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INSTRUCTION MANUAL FOR BAS EPSILON LC SYSTEMS

VERSION 2.50

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INTRODUCTION



Welcome to the BAS electronic manual for the epsilon LCEC detector and associated hardware. You have purchased a fundamentally new and exciting LC instrument incorporating the latest advances in hardware and software. These features include:

- Optically isolated circuitry for noise reduction
- 100 pA to 5 mA gain ranges
- 24-bit data resolution
- Up to 4-channel EC capability
- Provision for inputs from two external LC detectors
- Pulsed amperometric detection (PAD)
- Remote control of BAS pumps
- Upgradable to perform EC techniques such as cyclic voltammetry
- Upgradable over the Internet
- Built-in data processing
- Both analog and digital filtering for maximum noise reduction

We designed the epsilon platform with the Internet in mind. epsilon-based instruments have their own web site (http://www.epsilon-web.com) where users can:

- Upgrade their instrument via the Internet
- Monitor their instrument from remote locations via the Internet
- Access operation and application information via the Internet

For information on our full line of applications, products and services, visit the main BAS web site (http://www.bioanalytical.com).

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HOW TO CONTACT BAS

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Address	Bioanalytical Systems Inc. 2701 Kent Avenue West Lafayette IN 47906 USA	BAS Instruments, Ltd. Building 28 Stoneleigh Deer Park Stareton, Kenilworth, Warwickshire CV8 2LQ U.K.
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Service Coordinator	× 807	+44 2476 639 574
Fax	(765) 497-1102	+44 2476 639 568

Internet

epsilon web site
main BAS web site
maintenance and repair web site

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FREQUENTLY ASKED QUESTIONS

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- What can I do about pump noise?
- Why did my peaks get smaller?
- Why did my pressure go up?
- How to I run an electronic self-test?

GENERAL

What is an electrochemical (EC) detector? An EC detector for a liquid chromatograph detects analytes based on their electrochemical properties. The detector either removes an electron from (oxidation), or donates one to (reduction) the analyte molecule . The amount of current generated by the redox reaction is proportional to the amount of analyte present. See PRINCIPLES OF EC DETECTION for a detailed explanation.

Is an EC detector useful for all analytes? No. Many analytes are not easily oxidized or reduced. An EC detector is very good at what it does, but it is by no means a universal detector.

How do I know which analytes can be determined by LCEC? Published literature is a good source of information about routine analytes. BAS Application Capsules have specific information for determining many analytes. Electrochemical data for many other compounds are presented here. Finally, you may contract with BAS to determine the electrochemical properties of any molecule of interest.

How can I learn more about EC detection?Read our PRINCIPLES OF EC DETECTION tutorial.

INSTALLATION

How do I hook up this thing? It would be best to follow the detailed INSTALLATION instructions. Briefly, the epsilon detector is connected to the computer's serial port by a standard RS-232 cable. The cell leads plug into the back of the epsilon detector, and to the flowcell at the other end. The flowcell connections are coded by size, gender, and color, so there should be no confusion there. Finally, the flowcell is plumbed into the LC flow stream with standard 1/16"

fingertight connectors.

Do I need to use your software? I have my own data-analysis software. You can use your own software for data collection and analysis. However, you will have to use BAS Control software to turn on and set the parameters of the epsilon detector. Instructions can be found here.

ELECTRODES

What is a working electrode? A working electrode is a piece of conducting material (glassy carbon, gold, platinum, etc.) embedded in a plastic block. It is positioned so the eluent from the column will flow across its surface. Electrical connections on the back side allow the electronics to apply a potential and measure the resulting current.

When should I polish the working electrode? Usually only when your analyte peaks have gotten smaller and you suspect that the surface of the electrode is coated with something nasty.

How do I polish the working electrode? The simplest way is to wipe it hard with a laboratory tissue wetted with methanol. If that doesn't help, mild abrasives and pads are provided in the polishing kit.

How do I store the working electrode? When not in use the working electrode should be cleaned with methanol and stored in its box.

What is a reference electrode? A reference electrode is an electrochemical device that produces a known (reference) potential. Its functioning is critical because the potential applied to the working electrode is based on the potential of the reference electrode. A BAS reference electrode is a tube with a porous frit at one end, containing a silver-chloride-coated silver wire in a sodium-chloride gel solution.

How do I store the reference electrode? Because the reference electrode has a porous frit at one end, it is subject to drying out. To prevent this, carefully remove the rubbery shipping coat from the end of the electrode and store it with its tip immersed in 3M sodium chloride.

When should I replace the reference electrode? We recommend getting a fresh set of three every three months. A weak reference electrode will result in decreased peak height.

What is an auxiliary electrode? The auxiliary electrode sits across the mobile-phase stream from the working electrode. It serves to complete the electrical circuit.

SETTINGS

What is potential? Potential is the voltage applied to the working electrode. Think of it as the force required to drive the

oxidation or reduction of the analyte molecule.

What potential should I use? In general you should use as high a potential as you need to oxidize or reduce your analyte. But the higher the potential you use, the higher will be your background, and the more extraneous compounds will be detected. BAS Application Capsules detail the required potentials for many analytes. Electrochemical data for many other compounds are presented here. Finally, you may contract with BAS to determine the electrochemical properties of any molecule of interest.

What is range? Range is the amplification of the detector, expressed as the amount of current produced by a peak. For example, a peak that generates 100 nanoamperes of current would be half scale on a range of 200 nAfs (nanoamperes full scale). And a range of 20 nAfs is ten times more sensitive than one of 200 nAfs.

What range should I use? Use a range that will keep your largest peaks on scale. When in doubt, use a less-sensitive range, because the data-analysis system can easily resolve small peaks. A peak that goes off scale, on the other hand, cannot be quantitated.

What is filter? A filter is an electronic way of filtering out unwanted noise in detector output. Most detectors have built in filtering, sometimes called rise time. BAS filters are calibrated in Hertz (Hz). Large Hz values give less filtering than small values.

What filter should I use? For challenging determinations, use the filter that gives you the best signal-to-noise ratio. This must be determined by trial and error. For routine determinations, 0.1 Hz works well.

What is background? Background is the electrochemical activity of your system when analytes are not passing through the detector. It has contributions from the mobile phase, contaminants, electrodes, etc. High backgrounds contribute to baseline noise.

How do I offset (zero) background? Use the zeroing functions of the epsilon detector. When not doing programmed runs, the detector can be zeroed through a button in the Detector Status box. During programmed operation you can still use the zero button, or you can insert automatic rezeroes in the EC Detector Schedule.

DATA ANALYSIS

How do I import information about the samples?You need to enter this information in a special file called a RAN file, then tell ChromGraph Control to use this file.

What are all these files on my disk? There are a lot of files associated with instrument control, data acquisition, data processing, and results presentation. Here's a list of the files you may encounter:

FILES CREATED OR USED BY CHROMGRAPH CONTROL

Extension	Name	Properties

.mth	Method	Instrument control and data-collection options
.mth	Backup	Auto-backup of main Method
.dat	Data	Raw data and comments
.ini	Initialization	Configuration (Control.ini)
.log	Log	Log of injection times, run numbers, etc.
.lok	Backup Log	Log file cleared and copied here when it gets > 100 KB
.baq	Queue	Advanced programming for on-the-fly changes in Methods
.ran	Ran	Import sample info and save with data file

FILES CREATED OR USED BY CHROMGRAPH REPORT

Extension	Name	Properties
.met	Method	Instructions to identify and quantify peaks, print reports
.dat	Data	Raw data (generated by Control, used by Report)
.ini	Initialization	Configuration (Report.ini)
.bln	Baseline	Graphical representation of each run
.rep	Report	Text file of results (peaks, retention times, areas, etc.)
.sta	Standards	Info about standards for quantitation of unknowns
.prn	PRN	Processed data for exporting to spreadsheets
.csv	CSV	Processed data for exporting to spreadsheets
.tab	TAB	Processed data for exporting to spreadsheets

How can I make the graph from my UV detector read in AU? ChromGraph can receive range settings from the on-board EC detectors, but not from external detectors such as UV/Vis and Fluorescence detectors. This has no effect on quantitation, but graphs look better when the units are correct for the detector used. ChromGraph allows you to enter the relevant detector settings so the graph is correct.

How do peak heights and areas relate to amperes and coulombs? Peak heights are reported in counts, a digitized representation of detector response. Peak areas are counts summed over the width of the peak. Most people do not need to know about converting to amperes and coulombs, but if you do, the formulas are here.

MAINTENANCE

What periodic maintenance is required? You should clean the fan filter every three months. The reference electrodes should be stored in fresh 3M NaCl and a fresh one switched with the one in the instrument occasionally (whenever convenient).

What's the best way to shut down the system? You should flush out the mobile phase (which can be corrosive, and will leave abrasive salt deposits as it evaporates), remove and cap the column, disassemble the flowcell and store the electrodes. Details in the SHUTDOWN section.

TROUBLESHOOTING

How do I reduce a high background? In LCEC, cleanliness is important. Details here.

What can I do about pump noise? See the extensive troubleshooting sections on baseline noise.

Why did my peaks get smaller? There are many possibilities. Did you make a dilution error? Are your standards fresh? Is the working electrode coated with nasty stuff? Is the reference electrode weak? Is the injector bad? Is the column losing efficiency? See the TROUBLESHOOTING section.

Why did my pressure go up? Has the temperature gone down? Did you change to a higher viscosity solvent such as methanol? If neither of these, there's likely a partial clog somewhere. Disconnect things one at a time, starting at the column and working back to the pump. In-line filters are a good possibility, as this is what they're supposed to do. See the TROUBLESHOOTING section.

How do I run an electronic self-test? This section pertains to the epsilon electrochemical detector only. The epsilon contains two built-in resistors that can be substituted for the flowcell, thus providing a known input for testing the electronic circuits. See the SELF-TEST section.

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PRINCIPLES OF ELECTROCHEMICAL DETECTION

It is certainly possible to get acceptable results from your epsilon system without a firm grasp of electrochemical fundamentals. But those who wish to get the most out of their system, to routinely work at the limit of detection, to handle new problems with assurance, will benefit from an understanding of the underlying principles covered in these chapters.

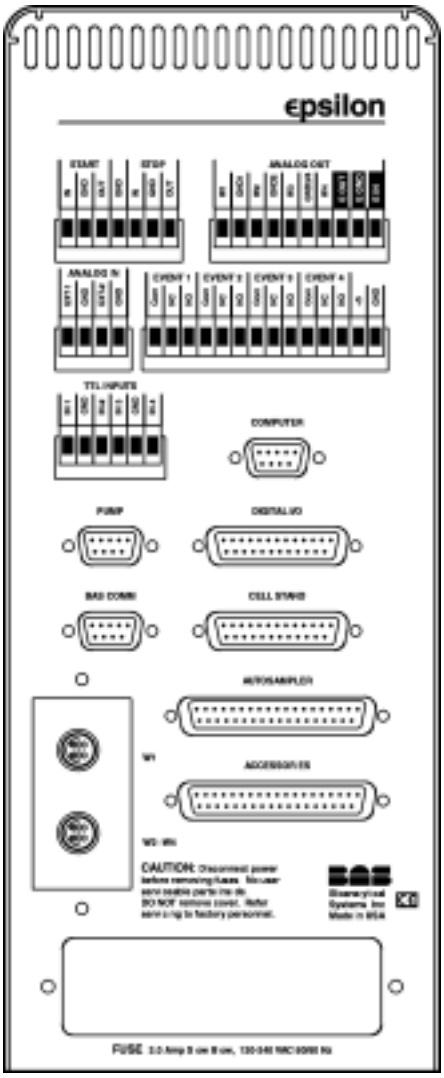
- **BASIC PRINCIPLES**
- **DETECTOR ELECTRODES**
- **TEMPERATURE-STABILIZED OPERATION**
- **OXIDATIVE-MODE LCEC**
- **REDUCTIVE-MODE LCEC**
- **ELECTROCHEMICAL CHARACTERISTICS OF SELECTED MOLECULES**

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HARDWARE INSTALLATION

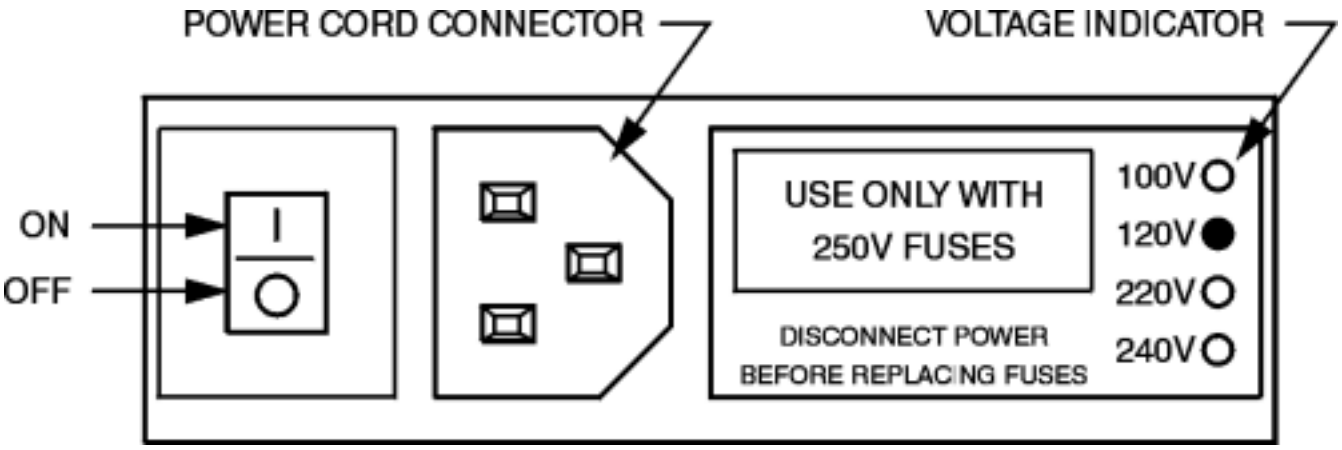
All cable connections to the epsilon system are made at the back panel:



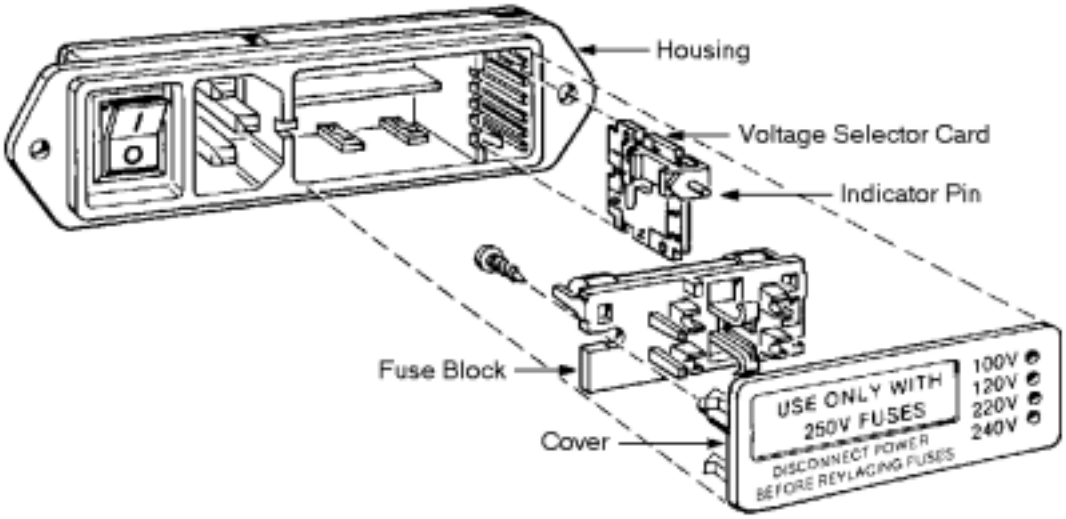
- **POWER**
- **COMPUTER**
- **ELECTROCHEMICAL DETECTORS**
- **EXTERNAL DETECTORS**
- **PUMP**
- **AUTOSAMPLER**
- **ANALOG OUTPUTS**
- **EXTERNAL REZERO**
- **REMOTE START/STOP**
- **TIMED EVENTS**

POWER

The epsilon system requires a 3-prong grounded power supply, providing either 120 or 240 V at 50/60 Hz. Before connecting the power cord, check that the indicator next to the power connection shows the correct voltage.



If the indicator does not show the correct voltage, change it as follows: Pry open the cover to the right of the connector and pull out the voltage selector card:



Orient the card so that the label for either 120 V or 240 V (do NOT use 100 V or 230 V) gets inserted first, with the writing facing towards your left. Slide the plastic indicator around so it nestles in the correct slot opposite the indicated voltage, then reinsert the circuit board. Install the cover and make sure that the correct voltage is indicated.

COMPUTER

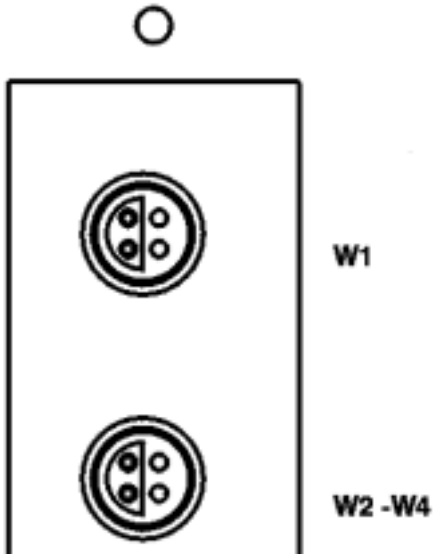
The epsilon system requires at least a 166 MHz Pentium computer with 32 MB RAM. Connect a standard 9-pin, RS-232

serial cable (BAS Communications, P/N ER-9534) between any COMM port on the computer and the COMPUTER port on the epsilon detector:



ELECTROCHEMICAL DETECTORS

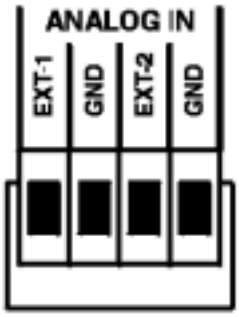
WARNING: NEVER CONNECT THE CELL LEADS WHEN THE EPSILON SYSTEM IS TURNED ON, AND NEVER TOUCH THE EXPOSED PINS OF THE CELL LEADS WHEN THE INSTRUMENT IS ON. DOING SO COULD DAMAGE THE SENSITIVE AMPLIFIERS AND VOID YOUR WARRANTY.



Connect the specialty LEMO connectors of the cell leads here. Note that the W1 cell lead has the smaller connector. If your instrument is multi-channel there also will be a lead for the larger W2-W4 connector. The connectors must be oriented so their half-moon inserts mate with those in the sockets. Connection of the other ends of these leads to the electrodes is described in the Flowcell section.

EXTERNAL DETECTORS

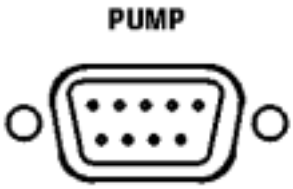
Connect the analog outputs for any external detectors to the ANALOG IN connector:



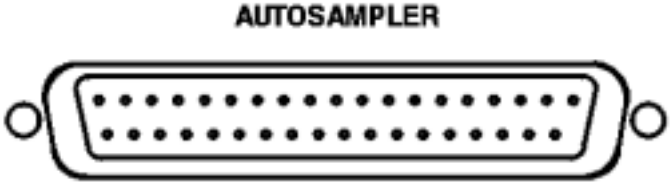
This allows the output of these detectors to be digitized, saved, and analyzed. The output from the external detectors must be either 0.1, 1, or 10 V full scale, and this voltage must be specified in the Detector Channel Options section of the ChromGraph Control Method.

PUMP

The epsilon system can control current model BAS pumps through the PUMP section of ChromGraph Control. However, many users prefer to control the pump independently. If integrated control of the pump is desired, connect a 9-pin (male-male) cable (BAS epsilon/Pump, P/N ER-9548) between any COMM.NET or COMMUNICATIONS NETWORK port on the pump and the PUMP port on the epsilon detector:



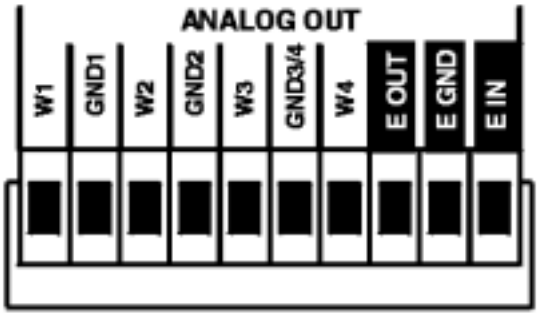
AUTOSAMPLER



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A BAS autosampler can be connected to this 37-pin port. The cable carries both triggering information and vial position, so no other connections are needed. Use cable number EW-4454 for the BAS Sample Sentinel Autosampler. See the section on AUTOSAMPLERS for additional information.

ANALOG OUTPUTS



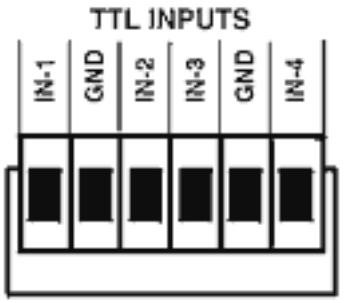
W1-W4. Analog outputs are provided for connection to chart recorders, integrators, or other data-acquisition devices. There are connections for up to four channels of data, corresponding to the active channels configured in the Detector Channel Options section of the Control Method. Only three grounds are provided, so if all four analog outputs (W1-W4) are being used, one of the grounds must be used for two of the outputs.

The analog outputs are all 1 Volt full scale. Thus, when connecting to a chart recorder or other device where the input voltage is selectable, specify a 1 V input. For an integrator where attenuation must be set, use a value of 10 (corresponds to 1.024 V). Connect the W1-4 terminals to the 'high' or '+' input of the peripheral device, and the GND terminals to the 'low' or '-' input. Do not use any additional grounding that may be present on the peripheral device.

E_{out} E_{gnd}. (Not for routine use). An output is provided to monitor the potential applied to detector 1 (W1).

E_{in}. Not used.

EXTERNAL REZERO



An external rezero is provided on the TTL-INPUTS connector, for users who wish to collect data with a non-BAS system. A momentary switch closure between IN-4 and GND, or a TTL-low, will rezero the epsilon.

The majority of users will not use this function: if you are using BAS software you should program an internal rezero using the EC SCHEDULE

REMOTE START/STOP



The remote start and stop connections provide several alternatives for sending and receiving signals to and from other instruments. These functions are fixed in time and unmodifiable, as detailed below. For *programmable* triggers to remote instruments, see TIMED EVENTS below.

START IN allows an external device such as a non-BAS autosampler to start data collection. A switch closure or TTL-low across the START IN terminal and its ground will trigger the epsilon system when the appropriate trigger has been selected in Data Acquisition Options.

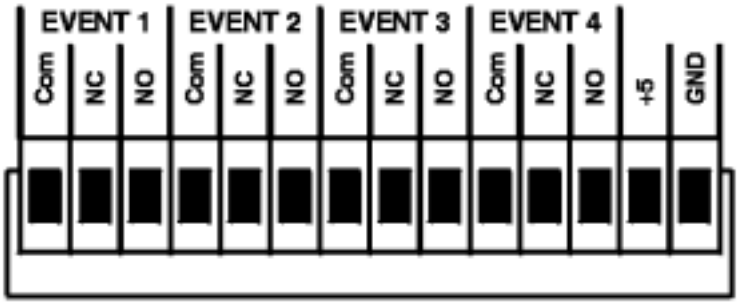
START OUT is used to trigger other instruments, such as an external detector or a non-BAS autosampler, when a chromatographic run begins. It provides a TTL-low when the run starts.

STOP IN allows a remote instrument to stop the chromatographic run. A switch closure or TTL-low across the STOP IN terminal and its ground will end the chromatographic run and save the data collected up to that point.

STOP OUT is used to trigger other instruments, such as an external detector or a non-BAS autosampler, when a chromatographic run ends. It provides a TTL-low at the end of the run.

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TIMED EVENTS



TIMED EVENTS are programmable switch closures that provide exceptional flexibility for controlling peripheral instruments. Four switches are provided, and each can be connected in a normally-open or normally-closed configuration. In most cases use the ground and normally-open terminals for each switch. The switches are programmed in the Switch Schedule section of the ChromGraph Control Method.

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INSTALLATION OF CHROMGRAPH™ SOFTWARE

ChromGraph is a sophisticated set of programs for the control of BAS instruments and the analysis of data generated by Liquid and Gas Chromatographs. The software runs in the Windows environment, thus providing all the flexibility and multitasking inherent in this operating system.

ChromGraph will run on almost any modern computer. The minimum specifications call for a Pentium®-class CPU with a 166 MHz clock speed. There should be at least 32 MB RAM and the operating system should be Windows 95 or most newer version of Windows.

ChromGraph comes as two sister programs, ChromGraph Control and ChromGraph Report. Control sets the instrument parameters and oversees the collection and storage of data. Report processes the data after it has been collected. The two programs can run independently or can be configured so Control automatically passes the data to Report for immediate processing.

If you purchased ChromGraph in a package that included a computer, the software will already be installed. If you are doing your own installation, follow the instructions for installing ChromGraph software and for setting the power-management options of your computer.

- **INSTALLING CHROMGRAPH SOFTWARE**
- **SETTING POWER-MANAGEMENT OPTIONS**

INSTALLING CHROMGRAPH SOFTWARE

1. Double-click on the 'My Computer' icon on the desktop, then double-click on the CD-ROM icon (usually drive 'D'). Double-click on the setup icon for your operating system (either the Windows 9x/ME family, Windows NT4, or the Windows 2000/XP family). **NOTE:** You may have to hold the mouse over the icons to see the full name of the file, which will indicate the operating system, the version of ChromGraph, and the word 'setup'.

2. You should accept all the default installation options as they are presented to you.

3. Shortcuts to the BAS programs and the on-line manual will be installed in the Start Menu, under Programs/ ChromGraph. Icon shortcuts for the programs and the manual will be installed to the Windows desktop.

4. The setup program will ask whether you wish to restart the computer. You need not restart immediately, but must restart before running ChromGraph Control software.

5. If you ever wish to uninstall ChromGraph software, go to the Windows Control Panel (Start, Settings, Control Panel), double-click on the 'Add/Remove Programs' icon, highlight 'ChromGraph' in the list of programs, and press the Add/Remove button.

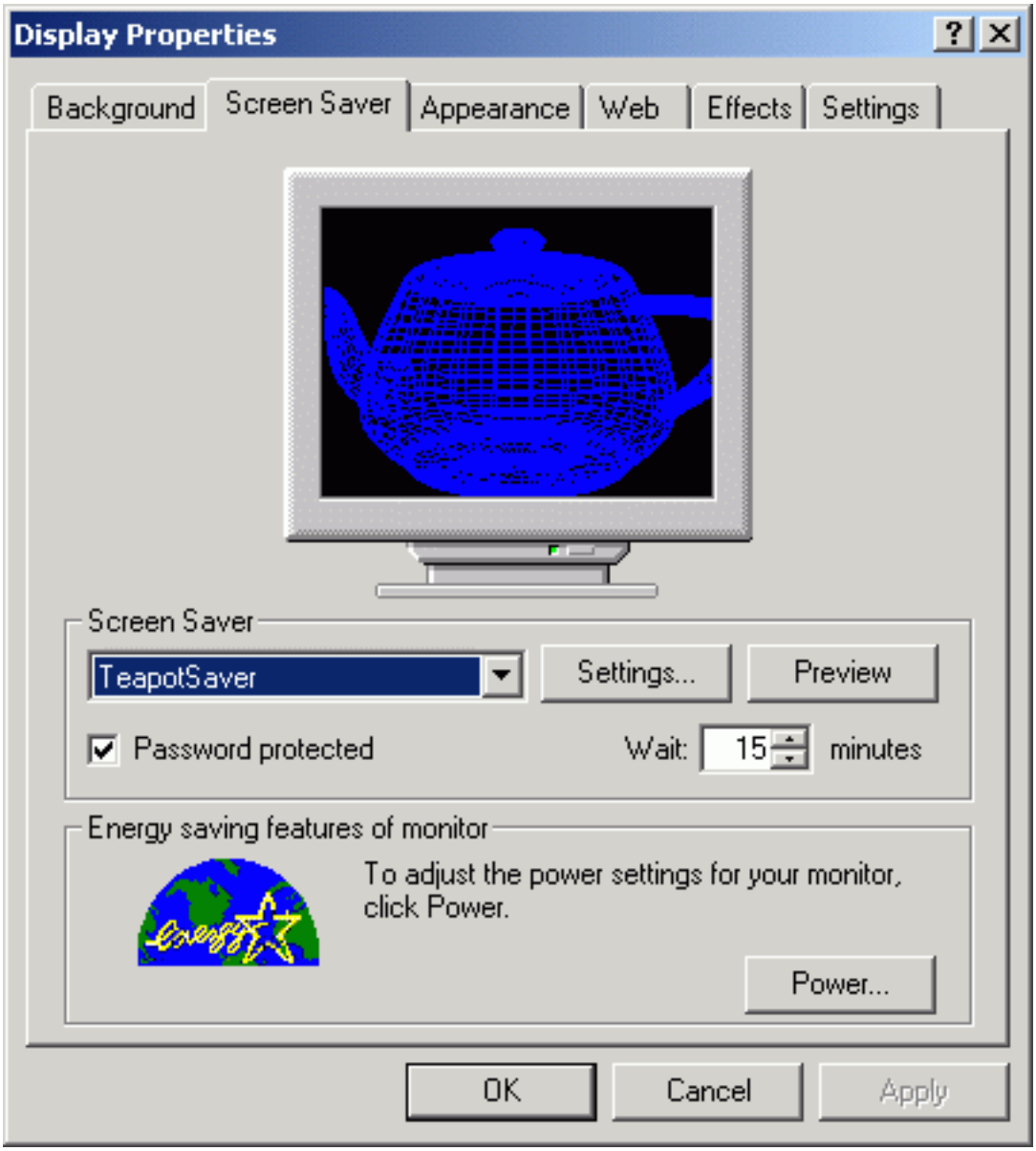
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SETTING POWER-MANAGEMENT OPTIONS

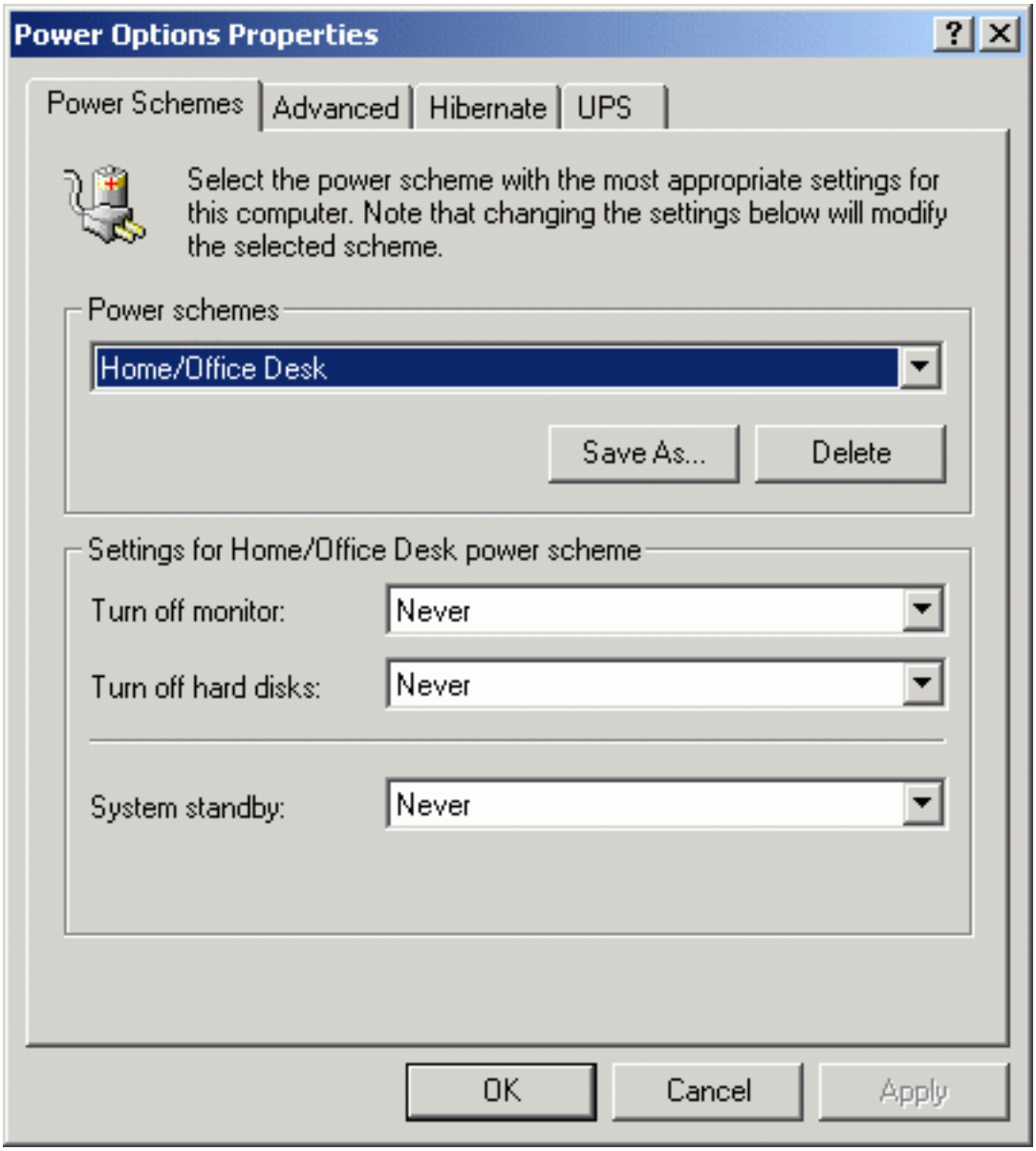
Windows computers usually are set to reduce power to hard drives and monitors after a certain amount of inactivity. This could prevent ChromGraph from saving the chromatography data to disk after long runs, or during overnight operation with an autosampler. We therefore recommend that you turn off any power-saving features of your computer. The procedure that follows is easy to do and works for most computers. In some cases, however, you must disable power-saving features in the computer's BIOS — consult the computer manufacturer if this is the case.

1. Right-click on the desktop and choose 'Properties'.

2. Click on the 'Screen Saver' tab.



3. Click on the 'Power' button.



4. Change each setting to 'Never'.

5. Use the 'Save As' button to save this scheme under the default name.

6. Close all the screens with the 'OK' button.

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- PLUMBING CONNECTIONS
- ELECTRICAL CONNECTIONS

FLOWCELL TYPES

BAS makes an assortment of flowcell types for the epsilon platform. You may choose from single, dual, or quad working electrodes with radial or cross-flow patterns. The main types are shown in the following diagram. When reading the diagram, start at the top with a choice of flow pattern, then read down to see the possible combinations of auxiliary electrode, gasket, and working electrode that correspond to that flow type.

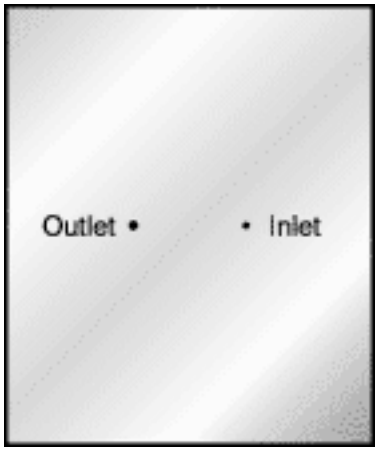
Flow Pattern



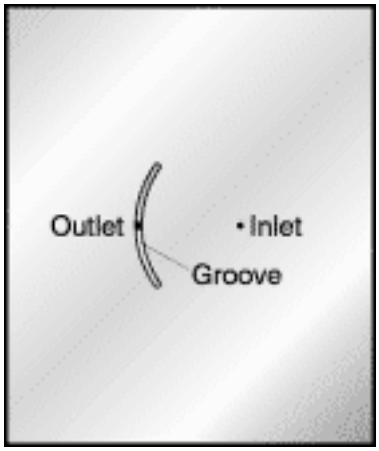
Cross Arc Radial

Auxiliary Electrode Blocks

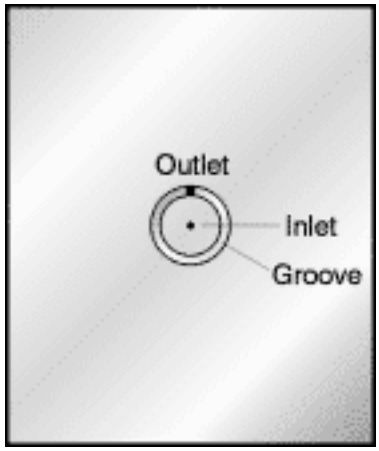
(Includes phenolic base, arms, and reference retainer.)



MF-1093

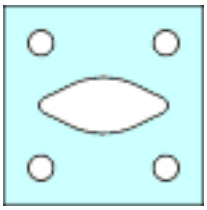


MF-1087

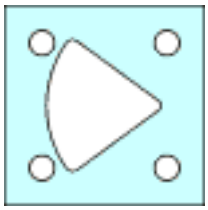


MF-1091

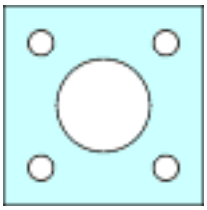
Cell Gaskets



0.5mil MF-1044
2.0mil MF-1046
5.0mil MF-1047

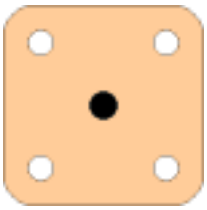


0.5mil MF-1067
2.0mil MF-1066



0.5mil MF-1068
2.0mil MF-1069

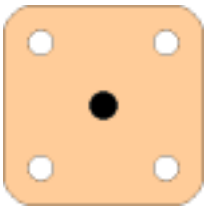
Working Electrode Blocks



MF-1095



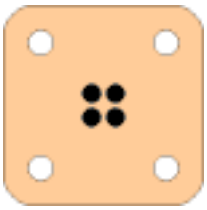
MF-1086



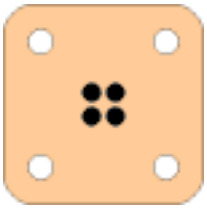
MF-1095



MF-1000



MF-1085

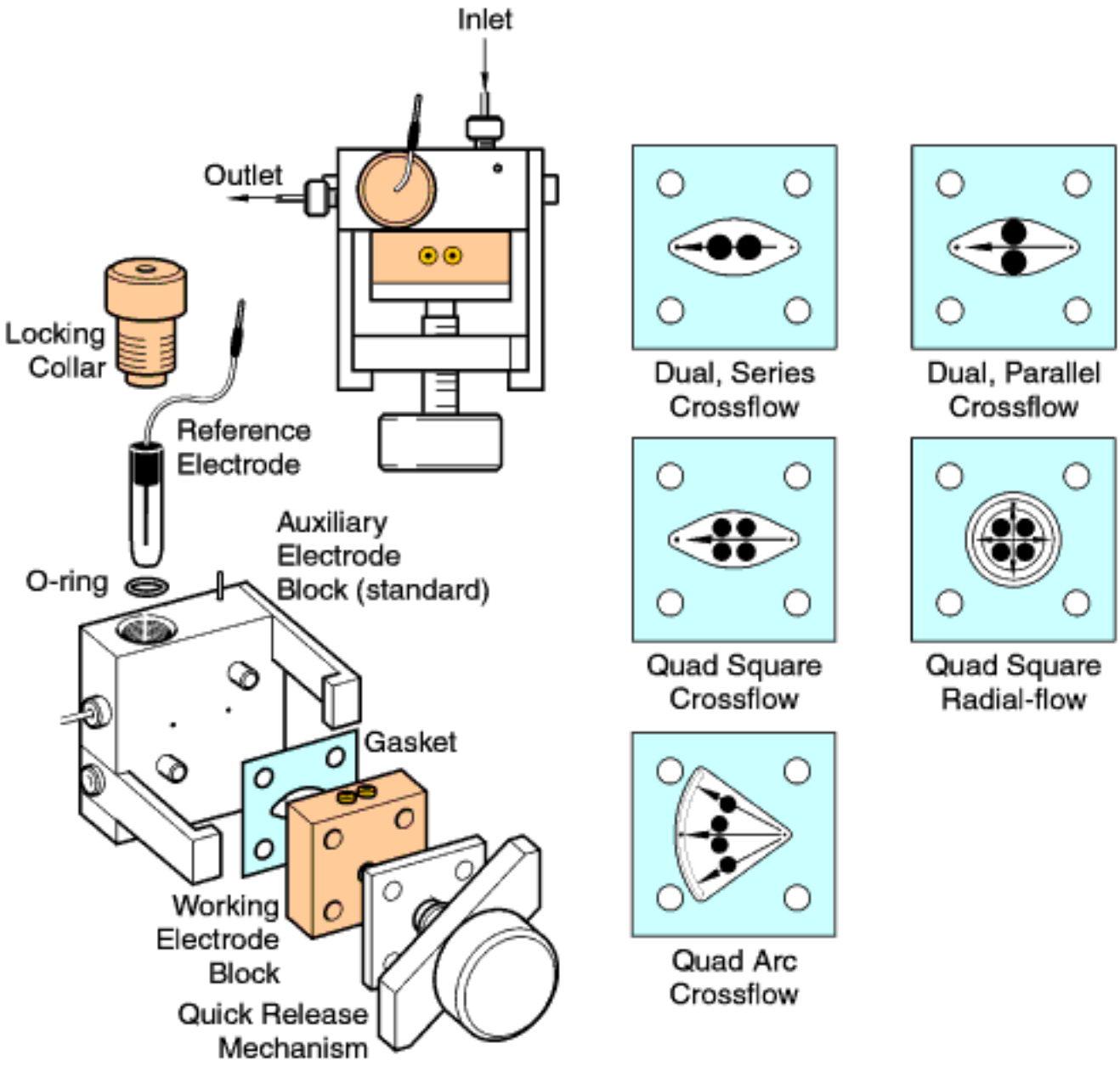


MF-1085

These part numbers are for glassy carbon working electrodes. Working electrodes also can be made from carbon paste, copper, gold, platinum, and silver. Many of these are available off-the-shelf, while others can be custom fabricated.

PLUMBING CONNECTIONS

Please refer to this diagram when assembling the flowcell. Bear in mind that your flowcell may differ slightly from the one pictured, depending on what configuration was purchased.



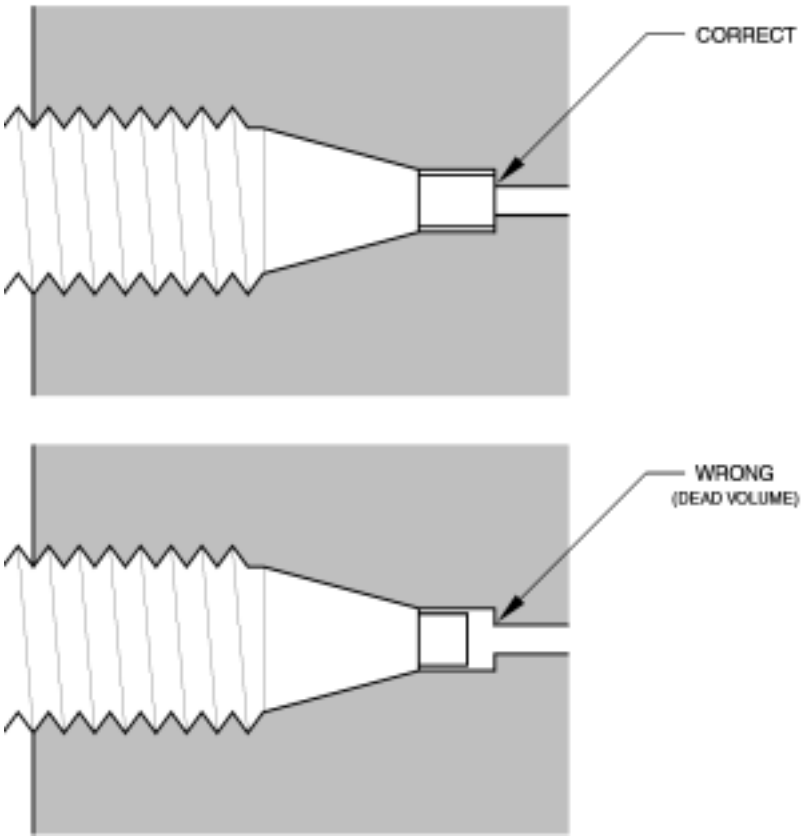
Place the thin-layer gasket over the pins of the auxiliary electrode, taking care that the cutout section matches both the flow pattern inside the cell, and the orientation of the working electrodes.

NONE OF THE TINY FLOW HOLES ON THE SURFACE OF THE AUXILIARY ELECTRODE, AND NONE OF THE ELECTRODE SURFACES ON THE FACE OF THE WORKING ELECTRODE, SHOULD BE COVERED BY THE GASKET.

Place the working electrode over the gasket, with the individual electrodes facing the gasket and the large brown circle of sealant facing out. Put the steel backing plate over the working electrode, taking care that its two pins go into the two empty holes (the other two holes are occupied by the pins of the auxiliary electrode). Attach the quick-release mechanism so its arms engage the arms of the auxiliary electrode. Tighten securely. Do not install the reference electrode at this time.

Before connecting the column to the flowcell, consider the flow direction through the cell. In the flowcell pictured above, in which the reference electrode compartment is offset to one side, that side must be the exit, because if your sample entered this way it would be diluted in the reference compartment. But for some of our other flowcells, in which the reference compartment is centered, and there's no flow through it, flow direction makes no difference.

Connect the column to the inlet of the flowcell using a fingertight plastic connector. Be sure to slide the connector back up the tubing first, so you can completely bottom out the tubing into the socket of the flowcell:



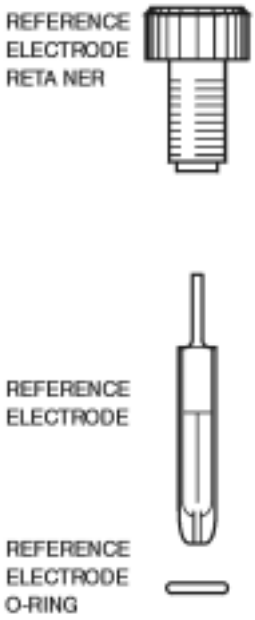
Connect the outlet of the flowcell to a waste line in the same manner.

IMPORTANT! The flowcell must not be grounded to the chassis or your detector will not work. Grounding can occur if steel tubing is used to make the connections to the flowcell. For example, the flowcell can ground to the injector if steel tubing and a metal column are used. (Steel tubing and a plastic column are all right, as are plastic tubing and a metal column.) If you must use steel, you can back the tubing out of the flowcell just enough so no contact is made -- use an ohmeter to check this.

Start the pump and allow mobile phase to flow through the cell and fill the reference electrode compartment. Stop the pump once the fluid has risen above the level of the shelf on which the o-ring will sit (you may have to block the flow of the exit tubing to get the fluid to rise this high). If there are bubbles in the well, remove the liquid with a disposable pipet and refill the well several times.

The reference electrode will be installed next. This closeup shows the reference electrode and its associated parts:

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With the pump stopped, place the o-ring on its shelf. Remove the yellow rubbery coating from the tip of the reference electrode if you haven't already done so (see REFERENCE ELECTRODES.) Then insert the reference electrode into the well at a slight angle, to avoid trapping an air bubble under it. Insert fully, then mop up any liquid above the o-ring or behind the flowcell with a laboratory tissue. Screw down the locking collar finger tight.

ELECTRICAL CONNECTIONS

The three electrodes of the flowcell (working, auxiliary, and reference) are connected to the epsilon controller by the terminal ends of the cell lead(s). The connections are differentiated by color, size, and sex. The other end of the cell lead (s) is connected to the back of the epsilon controller.

WARNING: NEVER CONNECT THE CELL LEADS WHEN THE EPSILON CONTROLLER IS TURNED ON, AND NEVER TOUCH THE EXPOSED PINS OF THE CELL LEADS WHEN THE INSTRUMENT IS ON. DOING SO COULD DAMAGE THE SENSITIVE AMPLIFIERS AND VOID YOUR WARRANTY.

The black connectors with male pins are for the working electrodes. They are labeled W1-W4, and get plugged into the recessed sockets on the edges of the working electrode block. In multi-electrode systems there may be a preferred location for each electrode, so pay careful attention to the application when making these connections.

The red female connector is for the auxiliary electrode. It connects to the gold pin atop the auxiliary block.

The white female connector is for the reference electrode. It connects to the silver pin at the end of the white wire atop the reference electrode.

The grounding lug should be securely bolted to the threaded stud on the inside rear of the flowcell compartment.

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ABOUT CHROMGRAPH CONTROL SOFTWARE

ChromGraph Control is a sophisticated set of programs for automated control of the epsilon chromatography platform and other BAS instruments. The software runs in the Windows environment, thus providing all the flexibility and multitasking inherent in this operating system.

Control provides all the features needed to operate the epsilon system, both in immediate mode and programmed operation. Up to four detectors may be controlled and monitored. Methods are easily chained together and integrated with the BAS Sample Sentinel autosampler. All details of data collection and storage are defined by the software, but actual processing of the data (peak identification, quantitation, etc.) is provided by a sister program, ChromGraph Report.

Report also is a Windows-based program, and it may run concurrently with Control. Control can pass data directly to Report, or it may store the data for later processing. Please refer to the **DATA ANALYSIS** section for a thorough discussion of this advanced data-processing software.

This manual is intended to be a simple introduction to Control. Where there are several paths to accomplish the same task, usually only one will be described. For example, many operations can be carried out either by clicking on a pushbutton with the mouse, or by selecting items from a menu with the keyboard. Only the mouse operations are described, to keep the manual as simple and undaunting as possible. Those who wish to explore other alternatives are free to experiment, using the comprehensive Help screens and a knowledge of Windows.

Both ChromGraph Report and Control run in the Windows operating system. Wherever possible, we have maintained the style of Windows in the look and feel of our software. We use similar drop-down menus, list boxes, dialog boxes, radio buttons, help screens, etc. If you are already familiar with Windows, the transition to ChromGraph software will be smooth. If Windows is new to you, we urge you to learn it first, using the Windows tutorial provided with your operating system.

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STARTING AND EXITING CHROMGRAPH CONTROL SOFTWARE

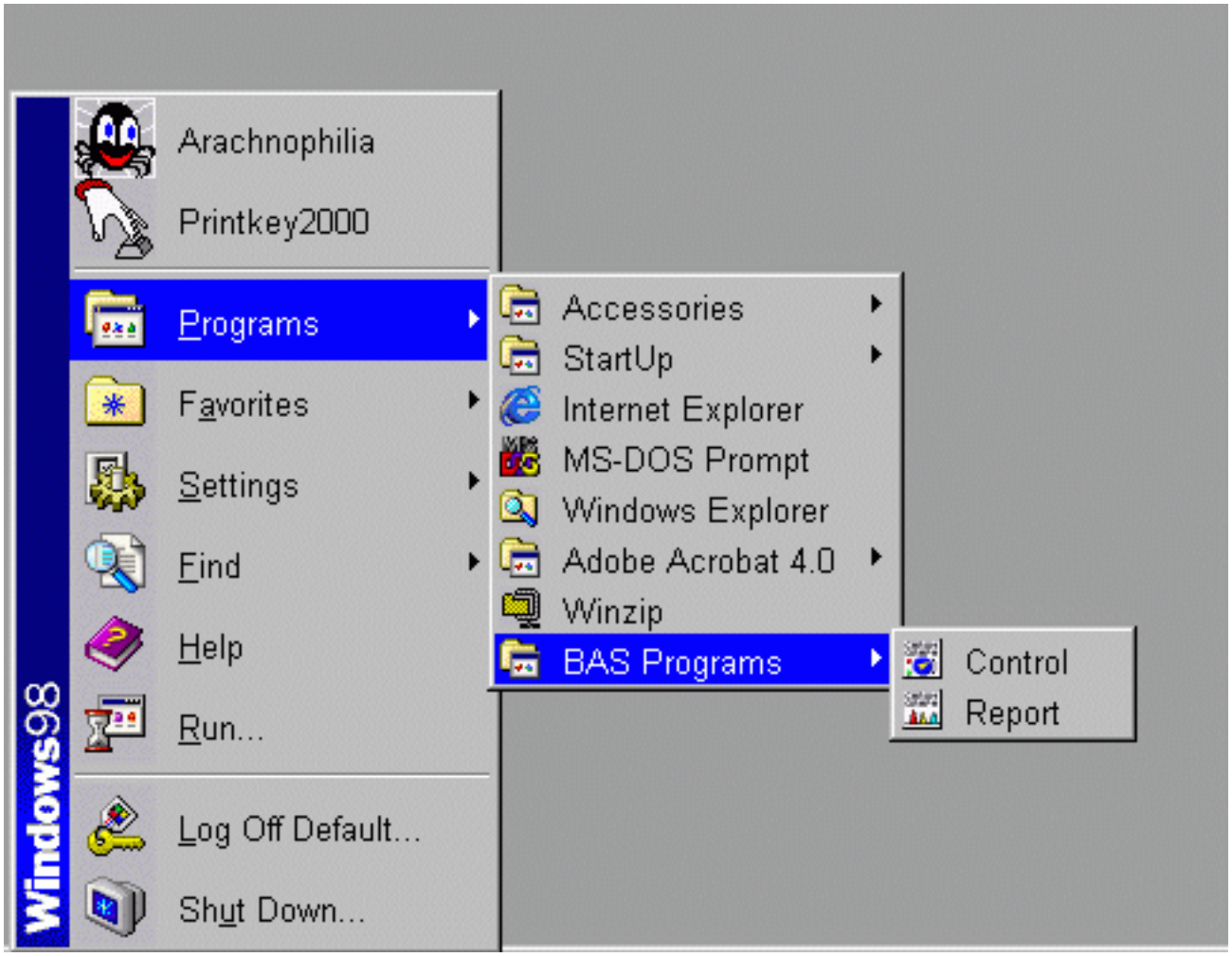
Before starting the system, make sure that your epsilon chromatograph and its software have been installed as specified in the INSTALLATION section. All peripheral equipment such as pump, external detectors, heaters, etc. should be turned on and connected as appropriate. Allow the instruments to complete their self checks before starting ChromGraph Control software.

- **STARTING CONTROL**
- **EQUIPMENT OPTIONS**
- **SETUP OPTIONS**
- **SAVING THE OPTIONS AND EXITING**

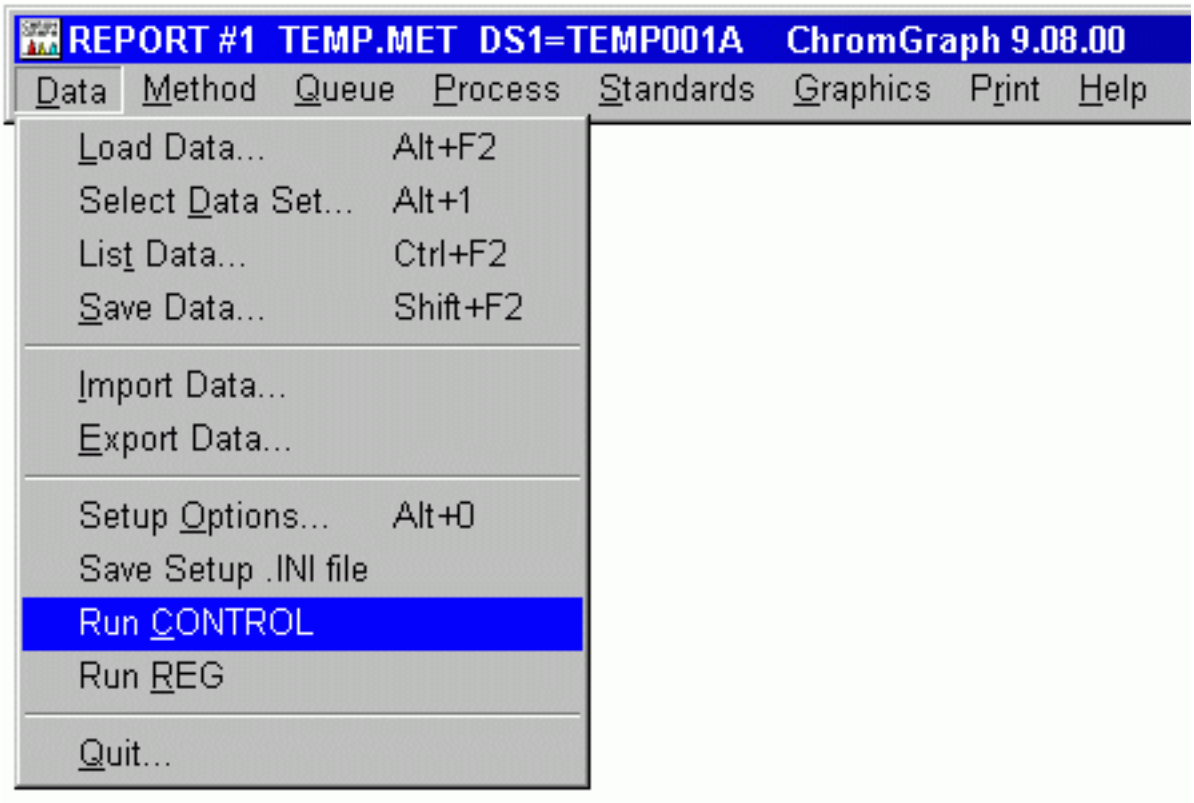
STARTING CONTROL

Start ChromGraph Control with either a desktop shortcut icon or the Windows Start Menu:

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Alternatively, you can start Control (or bring it to the forefront if it was minimized) with the 'Run CONTROL' option in the Data drop-down menu of ChromGraph Report:

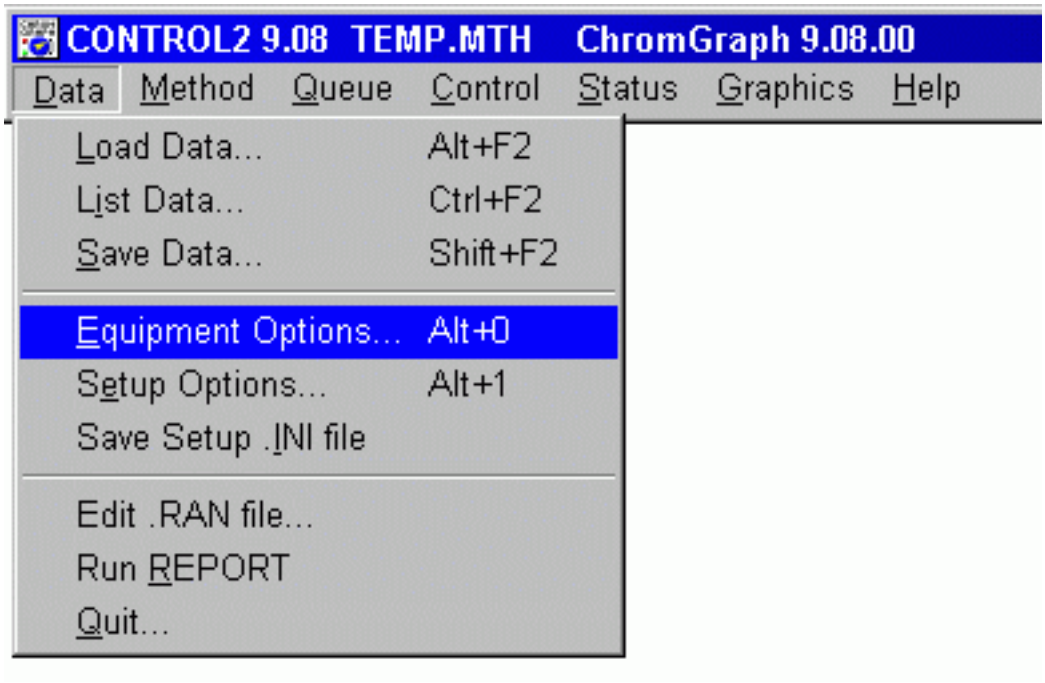


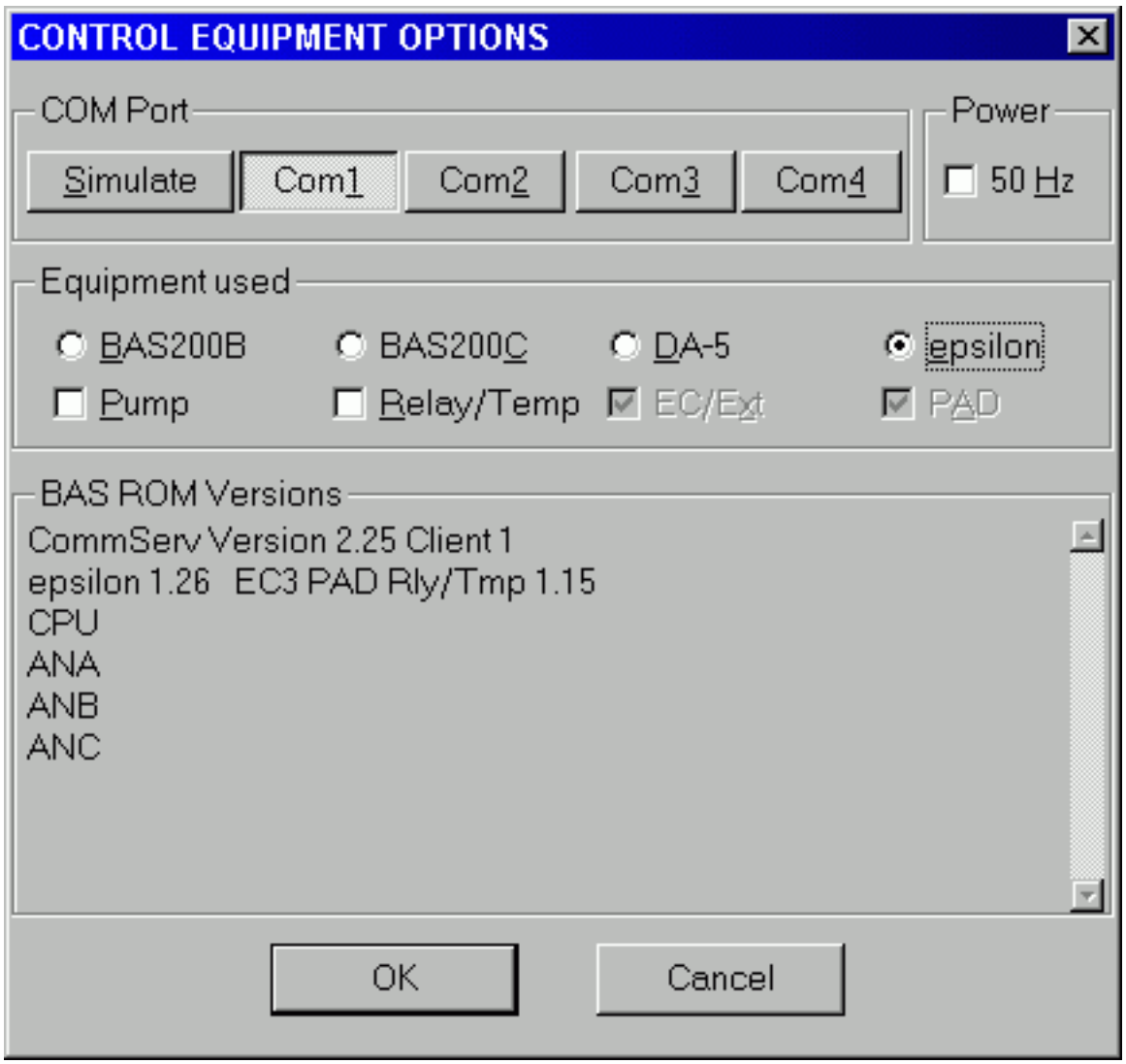
One of two things will happen at this point. If everything is connected properly, a picture of a chromatograph will appear. But if the computer cable is plugged into the wrong communications port, or is unplugged, the EQUIPMENT OPTIONS box will appear.

EQUIPMENT OPTIONS

To get to the options box if it does not come up, take the EQUIPMENT OPTIONS alternative in the Data menu:

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The following sections should be filled out:

COM Port: Com1, Com2, Com3, or Com4 should be depressed, depending on which port you've used when connecting the epsilon system to the computer. (Click to review the INSTALLATION section.)

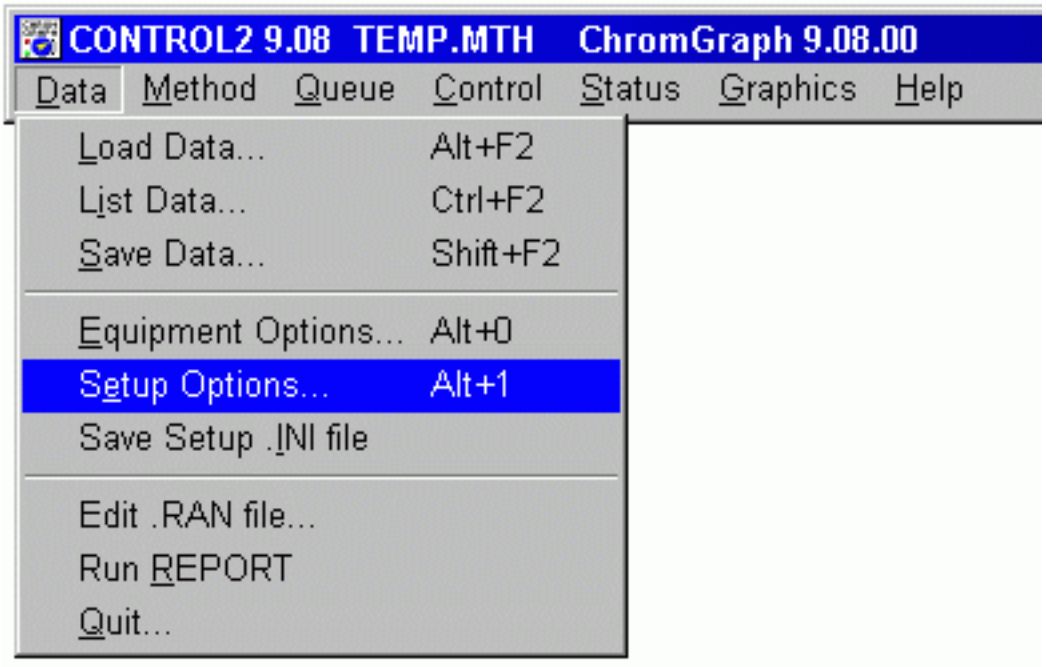
Power: Unchecked = 60 Hz (USA). Checked = 50 Hz.

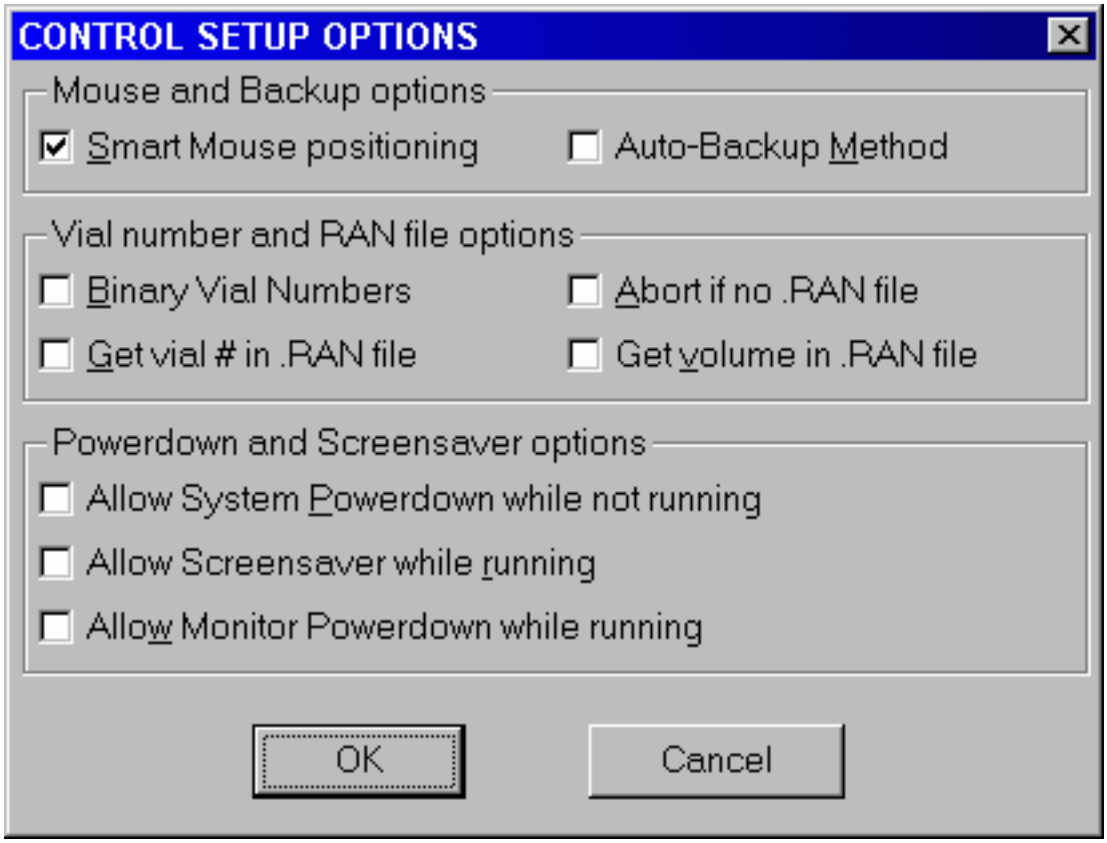
Equipment Used: Click the epsilon radio button.

Press the OK button next. The Equipment Options screen should come back with entries in the 'BAS ROM Versions' section, indicating that the software is communicating with the epsilon system. Press OK again and this screen will be replaced by a picture of a chromatograph.

SETUP OPTIONS

SETUP OPTIONS allows you to set some user preferences. This screen is reached as follows:





Check the options that you wish to use:

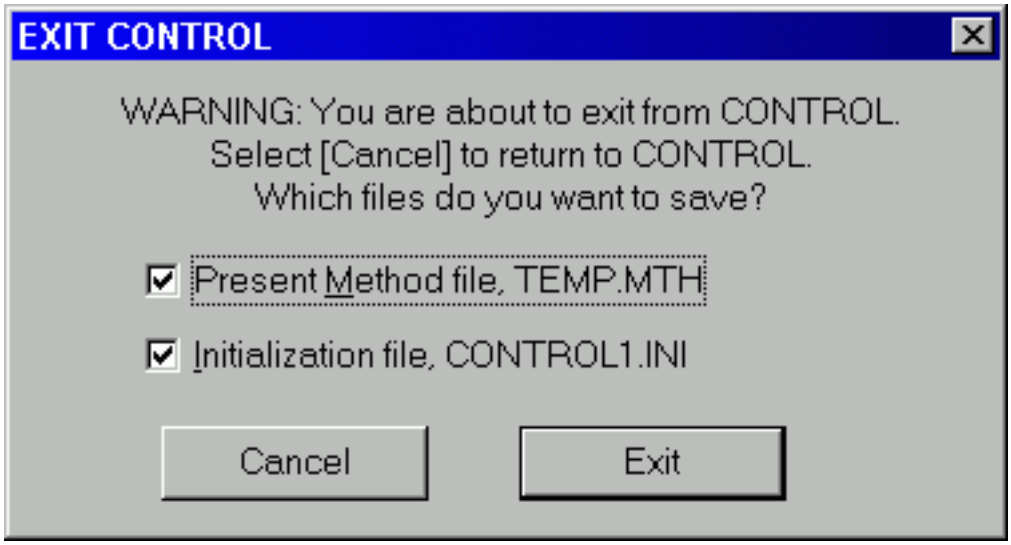
SMART MOUSE positioning puts the mouse cursor on the next OK >> or << OK button when you switch among the Method screens . It is an aid to rapidly scrolling through the Method.

AUTO-BACKUP Method saves a reference Method with each series of runs. Most users do not wish to store all these extra methods, so we recommend this feature only for those in regulated environments where every run must be documented.

The remaining options define how information is transferred from the BAS Sample Sentinel autosampler when importing sample-identifying information for attachment to the run data. Click here for a discussion of this advanced topic.

SAVING THE OPTIONS AND EXITING

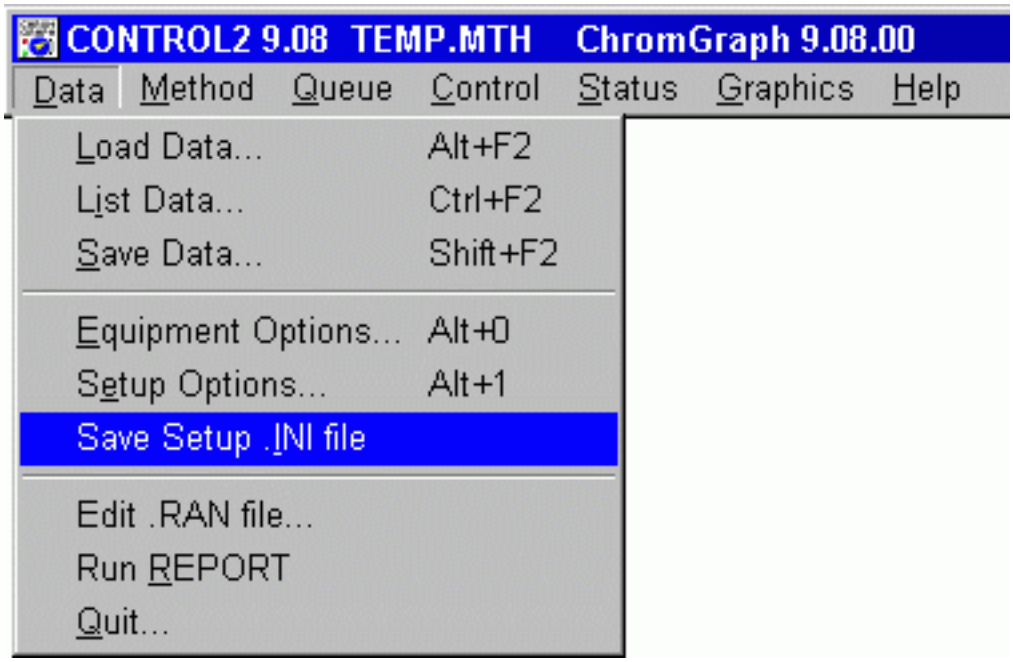
You can exit by clicking the X-button at the top right of the screen, or by taking the QUIT option in the DATA drop-down menu. In either case you'll be asked about saving certain items:



It's a good idea to check both items:

PRESENT METHOD FILE allows the current Method to be saved as temp.mth every time you exit, and therefore reloaded automatically at the next session. This temporary Method differs from your original Method in that it remembers the last run number and comes up ready to do the next run in the sequence.

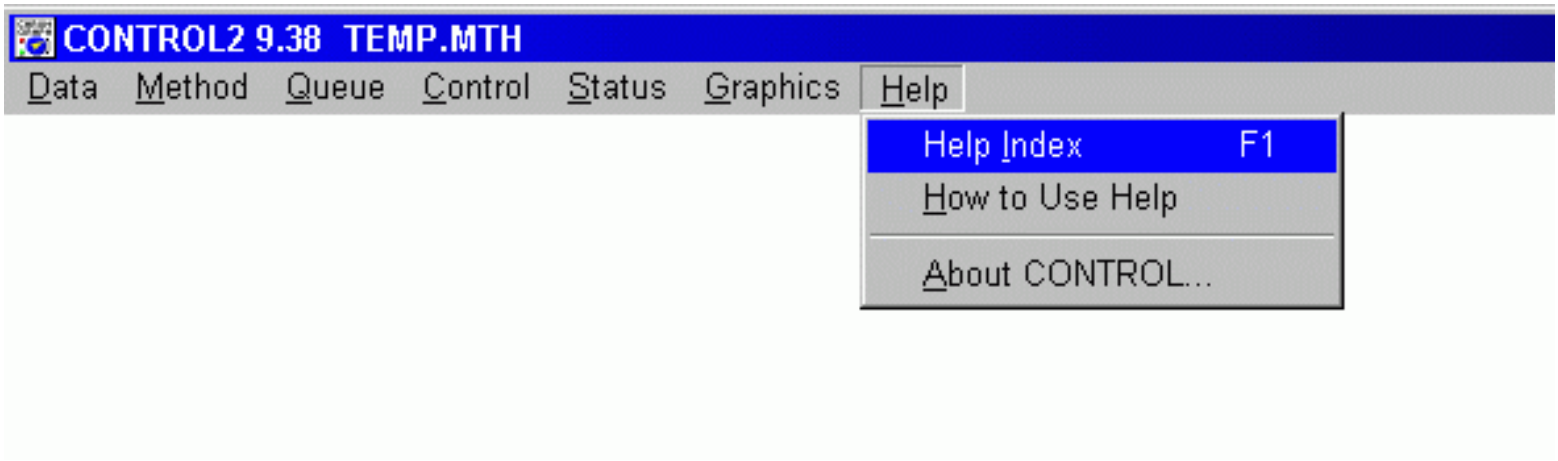
INITIALIZATION FILE saves the equipment options, setup options, screen colors and sizes, and default data directories so they will automatically go into effect at the next session. Any time you change these options during a session you must decide whether to make the changes permanent. Exit and save the initialization file to make them permanent. Exit and do not save the initialization file to forget the changes. If you don't want to exit, you can save the initialization file through an option under the Data drop-down menu:



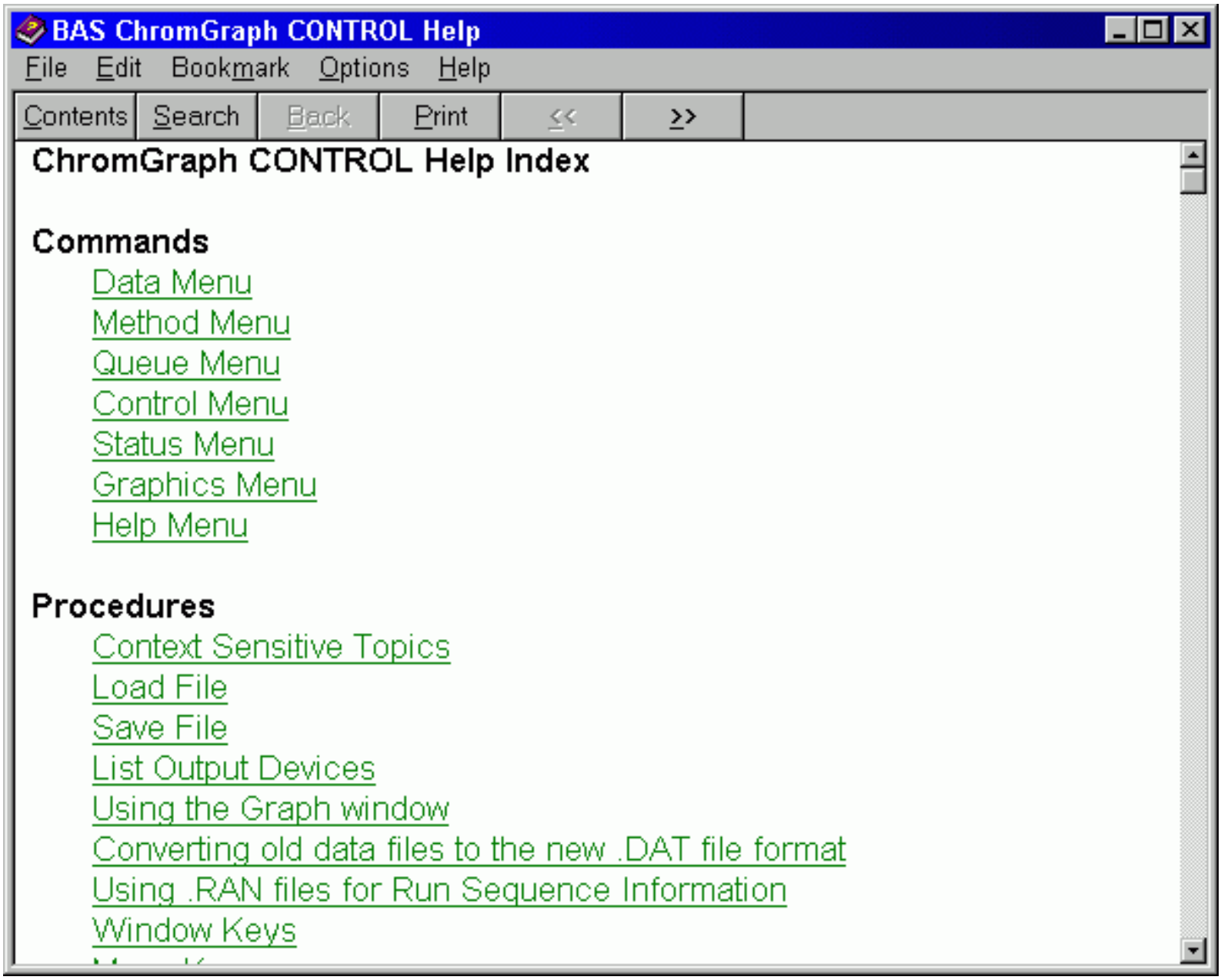
HELP SCREENS

ChromGraph software contains a complete on-line, context-oriented system of help files. Information is provided for virtually any aspect of the software, and for any entry that must be made. Help information can be obtained in several ways which parallel the help screens of Windows.

For general access to help screens, select Help from the menu bar across the top of the screen. The HELP INDEX option opens up a screen showing all the main help topics . Select whichever topic is of interest.



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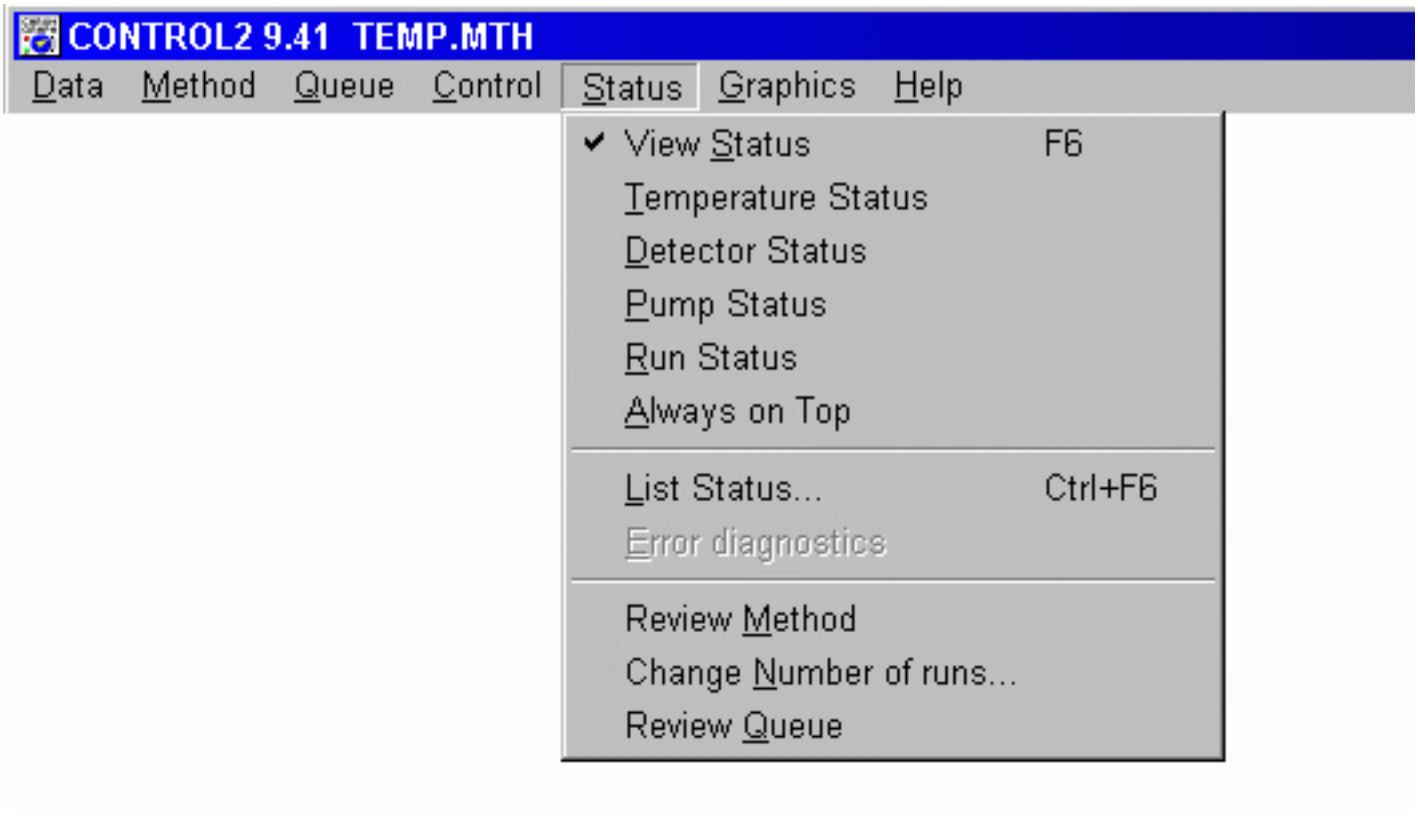
For more specific help when using the software (e.g., when you're not sure what to enter for an option in the Method), use the context-oriented feature of the help screens. Pressing F1 will bring information about whatever option is highlighted on the screen. Highlights can be moved around the screen using the tab and arrow keys.

A faster way to get information about any option is to use the mouse. If the option of interest requires a text entry, simply click on it, then press F1. If the option of interest is a pushbutton that will cause immediate action, you must prevent that action from occurring in order to get help. Press the mouse button but do not release it; then press and release F1. Now you can release the mouse button; instead of activating the button, a help screen will appear.

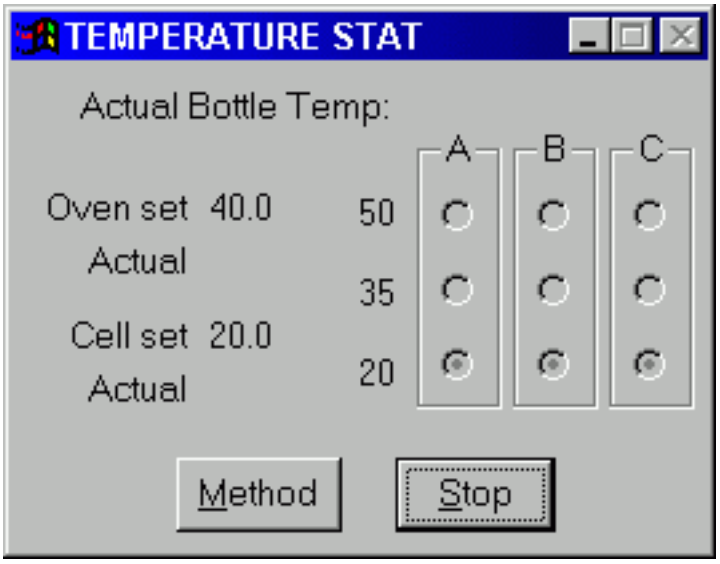
- **CONTENTS**

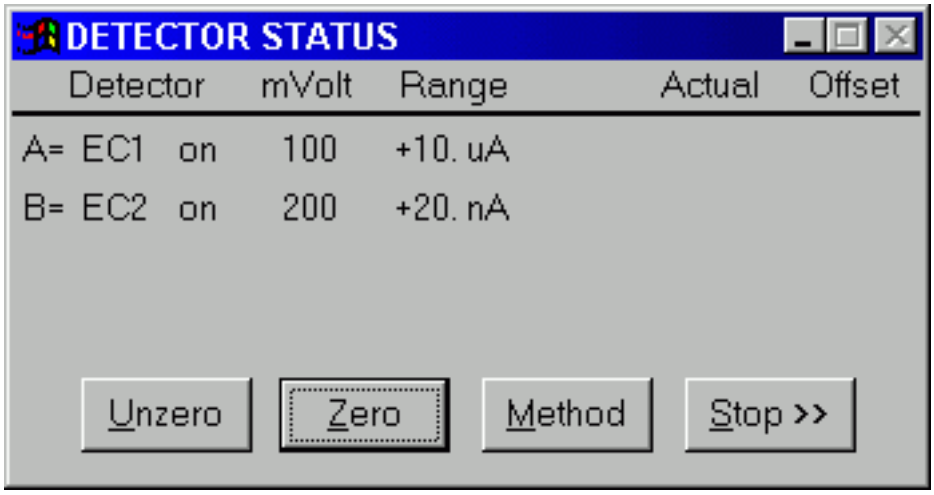
STATUS BOXES

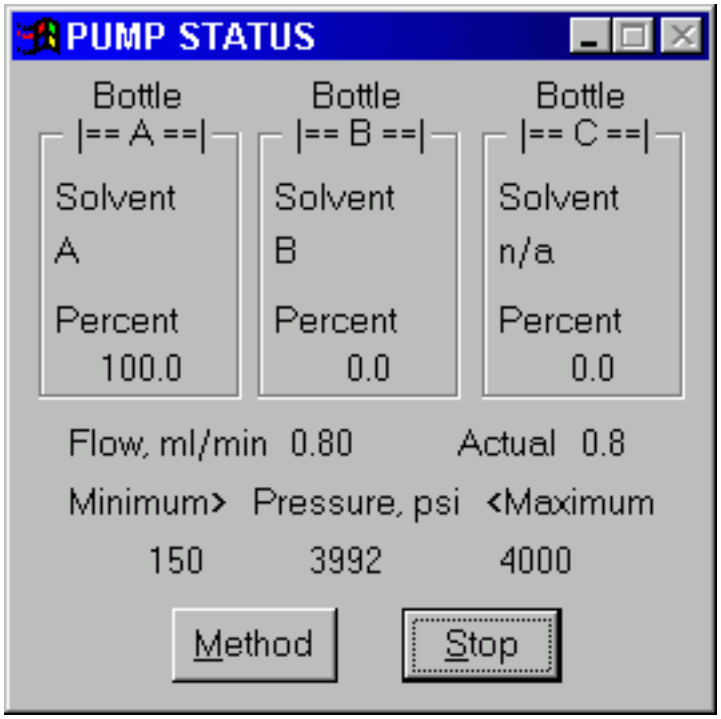
Status box icons appear along the bottom of the screen. To activate any status box, simply double-click on its icon. Pressing F6 will activate or minimize all the boxes at once, as will taking the View Status option in the Status drop-down menu:



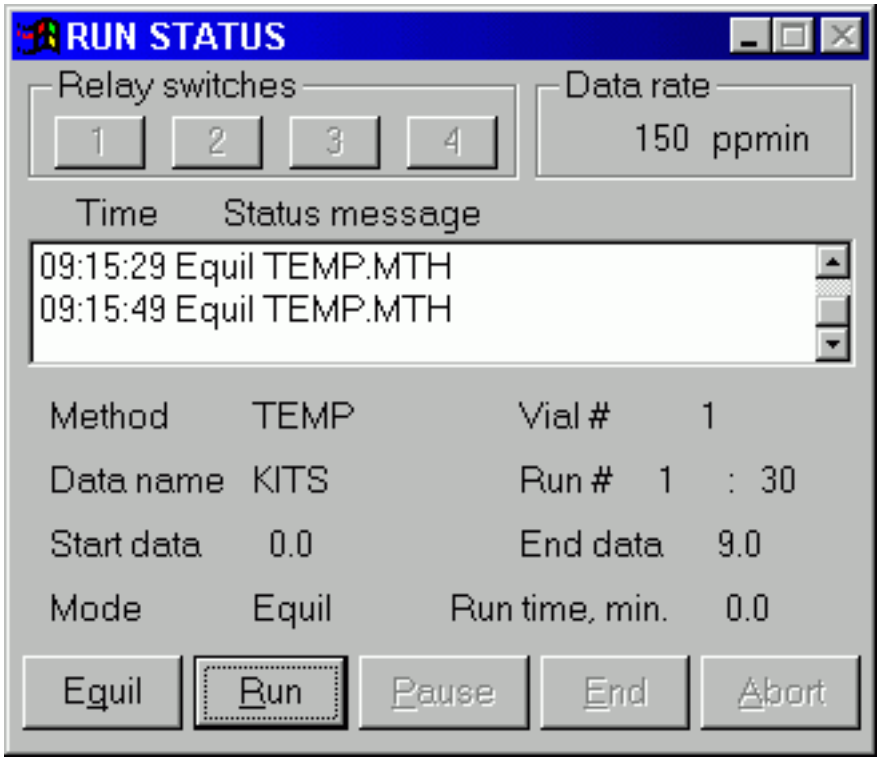
Depending on your particular hardware configuration, up to four status screens are available:







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The status boxes show real-time conditions of the various components of the system. The pump and temperature status boxes will only appear if the relevant BAS instruments are attached.

The status boxes have buttons that allow some control of the equipment, as well as a Method button that opens the Control Method for full control and editing.

The RUN STATUS box shows conditions during data collection, and also allows you to begin and end data collection. This is discussed in the STARTING A CHROMATOGRAPHIC RUN Section.

We recommend that status boxes be left as icons when not needed, and especially during automatic operation. When the status boxes are activated there is a flurry of communication between the computer and the epsilon system, which ties up computer memory and makes screen operations appear sluggish. Individual status boxes can be iconized by clicking on the down arrows at their upper right corners.

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USING THE METHOD SCREENS

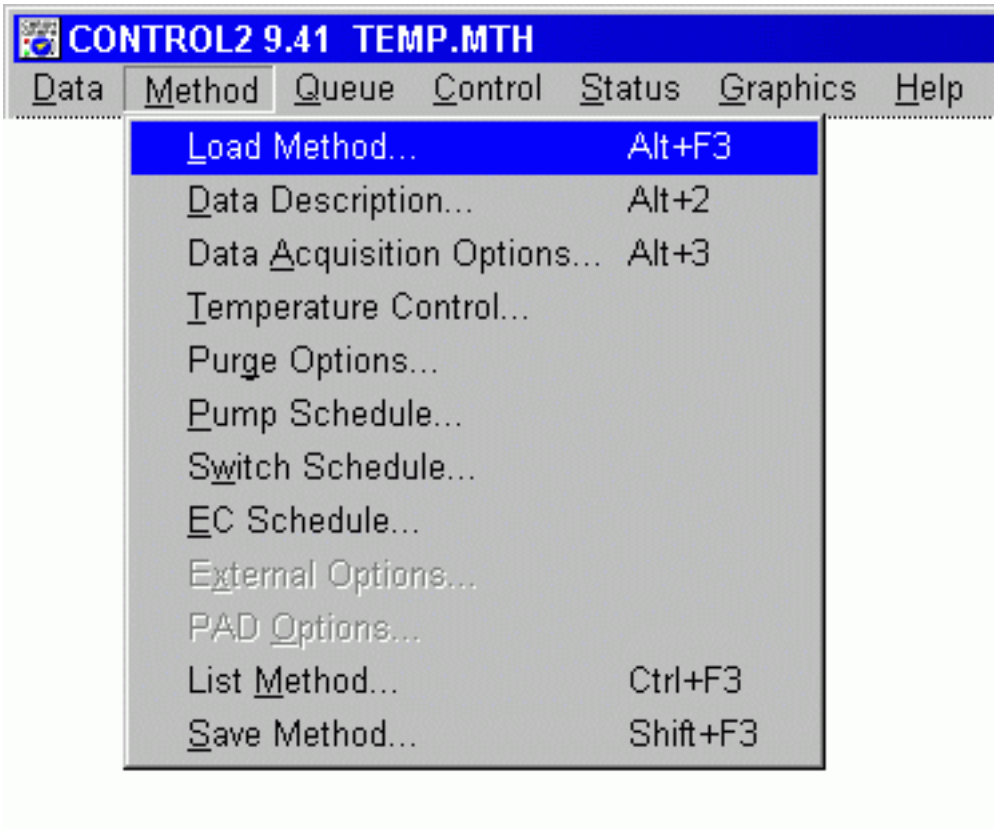
The nerve center of ChromGraph Control is the Method file. The Method contains all the information about how to do a series of runs: how many runs, which detectors and which filter settings to use, what pump settings to use, the duration of the run, the data collection rate, etc. In addition, the Method screens provide the means to manually control the various instruments -- you can turn detectors on and off, start and stop the pump, and set all the conditions so the system can equilibrate.

- **LOADING AND SAVING A METHOD**
- **OPENING THE METHOD SCREENS**
- **INSTRUMENT CONTROL VIA THE METHOD SCREENS**

LOADING AND SAVING A METHOD

A temporary Method is always loaded into RAM at startup, so a Method is always present. You can make changes to this Method or load an existing one.

To load an existing Method, select the LOAD METHOD option from the Method menu at the top of the screen:

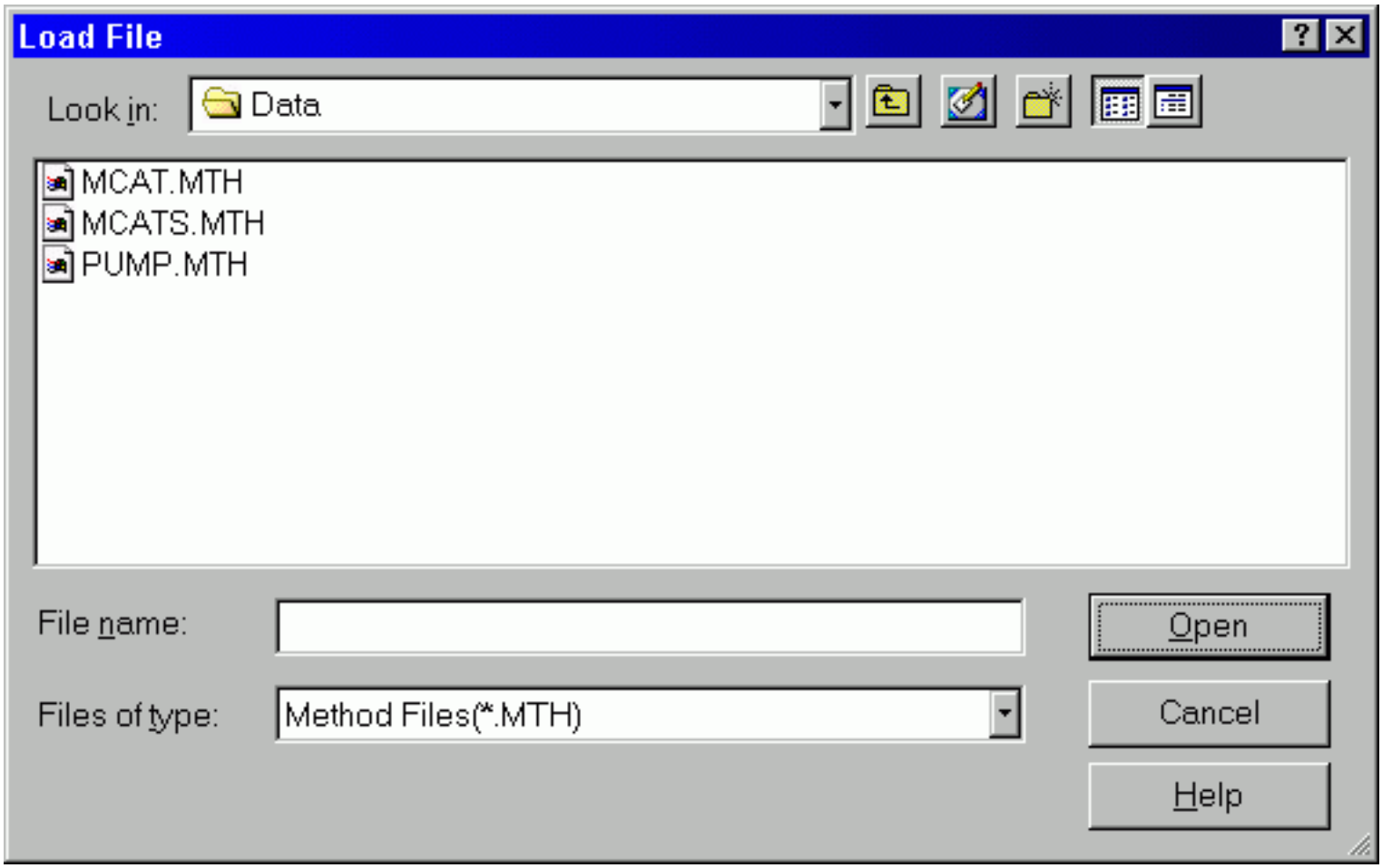


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A directory of existing Methods that reside in the default data location will appear.

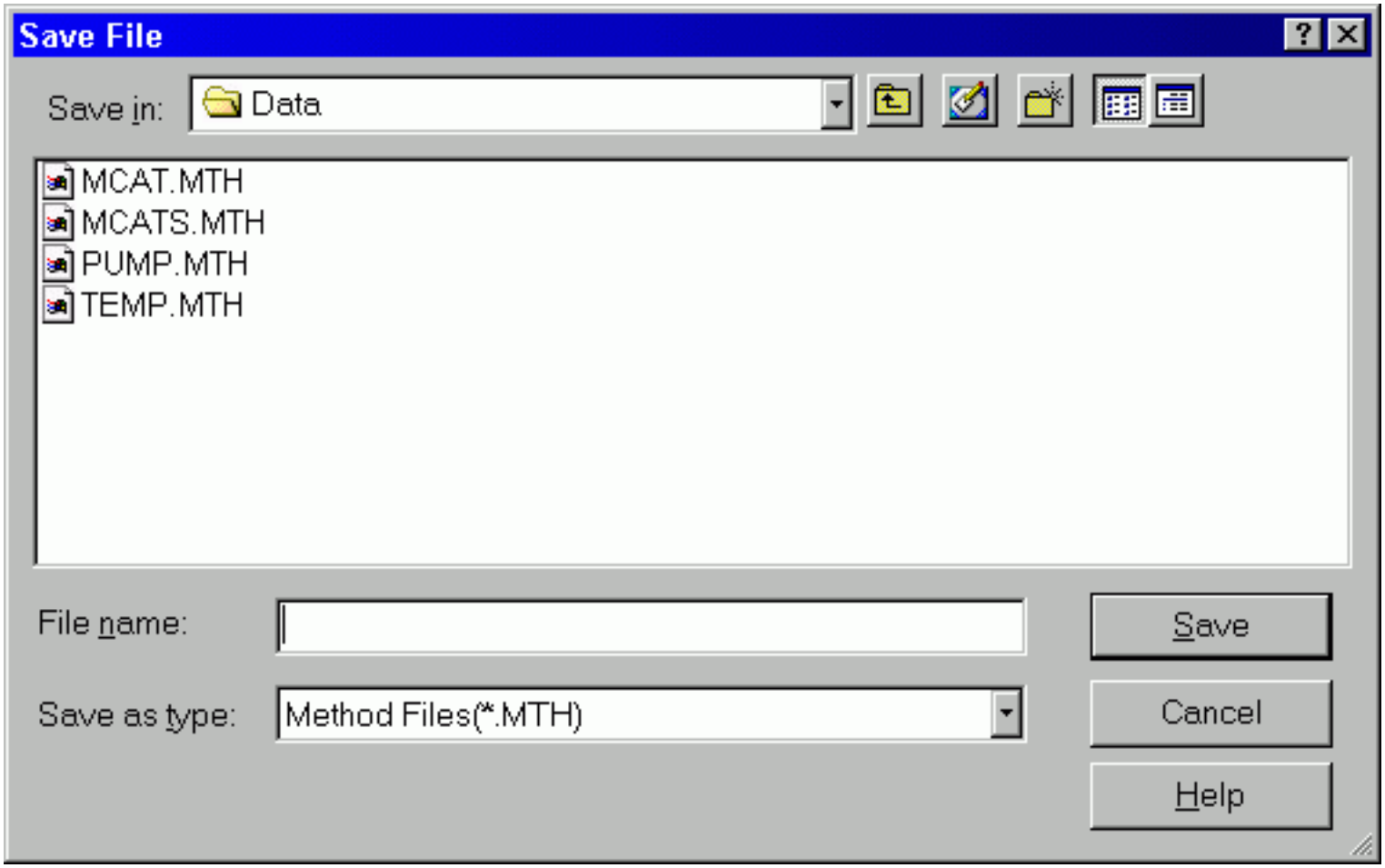
The **DEFAULT DATA LOCATION** is the subdirectory on the hard drive into which Data, Method, and other files are saved. Initially defined as C:\BAS\Data, the default directory may be changed by loading or saving data to another directory, then exiting with the save initialization file option.

To load a Method, either double click on its name or highlight it and click the OK button.



Methods can be loaded from other locations by using the mouse to select other drives or subdirectories. Such a selection automatically becomes the new default data location. The default location will remain in effect throughout the session, unless changed by another LOAD or SAVE operation. When you finish your session and quit, saving the CONTROL.INI file will save the new default data location so it will be used at the next session. If you do not save this file, the original default data location will be used for subsequent sessions.

To save a Method, either click the Save button from any of the Method screens, or take the SAVE option from the Method drop-down menu. A dialog will open up allowing you to save to the location of your choice with the name of your choice:



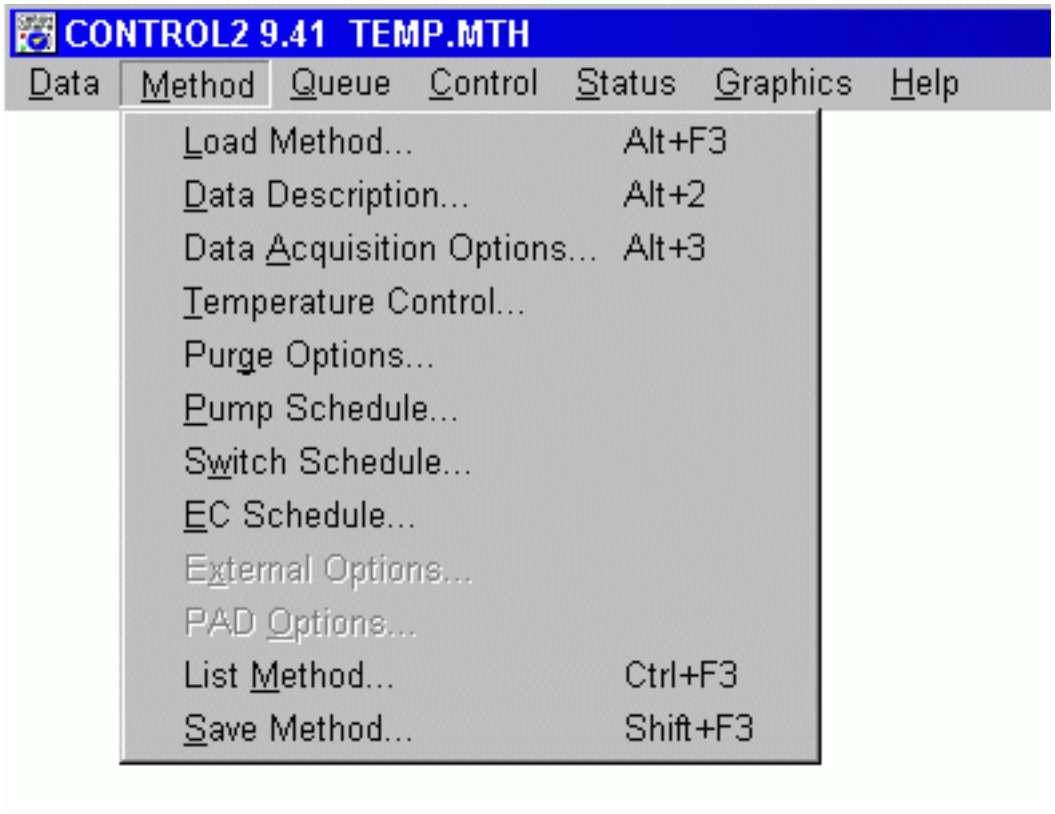
A Method name can have any combination of characters and spaces. The program will automatically append the extension .MTH to the name you choose. Method names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\mymethod.mth = 25 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a Method name may be truncated somewhat in various places in the program.

As with a load operation, saving to a new location will change the default data directory for the remainder of the session.

OPENING THE METHOD SCREENS

To edit or review the Method you must open the Method screens. Start by clicking on the METHOD section of the main menu. A list of options will appear:

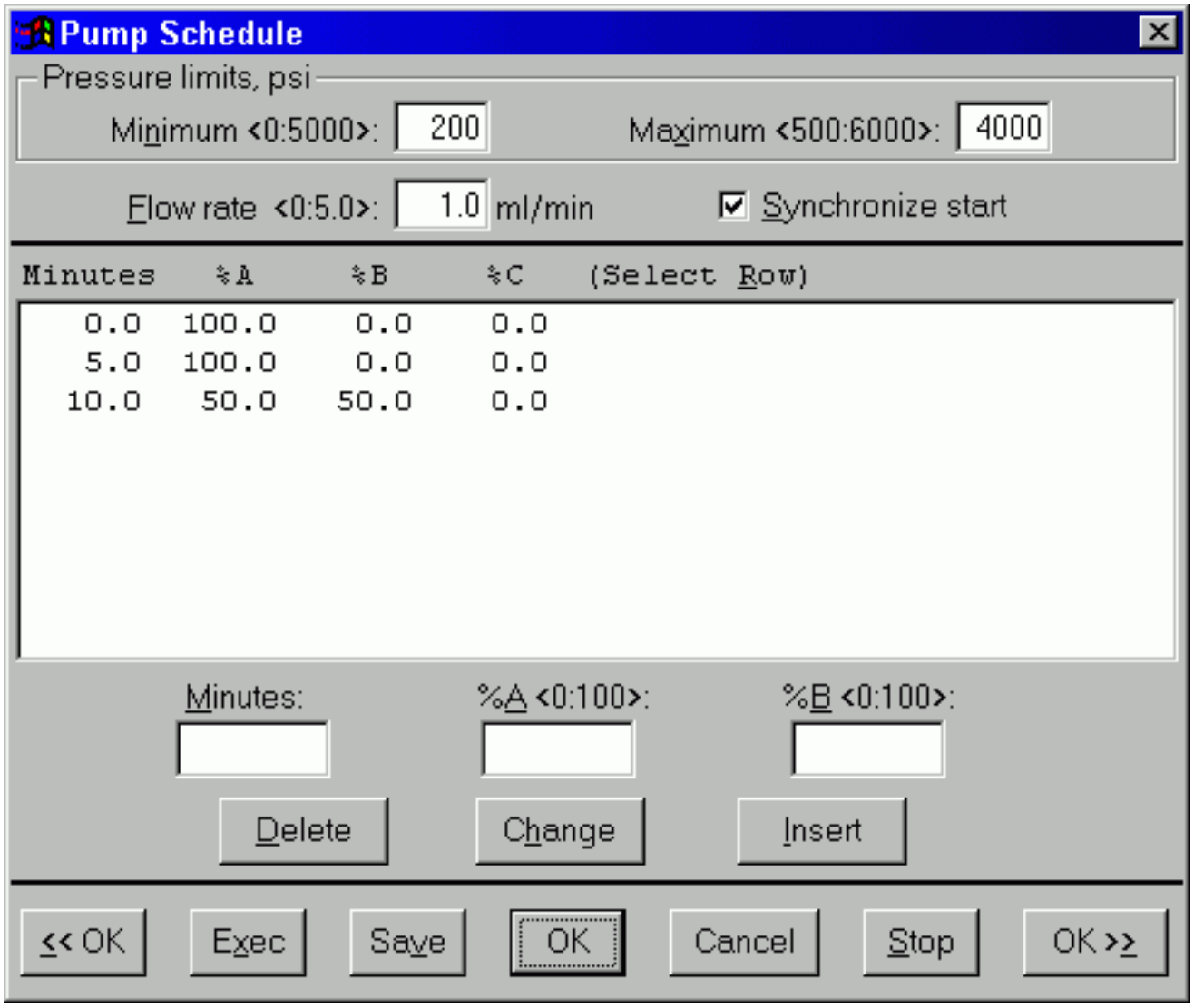


Except for the LOAD, LIST and SAVE options, each option will open a screen for a section of the Method. Simply click on the desired section with the mouse. You can access each screen through this drop-down menu. Once a screen is opened, you can use the << OK and OK>> buttons to move from screen to screen.

INSTRUMENT CONTROL VIA THE METHOD SCREENS

At the bottom of each Method screen (more correctly called a dialog box) are pushbuttons for subsequent action:

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OK accepts any changes and saves them to RAM. These changes are then used in subsequent runs of the Method. To permanently save these changes to disk, use the SAVE option.

<< **OK and OK** >> These have the same function as OK, except they automatically bring up the previous (<<) or next (>>) screen of the Method.

CANCEL closes the dialog box without accepting any of the changes. The original information is thus preserved.

EXEC sends a command to the epsilon hardware to set the conditions called for in the dialog box (e.g., pump speed, detector conditions). For screens with multiple time lines (e.g., Pump Gradient Schedule) the conditions on the 0.0 time line are activated.

STOP sends a command to the epsilon hardware to stop or turn off the appropriate device.

DELETE removes the highlighted line from a dialog box with time lines.

CHANGE puts edited information into the highlighted line of a dialog box with time lines.

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INSERT inserts a new line into a dialog box with time lines.

SAVE opens a dialog to save the Method with a name and location of your choice. As with a load operation, saving to a new location will change the default data directory for the remainder of the session.

Screens concerned with time schedules (pump, detector, timed events) contain listboxes -- lists of up to 10 time lines that change the operating conditions during a run. Time is measured in minutes from the start of a run. Time lines are automatically sorted by time as they are entered. The maximum time is 999.9 minutes, and lines must be at least 0.1 minutes apart.

Only the 0.0 time line can be put into effect from these screens. To run the entire schedule you must start a programmed run.

Under the listbox is found a set of edit boxes, used to enter, delete, and modify the time lines. To insert a new time line, enter the information in the edit boxes, then press **INSERT**. To modify the information in an existing line, click on the line to make it appear in the edit boxes. Make the changes, then press **CHANGE**. To delete a line from the listbox, click on it, then press **DELETE**.

- CONTENTS

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DATA ACQUISITION OPTIONS AND DETECTOR SELECTION

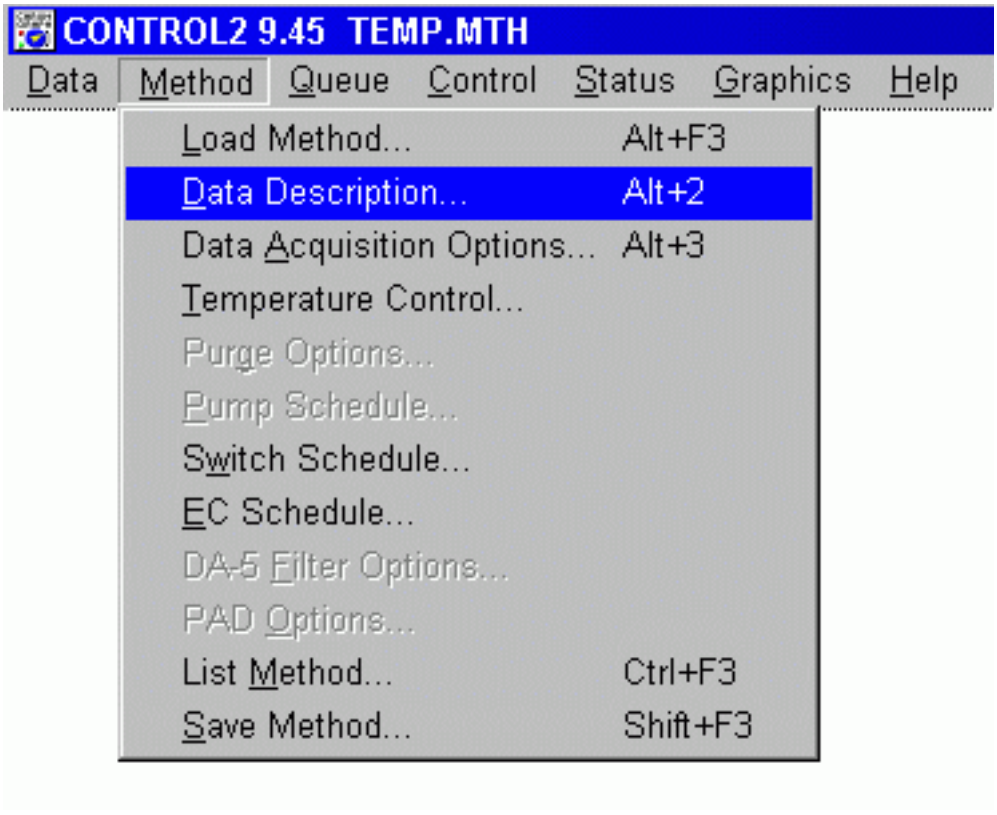
These sections of the Method allow you to control how data collection is initiated, the duration and frequency of data acquisition, and the way the runs are named and numbered.

The Channel Selection section allows you to select which detectors will be used for the run. The Epsilon can be configured for up to four simultaneous channels of data collection. Depending on the configuration purchased, you can select various configurations of onboard electrochemical (EC) and external (EXT) detectors.

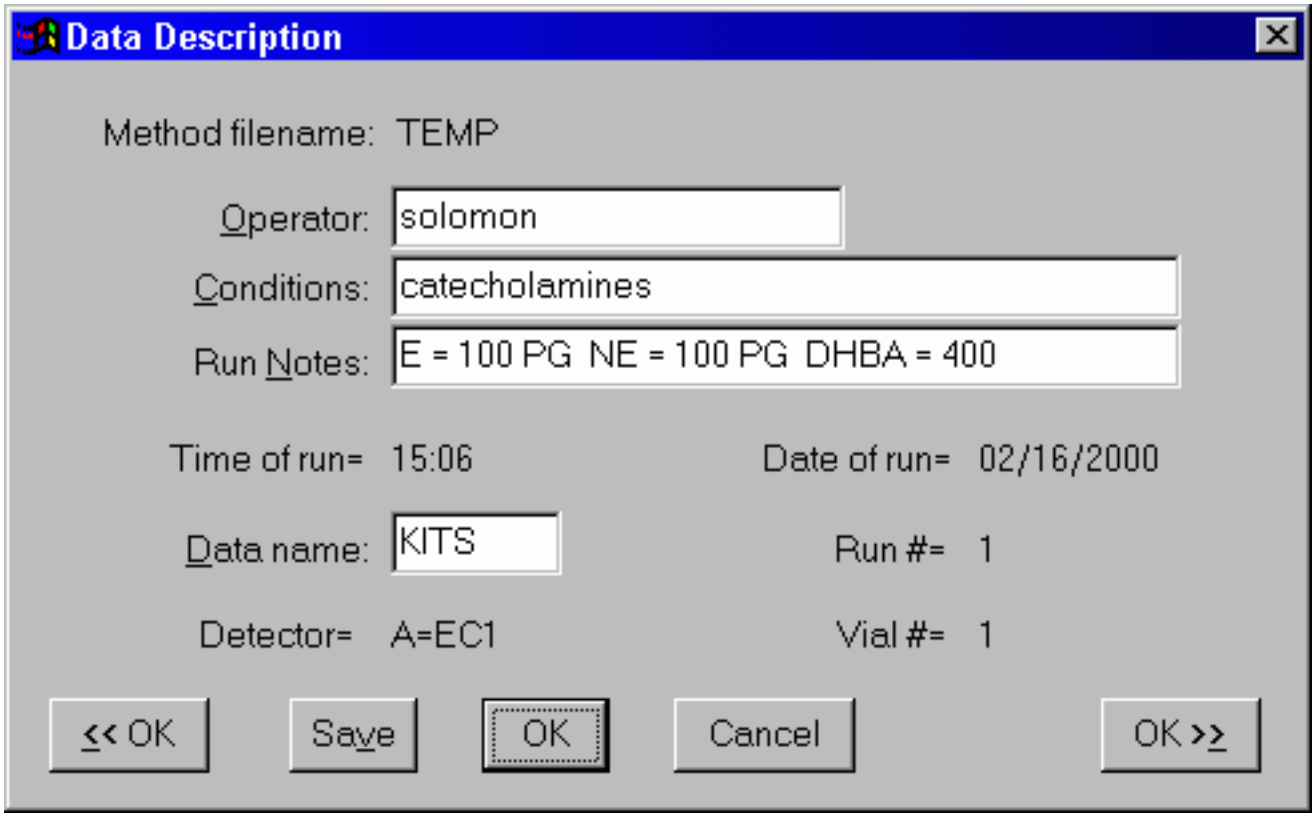
- **DATA DESCRIPTION**
- **DATA ACQUISITION OPTIONS**
- **DETECTOR CHANNEL SELECTION**

DATA DESCRIPTION

The Data Description section can be accessed through the Method drop-down Menu:



This section also can be reached by clicking the <<OK or OK>> buttons from other Method screens.



OPERATOR, CONDITIONS and RUN NOTES are user-inserted comments entered into the Method. This information is saved along with every Data file generated by CONTROL. CHROMGRAPH REPORT imports this information and includes it in the final processing report that it generates. By using these fields when you collect data, you will always be able to identify any particular Data file.

During unattended operation you can automatically import sample identifying information into the RUN NOTES section. Click here for details.

DATA NAME is the name used to store the data file. It can have any combination of characters and spaces (the last position must be a letter, to avoid confusion with the run number, which will be appended).

