

The Re-Integrate Option

The Re-integrate option is used to fully re-integrate a baseline in PeakSimple. When changes are made to a baseline often a partial integration will occur, selecting Re-integrate will perform a full integration on the baseline. The Re-integrate option can be selected by clicking on Edit in the PeakSimple menu bar and then Re-integrate from the list of options.

The View-Results Window

The Results window displays the results of the chromatogram runs performed in PeakSimple. The Results window is opened up by clicking on View in the PeakSimple menu bar and then selecting Results from the list of options.

The Channel option scrollbar specifies which of the four channels the results data should be displayed for. When the **Recognized peaks only** checkbox is selected only the results for named peaks will be displayed. The **Undetected components also** checkbox displays the results for the undetected components as well as the detected components in the chromatogram run when the option is selected.

Update

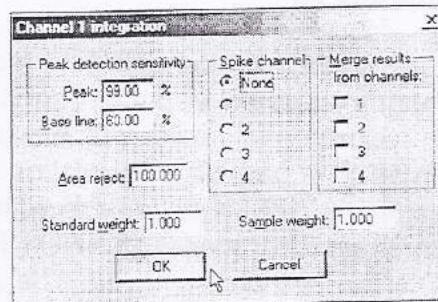
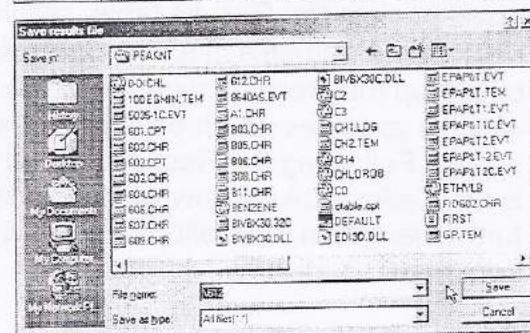
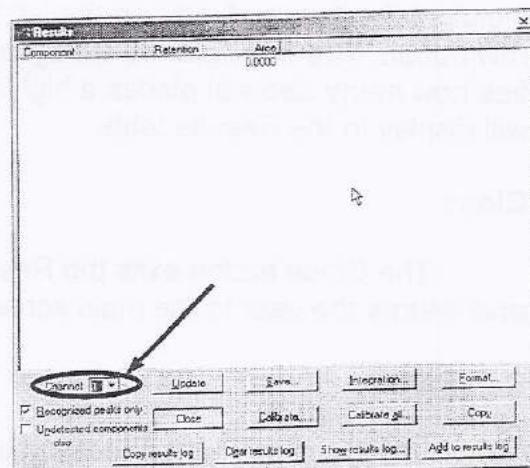
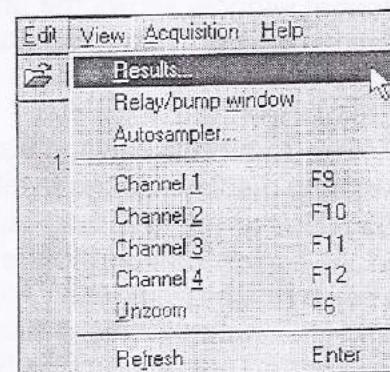
The Update button in the Results window updates the DDE link between the Results data and the DDE host program (typically Excel).

Save

Selecting the Save button in the results window opens up the Save results file window. In the Save results file window the results file is saved with a .res extension. The file is an ASCII file and not the raw chromatogram data.

Integration

As a convenience the integration button in the results window opens up the same Integration window that can be accessed in the Channels window. For more information on the Integration window consult the Channels-Integration portion of this manual.



The View-Results Window (cont.)

Format

Selecting the Format button in the Results window opens up the Edit format window. The Edit format window allows the user to specify the information that is to be included in the Results table.

The Available options box in the Edit format window displays all the available options that can be included in the results but that aren't selected. An option is added to the Selected options box by highlighting the item in the Available box and clicking on the right facing arrow button. To deselect an option from the Selected box highlight the item and click on the left facing arrow button. The Dec. places dialogue box specifies how many decimal places a highlighted unit will display in the Results table.

Close

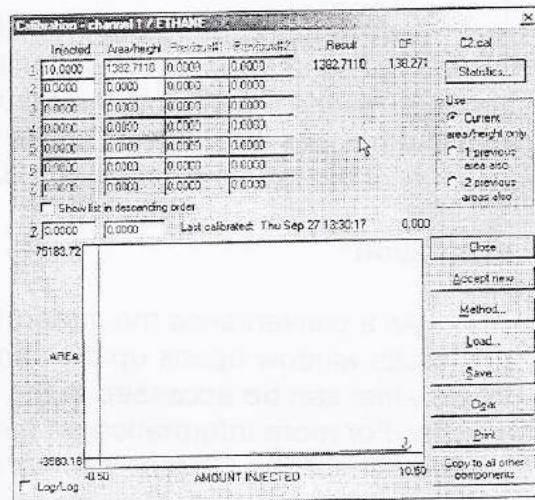
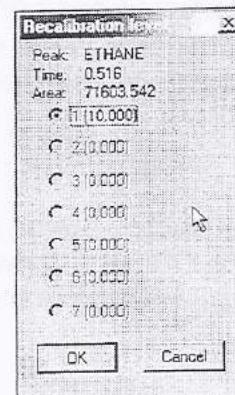
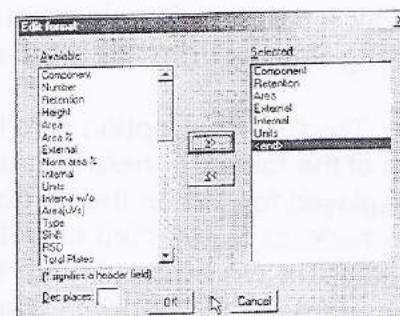
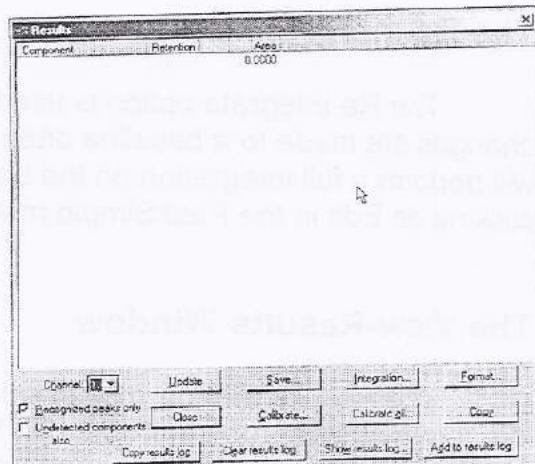
The Close button exits the Results window and returns the user to the main screen.

Calibrate

The Calibrate button recalibrates a recognized peak in the Results table. Highlighting a peak name and selecting the Calibrate button opens up the Recalibration Level window. The window specifies which peak level should be calibrated. Following the Recalibration level window is the Calibration window which is discussed at further length in the Calibration section of this document.

Calibrate All

The Calibrate all button recalibrates all the recognized peaks at once. The Calibrate all button calibrates all peaks with existing calibration curves on a particular calibration level. If named peaks are in the results table without calibration curves an error message, (NOT ENOUGH DATA POINTS), will be displayed. The calibration will



The View-Results Window (cont.)

Copy

The Copy button in the results window copies the results report to the Clipboard. Once the report is copied it can be pasted into other programs i.e. Excel.

Copy Results Log

The Copy results log button copies the .log file for the results to the Clipboard. This log file can be pasted into any Windows program. A certain number of lines in the results log will always be copied, by default the number is 20. If more than 20 lines are needed for an application the user must modify the peakwin.ini file located in the Windows folder. The default entry in the file is (SpareLines=20), delete the number 20 and insert the number of lines that are needed (up to a maximum of 100).

Clear Results Log

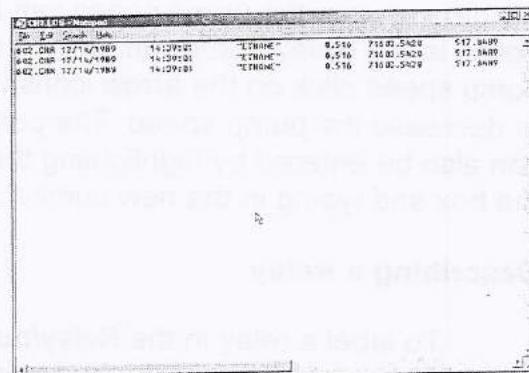
Clicking on the Clear results log button erases the results log file.

