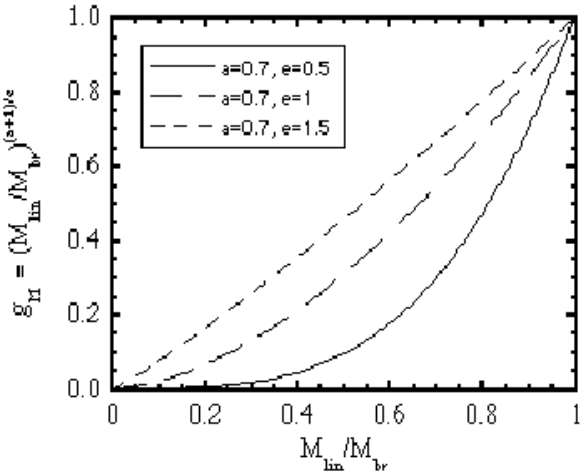

Figure D-1: Branching ratio g_M as a function of a and e for the Mass Method

For this method, the volumes should have a large region of overlap for an effective plot. To use this method, select the Mass method in the Branching properties view for each branched file. See “Branching” on page 8-80”.

Branching Ratio: Viscosity Method

The branching ratio $g'M$ is formally defined as:

(Equation 38)

$$g'(M) = \frac{[\eta(M)]_{br}}{[\eta(M)]_{lin}}$$

where η_{br} and η_{lin} are the intrinsic viscosities of branched and linear (unbranched) polymer samples to be compared. Note that the ratio is taken at the same molar mass, *not* at the same volume. In general, for a given molar mass, the branched polymer will have a smaller radius, so $g'M$ will lie between 0 and 1.

The Radius and Viscosity branching ratios are related by a term called the “drainage parameter” (e), as follows:

$g^e = g'$

1. B.H. Zimm and R.W. Kilb, *J. Polym. Sci.,* **37**, 19 (1959).

ASTRA uses the raw intrinsic viscosity vs. MM data for both the linear and branched files. For a number of points (300 points per decade of molar mass), Eq. 38 is applied.

In order to obtain useful branching information, the two files (linear and branched) should overlap as much as possible in molar mass. The branching ratio $g'M$ can only be calculated in this region of overlap, since only in this region can intrinsic viscosity be found at the same molar mass.

To use this method, select the Viscosity method in the Branching properties view. See "Branching" on page 8-80.

Branching Per Molecule

The number of branches per molecule is related to the branching ratio, but some knowledge of the type of branching is necessary. You can choose either trifunctional (Y or T) or tetrafunctional (X) branching, and monodisperse or polydisperse slices. These formulas[1] relate g_M to B *for randomly branched polymers:*

Trifunctional

- Polydisperse:

(Equation 39)

$$g_M = \frac{6}{B_{3w}}\left\{\frac{1}{2}\left(\frac{2+B_{3w}}{B_{3w}}\right)^{1/2}\ln\left[\frac{\left(2+B_{3w}\right)^{1/2}+B_{3w}^{1/2}}{\left(2+B_{3w}\right)^{1/2}-B_{3w}^{1/2}}\right]-1\right\}$$

- Monodisperse:

(Equation 40)

$$g_M = \left[\left(1+\frac{B_{3n}}{7}\right)^{1/2}+\frac{4B_{3n}}{9\pi}\right]^{-1/2}$$

Tetrafunctional

- Polydisperse:

(Equation 41)

$$g_M = \frac{\ln\left(1+B_{4w}\right)}{B_{4w}}$$

- Monodisperse:

(Equation 42)

$$g_M = \left[\left(1+\frac{B_{4n}}{6}\right)^{1/2}+\frac{4B_{4n}}{3\pi}\right]^{-1/2}$$

For each of these relations, the left hand side, g_M, is known already (see the previous sections). The appropriate equation is solved for B for each slice which produced a reasonable value of g_M. Note that if g_M falls outside the range 0 to 1, no value of B will be calculated for that slice.

1. B.H. Zimm and W.H. Stockmayer, *ibid*.

When plotted in ASTRA, the legend is labeled with the specified functionality: “3” for Trifunctional branching or “4” for Tetrafunctional branching; “n” for Monodisperse slices or “w” for Polydisperse. For each branched file to be plotted, select the branching functionality and whether the slices are monodisperse or polydisperse in the Branching property view.

The next figure, a plot of Eqs. (41)–(44), shows how the B's are related to g_M for the various branching options. Note that these relations assume randomly branched polymers. Also note that different assumptions about functionality and dispersity yield quite different values of B for the same value of g_M. Thus some knowledge of the type of branching is necessary for a plot of branches per molecule to have any meaning.

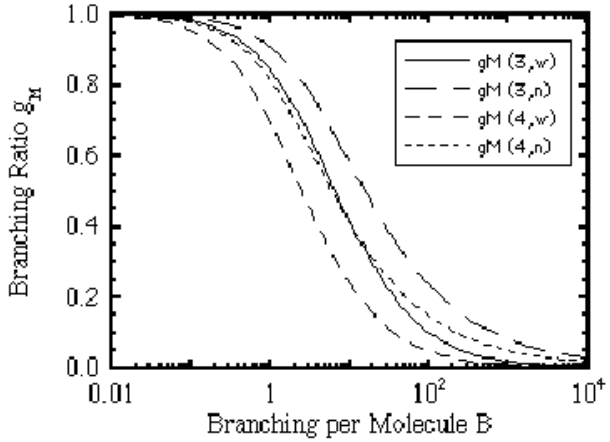

Figure D-2: Branching ratio g_M as a function of B for various branching options

Long Chain Branching

The long chain branching per 1000 repeat units is defined for each slice as

(Equation 43)

$$\lambda = 1000B\frac{R}{M}$$

where B is the branching per molecule for the slice (as calculated above), R is the repeat unit molar mass, and M is the branched molar mass for the slice. You must enter the repeat unit molar mass in the Unit MW box in the Branching property view for each branched file to be plotted.

