



DA-5 Control

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MF-9005

INSTRUCTION MANUAL

ChromGraph™ DA-5 CONTROL

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West Lafayette
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MANUFACTURER'S NOTE

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SECTION 1. INTRODUCTION

ChromGraph CONTROL is a sophisticated set of programs for automated control of the DA -5 Chromatography Control and Data System (Figure 1.1), BAS-200B Chromatograph, PM-80 Gradient pump, LC-4C Amperometric Detector, Pulsed Amperometric Detector, and other BAS instruments. The software runs in the Windows™ environment, thus providing all the flexibility and multitasking inherent in this operating system.

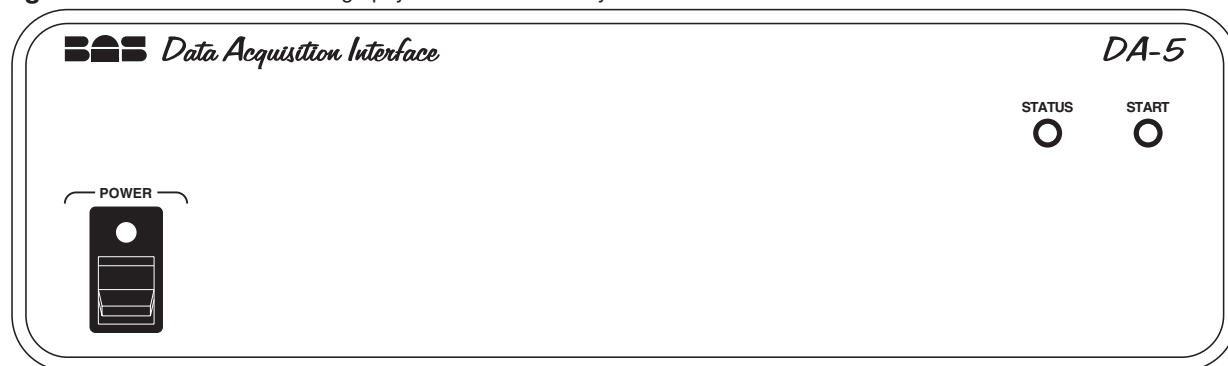
CONTROL provides all the control features needed to operate the DA-5, both in immediate mode and programmed operation. Up to four detectors may be monitored. Methods are easily chained together and integrated with the BAS Sample Sentinel autosampler. All details of data collection and storage are defined 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 ChromGraph REPORT manual (MF-9071) 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.

Figure 1.1. The DA-5 Chromatography Control and Data System



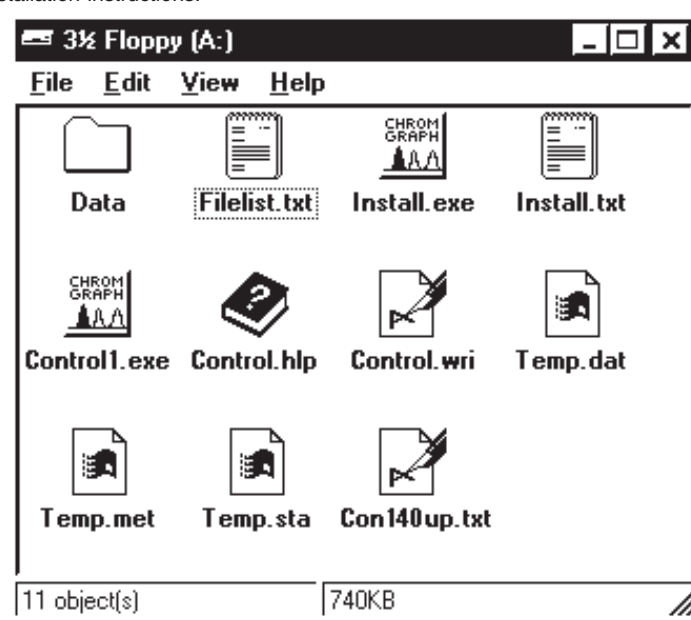
SECTION 2. INSTALLATION

NOTE: If you purchased your computer from BAS along with the software, this installation has been done for you. You may proceed to section three.

ChromGraph CONTROL and REPORT are complex programs that require a moderately powerful computer for optimal performance. In theory, any 80286 or larger computer that can run Windows will run ChromGraph. However, speed and multitasking ability will suffer with too small a computer. The minimum computer size we recommend is a 25 MHz 386SX, with a 4 MB RAM and an 80 MB Hard Drive. The Windows version should be 3.1 or higher. These instructions assume you are using Windows95.

Begin the installation of ChromGraph CONTROL by inserting the disk into the A: drive of your computer. Double-click on the "My computer" icon, and then on the "3½ floppy" icon. You should now see the folders on the A: disk (Figure 2.1).

Figure 2.1. Obtaining installation instructions.



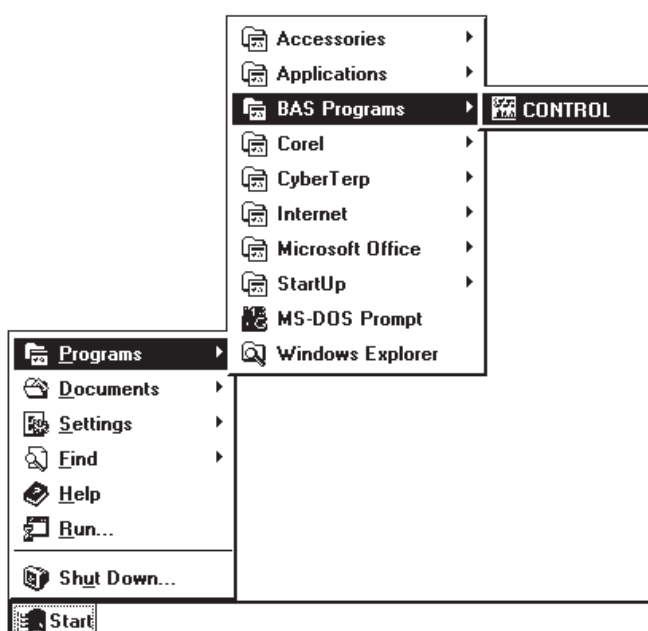
NOTE: Depending on how Windows95 is configured, you may not see the 3-letter extensions (e.g., .exe, .txt) following the folder names.

Double-click on the notebook labeled "INSTALL" or "INSTALL.TXT", which brings up complete instructions for installing CONTROL. It is helpful to print out these instructions, using the PRINT command found in the FILES menu at the upper left of the screen.

Begin the installation by double-clicking on the peaks icon labeled "INSTALL" or "INSTALL.EXE".

During the installation, CONTROL will be installed into a subdirectory called C:\BAS. Demonstration data files and methods will be installed into C:\BAS\DATA. The Windows95 START Menu will be modified so that CONTROL will appear in a new program group called BAS PROGRAMS (Figure 2.2).

Figure 2.2. BAS PROGRAMS group in Windows95.



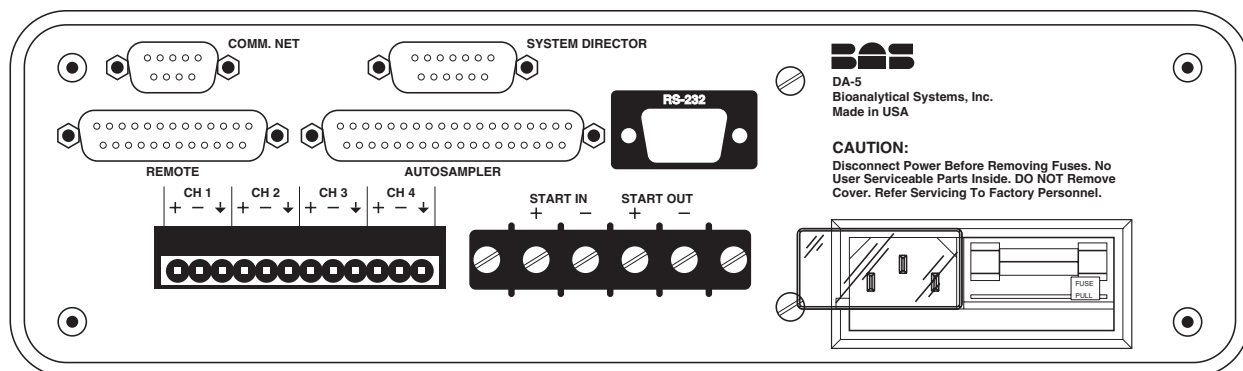
NOTE: The demonstration data sets in C:\BAS\DATA can be deleted once you have learned the system. If you ever wish to see them again they can be easily copied from the installation disk.