Show Results Log

The Show results log button when selected opens up Windows Notepad to view the results log.

Add to Results Log

To add the current report to the results log click the Add to results log button. The report can automatically be added to the results log at the end of each chromatogram run by checking the Add to results log checkbox in the Postrun window.



The View-Relay/Pump Window

The Relay/pump window manually controls the actions of the relays in PeakSimple. The Relay/pump window is opened up by opening the View menu and then selecting Relay/pump window from the list of options.

Selecting/Deselecting a Relay

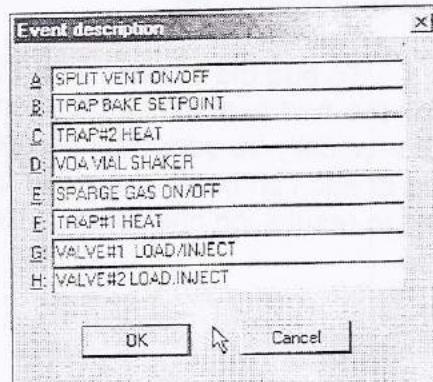
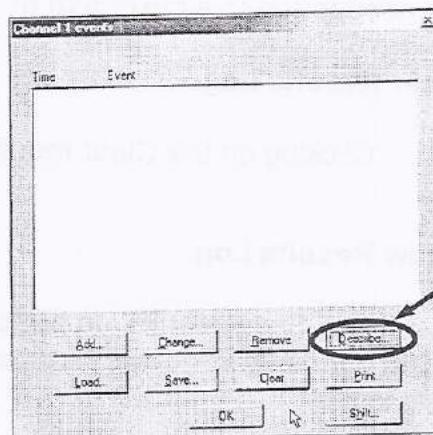
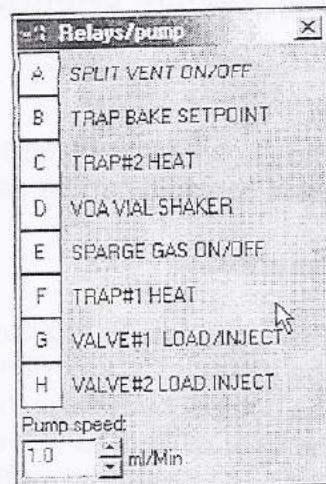
To manually activate a relay click on the letter next to the relay label to make the button dark. To deactivate a relay select the specified lettered button to turn it black. Pressing the control button and the letter corresponding to the relay together also selects/deselects the relay.

Pump Speed

When an SRI HPLC pump is connected to the data system the pump speed can be controlled in the Relay/pump window. To change the pump speed click on the arrow icons to increase or decrease the pump speed. The pump speed can also be entered by highlighting the value in the box and typing in the new number.

Describing a Relay

To label a relay in the Relay/pump window right click on the main screen and select Events from the list of options. Once the Events window is opened up clicking on the Describe button opens up the Event description window. To enter a relay description click on the specified relay's dialogue box and type in the information. The description of the relays has no effect on the relay function and will not affect hardware.

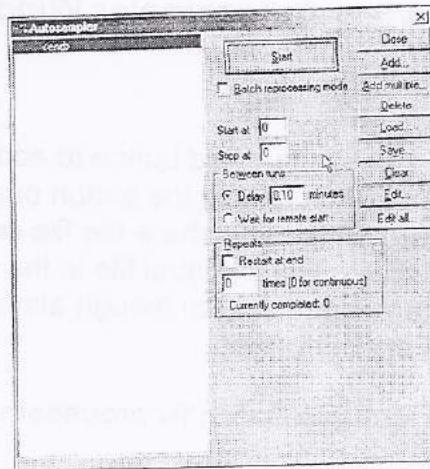


The View-Autosampler Window

The Autosampler window allows a list of control files to be run automatically. Control files are the master files which specify all parameters including temperature programming, component, and event files. These control files run tasks in PeakSimple. To open up the Autosampler window click on the View menu in the menu bar and then select Auto-Autosampler from the available options.

Start/Stop

The Start button when pressed begins the operation of the autosampler queue or reprocessing queue. A queue must be created or loaded before the control files can run. Once the autosampler is in operation the Start button changes into the Stop button. The Stop button ceases the autosampler operations that were previously running.



Batch Reprocessing Mode

To select Batch reprocessing mode click on the check box to the options left. While using the Batch reprocessing mode the user loads a list of previously stored chromatogram files in the list box to the left and then selects a control file which will reprocess the data files. When the operation begins PeakSimple will load each data file in the list into channel 1, perform the specified functions, and then increment to the next data file in the list.

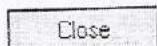
The **Start at** dialogue box specifies which control file number to begin operation at first. If no number is entered the autosampler will begin at the first control file. The **Stop at** dialogue box specifies the last control file to be run before operations of the autosampler cease. If no number is entered in the dialogue box the autosampler will end after the last control file in the list is run.

The **Delay "x" minutes** radio button when selected specifies how many minutes PeakSimple will wait before running the next control file in the list box. The **Wait for remote start** radio button when selected instructs the autosampler to wait for a remote start signal before advancing to the next control file.

The **Restart at end** checkbox restarts the queue after getting to the end of the control files in the list box. In the **"x" times** the user enters the number of times the control files in the list box should be cycled if the Restart at end checkbox is selected. If the value 0 is selected the queue will be cycled continuously.

Close

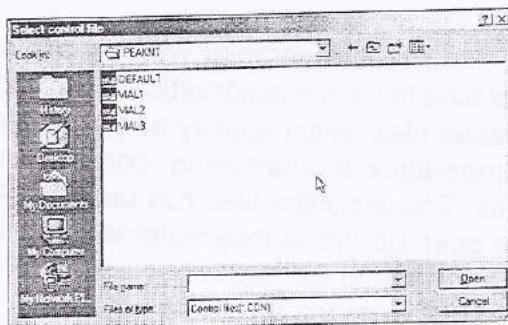
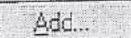
The Close button closes the Autosampler window when it is selected.



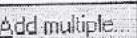
The View-Autosampler Window (cont.)

Add

Select the Add button to add a control file to the queue. Selecting the button opens up the Select control file window where the file can be loaded into the list box. Each control file in the queue must have a different name even though almost identical actions are performed.

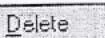


Add Multiple/Batch Reprocessing



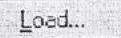
The Add multiple button allows the user to load multiple data files into the list box. Click on the button to open up the Select control file window and then click on a control file name to open up the Select data filenames window. Select as many data files as needed by pressing the shift button and clicking with the mouse cursor and then click on OK to load them into the queue. The Add multiple button is only useful for use with the Batch reprocessing mode.

Delete



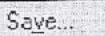
After highlighting a control file in the list box to the left select the Delete button to remove that control file from the queue.

Load



Select the Load button to open up a previously saved queue file. Clicking on the Load button opens up the Load autosampler queue window where the queue file can be selected and loaded.

Save

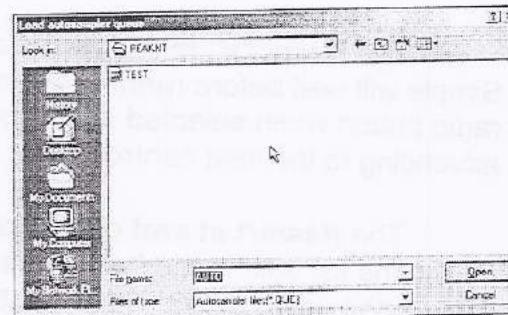
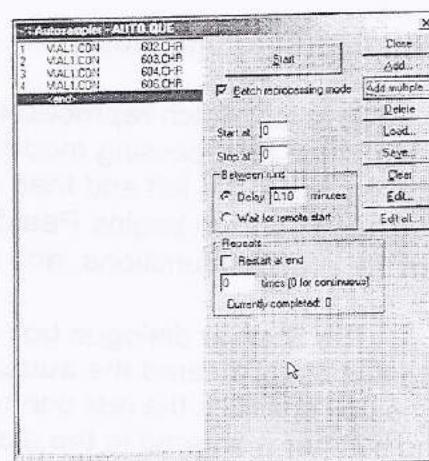
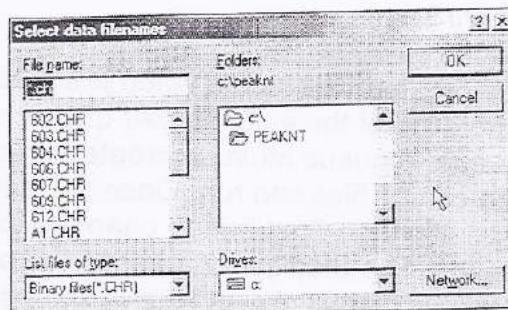


Selecting the Save button opens up the Save autosampler queue window. Save the queue in the file box by naming the file and selecting save. It is recommended that all files be saved to the Peak-Simple directory.