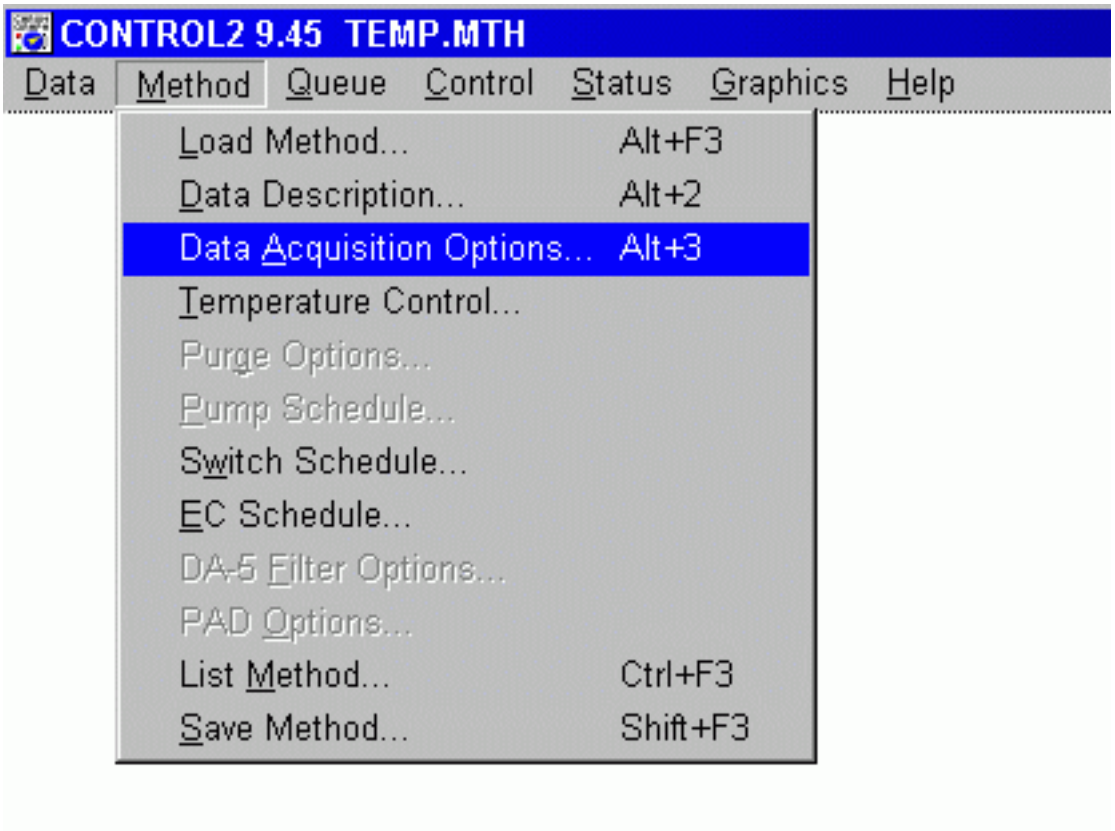
Run numbers (three digits, up to 999) and a letter (A through D, for detector 1 through 4) are automatically appended to the data name by the data-acquisition software. When saved to disk, the name is given the extension .DAT. Thus, CATS003A.DAT is the third run in a series called cats, and contains data from detector A.

Data names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\mydata002A.dat = 27 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

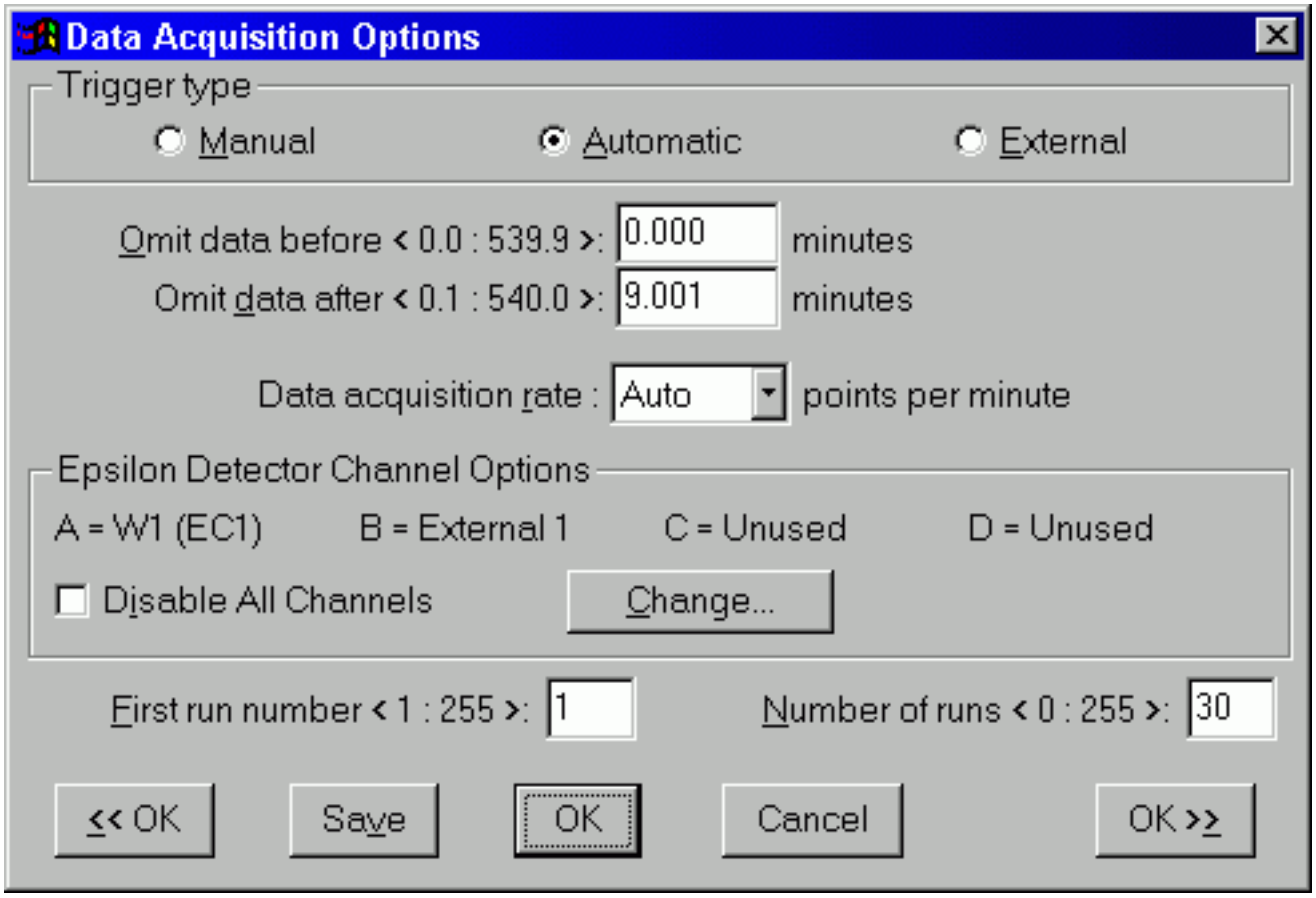
For display purposes, a filename may be truncated somewhat in various places in the program.

DATA ACQUISITION OPTIONS

The Data Acquisition Options section can be accessed through the Method drop-down Menu:



This section also can be reached by clicking the <<OK or OK>> buttons from other Method screens.



The following options are available:

TRIGGER TYPE determines how a chromatographic run will start. There are three types of triggers:

- **Manual** means you start each run by clicking the RUN button in the Run Status box.
- **Automatic** means you start the first run by clicking the RUN button in the Run Status box, but that subsequent runs start automatically.
- **External** means that an external device (e.g. an autosampler) will initiate each run. You must connect the external device to the epsilon's back panel. Before the first trigger arrives you must put the epsilon in the 'waiting for trigger' mode by clicking the RUN button in the Run Status box.

OMIT DATA BEFORE/AFTER allows you to specify the time during which data will be collected and saved. In most cases this is equal to the length of the run, but you may want to shorten the data-collection time if you have a column wash or re-equilibration programmed into the pump schedule.

DATA ACQUISITION RATE is the number of times per minute that detector response is recorded. Too small a rate will result in poor peak resolution. Too large a rate wastes computer resources. We recommend the AUTO setting, in which the optimum rate is decided for you. Alternatively, you may open up this listbox and select rates between 75-1200 points per minute. In all cases the actual data-collection rate is shown in the Run Status box.

FIRST RUN NUMBER is the number that will be given to the first run of a series. This value is incremented by one each

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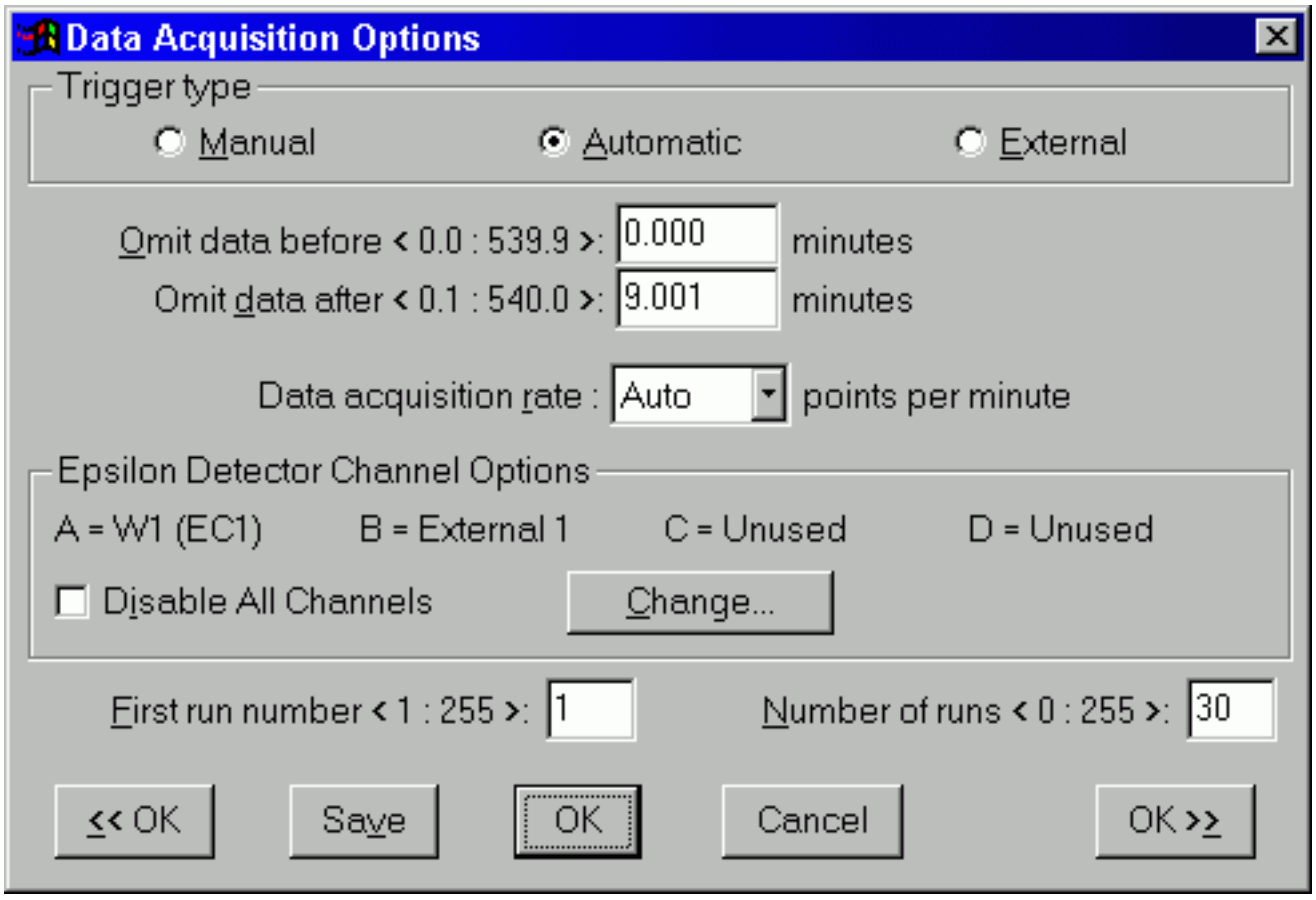
time a run is completed. If a series of runs is aborted during a run, the number assigned to the aborted run will remain, so the series can be restarted where it left off.

NUMBER OF RUNS is the number of runs (maximum = 999 for any Data Name) to be made in programmed operation. This number will be decremented by one each time a run is completed. If a series of runs is aborted during a run, the NUMBER OF RUNS will remain at its last decremented value so the series can be restarted where it left off.

If runs are being made manually, one by one, use either '0' or '1' for this parameter.

DETECTOR SELECTION

The number of detectors enabled, and their type, is shown in the Data Acquisition Options screen:

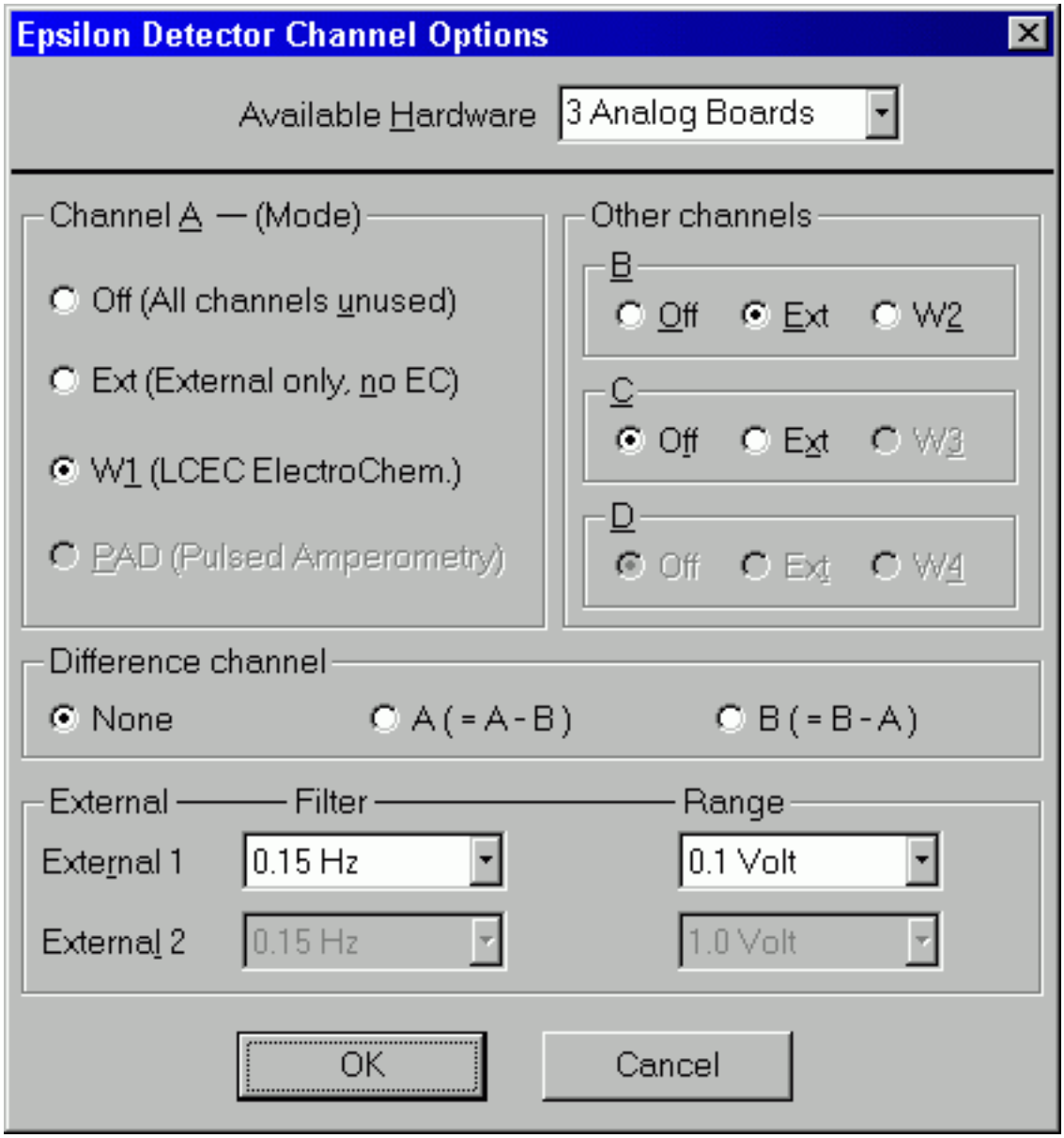


DISABLE ALL CHANNELS allows you to deconfigure all detectors at once. You must do three things to put this change into effect:

1. Check the 'Disable' box
2. Click the OK button
3. Click the EQUIL button in the Run Status box.

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CHANGE ... If the detector configuration is not appropriate for the analysis to be done, press the CHANGE button. This brings up the Detector Channel Options screen:



AVAILABLE HARDWARE shows the number of analog circuit boards installed in the epsilon detector. The detector channels available are determined by the number of circuit boards as follows:

Boards	Channels
1	1
2	2
3	3 and 4

Click on the configuration that is required for your analyses. The software will not allow you to make selections that are inconsistent with the installed hardware or the requirements of certain modes. In particular:

- Pulsed Amperometric Detection (PAD) precludes the use of any other detector.
- Electrochemical Detectors (W1-W4) must be selected before External Detectors.
- The number of available channels is limited by the number of analog boards purchased with the instrument.
- Detectors are added sequentially; upper channels become available only as the lower channels are filled.

After the detectors are enabled, their conditions must be set in the relevant detector schedules. Detector conditions for on-board EC detectors are set in the EC Detector Schedule and those for PAD are set in the PAD Options section.

Conditions for the External Detectors are set in this screen, but are discussed in the External Detectors LINK section.

DIFFERENCE CHANNEL. The epsilon detector provides a means for monitoring the difference between two chromatograms, in real time. When you click on one of the difference options, the system will do the requested subtraction during the run and graph it continuously. The data file that is generated is saved as a normal file and may be processed and graphed with ChromGraph Report. Please note the following:

- The difference channel will replace one of the original channels.
- An offscale peak in one of the original channels (particularly the one that is replaced, so you can't see it) can have some odd effects on the difference channel.
- Use an insensitive range to keep the peaks on scale.
- Rezero before each run, to keep the peaks on scale.

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PUMP OPERATION

This section describes control of BAS pumps attached to the epsilon platform. If you have another brand of pump it may be used with epsilon hardware, but you will not be able to control it with this software.

Two screens control all pump operations: the Purge Options screen allows mobile phase or cleaning solutions to be automatically pumped through the system at a high flow rate; the Pump Schedule controls isocratic and gradient flows during normal operation. Refer to the pump manuals for specifics on pump operation and maintenance.

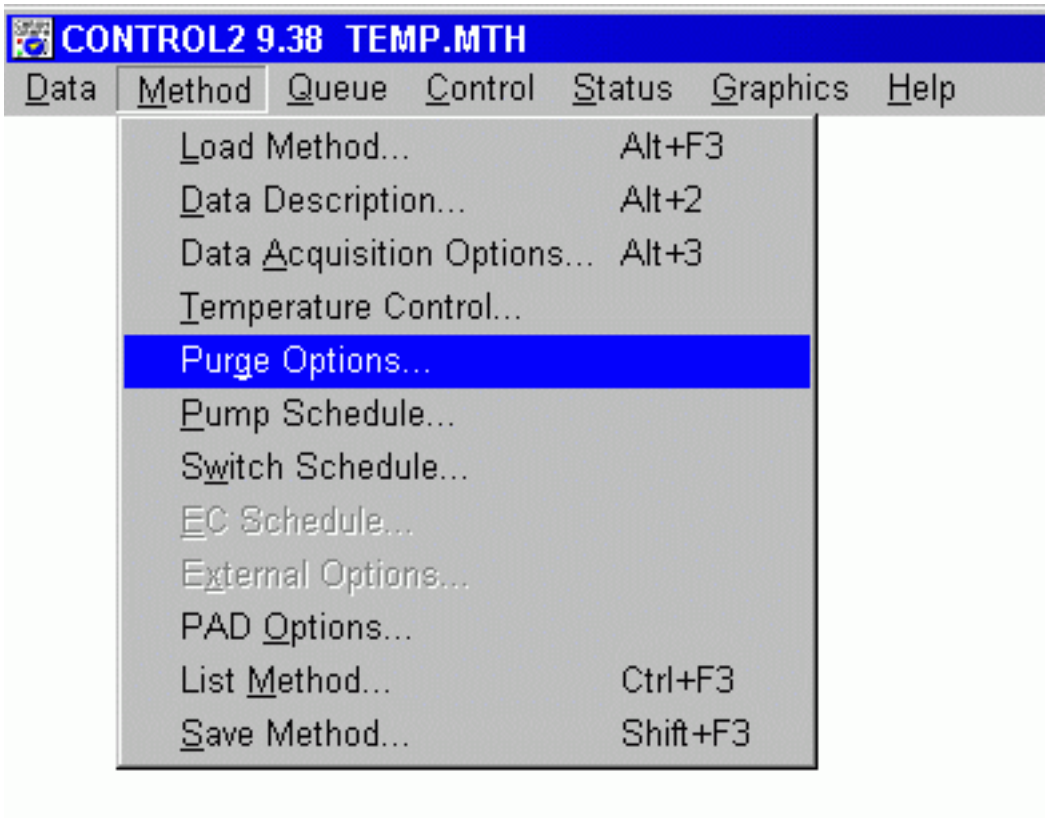
- **PURGE OPTIONS**
- **PUMP SCHEDULE**
- **MONITORING PUMP PRESSURE**

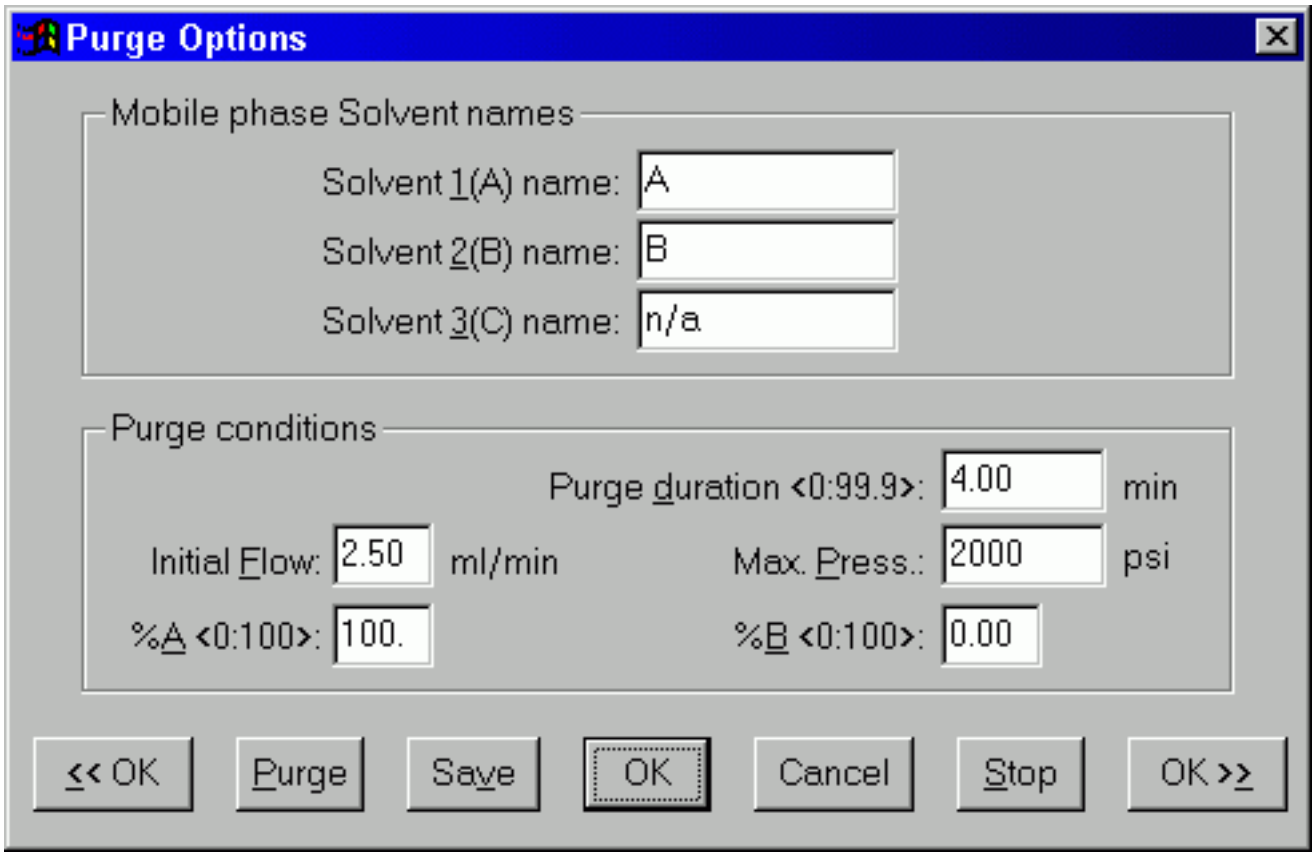
PURGE OPTIONS

A purge can be used to bring fresh mobile phase rapidly through the system. The pump will attempt to maintain a user-chosen pressure by varying the flowrate. If the chosen pressure limit is not reached (e.g., if the purge valve is open) the pump will increase flow to its maximum, 5 mL/min, and hold that flow rate for a user-chosen duration.

The PURGE OPTION screen can be accessed through the Method Menu or by clicking the <<OK and OK>> buttons from other Method screens.

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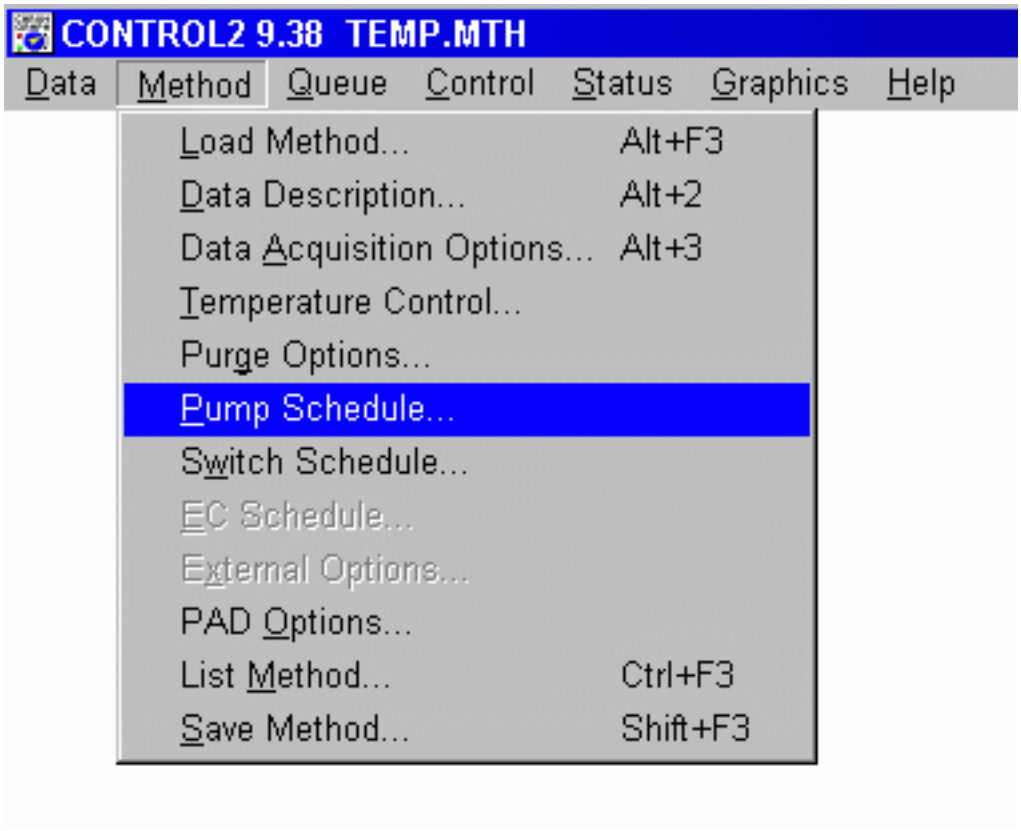
To program a purge, enter the **PURGE DURATION**, **MAX. PRESSURE**, and **SOLVENT %** (enter percentages for bottles A and B; bottle C will be calculated as the remainder). **INITIAL FLOW** is the starting flow rate, and should generally be about 1/2 of the desired flow rate. For a purge through the purge valve it should be 2.5 mL/min; for a purge through a column it should be 1/2 of the usual flow rate of the column.

Start the purge by clicking on the **PURGE** button. During a purge, the RUN STATUS box displays elapsed time. The pump will stop when elapsed time reaches zero.

PUMP SCHEDULE

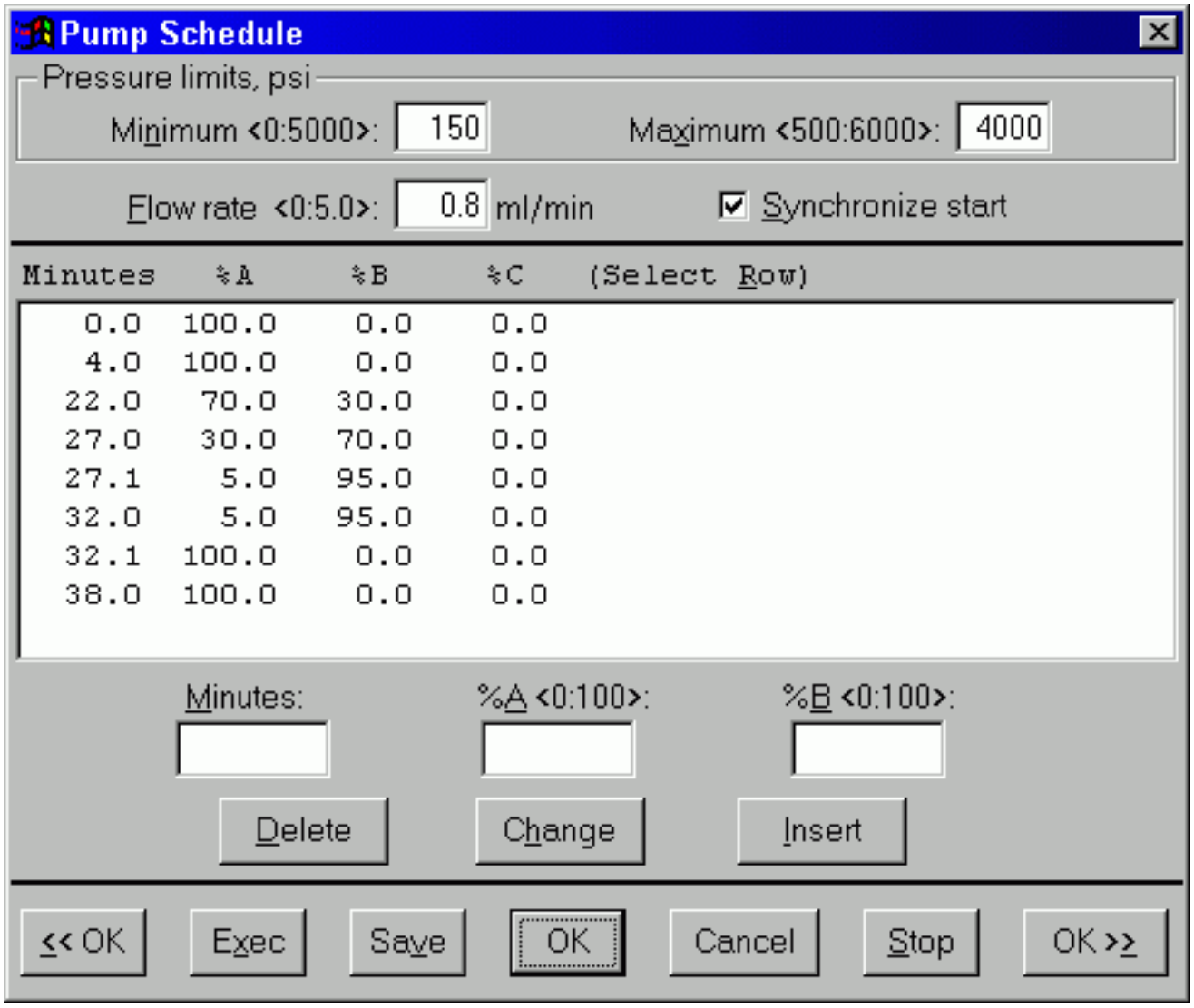
The PUMP SCHEDULE contains all the operating information for the pump, as well as pushbuttons for immediate-mode control. Note that your PUMP SCHEDULE may vary from the ones shown below, depending on the features of your pump.

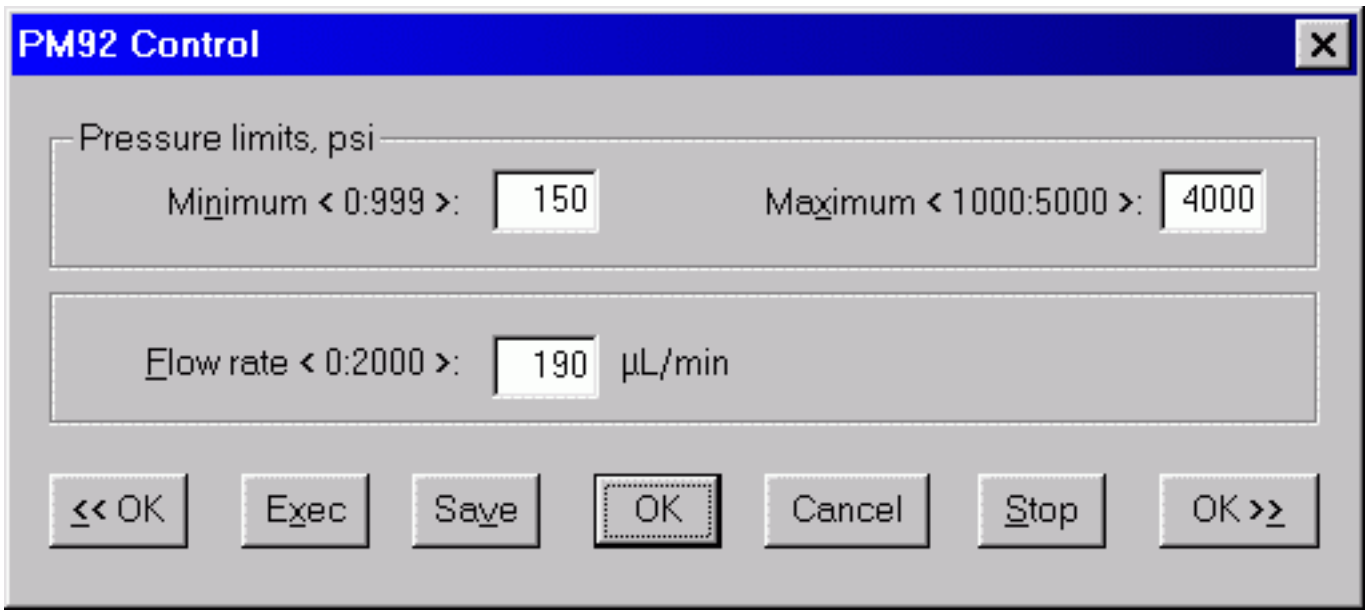
The PUMP SCHEDULE is accessed through the Method Menu or by clicking the <<OK and OK>> buttons from other Method screens.



Two typical pump control screens are shown below. Gradient pumps will have the first type. Isocratic pumps can have either type, depending on the model.

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MAXIMUM and **MINIMUM PRESSURE** are the safety limits for the pump. For most chromatography, we recommend a pressure minimum of 200 PSI and a maximum of 4000 PSI. If the pressure falls below the set minimum, or rises above the set maximum, the pump will stop and report the appropriate error.

FLOW RATE can be set from 0 to 0.5 mL/min in 0.01 mL increments, and from 0.5 to 5 mL/min in 0.1 mL increments.

SYNCHRONIZED START checkbox should be checked for gradient runs, and blank for isocratic runs. Synchronization ensures precise gradients by delaying the start of a run until a pre-set piston position has been reached.

GRADIENT SCHEDULE LISTBOX contains the pump instructions for a timed run. There must be at least two lines here: a 0.0 minute start line and an end line. Up to 10 lines may be entered in the gradient schedule; they will be automatically sorted by time. The first event must have time 0.0, and subsequent events must be at least 0.1 minutes apart. For each line of the listbox, enter the time and the percentages for bottles A and B (C is automatically calculated as the remainder). When bottle percentages differ between lines, the software will calculate the appropriate linear gradient between the two lines.

The chromatographic run will end at the longest time in any of the following modules: Pump, EC Detector, External Detector, PAD, or Switch Schedule. (Click here for a review of the other Method screens.) If the pump's maximum time is shorter than another time in the Method, the pump will revert to its time 0.0 conditions after completing this schedule.

PUMP DURATION is the equivalent of the last line of the gradient schedule, for pumps that use the second type of control screen. This is a non-critical entry, generally set to the length of the chromatographic run, or shorter. When the specified time is reached, the pump's clock stops and the pump continues to run at the entered conditions.

EXEC and **STOP** pushbuttons are provided for manual control of the pump. EXEC puts the 0.0 time line into effect.

Note that a pump program cannot be initiated from the Pump Schedule; you must start a programmed run to begin the program.

MONITORING PUMP PRESSURE

Certain troubleshooting procedures require a printout of pump pressure over time. This can be easily achieved with a BAS pump by feeding the pump's pressure output into the epsilon system as an external detector.

Proceed as follows:

1. Connect the 'Pressure' and 'Gnd' terminals on the back of the pump to either 'EXT-1' or 'EXT-2' and 'Gnd' on the back of the epsilon detector.

2. Set the input voltage Range to 10 Volts.

3. Modify the graph scaling so Full Scale Y is 10,000 and Label Y Units is PSI. Be careful to change the scaling only for the detector channel assigned to the pump.

4. Start a run and collect data for 10 minutes.

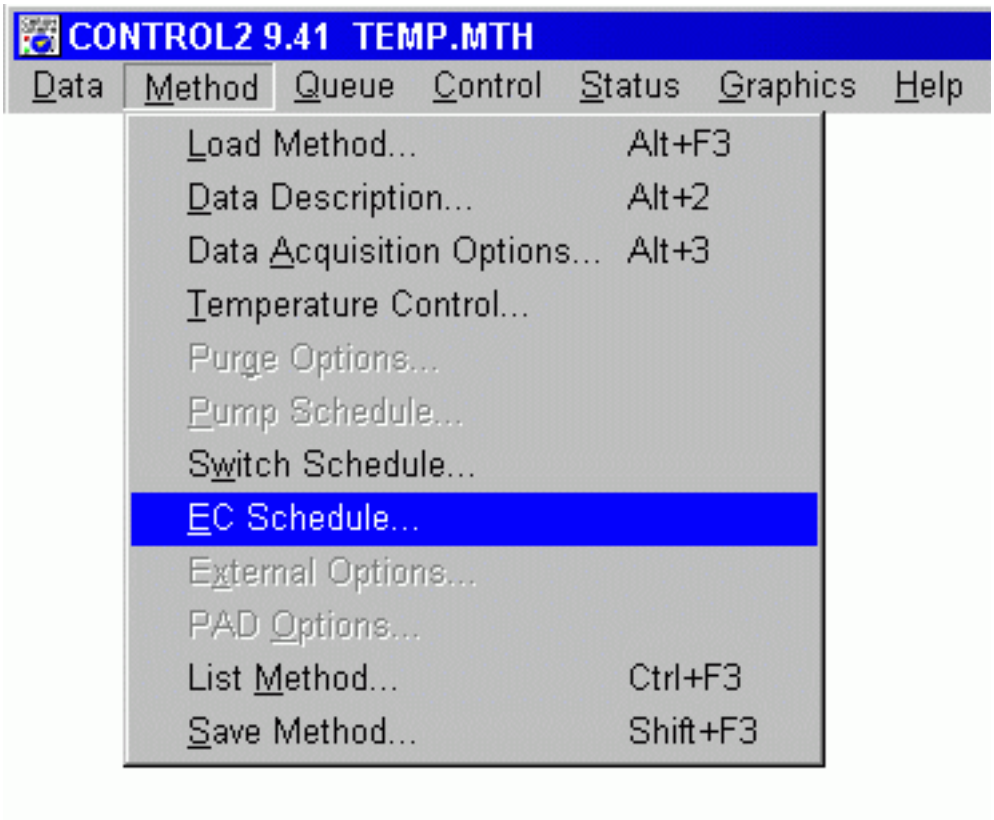
EC DETECTORS

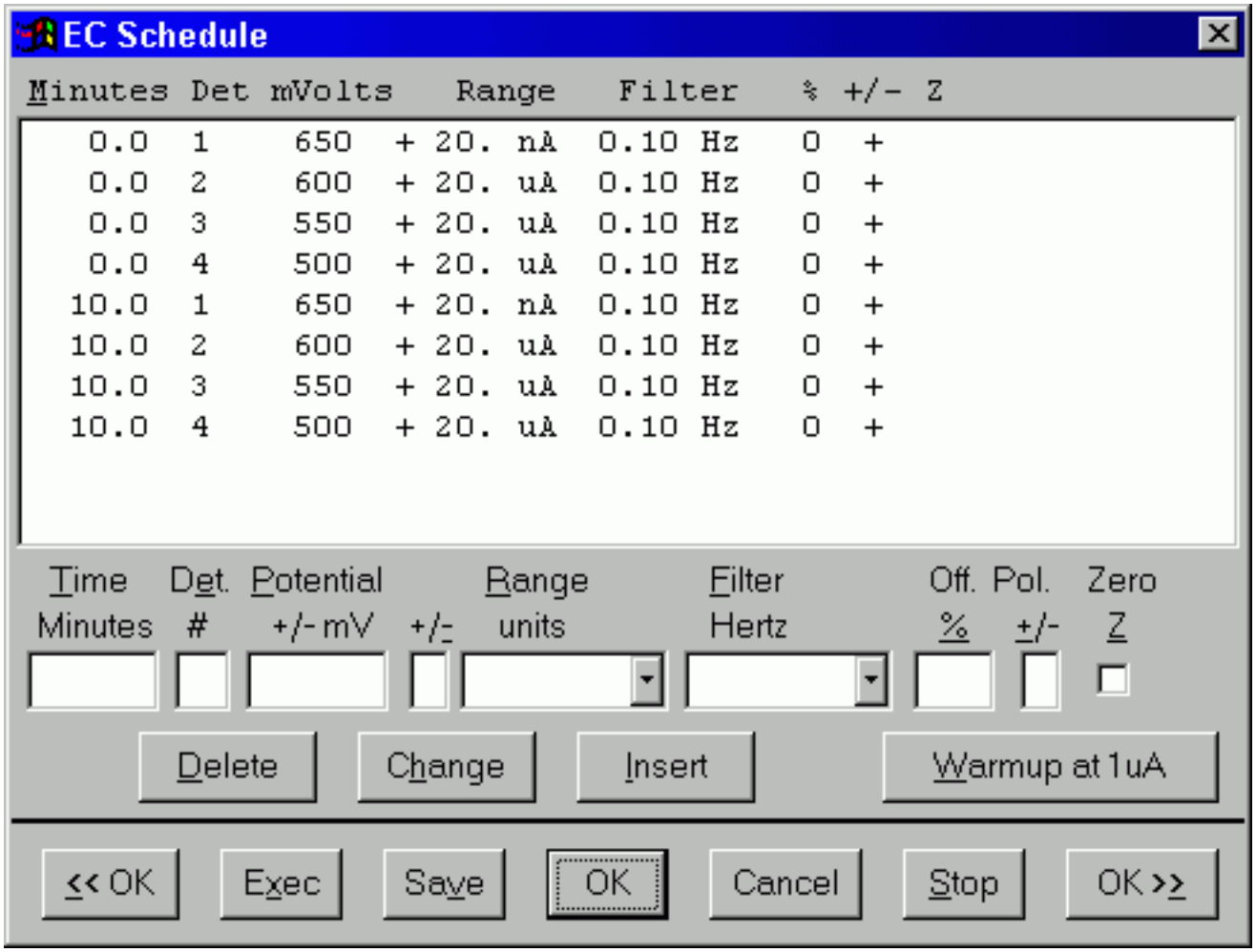
The EC Detector Schedule contains all the information for control of the EC detectors in their constant-potential mode (see the PAD section for the Pulsed Amperometric mode). The Detector Schedule contains instructions used during programmed runs, and also allows manual control of the detectors. Note that the EC detectors must be enabled in the Detector Channel Options section before this detector schedule can be accessed.

- **EC SCHEDULE**
- **MANUAL OPERATION**

EC SCHEDULE

The EC Detector Schedule can be accessed through the Method drop-down Menu:





The schedule also can be reached by clicking the <<OK or OK>> buttons from other Method screens.

The schedule consists of time lines, edit boxes, and pushbuttons. For every time line there will be a subline for each EC detector enabled under Detector Channel Options. These will be labeled 1-4, corresponding to detectors W1-W4.

TIME is set in minutes, with a minimum interval of 0.1 minutes between lines. Every detector schedule must have at least two time lines: a 0.0 line and an end line. The length of a run is determined by the longest time line in any of the following modules in the Method: Pump, EC Detector, External Detector, PAD, or Switch Schedule. (Click here for a review of the Method modules.) At the end of the run, the detectors revert to the time 0.0 conditions.

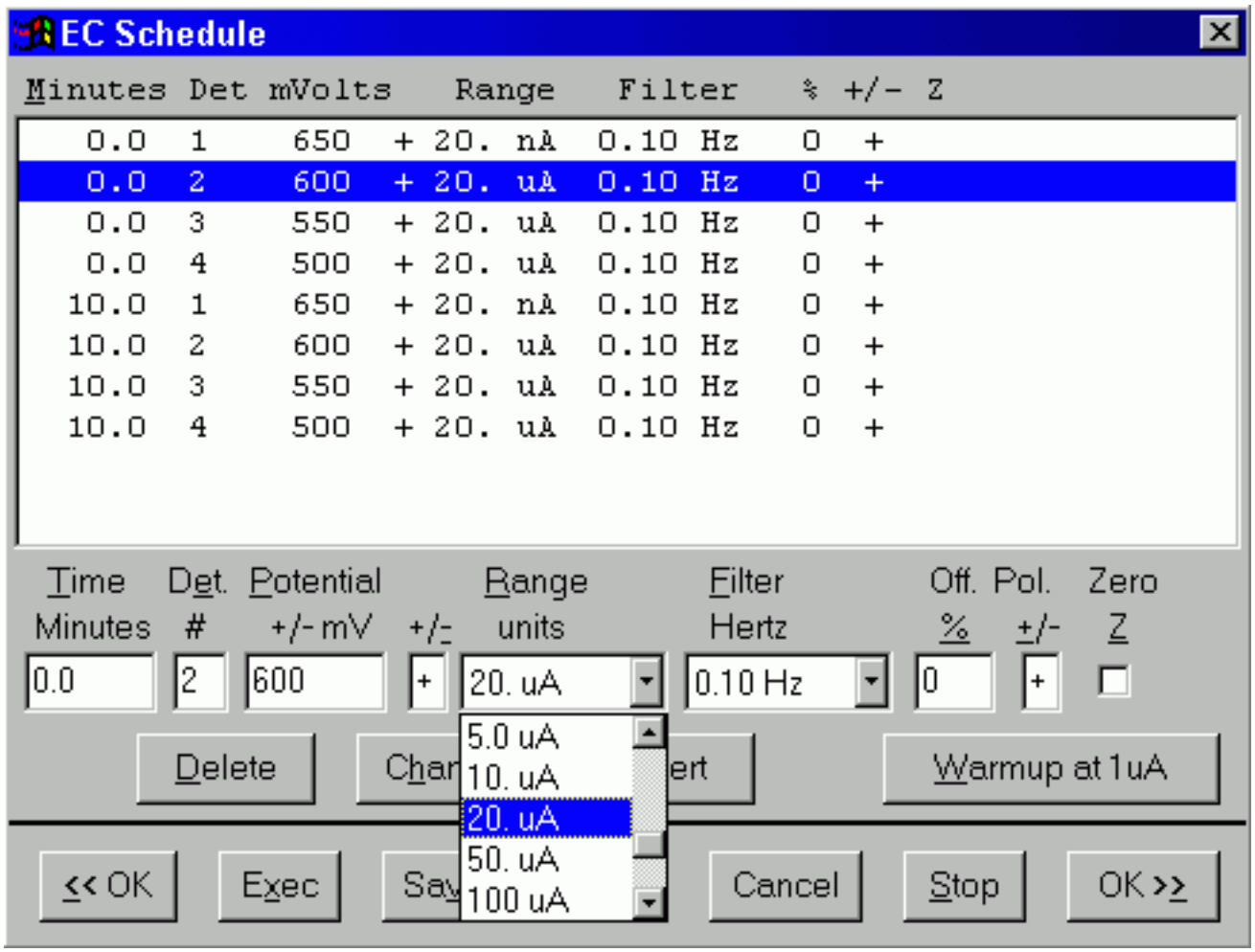
DET. is the detector number, corresponding to detectors W1-W4.

POTENTIAL is set by entering the voltage, in millivolts, in the edit box provided. Potentials must be in 10 mV increments. Use a minus sign (-) to indicate negative potentials.

+/- is the sign for the Range. Use a minus sign (-) for reductions, to make the peaks positive-going on the screen.

RANGE is the full-scale sensitivity of the detector, in Amperes full scale. Large values are less sensitive than small values. When in doubt, use a less-sensitive value, as small peaks can still be quantitated by ChromGraph Report. If you use a sensitive range and the peaks go offscale, that run is useless. Range is set via a drop-down listbox:

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FILTERis the noise-filtering setting for the detector, in Hertz. Large values give less filtering than small values. A value of 0.1 Hz is common. For determinations at or near the detection limits of the detector you should determine which filter setting provides the greatest signal-to-noise ratio (peak height / baseline noise) for your particular conditions. Filter is set via a drop-down listbox.

OFF. is an offset (in % of full scale) that can be applied to raise the baseline on the graph. Its most common use is to separate the traces of different detectors on the screen. Offset takes effect after a manual or programmed rezero.

POL. is the polarity of the analog signal that's available from the back-panel connectors. It's most common use is to change the pen direction on an external chart recorder. Changes here have no effect on the screen or on the data.

ZERO allows you to program an automatic rezero as each time line is reached during a programmed run. To rezero manually, use the Zero button in the Detector Status box.

The pushbuttons have their usual meanings as explained in the Using The Method Screens section. Two additional pushbuttons, Warmup and Stop, are explained below.

MANUAL OPERATION

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Manual control is needed when setting up, equilibrating, or performing maintenance on the detectors. The following functions are available:

WARMUP turns on the detectors at the insensitive range of 1 uAfs. ALWAYS turn on the detectors using the WARMUP mode, as this range can handle the surge of current at initial startup. The detectors will equilibrate faster if you use WARMUP.

When the Warmup button is pressed, a dialog opens that allows you to choose which detectors to turn on:



NOTE: No detector can be turned on unless its predecessor also is on (e.g., you can't turn on W2 unless W1 is turned on at the same time or already is on).

As always, the Detector Status box displays the current condition of the detectors.

EXEC. Once the detectors are equilibrated, then use EXEC to send the final conditions (line 1 of the detector schedule) to the detectors.

ZERO To rezero manually, use the Zero button in the Detector Status box.

STOP opens a dialog to turn off the detectors:



NOTE: No detector can be turned off unless subsequent detectors also are turned off (e.g., you can't turn off W1 unless W2 is turned off at the same time or is already off).

ALWAYS TURN OFF THE DETECTORS BEFORE TOUCHING THE CELL LEADS OR DISASSEMBLING THE ELECTRODES. STATIC DISCHARGES FROM YOUR FINGERS CAN DAMAGE THE UNIT'S AMPLIFIERS.

PULSED AMPEROMETRIC DETECTION

- **ABOUT PAD**
- **SETTING PAD OPTIONS**
- **PAD WAVEFORM FOR SUGARS**

ABOUT PAD

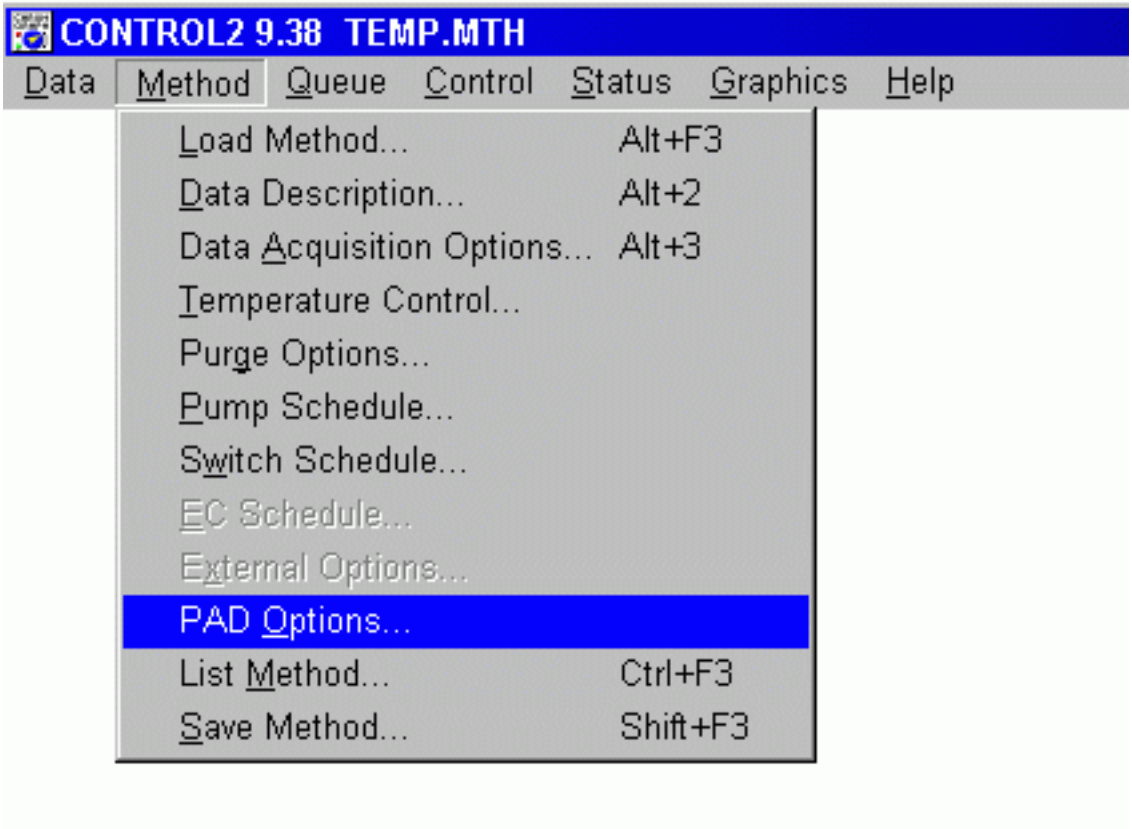
Pulsed Amperometric Detection (PAD) is a technique used to detect certain classes of compounds, notably sugars and polyalcohols, among others. These compounds tend to foul the surface of an electrode, making ordinary constant-potential amperometric detection difficult.

In PAD, cleaning potentials are applied to the electrode roughly once per second, interspersed with the detecting potential. The result is a waveform like the one pictured below.

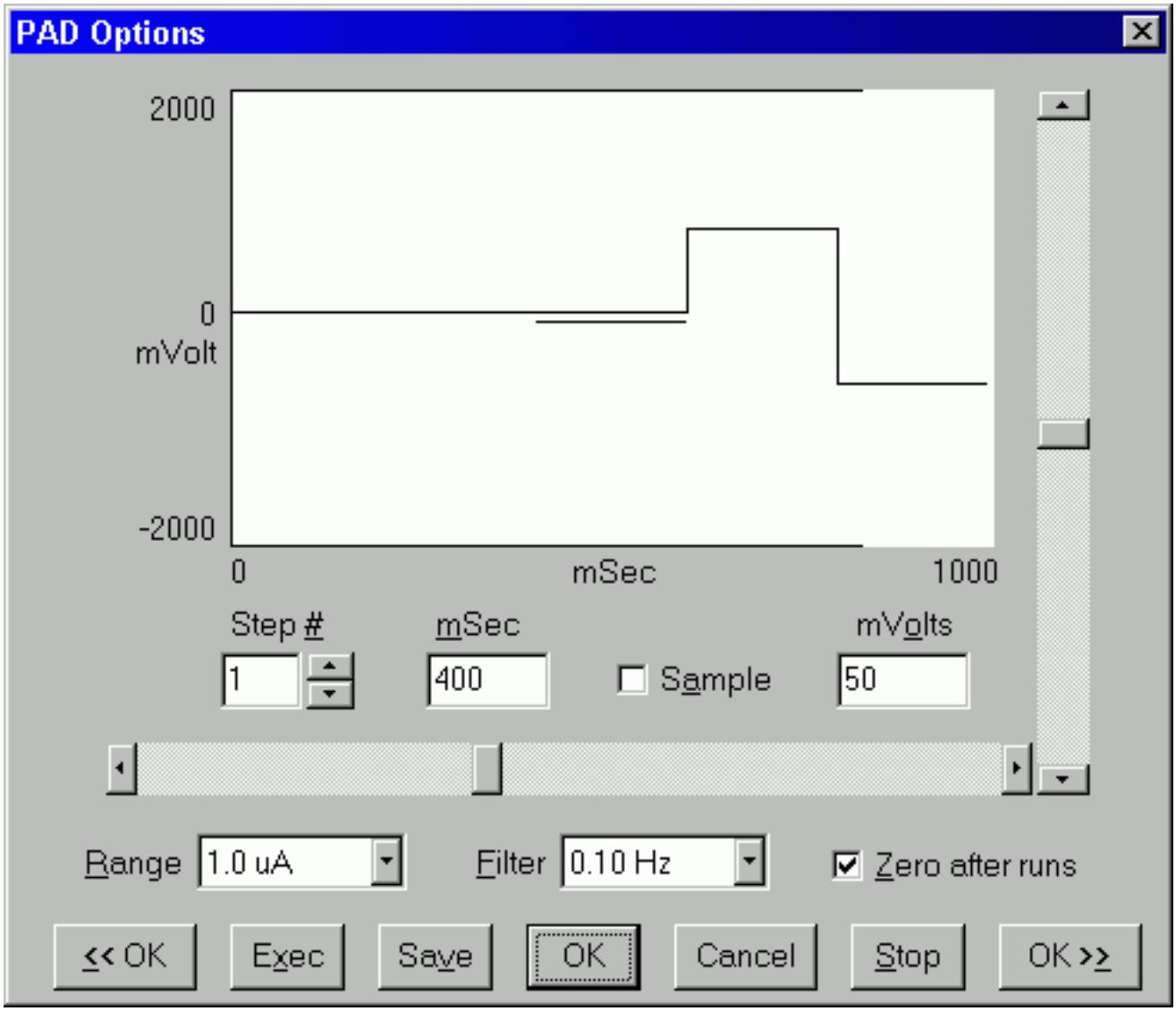
The application of cleaning potentials increases the background current and noise compared to what we've come to expect with constant-potential amperometric detection. This is because the electrode is never allowed to equilibrate for more than a few hundred milliseconds before it is pulsed again. Consequently, PAD is inherently unsuited to high-sensitivity analyses. We recommend using a range of no less than 1 µAfs for most PAD applications. This may result in extremely small peaks being visible during data collection. But there is enough resolution in ChromGraph that these peaks can be resolved and quantified in the data-analysis section, Report.

SETTING PAD OPTIONS

The PAD OPTIONS screen is reached through the PAD OPTIONS section of the Main Menu or by using the <<OK or OK>> buttons from other screens. If this section is grayed out you must first enable PAD in the Detector Channel Options section.



From the PAD OPTIONS screen you can interactively enter the PAD waveform, the range, and the filter:



STEP # is the sequential number of steps in the cycle, with each step having independent control of applied potential during its duration. Up to 16 steps are allowed. Enter a step number directly, or scroll to the desired step by clicking the up and down arrows with the mouse. Alternatively, clicking on any section of the graph brings its step into the edit boxes.

mSEC is the duration of a step, from 0-1000 milliseconds in 5 ms increments. A duration of 0-4 milliseconds will be interpreted as the end of a cycle.

SAMPLE, if checked, indicates that this is the step during which data will be collected.

mVOLTS is the potential to be applied during the step, from -2000 to +2000 millivolts in 10 mV increments.

RANGE is the gain range to be used during the SAMPLE step. We recommend a gain of no less than 1 µAfs for obtaining a stable baseline.

FILTER is the noise-reduction filtering to be applied to the data. The optimum filter setting can be determined by measuring signal-to-noise ratio (peak height ÷ baseline noise) at various filter settings, and choosing the filter setting that provides the highest ratio. But for most uses a value of 0.1 Hz. is adequate. (Large numbers provide less filtering than small numbers.)

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ZERO AFTER RUNS programs an automatic rezero at the end of each run. A rezero is needed if the peaks go offscale because of a high background. An alternative to using an automatic rezero is to use a sufficiently insensitive range (no less than 1 µAfs) so that peaks remain onscale.

The PAD waveform can be modified directly on the graph, by clicking on any section of the line and dragging it, or by using the scroll bars. During the dragging operation the values in the edit boxes change, allowing you to locate the line more precisely.

PAD WAVEFORM FOR SUGARS

A typical three-step pulse must be configured in four steps with the BAS PAD. This is because we allow more flexibility in setting the sampling duration and potential, so an extra step is required. For example, a typical carbohydrate waveform for a gold electrode requires the following four steps:

STEP	POTENTIAL	DURATION	FUNCTION
	(mV)	(mSec)	
1	50	400	Equilibration
2	50	200	Data
3	800	200	Cleaning
4	-600	200	Regeneration

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EXTERNAL DETECTORS

The epsilon LC detector provides inputs for two external detectors. Analog signals from the external detectors are digitized and processed in a similar fashion to the data from the EC detectors.

- **CONNECTING THE EXTERNAL DETECTORS**
- **CONFIGURING THE EXTERNAL DETECTORS**
- **SCALING THE CHROMATOGRAM**

CONNECTING THE EXTERNAL DETECTORS

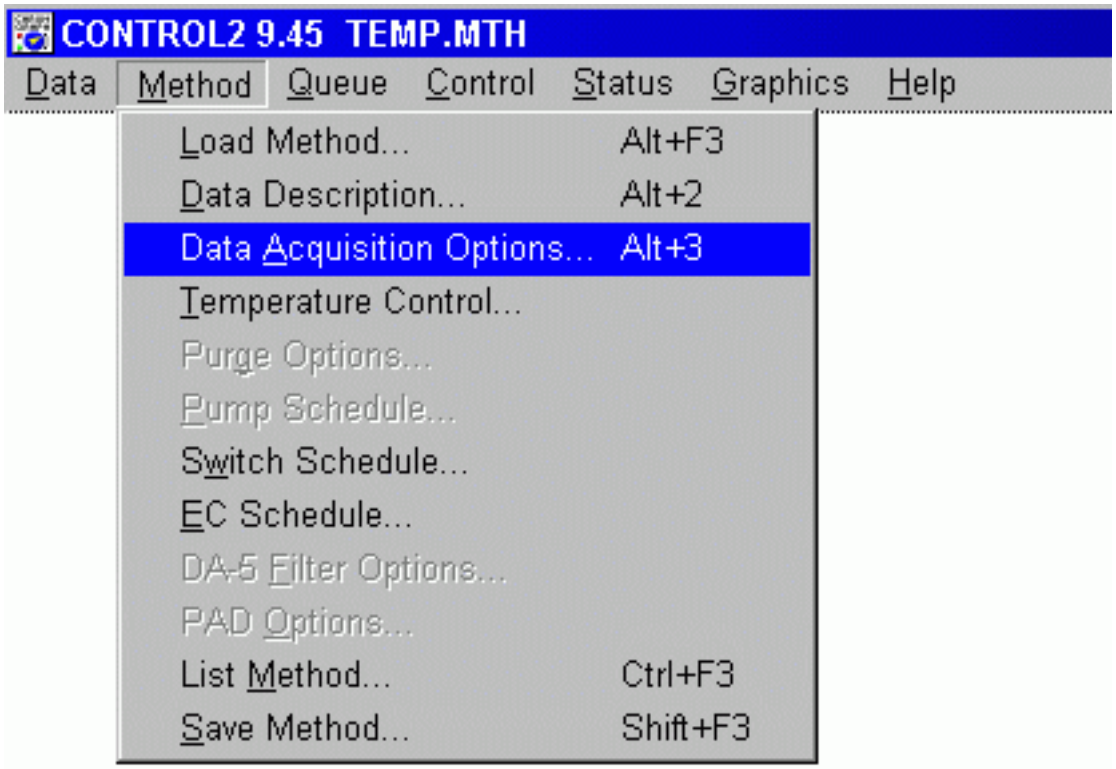
The epsilon detector will accept analog signals of either 0.1, 1.0, or 10.0 volts full scale. Connect the external detectors to the epsilon's back panel as shown here.

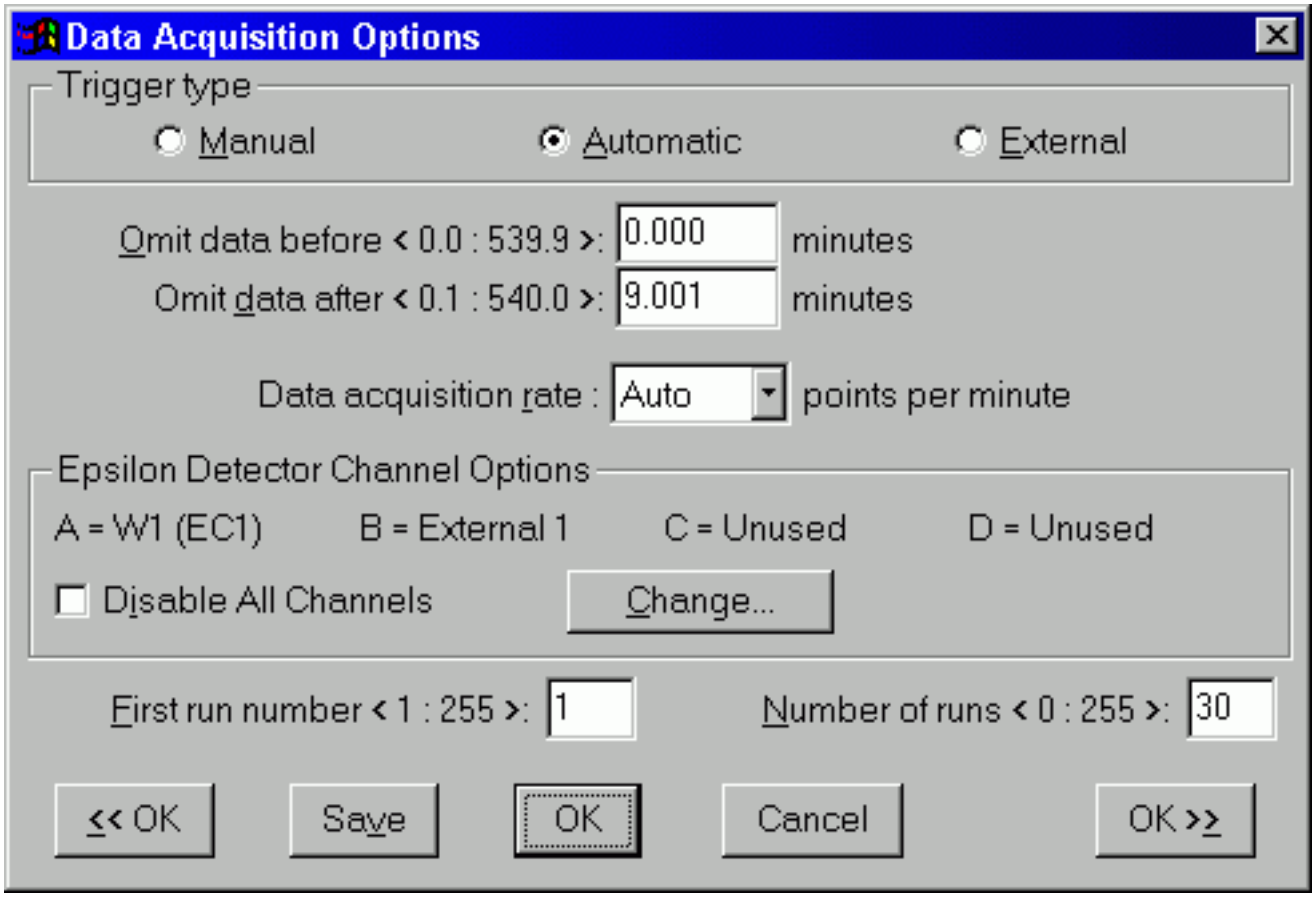
CONFIGURING THE EXTERNAL DETECTORS

External detectors are enabled and configured in the Detector Channel Options screen, which is reached as follows:

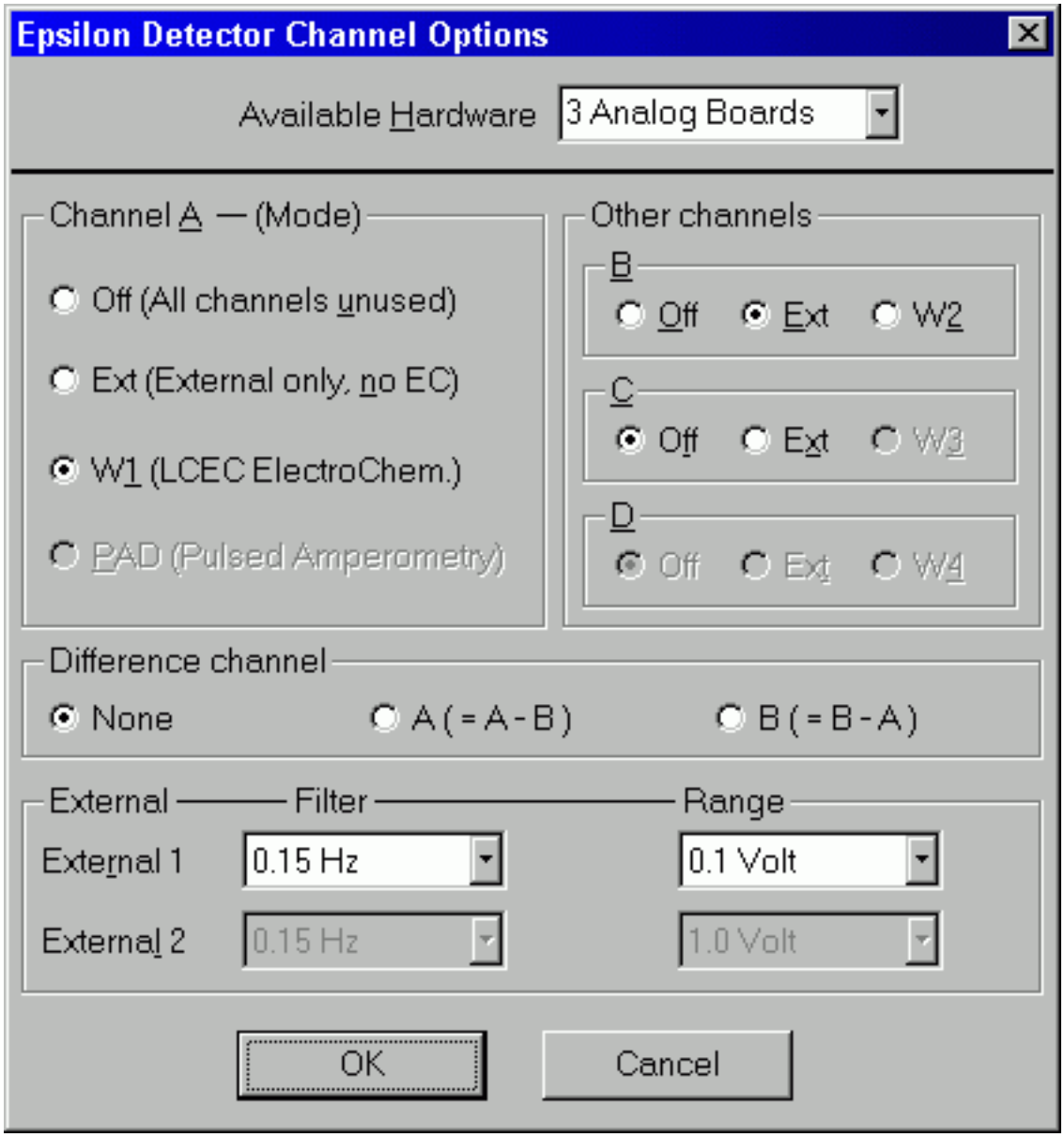
Open the Data Acquisitions Options screen, either through the Method drop-down menu, or by taking the <<OK or OK>> buttons from other Method screens:

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Click the CHANGE button to open the Detector Channel Options screen:



This screen is discussed in depth in the Data Options and Detector Selection section. The following items relate to configuring the external detectors:

FILTER is the noise-reduction filtering to be applied to the data. The optimum filter setting can be determined by measuring signal-to-noise ratio (peak height ÷ baseline noise) at various filter settings. But for most purposes a value of 0.1 Hz. is adequate. (Large numbers provide less filtering than small numbers.)

If your External Detector has its own filtering, you may disable the Epsilon filtering by setting it to the lowest setting (1.0 Hz.).

RANGE is the full-scale voltage to be expected from the incoming signal. Inputs of 0.1, 1.0, and 10 volts are provided. You should match the input range of the Epsilon to the output range of your External Detector. When in doubt, use a larger range to ensure that the detector's signal does not go off scale.

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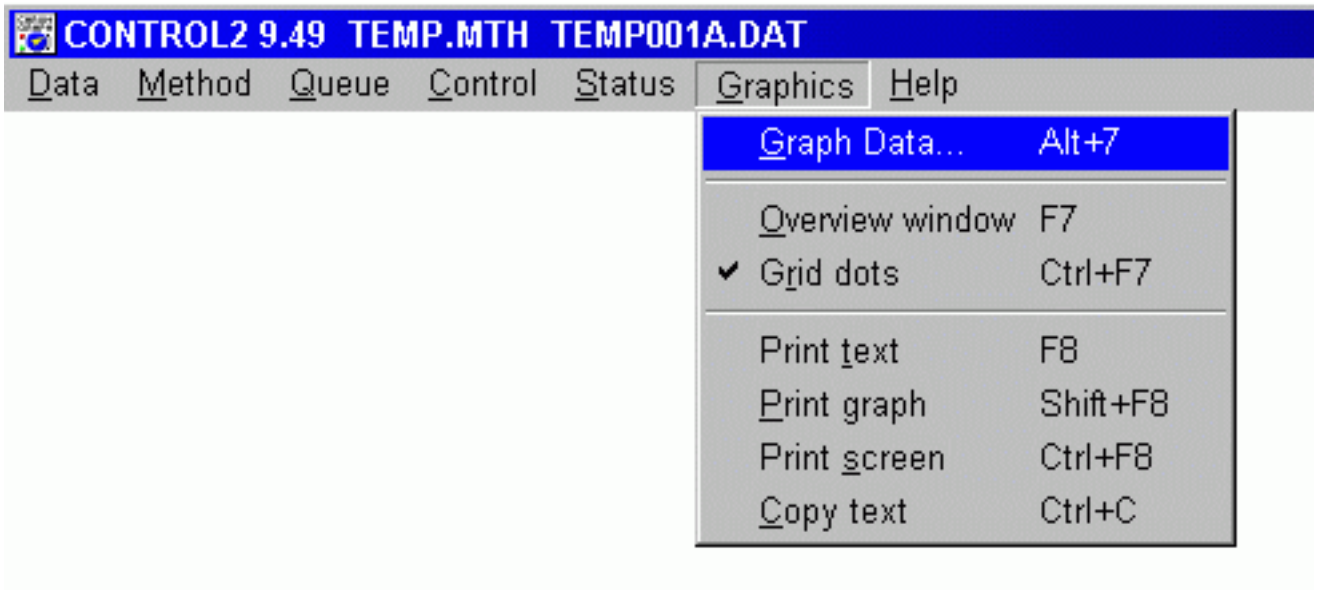
SCALING THE CHROMATOGRAM

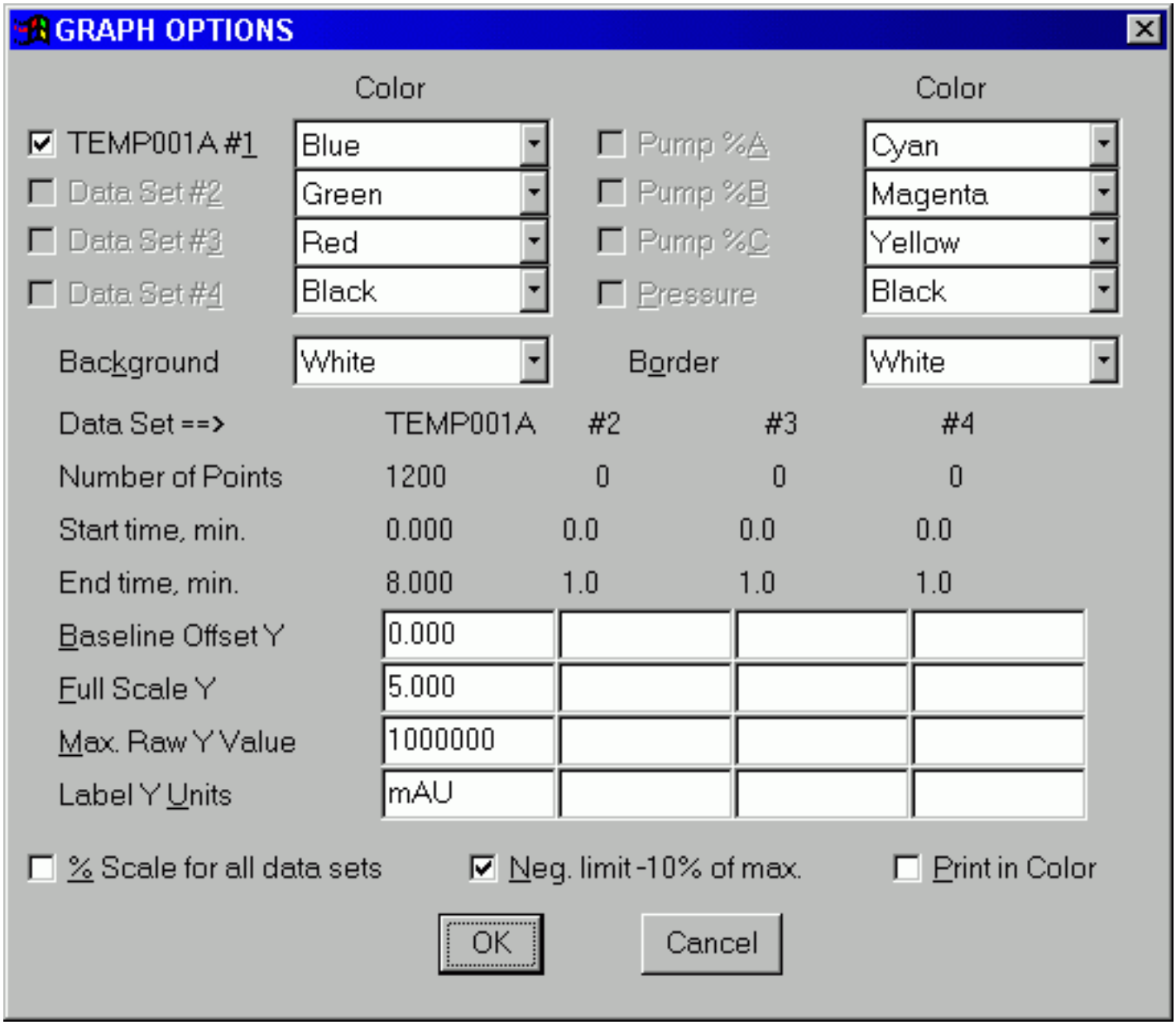
If not specified, the default scale for a single external detector will be Volts (or % Scale, for several detectors). For esthetic reasons you may wish to have the graph scaled in detector units. Proceed as follows:

First, the detector must be properly connected as detailed in the Installation section and configured as detailed above. Scaling will not be correct if the voltage output of the detector and the voltage input of the epsilon system are not matched.

Next, you must have one run in memory. Set the detector to the range you will use and start a run. You need not inject anything.

When the run is complete, take the Graph Data option under the Graphics menu:





For whichever data set corresponds to the external detector(s), change the Full Scale Y value to indicate the range of the detector (e.g., 5 if the detector is set to 5 mAU). Then change the Label Y Units as appropriate (e.g., mAU).

The new scale will remain in effect until changed again, or until you exit and start a new session.

The scaling changes you've entered will be saved with the data files and carried over into Report, so any graphs generated by Report will have the correct detector units as well.

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AUTOSAMPLERS

The epsilon LC detector can communicate with autosamplers for automatic operation. Back-and-forth triggers are provided to start either instrument from the other. In addition, ChromGraph Control can read the vial position from an appropriately equipped BAS autosampler and save it, along with user-provided sample information, with the data for each run.

For successful automatic operation, you must ensure that the computer does not switch to an energy-saving mode. Please read the section on Setting Power-Management Options.

- **THREE WAYS TO COMMUNICATE**
- **METHOD 1: epsilon IN CHARGE**
- **METHOD 2: AUTOSAMPLER IN CHARGE**
- **METHOD 3: FULL HANDSHAKE**
- **IMPORTING SAMPLE INFORMATION FROM A .RAN FILE**

THREE WAYS TO COMMUNICATE

There are three basic methods of coordinating injections with an autosampler. Each has its advantages and disadvantages. The three methods are:

- **METHOD 1.** epsilon is in charge.
- **METHOD 2.** Autosampler is in charge.
- **METHOD 3.** Full handshake -- neither instrument can start without a trigger from the other

The advantages and disadvantages relate to how easy it is to hook up and troubleshoot the connection, and to the consequences if something goes wrong with your equipment. What can go wrong? What if a momentary power outage causes the computer to reset, but not the autosampler? What if you miscalculate and run out of mobile phase overnight? What if you put one vial in the wrong place and the autosampler stops with a 'missing vial' error?

The advantages and disadvantages of each method are as follows:

- **METHOD 1.** Simple to connect and troubleshoot. If the chromatograph runs into trouble, the autosampler does not inject the remaining samples. If the autosampler runs into trouble, the chromatograph continues to make runs, but they are all blank.
- **METHOD 2.** Simple to connect and troubleshoot. If the autosampler runs into trouble the chromatograph will stop making runs. But if the chromatograph runs into trouble the autosampler continues to inject until ALL THE SAMPLES ARE LOST.
- **METHOD 3.** Complex to connect and troubleshoot. If either instrument runs into trouble, the other will stop making runs or injecting samples.

We recommend Method 1 for routine use, and Method 2 for the BAS Pollen-8 On-Line Injector.

METHOD 1: epsilon IN CHARGE

Method 1 can be used with any autosampler that will load a sample and wait for an inject trigger. This includes the BAS Sample Sentinel autosampler. Method 1 is a safe method of communication because the autosampler cannot inject the samples on its own.

Connect the Sample Sentinel autosampler to the epsilon system as described in the Installation section.

For a non-BAS autosampler, connect a two-wire cable from the START-OUT screw terminal and its ground on the rear panel of the epsilon detector:

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The START-OUT connection provides a TTL-low at the start of each run. Connect the other end of the cable to the SAMPLE ENABLE terminals on the autosampler.

Program the autosampler so it will end its run and load the next sample before the epsilon system has completed its run (about a minute shorter than the epsilon system's maximum run length). Run length is the longest time of any of the following modules in the Method: Pump, EC Detector, External Detector, PAD, or Switch Schedule. Click here for a review of the Method modules.

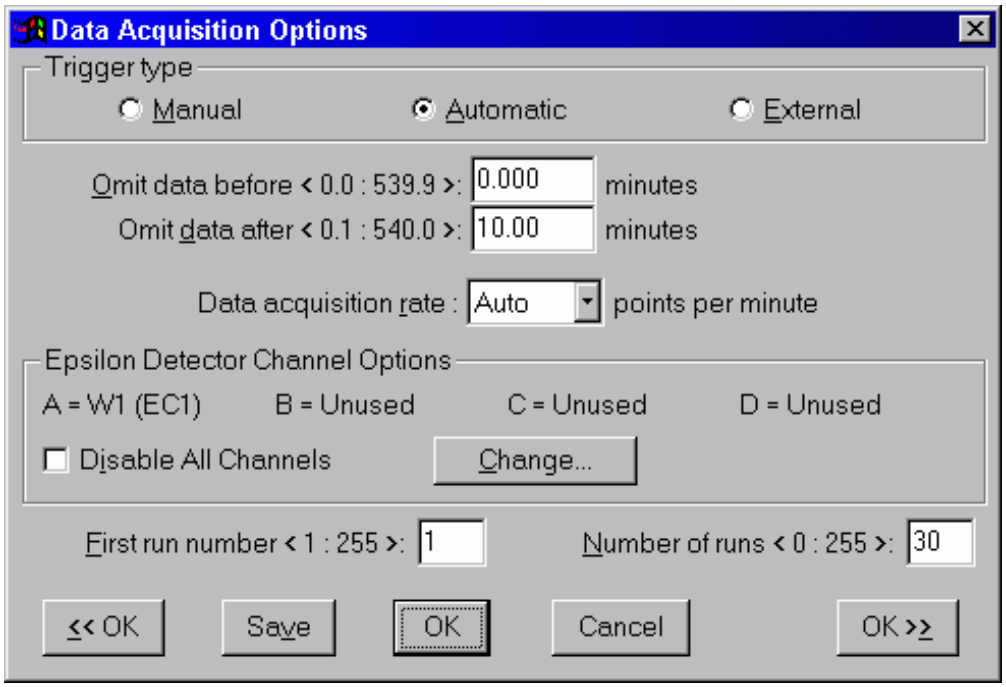
The autosampler must be instructed to wait for an inject trigger before injecting. For the BAS Sample Sentinel, this is done as follows:

Select **OPTIONS** from the Main Menu

Select **INPUT POLARITY** from the Options Menu

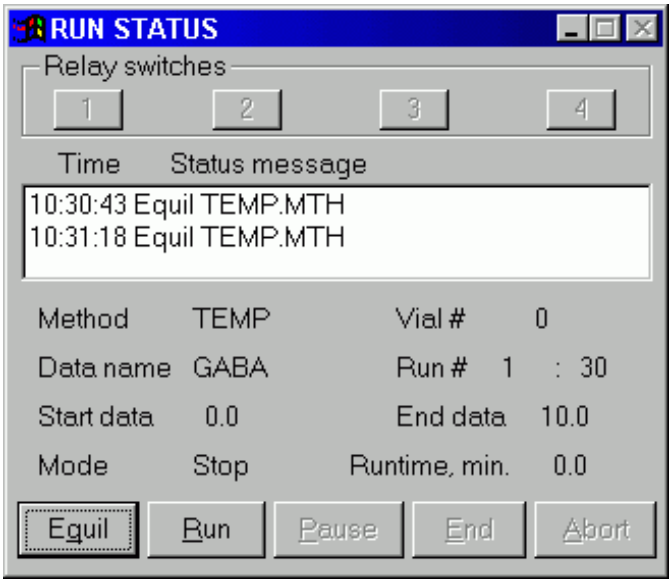
Change **INJECT HOLD ACTIVE** from **LO** to **HI**

Now program the epsilon system to run automatically. Set the Trigger Type to automatic in the Data Acquisitions section of the Method, and specify the number of runs:



Start the Method from the RUN STATUS box by clicking EQUIL, and then RUN:

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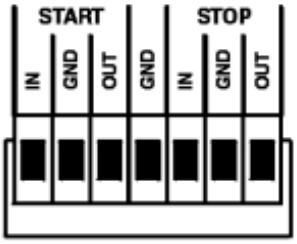


The epsilon system will proceed automatically from one run to the next, and send a start signal to the autosampler each time. It is imperative that the autosampler end its previous run, load the next sample, and be in a 'hold' mode when the epsilon system sends the trigger.

METHOD 2: AUTOSAMPLER IN CHARGE

If the autosampler will not accept an inject trigger, then the autosampler must be in charge of the run. Use this approach for the BAS Pollen-8 On-Line Injector. One drawback here is that the autosampler will have no input from the chromatograph, so it will continue to inject samples if the chromatograph malfunctions.

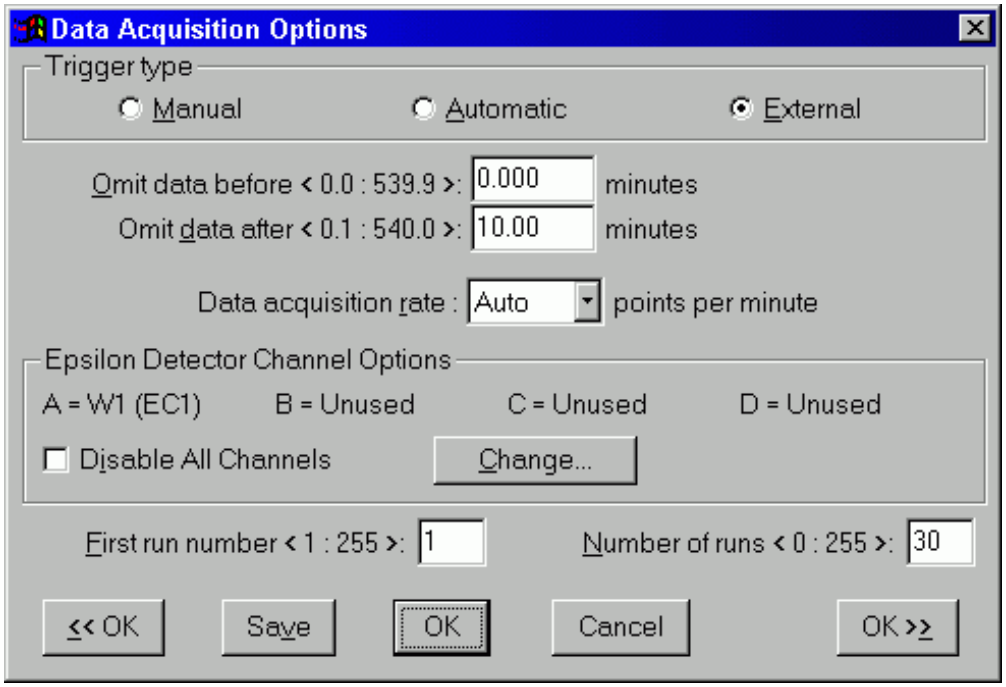
To configure the system with the autosampler in charge, use a two-wire cable. Connect one end to the START-IN screw connector and its ground on the rear panel of the epsilon detector:



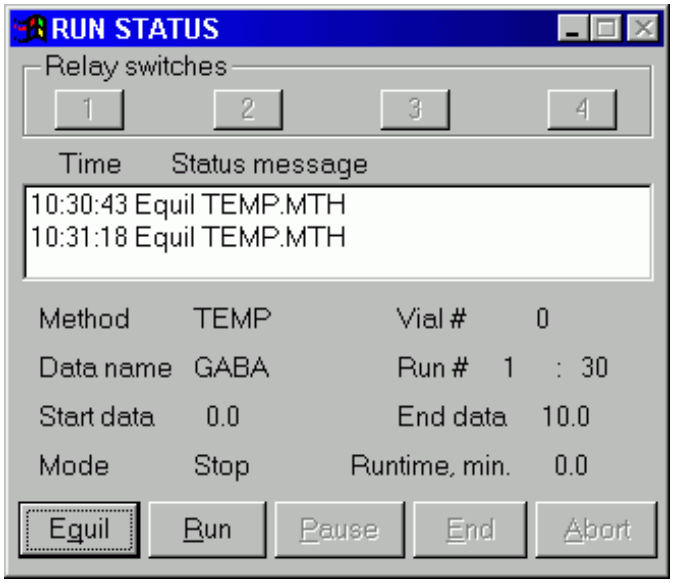
The START-IN connector accepts either a switch closure or a TTL-low signal. Connect the other end of the cable to the appropriate terminals on the autosampler. The autosampler's run must be about a minute longer than the maximum run length of the epsilon system, so the chromatograph is waiting for a trigger when the autosampler is ready to inject. Run length is the longest time of any of the following modules in the Method: Pump, EC Detector, External Detector, PAD, or Switch Schedule. Click here for a review of the Method modules.

Program the epsilon system to accept an external trigger in the Data Acquisition Options section of the Method, and set the number of runs as appropriate:

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Start the system by clicking EQUIL and then RUN in the RUN STATUS box:



The message 'waiting for trigger' will appear. Then start the autosampler.

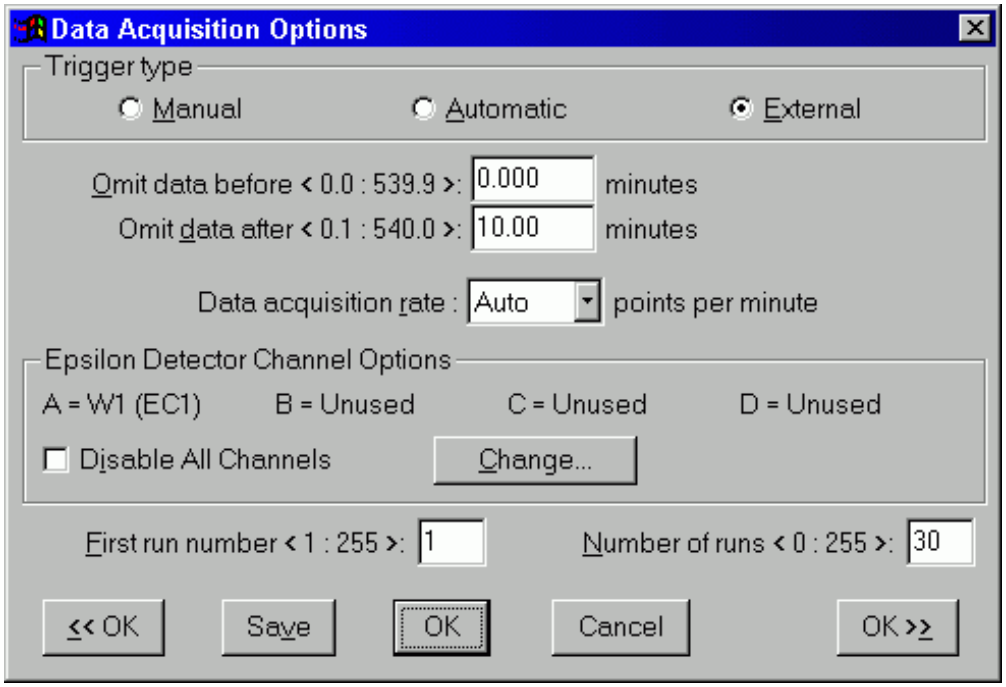
METHOD 3: FULL HANDSHAKE

The BAS Sample Sentinel autosampler is capable of two-way communication with the epsilon system. The autosampler will not inject the sample until the chromatograph is ready to start its run, and the chromatograph will not start its run until the autosampler is ready to inject. Samples are thus safeguarded in the event of mechanical failure or depletion of mobile phase.

Connect the Sample Sentinel autosampler to the epsilon system as described in the Installation section. Once this is done the two units must be programmed to accept signals from each other, as follows:

The epsilon system should be set to accept an External trigger in the Data Acquisition Options section of the Method, and the number of runs must be specified:

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The epsilon system's run length should be about a minute shorter than the autosampler's. Run length is the longest time of any of the following modules in the Method: Pump, EC Detector, External Detector, PAD, or Switch Schedule. Click here for a review of the Method modules.

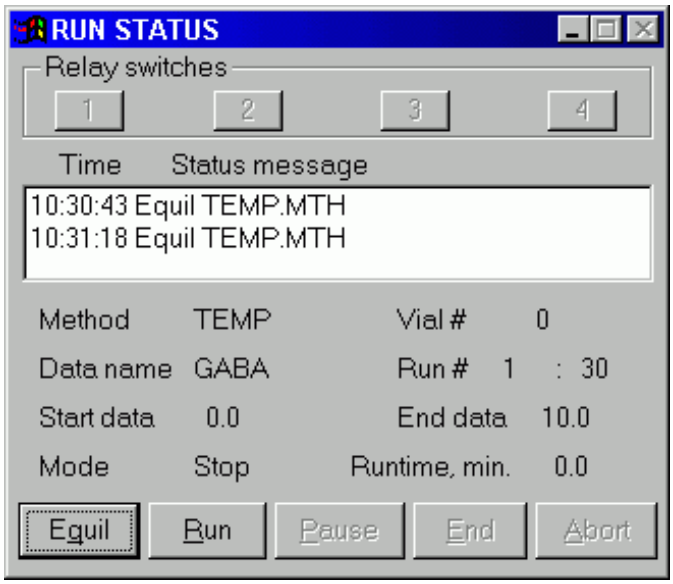
The Sample Sentinel must be set so that it waits for a signal from the epsilon system before injecting. Change INJECT HOLD ACTIVE to HI in the OPTIONS sections of its menu, as follows:

Select **OPTIONS** from the Main Menu

Select **INPUT POLARITY** from the Options Menu

Change **INJECT HOLD ACTIVE** from **LO** to **HI**

For the timing to work properly, it is imperative that the epsilon system be in the 'waiting for trigger' mode when the Sample Sentinel injects. Therefore, you must start the epsilon system first by clicking EQUIL, then RUN, in the RUN STATUS box:



Then start the autosampler. The complete sequence is as follows:

- epsilon system goes into 'waiting for trigger'.
- Sample Sentinel loads the sample, goes into hold, and sends a trigger to the epsilon system.

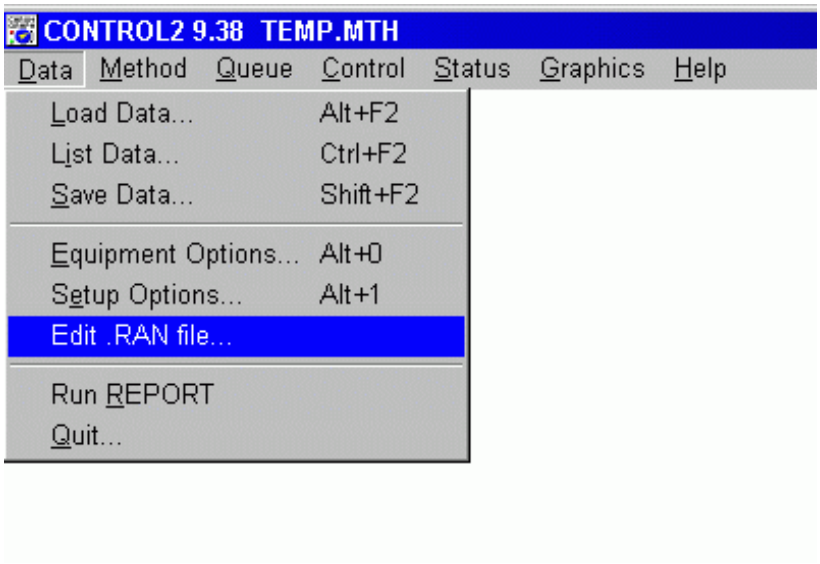
- epsilon system starts, sending a trigger to Sample Sentinel, which injects sample.
- epsilon system ends its run before the Sample Sentinel, goes into 'waiting for trigger' for next run.
- Sample Sentinel loads its next sample, signals epsilon system.

IMPORTING SAMPLE INFORMATION WITH A .RAN FILE

ChromGraph Control will take input from a text file with a .ran extension and save this information along with the data. This allows you to permanently associate sample information such as patient name with the data.

The positioning of information in this file is critical, so we recommend that you copy our template, TEMPLATE.RAN, from the BAS directory and rename it in your data directory. You can then edit it to add your own information.

It is important that the .ran file be in text format, so all characters and spaces have the same width. Use any text editor to make the modifications. Alternatively, Control will call Windows Notepad if you take the EDIT .RAN FILE option under the DATA menu:



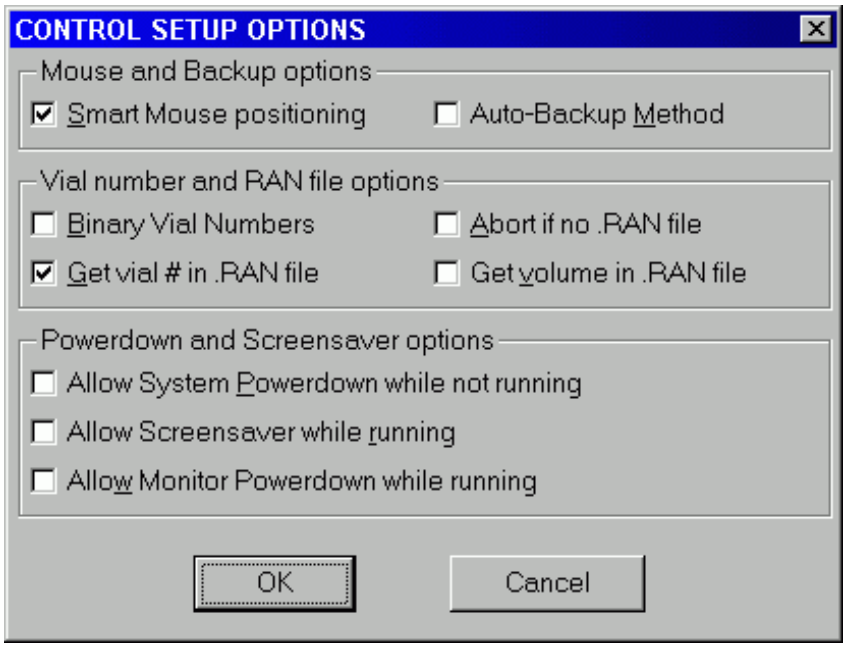

```
BAS - ChromGraph Sequence.RAN file (created:01/16/99 - 4:00:00 PM)
-------------------------------------------------------------------
TITLE: Demo .ran file                       PROJECT #:
ANALYST: Solomon                            RANDOM FILE:
# OF SAMPLES: 7                             AUTOSAMPLER CAPACITY:
NORMAL VOLUME: 10 uL                        NOTEBOOK #:
                                            PAGE(S):

<--RUN--> <--VIAL-> <VOLUME-> <----------SAMPLE ID---------------->
1         1         0         Low Standards
2         2         0         High Standards
3         3         0         Low QC Sample
4         4         0         High QC Sample
5         5         0         Unknown #298
6         6         0         Unknown #237
7         7         0         Unk own #272
```

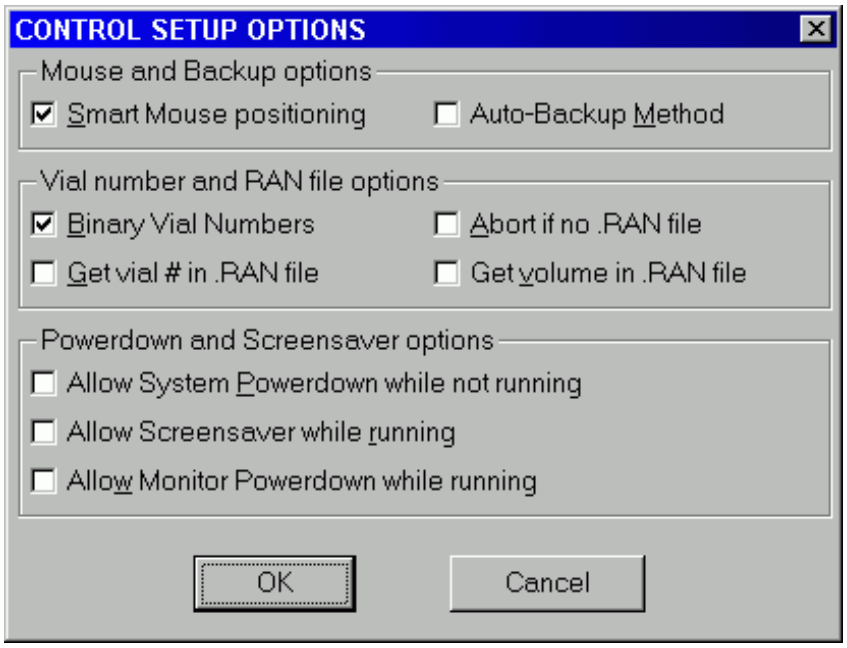
ChromGraph ignores the first 10 rows and 15 columns of the .ran file, including the heading, where you can enter comments. Information about the samples begins in row 11, column 16. The following information MUST be found in the specified columns, left justified.

Run Number: (Columns 16-25) If you do not have the vial-position option that sends the vial number to Control, Control will examine this column at the start of each run. If a run number anywhere in this column matches the current run number, Control will incorporate the

relevant sections of that line into the data file. (Including the vial number, which you should ensure is accurate.) You must configure SETUP OPTIONS under the Data menu as follows for this to work properly: check the 'Get vial # in .RAN file' option.



Vial Number: (Columns 26-35) If you do have the vial-position option, then Control will examine this column instead of the Run Number column, at the start of each run. If a vial number anywhere in this column matches the number being sent by the autosampler, Control will incorporate the relevant sections of that line into the data file. You must configure SETUP OPTIONS under the Data menu as follows for this to work properly: check the 'Binary Vial Numbers' option.



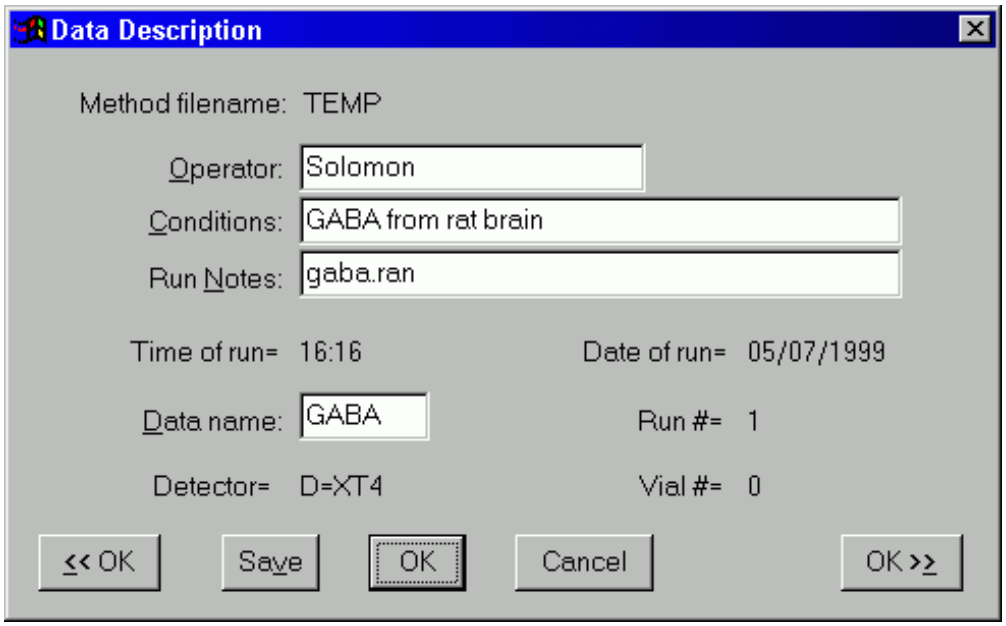
Volume: (Columns 36-45) These numbers allow ChromGraph Report to adjust the results for each sample to account for variable volumes or sample weights. If this is not the case, put a zero in this column.

If volume does differ among samples, you can enter the volume for each sample. You must also indicate this in Setup Options (see figure immediately above). See ChromGraph Report for information on how these volumes are used. If a volume is entered, Control will import it, and any text that follows it, into the Run Notes section of the data. This reduces the space available for Sample ID (see below) unless you move the ID's over so they follow the volumes, with one space between.

Sample ID (Columns 46-80) Unless variable volumes are used, these columns are imported into the Run Notes section of the data and are appropriate for appending identifying information about each sample.

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The .ran file should be placed in the default data directory where the Method and data files reside. Then edit the Method to insert the name of the .ran file into the Run Notes section of DATA DESCRIPTION:



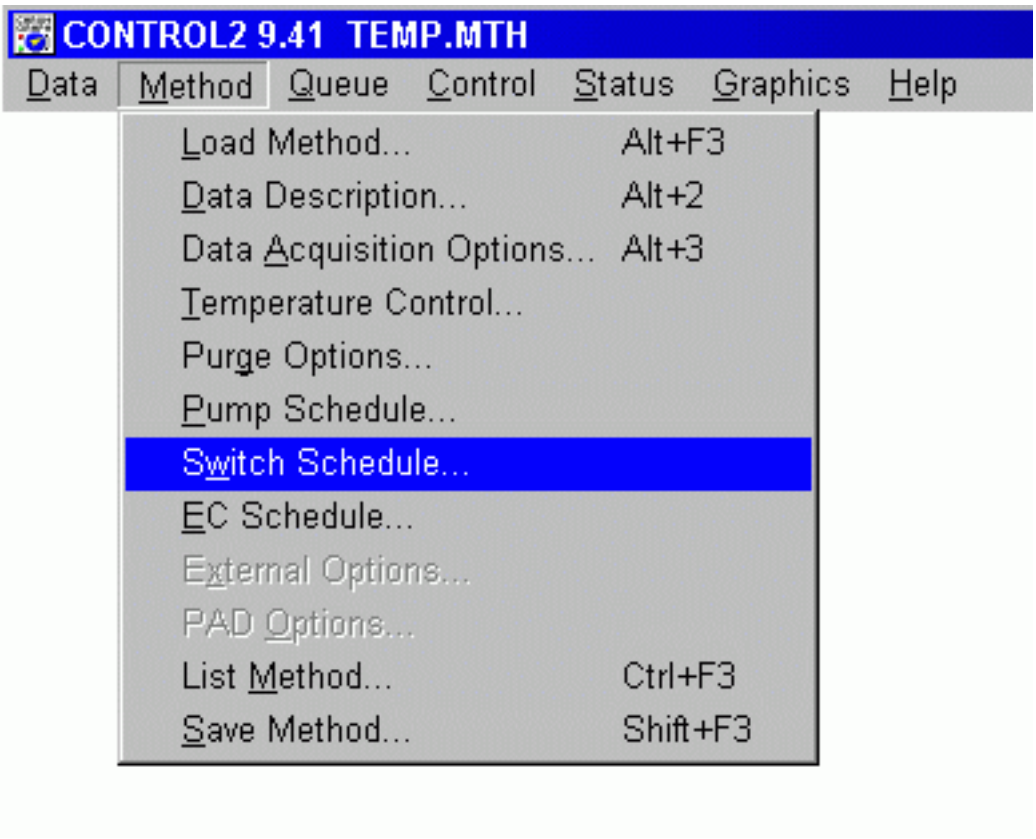
As each run is started, Control will import the relevant information from the matching line in the .ran file.

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SWITCH SCHEDULE

The epsilon system provides a set of four relay switches that may be opened or closed to control peripheral equipment such as external detectors. All connections are made to the Event Connector on the back panel of the epsilon detector.

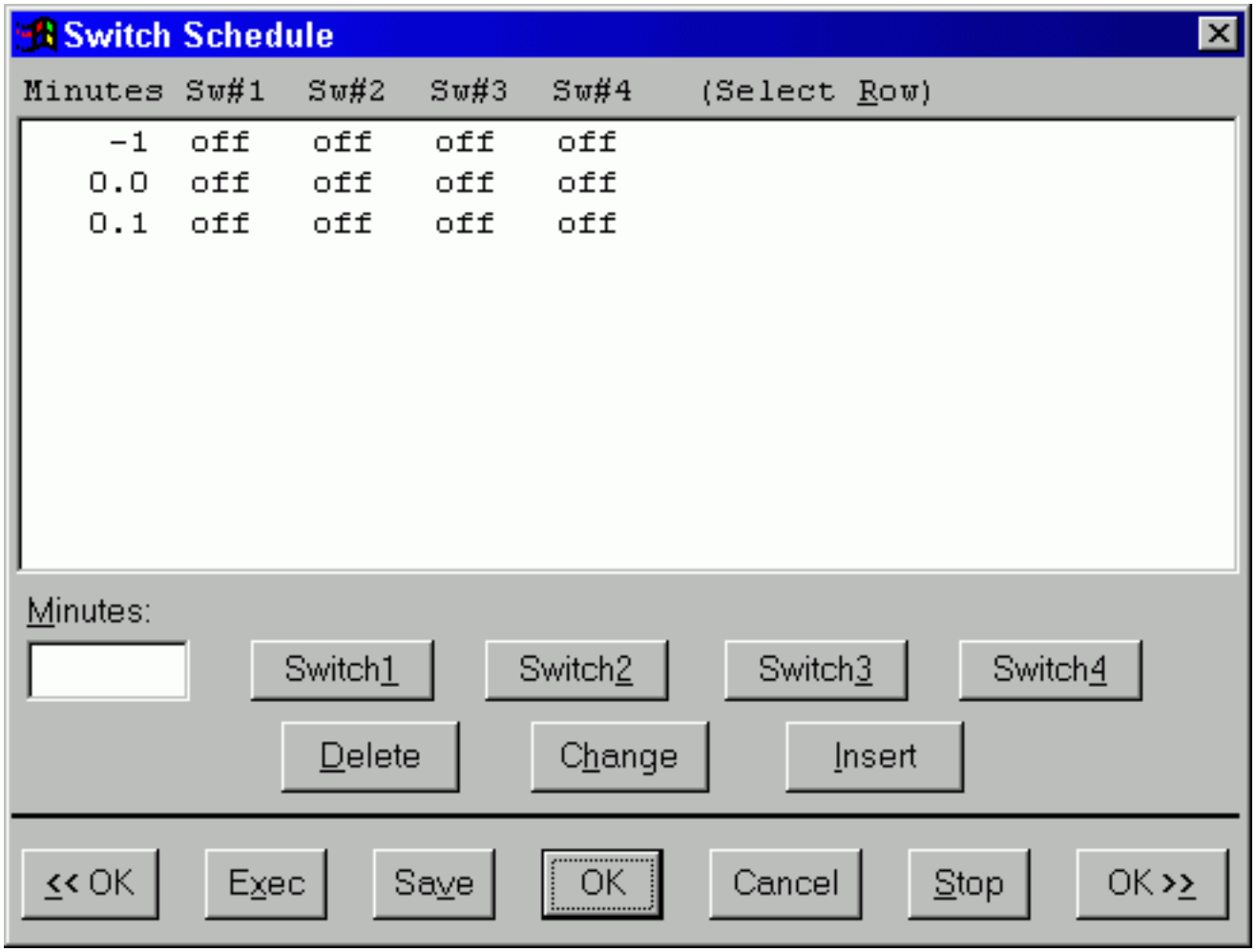
Control of the switches is via the Switch Schedule section of the Method, which can be accessed through the Method drop-down menu:



Alternatively, the Switch Schedule can be reached from other Method screens by pressing the <<OK or OK>> buttons.

The Switch Schedule is a listbox of times, each line indicating the on/off (closed/open) status of each switch:

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The -1 line is necessary to establish initial conditions, allowing the switches to change their state at the start (0.0 min) of a run . Without the -1 line, the system could not send a start trigger to a peripheral. For most purposes all the switches on the -1 line should be off.

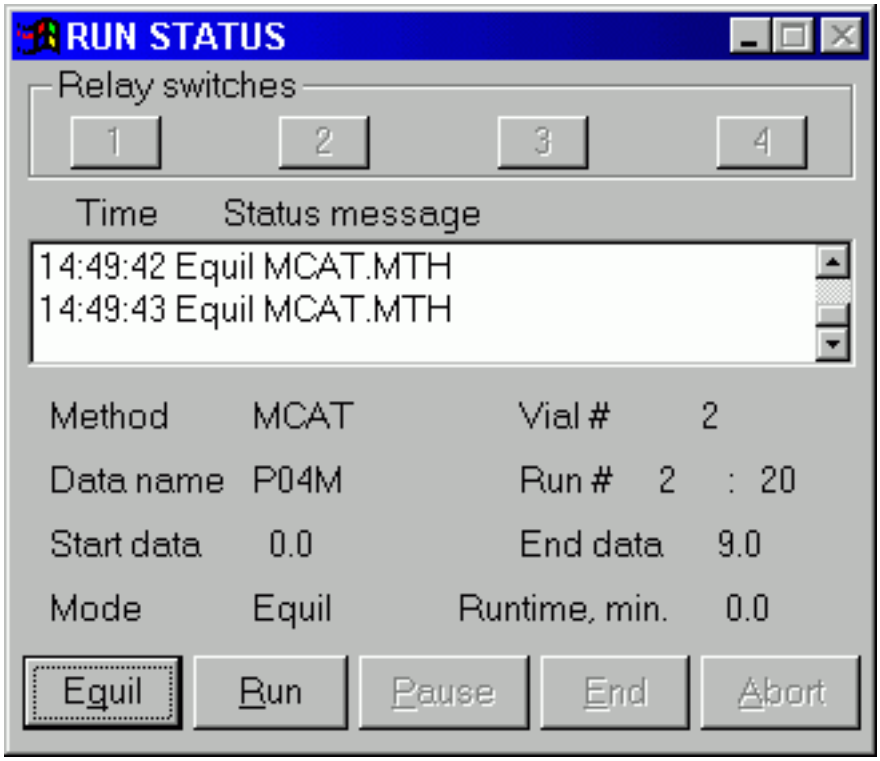
Use the listbox and its editing features to turn switches on or off at particular times. Click on a line to edit it, or enter a new time in the box. Depress (on) or release (off) the appropriate switches and press Change to enter the change. If it's a new line, press Insert.

The buttons have the usual features as detailed in the USING THE METHOD SCREENS section. However, the Exec button puts the -1 line into effect rather than the 0.0 line.

For most applications, turning a switch on at the start of a run (0.0 min) and off six seconds later (0.1 min) provides a sufficient signal. Similar logic governs signals that are sent during or at the end of the run.

The Run Status box shows the state of the switches at all times (depressed = on, released = off).

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RUN STATUS
Relay switches
1
2
3
4
Time
Status message
14:49:42 Equil MCAT.MTH
14:49:43 Equil MCAT.MTH
Method
MCAT
Vial #
2
Data name
P04M
Run #
2
: 20
Start data
0.0
End data
9.0
Mode
Equil
Runtime, min.
0.0
Equil
Run
Pause
End
Abort

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ELECTRONIC SELF-TEST FOR EPSILON DETECTORS

The epsilon detector contains two built-in resistors that can be substituted for the analytical flowcell. This will allow you, in conjunction with BAS technicians, to verify that the instrument's circuits are performing properly. Such a test can aid in determining whether performance problems are related to the application, the flowcell, or the instrument's electronics.

The test involves switching to the test resistors, running a set of pre-configured test methods, and comparing the resulting chromatograms with expected results. **YOU MUST THEN SWITCH BACK TO THE ANALYTICAL FLOWCELL** to return to normal operation.

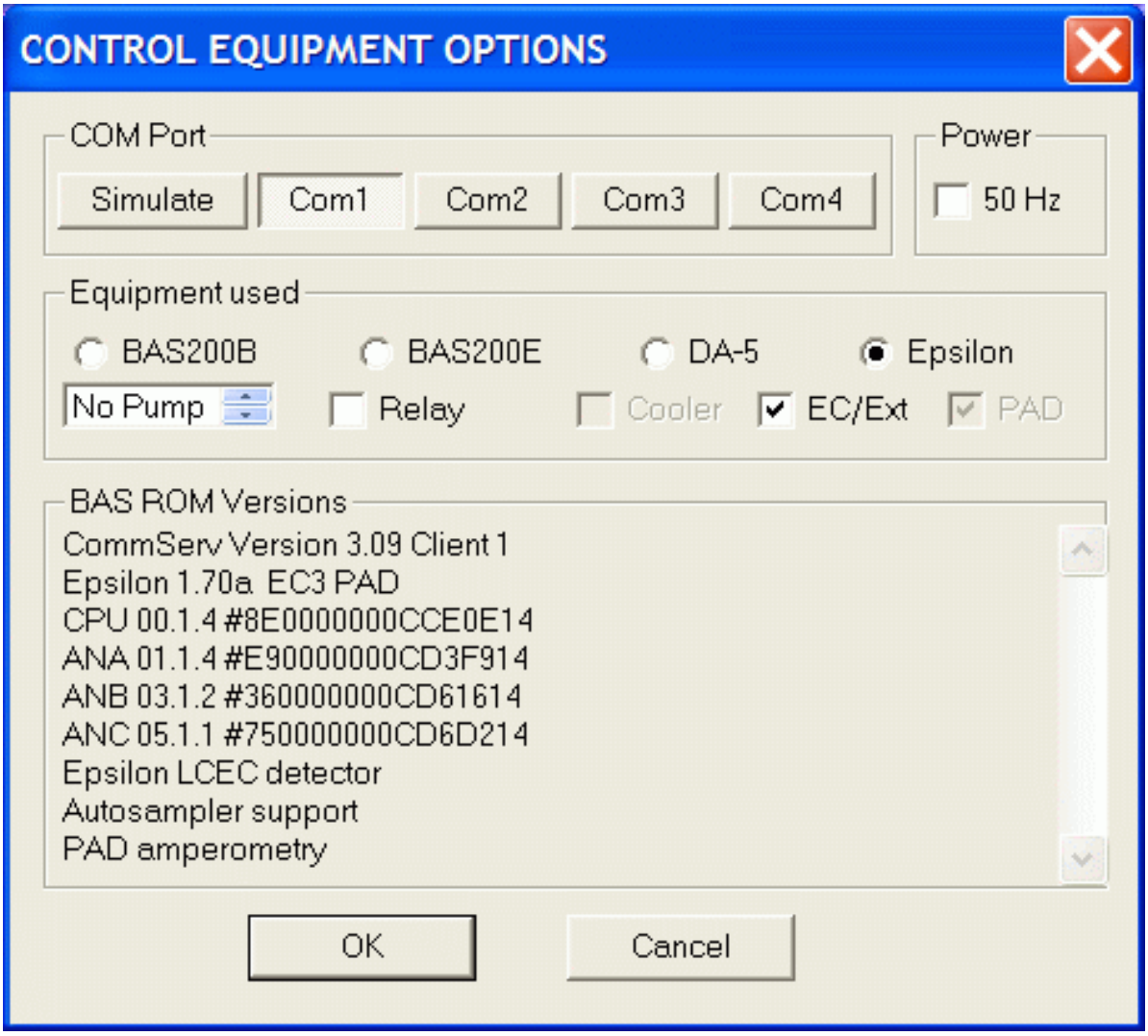
- **PREPARING FOR THE TEST**
- **RUNNING THE TEST**
- **EVALUATING THE RESULTS**

PREPARING FOR THE TEST

Before running the test you must obtain some basic information about the hardware and software versions in the epsilon (Equipment Options), and the number of channels of data collection available (Number of Analog Boards).