E Particles Theory

Particles support is an add-on option for ASTRA. This option provides a procedure to calculate number density (see "Number from LS Data" on page 8-69). Particles include lattices, liposomes, and vesicles. Particle measurements are especially suited for use when a light scattering instrument is coupled to a fractionation technique such as Field Flow Fractionation (FFF) or Capillary Hydrodynamic Fractionation (CHDF), but concentration is not measured.

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Determination of Sizes

As discussed in Appendix D, "Light Scattering Theory", data collected by a DAWN system can be used to derive molecular parameters. In Particles mode, concentration is not measured—instead the angular variation of the scattered light is measured and used to characterize particle shape.

As in Appendix D, we start with the following equation from Zimm[1]:

(Equation 1)

$$\frac{R_\theta}{K^* c} = MP(\theta) - 2A_2 cM^2 P^2(\theta)$$

where:

- c is the mass concentration of the solute molecules in the solvent (g/mL).
- M is the weight average molar mass (g/mol).
- A_2 is the second virial coefficient (mol mL / g2).
- $K^* = 4\pi^2 n_0^2 (dn/dc)^2 \lambda_0^{-4} N_A^{-1}$ is an optical constant where n_0 is the refractive index of the solvent at the incident radiation (vacuum) wavelength, λ_0 is the incident radiation (vacuum) wavelength, expressed in nanometers, N_A is Avogadro's number, equal to 6.022 x 10^{23} mol^{-1}, and dn/dc is the differential refractive index increment of the solvent-solute solution with respect to a change in solute concentration, expressed in mL/g (this factor must be measured independently using a dRI detector).
- $P(\theta)$ is the theoretically-derived form factor, approximately equal to $1-2\mu^2\langle r2\rangle/3! + \ldots$, where $\mu = (4\pi/\lambda)\sin(\theta/2)$, and $\langle r^2\rangle$ is the mean square radius. $P(\theta)$ is a function of the molecules' z-average size, shape, and structure.
- R_θ is the excess Rayleigh ratio (cm^{-1}).

In the absence of a concentration detector, the molar mass is not determined. However, the angular dependency of the scattered intensity can be used to determine particle shape. As in regular light scattering analysis, different formalisms may be used to derive these measurements.

Debye Fit Method

First, construct a Debye plot excluding the optical constant and concentration terms, that is, a plot of R_θ vs. $\sin^2(\theta/2)$. Second, fit a polynomial in $\sin^2(\theta/2)$ to the data, and thereby obtain the intercept at zero angle, R_0, as well as the slope at zero angle, $m_0 = d[R_\theta] / d[\sin^2(\theta/2)]$.

(Equation 2)

$$\left\langle r^2 \right\rangle = \frac{-3m_0\lambda^2}{16\pi^2 R_0}$$

1. *J. Chem. Phys.* 16, 1093-1099 (1948)

Zimm Fit Method

To compute mean squared radius from the Zimm fit, we must fit $1/R_\theta$ vs. $\sin^2(\theta/2)$. Fit the Zimm equation to these data, and thereby obtain the intercept at zero angle (R_0) and the slope at zero angle, $m_0 = d[\,1/R_\theta\,] \,/\, d[\,\sin^2(\theta/2)\,]$.

(Equation 3)

$$\left\langle r^2 \right\rangle = \frac{3m_0\lambda^2 R_0}{16\pi^2}$$

Berry Fit Method

To perform calculations with the Berry method, we must fit to $\sqrt{1/R_\theta}$ vs. $\sin^2(\theta/2)$, and thereby obtain the intercept at zero angle (R_0) and the slope at zero angle, $m_0 = d[\,\sqrt{1/R_\theta}\,] \,/\, d[\,\sin^2(\theta/2)\,]$.

(Equation 4)

$$\left\langle r^2 \right\rangle = \frac{3m_0\lambda^2 \sqrt{R_0}}{8\pi^2}$$

Random Coil Fit Method

To derive the Random Coil fit method, we go back to Eq. 1, but instead of fitting a polynomial to R_θ vs. $\sin^2(\theta/2)$, as in the Debye method, we insert into Eq. 1 the theoretical form factor $P(\theta)$ for random coils, which was first derived by Debye[1]:

(Equation 5)

$$P(\theta) = \frac{2}{u^2}\left(e^{-u} - 1 + u\right)$$

where $u = (4\pi/\lambda)^2 \sin^2(\theta/2)$

Since $P(\theta)$ is a nonlinear function of its parameter $\langle r^2 \rangle$, we use an iterative nonlinear least squares fit to the Zimm formalism. Unlike the other fit methods, the Random Coil method assumes the polymers are approximately random coils. This can be an advantage for large random coil molecules, because it allows the fit to proceed with fewer parameters than would otherwise be required in a simple polynomial fit, and the result can be lower estimated errors.

Model Fit Method

To derive the fit method for the various "modeled" form factors (sphere, coated sphere, rod), we fit the Zimm equation to R_θ vs. $\sin^2(\theta/2)$. To derive the specific equation for the desired model, we insert into Eq. 1 the theoretical form factor $P(\theta)$ for the specific model being analyzed. Form factor models have been derived for spheres, coated spheres, and rods.

1. P. Debye, *J. Phys. Coll. Chem.* 51, 18 (1947)

They are covered in the text by van de Hulst[1]. Note that the sphere and coated sphere models yield geometric radius, while the rod model produces a length.

Sphere

(Equation 6)

$$P(\theta) = \frac{3}{u^3}\left(\sin u - u\cos u\right)$$

Rods

(Equation 7)

$$P(\theta) = \int_0^{2u} \frac{\sin t}{t}\,dt - \frac{\sin^2 u}{u^2}$$

where u = $(2\pi/\lambda)$L sin$(\theta/2)$, and L is the rod length (L >> rod radius).