Clear



The Clear button erases the entire queue.



The View-Autosampler Window (cont.)

Edit

After highlighting a control file select the Edit button to modify that control file. Selecting the Edit button loads the control file on the PeakSimple main screen. To make any changes click on the main screen, do all modifications, and then select Save all from the PeakSimple file menu.



Edit All



To edit all the control files in the queue at once click on the Edit all button to open up the Autosampler queue spreadsheet. Many of the commonly adjusted control file parameters are displayed in the spreadsheet enabling the user to input changes to the queue. Not all control file parameters can be modified using Edit all (only the parameters that are selected in Format) and so must be done individually with the Edit function.

Autosampler Queue Window

Close



The Close button exits the window after prompting the user to save the spreadsheet.

Cancel



The Cancel button exits the spreadsheet window without prompting the user to save.

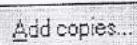
Num	Control file	Data file	Sample	Print
1	VIAL1.CON	high1.chr	VIAL1	0
2	VIAL1.CON	high1.chr	VIAL1	0
3	VIAL1.CON	high1.chr	VIAL1	0
4	VIAL1.CON	high1.chr	VIAL1	0
5	VIAL1.CON	high1.chr	VIAL1	0
6	VIAL1.CON	high1.chr	VIAL1	0
7	VIAL1.CON	high1.chr	VIAL1	0

Add

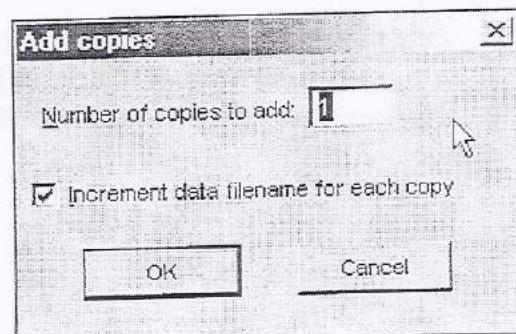


Selecting the Add button opens up the Select control file window where an existing control file can be added to the queue.

Add Copies



After highlighting a control file in the spreadsheet select the Add copies button to add copies of the file to the list. Once the Add copies window pops up input the number of copies to be made in the dialogue box and specify whether the file names should be incremented. The Add copies button is useful for creating a queue from scratch with a single control file.



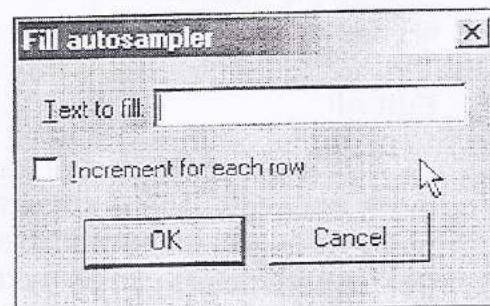
Autosampler Queue Window (cont.)

Delete

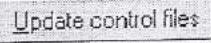
The Delete button deletes a highlighted control file off the list. If no file is highlighted then the last file will be deleted from the queue.

Fill

The Fill button fills a spreadsheet column, row, or cell with selected text. Once the desired cells are highlighted clicking the Fill button opens up the Fill autosampler options box. Input the text to fill in the information field and specify whether the text should be incremented for each row.



Update Control Files



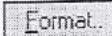
Selecting the Update control files button saves all changes to the control files in the list.

Print

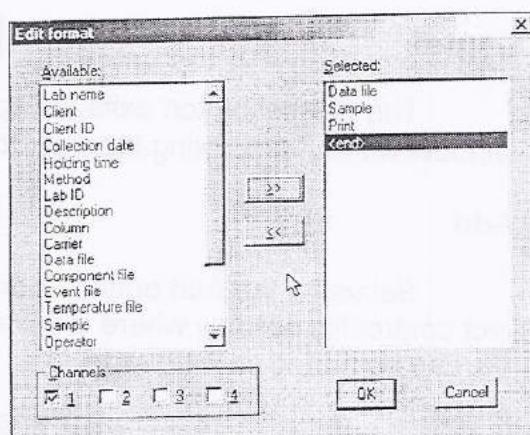


The Print button prints the queue spreadsheet.

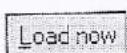
Format



To change the format of the queue spreadsheet and open up the Edit format window select the Format button. In the Edit format window a format type can be added by selecting it in the Available window and then hitting the right facing arrow button. To remove a format type from being displayed in the spreadsheet highlight the format type in the Selected box and click on the left facing arrow.



Load Now



After highlighting a control file select the Load now button to load that control file to the main PeakSimple screen. Click on the screen and make any changes to the control file and then select Save all to save the changes.

The View-Channel "X" Options

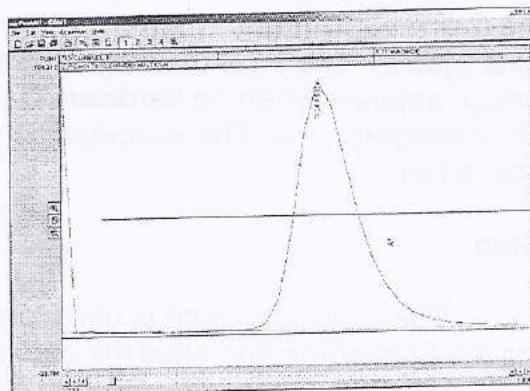
1 2 3 4

To view a specified chromatogram channel open the View menu in the PeakSimple menubar and select a channel to be viewed; either 1, 2, 3, or 4. Keyboard shortcuts can also be used to alternate viewing between chromatogram channels. Hitting F9 displays channel 1, F10 displays channel 2, F11 displays channel 3, and F12 displays channel 4. Furthermore the numerical icons in the PeakSimple toolbar can be used to toggle between chromatogram channels.

Unzoom

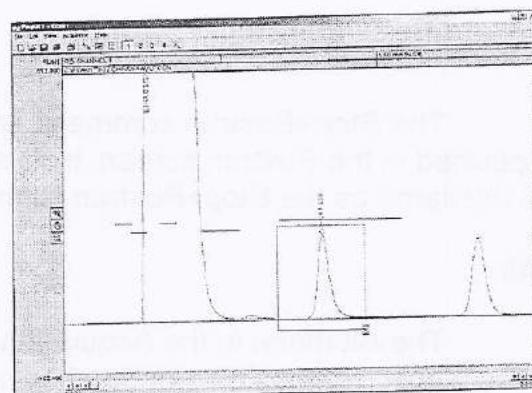


To unzoom from a close up view of a chromatogram select the Unzoom tool from the View menu or hit F6. PeakSimple will zoom out to the first level with the original display units of the chromatogram when the Unzoom tool is used. The Unzoom button in the PeakSimple toolbar can also be used to unzoom a chromatogram or F6 on the keyboard.



Refresh

The Refresh tool in the View menu redraws the chromatogram screen to fix any glitches or resolve an error. Pressing Enter on the keyboard also refreshes the screen.



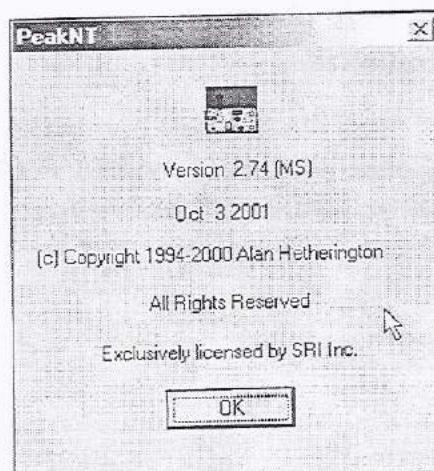
The Help Menu

About PeakNT

To view program information about PeakSimple click on the About PeakNT option in the Help menu. The PeakNT window will pop up and display the information.