EQUIPMENT OPTIONS. Obtain the list of equipment options by opening the Data/Equipment Options drop-down menu from the main screen of Control:

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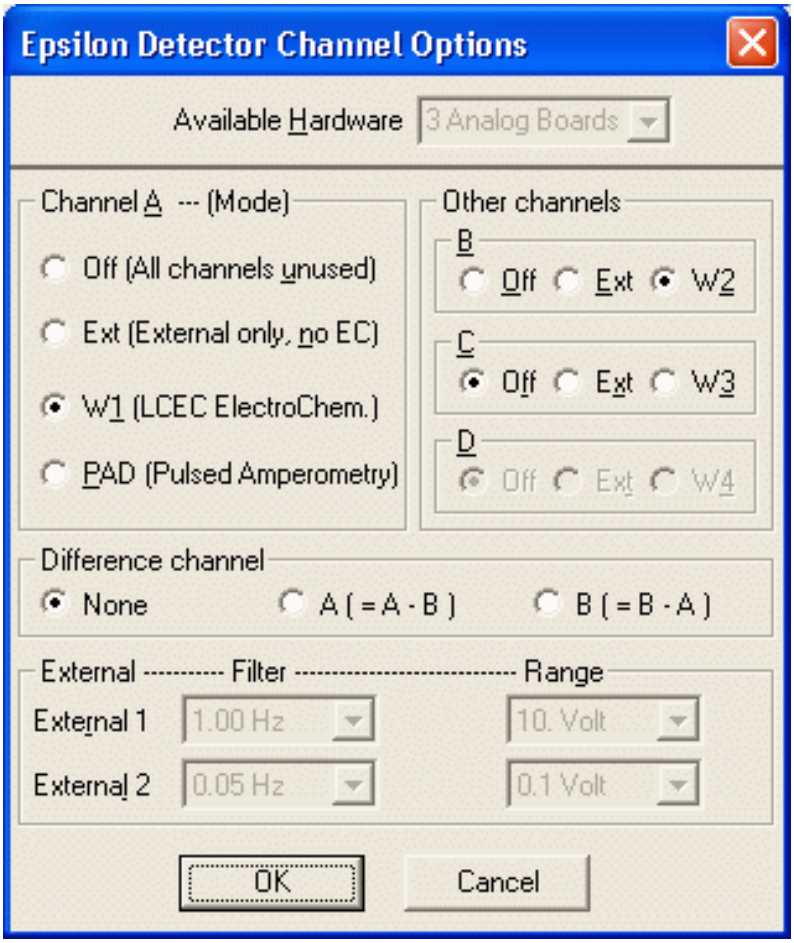


Make sure that the list indicates ROM version Epsilon 1.70 or greater. **The self-test will not run on earlier versions.**

Make a copy of this information by holding down the Alt-key and pressing the Print Screen key of your keyboard. Then open either a word-processing program, a spreadsheet program, or an image-manipulation program and paste (Ctrl-V) the copied image. You can then save and/or print the image.

NUMBER OF ANALOG BOARDS. Obtain this information by opening the Method/Data Acquisition Options drop-down menu from the main screen of Control, then pressing the 'Change' button:

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You need not copy this information, just note the number of analog boards indicated at the top of the screen. It will be either one (one-channel of data collection), two (two channels) or three (four channels).

RUNNING THE TEST

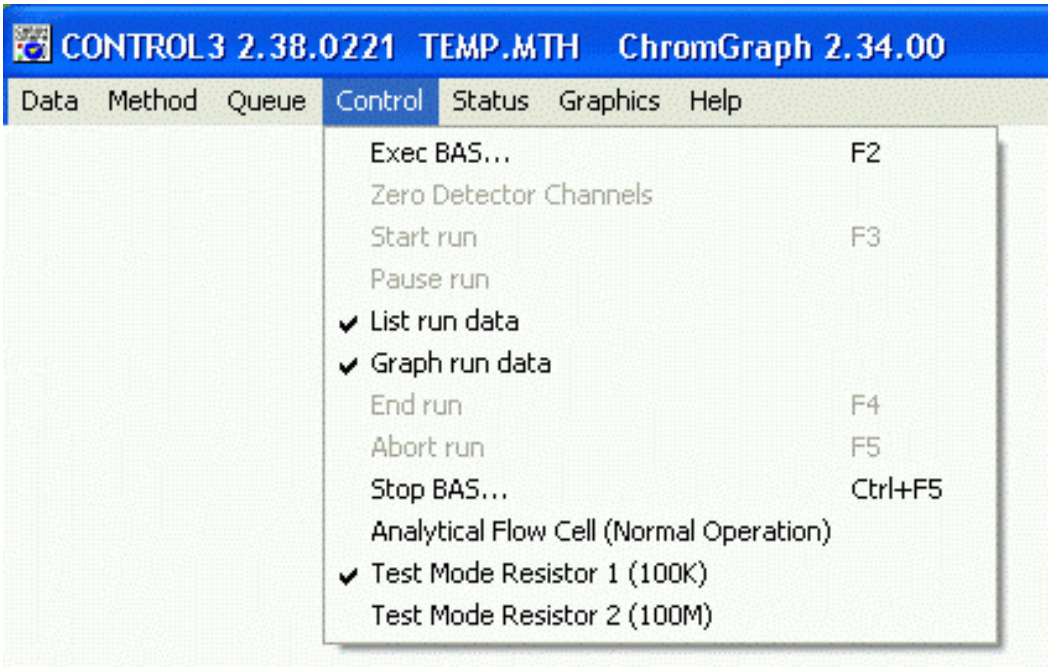
Running the self-test requires five steps:

1. **Switch to test resistor 1**
2. **Load, Equil, and run a Method for resistor 1**
3. **Switch to test resistor 2**
4. **Load, Equil, and run a Method for resistor 2**
5. **Switch back to the analytical flowcell**

SWITCH TO TEST RESISTOR 1

Open the Control drop-down menu and select 'Test Mode Resistor 1':

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NOTE: If you are controlling a BAS pump, your previous flow rate will remain in effect during the test. You will not be able to change any pump parameters until you exit Test Mode and resume normal operation.

LOAD AND RUN A METHOD FOR RESISTOR 1

Open the Method/Save Method drop-down menu and save your current Method to the directory you normally save Methods to.

Open the Method/Load Method drop-down menu and migrate to the Self-Test directory, typically C:\BAS3\Selftest. There will be three Methods for test_one, labeled for the number of analog boards:

Boards	Method
1	one_board_test_one.MTH
2	two_board_test_one.MTH
3	three_board_test_one.MTH

Load the Method that corresponds to the number of analog boards in your instrument, as determined above.

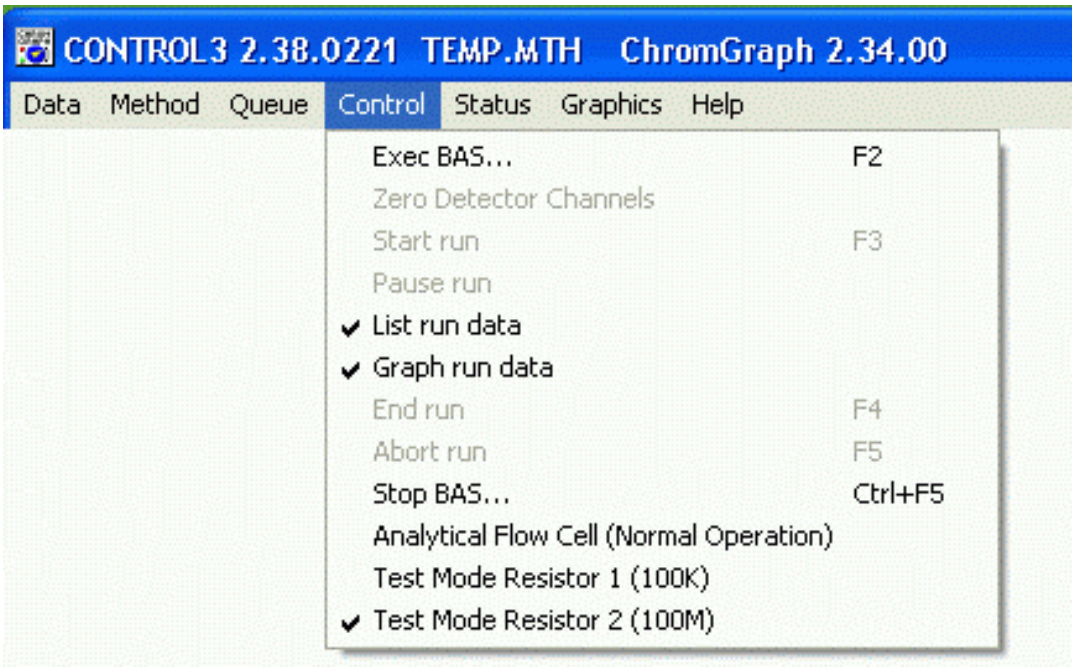
Optional — Change the Run Number: The Method is configured to save the data as run 1. If you have used the self-test before, and wish to retain the previous results, open the Method/Data Acquisition Options drop-down menu and change 'First run number' to whatever you wish (except 999, the run number under which we've saved specimen 'normal' runs).

Run the Test: Press the 'Equil' button in the Run Status box, then the unzero button in the Detector Status box, then 'Run' in the Run Status box.

Data collection will begin, and last for slightly more than a minute. The one-board Method will collect one channel of data (A), the two-board Method will collect two channels simultaneously (A and B), and the three-board Method will collect four channels simultaneously (A, B, C, and D).

SWITCH TO TEST RESISTOR 2

Open the Control drop-down menu and select 'Test Mode Resistor 2':



NOTE: If you are controlling a BAS pump, your previous flow rate will remain in effect during the test. You will not be able to change any pump parameters until you exit Test Mode and resume normal operation.

LOAD AND RUN A METHOD FOR RESISTOR 2

Open the Method/Load Method drop-down menu. There will be three Methods for test_two, labeled for the number of analog boards:

Boards	Method
1	one_board_test_two.MTH
2	two_board_test_two.MTH
3	three_board_test_two.MTH

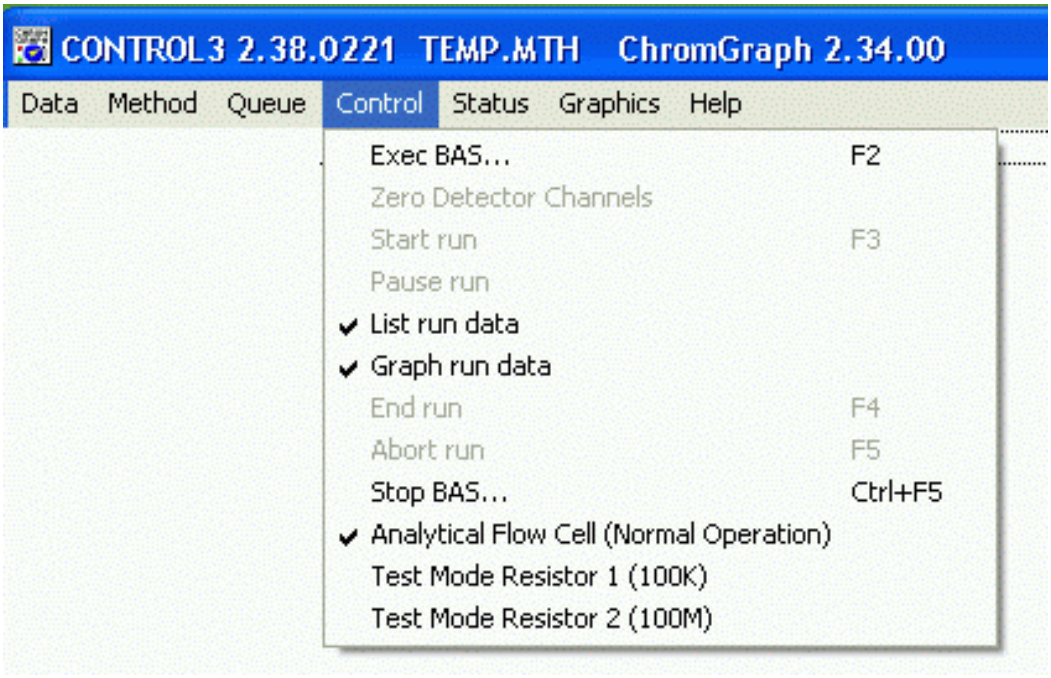
Load the Method that corresponds to the number of analog boards in your instrument, as determined above.

Optional — Change the Run Number: The Method is configured to save the data as run 1. If you have used the self-test before, and wish to retain the previous results, open the Method/Data Acquisition Options drop-down menu and change 'First run number' to whatever you wish (except 999, the run number under which we've saved specimen 'normal' runs).

Run the Test: Press the 'Equil' button in the Run Status box, then the unzero button in the Detector Status box, then 'Run' in the Run Status box.

SWITCH BACK TO THE ANALYTICAL FLOWCELL

Open the Control drop-down menu and click on the 'Analytical Flowcell (Normal Operation)' line:

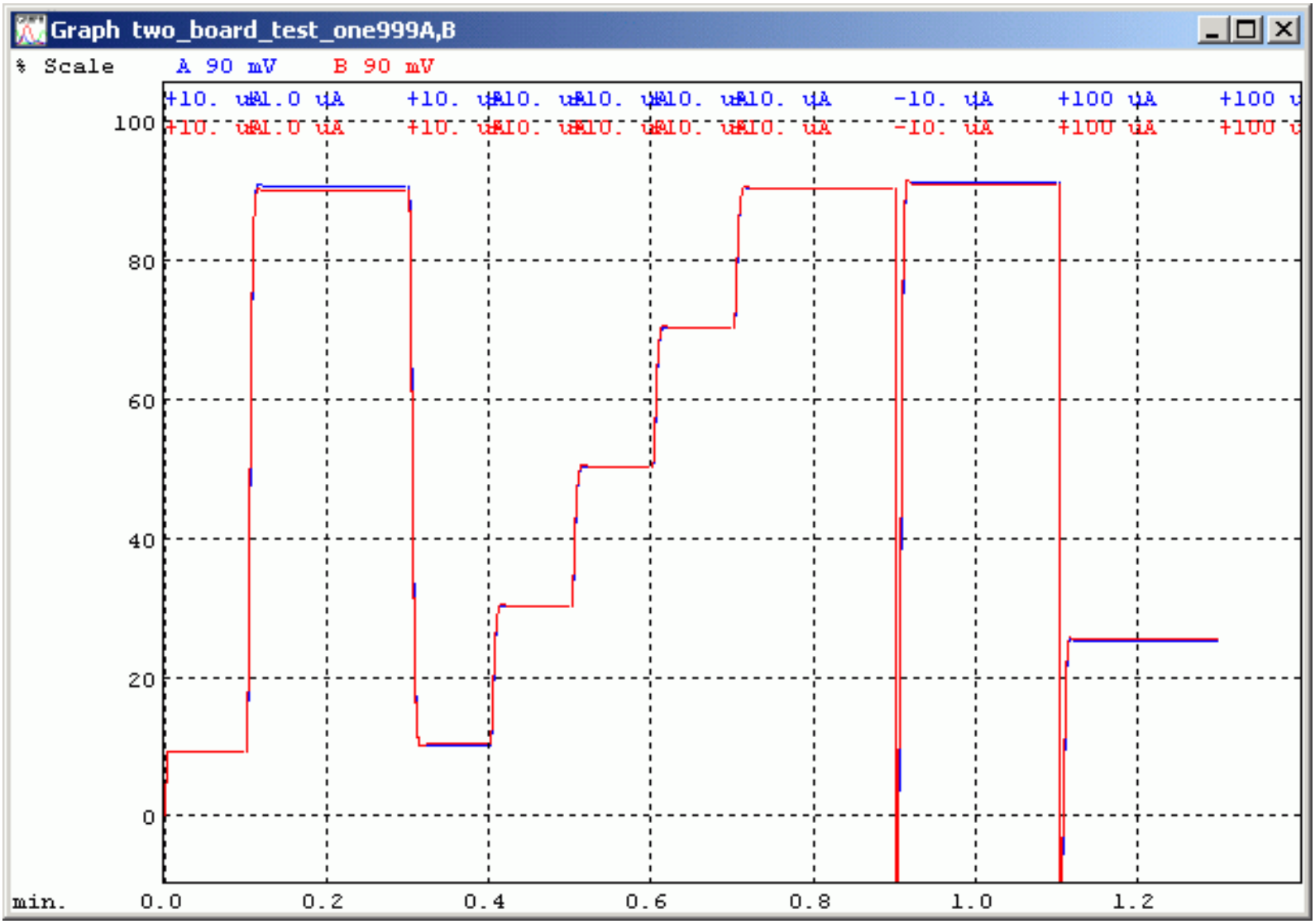


Then open the Method/Load Method drop-down menu, change to the directory where you save your Methods, and load your original Method. Use the warmup feature to equilibrate your flowcell.

EVALUATING THE RESULTS

A detailed analysis of the results should be conducted by BAS technicians. But in general, if the output for all channels looks like this, the epsilon electronics are most likely working properly:

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For comparison, the following specimen data sets have been placed in the Selftest directory. Use ChromGraph Report to load and compare your data set against the matching specimen data set:

Boards	Channel	Data Name
1	A	one_board_test_one999A.DAT
1	A	one_board_test_two999A.DAT
2	A	two_board_test_one999A.DAT
2	A	two_board_test_two999A.DAT
2	B	two_board_test_one999B.DAT

2	B	two_board_test_two999B.DAT
3	A	three_board_test_one999A.DAT
3	A	three_board_test_two999A.DAT
3	B	three_board_test_one999B.DAT
3	B	three_board_test_two999B.DAT
3	C	three_board_test_one999C.DAT
3	C	three_board_test_two999C.DAT
3	D	three_board_test_one999D.DAT
3	D	three_board_test_two999D.DAT

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STARTING A CHROMATOGRAPHIC RUN

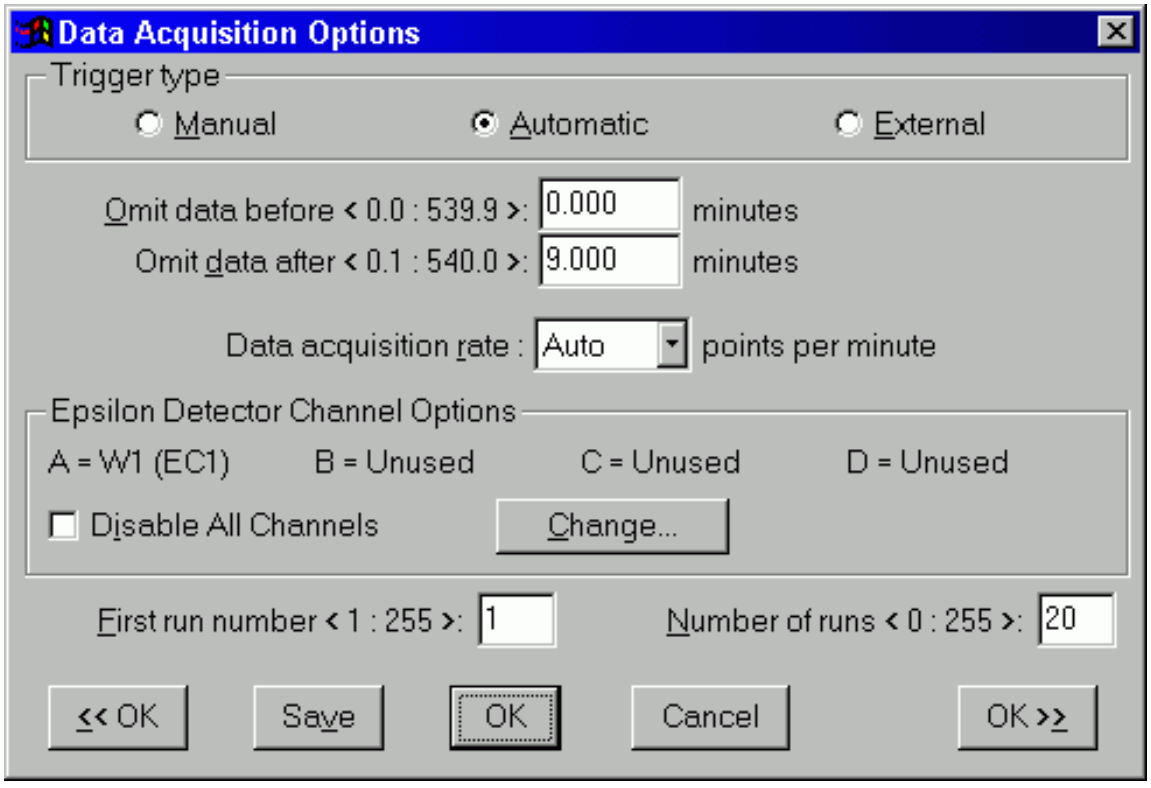
Some activities and maintenance procedures (e.g., pumping in isocratic mode, turning the detectors on and off) can be accomplished through the individual Method screens. But most operations (gradients, automatic runs with autosamplers, data collection, PAD) require that a programmed run be initiated.

- **SETTING THE RUN PARAMETERS**
- **STARTING A RUN**
- **ADDING SAMPLES TO A RUNNING METHOD**
- **REVIEWING A RUNNING METHOD**
- **FILES CREATED**

SETTING THE RUN PARAMETERS

First double-check the Method to make sure it is correct. In particular, check that the Detector Schedule has the correct detector conditions and run time, that the Pump Gradient Schedule has the correct solvent composition and flowrate, and that the Data Name and Data Collection Rate are satisfactory.

Next open the DATA ACQUISITION OPTIONS section of the Method:

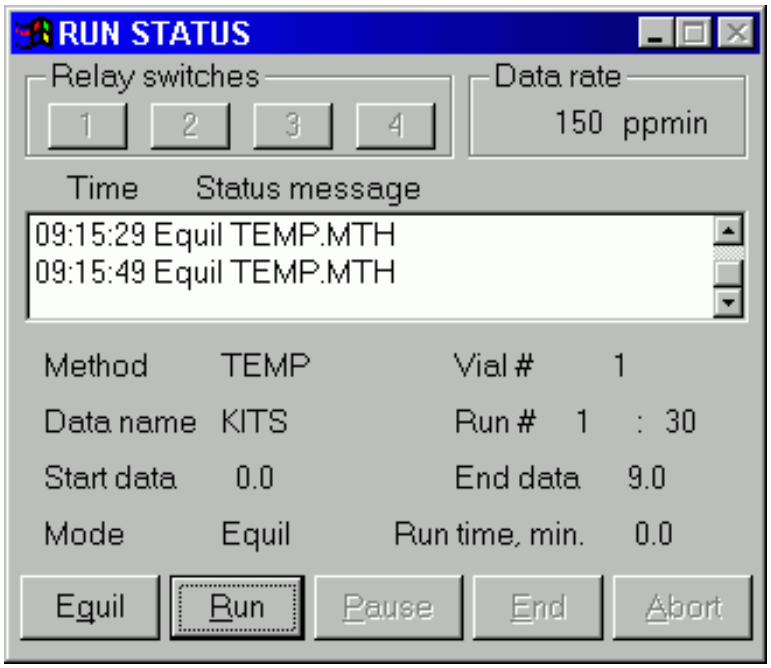


Set the TRIGGER TYPE to Manual if you will be using a manual injector. If using an autosampler, set it to Automatic or External as appropriate (See the Autosampler section to determine which to use.)

Next enter the number of the first run, and the number of runs in the series (maximum = 999). Then click OK. The system is now ready to run.

STARTING A RUN

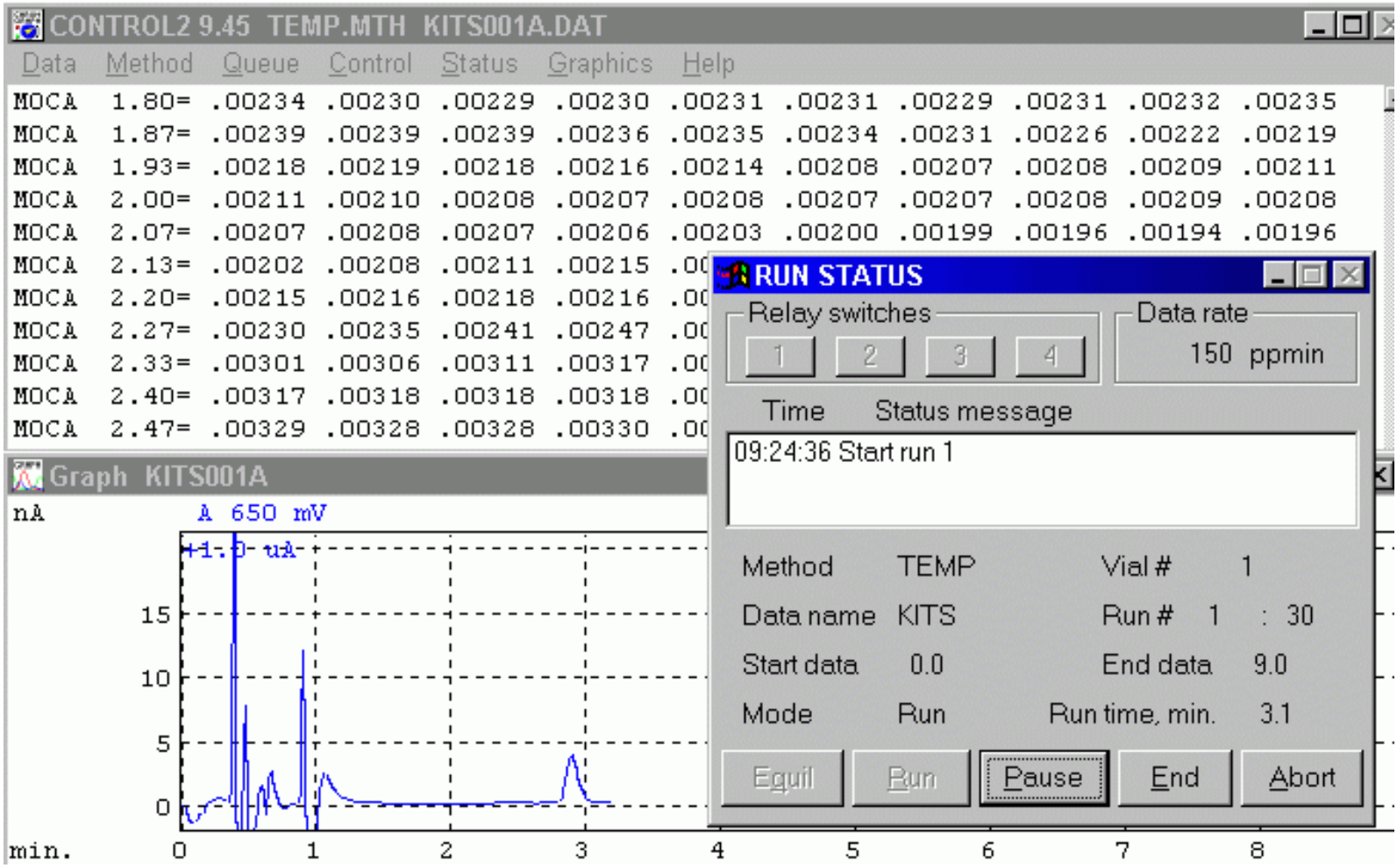
Double-click on the RUN STATUS icon, which will open up the RUN STATUS box:



Click EQUIL to send the information from the Method to the epsilon system. EQUIL puts the conditions in each Method module into effect. Where there are time lines, as in the Detector Schedule, it puts the 0.0 time line into effect. Note that the EQUIL function here is just a formality to ensure that the starting conditions from the Method are in effect -- the system should have been equilibrating for several hours before serious data collection is attempted.

To begin the series of runs, click RUN. The run will start within a few seconds if an automatic or manual trigger has been selected. If an external trigger has been selected, the run will await a signal from the autosampler . When the run starts, a graph will appear showing the chromatogram in real time:

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The EQUIL and RUN functions in the RUN STATUS box will be unavailable, and the PAUSE, END and ABORT buttons will become available.

PAUSE stops the timers from all modules. Thus, the current gradient conditions at any time during the run can be held constant. This feature can be used during method development, for example, to hold the gradient constant while peaks elute. A CONTinue button appears when PAUSE is pressed, allowing resumption of the run.

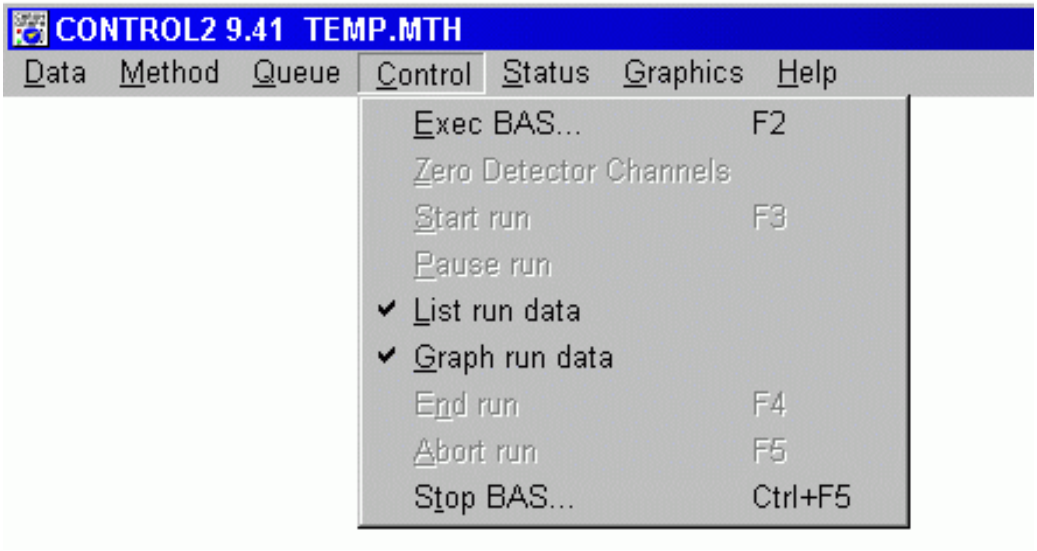
END stops data collection and saves the data to disk. END does not end the pump's run, in case there is a gradient or column wash programmed in. If you've ENDed the data and are sure you want to terminate the pump's run, press ABORT to terminate the run.

ABORT cancels all timed events and returns the system to 0.0 time conditions.

Several events occur onscreen while the run is in progress. Data and messages will scroll upwards on the output screen, which appears in the background. Superimposed on this output screen are the graph, which shows a chart-recorder style image of the detector output, and the RUN STATUS box. In addition to the pushbuttons described above, the RUN STATUS box displays the elapsed time and messages.

The listing and graphing of data during the run are turned on and off by options in the CONTROL MENU:

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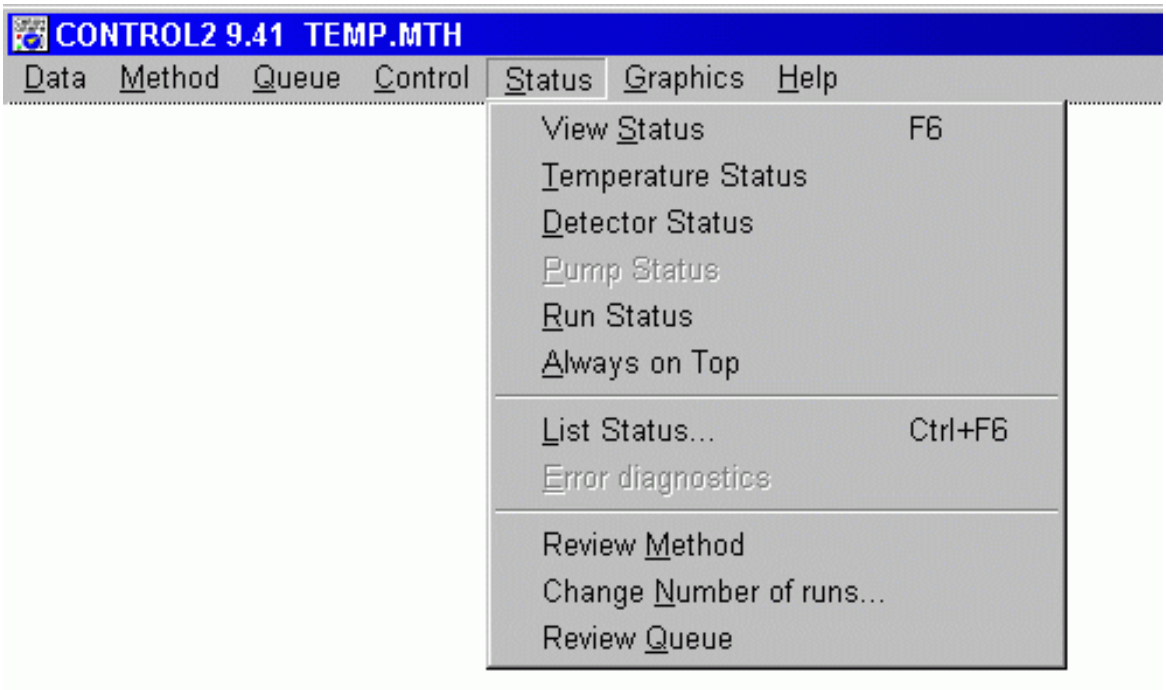


Graph colors are selected in the GRAPHICS MENU. The graphics capabilities of ChromGraph Control are not as extensive as those of ChromGraph Report, but the Zoom Cursor, Overview Window, and Grid Dots are available.

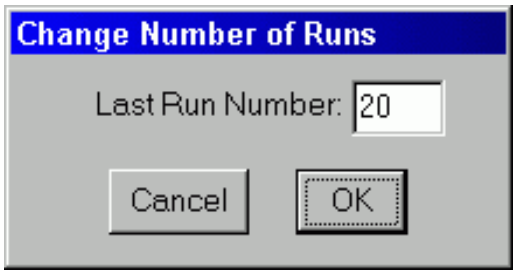
When the first run is complete, the system prepares for the next run in the series. The instruments return to time 0.0 conditions. The data are saved, the RUN NUMBER is incremented by one, the NUMBER OF RUNS is decremented by one, and the new run is either started or waits for a trigger.

ADDING SAMPLES TO A RUNNING METHOD

Additional runs may be scheduled while a batch of runs is in progress. Simply open the STATUS menu and take the CHANGE NUMBER OF RUNS option:

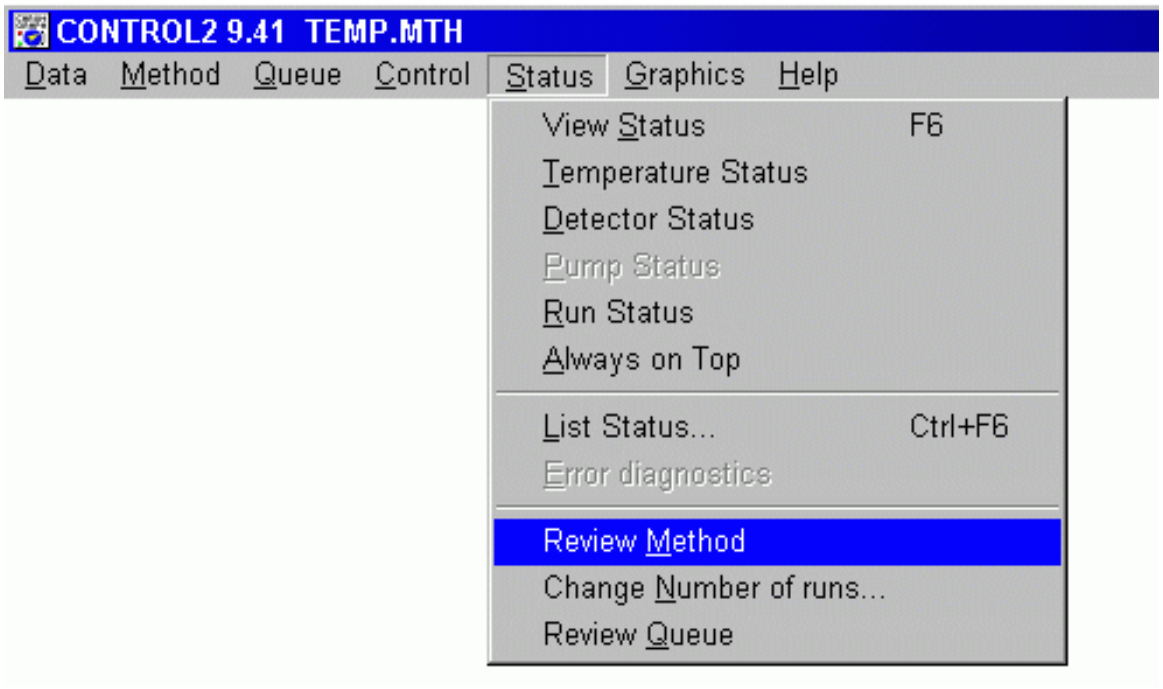


Then enter the updated LAST RUN NUMBER.



REVIEWING A RUNNING METHOD

If you've started a bunch of runs and need to check that everything in the Method is correct, you can do this without stopping the runs. Take the REVIEW METHOD option under the STATUS menu:



A copy of the Method will appear in the output window, and you can review it there. For this to occur, the scrolling of data on the output window must stop (no data points are lost, however). To turn data scrolling back on, take the LIST RUN DATA option under the CONTROL menu.

FILES CREATED

Several files are saved to the default data location in conjunction with a programmed run. These are:

DATA FILES contain the collected digitized data from the detectors. These have the format NAME###X.DAT. NAME is the data name, any combination of spaces and characters, ending in a letter. ### is the run number from 1-999. X is the detector code, from A-D, corresponding to detectors 1-4. Each detector will produce its own data file. The extension ".DAT" stands for data, and will automatically be appended.

Data names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\mydata002A.dat = 27 characters).

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Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a filename may be truncated somewhat in various places in the program.

LOG FILES have the Method name, followed by a ".LOG" extension. They record the runs made under each method. Log files are created automatically whenever you run a Method. They can be found in the startup directory (usually C:\BAS2 or C:\BAS3) and viewed with any text editor or word processor. When a log file reaches 100 KB it is automatically cleared, and its contents transfered into a backup file with the same name and a ".LOK" extension.

BACKUP METHOD FILES are copies of the Method that are associated with data files for future reference. If you made runs 1-20 from the Method MCAT.MTH, for example, you would find the following files in your default data location:

- MCAT.MTH (current Method file)
- MCAT.LOG (log file)
- MCAT001A.DAT through MCAT020A.DAT (20 data files)
- MCAT001A.MTH (Backup Method File)

The last file is a copy of MCAT.MTH. Because of its hybrid name (DATA name + .MTH) it will not normally be used as a Method file, and thus will never be modified. It serves as a record of the conditions under which the runs were made, and thus should not be altered.

Only one Backup Method File will be made for any sequence of runs. However, if you start runs individually, one by one, a Backup Method will be made for each. To turn off the backup feature, click off the Auto-Backup .MTH feature under Setup Options.

A complete list of all file types used by ChromGraph Control and ChromGraph Report is available here.

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QUEUES -- CHAINING METHODS TOGETHER

Methods can be chained together using the QUEUE feature from the main menu. Chaining can be useful in at least three situations:

1. When there are more than 999 runs in a series (the maximum for any data name) the data name can be changed with a second Method that allows another 999 runs, etc.

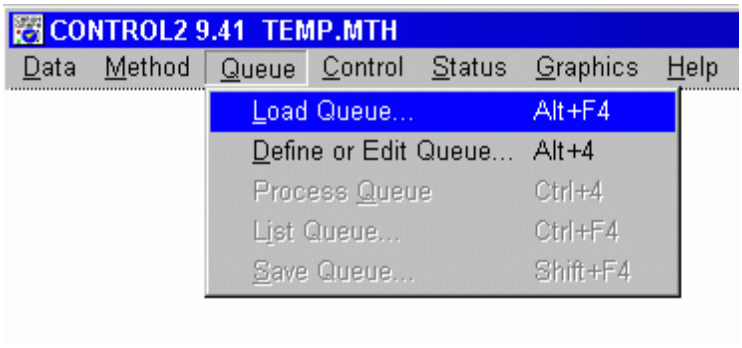
2. When two types of analyses are being conducted, a second Method can change any run parameters, including which detectors are enabled, detector filtering, and pump conditions.

3. To slow down the pump once a series of runs has been completed (a SHUTDOWN Method).

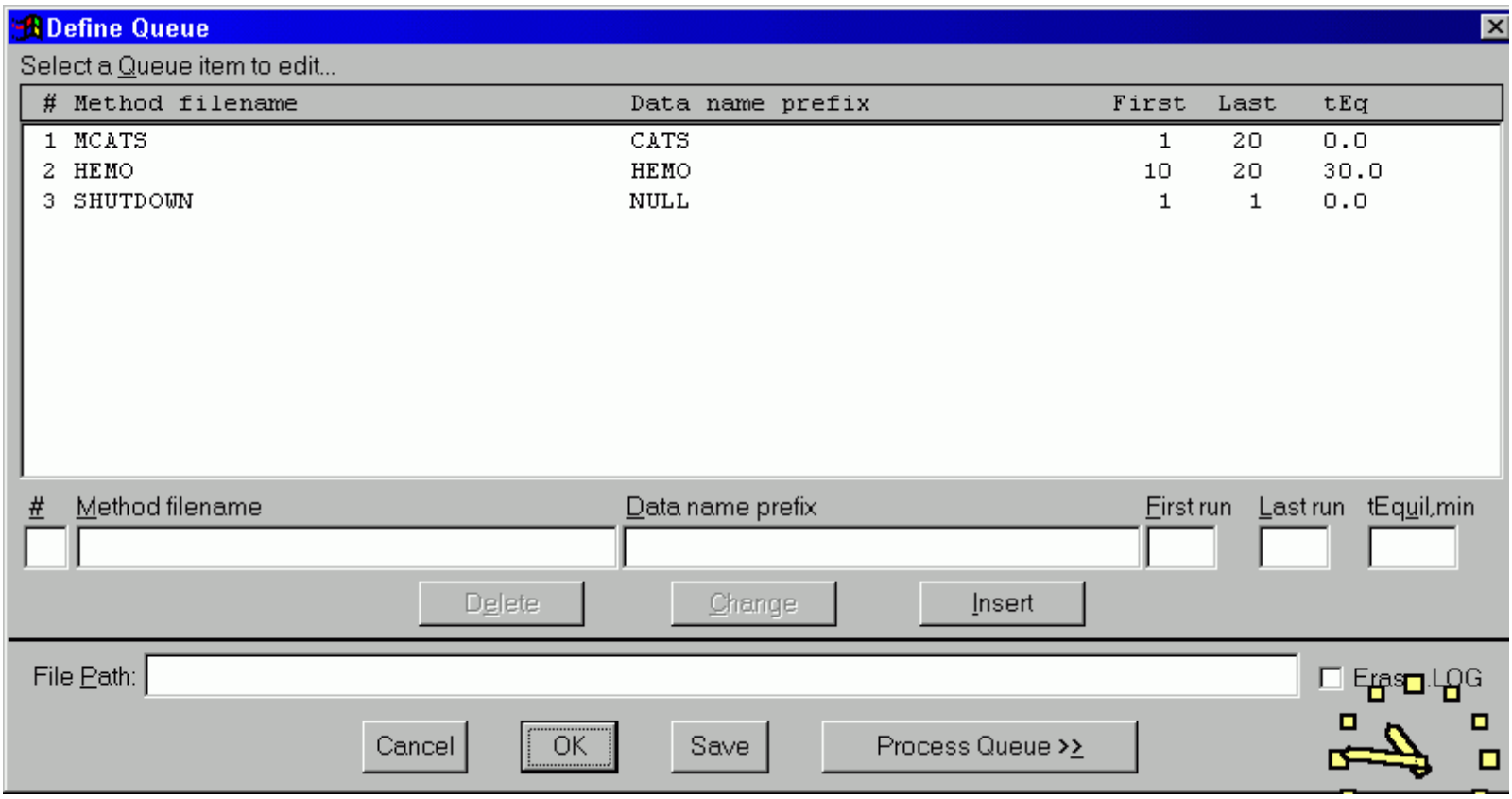
- **DEFINING A QUEUE**
- **CHAINING A SHUTDOWN METHOD**

DEFINING A QUEUE

To begin setting up a queue, open the QUEUE drop-down menu:



You can either load an existing queue or define a new one. Either way, a dialog box will appear:



Up to 20 methods can be chained together in the QUEUE listbox, each one occupying a line.

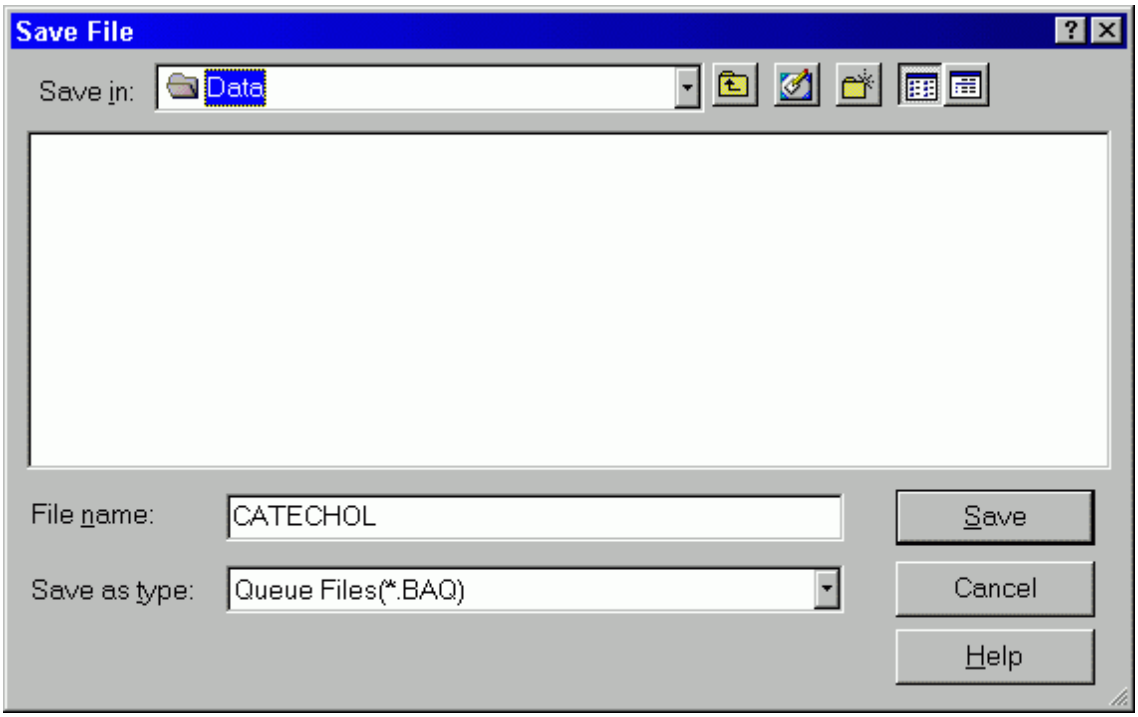
For each line in the listbox, enter a line number, the name of the Method to be run, the name of the data files to be saved, and the first and last run numbers of the sequence to be performed. The data filenames and run numbers in the listbox supersede those in the Method.

The tEQUIL entry allows you to program a time delay, in minutes, between Methods. This could be used, for instance, to allow the EC detectors to equilibrate, or to allow a new flowrate to stabilize. For most purposes, however, set the time to zero for immediate execution of the Method.

Enter a FILE PATH only to send data to a location different from the default data location. For most purposes, leave this section blank.

Use the ERASE .LOG feature to automatically erase any previous .LOG file when the Queue is started. Since the LOG file contains a record of each run, it's best not to erase it. Rather, rename the LOG file and archive it with the data.

If desired, SAVE the Queue by pressing the SAVE button. This opens a dialog for saving the Queue:

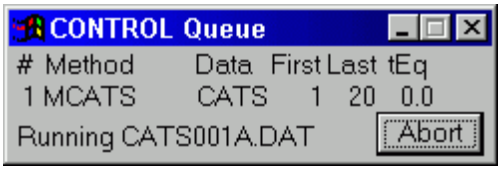


A Queue name can have any combination of characters and spaces, and will automatically be assigned a .BAQ extension. Report Queues, used for data processing, have a .QUE extension to distinguish them from Control Queues.

Names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\myqueue.baq = 24 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a queue name may be truncated somewhat in various places in the program.

To start the sequence of chained Methods, click the PROCESS QUEUE >> button. The Methods will begin running according to their individual directions. While the Queue is processing, a status box will indicate the current Method and the scheduled runs:

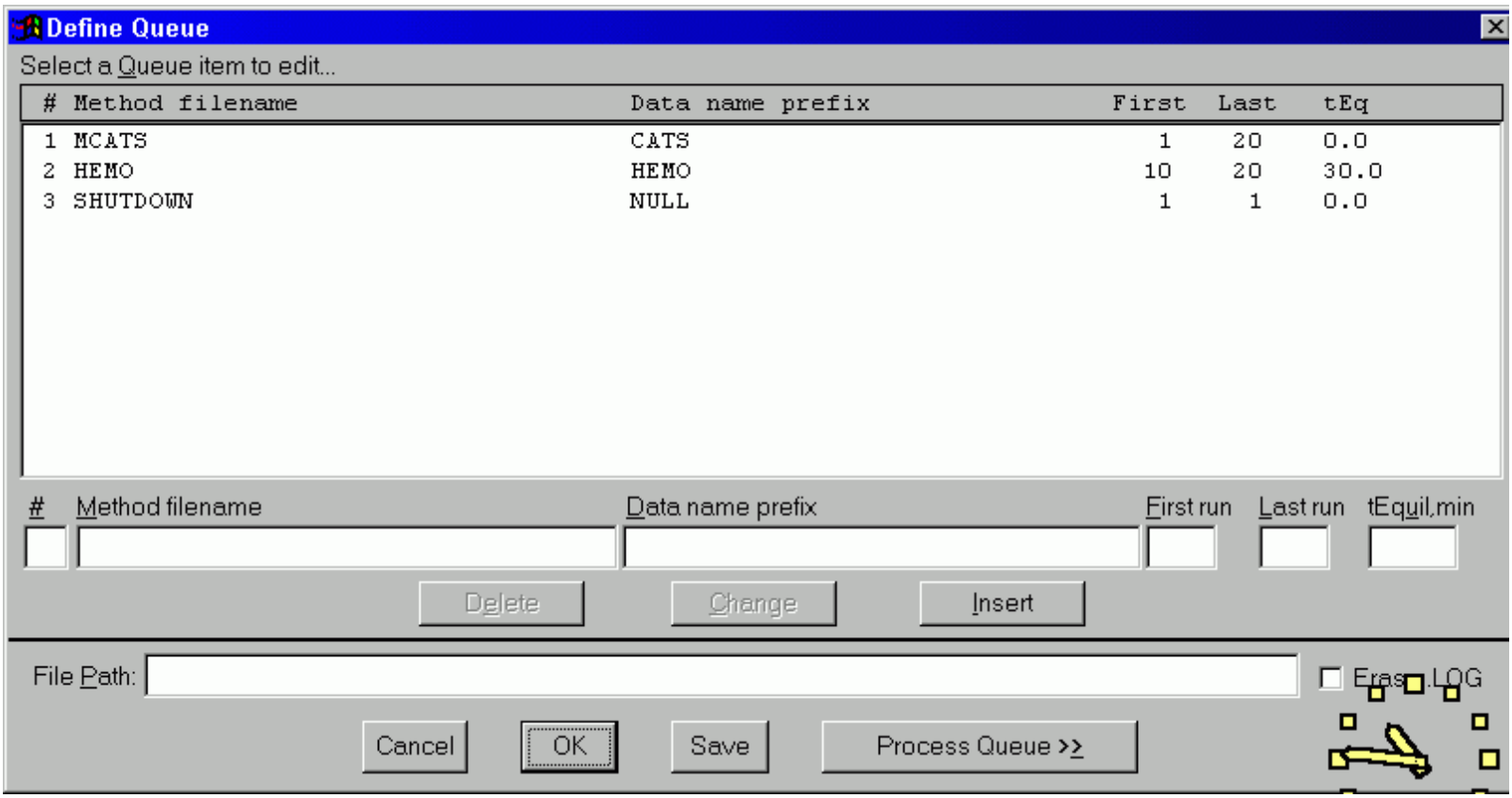


CHAINING A SHUTDOWN METHOD

The last Method in the queue can be a SHUTDOWN Method. This allows the pump to be slowed down after all the samples have run, thus conserving mobile phase. Note that the pump should be slowed down, not stopped: because of the potential for corrosion, pumps should never be shut down with mobile phase in the system.

To create a SHUTDOWN Method to slow the pump, create a method with a lower flow rate (PUMP GRADIENT SCHEDULE). Save this Method under the name SHUTDOWN, and add this name at the end of the Queue:

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To start the sequence of Methods, choose the PROCESS QUEUE button. The Methods will run in sequence, each performing its appropriate number of runs. When the SHUTDOWN method is reached, it will make the appropriate changes to the pump, then maintain this new setting.

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LINKING CONTROL TO REPORT

The data-analysis companion to ChromGraph CONTROL is called ChromGraph REPORT. Detailed instructions for using REPORT are provided here. It is imperative that you learn Report thoroughly before you attempt to link the two programs.

Linking Control and Report allows data analysis and printing to occur immediately after each sample is run. This saves a little time if you have a large numbers of samples and a slow printer. For most situations, however, we recommend the unlinked Batch-Process mode.

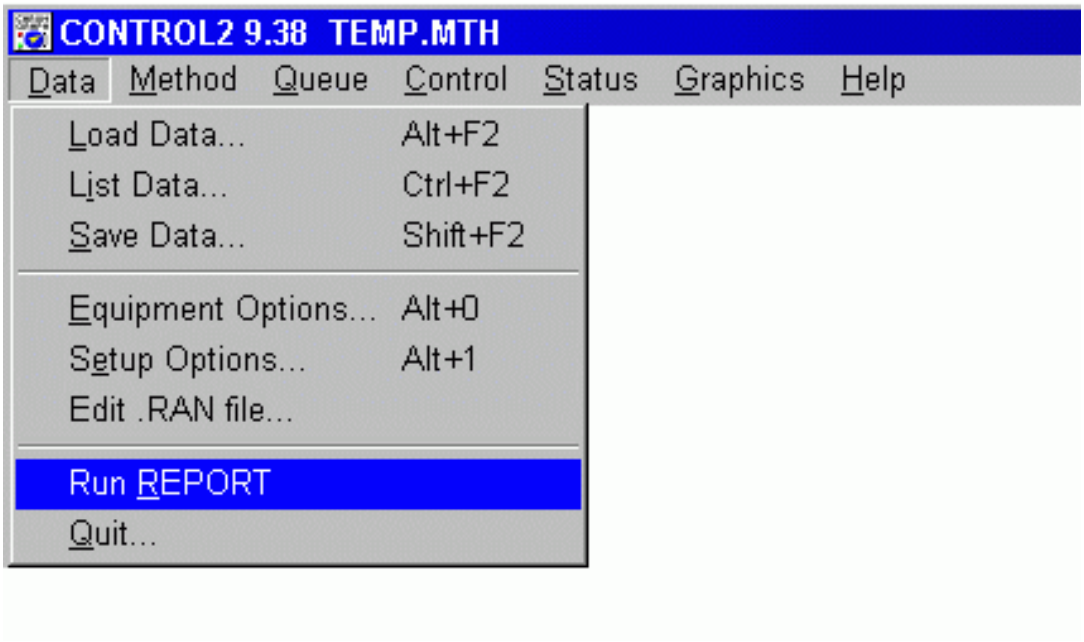
- **REPORT IN THE BATCH-PROCESS MODE**
- **REPORT IN THE LINKED MODE**

REPORT IN THE BATCH-PROCESS MODE

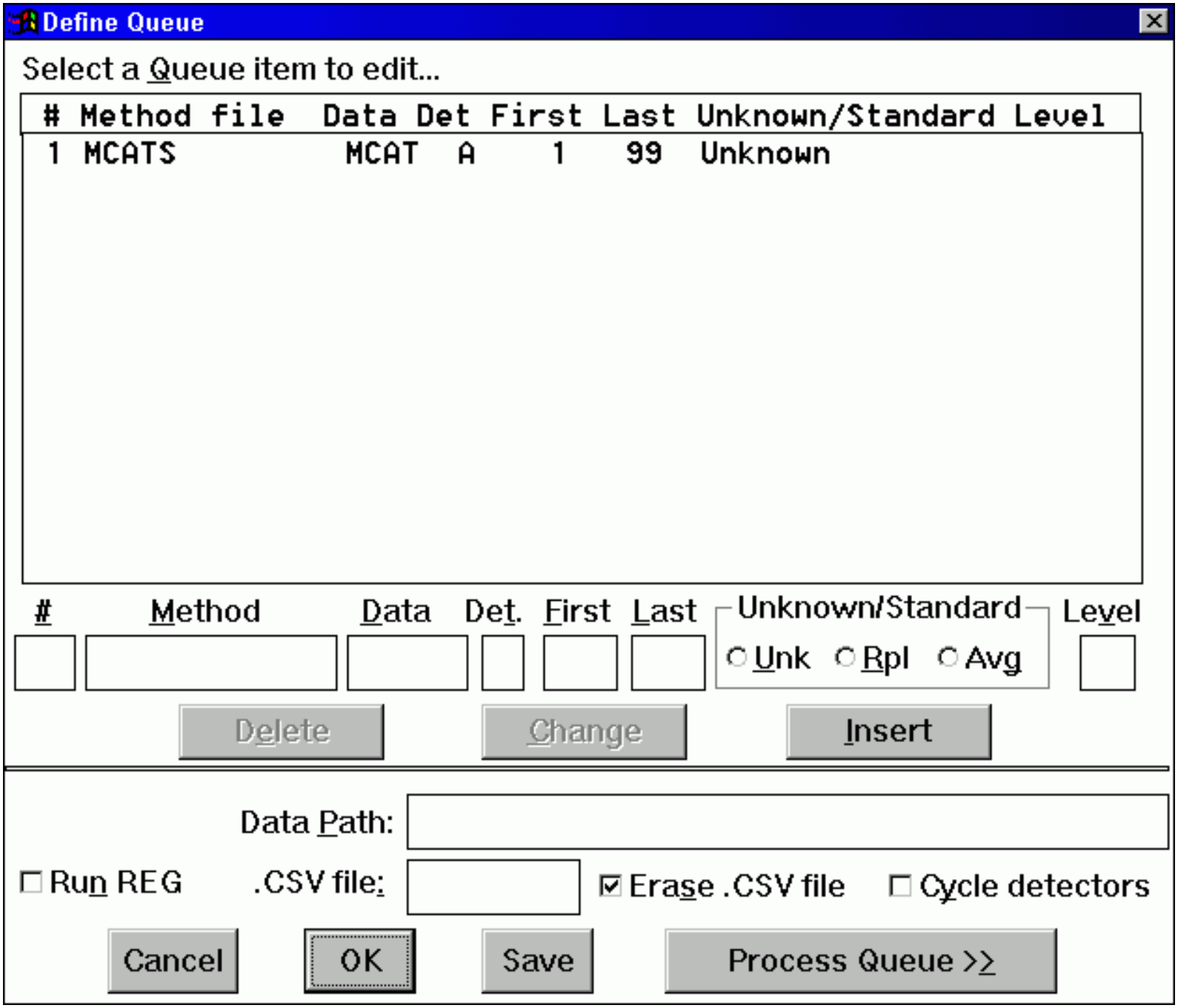
The easiest way to interface the two modules is not to interface them at all. Many users prefer to separate data collection and data analysis into two separate activities. This reduces the array of things one must do before starting a series of runs, and reduces the chance of making mistakes.

In the batch-process mode you first do the chromatography. For example, you may have 30 microdialysis samples in which you are determining amino acids. Create an appropriate Method within CONTROL, paying particular attention to the default data location to which the data will be saved. Configure the autosampler appropriately, start the system, and let the chromatograph and autosampler process the samples overnight.

In the morning you will have a set of 30 data files in the default data location, plus a log file detailing the runs that were made and recording any error messages. (The log file has your Method's name followed by a ".log" extension, and can be found in the startup directory, usually C:\BAS2 or C:\BAS3). Now load REPORT. This can be done by double-clicking on the REPORT icon in the Start Menu or Desktop, or by taking the RUN REPORT option from Control:



When REPORT is active, load a data file for any one of the runs of interest. This will both set the appropriate default data location, and allow you to process a sample run and tailor the report to your needs. Next use REPORT's QUEUE dialog box to instruct the software on the Method(s) to use and the runs to process:



Press the PROCESS QUEUE button, and all the runs will be analyzed.

REPORT IN THE LINKED MODE

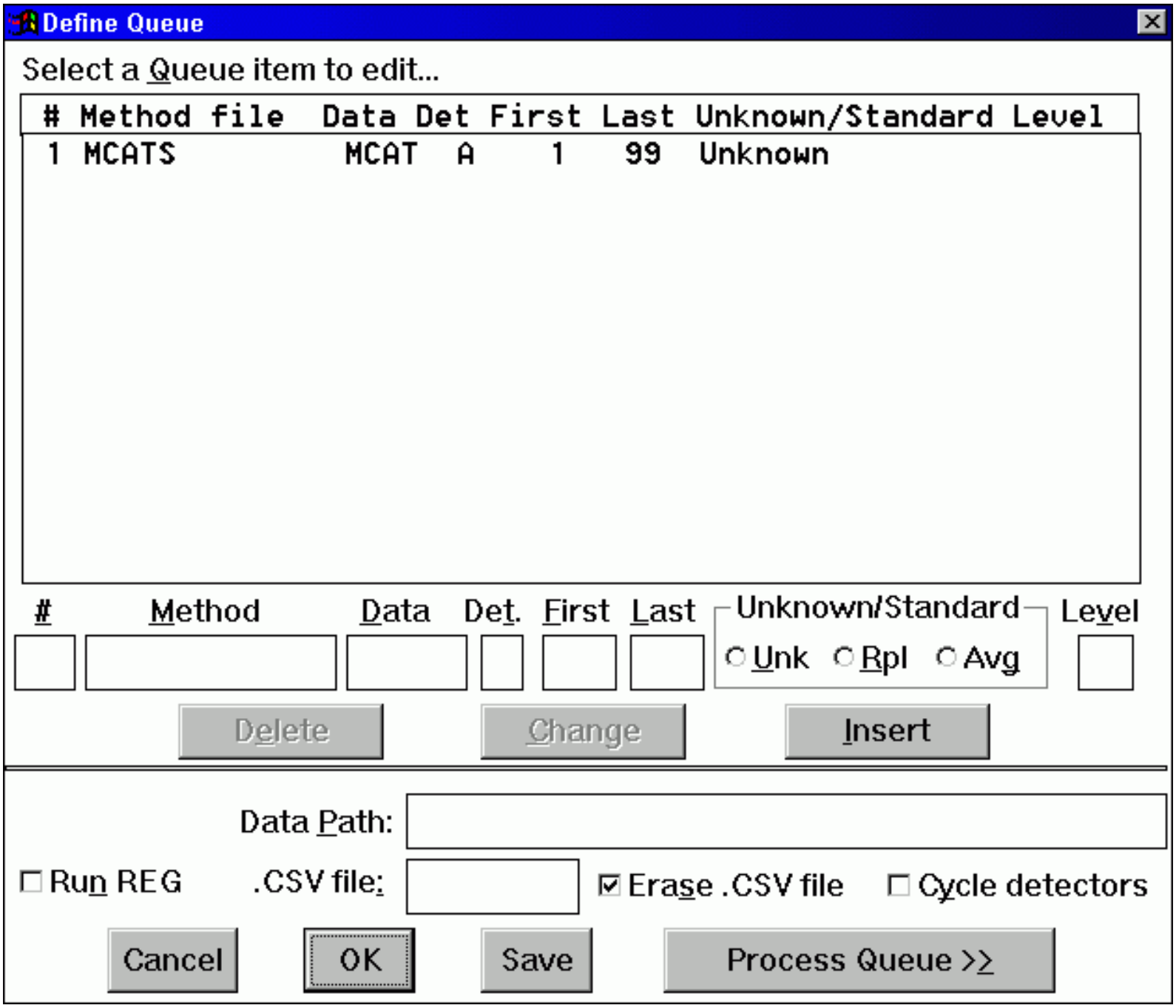
REPORT can be configured to process the data from each run immediately after the run is completed. This is a more complex procedure than the batch-process mode. The advantage to the linked mode is that printed output from early runs can be examined while later runs are still in process. And if you do the runs overnight, the printed reports will be waiting for you the next morning.

We recommend that you attempt the linked mode only after you are thoroughly familiar with both Control and Report.

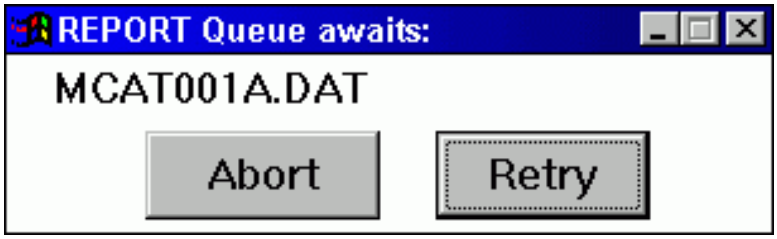
The first step in setting up the system for linked data processing is to erase, rename or move any data files with the same name and run numbers as those you will be creating with the new runs.

We recommend that old data files be archived on a floppy disk with the Method and LOG files associated with them. Use Window's Explorer or My Computer to copy these files for storage.

To ready REPORT to receive data, first load some data from the directory in which Control will save it. This sets the default data location appropriately. Next, set up a Report QUEUE listing the Method and run numbers to use:



If more than one detector is configured, you need a line for each one. Also check the CYCLE DETECTORS box, which tells Report to process all the detectors for any run before going on to the next run. Then click the PROCESS QUEUE button. Since the data files to be processed do not yet exist, the message "REPORT Queue Awaits:" will appear:



Report will do nothing until Control writes the data files to disk.

Now switch back to Control. Start the sequence of runs or the Queue. After the data for each run are saved, Report will become active and produce a report for the run.

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DATA COLLECTION WITH NON-BAS SOFTWARE

Some users would prefer to use their own data-collection systems, either because they are in a regulated environment, or because they are using the epsilon detector in an existing LC system that has its own data collection and processing. This can be accomplished by using the analog outputs of the epsilon to send the signal to the other system, and configuring the epsilon to accept an external rezero from the other system. You will have to use BAS Control software to set the epsilon's parameters, turn on the cell, etc.

- **CONNECTING THE ANALOG OUTPUTS**
- **EXTERNAL REZERO**
- **CONTROLLING THE EPSILON**
- **PUTTING IT ALL TOGETHER**

CONNECTING THE ANALOG OUTPUTS

The epsilon provides a 1-Volt full scale analog output (pictured here) for connection to an external data system. Please be sure to:

1. Connect W*n* on the epsilon to (+) or High on the data system.
2. Connect Gnd on the epsilon to (-) or Low on the data system.
3. Set the data system for an input of 1 Volt (or an attenuation of 10).
4. **NOT** use any additional ground connections at the data system.

EXTERNAL REZERO

It is important to rezero the epsilon during unattended operation, to prevent the baseline from drifting offscale. This is most conveniently done just before each injection. Most autosamplers and data systems provide a means to send a suitable trigger to peripheral equipment. The epsilon detector requires a momentary switch closure, or a TTL-low, to its TTL-INPUTS connector to accomplish this.

CONTROLLING THE EPSILON

The epsilon detector must be controlled by BAS Control software. You will be using MANUAL OPERATION in the EC

SCHEDULE to WARMUP, and then EXEC, the detector with the parameters found in the first line of the EC Schedule.

Be sure to read the entire section on OPERATION WITH CHROMGRAPH CONTROL to fully understand BAS Control software. You will generally not be using the procedures discussed in DATA COLLECTION WITH CHROMGRAPH CONTROL, nor those in DATA ANALYSIS WITH CHROMGRAPH REPORT.

PUTTING IT ALL TOGETHER

The sequence for making this work is as follows:

1. Assemble and connect the FLOWCELL.
2. Start the pump.
3. WARMUP the detector and allow it to equilibrate for as many hours as necessary.
4. EXEC and ZERO the detector.
5. From this point on the autosampler and data system take control: the autosampler triggers a rezero, triggers the data system, and injects.

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ABOUT CHROMGRAPH REPORT SOFTWARE

ChromGraph is a sophisticated set of programs for the control of BAS instruments and the analysis of data generated by Liquid and Gas Chromatographs. The software runs in the Windows environment, thus providing all the flexibility and multitasking inherent in this operating system.

The data processing sections of ChromGraph (ChromGraph Report) include the following functions: peak identification, peak integration, comparison to internal and/or external standards, and report generation. Reports may be output to printer, disk, or to special files configured for easy manipulation by spreadsheet programs. Special features include automatic updating of standards and superb graphics.

ChromGraph REPORT may operate independently in a batch-process mode for already collected data, or it may be linked to its sister program, ChromGraph CONTROL, for linked processing of current data. Please refer to the OPERATION and DATA COLLECTION sections for detailed instructions on using CONTROL.

This manual is intended to be a simple introduction to REPORT. Where there are several paths to accomplish the same task, usually only one will be described. For example, many operations can be carried out either by clicking on a pushbutton with the mouse, or by selecting items from a menu with the keyboard. Only the mouse operations are described, to keep the manual as simple and undaunting as possible. Those who wish to explore other alternatives are encouraged to experiment, using the comprehensive Help screens and their knowledge of Windows.

Both ChromGraph REPORT and CONTROL run in the Windows operating system. Wherever possible, we have maintained the style of Windows in the look and feel of our software, including drop-down menus, list boxes, dialog boxes, radio buttons, etc. If you are already familiar with Windows, the transition to ChromGraph will be smooth. If Windows is new to you, we urge you to learn it first, using the tutorial provided with Windows.

- **CONTENTS**

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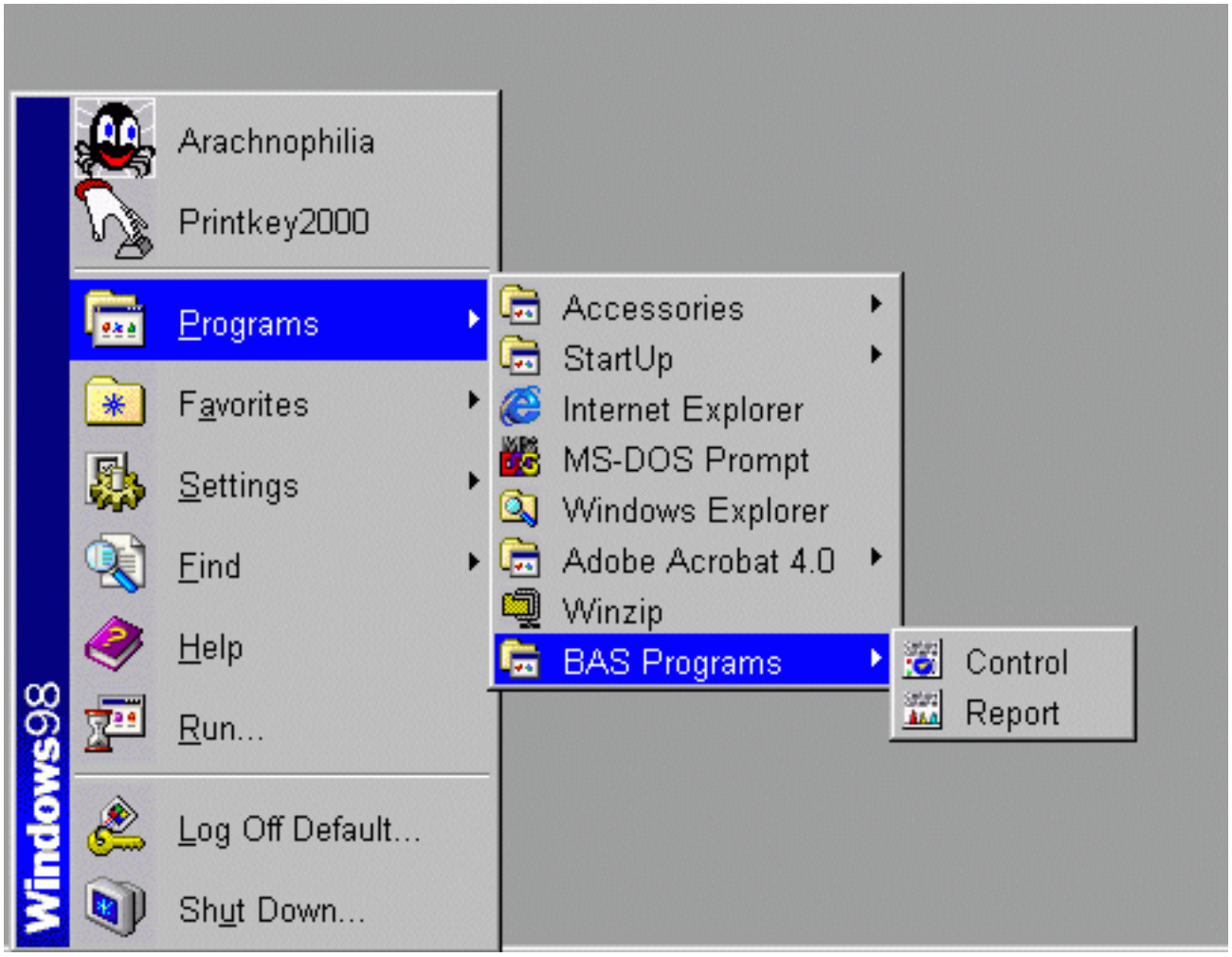
STARTING AND EXITING CHROMGRAPH REPORT SOFTWARE

Report software can be run independently of the epsilon system to analyze data that have already been collected. It can also be installed on a remote computer, in which case the data generated by ChromGraph Control must be transferred to it.

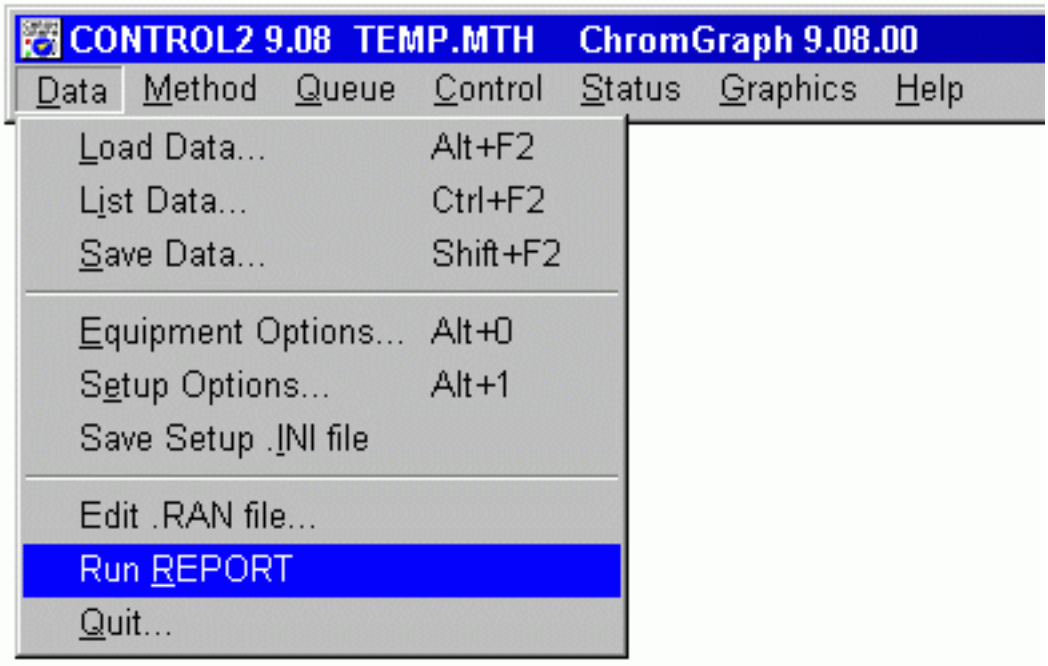
- **STARTING REPORT**
- **SETUP OPTIONS**
- **SAVING THE OPTIONS AND EXITING**

STARTING REPORT

Start ChromGraph Report with either a desktop shortcut icon or the Windows Start Menu:

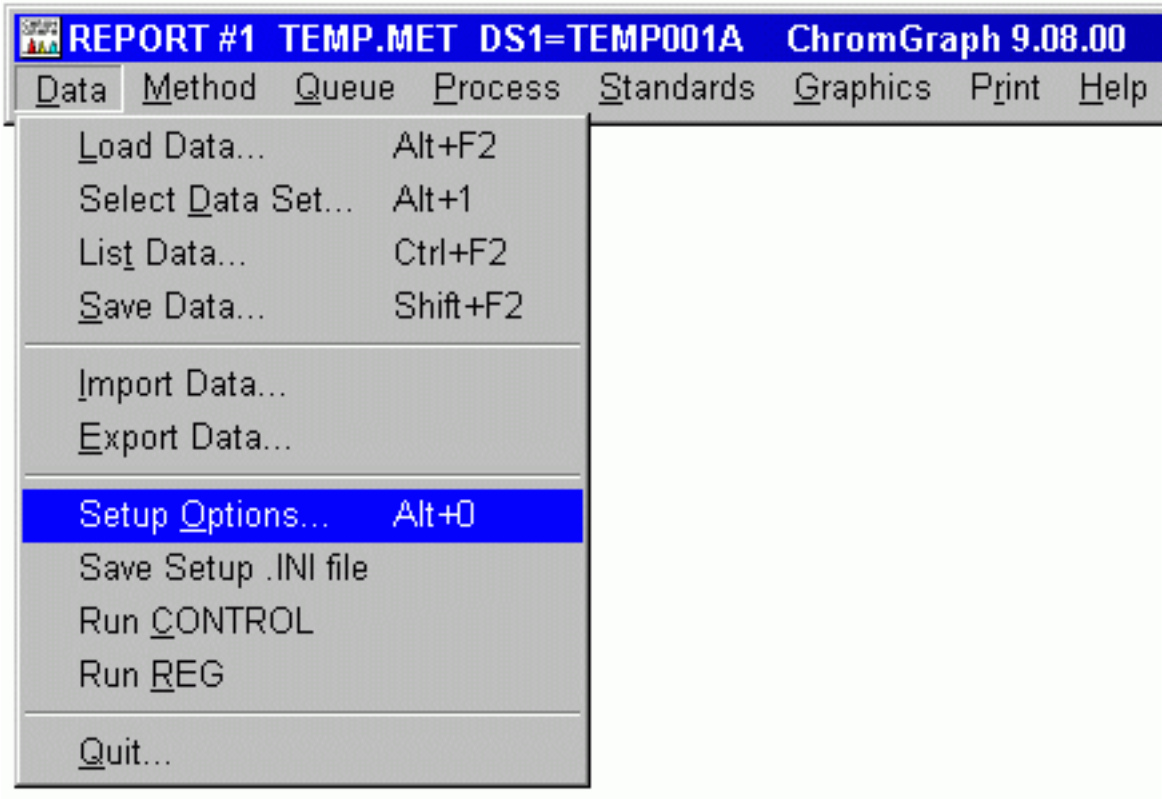


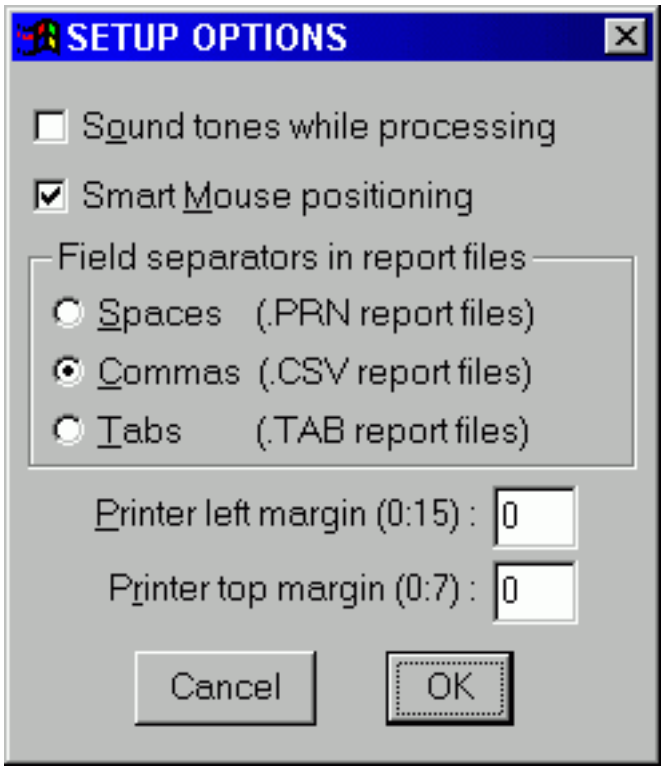
Alternatively, Report can be started (or brought to the forefront if it was minimized) by the 'Run REPORT' option under the Data Menu of Control.



SETUP OPTIONS

SETUP OPTIONS allows you to set some user preferences. This screen is reached as follows:





Check the options that you wish to use:

TONES enables a set of sounds associated with the various steps in processing data. They may be used as an audible cue to indicate analyses in progress.

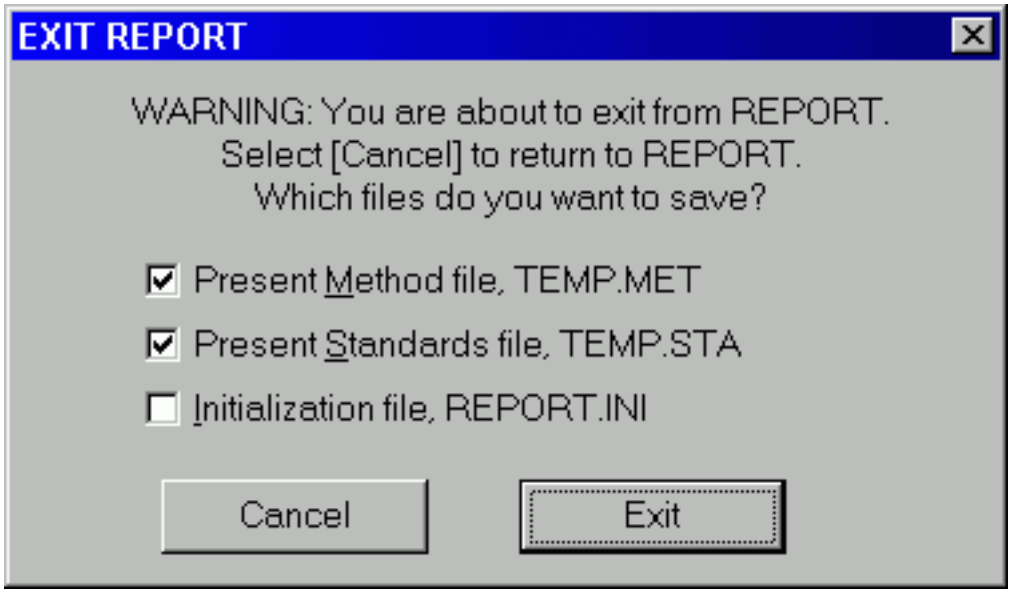
SMART MOUSE POSITIONING puts the mouse pointer at the most likely next step when screens change.

The **FIELD SEPARATOR** options determine the data format for export to spreadsheets. Click here for additional information about this topic.

PRINTER MARGIN moves printing to the right by up to 15 spaces, and down by up to 7 lines, for better control of the appearance of printed output.

SAVING THE OPTIONS AND EXITING

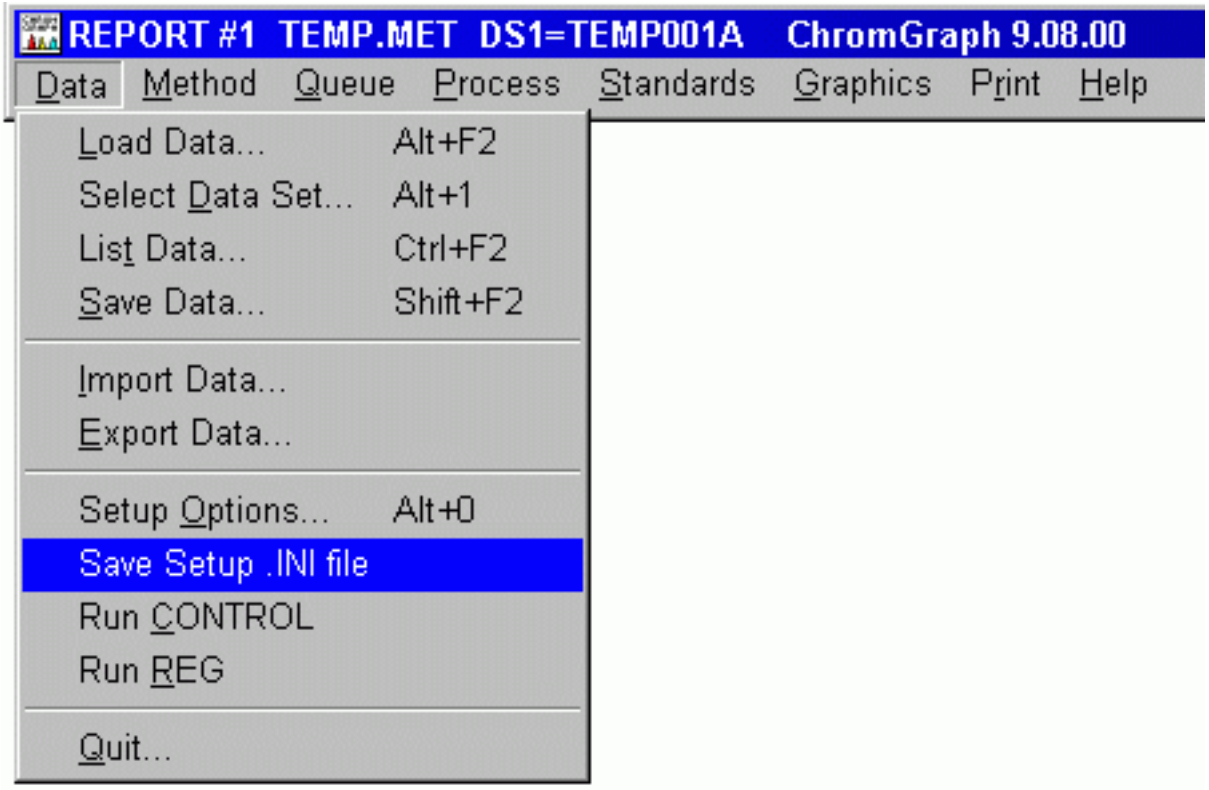
You can exit by clicking the X-button at the top right of the screen, or by taking the QUIT option in the DATA drop-down menu. In either case you'll be asked about saving certain items:



PRESENT METHOD FILE allows the current Method to be saved as temp.met every time you exit, and therefore reloaded automatically at the next session.

PRESENT STANDARDS FILE allows the current standards to be saved as temp.std every time you exit, and therefore reloaded automatically at the next session.

INITIALIZATION FILE saves the setup options, screen colors and sizes, and default data directories so they will automatically go into effect at the next session. Any time you change these options during a session you must decide whether to make the changes permanent. To make them permanent, exit with this box checked. To forget the changes, exit without checking this box. If you don't wish to exit, you may save the initialization file using an option in the Data drop-down menu:


REPORT #1 TEMP.MET DS1=TEMP001A ChromGraph 9.08.00
Data Method Queue Process Standards Graphics Print Help
Load Data... Alt+F2
Select Data Set... Alt+1
List Data... Ctrl+F2
Save Data... Shift+F2
Import Data...
Export Data...
Setup Options... Alt+0
Save Setup .INI file
Run CONTROL
Run REG
Quit...

- **CONTENTS**

THE REPORT METHOD

ChromGraph REPORT data processing Methods are concerned only with the analysis of acquired data. We will call this analysis PROCESSING. Data collection and instrument control are the provinces of ChromGraph CONTROL. To distinguish the two types of Methods, the extensions .MTH (CONTROL) and .MET (REPORT) are automatically appended to the Method names.

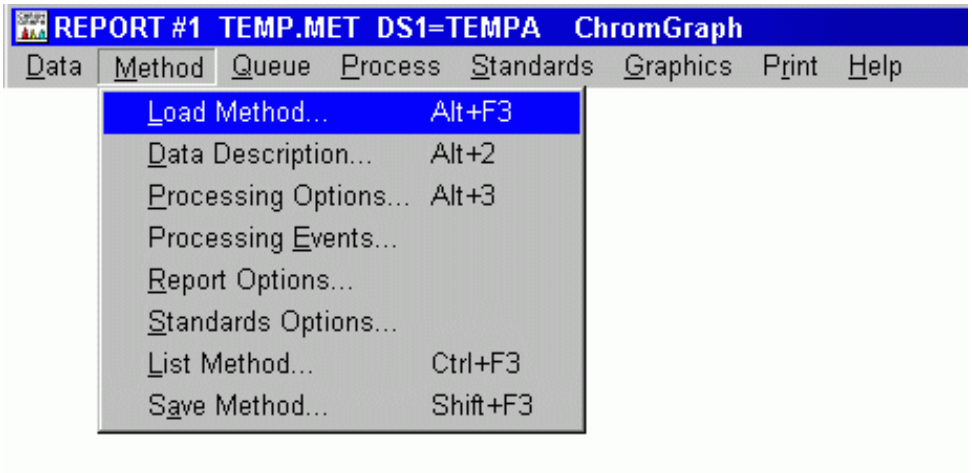
All the information necessary to process a Data file is contained within a REPORT Method. This information includes the sensitivity for discriminating peaks from baseline, the times during which to search for peaks, which set of standards to use, what items to include in the report, etc. We will go through the development of an entire Method here. This will rarely be needed, since in most cases an existing Method can be edited slightly to create new Methods.

- **LOADING AND OPENING A METHOD**
- **PUSHBUTTONS FOUND IN THE METHOD SCREENS**
- **SECTIONS OF THE METHOD**
  - **DATA DESCRIPTION**
  - **PROCESSING OPTIONS**
  - **PROCESSING EVENTS**
  - **REPORT OPTIONS**
  - **STANDARDS OPTIONS**
- **FILES CREATED**
- **LISTING A METHOD**
- **SAVING A METHOD**

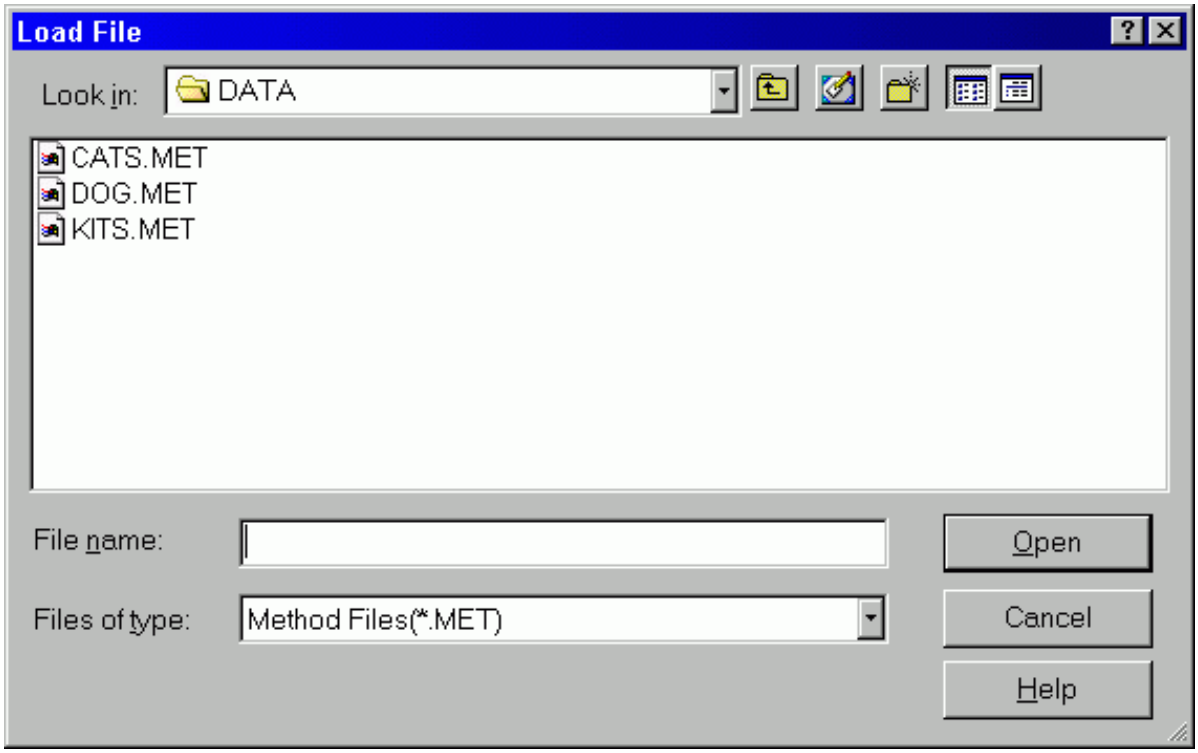
LOADING AND OPENING A METHOD

Begin creating a Method by loading an existing Method which we will modify. (Loading is not strictly necessary: a temporary Method is always loaded into RAM at startup, so a Method is always present. But this is a good place to learn how to load a Method.)

Select the Method drop-down menu by clicking on the METHOD section of the MAIN MENU:



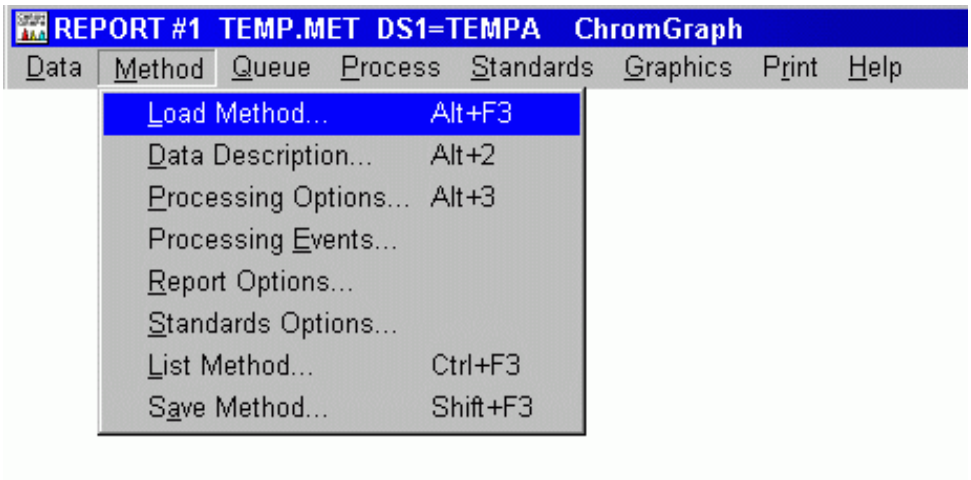
Select the LOAD METHOD option, and a dialog box containing a directory of existing Methods in the default data location will appear. To load a Method, either double click on its name or highlight it and click the OK button.



Methods can be loaded from other locations by browsing through the 'Look in' section of the dialog box until you find the correct location. All the examples in this manual were taken from Methods and Data files in the C:\BAS\DATA directory.

If you load a file from a different directory, this new directory automatically becomes the default data location. The default data location will remain in effect throughout the session, unless changed by another LOAD or SAVE operation. When you finish your session and QUIT, saving the REPORT.INI file will save the new default data location for subsequent sessions. If you don't save the REPORT.INI file, the new data location will be forgotten.

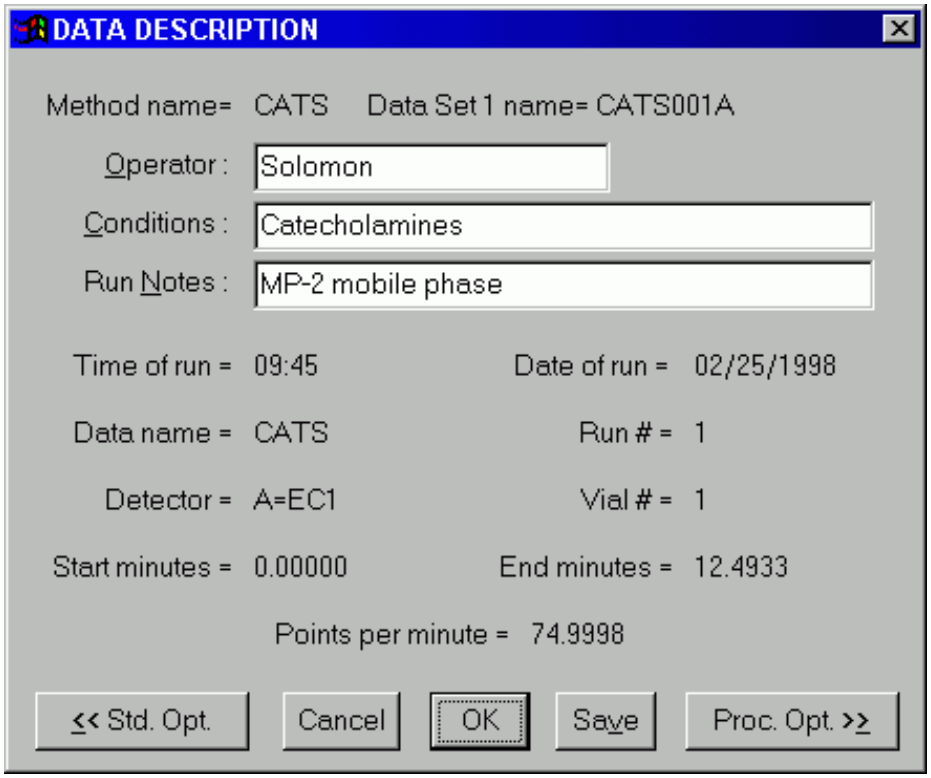
To edit or review the Method you must open the Method screens (technically they are called dialog boxes). Start by clicking on the METHOD section of the MAIN MENU. A list of options will appear:



Except for the LOAD, LIST and SAVE options, each option will open a section of the Method. Simply click on the desired section with the mouse.

PUSHBUTTONS FOUND IN THE METHOD SCREENS

At the bottom of each Method screen are five pushbuttons for subsequent action:



OK accepts any changes and saves them to RAM. These changes are then used in subsequent runs of the Method. To permanently save these changes to disk, use the SAVE option.

<< **(destination)** and **(destination)** >>. These have the same function as OK, except they automatically bring up the previous (<<) or next (>>) screen of the Method.

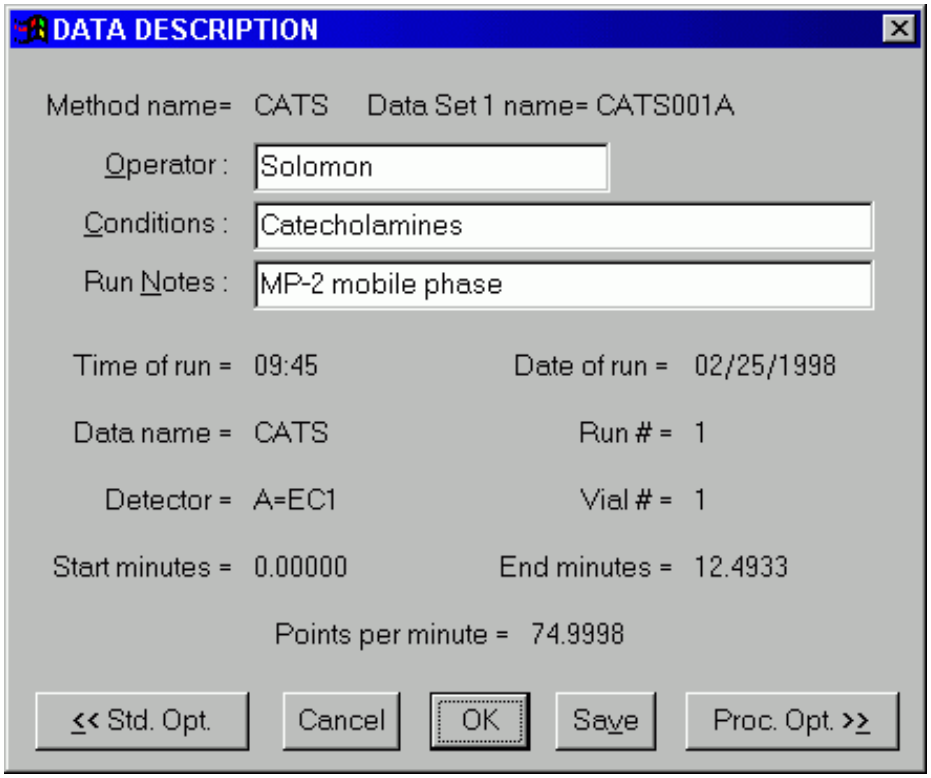
CANCEL closes the dialog box without accepting any changes that were made in the box. The original information is thus preserved.

SAVE opens a dialog to save the Method with a name and location of your choice. Valid Method names are discussed here.

DATA DESCRIPTION

Choosing the DATA DESCRIPTION option under METHOD brings up the edit screen for the first section of the Method. The Data Description section shows information about the Data file currently loaded; as a consequence, it is not a permanent part of the Method and will change as new Data files are loaded.

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OPERATOR, **CONDITIONS** and **RUN NOTES** are user-inserted comments entered into the ChromGraph Control software Method at the time of data collection. This information is saved along with every Data file generated by CONTROL. REPORT imports this information and includes it in the final processing report that it generates. By using these fields when you collect data, you will always be able to identify any particular Data file. You can fill out a table of information that will be imported to the RUN NOTES section during automatic operation: click here for details.

A **SLASH (/)**, followed by a number, in the RUN NOTES field indicates a volume or mass adjustment for the sample. Adjustments are generally attached during data collection by ChromGraph Control (details here). Adjustments allow the results to be reported on a per mass or per volume basis when the weight or volume of the original sample is known. When a number appears here, the amount reported for this sample in the final report will be the calculated amount divided by the number.

The following items are entered automatically by Control at the time of data collection:

TIME and **DATE** of run indicate when the data were collected.

VIAL# refers to the position of the sample in the BAS Sample Sentinel autosampler, if used.

DATA NAME is the name of the data file.

RUN# is the run's number in the sequence of samples.

DETECTOR is the detector code letter, A-D.

The Data file name begins with the data name, which can have up to four numbers and letters (the last position must be a letter, to avoid confusion with the run number, which follows). Run numbers (three digits, up to 999) and a letter (A through D, for detector 1 through 4) are automatically appended to the data name by the data-acquisition software. When saved to disk, the name is given the extension .DAT. Thus, CATS003A.DAT is the third run in a series called cats, and contains data from detector A.

The information above will print out with the report. Three remaining items do not print out:

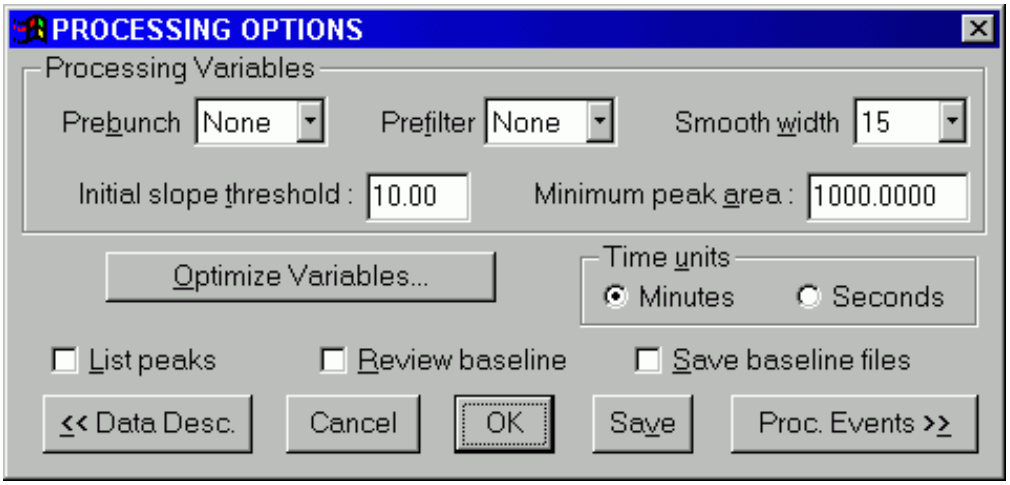
START and **END** minutes indicate the time from the start of the run during which data were collected.

POINTS PER MINUTE is the rate of data collection.

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PROCESSING OPTIONS

This next section of the Method can be accessed by either choosing it from the Method drop-down menu or by clicking the << or >> buttons from other screens. The options in this screen define how peaks are detected and specify some aspects of the report. Try the OPTIMIZE VARIABLES option initially. Optimize will decide on appropriate values for all the processing variables. You can then observe the peaks and modify the values if necessary.



IMPORTANT NOTE REGARDING THE PREBUNCH AND PREFILTER PARAMETERS: These parameters are intended for advanced users who have a firm understanding of peak-finding algorithms. If you use them, keep the following points in mind:

- Prebunch and Prefilter modify the data in RAM. Therefore, the data will be automatically renamed to preserve the integrity of the original data.
- Prebunch and Prefilter are intended to be used once. If you process the data a second time without turning them off, the data will be changed once more. A warning will appear if you attempt this.
- The original data files, on disk, will never be modified by any aspect of ChromGraph software.

PREBUNCH attempts to reduce the number of data points to some optimum number for peak detection. It does this by averaging every x number of points, with x selectable from 2-16. An optimum sampling rate would produce at least 20 data points for the narrowest peak and no more than 500 data points for the widest peak. In most cases the sampling rate automatically set by ChromGraph Control will provide excellent peak detection.

PREFILTER applies additional filtering to the data. Filter rates are in Hertz, with smaller numbers providing greater filtering than larger numbers. As filtering increases, small details become smoothed over and unresolved peaks tend to merge.

SMOOTHING WIDTH compensates for noisy baselines. Use a small value for smooth baselines, and a larger value if baseline noise is being detected as peaks. Peak detection takes longer with large values than with small values.

INITIAL SLOPE THRESHOLD is the sensitivity of the peak-finding algorithm. A small value is very sensitive, whereas a large value is very insensitive. Set this option so the important peaks are detected, while small extraneous peaks are not.

MINIMUM PEAK AREA enables you to reject peaks below a certain area during peak detection. Use this option to exclude small peaks that are of no interest, but still large enough to be detected. Unless you are thoroughly familiar with your data, use a small value so that all detected peaks appear in the report.

TIME UNITS selects minutes or seconds as time units in tables and graphs of the report.

LIST PEAKS allows one of the intermediate steps of peak detection, the finding of start and stop points for the peaks, to be listed to the screen during interactive data processing.

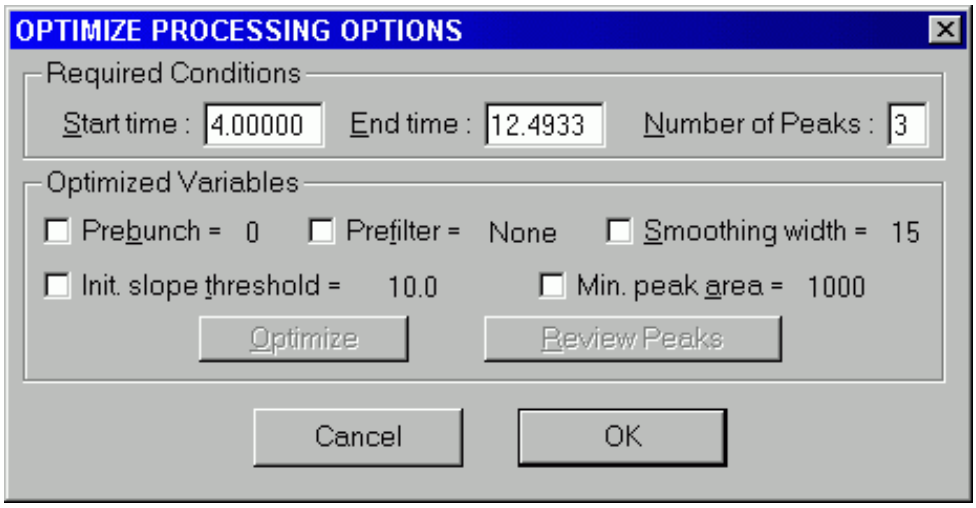
REVIEW BASELINE causes a graph of the peaks, with their start and stop points marked, to appear on the screen during data processing. Processing halts until the user reviews the graph and chooses to cancel or continue.

The **SAVE BASELINE** checkbox causes a copy of the chromatogram to be saved to the default data location each time a run is processed. The file will have a name composed of the data name and run number, followed by a .BLN extension. These files take up a lot of disk space, and

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are redundant in that the chromatogram can be easily redrawn at a later time from the raw data. However, if the regulations you work under require the archiving of the original chromatogram, check this box.

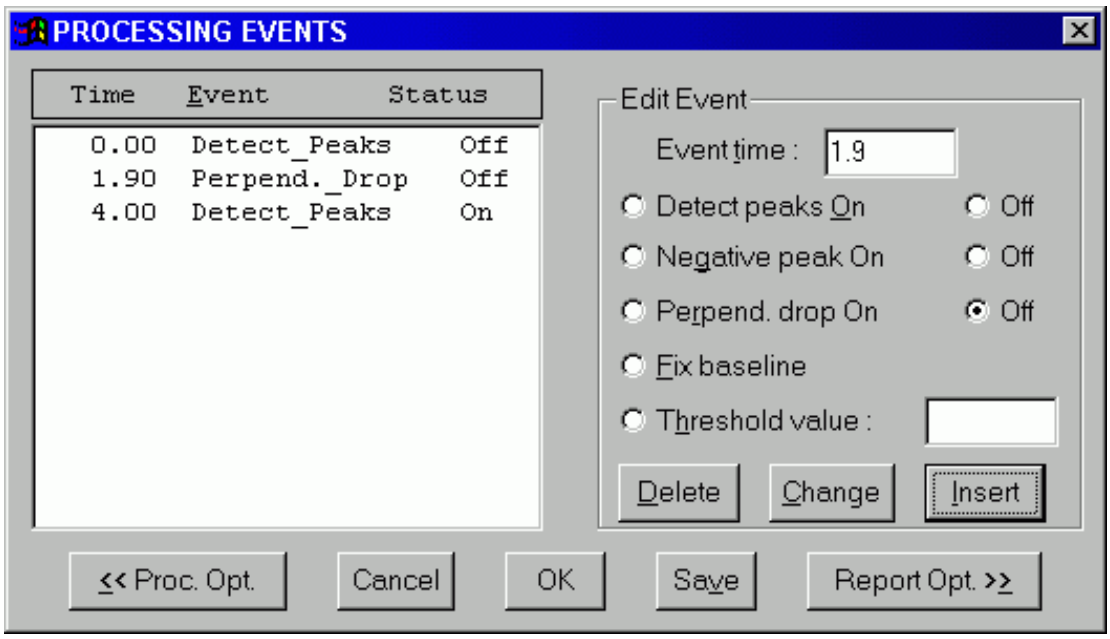
OPTIMIZE VARIABLES opens an iterative procedure that finds suitable values for the processing options:



Indicate the time during which your peaks elute, and the number of peaks expected. Then check one or more of the variables to be optimized. Click the OPTIMIZE button and the software will calculate the values and insert all except Prebunch and Prefilter into the Method. Click REVIEW PEAKS to see the results of peak detection with the optimized variables. Prebunch and Prefilter are excluded here because they alter the data. Optimized values for these two parameters are calculated, but the user must decide whether to use them.

PROCESSING EVENTS

This section of the Method can be reached by taking the PROCESSING EVENTS option from the Method drop-down menu or by clicking the << or >> buttons from other screens:



The left side of this section contains a listbox of instructions that determine how peaks are detected. These instructions are called events because they can be activated or disabled at specified times. (In this manual, time will always refer to elapsed time from the start of a chromatographic run.) The following events are available:

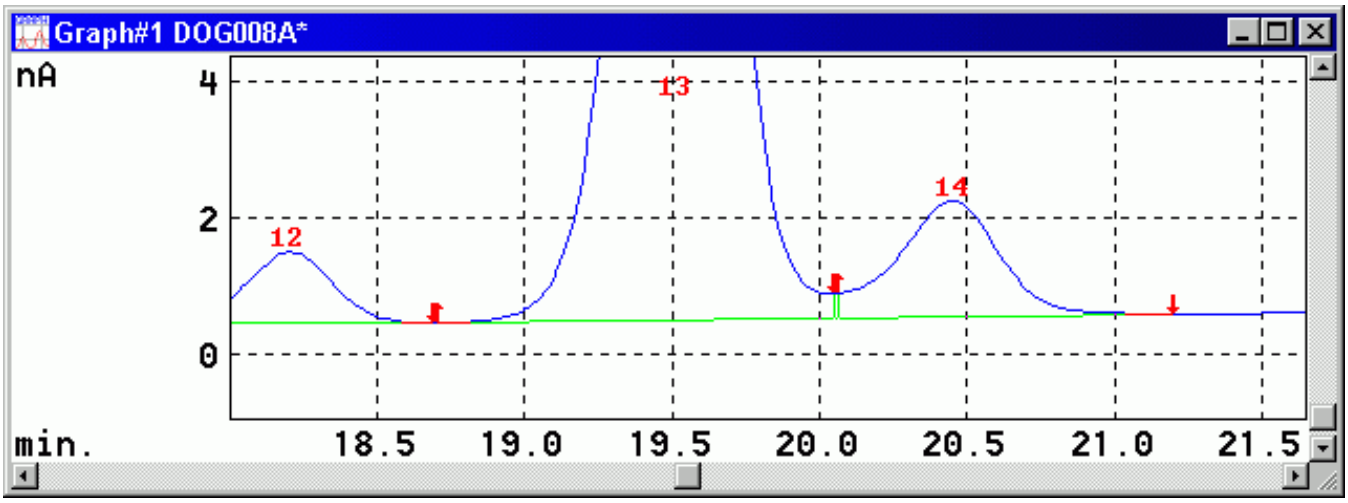
DETECT PEAKS (ON or OFF) determines the times during which ChromGraph searches for peaks. All Methods must have at least one

Peak Detect ON (usually at time 0.0) in order to process data. A more sophisticated use is to turn Peak Detect OFF at time 0.0 to ignore the baseline disturbance at the beginning of the run, and turn it ON before the first peak of interest.

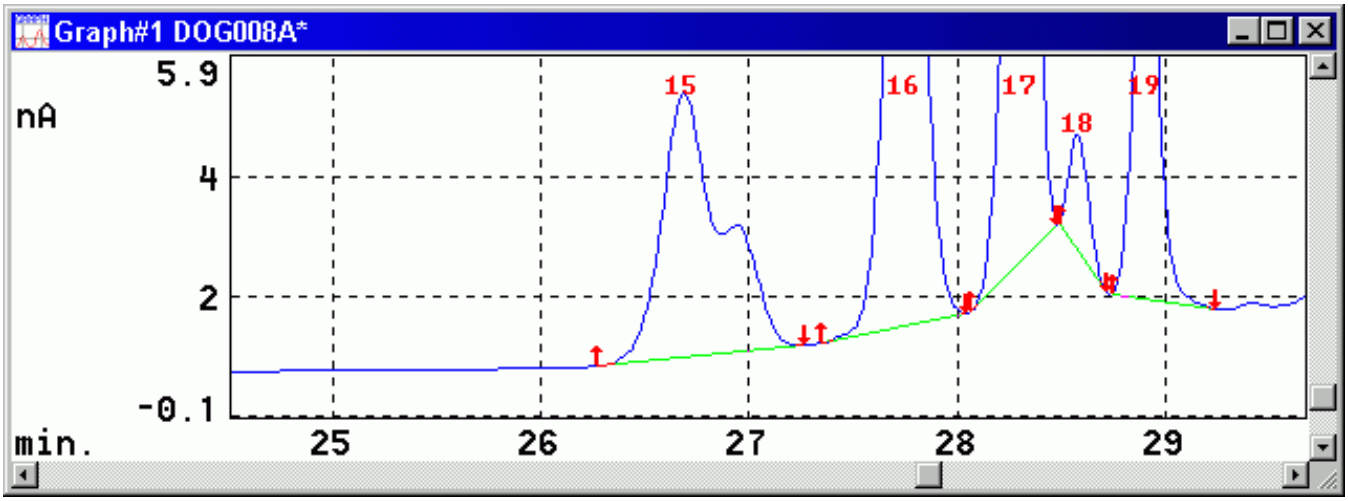
NEGATIVE PEAK (ON or OFF) may be useful in vacancy chromatography. It serves to reverse a negative-going peak so it can be processed like a normal peak. Most users will not use this option.

PERPENDICULAR DROP (ON or OFF) determines how the baseline is drawn under two peaks that are not completely resolved:

ON draws a straight baseline between the start of the first peak and the end of the second, and drops a perpendicular divider from the valley between the two peaks to the baseline. In this figure the green lines indicate the area boundaries of peaks 13 and 14 when perpendicular drop is on:



OFF draws the baseline for each peak independently, resulting in a 'tangent skimming' approach that is appropriate for quantifying small peaks on the shoulders of larger ones. The figure below shows this approach. Had perpendicular drop been on in this case, the area under peak 18 would have nearly doubled.



The default state for Perpendicular Drop is ON.

FIX BASELINE forces the software to accept the baseline value at any given time as a true baseline. Occasionally the baseline at the beginning of a run has such a steep slope that the software won't recognize it as a baseline, and therefore won't recognize the first peak. Inserting a Fix Baseline command before the first peak will solve this problem.

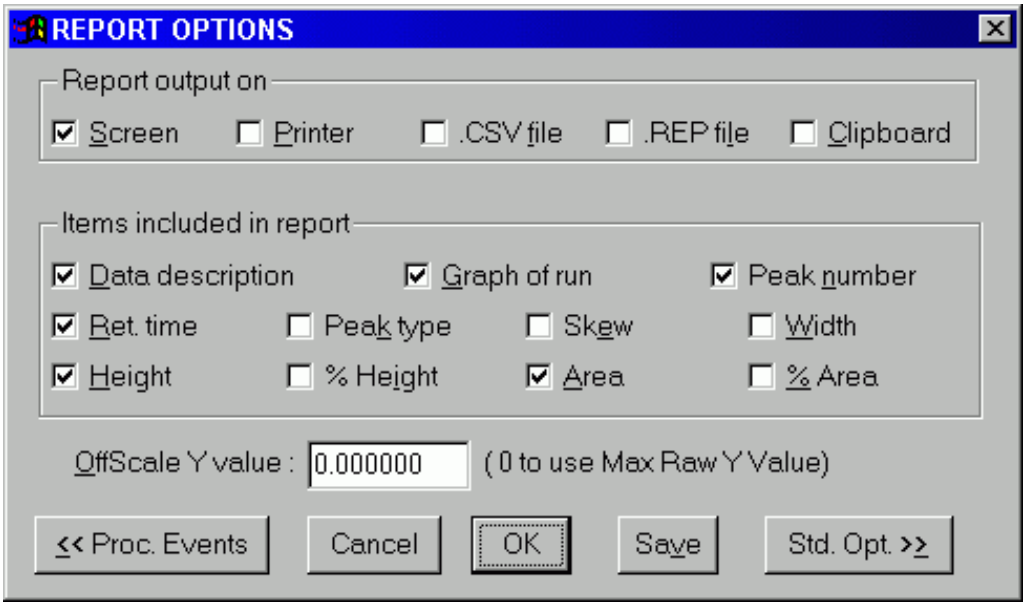
THRESHOLD VALUE allows the Initial Slope Threshold to be changed at a given time. This feature can be used to increase the sensitivity for late-eluting, broad peaks. In most cases, however, the Initial Slope Threshold will be sufficient to detect all the peaks.

To edit an existing event, click on it so its values appear in the edit boxes on the right side of the screen. Make the appropriate changes and click the CHANGE button. To insert a new event, enter the time and event in the edit boxes, then click the INSERT button. To delete an event, highlight it and click the DELETE button. In addition to this editing menu, Processing Events can be edited graphically, using the Event Cursor.

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REPORT OPTIONS

The REPORT OPTIONS screen is accessed through the Method drop-down menu or by clicking the << or >> buttons from other screens. This screen customizes the report of the analysis, detailing which output devices are to be used and which items are to be included:



Output can be routed to the SCREEN and the PRINTER. The remaining output options are intended to transfer reports to spreadsheets or word processors:

PRN, **CSV** and **TAB** files are ASCII files, formatted so their information can be readily imported into spreadsheets . As ASCII files, they contain only text (not graphs). To select PRN, comma-delimited (CSV) or tab-delimited (TAB) files, see the SETUP OPTIONS dialog.

> In INTERACTIVE MODE, requesting a PRN, CSV or TAB file will generate one ASCII file for each Data file (each run). The file will have the Data file name followed by .PRN, .CSV or .TAB (e.g., CATS005A.PRN). This is not very useful, but the ASCII files were designed to be used in automatic mode.
>
> In AUTOMATIC MODE, you can specify one file to which reports for all Data files will be sent. Thus, the results from hundreds of runs can be put in a format that's easily manipulated by a spreadsheet.

REP files also are ASCII files. These are exact images of the reports that are generated on the screen or printer. If selected, one REP file will be generated per Data file, and named with the data file name followed by .REP (e.g., CATS002A.REP). Only text can be stored in a .REP file. REP files are recommended only for those who's regulatory environment requires permanent storage of the original results for each data file.

CLIPBOARD sends an image of the report that is generated on the screen or printer to Clipboard, Windows' internal message board. From Clipboard the image can be pasted to other Windows programs. Since Clipboard can hold only one report or image at a time, reports from subsequent Data files will overwrite those of earlier Data files.

On the remainder of this screen, check the items you wish printed in the final report. The screen and printer have only a finite width; if more items than can fit on a line are selected, the line will wrap around and print on a second line. For neatness, we suggest you select only the items that are important for your analysis. The available items include the following:

DATA DESCRIPTION is the identifying information about the chromatographic run.

GRAPH OF RUN plots the chromatogram.

PEAK NUMBER is a sequential numbering of the peaks in the chromatogram, ordered by retention time.

RETENTION TIME is the elution time of the apex of each peak. Time units are minutes or seconds, as selected in Processing Options.

PEAK TYPE describes how well a peak was resolved from its neighbors. The first letter of this two-letter code indicates whether the peak started at the baseline (B) or in a valley (V) between peaks, and the second letter indicates the same for the end of the peak. Thus, a BV peak is one that began at the baseline, but was not well separated from the following peak.

SKEW is a number from 0 to 2 that represents the symmetry of the peak. A symmetrical peak has a skew of 1. Peaks with skews below 1 have a majority of their area before the apex, while those with skews greater than 1 have a majority of their area after the apex.

WIDTH is the peak width at half height, in the time units selected in Processing Options.

HEIGHT is the height of the peak.

% HEIGHT is the percent contribution made by any one peak to the sum of the heights of all peaks in the chromatogram.