To start the data processing section of ChromGraph, click on CONTROL as in Figure 2.2. The initial screen for CONTROL will appear.

SECTION 3. CONNECTIONS

All connections to the DA-5 are made via the rear panel (Figure 3.1).

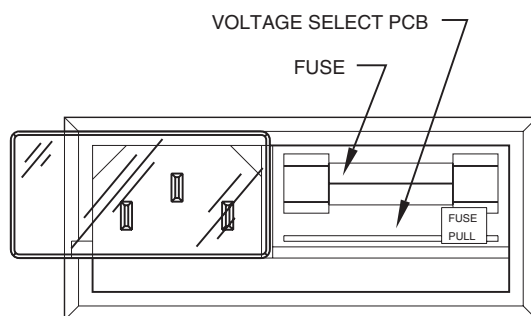
Figure 3.1. Rear panel connectors.



3.1 Connecting the DA-5

The DA-5 can operate on either 100, 120, 220 or 240 VAC and 50 or 60 Hz, but the correct voltage must be selected before use. With the power cord unplugged, examine the power receptacle on the back of the instrument (Figure 3.2). If the correct voltage is not visible on the edge of the printed circuit board (PCB), do the following:

Figure 3.2. Main voltage selection.



Pull out the PCB and turn it so that the desired voltage will be readable after reinsertion. Then push the PCB back into its socket. Next check to see that the fuse is of the proper rating (Table 3.1).

Table 3.1. Correct Fuse Selection

<u>Voltage</u>	<u>Fuse</u>
100-120	1 A SloBlo
220-240	500 mA SloBlo

Once the power level is selected, plug the power cord into the power receptacle on the DA-5, and into a power source of known high quality. We strongly suggest that all components of the LC system be plugged into the same power strip, so they all get the same high-quality power and have a single good ground.

Next connect the DA-5 to the computer with an RS-232 cable. Use the RS-232 socket on the back of the DA-5 (Figure 3.1) and a serial port (COM 1, 2, 3 or 4) on the computer. Make a note of which COM port you used.

The PM-80 Gradient Pump communicates with the DA-5 via a 15-pin cable (EW-8086). Connect the cable to the sockets labeled SYSTEM DIRECTOR on the rear panels of the DA-5 (Figure 3.1) and PM-80.

If you wish to control one or two LC-4C Amperometric Detectors, then the detector(s) must be connected to the DA-5 via a control cable (ER-9530). Plug the cable into the ports labeled REMOTE on the backs of the detector(s) and the DA-5.

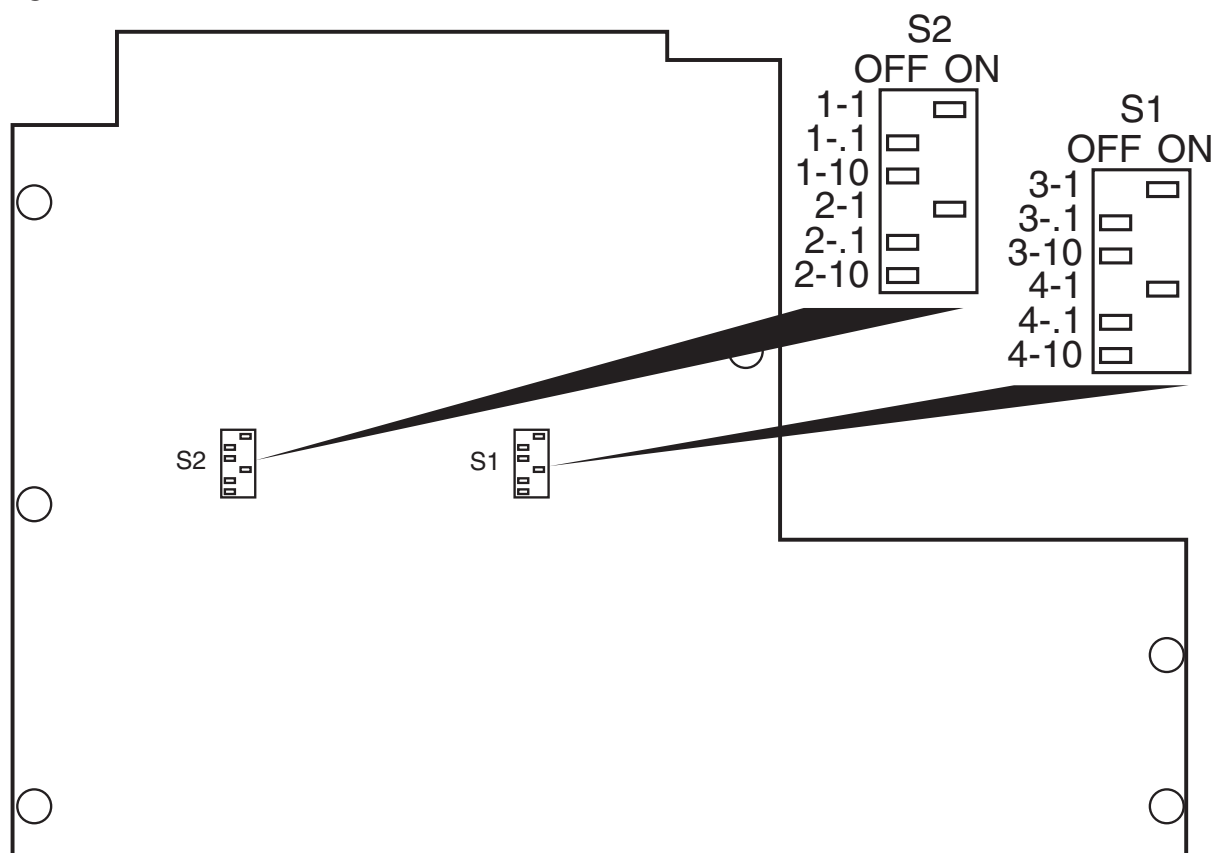
If you are controlling two LC-4Cs, the address of the second one must be changed from W1 (default) to W2: Move dipswitch M on the rear panel to the down position.

All the functions of newer (serial numbers 9000 and above) LC-4Cs can be controlled with this software. Older LC-4Cs can be factory updated for full computer control (EF-1016). Alternatively, control of all but potential on an older LC-4C can be achieved with cable ER-9530 and an isolator (EW-8105).

3.2 Connecting The Detector Output

The DA-5 accepts analog signals from up to four detectors. Each of the detector inputs can be set to receive a full-scale signal of 0.1, 1.0 or 10 V. The factory setting is 1.0 V. If you need to input a signal of 0.1 V or 10 V, follow the instructions for changing the input voltage.

CHANGING THE INPUT VOLTAGE. Remove the power cord from the DA-5, then turn the unit upside down. Remove the bottom cover. You will see two DIP switches, labeled S1 and S2, on the printed circuit board (Figure 3.3). The switches are labeled with the detector number followed by a voltage range (e.g., 1-10 means detector 1, 10 V). Simply slide the switch to the ON position for the voltage range you wish to select. **IMPORTANT: MAKE SURE THAT ONLY ONE SWITCH IS ON FOR EACH DETECTOR!**

Figure 3.3. Input voltage selection.

CONNECTING THE DETECTORS. Connect the detector leads to the terminal strip on the back of the DA-5 (Figure 3.4). A positive (+), negative (-) and ground (arrow symbol) connection are provided for each detector. Most UV and fluorescence detectors require only a (+) and (-) connection.