Show Tooltips

The Show tooltips option in the Help menu toggles the PeakSimple tooltips off or on. When Show tooltips is checked a helpful text tip will appear when the mouse cursor is held over a tool or button in PeakSimple. The tooltips provide relevant information to the operation and use of the PeakSimple data system.



The Acquisition Menu

The Acquisition menu contains the commands to run a chromatogram run when hardware is connected to the PeakSimple data system. All Acquisition menu commands have corresponding keyboard hotkeys for convenience.

Run

The Run command begins a chromatogram run on the main trigger group when hardware is connected to the data system. The error message "No active channels in group" appears when no hardware is available to make a chromatograph run. The spacebar can also be used to start a run.



Stop

The Stop command is used to end a chromatogram run once it has been started. Using the Stop command ends the chromatogram run without running any of the Postrun operations. The End button can also stop a chromatogram run.

Stop+Postrun

The Stop+Postrun command ends a chromatogram run and executes the operations specified in the Postrun screen. Holding the Control button and pressing End on the keyboard is the same as the Stop+Postrun command.

Alt

The Alt menu in the Acquisition menu controls the acquisition commands for the alternate trigger group. The + button begins the alternate trigger chromatogram run, the - button stops the alternate trigger run, and the / button on the keyboard stops the run and begins the Postrun operations for the alternate trigger group.

Re-initialize

The Re-initialize command reestablishes the connection between the hardware and the PeakSimple data system. A connection between hardware and the data system has to exist for re-initialization to occur.

Loopback Test: For Data Validation

A loopback test may be performed if you are required to validate the precision of the G.C. or Data System's analog to digital conversion. This test requires the user to install a jumper wire on the A/D board inside the G.C. or Data System.

Description of Test:

A jumper wire is installed on the A/D board between 'temperature program one', (TP1), and 'channel one signal input', (Sig. 1+). A data file is then loaded into channel four. When the 'loopback' mode is selected in PeakSimple, the data on channel four is routed out TP1 to the channel one signal input. When a chromatogram run is started, channel one will begin to reproduce the data loaded into channel four. After the run has completed, area counts from a specific data peak may be collected and the run repeated several times. After at least three runs, the user may then calculate the average area counts and the percent relative standard deviation,(%RSD) and thus the precision of the A/D converter. Less than 0.5% RSD is typical for the SRI Model 202 and 203 A/D boards.

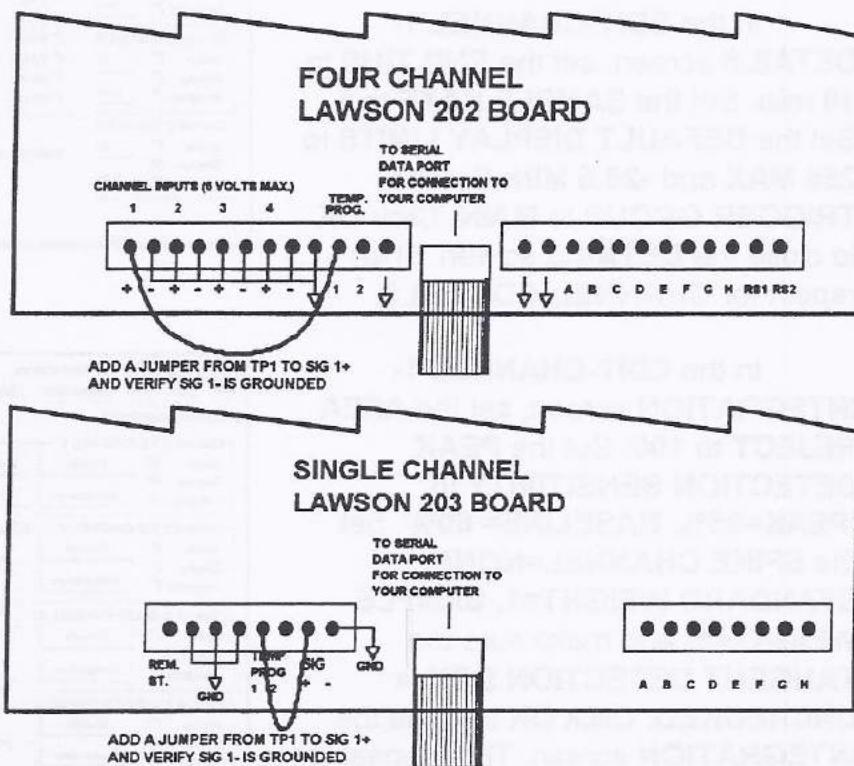
Setting Up The Hardware:

With the G.C. unplugged, remove the six screws securing the bottom cover. Flip the G.C. on its back and locate the A/D board on the right-hand side. Remove any wires from 'TP1' and 'SIG 1+' and add an insulated 22 AWG wire between TP1 and SIG 1+. Refer to the diagram below for jumper placement.

Most systems will contain the Four Channel 202 Board. Also, verify that SIG 1- is grounded. Add another jumper if needed.

Some systems will contain the Single Channel 203 Board. Also, verify that SIG 1- is grounded. Add another jumper if needed.

You could also run the TP1 wire to SIG 1+ through a relay for automatic hardware setup.



Loopback Test: (continued)

Setting Up The Software:

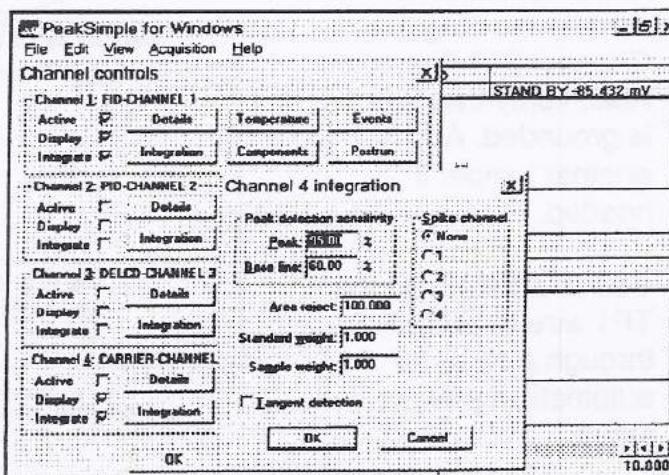
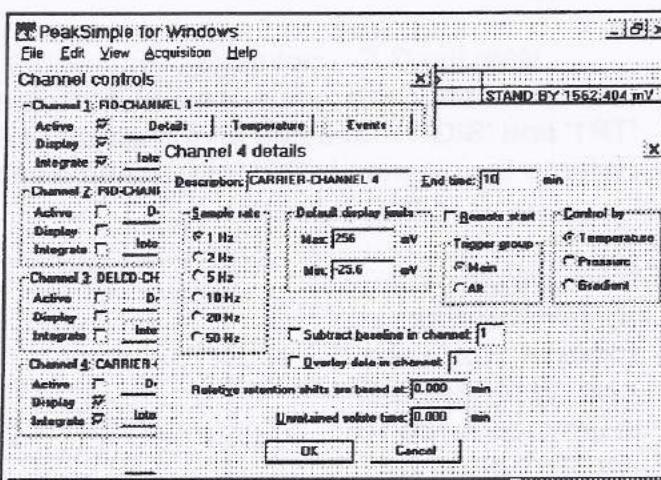
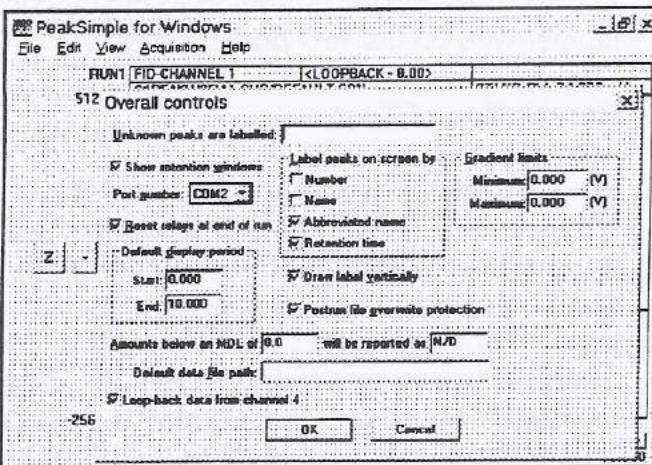
Re-attach the bottom cover and plug the G.C. back in. Turn the G.C. power on and start PeakSimple. Verify that the computer is communicating properly with the G.C..