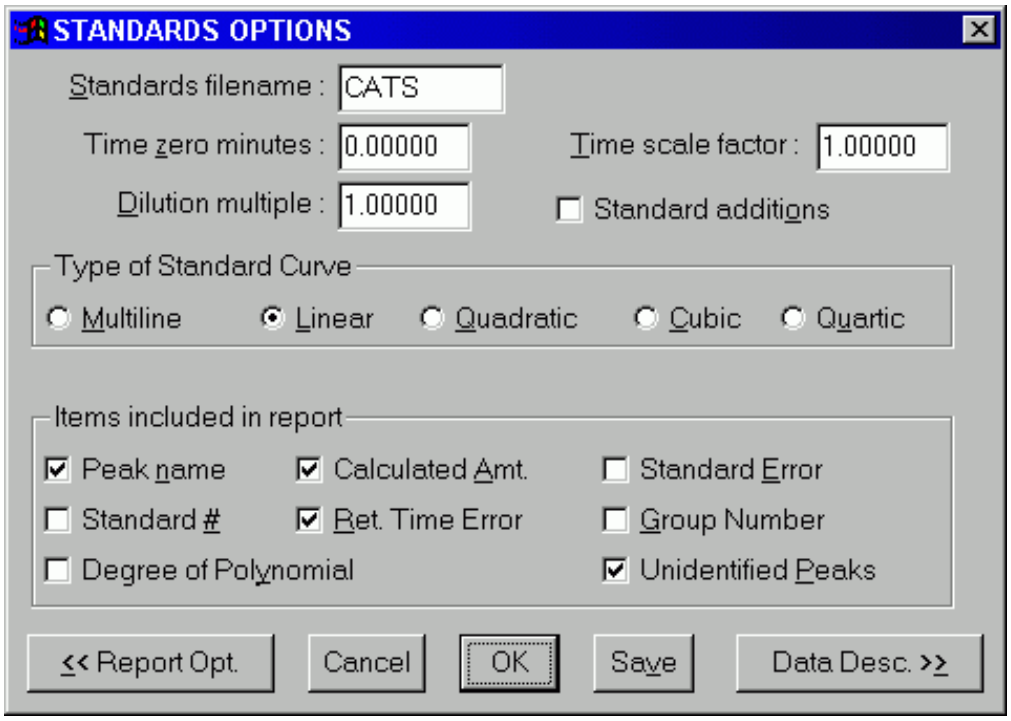
AREA is the area delimited by the peak and the baseline drawn under it.

% AREA is the percent contribution made by any one peak to the sum of the areas of all peaks in the chromatogram.

OFFSCALE Y VALUE can be used under certain circumstances when a detector has a lower full-scale range than does ChromGraph. In this rare situation, an offscale peak on the detector might appear flat topped in ChromGraph, but since it doesn't go off the ChromGraph scale it is not reported as offscale. To remedy this situation, enter a maximum peak height here — peaks having a greater height will be reported as offscale. For most uses, enter a zero here to disable this feature.

STANDARDS OPTIONS

The STANDARDS OPTIONS screen is reached through the Method drop-down menu or by clicking the << or >> buttons from other Method screens. STANDARDS OPTIONS define how the peaks are to be quantitated and which items will appear in the report. Creation and use of standards files is discussed in the section on QUANTITATION.



STANDARDS FILENAME is the name of the Standards file to which the sample results are to be compared. When a new Standards file is created and saved, its name will automatically be inserted here. To skip the standards-comparison step of data processing, delete the name from this box.

TIME ZERO minutes (or seconds) compensates for data whose injection time do not correspond to the injection time of the standard. Time units are those specified in Processing Options This option will rarely be used, and usually should be set to 0.

TIME SCALE FACTOR compensates for runs in which elution was faster or slower than that of the standard. The Time Zero minutes is subtracted from the retention time of each peak, and the result is multiplied by the Time Scale Factor, to make the correction. This option will rarely be used, and usually should be set to 1.

DILUTION MULTIPLE corrects calculated amounts for any known dilutions of the samples. Thus, if you diluted your samples in half before analyzing, a dilution multiple of 2.0 will double the calculated amounts so they are corrected to the original sample. The default value of 1.0 eliminates the correction. The correction is **applied equally to all samples**. To apply corrections when individual samples differ in volume or mass, see the discussion above and in the section about importing sample information into ChromGraph Control.

The **STANDARD ADDITIONS** checkbox indicates whether you have used standard additions (endogenous) calibration. This occurs when a pooled matrix (such as plasma or urine) containing an unknown but constant amount of an endogenous analyte is spiked with known amounts of the analyte to create a series of calibrators. In this situation the size of the peak is determined by both the endogenous amount and the spiked amount. The endogenous amount is represented by the y-intercept of the regression equation, and the spiked amounts by the slope. When you check this box the y-intercept will be dropped from the formula, thereby correcting for the endogenous component of the calibrators.

TYPE OF STANDARD CURVE: Choose MULTILINE (point-to-point), LINEAR, QUADRATIC, CUBIC or QUARTIC. Most standard curves are linear (if there are three or more levels of standards) or multiline (two or fewer levels).

The following items will be included in the report if checked. Since there are too many items to fit neatly across the width of a standard page, we suggest that you select only those that are useful in the report.

PEAK NAME is the name assigned to a peak with a given retention time. Names are entered when creating a Standards file.

CALCULATED AMOUNT is the result of comparison of a sample component to internal and/or external standards.

STANDARD ERROR is the standard error of the calculated amount.

STANDARD # is the sequential number of the peak with this retention time in the Standards file.

RET. TIME ERROR is the difference in time between the unknown peak and the standard peak. Time units are those selected in Processing Options.

GROUP NUMBER allows the standards to be assigned to categories for pooled area reports. Standard peaks are assigned to categories when the Standards file is created.

DEGREE OF POLYNOMIAL (0 = multiline, 1 = linear, etc.) is the coefficient of the standard curve that was actually used for quantification. This may be lower than the degree requested above (Type of Standard Curve) if some standard peaks or levels are missing.

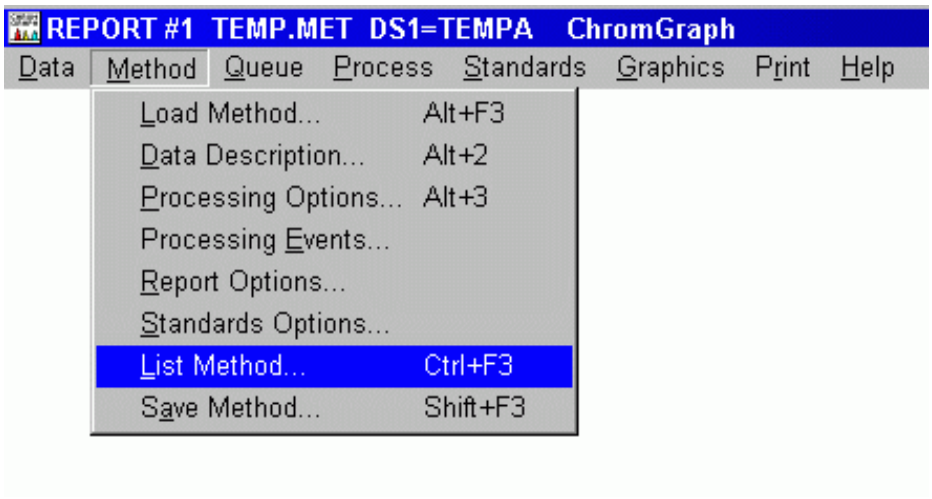
UNIDENTIFIED PEAKS are those that do not match any peak in the Standards file. They may be included in the report by checking this option.

FILES CREATED

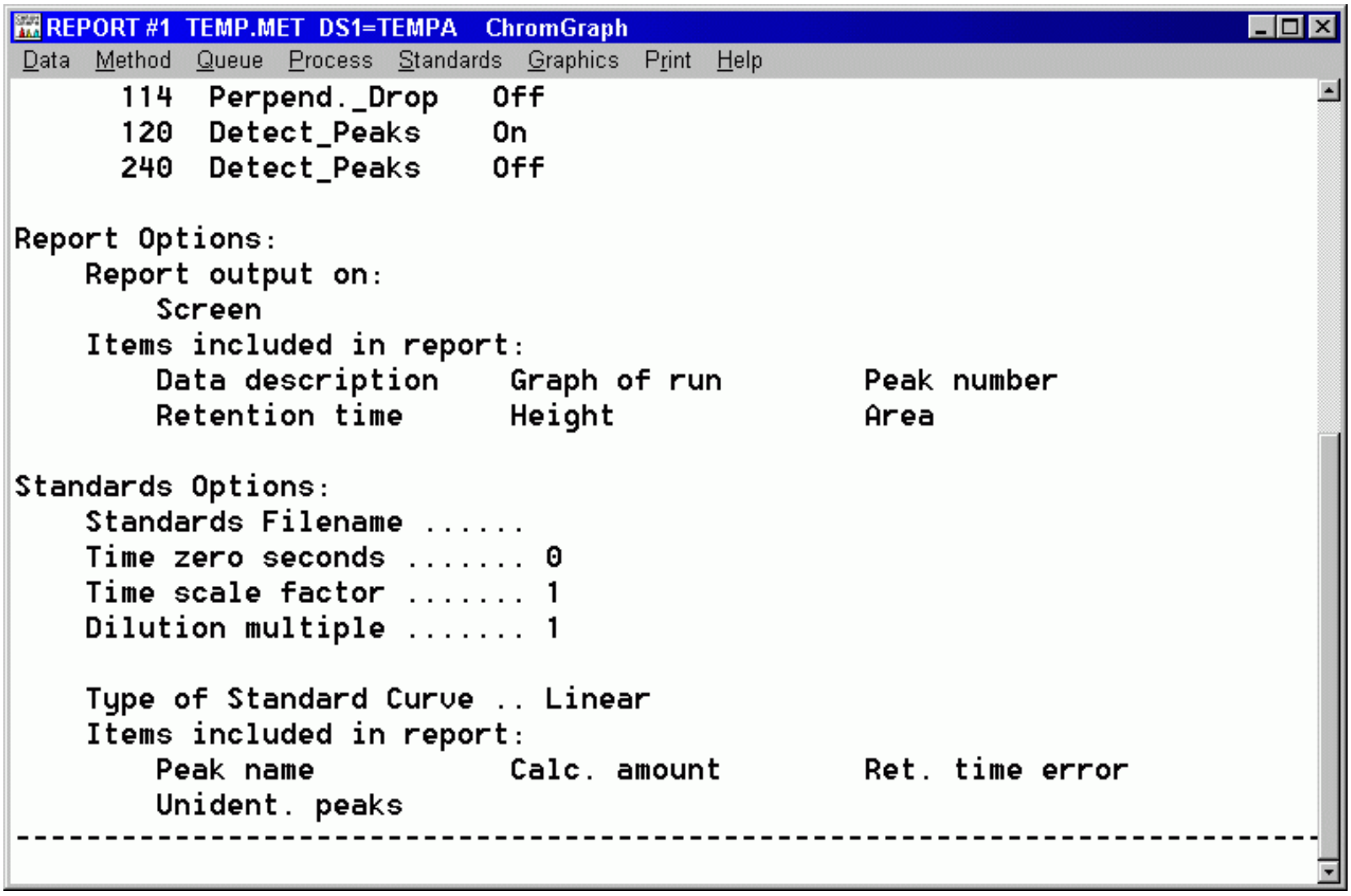
A complete list of the files created by both ChromGraph Control and ChromGraph Report is available here.

LISTING A METHOD

To review the Method, you may scroll forward or back through all the screens using the << and >> buttons, or choose LIST METHOD from the Method drop-down menu:



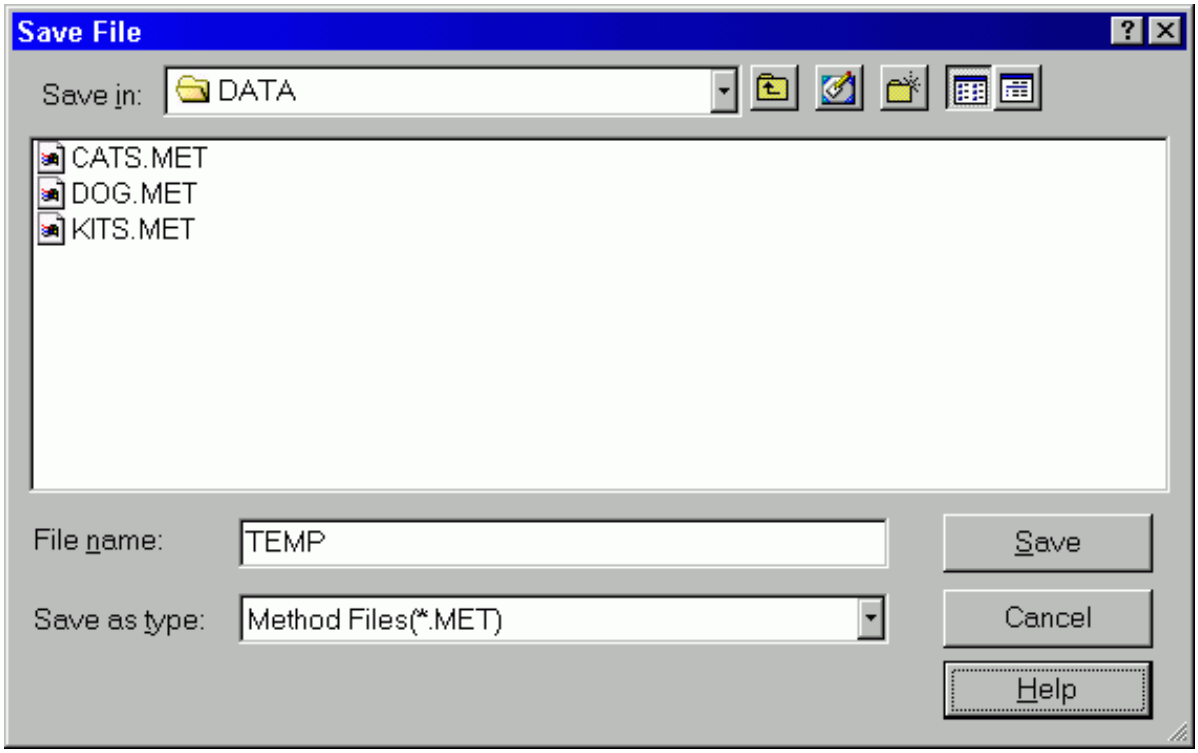
Methods may be listed to the screen, printer, disk and Clipboard. If listed to the screen, they scroll along the rearmost section of the screen, the OUTPUT WINDOW. After the listing is finished, you may scroll through the Method by using the scroll bars on the right side of the Output Window:



SAVING A METHOD

Choose the SAVE button from any of the Method screens, or SAVE METHOD from the Method drop-down menu. A SAVE screen will

appear, showing the default Method name and the location to which it will be saved:



A listbox will show the existing Methods in the default data location. Change the Method name or data location as appropriate and click on the OK button to save it. The extension .MET will automatically be appended to the name you choose. If a file of the same name already exists, a warning will appear.

Method names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\mymethod.met = 25 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a Method name may be truncated somewhat in various places in the program.

ChromGraph CONTROL Methods have the extension .MTH to distinguish them from ChromGraph REPORT Methods. They can therefore be given the same name, to associate them. For example, Methods to run and process catecholamine samples can be named CATS.MTH and CATS.MET, respectively, while those to run and process amino acids can be named AMINO.MTH and AMINO.MET.

- **CONTENTS**

INTERACTIVE DATA PROCESSING

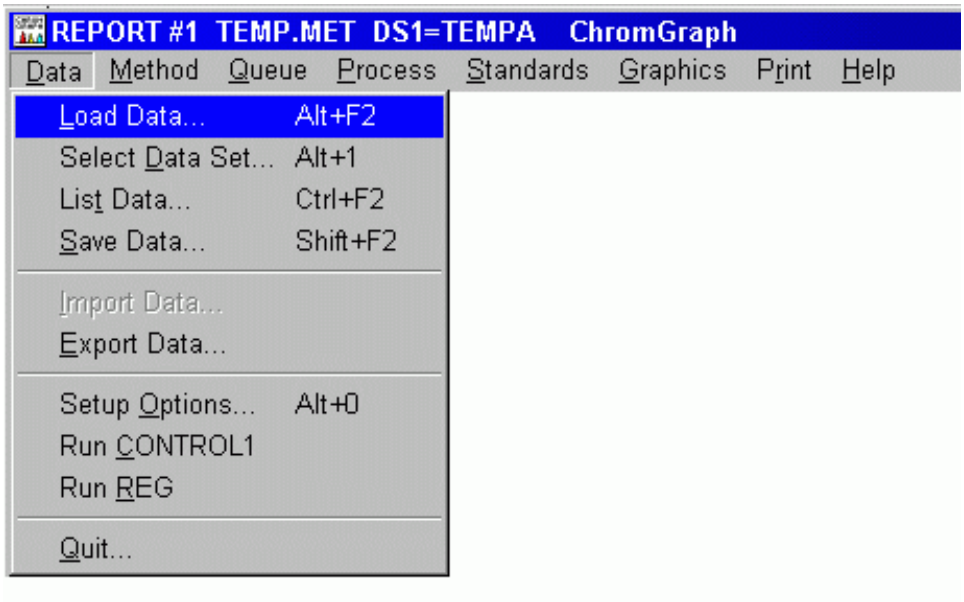
ChromGraph REPORT can rapidly process an almost unlimited number of Data files in the fully AUTOMATIC MODE. A slower interactive approach is useful, however, when optimizing a Method for a particular analysis, and when learning to use the software.

INTERACTIVE DATA PROCESSING includes three main functions: peak detection, integration and quantitation. During peak detection the software determines where peaks start and stop. During integration, which can only occur after peak detection, the areas under the peaks are determined. These peak areas are compared to the areas of known standards during quantitation, which estimates the amount of each analyte in the injection.

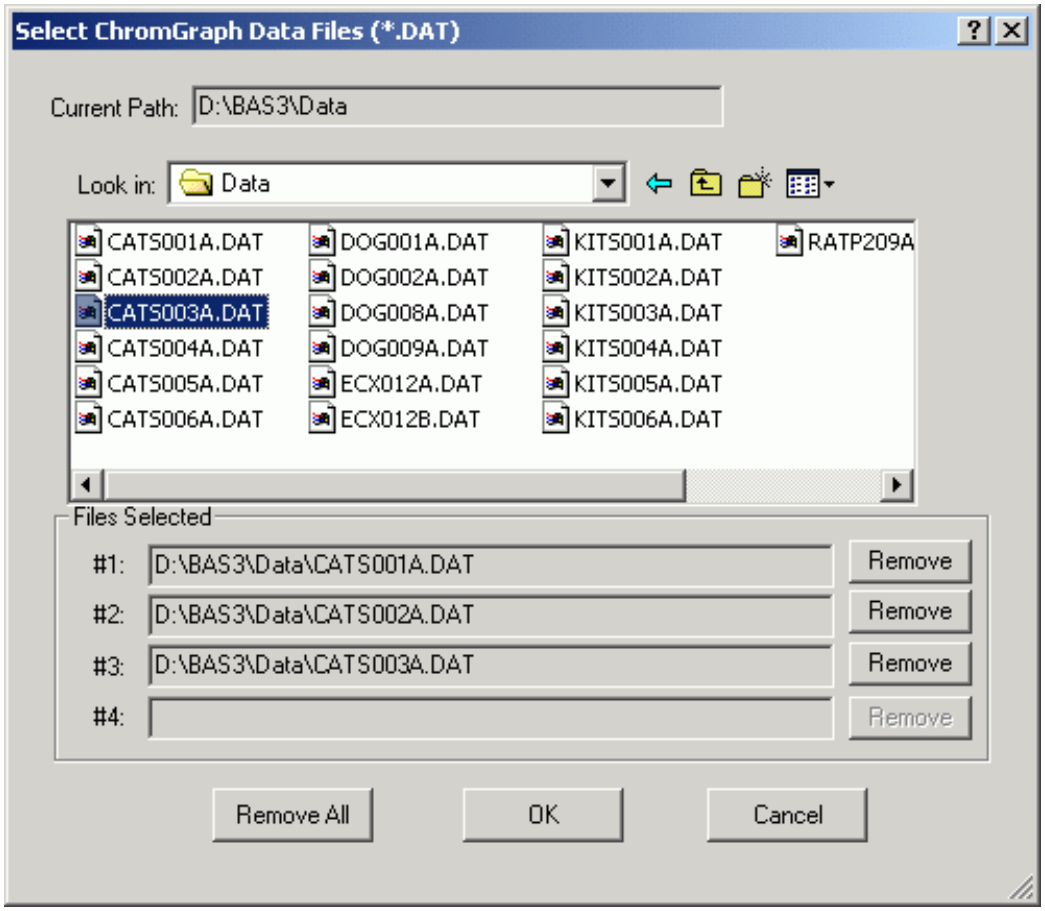
- **LOADING DATA**
- **DETECTING PEAKS**
- **INTEGRATION**
- **QUANTITATION**
- **SEMI-AUTOMATIC PROCESSING**
- **CONVERTING PEAK AREAS TO COULOMBS**

LOADING DATA

Begin the interactive processing mode by loading the Data files to be processed. Take the LOAD DATA option from the Data Menu:



The SELECT CHROMGRAPH DATA FILES dialog box appears:



In interactive mode only four Data files (runs) can reside in RAM simultaneously. The Files Selected section of this window gives the name and slot number for each Data set that is already present in RAM. If you wish to keep these in RAM, do nothing. If you wish to replace these with other Data files, click the Remove button.

Click on the Data files you wish to load and their names will be transferred to the Files Selected section. To follow along in this section, load the files indicated in the figure above. These will be present on your disk if you followed the installation instructions (link to software installation). Click on OK and the files will be sequentially loaded into the designated slots. Also load the appropriate Method for these Data files, CATS.MET (if necessary, review the LOAD METHOD procedure).

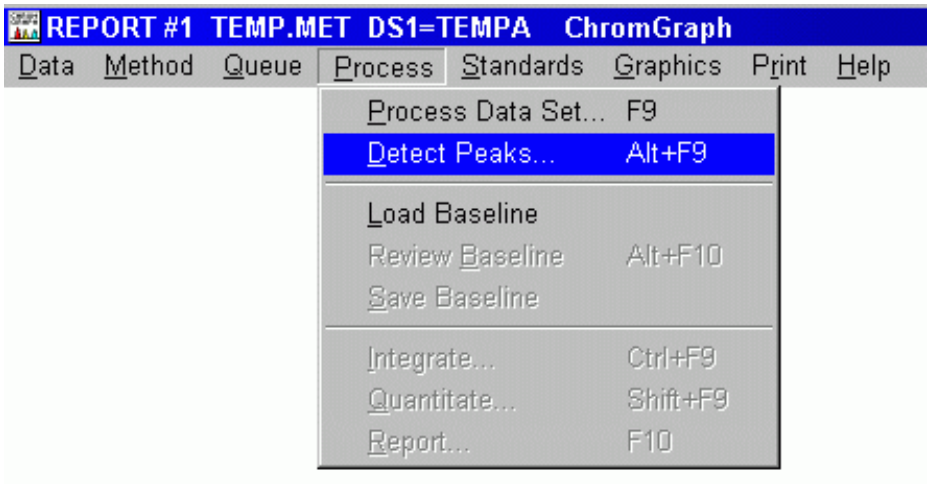
Data or Method files can be loaded from other locations by using the mouse to select other drives or subdirectories in the Look in: section of this window. Such a selection automatically becomes the new DEFAULT DATA LOCATION. The default location will remain in effect throughout the session, unless changed by a LOAD or SAVE operation. When you finish your session and QUIT the software, saving the REPORT.INI file will save the new default data location for subsequent sessions. If REPORT.INI is not saved, the new data location will be forgotten; the original default location will be used for subsequent sessions.

When you load multiple Data files, the last-loaded file automatically becomes the focus for subsequent operations. You can change this focus with the SELECT DATA SET option under the Data Menu, or with some of the options under the Process Menu.

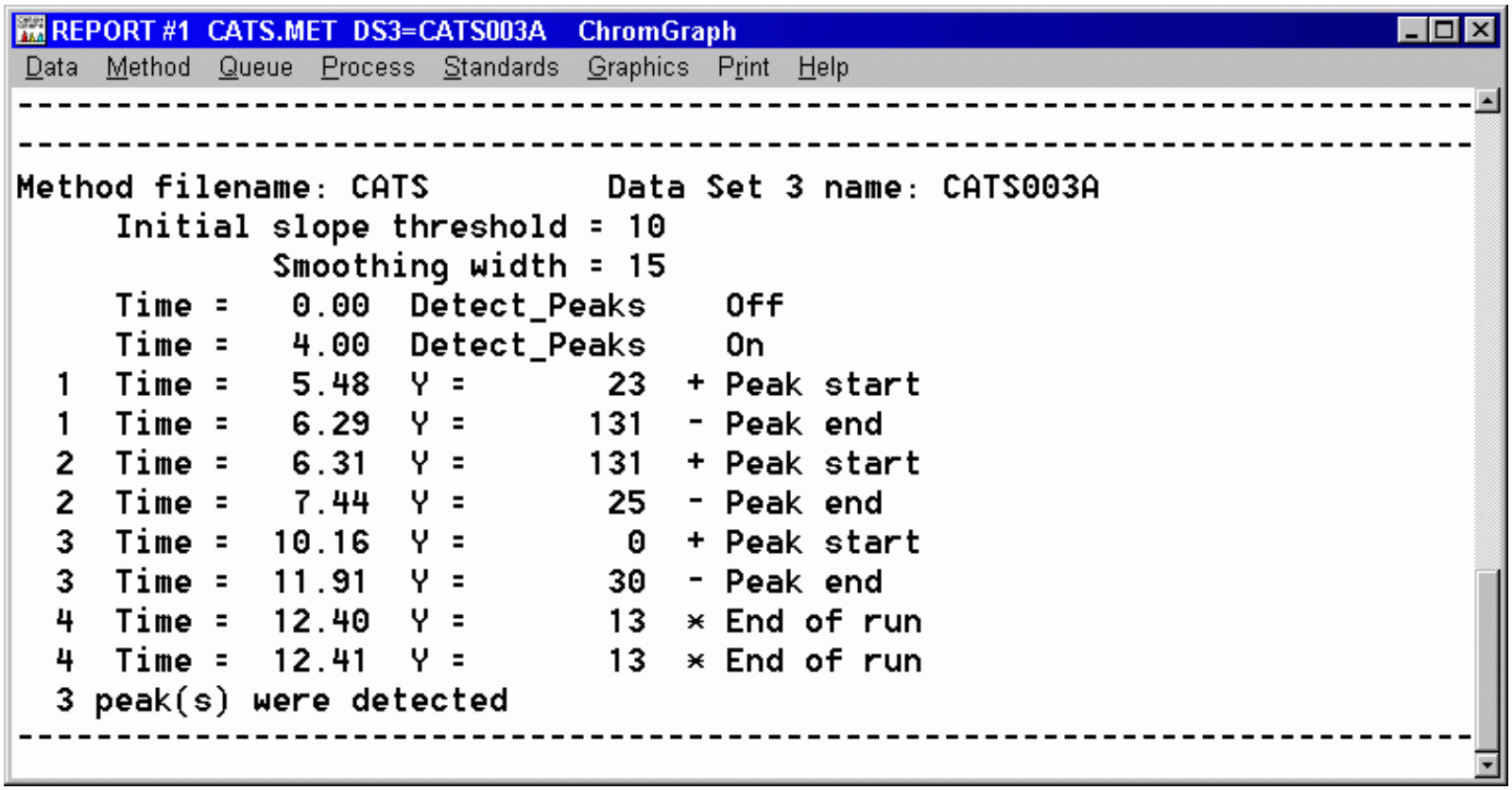
DETECTING PEAKS

The first step in interactive processing is to identify the peaks that are present. In this step the software determines what a normal baseline is (a consecutive set of data points whose values don't change significantly) and then determines where peaks start and stop (consecutive sets of data points whose values change markedly). The Processing Options involved in these decisions (INITIAL SLOPE THRESHOLD, SMOOTHING WIDTH, PREBUNCH, PREFILTER, and PROCESSING EVENTS) are discussed in the Method section.

To begin peak detection, first turn on the List Peaks option in Processing Options. Then select the DETECT PEAKS option from the Process Menu:



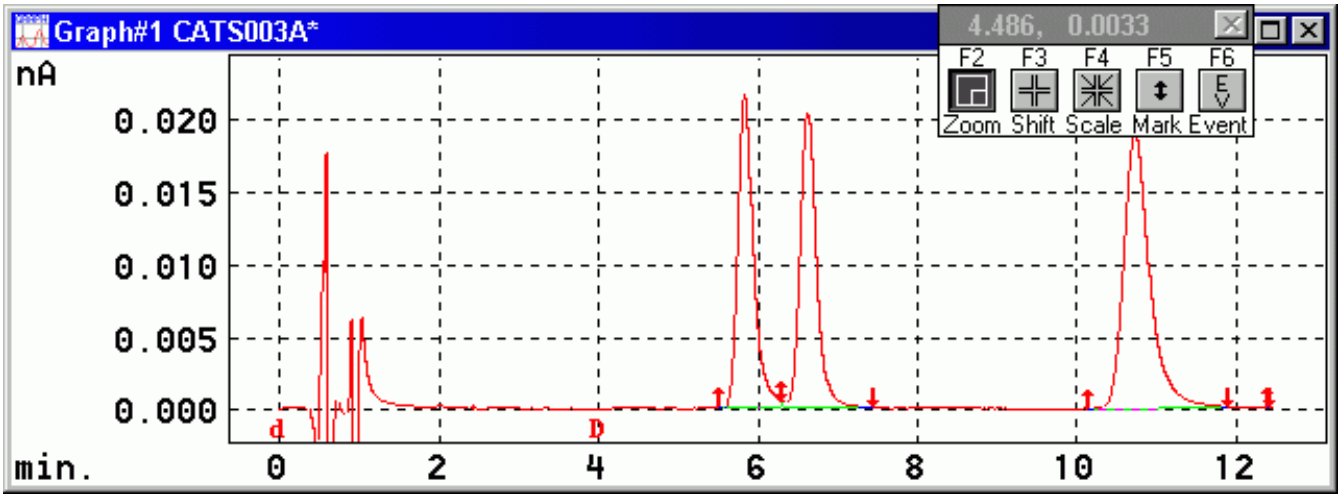
Select the screen as LIST DEVICE when prompted. As peak start and stop points are detected, a list of times and baseline values will be posted to the output window:



These can be reviewed by using the scrolling feature of the Output Window.

If you wish to see the chromatogram at this point, take the REVIEW BASELINE option from the Process Menu:

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The graph shows the chromatogram, with up- and down-arrows indicating the start and stop points of peaks. All the interactive features of ChromGraph graphics are available in this screen.

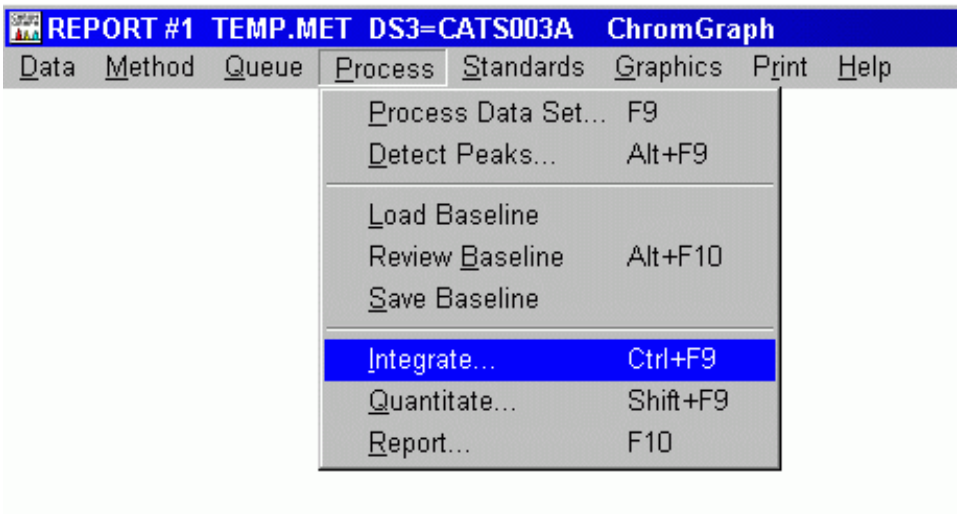
At this point you can save the graph by taking the SAVE BASELINE option from the Process Menu. The file will be saved with the data name and run number, and a .BLN extension. Baseline files take up a lot of disk space, and can easily be recreated from the raw data using the REVIEW BASELINE option. Therefore, we recommend that they only be saved if you are required by regulation to archive the original.

You can also LOAD a BASELINE from the Process Menu, which brings up a choice of previously saved baseline files for review.

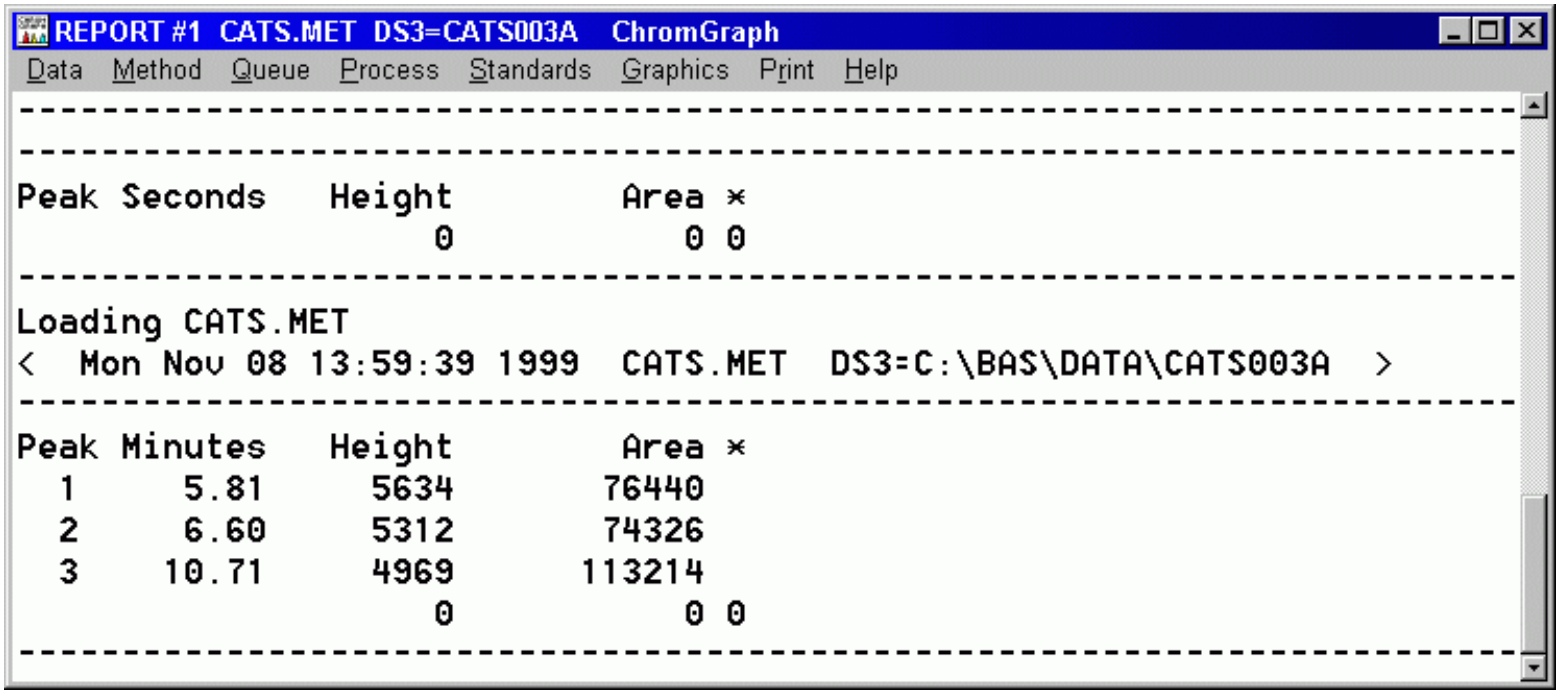
It's likely that the first attempt at peak detection for an unknown Data file will not provide entirely satisfactory results. There may be peaks that have not been detected. Or every little blip in the baseline may have been interpreted as a peak. If this is the case, return to the PROCESSING OPTIONS and PROCESSING EVENTS sections of the Method, adjust the relevant parameters, and detect the peaks again. Feel free to enter extreme values to see what will happen; it's a good way to gain familiarity with the software. Or use the OPTIMIZE VARIABLES procedure to let Report suggest some values.

INTEGRATION

Integration can proceed only after the peaks have been detected. The integration function determines the area under each peak between the start and stop points. To integrate the peaks, select INTEGRATE from the Process Menu and choose the screen as LIST DEVICE when prompted:



A list of sequentially numbered peaks, with retention times, heights and areas, will be posted to the Output Window. These may be reviewed with the scrolling feature of the Output Window:



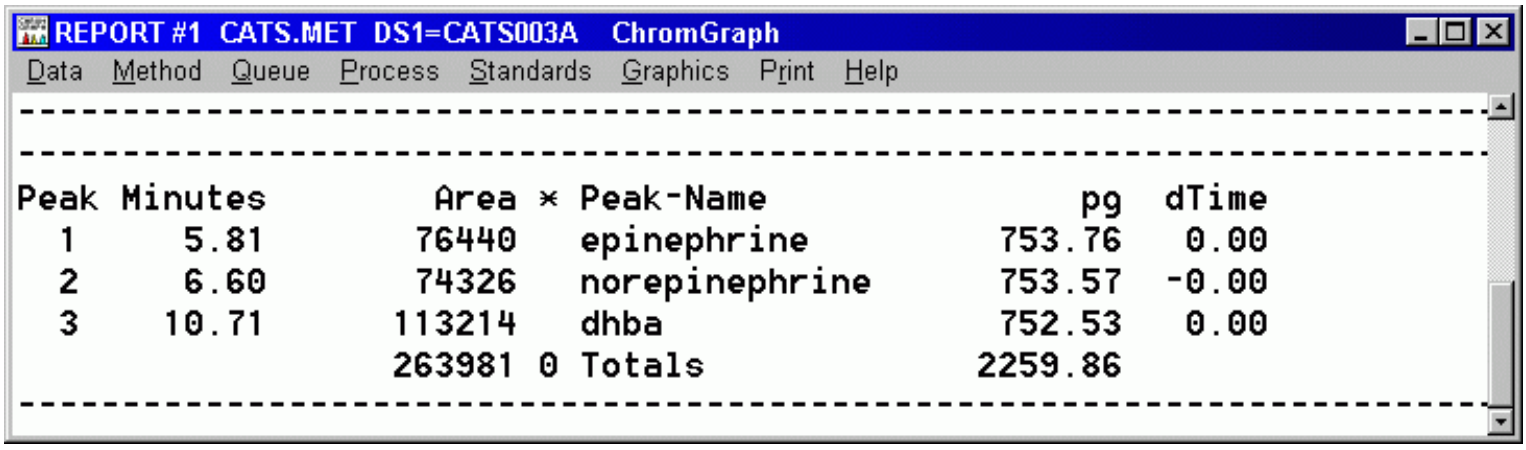
Small peaks of little interest may be deleted from the list of peaks by raising the MINIMUM PEAK AREA. This change requires that you re-detect the peaks.

QUANTITATION

Quantitation is the comparison of the integrated data to a pre-defined set of integrated standards, called a STANDARDS FILE. The Standards file must be created as described in the STANDARDS section.

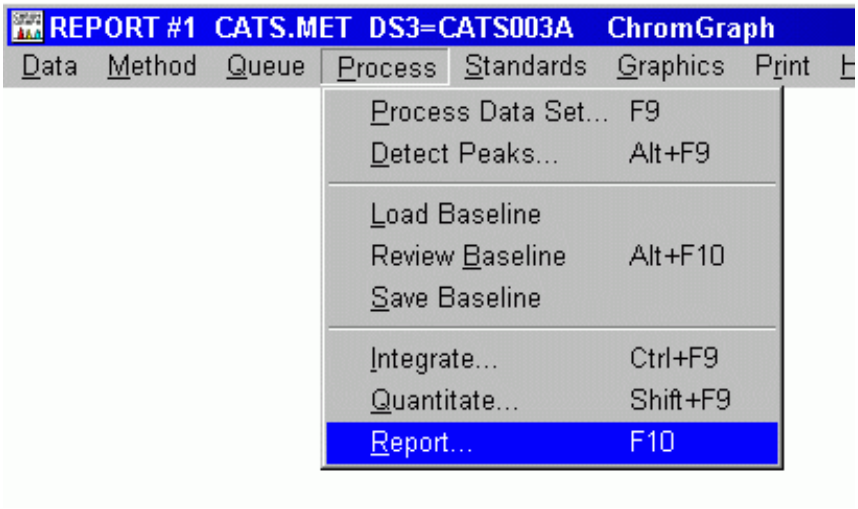
In order to quantitate in the interactive mode, you must have integrated the current Data file and have a Standards file in RAM. If a Standards file is specified in the Method, as is the case with CATS.MET, it will automatically be loaded when the Method is loaded. Otherwise, Standards files may be loaded through the LOAD STANDARDS option of the Standards Menu.

To quantitate, select QUANTITATE from the Process Menu, and choose the screen as LIST DEVICE when prompted. A sequential list of peaks, and the results of the quantitation, will appear on the Output Window:



The items that appear in the list are those checked in the REPORT OPTIONS and STANDARDS OPTIONS sections of the Method The column under the asterisk (*) is a comment column: a question mark (?) here indicates that the calculated amount was extrapolated more than 10% from the standard curve, and may be questionable. A pound sign (#) indicates that a peak specified in the standards file was not found in this sample.

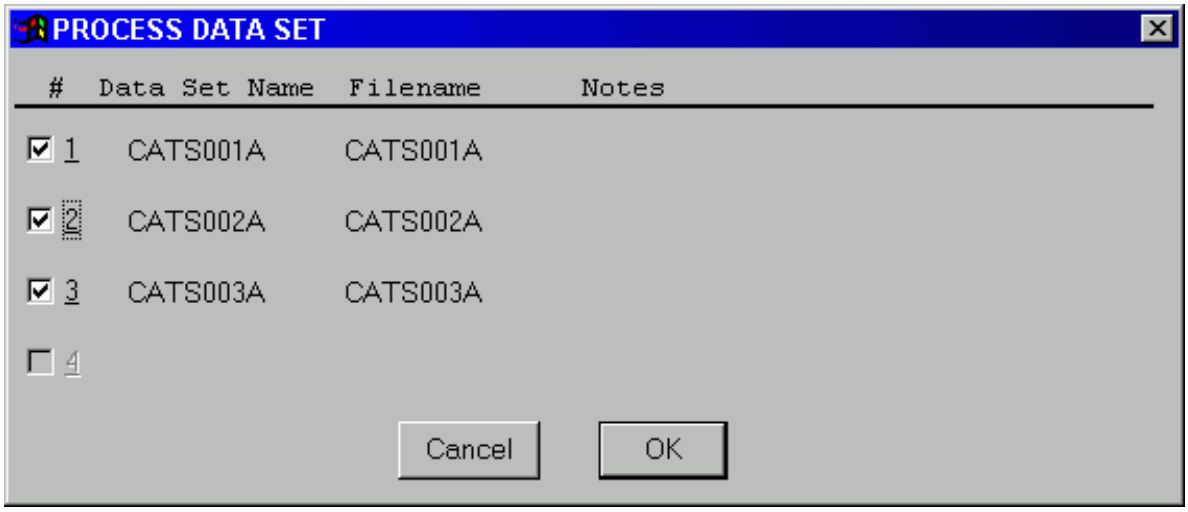
A complete report, including a descriptive header and all items (including the graph) selected in the Method, can be obtained by selecting the REPORT option in the Process Menu:



Once the data analysis and reporting options are properly set, be sure to SAVE the updated version of the Method.

SEMI-AUTOMATIC PROCESSING

From one to four runs can be processed fairly rapidly, once a satisfactory Method has been developed. Select PROCESS DATA SET from the Process Menu. The Process Data Set dialog box will appear, allowing the selection of any (or all) Data files in RAM for processing:



Click on the Data files to be processed, and then on OK. The Data files will be processed sequentially according to the options specified in the Method.

CONVERTING PEAK AREAS TO COULOMBS

****** This is a special topic that most users need not read. ******

The digitizer in epsilon and DA-5 systems from BAS divides a full-scale signal into 1,000,000 subdivisions, informally called counts. Peak heights are reported in counts, and areas are reported as the summed counts for all the data-collection times under the peaks.

This is an excellent procedure for most purposes, including quantification — counts of an unknown are compared to counts of a known, and the units of 'counts' don't matter.

An occasional user who's well-versed in electrochemistry might want to convert counts to amperes and area to coulombs. Here's how it's done:

COUNTS TO AMPERES

Simply divide counts by 1,000,000 to get the proportion of full scale that the peak represents, then multiply by the Range setting:

amperes = (counts ÷ 1,000,000) × Range

Be sure to use the correct units of Range (e.g., nA, µA).

AREA TO COULOMBS

Use the formula:

coulombs = (area × Range) ÷ (pps × 1,000,000)

where pps = points per second = data-collection rate, in seconds.

Again, be sure to use the correct units of Range.

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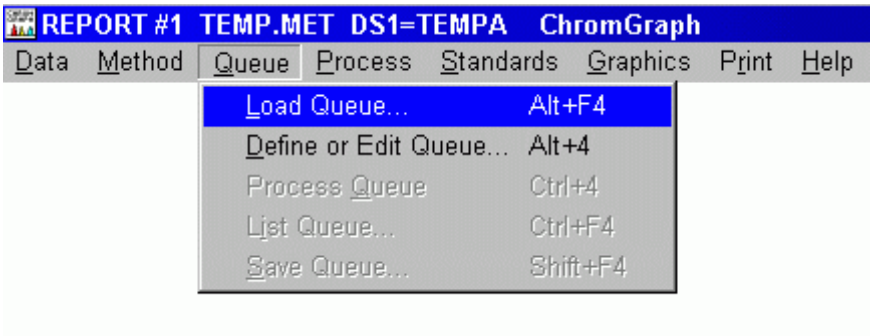
QUEUES -- AUTOMATIC PROCESSING

A QUEUE is a list of Data files to be processed and the Methods to process them with. It is used for automatic data processing, for export of processed data to spreadsheets, and for automatically receiving data from ChromGraph CONTROL (see the section on linking).

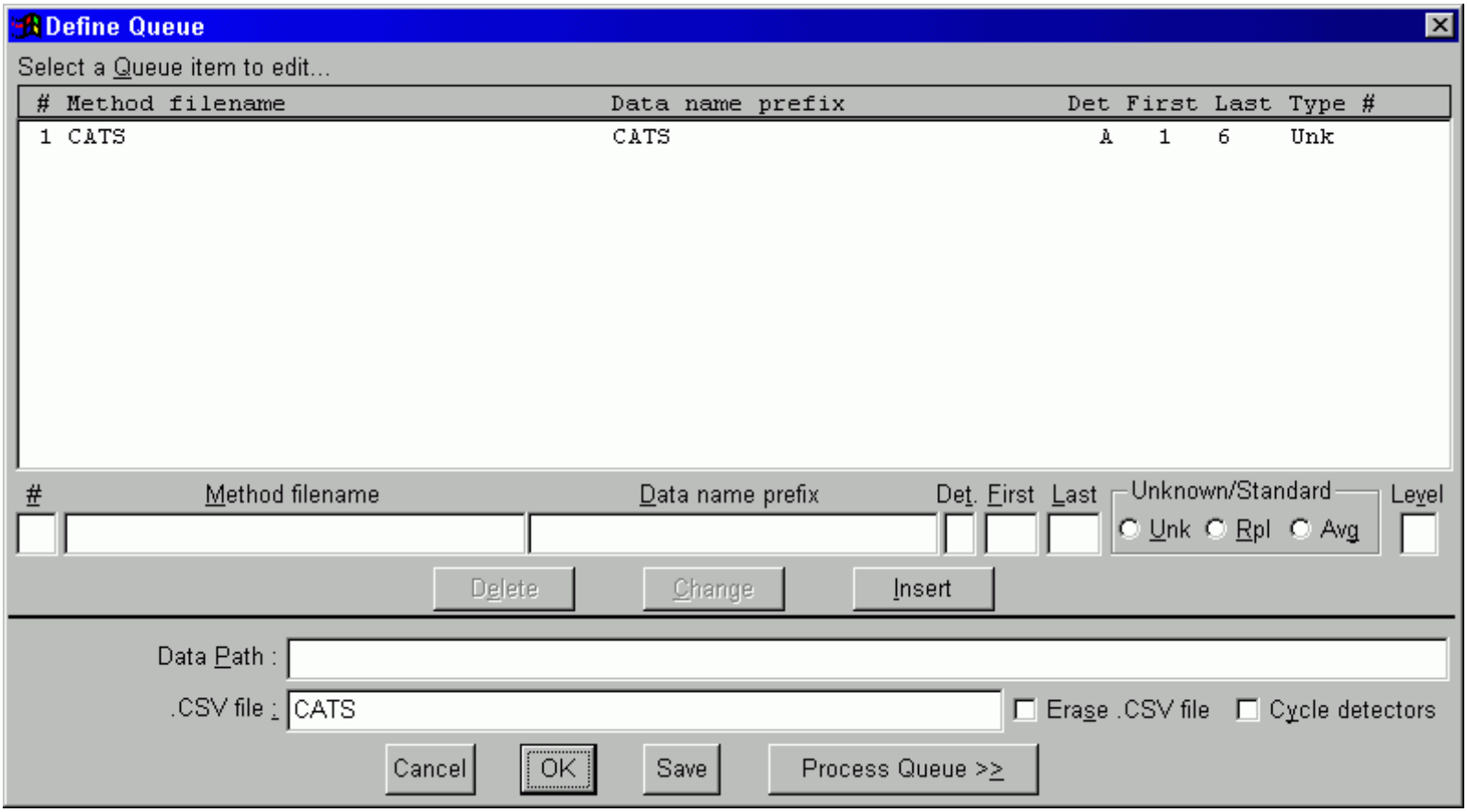
- **USING A QUEUE**
- **EXPORTING TO SPREADSHEETS**

USING A QUEUE

Queues may be accessed through the QUEUE MENU:



To begin setting up a Queue, either LOAD a previously saved Queue from disk, or take the DEFINE OR EDIT QUEUE option. This opens the DEFINE QUEUE dialog box:



The Define Queue dialog box contains a listbox of up to 20 lines. Each line directs ChromGraph to use a specific Method to process specific

Data files. The Queue is not limited to just 20 lines: you can chain Queues together by entering the name of a subsequent Queue as the Method name in the last line of any Queue.

Queue instructions are edited in the row of edit boxes across the middle of the screen, just above the row of DELETE, CHANGE and INSERT buttons. To edit an existing line in the Queue, first click on the line in the listbox. That line will then appear in the edit boxes. Make the appropriate changes, then click on the CHANGE button. The modified line will then be posted to the listbox.

To insert a new line in the Queue, first enter the information in the edit boxes. Then click the INSERT button.

To delete a line from the Queue, click on the line, then click DELETE.

The following items appear in the lines of the Queue:

is the sequential line number, 1-20.

METHOD is the ChromGraph Report Method to use to process the data. This Method must exist in the Default Data Location.

DATA is the Data file name.

DET. is the detector letter code, A-D.

FIRST and **LAST** define the sequence of run numbers to be processed.

UNKNOWN/STANDARD indicates whether these Data files are from standards or unknowns. If they are standards, indicate whether the old standards are to be replaced (RPL) by the new data, or averaged (AVG) with them. For standards, also indicate which level (concentration) of standards is being processed. The Quantitation section contains detailed information on automatically updating Standards files using a Queue.

The options at the bottom of the screen provide general instructions for processing the Queue:

DATA PATH can be used to specify a non-default location from which to get Data files. Leave this box blank to get data from the Default Data Location. The Method files must always be in the Default Data Location.

PRN FILE and **ERASE .PRN FILE** are used for exporting to spreadsheets.

CYCLE DETECTORS can be useful if more than one detector was employed. If there are 20 runs for detector A on the first line of the Queue, and 20 runs for detector B on the second line, the Queue will usually process all the runs for detector A before going on to B. If the CYCLE DETECTOR box is checked, however, ChromGraph Report will process run 1 detector A, then run 1 detector B, then run 2 detector A, and so on.

Once editing is complete, the Queue may be saved for future use with the SAVE QUEUE option on the Queue Menu or the SAVE button in the Define Queue dialog box. A Queue name can have any combination of characters and spaces; the extension .QUE will automatically be appended.

Queue names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\myqueue.que = 24 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a filename may be truncated somewhat in various places in the program.

ChromGraph CONTROL QUEUES, used for automatic data collection, have the extension .BAQ to distinguish them from ChromGraph Report Queues, used for automatic data processing. To save time when working with a complex Queue, the ChromGraph CONTROL Queue can be loaded directly into ChromGraph REPORT (some editing will be necessary).

To begin processing the Queue, click on PROCESS QUEUE in the Define Queue dialog box or in the Queue Menu. As each run is processed, the FIRST entry in the current line of the Queue is incremented by one. If you wish to restart the Queue from the beginning, either change FIRST back to its original number or load the SAVEd version of the Queue.

If a Data file specified in the Queue is not present in the Default Data Location (or in the Data Path, if specified), the message REPORT QUEUE AWAITS, with the complete Data file name, will appear. This is normal behavior if ChromGraph REPORT is linked to ChromGraph CONTROL: when the run is completed, the DATA file will be passed to REPORT and processing will begin. If REPORT is not linked to CONTROL, the message indicates that the specified Data file cannot be found. Cancel the Queue and check for errors in the name

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or location of this Data file.

EXPORTING TO SPREADSHEETS

The PRN/CSV/TAB option allows you to easily import your data into spreadsheet programs. Spreadsheets are designed to import delimited ASCII files with, depending on the spreadsheet and version, a PRN, CSV, or TAB delimited format.

Consult your application's documentation to determine which type of field-delimited file it can import. Individual data fields in a PRN-style file are typically separated by a comma, space, or semicolon, with character strings contained within quotation marks. Each line in a PRN file begins with an apostrophe:

```
'"Peak Minutes   Height        Area * Peak-Name                pg  dTime Data Run D
'"0000 0000000 00000000 000000000 * ---Peak-Name--- 0000000000 000000 Data 000 D
'"  1     5.82     2022       27114   epinephrine         253.76   0.01 CATS   1 A
'"  2     6.61     1906       26020   norepinephrine      253.57   0.01 CATS   1 A
'"  3    10.72     1767       39580   dhba                252.53   0.01 CATS   1 A
'"                5694       92714 0 Totals              759.86        CATS   1 A
'"  1     5.82     3763       50665   epinephrine         492.49   0.00 CATS   2 A
'"  2     6.61     3545       49138   norepinephrine      492.86   0.00 CATS   2 A
'"  3    10.72     3329       75279   dhba                494.94   0.01 CATS   2 A
'"               10637      175082 0 Totals             1480.28        CATS   2 A
'"  1     5.81     5634       76440   epinephrine         753.76   0.00 CATS   3 A
'"  2     6.60     5312       74326   norepinephrine      753.57  -0.00 CATS   3 A
'"  3    10.71     4969      113214   dhba                752.53   0.00 CATS   3 A
'"               15914      263981 0 Totals             2259.86        CATS   3 A
'"  1     5.82     1845       25470   epinephrine         237.10   0.00 CATS   4 A
'"  2     6.60     1708       23675   norepinephrine      229.30  -0.00 CATS   4 A
'"  3    10.71     1575       34366   dhba                217.13  -0.00 CATS   4 A
'"                5128       83512 0 Totals              683.52        CATS   4 A
'"  1     5.81     3715       50679   epinephrine         492.63  -0.00 CATS   5 A
'"  2     6.60     3506       49147   norepinephrine      492.95  -0.00 CATS   5 A
'"  3    10.70     3282       73440   dhba                482.45  -0.01 CATS   5 A
'"               10503      173266 0 Totals             1468.03        CATS   5 A
'"  1     5.81     5567       74787   epinephrine         737.00  -0.00 CATS   6 A
'"  2     6.60     5247       72870   norepinephrine      738.50  -0.01 CATS   6 A
'"  3    10.70     4952      111500   dhba                740.89  -0.01 CATS   6 A
'"               15767      259157 0 Totals             2216.39        CATS   6 A
```

Since the PRN file format is the oldest, the importation of PRN files is supported by a wide variety of applications.

As the name might suggest, TAB delimited files contain data fields which are separated by the Tab character. This format may be most widely used by database programs.

CSV indicates a third type of field delimiter. CSV stands for Comma Separated Values. This format separates the contents of each data field with a comma, and leaves it up to the application to determine if characters in a field are text or numeric data:

```
Peak,Minutes,Height,Area,*,Peak-Name,pg,dTime,Data,Run,D,Method,Operator,Conditions,
1,5.82,2022,27114,,epinephrine,253.76,0.01,CATS,1,A,CATS,,,,1,CATS,1.00000,0.00000,1
2,6.61,1906,26020,,norepinephrine,253.57,0.01,CATS,1,A,CATS,,,,1,CATS,1.00000,0.0000
3,10.72,1767,39580,,dhba,252.53,0.01,CATS,1,A,CATS,,,,1,CATS,1.00000,0.00000,1.00000
,,5694,92714,0,Totals,759.86,,CATS,1,A,CATS,,,,1,CATS,1.00000,0.00000,1.00000,
1,5.82,3763,50665,,epinephrine,492.49,0.00,CATS,2,A,CATS,,,,2,CATS,1.00000,0.00000,1
2,6.61,3545,49138,,norepinephrine,492.86,0.00,CATS,2,A,CATS,,,,2,CATS,1.00000,0.0000
3,10.72,3329,75279,,dhba,494.94,0.01,CATS,2,A,CATS,,,,2,CATS,1.00000,0.00000,1.00000
,,10637,175082,0,Totals,1480.28,,CATS,2,A,CATS,,,,2,CATS,1.00000,0.00000,1.00000,
1,5.81,5634,76440,,epinephrine,753.76,0.00,CATS,3,A,CATS,,,,3,CATS,1.00000,0.00000,1
2,6.60,5312,74326,,norepinephrine,753.57,-0.00,CATS,3,A,CATS,,,,3,CATS,1.00000,0.000
3,10.71,4969,113214,,dhba,752.53,0.00,CATS,3,A,CATS,,,,3,CATS,1.00000,0.00000,1.0000
,,15914,263981,0,Totals,2259.86,,CATS,3,A,CATS,,,,3,CATS,1.00000,0.00000,1.00000,
1,5.82,1845,25470,,epinephrine,237.10,0.00,CATS,4,A,CATS,,,,4,CATS,1.00000,0.00000,1
2,6.60,1708,23675,,norepinephrine,229.30,-0.00,CATS,4,A,CATS,,,,4,CATS,1.00000,0.000
3,10.71,1575,34366,,dhba,217.13,-0.00,CATS,4,A,CATS,,,,4,CATS,1.00000,0.00000,1.0000
,,5128,83512,0,Totals,683.52,,CATS,4,A,CATS,,,,4,CATS,1.00000,0.00000,1.00000,
1,5.81,3715,50679,,epinephrine,492.63,-0.00,CATS,5,A,CATS,,,,5,CATS,1.00000,0.00000,
2,6.60,3506,49147,,norepinephrine,492.95,-0.00,CATS,5,A,CATS,,,,5,CATS,1.00000,0.000
3,10.70,3282,73440,,dhba,482.45,-0.01,CATS,5,A,CATS,,,,5,CATS,1.00000,0.00000,1.0000
,,10503,173266,0,Totals,1468.03,,CATS,5,A,CATS,,,,5,CATS,1.00000,0.00000,1.00000,
1,5.81,5567,74787,,epinephrine,737.00,-0.00,CATS,6,A,CATS,,,,6,CATS,1.00000,0.00000,
2,6.60,5247,72870,,norepinephrine,738.50,-0.01,CATS,6,A,CATS,,,,6,CATS,1.00000,0.000
3,10.70,4952,111500,,dhba,740.89,-0.01,CATS,6,A,CATS,,,,6,CATS,1.00000,0.00000,1.000
,,15767,259157,0,Totals,2216.39,,CATS,6,A,CATS,,,,6,CATS,1.00000,0.00000,1.00000,
```

If your application supports importing comma separated values, this is usually the best choice. In the unlikely event your application is misidentifying whether data are text or numeric, then try the PRN format. Regardless of the type of field delimiter, some applications may default to expect any importable file to use a PRN filename extension, while others distinguish field-delimiter types by filename extension. When ChromGraph Report creates a spreadsheet-importable file, the delimiter format used is indicated by either a PRN, CSV, or TAB filename extension

There are several places where you must indicate that you want Report to create a spreadsheet-importable file, and which type you want:

First, SETUP OPTIONS, found under the Data Menu, must be configured. Check the PRN, CSV or TAB option as appropriate. NOTE: The Setup Option, and occasionally this documentation, uses the term PRN files to generically refer to spreadsheet-importable files which may actually be either CSV or TAB delimited files or classic PRN-style files.

Next, you must specify that you want the selected file type created by the current Method. To do so, go to REPORT OPTIONS in the Method Menu and check the box that says either .PRN, .CSV, or .TAB in the 'Report output on' section.

Finally, the following two options in the Define Queue dialog box must be entered:

> PRN FILE allows you to specify a name for the PRN, CSV or TAB file to which output from the entire Queue will be sent.
>
> ERASE .PRN FILE box allows you to automatically erase any existing PRN, CSV or TAB file of the same name at the start of Queue processing. If not checked, and a PRN, CSV or TAB file of the same name exists, the report will be appended to that file.

In interactive mode ChromGraph will create one importable PRN, CSV or TAB file for each run. If you have lots of samples, this can be somewhat cumbersome. In contrast, automatic processing with a QUEUE creates just one PRN/CSV/TAB file for the whole series of runs. In this case, the data from each sample will be added to the PRN/CSV/TAB file sequentially, one run at a time. Thus it is much more efficient to use a Queue, since only one all-inclusive file needs to be imported into the spreadsheet.

With the variety of different importation formats and varying capabilities of different spreadsheets, it is beyond the scope of this manual to provide instructions on how to generate a report once the processed data file has been imported into a spreadsheet. In general, however, you want to use the spreadsheet's formatting functions to generate a table of analyte concentrations ordered by runs:

	A	B	C	D	
1		picograms of			
2	**Run**	**Norepi**	**Epi**	**DHBA**	
3	1	253.57	253.76	252.53	
4	2	492.86	492.49	494.94	
5	3	753.57	753.76	752.53	
6	4	229.3	237.1	217.13	
7	5	492.95	492.63	482.45	
8	6	738.5	737	740.89	
9					

- **CONTENTS**

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QUANTITATION WITH INTERNAL AND EXTERNAL STANDARDS

A STANDARDS FILE is simply a set of peak names, retention times, peak areas and amounts (or concentrations) used to quantitate unknown samples. A typical Standards file will have at least two concentrations of standards, generally a high and a low, to bracket the expected concentrations of the samples. A one-level Standards file cheats somewhat by assuming the origin (0 concentration, 0 area) as the lower limit. This works mathematically, but is subject to extrapolation errors. We recommend using at least a two-level Standards file.

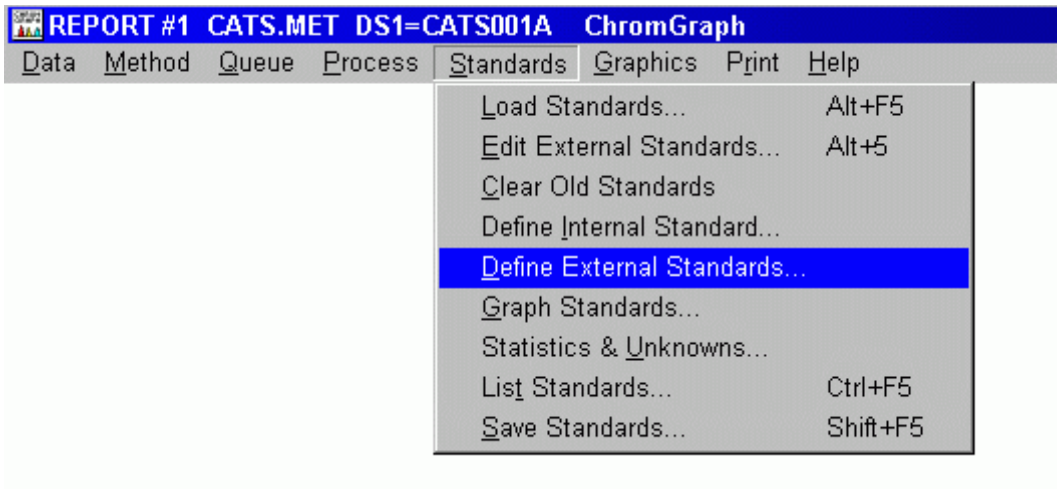
ChromGraph allows you to construct a Standards file of up to ten levels, including both external and internal standards, and to update this Standards file automatically. The Standard Addition method for endogenous analytes is supported.

- **CREATING A ONE-LEVEL EXTERNAL STANDARDS FILE**
- **CREATING A MULTI-LEVEL EXTERNAL STANDARDS FILE**
- **USING AN INTERNAL STANDARD**
- **INTERACTIVE AND AUTOMATIC STANDARDS UPDATING**
- **STANDARDS FOR MULTIPLE DETECTORS**
- **UTILITIES FOR STANDARD**S

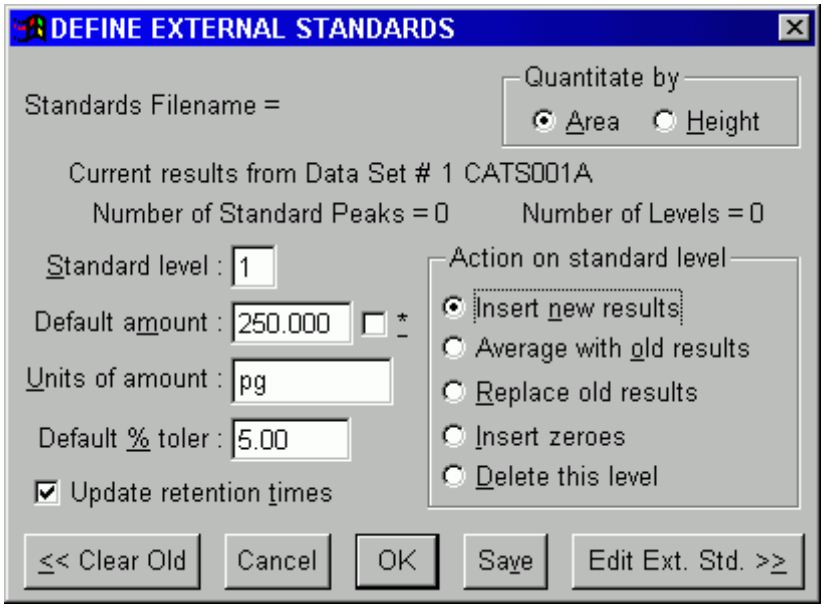
CREATING A ONE-LEVEL EXTERNAL STANDARDS FILE

A Standards file is constructed from processed data (retention times, peak heights, peak areas), so before creating the Standards file, load the appropriate Data file and process it. (CATS001A.DAT for this example.)

Next choose the DEFINE EXTERNAL STANDARDS section from the Standards Menu:



The DEFINE EXTERNAL STANDARDS dialog box will appear:



Since this will be a new Standards file, click on the << CLEAR OLD button, to erase any old Standards file that may be in RAM. Next enter the following information:

STANDARD LEVEL should be 1, since this is the first level (concentration) of a one-level file.

DEFAULT AMOUNT. If all peaks in the standard injection have the same amount or concentration, enter it here. This is a convenience, as this amount will be brought up automatically in the EDIT STANDARDS section. If all peaks have different concentrations, just ignore this section.

The ***-box** to the right of Default Amount makes it easier to add new levels to Standard files whose peaks have different concentrations.

UNITS OF AMOUNT. This is a label for the final results. Enter whatever is appropriate (e.g., pg).

DEFAULT % TOLERANCE is the window in time within which a sample peak must fall in order to be identified. For example, if peak 1 in the Standards file has a retention time of 5.0 minutes and a tolerance of 5%, any unknown peak between 4.75 and 5.25 minutes (5 minutes ± 5%) will be identified as peak 1. Default % Tolerance must be greater than zero in order to identify peaks.

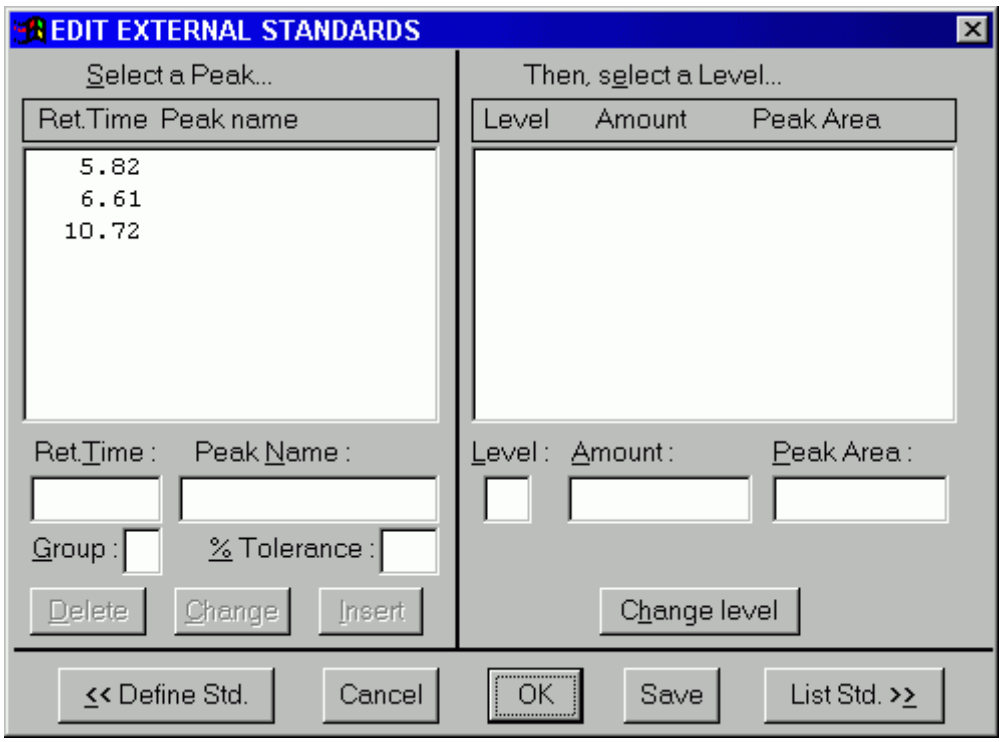
UPDATE RETENTION TIMES is used during interactive or automatic updating. It allows the retention times of the new standard injection to be incorporated into the Standards file, and should generally be turned on.

QUANTITATE BY (choose area or height). This option becomes fixed after the first standard is entered; all components of the Standards file must use the same criterion.

ACTION ON STANDARD LEVEL selects the current operation. In this case we will INSERT NEW RESULTS. When updating an existing Standards file with a new injection, use REPLACE OLD RESULTS. DELETE THIS LEVEL is useful when you've accidentally added an extra level, or to clear all levels without losing peak names.

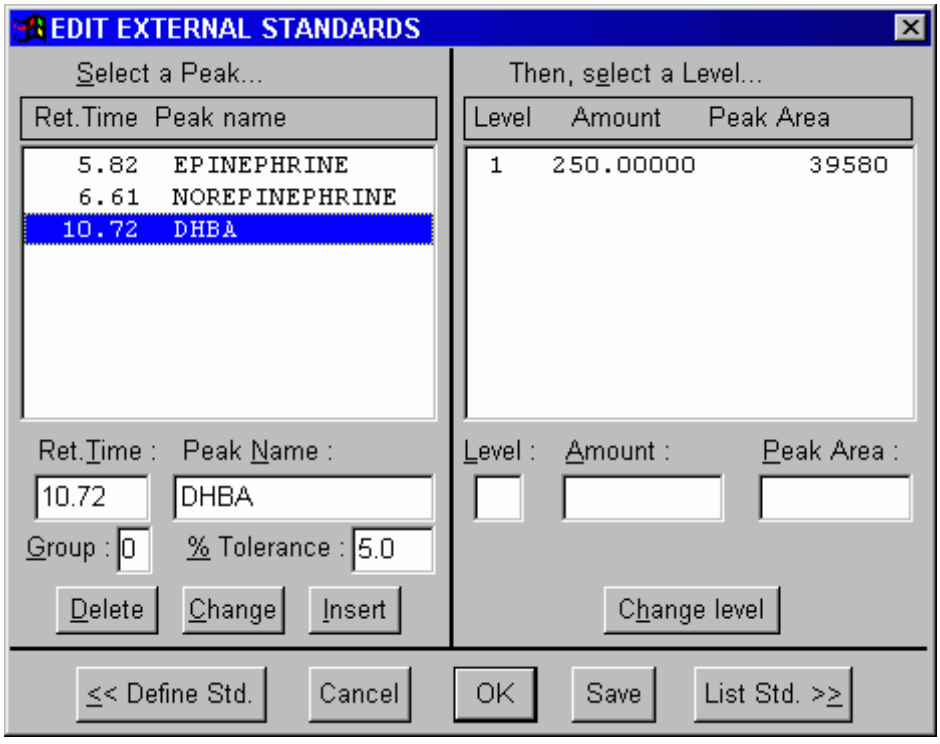
To enter the specified information into the Standards file, click the EDIT EXT. STD. >> button. (OK also enters the information, but does not proceed to the next section.) Information on the level-one standards is automatically entered from the most recently processed Data file, but must be edited. The EDIT EXTERNAL STANDARDS dialog box appears next:

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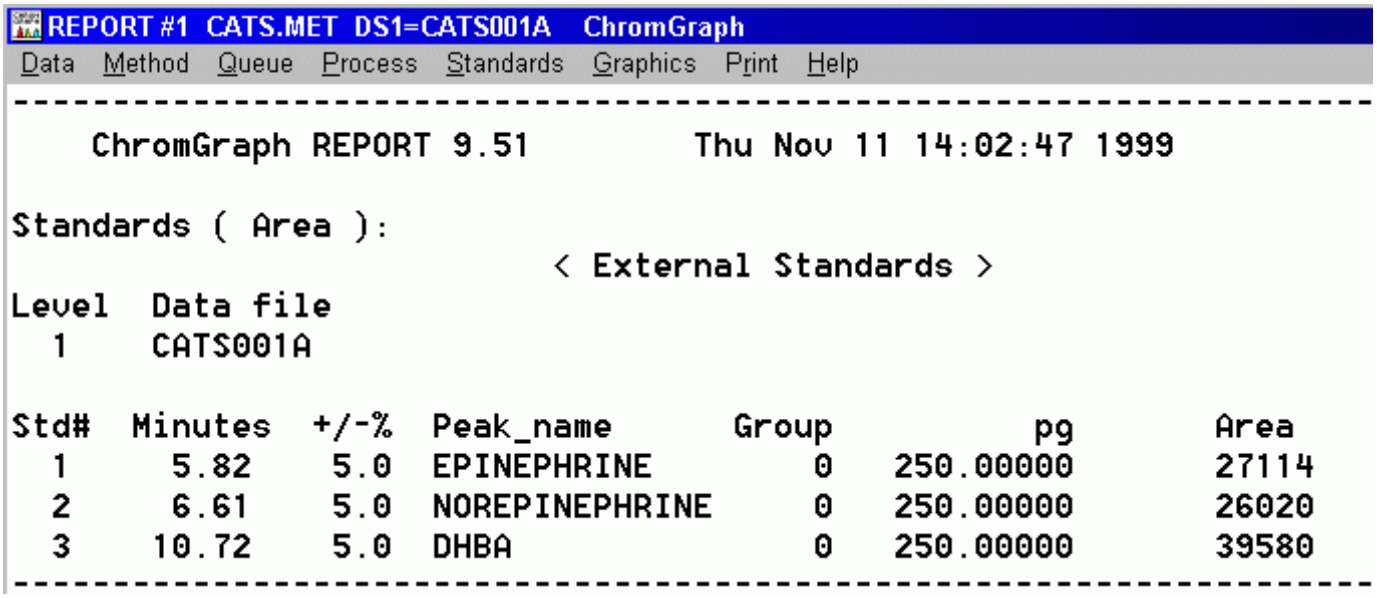


Although the EDIT EXTERNAL STANDARDS dialog box has many options and features, for most purposes the only thing that has to be done here is to name the peaks and perhaps enter an amount. Click on the retention time for the first peak that you wish to incorporate into the Standards file: the retention time will appear in the edit boxes below, and the amount and area will appear on the right. Enter the peak's name in the edit box, and press CHANGE to post it to the listbox. If you used a Default Amount in the Define External Standards dialog, then AMOUNT should be correct. If not, click on it and enter the correct amount in the edit boxes on the right. Click CHANGE LEVEL to post the corrected amount to the listbox.

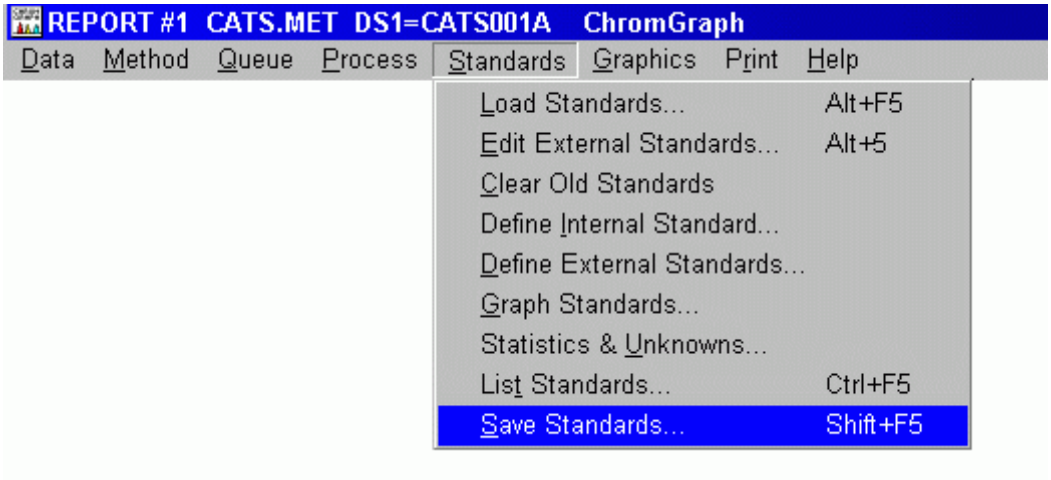
Continue to name each peak of interest. If there are other peaks present, they may be deleted by highlighting their retention times and clicking the DELETE button. (If you accidentally delete a peak, press the INSERT button immediately to recall it.) The completed dialog box should look like this:



As a final check that all is correct, click the LIST STD. >> button for a listing of your completed Standards file:



Verify that each peak of interest is included in the list, with accurate amounts and areas or heights. Then take the SAVE STANDARDS option from the Standards Menu to name and save the Standards file:



Standards file names can have any combination of characters and spaces. The extension .STA will be appended to the name you choose. Names can theoretically be up to 127 characters long, including the path (thus, C:\BAS2\DATA\mystandards.sta = 28 characters). Some advanced procedures may require shorter names, so we recommend using names with total lengths (including the path) of less than 33 characters.

For display purposes, a filename may be truncated somewhat in various places in the program.

The name of the Standards file will automatically be recorded in the Method, under STANDARDS OPTIONS. Once this is done, all data files processed with this Method will be compared to the standards file, and calculated amounts will be printed in the report.

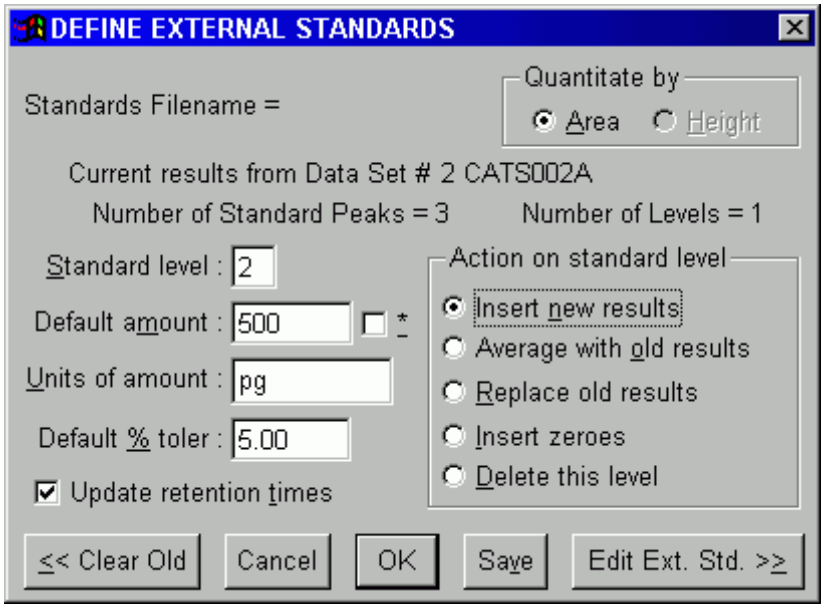
CREATING A MULTI-LEVEL EXTERNAL STANDARDS FILE

Building a multi-level Standards file is a stepwise extension of building a single-level Standards file. Editing or adding subsequent levels is easier, however, since the peak names have already been entered. (If you've just built the one-level standards file above, skip the next paragraph.)

Begin by loading and processing the Data file for the first level. (Most people find it easier to build the Standards file in ascending order of concentration, so we will adopt that convention.) Then build a one-level Standards file, exactly as above. Save the Standards file, as a precaution.

Next load and process the Data file for the second level. To follow along here, load and process CATS002A.DAT. Then select DEFINE EXTERNAL STANDARDS from the Standards Menu.

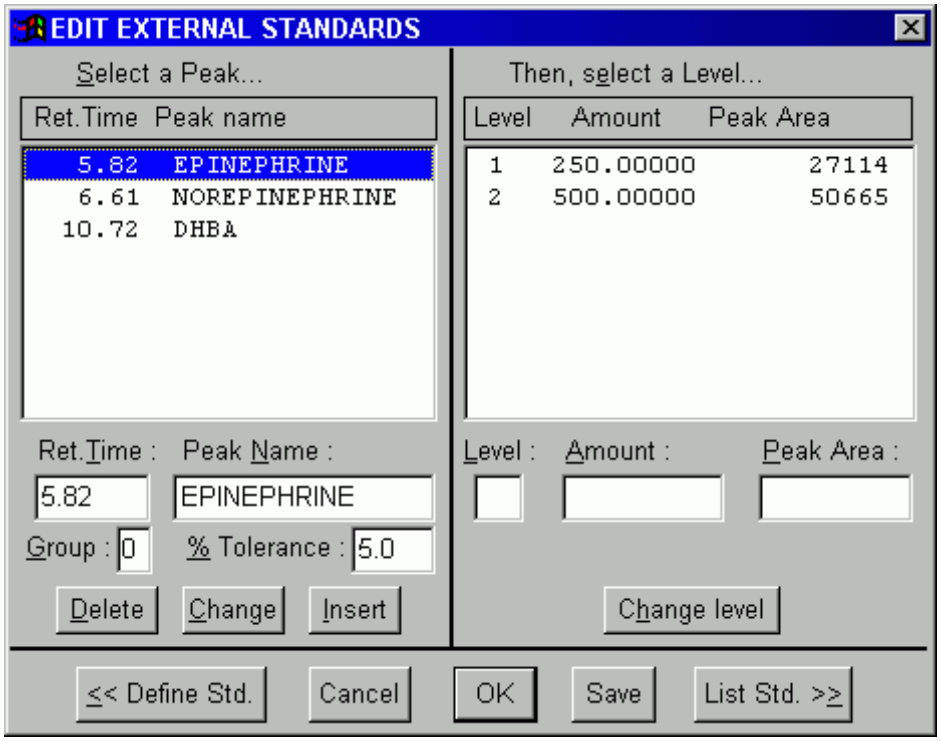
IMPORTANT: DO NOT CLEAR THE OLD STANDARDS FOR THE SECOND AND SUBSEQUENT LEVELS! WE WANT TO ADD TO THE PREVIOUS LEVEL, NOT REPLACE IT.



There are only two changes from the first level: STANDARD LEVEL changes from 1 to 2, indicating that this will be the second level, and DEFAULT AMOUNT (if used) changes to reflect the concentration of standards in the second level.

The *-box to the right of Default Amount makes adding levels easier when 1) peaks within levels have different amounts, and 2) all peak amounts in a subsequent level are a constant multiple of amounts in the immediately preceding level. To use this feature, click on the *- box and enter the multiple as the Default Amount. For example, assume level one has four peaks with the following amounts: 100, 50, 250, 75 ng. Clicking the *-box and entering 2.0 as Default Amount causes the following amounts to be automatically entered for the four peaks in level two: 200, 100, 500, 150 ng. This feature should not be checked for the tutorial example.

When the Define External Standards section is completed, click on the EDIT EXT. STD. >> button to add the second level and switch to the editing dialog. The EDIT EXTERNAL STANDARDS dialog box will appear much as it did in level one. But when you click on one of the peak names, both levels of amounts and areas will appear on the right side of the screen:



As in level one, if the peaks comprising level two do not have the same concentrations you may have to do some editing of the amounts. When this is completed, click the LIST STD. >> button. The listing should look somewhat like this:

REPORT #1 CATS.MET DS2=CATS002A ChromGraph

Data Method Queue Process Standards Graphics Print Help

Standards (Area):

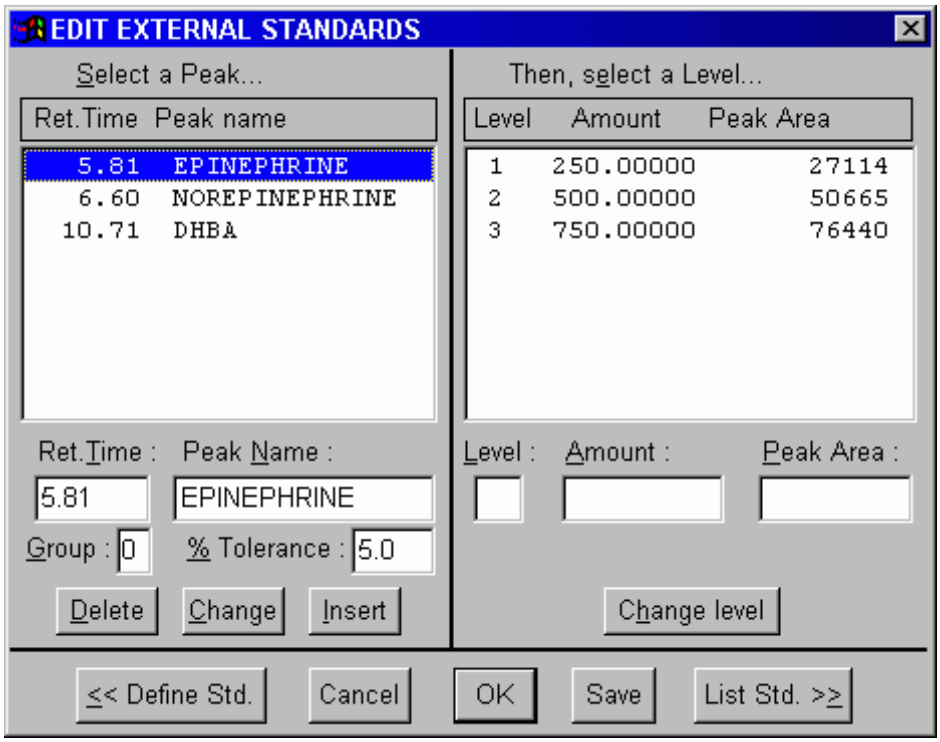
< External Standards >

Level Data file
1 CATS001A
2 CATS002A

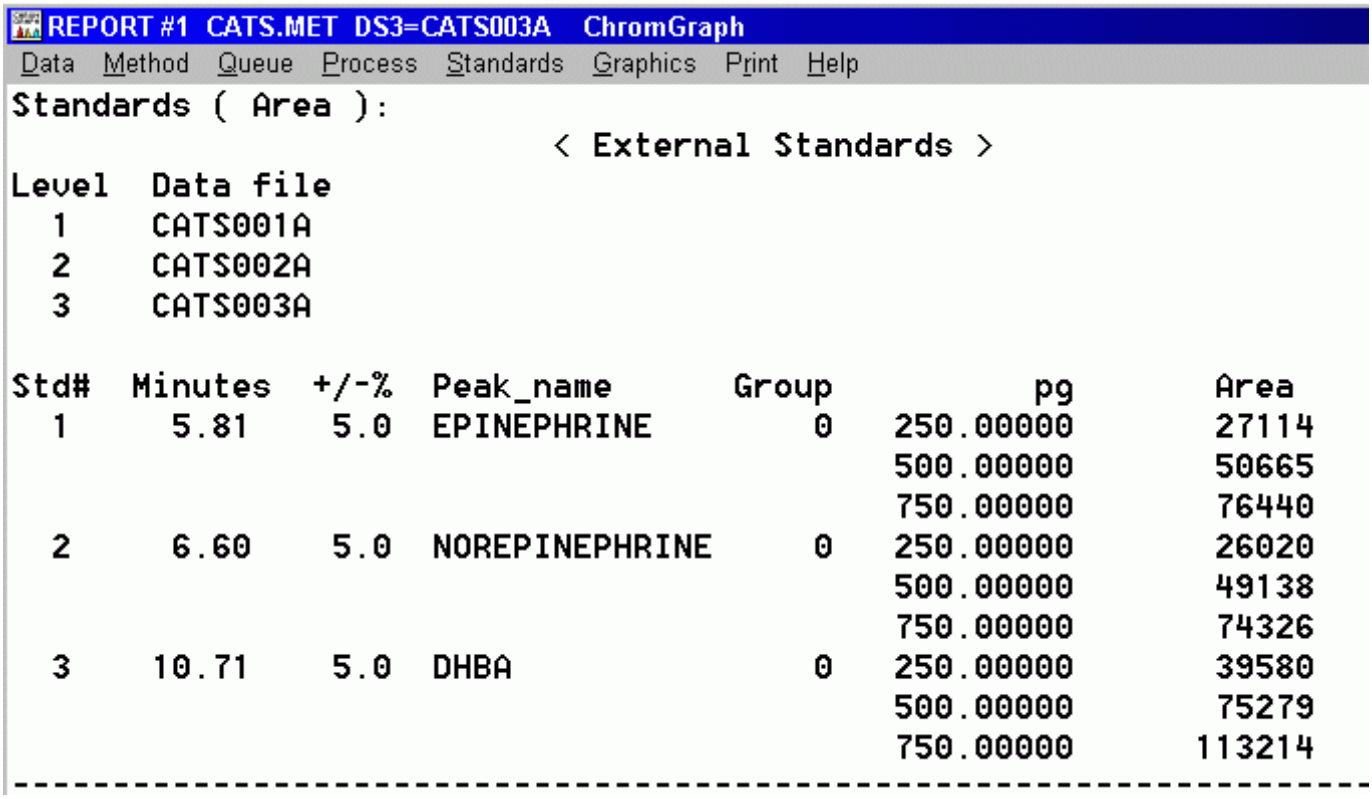
Std#	Minutes	+/-%	Peak_name	Group	pg	Area
1	5.82	5.0	EPINEPHRINE	0	250.00000	27114
					500.00000	50665
2	6.61	5.0	NOREPINEPHRINE	0	250.00000	26020
					500.00000	49138
3	10.72	5.0	DHBA	0	250.00000	39580
					500.00000	75279

Again, save these intermediate standards as a precaution.

Subsequent levels can be added exactly as level two was added. For a three-level Standards file, load and process CATS003A.DAT. Then choose DEFINE EXTERNAL STANDARDS from the STANDARDS MENU. Change STANDARD LEVEL to 3 and DEFAULT AMOUNT to 750. Click the EDIT EXT. STD. >> button. The EDIT EXTERNAL STANDARDS dialog box will look like this:



A listing of the Standards file (LIST STD. >>) now looks like this:



Be sure to save the final version of the Standards file.

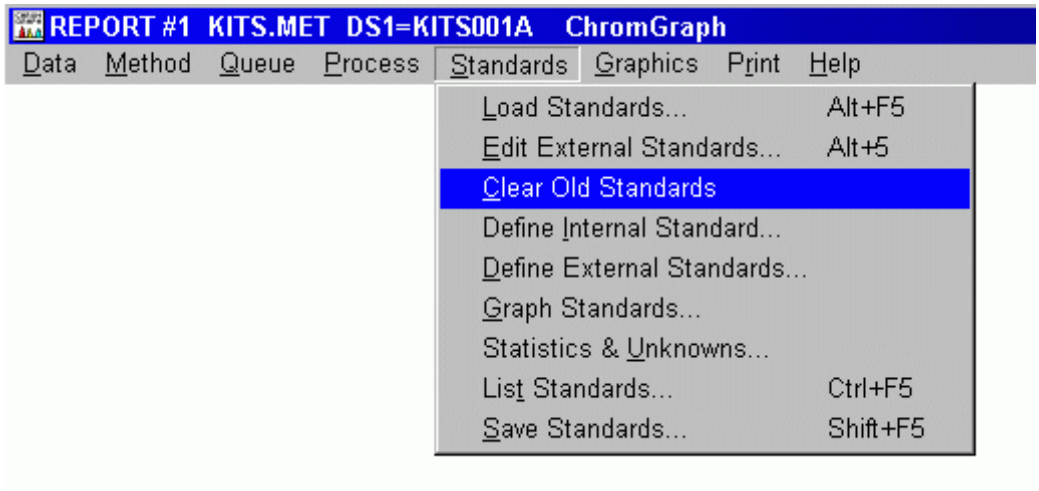
USING AN INTERNAL STANDARD

An internal standard is used to correct for variable recovery during an extraction step in sample preparation. (If you're not using an extraction step, an internal standard may not be necessary.) A fixed amount of an internal standard is added to each sample before extraction, and to the standards. During processing, the area (or height) of each peak in every run is divided by the area (or height) of the internal standard for that run, to produce a ratio. This process automatically corrects for run-to-run variation in extraction efficiency and chromatographic response (Snyder, L.R. and J.J. Kirkland. 1979. Introduction to modern liquid chromatography. Wiley- Interscience, NY).

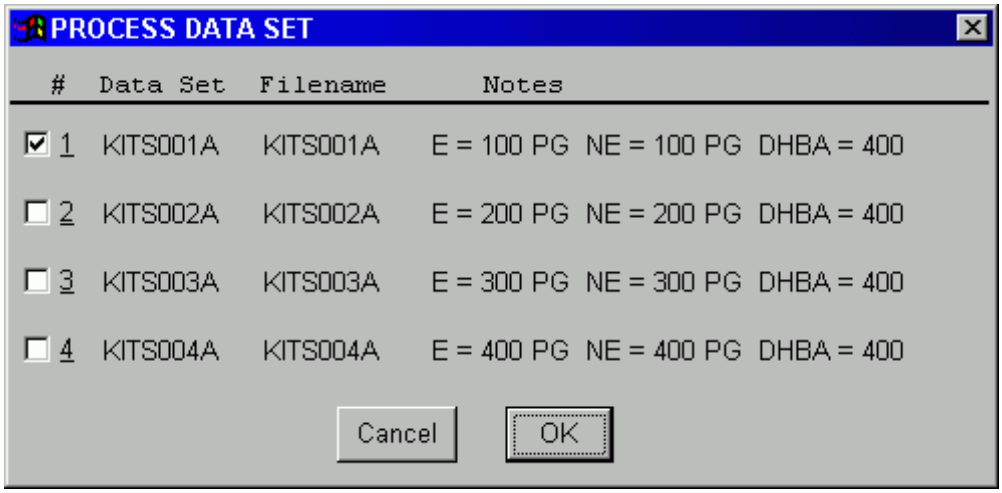
For the standard runs, these ratios are fitted to a regression with amount as independent variable. For the samples, the ratios are used to predict amount from the regression. The report from an analysis using an internal standard will contain a column for HeightCF or AreaCF which contains these ratios (times 10,000, for precision).

Begin defining an internal standard by loading the appropriate Method (KITS.MET for this example). Then load the data sets for the standards. For this example, load KITS001A.DAT through KITS004A.DAT.

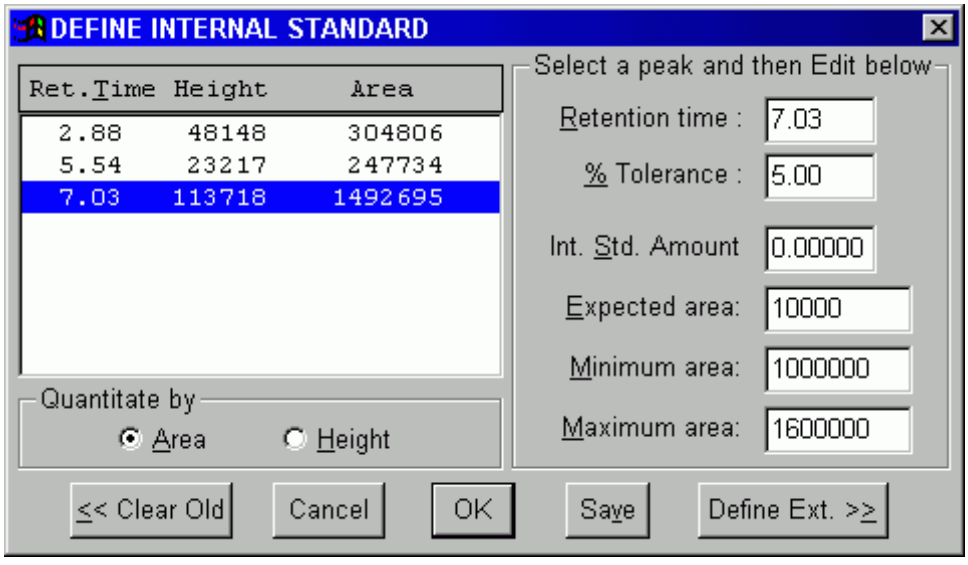
Next take the CLEAR OLD STANDARDS option from the Standards Menu:



Then process the KITS001A.DAT Data file:



When the report appears, take the DEFINE INTERNAL STANDARD option from the Standards Menu:



In the INTERNAL STANDARD dialog box, click on the retention time of the internal standard (7.03 minutes in this example). Configure the remaining options as follows:

Select **AREA** or **HEIGHT** as the parameter for quantitation.

% TOLERANCE is the window of time within which the software will accept a peak as being the internal standard (retention time ± %).

INT. STD. AMOUNT should be set at zero. This tells the software that amounts are to come from external standards, and that the internal standard is to be used for calculating peak ratios within runs.

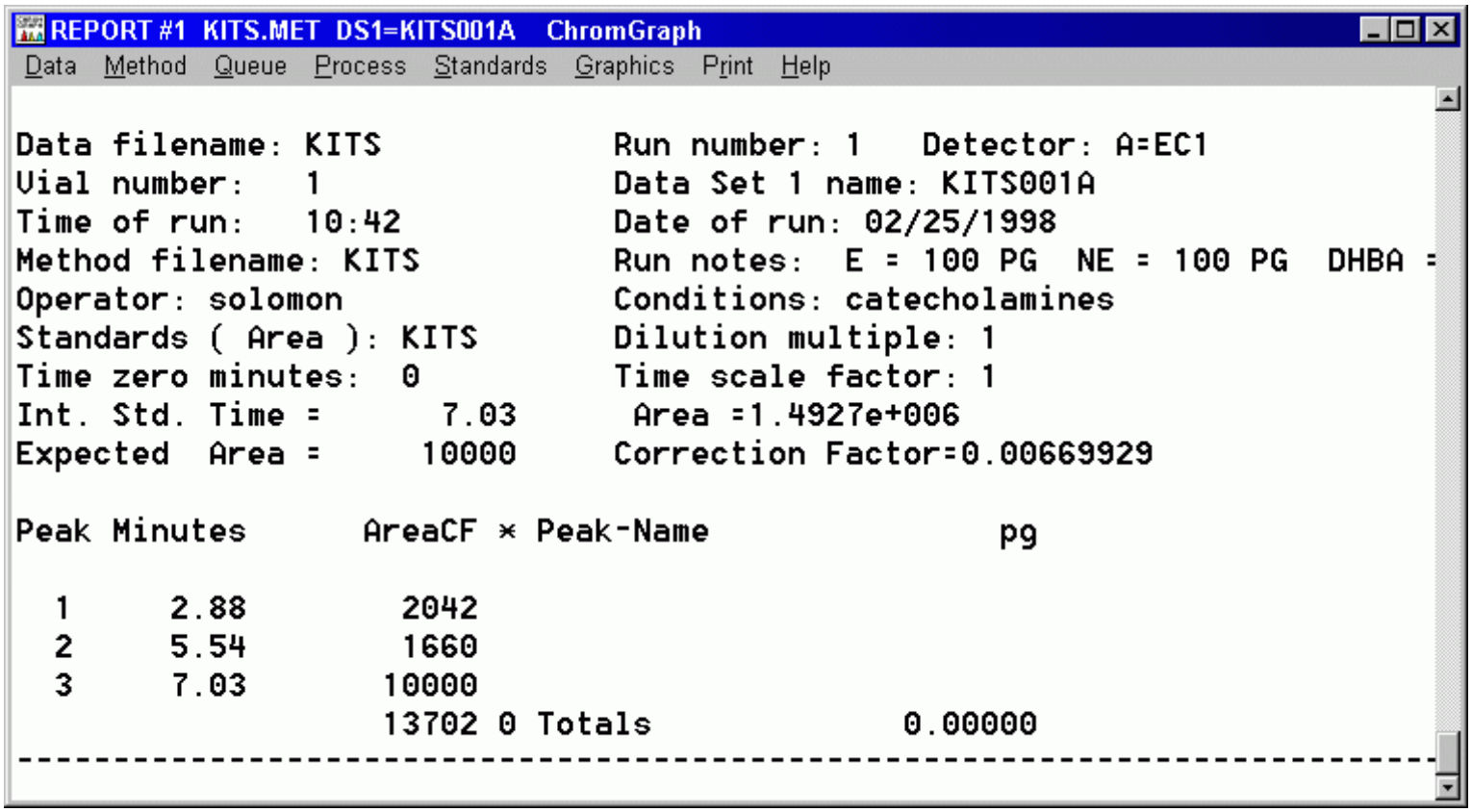
EXPECTED AREA (or height) is a correction factor that can be used to format the AreaCF (or HeightCF) column of the report. Set this at 10000, and the AreaCF column will report RATIO x 10000. As a verification that the standards have been set up properly, the internal standard identified in each run will have an AreaCF or HeightCF of exactly 10000 (read this as 1.0000).

MINIMUM and **MAXIMUM AREAS** (or heights) serve both to reject extraneous peaks and to warn you if there was an error in the extraction procedure. The software will not identify a peak in a sample as being the internal standard unless it 1) falls within the window of tolerance, 2) is no larger than the maximum area and 3) is no smaller than the minimum area.

> Set the MAXIMUM AREA (or height) slightly greater than the area of the internal standard peak. If the internal standard peak in a sample is larger than this, it indicates an error in the extraction procedure, or a coeluting contaminant. The message 'Internal standard was not found' will appear above the report.
>
> Set the MINIMUM AREA (or height) to a value corresponding to a minimally acceptable extraction efficiency. For example, if you consider anything less than 50% extraction efficiency to indicate an error in the extraction procedure, set the MINIMUM AREA at 50% of the area of the internal standard peak in the first standard. If the internal standard peak in a sample is smaller than this, the message 'Internal standard was not found' will appear above the report.

After defining the internal standard, press the SAVE button and name the standard. This saves the standard and, more importantly, enters its name in the Method, to enable the ratio calculations to proceed.

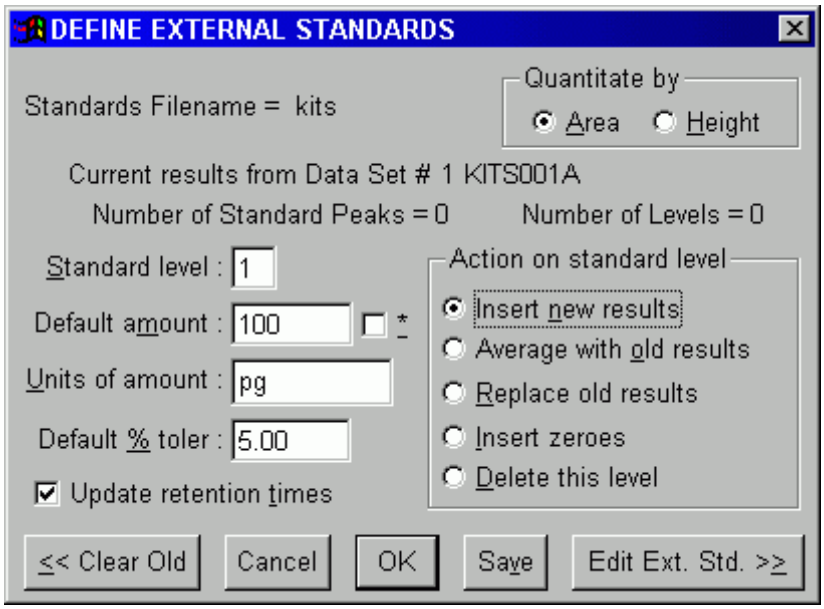
Since the level-one standard was not processed against the internal standard, this Data file must be processed again. After KITS001A.DAT is reprocessed, the report will contain an AreaCF column:



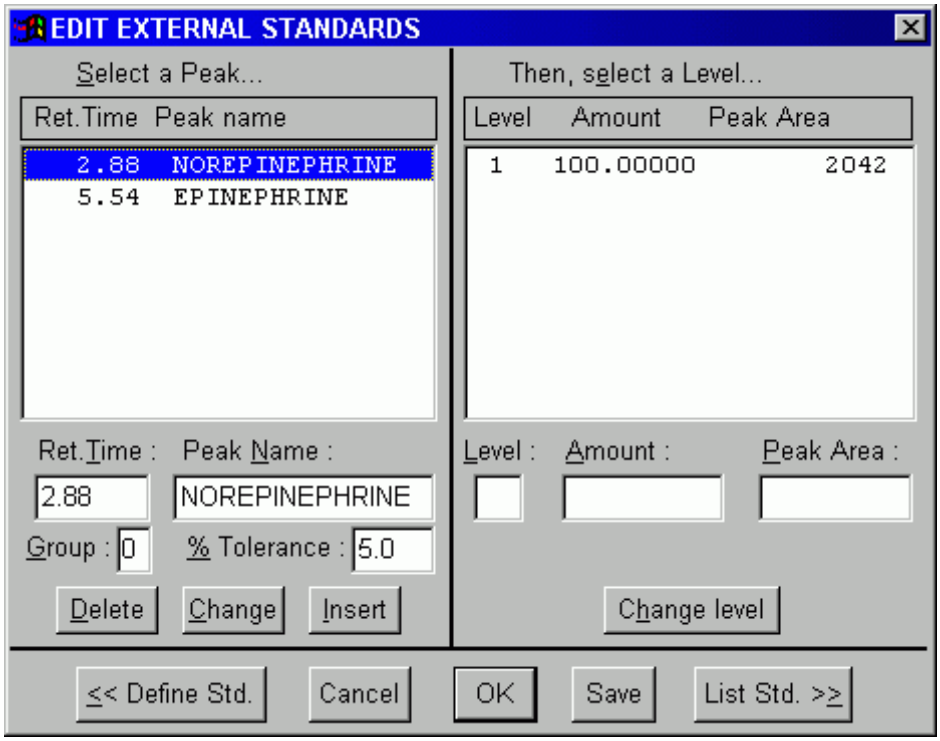
Remember that these values are ratios x 10000, so the value for the internal standard (peak 3) is read as 1.0000, and the value for peak 1 is read as 0.2042.

Next you must build the external standard file as in the Section on multi-level standards. Choose DEFINE EXTERNAL STANDARDS from the Standards Menu.

IMPORTANT: DO NOT CLEAR OLD STANDARDS NOW!



For the level-one standard, change STANDARD LEVEL to 1, DEFAULT AMOUNT to 100, UNITS OF AMOUNT to pg, DEFAULT % TOLERANCE to 5, and click on UPDATE RETENTION TIMES and INSERT NEW RESULTS. Then press the EDIT EXTERNAL STD. >> button.



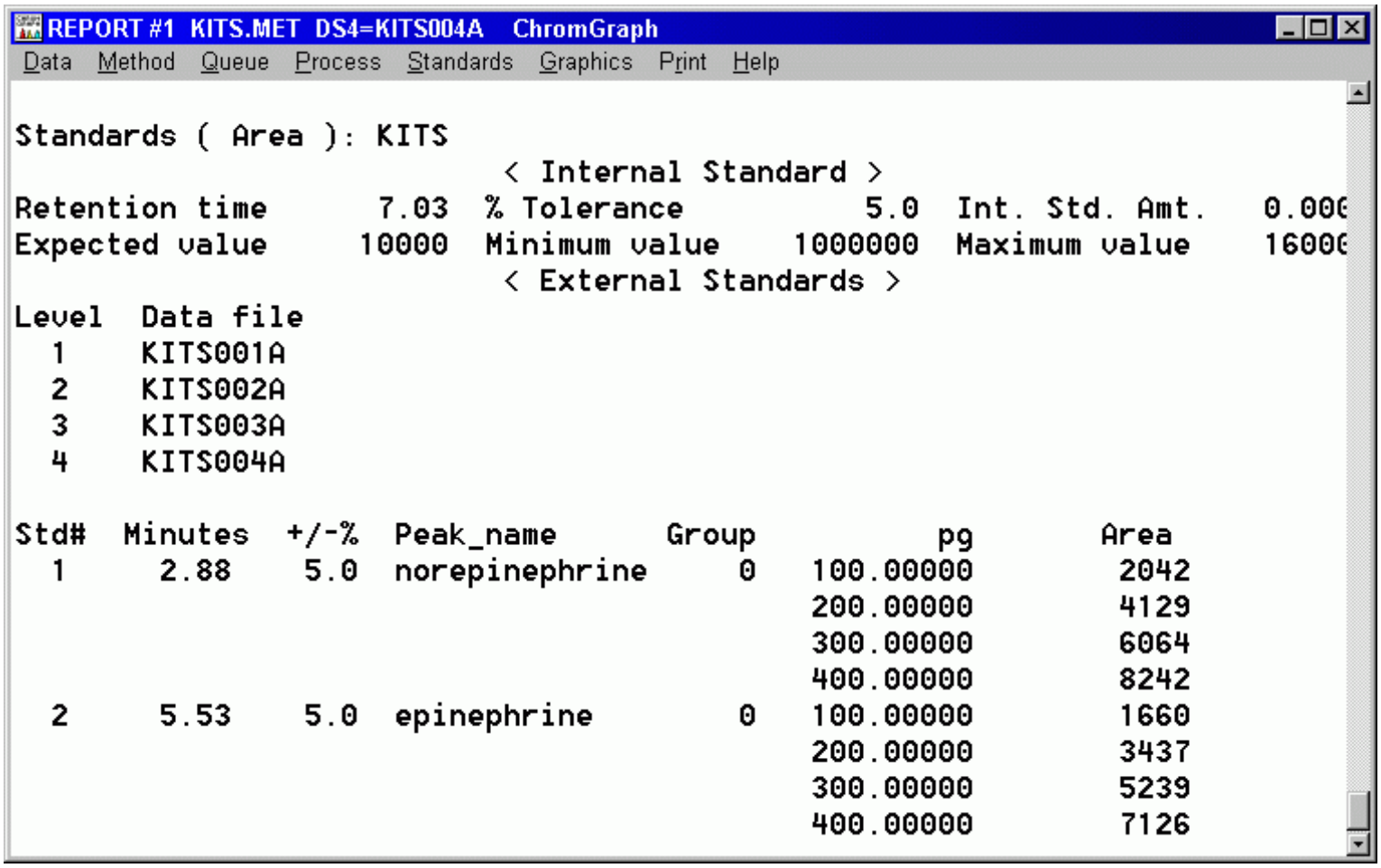
In the EDIT EXTERNAL STANDARDS screen above, click on the retention time of each peak of interest, enter its name in the edit boxes, and change the % tolerance or amount if necessary. Delete any peaks of no interest, including the internal standard. Then press the OK button.

Process each subsequent level of the standards, and insert each into the Standards file via the DEFINE EXTERNAL STANDARDS dialog box. The run numbers and amounts (in pg) are as follows for these runs:

RUN	EPINEPHRINE	NOREPINEPHRINE	INTERNAL STD (DHBA)
KITS001A	100	100	400
KITS002A	200	200	400
KITS003A	300	300	400

KITS004A	400	400	400

When the last level is entered, save the standards file and list it. The listing should look like this:

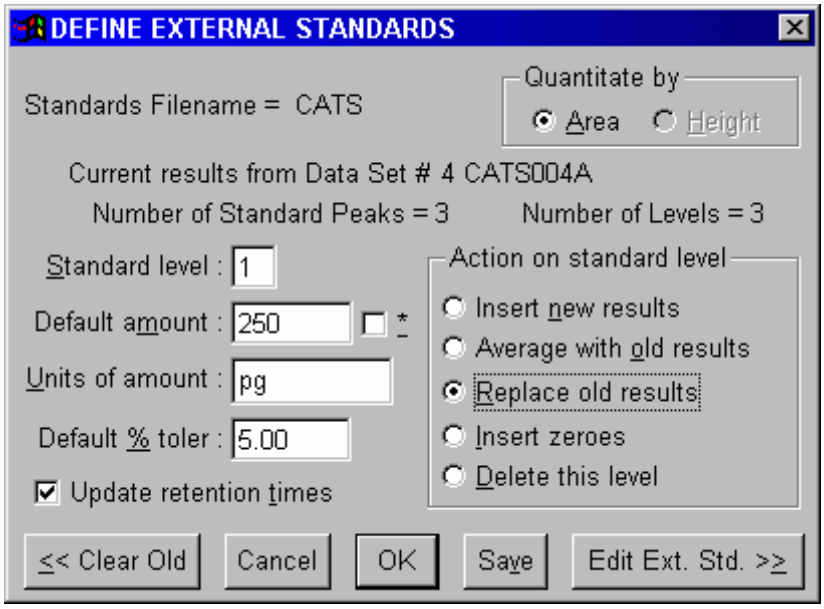


You can now load and process KITS005A.DAT and KITS006A.DAT, which are simulated unknowns. One has about 150 pg norepinephrine and 350 pg epinephrine, and the other has the opposite amounts.

INTERACTIVE AND AUTOMATIC STANDARDS UPDATING

Once set up, even multi-level Standards files containing internal and external standards may be easily updated with information from fresh standard injections.

To INTERACTIVELY UPDATE a Standards file, first do the relevant chromatographic runs, then load the Data files into RAM. Process the first-level Data file, then take the DEFINE EXTERNAL STANDARDS option in the Standards Menu:

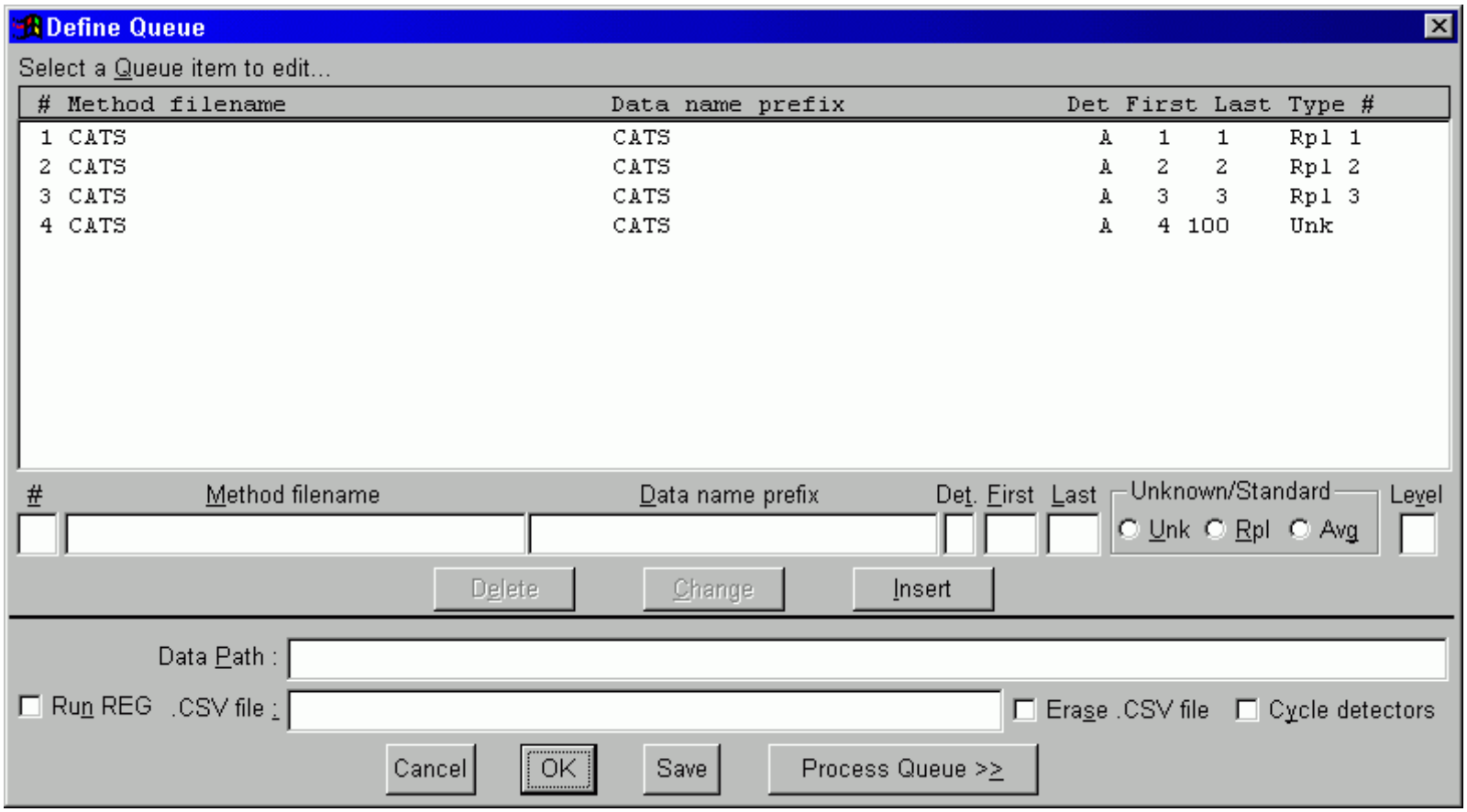


IMPORTANT: DO NOT CLEAR THE OLD STANDARDS!

Change the STANDARD LEVEL to 1, and click on REPLACE OLD RESULTS. Click the OK button and level one is done. All the peak names, amounts, and tolerances from the old Standards file are carried into the new file. Only areas (or heights) and retention times are updated. For updating to work properly, the retention times of the new standards must fall within the % Tolerance of the old standards. Information about the internal standard does not require updating.

Repeat the updating procedure for the remaining levels, remembering to first process the Data files. Be sure to indicate the appropriate level of each update. After the last level is entered, LIST the new Standards file to check for errors, then SAVE it.

AUTOMATIC UPDATING of standards can be accomplished with a Queue. The Queue can be set up so the first few Data files update the Standards file, and the remaining Data files are quantitated against the updated Standards file:



This Queue is set up to update a three-level Standards file, then process 97 samples. For clarity, use a separate line in the Queue for each standard level to be updated. For each level, indicate the Data file name and run number, and which level is to be replaced, then click on the RPL (replace) button. Click the UNKnown button when entering lines for unknowns.

NOTE: The run numbers and standard levels in the queue will increment as the queue is run. Therefore, you should save the queue just after you create it, so its original form will be available for subsequent use.

STANDARDS FOR MULTIPLE DETECTORS

There are often several detectors in use, and therefore several channels of data, for each sample. These may be different types of detectors (e.g., electrochemical, ultraviolet and fluorescence) or the same type of detector at different gains. In either case, response will differ among detectors, so you need a separate Standards file for each detector.

In order to make a separate Standards file for each detector, simply build each one as before. Start with detector A, since it's easier to build the Standards files in a logical sequence. Load the Data files for detector A, process the Data files and build the Standards file.

Both the Standards file for detector A, and the Method for detector A, must be differentiated from those for the remaining detectors. The Standards file must be separated because response differs among detectors. The Methods must be separated because 1) processing must be optimized for each detector individually, and 2) since the Method specifies which Standards file to use, the only way to ensure that the data from each detector are compared to the appropriate standards is to use a different Method for each detector.