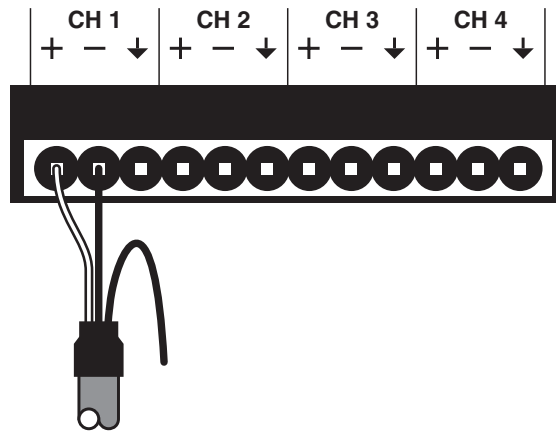
IMPORTANT GROUNDING INFORMATION FOR EC DETECTORS

An electrochemical (EC) detector, such as the BAS LC-4C, requires one (and only one) good ground for quietest operation. The best ground is the case of the LC-4C itself. Connect a wire from screw terminal 8 (OUTPUT GND) on the rear panel of the LC-4C to the threaded hole at the upper left of the rear panel.

If several LC-4Cs are being connected, ground only one to its case as described above. Then connect each detector to the next via screw terminal 8.

Once the grounds are established, connect the output of the detectors to the DA-5 using only the (+) and (-) connectors.

Figure 3.4. Connecting detectors to the DA-5. For most applications, the ground wire is not used.



SECTION 4. STARTING THE SYSTEM

Once the hardware is connected, turn on the DA-5 and (if part of the system) the PM-80 Gradient Pump and LC-4C Detectors. Always allow these instruments to do their self checks before trying to access them with the computer. The pump self check is complete when its pressure display stops flashing, and the DA-5 self-check is complete when the STATUS LED changes from red to blank.

To bring up the software, select CONTROL from the Windows Start Menu (Figure 2.2, page 3). As CONTROL loads, a serial interface screen may appear (Figure 4.1). (If the software has been installed for you at the factory, this screen may not appear.) If it does appear, select the communications port to which you connected the DA-5.

Figure 4.1. Setup Options screen showing settings for normal operation.

The screenshot shows a dialog box titled "CONTROL SETUP OPTIONS". It contains the following settings:

- COM Port:** Simulate, COM1, COM2, COM3, COM4
- Baud Rate:** 9600, 19200, 38400
- BAS ROM Versions:** DA-5 3.03e
- Simulated equipment:** Pump, Utility, LC4C, 4C/PAD, DA-5
- Smart Mouse positioning:**
- Binary Vial Numbers:**
- Get vial # in .RAN file:**
- Auto-Backup .MTH:**
- Abort if no .RAN file:**
- Get volume in .RAN file:**

Buttons: OK, Cancel

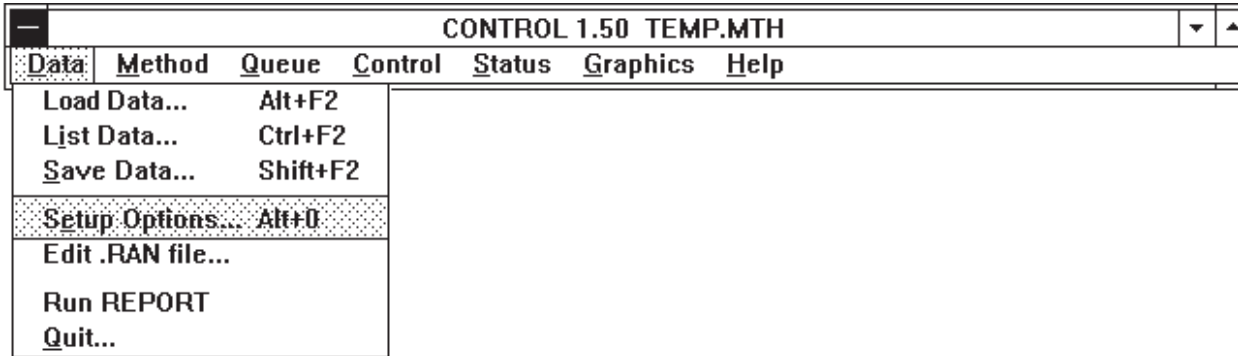
Once the ComPort is selected, click on the OK button, and a message giving several ROM versions should appear. We recommend that you start with the configuration of Figure 4.1. Smart Mouse positioning puts the mouse cursor on the next OK >> or << OK button when you switch screens. Auto-Backup .MTH saves a reference Method with each series of runs (Section 8.5, page 32).

The remaining options define how information is transferred from the BAS Sample Sentinel autosampler (Section 11).

Once the options in this screen are selected, click OK again and the serial interface screen will disappear. You're now ready to operate the system. (If the message "BAS hardware isn't responding" appears, check that the cable is tightly plugged into the proper ports, and try the OK button again.)

The serial interface screen will not appear again unless there is a communication problem between the DA-5 and the computer. If you wish to review or edit this screen, choose "Setup Options" in the DATA menu (Figure 4.2). When you exit the system, save the CONTROL.INI file (Section 13, page 48) to permanently save these setup options.

Figure 4.2. Selecting the Setup Options screen from the DATA menu.



SECTION 5. HELP SCREENS

ChromGraph CONTROL 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 (Figure 5.1). The INDEX option opens up a screen showing all the main help topics (Figure 5.2). Select whichever topic is of interest.

Figure 5.1. Using the HELP menu.

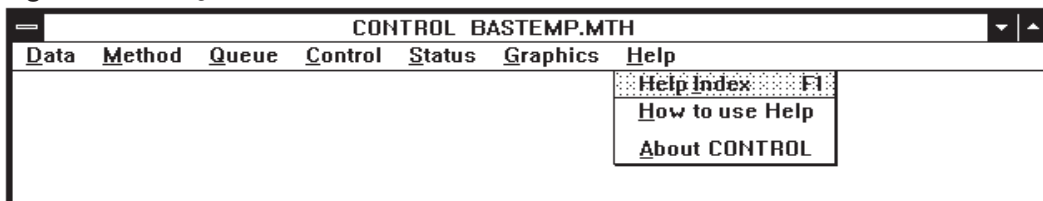
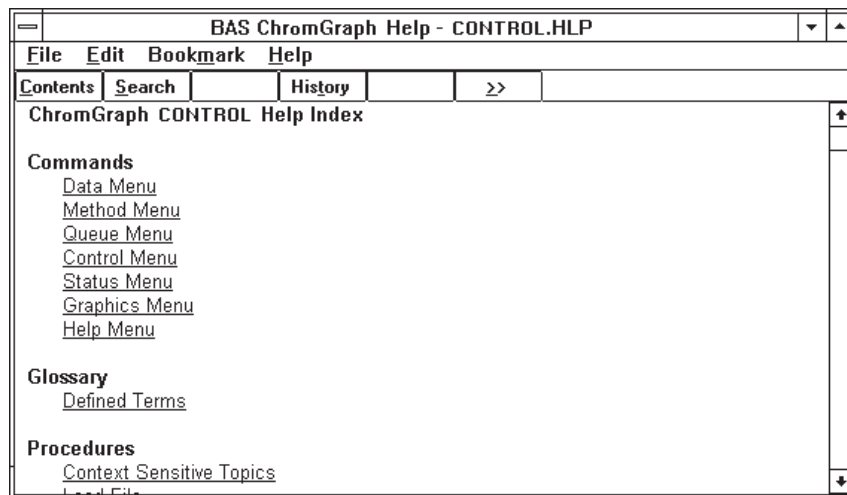


Figure 5.2 The HELP index.



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.

SECTION 6. CREATING A METHOD FILE

The nerve center of both immediate-mode and automated operation 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. 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.

6.1 Loading A Method

Begin creating a Method by loading an existing Method which we will modify. (This 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 LOAD METHOD option from the Method menu at the top of the screen (Figure 6.1). A directory of existing Methods that reside in the default data location will appear (Figure 6.2). To load a Method, either double click on its name or highlight it and click the OK button.

Figure 6.1. Accessing the Method and its subsections.