In the EDIT-OVERALL screen, check the LOOPBACK box. Set the START TIME to 0 minutes and the END TIME to 10 minutes. Also verify that the SHOW RETENTION WINDOWS box is checked.

In the EDIT-CHANNELS screen, check the ACTIVE, DISPLAY and INTEGRATE boxes for channel 1. And check the DISPLAY and INTEGRATE boxes for channel 4.

In the EDIT-CHANNEL 1- DETAILS screen, set the END TIME to 10 min. Set the SAMPLE RATE to 1. Set the DEFAULT DISPLAY LIMITS to 256 MAX and -25.6 MIN. Set the TRIGGER GROUP to MAIN. Click OK to close the DETAILS screen. Then repeat for CHANNEL 4-DETAILS.

In the EDIT-CHANNEL 1- INTEGRATION screen, set the AREA REJECT to 100. Set the PEAK DETECTION SENSITIVITY to 'PEAK=95%, BASELINE= 60%'. Set the SPIKE CHANNEL=NONE, STANDARD WEIGHT=1, SAMPLE WEIGHT=1, and make sure the TANGENT DETECTION BOX is UNCHECKED. Click OK to close the INTEGRATION screen. Then repeat for CHANNEL 4-INTEGRATION.



Loopback Test: (continued)

Software Setup: (continued)

In the **EDIT-CHANNEL 1-COMPONENTS-LOAD** screen, highlight the **602.cpt** sample components file and click **OPEN**. Click **OK** again to close the **COMPONENTS** screen. Then repeat for **CHANNEL 4-COMPONENTS**.

In the **FILE-OPEN** screen, select **CHANNEL 4** at the bottom of the window and then highlight the **602.chr** sample chromatogram file and select **OPEN**.

The **602.chr** sample chromatogram that is now displayed on channel four represents the data that will be fed back through the A/D converter.

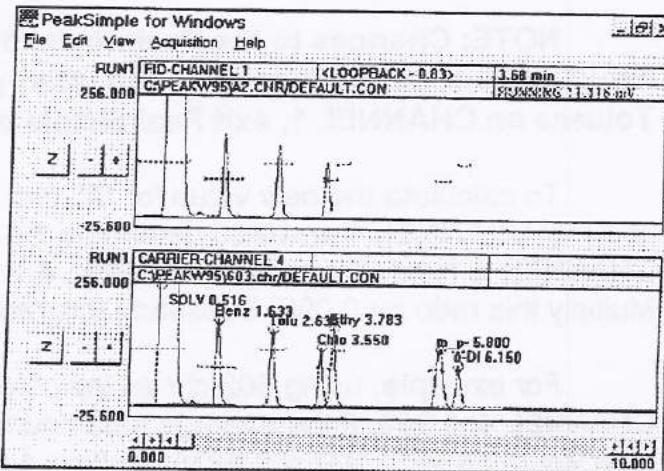
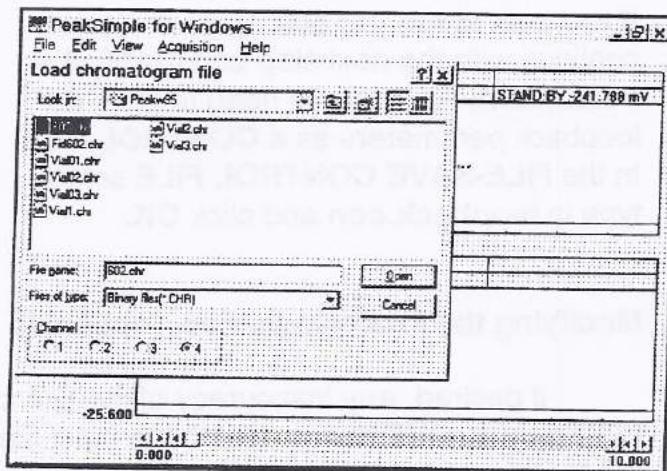
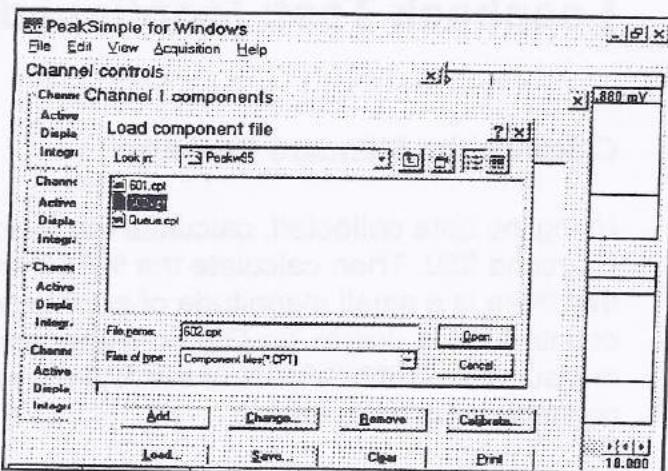
Starting the Run:

Auto-zero channel 1 by clicking the '**Z**' button. Depress the **SPACEBAR** and the chromatogram will start running. The data on **CHANNEL 1** should appear to be an exact replica of the data that was fed into **CHANNEL 4**.

Collecting the Data:

After the run has completed, make note of the area counts of one of the peaks by left-clicking on one of the peaks. Toluene, for example, may have an area count of 931.

Repeat the run three or more times; for each run, record the area counts of the same peak. Once the data has been collected from at least three runs; an average area count can be calculated as well as the percent relative standard deviation.



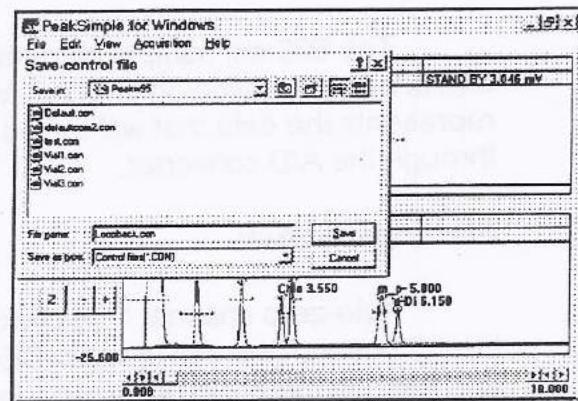
Loopback Test: (continued)

Calculate the Standard Deviation:

Using the data collected, calculate the average area counts for Toluene. Typically this value is around 950. Then calculate the %RSD which is usually less than 0.5%. You may notice that there is a small magnitude of error between the **CHANNEL 1** and **CHANNEL 4** area counts. This is due to the D/A converter and not the A/D converter. Since the loopback test measures the **PRECISION** of the A/D converter and not its **ACCURACY**, this minor discrepancy is insignificant.

Save Your Loopback Test as a CONTROL FILE:

If you wish to run this test again or if you continue with the next step and modify the **Peakwin.ini** file, you will need to save the loopback parameters as a **CONTROL FILE**. In the **FILE-SAVE CONTROL FILE** screen, type in **loopback.con** and click **OK**.



Modifying the Peakwin.ini File, (optional)

If desired, any inaccuracy of the D/A converter can be adjusted by attenuating the **LOOPBACK OUTPUT** to match the input signal. This adjustment can be made by entering the line "**LoopbackFactor=X**" in the [Lawson] section of the **PEAKWIN.INI** file located in the **WINDOWS** directory. The default value of '**X**' is **0.098**.

NOTE: Changes to the Peakwin.ini file will not be recognized unless the PeakSimple application is restarted. After you have obtained the average area count for Toluene on CHANNEL 1, exit PeakSimple by pressing 'Q', then 'Y'.