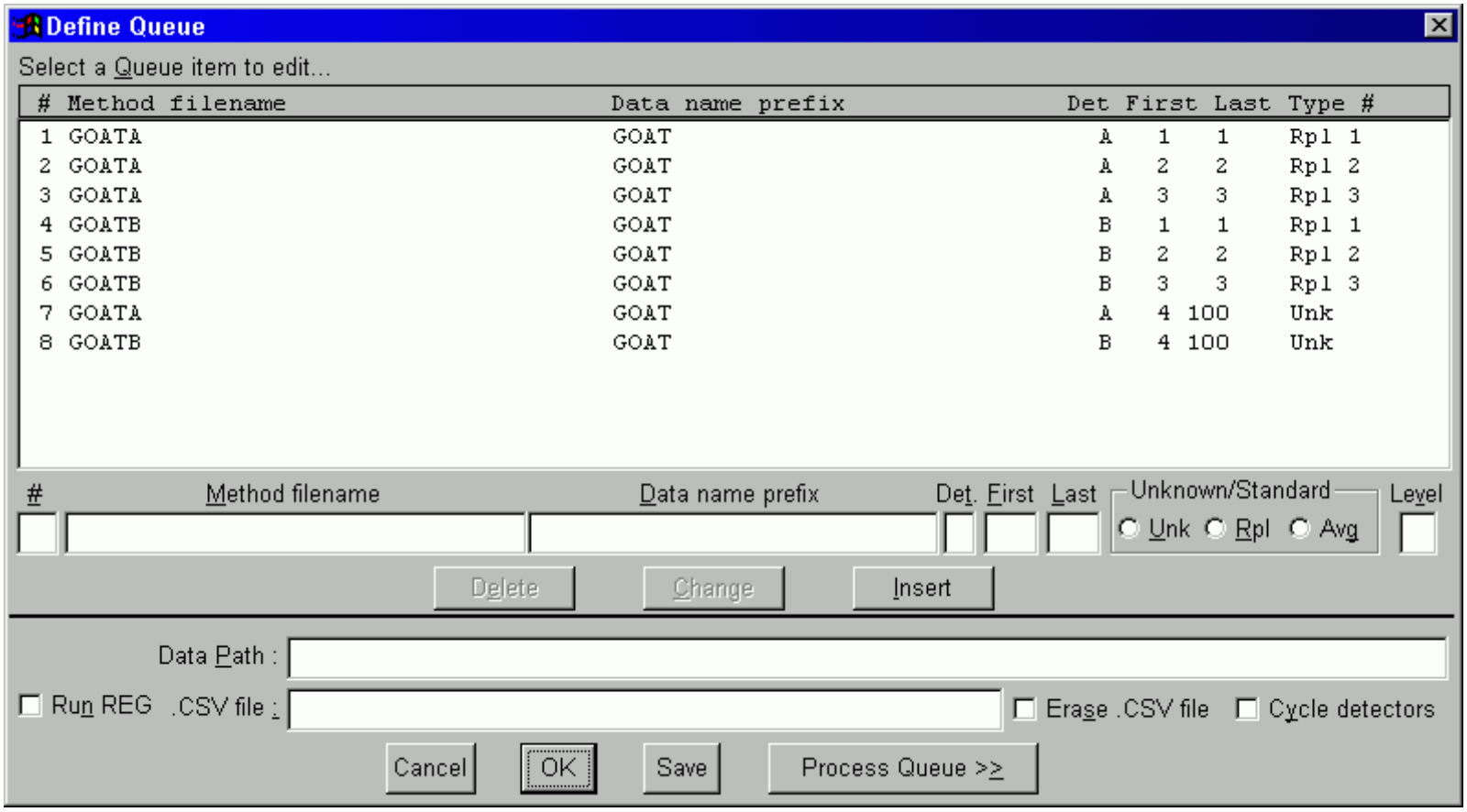
Use an easily remembered code to differentiate Methods and Standards files by detector. For example, after building the Standards file for detector A, save it with a name associated with detector A, let's say GOATA. This automatically enters its name in the Standards Options section of the Method. Now save the Method with the name GOATA.

Next load the Data files for detector B, optimize processing for this detector, and build a Standards file. Save this file as GOATB, and save the Method file as GOATB. Do the same for detector C, calling the relevant Standards and Method files GOATC.

There will now be three Method files on disk: each one is optimized for processing the output of its associated detector, and each one will call the appropriate Standards file for quantitation. To start processing, simply define a Queue listing each Method and the appropriate Data files for each Method to process.

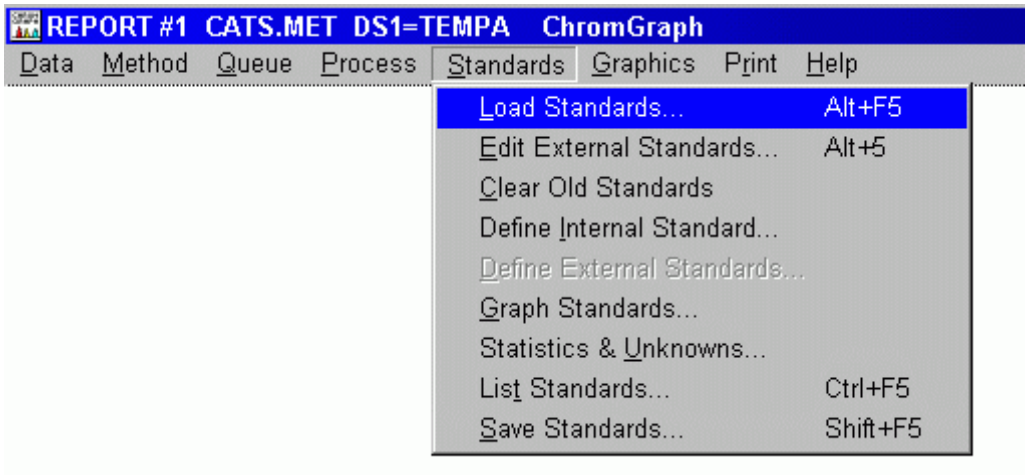
The following Queue adds a level of complexity to this; it will automatically update a three-level Standards file for each of two detectors, and then process sample Data files:

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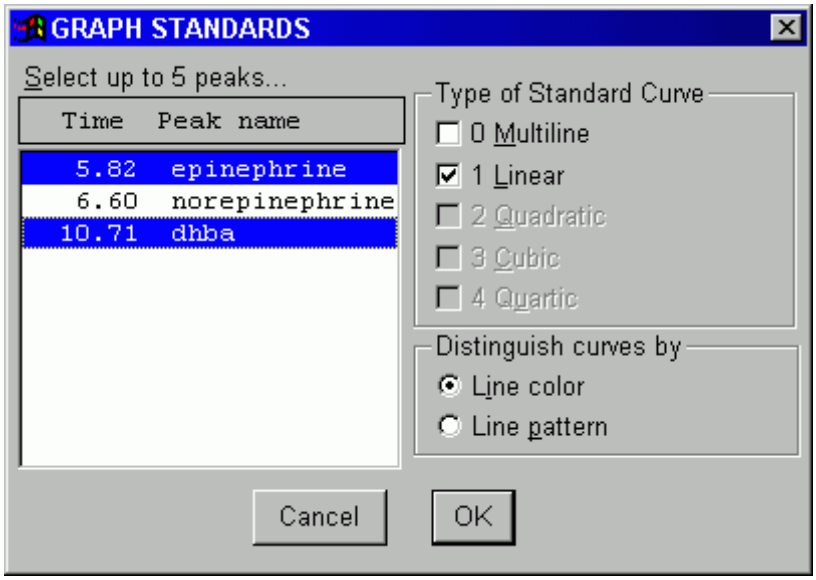
UTILITIES FOR STANDARDS

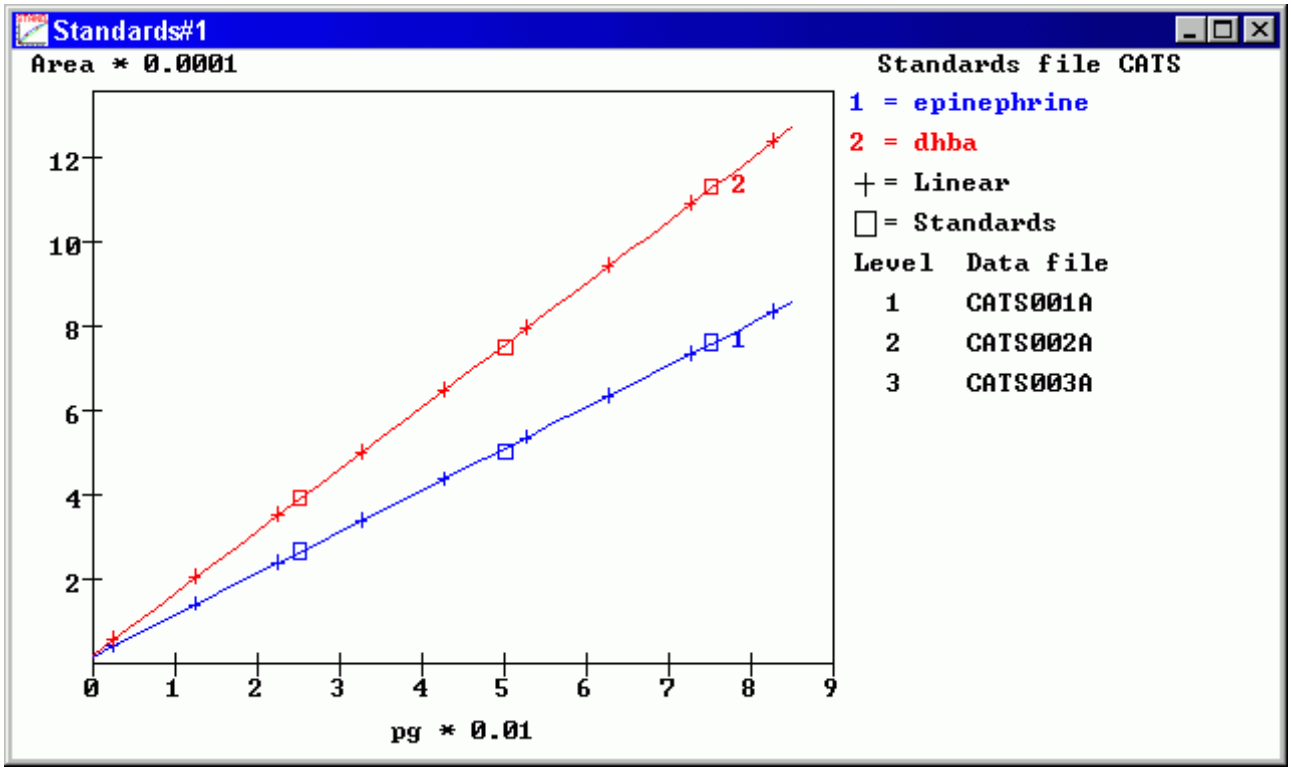
The Standards Menu contains several options that facilitate working with Standards files:



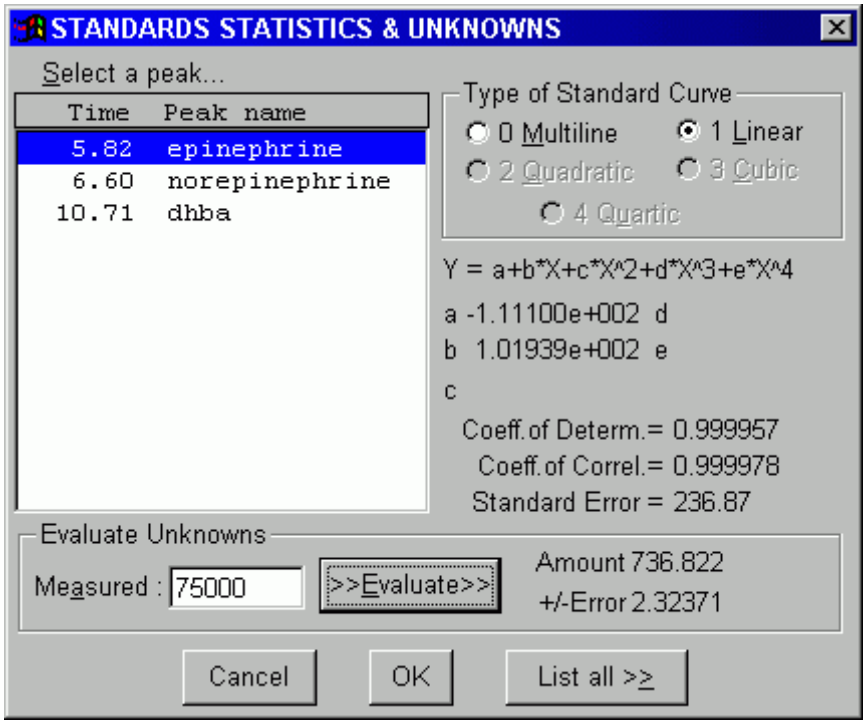
LOAD, LIST and SAVE have the usual meanings. GRAPH STANDARDS and STATISTICS & UNKNOWNS allow you to interactively work with the Standards file.

GRAPH STANDARDS opens a dialog box to graph the completed Standards file. This is used as a visual check for linearity or errors. Click on up to 5 peaks, select the line options, and click on OK:





STATISTICS & UNKNOWNS opens a dialog box that reports statistical information about the standard curve for each peak, and allows you to enter areas or heights of unknowns and obtain calculated amounts:



Simply click on the peak of interest to obtain statistics on the standard curve. To estimate the amount of an unknown, enter its height or area in the edit box, and click EVALUATE >>.

- **CONTENTS**

GRAPHICS AND PRINTING

The GRAPHICS MENU allows you to control the appearance of the graph, including selection of Data files, labels, colors, scaling, etc. This section is interactive, but settings made here will carry over into graphs produced by automatic processing. Changes made to the graph options can be made permanent by saving the REPORT.INI file.

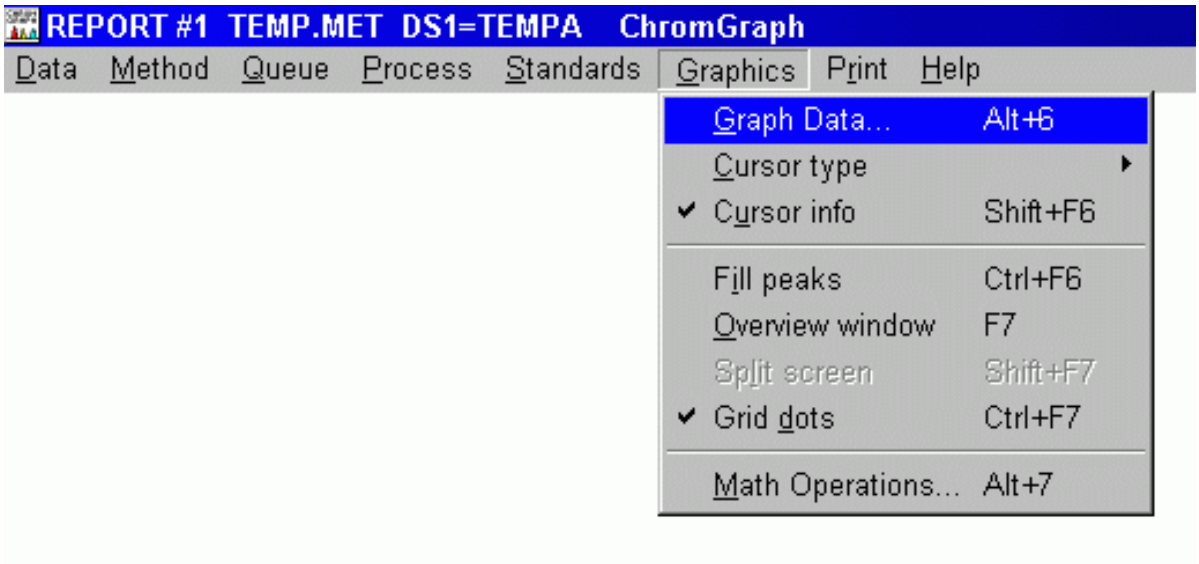
The MATH OPERATIONS section allows you to manipulate the data in various ways (e.g., baseline subtraction).

PRINTING options provide flexibility for setting up printers and selectively printing all or parts of reports.

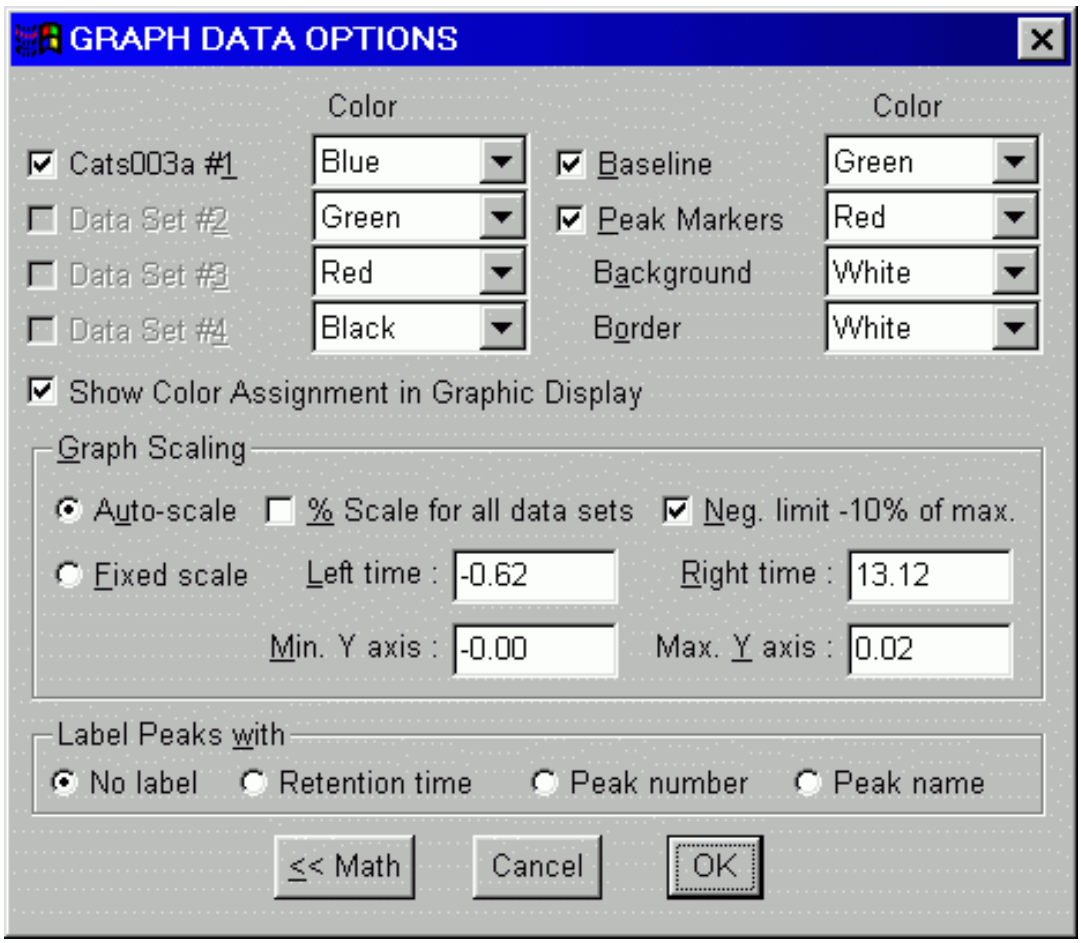
- **GRAPHING DATA**
- **FORMATTING OPTIONS**
- **INTERACTIVE CURSORS**
- **MATH OPERATIONS**
- **PRINTING**

GRAPHING DATA

Begin by taking the GRAPH DATA choice from the Graphics Menu:



The GRAPH OPTIONS window opens, allowing you to select the options controlling the appearance of the graph:



Each Data file in RAM will be listed in the dialog box. Simply click on the check box to request that one or more of the Data files appear in the graph. A drop-down listbox allows selection of a unique color for each Data file.

Color selection also is provided for other aspects of the graph: you may select whether or not the calculated baseline is drawn underneath peaks, whether or not peak start and stop markers are included, and the colors for these options. Background and border colors also are provided.

The Show Color Assignment in Graphic Display checkbox allows the name for each data set to be printed below the graph, in the appropriate color, when more than one data set is shown on the same graph (i.e., when SPLIT SCREEN is turned off).

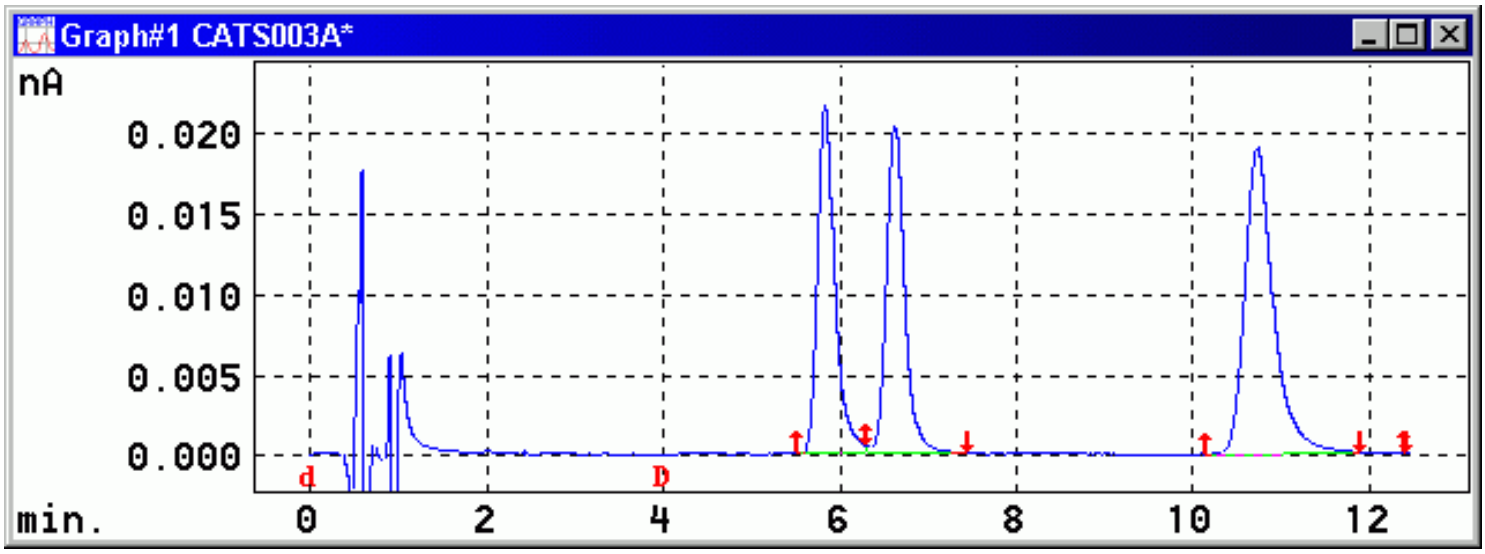
Depending on the hardware used during data collection, the graph might already be scaled in appropriate detector units. With external detectors, however, the graph might be scaled in arbitrary Y-units. The units of the Y-scale are purely esthetic: they have no effect on quantitation. However, you may change the units and the axis label to whatever seems appropriate. Press the MATH button, and change FULL SCALE Y and LABEL Y UNITS on the Math Operations screen to something suitable for the detector that was used. (If you find yourself doing this very often, you can change the scaling parameters in Control so data from external detectors are saved with the appropriate information.

Press the OK button and the graph will be drawn. The size of the graph on the screen (and printer) may be adjusted by dragging its borders in or out with the mouse.

The size of the peaks within the graph may be scaled in several ways. For temporary rescaling that only affects the current Data file, see CURSOR TYPES. For rescaling that affects all the Data files to be processed, use the GRAPH SCALING options in the GRAPH DATA OPTIONS dialog box as follows:

AUTOMATIC SCALING is the simplest method of formatting the graph. In general terms, automatic scaling fits the length of the Data file into the X (time) axis, offsets the baseline about 15% above the bottom of the graph, and puts the highest peak near the top of the graph. This generally produces an acceptable graph:

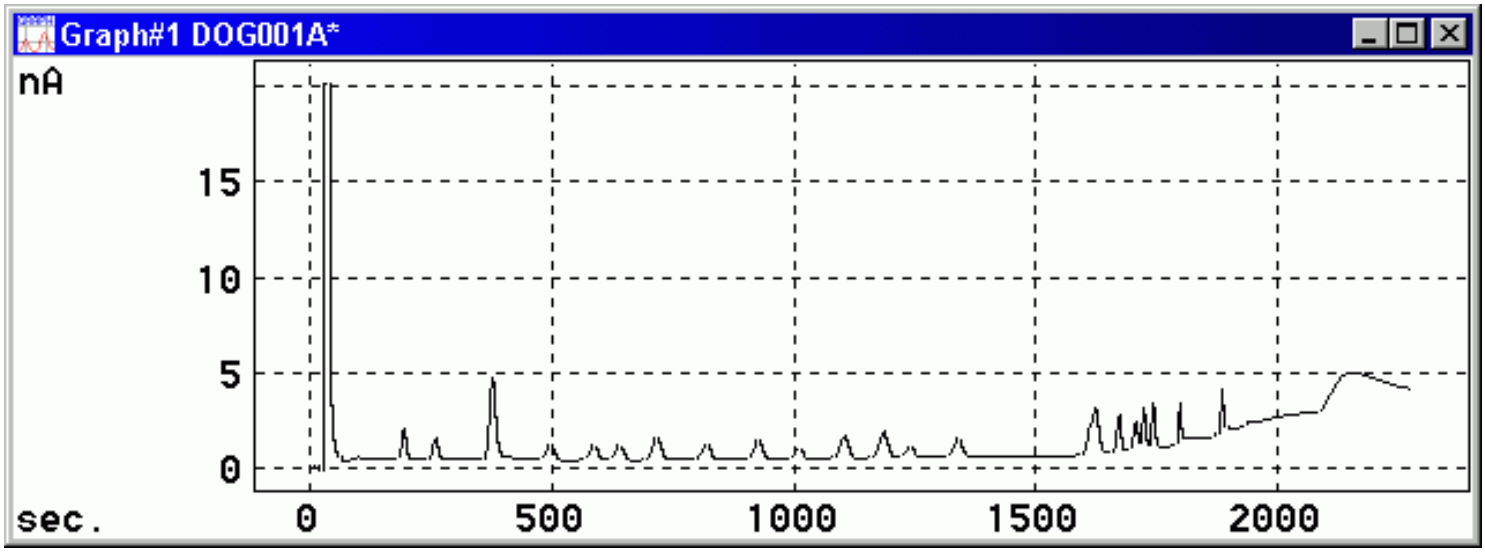
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If the baseline is very negative and offscale, uncheck the Neg.Limit-10% box. The box is usually checked, to prevent large negative excursions of the baseline (e.g., the injection disturbance) from affecting scaling.

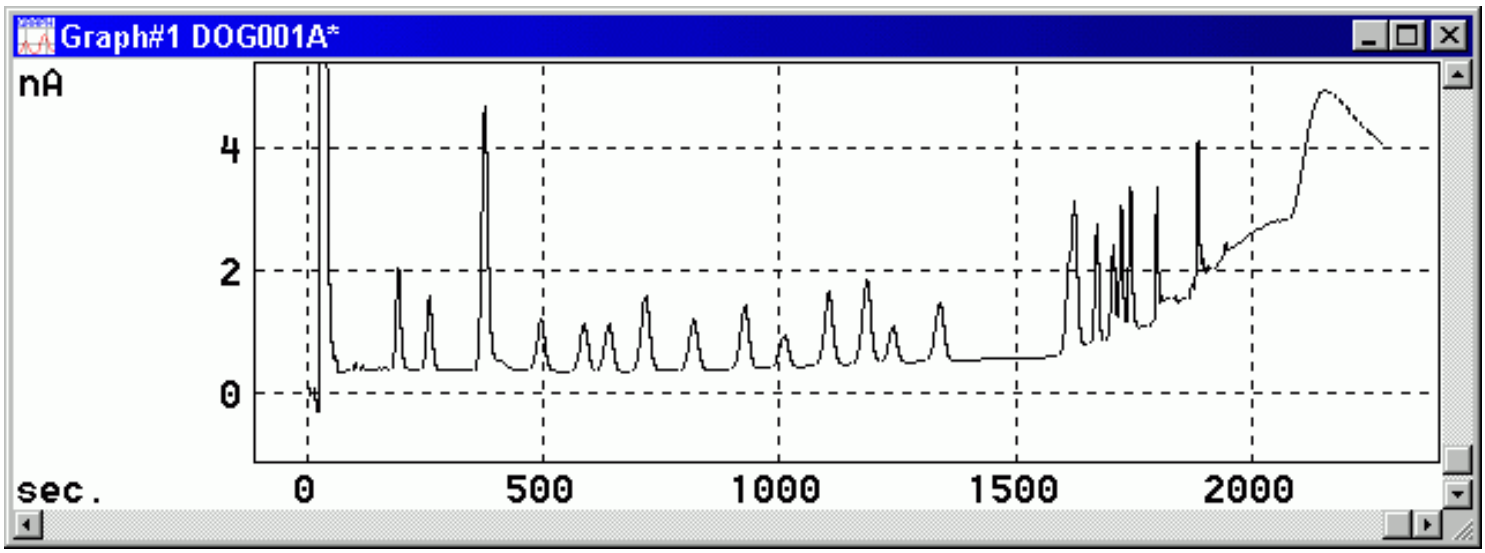
There are two drawbacks to automatic scaling. First, since each graph is separately scaled, it's hard to do visual comparisons of several Data files processed in a batch: as each file is processed and graphed, it will be maximized to fit the graph. (NOTE: peak heights, areas, and calculated amounts from the report are unaffected by scaling. These numbers may be used for comparing Data files.)

The second drawback to automatic scaling is that the scaling algorithms cannot distinguish between the void-volume response and a peak. At sensitive gains, the detector may show a full-scale deflection in response to the injection, while the true peaks may be quite small. Automatic scaling will fit the void-volume response to the graph, and consequently the peaks may be tiny:



FIXED SCALING allows you to specify the upper and lower limits for the Y (and less commonly, the X) scale. The figure below shows an improved graph of the same Data, achieved by using fixed scaling. The fixed scale is retained in RAM, so every subsequent Data file will be graphed on the same scale. The fixed scale can be saved for subsequent use by saving the REPORT.INI file.

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To use fixed scaling, first load the Data file with the largest peaks from your series of runs (this is generally the high standard). Process it with automatic scaling, then observe the graph. Use the Y-scale on the automatically scaled graph to estimate the appropriate upper and lower limits for the fixed scale. Then select GRAPH DATA. Click on the Fixed scale option, and enter the Min. and Max. Y-axis values. Click on OK to see the results, and if necessary, further refine the upper and lower limits. The X-axis can be scaled using the Left and Right time boxes.

The LABEL PEAKS WITH option allows the peaks to be labeled with their retention time, number (sequential number from the integration report or name (from the Standards file). These options are only available when the Data file has been processed. Unprocessed data may be graphed but not labeled.

FORMATTING OPTIONS

The formatting options include the following choices for controlling the appearance of the graph:

FILL PEAKS colors in the area under each peak. Peaks will be filled with the line color selected for that Data file. If several Data files are graphed simultaneously, only the most recently processed Data file will have filled peaks.

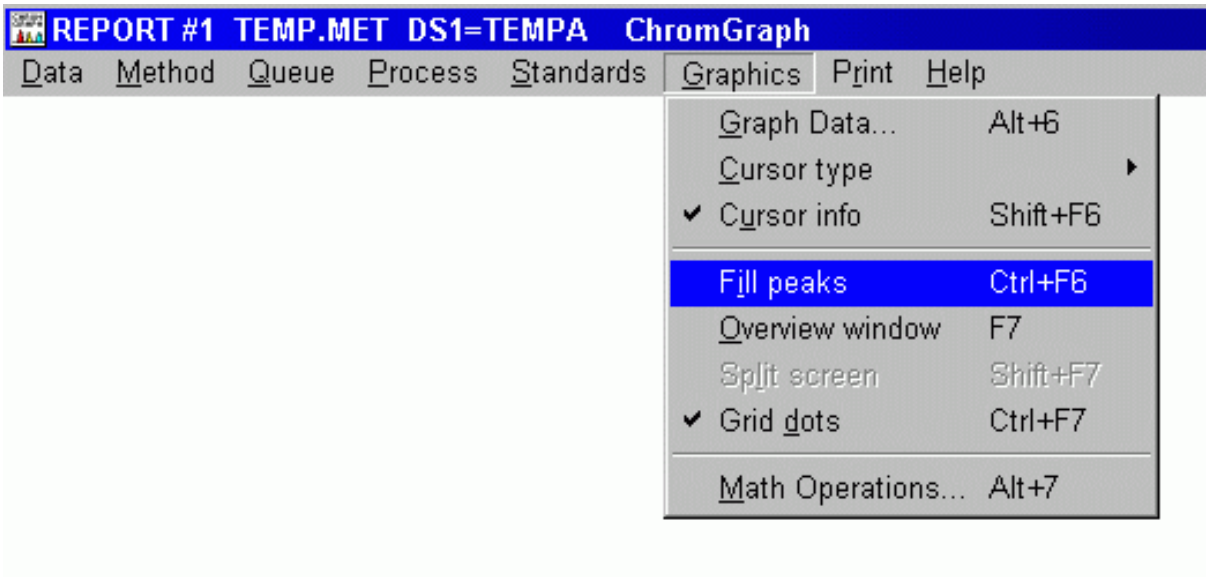
OVERVIEW WINDOW enables a second window which always shows the entire chromatogram. When a section of the main graph has been enlarged with the Zoom Cursor, the enlarged section is indicated on the Overview Window by a rectangle. The relative sizes of the Overview Window and the main graph can be adjusted by dragging on the border between them with the mouse.

SPLIT SCREEN divides the screen horizontally so that Data files will graph separately rather than overlap.

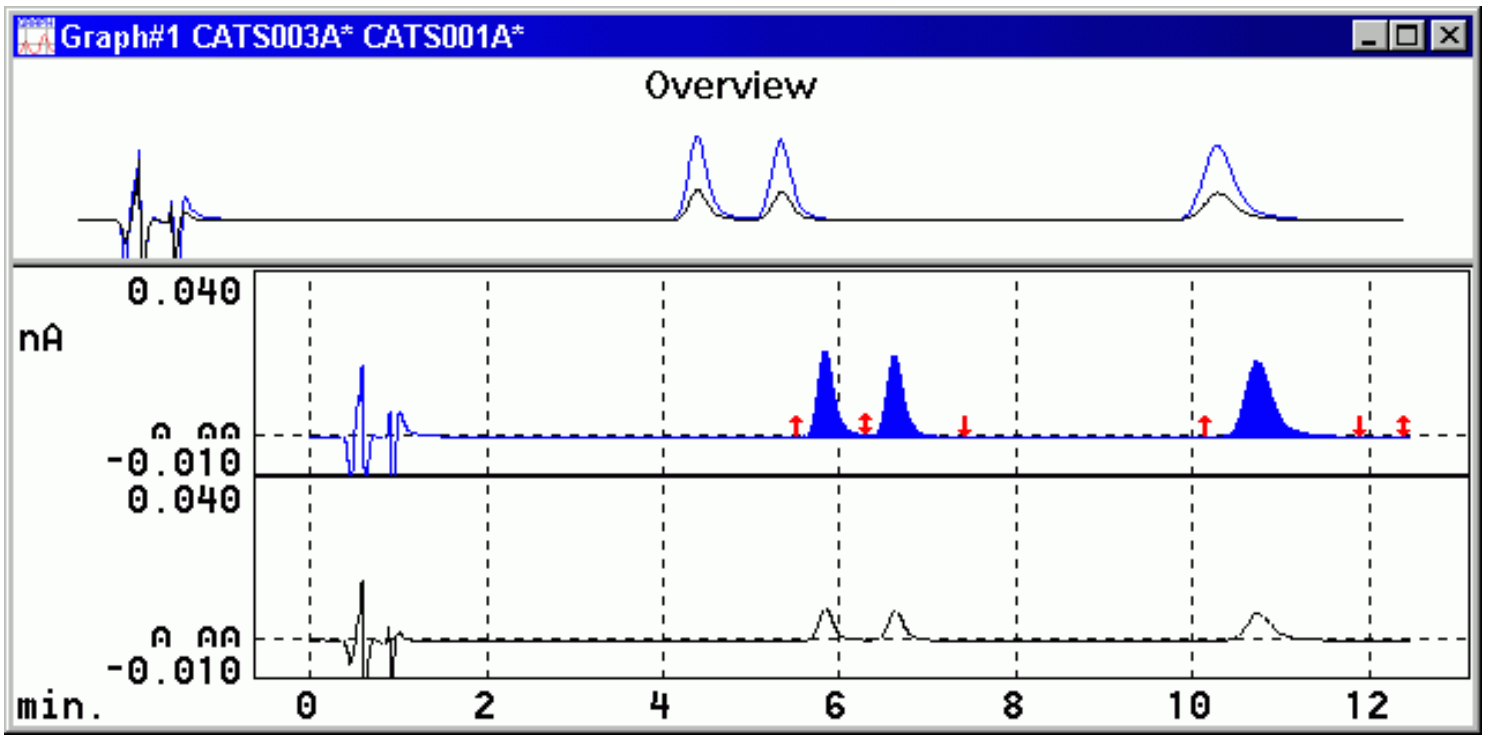
GRID DOTS overlays a grid on the graph, to facilitate visual estimations of peak height and retention time. For more accurate estimates, enlarge the peak of interest with the Zoom Cursor so the grid switches to a finer scale.

These options can be turned on or off by clicking on their names in the Graphics Menu:

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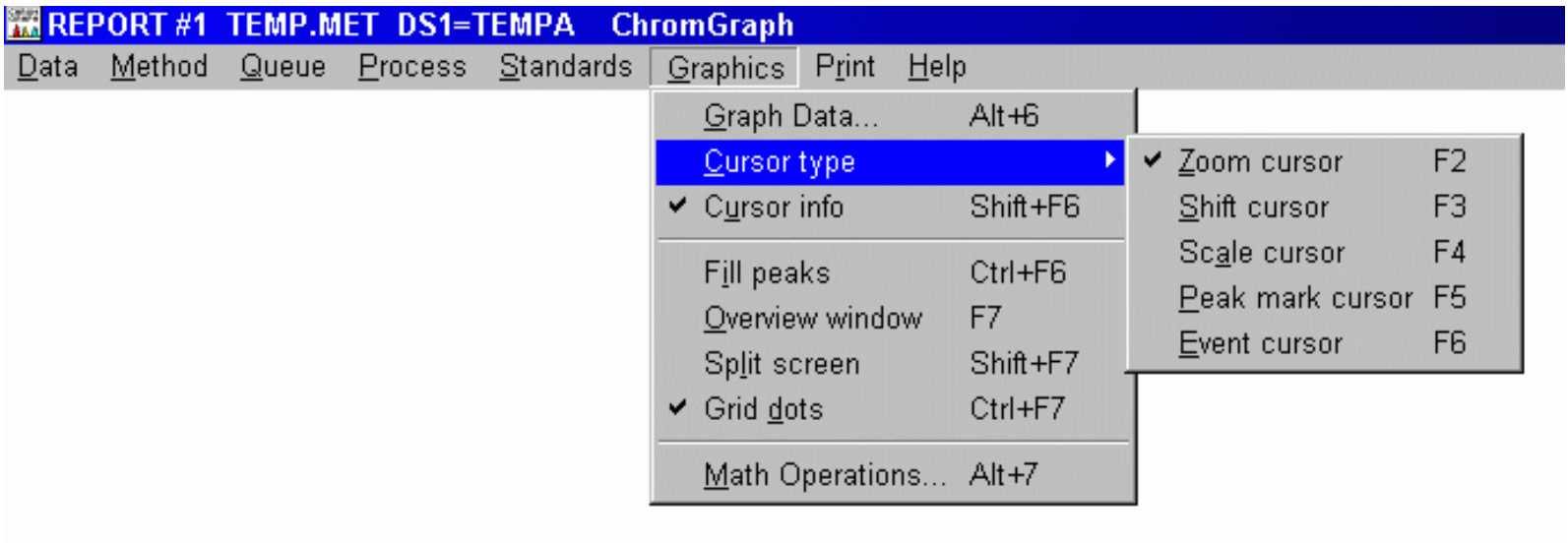


The figure below shows a graph with Split Screen, Overview Window, Fill Peaks and Grid Dots turned on:

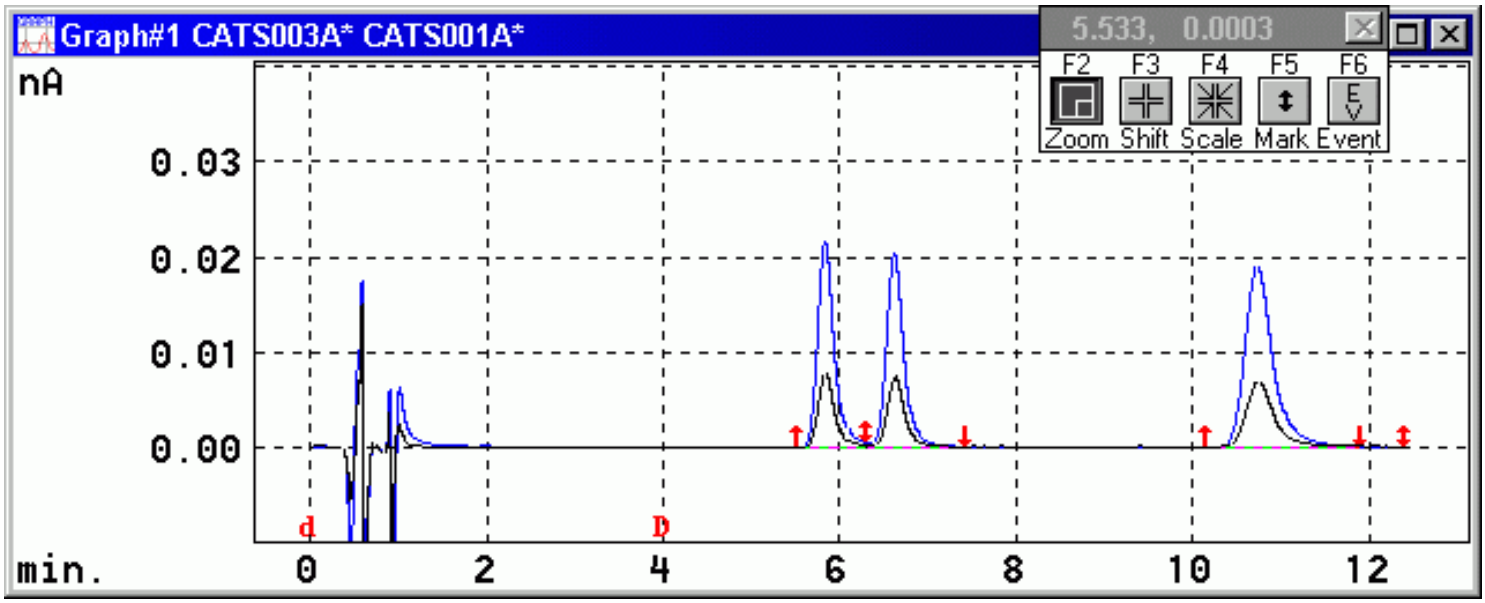


INTERACTIVE CURSORS

Five cursor types allow interactive manipulation of the graph. These are ZOOM, SHIFT, SCALE, PEAK MARK and EVENT. The cursor type may be selected from the Graphics Menu:



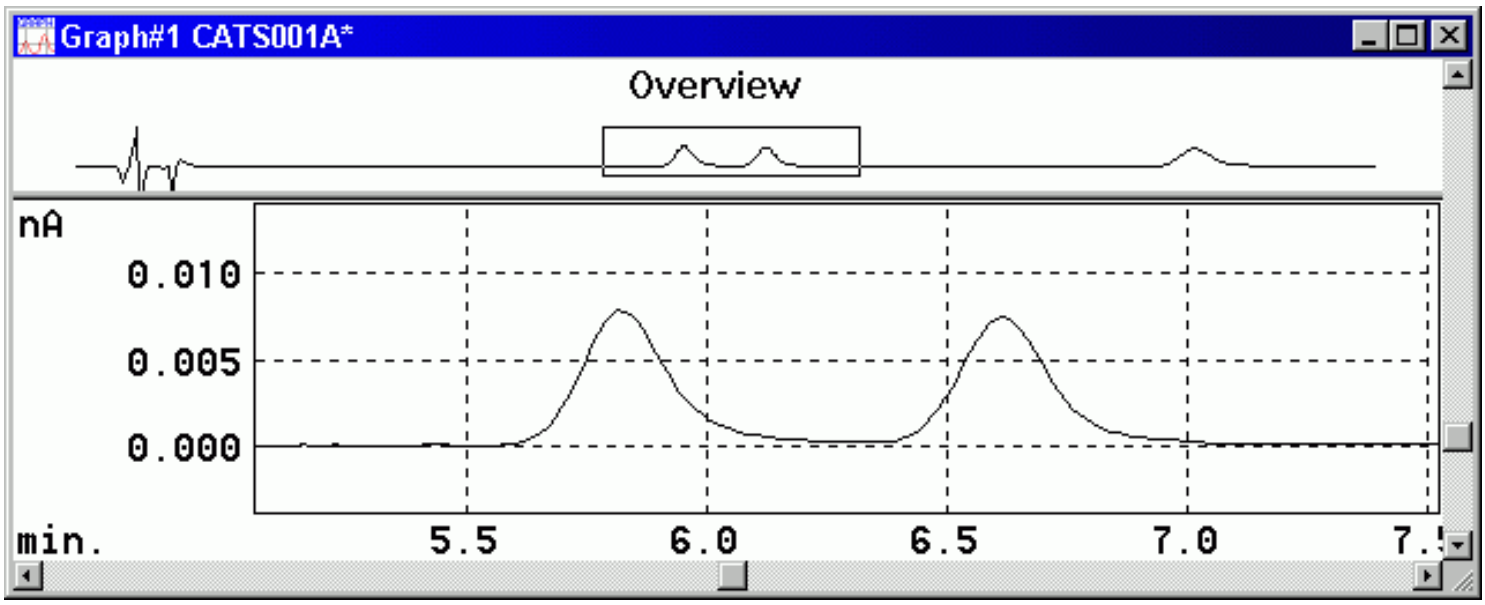
Alternatively, if CURSOR INFO is checked in the Graphics Menu, cursor type may be chosen from a selector box that appears near the graph:



Each cursor type has a distinctive shape when positioned on the graph. In addition to allowing cursor selection, this box provides a continuous display of the cursor's position on the X and Y axes. To select a cursor type, click on the appropriate button in the selector box.

ZOOM CURSOR. Parts of the graph may be enlarged using the Zoom Cursor, which is the default cursor type. You can select the Zoom Cursor, if necessary, by clicking on its button. To use the zoom feature, simply click and hold the left mouse button on a section of the graph, drag the cursor to another section (thus making a rectangle) and release the button. The rectangle can be drawn either on the main graph or on the Overview Window, but only the main graph shows the enlargement:

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Notice that the Overview Window retains the original graph, with a rectangle delineating the enlarged area. This rectangle can be dragged along the Overview Window to enlarge other sections of the graph. To return from an enlarged graph to the previous view, position the cursor on the main graph and click the right mouse button.

SHIFT CURSOR. The Shift Cursor may be used to move the peaks of the most recently processed chromatogram to the right or left, or to line up peaks on two chromatograms. A shift to the right or left actually changes the retention times of the data in RAM. To use the Shift Cursor, first select it by clicking on its button. Then click and hold the left mouse button anywhere on the graph. Drag the cursor to make a line in the desired direction of movement, then release the mouse button. If the shift affects the time axis, a warning message will appear, indicating that the data must now be reintegrated. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination. Click on OK and the new graph will be drawn. A shift cannot be easily undone. If this becomes necessary, reload the original Data file.

SCALE CURSOR. The Scale Cursor allows the peaks in the most recently processed chromatogram to be stretched or shrunk in any direction. Select this cursor by clicking on its button. To rescale the chromatogram, click and hold the left mouse button anywhere on the graph, then drag a line in the desired direction. The length of this line is proportional to the amount of enlargement or reduction. Lines drawn up and to the right enlarge, while those drawn down and to the left reduce. When the mouse button is released the new graph will be drawn. If the scale operation occurs along the time axis, a warning that the data must be reintegrated appears. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination. The most common use of the Scale Cursor is to enlarge the peaks prior to printing the graph. A change in scale is not easily undone. If this becomes necessary, reload the original Data file.

PEAK MARK. The Peak Mark (Mark) Cursor allows interactive adjustment of peak start and stop markers on the most recently processed chromatogram. To move a peak marker, first select the Mark Cursor by clicking on its button. Then simply click and hold the left mouse button on a marker, drag it to another location, and release the mouse button. You must move the cursor to the precise location in the horizontal direction. Vertically, however, it will jump to the baseline when you release the mouse button. A warning that the data must now be reintegrated will appear. Reintegration is needed only if you wish to analyze the modified data; it is not needed for replotting and visual examination.

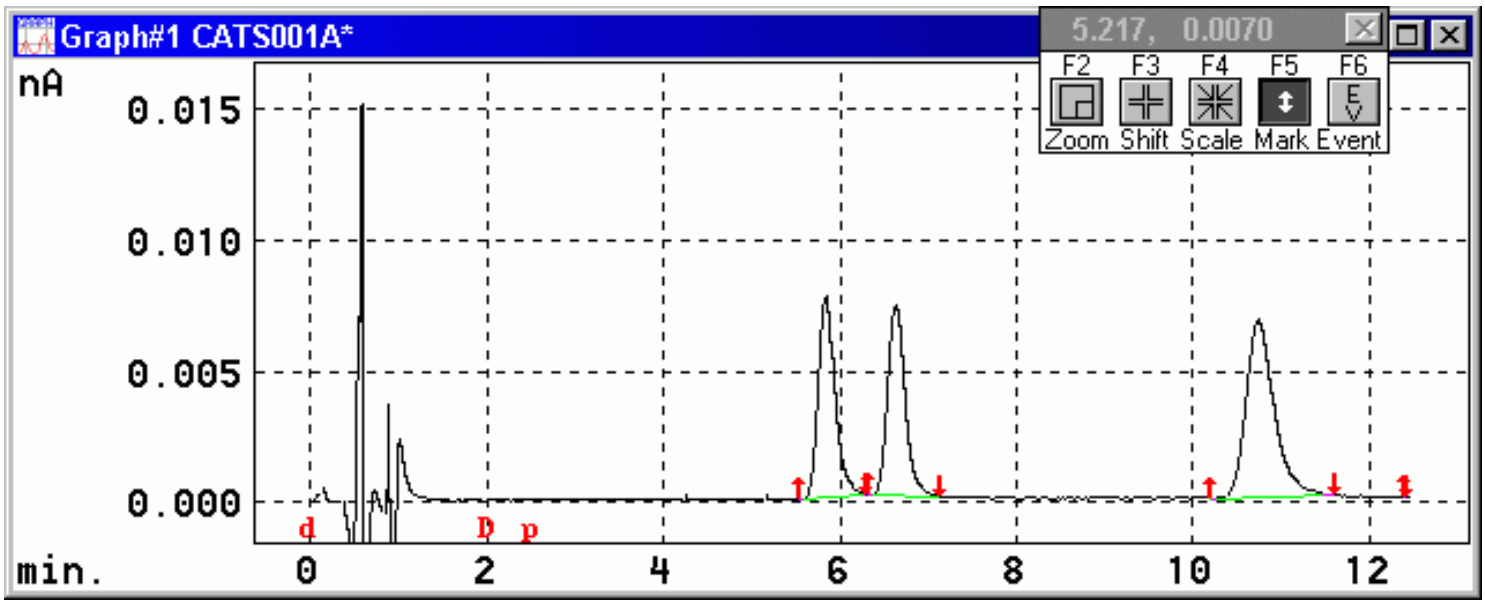
To remove a pair of peak markers, position the cursor between the start (up arrow) and end (down arrow) markers and press the delete key. To insert a pair of markers, position the cursor above the baseline, near where you want them. Press the insert key to insert the pair of markers, then move them with the mouse. It is helpful to enlarge the chromatogram with the Zoom Cursor before moving baseline markers.

A peak marker may not be moved across another peak marker. For example, an up-arrow (peak start) may not be moved to the right of its down-arrow mate, nor to the left of any previous markers.

Moving the baseline markers does not change either the Data file or the Method. The Data file will have to be reintegrated in order for the modified peak markers to be recognized. If you redetect peaks the original placement of the markers will be restored.

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EVENT CURSOR. The Event Cursor allows processing events to be interactively inserted and deleted. Existing events are indicated by letters along the lower margin of the graph:



A corresponding letter over the cursor indicates the type of event to be edited:

E (e)	The initial state (can delete any event)
D	Peak detect on
d	Peak detect off
N	Negative peak on
n	Negative peak off
P	Perpendicular drop on
p	Perpendicular drop off
F (f)	Fix baseline
T (t)	New threshold

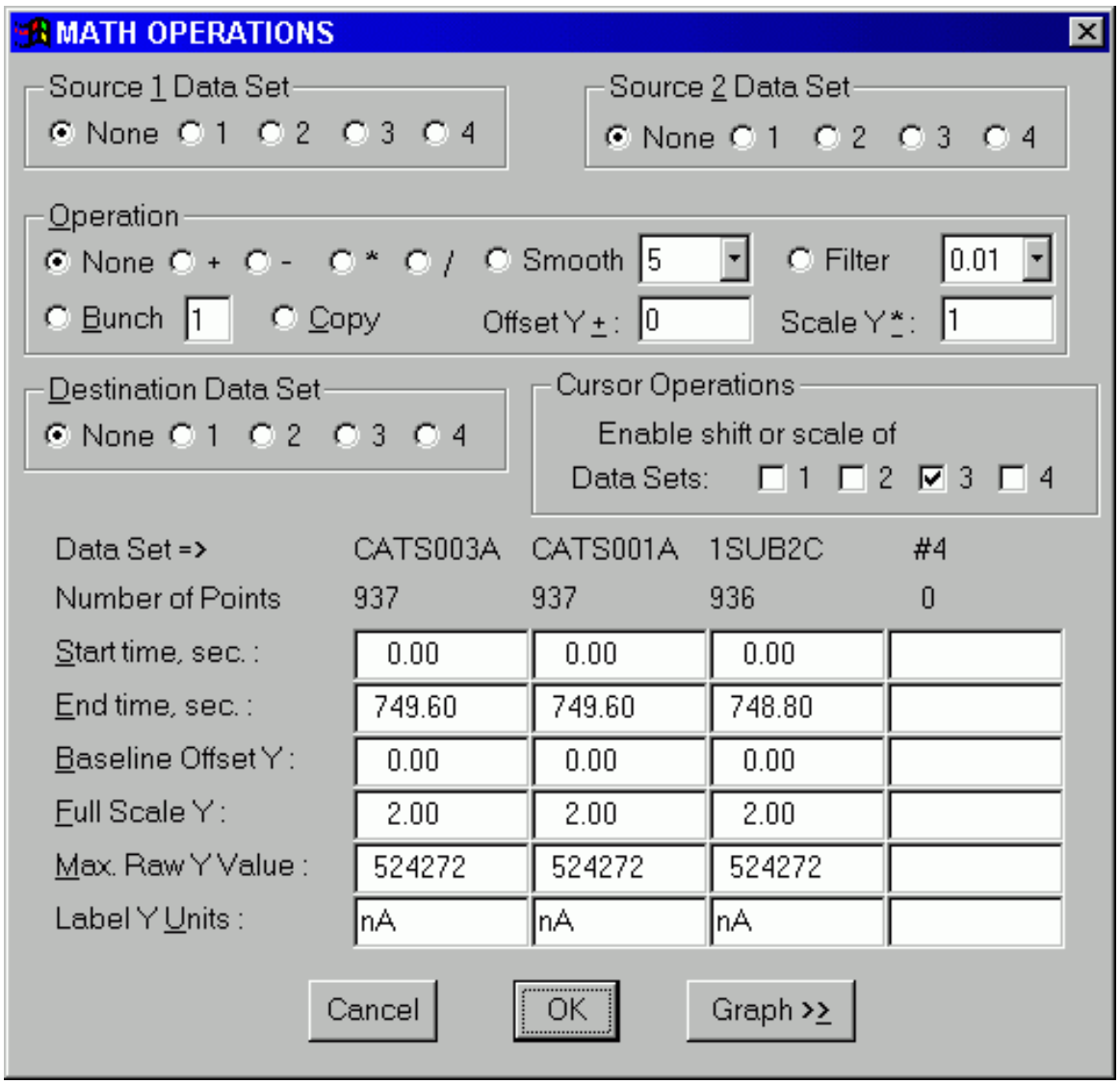
To use this feature, first click the Event button. Then press the appropriate key so the letter code for the function of choice appears above the cursor. Position the cursor over the baseline, then press the insert key to insert an event, or the delete key to remove an existing event. The events are inserted or deleted from the Method in RAM, and any changes will appear in the Processing Events dialog box. The Data file must be processed for the changes to be put into effect. Subsequent Data files also will be processed with the changed events. SAVE the Method to make the changes permanent. If it becomes necessary to restore the original Events, do not save the changed Method: instead, reload the original Method.

MATH OPERATIONS

A number of mathematical operations are available through the MATH OPERATIONS section of the Graphics Menu. These include addition and subtraction of Data files, normalization, smoothing, derivatization, etc. Most of these are beyond the scope of this introductory manual, and additional information about them can be found in the Help screens. One feature of more general interest is subtraction, which can be used to remove from the samples any regular and predictable disturbances in the baseline. These disturbances may include slope (e.g., from gradients) or peaks from contaminants or derivatization reagents.

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To perform a baseline subtraction, first perform the chromatography and collect the data. You should have a sample run and a relevant blank run. Next load the two runs into data slots in Report. Select the Math Operations section of the Graphics Menu:

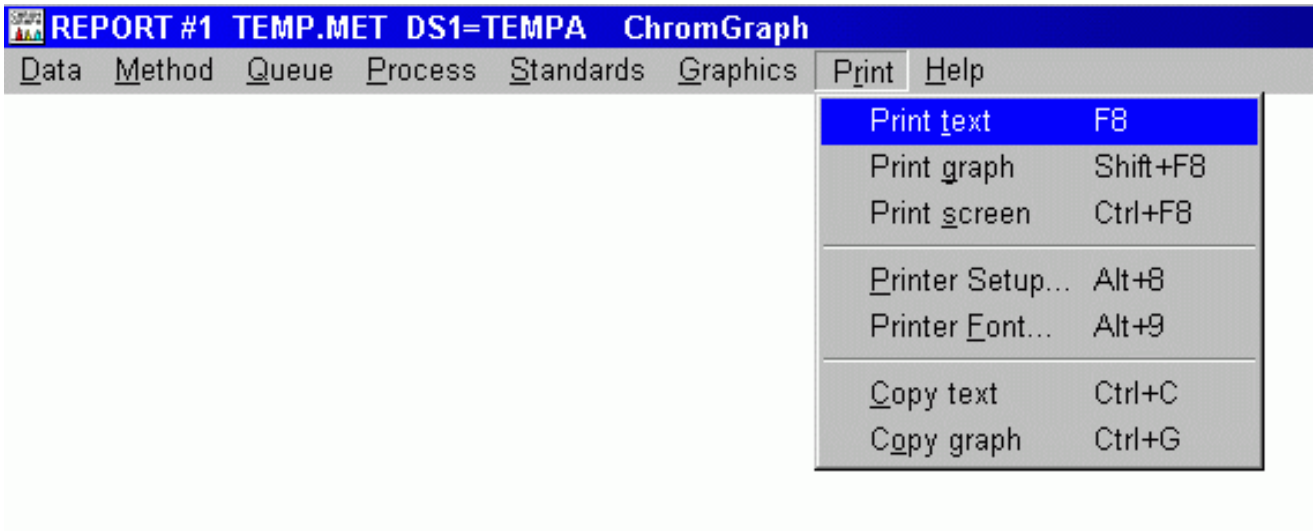


Designate the sample Data file as Source 1 and the blank Data file as Source 2. Select subtract (-) as the Operation, and any unoccupied data slot as the Destination Data file. Press OK and the operation will be performed. The new Data file will be called 1Sub2, and can be processed like a typical Data file.

PRINTING

ChromGraph offers various options for printing. For example, via the Report Options section of the Method you can instruct Report to send all output to the printer. Also, various other menu options employ LIST DEVICE boxes which permit you to send that task's output to the printer. Occasionally you may want to print or capture only the screen currently displayed. Options in the PRINT MENU afford that opportunity:

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Text on the output screen can be sent either to Windows' Clipboard, for pasting to other Window's programs, or to a printer. First highlight the text by dragging the mouse over the lines of interest. Then click on either COPY TEXT (to send it to Clipboard) or PRINT TEXT (to send it to the printer). To print graphics, select PRINT SCREEN to send the entire screen (text and graphics) to the printer, or PRINT GRAPH to send only the graphics. To send only the graph to Clipboard, press COPY GRAPH. To send the entire screen to Clipboard, press the PRINT SCREEN key on the keyboard.

PRINTER SETUP opens a dialog for choosing among several printers and selecting certain options. This need not be done if a single printer has been configured under the Windows system.

PRINTER FONT opens a dialog for selecting printer font type and size. We have found the True Type (TT) fonts to work well under all conditions.

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WORKING ELECTRODES

The working electrode consists of a carbon or metal rod embedded in a plastic block. Glassy carbon is the most common electrode for LCEC. Specialty materials such as platinum, gold, silver, nickel, and carbon paste are used for specific assays described in BAS applications capsules.

The block is made of PEEK (polyetheretherketone) and is resistant to solvents and temperatures normally used in liquid chromatography/electrochemistry. PEEK will be chemically degraded by concentrated nitric and/or sulfuric acids, but diluted HNO_3 may be used to remove mercury from gold amalgam electrodes without harming the PEEK surface. Working electrodes are maintained by polishing as needed, using the instructions given in this manual. Instructions differ according to working electrode material. We do not recommend polishing methods or materials other than those described in this manual.

- **INTRODUCTION TO POLISHING**
- **POLISHING GUIDELINES**
- **POLISHING GLASSY CARBON, SILVER, AND NICKEL**
- **POLISHING PLATINUM AND NATIVE GOLD**
- **ELECTROCHEMICAL CLEANING OF PLATINUM**
- **GOLD/MERCURY AMALGAM ELECTRODES**
- **CARBON PASTE ELECTRODES**
- **POLISHING THE UNIJET ELECTRODE**
- **WORKING ELECTRODE STORAGE**

INTRODUCTION TO POLISHING

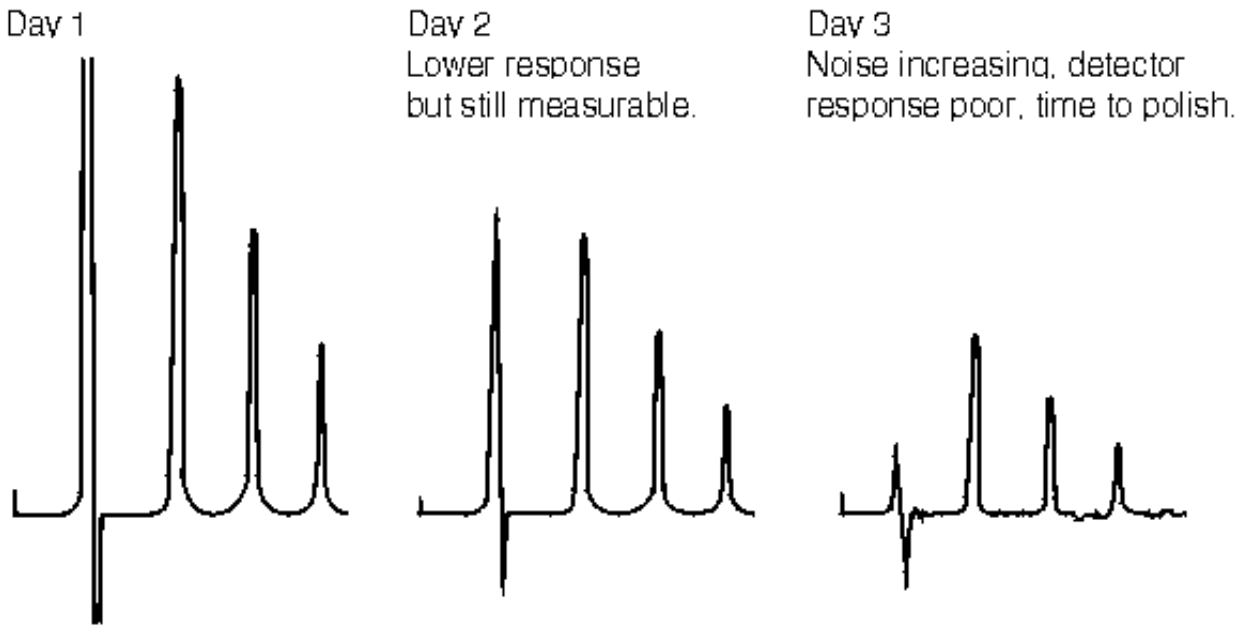
The objective of polishing the electrode is to remove the redox reaction products that accumulate during some experiments. The rate of electrode coating (and corresponding decrease in responsiveness) depends on:

- The analyte molecule
- The concentration of the analyte molecule
- The composition of the electrolyte solution (including pH)
- The applied potential
- The frequency of use

Electrodes used in liquid chromatography may last for several months without repolishing in some applications. LCEC has the advantage of using a flowing stream which helps to remove redox products, and deals with much lower concentrations of analytes than does voltammetry. Electrodes also can accumulate material by adsorption from the atmosphere. For example, components in cigarette smoke and aerosols of various compositions can affect electrode performance. Silver is easily oxidized under such conditions and should be carefully repolished to remove oxides prior to use.

There are many rumors and horror stories about electrode polishing. There are also some homemade polishing recipes that we specifically discourage. The use of concentrated acid is one. Use of jeweler's rouge, toothpaste, Aunt Ruth's fruitcake (vintage 1972) and other abrasive compounds should be fervently avoided. Many of these home remedies do not work and may harm the electrode. One thing is certain: The need for polishing varies dramatically with the application.

There are many potential causes for that "glitch" or noise in the baseline. Don't routinely conclude that polishing the electrode will cure all. As a rule, polishing is justified when you see a gradual decrease in the response of the electrode, as shown here:



You can live with decreased response if you are still able to quantitate the peaks of interest and if you periodically inject a set of standards. In many cases, a light buffing of the working electrode surface with a methanol-soaked lab tissue is all that s needed to restore the electrode. Try this first! If such a treatment improves the response only slightly, then the use of polishing abrasives is the next logical step.

A series of one to three abrasives is used for polishing, progressing from coarser materials to very fine polishes used on soft-surfaced pads. The polishing process should remove a negligible amount of electrode material. In most cases, a single abrasive is used to lightly erode the surface and physically remove the contaminants. Precious metal electrodes or chemically modified electrodes (e.g., gold/mercury amalgam) tend to require a series of two or three abrasives to restore the original mirror-like finish.

All polishing steps require **extensive rinsing** and flushing of the electrode before moving on to the next stage. Without this washing, minute particles from the previous polishing step will be carried over and will hinder the progression towards smaller-sized particles of abrasive. Plastic squeeze bottles with thin nozzles are best for rinsing. Fill one with clean, distilled water for rinsing alumina polish. Fill another with methanol for rinsing diamond polishes. Label the bottles.

A major concern of polishing is that you do not erode the softer electrode below the surface of the surrounding plastic or glass. This can be avoided by polishing the electrode on a hard flat surface. Follow the general guidelines below when handling your working electrode.

POLISHING GUIDELINES

- It is important to use the polishing materials provided by BAS and to follow the recommended procedure. The polishing kit provided with your BAS instrument gives you all materials needed for all polishing procedures. Individual polishes, pads, or the entire kit can be purchased separately.
- Before use, shake all bottles of polishing slurry well to ensure that all the grit is evenly suspended.
- Only one grade of polishing slurry is to be applied to the pad; that is, use a different pad for each polishing step. Do not add fresh polishing compound to pads once a particular polishing step has been initiated; add sufficient slurry at the beginning. If additional lubrication is required, add water to the pad.
- While most individual nylon (white) polishing pads may be reused, the fine grit polishing pad (black to dark gray in color) should be used one time only. If used more than once, excess scratching may occur due to the spaces between the grit being filled in with PEEK, causing a deeper pressure scratch to occur.
- The diamond slurries (15-µm blue, 6-µm orange, 3-µm yellow, and 1-µm gray) have an oil base, so a methanol or acetone flush of the electrode

is required.

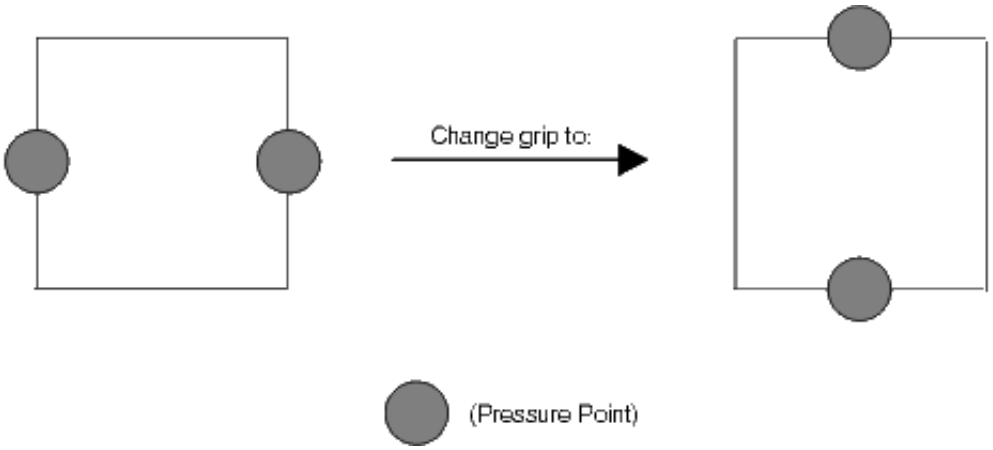
- The pads have self-adhesive backs and are less likely to rise off the glass when wetted if applied to the glass plate the previous day. Clean the glass plate with methanol first. Then peel the pad and push down from the center out to the edge so no air pockets are trapped between pad and plate (so no high spots are produced). Label each plate for the appropriate grade of slurry to be used.

- Moisten the pads with distilled water a few minutes prior to use. This will aid in breaking up any clumps of grit that were not rinsed off from a previous use. The excess polishing grit should always be rinsed off the pads after use to lessen the chances of clumping. Large clumps of grit will excessively scratch the gold and PEEK.

- Rinse all surfaces of the electrode block free of any polishing compound (grit). Since the polishing schedule progresses from coarse to fine, it is very important not to mix large and small particle sizes.

- Polish the electrode on pads attached to the heavy glass plates provided in the polishing kit. Hold the electrode surface as parallel to the surface of the glass as possible. This is especially important with voltammetry electrodes and will ensure that the surrounding plastic is not worn unevenly.

- Moving the block in a figure-eight pattern is optimal for uniform polishing. However, an alternating clockwise/counterclockwise motion is also acceptable. During all polishing steps, apply even pressure to the electrode block. This may be accomplished by alternating the position on the block to which you are applying pressure:



- Never attempt to remove the actual electrode material from the supporting plastic. This will destroy the electrode.

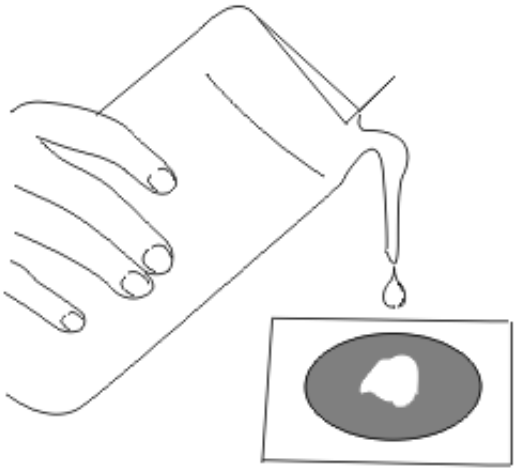
- Do not heat the electrode when drying. Allow it to dry at room temperature. Any heating of the electrode should be attempted with caution due to the difference in the coefficients of expansion of the electrode and the supporting plastic or glass.

POLISHING GLASSY CARBON, SILVER, AND NICKEL

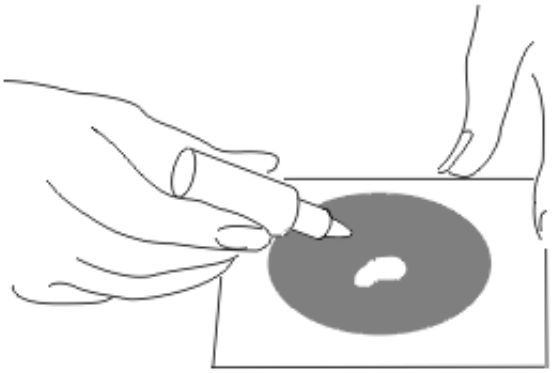
Remove a new microcloth disk from the polishing kit. (Microcloth is brown and has a soft, velvety texture.) Peel away the backing to expose the adhesive, and attach the disk to the glass plate. Use a permanent marker to label the glass with the type of polishing fluid used. It is possible to reuse the disks several times, but it is important to use the same type of polish each time. Your label will remind you which polish you used. Now follow these steps:

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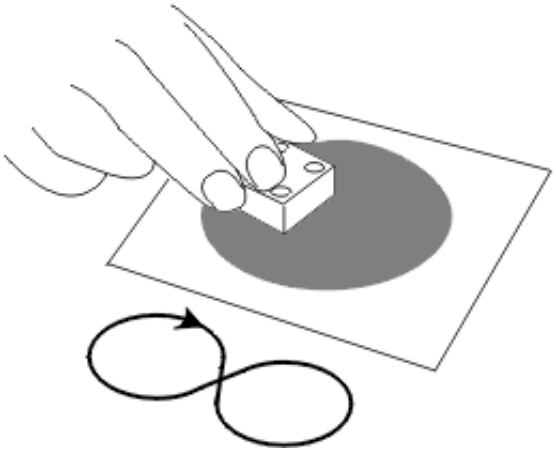
Wet Polishing Pad Before Adding Polish



Add a Small Amount of Polish to the Wet Pad



Use Smooth Motion and Even Pressure when Polishing



- Rinse the electrode surface with water to flush away any encrusted material on the surface. Follow this with a methanol rinse. Wipe dry with a fresh lab tissue.
- Wet the disk surface with clean DISTILLED water. Shake the alumina suspension and add several drops of alumina polish, spacing them evenly around the pad surface.
- Place the electrode face down on the pad. Using a smooth, circular motion, and even pressure, move the electrode all over the pad. By reversing the polishing direction and rotating the electrode 90° at regular intervals, you will prevent uneven wear of the electrode. After 1-2 minutes, remove the electrode and rinse it well with DISTILLED water.

- Electrodes may be sonicated to remove residual abrasive particles. Immerse the electrode **surface** in a **shallow** amount of DISTILLED water in a beaker that has been placed in the water of a low-power (150 watts or less) ultrasonic cleaner. Sonicate for no more than 5 minutes (longer periods may overheat and damage the electrode). Rinse again with distilled water from the squeeze bottle and shake off the remaining water.
- Rinse the electrode briefly with methanol and wipe it dry. The electrode is now ready to use.
- Do not touch the electrode surface with your fingers, or place the surface in contact with sharp objects or other materials which could scratch it.

POLISHING PLATINUM AND NATIVE GOLD

Remove a new microcloth disk from the polishing kit. (Microcloth is brown and has a soft, velvety texture.) Peel away the adhesive back and attach the disk to the glass plate. Next, remove a nylon disk from the kit. (Nylon disks are white with a tight, woven texture.) Peel away the backing to expose the adhesive, and attach the disk to the OPPOSITE side of the glass plate. There are rubber feet on both sides of the glass so you can use both sides without having to place the polishing surface on your benchtop.

Label each side of the glass with the type of polishing fluid used. Use the brown microcloth with alumina polish, and the white nylon pad with the diamond slurry polish. It is possible to reuse the disks several times, but it is important to use the same type of polish each time. Your label will remind you which polish you used. Shake polishing slurries thoroughly before using. Now follow these steps:

- Rinse the electrode surface with water followed by methanol to flush away any encrusted material on the surface. Gently wipe dry using a fresh lab tissue.
- Wet the white nylon disk with DISTILLED water and apply a few drops of the 1-µm diamond polish slurry.
- Place the electrode face down on the pad. Using a smooth circular or figure-eight motion and even pressure, move the electrode all over the pad. Reverse direction and rotate the electrode 90° at regular intervals to prevent uneven wear of the electrode. After 1-2 minutes, remove the electrode and rinse away all remaining polishing grit with methanol, using a squeeze bottle.
- Next, turn over the glass and wet the microcloth disk surface with DISTILLED water. Shake the alumina suspension and add several drops of alumina polish, spacing them evenly around the pad surface.
- Place the electrode face down on the pad. Polish as above. After 1 2 minutes, remove the electrode and rinse well with DISTILLED water.
- Electrodes may be sonicated to remove residual abrasive particles. Immerse the electrode **surface** in a **shallow** amount of DISTILLED water in a beaker that has been placed in the water of a low-power (150 watts or less) ultrasonic cleaner. Sonicate for no more than 5 minutes (longer periods may overheat and damage the electrode). Rinse again with distilled water from the squeeze bottle and shake off remaining water.
- Rinse the electrode briefly with methanol and wipe dry. The electrode is now ready to use.
- Do not touch the electrode surface with your fingers, or place the surface in contact with sharp objects or other materials which could scratch it.

ELECTROCHEMICAL CLEANING OF PLATINUM

Often, the gradual loss in electrode response is due to slow oxidation of the electrode surface. Sometimes, the redox chemistry depends on the presence of certain catalytic moieties on the surface which must be regenerated. This process can usually be performed electrochemically, without disassembling the cell. For many applications, the need to use an abrasive polish on a platinum electrode is rare.

Alternate the polarity of the working electrode by setting the potential to 500 mV and switching the polarity switch between (+) and ( ). Perform at least 10 cycles, pausing at each potential for a few seconds. Return the potential to the desired value and test the response with a standard solution.

If the response does not improve, disassemble the cell and polish the electrode with a methanol-soaked lab tissue. Use firm pressure. Rinse the

block with methanol and reassemble. Proceed polishing with abrasives only if the response is still too low compared to earlier performance.

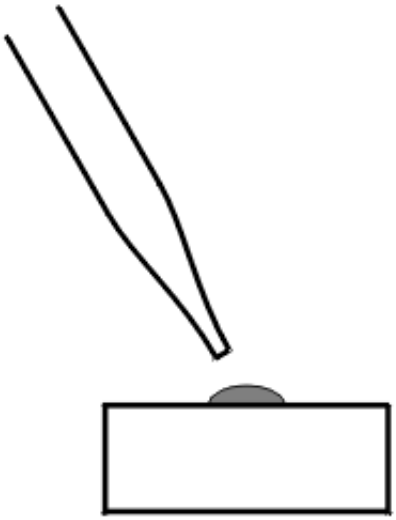
GOLD/MERCURY AMALGAM ELECTRODES

Gold amalgam electrodes consist of a solid gold disk that has been covered with a thin mercury film. When the mercury is gone, the gold must be repolished in several steps. In certain applications where a gold amalgam is used, the mercury is critical to the electrochemical reaction. In determination of thiols, for example, the R-SH complexes with mercury to form a product which is then oxidized. This process will tend to gradually strip away the mercury film. A gold tinge is often noticeable on the surface of the working electrode when the electrode loses responsiveness. When the electrode performance deteriorates, you can initially try to revive the electrode by reapplying more mercury.

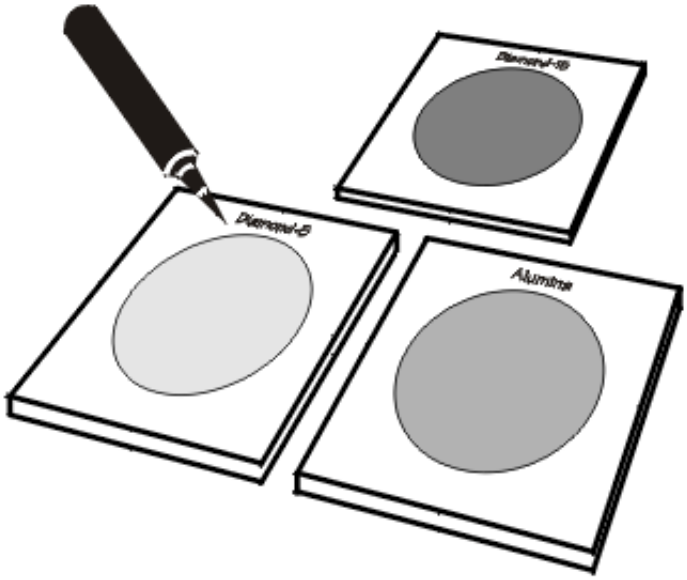
To apply more mercury, clean the surface of the electrode with distilled water and wipe it with a methanol-soaked lab tissue. Follow this with more squirts of methanol and then air dry. Add new mercury. This procedure will often buy some extra time before a full repolishing is required. To fully repolish a gold amalgam electrode, follow the instructions below.

The final performance of thin-film mercury amalgam electrodes is very dependent on the finish of the gold substrate. A smooth, mirror-like finish produces the best results. A combined acid/abrasive procedure will achieve that goal. The acid treatment quickly removes residual mercury from the electrode surface. The grit pad and diamond polishes do the majority of the polishing, and the alumina polish burnishes the gold back to a shiny appearance in preparation for reapplication of the mercury.

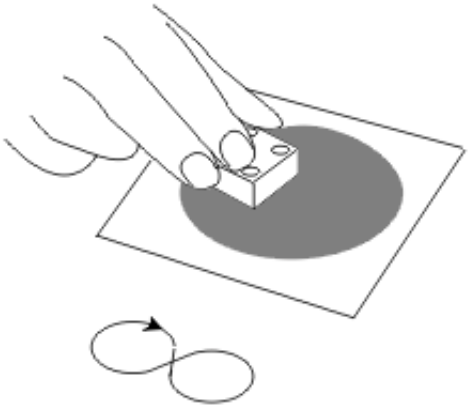
CLEANING AND POLISHING PROCEDURE

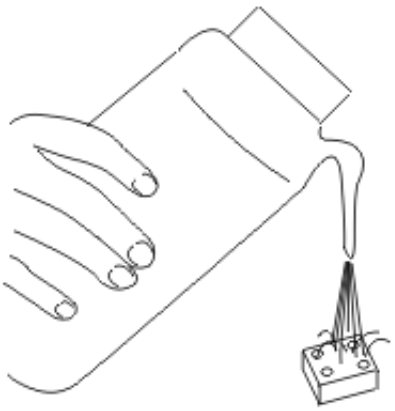


Apply nitric acid with a glass pipette.



Use fresh polishing pads and label the glass with the type of polishing fluid used.







Use a figure-8 motion when rubbing the electrode on the polishing pad.



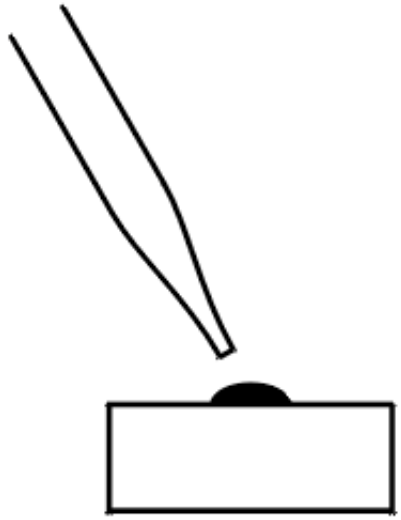
Rinse electrode frequently and extensively with distilled water.

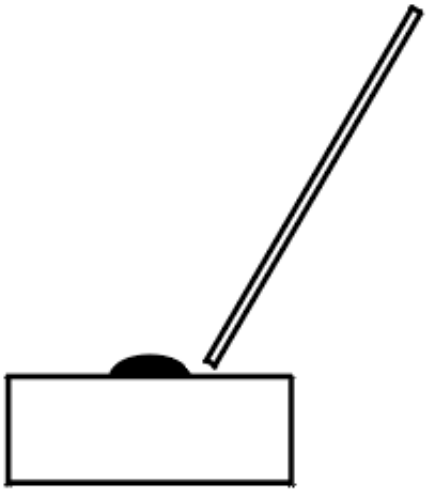
- Rinse the electrode surface with water followed by methanol to flush away any encrusted material on the surface. Shake off the liquid and wipe the surface dry with a lab tissue.
- Prepare for the next step using the normal precautions required when working with strong acids. Wear rubber gloves, safety glasses, and an apron, and work under a fume hood.
- Using a Pasteur pipette, apply a few small drops of 6 N NITRIC ACID (HNO_3) to the electrode surface. When the color changes from grayish-black to rusty yellow, the old amalgam has been destroyed. Repeat with a second drop of 6 N nitric acid. **Do not use concentrated acid**, which will damage the plastic. After the color turns, rinse thoroughly with water and proceed.
- Use the fine grit pad (dark gray) wetted with copious amounts of water. Polish for 5 minutes or less, until the gold appears to be of an even matte texture. The mercury has removed some of the gold, so the electrodes are recessed. The PEEK must be removed (polished away) to expose the gold electrodes to the polishing schedule. This is a critical step, so slight overpolishing is preferable to underpolishing. The gold must be exposed to the subsequent polishing and smoothing steps. Flush all surfaces of the electrode with a stream of DISTILLED water to remove grit. Use the fine grit pad only once and then discard.
- Immerse the electrode **surface** in a **shallow** amount of DISTILLED water in a beaker that has been placed in the water of a low-power (150 watts or less) ultrasonic cleaner. Sonicate for no more than 5 minutes (longer periods may overheat and damage the electrode). Rinse again with distilled water from the squeeze bottle and shake off remaining water.
- Apply a few drops of 15-µm diamond slurry (blue) to a water-moistened nylon pad (white). Polish until gold electrodes and PEEK are each of a uniform texture; this should take 3-5 minutes. This intermediate polishing step is important to impart a smooth finish to both the PEEK and the gold. Overpolishing is preferable to underpolishing. The diamond slurry is oil-based, so flush the electrode surfaces with a stream of methanol, ensuring that all grit has been removed. Sonicate as before. Wipe dry.
- Apply a few drops of 3-µm diamond slurry (yellow) to a water-moistened nylon (white) pad. Polish the electrode for about 2 minutes. Flush and sonicate the electrode as before. Wipe dry.
- Apply a few drops of 1-µm diamond slurry (gray) to a water-moistened nylon(white) pad. Polish, flush, and sonicate the electrode as before.
- Apply a few drops of polishing alumina (white) to a water-moistened nylon pad. Polish for about 2 minutes. Flush all electrode surfaces with a stream of water and then methanol.
- Stand the electrode on one side in a small beaker (100 mL). Add just enough distilled water to cover the plastic block. Clean in a bath sonicator of less than 150 watts power for 2 minutes. Flush the electrode surface thoroughly and sonicate for an additional 2 minutes. Flush the surface again with water and then methanol.
- The electrode may be wiped dry and stored, or amalgamated for use. Prior to the addition of mercury, the gold surfaces must be rinsed with methanol and wiped dry.

AMALGAMATING PROCEDURE

Use a figure-8 motion when rubbing the electrode on the polishing pad.

Rinse electrode frequently and extensively with distilled water.





Apply mercury drop to polished surface.

Scrape off excess mercury with a clean index card.

- To form the amalgam, place a drop of TRIPLE-DISTILLED MERCURY on the gold electrode. Slightly roll the drop around to make sure that it evenly touches the gold in all places. Wait 5 minutes, then use an index card to push off the excess mercury into a waste vessel. Polish the mercury surface with a lab tissue to produce an even layer.
- Allow the electrode to rest face-up. If the surface becomes dull in 10 minutes or less, you have removed too much mercury, and your amalgam will be short-lived. If the surface remains shiny after 60 minutes, you have not removed enough mercury, and your baseline will be noisy.
- Allow new amalgam to rest for at least 6 hours, or overnight. The electrode may be installed on a flowing LC system during this rest period, but do not apply a potential (keep the detector on the STANDBY setting). Plan on waiting overnight before using the detector for assays. If you try to use the electrode before this time, you may get a very high background and a change in electrode response over time.
- When the electrode is not in continuous use, or you plan to store it for an extended period, remove the mercury film using 6 N nitric acid. Polish the electrode again prior to amalgamation.

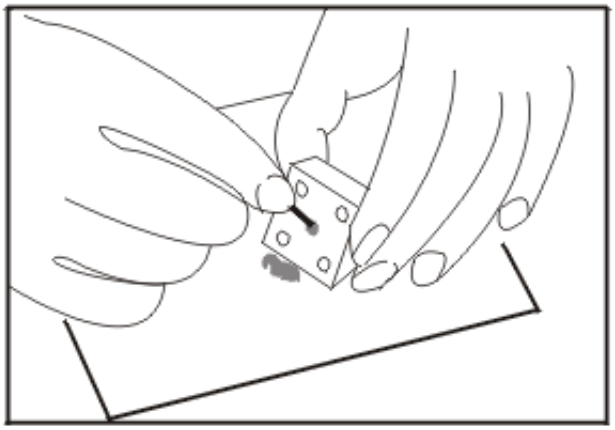
CARBON PASTE ELECTRODES

The nice thing about carbon paste is that you don't actually polish it. Instead, you make an entirely new electrode every time. The disadvantage of paste is that it will permanently stain any piece of cloth it encounters.

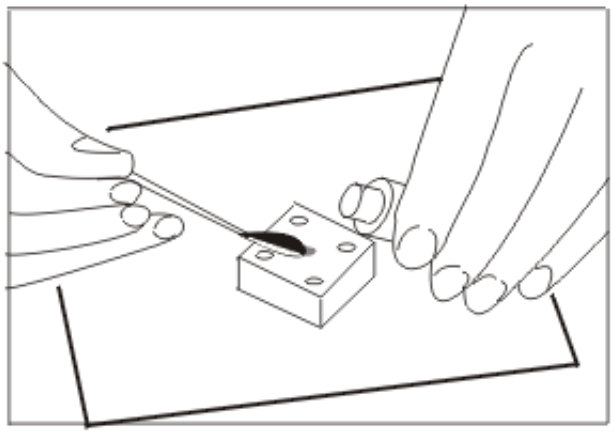
Carbon paste offers several interesting possibilities when studying compounds that are soluble in aqueous media. The electron transfer kinetics of carbon paste are markedly different than those of glassy carbon, and some users feel that the electrode offers slightly better sensitivity in some cases. It cannot be used with a high concentration of organic solvents in the mobile phase since this will erode the surface of the paste. Carbon paste compositions vary widely, including combinations of polyethylene or Kel-F powders pressed with carbon. This discussion is limited to handling of pastes currently manufactured by BAS.

Keep the vial of carbon paste closed when it is not in use. Like other forms of graphite, it can absorb contaminants from the laboratory environment.

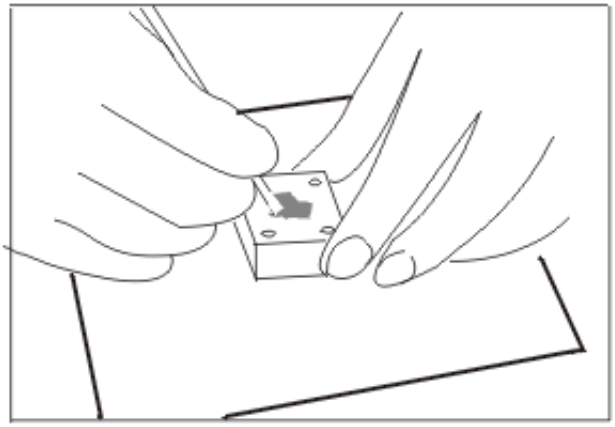
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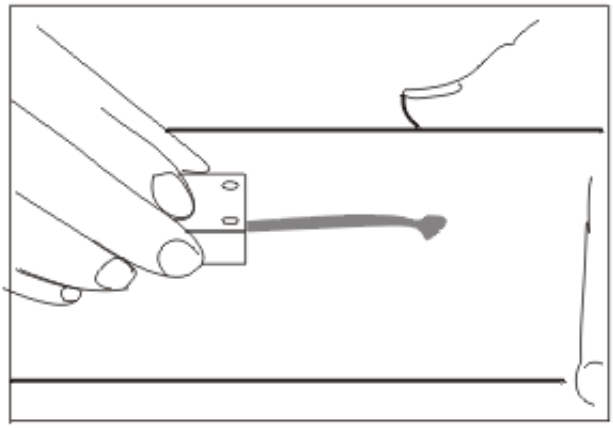
Carefully remove old paste from electrode well.



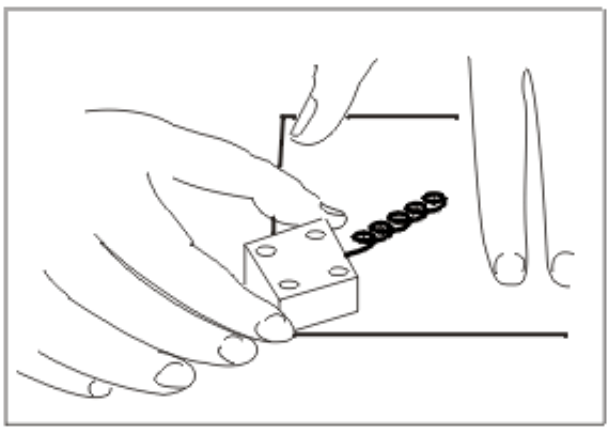
Add a small scoop of new paste to well.



Tamp into well with a small glass rod,
leaving a slight mound of paste on electrode.



Rub off excess paste on a clean index card.



Keep moving to a clean area of card and use
circular motion to finish polishing the paste surface.

- With a new electrode block (not previously filled with paste), begin by scooping some paste from the vial and dropping it into the open well(s) in the electrode block. In a thin-layer electrode, each well is attached to a gold connector. In a voltammetry electrode, there is an open hole at the end of the electrode rod.

- Use the end of a glass rod to tamp down the paste evenly into the well, eliminating air spaces. Keep filling and tamping it down until the well is filled with carbon paste and is mounded up slightly above the surface of the plastic block. Don't tamp too hard. You should not be squeezing the paste to the point where it begins to ooze oil.

- Obtain a smooth, CLEAN card. Large index cards work well. Place the card on a flat, hard surface like a large piece of thick, clear glass or a seamless benchtop.

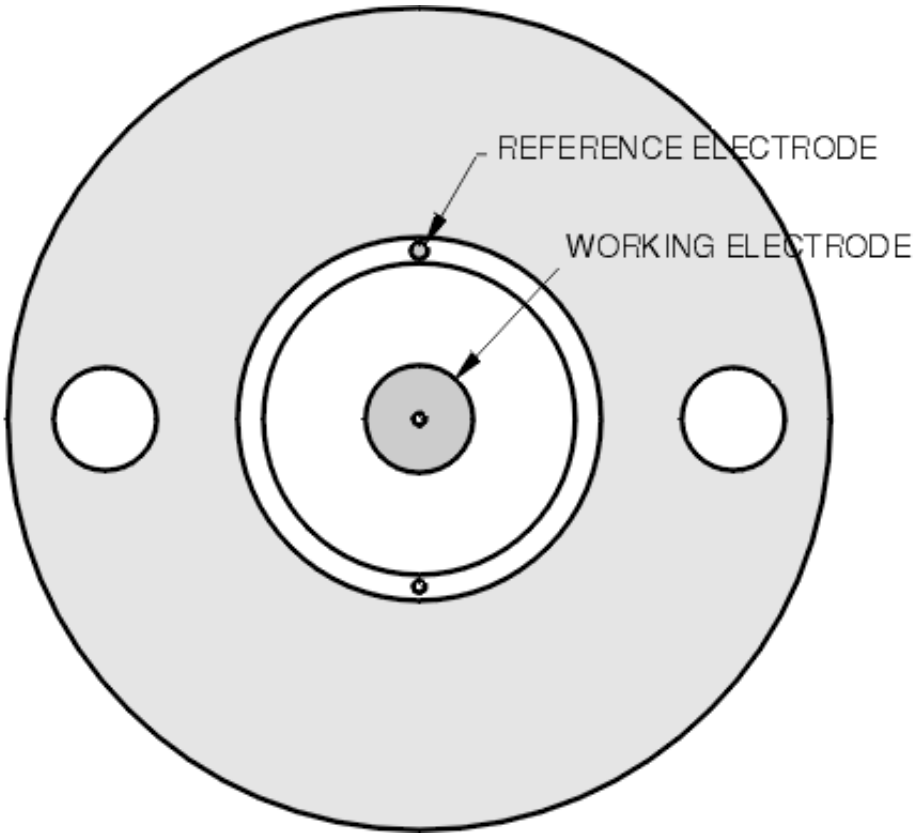
- Turn the electrode over and draw it gently across the card, leaving a line of carbon on the paper. Then go to a clean section of the card and move the electrode in tiny, continuous circles on the surface of the card. Move to another section and repeat this until little or no paste is left on the paper surface by this action.

- Turn the electrode over and inspect it. The surface should be even and smooth with no cracks apparent in the surface. Small dark speckles are normal parts of the paste. If any crack or obvious discontinuity is present, return the electrode to a section of the card marked with paste and continue rubbing it in this section to help fill up the cracks and smooth out the surface.

- If you have been using an electrode and wish to resurface the paste, simply remove about a millimeter of the paste by wiping it away with a lab tissue. Then proceed by dropping a little lump of paste onto a card, pressing the electrode down onto this lump and proceeding as above.

POLISHING THE UNIJET ELECTRODE

The surface of the UniJet electrode contains both the working electrode and the silver reference electrode. You have a choice of polishing one or both of these electrodes.



The working electrode needs polishing only if response has declined and you suspect that the electrode is fouled. The reference electrode needs polishing if response has declined and the bronze-colored coating has worn off. After polishing, the reference electrode must be treated to create the AgCl coating.

To polish both the working electrode and reference electrode simultaneously, simply follow the instructions for whatever material the

working electrode is composed of. Invert the electrode assembly onto the polishing pad so that both electrodes are polished.

To polish either the working electrode or the reference electrode independently, apply the appropriate polishing compound with a wooden cotton-tipped applicator. Use a circular motion for even polishing. Rinse with water, then methanol, and allow to air dry.

WORKING ELECTRODE STORAGE

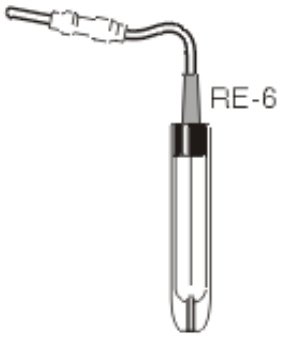
Although the working electrode is a solid-state device, it can be damaged by mechanical or chemical stress. Therefore, when not in use it should be removed from the system, cleaned, and dried. Then store it in its plastic box, or some other place where it will not get banged up.

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REFERENCE ELECTRODES

BAS offers several models of Ag/AgCl reference electrodes. Each reference electrode is individually inspected and tested for adherence to an acceptable range relative to a standard calomel electrode. The model **RE-6** is used by the epsilon system when configured with any of the standard flowcells. It features glass-body construction with a porous ceramic frit at the solution interface. The filling solution is 3 M NaCl gel that has been saturated with AgCl; the gel is semi-solid and will appear cloudy with occasional particles.



MF-2078

- **WHAT IS A REFERENCE ELECTRODE?**
- **REMOVING THE SHEATH**
- **STORAGE**
- **ROTATION**
- **TESTING**
- **UNIJET REFERENCE ELECTRODE**

WHAT IS A REFERENCE ELECTRODE?

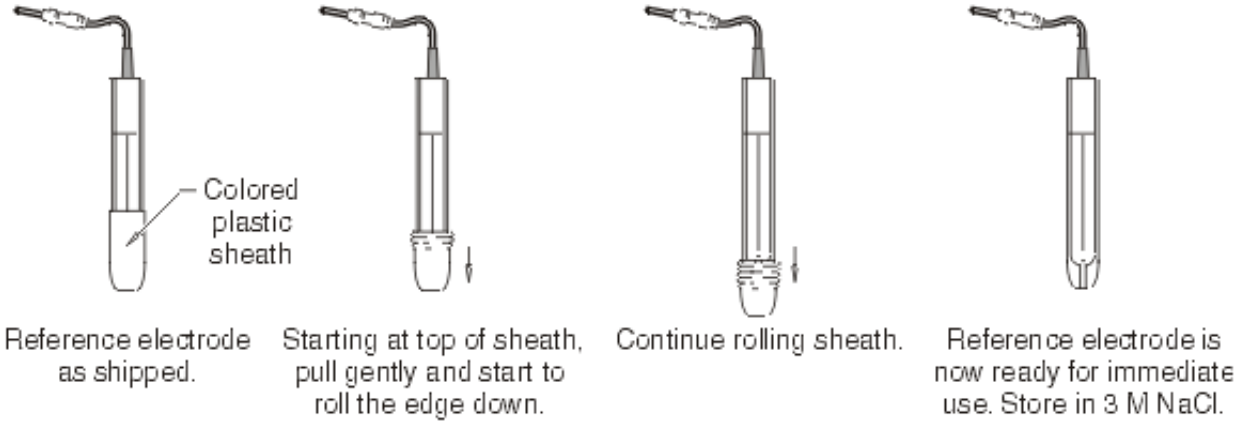
The purpose of the reference electrode is to provide a stable, reproducible voltage to which the working (detector) electrode potential may be referenced (see PRINCIPLES). A reference electrode may be considered a small battery whose voltage (potential) is determined by the chemistry taking place between a solid conductor (usually a metal salt) and the electrolytic solution around it. Ideally, if a small current is passed through the electrode, the potential change is negligible, and in any case, returns to the initial value when the current ceases. In addition, the potential value should not vary with time and should be reproducible from electrode to electrode. The most common reference electrodes meeting these criteria are the mercury/mercurous chloride (calomel) and the silver/silver chloride (Ag/AgCl) electrodes.

REMOVING THE SHEATH

Every Ag/AgCl reference electrode is shipped with a colored plastic sheath that covers the porous tip and retards drying. Immediately upon receipt, remove this sheath by rolling it down from the glass body to the tip. The plastic will roll down and slide off the end of the electrode. Do not tug at the sheath or hold the electrode by its lead while you are doing this. If you have trouble removing the sheath, make a small cut at the upper edge of the sheath using small scissors, and try again.

BE CAREFUL NOT TO BREAK OFF THE END OF THE ELECTRODE!

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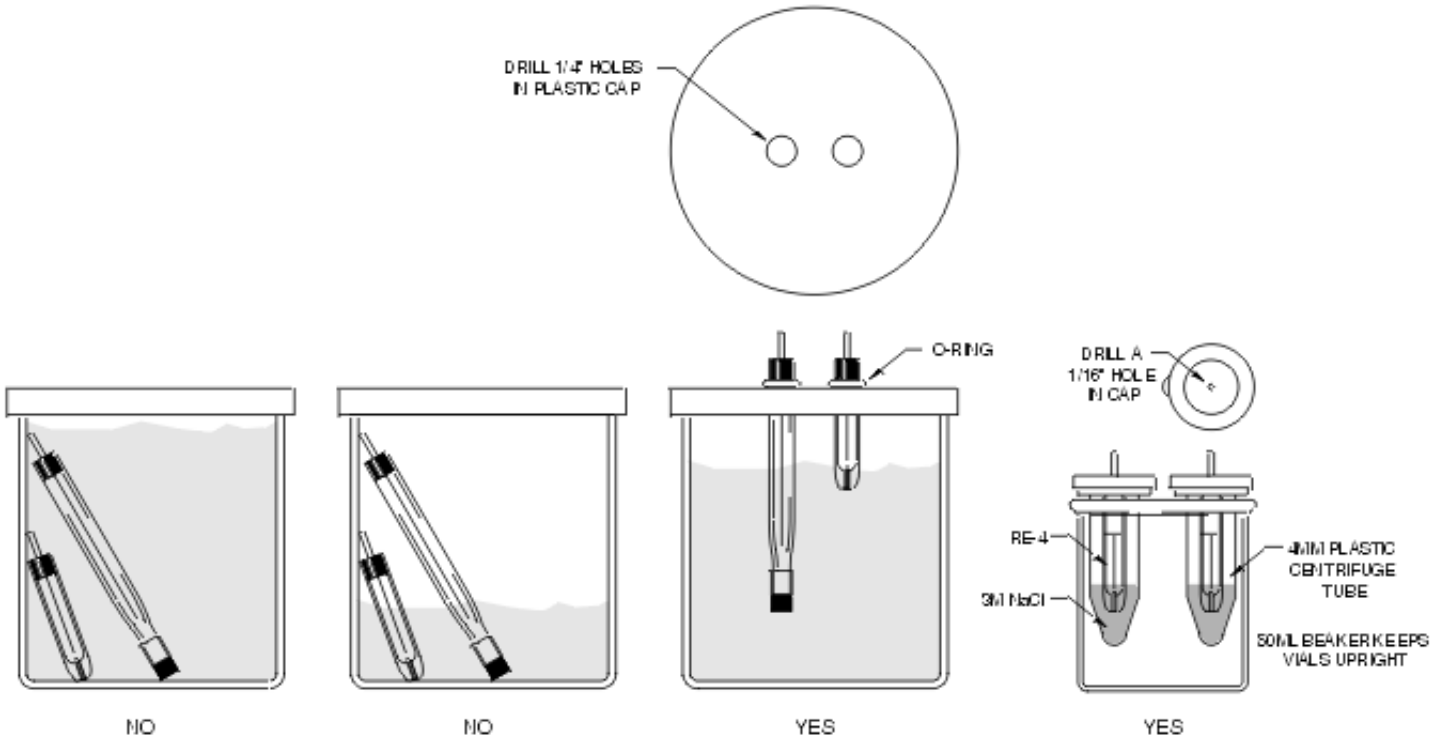


ONCE THE SHEATH IS REMOVED, STORE THE ELECTRODE TIP IN 3 M NaCl AS SHOWN BELOW.

The Ag/AgCl reference electrodes are easily ruined by drying. Keep the tips wetted at all times and store in 3 M NaCl when not in use.

STORAGE

The Ag/AgCl reference electrodes are easily ruined by drying. Keep the tips wetted at all times and store in 3 M NaCl when not in use. A reference electrode storage vial is available from BAS (MR-5275. Be sure that you check the electrodes periodically and replace the solution in the storage vessel with fresh 3 M NaCl to keep the tips wet. DO NOT ENTIRELY IMMERSE REFERENCE ELECTRODES. Keep the connecting pins dry, or they will corrode and contaminate the reference electrode.



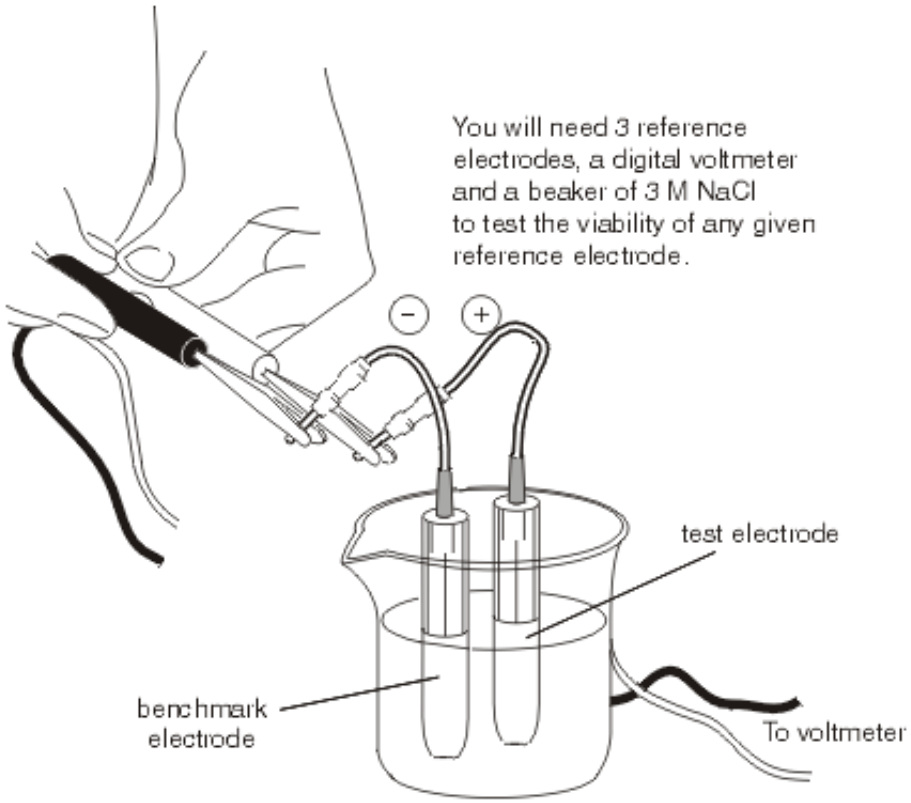
Reference electrodes will naturally change with use due to the transport of ions and solvent across the junction. The rate of change is a function of the difference in composition between the sample solution (i.e., mobile phase in liquid chromatography) and the filling solution (3 M NaCl gel). In LCEC usage, it is advisable to change to a new reference electrode frequently, at least once per month. Storing the reference electrode in 3 M NaCl between experiments will extend its lifetime. In spite of all attempts to extend their lifetimes, reference electrodes are still expendable items, so be certain to have spares on hand as needed.

ROTATION

We recommend that three reference electrodes be rotated in your LCEC system. Keep one electrode in your system for about two weeks. Turn the detector to STANDBY before removing and replacing the electrode. (Failure to do so can ruin a glassy carbon working electrode.) Rinse excess 3 M NaCl storage solution off the replacement reference electrode before inserting it into the cell. Replace the bushing and O-ring if they show signs of wear. Turn the cell on. Place the first electrode into the storage container. In another two weeks, replace the reference electrode with the third reference electrode provided in the kit. By rotating the three reference electrodes provided with your detector on a continuous basis, you can maximize their lifetimes. Depending on the mobile phase conditions and detector use, the reference electrodes can last from 3-6 months. When you replace reference electrodes, replace all three of them at the same time.

TESTING

If you are concerned about the viability of a particular Ag/AgCl reference electrode, you can test it using a simple voltmeter, additional reference electrodes of the same type (or a calomel reference electrode), and a small beaker of 3 M NaCl.

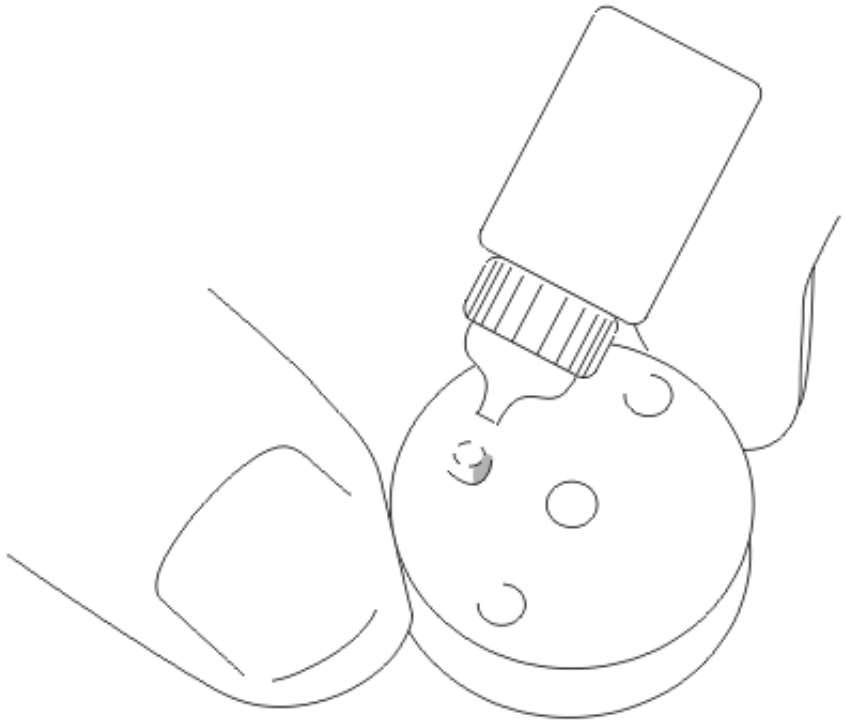


Read the potential difference between the electrodes on the voltmeter. Ideally the difference between two electrodes of the same type would be zero. However, in actual practice there is commonly some variation. If the two electrodes are of the same type (e.g., Ag/AgCl vs. Ag/AgCl, or calomel vs. calomel) the meter should read 0 ± 20 mV. If your reading for any pair of electrodes is significantly different, you should have another electrode of the same type handy to help distinguish which of the two is bad. When comparing an Ag/AgCl reference electrode to a calomel electrode, make the calomel the black (negative) input on the voltmeter. The meter should then read -35 ± 20 mV. A single-probe combination pH electrode is not suitable for this test.

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UNIJET REFERENCE ELECTRODE

The UniJet detector is shipped with an embedded silver wire, which gets coated with AgCl to form the Ag/AgCl reference electrode. It should be regenerated each time the mobile phase is changed or the electrode is polished . After polishing and rinsing with water and methanol, apply a drop of the reference electrode coating solution (CF-2200) to the electrode surface:



Do not allow the solution to get on the working electrode or on your skin. Leave the solution on the electrode for 5 minutes. Rinse the electrode with water. The reference electrode should be a dull bronze color and uniform in appearance.

The UniJet reference electrode is a pseudo-Ag/AgCl reference electrode. The reference electrode potential is determined by the quantity of Cl^- in the mobile phase. The higher the Cl^- concentration, the closer the reference will become to a standard Ag/AgCl. We recommend using 10 mM NaCl in any mobile phase to increase the stability of the reference electrode and prevent alterations in the potential. The UniJet reference electrode is about 100 mV more positive than the standard RE-6 Ag/AgCl reference electrode. Thus, the potential set on the detector should be 100 mV less than was used with an RE-6.

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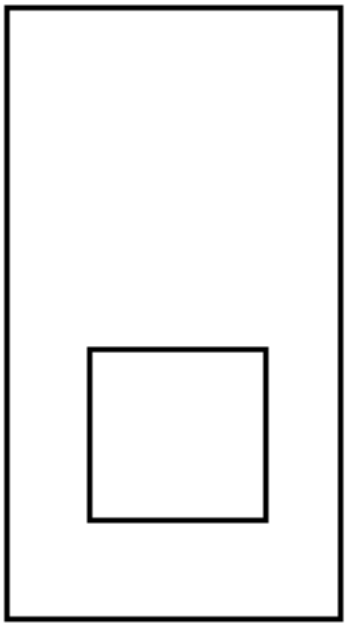
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FAN FILTER

The epsilon controller and other BAS instruments use cooling fans to prevent overheating. These fans have filters which prevent dust from entering the instrument. As dust accumulates the volume of airflow decreases, reducing the cooling efficiency.

CLEAN THE FAN FILTER EVERY THREE MONTHS, OR MORE OFTEN IF VISIBLY DIRTY.

Filter location on the epsilon controller:



Clean the filter as follows:

1. Pull off the front panel.
2. Grab the filter retaining grid by pinching one of the slots and pull it off. (Do not undo any screws -- these hold the entire fan in place!)
3. You may vacuum the filter or wash it in warm sudsy water. Be careful not to tear it.
4. If you've washed the filter, blot it well between sheets of paper towels, then allow it to dry.
5. Reinstall the filter by holding it in place over the fan opening, then snapping the retaining grid in place.
6. Replace the front cover

SHUTDOWN

Mobile phases are corrosive. Turning off an LC system without carefully removing the mobile phase from the pump, tubing, and detectors will result in corrosion and microbiological contamination. Follow these instructions to prepare your epsilon system for idle periods.

If the system will be used every day, or every few days, leave it running. It will then be ready and equilibrated when you wish to use it. Electrochemical detectors are solid-state devices — they do not get used up with use. (Lamps in UV-Vis and fluorescence detectors should be turned off, however.) Pump seals generally last longer if the pump is kept running than if it is repeatedly started and stopped, so leave the pump running as well.

For inactive periods of a week or more, we recommend that the system be flushed clean of mobile phase salts and disassembled as follows:

- EC DETECTOR
- PUMP
- COLUMN

EC DETECTOR

THE DETECTOR CAN BE DAMAGED IF YOU TOUCH LIVE CELL LEADS — BE SURE TO TURN THE CELL OFF BEFORE DISCONNECTING!

Use the following sequence to properly shut down the detector:

1. Turn the detectors off using the STOP button in either the RUN STATUS box or the EC SCHEDULE.
2. Flush the pump, column, and flowcell with 40:60 acetonitrile:water.
3. Disconnect the cell leads from the electrodes.
4. Remove the working electrode, rinse with methanol, dry with a lab tissue, and store it in it's plastic box.
5. Remove the reference electrode, rinse with deionized water, and store in 3 M NaCl.
6. Remove the thin-layer gasket, rinse with deionized water and dry it.
7. Wipe the auxiliary electrode with methanol and dry it.

PUMP

THESE INSTRUCTIONS ASSUME THAT THE COLUMN CAN BE STORED IN 40:60 ACETONITRILE:

WATER. IF THIS IS NOT THE CASE, REMOVE THE COLUMN BEFORE FLUSHING THE PUMP.

Proceed as follows to flush out the mobile phase:

1. Turn the detector off.
2. Replace the mobile phase with 40:60 acetonitrile:water.
3. Purge the pump with at least 20 mL to remove the old mobile phase.
4. Pump through the entire system (injector, column, flowcell) for at least 15 minutes.
5. Stop the pump and allow the pressure to decrease to 0 psi.
6. Flush deionized water through any irrigation holes and around the pump heads.
7. Remove the column and cap it for storage.
8. Follow the instructions for disassembling the flowcell.

COLUMN

In most cases the column can be simultaneously flushed along with the pump. But if the column manufacturer recommends storage in some other solvent, follow those recommendations.

Be careful to separate incompatible solvents. If the column is to be stored in 100% organic solvent, flush out the mobile phase with 40:60 acetonitrile:water before switching to the organic solvent.

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FLOWCELL LEAKS

A small amount of salts accumulating around the juncture between the auxiliary and working electrodes is normal. But any visible liquid or dripping should be dealt with. The most common reasons for this are:

- Torn or scratched gasket. Replace.
- Scratched surface on working electrode or auxiliary electrode. Polish or replace.
- Improperly assembled flowcell. Pay particular attention that all parts are mating properly and are in the correct order, and that the two alignment pins on the auxiliary electrode DO NOT sit in the same holes as the two on the backing plate. Review assembly instructions.
- Excessive pressure in the waste line. This could be caused by a combination of clogging, crushing, too narrow a bore, too long a length, and a high flow rate. We suggest using no more than 12" of 0.010" ID tubing. Try switching to 0.020" ID tubing.

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HIGH PRESSURE

Pressures as high as 4000 psi are not necessarily a problem. However, a rise in pressure above what is typical for a given column and system may indicate a problem such as clogged tubing or an occluded column. If a high-pressure problem develops, look at the following:

- Is the injection valve fully turned to the 'inject' or 'load' position? In between these positions the passages may be partially to fully blocked.
- Is the column, precolumn, or in-line filter clogged? Disconnect these sequentially from the detector towards the pump (allow pressure to drop to zero before disconnecting the column). Run the pump and note the pressure after each removal. A return to normal pressure indicates that the clogged component has just been removed (knowledge of typical pressures for each component is needed for this troubleshooting.) The clogged component should be cleaned or replaced as necessary.
- Is the tubing clogged? If there is excessive pressure with the column, precolumn, and in-line filter removed, there may be a clog in the lines. Begin removing tubing and work back towards the pump until the section with high pressure is identified.
- Has the mobile phase changed? In particular, methanol will give much higher pressures than acetonitrile.
- Is there something wrong with the column? Check with the manufacturer of the column, or compare with a known good column. The type of packing may have changed, or certain lots may have higher pressures than others.
- Does the pressure gauge work? This is harder to check, but worth keeping in mind.
- Has the column temperature gone down? The lower the temperature, the higher the column backpressure.

LOW PRESSURE

As with high pressure, low pressure is only a problem if it is unusual for a given system and column. A drop in pressure can indicate a problem with the pump, or a massive leak in the system. Examine the following items if low pressure develops:

- Are there any obvious leaks? Look for dripping. Salt accumulations indicate small leaks that should be corrected, but they are unlikely to cause low pressure.
- Is the flow rate within specifications? Measure the flow from the column with a graduated cylinder. If it is lower than expected there might be a large leak, the pump motor or gearbox may need repair, or a check valve might not be seating properly. Although rare, a piston may be broken.

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- Are the check valves working? Low pressure, especially in conjunction with pressure fluctuations, can be caused by check valves that do not seal properly because of an air bubble or particle. Check valves can be sonicated or replaced to diagnose and correct the problem.

- Is there a problem with the column? Substitute a known good column, or contact the manufacturer to inquire about problems with the lot.

PRESSURE FLUCTUATION

Pressure fluctuations are a consequence of the alternating pulsations of the piston pump. They are minimized by good system design, such as the size and stroke length of pistons and the use of a pulse damper. Leaks and air bubbles are the major causes of excessive pressure fluctuation. Check the following items:

- Is a pulse damper being used? Sensitive gains require pulse-dampened flow.

- Are any fittings leaking, even slightly? Leaks allow pressure to drop as fluid is forced out in response to each piston stroke.

- Is there air in the system? Air can lodge almost anywhere, but check valves and pulse damper are likely locations. Purge the system at a high flow rate with freshly degassed, warmed mobile phase. If air bubbles recur consider their source: inadequate degassing of the mobile phase, restricted flow in the mobile-phase intake line, a loose connection in the intake line that allows air to be sucked in, or on-line mixing of aqueous and organic phases.

- Are the check valves working properly? Pressure drops that occur during the stroke of only one piston of a dual-piston pump suggest an improperly seating check valve. The source of the problem could be either the inlet check valve on the side with the pressure drop, or the outlet check valve on the opposite side. The check valves could be damaged or merely dirty. Soncicate the suspected check valves to clean them. If this doesn't help, replace them with known good ones.

- Are the seals leaking? Pressure drops that occur during the pressure stroke of only one piston of a dual-piston pump suggest a leaking plunger seal. Replace and break in (if necessary) in accordance with the manufacturer's directions.

- Are the pistons scratched? Scratches where the piston engages the seal will cause leaks. An indicator of scratched pistons is the rapid return of leaks after seal replacement. Replace any scratched pistons.

- Is the pump itself malfunctioning? Worn or broken gear teeth, cams, bearings, etc. could cause pressure fluctuations.

HIGH BACKGROUND

The background reading represents the electrochemical activity of the mobile phase, including all its components and contaminants. It is measured as the detector output when all offsets (zeroing) have been turned off. Every combination of mobile phase, electrode material, and applied potential has a characteristic background. The typical background current for your application is of diagnostic value — it should be noted each time the system is used and when mobile phase or hardware is changed. Backgrounds increase with temperature, applied potential, and contamination, and can decrease if the working electrode becomes coated or the reference electrode becomes depleted.

Many chromatographers don't understand the importance of a low background. They think that rezeroing gives them a zero background. But zeroing simply moves the frame of focus — the high background is still there, and it contributes to noise and distortion. This is true for both electrochemical and optical detectors.