Mie Fit Method

In the Lorenz-Mie Fit method, Maxwell's equations for electromagnetic radiation are solved under the assumption of spherical particles. The Mie solution does not require the particle to satisfy the Raleigh-Gans-Debye criteria, and is therefore the most general method for analyzing spheres of any size.

To derive the fit method for the various "modeled" form factors (sphere, coated sphere, rod), we fit the Lorenz-Mie equation to R_θ vs. sin$(\theta/2)$.

Note that the Mie fit yields a geometric radius.

1. *Light Scattering by Small Particles*, Wiley, New York [1957]

Radius Moments

ASTRA calculates the following radius moments for each peak. The specific type of radius (RMS radius, geometric radius, or hydrodynamic radius) depends on the type of analysis being performed. All summations are taken over one peak.

RMS Radius

Number Average

(Equation 8)

$$\left\langle r^2 \right\rangle_n = \frac{\sum \frac{R_0 \left\langle r^2 \right\rangle_i}{V_i^2}}{\sum \frac{R_0}{V_i^2}}$$

Weight Average

(Equation 9)

$$\left\langle r^2 \right\rangle_w = \frac{\sum \frac{R_0 \left\langle r^2 \right\rangle_i}{V_i}}{\sum \frac{R_0}{V_i}}$$

Z-Average

(Equation 10)

$$\left\langle r^2 \right\rangle_z = \frac{\sum R_0 \left\langle r^2 \right\rangle_i}{\sum R_0}$$

The quantities R_0, $\langle r^2 \rangle_i$, and V_i in these equations are respectively the Rayleigh Ratio, mean square radius, and volume of the i^{th} slice. The RMS radii are simply the square roots of the appropriate mean square radii.

Uncertainty Weighted Average

(Equation 11)

$$\left\langle r^2 \right\rangle_{avg} = \frac{\sum \left\langle r^2 \right\rangle_i \sigma^2_{\left\langle r^2 \right\rangle_i}}{\sum \sigma^2_{\left\langle r^2 \right\rangle_i}}$$

Where $\langle r^2 \rangle_i$ is as defined previously, and $\sigma_{\left\langle r^2 \right\rangle_i}$ is the uncertainty in the mean square radius measurement. The error in this calculation is defined as follows:

(Equation 12)

$$\sigma_{\left\langle r^2 \right\rangle_{avg}} = \frac{1}{\sum \sigma^2_{\left\langle r^2 \right\rangle_i}}$$

Geometric and Hydrodynamic Radius

Number Average

(Equation 13)

$$R_n = \frac{\sum \frac{R_0 r_i}{V_i^2}}{\sum \frac{R_0}{V_i^2}}$$

Weight Average

(Equation 14)

$$R_w = \frac{\sum \frac{R_0 r_i}{V_i}}{\sum \frac{R_0}{V_i}}$$

Z-Average

(Equation 15)

$$R_z = \frac{\sum R_0 r_i}{\sum R_0}$$

The quantities R_0, r_i, and V_i in these equations are respectively the Rayleigh Ratio, radius (either geometric or hydrodynamic), and the volume of the i^{th} slice.

Uncertainty Weighted Average

(Equation 16)

$$r_{avg} = \frac{\sum r_i \sigma_{r_i}^2}{\sum \sigma_{r_i}^2}$$

where r_i is as defined previously, and σ_{r_i} is the uncertainty in the radius measurement. The error in this calculation is defined as follows:

(Equation 17)

$$\sigma_{r_{avg}} = \frac{1}{\sum \sigma_{r_i}^2}$$

Theory

This section discusses how the distribution plots are calculated and why a model is needed.

The mean square radius is given by Eq. 18, where the distances r_i are measured from the particle's center of mass to the mass element m_i.

(Equation 18)

$$< r^2 >= \frac{\sum_i r_i^2 m_i}{\sum_i m_i} = \frac{1}{M}\int r^2 dm$$

Eq. 18 refers to a single particle whereas the quantity actually measured from an ensemble of particles may be shown to be a so-called LS-average mean square radius.[1] Were the particles random coils in a theta solvent, then this would be the so-called z-average mean square radius. We assume that the particle size distribution within each slice of an eluting sample following separation is essentially monodisperse. Therefore the particles in slice i, each of mass M_i, are assumed to have the same mean square radius. We define the root mean square radius as the square root of the mean square radius or, simply, $r_g = < r^2 >^{1/2}$.

The Rayleigh-Gans-Debye approximation (RGD):

(Equation 19)

$$\frac{K^* c}{R(\theta)} \approx \frac{1}{M_w P(\theta)}$$

can be re-written in the limit as $\theta \rightarrow 0$, P(0) = 1, and we have:

(Equation 20)

$$R(0) = K^* c_i M_i = K^* n_i M_i^2$$

since the concentration of mass in the ith slice is $c_i = n_i M_i$. If the elements of the particle whose molar mass is M_i are of uniform density and occupy a volume V_i, then the number of particles per mL in the ith slice, n_i, is proportional to the extrapolated zero-angle Rayleigh ratio divided by the square of the particle's volume, i.e.:

(Equation 21)

$$n_i \propto R(0) / V_i^2$$

Therefore we can write the *number fraction* of particles within slice i as n_i / D where:

(Equation 22)

$$D = \sum_j n_j$$

1. P.J. Wyatt, "New Insights into GPC Combined with MALS," Waters Corporation GPC Symposium Proceedings (San Diego, 1996).

is the summation taken over all slices in the selected region (or peak) of the eluting fractions. Note that although M_i is the *molar* mass of the particles, that value is proportional to the *mass* of the particles. Both are proportional to the *volume* of the particles if the volume is of uniform density.