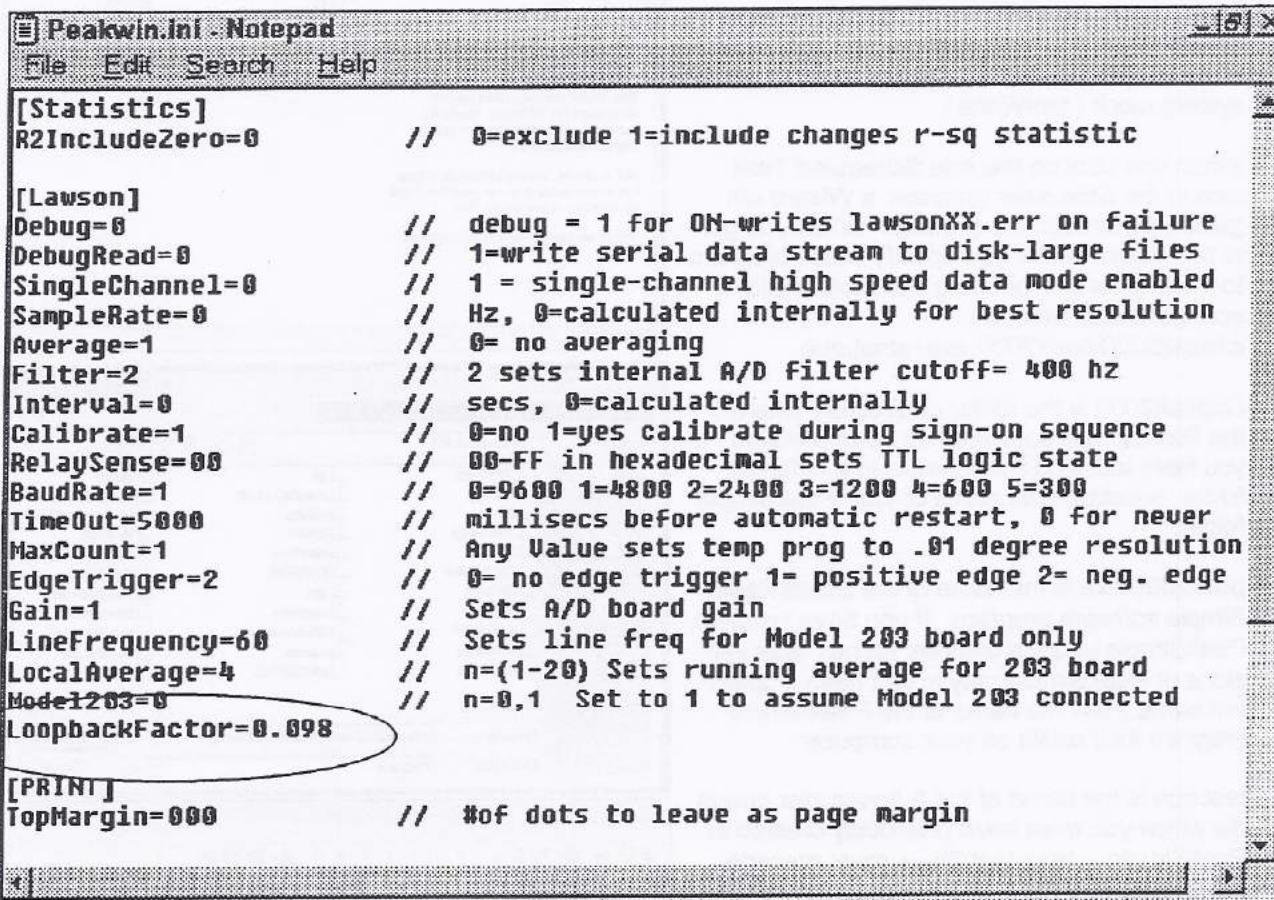
To calculate the new value for '**X**', first determine the average area counts of a specific peak for **CHANNEL 1** and also determine the area count of the corresponding peak on **CHANNEL 4**. Next, divide the **CHANNEL 4** area count by the **CHANNEL 1** area count. Multiply this ratio by 0.098. Substitute this new value for '**X**'.

For example, using **602.chr** as the chromatogram file, the toluene area count for **CHANNEL 4** is **953**. If the average area count of toluene on **CHANNEL 1** is **931** then the ratio would be **953 / 931 = 1.0236**. Multiply **1.0236 x 0.098**, the answer is **0.1003128**. Round this new value for '**X**' to **0.1003**.

Loopback Test: (continued)

Modifying the Peakwin.ini File, (continued)

Find the PEAKWIN.INI file in the WINDOWS sub-directory. Double-click to open it. Scroll down until you find the [Lawson] section. Place the cursor at the last line of the [Lawson] section and type "LoopbackFactor=X", and then press ENTER. X is the value you calculated earlier. For example "LoopbackFactor=0.1003".



```
[Peakwin.ini - Notepad]
File Edit Search Help

[Statistics]
R2IncludeZero=0      // 0=exclude 1=include changes r-sq statistic

[Lawson]
Debug=0              // debug = 1 for ON-writes lawsonXX.err on failure
DebugRead=0           // 1=write serial data stream to disk-large files
SingleChannel=0       // 1 = single-channel high speed data mode enabled
SampleRate=0          // Hz, 0=calculated internally for best resolution
Average=1             // 0= no averaging
Filter=2              // 2 sets internal A/D filter cutoff= 400 hz
Interval=0            // secs, 0=calculated internally
Calibrate=1           // 0=no 1=yes calibrate during sign-on sequence
RelaySense=00          // 00-FF in hexadecimal sets TTL logic state
BaudRate=1             // 0=9600 1=4800 2=2400 3=1200 4=600 5=300
TimeOut=5000           // millisecs before automatic restart, 0 for never
MaxCount=1             // Any Value sets temp prog to .01 degree resolution
EdgeTrigger=2           // 0= no edge trigger 1= positive edge 2= neg. edge
Gain=1                // Sets A/D board gain
LineFrequency=60         // Sets line freq for Model 203 board only
LocalAverage=4           // n=(1-20) Sets running average for 203 board
Model203=0              // n=0,1 Set to 1 to assume Model 203 connected
LoopbackFactor=0.098

[PRINT]
TopMargin=000           // #of dots to leave as page margin
```

Press ALT-F then S to save the file. Press ALT-F then X to exit. Restart PeakSimple and load the loopback.con control file you saved earlier. Run the loopback test again. The accuracy of the D/A converter should be improved. (Channel 1 toluene area counts should closely match the channel 4 toluene area counts).

End of Test:

Turn off G.C. power and re-connect the original A/D board wiring. The loopback test is completed.

Chapter: PeakSimple

Topic: Using the Windows Scheduler program to trigger PeakSimple's Autosampler queue

The Windows Task Scheduler program is supplied with the Windows operating system. It is found under Programs/Accessories/System Tools. The Scheduler allows you to trigger a PeakSimple Autosampler Queue or a specific control file at a scheduled time and date, or on a regular repeating basis using the computer's system clock (time/date).

When you click on the Add Scheduled Task icon in the Scheduler program, a Wizard will guide you through the process. When you get to the screen where you specify which program to start, enter the following line modified for your particular situation:

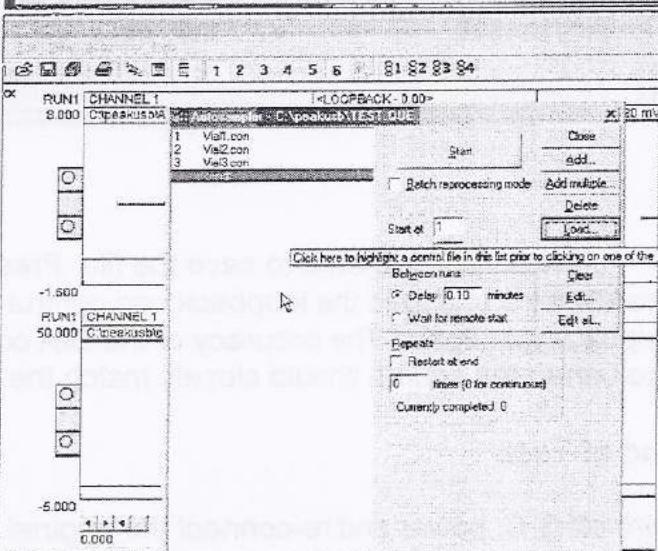
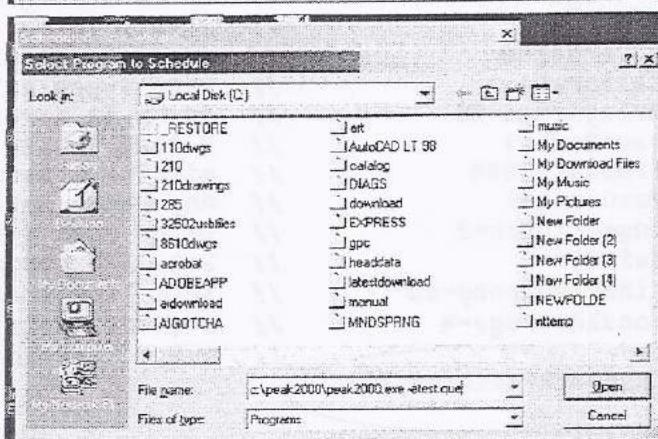
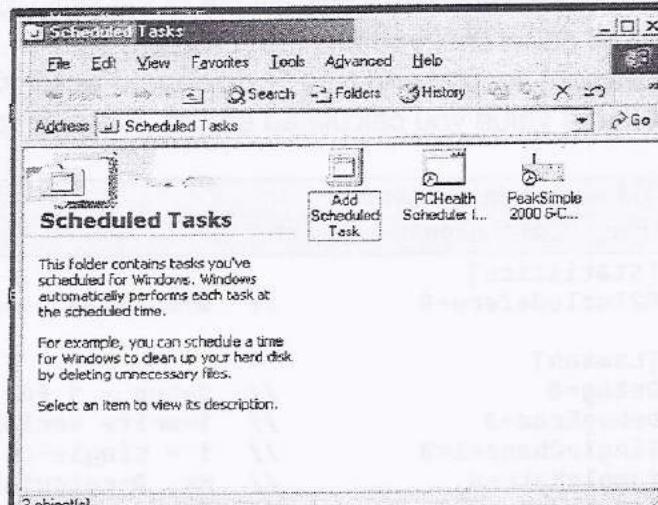
c:\peak2000\peak2000.exe -atest.que