Whether it be UV or EC detection, a high background makes it difficult to distinguish small peaks, as they represent only a tiny fraction of the total signal. When the background is low, however, these same small peaks are easier to distinguish because they represent a greater proportion of the total signal. High backgrounds can cause increased baseline noise, and are an indication that something is wrong with the system. Check the following if high backgrounds become a problem:

- Is the working electrode dirty? Electroactive material may have built up on the electrode. Try wiping it with methanol or acetonitrile, and if this doesn't work, polish it.
- Is the mobile phase old? Contaminants or microbial metabolites may be accumulating. Try a fresh batch of mobile phase. If this doesn't help, clean the system with solvents and try again with fresh mobile phase.
- Is the mobile phase new? If the problem began with a change of mobile phase, the mobile phase may have been improperly made, or a contaminant could have been introduced. Does the deionized water have > 15 MOhms resistivity? Was a different bottle of one component used? Was only clean glassware used?
- Is the column dirty? Try bypassing it. If the background goes down, the column should be cleaned.
- Is the mobile-phase uptake frit dirty? Remove it to see if the background goes down.
- Is Fe^{2+} being oxidized to Fe^{3+}at the electrode? Try adding a metal chelator (e.g., 1 mM ethylenediaminetetraacetic acid) to the mobile phase.
- Is the potential set correctly? Higher potentials will produce higher backgrounds.
- Is the working electrode damaged? Try substituting a known good electrode.

LOW BACKGROUND

Low backgrounds may seem desirable, but atypically low ones may indicate a problem. Be suspicious of a low background, especially when coupled with an unnaturally quiet baseline and small peaks. Examine the following items if backgrounds are low:

- Is the mobile phase new? The concentration of buffer may be too low.
- Is the working electrode coated with something? Try wiping it with methanol or acetonitrile, and if this doesn't help, try polishing.
- Is the reference electrode depleted? Substitute a known good one.
- Is the potential set correctly? Lower potentials give lower backgrounds.

REGULAR BASELINE NOISE

Regular baseline noise (fluctuation) has a constant period that usually can be traced to a system component, especially if the period matches that of the pump. Air bubbles (and leaks) will produce baseline noise as they compress (or leak) at each pump stroke. To test whether the noise is flow related, change the pump speed. The period of the baseline noise should change proportionately. Consider the following items when tracking down baseline noise:

- Is there air in the flowcell? Purge the air as follows: turn off the detector, remove the reference electrode, allow fresh mobile phase to fill up the reference well, then reassemble.
- Is there enough backpressure on the flowcell? Backpressure reduces outgassing in the cell. Try using two feet of 0.010" tubing as the exit line from the cell. Alternatively, use a commercial backpressure regulator that provides 100 psi.
- Is the flowcell leaking? Clean and dry the mating surfaces, and use a fresh cell gasket.
- Is the reference electrode cracked? Replace it.
- Is there fluid leaking around the reference electrode? Replace the o-ring.
- Is there air in the check valves, pulse damper, or tubing? Purge at high flow rate with freshly degassed warm mobile phase.
- Are you mixing buffer and organic solvent on line? Incomplete mixing can cause a regular noise pattern. Try mixing and filtering the mobile phase before putting it on the LC system.
- Are there excessive pressure fluctuations? See this section.
- Is there a ground loop? Ground loops can cause regular or irregular noise. Check that the EC detector is properly grounded.

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- Is the period very long (many minutes or hours)? See the section on baseline drift, especially temperature fluctuations.

IRREGULAR BASELINE NOISE

Irregular baseline noise (fluctuation) can be difficult to track down. Sources can be internal or external to the LC system. Consider the following items:

- Is there a ground loop? Ground loops can cause regular or irregular noise. Check that the EC detector is properly grounded.
- Is there electrical interference from other equipment? Try running a heavy-duty extension cord from another location. If the baseline improves, consider a dedicated power line for the LCEC system.
- Is radio interference from pagers causing intermittent problems? Consider shielding the detector, or moving the system to another location.
- Does the baseline change if you touch or stand in front of the system? Check the grounding, and make sure the flowcell is within an enclosure that acts as a Faraday cage.
- Is the noise associated with high background? Solving the background problem may reduce the noise.
- Is the column dirty? Continual elution of small peaks may look like noise. Clean the column or substitute a known good one.
- Are the electrodes damaged? Substitute known good reference and working electrodes.
- Is the period very long (many minutes or hours)? See the section on baseline drift, especially temperature fluctuations.

BASELINE SPIKES

Spikes are fast deflections in the baseline, either positive, negative, or both. They usually occur at irregular intervals, but depending on their source can be regular as well. Evaluate the following possible causes:

- Is there a ground loop, or no ground? Ground loops can cause regular or irregular noise. Check that the EC detector is properly grounded.
- Are all electrical connections clean and tight? Check cell leads, ground wire, and connections to chart recorder or

data system.

- Is there electrical interference from other equipment? Try running a heavy-duty extension cord from another location. If the baseline improves, consider a dedicated power line for the LCEC system.

- Is radio interference from pagers causing intermittent problems? Consider shielding the detector, or moving the system to another location.

- Does the baseline change if you touch or stand in front of the system? Check the grounding, and make sure the flowcell is within an enclosure that acts as a Faraday cage.

- Are there bubbles passing through the detector? Submerge the exit line from the cell in a beaker of water and watch for bubbles emerging from the end. If found, track down the source and correct it. Sources include inadequate degassing of the mobile phase, restricted flow in the mobile-phase intake line, a loose connection in the intake line that allows air to be sucked in, or on-line mixing of aqueous and organic phases.

BASELINE DRIFT

Baseline drift is a change in background over a long period of time, usually hours. A decrease in background after initially turning on the cell is normal, as the detector equilibrates. For other causes, consider the following items:

- Has the composition of the mobile phase been changed? Be sure to purge the system to remove the old mobile phase. The column will need time to equilibrate with the new mobile phase.

- Does the baseline continually drift down? Are peak heights also decreasing? The working electrode may be becoming coated as the mobile phase passes over it. The coating can be removed by wiping the electrode with methanol or acetonitrile at regular intervals. Higher-quality mobile phase components, or better sample pretreatment, could eliminate the problem.

- Does the baseline drift up or down after a new bottle of mobile phase is put on the system? The temperature of the fluid could be changing.

- Does the baseline continually drift up? This could signify a buildup of contaminants from the mobile phase or samples. Do not recycle the mobile phase into the solvent reservoir while injecting samples. Change the mobile phase every three days if it is acetate- or phosphate-based, as these buffers are good growth media for bacteria. The electrode can be accumulating an electroactive coating — this can be removed by wiping with methanol or acetonitrile at regular intervals.

- Does the baseline drift up and down over a period of hours? This could be a response to the cycling of room temperature. Try operating the flowcell 5 °C above ambient temperature to isolate it from fluctuations.

- Does the baseline increase during the day and decrease at night? This pattern, or its inverse, could be a response do different day and night temperatures in the building. Try operating the flowcell 5 °C above ambient

temperature to isolate it from fluctuations.

- Does the baseline drift up during gradient runs? A certain amount of drift is unavoidable, but a larger amount may be caused by old or impure components of the mobile phase. Try using the best grades available (at least AR grade, but preferably HPLC grade).

- Does the baseline drift up only during the first gradient of the day? Impurities in the initial mobile phase may be collecting on the column during overnight recycling. Always run the gradient once to clean the column before injecting samples.

SMALL PEAKS

If peaks have normal shape and retention times but are smaller than expected, check the following:

- Have the standards been made/diluted properly? Have they degraded?

- Has the mobile phase been changed? Was it made properly?

- Is the chart recorder or data system set properly?

- Are the detector range and potential set properly?

- Is the working electrode coated? Try wiping with methanol or acetonitrile, or polishing.

- Is the reference electrode depleted? Try substituting a known good electrode,

- Is the electrochemical detector functioning properly? Perform the self test if available for this detector.

NO PEAKS

Lack of peaks can be either a separation or a detection problem. Consider the following:

- Have the standards been made/diluted properly? Have they degraded?

- Is the detector on? Are the range and potential set properly?

- Is the detector responding to anything that is injected? A normal response to the injection, and normal baseline noise, suggest a separation or injection problem rather than a detector problem.

- Is the chart recorder or data system set properly?
- Is the working electrode coated? Try wiping with methanol or acetonitrile, or polishing.
- Is the reference electrode depleted? Try substituting a known good electrode,
- Is the electrochemical detector functioning properly? Perform the self test if available for this detector.
- Is there something wrong with the mobile phase? If the problem developed after the mobile phase was changed, try remaking it.
- Are the peaks eluting in the void? This is common when developing a new method. Compare the void produced by an injection of mobile phase with that produced by standards dissolved in mobile phase. A larger void in the latter case suggests the presence of unretained analytes. Try using less solvent in the mobile phase to increase their retention.
- Are the peaks eluting very late? Leave the chart recorder or data system running and go to lunch. Once late peaks are located, the solvent strength of the mobile phase can be increased to elute them earlier.

CONTAMINANT PEAKS

Contaminants are not necessarily a problem, unless they interfere with an analyte peak. If a contaminant does cause a problem, its source must be known before it can be dealt with. Common sources include the sample, chemicals used in extraction or purification, and mobile-phase components. Consider the following when tracking down a contaminant problem:

- Are the contaminants present in the system? Do they appear when the injector is thrown to the 'inject' position with only mobile phase in the loop? If so, try cleaning the column and the loop. The contaminants may have been introduced with the mobile phase. Consider the quality of each mobile-phase component. Ion-pair reagents in particular have been known to cause these problems. Try another source for these reagents if you suspect a problem.
- Are the contaminants in the syringe? If no peaks eluted when the injector was thrown to the 'inject' position, fill the loop with mobile phase using the syringe, then inject. If the contaminants appear now, the syringe may be dirty. Alternatively, the waste line of the injector may be clogged, allowing contaminants to wash back into the loop.
- Are the contaminants in the standards? Inject freshly diluted standards.
- Are the contaminants in the extraction procedure? Extract a blank sample. If contaminant peaks appear now, change the extraction components one by one until the problem is found. Old buffers are likely suspects. In catecholamine assays, alumina that is old or has not been stored in a desiccator may have adsorbed contaminants that elute with the analytes.

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- Are the contaminants in the samples? Only a better cleanup or extraction procedure will solve this.

LATE ELUTERS

These are normal sample components that elute much later (even hours later) than the analytes of interest. Consider the following:

- Can the sample preparation scheme be modified to remove these? Solid-phase extraction may be useful here.
- Can the injection schedule be timed so that the late eluters from the previous sample elute in a clear area of the current sample? Timing is critical here.
- Can a certain number of injections be performed, followed by a quick wash of the column to remove the late eluters? Alternatively, can a wash be programmed after each injection?

SPLIT PEAKS

A split peak is one peak that appears to be two poorly separated peaks. Consider the following:

- Is the peak truly split, or are there really two compounds? If only one peak in the chromatogram appears to be split and the others appear normal, it is likely there really are two closely eluting peaks. Try injecting a fresh dilution of standards.
- Is there a poor connection at the top of the column? Try remaking this connection so there is no dead space.
- Has the column bed developed a void or channels at the top? If this occurs, some of the analyte travels through the "open" spaces, thus eluting slightly before the rest of the analyte that travels in the bed. All peaks in the sample will be split. Try reversing the column. A temporary improvement suggests that the column bed has developed problems. Replace the column.
- Is the injection fluid too strong (organic strength or pH)? Try injecting a standard dissolved in mobile phase. If this helps, adjust the solvent strength or pH of the injection fluid, or reduce its volume. If pH is a problem try increasing the buffering capacity of the mobile phase.

BROAD PEAKS

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The lowest detection limit is achieved when an analyte is in as compact a band as possible within the flow stream. We see this compactness as a narrow peak on the chromatogram. In isocratic elution peaks broaden as retention time increases, so it's best to elute the analytes as early as possible, consistent with good separation. Unusually broad peaks suggest the following:

- Has the loop been changed? Larger injection volumes lead to more dispersion of the sample, hence broader peaks.
- Has new tubing been installed somewhere between the injector, column, and detector? tubing with an internal diameter larger than 0.010" can cause sample dispersion.
- Is the column losing active sites or developing voids? Compare to a known good column.
- Is the flowcell volume large compared to the volume of the peak? Dead volume in the flowcell causes peak dispersion. Microbore systems require lower flowcell volume than do standard-bore systems.
- Is the pH of the mobile phase near the pK_a of the analyte? This could produce two species of the analyte that differ in hydrophobicity, and hence in retention time.

RETENTION TIME CHANGES

Retention times can vary randomly, or steadily increase or decrease. Consider the following:

- Is the column thoroughly equilibrated with the mobile phase? Mobile phases containing ion-pair reagents require longer time (greater volume) to reach equilibrium.
- Is the pump malfunctioning? Check the flow rate several times to make sure it is consistent.
- Is the composition of the mobile phase changing? Evaporation of organic solvent will cause retention times to progressively increase. Compare with fresh mobile phase.
- Is the column losing bonded phase? Column deterioration will cause peaks to elute sooner. Compare to a known good column.
- Is ambient temperature fluctuating? Do day temperatures differ from night temperatures? Retention varies inversely with temperature. Keep the column in a thermostatted oven at least 5 °C above ambient.
- Is the sample being injected at the proper time? Injection and the start of data collection must be simultaneous.
- Is this a gradient separation? Slight differences in piston position at the start of a run can cause variations in gradient composition from run to run, hence retention times vary randomly. Use a pump that synchronizes piston position for the start of each run.

WARRANTY AND SERVICE INFORMATION

- **PRODUCT WARRANTY**
- **DAMAGED SHIPMENTS**
- **SERVICE INFORMATION**

PRODUCT WARRANTY

Bioanalytical Systems, Inc. products are fully warranted against defects in material and workmanship. Epsilon hardware is unconditionally warranted for one year from date of shipment, except when failure is due to obvious abuse or neglect, unauthorized tampering, procedures not described in manuals, or improper connection of electronic units to other components. Electrochemical cells are warranted for 60 days from date of shipment under the same exclusions. Chromatographic columns and injection valves are warranted for 30 days. The following items are not covered under any warranty: carbon paste, activated aluminum oxide, lamps, panel lights, fuses, pump seals, valve seals, reference electrodes.

For any product expressly covered under this warranty, Bioanalytical Systems is liable only to the extent of replacement of defective items. Bioanalytical Systems, Inc. shall not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever. The foregoing warranty is in lieu of all other warranties of merchantability and fitness for a particular purpose.

To activate your warranty, and receive product update information news and valuable information related to this and other BAS products, fill out and return the Warranty Enrollment Card which was shipped with the instrument.

DAMAGED SHIPMENTS

Breakage of any part of this instrument during shipping should be reported immediately to the BAS Service Coordinator. It will be necessary to retain the original packing box and contents for inspection by the freight handler. BAS will replace any new instrument damaged in shipping with an identical product as soon as possible after the claim filing date. Claims not filed within 30 days after shipping date will be invalid.

Do not return damaged goods to Bioanalytical Systems without first contacting the BAS Service Coordinator for a Return Authorization Number (RA#). When a defective part is returned to BAS, the RA# immediately identifies you as the sender and describes the item being returned. Bioanalytical Systems refuses all unauthorized return shipments.

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SERVICE INFORMATION

Bioanalytical Systems provides a skilled service staff available to solve your equipment-oriented problems. For further details call the BAS Service Coordinator, who may choose to route your problem to the correct individual.

Following discussion of your specific difficulties, an appropriate course of action will be described and the problem resolved accordingly. Do not return any products for service until a RETURN AUTHORIZATION NUMBER (RA#) has been obtained. The RA# identifies you as the sender and describes to us the problem you are having in full detail.

Turnaround time on service can be quoted to you at the time your RA# is issued, although we can not determine the actual amount of service required until we have received your unit and diagnosed the problem. All correspondence and shipments should be sent to:

RA #, Service Department
Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

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ELECTROCHEMICAL CHARACTERISTICS OF SELECTED MOLECULES

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- NOMENCLATURE
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- ELECTROCHEMICAL CHARACTERISTICS OF SELECTED MOLECULES
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WHAT ARE CYCLIC VOLTAMMETRY DATA CHARTS?

The following tables will help to summarize a great deal of information about the redox characteristics of a variety of molecules. This information can be used as a guideline when evaluating the feasibility of detecting these compounds using LCEC techniques. Information concerning the applied potential is not absolute; you will need to confirm the optimum using hydrodynamic voltammograms and the electrode and mobile phase necessary for your assay.

NOMENCLATURE

The potential axis runs from left to right for reductions and from right to left for oxidations, per the traditional American convention.

For REDUCTIONS: the left end of the rectangle represents the potential where the current is equal to 1/2 of the peak current. The right end of the rectangle represents the potential at the peak maximum. The opposite is true for OXIDATIONS.



An empty rectangle indicates that only one forward peak is observed and no other peak is obtained on the reverse scan between limiting potentials of the medium.



A shaded rectangle indicates that there is one reduction (or oxidation) peak and that an oxidation (or reduction) peak is observed when the scan is reversed. However, the electrochemical reaction is CHEMICALLY IRREVERSIBLE.

For example,

$$ArNO_2 + 4e^- \rightarrow ArNHOH + H_2O$$

(Forward Scan = A Reduction)

$$ArNHOH \rightarrow ArNO + 2e^- + 2H^+$$

(Reverse Scan = An Oxidation, but not resulting in the original compound, and irreversible)



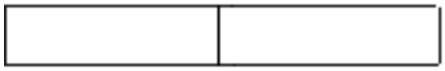
A solid rectangle means that the system is CHEMICALLY REVERSIBLE (and that the electron transfer system is sufficiently fast that both forward and reverse processes occur on the time scale of the CV experiment).



A rectangle without the right (or left for an oxidation) bar indicates that a poorly defined forward peak was obtained (a shoulder on the second wave) and that the $E_{1/2}$ value was estimated by setting $E_{1/2}$ at the potential equal to 1/2 of the current at the peak shoulder.



A solid bar following a rectangle indicates the peak potential of additional forward peaks that exist before the background limit. However, the $E_{1/2}$ value cannot be determined due to the poor resolution between adjacent peaks.



For differential pulse and other small-amplitude voltammetric techniques, this symbol is used. The central vertical bar represents the peak potential and the box indicates the width of the curve at half the peak current. The same symbol is used for polarography and hydrodynamic voltammetry where the three vertical bars indicate the $E_{1/4}$, $E_{1/2}$ and $E_{3/4}$ potentials. Again, a narrow box suggests a fast heterogeneous electron-transfer rate. An asymmetric box may indicate complications in the mechanism.

CONDITIONS

All cyclic voltammograms used to obtain data on the following 9 figures used identical conditions:

Electrolyte:	0.1 M Citrate:10% Ethanol (V:V)
Scan:	200 mV/sec
Working Electrode:	Carbon Paste type CPO (for oxidative CV)
	Mercury/Gold Amalgam (for reductive CV)
Conditions:	Deoxygenation of solution was required for Reductive CV

Figure 8.1. Oxidative Cyclic Voltammetric Data for Selected Aminophenols.

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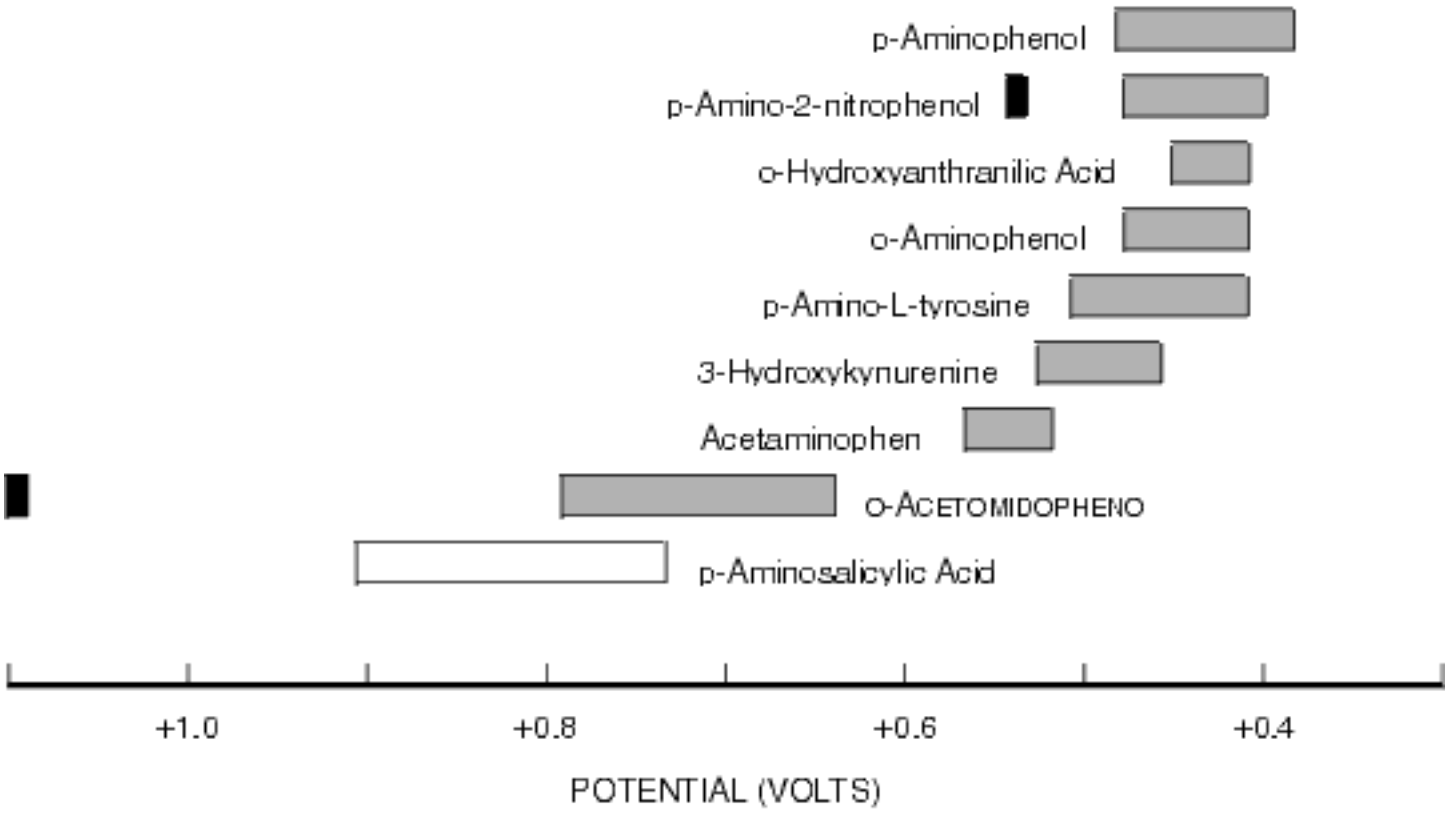


Figure 8.2. Oxidative Cyclic Voltammetric Data for Selected Aromatic Amines.

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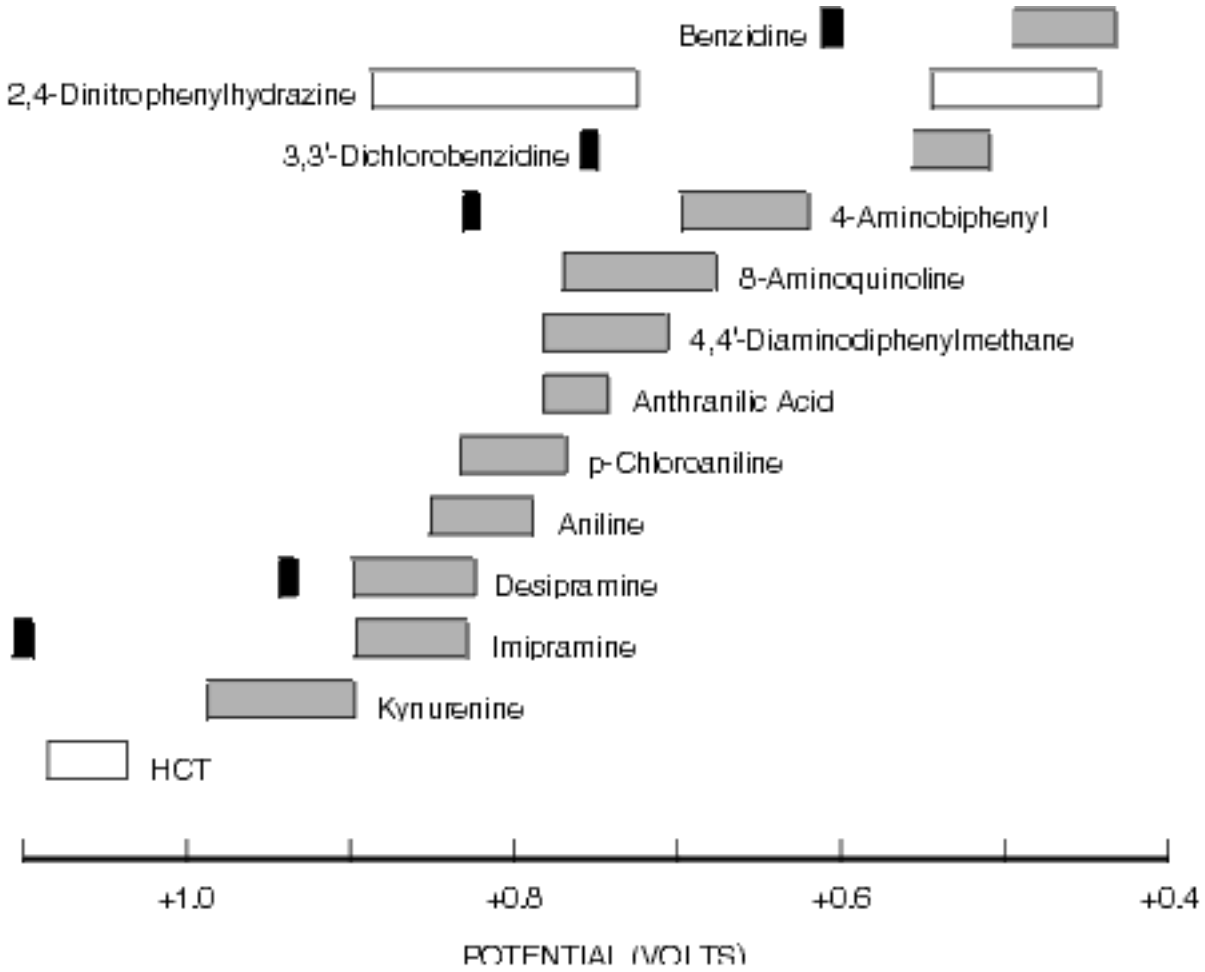


Figure 8.3. Oxidative Cyclic Voltammetric Data for Selected Alkylphenols of Environmental Interest.

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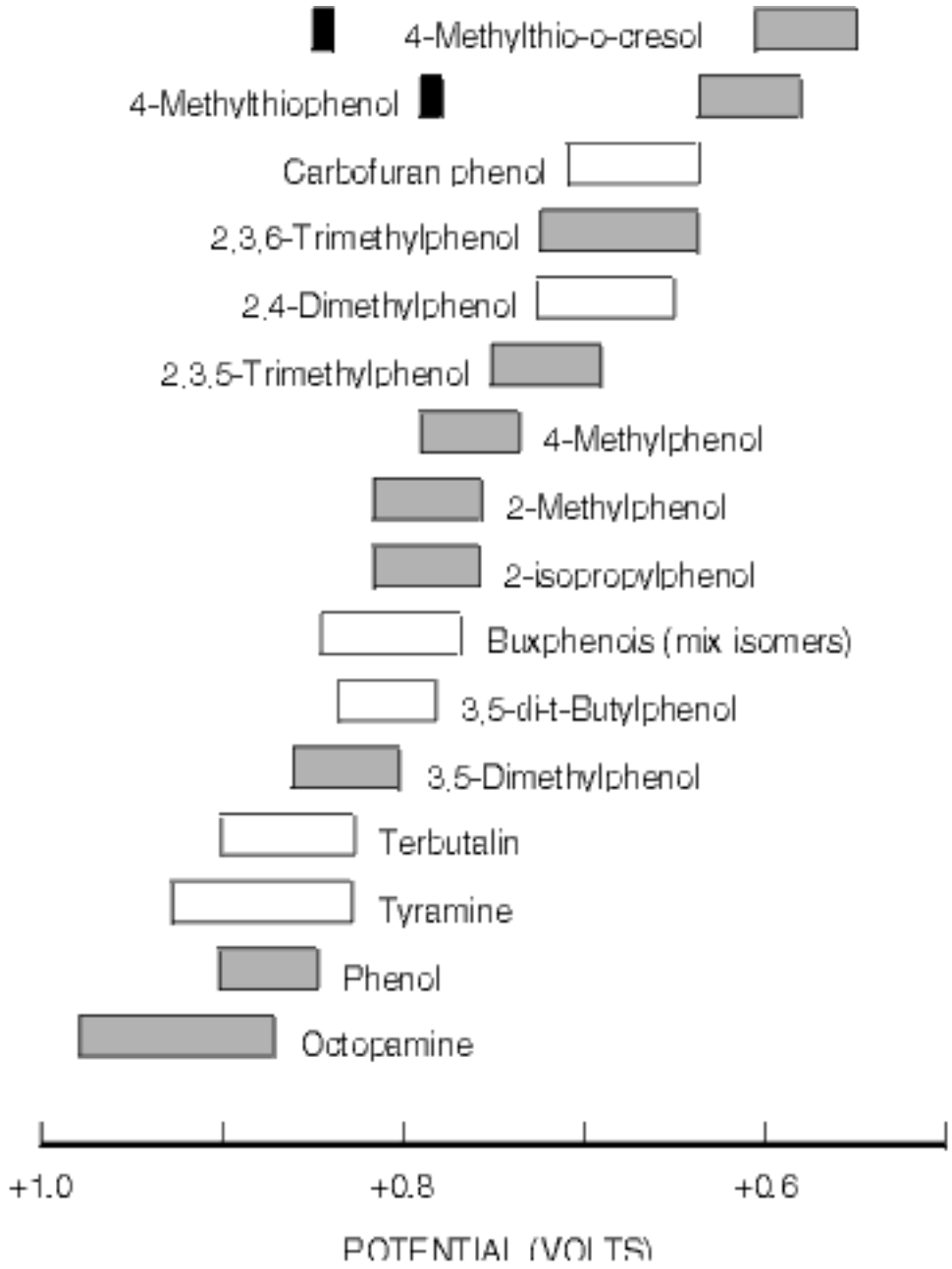


Figure 8.4. Oxidative Cyclic Voltammetric Data for Selected Chlorophenols of Environmental Interest.

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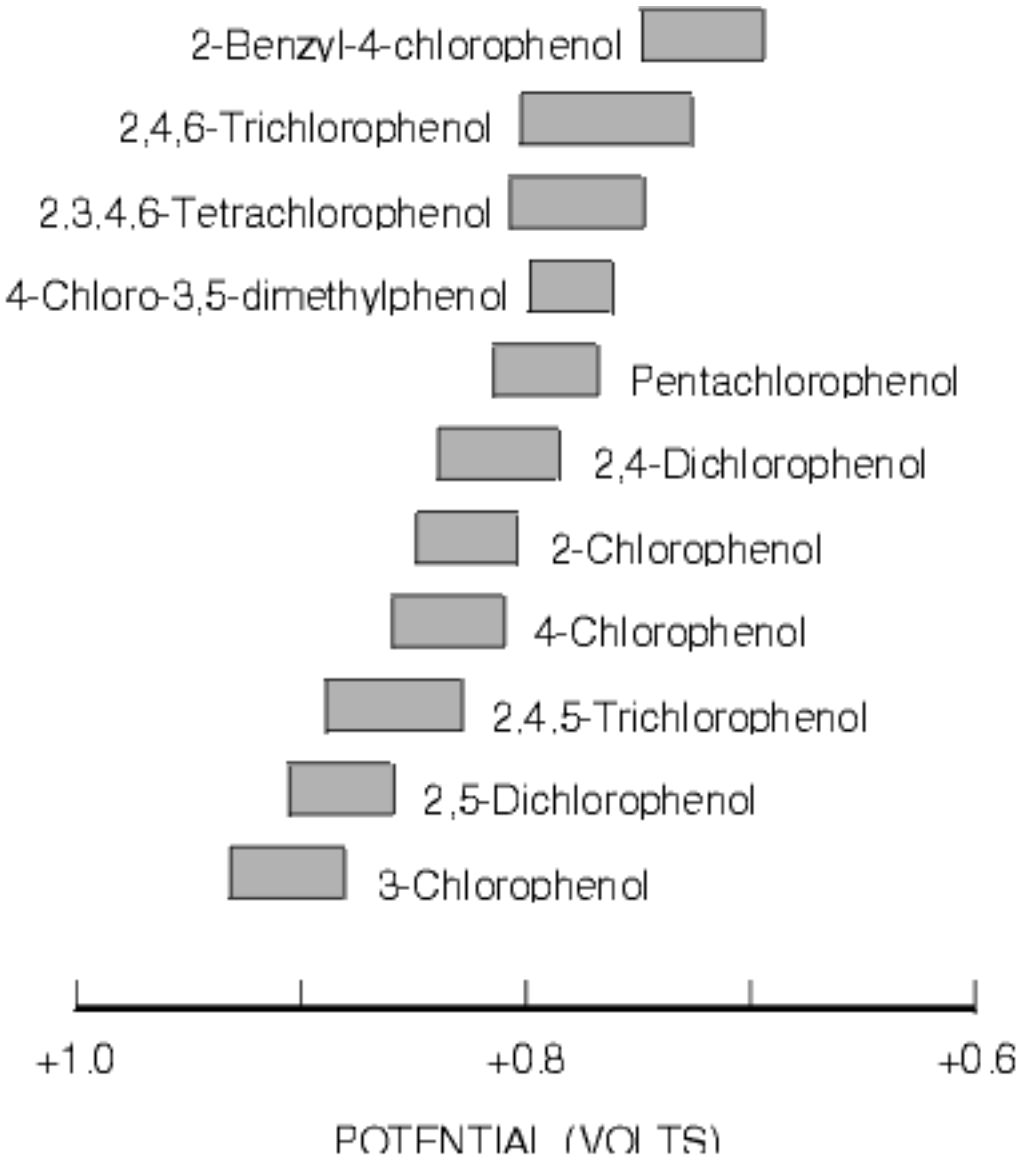


Figure 8.5. Oxidative Cyclic Voltammetric Data for Some Vanillyl Metabolites of Tyrosine and Related Compounds.

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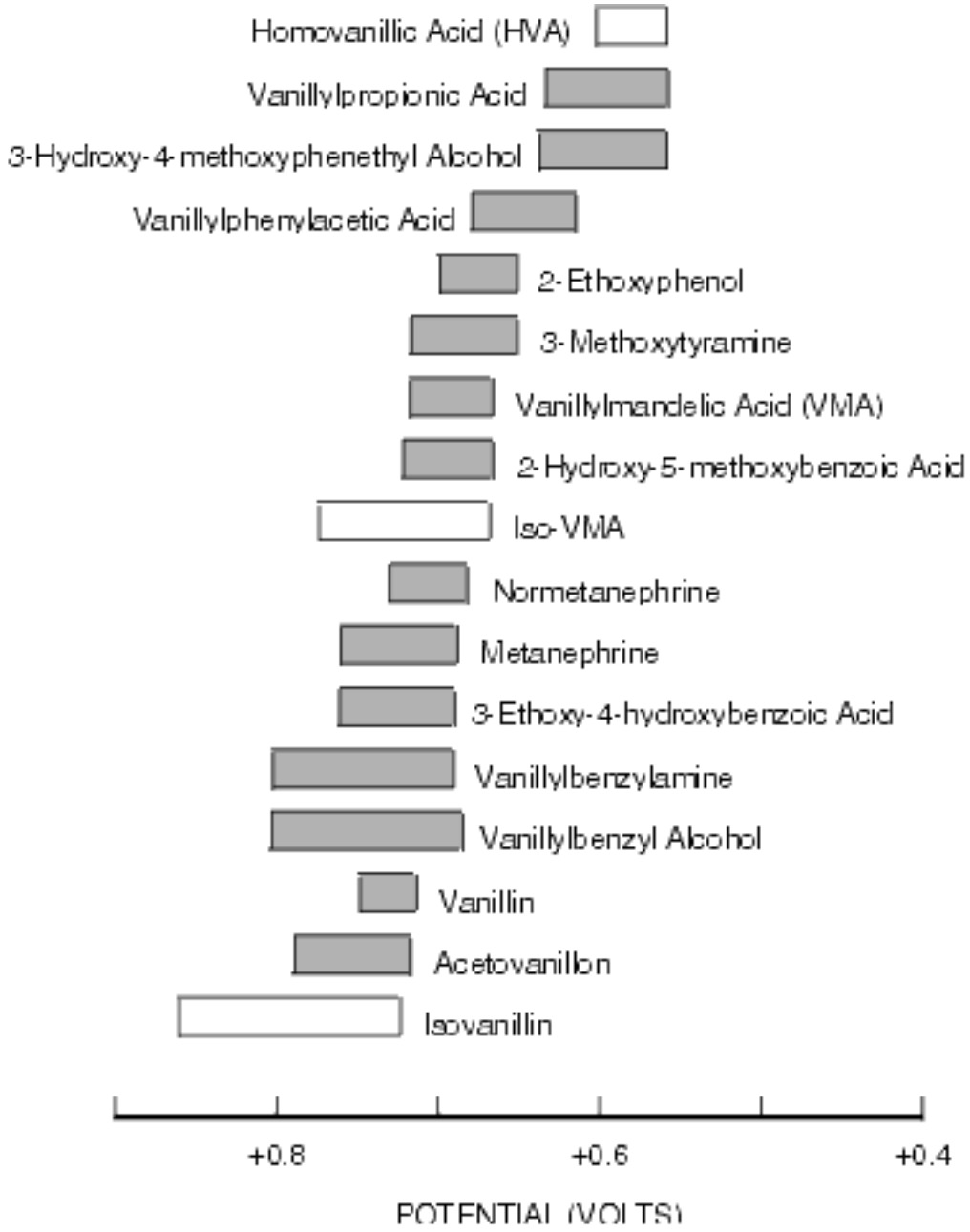


Figure 8.6. Oxidative Cyclic Voltammetric Data for Some Indole Metabolites of Tryptophan and Related Compounds.

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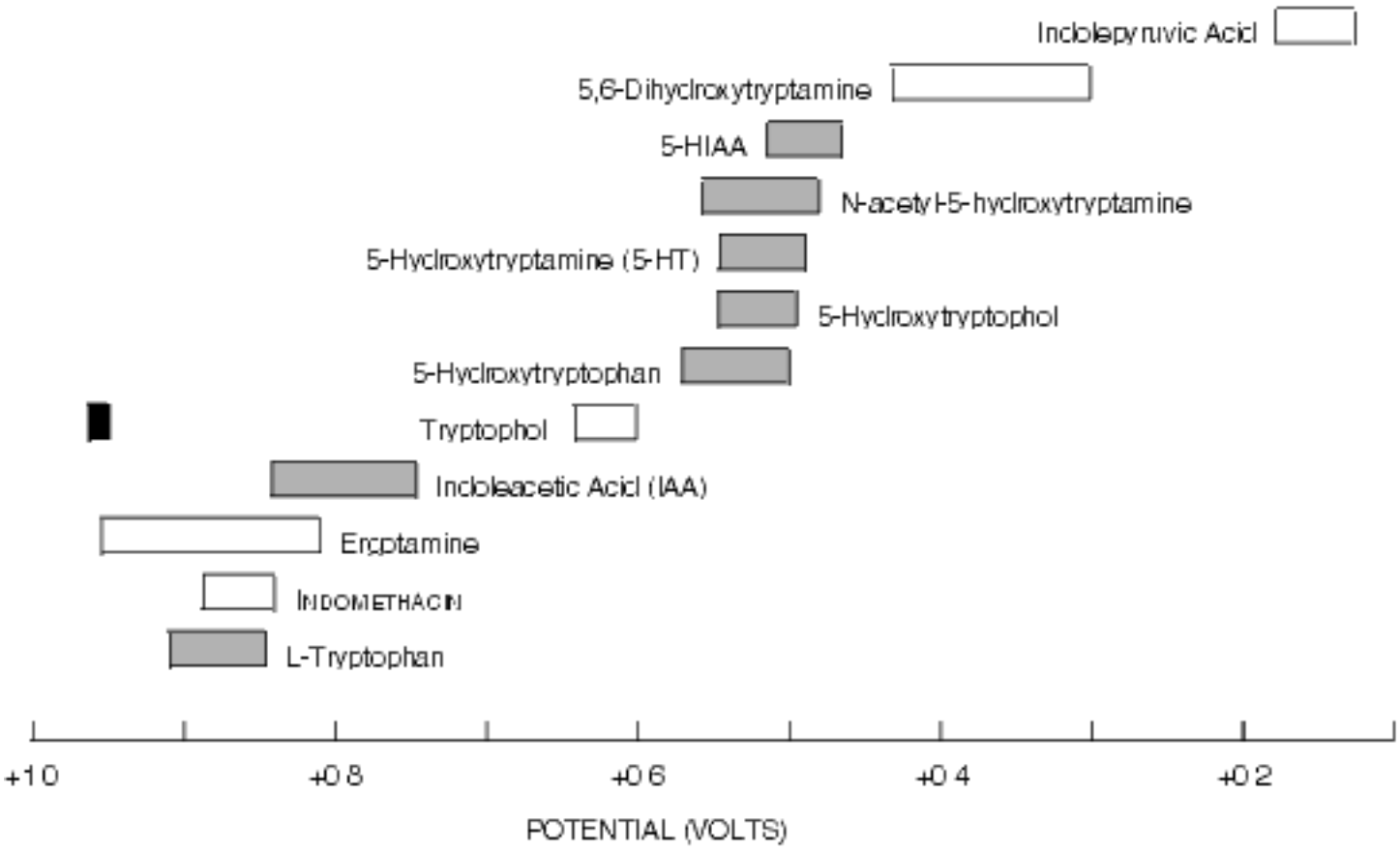


Figure 8.7. Oxidative Cyclic Voltammetric Data for Biologically Important Catecholamines and Other Easily Oxidized Diphenols.

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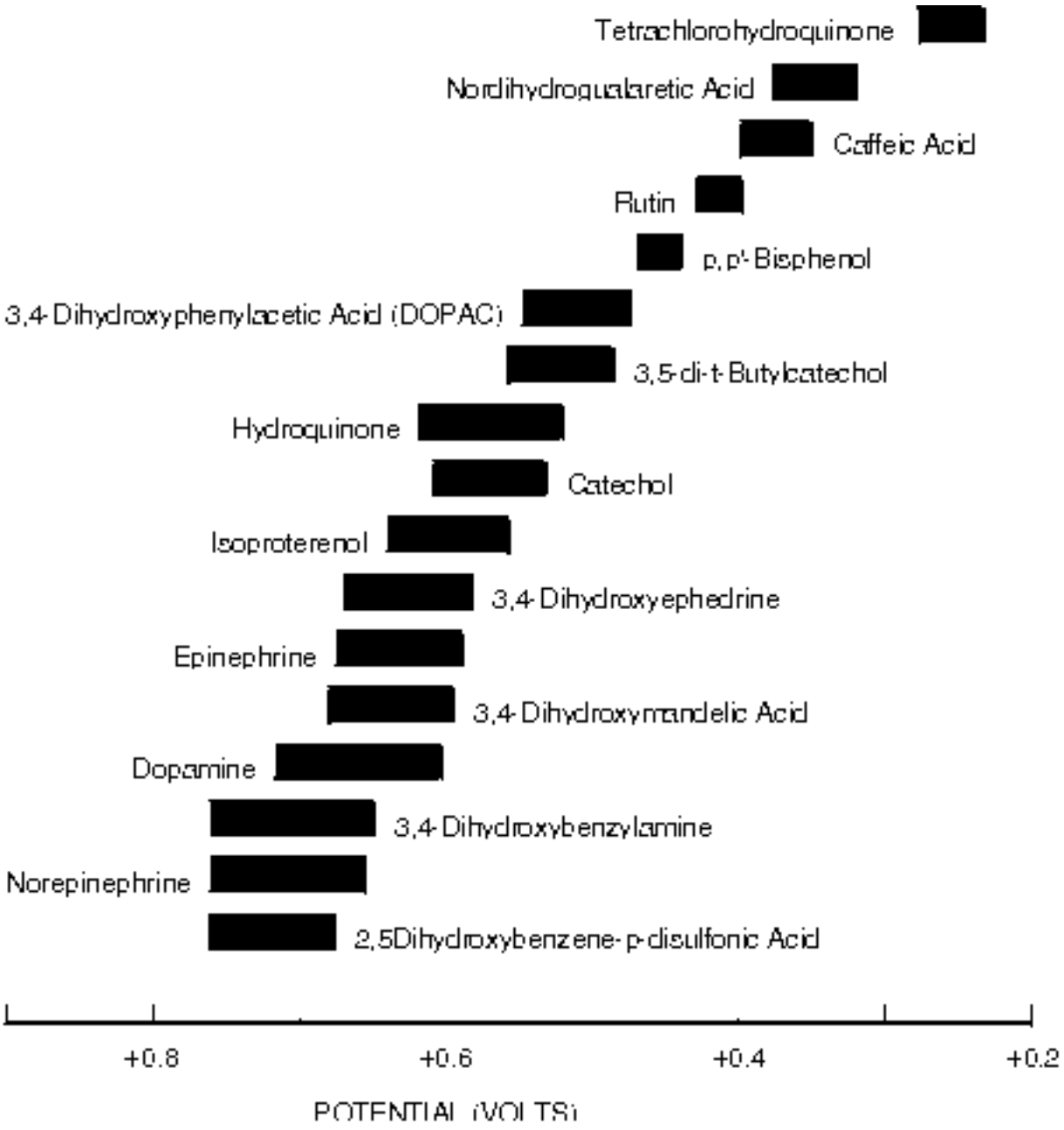


Figure 8.8. Oxidative Cyclic Voltammetric Data for Some Natural Phenolic Acids.

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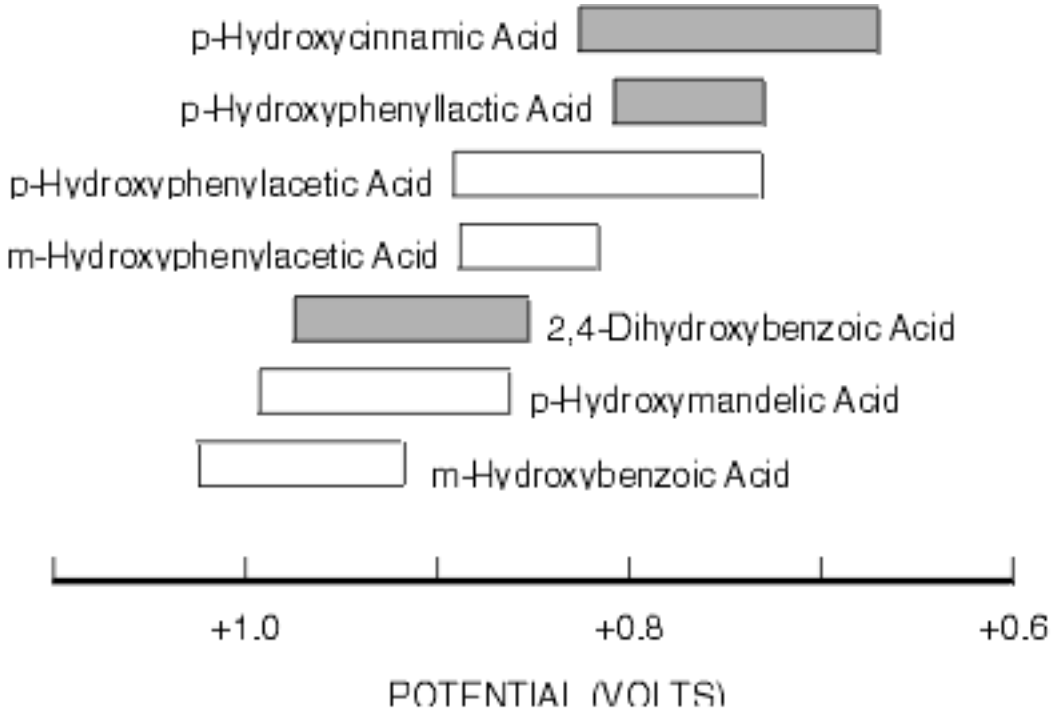
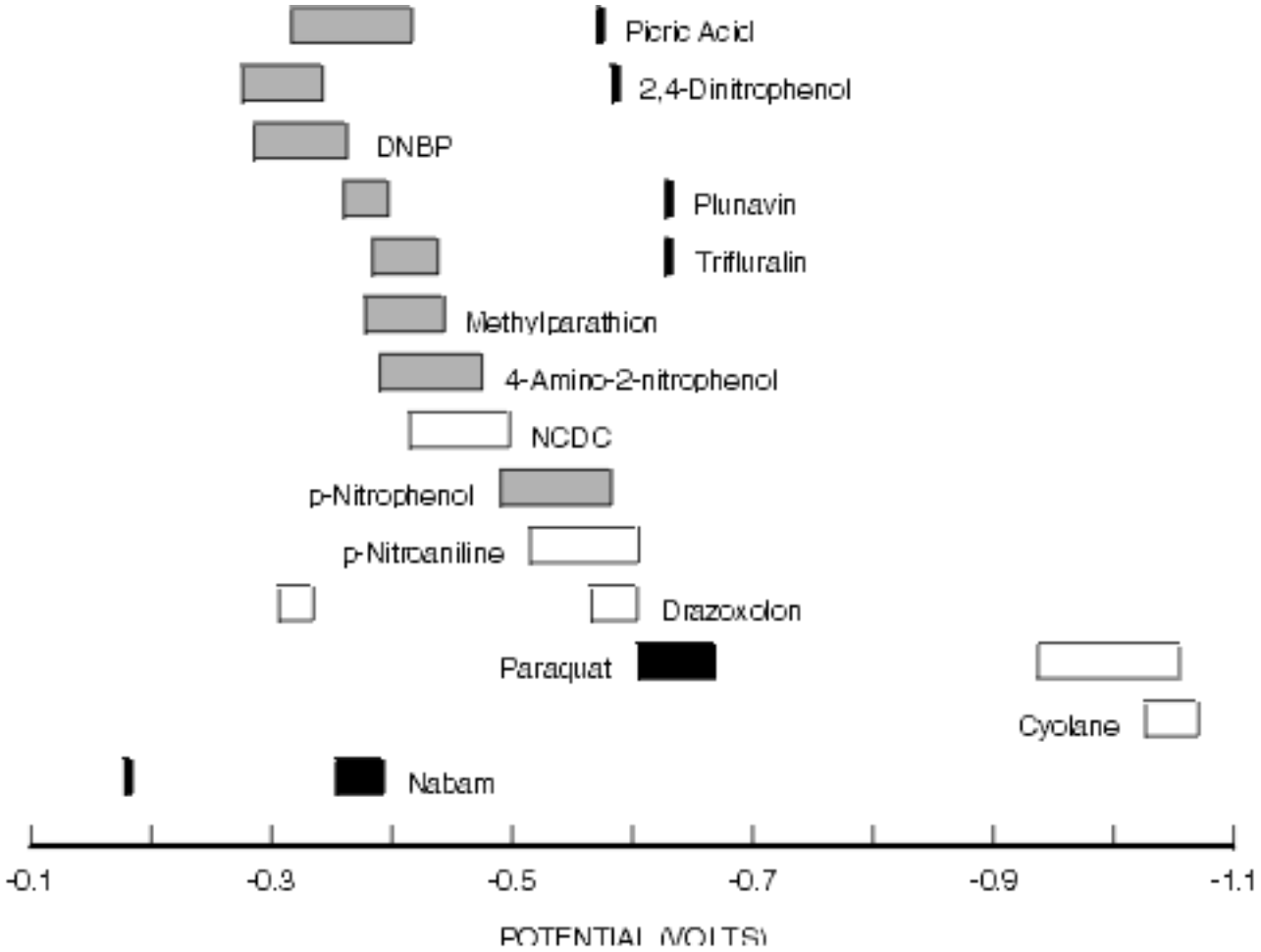


Figure 8.9. Reductive Cyclic Voltammetric Data for Selected Compounds of Environmental Interest.



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BASIC PRINCIPLES

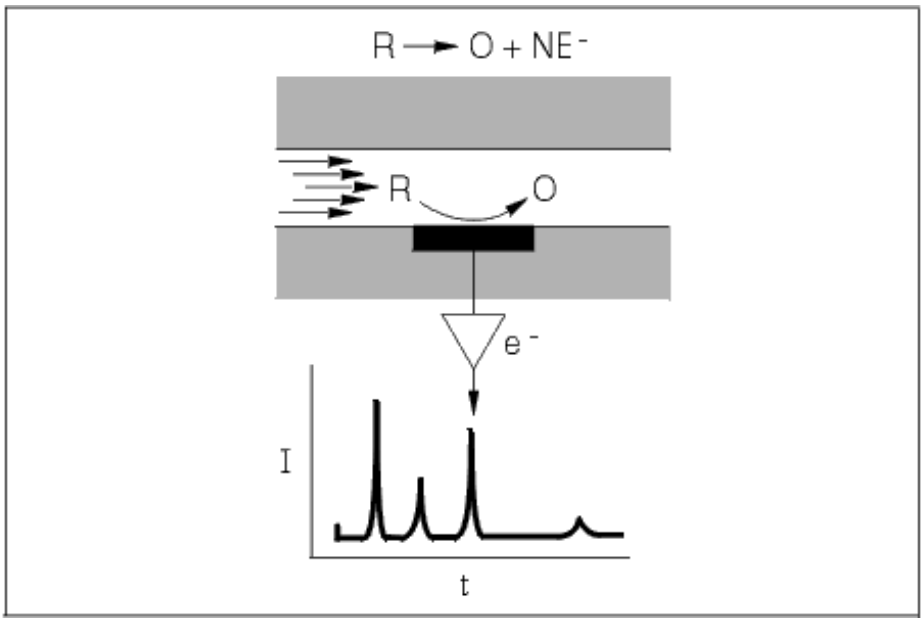
This section includes basic background information on the theory of electrochemical detection. It is not mathematically oriented. Concepts important to the LCEC technique are explained. Bioanalytical Systems commercialized these concepts in 1974. While the instrumentation has evolved a great deal since then, the basic principles remain the same.

- **ELECTROCHEMICAL FUNDAMENTALS**
- **HYDRODYNAMIC VOLTAMMOGRAMS**
- **OXIDATION OR REDUCTION**
- **REFERENCE ELECTRODES**
- **MOBILE PHASE LIMITATIONS**
- **UNCOMPENSATED RESISTANCE**
- **THE CURRENT RESPONSE**
- **THE BACKGROUND CURRENT**
- **NOISE**
- **ATTAINING GOOD SIGNAL-T0-NOISE RATIOS**
- **DUAL-MODE LCEC**

ELECTROCHEMICAL FUNDAMENTALS

LCEC may be considered in terms of electrolysis at a fixed point along a flowing stream. The stream in this case is the eluent from the analytical LC column and a sequence of solute ("analyte") zones separated with varying degrees of resolution. These zones pass into a very low-volume thin-layer cell, where the flow is constrained to a thin film passing over a planar electrode held at a fixed potential. Figure 1.1 illustrates an exploded view of this region. If the potential is greater (more positive for oxidation, more negative for reduction) than that required for the electrolysis of the analyte, a measurable charge passes from electrode to analyte (or vice versa). The resulting current is directly proportional to the concentration of solute passing through the channel.

Figure 1.1. Exploded view of electron transfer at the surface of a thin-layer electrode. A laminar flow profile passes over the electrode in the thin-layer zone containing reduced analyte R. Oxidation to O at the electrode surface releases electron(s) to the surface. This current is subsequently converted to a voltage, which drives the recorder and produces the chromatogram.



The electrode may be thought of as a chemical reagent. The more positive its potential, the stronger an oxidizing agent it becomes; when the potential is made more negative, it becomes a stronger reducing agent. In either case, as the concentration of solute rises and falls in passing through the thin-layer cell, the electrolysis current proportionately follows these changes. This current, as a function of time, is amplified and sent to a recorder to yield a chromatogram.

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LCEC is an AMPEROMETRIC determination. Unlike measurements (such as pH) of a potential difference under zero-current conditions, LCEC measures current at a fixed potential. It must always be remembered that this experiment involves heterogeneous electron transfer (from one phase to a different phase), and the success of the experiment depends in large part upon the care with which these components are chosen and operated.

Crucial to all amperometric determinations is FARADAY'S LAW, which states that:

(1) **Q = nFN**

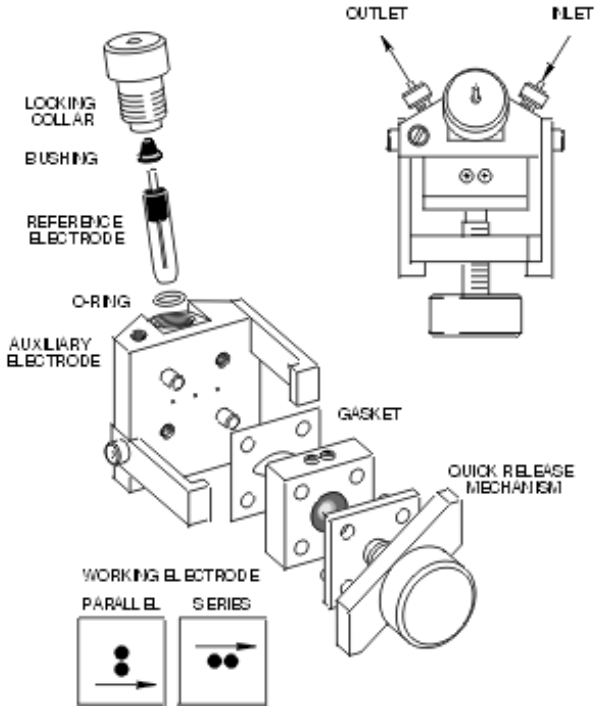
Q is the number of coulombs (a unit of charge) used in converting N moles of material, n is the number of moles of electrons lost or gained in the transfer process per mole of material, and F is Faraday's constant (96,500 coulombs/mole of electrons). Differentiation of (1) with respect to time yields the current, which is the measure of the rate at which material is converted:

(2) **dQ/dt = i = nF dN/dt**

Equation (2) therefore relates a measurable quantity, the current, to the fundamental redox process occurring in the cell.

Bioanalytical Systems established a new standard for its cross-flow electrochemical transducers in the BAS 200A and BAS 480 liquid chromatography systems (Figure 1.2). As from the beginning, the proven thin-layer concept remains central to this design. In it, two blocks form a sandwich around a thin fluoropolymer gasket which defines a microliter flowcell. Two working electrodes (glassy carbon or other material) are embedded along one wall of the channel, whereas the reference and auxiliary electrodes are directly opposite, typically only 50 micrometers distant.

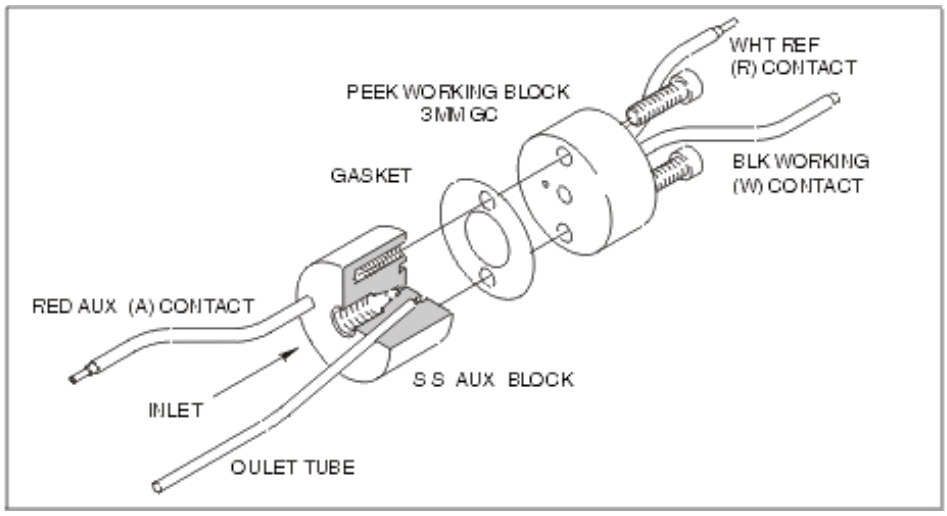
Figure 1.2. Cross-flow thin layer cell for LCEC.



In 1994, BAS introduced the UniJet cell (Figure 1.3), specifically for microbore LCEC. The UniJet utilizes a radial flow thin-layer cell which has some advantages in low-flow, low-dead-volume situations.

Figure 1.3. UniJet cell design for BAS microbore chromatography.

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The nature of the working electrode, particularly its bulk composition and surface treatment, is critical to detector performance. Many organic compounds react at significantly different rates depending on the electrode used. It is normally desirable to carry out the electrode reactions at the greatest possible rate in order that the current be limited only by mass transport of molecules to the surface and not by their reaction rate at the surface. This situation affords the greatest sensitivity and stability without sacrificing selectivity.

Carbon paste (a mixture of spectroscopic graphite powder and a dielectric material such as mineral oil, silicone oil, and paraffin wax) was used for initial LCEC experiments (ca. 1972) due to its excellent properties for organic electrochemical reactions. For most published applications of carbon paste electrodes, the useful lifetime of the electrode surface can extend to many months. However, if extremes of potential are used (> 1 V vs. Ag/AgCl) and/or if the mobile phase contains high concentrations of organic solvent, the electrode surface must be renewed more frequently. Carbon paste is rarely used now, since most users prefer the convenience of glassy carbon. However, it is still hard to match the performance of a well-packed carbon paste electrode.

Glassy carbon falls more closely in line with our desire for a universal electrode material. Glassy carbon is a hard, amorphous carbon material capable of being polished to a mirror-like finish. When housed in a PEEK block, a glassy carbon cell offers good solvent resistance, a feature particularly useful with mobile phases containing acetonitrile or large percentages of methanol. It has been used in entirely nonaqueous systems with good success.

It is widely believed that sample molecules, lipids, or proteins will "poison" the electrode surface after a few samples have been injected. This notion undoubtedly arises from classical voltammetry (including polarography), where the presence of molecules of high molecular weight leads to serious problems. Voltammetric measurements quite often involve sample concentrations over the range 10^{-5} to 10^{-3} moles/liter. In some cases, the electrode reaction itself leads to polymer formation, which "passivates" the surface, causing unreliable results.

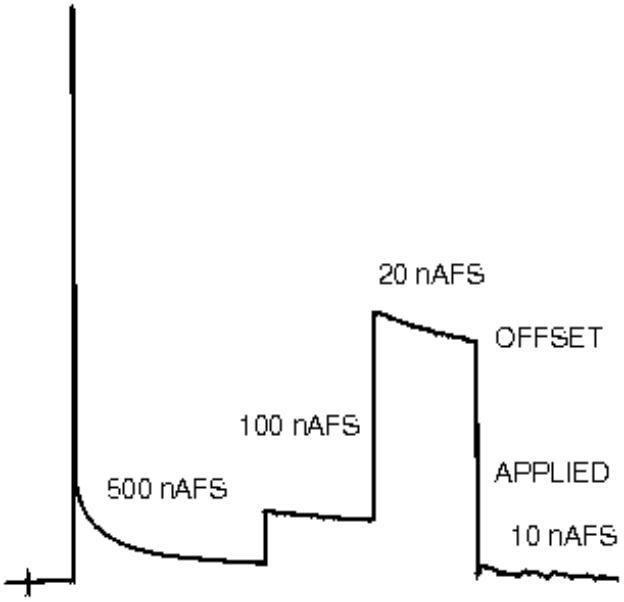
In the case of amperometric detection, the column isolates the electrode from many contaminants, the mobile phase continuously cleanses the surface, and the usual sample concentrations are in the range of 10^{-8} to 10^{-6} moles/liter. Furthermore, the electrode is only exposed to individual compounds for a minute or less due to the narrow elution zones encountered in liquid chromatography. All of these factors heavily favor the LC electrode in comparison with direct (i.e., no chromatography) electrochemical measurements. Hence, with LCEC experiments, electrode "life" is extended much longer than with traditional voltammetric experiments.

The LC-4C and LC-3C detectors set the reference electrode at ground potential and the working potential in reference to this. The epsilon system sets the working electrode to ground potential and the reference electrode at -E. This situation often baffles those who have not been introduced to the simple fact that it is the POTENTIAL DIFFERENCE BETWEEN AN ELECTRODE AND THE SOLUTION which is of importance in electrochemistry and NOT the potential of the electrode material itself. Normally, this potential difference extends across an interphase region (the "electrical double layer") which is quite thin. It is the purpose of the electronics to control this potential difference while at the same time converting the current (resulting from the electrode reaction) into a voltage which is easily recorded or processed by a computer.

Figure 1.4 illustrates a recorder tracing encountered following initial application of a predetermined potential difference to a thin-layer cell containing a freshly prepared carbon paste electrode. The mobile phase is passing though the detector cell, but no sample has been injected into the chromatograph. Why then is so much current measured? Initially, some charge is required for the electrode/solution interface to achieve the applied potential difference. Additional charge will pass as the oxidation states of functional groups on the graphite are altered to a new state of equilibrium. Finally, impurities in the mobile phase and the mobile phase itself will oxidize (or reduce) resulting in a steady-state background current. Water will oxidize (or reduce) at all potentials although the rate of the reaction is quite slow except at very positive (or negative) extremes.

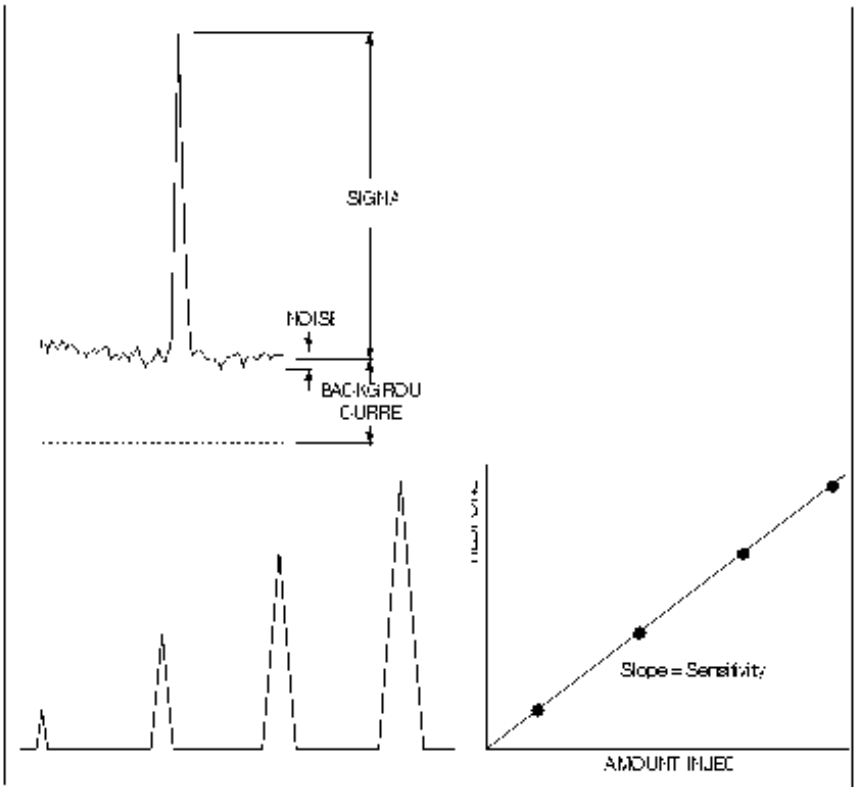
Figure 1.4. Typical equilibration response of electrode when it is first turned on.

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The background current is electronically canceled by the offset current applied by the user. This results in a steady baseline at recorder zero (Figure 1.4). It is always desirable to operate with the minimum absolute background current to optimize sensitivity. The chromatogram is recorded "on top" of a current which often exceeds the peak heights for eluted compounds. High background currents increase the susceptibility of the instrument to noise (e.g., pump pulsations) and can result in serious nonlinear (negative) deviations in calibration curves.

Figure 1.5. Performance parameters for detector evaluation.



It is useful to evaluate the peak-to-peak baseline noise over a time period about ten times the width of the chromatographic peak (Figure 1.5). The frequency content of the noise relative to frequencies much higher or lower can usually be removed. The high frequencies are easily filtered out and the very low frequencies are, in effect, baseline drift which is eliminated when the peak is quantitated. The amount injected that would give a peak height equal to the background current noise is clearly below the limit of usefulness. An amount that would give a peak height five times the background current noise is useful under many circumstances.

Figure 1.5 summarizes in graphic form the various parameters useful in evaluating detector performance. These are often confused in real practice. Sensitivity is the slope of the response versus amount. Detection limits refer to the amount of analyte required to give a signal X times greater than the noise (usually X = 3). The two terms are not synonymous. The reader should be able to foresee situations in which

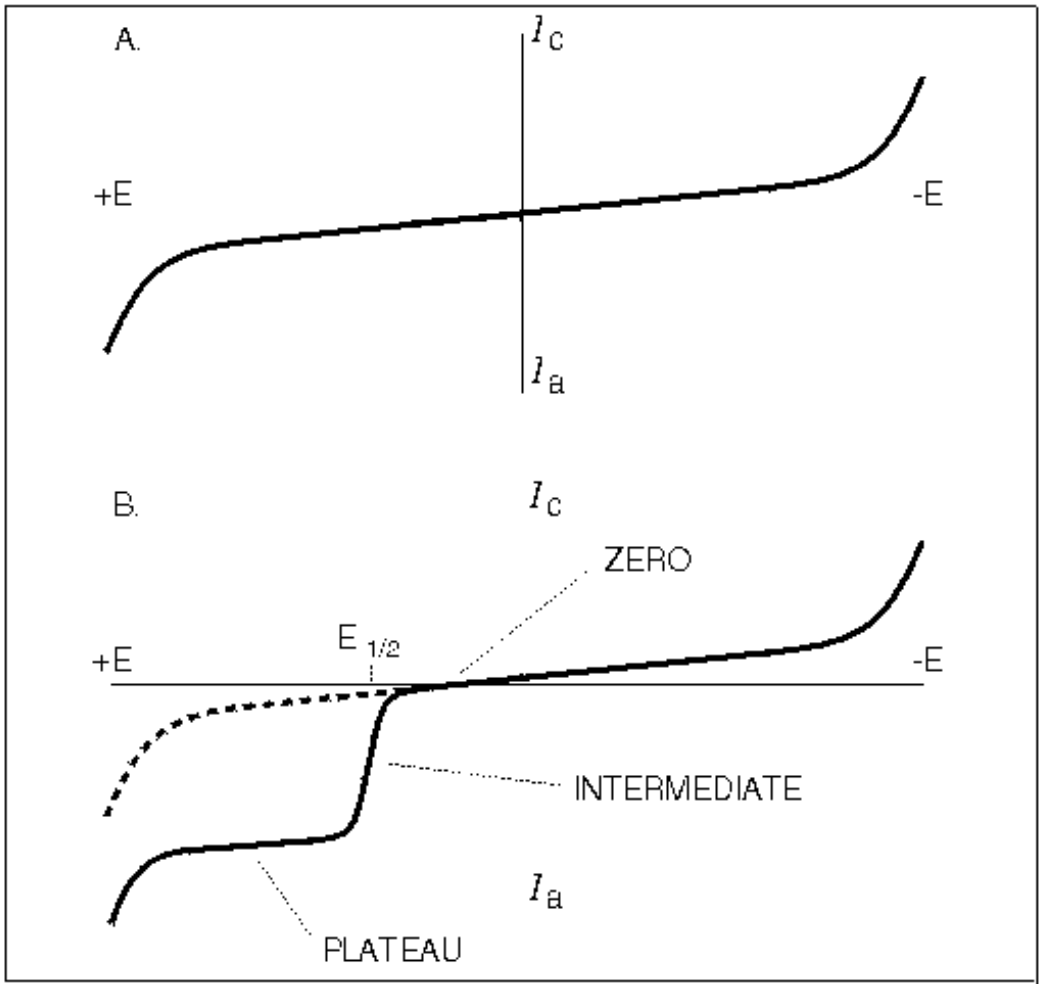
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two detectors demonstrate equal sensitivity but unequal detection limits. The only difference necessary is the noise. We recommend that LCEC users report sensitivity for each peak of interest as well as the noise. With this information, it is possible for the reader to predict a reasonable detection limit for each substance being determined.

HYDRODYNAMIC VOLTAMMOGRAMS

Let's begin our discussion by using a hypothetical electrochemical detector. Suppose we are operating the unit in an oxidative mode with a carbon electrode, and have initially chosen a potential low enough so that no detection of our test analyte should be possible. An injection of the test analyte is made; as predicted, no response is seen at the expected time. The potential is raised another 100 mV, the injection repeated, and the current response noted. This process is repeated until the potential becomes so high that the background current is prohibitive (usually +1.2 V). Suppose our test analyte were electroactive in the range examined. Then a plot of peak height versus applied potential would look similar to the solid line in Figure 1.6B.

Figure 1.6. Hydrodynamic voltammogram for mobile phase alone (A) and solute in mobile phase (B). The waveform for the solute is characterized by the half-wave potential $E_{1/2}$.



Three zones along this curve would become apparent:

1. Zero current region. The potential was not sufficient to force oxidation to occur.

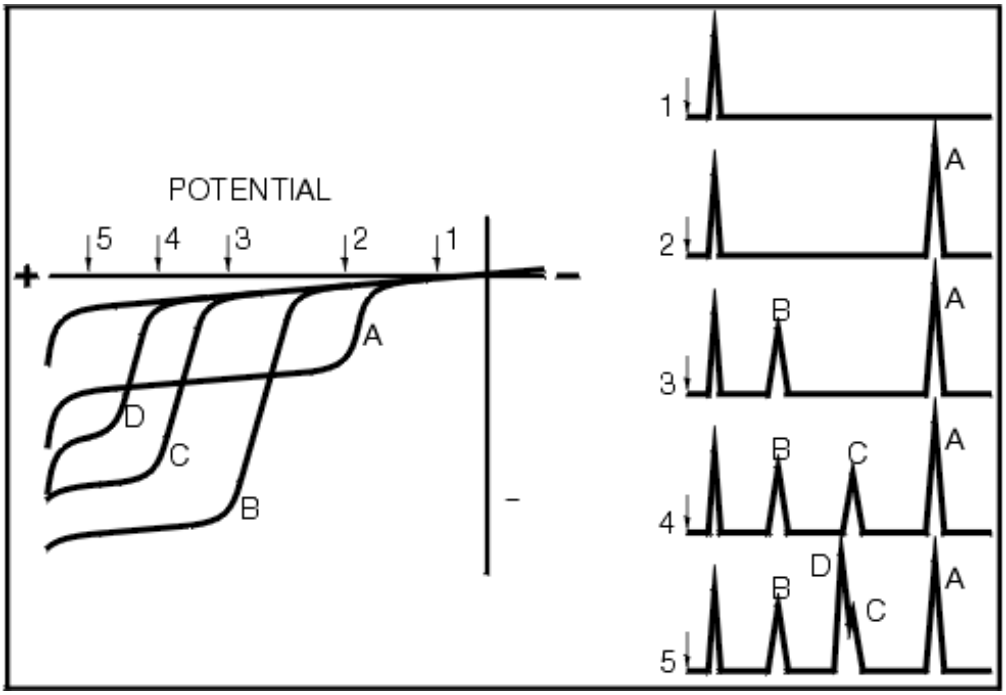
2. Intermediate region. The peak height is rising with increasing potential. Here the potential is controlling the kinetics of the heterogeneous electron transfer from the solute to the electrode surface.

3. Plateau region. In this zone, the peak height is independent of the potential. Diffusion to the electrode surface is the rate-determining factor; that is, the current is proportional to the rate of transport of molecules per unit surface area and per unit time.

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In most situations, it is advantageous to operate the detector at a potential in the plateau region within 50-100 mV of the final break in the curve. Such a choice will offer maximum selectivity. The background current will be minimal and fewer interferences are likely. These advantages diminish when high potentials (> 1 V) are required. This point is illustrated in Figure 1.7 where the voltammograms are shown for equimolar solutions of four oxidizable compounds (A-D).

Figure 1.7. Left: Hypothetical hydrodynamic voltammograms for four oxidizable solutes A, B, C, and D. Right: Hypothetical chromatograms for EC detector operated at potentials 1-5.



The curves have different oxidation potentials, as exemplified by the wave locations. Each compound also has its own particular limiting current, depending on such factors as the number of electrons transferred, the rate of that transfer from bulk solution to the surface, and the type of electrode surface used. Substance A, for example, is easier to oxidize than C, but yields a greater current response due to some combination of the above factors. Detecting A is very simple via LCEC. By setting the potential at 2, A may be clearly measured with no response from B, C, or D. In order to detect C, however, the potential must be set at a value which is sufficient for oxidation of B and A as well. Selectivity in this case is also dependent on the chromatography. Figure 1.7 also illustrates the chromatograms that would be obtained for potentials 1 through 5. Note that once in a plateau region, the current response for that compound is the same for any higher potential. Also, the farther one moves out in potential, the greater the number of compounds that will show up on a complex chromatogram. At potential 5, the selectivity (both electrochemically and chromatographically) is inadequate to permit separation of C and D.

Dual-electrode LCEC can overcome some of the limitations described above. Since BAS dual electrodes are interchangeable between parallel and series arrangements, and since different electrode materials can be used together in one cell, several alternatives not available in single-electrode cells become possible. In the previous example, it might be possible to distinguish between C and D based on a difference measurement in a dual series configuration. Detailed examples of the use of dual electrodes for complex problems are available in BAS Application Capsules

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OXIDATION OR REDUCTION?

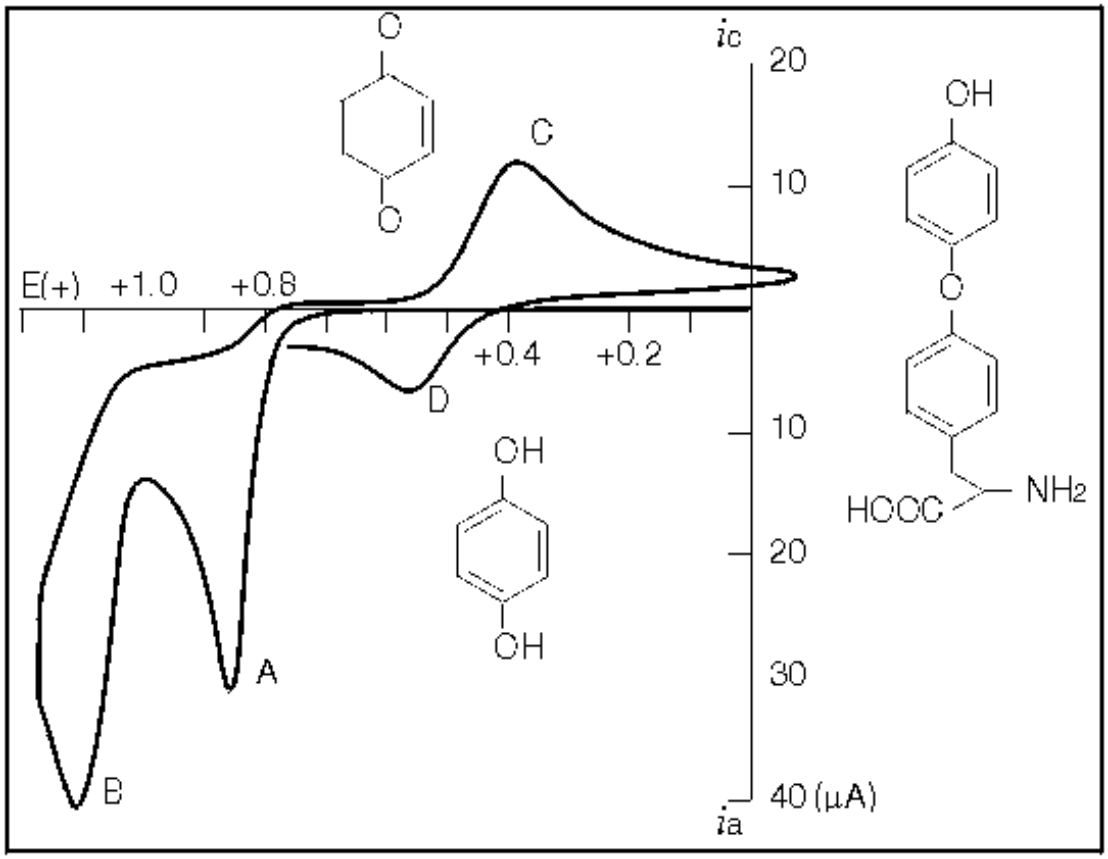
Most of the discussion thus far assumes that you have some idea of whether you will be looking at either an oxidative or a reductive electrochemical reaction. What if you don't know? Don't panic! It is not always obvious. Hydrodynamic voltammograms can give you some information, but they require several injections onto your LCEC analyzer, use mobile phase, and put wear on the column. There is an easier way.

Cyclic voltammetry (CV) is a quick and inexpensive means of learning the electrochemical behavior of the analyte in the mobile phase you plan to use. Mobile phase changes can cause shifts in applied potential. To keep the assay optimized, you should either do an HDV as described, or run a cyclic voltammogram on the sample in your new mobile phase. A sample cyclic voltammogram is illustrated in Figure 1.8. CV data for a wide range of compounds can be found here. If you are continually faced with new analytical problems, you will find a CV instrument an invaluable accessory for LCEC.

Figure 1.8. This cyclic voltammogram of thyronine tells us that a potential of +0.85-0.90 V is necessary to detect it. It also undergoes an

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interesting follow-up reaction which could be detected by dual-electrode LCEC.



REFERENCE ELECTRODES

The role of the reference electrode in any electrochemical cell is to provide a stable half-cell potential. Recall the Nernst equation, which states that the half-cell potential, E, is a function of the thermodynamic E°, measured at unit activity for all species involved, and the various activities of the components of the half-cell reaction.

$$\mathbf{E = E° + (RT/nF)logK}$$

K is the equilibrium constant for the half-cell reaction, written as an oxidation.

For the Ag/AgCl reference electrode commonly used in LCEC detection, the reaction and corresponding Nernst equation are as follows:

$$\mathbf{Ag° + Cl^- \rightarrow \underline{AgCl} + e^-}$$

$$\mathbf{E = E° + 0.059 \log (1/[Cl^-])}$$

The activities of the Ag wire and AgCl are unity and, therefore, to a good first approximation, only the chloride ion concentration determines the E value.

What affects the stability of the E value? From our experience, any minor drift in reference electrode E is usually due to a change in Cl^-
The concentration inside BAS reference electrodes is initially 3 M. Since the chloride ion concentration in the mobile phase is typically zero, a

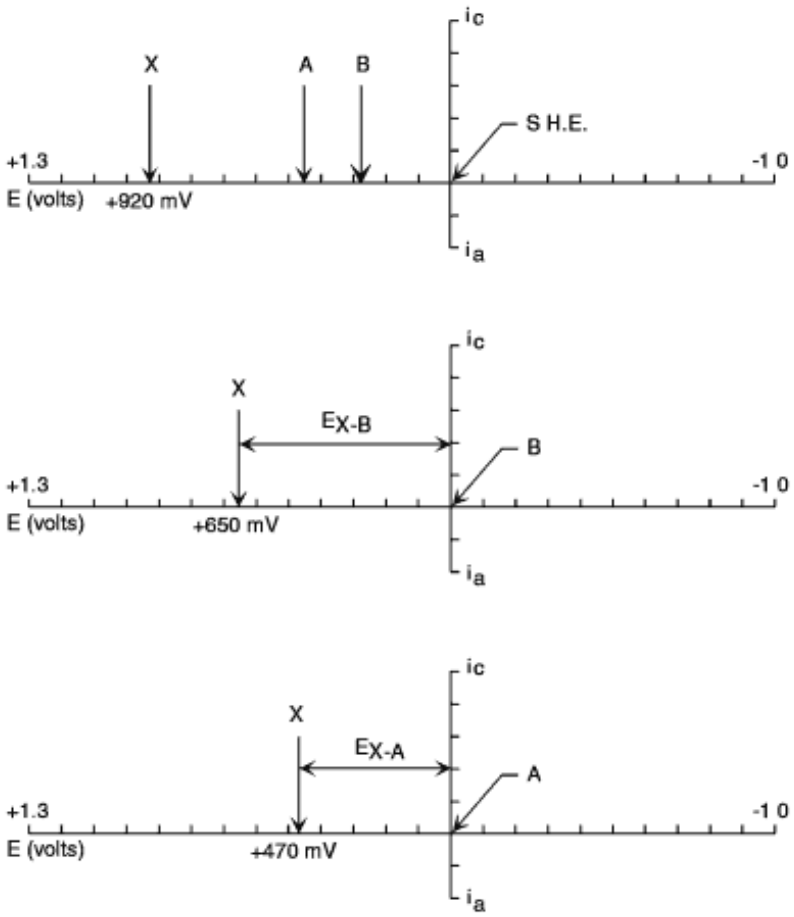
steep concentration gradient exists along the porous frit (at the tip) separating these two solutions. Since the concentration gradient allows the chloride ion concentration to decrease by continuous diffusion into the mobile phase, the potential must necessarily change as dictated by the Nernst equation. Although other chloride concentrations could be used with a reference electrode half cell, the problem of the concentration gradient would still exist, and drift in the reference electrode potential would likewise occur.

On a time scale of several weeks, the potential of the reference electrode half cell may drift to values on the order of 40 to 50 mV from when the reference electrode was originally installed in the chromatograph. On a short-term basis, this type of drift is not objectionable, because the change per day would only amount to a few millivolts. Since the applied potential required to force the LCEC oxidation or reduction to occur is generally set at least 20 to 50 mV in excess of that required, the detector's response factors are maintained at an adequate level. In isolated cases, however, the reference electrode may experience catastrophic failure. This degree of failure is indicated by the magnitude of drift in the reference electrode's potential, and the error normally amounts to +0.5 to +1.0 V. Obviously the reference electrode is not providing a stable potential. Usually, the cause of such deterioration is mechanical, and the reference electrode will require replacement.

In terms of caring for reference electrodes, a few guidelines are important. First, when using a reference electrode, always keep its tip wet. When not in use, the electrode should be stored in 3 M sodium chloride to prevent the problem of chloride depletion mentioned earlier. To prevent clogging of the porous electrode tip, do not keep the reference electrode in strongly nonaqueous solutions for any period of time. In reversed-phase liquid chromatography, however, mobile phases containing nonaqueous solvents are not unusual and some trade-offs must be made. In our laboratories, it is common practice to use a given electrode over a period of two weeks. When the reference electrode potential has drifted to an objectionable value, it is replaced with a new electrode. The old electrode may be partially restored by soaking it in a solution of 3 M sodium chloride, using the recommended storage container.

What value does the reference electrode have in the electrochemical cell? The half-cell potential of this electrode serves as the reference point along the potential axis by which we judge the oxidizing or reducing power of the working electrode in the vicinity of the interfacial region between the working electrode and the electrolytic solution. Suppose we have two different reference electrodes (Figure 1.9).

Figure 1.9. Potential axes for two reference electrodes (A and B) vs. the standard hydrogen electrode (SHE) (top). Referencing all potentials relative to reference electrode B would translate the potential axis (bottom left). The applied potential for a hypothetical analyte X will now be less when expressed versus reference electrode B. If reference electrode A were used, even less applied potential would be necessary (bottom right). Both situations provide equivalent oxidizing power.



Reference electrode A is situated at a potential more positive than that of reference electrode B. These potentials are dictated by variables such as the basic half-cell reaction, the concentration (formally the activities) of the participants in the half cell, temperature, etc. The potential axis

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upon which we have placed these two reference electrode half cells is a variable scale of oxidizing or reducing power. As we go to more positive potentials, the oxidizing power of the electrochemical detector increases; conversely, at more negative potentials, the power of the electrode to serve as a reducing agent improves. Suppose we impress between the reference and the working electrodes a potential difference of +0.5 V. As an example, let's take the compound caffeic acid, which oxidizes at approximately +500 mV with respect to the Ag/AgCl reference electrode. If we were to select a reference electrode whose potential relative to Ag/AgCl were 200 mV more positive than that of the Ag/AgCl couple, the applied potential required on the electrochemical controller to achieve equivalent oxidizing power would only be +300 mV. It is important to realize that the electrochemical potential axis is arbitrary in the sense that the reference electrode sets the zero point for this axis. For this reason, the reporting of electrochemical detector potentials must be referenced against the type of reference electrode used to complete the electrochemical cell. For example, it is commonplace to say caffeic acid oxidized at a potential of +500 mV vs. Ag/AgCl.

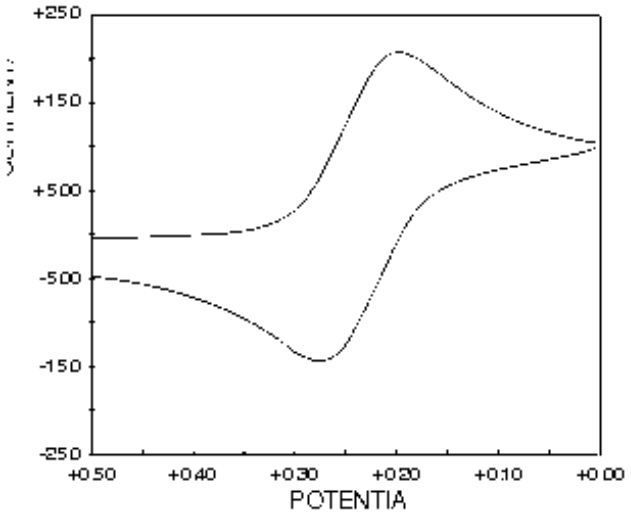
In the case of catastrophic failure, where the reference electrode potential might shift to a value of +500 mV with respect to its proper value, the performance of the electrochemical detector can be catastrophic as well. The electronic controller is still applying the same potential between the reference and working electrodes; it does not sense that a change in the reference electrode potential has taken place. Therefore, due to the shift, the actual oxidizing power of the working electrode has been increased to a sufficiently high level that objectionable and excessive oxidation of the mobile phase may occur (i.e., very large background current). You may not immediately discern whether the problem is due to the oxidation of some impurity in the mobile phase, deterioration of the working electrode surface, or a catastrophic change in the half-cell potential of the reference electrode couple. The problem must be diagnosed using a trial and error procedure where each of the above variables is tested independently of the others.

Since the half-cell potential of the reference electrode is taken as the arbitrary zero point along the electrochemical potential axis, it should not be surprising that oxidations may take place at negative potentials and reductions may take place at positive potentials. The positive or negative sign is merely an indication of where, in terms of absolute oxidizing or reducing power, the working electrode sits relative to the reference electrode. For example, if we selected a reference electrode whose potential was +1.0 V with respect to the present Ag/AgCl reference electrode potential, then nearly all electrochemical oxidations and reductions would be reported at negative potentials versus this new reference electrode. Specifically, in the case of pentachlorophenol whose oxidation potential vs. Ag/AgCl is approximately +800 mV, the new oxidation potential for an equivalent response would now be -200 mV because we would need to lower the working electrode's potential by that amount to make its oxidizing power equivalent to that when Ag/AgCl was used. For the reduction of a compound such as nitroglycerin where the applied potential vs. Ag/AgCl was -1.0 V, the new potential would be -2.0 V versus the new reference electrode.

Thus, we have transformed all of our oxidation and reduction potentials to a series of electrochemical potentials that are all negative, even though some of these are oxidation reactions where electrons will be passed into the electrode surface. Although it may seem contradictory that electrons would flow into an electrode with a negative potential, the issue here is not the charge on the electrode surface, but the establishment of a gradient of potential at the electrode/solution interface that is sufficient to cause the oxidation or reduction to occur. This *difference in potential across the interface* is what concerns the electrochemist! In all the cases we have described, the same interfacial potential difference would exist. A few more examples may be in order.

Let's take the case of the well-behaved electrochemical couple ferricyanide/ferrocyanide. This is a one-electron oxidation between two forms of iron held in a very stable hexacyano complex. Figure 1.10 demonstrates the oxidation and reduction of these complexes by cyclic voltammetry. Both reactions occur at positive applied potential.

Figure 1.10. Oxidation and reduction of iron hexacyano complex using cyclic voltammetry.

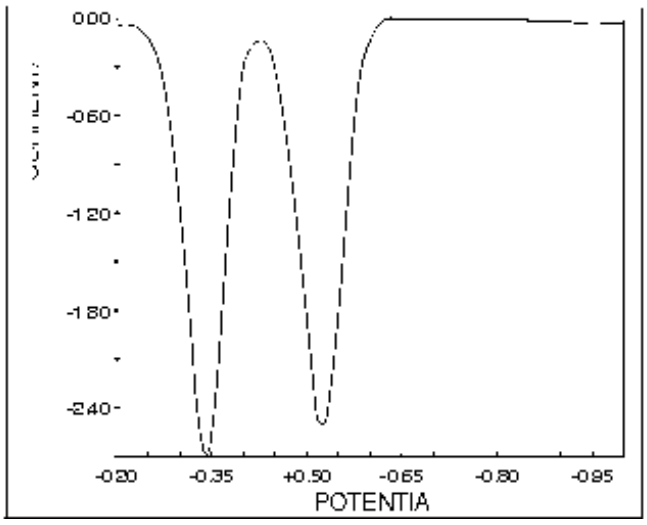


Another application familiar to all electroanalytical chemists is anodic stripping voltammetry. In this technique, the potential of the working electrode usually mercury or mercury-coated graphite is held at a sufficiently negative potential, typically -1.0 V vs. Ag/AgCl, while trace metals are deposited to form a concentrated amalgam of metallic atoms in the zero oxidation state. After a sufficient deposition period, the potential is scanned in the positive direction, and the metal atoms are characteristically stripped from the mercury into solution via a

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forced oxidation to their ionic states. The potential of each of these stripping reactions occurs at a value characteristic of the metal ion being analyzed (Figure 1.11). Note that all of these oxidation reactions are done at negative potentials versus the reference electrode implied.

Figure 1.11. Anodic stripping voltammetry of trace metal ions using a BAS 100B/W Electrochemical Workstation and an Hg/Au voltammetry electrode. The stripping peaks correspond to the oxidation (all at negative potentials) of trace metals electrodeposited in the mercury.



In summary, we should make clear the following points about reference electrodes and their use:

1. The reference electrode serves as our electrochemical zero point on the applied potential axis, and for this reason, when potentials are reported either for cyclic voltammetry, LCEC, or other purposes, the reference electrode must be specified.

2. The potential of the reference electrode is determined by the half-cell couple involved. In the case of Ag/AgCl, the reference electrode potential is determined by the integrity of the Ag/AgCl wire in the electrode and the concentration of chloride ion in the reference electrode filling solution. The concentration of chloride ion in the reference electrode will necessarily change with time due to the concentration gradient across the reference electrode frit. This small change in the reference electrode potential may be retarded by careful storage of the reference electrode in 3 M sodium chloride. Alternately, the change may be compensated by a corresponding change in the potential applied to the cell by the electrochemical controller.

3. The sign of the applied potential is determined by the relative magnitude of the oxidizing or reducing power desired, relative to the reference electrode's potential. There are several types of reductions that may be carried out at positive potentials and an equally diverse number of oxidations that may be done at negative potentials with respect to the Ag/AgCl couple. Keep in mind that these sign conventions are relative and not fixed.

MOBILE PHASE LIMITATIONS

Since amperometric detection depends on the transfer of electrons between a solute and the electrode surface, it is important to choose a solvent (mobile phase) that effectively permits the electrode reaction to occur. The primary limitations on the mobile phase are:

1. ELECTROLYTE MUST BE PRESENT, usually at 0.01 M to 0.1 M concentrations, to convey charge through the electrochemical cell.

2. The solvent must have a sufficiently high dielectric constant to freely permit IONIZATION OF THE ELECTROLYTE.

3. The mobile phase (electrolyte + solvent) must be ELECTROCHEMICALLY INERT at the electrode surface; that is, the background current at the applied potential should be negligible, with no chemical deterioration of the surface.

Even under the above restrictions, the scope of LCEC is very wide, since all ion-exchange and most reversed-phase separations employ these types of mobile phases. Initially all reports on LCEC dealt exclusively with ion-exchange columns using aqueous buffers. A majority of the separations performed with LCEC are now done with reversed-phase packing materials (e.g., BAS Biophase ODS or Phase II bonded phases). By bonding silyl hydrocarbon chains to a particle of silica, one fashions a packing capable of retaining nonpolar and weakly polar solutes from polar mobile phases. The versatility of reversed-phase materials can be further extended to ionic solutes by adding small amounts of ion pairing reagents to the mobile phase (e.g., BAS P/N CF-1090 sodium octyl sulfate). Polar bonded phases (diol or nitrile) have also been used. Few applications with silica gel columns have been reported, since the mobile phases needed to effect separations on these materials

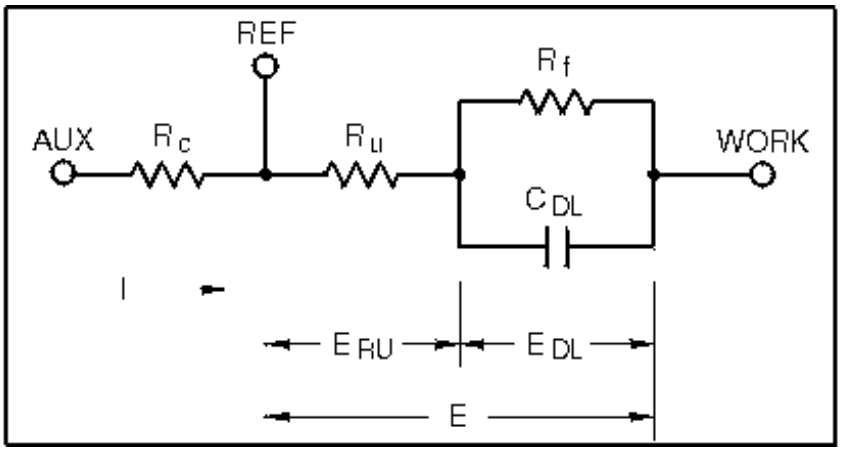
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(nonpolar solvents with low dielectric constants) are incompatible with the requirements of LCEC. There are some ways to get around this problem with nonaqueous solvents.

UNCOMPENSATED RESISTANCE

The simplest electronic equivalent of a three-electrode electrochemical cell would resemble Figure 1.12. The goal of the instrumentation is to impress all of the potential applied by the potentiostat across the interface between the electrode surface and the solution. This presents the sharpest potential gradient to the molecules.

Figure 1.12. Simple electronic equivalent of a detector cell.



Since the potential is applied between the reference and working electrodes, the user's control of the distribution of this potential between R_u and the interfacial double-layer impedance is impossible. The reference electrode may be moved closer to the working electrode (thereby minimizing R_u, but the term R_u will always be present to some degree. For this reason, R_u is termed the uncompensated resistance. C_{DL} is the double-layer capacitance (this capacitance is charged up when the detector is turned on, and is the cause of the initial off-scale transient) while R_f is the faradaic resistance. The latter term represents the resistance to charge transfer across the interfacial region.

Since uncompensated resistance is always present, the simplest expression possible for the applied potential, E, is

$$\mathbf{E = E_{RU} + E_{DL}}$$

Figure 1.12 describes this distribution. The difference between E and real applied potential E_{DL} is the term E_{RU} Since the electrical equivalent in this region is a simple resistor,

$$\mathbf{E_{RU} = iR_u}$$

where i is the current passing through R_u (and the double layer) at any time. This factor, dependent on both the current and the resistance in the cell, is referred to as "iR drop."

The cells described in Figures 1.2 and 1.3 possess very low uncompensated resistance. The electrodes face each other directly across the thin-layer channel. Hence, even if cell currents reach hundreds of microamperes, E_{RU} remains negligible and the interfacial potential remains constant.

The "curse" of high uncompensated resistance is narrow dynamic range. Spatial relationships within electrochemical cells are critical, and the compact nature of these two updated thin-layer designs is optimal in this regard.

THE CURRENT RESPONSE

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Two principal contributions to the current response of a thin-layer electrochemical cell are encountered. These are:

1. The FARADAIC RESPONSE, due to redox processes either from analyte or solvent impurities, and

2. The CHARGING CURRENT, required to charge the double-layer capacitance at the solution/electrode interface.

Normally, the charging current is not an issue since the detector is operated at fixed potential. Once the potential is supplied to the cell, and the background decays to an acceptably flat level, only faradaic contributions exist.

The fundamental relation for LCEC operation,

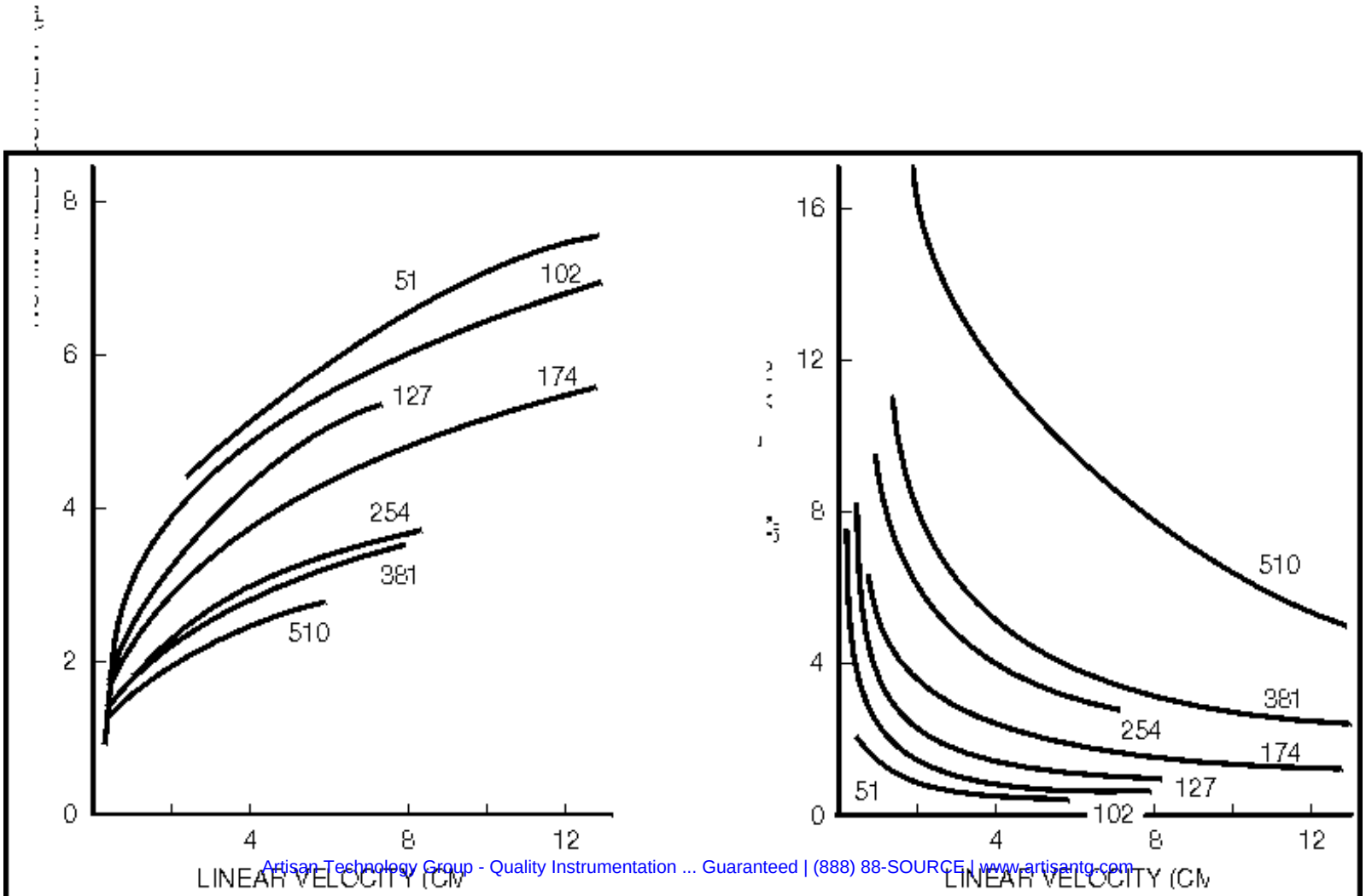
$$\mathbf{i = nFAJ_{x=0}}$$

describes the current in terms of the flux J and various constants. The flux is dependent on the flow rate, cell dimensions, and the diffusion coefficient and concentration of the analyte being oxidized or reduced. A rigorous derivation incorporating a detailed expression for the flux permits the following equation:

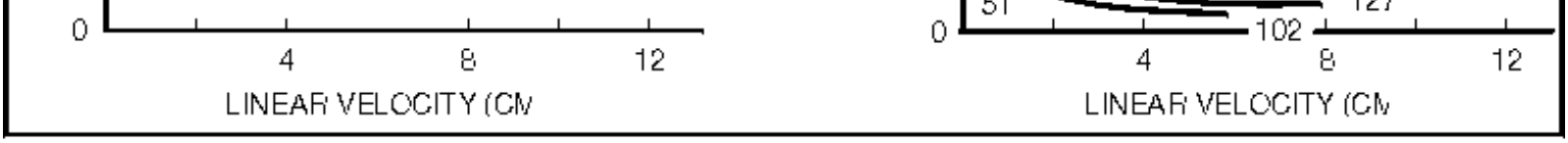
$$\mathbf{i_{lim} = 1.467\ nFAC_0(D/h^{2/3})(U_v/d)^{1/3}}$$

where n is the number of electron equivalents/mole, F is Faraday's constant, A is the area of the electrode (cm^2), C_0 is the concentration of reactant in the bulk eluent (mol/cm^3), D is the diffusion coefficient (cm^2/s), h is the thickness of the channel, U is the volume flow rate (cm^3/s), and d is the width of the channel. From this expression, it is evident that the diffusion-limited current should be proportional to the concentration of the analyte, the area of the electrode, and the cube root of the velocity through the cell, and inversely related to the cube root of the channel thickness.

Figure 1.13. Peak height (left) and peak area (right) versus linear velocity for various channel thicknesses (indicated in mm).



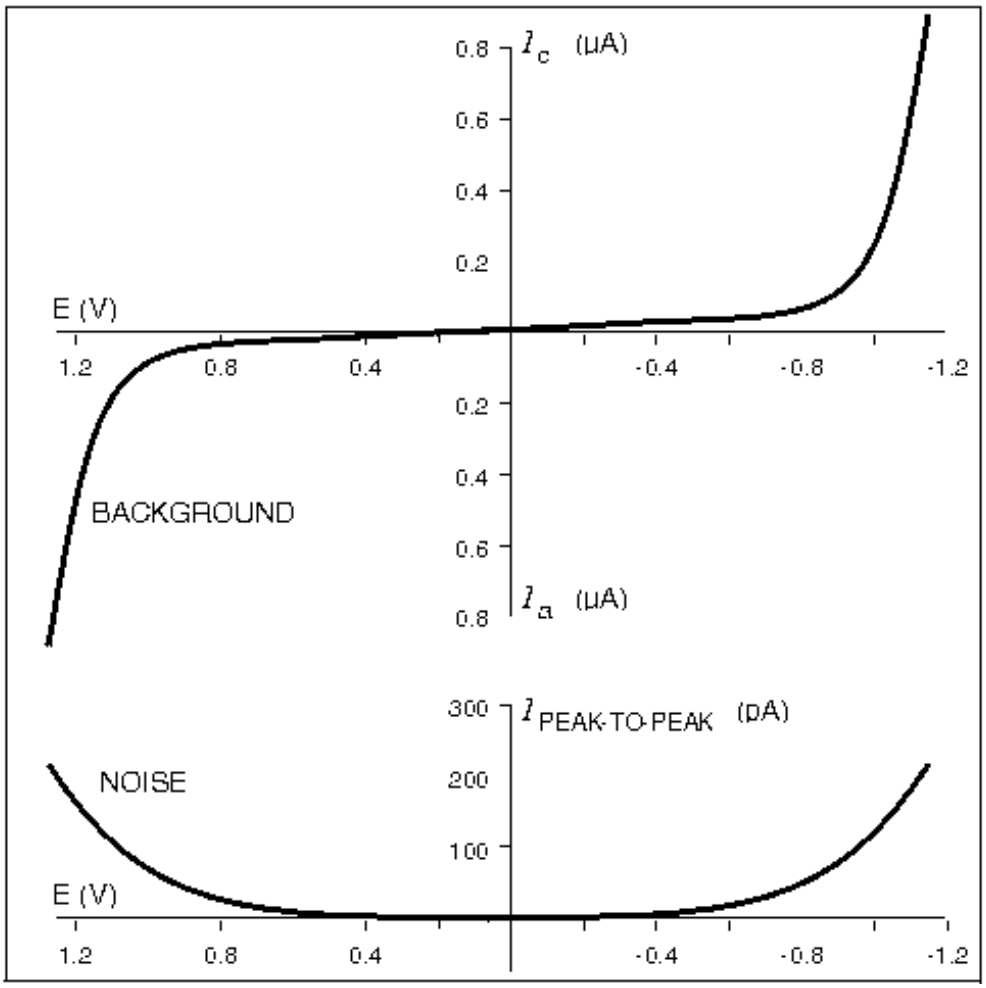
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THE BACKGROUND CURRENT

The background current is principally faradaic current arising from the oxidation (or reduction) of electroactive impurities in the mobile phase. It is entirely analogous to the background absorbance in ultraviolet detector systems. Common sources of background current are oxidation or reduction of the mobile phase solvent or buffer salts, oxygen (reductive), ferrous iron (oxidative), metal ions (reductive), etc. For the simple and usual case where the background current is caused mainly by the mobile phase, a plot of background current versus applied potential would resemble Figure 1.14. Note that the current is exponentially related to the applied potential at both a positive and a negative limit. Within these limits, however, a fairly flat low background response is typical.

Figure 1.14. Generally, the greater the background, the greater the noise. Both follow the same trend.



NOISE

Noise is the random or periodic pattern superimposed on the steady-state background signal. Usually measured from peak to peak, the noise represents the summation of spurious contributions from pump pulsation, flowcell hydrodynamics, surface reactions, static electricity, power-line noise, and electronic amplification. Obviously it is desirable to minimize noise. Specific guidelines are given below; detailed advice is outlined in the TROUBLESHOOTING section.

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- Select a pump capable of delivering pulseless flow relative to the detection limits you desire. Use pulse dampers. Use a complete BAS LCEC Analyzer system.

- Passivate your liquid chromatograph frequently. Check with the manufacturer for recommendations on this. Usually, an acid washing (e.g., 6 N HNO_3 is employed. Be sure to disconnect your column and cell before attempting this procedure.

- Follow good laboratory practice and maintain the pump seals and check valves in top working order. Lubricate the pump or maintain as specified by the manufacturer. Be fastidious about flushing the system thoroughly (with a mobile phase devoid of salts, e.g., 40% CH_3CN:H_2O) when you don't plan to use it for a long period. This helps eliminate random flow fluctuations.

- Connect all components in your system to the same electric circuit to avoid ground loops.

- Avoid solvents capable of destroying the electrode surface. This is of concern primarily for carbon paste or chemically modified electrode surfaces.

How do the background current and noise affect performance? As with most other quantitative measurements, the noise with an electrochemical detector is dependent on the magnitude of the background signal. Generally, the greater the background, the greater the noise, and the ratio of noise to background current stays approximately the same. This trend is true for the amperometric detector and glassy carbon materials. The noise will follow the same trend as the background current (Figure 1.14). It is impossible to operate at extremely high gain with high background currents if trace measurements are to be achieved.

ATTAINING GOOD SIGNAL-TO-NOISE RATIOS

The parameter most useful for analytical comparison is the signal-to-noise ratio (SNR or S/N). An extremely responsive electrode may be equally noisy, just as the apparent baseline quiescence of another electrode may be due to passivation, thus rendering it worthless. Neither situation may be evaluated by comparing just the signal (the response from injection of analyte) or just the noise. For this reason, the SNR is most pertinent.

Response should be directly proportional to surface area. Therefore, if we were to use an electrode of 10 times the area, it should give 10 times the response. Such improvement would be highly significant, if the cell noise were to remain at its original value. However, if the noise were to increase proportionately, the use of a larger surface would be questionable.

In actual practice, larger surface area electrodes are less useful. Figures 1.15 and 1.16 illustrate this trend.

Figure 1.15. Signal and noise for electrodes of different surface areas. The solid lines indicate the increase in current expected for a linear response in area.

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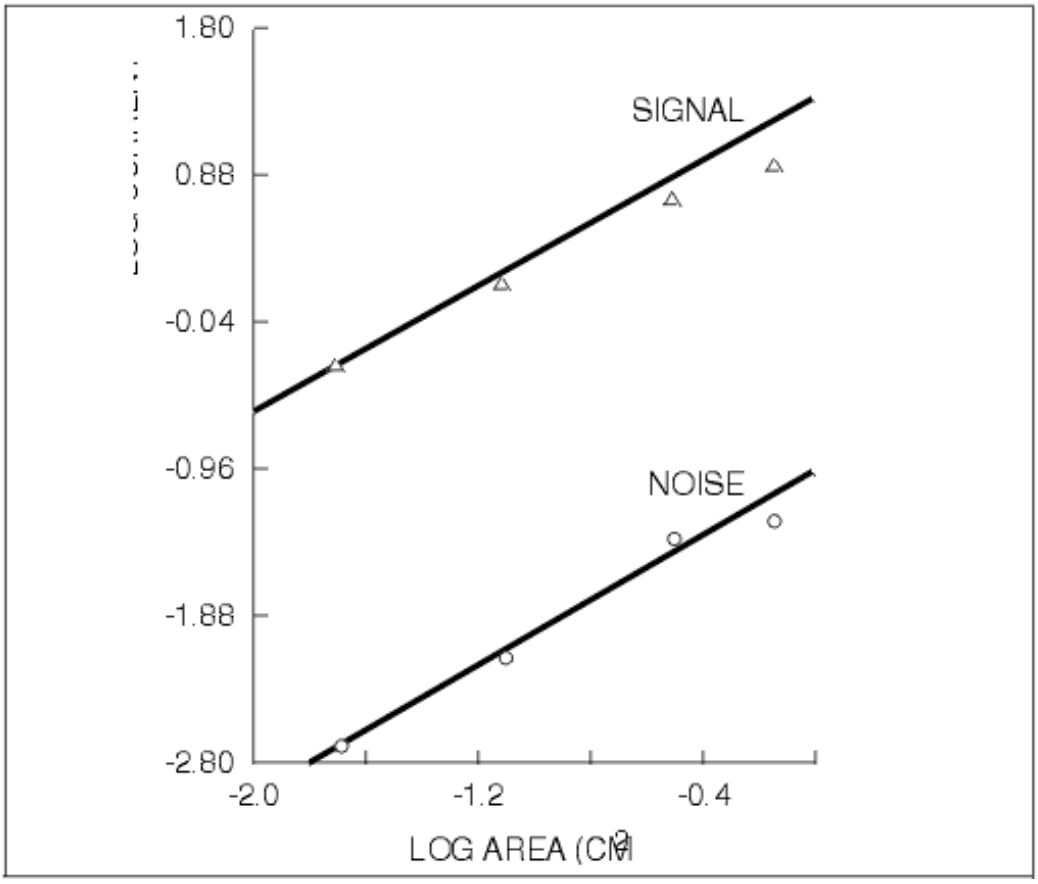
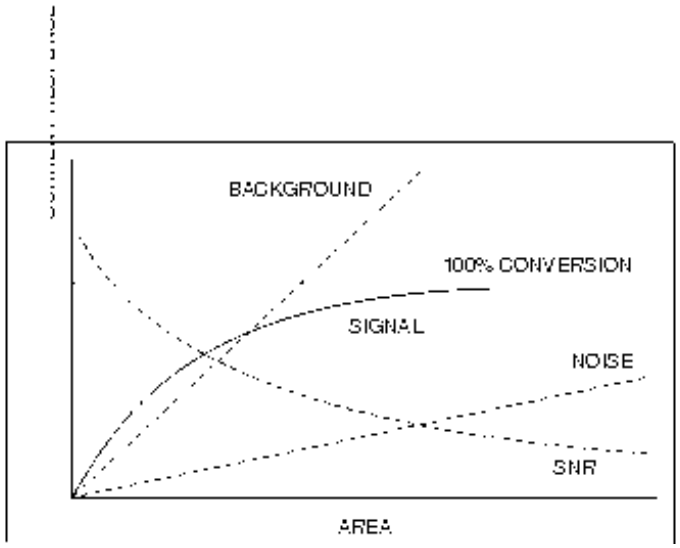


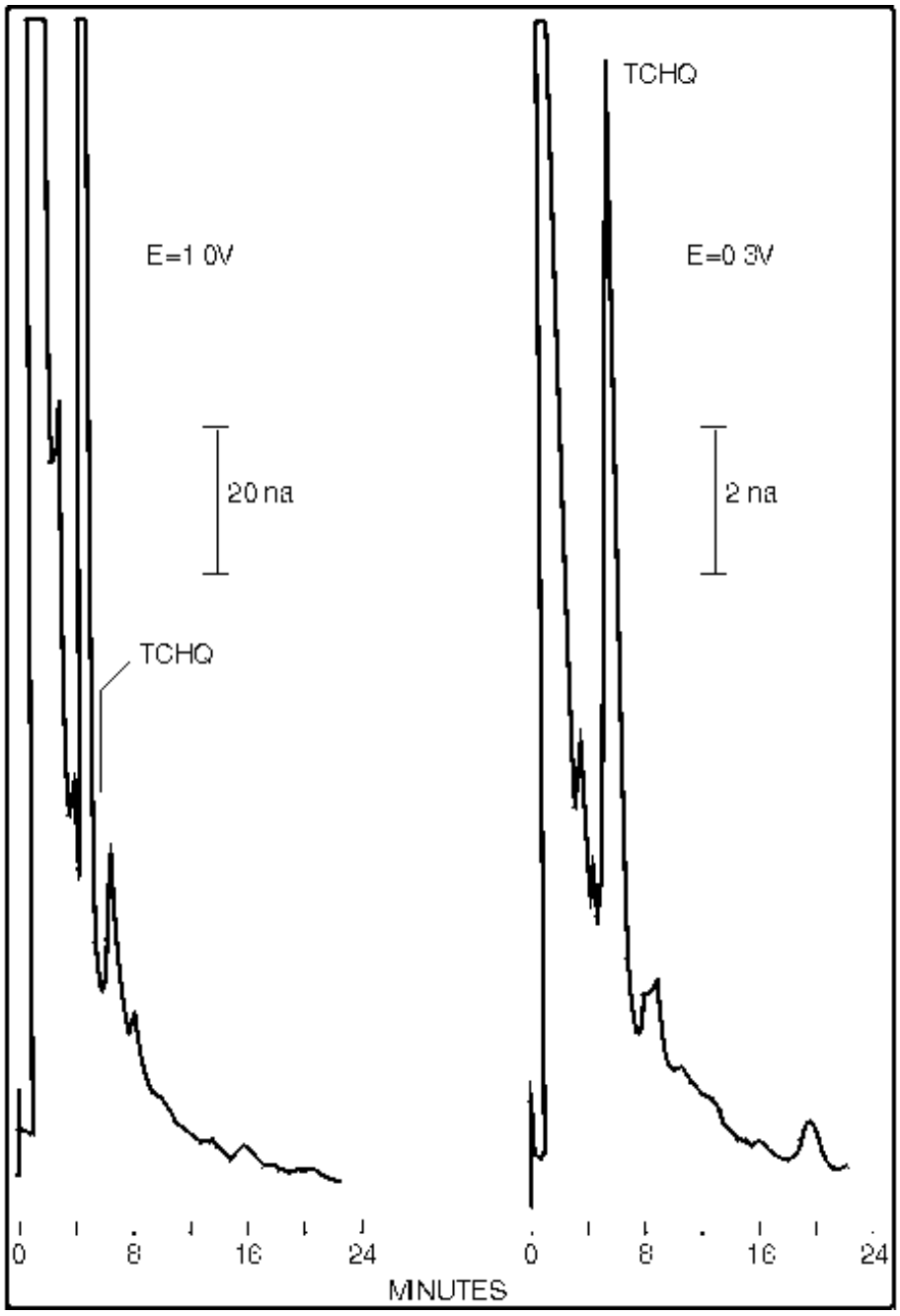
Figure 1.16. As the electrode surface area increases, the signal-to-noise ratio becomes unfavorable.



Both the analyte signal (peak height) and peak-to-peak noise are plotted. Each increment of added area adds proportionately less to the output signal, but adds linearly to the output noise. The smaller the electrode, the greater the signal-to-noise ratio. For this reason, large plate electrodes, porous flow-through cells, and other large surface electrodes demonstrate no advantages in terms of the SNR. If you intend to use the "Jumper" to couple the dual electrodes together, you should expect to see a less favorable SNR. The experiment might still be useful if you are trying to differentiate a particularly small peak and need a larger signal. Since noise depends on potential, it follows that the smallest minimum detectable quantities will occur in cases in which the substances of interest are easily oxidized or reduced. Figure 1.17 demonstrates the value of judicious potential selection.

Figure 1.17. Detection of tetrachlorohydroquinone (TCHQ) at two different applied potentials. This illustrates the specificity of electrochemical detection, which can in turn influence the sensitivity. Determination of the optimum applied potential is not unlike determination of the optimum wavelength used in UV detection methods.

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DUAL MODE LCEC

The electrochemistry for dual-electrode LCEC is governed by the same variables described previously. With this instrumentation, the electrochemistry can be manipulated to improve sensitivity and extend the applications of electrochemical detection.