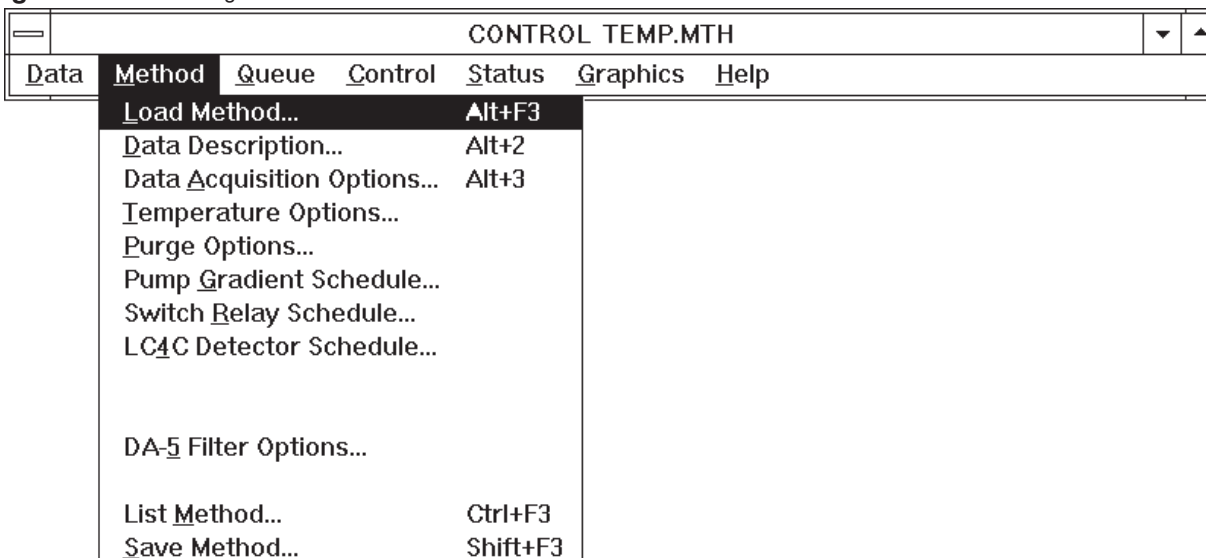
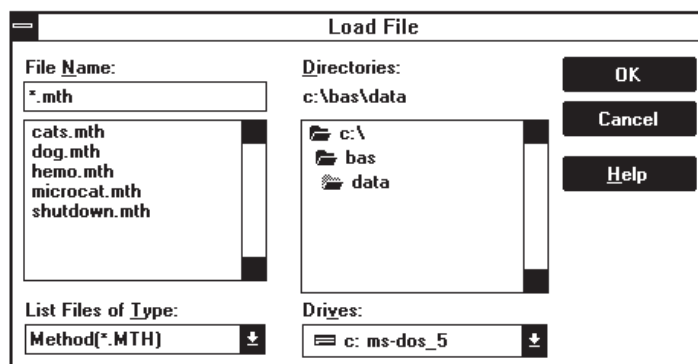


Figure 6.2. Loading a Method file.

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 operation. When you finish your session and QUIT the software, saving the CONTROL.INI file will save the new default data location for subsequent sessions. If you do not save this file, the original default data location will be used for subsequent sessions (see Section 13, page 46).

6.2 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 (Figure 6.3). 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.

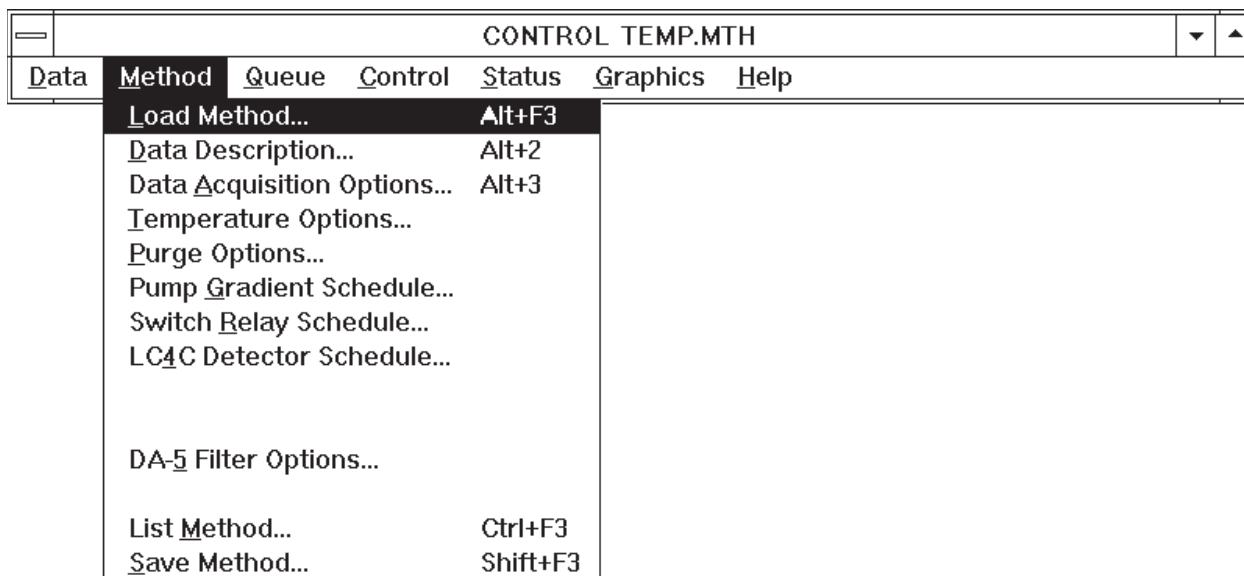
Figure 6.3. Accessing the Method and its subsections.

Figure 6.4. Sample subsection of the Method.

PUMP GRADIENT SCHEDULE				
Pressure limits, psi				
Minimum <0:5000>:		<input type="text" value="200"/>	Maximum <500:6000>: <input type="text" value="4000"/>	
Flow rate <0:5.0>:		<input type="text" value="1.00"/>	ml/min	<input type="checkbox"/> Synchronize start
Minutes	%A	%B	%C (Select a Row)	
0.0	100.0	0.0	0.0	
10.0	100.0	0.0	0.0	
Minutes:		%A <0:100>:	%B <0:100>:	
<input type="text"/>		<input type="text"/>	<input type="text"/>	
<input type="button" value="Delete"/>		<input type="button" value="Change"/>	<input type="button" value="Insert"/>	
<input type="button" value="OK <<"/>	<input type="button" value="Exec"/>	<input type="button" value="Save"/>	<input type="button" value="OK"/>	<input type="button" value="Cancel"/>
<input type="button" value="Stop"/>		<input type="button" value="OK >>"/>		

6.3 Pushbuttons Found In Method Screens

At the bottom of each Method screen (more correctly called a dialog box) is a variable number of pushbuttons for subsequent action (Figure 6.4). These are:

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 DA-5 or PM-80 Gradient Pump to set the conditions called for in the dialog box (e.g., pump speed). 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 DA-5 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.

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.

6.4 Listbox In Pump and Detector Schedules

The Pump Gradient Schedule and LC-4C Detector Schedule contain listboxes - lists of up to 10 time lines for control of the gradient pump or detectors during a run (Figure 6.4). 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.

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.

6.5 Data Description

Choosing the DATA DESCRIPTION option under METHOD brings up the edit screen for the first section of the Method (Figure 6.5). You may enter information about the run under OPERATOR, CONDITIONS and RUN NOTES. These are all comments and do not affect chromatography.

Figure 6.5. Data Description section of the Method.

DATA DESCRIPTION

Method filename: BASTEMP

Operator: solomon

Conditions: microbore cats

Run Notes:

Time of run = 16:14 Date of run = 02/11/93

Data name: mcat Run # = 6

Detector = A=EC1 Vial # = 64

<< OK Save OK Cancel OK >>

The entries for TIME OF RUN, DATE OF RUN, RUN NUMBER, VIAL NUMBER, and DETECTOR will be automatically entered during a run and saved with the data. Each time you load an existing data set, its values for these parameters will appear.

DATA NAME is the most important parameter in this section. It is the name under which the data file will be saved. Enter up to four numbers or letters (the last position must be a letter, to avoid confusion with the run number). Run numbers (three digits) and a letter (A through D, for detector 1 through 4) will be automatically appended during a run. On disk, the data file will have the extension ".DAT." Thus, MCAT006A.DAT is the sixth run of a data file called mcat, and contains data from detector A.

6.6 Data Acquisition Options

This next section of the Method can be accessed by either choosing it from the Method Menu (Figure 6.3) or clicking the OK >> button from the DATA DESCRIPTION screen. The parameters in this screen (Figure 6.6) define how the data are collected:

Figure 6.6. Selecting Data-Acquisition Options. The Detectors Enabled section will vary, depending on which options were purchased.