Although the analysis of each slice results in a corresponding value of r_g, there may be other slices with similar sizes due to experimental fluctuations in the derived values. The expected monotonic variation of r_g with elution volume may be obtained by fitting the calculated values to a selected functional form using a least squares procedure. Alternatively, the slice data may be sorted into a set of size bins to obtain the differential number fraction after dividing each such fraction by the bin size. The fractions may also be distributed over the range of size bins included within the measured standard deviation associated with the particular contributing fraction.

In any event, the differential number fraction $n(r)\ dr$ of particles in the selected peak region between r and $r + dr$ now may be calculated explicitly without any *a priori* knowledge of the mass concentration at each slice provided we know the particle structure and that the RGD approximation is valid. For example, if we know that the particles are homogeneous spheres, we may replace V_i^2 by r_g^6. There are many other particle shapes where the relation between r_g and V_i^2 is known. The differential mass fractions may be generated in a similar manner without reference to a second detector.

What about particles whose shape is not known *a priori*? Although we may still calculate r_g as a function of elution volume (the r_g "calibration curve"), we cannot determine the differential number or mass fractions. Indeed, if we do not know the relation between the measured r_g and the particle's hydrodynamic radius, we cannot generate differential distributions. Were we to add a concentration detector following the LS detector, we could easily generate the differential mass fraction distributions of r_g.

A few other points must be discussed; most important among them is the applicability of the RGD approximation assumed in the preceding analysis. The simplest particles most frequently measured by particle sizing procedures are the polystyrene latex (PSL) spheres (emulsions) whose refractive index at wavelengths in the visible is about 1.59. Relative to water, whose refractive index is about 1.33, these spheres have a relative refractive index $m = 1.59/1.33 \approx 1.2$. Rigorous application of the RGD theory requires that $m - 1 << 1$, which is a slight stretch for these PSL spheres. Perhaps more importantly, the phase shift of a wave passing through the particle, $2\pi a[m\text{-}1]\ n_0/\lambda_0$, where a is the sphere radius, also must be << 1. Even if we make the assumption that $0.2 << 1$, attempting to size larger submicron particles using this approximation will quickly lead us out of the range of RGD applicability!

The saving grace of this approach is twofold: first, the theory happens to work significantly better than one might expect, even when the RGD requirements are not strictly satisfied, and second, the pertinent values are calculated in the limit $\theta \rightarrow 0$ (as shown in Eq. 20), a regime where the RGD requirements are much more easily satisfied. As the scattering angle becomes very small, the RGD approximation becomes more valid as was confirmed vividly by the analyses of Kerker et al.[1] The result is that values of $R(0)$ may be generated directly from the measurements if the particle's structure is known, or from the more general expansion of the form factor $P(\theta)$.[2]

Furthermore, many of the calculated results for the analyses of distributions of PSL spheres may be checked with more exact LS theory to confirm the precision of the sizes measured using the RGD approximation. Applying the Lorenz-Mie theory confirms the results derived by the present treatment. In addition, average values measured by photon correlation spectroscopy (PCS) at individual slices also confirm the average values generated by the present implementation of RGD theory.

1. M. Kerker, W.A. Farone, and E. Matijevic, *J. Opt. Soc. Am.* **53**, 758 (1963).
2. P.J. Wyatt, *Analytica Chimica Acta* **272**, 1-40 (1993).

F QELS Theory

This appendix gives a quick overview of the theory behind cumulants and regularization, which are analysis techniques used with QELS data. This includes descriptions of the implementation in ASTRA V and interpretation of results.

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Cumulants

The analysis of QELS data is straightforward for a monodisperse sample. For unfractionated, polydisperse samples, however, the analysis becomes much more complicated. The simplest approach to analyzing data from polydisperse samples is to assume that the sample is monodisperse, apply the analysis from ASTRA, and come up with some sort of mass-averaged result for the hydrodynamic radius. The measured correlation function for a polydisperse sample actually contains more information than this, and several strategies have been developed to extract more information about the underlying size distribution from the correlation function.

The next level of sophistication in QELS analysis for polydisperse, unfractionated samples is the method of cumulants. In a nutshell, the method of cumulants involves fitting the correlation function not to a single decay time, but to a Gaussian distribution of decay times. The method of cumulants retrieves the mean and variance for this distribution.

Theory

The result of a QELS measurement is a second order correlation function:

(Equation 1)

$$g^{(2)}(\tau) = \frac{\langle I(t)I(t+\tau)\rangle}{\langle I(t)\rangle^2}$$

where $I(t)$ is the intensity of the scattered light at time t, and the brackets indicate averaging over all t. The correlation function depends on the delay τ, that is, the amount that a duplicate intensity trace is shifted from the original before the averaging is performed. A typical correlation function for a monodisperse sample is shown in Figure F-1.

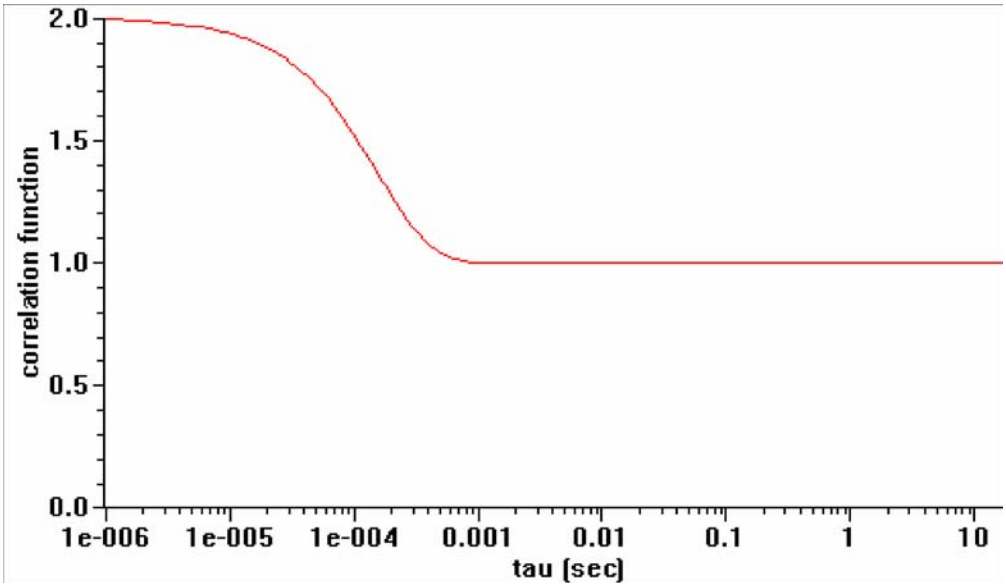

Figure F-1: Correlation function for a multi-tau correlator like that in WyattQELS