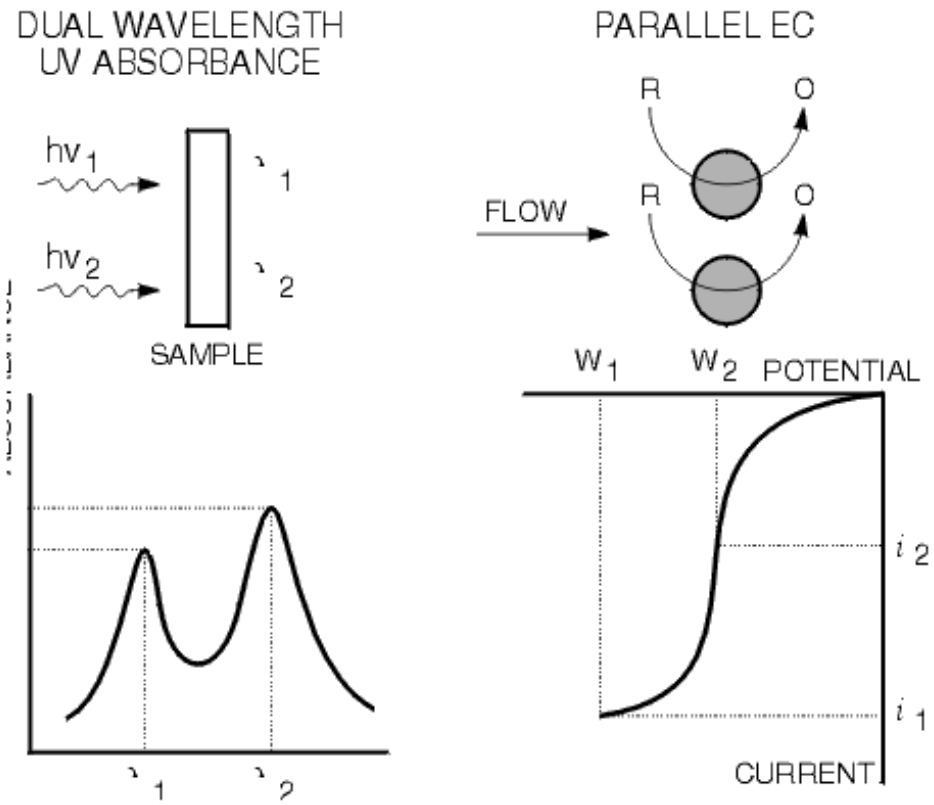
To understand these options, the following discussion will make reference to common spectroscopic techniques that are familiar to the reader. Analogies can be made on a number of points. In spectroscopy, the response of a compound to light energy is recorded as an absorbance spectrum, or in the case of fluorescence, as an excitation/emission spectrum. In electrochemistry, the current response is plotted against the applied potential. When the potential scan is reversed and plotted, the electrochemical equivalent to a fluorometric emission spectrum is obtained.

How does this apply to dual-electrode LCEC? The analogies are shown in Figures 1.18 and 1.19. In the DUAL-PARALLEL (adjacent) configuration, the detection options available are similar to those available when using a dual-wavelength photometric detector. The eluent from the LC can be monitored at two independent applied potentials. A simultaneous profile of reducible and oxidizable analytes can be obtained. Also, response ratios can be calculated to provide qualitative information on a particular chromatographic peak. Going back to our spectrophotometric analogue, the current ratio i_1/i_2 is conceptually the same as the absorbance ratio A_1/A_2. Both are constants which can be

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used for peak identification.

Figure 1.18. An analogy can be made between DUAL-PARALLEL amperometric detection and spectroscopic measurements. Enhanced selectivity is often the end result.



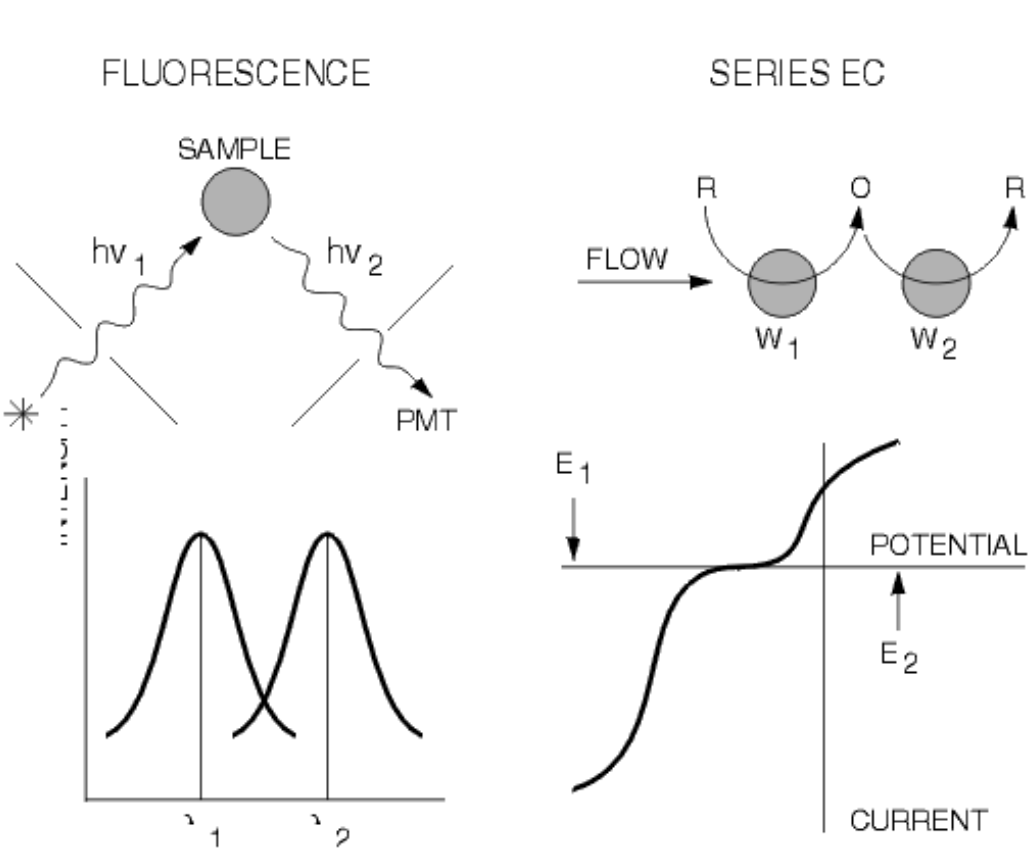
Simultaneous measurement in the DUAL-PARALLEL amperometric detection mode can be used to determine more than one compound in a chromatogram. For example, the detector potential at one electrode may be set sufficiently positive to oxidize all compounds of interest and the second electrode may be set at a substantially lower oxidizing potential to only react with those compounds that are electrochemically active at these lower potentials. Thus, the resultant dual tracing will allow quantitation at both applied potentials, and the lower-potential chromatogram will be more discriminating, improving the selectivity of the measurement.

Another application of the DUAL-PARALLEL mode is to measure the difference chromatogram. This would be similar to choosing a particular potential window to monitor. In this mode, the working electrodes are poised in a potential region where the response of the analyte of interest is dramatically different; that is, where the slope of the current-potential curve is large. The difference chromatogram enhances specificity by only displaying those compounds that are electrochemically active in the region between the two set potentials; all other responses, whether noise or analyte signal, are subtracted out. Of course, one electrode can be measuring an oxidation while the second can be monitoring a reductive reaction.

The DUAL-SERIES electrode configuration most closely resembles fluorometry. This analogy is shown in Figure 1.19.

Figure 1.19. DUAL-SERIES amperometric detection methods can also enhance selectivity and thus improve detection limits. An analogy can be made to fluorescence measurements for applications that require a reaction at the upstream electrode to create a product detectable at the downstream electrode.

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As in fluorescence, a reactive "intermediate" is produced by some excitation function which yields a product that generates some measurable response. In the case of fluorescence, it is the emission signal; in the case of the electrochemical detector, it is the redox couple's follow-up reduction (oxidation) reaction. In fluorescence, "quantum efficiency" is the measure of the maximum amount of fluorescence available from a given input intensity. A similar term in series-dual electrochemical detection is "collection efficiency," the ratio of the current at the downstream electrode to the current at the upstream electrode:

As quantum efficiency is a statement of the expected response for a given compound under a specified set of conditions, so is collection efficiency. A range of collection efficiencies can be obtained depending on cell dimensions (distance between electrodes and the ratio of lengths of the electrodes along the flow axis) and the homogeneous chemistry (hydrolysis, coupling, etc.) that might take place prior to arrival at the downstream electrode. The maximum range of values is 0.37 to 0.42 for reversible compounds at equal-surface-area planar electrodes in a standard cross-flow thin-layer cell at normal flow rates (1 mL/min). In a standard cross-flow thin-layer cell configured for microbore chromatography (16 mm thin-layer gasket and < 100 mL/min flow rate), a collection efficiency of > 0.85 is observed.

DUAL-SERIES electrochemical detection can, in certain cases, improve selectivity and detection limits. Compounds with higher collection efficiencies will dominate the response at the downstream electrode and can be measured with improved selectivity. Not all compounds have a reversible redox couple; these will not react at the downstream electrode. This selectivity may be advantageous. The upstream electrode functions as a derivatizing (GENERATOR) electrode, while the downstream electrode detects the product(s) created upstream. There are many applications of these schemes, but the major use is to improve the detection of compounds by generating electrochemical products that can be detected at a more favorable potential (where noise and interferences are less). This is successful because most background (media) reactions are chemically irreversible (e.g., reduction of oxygen in aqueous media, reduction of hydrogen ion, and oxidation of water).

When the same two SERIES electrodes are operated at the same potential, and the difference response is recorded, the result will be approximately 40% of the difference between the upstream and downstream electrode responses. Responses that would be equal at the same potentials, such as background currents and other factors contributing to noise, are nulled out.

- **PRINCIPLES CONTENTS**
- **EPSILON CONTENTS**

DETECTOR ELECTRODES

In electrochemical detection, the signal being monitored is a direct response to an ACTUAL CHEMICAL REACTION, as compared to the physical measurement occurring in other LC detectors (e.g., refractive index, absorbance, fluorescence). Electrochemical detectors sometimes get the reputation of being "finicky" compared to optical detectors, but it is important to realize that they behave in a very different manner, and this difference is responsible for the higher degree of sensitivity you can expect from EC methods. You will learn to handle electrochemical detection the same way you would handle any chemical reaction, by considering several variables which can influence the outcome of the reaction. In LCEC, the reaction product that concerns us is the current (i.e., the response, or signal).

- **ELECTRODES AS ACTIVE SURFACES**
- **UNIJET DETECTOR CELL**
- **LEAVING THE ELECTRODE "ON"**

ELECTRODES AS ACTIVE SURFACES

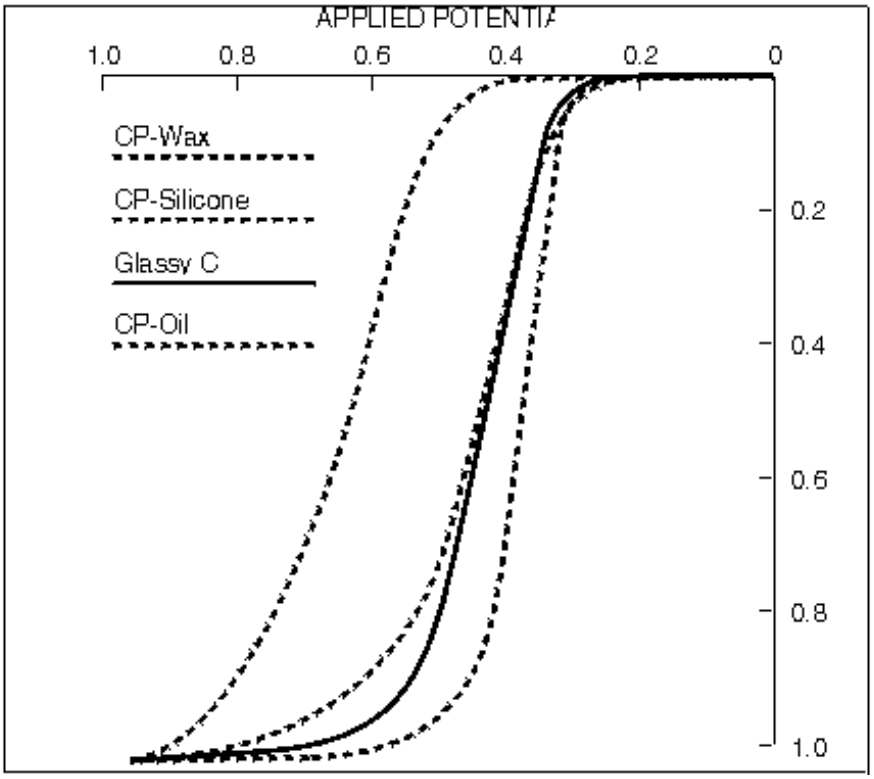
The response of an electrode is dependent on the chemical (electrochemical) reaction variables. These include the electrode surface where the reaction is taking place, the mobile phase (reaction medium), and the compound undergoing the reaction. The fundamental electrochemical relationships for this mode of detection were discussed in the BASIC PRINCIPLES section. This section will go into greater detail on some of the practical aspects of the electrodes, including electrode materials, detector cell design, solvent considerations, and the maintenance, service, and performance of each.

The general requirements for electrochemical detection are that the mobile phase be conducting, the working (detector) electrode be chemically inert, and the analyte be electrochemically oxidizable or reducible at the electrode surface in the chosen solution. Solution conductance is met by having an electrochemically inert salt (an ionic conductor) dissolved in the mobile phase. This places some restrictions on the mobile phase composition. Usually, aqueous or partially nonaqueous solutions are used, though a totally nonaqueous solution can be used as long as an appropriate salt is dissolved in it. Since the majority of the liquid chromatographic separations being performed today use reverse-phase packing materials, this requirement is easily met. It is also advisable that the mobile phase be a buffer solution for both electrochemical and chromatographic reasons.

Ideally, the working electrode should be inert to the electrolytic solution and only respond to the analyte in a thermodynamically defined, potential-dependent fashion. Many times this is not the case. The kinetics of heterogeneous charge transfer between the electrode and the analyte, in addition to the reactivity of the electrode itself, enter into the situation. For example, Figure 2.1 shows actual current-voltage curves (normalized hydrodynamic voltammograms; click here for further discussion of these waveforms) for the oxidation of a substituted o-hydroquinone on four carbon-based electrodes.

Figure 2.1. Hydrodynamic voltammograms for various carbon pastes and glassy carbon working electrodes.

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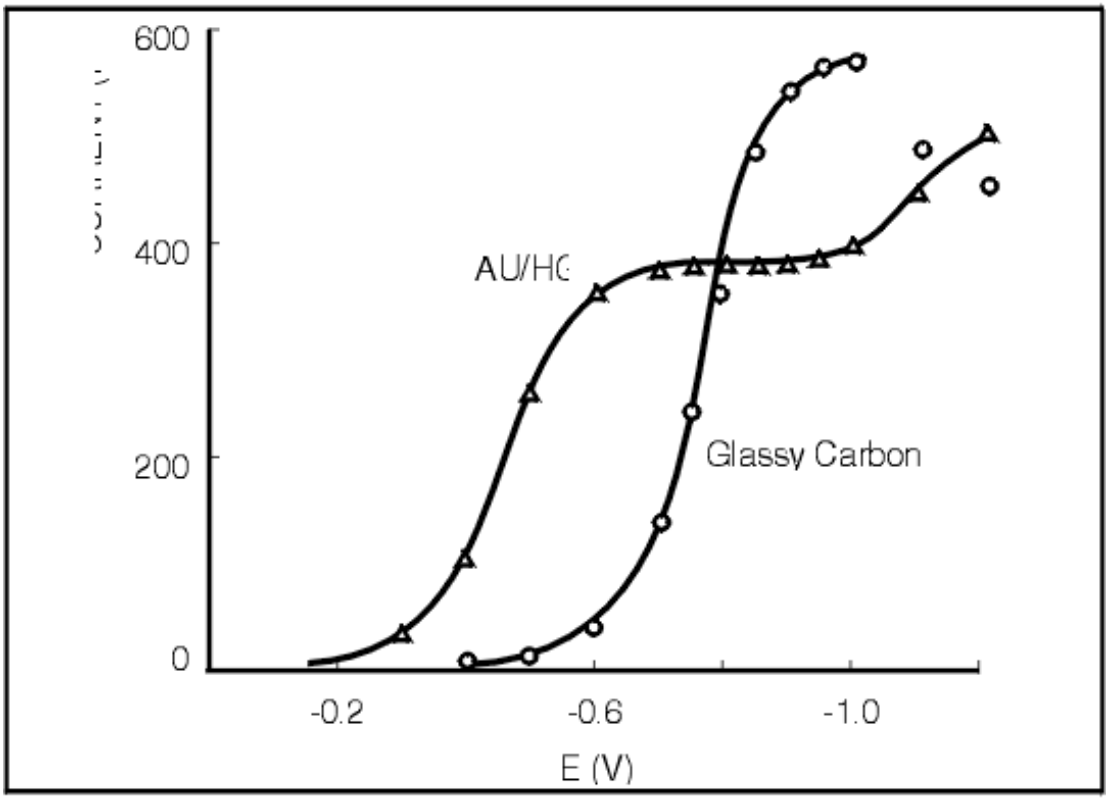


The sharpest break with potential occurs on the CP-O while the broadest voltammogram occurs on the CP-W, indicating that faster electron-transfer kinetics occur on CP-O relative to CP-W. The CP-S and glassy carbon electrode materials exhibit kinetics similar to CP-O. Fast electron-transfer kinetics characterized by sharply rising voltammograms improve the selectivity of the overall determination. Some carbon paste formulations (graphite mixed with hydrocarbon and fluorocarbon polymers) show slower kinetics, similar to the CP-W described above. The sensitivity or response for a given amount injected is approximately the same in the diffusion-limited region, but for these materials, a greater potential is usually required for equivalent response.

Electrochemical reactivity can be altered considerably by changing the electrode material. In many cases, this can be highly advantageous. The large hydrogen overpotential characteristic of mercury electrodes in protic solutions extends the attainable negative potential range (past carbon) and makes difficult reduction reactions possible. For this reason, mercury remains the material of choice in these potential regions. However, the reduction of dissolved oxygen, a very facile reaction on mercury over a wide potential range, does not occur until well into the negative-potential region on a glassy carbon electrode. Thus, the oxygen overpotential of glassy carbon is much better than that of mercury (Figure 2.2) and precludes the need for oxygen purging at moderately negative potentials.

Figure 2.2. Hydrodynamic voltammogram for oxygen on glassy carbon and mercury electrodes.

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Not all electrode materials will withstand solvents. All carbon-paste formulations are limited to varying degrees (usually not more than 10% organic solvent), depending on the graphite binder. Glassy (vitreous) carbon, platinum, and mercury (amalgamated gold) are far more resistant to organic solvents.

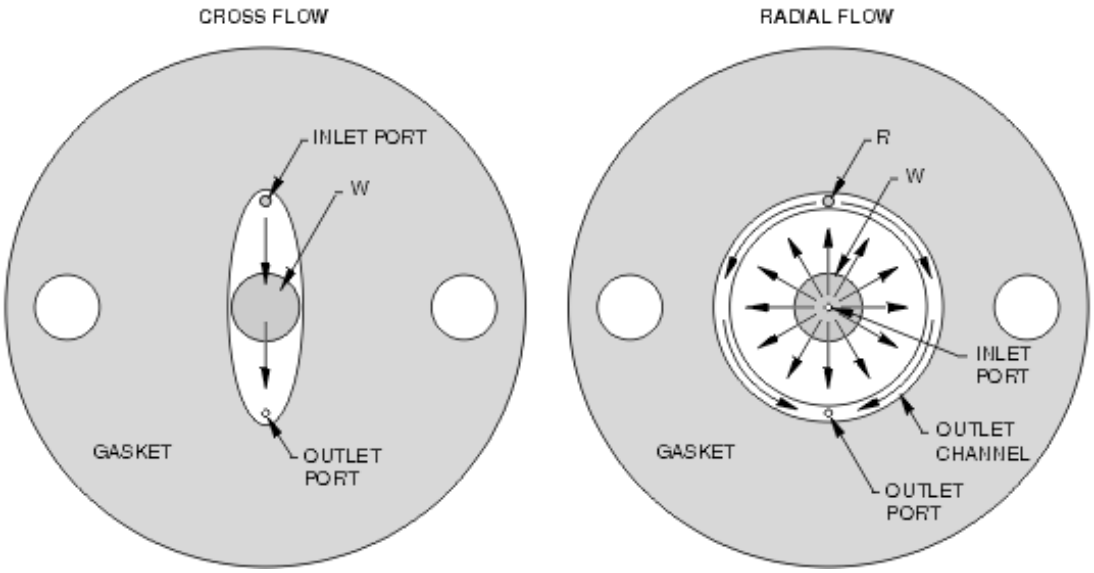
All electrode materials require some surface conditioning or modification before they stabilize to a constant background current level. The conditioning process is observed as a slowly decaying current output from the detector after the electrode is turned on. This may take only a few minutes for an electrode that has been switched off momentarily to as long as a few hours for a freshly prepared glassy carbon electrode at high negative or positive potentials. Longer stabilization times will be required for high-sensitivity operation.

UNIJET DETECTOR CELL

The UniJet detector is a new addition to the BAS line of amperometric detectors for liquid chromatography. The detector has been designed with microbore chromatography in mind. Due to the stringent requirements of minimal dead volume in microbore systems, the UniJet detector was designed as the end fitting of the SepStik microbore column. In order to keep the overall size and the internal volume to a minimum, a radial flow pattern was used (Figure 2.3; this is not wall-jet hydrodynamics).

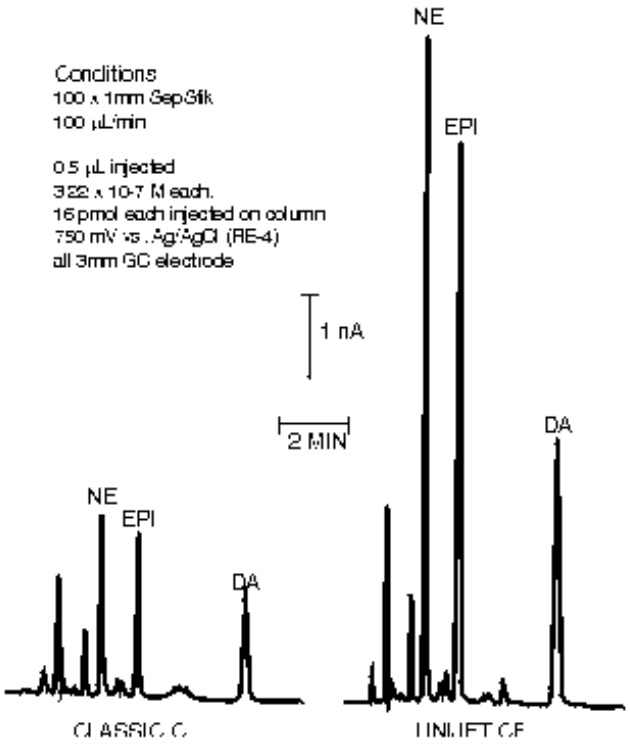
Figure 2.3. Flow patterns for cross-flow and radial cells.

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In comparison to the more traditional approach of a cross-flow cell, the radial flow cell gives improved response at microbore flow rates (< 200 mL/min) and less dilution of the sample before the detector. Figure 2.4 illustrates the improved response for the UniJet cell compared to the classical BAS detector cell under microbore conditions. In addition to the above improvements, the radial flow profile allows for more rapid equilibration of the electrode. The UniJet detector cell does allow for a variety of reference electrodes used without a liquid junction (salt bridge).

Figure 2.4. Comparison of chromatograms from BAS classic and UniJet cells at low flow rates.



LEAVING THE ELECTRODE "ON"

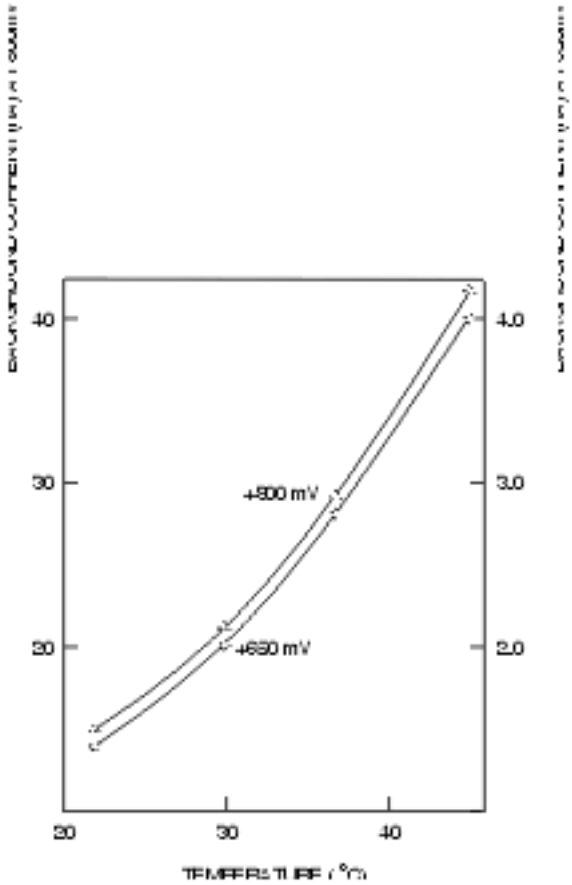
If the LC system is being used on a daily basis, the electrode can be left "ON" continuously. Make sure you have plenty of mobile phase in the reservoir, or route the outlet from the LCEC cell to the reservoir so you recycle the mobile phase until you return. The startup time the following morning is substantially reduced.

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TEMPERATURE-STABILIZED OPERATION

Thermostatted control of the eluent temperature before it reaches the cell reduces the effects of ambient temperature change on electrochemical detection. Furthermore, temperature control of the column protects the separation (peak retention and width) from the effects of temperature change, and when the LC-23C cartridge column heater is used with the cross-flow cell package, the distance between the heated column and cell inlet is quite small and there will be sufficient temperature control to minimize effects on detection. However, in extreme environments (e.g., lab temperature changes due to automated heating/cooling shutdown after business hours, or proximity to vents, ducts, or drafts) or at high detector gain, small fluctuations in temperature at the detector cell can still produce marked deviations in the detector baseline. In these cases, both a column heater and a cell preheater may be needed. Figure 3.1 shows plots of EC detector background current versus temperature. The slope of the curve (di/dT) is significant, typically 0.5 1.5 nA per °C. Hence it is easy to see how a small change in eluent temperature (e.g., 0.1 °C) could still cause appreciable shifts in the baseline.

Figure 3.1. Plot of electrochemical background current versus mobile phase temperature on a glassy carbon electrode operated at 650 and 800 mV applied potential.



What phenomena are responsible for this dependence? The background current in electrochemical detection derives from several contributions, the majority component being the oxidation or reduction of the solvent itself.

For water, the reaction is sluggish at moderate potentials; this is due to poor heterogeneous electron-transfer kinetics at the electrode surface. Elevations in temperature increase the heterogeneous rate constants, and the background current (the measure of the rate of electron transfer) correspondingly increases. From a noise standpoint, if we must operate at

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elevated temperature, we must do so precisely. In many cases, only a small rise over ambient is a good compromise. In doing so, one gains control of environmentally induced baseline drift without fighting large temperature coefficients.

Elevated temperatures similarly affect the magnitude of the peak current. It is not unusual to increase peak currents 50 70% (over ambient) by elevated-temperature operation. Although the temperature dependence of diffusion coefficients alone cannot explain this, it is probable that the diffusion layer thickness decreases as the viscosity drops. The concentration gradient at the electrode surface is accentuated, and the end effect is larger peak currents.

Taken separately, the trends in both background current and peak current versus temperature are inadequate in predicting the effect, if any, on detection limits. When the pertinent data are properly expressed in terms of the signal-to-noise ratio, the improvement is not so dramatic. For example, operation at 55 °C requires more vigorous temperature precision than at 35 °C. Thus, a 1.8-times increase in peak current is counterbalanced by a 23-times increase in baseline noise. A small increase in temperature (35 °C vs. ambient) makes the most sense in terms of signal/noise (Table 3.1).

Table 3.1. Signal and Noise vs. Temperature Setpoint on CC-5 Preheater Module. Conditions: 2.2 mL/min, +750 mV/GC/RE-4 reference, reversed-phase ion-pair separation, norepinephrine is test solute.

Temperature	Peak Height	Noise	SNR
ambient	1.9	0.1	19
35	2.7	0.1	27
45	3.7	0.2	18
55	4.9	0.25	20

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OXIDATIVE MODE LCEC

The oxidation of a compound involves the electron transfer from a molecule to the electrode surface. The two major advantages of most oxidative applications are (1) that oxygen is not electrochemically active, and (2) that solid electrodes can be used. Of course, both of these are definite advantages in the LCEC experiment as well. The importance in removing oxygen for reductive electrochemistry was discussed in the BASIC PRINCIPLES Section, and the LC system modifications required for these determinations will be discussed in the REDUCTIVE MODE Section.

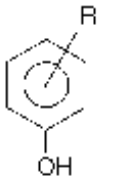
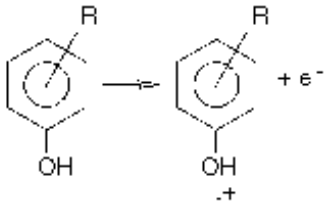
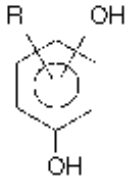
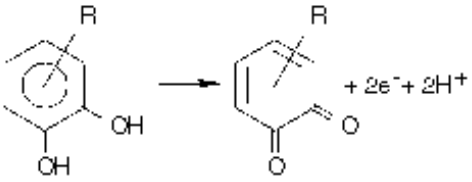
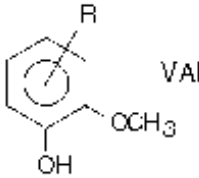
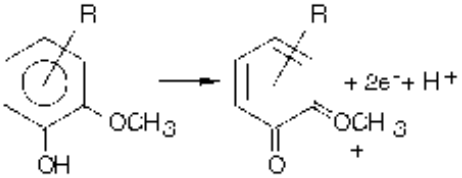
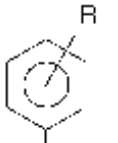
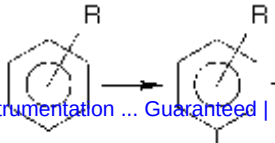
The working electrode materials most commonly used are based on a carbon matrix, typically an anisotropic solid such as glassy carbon. Obviously, other materials exist and can be used for the LCEC determination. For example, gold, nickel, silver, platinum, and a thin mercury amalgam can all be used for specific measurements.

This section will primarily deal with typical functional groups that can be oxidized, but the list generated below is by no means exhaustive. The only conclusive way of knowing if a compound is electrochemically active is by a technique such as cyclic voltammetry. Even with our experience, we cannot always guess at how a specific compound may behave. A voltammetric assessment can provide information about the reversibility of the reaction as well, which can influence a decision on which mode of LCEC to use (e.g., Dual Series). In general, for electrochemical oxidation to take place, one can look for the same features and reactive centers as one would for a homogeneous oxidation reaction (e.g., delocalized electrons, stability of product, etc.). This does not mean that if a homogeneous chemical reaction does take place then this is directly applicable to the heterogeneous electrochemical reaction. The correlation is often thought to occur, and is many times an incorrect assumption.

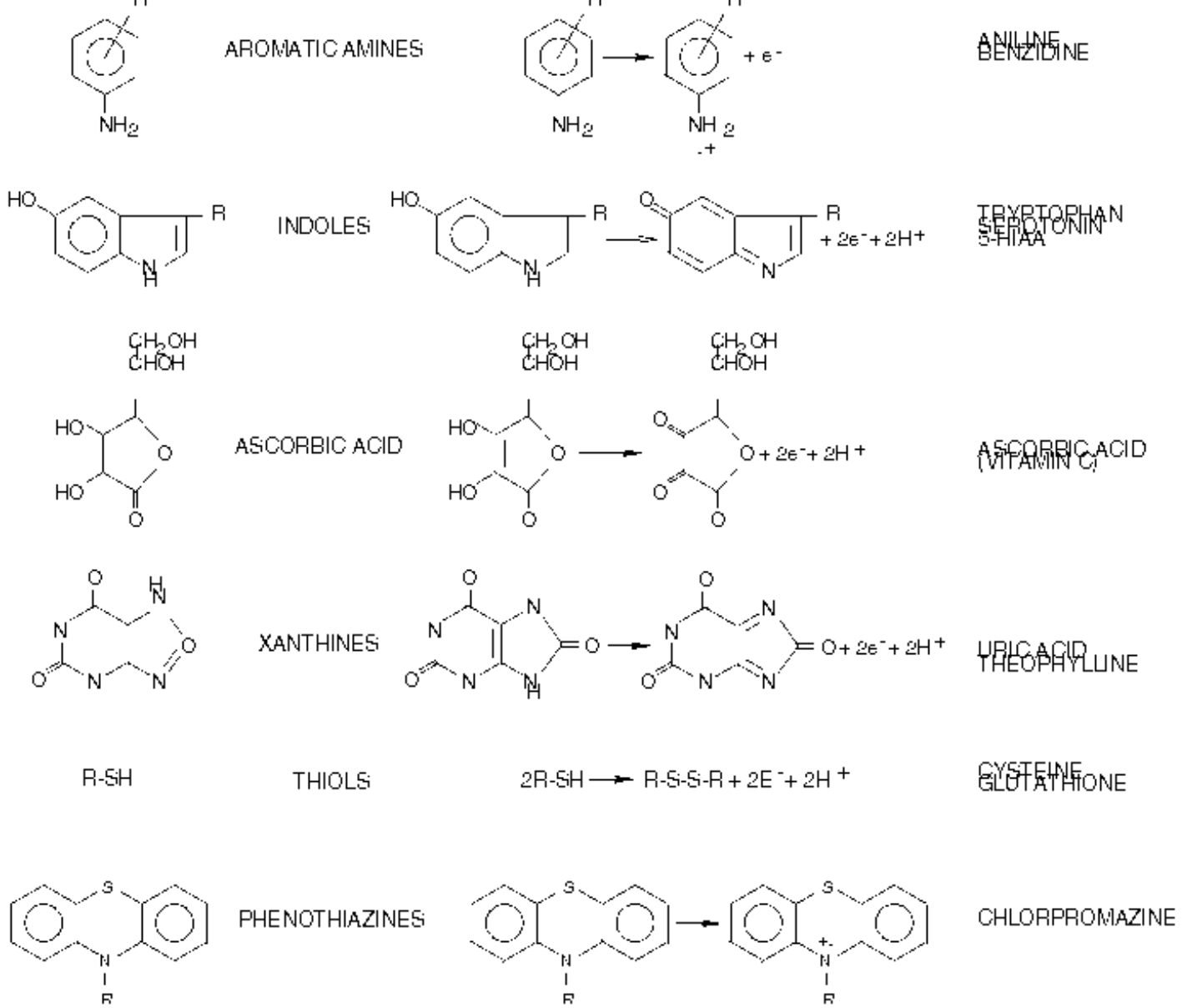
The listing in Table 4.1 is not all-inclusive but only indicates some typical functional groups and compounds that are known to be electrochemically active. Later sections in this manual contain additional information based on cyclic voltammetry studies. If you have any questions regarding the electrochemical activity of your particular compound under a given set of conditions, it is best to do the voltammetry experiment.

Laboratories that are often investigating new compounds would find a BAS cyclic voltammetry instrument or the BAS 100B Electrochemical Analyzer a worthwhile investment.

Table 4.1. Functional Groups Suitable for Oxidative Electrochemical Detection.

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	PHENOLS		PHENOL PENTACHLOROPHENOL DOPAMINE TYROSINE
	HYDROQUINONES		CATECHOLAMINES
	VANILLYL COMPOUNDS		HOMOVANILLIC ACID VANILMANDELIC ACID FERULIC ACID
	AROMATIC AMINES		ANILINE BENZIDINE

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REDUCTIVE-MODE LCEC

This section details specific liquid chromatographic procedures for reductive mode LCEC. The section is divided into five parts:

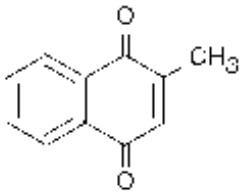
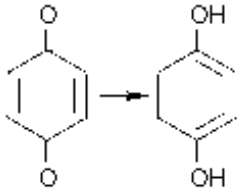
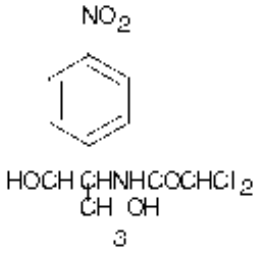
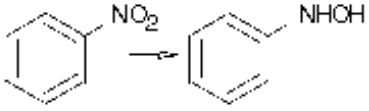
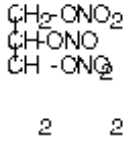
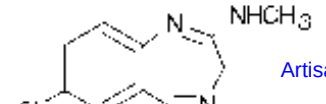
- **TYPICAL FUNCTIONAL GROUPS**
- **SYSTEM MODIFICATIONS FOR REDUCTIVE LCEC**
- **PROCEDURES FOR DEGASSING THE MOBILE PHASE**
- **PROCEDURE FOR DEGASSING SAMPLE**
- **GLASSY CARBON vs. MERCURY/GOLD ELECTRODES FOR REDUCTIVE LCEC**

The preparation of both glassy carbon and mercury/gold electrodes is described in detail in the section on WORKING ELECTRODES. Users with questions concerning the cell itself should read this material first.

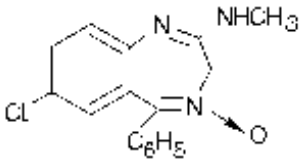
TYPICAL FUNCTIONAL GROUPS

By far the majority of the LCEC literature deals with oxidative mode electrochemical detection, but considering strictly the electrochemical literature of organic compounds, reduction processes have been examined in much greater detail. Table 5.1 describes some functional group candidates capable of being analyzed by reductive LCEC. Note that within a given functional group, a broad range of reduction potentials may exist due to the effects of substituent groups. Generally, the more delocalized the electrons become, the more easily reducible the substance. In addition, electron-withdrawing groups on an aromatic ring will enhance the reduction reaction.

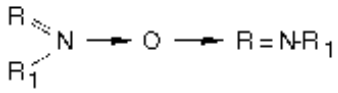
Table 5.1. Functional Groups Suitable for Reductive Electrochemical Detection.

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	QUINONES		VITAMIN K3
	AROMATIC NITRO		CHLORAMPHENICOL
	ALIPHATIC NITRO		NITROETHANE NITROGLYCERIN
	ORGANOMETALLICS		TRIPHENYLLEAD CIS-PLATINUM
	N-OXIDES		CHLORDIAZEPOXIDE

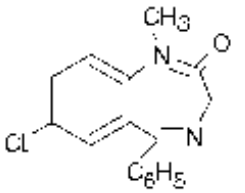
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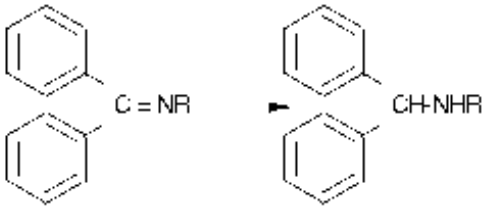
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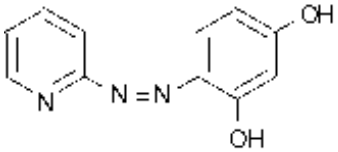
CHLORDIAZEPOXIDE



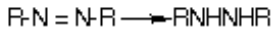
AZOMETHINE



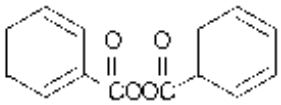
DIAZEPAM
NITRAZEPAM



AZO COMPOUNDS



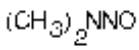
(4-(2-PYRIDYLAZO)RESORCINOL)



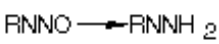
PEROXIDES



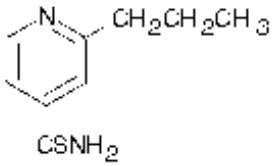
BENZOYL PEROXIDE



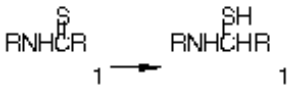
NITROSAMINES



DIMETHYLNITROSAMINE



THIOAMIDES



PROTHIONAMIDE

SYSTEM MODIFICATIONS FOR REDUCTIVE LCEC

Reductive mode LCEC requires some mechanical modifications to remove dissolved oxygen from both the mobile phase and sample; it is just as necessary to make sure oxygen does not reenter the system. Oxygen is removed from the mobile phase and sample by bubbling an inert gas (e. g., helium) through the solutions. Refluxing the mobile phase at the same time will thoroughly deoxygenate the system. A mechanical arrangement such as that shown in Figure 5.1 can be set up and dedicated to a specific LC system for this purpose. Table 5.2 details specific parts needed for this modification to modular BAS LCEC systems.

Figure 5.1. Deoxygenation apparatus for LCEC; both mobile phase and sample are deoxygenated.

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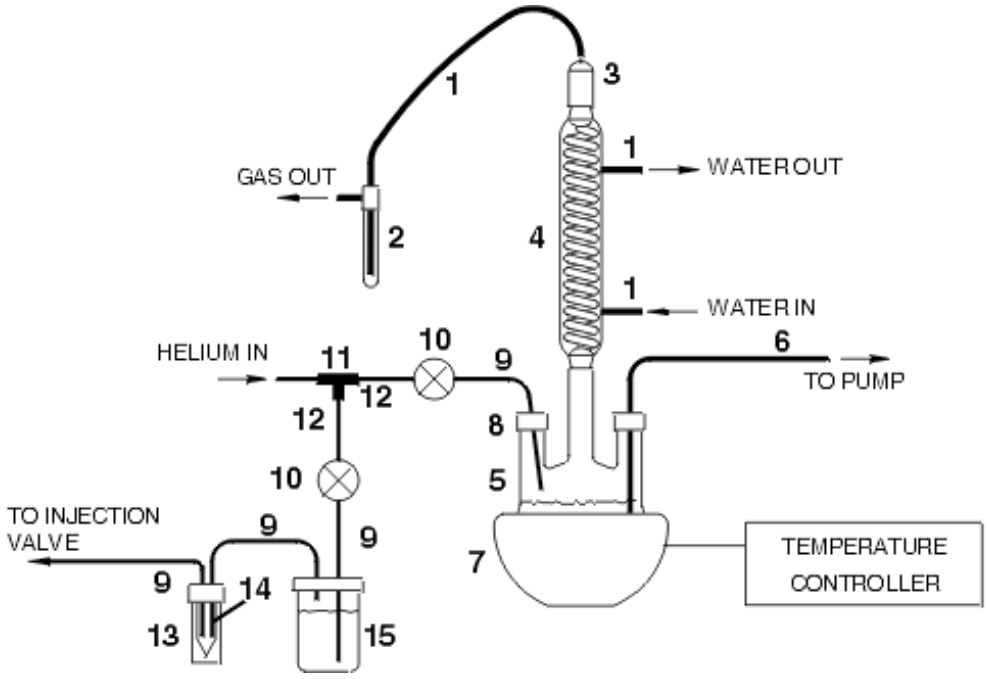


Table 5.2. Parts list for reductive LCEC.

Ref.	Description	Source
1	Connecting plastic tube	Common Lab Supplies
2	Gas-trap bubbler	Reliance Glass
3	Condenser adapter	Reliance Glass
4	Condenser	Reliance Glass
5	3-neck, 1 liter, round-bottom flask	Kimble
6	Stainless steel inlet tube for pump Model PM-80	BAS
7	Heating mantle	Scientific Products
8	Rubber septum	Common Lab Supplies
9	1/16" o.d. stainless steel tubing (when attaching to valve you may need extra fittings)	BAS
10	Needle valve	Swagelok
11	Tee to He line	Swagelok
12	1/8" o.d. copper tubing	Common Lab Supplies
13	Sample vial with rubber septum	BAS
14	Vent needle	Scientific Products
15	Gas humidifying chamber	Common Lab Supplies

All plastic tubing in the system must be replaced with stainless steel, because most plastic tubing is permeable to oxygen (PEEK tubing is less permeable than most, and may be an acceptable substitute for stainless steel). A one- or two-liter round-bottom flask with reflux condenser and heating mantle serves as the mobile phase reservoir. One neck is used for the condenser, and the other two necks for the helium inlet and pump outlet. Ordinary laboratory grade helium supplied at ca. 20 psi is tapped at two separate needle valves for independent control of both solvent and sample degassing. A 1/8"-o.d. stainless steel outlet line runs to the inlet check valve of the pump. Large rubber septa seal off the mobile phase from the outside environment. Temperature of the heating mantle can be controlled electronically.

Samples are degassed with the second needle valve. An optional helium saturation chamber filled with water wets the gas thoroughly before it enters the sample container.

The outlet tube from the column to the detector must be replaced with a special steel connector (P/N MF-1029). No plastic tubing may be used! This connector uses special plastic end fittings to electrically isolate the cell from the rest of the LC system (which is grounded). The steel tubing prevents gas intrusion. If you are using a preheater module, the steel tubing in this is appropriate.

Modifications are now completed for reductive LCEC.

If you think this seems like a lot of preparation, you are correct. After the initial setup, a system like this can be fairly reliable for reductive LCEC work, but it has limitations. For example, no gradient elutions can be run under these conditions. For customers requiring a full-featured liquid chromatograph with solvent deoxygenation capabilities, the BAS 200e Analyzer is the instrument of choice.

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PROCEDURE FOR DEGASSING THE MOBILE PHASE

The following chromatographic startup procedure is recommended for reductive LCEC applications. Before initiating flow through the LC system, heat the mobile phase to 30 35 °C and bubble the inert gas rather vigorously through it for 30-60 minutes. Start pumping the mobile phase through the LCEC system during the last 15 minutes of vigorous bubbling. Even though the mobile phase is degassed, the entire system is not. Oxygen penetrates the stationary phase pores, and it must be flushed out of these pores by initiating the flow of degassed mobile phase through the LC system. This may take several hours.

Degassing of the system requires at least 100-200 mL of preheated mobile phase. Be liberal and flush thoroughly. Turn on the working electrode after flushing and keep the detector at the lowest gain (highest RANGE setting) until the background current has stabilized. Reduce the flow of helium through the mobile phase. Some flow must be maintained to keep the oxygen out, but this need not be as vigorous as during the initial deoxygenating. If the baseline response (background current) begins to gradually increase, the rate of bubbling inert gas through the mobile phase is insufficient to keep the oxygen out of the system and must be increased until the background stabilizes. NOTE: Valuable time can be saved if degassing of the system is performed overnight at a minimal flow rate (0.2-0.3 mL/min). You can turn on the working electrode before leaving the lab in the evening to provide you with a stable system in the morning.

PROCEDURE FOR DEGASSING SAMPLES

Sample degassing is necessary when working at potentials more negative than 0 to -0.1 V for mercury/gold and -0.3 to -0.4 V for glassy carbon electrodes, as illustrated by hydrodynamic voltammograms in Figure 2.2. The potentials at which the electrodes will be insensitive to dissolved oxygen may vary, depending on the pH, concentration and type of nonaqueous solvent, and the previous history of the electrode surface.

Care must be taken while degassing a sample in order to preserve its original composition. This is extremely important when handling volumes smaller than 500 mL. Presaturating helium gas with mobile phase and maintaining a gentle flow of helium gas through a sample will minimize evaporation of a sample. A presaturation device is pictured in Figure 5.1.

To degas, pass helium into the sample for about 3-5 minutes. This should be regulated at a flow rate as vigorous as allowed by the sample volume (smaller volumes will have to be degassed more gently than larger ones).

To inject, it is best to draw the sample slowly into the injection loop by gentle suction. Exposure to the atmosphere is avoided, and the integrity of the closed system, particularly at the needle seal, is preserved. On BAS chromatographs, you would immerse the waste port of the injection valve into the deoxygenated sample, and fill the loop by aspiration with a syringe mounted in the front (sample injection) port.

GLASSY CARBON vs. MERCURY/GOLD ELECTRODES FOR REDUCTIVE LCEC

Both glassy carbon and mercury/gold electrode surfaces are useful in reductive LCEC. Although mercury is usually the surface of choice with electrochemists, there are fewer reasons for using it in LCEC. In polarography, mercury provides a repeatable, fresh electrode surface, a high hydrogen overvoltage (overpotential), and fast setting times. In liquid chromatography, however, dead volume must be minimized, thereby obviating the use of the usual dropping mercury electrode. For reductive LCEC, the optimal approach is to employ a thin-layer cell. The dropping mercury electrode is replaced by a glassy carbon surface or a mercury film.

To select the proper electrode, follow these guidelines:

1. For reductions requiring potentials between +1.00 and 0.90 V (vs. Ag/AgCl), try glassy carbon first. Glassy carbon offers better long-term stability than mercury/gold. Background currents should be less than 100 nA throughout this range. Usable performance may be obtained at more negative potentials, depending on the conditions.

2. For reductions at potentials between 0.90 and 1.1 V, a freshly prepared mercury/gold surface will probably be necessary for sufficient hydrogen overvoltage protection. Do not expect subpicomole detection limits, however! Remember, at these applied potentials, you are dealing with a high-energy situation (not unlike low-wavelength UV detection). Everything will be noisier and your detection limits will be hindered because of it.

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3. Some applications will intimately involve the surface chemistry of one electrode material, thereby favoring that material. For example, although this is not an electrochemical reduction, the detection of sulfhydryls on mercury occurs at a potential about 600 mV less positive than on carbon due to the following mechanism:

$$Hg + RSH \rightarrow HgSR + e^- + H^+ \qquad E_p = +0.2 \text{ to } +0.4 \text{ V}$$

$$2\ HgSR \rightarrow Hg + Hg(SR)_2$$

It is the complex of mercury and thiol that is actually undergoing the oxidation.

On carbon, it has been shown that disulfides are the usual product, with the reaction taking place at a much higher applied potential:

$$2\ HgSR \rightarrow RSSR + 2H^+ + 2e^- \qquad E_p = +1.0 \text{ V}$$

4. If permitted under the constraints of guidelines 1 3 above, use glassy carbon for longer service lifetimes. A mercury/gold amalgam is a solid solution at the interface between the gold substrate and the thin mercury film; eventually the gold will diffuse through the film to the surface. The advantageous hydrogen overvoltage will eventually vanish.

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- **CONTENTS**

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INTRODUCTION AND SPECIFICATIONS

This manual explains the installation and operation of the PM-80 Pump, as well as procedures for routine maintenance and troubleshooting. Read the entire manual prior to installation and start-up.

The PM-80 is a reciprocating, dual-piston unit. It is designed for precise and reliable solvent delivery for isocratic and (optional) gradient elution at flow rates between 0.01 and 5.0 mL/min. Advanced features of the PM-80 pump include:

- Type 316 stainless steel tubing throughout.
- A microprocessor-controlled, computer-designed, dual-piston pump. Each head is removable as a single, precision-engineered assembly, which may be interchanged in seconds. The construction of the pump is solid (cast aluminum and machined 316 stainless steel); no sheet metal is included. The end result is greater durability, mechanical reliability, and serviceability.
- Solid-state pressure transducer, to monitor system backpressure in real time.
- Pulse damper, installed as a standard feature for use at high sensitivity.
- Purge valve, for bypassing the column and injector while flushing the solvent delivery system.
- High- and low-pressure limit controls, for safety.
- Analog output for pressure monitoring.
- Provision for remote-control operation.
- Optional ternary gradient capability. Contact BAS for information on upgrading your isocratic PM-80 to a gradient system.

The PM-80 has been engineered for durability, and with proper maintenance should provide years of reliable service.

SPECIFICATIONS

HEIGHT	6.6 inches
LENGTH	17 inches

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WIDTH	11 inches
WEIGHT	38 pounds
FLOW RATE	0.1 - 5.0 mL/min
FLOW PRECISION	better than 0.3% RSD
FLOW REPEATABILITY	0.3%
PRESSURE RANGE	0-6000 PSI (0-40 bar) with selectable limits
INPUT VOLTAGE	100/120/220/240 vac 50/60 Hz
POWER	3A (max) @ 120 VAC 50/60 Hz

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PM-80 PUMP INSTALLATION

- **UNPACKING**
- **IDENTIFICATION OF PARTS**
- **LOCATION IN THE LAB**
- **POWER REQUIREMENTS**
- **FLUID CONNECTIONS (ISOCRATIC)**
- **FLUID CONNECTIONS (GRADIENT)**
- **COMMUNICATIONS**

UNPACKING

Please retain the shipping box and packing materials until the unit has been fully tested. The shipping materials will be needed if you discover damage incurred during shipping.

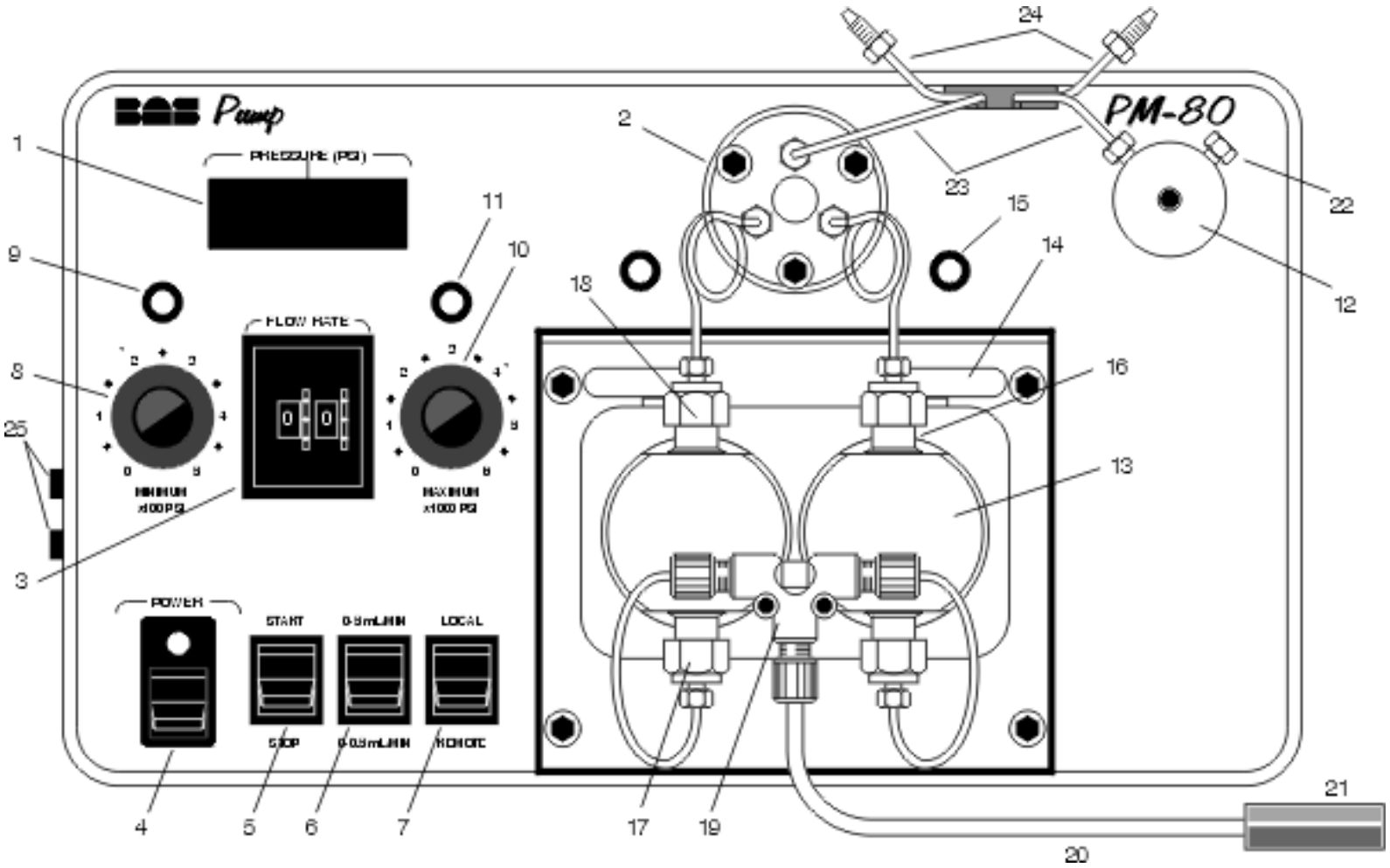
The shipping box should contain the following items:

- PM-80 Pump
- PM-80 Accessories (includes: power cord, 1/8" PTFE inlet lines with fittings, 50 mL syringe).
- PM-80 Manual
- Optional Gradient Controller

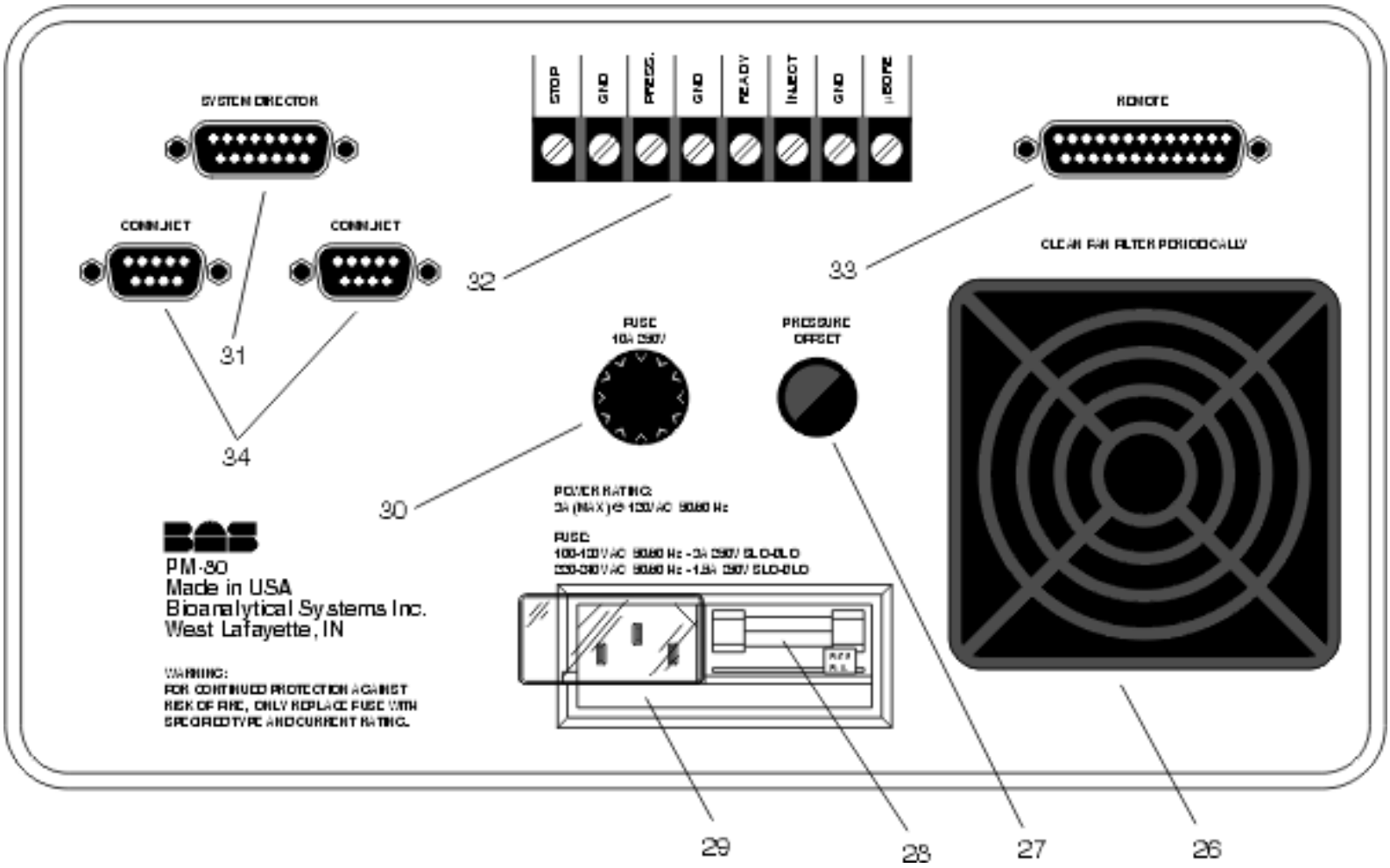
If any discrepancies exist, retain the packing slip and contact BAS Customer Service for assistance.

IDENTIFICATION OF PARTS

The following two figures show front and back views of the PM-80 pump. The parts identified by number are described below.



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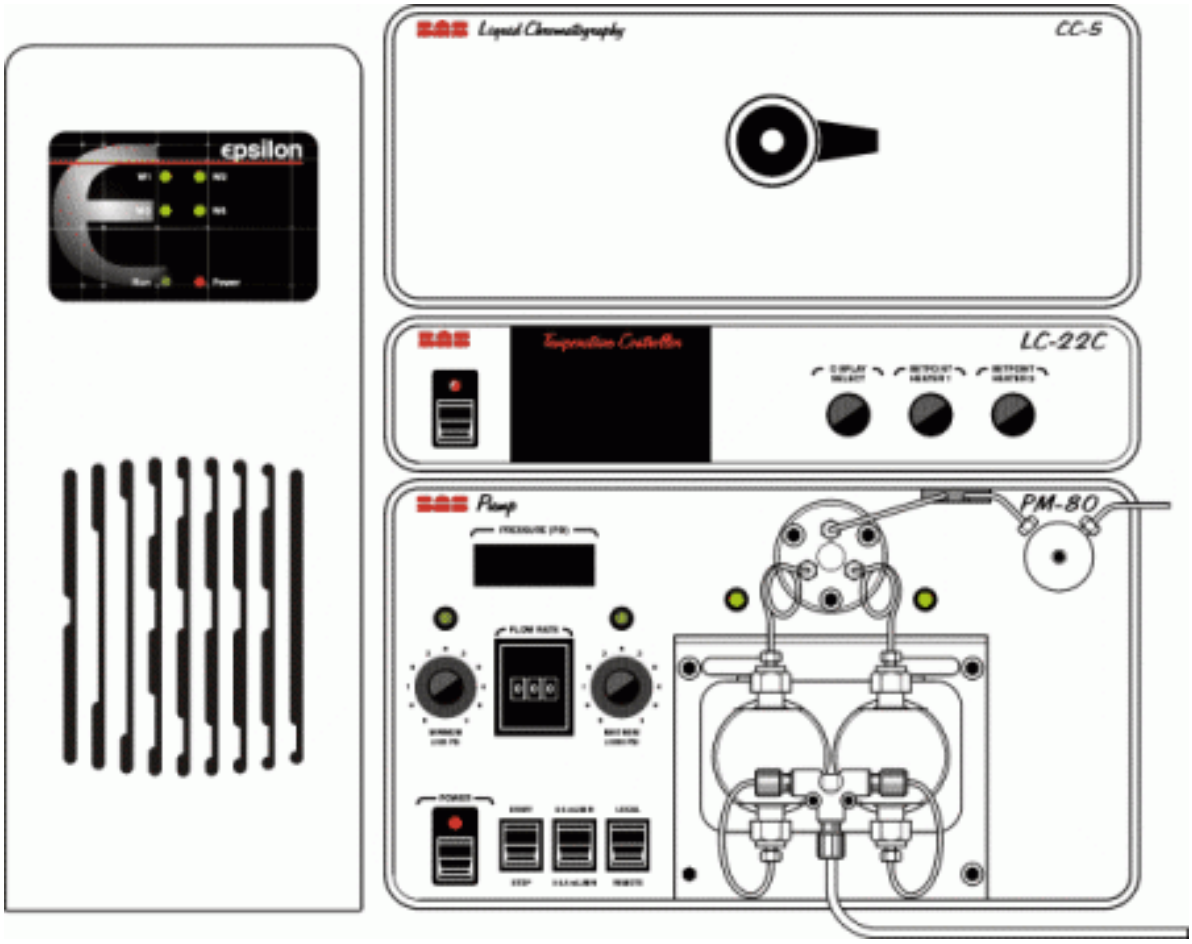
1. Pressure display
2. Pressure transducer
3. FLOWRATE control
4. POWER switch
5. START/STOP switch
6. FLOWRANGE switch
7. LOCAL/REMOTE switch
8. MINIMUM pressure (low limit)
9. Minimum pressure warning LED
10. MAXIMUM pressure (high limit)
11. Maximum pressure warning LED
12. Prime/purge valve
13. Pump head
14. Pump-head release lever
15. Pump-head indicating LED
18. Outlet check-valve assembly
19. Inlet tee
20. Solvent-uptake line (Isocratic version)
21. Solvent-uptake frit (Isocratic version)
22. Outlet port to injection valve
23. Connections to pulse damper
24. Optional connections to mixer
25. Solvent intakes (gradient version)
26. Air filter
27. Offset adjustment for pressure output
28. Voltage selector
29. Power input
30. Fuse
31. LCD Controller connector
32. Terminal strip

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16. Plunger-irrigation port (hidden)
17. Inlet check-valve assembly
33. REMOTE connector
34. BAS Instrument Control connectors

LOCATION IN THE LAB

The PM-80 pump is designed to serve as the base for a stack of BAS chromatography instruments. A typical setup is illustrated by the BAS-480e chromatograph. The components of this system are (from the bottom up) the PM-80 pump, LC-22C temperature controller, and CC-5 flowcell compartment. The epsilon LC detector is on the left.



Location considerations for the pump must, of necessity, include the requirements for all the component instruments in the stack. Use the following guidelines:

- Provide a surge-free power source which can be dedicated to the chromatograph. Other laboratory instruments such as ovens, vortex mixers, centrifuges, and large motors may cause spikes in the power supply.

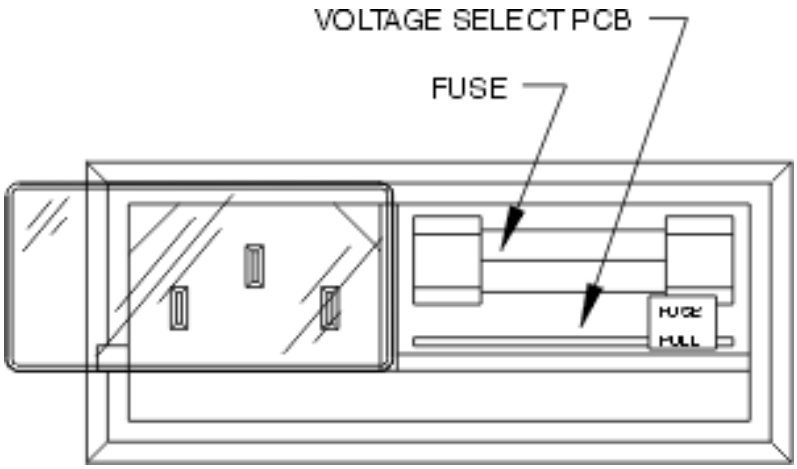
- Ensure that all components of the chromatograph share the same ground circuit. This can best be accomplished by plugging all components into a multi-outlet power strip. Plugging the components into independent outlets can produce ground loops (current that flows between ground circuits at slightly different potentials) which can produce baseline noise.

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- Locate the chromatograph on a stable bench. Vibrations can hamper the performance of any sensitive instrument.
- Select a room where temperature remains stable throughout the day. Avoid installing the chromatograph near windows, air ducts, ovens, or refrigerators.
- Place the chromatograph away from busy, congested areas. Remote, isolated areas are best for high-sensitivity work.
- Avoid very dry areas and areas that are carpeted. Static electricity can affect instrument performance. Anti-static floor mats and bench mats are useful if spiking caused by static charge is a problem.
- Avoid areas where radio-frequency interference is likely. Beeper-type paging devices can be a problem in some installations.

POWER REQUIREMENTS

The power cord attaches to the PM-80 via the receptacle on the rear of the instrument. The pump can be operated with either 100, 120, 220, or 240 VAC and 50 or 60 Hz power, but the correct voltage must be selected before use at the cord connector:

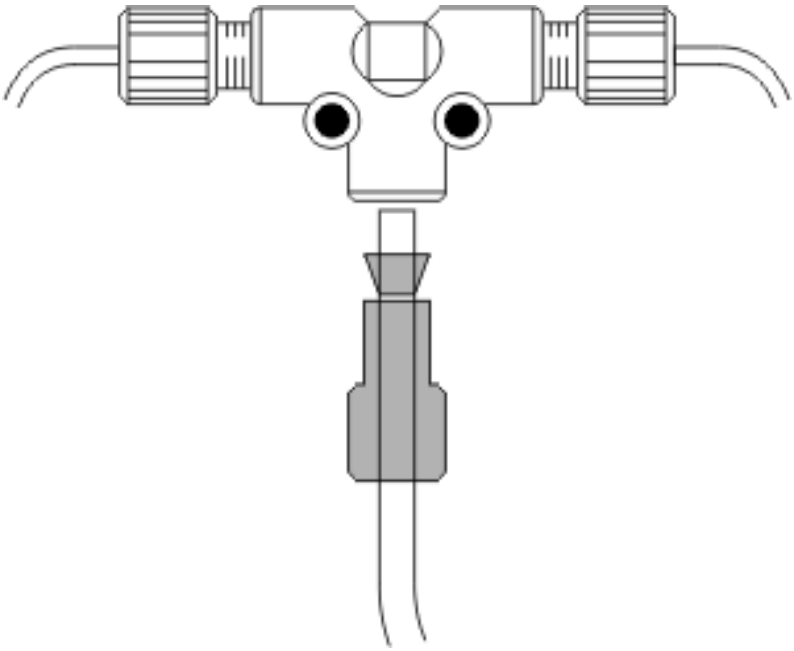


Should the power option need to be changed, unplug the line cord and slide the plastic window to the left. The orientation of the small circuit board now exposed in this socket determines the voltage option. If the voltage labeled on the outer edge of this board is not that required, pull out the board and turn it such that the desired voltage is readable. Reinsert the board and push the fuse holder back into the cavity. Also check to see that the fuse is the proper rating:

Voltage	**Fuse**
100-120V	3 A/SB
220-240V	1.5 A/SB

FLUID CONNECTIONS (ISOCRATIC)

The PM-80 is factory plumbed, and ready as received for final connection of the inlet and outlet solvent lines. Unwrap the PTFE tubing carefully and remove the protective cover from the solvent-uptake frit. The uptake tubing connects to the tee at the pump head with a flangeless fitting. Be sure to install this with the flat face of the ferrule entering the tee:



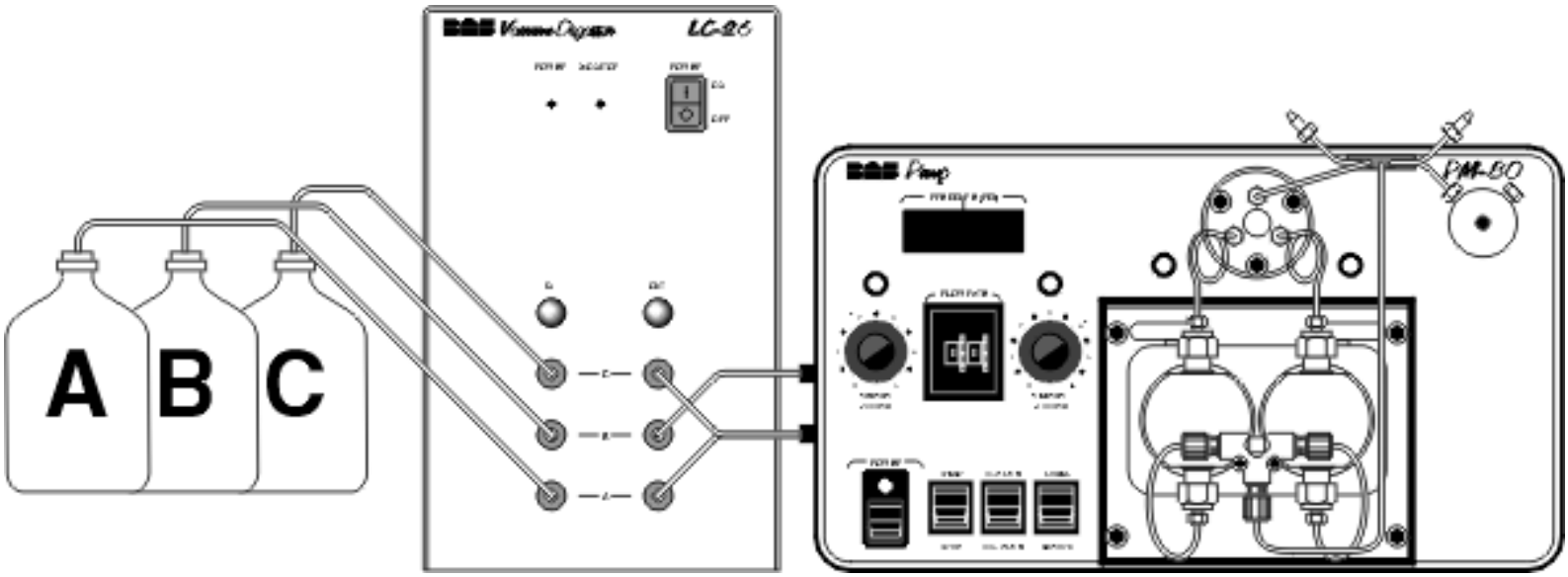
Connect a solvent-outlet line between the prime/purge valve and the pump input of your injection valve. A nut and ferrule are provided for connection to the prime/purge valve. We recommend 1/16" OD, 0.015" ID, #316 stainless steel for this line. An in-line filter assembly (MR-4135) is recommended between this tubing and the injection valve.

Installation is now complete. Refer to the STARTUP section to begin purging the pump.

FLUID CONNECTIONS (GRADIENT)

The gradient version of the PM-80 pump differs from the isocratic version in the number and location of solvent uptake lines, and in the presence of an internal high-pressure mixer.

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Connect the three solvent-uptake lines to the ports marked 'A', 'B', and 'C' on the left side of the pump. Be sure to observe the correct orientation for the flangeless ferrules. The solvent-uptake frits should be placed into three bottles of freshly filtered mobile phase. (For initial startup, cleaning, etc., you may put all three uptake lines into the same bottle of 40:60 acetonitrile:water or other appropriate solution.) For best results, elevate the mobile-phase bottles to provide hydrostatic pressure to the pump.

WE STRONGLY RECOMMEND the use of the LC-26 DEGASSER. Use of an on-line degasser is required for dependable and continuous performance during gradient operation.

The **HIGH-PRESSURE MIXER** is located inside the cabinet, at the front, upper-right side. Four steel tubes emerge from the cabinet at this point: the two labeled 'M' come from the mixer, while the two unlabeled tubes come from the pulse damper. The two uses are mutually exclusive. Isocratic operation will benefit from a pulse damper, and does not require a mixer. Gradient operation does require a mixer, and will be impaired by the large dead volume of a pulse damper.

To convert from isocratic to gradient operation, remove the tubes from the pulse damper at the pressure transducer and the purge valve, and replace them with those from the mixer.

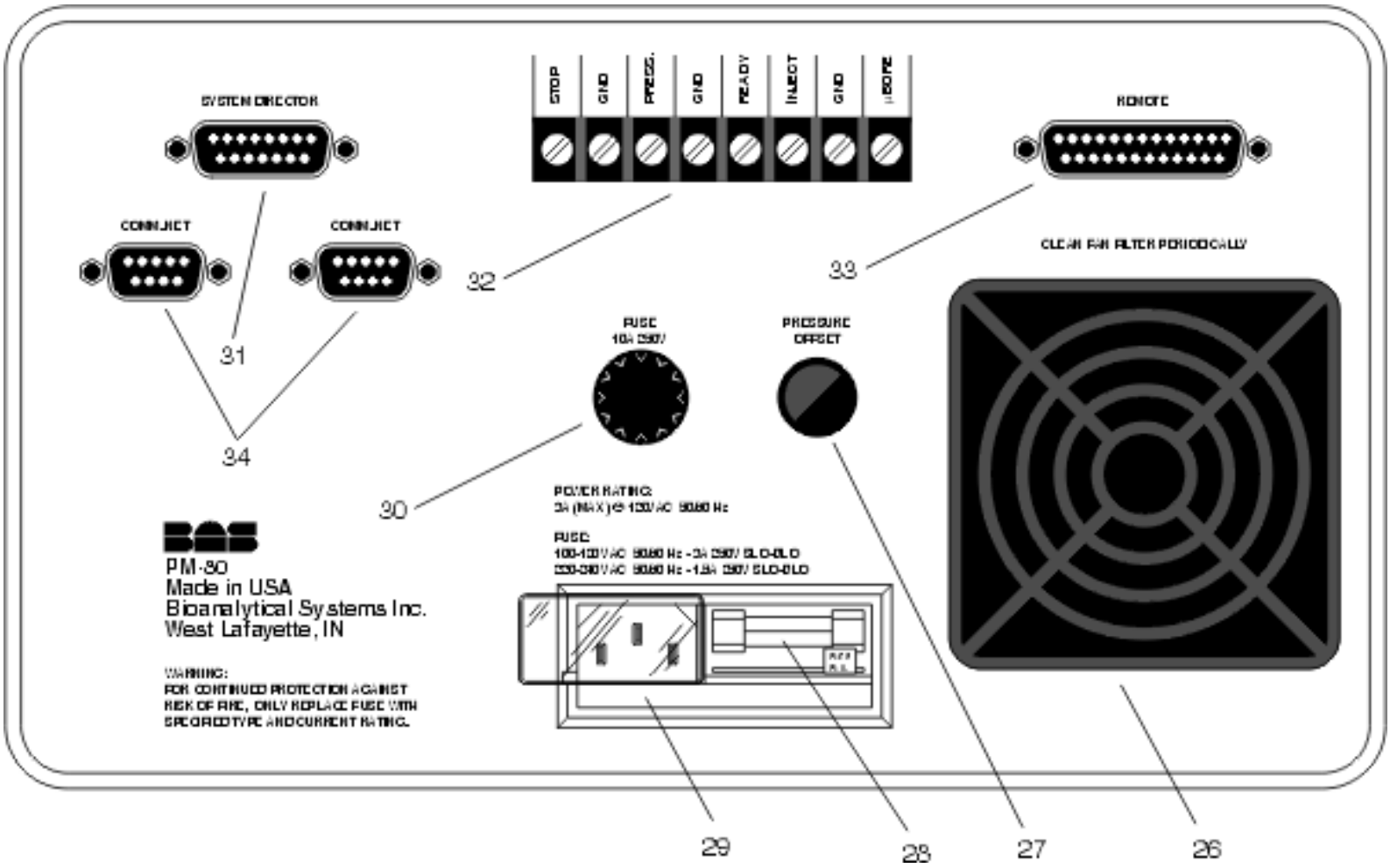
BE SURE TO FLUSH OUT THE PUMP WITH 40:60 ACETONITRILE:WATER BEFORE CHANGING BETWEEN THE MIXER AND THE PULSE DAMPER, so stagnant mobile phase doesn't corrode the unused component.

The mixer motor is software controlled: it turns on whenever a method calls for more than one bottle of mobile phase.

COMMUNICATIONS

Communication with other instruments is accomplished via connections to the rear panel:

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The only connection that needs to be made to an epsilon system is via the **COMM NET** port. Insert the female end of the 9-pin cable provided into either COMM NET port. The other end of this cable is connected to 'PUMP' connector on the rear panel of the epsilon controller. This connection needs to be made only if you wish to control the pump from the computer. Many users prefer to control the pump from its front panel (see the section on Manual Controls).

Additional connections are provided for other applications:

TERMINAL STRIP CONNECTIONS

Pressure. Provides a 1 V per 1000 PSI output to monitor pressure. The output indicates absolute pressure when the offset adjustment is fully counter-clockwise.

To monitor pressure with an epsilon system, connect the 'PRESS.' terminal and its 'GND' as an external detector with a 10V input. Click here for details.

To monitor pump pressure with a chart recorder, first set your chart recorder for an input of 10 V. Connect two wires from the PRESS. and GND connectors on the terminal strip of the PM-80 to the input of the chart recorder. Adjust the PRESSURE OFFSET potentiometer on the rear panel of the PM-80 until the chart-recorder pen is about mid-scale. Now reduce the input voltage of the chart recorder in steps, adjusting the PRESSURE OFFSET control as necessary to keep the pen on the chart. Reduce the input voltage of the chart recorder until the pressure fluctuations can be measured on the chart (a 0.1 V input range should be about right). The analog pressure output produces 1 volt per 1000 PSI. The chart can be calibrated with the following formula:

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P = (1000 x D x V) / W

where:

P = pressure fluctuation in PSI

W = width of chart paper (mm)

D = magnitude of pen deflection (mm)

V = input range of chart recorder (Volts)

STOP. A switch closure or low-level TTL across the STOP and GND terminals will stop the pump when it's in the LOCAL mode

READY. A switch closure or TTL-low across the READY and GND terminals will trigger the start of a timed run. Used to accept a 'ready-to-inject' signal from an autosampler (gradient versions).

INJECT. Sends a six second TTL-low signal at the start of a timed run. Used to trigger an autosampler to inject (gradient versions).

µBore. A switch closure or TTL-low across the µBORE and GND terminals will switch the pump from normal flow ranges (0-5 mL/min) to µbore ranges (0-0.5 mL/min). Used to slow down the flow rate after a series of runs, to conserve mobile phase. For example, a switch closure signal from an autosampler after the last sample has been injected will change a 1 mL/min flow rate to 100 µL/min. WARNING: running at low pressure might decrease plunger-seal life.

REMOTE. This 25-pin 'D' connector is not currently used.

SYSTEM DIRECTOR. This 15-pin 'D' connector is provided for the LCD gradient controller or the DA-5/ChromGraph computer system.

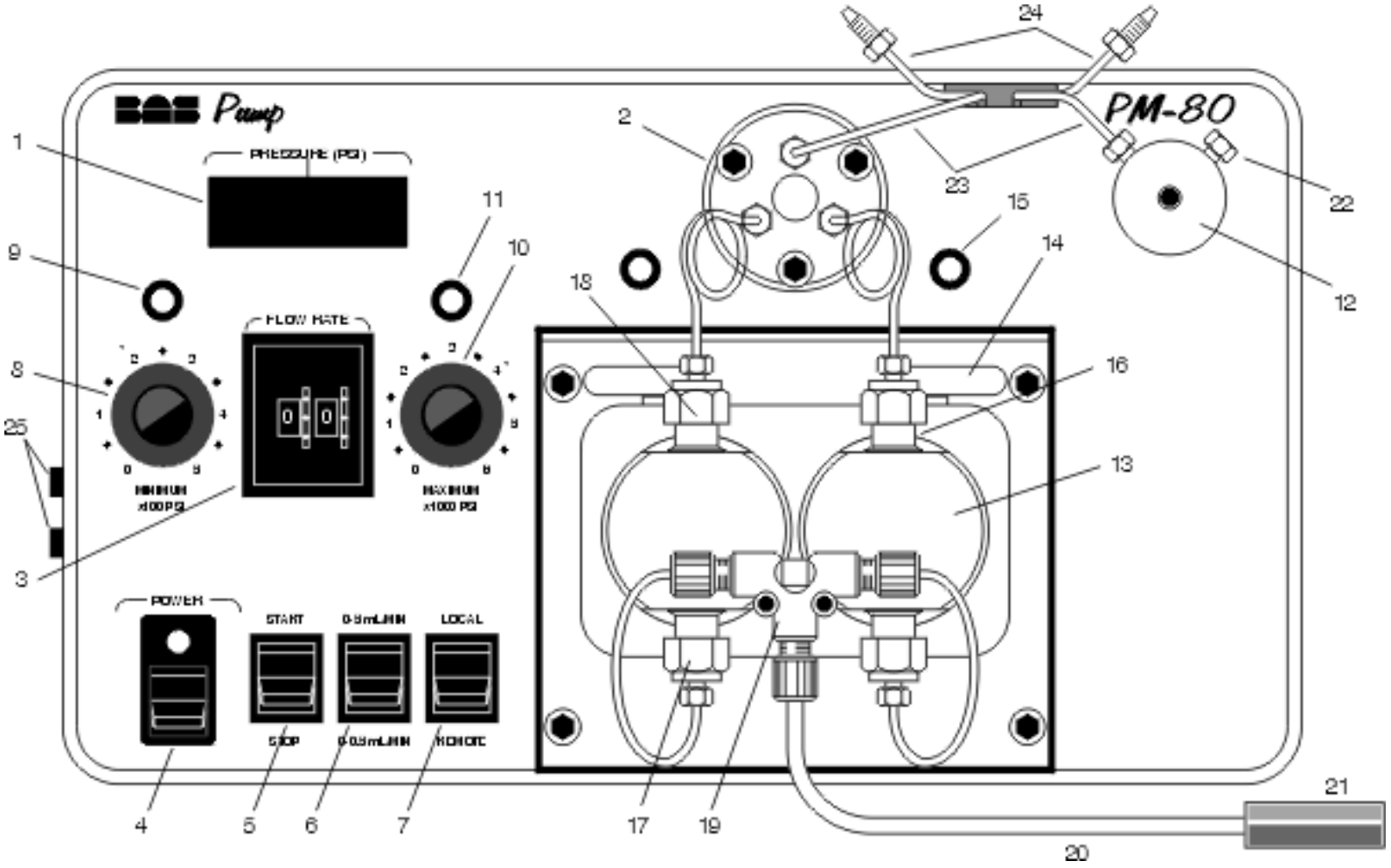
PRESSURE OFFSET. This potentiometer is used to adjust pen position when recording pump pressure.

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MANUAL CONTROLS

The PM-80 pump can be controlled from its front panel as detailed below, by an LCD Controller, or by the pump module of ChromGraph Control software. The front panel allows only isocratic operation.



The following controls are available on the front panel:

POWER. An on/off toggle switch that applies power to the pump. When the power switch is turned on, the microprocessor performs a self test. During the self test the pressure display will alternately flash BAS and the software version number.

START/STOP. A toggle switch that starts or stops the pump. This control is used to start or stop solvent flow without turning off the main power.

LOCAL/REMOTE. A toggle switch that selects between flow control by the front-panel switches or by a remote computer or optional gradient controller.

MINIMUM. A potentiometer that sets the low-pressure limit (in PSI) for pump operation. It is **EXTREMELY IMPORTANT** that this limit be set to some reasonable non-zero value (e.g., 200 PSI, as indicated by the asterisk on the

instrument) during normal operation. If a fitting develops a leak, or if the mobile-phase reservoir runs dry during unattended operation, the low-pressure cutoff will stop the pump as pressure drops. Proper setting of this control will avoid pump damage.

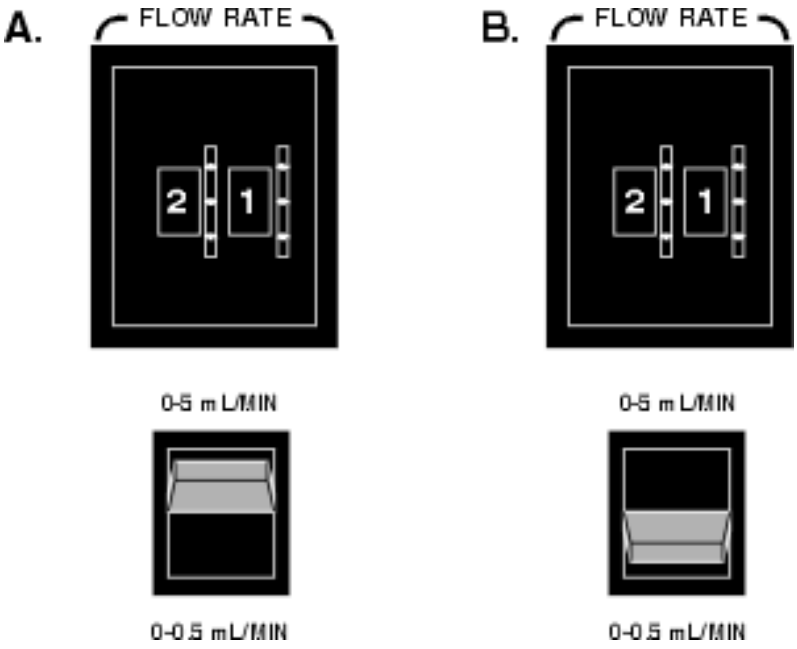
If the low-pressure limit is reached, the pump will stop and the red LED above the potentiometer will light. After correcting the problem, reset the protective circuitry by cycling the START/STOP toggle switch through STOP and START.

The low-pressure limit is enabled only after pressure exceeds its set value. Thus, you can run the pump with the prime/purge valve open (pressure will be 0 PSI) even though the low-pressure limit is set to 200 PSI. Only after the prime/purge valve is closed, and pressures get above 200 PSI, will a drop in pressure trigger the protective circuitry.

MAXIMUM. A potentiometer that sets the high-pressure limit (in PSI) for pump operation. It is **EXTREMELY IMPORTANT** that this limit be set to some reasonable value; if a clog develops anywhere in the flow stream between the pump and the detector, the high-pressure cutoff will stop the pump as pressure increases. We suggest setting the cutoff 1000 PSI above your typical operating pressure. A general-purpose setting is 4000 PSI, as indicated by the asterisk on the face of the instrument.

If the high-pressure limit is reached, the pump will stop and the red LED above the potentiometer will light. After correcting the problem, reset the protective circuitry by cycling the START/STOP toggle switch through STOP and START.

FLOW RANGE. A toggle switch that chooses between standard- and micro-flow rates. In the 0-5 mL/min position, the FLOW RATE controls have an imaginary decimal point between the two digits. In the 0-0.5 mL/min position, the flow rate controls have an imaginary decimal point to the left of the digits.



FLOW RATE. These two control switches change the flow rate in conjunction with the FLOW RANGE toggle switch. With the toggle switch in the 0-5 mL/min position, the digits of the control are to be read with an imaginary decimal point between them (Figure 4.1). With the toggle switch in the 0-0.5 mL/min position, the digits are to be read with the decimal point to the left of both digits.

PRIME/PURGE. This valve provides a tap into the solvent flow line between the pulse damper and the injection valve.

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Suction can be applied here with a syringe to prime the pump, or new mobile phase can be rapidly brought through the pump and pulse damper.

NOTE: Always allow the system pressure to fall below 30 PSI before opening the prime/ purge valve. This will prevent pressure shocks from damaging the column or pulse damper.

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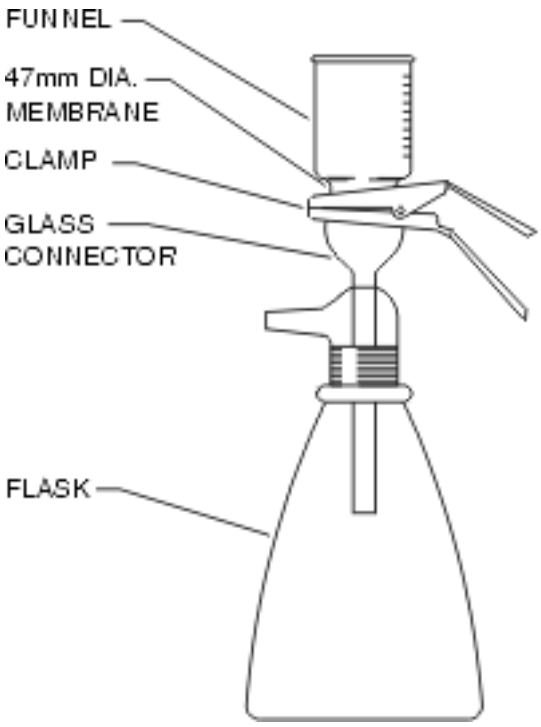
MOBILE PHASE PREPARATION

Use only high-purity solvents and buffer salts for preparing mobile phases. Water should be type I reagent grade, with a resistivity of >15 megohms/cm^2. Don't forget to thoroughly wash all glassware used to prepare and hold the mobile phase.

Filter all mobile phases through a 0.2 µm membrane filter. The figure below illustrates the filtering device recommended by BAS for doing this. It is borosilicate glass throughout, and uses ground glass joints to avoid contamination from stoppers. The filtration step will reduce problems with column degradation significantly.

CLEAN, PARTICLE-FREE MOBILE PHASES ARE CRITICAL TO ANY LC METHOD!

MF-6126 Filtration Apparatus



Mobile phases should be degassed prior to use. Vacuum filtering may be sufficient to degas the mobile phase. But if bubbles are a problem in your system, a more-thorough degassing is required. Allowing the solution to sit under the vacuum generated by an aspirator for 5-15 minutes should be sufficient. The MF-6126 kit also can be used for degassing, by replacing the filtration apparatus on the top of the unit with a stopper. Small amounts of volatile organic modifiers may be lost, but not enough to cause any difficulty.

The easiest and most effective method of degassing is the LC-26 on-line degasser. This highly effective instrument reliably and consistently delivers bubble-free mobile phase to the PM-80 pump. We strongly recommend its use, particularly for gradient applications.

Whichever way you make your mobile phase, be consistent; consistency in mobile phase preparation is critical for

reproducible chromatography.

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STARTUP

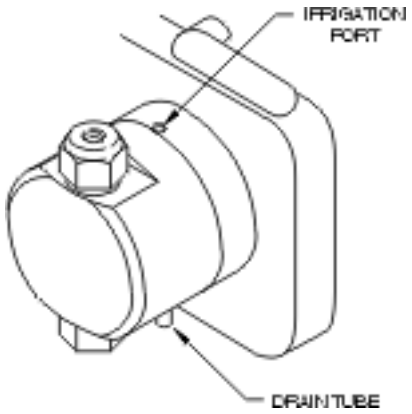
Proper startup and shutdown procedure is vital for long and dependable pump life. The two most important considerations are:

- **NEVER START THE PUMP WITH DRY PLUNGERS**
- **NEVER LEAVE STAGNANT MOBILE PHASE IN THE PUMP**

Correct startup procedure is to purge the pump as follows:

1. Put the START/STOP toggle switch in the STOP position and turn the POWER on.

2. Squirt a few drops of water into the plunger-irrigation ports.



3. Place the solvent-uptake frit into a glass reagent bottle containing 40:60 (v:v:) acetonitrile:water. Elevating the reservoir to provide hydrostatic pressure will be helpful.

   NOTE: ALL SOLVENTS AND MOBILE PHASES USED IN THE PM-80 PUMP SHOULD BE FILTERED THROUGH 0.2 µm MEMBRANE FILTERS. USE ONLY LC-GRADE SOLVENTS AND TYPE I REAGENT-GRADE WATER. SEE THE SECTION ON MOBILE PHASE PREPARATION.

4. Attach a 50-mL disposable syringe to the prime/purge valve and open the valve one turn (don't forget that system pressure must be below 30 PSI before opening the valve). Aspirate until solvent comes through the pump. Leave the syringe attached with the prime/purge valve open.

5. Set the FLOW RATE control to 5 mL/min. Then turn the START/STOP switch to START and pump for 5 minutes. During this purge you may alternately draw back and release the syringe plunger to help dislodge air bubbles from the system.

6. Stop the pump. Set the FLOW RATE control to 1 mL/min. Close the prime/purge valve.

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7. If there is no column attached, you may wish to pump solvent through the injection valve to displace any air and old solvent. Simply start the pump again and collect the waste solvent at the outlet of the injection valve.

8. To change solvents, repeat steps 3-7 as appropriate.

9. If necessary, reset the FLOW RATE control to 1 mL/min or other appropriate setting.

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SHUTDOWN

The most important principle to remember when turning off your pump for storage is to remove all mobile-phase salts. Salts in the system will lead to corrosion of the stainless steel lines (yes, they corrode, but at a slow rate), and the presence of abrasive crystals in the pump heads. Abrasives will scratch the seals and plungers during subsequent startup.

The shutdown procedure is as follows:

1. Switch the START/STOP toggle switch to STOP.

2. Place the solvent-pickup frit in a reagent bottle containing 40:60 (v:v:) acetonitrile:water. Elevating the reservoir to provide hydrostatic pressure will be helpful.

   NOTE: ALL SOLVENTS AND MOBILE PHASES USED IN THE PM-80 PUMP SHOULD BE FILTERED THROUGH 0.2 µm MEMBRANE FILTERS. USE ONLY LC-GRADE SOLVENTS AND TYPE I WATER.

3. Place a 50-mL syringe on the prime/purge valve. Check to make sure that system pressure is below 30 PSI, then open the valve one turn. Set the FLOW RATE to 5 mL/min.

4. Switch the START/STOP toggle switch to the START position and pump for 5 minutes at 5 mL/min. This will bring fresh solvent through the pump and pulse damper.

   If the column is to be cleaned with this solvent, proceed to step 5. If the column has been removed, go to step 6.

5. (To clean both the column and the pump.) Set the flow rate to 1 mL/min or other appropriate range. Close the prime/purge valve. Pump 100 mL solvent through the column. Shut the system off, remove and cap the column for storage, and you're done.

6. (To clean only the pump.) Remove the column. Set the FLOWRATE to 2 mL/min. Pump 100 mL solvent through the pump, pulse damper, and injector (in INJECT position to flush the loop). Then shut the system off.

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GRADIENT CONTROLLER

This section covers the use of the LCD CONTROLLER for stand-alone gradient pumps. For front-panel control of isocratic pumps, see the MANUAL CONTROLS section. For computer control see the PUMP section under Operation With ChromGraph Control.

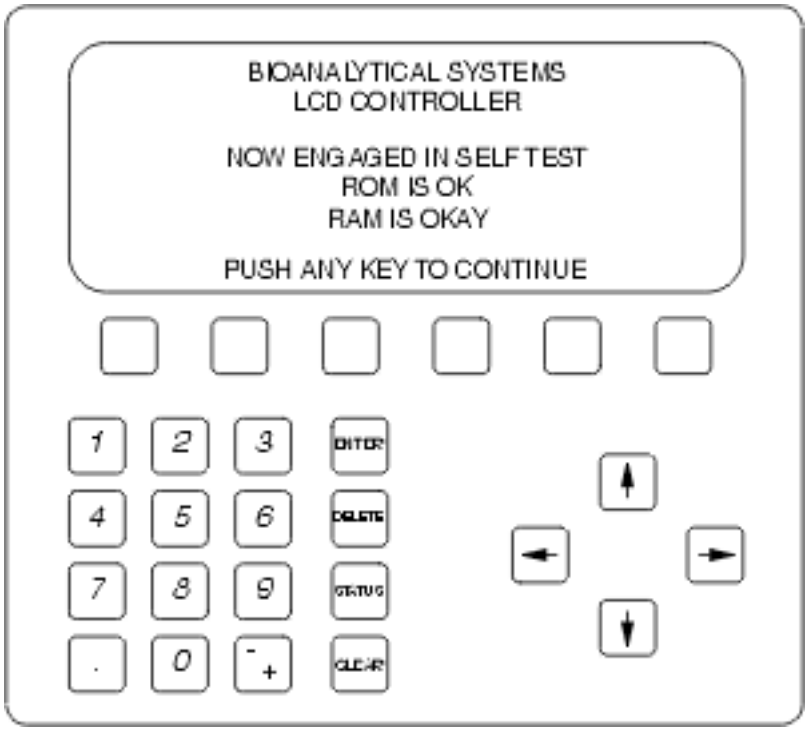
Before operating in the gradient mode you must connect the mobile phases and switch from the pulse damper to the mixer. See the INSTALLATION section for details.

- **LCD CONTROLLER**
- **MANUAL OPERATION**
- **PURGE**
- **PROGRAMMED AND GRADIENT OPERATION**
- **HARDWARE CONFIGURATION**
- **AUTOSAMPLERS**

LCD CONTROLLER

The LCD controller connects to the SYSTEM DIRECTOR port on the back panel. It takes over all functions from the front panel when the LOCAL/REMOTE switch is in the REMOTE position. The controller can edit and store up to seven methods. Programming the controller is best learned by the hands-on approach. The following guidelines will make this process smoother.

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The row of blank buttons directly under the display become active when function labels appear in the display just above them.

Use the **ENTER** key to scroll horizontally across lines of information.

Use **CLEAR** to remove an erroneous entry.

The cursor cannot back up. If you enter an erroneous entry, complete the line and come back to it with the UP arrow key.

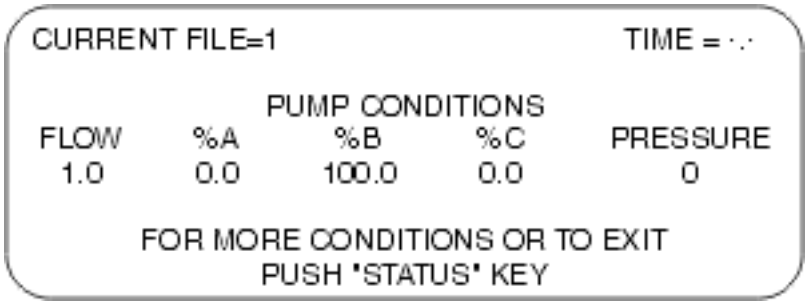
Use **DELETE** to remove an entire line of information. The cursor must be on the left side of the line for DELETE to be active.

Use **UP** and **DOWN** arrows to scroll vertically among lines. The cursor must be on the left side of the line for these keys to be active.

To **SAVE** a modified method, you must press two save functions. The first saves the method to RAM, the second saves it to permanent storage under a file number from 1-7.

STATUS will toggle to a status screen that shows pump conditions and run time:

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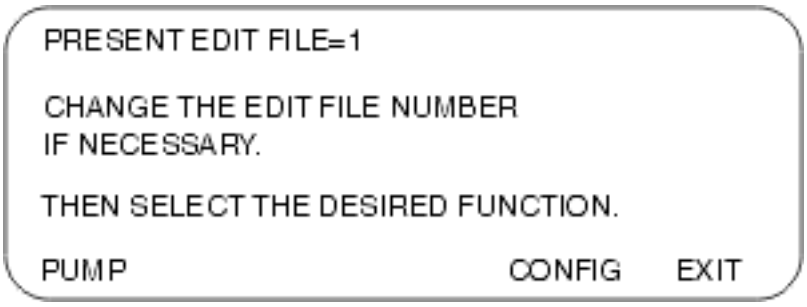


Press STATUS again to return to the previous screen. STATUS is active at all times.

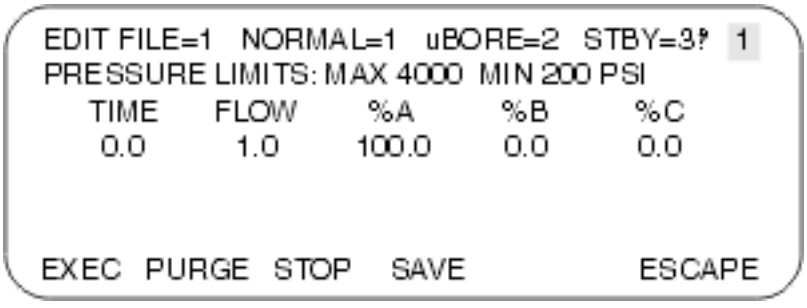
MANUAL PUMP OPERATION

Gradient operation generally requires a timed program in which the proportion of mobile phase from the bottles varies with time. However, many routine operations, such as cleaning columns and setting up initial conditions, require only that the pump be started at a specified flow rate and mobile-phase mix. These manual operations can be set into effect with a few keystrokes. For example, to set up the initial conditions of 80% A, 20% B, 1 mL per minute, do the following:

1. Turn on the main power and set the pump for REMOTE operation. The LCD will come on, do some self checks and ask you to press any key. The screen will then look like this:



2. Press the key below the word PUMP, and the screen below will appear. This is the main programming screen for the pump.



3. The cursor will appear at the top right of the screen, with a default of 1. The two valid choices here are:

    - 1 = normal flow rates, 0-5 mL per minute in 0.1 mL increments.

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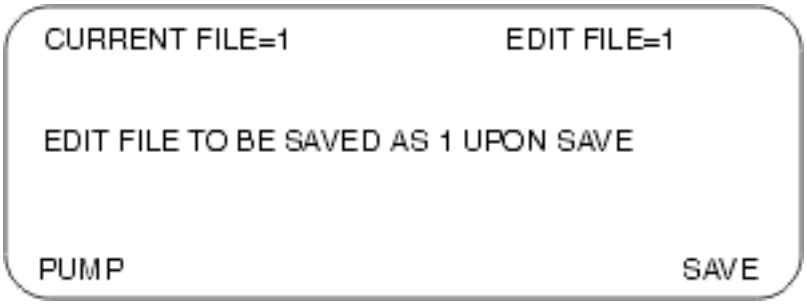
- 2 = microbore flow rates, 0-0.5 mL per minute in 0.01 mL increments.

Choose normal flow rates by pressing ENTER. (To choose microbore, key in a "2", then press ENTER.)

4. The cursor then moves down to the high- and low-pressure cutoffs, which should be set for most uses at 4000 and 200 PSI. Press ENTER to accept these defaults.

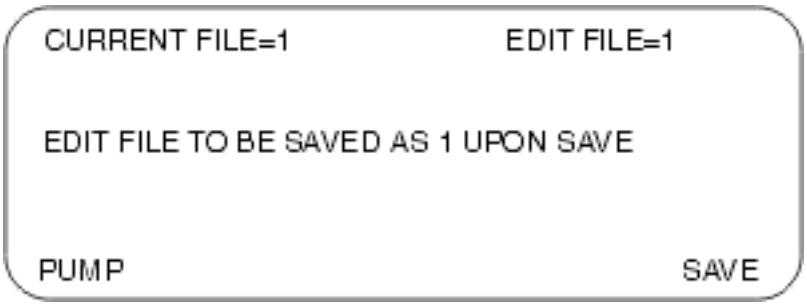
5. The cursor is now at the 0.0 time line. Press ENTER to move to the FLOW column, and change it, if necessary. Then press ENTER to move to the % A column. Press ENTER if it is correct (80.0% in this example), or key in the correct percentage, then press ENTER. The % B column is done similarly. The % C column is calculated automatically as the difference between A+B and 100%.

6. The cursor should now be at the start of line 2 (even if it is a blank line). Press EXEC to start the pump with the conditions set in the 0.0 time line. The STOP button will stop the pump, and the SAVE button is the first of the two-step process to make the file permanent. Pressing SAVE will bring up the Save File Screen:

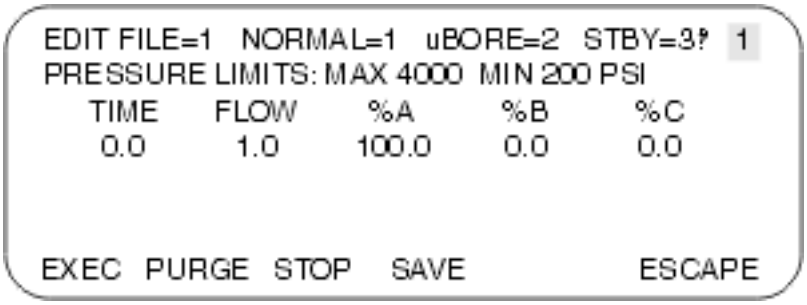


File numbers 1-7 are valid entries for up to 7 stored methods. Change the file number if necessary, then press SAVE again to complete the process.

7. If you do not want the current pump conditions to become part of a permanent method (e.g., you are flushing the column with an organic solvent), do not SAVE the instructions. When you wish to recall the original program, use the ESCAPE button. The following LCD screen will appear:



Press PUMP and the following screen will appear:

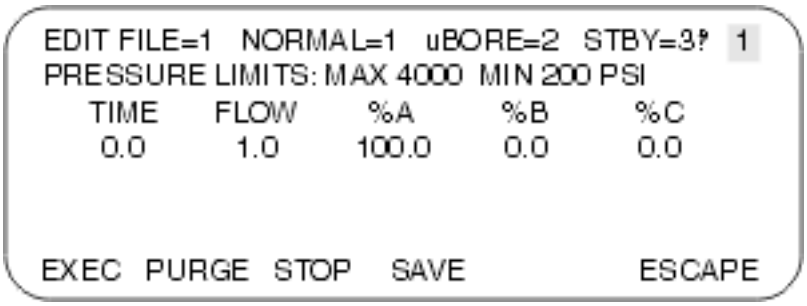


The temporary instructions are lost and the original instructions have reappeared. The pump will still be carrying out the temporary instructions, but pressing EXEC will reinstate the original instructions.

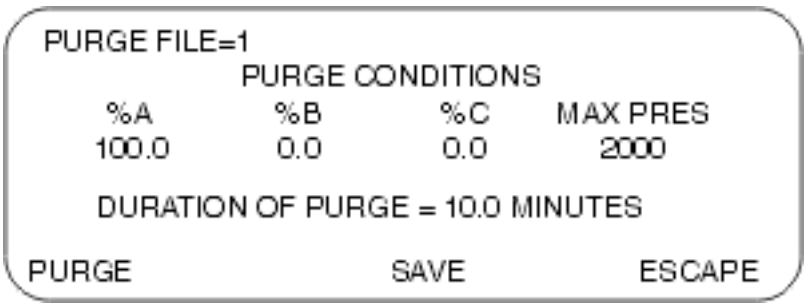
PURGE

Purge instructs the pump to automatically modify flow rate to maintain a given pressure. If the preset pressure is not reached, the pump will gently increase flow rate to its maximum of 5 mL per minute. A purge is useful to wash a column, or to bring fresh mobile phase from the solvent reservoirs up to the prime/purge valve. To begin a purge:

1. Start from the main programming screen:



2. Press the key under the PURGE label. The purge control screen will appear:



3. Enter the percentages to be metered from each solvent reservoir, and the pressure that the pump should attempt to hold. A valid pressure range is between 0 and 5000 PSI.

4. Enter the duration of the purge (0.1 - 99.9 minutes).

5. The purging information can be saved, if desired, by pressing SAVE in this screen, SAVE in the main programming screen, and SAVE in the Save File screen.

6. ESCAPE will return to the main programming screen.

7. Before beginning the purge:

    - If the purge is to bring fresh mobile phase up to the prime/purge valve, put a 50-mL disposable syringe on the valve, and open the valve one turn.

    - If the purge is to wash a column, make sure the maximum pressure setting does not exceed the working pressure of the column, and that the prime/purge valve is closed.

8. Press PURGE to start the purge cycle. The pump will slowly increase flow rate until it hovers around the preset pressure. If the preset pressure is not reached, the pump will increase its flow rate to 5 mL per minute.

9. At any time during the purge, ESCAPE will end the purge, and STATUS will bring up the status screen.

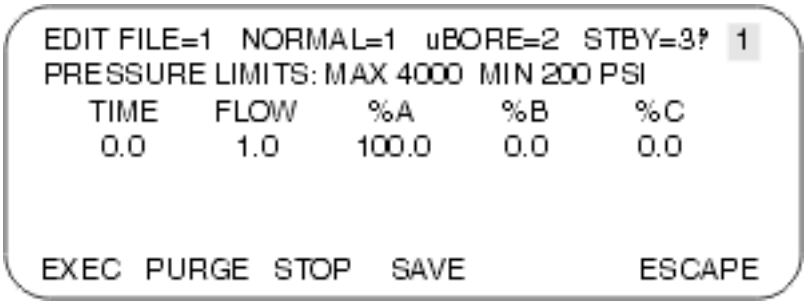
PROGRAMMED AND GRADIENT OPERATION

A program (a series of timed events) must be used whenever a gradient is needed, and whenever the pump must be synchronized with peripheral equipment (such as an autosampler).

Before beginning a gradient, check that pump synchronization is turned on. Synchronization ensures precise, repeatable gradients by delaying the start of each run until a specific piston position is reached.

Program a gradient as follows:

1. Start at the main programming screen:



2. Assuming the FLOW RANGE, MAX and MIN settings are correct, press ENTER three times to put the cursor at the left of the 0.0 time line.

3. Enter the FLOW and bottle percentages as appropriate for the 0.0 time line (initial conditions).

4. The cursor will automatically begin the second time line. Enter the time (minutes) and bottle percentages for this line (there is no FLOW programming, so the FLOW column will be blank).

5. A program must have at least two time lines: a start (0.0) line and an end line. Up to ten lines are permitted. Note that:

    - A linear gradient will be performed between any two time lines that differ in bottle percentages.
    - Any time period in which the solvent mix does not change must be indicated by bracketing it with time lines that do not differ in bottle percentages.

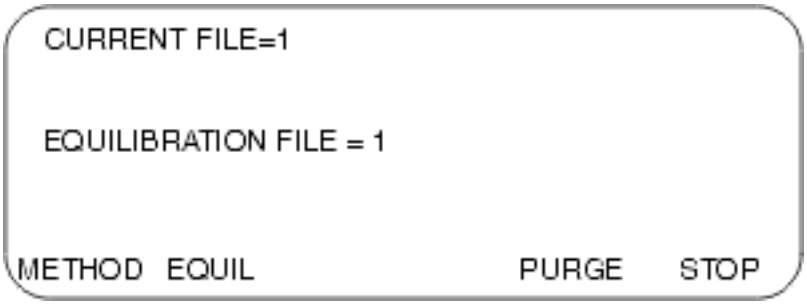
6. Only three time lines will fit on the screen simultaneously. The lines will scroll up during entry of subsequent lines. For review of hidden lines, move the cursor to the left side of a line and use the UP and DOWN arrows.

7. Use DELETE to remove an entire line, use CLEAR to change an erroneous entry.

8. Lines can be entered in any order and will sort themselves out on the screen.

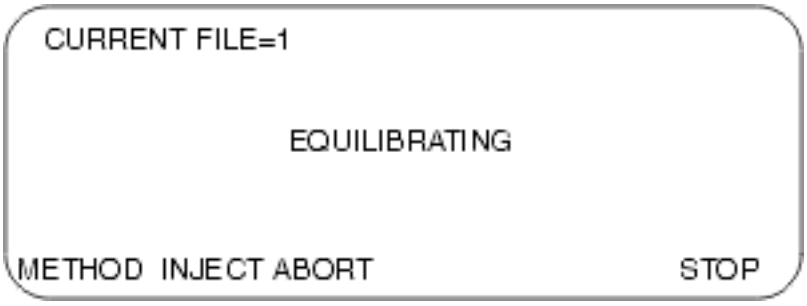
9. After the last entry, press SAVE in the programming screen and SAVE in the Save File screen.

10. To start the pump under the conditions in the 0.0 time line, press EXEC in the main programming screen or EQUIL in the equilibration screen:



Begin gradient operation as follows:

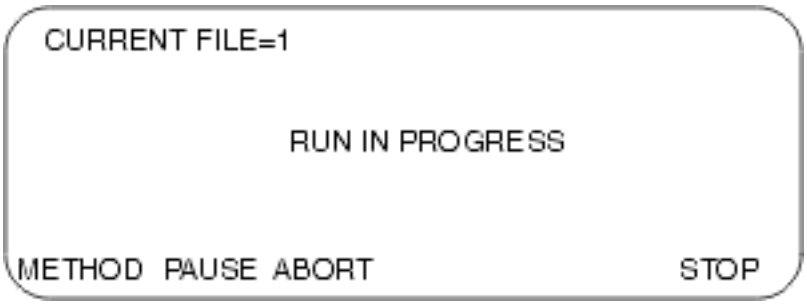
1. From the equilibration screen press EQUIL. The pump will start, and the Equilibrating Screen will appear:



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This indicates that the program is ready to begin. Note that there is a difference between the Equilibration screen (i.e., ready to equilibrate) and the Equilibrating screen (i.e., already equilibrating, ready to run).

2. Press INJECT to start the run. Depending on the options you have selected under CONFIGURATION, the run may start immediately, or after a delay for synchronization. If AUTO RUNS was selected, you will be asked to enter the number of runs desired. When the run starts, the Run in Progress screen appears:



3. STATUS may be pressed at any time during a run. However, the RUN IN PROGRESS screen must be visible for the run to end properly and for subsequent runs to begin (i.e., do not leave the controller in status mode).

4. ABORT terminates the gradient run and returns to the 0.0 time conditions.

5. STOP terminates the gradient run and stops the pump.

6. PAUSE will halt the clock and maintain the current mobile phase composition indefinitely. The PAUSE button will change to CONTinue, which should be pressed to resume the run.

7. You can make changes to the pump program and save it at any time. However, the old program will still be in effect. To put the new program into effect, press ABORT then EQUIL.

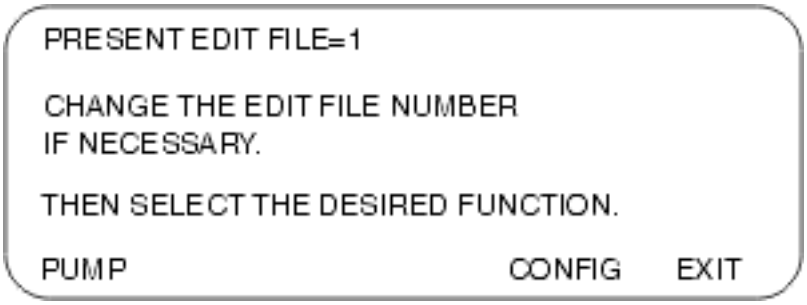
8. The run will end when the last time line of the program is reached. The Equilibrating screen will appear, and the zero-time conditions will be maintained. If AUTO RUNS was selected, the next run will start. If not, the pump will await a signal from the operator or autosampler.

HARDWARE CONFIGURATION

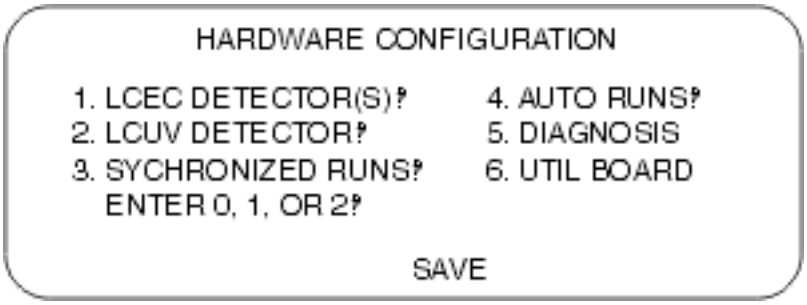
Certain options are available to control the behavior of the pump in gradient and non-gradient operations, and for communication with an autosampler. To reach the hardware configuration screen, do the following:

- From initial startup, follow the PUSH ANY KEY TO CONTINUE instruction. A screen with software versions will appear. Press any key to continue. The next screen says PRESENT EDIT FILE=1" at the top left:

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- Press the CONFIG key. The Configuration Screen appears:



- To get to the configuration screen from the main programming screen, press SAVE, then SAVE in the next screen, then METHOD in the equilibration screen, then the CONFIG button.

- Options 1 and 2 (detector options) are invalid in this application. Set them to "0".

- Option 3: **PUMP SYNCHRONIZATION**. Turn synchronization on with a "1", off with a "0". Pump synchronization ensures that each gradient run starts with the pistons in the same relative positions, for precise control of retention times. ALWAYS TURN SYNCHRONIZATION ON FOR GRADIENT OPERATION. Synchronization may be left on or off for isocratic operation. The synchronization process causes a slight delay (up to 12 seconds at 1 mL/min) before each run starts. At microbore flow rates this delay will be noticeable (up to 120 seconds at 100 µL/min).

- Option 4: **AUTO RUNS**. With certain kinds of autosamplers, we want the PM-80 pump to be in control and signal the autosampler to inject. (See AUTOSAMPLERS below.) In this situation, turn AUTO RUNS on ("1") so the pump will initiate subsequent runs automatically. When AUTO RUNS is on, you will be asked "HOW MANY RUNS" when you press INJECT to start the runs. You will also be asked for the "NEXT FILE NUMBER", which allows the chaining of several methods.

- Option 5 provides onscreen diagnostics for use by our engineers. May be left on or off.

- Option 6 is invalid in this application. Set it to "0". Press ENTER to continue.

- Press SAVE to exit and institute whatever changes were made.

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AUTOSAMPLERS

The PM-80 gradient pump can communicate with autosamplers for automatic operation. The specific procedure to be used depends upon the type of autosampler that is available. (This discussion assumes a stand-alone pump. If this is an epsilon computer-controlled system, see the AUTOSAMPLER section.)

BAS Sample Sentinel Autosampler

The Sample Sentinel autosampler is capable of two-way communication with the PM-80 pump. Two-way communication ensures that no injections will occur if either unit malfunctions. Connect the units as follows (click here for a view of the pump's rear panel).

Autosampler Pin	Pump Terminal
1	Gnd
7	Inject
8	Ready

When programming the autosampler, make sure that the it's run length is a minute or two longer than that of the pump. This ensures that the pump is in the EQUILIBRATING screen when the autosampler sends its READY signal. You also must change 'Inject Hold Active' to HI on the autosampler's input polarity menu.

In the pump's configuration screen, AUTO RUNS should be off. To start the sequence of runs, program the pump. Then press SAVE, SAVE, and EQUIL. You're now in the EQUILIBRATING screen. Start the autosampler. When it has loaded the sample it will send a READY signal, which triggers the PM-80 to begin the run. The PM-80 sends the INJECT signal to the autosampler when the run begins.

Other Autosamplers: PM-80 In Charge

If an autosampler is not capable of two-way communication, but will accept an INJECT signal, the system can be automated with the PM-80 pump in charge. Program the autosampler so it will end its run and load the next sample before the PM-80 has completed its run. The autosampler must then wait for a trigger from the PM-80. Connect leads from the INJECT and GND terminals on the PM-80 to the SAMPLE ENABLE terminals on the autosampler. Turn AUTO RUNS on, which will allow the PM-80 to do a sequence of runs without waiting for a trigger.

To start the sequence, first start the autosampler. When it has loaded the sample and is waiting, bring the PM-80 to the EQUILIBRATING screen. To get here from the main programming screen press SAVE, SAVE, EQUIL). Now press INJECT. Enter the number of runs in the sequence, and the next file number (typically the same as the current file). Press ENTER to accept the file number, and the sequence of runs will begin.

Other Autosamplers: Autosampler In Charge

If the autosampler will not accept an INJECT signal, then the autosampler must be in charge of the run. Pump

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synchronization must be off, since there is no way to communicate the variable start times to the autosampler. For isocratic runs, simply turn on the pump in the local mode, and let it run while the autosampler injects. For gradients, attach leads to the autosampler terminals that signal when an injection has occurred. Connect these to the READY terminals on the PM-80. Put the PM-80 in the EQUILIBRATING screen, then start the autosampler.