C:\peak2000 is the folder or directory where the PeakSimple software has been installed. If you have installed PeakSimple in a different folder, substitute the name of your PeakSimple folder.

peak2000.exe is the name of the actual PeakSimple software program. If you have installed PeakSimple under a different name (later versions of PeakSimple may in fact have a different name) use the name of the PeakSimple program as it exists on your computer.

test.que is the name of the Autosampler queue file which you must have previously created in PeakSimple. Note that the -a must precede the name of the .que file. When you create the .que file in PeakSimple you can save the que under any name you want. The -a is a Windows programming convention and must precede the name of the que file you want to run.

When the Scheduler starts PeakSimple, the specified queue will begin. At the end of the queue, PeakSimple will wait for the delay time specified in the queue, and then PeakSimple will Close automatically.



GPC From PeakSimple Data Acquisition

Introduction

The following is an outline of how PeakSimple data acquisition software/hardware can be used to acquire and analyze (in conjunction with an appropriate spreadsheet) gel permeation chromatography data. At this time, two different version of PeakSimple software were required for successful analysis. Version 2.08 was used to collect the data and obtain result tables for narrow polymer standard chromatograms, while version 2.09 was used to obtain the peak slice information for broad unknown polymers. That is, using 2.09, the voltage difference between the detector output and the subsequently drawn baseline was obtained for each data point and saved as an ASCII file, which was then imported into Excel for in-depth GPC analysis. Ultimately, it would be preferred to use only one version of PeakSimple. However, 2.09 (the latest version) was not stable while acquiring data. The program would crash after approximately 5 minutes. Furthermore, the time display in the upper right hand corner did not appear to work and retention windows were not visible on the screen although a component file was active. Thus, 2.09 was used only for obtaining slice information with non-active channels.

To illustrate how PeakSimple can be used for GPC analysis, I have included 3 narrow polystyrene standard chromatograms (4 standards per chromatogram) and two broad unknown polymer chromatograms. Chromatograms were obtained using a Waters 510 pump (U6K injector), an ethyl acetate mobile phase (1 mL/min), a series of Ultrastyrogel® columns (Waters 10⁶, 10⁴ and 500 Å) and Waters 2410 refractive index detector. All polymers were pre-dissolved in ethyl acetate and chromatograms were collected at 1 Hz. Polystyrene standard concentrations were 0.1 % w/w or less (50 µL injection volume) while broad unknown polymers were approximately 1 % w/w (75 µL injection volume). Also included are component files, containing the standard identities and expected retention windows, an event file for integration, and two ASCII data files containing slice information for the broad unknown polymers, and an Excel file with in-depth GPC analysis.

Obtaining a Calibration Curve

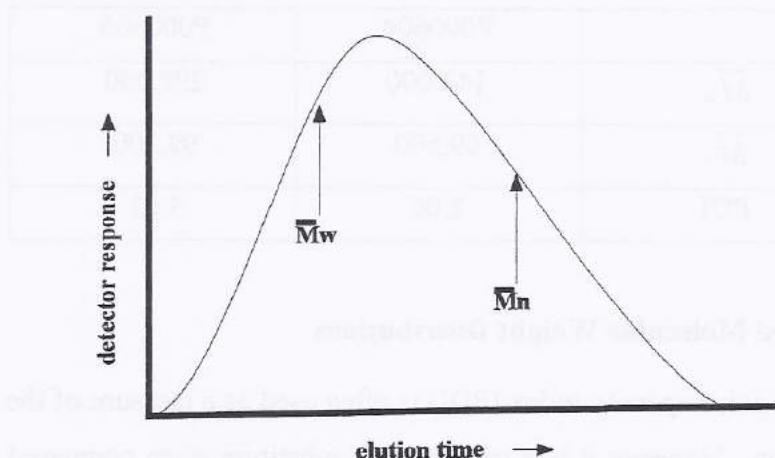
Polydisperse polymers in solution are fractionated according to size or hydrodynamic volume during GPC, which is also known as size exclusion chromatography. Molecular weight is related to the hydrodynamic volume. In GPC a dilute polymer solution is injected into a solvent stream which then flows through a series of columns packed with porous gel beads. Smaller molecules pass through and around the beads while larger molecules are excluded from all but the largest pores. Thus fractionation occurs with the largest molecules eluting first. The molecular weight of an eluting polymer molecule varies exponentially with eluting volume, the latter of which is proportional to time under constant flow rate conditions. To obtain molecular weight data and convert the GPC chromatogram into a molecular weight distribution, the relation between molecular weight and elution time is obtained from a series of polymer standards of known molecular weight. The calibration curve is thus obtained from a plot of the logarithm of molecular weight versus time. Given that GPC is a comparison of hydrodynamic volumes, unknown molecular weight determinations will be relative to the calibration standards. For a good introductory reference to polymer science, see R. J. Young and P. A. Lovell, Introduction to Polymers.

Using PeakSimple 2.08, the result table for each of the three polystyrene standard chromatograms was copied using DDE into Excel. The natural logarithm of molecular weight versus time was plotted and a best fit analytical approximation to the curve was obtained from a third order polynomial, $P(t_e)$. This is the calibration curve relating molecular weight to elution time.

Obtaining Molecular Weight Averages

The most common and convenient way to characterize a distribution of molecular weights making up a polymer sample is using molecular weight averages such as, number average molecular weight (\bar{M}_n), and weight average molecular weight (\bar{M}_w), as shown in the following figure for a typical polymer chromatogram. \bar{M}_n is defined as a sum of products of the molecular weight of each fraction multiplied by its mole fraction. That is: $\bar{M}_n = \sum X_i M_i$ where X_i is the mole fraction of molecules of molecular weight mass M_i . The weight average molar mass is

defined as a sum of the products of the molecular weight of each fraction multiplied by its weight fraction, w_i . That is: $\overline{M}_w = \sum w_i M_i$. Additionally, it can be shown that the number average molecular weight, in terms of weight fraction, is equal to: $\overline{M}_n = 1/\sum(w_i/M_i)$. The ratio $\overline{M}_w/\overline{M}_n$ is known as the polydispersity or polydispersity index (PDI). The PDI is often used as a measure of the breadth of the molecular weight distribution. Polymers that are monodisperse (i.e. all chains have the same molecular weight) would have a PDI of 1.



A typical polydisperse polymer molecular weight distribution showing the approximate locations of \overline{M}_n and \overline{M}_w .