As described in various light scattering texts[1], the correlation function for a monodisperse sample can be analyzed via the equation:

(Equation 2)

$$g^{(2)}(\tau) = B + \beta \exp(-2\Gamma\tau)$$

where B is the baseline of the correlation function at infinite delay, β is the correlation function amplitude at zero delay, and Γ is the decay time. A nonlinear least squares fitting algorithm can be applied to Eq. 2 to retrieve the correlation function decay time Γ. This is exactly what is done in the ASTRA QELS analysis.

From this point, Γ can be converted to the diffusion constant D for the particle via the relation:

(Equation 3)

$$D = \frac{\Gamma}{q^2}$$

Here, q is the magnitude of the scattering vector, and is given by

(Equation 4)

$$q = \frac{4\pi n}{\lambda_0}\sin(\theta/2)$$

where n is the solvent index of refraction, λ_0 is the vacuum wavelength of the incident light, and θ is the scattering angle.

Finally, the diffusion constant can be interpreted as the hydrodynamic radius r_h for a diffusing sphere via the Stokes Einstein equation:

(Equation 5)

$$r_h = \frac{kT}{3\pi\eta D}$$

where k is Boltzmann's constant and η is the solvent viscosity.

The previous equations provide the tools for analyzing a correlation function from a monodisperse sample, but do not address the effects of polydispersity on the correlation function. One of the first attempts to analyze such data was the method of cumulants. First proposed by Koppel[2], the method of cumulants involves expanding Eq. 2 into the various moments of a distribution. In its simplest expression, this expansion turns Eq. 2 into the following:

(Equation 6)

$$g^{(2)}(\tau) = B + \beta \exp\left(-2\overline{\Gamma}\tau + \kappa_2\tau^2 - \frac{\kappa_3}{3}\tau^3 \ldots\right)$$

Here, the decay time is now the average for the distribution, while the higher moments correspond to the variance, or width of the distribution (K_2), the skewness of the distribution (K_3) and so on.

1. B. Chu, *Laser Light Scattering: Basic Principles and Practice*, (Academic, Boston, 1991).
2. D.E. Koppel, "Analysis of macromolecular polydispersity in intensity correlation spectroscopy: The method of cumulants," *J. Chem. Phys.* 57, 4814-4820 (1972).

In practice, it usually only possible to determine the first two moments of the expansion in Eq. 6, that is, the average and variance. These are often referred to as the first and second cumulant. In this simplest form, the method of cumulants then boils down to fitting the correlation function to a Gaussian distribution of decay times; only the average and width of the distribution are obtained.

Application of the Method of Cumulants

In the ASTRA software, a variant of Eq. 6 is used to obtain the first and second cumulants in a nonlinear least squares fit of the correlation function. This variation was derived by Frisken[1], and is given by:

(Equation 7)

$$g^{(2)}(\tau) = B + \beta \exp\left(-2\bar{\Gamma}\tau\right)\left(1 + \frac{\mu_2}{2!}\tau^2 - \frac{\mu_3}{3!}\tau^3 \ldots\right)^2$$

Here, the moments μ_n correspond to the K_n terms in Eq. 6, and are the physical moments about the mean $\bar{\Gamma}$. Eq. 7 is inherently more stable than Eq. 6 when fitting at large delay times τ, thus leading to a more robust analysis of the correlation function than has traditionally been obtained from the method of cumulants.

The results obtained from the fit in the QELSBatch cumulant analysis are the first two moments, $\bar{\Gamma}$ and μ_2 in Eq. 7, as well as the baseline B and amplitude β. The baseline and amplitude values are used in the data filtering algorithm to reject QELS correlation functions after the initial cumulants analysis. However, the first two cumulants are the quantities of interest for assessing the polydispersity of the sample.

The first two moments define a Gaussian distribution in decay times, where the first cumulant gives the mean of the distribution, and the square root of the second cumulant gives the standard deviation. In terms of a distribution for sizes, the decay time distribution can be converted to hydrodynamic radius via equations 3 through 5. Since the radius is inversely proportional to the decay time, the distribution in radius is no longer a symmetric Gaussian. This can be seen in Figure F-2.

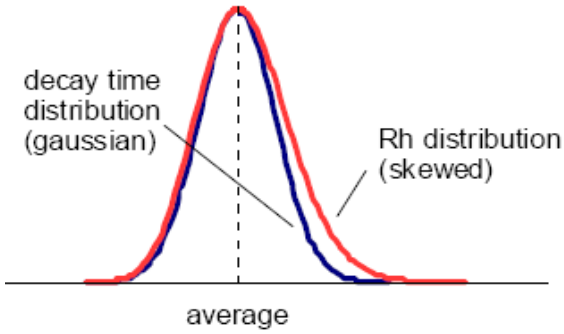

Figure F-2: Cumulants distributions in decay time and hydrodynamic radius

1. B.J. Frisken, "Revisiting the method of cumulants for the analysis of dynamic light-scattering data," *Applied Optics 40*, 4087-4091 (2001).