TRIGGER TYPE determines which piece of equipment starts the chromatographic run. Choose MANUAL if you will use the mouse to start the run. Choose AUTOMATIC if the DA-5 will be in charge of an autosampler, and will send a start signal to the autosampler (see USING AUTOSAMPLERS, Section 11, page 40). In the AUTOMATIC mode, the DA-5 will perform its designated number of runs, one after another. Choose EXTERNAL if the DA-5 should wait for a start signal from an autosampler before beginning its run. This is the appropriate mode for interaction with the BAS Sample Sentinel autosampler.

EXTERNAL forces the system to go into a waiting mode when RUN (Section 8.2, page 30) is pressed. The run will not start until the front-panel start switch is pressed, or a signal is received on either the rear-panel START-IN terminals or via the AUTOSAMPLER connector (Section 11, page 42).

START DATA TIME and END DATA TIME refer to the duration of data collection. These values may be the start and end times of the chromatographic run, or some subset of the entire run. (The length of the chromatographic run is determined by either the END DATA TIME or the last time line of the pump or detector, whichever is longer.)

POINTS PER MINUTE determines how much of the data are actually stored. The digitizer acquires data at a much greater rate than needed; storing all this data uses excess space, and processing it takes too much time. This parameter reduces the data to a more reasonable level by summing a given number of points before storing the summed value. Table 6.1 shows recommended data acquisition rates.

Table 6.1. Data Acquisition Rates

<u>RUN LENGTH</u> (minutes)	<u>POINTS PER MINUTE</u>
1 - 5	300
6 - 10	150
11 - 18	75
19 - 40	50
41 - 60	25

Set the points per minute by clicking on the down arrow. A listbox of choices will appear.

DETECTORS ENABLED. To enable a detector for data collection, click on its DA# box (Figure 6.6). For DA#1 and DA#2 you must also select VOLTS, to indicate that only data collection will occur. For each detector enabled, you may rely on the detector's internal filtering, or select an electronic filter setting (Section 6.10, page 21).

You can control the operation of LC-4C amperometric detectors via this software. For computer control of the LC-4Cs, select the LC-4C button. You can control one or two LC-4C detectors. For Pulsed Amperometric operation, select the PAD button. You can control only one pulsed amperometric detector, and taking this option precludes controlling a second LC-4C.

FIRST RUN NUMBER is the number that will be given to the first run of a series. This value is incremented by one each 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 = 255 for any one Method) to be made in programmed operation. It 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.

For manual operation (starting each run with the mouse), the NUMBER OF RUNS can be ignored: run numbers will increment properly each time a run is completed.

6.7 Purge Options (PM-80 Gradient Pump Only)

The Purge Option screen (Figure 6.7) can be accessed through the Method Menu (Figure 6.3) or by clicking the <<OK and OK>> buttons from other Method screens. A purge can be used to bring fresh mobile phase rapidly through the system. The DA-5 will attempt to maintain the set pressure by varying the flowrate. If the set pressure limit is not reached (e.g., if the purge valve is open) the pump will increase flow to its maximum, 5 mL/min.

Figure 6.7. Purge Options.

PURGE OPTIONS	
Mobile phase Solvent names	
Solvent 1(A) name:	CAT'S M.P.
Solvent 2(B) name:	40% ACN
Solvent 3(C) name:	100% ACN
Purge conditions	
Purge duration <0:99.9>:	5.00 min
Initial Flow:	2.50 ml/min
Max. Press.:	3000 psi
%A <0:100>:	100.
%B <0:100>:	0.00
<input type="button" value="≤< OK"/> <input type="button" value="Purge"/> <input type="button" value="Save"/> <input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="Stop"/> <input type="button" value="OK >>"/>	

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 (Figure 8.2, page 29) displays elapsed time.

6.8 Pump Gradient Schedule (PM-80 Gradient Pump Only)

The Pump Gradient Schedule screen (Figure 6.8) is accessed through the Method Menu (Figure 6.3) or by clicking the <<OK and OK>> buttons from other Method screens. This screen contains all the operating information for the pump, as well as pushbuttons for immediate-mode control.

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.

Figure 6.8. Control of the pump.

PUMP GRADIENT SCHEDULE			
Pressure limits, psi			
Minimum <0:5000>:	200	Maximum <500:6000>:	4000
Flow rate <0:5.0>:	1.00 ml/min	<input type="checkbox"/> Synchronize start	
Minutes	%A	%B	%C (Select a Row)
0.0	100.0	0.0	0.0
10.0	100.0	0.0	0.0
Minutes: <input type="text"/> %A <0:100>: <input type="text"/> %B <0:100>: <input type="text"/>			
<input type="button" value="Delete"/> <input type="button" value="Change"/> <input type="button" value="Insert"/>			
<input type="button" value="≤< OK"/> <input type="button" value="Exec"/> <input type="button" value="Save"/> <input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="Stop"/> <input type="button" value="OK >>"/>			

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 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, and they are 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 DA-5 will calculate the appropriate linear gradient between the two lines. The run will end either at longest time line in this schedule, or, if longer, at the END DATA TIME. When the last time line is reached, the PM-80 pump reverts to time 0.0 conditions.

EXEC and STOP pushbuttons are provided for manual control of the pump. EXEC puts the 0.0 time line into effect. The gradient program cannot be started from this screen (see Section 8, page 29, PROGRAM CONTROL OF THE DA-5).

6.9 LC-4C Detector Schedule

The LC-4C Detector Schedule screen (Figure 6.9) is accessed through the Method Menu (Figure 6.3) or by clicking the <<OK or OK>> buttons from other Method screens. This screen contains all the information for control of LC-4C Amperometric Detectors in their normal (non-pulsed) mode. See Section 6.11 for control of the pulsed mode.

Figure 6.9. Controlling the LC-4C detectors.

LC4C DETECTOR SCHEDULE				
Minutes	Det	mVolts	Range	Filter
0.0	1	750	+50. uA	0.05 Hz
0.0	2	50	+20. uA	0.05 Hz
25.0	1	750	+50. uA	0.05 Hz
25.0	2	50	+20. uA	0.02 Hz

Time	Det.	Potential	Range	Filter
Minutes	1:2	+/- mV	+/- nA	Hertz
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

The screen consists of a listbox for time lines, edit boxes, and pushbuttons. If two LC-4C detectors have been enabled in the DATA ACQUISITION OPTIONS screen (Figure 6.6, page 15), then each time-line in the detector listbox will have two sublimes, one for each detector. These will be numbered 1 and 2, corresponding to the W1 and W2 connections of the cell lead.

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 the detector or pump schedule. At the end of the run, the detectors revert to the time 0.0 conditions.

There is an automatic rezero of the LC-4C detectors as each time line (except the 0.0 line) is reached during a programmed run. The MANUAL OFFSET toggle switch on the LC-4C must be set to OFF, and the AUTO ZERO toggle switch must be set to ON.

POTENTIAL is set by entering the voltage, in millivolts, in the edit box provided. The potential set here is added to whatever potential is dialed in from the front panel of the LC-4C. In most cases, YOU SHOULD TURN THE LC-4C POTENTIAL KNOB FULLY COUNTERCLOCKWISE, SO NO POTENTIAL IS BEING APPLIED OTHER THAN BY THE COMPUTER.

NOTE: Because of the noise inherent in a digital to analog converter, baseline noise will be greater when applying potential to the LC-4C from software than when applying it from its front panel. This should only be a problem when operating at high sensitivities. If baseline noise becomes a problem, set the potential to 0 in the detector schedule and dial in the appropriate voltage on the front panel.

RANGE and FILTER are set for each detector, using a listbox (Figure 6.10). You may scroll with the mouse or the up and down arrow keys to select one of the preset values for these parameters.

Figure 6.10. Setting the Range for detector 1.