- **PM-80 CONTENTS**
- **EPSILON CONTENTS**

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PM-80 MAINTENANCE

- **PUMP HEADS**
- **CHECK VALVES**
- **PRESSURE TRANSDUCER**
- **PRIME/PURGE VALVE**
- **FITTINGS**
- **IN-LINE SOLVENT FILTER**
- **FAN FILTER**
- **ROUTINE MAINTENANCE**

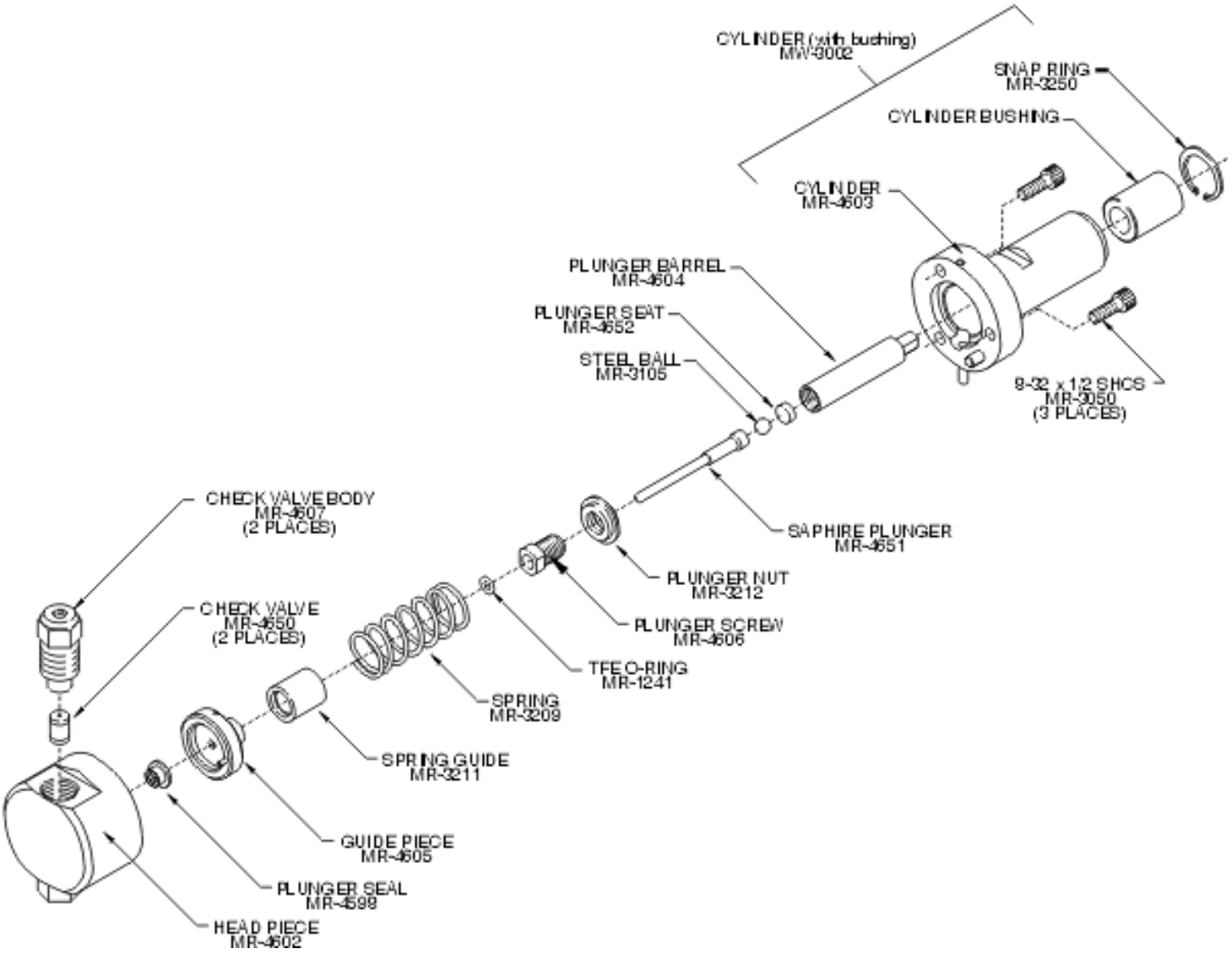
PUMP HEADS

The procedures detailed below encompass all user services that require removal of the pump heads. They include:

- Pump head replacement
- Plunger seal replacement
- Plunger spring replacement
- Plunger free-play adjustment
- Plunger replacement
- Plunger seal break-in

This section will detail the entire procedure, from head removal to re-insertion. The text will indicate which sections can be skipped when performing simple maintenance.

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Pump Head Removal

An entire pump head may be rapidly removed by the following procedure:

1. Observe the pressure display to make sure there is no residual pressure in the lines. Then turn the POWER off.

2. Hold one check-valve assembly stationary with a wrench and remove the inlet or outlet line with a second wrench. Repeat for the other check-valve assembly.

3. Hold the pump head against the body of the pump with the palm of one hand, and flip the locking lever towards the remaining pump head.

CAUTION: THE PUMP HEAD WILL BE EJECTED WITH SOME FORCE!

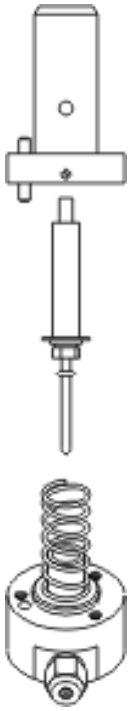
Pump Head Disassembly

Servicing or inspection of the plunger seal, plunger spring, and plunger require disassembly of the pump head. Proceed as follows:

1. Place the pump head face down on a clean surface, and locate the three hex-head screws on the back of the pump head. Loosen all three slightly with a hex wrench.

2. It is important to remove the three hex-head screws evenly; this will prevent spring pressure from cocking the rear part of the head and snapping the plunger. Remove these screws by alternately undoing each a few turns at a time.

3. Carefully lift off the rear part of the pump head. Pull the plunger assembly straight up to remove it:



4. Remove the plunger spring and examine it for corrosion, breaks or nicks. Replace the spring if damaged.

5. Lift the guide piece from the rear face of the pump head to expose the plunger seal:



Plunger Inspection and Servicing

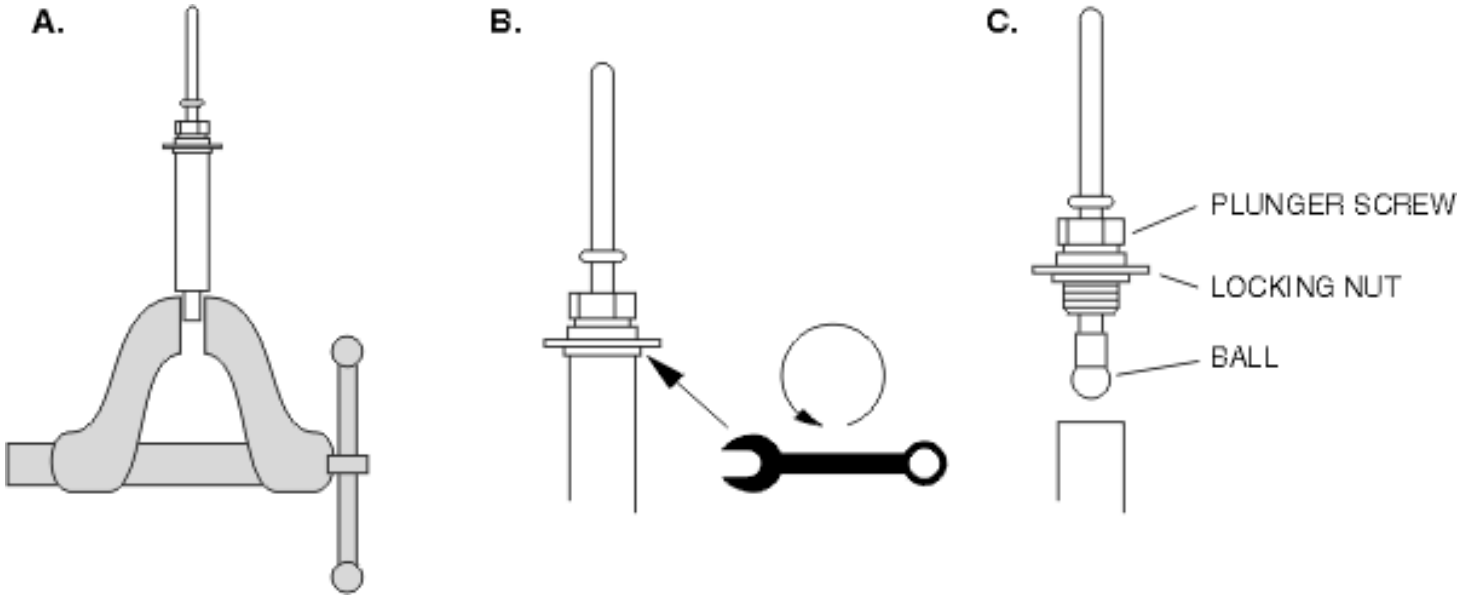
1. Examine the plunger carefully. Remove the white o-ring and wash the sapphire rod with water and methanol. Salts may be removed by gently scrubbing with a green Scotchbrite® scouring pad moistened with water. Replace the plunger if it is chipped, scored or scratched (if replaced, proceed to item 4).

2. Test the plunger free-play. Hold the plunger assembly in one hand with the sapphire rod pointing up. Wiggle the sapphire rod

from side to side with the other hand. It should move freely. Rotate it radially; it should move freely through 360°, much as you would roll your head on your shoulders to stretch your neck muscles. Now try to move it up and down; there should be no movement in the longitudinal direction.

3. If the rod passes the free-play test, proceed to the next section (seal replacement). If the sapphire rod is either too loose or too tight, perform the adjustment procedure beginning in item 4.

4. To replace or adjust the sapphire rod, place the end of the plunger assembly that is farthest away from the rod in a vise, with the rod facing up. The tip of the piston assembly has two flat surfaces that the vise can grip. Do not overtighten and damage the assembly.



5. Loosen the plunger nut by turning counterclockwise (with reference to the vise) with a wrench. Loosen this nut only enough to unlock the plunger screw above it.

6. To replace the sapphire rod, fully unscrew (by hand) the plunger screw, remove the rod, and replace with a new one. A small ball bearing sits at the base of the rod, and is held in place with a dab of grease. Do not lose the ball during assembly. Make sure that the rod is fully seated inside the plunger nut. Reinstall the plunger screw into the barrel, but do not fully tighten it.

7. To adjust the new or old sapphire rod, tighten or loosen the plunger screw by hand, while testing free-play. Wiggle the sapphire rod from side to side; it should move freely. Rotate it radially; it should move freely through 360°, much as you would roll your head on your shoulders to stretch your neck muscles. Now try to move it up and down; there should be no movement in the longitudinal direction. When the adjustment is correct, hold the plunger screw with a wrench and tighten the plunger nut (clockwise) to hold the adjustment.

8. Re-test the plunger free-play. There is a tendency for the rod to bind slightly when the plunger nut is tightened. You may have to loosen the plunger nut again, and back off the plunger screw a bit for a final adjustment.

Plunger Seal Replacement

1. Place the front portion of the pump head on a flat surface with the seal facing up.



2. Remove the seal by inserting a 6-32 threaded screw into the seal material several turns, then pulling out the seal. Be careful not to scratch the metal of the pump head with the screw.

3. Wash the pump head with water. Use a squeeze bottle to flush liquid through the check valves and all exposed ports.

4. Irrigate the pump head and new seal (part number MR- 4598) with methanol, and push the seal fully into its seat with your thumb or the guide piece. Do not scratch the seal with a fingernail.

5. Proceed with pump head reassembly. Be sure to follow the break-in procedure for new plunger seals (see below) after the pump is reassembled.

Pump Head Assembly

1. Wash all parts with water.

2. Place the front part of the pump head face-down on a flat surface.

3. Insert the guide piece, with its registration pin seated into the matching hole on the inner face (hint: insertion will be easier if you dry the hole with a lab tissue).

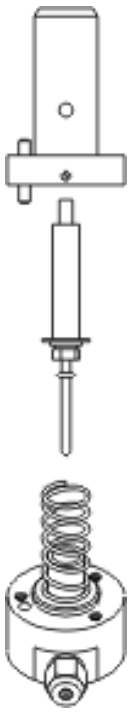


4. Place the old or new spring over the PTFE spring guide on the back of the guide piece. (If the spring guide has been removed, be sure to put it back with the chamfered inner edge on the guide piece.)

5. Re-install the o-ring on the sapphire rod.

6. Irrigate the plunger-seal area and the sapphire rod with methanol.

IT IS IMPORTANT THAT THE SEAL BE MOIST BEFORE INSTALLING THE PLUNGER ASSEMBLY!



7. Insert the plunger assembly with the sapphire rod entering the plunger seal.

8. Place the rear part of the pump head over the plunger assembly, and align the registration pin with the matching hole on the inner face of the front section.

9. Insert the three hex-head screws. Tighten these alternately, a few turns at a time, to avoid lateral stresses that could snap the sapphire rod.

Pump Head Installation

1. Push the pump head into place in the PM-80 pump.

2. Flip the locking lever into the locked (outward) position.

3. Hold one check-valve assembly stationary with a wrench and attach the inlet or outlet line with a second wrench. Repeat for the other check-valve assembly.

4. Follow the STARTUP procedure. It is very important to 1) ensure that the plunger seals are moist at startup, and 2) purge all air from the system.

5. If new plunger seals were installed, follow the break-in procedure below.

PLUNGER SEAL BREAK-IN

Plunger seals must seat properly for longest life. A salt-free solvent is recommended for break-in. Proceed as follows after installing new seals:

1. Wet the irrigation ports in the pump heads with a few drops of water.

2. Purge the system with filtered 40:60 (v:v) acetonitrile:water, as described in the STARTUP section.

3. Attach a column to the system. It need not be a good column, as its only purpose is to provide backpressure.

4. Run the pump for two hours at a pressure of 3000-3500 PSI. Adjust the FLOW RATE as necessary to achieve this pressure.

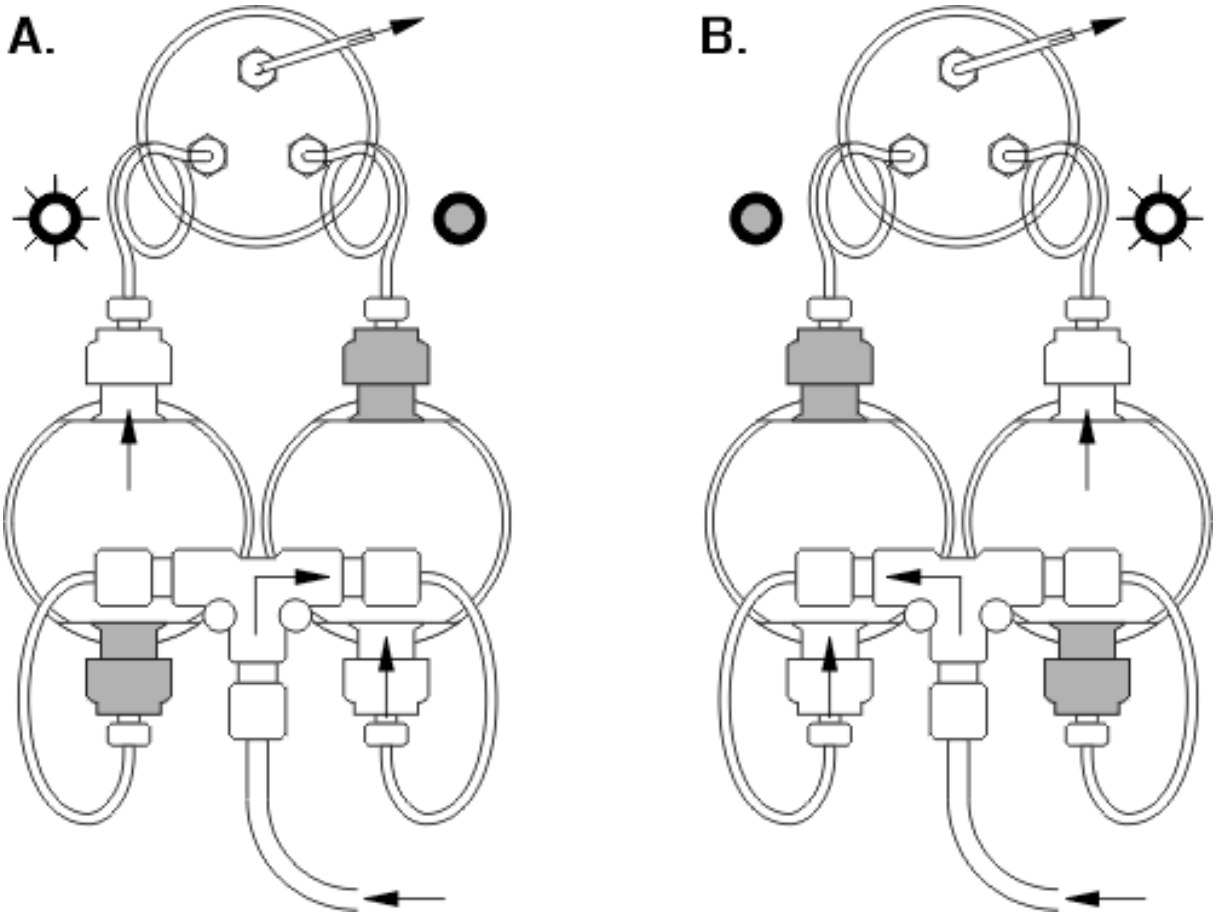
5. After two hours you may switch to mobile phase and begin chromatography.

SERVICING CHECK VALVES

The PM-80 pump uses four cartridge-type check valves (part number MR-4650) that do not contain removable parts. The same valve is used for both the inlet and outlet check valves on the pump. Each check-valve cartridge contains two precision-engineered balls and seats in series, for a reliable seal. You can determine the direction of flow through the cartridge by attempting to squeeze water or methanol through it in each direction. When installing, remember that flow is always up through the pump.

In most cases, faulty check-valve performance (as indicated by large pressure fluctuations) is due to microscopic debris, salt precipitation, or trapped gas pockets. Gas can be removed by purging with freshly degassed mobile phase or solvent as described in the STARTUP section. The check valves may be cleaned by sonication (see below), or (if necessary) replaced. When cleaning or replacing these cartridges, remember that any lint from towels, paper wipes, etc. may reintroduce the problem. The best approach is to flush the check valves with methanol and to reassemble them wet.

An understanding of flow through the pump and check valves is helpful in diagnosing check-valve problems. A full cycle consists of two pump strokes:



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When the left pump head is compressing, the green LED above it lights. The inlet check valve below it closes, preventing any backflow. The outlet check valve above it opens, allowing mobile phase to proceed towards the pressure transducer.

While this is occurring, the right pump head is aspirating. Its inlet check valve is open, allowing mobile phase to enter from the solvent reservoir. The outlet check valve is closed, which prevents any backflow into the right pump head from the pressure transducer.

After the left pump head finishes its stroke, the roles of the two pump heads reverse. The green LED above the right pump head lights, and the head begins compressing. The inlet check valve closes, and the outlet check valve opens. The left pump head begins aspirating; its inlet check valve opens and its outlet check valve closes.

Pump-related malfunctions that produce pressure fluctuations can be localized by observing the pump stroke and pressure readout. When the LED over the left pump head is on, the left head is compressing. Its inlet check valve must shut, and its outlet check valve must open. In addition, because the pressure transducer chamber is open to both pump heads, the outlet check valve of the right pump head must close. If it didn't, fluid would flow from the left pump head into the transducer, then down into the right pump head. The opposite argument applies to the right pump head.

Because of this relationship, there are three likely places to check when pressure drops as one pump head compresses: the pump head itself (plunger seal, plunger spring, plunger), the inlet check valve for that pump head, and the outlet check valve for the opposite head.

Check Valve Removal

1. Stop the pump and allow pressure to dissipate. Then turn off the POWER.

2. Hold the check-valve assembly stationary with a wrench and remove the inlet or outlet line with another wrench.

3. Remove the check-valve assembly, which contains the check-valve cartridge.

Check Valve Cleaning

1. Place the entire check-valve assembly in a solution of laboratory detergent warmed to 50 °C. We recommend a 50% solution of RBS-35® (Pierce Chemical Company). Soak for 1-2 hours. (Do not warm the solution above 60 °C, because plastic parts in the check valves may become distorted.)

2. Sonicate the check-valve assembly for 15 minutes in the detergent solution.

3. Flush with deionized water, then methanol.

4. Reinstall the check-valve assembly following the instructions below. If check-valve problems continue, install a new cartridge.

Check-valve Cartridge Replacement

1. Remove check-valve assembly from the pump head.

2. If the cartridge does not shake out of the assembly, push it out with a paper clip. This may require some force. Alternatively, you may be able to insert a small screw into the cartridge and pull it out of the assembly. Discard the old cartridge, as it is now damaged.

3. Clean the new cartridge, as above.

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4. Insert the new cartridge. Be certain to install the cartridge so it allows flow in the proper direction. Flow is always up through the pump; inlet check valves permit flow from the inlet line to the pump head, outlet check valves permit flow from the pump head to the outlet line. The cartridges may have an arrow to indicate flow direction. If not, the end with the small hole is the inlet, and the end with either several holes or a large hole is the outlet.

Check Valve Installation

1. Squirt some methanol through the check valve. This will both wet it and allow you to observe whether the direction of flow is correct.

2. Install the check-valve assembly onto the pump head with a wrench.

3. Hold the check-valve assembly stationary with the wrench and connect the inlet or outlet tubing with a second wrench.

4. Follow the startup procedure to prime the pump and remove all air from the check valves and lines.

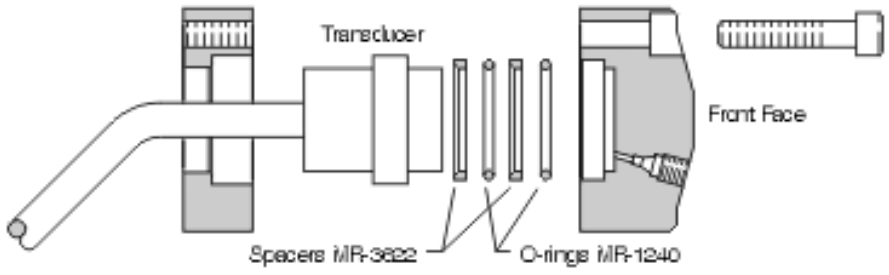
PRESSURE TRANSDUCER

The pressure transducer will rarely need attention. If a leak ever develops, there are two PTFE o-rings inside that need to be replaced (part number MR-1240). Follow these steps:

1. Stop the pump and allow system pressure to fall to zero. Turn the POWER off.

2. Loosen and bend aside all 3 tubes entering the front face of the pressure transducer. Mark the front and back halves of the transducer with a grease pencil, so they can be reinstalled in the original orientation.

3. Loosen and remove the 3 hex screws. Remove the front face of the housing and the two PTFE o-rings:



   Discard the o-rings. Save the steel spacers which are underneath the o-rings. Clean all parts that are to be reinstalled.

4. Install one steel spacer first. Slide it over the transducer, with the beveled edge facing the transducer and the flat edge facing out.

5. Install the first o-ring next. Use your fingers to slightly stretch the o-ring so it just fits over the transducer. Be careful not to scratch the o-ring with your fingernails or on the edge of the transducer. Slide the o-ring down on the transducer so it lies against the flat edge of the first steel spacer. Repeat with second steel spacer and second o-ring.

6. Wet the o-rings with water. Then install the front face of the assembly onto its back face, observing the original alignment.

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Tighten the three hex-head screws alternately to drive the face down evenly on the o-rings.

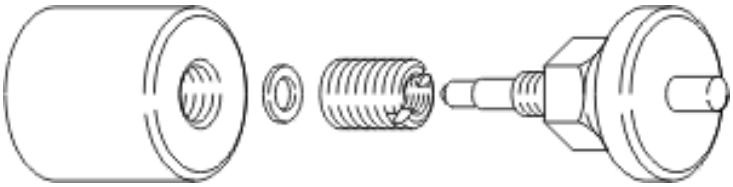
7. Purge the pump as described in the STARTUP section.

PRIME/PURGE VALVE

The prime/purge valve only rarely requires service. If the knurled knob loosens on its shaft, tighten with a hex wrench. If the valve-stem nut loosens, tighten gently with a wrench. If leaks develop, the internal seals must be replaced. The seal kit is part number MF-5406. The replacement procedure is as follows:

1. Switch the START/STOP switch to STOP and allow pressure to drop to zero. Turn the POWER switch off.

2. Open the valve-stem nut all the way, and unscrew the valve stem. The entire stem assembly can now be removed.



3. Place the metal tool provided in the kit across the slots in the seal retainer, and turn counterclockwise to remove. It may be necessary to grasp the tool with a pliers for greater torque. (Some seal retainers are designed to use a hex wrench.)

4. Straighten a paper clip, then form a 1/8" right-angle bend at its tip. Reach into the valve body and carefully remove the old PTFE seal. Do not scratch the seat. Clean all parts to be reinstalled.

5. Insert the new PTFE seal into the valve body so it rests against the seat.

6. Screw the seal retainer back into the body, and tighten firmly with the tool. Do not turn the tool with a pliers.

7. Remove the old plastic tip from the valve stem with forceps or pliers. Push the new tip onto the stem by hand.

8. Screw the stem back into the body, then tighten the valve-stem nut gently with a wrench. Then check that the valve stem turns without binding.

9. Purge the pump as described in the STARTUP section. Close the prime/purge valve firmly and check for leakage during normal operation.

FITTINGS

Check all fittings for leaks every day. Large leaks will be obvious. Small leaks will be apparent only by the accumulation of salt deposits around the fitting. It is not normal for fittings to have even small leaks, and these should be tended to.

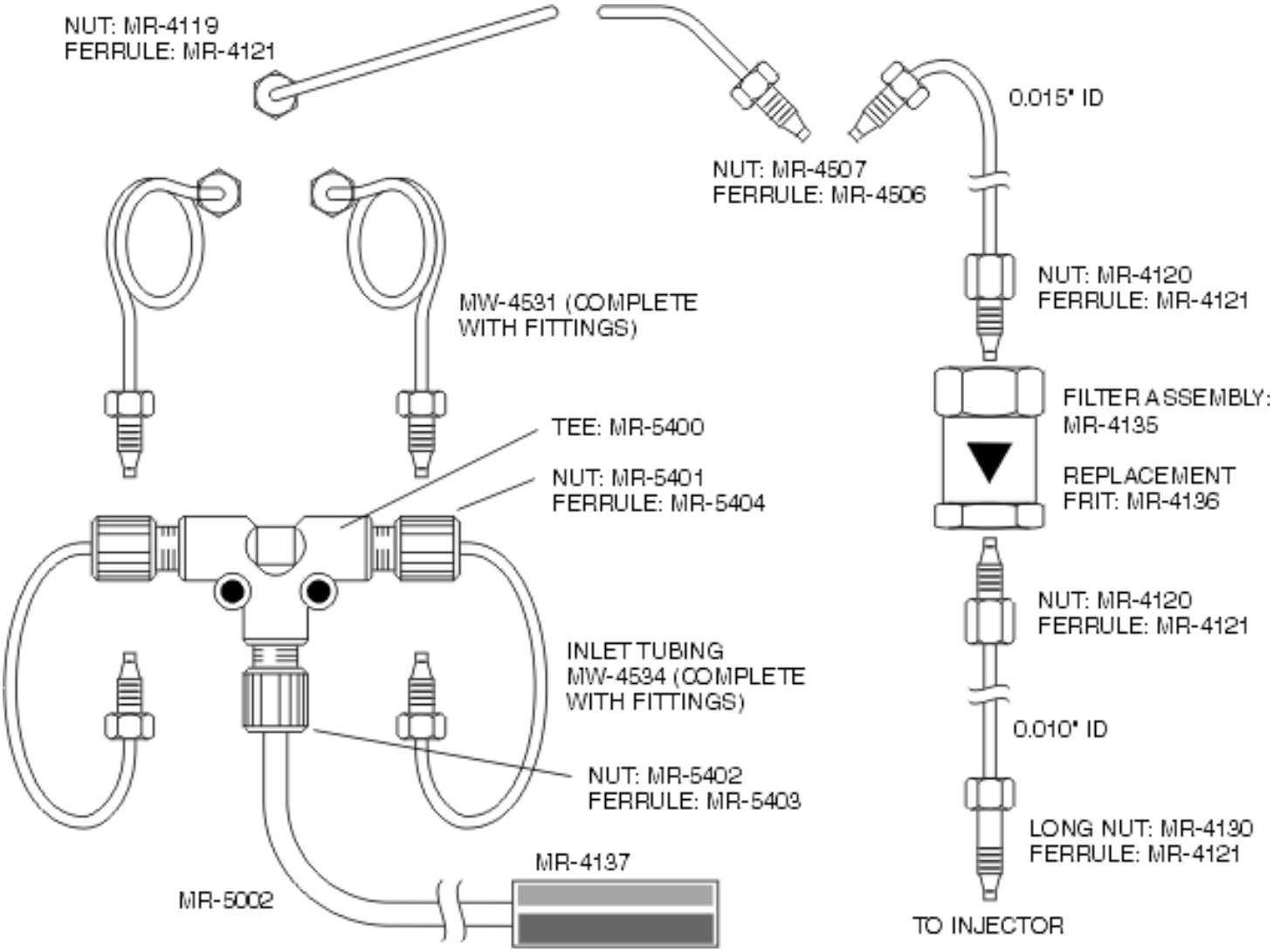
The first approach to treating a small leak is to clean up the salts with water and tighten the nut slightly. About 1/8 turn should do it,

unless the nut is loose. If this doesn't stop the leak, it's best to replace the fitting and line entirely. Why?

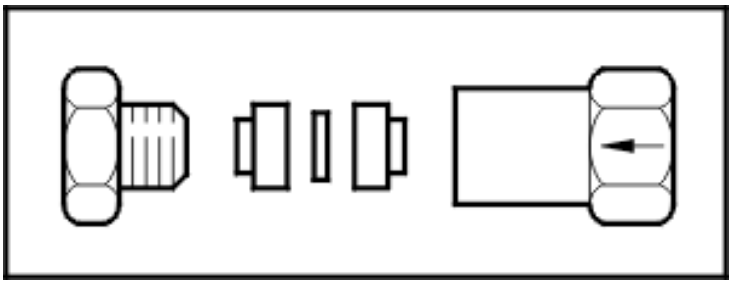
It's certainly tempting to use a lot of force to stop a small leak. But several bad things can happen when you overtighten a fitting:

- The nut may break off with its threads still in the hole. A machinist will have to get it out for you, and if you're very lucky the part won't be damaged.
- The nut may fuse in place, and break off the next time you try to open it.
- The tubing end may become crimped, restricting flow and causing performance problems.

It makes more sense to replace a fitting or line before any of these events occur. This figure gives part numbers and ferrule types for all tubing used in the PM-80 pump:



IN-LINE SOLVENT FILTER



The in-line filter should be suspected whenever system pressure rises above normal. The filter becomes clogged by doing its job: preventing particles from the mobile phase or pump seals from lodging in the injector or on the column. To replace the filter frit do the following:

1. Stop the pump and allow pressure to drop to zero.
2. Remove the inlet and outlet tubing to the filter assembly. Be sure to note the flow direction.
3. Loosen the two halves of the filter assembly with two wrenches, but do not separate them yet.
4. Hold the assembly vertically, with the smaller section on the bottom. Now unscrew the larger section and remove.
5. The two frit supports and the frit can now be removed from the smaller section. Keep the supports separate so they can be reassembled in the same order: they seal best when installed in the section from which they came.
6. Flush water through each support to ensure that its passageway is not clogged.
7. Place the appropriate support on the smaller section, then put a new filter frit (MR-4136) on top of it. Put on the remaining support, then screw on the larger section. Tighten with two wrenches.
8. Attach the inlet line from the pump to the assembly, observing proper flow direction. Pump some mobile phase through the unit to remove air, then attach the outlet tubing.

FAN FILTER



The fan filter should be removed for cleaning at least once per year (more often if it is visibly dirty.) Proceed as follows:

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1. Gently pry off the retaining grid with a screwdriver. **Do not unscrew the mounting screws!**

2. Carefully pull out the filter. You may either vacuum it clean, or wash it gently in warm sudsy water. Be careful not to tear the filter.

3. If you've washed the filter, blot it well between sheets of paper towels, then allow it to dry.

4. Reinstall the filter by holding it in place over the fan opening, then snapping the retaining grid in place. Position the grid with its ridges facing in towards the filter.

ROUTINE MAINTENANCE

Regular maintenance will keep pump performance up to specifications. We recommend:

Every Day	Inspect all fittings in the flow path for leaks.
Every Three Months	Replace plunger seals and inspect interior of pump head.
Every Year	Clean the fan filter.

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- **EPSILON CONTENTS**

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PM-80 TROUBLESHOOTING

This section contains troubleshooting tips specific to the PM-80 pump. For general troubleshooting see the main TROUBLESHOOTING page.

POWER WON'T TURN ON

- Check power connection.
- Check fuse and fuse type.

AUDIBLE NOISE

The drive train of the pump can be noisy when there is no load (no high pressure) on it. This audible cue is noticeable, for example, when the purge valve is open.

Drive-train noise during regular operation suggests that pressure is not being maintained. Observe the pressure display, and proceed to the Pressure Fluctuation section below if the difference between the highest and lowest pressures is greater than about 100 PSI.

NO SOLVENT FLOW

- **Green Led's Don't Alternate**
    - Check FLOW RATE and FLOW RANGE controls. Are they set for valid, non-zero ranges (0.1-0.5 or 0.1-5 mL/min)?
    - Is the START/STOP switch in the START position?
    - Is the LOCAL/REMOTE switch in the LOCAL position?
    - Is the high- or low-pressure LED on? If so, see the **Pump Stops** sections below.
    - Is there an error message on the display? If so, call BAS.
- **Green Led's Do Alternate**
    - Air in the pump. Check for loose connections on the inlet side of the pump. Purge with freshly degassed mobile phase as described in the STARTUP section.

- Mobile phase reservoir is empty. Purge with fresh mobile phase as described in the STARTUP section.
- Solvent-uptake frit is clogged. Remove temporarily and see if the pump now works.

PUMP STOPS: LOW-PRESSURE LIMIT

- The mobile-phase reservoir is empty. Make fresh mobile phase and purge as described in the STARTUP section.
- The MINIMUM potentiometer is set too high. Reset to 200 PSI or a reasonable value for your conditions.
- There's a leak in the system. Check all connections.

PUMP STOPS: HIGH-PRESSURE LIMIT

- The MAXIMUM potentiometer is set too low. Reset to 4000 PSI or a reasonable value (1000 PSI above your typical operating pressure) for your conditions.
- The injection valve is not fully in the inject or load position. Rotate it to one side.
- Have you changed to a more viscous mobile phase (e.g., one with methanol)?
- There's a clog in the flow path. It could be anywhere between the pump outlet and the detector. It may be the in-line solvent filter. Start opening fittings at the detector and work towards the pump. At some point the pressure will drop, pinpointing the location of the clog. (NOTE: There will be a normal drop in pressure when the column is removed.)

PRESSURE FLUCTUATIONS

- Purge the pump (see the STARTUP section) with freshly degassed mobile phase to remove air from the check valves. If performance improves, air bubbles were in the check valves. If the problem returns, degas the mobile phase more frequently, and check the connections to the inlet side of the pump, where air could be sucked in.
- Switch the inlet check valves between the two heads. If the pressure drop now occurs on the opposite pump head, it is associated with the inlet check valve on the head showing the problem. Clean or replace as described in the CHECK VALVES section.
- Switch the outlet check valves between the two heads. If the pressure drop now occurs on the opposite pump head, it is associated with the outlet check valve on the head that does not show a pressure drop. Clean or replace as described in the CHECK VALVES section.
- One or both pump heads may need an overhaul. See the PUMP HEADS section.
- One of the outlet lines from the pump to the pressure transducer may be clogged. Remove and examine. Replace

as necessary.

FLUID LEAKS

- Fluid leaks from (or salt accumulates around) fittings. Leaks indicate that FITTINGS need to be tightened or replaced.
- Fluid leaks (or salt accumulates) around pump heads or from irrigation waste ports. This indicates that the plunger seals are worn and leaking. Replace the PLUNGER SEALS to prevent internal corrosion of the pump head.
- Fluid leaks (or salt accumulates) around the junction of the front and back halves of the pressure transducer. Replace the PRESSURE TRANSDUCER O-RINGS.
- Fluid leakage (or salt accumulation) at the prime/purge valve. Replace the PRIME/PURGE SEALS.

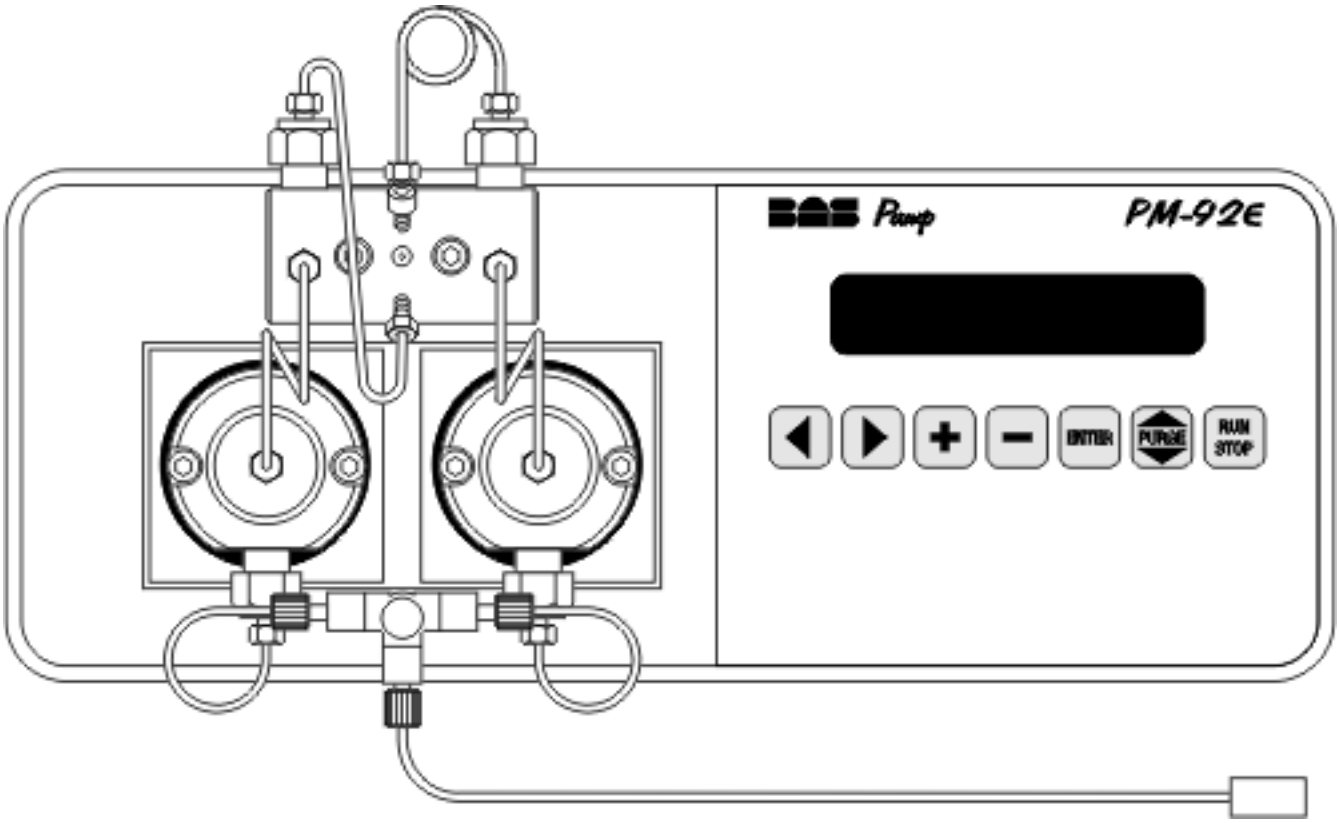
OVERHEATING

If the pump heads or cabinet feel warm, clean the FAN FILTER and check that the fan is operating.

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- **EPSILON CONTENTS**

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INTRODUCTION AND SPECIFICATIONS



This manual explains the installation and operation of the PM-92 dual-head and PM-91 single-head Pump, as well as procedures for routine maintenance and troubleshooting. Read the entire manual prior to installation and start-up.

NOTE: We will refer to the pump as the PM-90 when when the information refers to both models.

The **PM-92** is a reciprocating, dual-piston unit. It is designed for precise and reliable solvent delivery for isocratic elution at flow rates between 1.0 and 2000 µL/min. Advanced features of the PM-92 pump include:

- Dual independently controlled reciprocating pistons each driven by a microstepping motor through a high efficiency ballscrew.
- Solid-state pressure transducers monitor each piston's head pressure along with system pressure to provide smooth crossover from one piston delivery to the next.
- Minimal flow-through volume. No pulse damper required for all but the most demanding applications.
- Optimized for microbore flow rates.
- Piston velocity profile and stroke length is software controlled.
- Pump performance is independent of system backpressure.
- Each head is removable as a single, precision-engineered assembly which may be interchanged in seconds.
- High- and low-pressure limit controls, for safety.
- Analog output for pressure monitoring.
- Provision for remote-control operation.
- Wetted materials: 316 stainless steel, sapphire, UHMW polyethylene, Teflon, PEEK.

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The PM-91 is a reciprocating, single-piston unit. It is designed as an economical alternative to the PM-92 for isocratic elution at flow rates between 1.0 and 1200 µL/min. Advanced features of the PM-91 pump include:

- Reciprocating piston driven by a microstepping motor through a high efficiency ballscrew.
- Solid-state pressure transducer monitors head pressure and system pressure independently.
- Microprocessor control takes a 'snapshot' of system pressure just prior to refilling and drives the plunger to quickly refill and pressurize the mobile phase to the 'snapshot' pressure before resuming the desired delivery flow rate.
- Fast refill and pressurization in combination with a pulse damper provides minimal flow and pressure fluctuation.
- Optimized for microbore flow rates.
- Pump performance is independent of system backpressure.
- The pump head is removable as a single, precision-engineered assembly which may be interchanged in seconds.
- High- and low-pressure limit controls, for safety.
- Analog output for pressure monitoring.
- Provision for remote-control operation.
- Wetted materials: 316 stainless steel, sapphire, UHMW polyethylene, Teflon, PEEK.

The PM-90 series pumps have been engineered for durability, and with proper maintenance should provide years of reliable service.

SPECIFICATIONS

HEIGHT	4.6 inches
LENGTH	9 inches
WIDTH	11 inches
WEIGHT	15 pounds
FLOW RATE	1-2000 (PM-92) or 1-1200 (PM-91) µl/min
FLOW PRECISION	better than 0.3% RSD
FLOW REPEATABILITY	0.3%
PRESSURE RANGE	0-5000 PSI (0-34 bar) with selectable limits
INPUT VOLTAGE	100/120/220/240 vac 50/60 Hz
POWER	2A (max) @ 120 VAC 50/60 Hz

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- **EPSILON CONTENTS**

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PM-90 PUMP INSTALLATION

- **UNPACKING**
- **IDENTIFICATION OF PARTS**
- **LOCATION IN THE LAB**
- **POWER REQUIREMENTS**
- **FLUID CONNECTIONS**
- **COMMUNICATIONS**

UNPACKING

Please retain the shipping box and packing materials until the unit has been fully tested. The shipping materials will be needed if you discover damage incurred during shipping.

The shipping box should contain the following items:

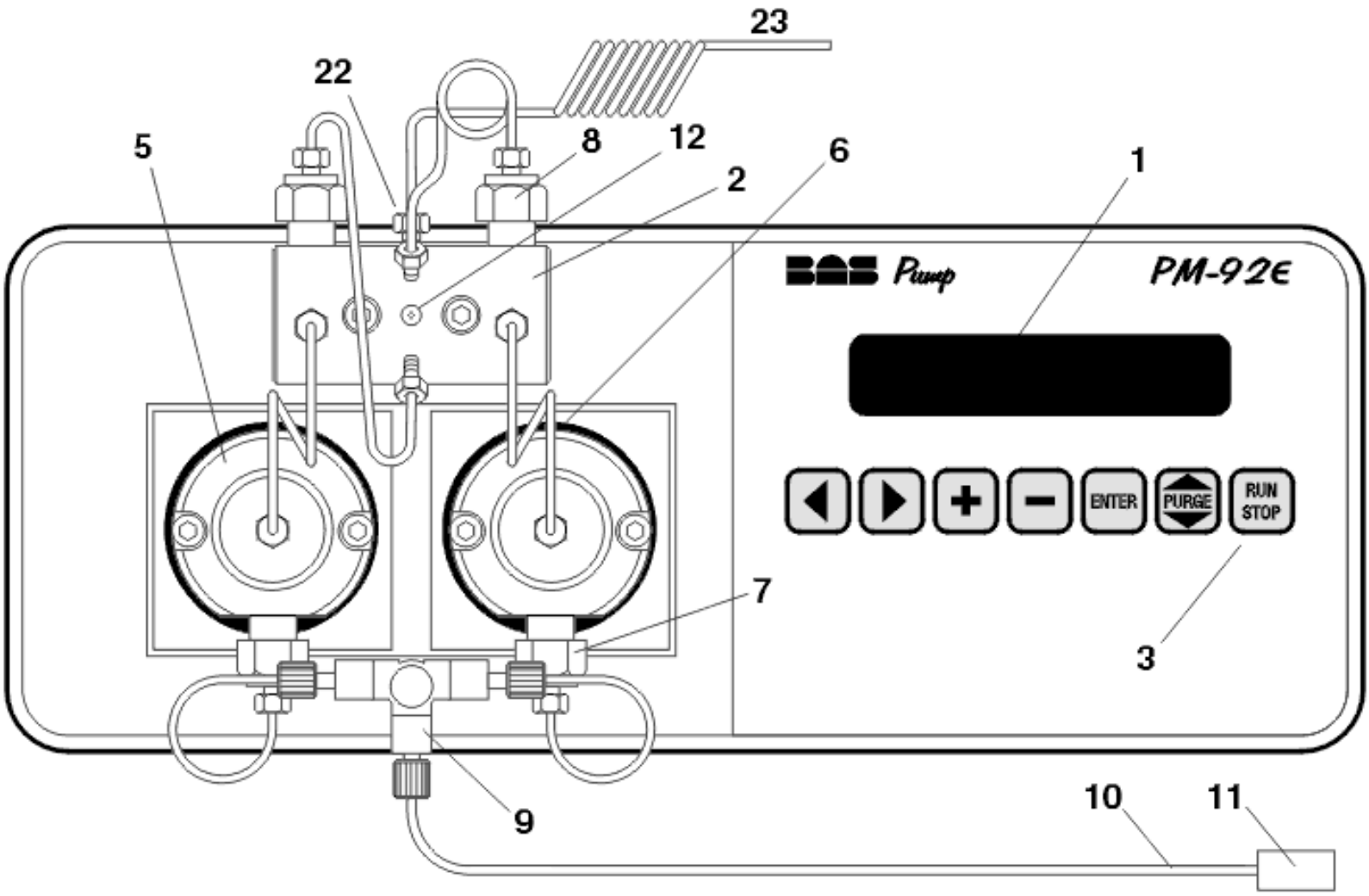
- PM-92 or PM-91 Pump
- PM-90 Accessories (includes: power cord, 1/8" PTFE inlet lines with fittings, 60 mL syringe, luer adapter).
- Pulse Damper (PM-91 ONLY)

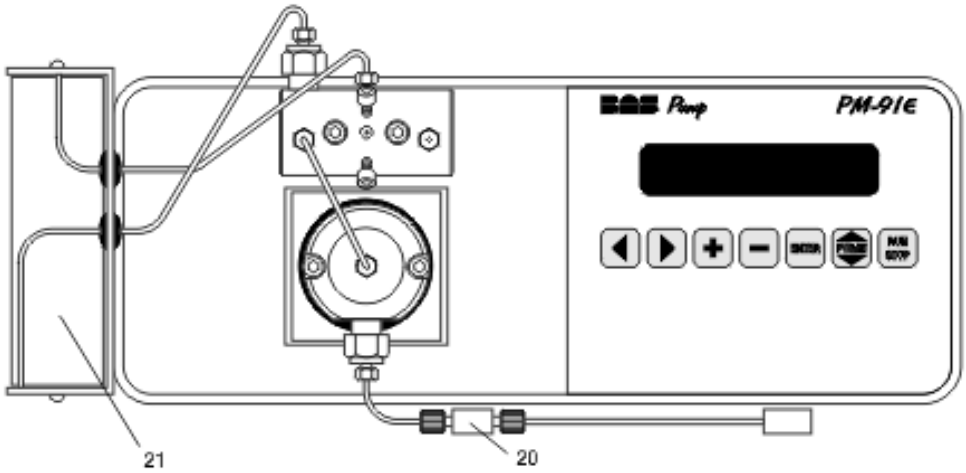
If any discrepancies exist, retain the packing slip and contact BAS Customer Service for assistance.

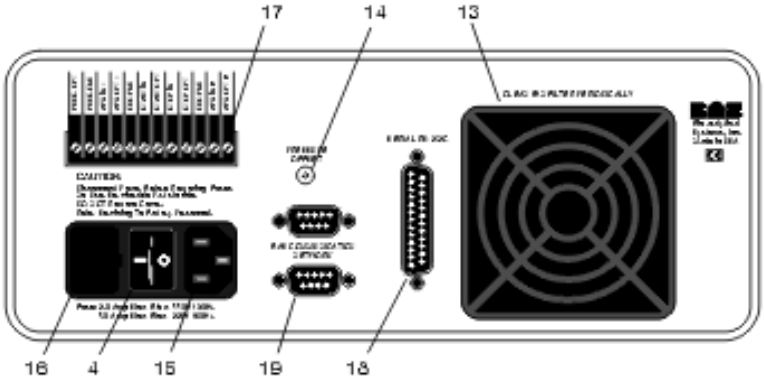
IDENTIFICATION OF PARTS

The following figures show front and back views of the PM-92 and PM-91 pumps. The parts identified by number are described below.

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23
22
5
8
12
6
2
1
BAS Pump
PM-92E
ENTER
PURGE
RUN
STOP
7
3
9
10
11


BAS Pump
PM-91E
21
20

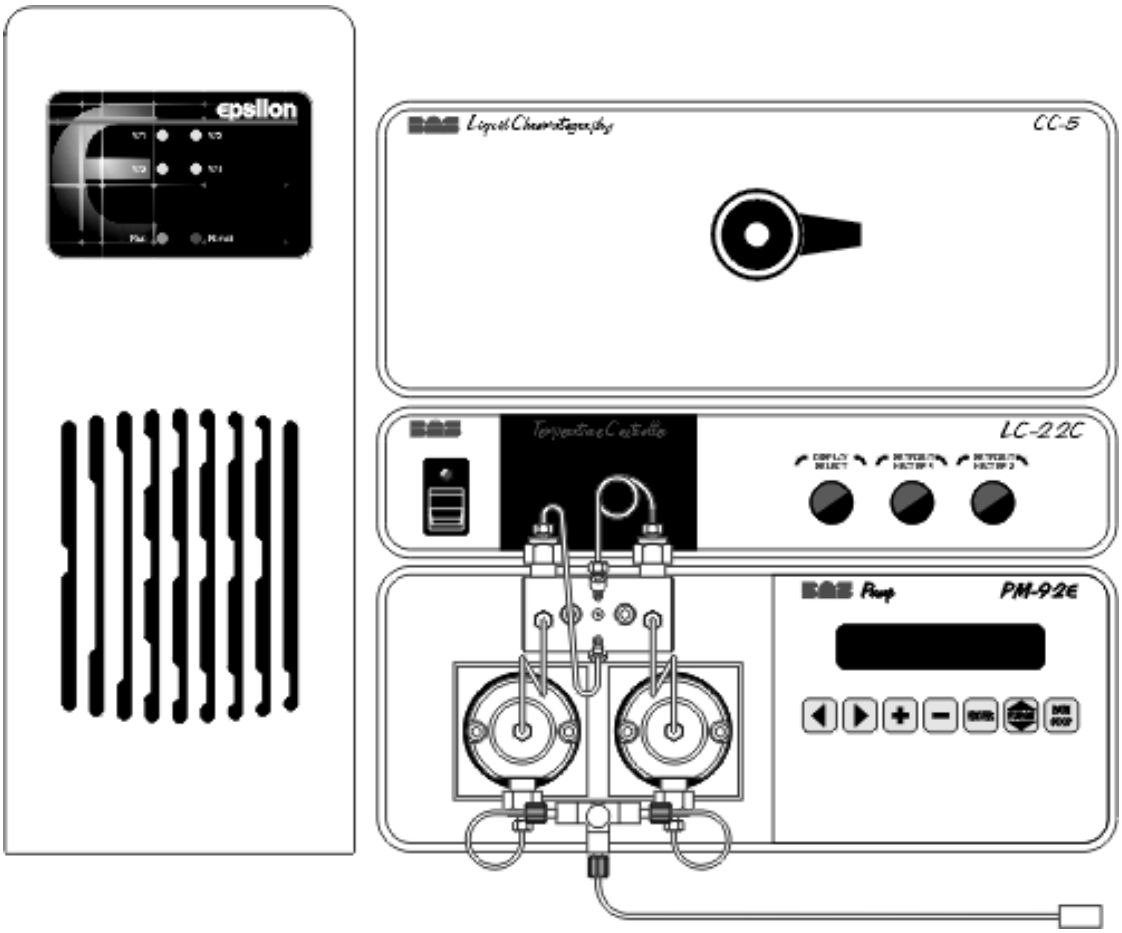


1. LCD display
2. Pressure manifold
3. Keyboard
4. POWER switch
5. Pump head
6. Plunger-irrigation port
7. Inlet check-valve assembly
8. Outlet check-valve assembly
9. Inlet tee (PM-92)
10. Solvent-uptake line
11. Solvent-uptake frit
12. Purge port
13. Air filter
14. Pressure offset adjustment
15. Power input
16. Fuse
17. Terminal strip
18. Serial RS-232c connector
19. BAS Communication Network connector
20. Inlet fitting (PM-91)
21. Pulse Damper (PM-91)
22. Outlet to injector
23. Mixing tube (PM-92)

LOCATION IN THE LAB

The PM-90 pump is designed to serve as the base for a stack of BAS chromatography instruments. A typical setup is illustrated by the BAS-502e chromatograph. The components of this system are the PM-92 pump, LC-22C temperature controller, and CC-5 flowcell compartment. The epsilon LC detector is on the left.

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Location considerations for the pump must, of necessity, include the requirements for all the component instruments in the stack. Use the following guidelines:

- Provide a surge-free power source which can be dedicated to the chromatograph. Other laboratory instruments such as ovens, vortex mixers, centrifuges, and large motors may cause spikes in the power supply.
- Ensure that all components of the chromatograph share the same ground circuit. This can best be accomplished by plugging all components into a multi-outlet power strip. Plugging the components into independent outlets can produce ground loops (current that flows between ground circuits at slightly different potentials) which can produce baseline noise.
- Locate the chromatograph on a stable bench. Vibrations can hamper the performance of any sensitive instrument.
- Select a room where temperature remains stable throughout the day. Avoid installing the chromatograph near windows, air ducts, ovens, or refrigerators.
- Place the chromatograph away from busy, congested areas. Remote, isolated areas are best for high-sensitivity work.
- Avoid very dry areas and areas that are carpeted. Static electricity can affect instrument performance. Anti-static floor mats and bench mats are useful if spiking caused by static charge is a problem.
- Avoid areas where radio-frequency interference is likely. Beeper-type paging devices can be a problem in some installations.

POWER REQUIREMENTS

The power cord attaches to the PM-90 via the receptacle on the rear of the instrument. The pump can be operated with either 100, 120, 220, or 240

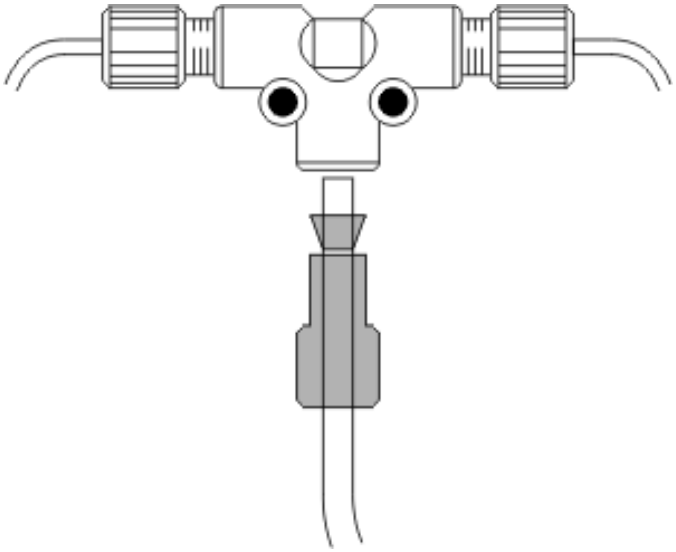
VAC and 50 or 60 Hz power. The instrument uses a fused self-sensing power supply. There is no need to make power related adjustments. You should check to see that the fuse is the proper rating for the voltage used:

Voltage	**Fuse**
100-120V	2.0 Amp Slow Blow
220-240V	1.0 Amp Slow Blow

FLUID CONNECTIONS

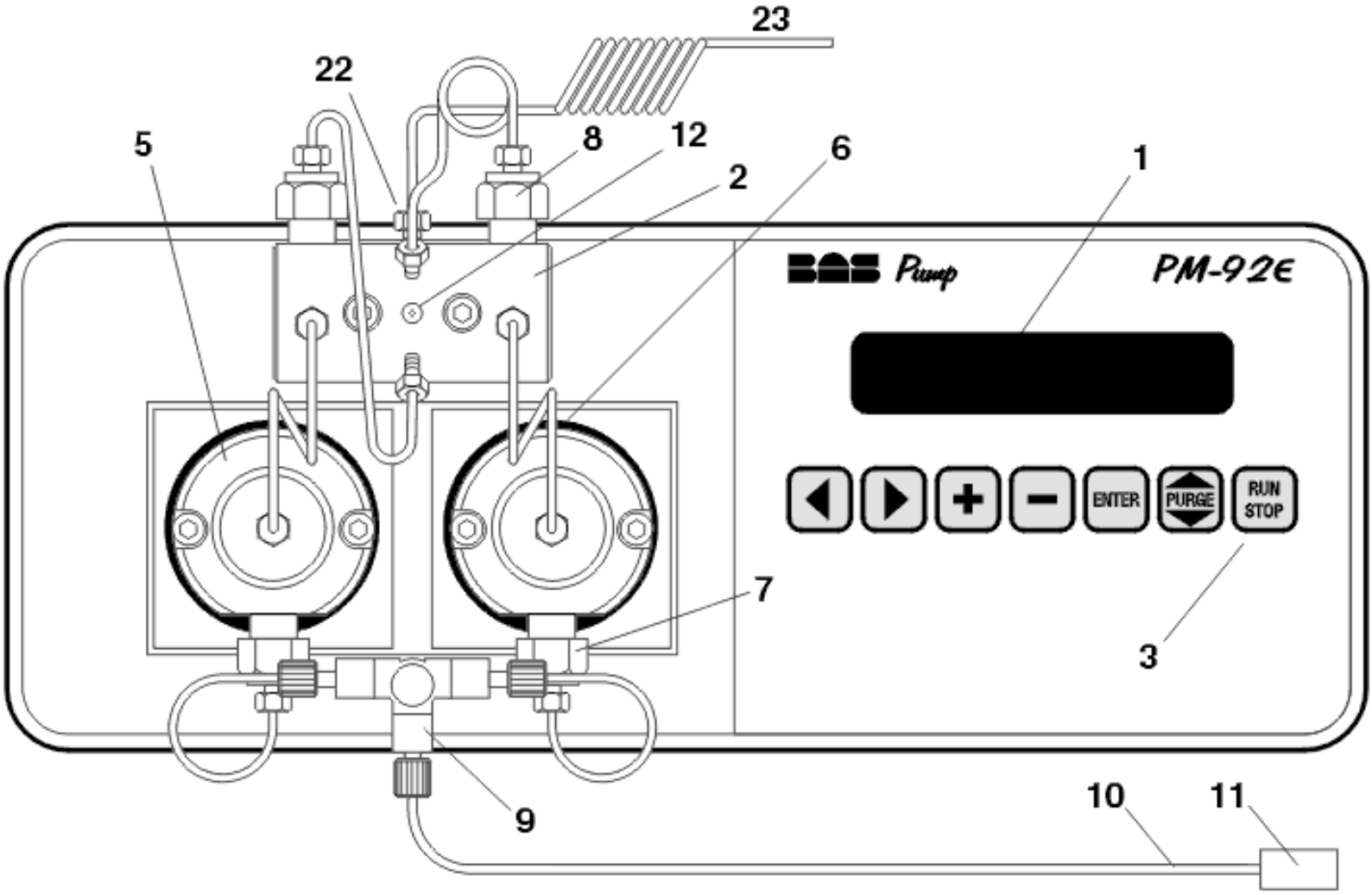
PM-92

The PM-92 is factory plumbed, and ready as received for final connection of the inlet and outlet solvent lines. Unwrap the PTFE tubing carefully and remove the protective cover from the solvent-uptake frit. The uptake tubing connects to the tee at the PM-92 pump head with a flangeless fitting. Be sure to install this with the flat face of the ferrule entering the tee:



Connect the mixing tube (#23) to the topmost pump manifold outlet (#22) with the nut and ferrule provided. Connect the other end of the mixing tube to an in-line filter assembly (MR-4135). Then connect the filter to the input of your injection valve. We recommend 1/16" OD, 0.015" ID, #316 stainless steel tubing for these connections. We strongly recommend using plastic fittings (MF-4166) to connect the in-line filter, as this is a frequently opened connection. Plastic fittings can be replaced when they start to leak; leaks in steel fittings require the replacement of the entire tube.

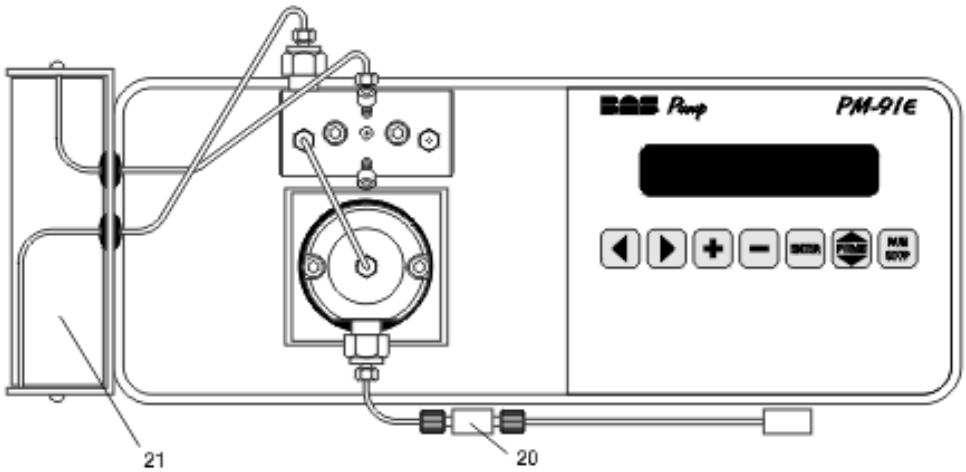
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PM-91

Unwrap the teflon tubing carefully and remove the protective cover from the solvent-uptake frit. The uptake tubing connects to the inlet fitting (#20) at the PM-91 pump head with a flangeless fitting. Be sure to install this with the flat face of the ferrule entering the inline fitting.

Mount the pulse damper (#21) to the left side of the pump cover using the two 6-32 flat head screws supplied. To ensure that any air bubbles that enter the system don't get trapped in the pulse damper, always have the flow from the pump (emerging from the upper check valve) enter the bottom of the pulse damper and emerge from the top.

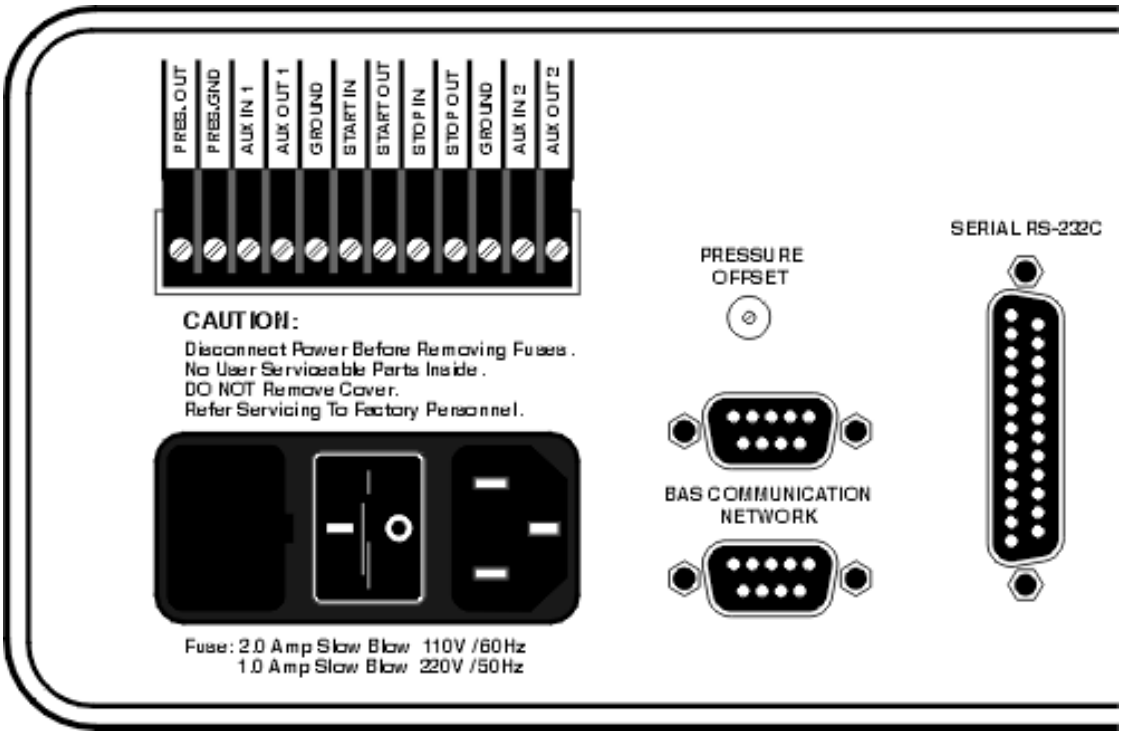


Connect the bottom line of the pulse damper to the outlet check valve assembly. Connect the top line of the pulse damper to the second highest port on the pressure manifold. The topmost port (#22) should be connected to an in-line filter assembly (MR-4135). Then connect the filter to the input of your injection valve. We recommend 1/16" OD, 0.015" ID, #316 stainless steel tubing for these connections. We strongly recommend using plastic fittings (MF-4166) to connect the in-line filter, as this is a frequently opened connection. Plastic fittings can be replaced when they start to leak; leaks in steel fittings require the replacement of the entire tube.

Installation is now complete. Refer to the STARTUP section to begin purging the pump.

COMMUNICATIONS

Communication with other instruments is accomplished via connections to the rear panel:



The only connection that needs to be made to an epsilon system is via a **BAS COMMUNICATION NETWORK** port. Insert the female end of the 9-pin cable provided into either port. The other end of this cable is connected to the PUMP connector on the rear panel of the epsilon controller (or the COMM. NET connector on a DA-5 Data System). This connection needs to be made only if you wish to control the pump from the computer. Many users prefer to control the pump from its front panel (see the section on Manual Controls).

Additional connections are provided for other applications:

PRESS. OUT and **PRESS. GND.** Provide a 1 V per 1000 PSI output to monitor pressure. The output indicates absolute pressure when the offset adjustment is fully counter-clockwise.

PRESSURE OFFSET. This potentiometer is used to adjust pen position when recording pump pressure.

To monitor pressure with an epsilon system, connect the 'PRESS.' terminal and its 'GND' as an external detector with a 10V input. Click here for details.

To monitor pump pressure with a chart recorder, first set your chart recorder for an input of 10 V. Connect two wires from the PRESS. and GND connectors on the terminal strip of the PM-90 to the input of the chart recorder. Adjust the PRESSURE OFFSET potentiometer on the rear panel of the PM-90 until the chart-recorder pen is about mid-scale. Now reduce the input voltage of the chart recorder in steps,

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adjusting the PRESSURE OFFSET control as necessary to keep the pen on the chart. Reduce the input voltage of the chart recorder until the pressure fluctuations can be measured on the chart (a 0.1 V input range should be about right). The analog pressure output produces 1 volt per 1000 PSI. The chart can be calibrated with the following formula:

P = (1000 x D x V) / W

where:

P = pressure fluctuation in PSI

W = width of chart paper (mm)

D = magnitude of pen deflection (mm)

V = input range of chart recorder (Volts)

START IN. A switch closure or TTL-low across the START and GROUND terminals will start the pump. This is the same as pressing the RUN key from the front panel

START OUT. Sends a 1 second TTL-low signal when the pump is started.

STOP IN. A switch closure or low-level TTL across the STOP and GROUND terminals will stop the pump. This is the same as pressing the STOP key from the front panel.

STOP OUT. Sends a 1 second TTL-low signal when the pump is stopped.

AUX OUT1. AUX OUT1 and it's ground produce a TTL-HI (5 V) when the pump is not pumping, and a TTL-LO (0 V) when the pump is running. This can be used to signal peripheral equipment, such as autosamplers, that the pump is in a ready (running) state.

AUX IN1, AUX IN2 and OUT2. Not currently used.

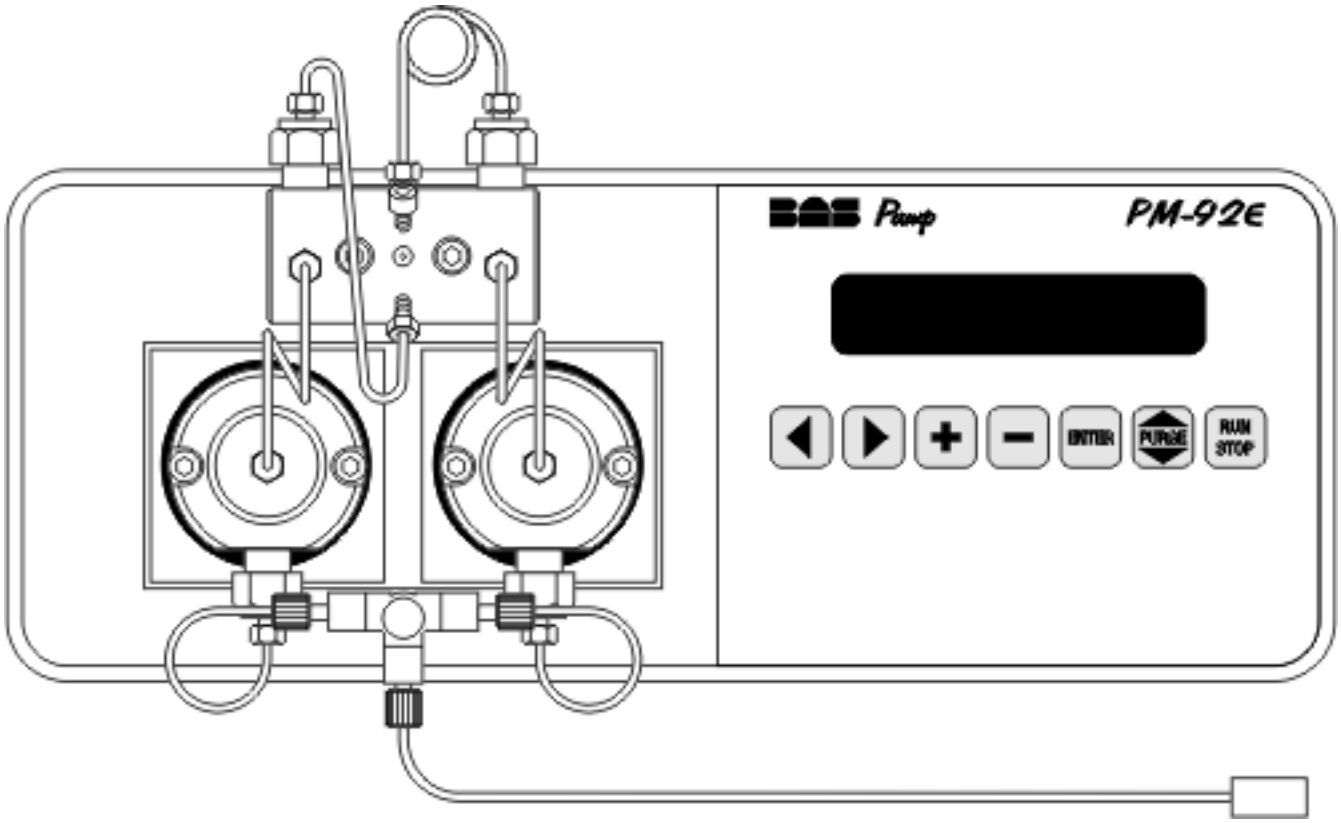
SERIAL RS-232C. This 25-pin 'D' connector is provided for remote control of the PM-90 pump. In most cases, however, the pump is connect to the epsilon detector (or the DA-5 Data System) via one of the BAS COMMUNICATIONS NETWORK ports.

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MANUAL CONTROLS

The PM-90 pump can be controlled from its front panel as detailed below, or by the pump module of ChromGraph Control software.



KEY FUNCTIONS



Move the cursor left and right within a line.



Increase or decrease a numerical value within a line.



Advances to the next input line — use this key to quickly navigate and review the two screens.



Sets the pump to operate at a flow rate of approximately 5 mL/min to aid in priming the pump or purging the system of air. You must press RUN/STOP to begin the purge.



Starts or stops purging after PURGE has been pressed — when purging is stopped both plungers are moved to the full forward position for head removal or installation.



Starts or stops the pump — this control is used to start or stop solvent flow without turning off the main power.

CONTROLS

There are three parameters involved in setting up the pump for operation:

1. Flow Rate
2. Low Pressure Limit
3. High Pressure Limit

Flow Rate. A flow rate ranging from 1-2000 (PM-92) or 1-1200 (PM-91) µL/min can be selected using the + and - keys to increment or decrement the value. After selecting the desired flow rate, press the ENTER key to advance to the next screen. If the pump is currently running, the flow rate will change as new values are entered.

Low Pressure Limit. If necessary, press the ENTER key to change to this screen. Then use the + and - keys to select the Low Pressure Limit (PSI). The Low Pressure limit can be adjusted to a value between 0 and 999 PSI. Press the ENTER key to advance to the next field.

If a fitting develops a leak, or if the mobile-phase reservoir runs dry during unattended operation, the low-pressure cutoff will stop the pump as pressure drops. Proper setting of this control will avoid pump damage. When the Low Pressure Limit is set to 0 PSI this function is disabled.

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If the low-pressure limit is reached, the pump will stop and the LCD will display a Low Pressure message. After correcting the problem, reset the protective circuitry by pressing the ENTER key. The pump can then be restarted by pressing the RUN key.

The low-pressure limit is enabled only after pressure exceeds its set value. Thus, you can run the pump with the prime/purge connection open (pressure will be 0 PSI) even though the low-pressure limit is set to a value such as 300 PSI. Only after the prime/purge connection is closed, and pressure rises above 300 PSI, will a drop in pressure trigger the protective circuitry.

High Pressure Limit. If necessary, press the ENTER key to change to this screen. Then use the + and - keys to select the High Pressure Limit (PSI). High Pressure limit can be adjusted to a value between 1000 and 5000 PSI. Press the ENTER key to return to the Flow Rate Screen.

It is **EXTREMELY IMPORTANT** that this limit be set to some reasonable value; if a clog develops anywhere in the flow stream between the pump and the detector, the high-pressure cutoff will stop the pump as pressure increases. We suggest setting the cutoff 1000 PSI above your typical operating pressure.

If the high-pressure limit is reached, the pump will stop and the LCD will display a High Pressure message. After correcting the problem, reset the protective circuitry by pressing the ENTER key. The pump can then be restarted by pressing the RUN key.

Purge. The PURGE key causes the pump to operate at a flow rate of approximately 5 mL/min to aid in priming the pump or to purge the system of trapped air. The purge plug on the manifold should be removed prior to purging the pump if a column is connected. The plug can be replaced by a luer adapter which allows a syringe to be connected to apply suction to aid in priming the pump.

To avoid inadvertant purging, the RUN/STOP key must be pressed after the PURGE key to begin purging.

Pressing the STOP key will stop purging and move both pistons to their full forward position. This is done to allow easy removal or installation of pump heads. It may take 1-2 seconds for the pump to come to a complete stop after the STOP key is pressed while purging. After the STOP key is pressed the pump will be reset to the flow rate prior to the start of the purge.

NOTE: Always allow the system pressure to fall below 30 PSI before opening the prime/purge plug on the manifold. This will prevent pressure shocks from damaging the column or pulse damper.

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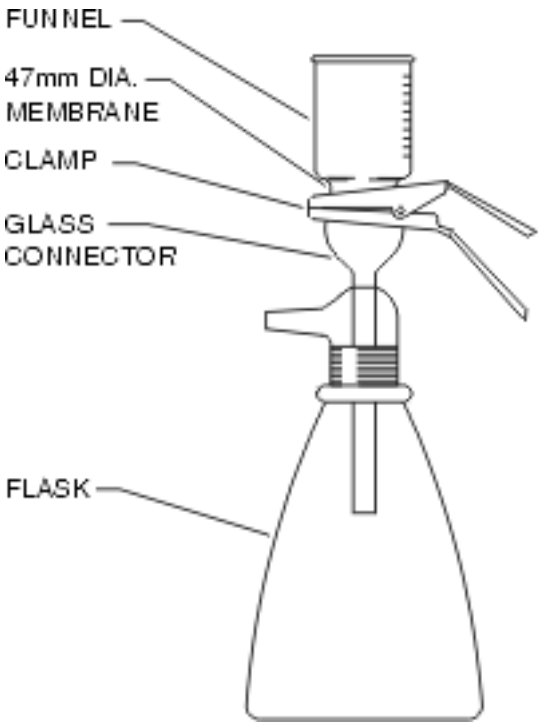
MOBILE PHASE PREPARATION

Use only high-purity solvents and buffer salts for preparing mobile phases. Water should be type I reagent grade, with a resistivity of >15 megohms/cm^2. Don't forget to thoroughly wash all glassware used to prepare and hold the mobile phase.

Filter all mobile phases through a 0.2 µm membrane filter. The figure below illustrates the filtering device recommended by BAS for doing this. It is borosilicate glass throughout, and uses ground glass joints to avoid contamination from stoppers. The filtration step will reduce problems with column degradation significantly.

CLEAN, PARTICLE-FREE MOBILE PHASES ARE CRITICAL TO ANY LC METHOD!

MF-6126 Filtration Apparatus



Mobile phases should be degassed prior to use. Vacuum filtering may be sufficient to degas the mobile phase. But if bubbles are a problem in your system, a more-thorough degassing is required. Allowing the solution to sit under the vacuum generated by an aspirator for 5-15 minutes should be sufficient. The MF-6126 kit also can be used for degassing, by replacing the filtration apparatus on the top of the unit with a stopper. Small amounts of volatile organic modifiers may be lost, but not enough to cause any difficulty.

The easiest and most effective method of degassing is the LC-26 on-line degasser. This highly effective instrument reliably and consistently delivers bubble-free mobile phase to the PM-90 pump. We strongly recommend its use, particularly for gradient applications.

Whichever way you make your mobile phase, be consistent; consistency in mobile phase preparation is critical for

reproducible chromatography.

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STARTUP

Proper startup and shutdown procedure is vital for long and dependable pump life. The two most important considerations are:

- **NEVER START THE PUMP WITH DRY PLUNGERS**
- **NEVER LEAVE STAGNANT MOBILE PHASE IN THE PUMP**

Correct startup procedure is to purge the pump as follows:

1. Turn the POWER on.
2. Squirt a few drops of water into the plunger-irrigation ports. Use only enough water to moisten the plungers.

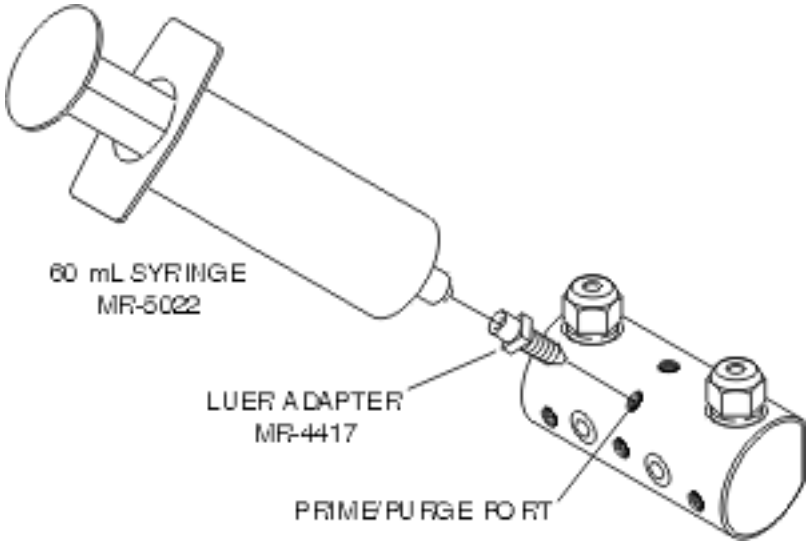


3. Place the solvent-uptake frit into a glass reagent bottle containing 40:60 (v:v:) acetonitrile:water. Elevating the reservoir to provide hydrostatic pressure will be helpful.

   NOTE: ALL SOLVENTS AND MOBILE PHASES USED IN THE PM-90 PUMP SHOULD BE FILTERED THROUGH 0.2 µm MEMBRANE FILTERS. USE ONLY LC-GRADE SOLVENTS AND TYPE I REAGENT-GRADE WATER.

4. Attach the luer adapter (supplied) to a 60 mL disposable syringe. Remove the purge plug from the manifold (don't forget that system pressure must be below 30 PSI before opening the plug) and replace with the luer adapter. Aspirate until solvent comes through the pump. Leave the syringe attached.

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5. Press the PURGE Key, then press the RUN/STOP key to start the purge. Purge for five minutes. During this purge you may alternately draw back and release the syringe plunger to help dislodge air bubbles from the system.

6. Press the RUN/STOP key to stop the pump. Note: The pump may take 1-2 seconds to come to a complete stop following purge. Set the flow to 1000 µl/min. Replace the luer adapter with the plug.

7. If there is no column attached, you may wish to pump solvent through the injection valve (in the 'inject' position) to displace any air and old solvent. Simply start the pump by pressing the RUN/STOP key and collect the waste solvent at the outlet of the injection valve.

8. To change solvents, or to switch to the mobile phase, repeat steps 3-7 as appropriate.

9. If necessary, reset the flow to 100 µl/min or other appropriate setting.

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SHUTDOWN

The most important principle to remember when turning off your pump for storage is to remove all mobile-phase salts. Salts in the system will lead to corrosion of the stainless steel lines (yes, they corrode, but at a slow rate), and the presence of abrasive crystals in the pump heads. Abrasives will scratch the seals and plungers during subsequent startup.

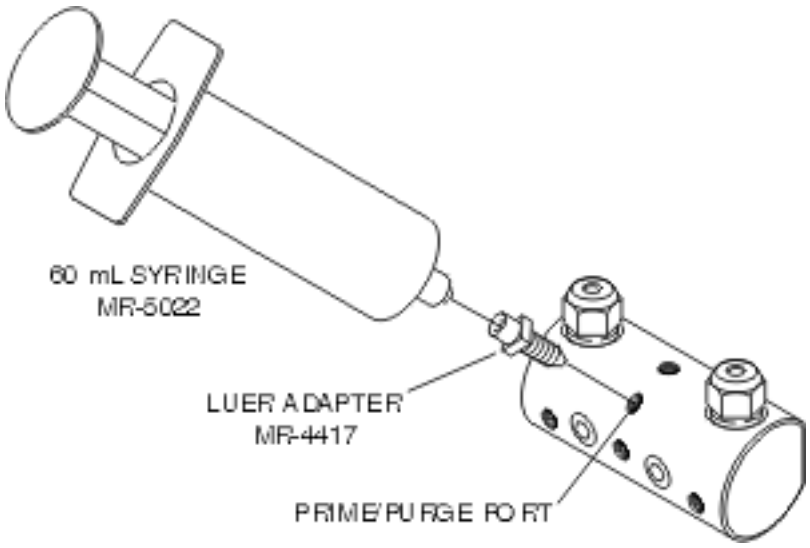
The shutdown procedure is as follows:

1. Turn the POWER on.

2. Place the solvent-pickup frit in a reagent bottle containing 40:60 (v:v:) acetonitrile:water. Elevating the reservoir to provide hydrostatic pressure will be helpful.

   NOTE: ALL SOLVENTS AND MOBILE PHASES USED IN THE PM-90 PUMP SHOULD BE FILTERED THROUGH 0.2 µm MEMBRANE FILTERS. USE ONLY LC-GRADE SOLVENTS AND TYPE I WATER.

3. Check to make sure that system pressure is below 30 PSI. Replace the prime/purge plug on the manifold with the luer adapter and connect a 60-mL syringe to the luer adapter.



4. Press the PURGE key then press the RUN/STOP key to begin purging the pump. Purge for five minutes. This will bring fresh solvent through the pump.

5. If the column is to be cleaned with this solvent, proceed to step 6. If the column has been removed, go to step 7.

6. (To clean both the column and the pump.) Set the flow to 100 µL/min or other appropriate range. Replace the luer adapter with the plug. Pump an appropriate amount of solvent through the pump, injector (in INJECT position to flush the loop), and column. Shut the system off, remove and cap the column for storage.

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7. (To clean only the pump.) Remove the column. Set the flow to 1999 µL/min. Pump an appropriate amount of solvent through the pump and injector (in INJECT position to flush the loop). Then shut the system off.

8. Squirt a few drops of water into the plunger-irrigation ports. Use only enough water to moisten the plungers.



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PM-90 MAINTENANCE

- **PUMP HEADS**
- **CHECK VALVES**
- **PRESSURE TRANSDUCERS**
- **FITTINGS**
- **IN-LINE SOLVENT FILTER**
- **FAN FILTER**
- **ROUTINE MAINTENANCE**

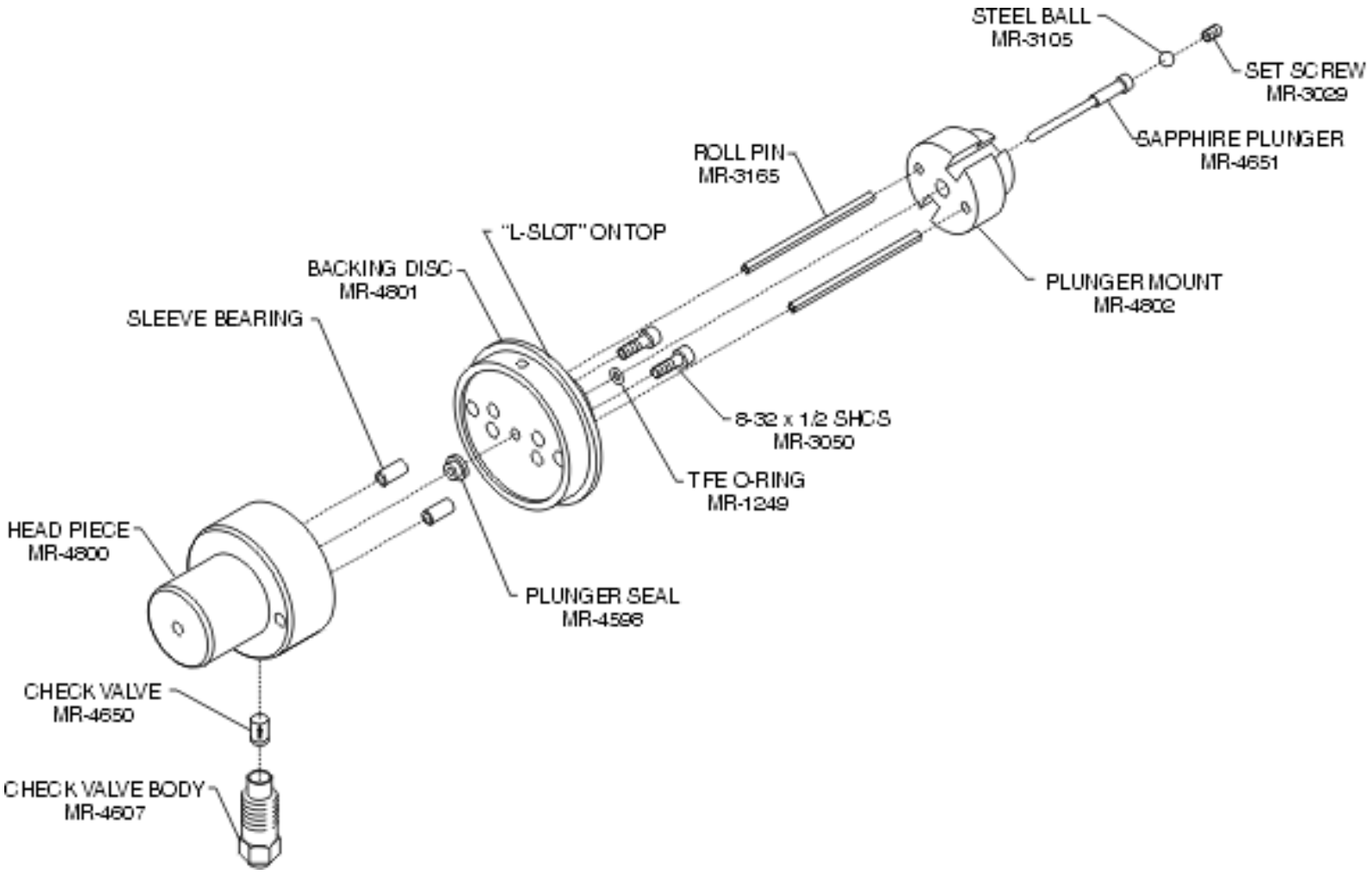
PUMP HEADS

The procedures detailed below encompass all user services that require removal of the pump heads. They include:

- Pump head replacement
- Plunger seal replacement
- Plunger free-play adjustment
- Plunger replacement
- Plunger seal break-in

This section will detail the entire procedure, from head removal to re-insertion. The text will indicate which sections can be skipped when performing simple maintenance.

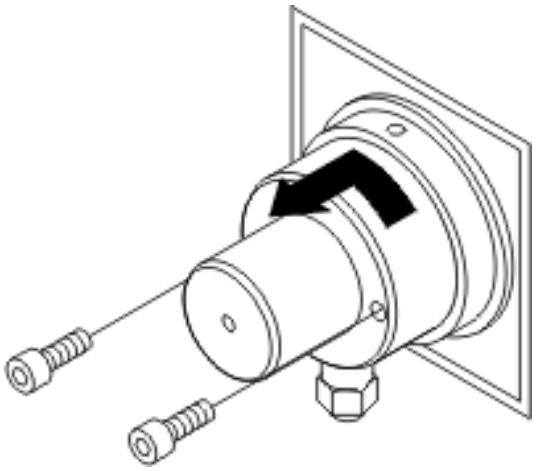
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Pump Head Removal

An entire pump head may be rapidly removed by the following procedure:

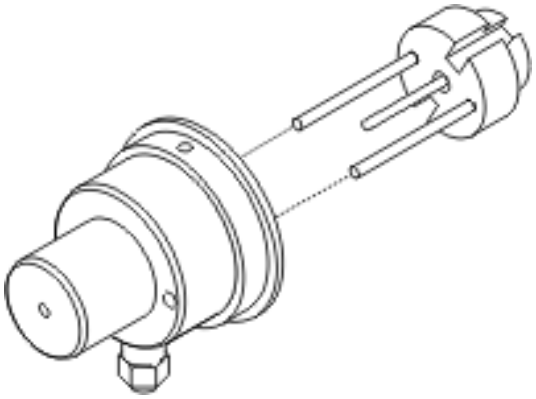
1. Observe the pressure display to make sure there is no residual pressure in the lines.
2. Open the purge plug. Press the PURGE key and purge the pump for 5 seconds. Then press the STOP key to stop the pump. This step moves the plungers to the full forward position for removal of the pump heads.
3. Turn the POWER off.
4. Remove the outlet line from the head using a 1/4" wrench.
5. Hold the inlet check-valve assembly stationary with a 1/2" wrench and remove the inlet line with a 1/4" wrench.
6. Using a 9/64" hex head driver, unscrew two 8-32 socket head cap screws from the pump head.
7. Rotate the head 30° counterclockwise until it stops, then gently pull the head straight out of the housing.



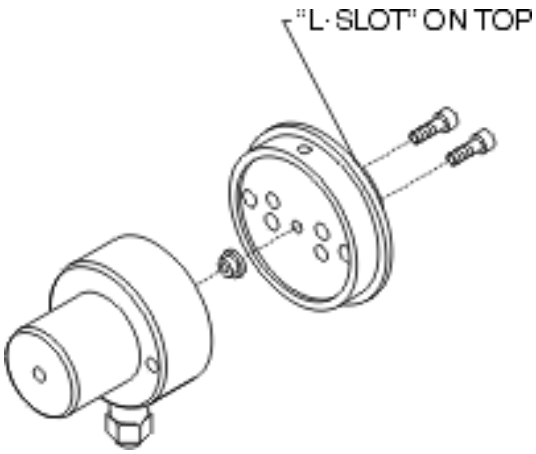
Pump Head Disassembly

Servicing or inspection of the plunger seal, plunger spring, and plunger require disassembly of the pump head. Proceed as follows:

1. Gently pull the plunger assembly straight back from the Head pulling the plunger out of the Head.



2. Locate the two 8-32 socket head cap screws on the back of the Head and remove using a 9/64" hex head driver. Pull the Backing Disk away from the head to expose the plunger seal.



Plunger Inspection and Servicing

1. Examine the plunger carefully. Wash the sapphire rod with water and methanol. Salts may be removed by gently scrubbing with a green Scotchbrite® scouring pad moistened with water. Replace the plunger if it is chipped, scored or scratched (if it needs to be replaced, proceed to item 4).

2. Test the plunger free-play. Hold the plunger assembly in one hand with the sapphire rod pointing up. Wiggle the sapphire rod from side to side with the other hand. It should move freely. Rotate it radially; it should move freely through 360°, much as you would roll your head on your shoulders to stretch your neck muscles. Now try to move it up and down; there should be no movement in the longitudinal direction.

3. If the rod passes the free-play test, proceed to the next section (seal replacement). If the sapphire rod is either too loose or too tight, perform the adjustment procedure beginning in item 6.

4. To replace the sapphire plunger, grasp the Plunger Mount firmly and unscrew the set screw from the back of the Plunger Mount. Remove the plunger by sliding it out of the back of the Plunger Mount and replace with a new one. A small ball bearing sits at the base of the plunger and is held in place with a dab of grease. Do not lose the ball during assembly.

5. A nylon patch locks the reusable set screw in position once the position is set.

6. To adjust the new or old sapphire plunger, tighten or loosen the set screw while testing free play.

7. Wiggle the sapphire plunger from side to side; it should move freely. Rotate it radially;it should move freely through 360°, much as you would roll your head on your shoulders to stretch your neck muscles. Now try to move it up and down; there should be no movement in the longitudinal direction.

Plunger Seal Replacement

1. Place the front portion of the pump head on a flat surface with the seal facing up.

2. Remove the seal by inserting a 6-32 threaded screw into the seal material several turns, then pulling out the seal. Be careful not to scratch the metal of the pump head with the screw.

3. Wash the pump head with water. Use a squeeze bottle to flush liquid through the check valves and all exposed ports.

4. Irrigate the pump head and new seal (part number MR- 4598) with methanol, and push the seal fully into its seat with your thumb. Do not scratch the seal with a fingernail.

5. Irrigate the Backing Disk if salt deposits have collected inside the irrigation ports.

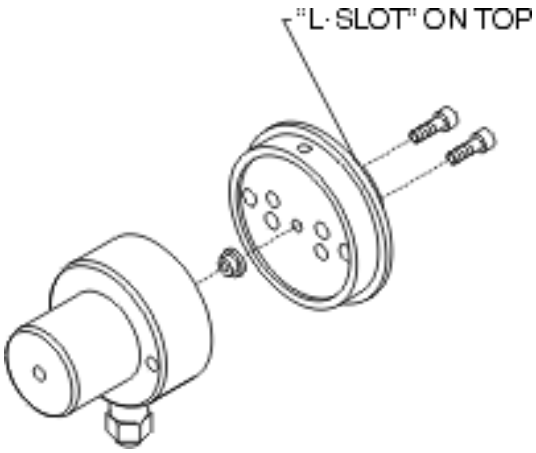
6. Proceed with pump head reassembly. Be sure to follow the break-in procedure for new plunger seals (see below) after the pump is reassembled.

Pump Head Assembly

1. Wash all parts with water.

2. Place the front part of the pump head face-down on a flat surface. Irrigate the plunger seal with methanol.

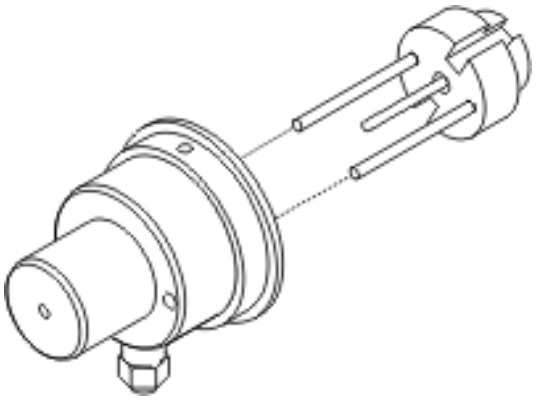
3. The backing disk has an L-shaped slot in its side for locking the head assembly to the pump housing. Orient the backing disk so the L-shaped slot is opposite the check valve assembly on the head. Insert the backing disk on the back of the head and screw down with two 8-32 socket head cap screws in the counterbored holes.



4. Irrigate the plunger-seal area and the sapphire rod with methanol.

   IT IS IMPORTANT THAT THE SEAL BE MOIST BEFORE INSTALLING THE PLUNGER ASSEMBLY!

5. Align the guide rods on the plunger assembly with the two holes in the backing disk and insert the plunger assembly with the sapphire plunger entering the plunger seal.



6. Insert the plunger assembly until it is about 1/4" away from the Backing Disk. Do not press the plunger assembly all the way down against the backing disk or the head cannot be reinstalled into the pump drive.

Pump Head Installation

To properly engage the plunger to the plunger drive mechanism, the plunger drive mechanism must be fully forward. This procedure assures that the drive mechanism is properly positioned for head installation or removal.

1. Turn Power on the pump.

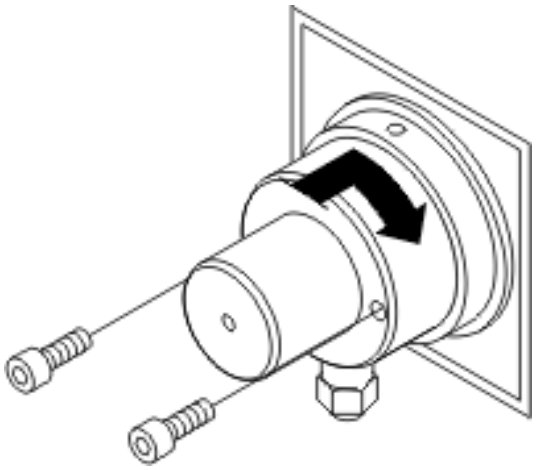
2. Press the PURGE key.

3. Press the RUN key. Purge for 5 seconds.

4. Press the STOP key. By stopping the pump following purging, the plunger drive mechanism(s) will automatically be moved to the full forward position for installation of the pump head.

   NOTE: THE PLUNGER DRIVE MUST BE FULLY FORWARD FOR THE PLUNGER TO ENGAGE THE DRIVE MECHANISM.

5. With the pump head inlet check valve facing down, align the slot in the plunger assembly with the pin in the housing and slide the head assembly straight into the housing. As the head plunger mount contacts the plunger drive in the pump, the resistance to insertion will increase as the plunger slides within the seal. Continue to slide the head forward until the head backing disk rests firmly against the face of the housing.



6. Rotate the head clockwise 30° until the check valve is vertically down and the head mounting holes align with the threaded holes in the housing. This engages the plunger mount to the plunger drive.

   Note: If the plunger assembly is closer than 1/4" to the Backing Disk, the head will be locked out from rotating into place. Remove the head and pull the plunger assembly away from the Backing Disk about 1/4" and repeat steps 5 and 6.

7. Tighten the head to the housing with two 8-32 socket head cap screws.

8. Hold the inlet check valve assembly stationary with a 1/2" wrench and attach the inlet line with a 1/4" wrench.

9. Attach the fitting on the pressure manifold line to the front of the head(s).

10. Follow the STARTUP procedure. It is very important to:

    1. ensure that the plunger seals are moist at startup

2. purge all air from the system

11. If new plunger seals were installed, follow the break-in procedure below.

PLUNGER SEAL BREAK-IN

Plunger seals must seat properly for longest life. A salt-free solvent is recommended for break-in. Proceed as follows after installing new seals:

1. Wet the irrigation ports in the pump heads with a few drops of water.

2. Purge the system with filtered 40:60 (v:v) acetonitrile:water, as described in the STARTUP section.

3. Attach a column to the system. It need not be a good column, as its only purpose is to provide backpressure.

4. Run the pump for two hours at a pressure of 3000-3500 PSI. Adjust the flow rate as necessary to achieve this pressure.

5. After two hours you may switch to mobile phase and begin chromatography.

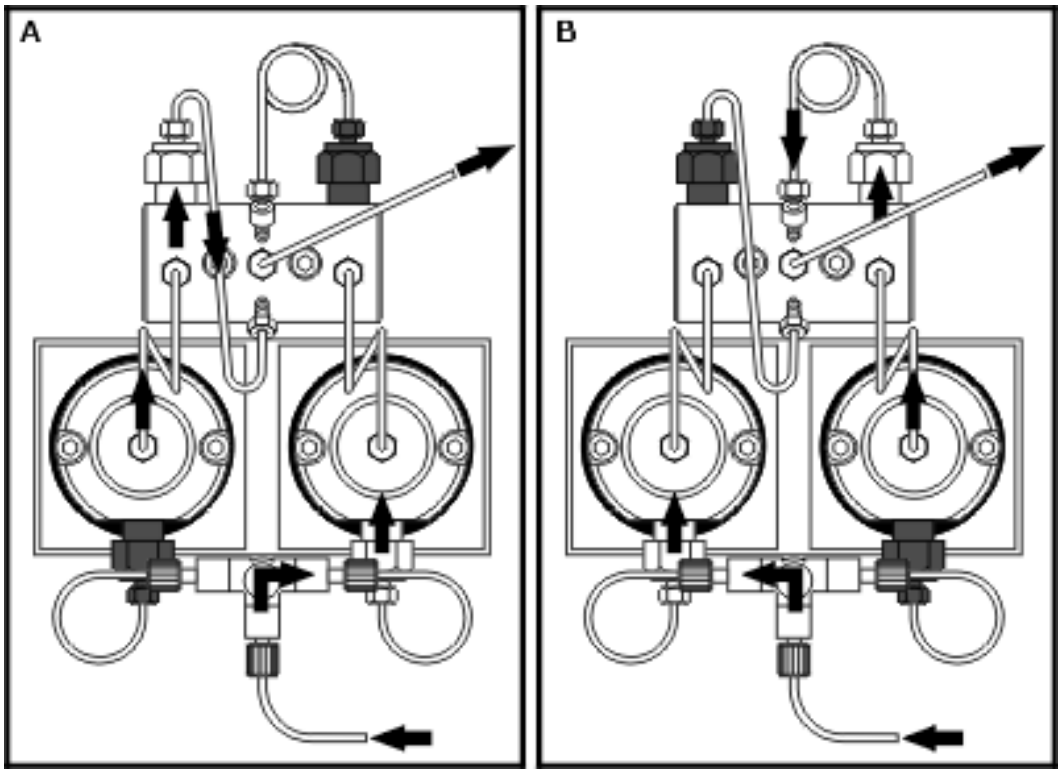
SERVICING CHECK VALVES

The PM-90 pumps use cartridge-type check valves (part number MR-4650) that do not contain removable parts. The same valve is used for both the inlet and outlet check valves on the pump. Each check-valve cartridge contains two precision-engineered balls and seats in series, for a reliable seal. You can determine the direction of flow through the cartridge by attempting to squeeze water or methanol through it in each direction. An arrow on the side of the cartridge also indicates flow direction.When installing, remember that flow is always "up" through the pump.

In most cases, faulty check-valve performance (as indicated by large pressure fluctuations) is due to microscopic debris, salt precipitation, or trapped gas pockets. Gas can be removed by purging with freshly degassed mobile phase or solvent, as described in the STARTUP section. The check valves may be cleaned by sonication (see below), or (if necessary) replaced. When cleaning or replacing these cartridges, remember that any lint from towels, paper wipes, etc. may reintroduce the problem. The best approach is to flush the check valves with methanol and to reassemble them wet.

An understanding of flow through the pump and check valves is helpful in diagnosing check-valve problems. Flow during a full cycle of two pump strokes for the PM-92 pump is as follows:

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When the left pump head is compressing, the inlet check valve below it closes, preventing any backflow. The outlet check valve above it opens, allowing mobile phase to proceed towards the system pressure transducer in the center of the pressure manifold, then out of the pump.

While this is occurring, the right pump head is aspirating. It's inlet check valve is open, allowing mobile phase to enter from the solvent reservoir. The outlet check valve is closed, which prevents any backflow into the right pump head from the left head and manifold. The LCD displays an "L" during this part of the pump cycle.

After the left pump head finishes its stroke, the roles of the two pump heads reverse. The right pump head begins compressing. The inlet check valve closes, and the outlet check valve opens. The left pump head begins aspirating; its inlet check valve opens and its outlet check valve closes. The LCD displays an "R" during this part of the pump cycle.

Pump-related malfunctions that produce pressure fluctuations can be localized by observing the pump stroke and pressure readout. When the display shows "L" while pumping, the left head is compressing. It's inlet check valve must shut, and it's outlet check valve must open. In addition, because the pressure manifold chamber is open to both pump heads, the outlet check valve of the right pump head must close. If it didn't, fluid would flow from the left pump head into the manifold, then down into the right pump head. The opposite argument applies to the right pump head.

Because of this relationship, there are three likely places to check when pressure drops as one pump head compresses: the pump head itself (plunger seal, plunger), the inlet check valve for that pump head, and the outlet check valve for the opposite head.

Check Valve Removal

1. Stop the pump and allow pressure to dissipate. Then turn off the POWER.

2. Hold the check-valve assembly stationary with a wrench and remove the inlet or outlet line with another wrench.

3. Remove the check-valve assembly, which contains the check-valve cartridge.

Check Valve Cleaning

1. Place the entire check-valve assembly in a solution of laboratory detergent warmed to 50 °C. We recommend a 50% solution of RBS-35® (Pierce Chemical Company). Soak for 1-2 hours. (Do not warm the solution above 60 ° C, because plastic parts in the check valves may become distorted.)

2. Sonicate the check-valve assembly for 15 minutes in the detergent solution.

3. Flush with deionized water, then methanol.

4. Reinstall the check-valve assembly following the instructions below. If check-valve problems continue, install a new cartridge.

Check-valve Cartridge Replacement

1. Remove check-valve assembly from the pump head.

2. If the cartridge does not shake out of the assembly, push it out with a paper clip. This may require some force. Alternatively, you may be able to insert a small screw into the cartridge and pull it out of the assembly. Discard the old cartridge, as it is now damaged.

3. Clean the new cartridge, as above.

4. Insert the new cartridge. Be certain to install the cartridge so it allows flow in the proper direction. Flow is always up through the pump; inlet check valves permit flow from the inlet line to the pump head, outlet check valves permit flow from the pump head to the outlet line. The cartridges may have an arrow to indicate flow direction. If not, the end with the small hole is the inlet, and the end with either several holes or a large hole is the outlet.

Check Valve Installation

1. Squirt some methanol through the check valve. This will both wet it and allow you to observe whether the direction of flow is correct.

2. Install the check-valve assembly onto the pump head with a wrench.

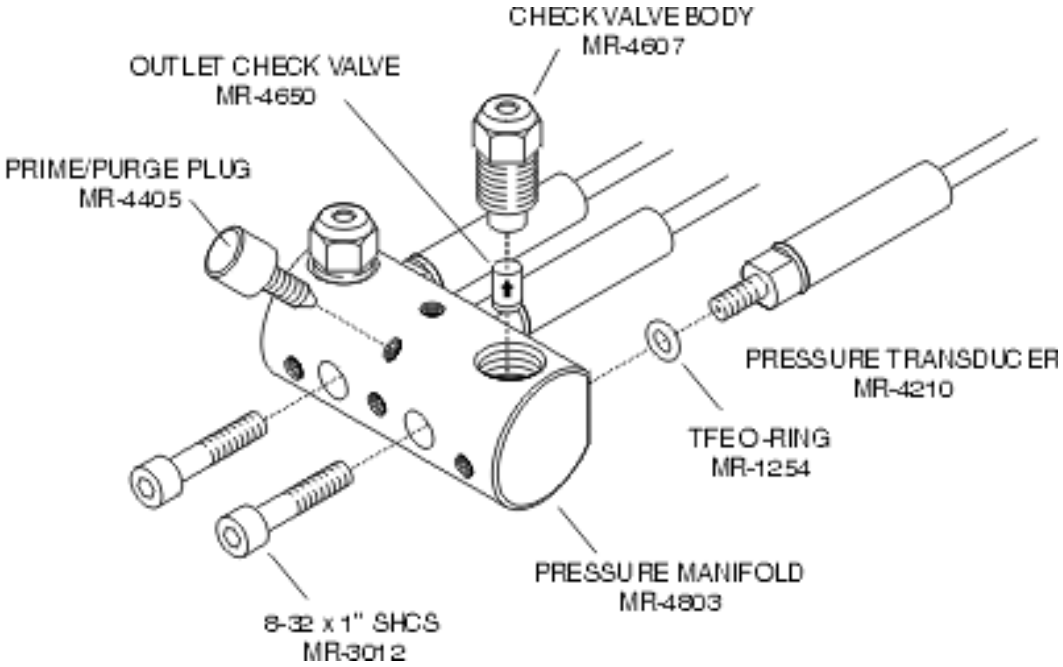
3. Hold the check-valve assembly stationary with the wrench and connect the inlet or outlet tubing with a second wrench.

4. Follow the STARTUP procedure to prime the pump and remove all air from the check valves and lines.

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PRESSURE TRANSDUCERS

There are three pressure transducers located in the pressure manifold of the PM-92 pump. Two pressure transducers are located in the manifold of the PM-91 pump. They should rarely need attention. If a leak ever develops, there is a PTFE o-ring on the pressure transducer that needs to be replaced (Part Number MR-1254). Follow these steps:



1. Stop the pump and allow system pressure to fall to 30 PSI or less. Turn the POWER off.

2. Using a 1/2" wrench to hold the outlet check valve, remove the fitting attached to the outlet check valve using a 1/4" wrench. Remove the fittings from the other outlet check valve.

3. Using a 1/4" wrench, remove all the fittings from the pressure manifold.

4. Remove the two 8-32 socket head cap screws which mount the pressure manifold to the front panel.

5. Pull the pressure manifold straight back. The pressure transducers are mounted to the back of the pressure manifold.

6. Use a 3/8" wrench on the flat of the leaking pressure transducer to unscrew the transducer from the pressure manifold. Pull the transducer straight back from the manifold.

7. Remove the o-ring from the transducer and replace with a new o-ring (Part Number MR-1254).

8. Before screwing the pressure transducer back into the pressure manifold, twist the pressure transducer four turns counter-clockwise so that when the transducer is screwed into the manifold, the transducer cable is unwound and is left untwisted when the transducer is mounted tightly to the pressure manifold.

9. Screw the pressure transducer into the manifold finger tight, so the o-ring is squeezed against the back of the

pressure manifold. Using a 3/8" wrench, tighten the transducer another 3/4 turn.

10. Mount the pressure manifold to the front panel with two 8-32 socket head cap screws.

11. Reconnect all fittings to the same ports they were connected to previously.

12. Purge the pump as described in the STARTUP section.

FITTINGS

Check all fittings for leaks every day. Large leaks will be obvious. Small leaks will be apparent only by the accumulation of salt deposits around the fitting. It is not normal for fittings to have even small leaks, and these should be tended to.

The first approach to treating a small leak is to clean up the salts with water and tighten the nut slightly. About 1/8 turn should do it, unless the nut is loose. If this doesn't stop the leak, it's best to replace the fitting and line entirely. Why?

It's certainly tempting to use a lot of force to stop a small leak. But several bad things can happen when you overtighten a fitting:

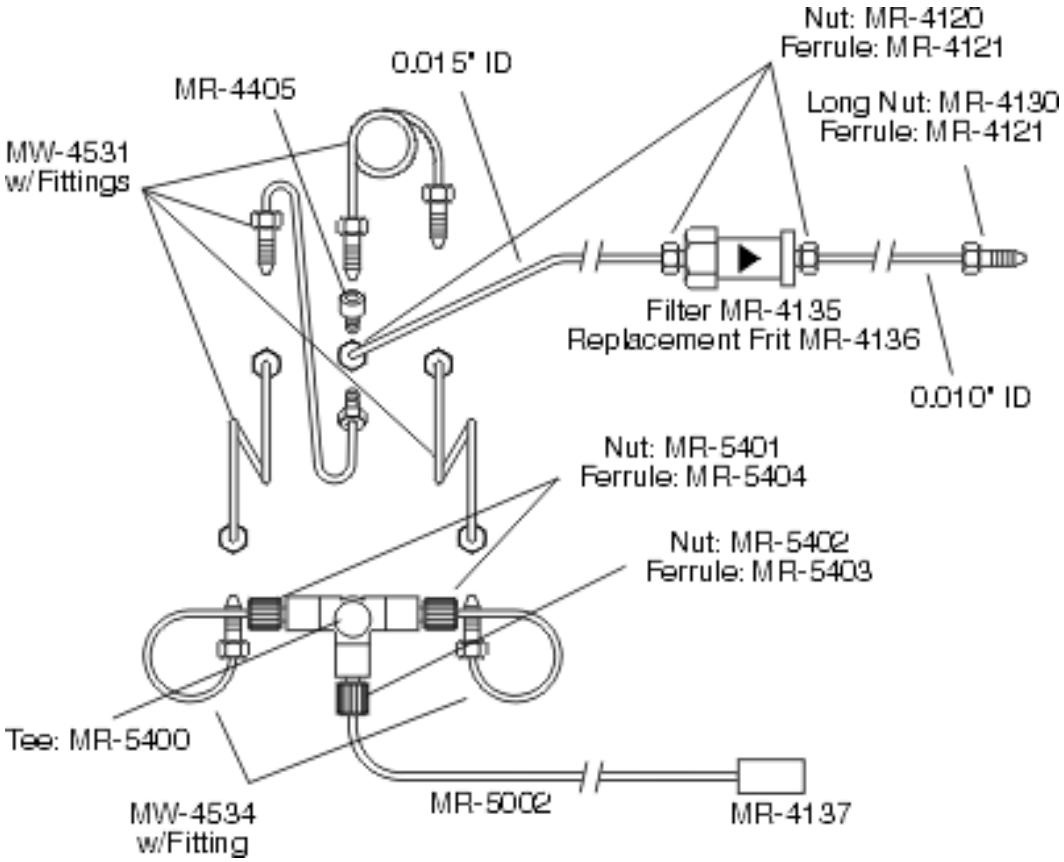
- The nut may break off with its threads still in the hole. A machinist will have to get it out for you, and if you're very lucky the part won't be damaged.

- The nut may fuse in place, and break off the next time you try to open it.

- The tubing end may become crimped, restricting flow and causing performance problems.

It makes more sense to replace a fitting or line before any of these events occur. This figure gives part numbers and ferrule types for all tubing used in the PM-90 pump:

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IN-LINE SOLVENT FILTER



The in-line filter should be suspected whenever system pressure rises above normal. The filter becomes clogged by doing its job: preventing particles from the mobile phase or pump seals from lodging in the injector or on the column. To replace the filter frit do the following:

1. Stop the pump and allow pressure to drop to zero.
2. Remove the inlet and outlet tubing to the filter assembly. Be sure to note the flow direction.
3. Loosen the two halves of the filter assembly with two wrenches, but do not separate them yet.
4. Hold the assembly vertically, with the smaller section on the bottom. Now unscrew the larger section and remove.
5. The two frit supports and the frit can now be removed from the smaller section. Keep the supports separate so they can be reassembled in the same order: they seal best when installed in the section from which they came.
6. Flush water through each support to ensure that its passageway is not clogged.

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7. Place the appropriate support on the smaller section, then put a new filter frit (MR-4136) on top of it. Put on the remaining support, then screw on the larger section. Tighten with two wrenches.

8. Attach the inlet line from the pump to the assembly, observing proper flow direction. Pump some mobile phase through the unit to remove air, then attach the outlet tubing.

FAN FILTER



The fan filter should be removed for cleaning at least once per year (more often if it is visibly dirty.) Proceed as follows:

1. Gently pry off the retaining grid with a screwdriver. **Do not unscrew the mounting screws!**

2. Carefully pull out the filter. You may either vacuum it clean, or wash it gently in warm sudsy water. Be careful not to tear the filter.

3. If you've washed the filter, blot it well between sheets of paper towels, then allow it to dry.

4. Reinstall the filter by holding it in place over the fan opening, then snapping the retaining grid in place. Position the grid with its ridges facing in towards the filter.

ROUTINE MAINTENANCE

Regular maintenance will keep pump performance up to specifications. We recommend:

Every Day	Inspect all fittings in the flow path for leaks.

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Every Three Months	Replace plunger seals and inspect interior of pump head.
Every Year	Clean the fan filter.

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PM-90 TROUBLESHOOTING

This section contains troubleshooting tips specific to the PM-90 pump. For general troubleshooting see the main TROUBLESHOOTING page.

POWER WON'T TURN ON

- Check power connection.
- Check fuse and fuse type.

AUDIBLE NOISE

- **Continuous Buzzing Sound**
    - Grasp each pump head with your hands. Is the audible noise significantly reduced? If so, the noise is due to motor-generated vibration. At certain flow rates, the stepper motor excites the pump module to greater vibrations. This can cause the pump module to contact the adjacent pump module or the sheet metal front panel.. This is more likely to happen when there is no load (no high pressure) and at higher flow rates.
    - Open the purge plug, reduce the flow rate to 50 µL/min. and observe the L and R indicator on the LCD screen. Does the pump make a continuous knocking sound the entire time while either L or R is displayed? If so, Contact BAS.
- **Intermittent Buzzing Sound**
    - Open the purge plug , reduce the flow rate to 50 µL/min. and observe the L or R indicator on the LCD screen to correlate the noise with Left or Right head. Does the buzzing resolve into a continuous knocking sound which only occurs with one head? Does the knocking sound occur only towards the end of stroke of one head? If so, the pump head is not engaged to the drive mechanism. Remove the head and reinstall it.

NO SOLVENT FLOW

- Check FLOW RATE setting. Is it set for a valid, non-zero range (1-1999 µL/min)?
- Has the Run/Stop key been pressed to start the pump? The LCD will display pumPing or iniTing while the pump is operating.
- Is the high- or low-pressure limit displayed on the LCD? If so, see below.

- Is there an error message on the display? If so, contact BAS.
- Check for loose connections on the inlet side of the pump. Purge with freshly degassed mobile phase.
- Is the mobile phase reservoir empty. Purge with fresh mobile phase.
- Is the solvent uptake frit clogged? Clean or replace.

NO SOLVENT FLOW FROM ONE HEAD ONLY

- Check for loose inlet connection to that head. Purge with freshly degassed mobile phase.
- Head not engaged to drive mechanism. Remove head and reinstall.
- Inlet check valve failure. Switch inlet check valves between the two heads. If the problem follows the check valve then clean or replace inlet check valve cartridge.
- Check the irrigation port outlet behind and under the head. If wet or buffer salt deposits have accumulated, a seal may be leaking. See the section for seal replacement.

PUMP STOPS: LOW-PRESSURE LIMIT

- The mobile-phase reservoir is empty. Make fresh mobile phase and purge as described in the STARTUP section.
- There's a leak in the system. Check all connections.
- Inlet check valve failure. Switch inlet check valves between the two heads. If the problem follows the check valve then clean or replace inlet check valve cartridge.
- Check the irrigation port outlet behind and under the head. If wet or buffer salt deposits have accumulated, a seal may be leaking. See the section on seal replacement.
- The low pressure limit is set too high. Reset to a reasonable value for your conditions.

PUMP STOPS: HIGH-PRESSURE LIMIT

- The high-pressure limit is set too low. Reset to 4000 PSI or a reasonable value (1000 PSI above your typical operating pressure) for your conditions.
- The injection valve is not fully in the inject or load position. Rotate it to one side.

- The injection valve is not rotated fast enough when switching from load to inject or inject to load. When making an injection the valve must be rotated quickly or pressure may exceed the high pressure limit.

- There's a clog in the flow path. It could be anywhere between the pump outlet and the detector. It may be the in-line solvent filter. Start opening fittings at the detector and work towards the pump. At some point the pressure will drop, pinpointing the location of the clog. (NOTE: There will be a normal drop in pressure when the column is removed.)

- Have you changed to a more viscous mobile phase (e.g., one with methanol)?

PRESSURE FLUCTUATIONS

- The most common cause of pressure fluctuations at microbore flow rates is the failure of check valves to seal. This can be caused by air bubbles or contaminants in the check valve. Purge the pump with freshly degassed mobile phase. If performance improves, air bubbles were in the check valves. If the problem returns, degas the mobile phase more frequently and check the connections to the inlet side of the pump where air can be sucked in. Note which head is compressing on the LCD display (L or R) when the pressure drops.

- Switch the inlet check valves between the two heads. If the pressure drop now occurs on the opposite pump head, it is associated with the inlet check valve on the head showing the problem. Clean or replace as described in the CHECK VALVES section.

- Switch the outlet check valves between the two heads. If the pressure drop now occurs on the opposite pump head, it is associated with the outlet check valve on the head that does not show a pressure drop. Clean or replace as described in the CHECK VALVES section.

- One or both pump heads may need an overhaul. See the PUMP HEADS section.

- One of the outlet lines from the pump to the pressure manifold may be clogged. Remove and examine. Replace as necessary.

- Check the irrigation port outlet behind and under the head. If wet or buffer salt deposits have accumulated, a seal may be leaking. See the section on seal replacement.

FLUID LEAKS

- Fluid leaks from (or salt accumulates around) fittings. Leaks indicate that FITTINGS need to be tightened or replaced.

- Fluid leaks (or salt accumulates) around pump heads or from irrigation waste ports. This indicates that the plunger seals are worn and leaking. Replace the PLUNGER SEALS to prevent internal corrosion of the pump head.

- Fluid leaks (or salt accumulates) around the junctions of the pressure transducers. Replace the PRESSURE TRANSDUCER O-RINGS.

OVERHEATING

If the pump heads or cabinet feel warm, clean the FAN FILTER and check that the fan is operating.

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