In the cumulants analysis results, the fitted first and second moments—that is the decay time distribution average and variance—are reported, as well as the uncertainties (one standard deviation) from the fit. The square root of the variance is used to determine the standard deviation in the decay time distribution. The average, the average plus the standard deviation, and the average minus the standard deviation are converted to hydrodynamic radius via Eq. 3 through 5, and are included in the results.

Interpretation of Cumulant Results

The cumulants method presents a challenge in displaying the results. ASTRA present the hydrodynamic radius results from the cumulants analysis, since size is more intuitive than decay time for most researchers. In the cumulant analysis graph, the average hydrodynamic radius and the distribution values at one standard deviation are presented. This creates an “error bar” appearance for the graph, but the error bars indicate the width of the fitted distribution. They are asymmetric because the hydrodynamic radius is inversely proportional to the symmetric decay time distribution, as shown in Figure F-2.

With cumulant results presented this way, it is important to remember that there is uncertainty in the first and second moments determined from the fit. This uncertainty translates into an uncertainty in the average hydrodynamic radius, but more importantly, an uncertainty in the effective width of the distribution implied by the cumulants analysis graph.

Therefore, an uncertainty in this width is estimated by calculating the spread in possible width values based on the fitted uncertainty in the second cumulant. The effective width implied by the cumulants is then compared to the spread of possible widths to derive a percentage uncertainty in the effective width. The average uncertainty in width is reported in the Width property, and should provide a good measure of how much to trust the widths that result from the analysis.

After all is said and done, the question remains how the cumulant analysis results relate to the actual polydispersity of the sample. Assuming that the size distribution in Figure F-2 reflects all samples is simply incorrect. Therefore, the cumulant results should be taken as a semi-quantitative estimate of the degree of polydispersity. It would probably be safe to assume that for two samples with the same average size, but different widths estimated from the cumulant analysis, that the sample with the greater width is more polydisperse. However, trying to define a rigorous polydispersity index from the cumulant analysis would probably lead to very inaccurate results when compared to a quantitative method such as fractionation followed by light scattering to determine the underlying distribution. Therefore, cumulant analysis results should only be used to assess the potential relative polydispersity of samples. Follow-up analysis, such as fractionation followed by light scattering, should be used to assess the reliability of the cumulant analysis results, particularly if they are to be used as the sole assay for polydispersity.

Regularization

Whereas the method of cumulants is one of the simplest approaches to analyzing QELS data from a polydisperse sample, the regularization analysis is one of the most sophisticated. There are many excellent references for the regularization method, and the theory is quite detailed.[1]

Theory

As opposed to the method of cumulants, the regularization analysis makes far fewer assumptions about the underlying distribution of sizes that make up the polydisperse sample. A simple predecessor of the regularization method—the histogram method—demonstrates this nicely. In the histogram method, the distribution of decay times is not assumed to be Gaussian, as it is for the cumulant method with only the first two cumulants. Instead, the decay time distribution is divided into bins. Consider, for example, the model correlation function in Figure F-3 for a bimodal distribution consisting of widely separated sizes:

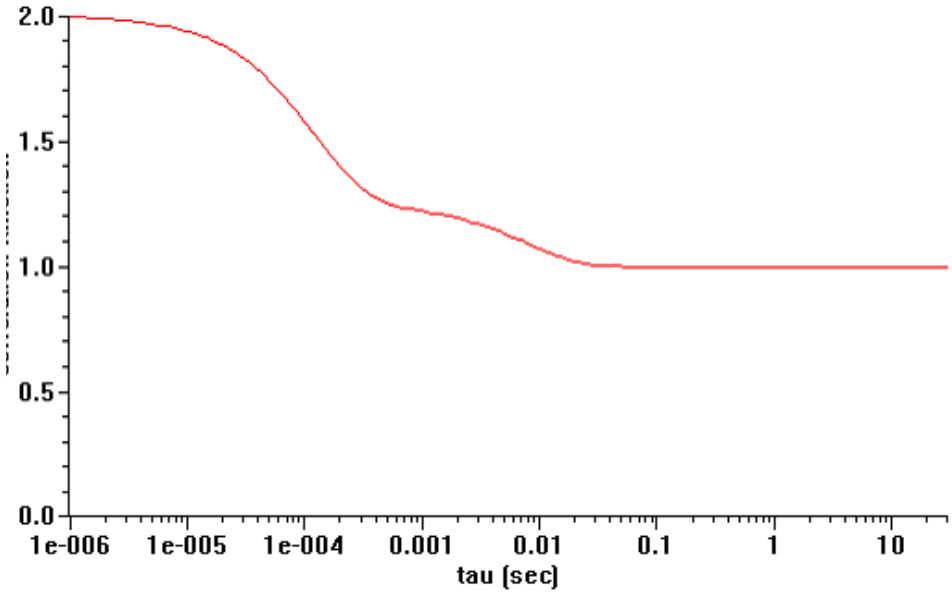

Figure F-3: Bimodal correlation function for mixture of 10 nm and 1 µm particles.

Clearly, the correlation function in Figure F-3 would best be fit by a "sum" of two separate correlation functions, one with a short decay time, and one with a long decay time. In terms of the histogram method, the underlying distribution would appear as in Figure F-4, that is, only two bins would have any intensity. The correlation function is then modeled by "adding" the correlation functions for the two separate bins. (It is more complicated than this, since there is cross-correlation between the various components, but for the sake of pedagogy, the concept of adding is adequate.)

1. S.W. Provencher, "Inverse problems in polymer characterization: Direct analysis of polydispersity with photon correlation spectroscopy," *Makromol. Chem.* 180, 201-209 (1979).