LC4C DETECTOR SCHEDULE				
Minutes	Det	mVolts	Range	Filter
0.0	1	750	+50. uA	0.05 Hz
0.0	2	50	+20. uA	0.05 Hz
25.0	1	750	+50. uA	0.05 Hz
25.0	2	50	+20. uA	0.02 Hz

Time	Det.	Potential	Range	Filter
Minutes	1:2	+/- mV	+/- nA	Hertz
0.0	1	750	+50. uA	0.05 Hz

The pushbuttons allow immediate-mode control of the EC detectors. EXEC activates the 0.0 time-line instructions, turning on the detectors. STOP opens a dialog to turn off the detectors Figure (6.11). Note that EC2 is electronically dependent on EC1, so the software will not allow EC2 to operate without EC1.

Figure 6.11. Stop detectors dialog.

STOP DETECTORS		
<input checked="" type="checkbox"/> EC1+EC2	<input type="checkbox"/> EC2	<input type="checkbox"/> DA-5
<input type="button" value="All"/>	<input type="button" value="Stop"/>	<input type="button" value="Cancel"/>

WARNING: ALWAYS KEEP THE *CELL* SWITCH ON THE FRONT PANEL OF THE LC-4C IN THE *STBY* POSITION, SO ONLY THE COMPUTER DETERMINES WHETHER THE CELL IS ON OR OFF.

WARMUP opens a dialog to turn on the detectors at the insensitive range of 1 μ Afs (Figure 6.12). 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. Once the detectors are equilibrated, then use EXEC to send the final conditions to the detectors.

Figure 6.12. Use the Warmup feature to turn on the LC-4C detectors.

WARMUP EC DETECTORS	
<input type="checkbox"/> EC1	<input checked="" type="checkbox"/> EC1+EC2
<input type="button" value="Warmup"/>	<input type="button" value="Cancel"/>

NOTE: EC2 cannot be used by itself: you can turn on EC1 or both EC detectors.

6.10 DA-5 Filter Options

The DA-5 Filter Options screen (Figure 6.13) is reached through the Method Menu (Figure 6.3) or by clicking the << OK or OK >> buttons from the other Menu Screens. This section sets the filters for the enabled detectors.

The DA-5 incorporates digital filters of the Finite Impulse Response (FIR) type. These filters are generally more accurate and perform better than the analog filters used in most detectors. You may choose to use either the analog filters in your detector, or the digital filters in the DA-5.

If your detector's filters are performing satisfactorily, continue to use them and set the DA-5 filters to NONE. If your detector's filters are not filtering adequately, then set your detector to its minimum filtering, and use the DA-5 filters. (If your detector's filtering is measured in units of seconds, minimum filtering occurs at the smallest value; if it is measured in Hertz, the largest value provides the least filtering.)

To set the DA-5 filters, click on the down arrow for the appropriate detector; a listbox of choices will appear (Figure 6.14). Click on the desired filter value to select it. Note that the available filter values are dependent on the data collection rate, so they will automatically change if the collection rate is changed.

Figure 6.13. Filter options.

DA-5 FILTER OPTIONS		
Detector	Filter, Hertz	Comment
<u>1</u>	None ↓	<p>The cutoff frequencies of the filters change with the Data acquisition rate by this formula: Hertz = c[filter #] * Pts/Min / 60 where c[0]=None, c[1]=0.05, c[2]=0.075, c[3]=0.10, c[4]=0.15, c[5]=0.25 for filters 0 to 5.</p>
<u>2</u>	0.250 ↓	
<u>3</u>	0.250 ↓	
<u>4</u>	0.250 ↓	
<input type="button" value=" << OK"/> <input type="button" value=" Exec"/> <input type="button" value=" Save"/> <input type="button" value=" OK"/> <input type="button" value=" Cancel"/> <input type="button" value=" Stop"/> <input type="button" value=" OK >>"/>		

Figure 6.14. Selecting filter options for detector 2.

DA-5 FILTER OPTIONS		
Detector	Filter, Hertz	Comment
1	None	The cutoff frequencies of the filters change with the Data acquisition rate by this formula: Hertz = c[filter #] * Pts/Min / 60 where c[0]=None, c[1]=0.05, c[2]=0.075, c[3]=0.10, c[4]=0.15, c[5]=0.25 for filters 0 to 5.
2	0.250	
3	None	
4	0.250	
	0.375	
	0.500	
	0.750	
	1.250	

6.11 Pulsed Amperometric Detection (PAD) Options

The PAD Options screen (Figure 6.15) is reached through the DA-5 PAD OPTIONS section of the Main Menu (Figure 6.3) or by using the <<OK or OK>> buttons from other screens. From this screen you can interactively enter a series of pulses (steps) of potential to apply to the electrochemical detector.

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. 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.

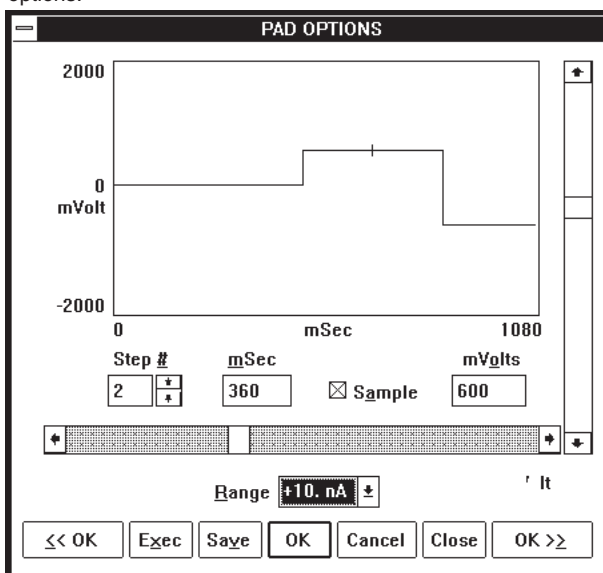
mVOLTS is the potential to be applied during the step, from -2000 to +2000 millivolts. Be sure to set the potential knob on the LC-4C fully counter-clockwise (0 volts).

RANGE is the gain range to be used during the SAMPLE step. See Section 6.9.

The PAD options can be edited directly on the graph, by clicking on any section of the line and dragging it to another section, 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.

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 cycle for a gold electrode requires the following four steps:

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

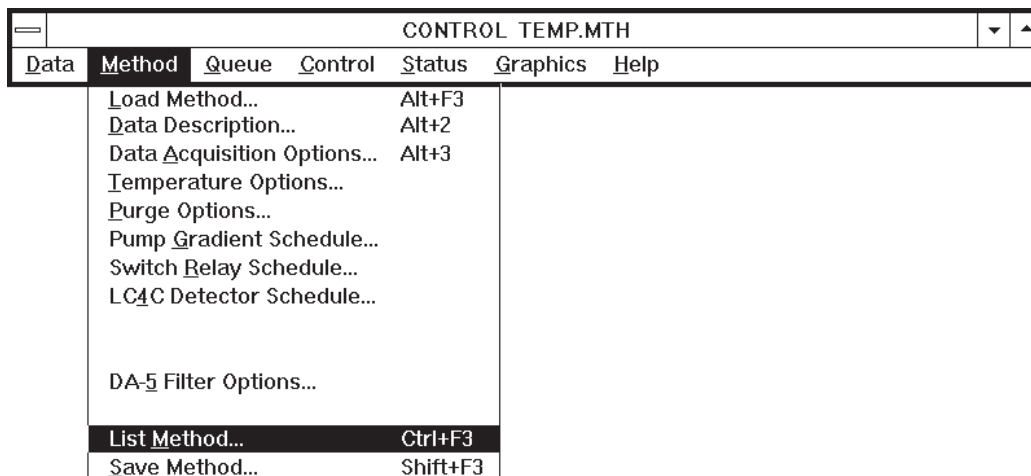
Figure 6.15. Setting the PAD options.

WARNING: ALWAYS KEEP THE *CELL* SWITCH ON THE FRONT PANEL OF THE LC-4C IN THE *STBY* POSITION, SO ONLY THE COMPUTER DETERMINES WHETHER THE CELL IS ON OR OFF.

NOTE: AUTOZERO cannot function in PAD mode. Turn it off on the LC-4C and use the manual offset to zero the baseline.

6.12 Listing A Method

To review the method, you may scroll forward or back through all the screens using the <<OK>> buttons, or choose LIST METHOD from the Method Menu (Figure 6.16). Methods may be listed to the screen and/or the printer. If listed to the screen, they scroll along the rearmost section of the screen, the output window.

Figure 6.16. The Method Menu.