Using PeakSimple 2.09, polymers p000604 and p000606 were integrated (using the GPC event file) and the results saved in ASCII files. The ASCII files were imported into Excel and the corresponding sample times were added as a third column of data starting at time equal to zero. Only slice and time data corresponding to the major peak of interest were retained (columns A,B and J,K respectively). For each time slice, a corresponding molecular weight, M_i , was calculated using the analytical equation fitted to the calibration curve (columns C and L, respectively). Note that extrapolation of a few minutes outside of the last standard ($MW = 1,000,000$) is usually not a problem. Furthermore, the refractive index response of the detector is proportional to the weight concentration of eluting polymer, independent of molecular weight. Thus, the weight fraction, w_i , of polymer in any slice is equal to the detector voltage response or height (baseline subtracted) divided by the sum of detector voltage responses for each polymer

elution slice (i.e. $w_i = \text{height}_i / \sum \text{height}_i$, columns D and M respectively). \overline{M}_w was obtained by multiplying w_i and M_i and summing the appropriate columns (see bottom of columns E and N) $1/\overline{M}_n$ was obtained by dividing each w_i by M_i and summing the appropriate columns (see bottom of columns F and O). Thus, the molecular weight averages for the two polymers were obtained and are summarized in the following table.

Polymer Molecular Weight Averages

	P000604	P000606
\overline{M}_w	143,000	299,000
\overline{M}_n	69,500	99,300
PDI	2.06	3.01

Obtaining Normalized Molecular Weight Distributions

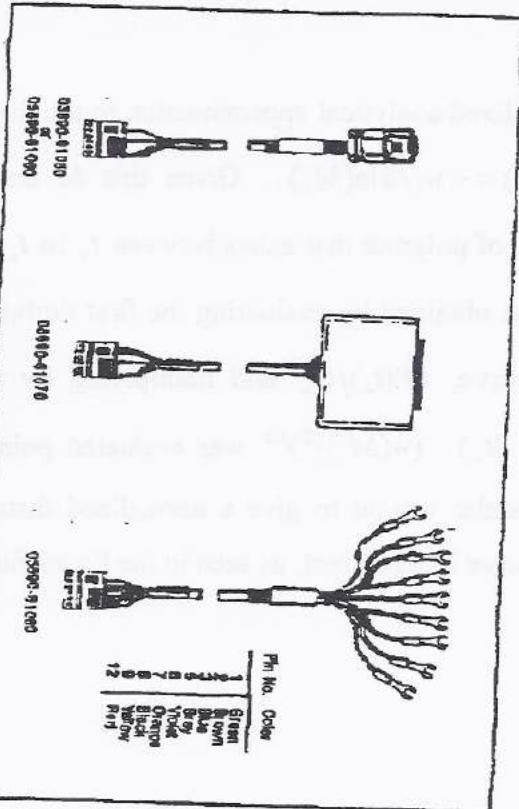
As mentioned the polydispersity index (PDI) is often used as a measure of the breadth of the molar mass distribution. However it is often a poor substitute when compared to a graphical representation of the complete molecular weight distribution curve, especially when comparing polymer distributions. To a first approximation, the raw chromatogram (a graph of detector response, $f(t_e)$, versus elution time, t_e) is a graphical representation of the distribution. However, the chromatogram height is injection concentration dependent, making comparisons difficult, and t_e is often non-linear with $\ln(M)$, as evidenced by a third order calibration curve.

A normalized molecular weight distribution function is given by $w(M) = -dw/d\ln(M)$. Conversion of $f(t_e)$ versus t_e to a normalized molecular weight distribution plot (i.e. $w(M)$ versus M or $\ln(M)$), is obtained by considering that the weight fraction, dw , of polymer which elutes between t_e and $t_e + dt_e$ is given by: $dw = f(t_e)dt_e / \int_0^\infty f(t_e)dt_e$ where the integral in the denominator is simply the area under the chromatogram. Thus, an analytical approximation of dw at the i^{th} slice is w_i , the weight fraction of polymer

A normalized analytical approximation to the distribution function, $w(M_i)$, is thus obtained from: $w(M_i) = -w_i/d\ln(M_i)$. Given that M decreases as t_e increases, the same weight fraction, dw , of polymer that exists between t_e to $t_e + dt_e$ also exists between $\ln(M) - d\ln(M)$. $d\ln(M_i)$ was obtained by evaluating the first derivative of the analytical equation fitted to the calibration curve, $dP(t_e)/dt_e$ and multiplying by the time interval (i.e. the 1 Hz sampling frequency $\sim dt_e$). $(w(M_i))^2)^{1/2}$ was evaluated point by point (columns H and Q) and plotted against molecular weight to give a normalized distribution that is injection concentration and calibration curve independent, as seen in the Excel file.

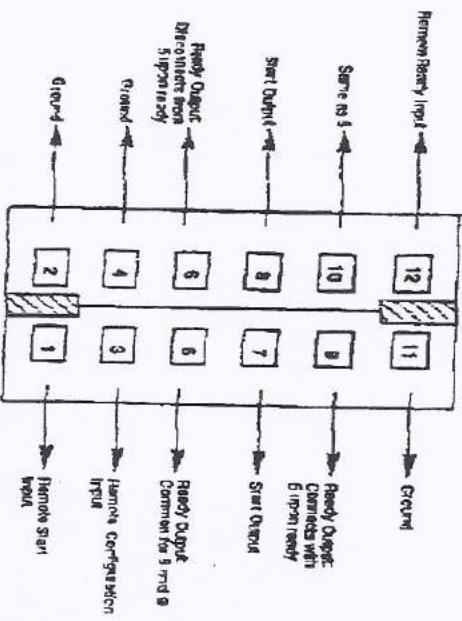
REMOTE RECEPTACLE

The REMOTE receptacle provides a function used primarily to start an Integrator or data system when an HP 5890 run begins, and also provides ready information.



Remote Start/HP 5890 Ready Cables

VIEW FROM THE TOP AND FRONT OF THE HP 5890
Available Functions, Remote Receptacle J2



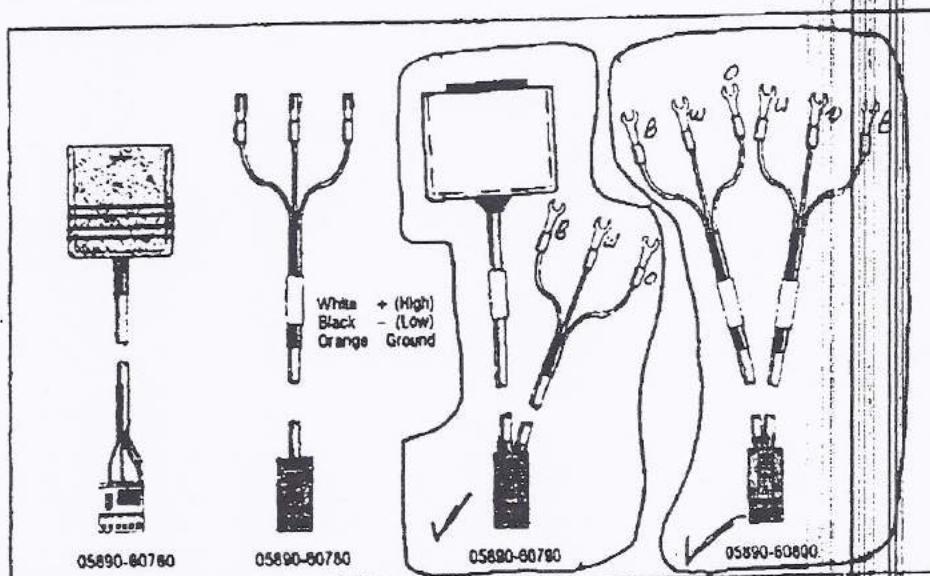
REMOTE RECEPTACLE

The 12-pin REMOTE receptacle provides a variety of functions, depending upon connections made via the cable. The figure below and the table following identify the function at each pin.

Available Remote Start/HP 5890 Ready Cables		
Part No.	Use	Length
03394-000600	HP 3394/338A Integrator/Controller	2 m
05890-010000	HP 3392A Integrator/Controller	2 m
05890-010500	HP 3380A Integrator	2 m
05890-010700	HP 3380 Series LAG	2 m
05890-010800	General Purpose	2 m

Analog Signal Output Cables

The following figure and table show cables available to connect an HP 5890 ANALOG output channel (variable DC signals, +1 V or +1 mV maximum) to a recorder, integrator, and/or A/D converter for a computer system. If a second output channel is installed, a second cable is also required.



Available Analog Signal Output Cables

Note that the general purpose and HP 3350 Series LAS analog output cable assemblies consist of two independent cables, terminating together at a common, single, female plug at the HP 5890. One cable is labelled 1 mV, the other +1 V output. In general, the +1 V cable is connected to an integrating device or A/D converter, and the 1 mV cable is connected to a chart recording device.

311

Installation 2-20