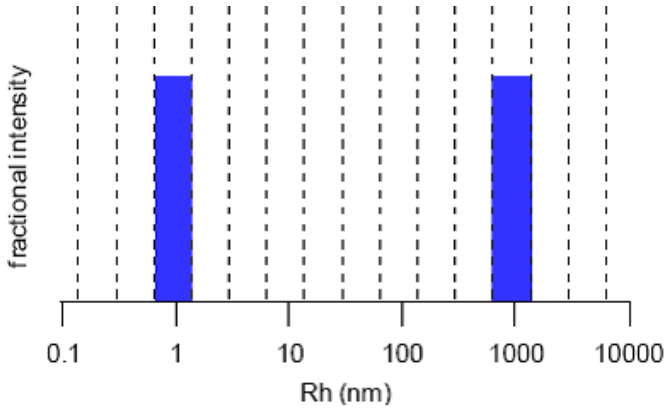

Figure B-2. Model histogram for bimodal size distribution.

Figure F-4: Model histogram for bimodal size distribution

More complicated correlation functions from more polydisperse samples could be modeled by the histogram method. Intensity would be shifted between bins until the right match was found. In so doing, the underlying distribution would be revealed, albeit in a somewhat jagged fashion from the bins. We can remedy this jaggedness by making our bin sizes smaller and smaller until we get the true distribution.

In reality, the histogram method breaks down long before enough bins can be added to accurately represent a distribution. The problem is that as more bins are added, the number of possible solutions explodes. There is not enough information in the correlation function to accurately distribute the intensity over hundreds of bins. In fact, for a standard correlator such as that in the WyattQELS instrument, the largest number of bins that can be handled is only about ten.

The regularization method makes it possible to have a finer mesh of bins. This is accomplished by constraining the types of distributions that can accurately reproduce the correlation function. The most common constraint, and the one employed in the DYNALS algorithm used in ASTRA V, is that the distribution be smooth. This is accomplished by adding a regularization term that penalizes solutions that are not smooth. The magnitude of the regularization term determines how smooth the final result must be. The trick of every regularization algorithm is to determine the optimal amount of regularization such that the final solution captures as many features of the true distribution as possible, while balancing out the effects of noise in the correlation function. Noise can add spurious components to the calculated distribution, hence as the noise increases, the regularization term needs to increase to damp these spurious components.

Implementation of Regularization in ASTRA V

The regularization algorithm in the ASTRA V software is the DYNALS 2.0 algorithm supplied by Alango, Ltd.[1] The DYNALS algorithm sets the regularization level—referred to as the resolution—to the most appropriate

value for the level of noise in the correlation function. The resolution value can range between 0 and 1, where 0 corresponds to the noisiest data, and 1 corresponds to the least noisy data. In ASTRA V, the optimal value of the resolution is taken from the DYNALS algorithm and reported in the data window for the regularization analysis window.

The results of the regularization are an intensity distribution in hydrodynamic radius. However, in light scattering, the intensity distribution does not give an accurate representation of the number distribution. Therefore, intensity information can be converted to relative number by choosing a mass model for the particles, and applying a correction factor for the intensity. The mass models in ASTRA V are sphere and random coil.

Interpreting Regularization Results

Regularization analysis results are more physical than results for the cumulants method. However, some care must be taken in interpreting these results. First, low size peaks (< 1 nm) often appear in the regularization results. These are sometimes attributed to solvent scattering, but are most likely due to avalanche photodiode afterpulsing picked up by the correlator. To exclude this from the correlation function, try setting a longer minimum delay time for the correlation function in ASTRA. Large size peaks are also common in the final distribution. These are usually real and correspond to dust.

Another issue of concern in interpreting regularization results is determining whether the resulting width of the distribution corresponds to an actual polydispersity. For example, applying the regularization analysis to a correlation function from a monodisperse sample often results in a distribution with some width. In general, the noisier the correlation function, the lower the optimal resolution of the regularization algorithm, and the broader the apparent width. Therefore, when interpreting distribution widths from regularization, always consider the resolution obtainable given the level of noise in the correlation function. Ideally, correlation functions for a monodisperse sample and the sample of interest can be obtained with comparable levels of noise, such that the regularization analysis resolution can be accurately assessed.

Finally, the smoothing nature of the regularization algorithm can mask features in the true distribution, even for correlation functions with very low noise. Therefore, if a very structured distribution in sizes is expected, regularization typically returns a much smoother distribution. In short, it is prudent to compare regularization results with a quantitative method such as fractionation followed by light scattering to determine the true distribution. In general, regularization provides the most accurate analysis for samples that are broadly polydisperse over several orders of magnitude in size and that have intrinsically smooth distributions.

1. A.A. Goldin, “Software for particle size distribution analysis in photon correlation spectroscopy,” website documentation at http://www.softscientific.com/science/WhitePapers/dynals1/dynals100.htm.

G Viscosity Theory

This appendix reviews the theory of viscosity-related calculations.

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Calculating Intrinsic Viscosity

ASTRA V can process a wide variety of input viscosity sources, ranging from simple devices producing only a single pressure differential to more sophisticated devices that measure specific viscosity directly.

Once specific viscosity is measured, it is useful to compute the intrinsic viscosity. Intrinsic viscosity is defined as the limit of:

(Equation 1)

$$[\eta] = \lim_{c \to 0} \frac{\eta_{sp}}{c}$$

Of course, all real instruments measure the specific viscosity at finite concentrations. The concentration dependency of the specific viscosity is typically described using one of three formalisms: the Huggins equation, the Kraemer equation, and the Solomon-Gatesman equation. In all cases, the concentration of the sample must be derived from a detector, such as the Optilab rEX or a UV absorption detector.