After the listing is finished, you may scroll through the method by using the scroll bars on the right side of the output windows (Figure 6.17).

Figure 6.17. A partial listing of the Method.

```

ChromGraph CONTROL           Wed Mar 23 10:22:15 1994

Method filename: TEMP
Operator:                    Conditions:
Data filename: CATS          Run notes:

Acquisition Options:
Trigger type ..... External
Start time, minutes ..... 0
End time, minutes ..... 5
Points per minute ..... 300
Detectors enabled:
  DA #1  DA #2  DA #3  DA #4
First run number ..... 1
Number of runs ..... 0

Purge Options:
Solvent A name ..... cat's m.p.
Solvent B name ..... 40% ACN
Solvent C name ..... 100% ACN
Purge duration, minutes . 4
Purge max. pressure, psi 2500
Purge percent A ..... 100
Purge percent B ..... 0
Initial flow, ml/min .... 2.5

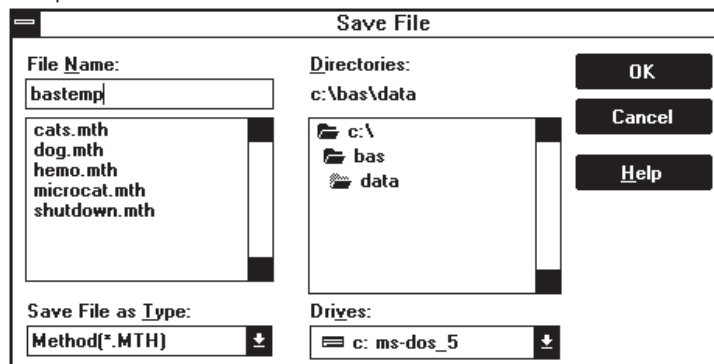
Pump Gradient Schedule:

```

6.13 Saving A Method

Choose the SAVE option from any of the Method screens, or from the Method Menu (Figure 6.16). A SAVE screen will appear, showing the default method name and the location to which it will be saved (Figure 6.18). 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. A Method name can have up to 8 characters. The extension ".MTH" will automatically be appended to the name you choose. If a file of the same name already exists, a warning will appear.

Figure 6.18. Saving the completed Method file.



SECTION 7. IMMEDIATE-MODE CONTROL OF THE PUMP AND DETECTORS

There are many instances where fully automatic chromatography is not needed. When developing a separation, for example, you may wish to set the pump for isocratic elution and observe the results of injections on a chart recorder. And during startup, shutdown or maintenance, you may wish to turn the pump on and off, change solvents, etc. The Method screens and the mouse provide a convenient way to control the pump and detectors. The EXEC button turns the pump on with the conditions found in the Pump Gradient Schedule (Figure 7.2). Only the 0.0 time line is executed. The STOP button turns the pump off. For the LC-4C detectors, EXEC turns the cell on with the time 0.0 conditions. WARMUP turns them on at the insensitive range of 1 μ Afs, and STOP opens a dialog to turn them off.

7.1 Operating The Pump

Let's assume a typical situation: you wish to start up the system which has been cleaned and disassembled for vacation. The column has been stored in 40% acetonitrile, and the electrochemical flowcell disassembled. Begin by turning on the DA-5, the PM-80 Gradient Pump, and the computer, starting Windows, and double-clicking on the CONTROL icon.

The first thing to do is pump fresh, filtered mobile phase, which is in bottle A, through the system. Flush the irrigation ports on the pump with water, then open the prime/purge valve and put a 50 mL syringe on it. Next open the Purge Options screen and set the system to purge for 5 minutes from bottle A at 3000 PSI (Figure 7.1). Click on the PURGE button. The pump will never reach 3000 PSI since the prime/purge valve is open, so it will increase its speed to 5 mL/minute and hold that until 5 minutes are up. Now fresh mobile phase is present in most of the system.

Figure 7.1. Setting a purge from bottle A.

PURGE OPTIONS	
Mobile phase Solvent names	
Solvent 1(A) name:	CAT'S M.P.
Solvent 2(B) name:	40% ACN
Solvent 3(C) name:	100% ACN
Purge conditions	
Purge duration <0:99.9>:	5.00 min
Initial Flow:	2.50 ml/min
Max. Press.:	3000 psi
%A <0:100>:	100.
%B <0:100>:	0.00
<input type="button" value="≤< OK"/> <input type="button" value="Purge"/> <input type="button" value="Save"/> <input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="Stop"/> <input type="button" value="OK >≥"/>	

Close the prime/purge valve and go to the Pump Gradient Schedule screen (Figure 7.2). If necessary, set the flowrate to 1 mL/minute and the 0.0 time line to 100% A. Put a beaker

under the tubing that leads to the column and press EXEC. Fresh mobile phase will now flow through this tube. After a minute or two press STOP. Now connect the column. Press EXEC again and mobile phase will flow through the column, to waste. Once the column is equilibrated, connect its outflow to the detector.

Figure 7.2. Pump conditions for the example in the text.

PUMP GRADIENT SCHEDULE				
Pressure limits, psi				
Minimum <0:5000>:		200	Maximum <500:6000>: 4000	
Flow rate <0:5.0>:		1.00 ml/min	<input type="checkbox"/> Synchronize start	
Minutes	%A	%B	%C (Select a Row)	
0.0	100.0	0.0	0.0	
10.0	100.0	0.0	0.0	
Minutes: <input type="text"/> %A <0:100>: <input type="text"/> %B <0:100>: <input type="text"/>				
Delete		Change	Insert	
<< OK Exec Save OK Cancel Stop OK >>				

7.2 Operating The Detectors

Immediate-mode control can be used when setting up, equilibrating, or maintaining the LC-4C detectors. For non-pulsed operation, use the LC-4C Detector Schedule (Figure 7.3). To turn on the detectors to allow equilibration, use the WARMUP feature. This turns on the detectors at the insensitive range of 1 μ Afs, which can handle the large startup currents produced by the cell. The potential and filter values come from the time 0.0 line of the schedule. The DA-5 STATUS screen (Figure 7.4) shows the condition of the detectors, and allows them to be turned off (STOP) or zeroed (ZERO).

Figure 7.3. Use the Detector Schedule for manual control of the LC-4C detectors.

LC4C DETECTOR SCHEDULE					
Minutes	Det	mVolts	Range	Filter	
0.0	1	750	+50. uA	0.05 Hz	
0.0	2	50	+20. uA	0.05 Hz	
25.0	1	750	+50. uA	0.05 Hz	
25.0	2	50	+20. uA	0.02 Hz	

Time	Det.	Potential	Range	Filter	
Minutes	1:2	+/- mV	+/- nA	Hertz	
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

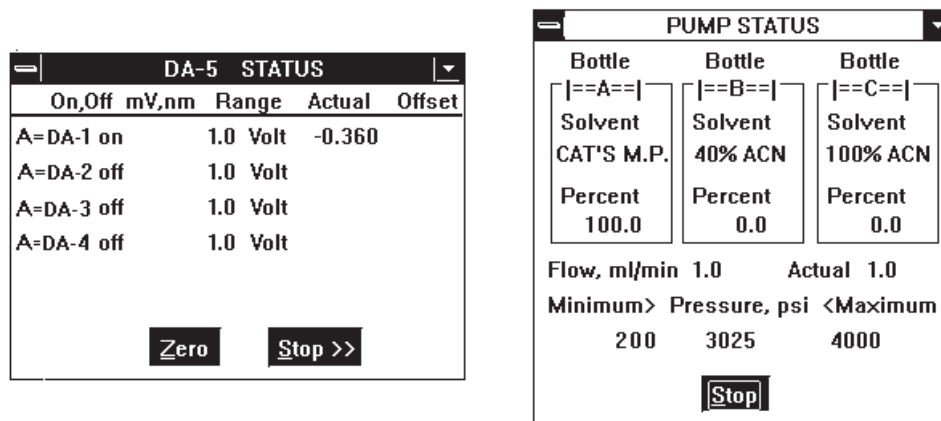
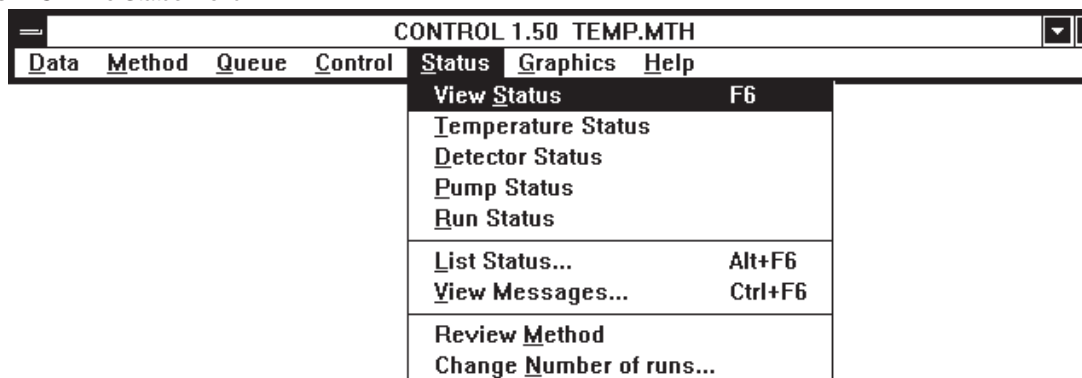
The EXEC button can be used after the detectors have equilibrated. It puts all the time 0.0 conditions into effect, including the Range. You can use EXEC to turn the detectors on if they have been off for a short while. But use WARMUP if the detectors have been off for several hours, or if they have been polished.