Huggins

The Huggins equation is specified as:

(Equation 2)

$$\eta_{sp} = [\eta]c + k'[\eta]^2 c^2 + O\left(c^3\right)$$

The coefficient k' is the Huggins constant. For random coil polymers in good solvents, the Huggins constant typically has a value between 0.0 and 0.3. In size exclusion chromatography, the concentration of the sample is usually so dilute that one can ignore the concentration terms of third power (or above) and use the approximation:

(Equation 3)

$$[\eta] = \eta_{sp} \Big/ c$$

Solving Eq. 2 for intrinsic viscosity yields:

(Equation 4)

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2 k'}{c} + O\left(\eta_{sp}^3\right)$$

Eq. 4 is simplified to the following for computation:

(Equation 5)

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4\eta_{sp} k'}}{2k'c}$$

Kraemer

The Kraemer equation is:

(Equation 6)

$$\frac{\ln(\eta_{sp}+1)}{c} \approx [\eta] + k''[\eta]^2 c$$

Solving Eq. 6 for intrinsic viscosity yields:

(Equation 7)

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{(1/2 + k'')\eta_{sp}^2}{c} + O(\eta_{sp}^3)$$

The expansion shows that for small values of specific viscosity, which is almost always the case for chromatography, the two formalisms are related:

(Equation 8)

$$k' = 1/2 + k''$$

Eq. 7 is simplified to the following for computation:

(Equation 9)

$$[\eta] \approx \frac{-1 + \sqrt{1 + 4k''\ln(1+\eta_{sp})}}{2k''c}$$

Solomon-Gatesman

The advantage of the Solomon-Gatesman equation is that it does not require empirical constants. However, for values of specific viscosity much less than one, it reduces to the Huggins Equation, with a value of k' = 1/3.

(Equation 10)

$$[\eta] \approx \frac{\eta_{sp}}{c} - \frac{\eta_{sp}^2}{3c} + O(\eta_{sp}^3)$$

Eq. 10 is simplified to the following for computation:

(Equation 11)

$$[\eta] \approx \frac{\sqrt{2\eta_{sp} - 2\ln(\eta_{sp}+1)}}{c}$$

Intrinsic Viscosity and Molecular Parameters

The simplest model of the intrinsic viscosity is due to Einstein and Simha[1]. They considered the case of noninteracting rigid particles. They found that the viscosity can be related to the volume fraction of the fluid occupied by the particles. They found:

(Equation 12)

$$\eta = \eta_0(1 + \gamma\phi)$$

where ϕ is the volume fraction and $\gamma = 2.5$ for spheres and larger for non-spherical particles.

If the weight concentration of the molecule is c, then the number of molecules per unit volume is $N_A c/M$, where N_A is Avogadro's number and M is the molar mass as measured by light scattering. Therefore Eq. 12 can be written in terms of the measured intrinsic viscosity as:

(Equation 13)

$$[\eta] = \frac{\gamma N_A V_h}{M}$$

where V_h is the hydrodynamic volume of the molecules. Note that M/V_h is the molecular density, so in some sense, the intrinsic viscosity is measuring the molecular density.

The intrinsic viscosity often differs from the bulk density due to molecular shape, molecular density, and the effects of adsorbed or immobilized solvent on the surface of molecule. This so-called hydration layer moves with the molecule, so it affects measurement of the molecular density. In addition, when the molecule has an extended shape, penetration of non-immobilized solvent into the interior of the molecule similarly affects this measurement.

If we set $\gamma = 2.5$, this can be used to define the equivalent spherical volume of a nonspherical molecule. Similarly, it can be used to define the hydrodynamic volume r_h as:

(Equation 14)

$$r_h = \left[\frac{3V}{4\pi}\right]^{1/3}$$

When defined in this way, r_h is the radius of a sphere with the same intrinsic viscosity as the molecule under study.

1. A. Einstein, *Ann.Physik*, 19, 289 (1906); 34, 591 (1911) and R.Simha, *J.Phys. Chem.*, 44, 25 (1940); *Science* 92, 132 (1940)

Flory-Fox Relation

While the Einstein-Simha relation can be used to define the hydrodynamic radius for solid molecules with adsorbed solvation layers, it not simply related to the molecular size of extended molecules such as random coil polymers. Several models have been developed to consider the effect the hydrodynamic drag on the intrinsic viscosity.

One of the most successful models comes from Flory and Fox who modeled the random coil as a series of "beads on a string" or a "jointed chain". The string is flexible, but beads are rigid. Flory and Fox considered that hydrodynamic friction causes the solvent near the center of the molecule to move with the same velocity as the center of mass, but solvent near the edges is free to flow into and out of the molecule. This led them to a relationship between the intrinsic viscosity and the mean square radius of the polymer chain in a theta solvent. Their model is:

(Equation 15)

$$[\eta] = \Phi \left\langle r^2 \right\rangle^{3/2} / M$$

where $<r^2>$ is the mean squared end-to-end distance of the chain, and Φ_0 is a universal constant having the value 2.87×10^{23}. In practice, this constant varies somewhat from polymer to polymer with an experimental value closer to 2.5×10^{23}.

The Flory-Fox relationship is valid for polymers in theta solvents. Ptitsyn and Eizner considered the modification required to model other solvents. They found the following relationship:

(Equation 16)

$$[\eta] = \Phi(\varepsilon) \left\langle r^2 \right\rangle^{3/2} / M$$

(Equation 17)

$$\Phi(\varepsilon) = \Phi_0 \left(1 - 2.63\varepsilon + 2.86\varepsilon^2\right)$$

where Φ is now a function of the polymer-solvent iteration parameter ε, and Φ_0 is the Flory-Fox constant. When $\varepsilon = 0$, it reduces to the theta solvent result.

The ε parameter is experimentally measurable with a Mark-Houwink analysis. To perform a Mark-Houwink analysis, the data for a random coil polymer is fit to:

(Equation 18)

$$[\eta] = KM^a$$

where M is the molar mass. The K and a are fit parameters, which depend upon the polymer, solvent, and temperature. Traditionally, this data is also plotted as Log[η] vs. Log[a]. If the data is fit well, this should be a straight line. The slope parameter a is related to ε by:

(Equation 19)

$$\varepsilon = (2a - 1)/3$$

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