The STOP button opens a dialog to allow the detectors to be turned off. Always turn off the detectors before touching the cell leads or disassembling the electrodes.

For PAD operation, use the PAD OPTIONS screen for immediate control (Figure 6.15, page 23). EXEC starts the pulses cycling, and STOP turns off the detector.

7.3 Status Screens

Status screen icons appear along the bottom of the screen. To activate any status screen, simply double-click on its icon. The status screens show real-time conditions of detectors (DA-5 Status) and pump (Figure 7.4). (The RUN STATUS screen will be discussed in the next section.) We recommend that status screens be left as icons when not needed, and especially during automatic operation. When the status screens are activated there is a flurry of communication between the computer and the DA-5, which ties up computer memory and makes screen operations appear sluggish. Individual status screens can be iconized by clicking on the down arrows at their upper right corners. All three screens can be activated or iconized together by taking the View Status option in the Status menu (Figure 7.5), or with the F6 key.

Figure 7.4. Real-time status screens.**Figure 7.5.** The Status menu.

7.4 Temporary vs. Permanent Changes

When operating in immediate mode, you have the flexibility of making changes that are temporary or permanent. For example, you can change the flow rate to 0.5 mL/minute by changing it in the Pump Gradient Schedule and pressing EXEC. To make this change permanent, click the OK button, so the change is saved to RAM. To keep it temporary, click CANCEL. When the screen is CANCELED, the pump will continue to run at 0.5 mL/minute. But the next time you open the Pump Gradient Schedule screen, the default flow rate will be listed as the original 1 mL/minute. This will take effect the next time EXEC is clicked.

SECTION 8. PROGRAM CONTROL OF THE DA-5

Program control of the DA-5 will be required for all the following applications: gradients, automatic runs with autosamplers, data collection and analysis, detector control and PAD.

8.1 Programming

To begin program control, first double-check the Method to make sure it is correct. Then open the DATA ACQUISITION OPTIONS section of the Method (Figure 8.1). Set the TRIGGER TYPE to “automatic” if the DA-5 will be sending a start signal to an autosampler, or to “external” if the autosampler will be sending start signals to the DA-5 (see section 11, page 40, USING AUTOSAMPLERS). Use “external” for the BAS Sample Sentinel autosampler.

Figure 8.1. Data Acquisition Options for automatic operation.

Next enter the number of the first run, and the number of runs in the series (maximum = 255). Then click OK. To start the program, double-click on the RUN STATUS icon, which will open up the RUN STATUS window (Figure 8.2). Click EQUIL to send the information from the Method to the DA-5.

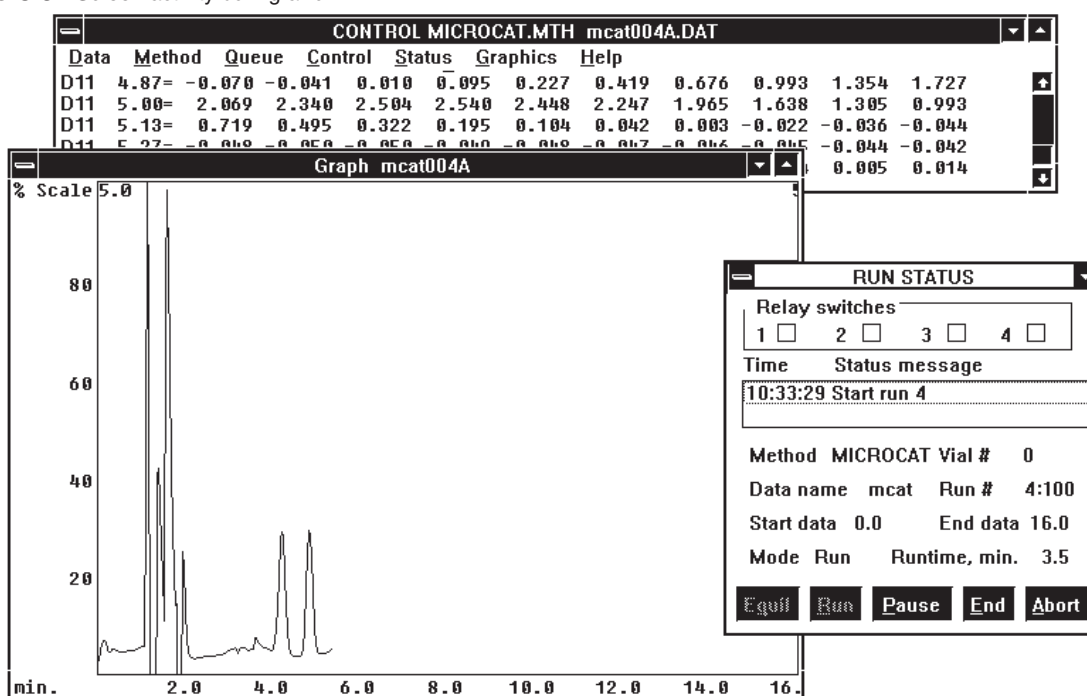
Figure 8.2. Programmed runs are started from the Run Status screen.

NOTE: ALWAYS CLICK THE EQUIL BUTTON IN THE RUN STATUS WINDOW BEFORE BEGINNING A SERIES OF RUNS. EQUIL puts any changes you made in the Method into effect.

8.2 Starting a Run

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 (see Section 11, Using Autosamplers). When the run starts, a graph will appear showing the chromatogram in real time (Figure 8.3). The EQUIL and RUN functions in the RUN STATUS window will be unavailable, and the PAUSE, END and ABORT buttons will become available.

Figure 8.3. Screen activity during a run.



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.

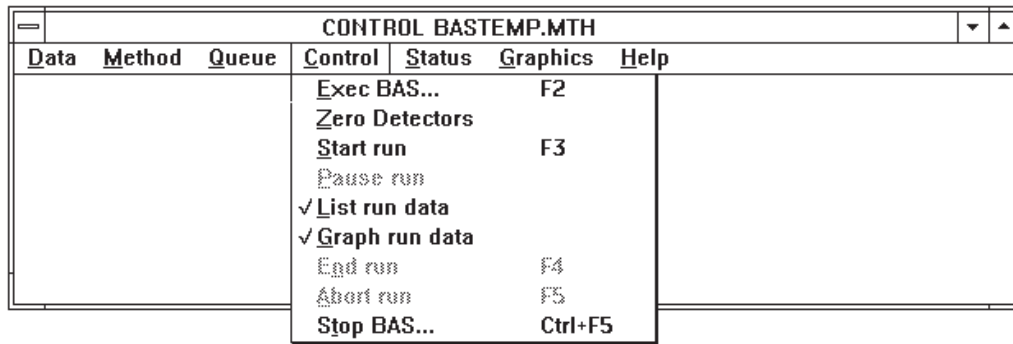
END stops data collection and saves the data to disk. END does not end the pump's run, in case there is a gradient or column wash programmed in. If you've ENDED the data and are sure you want to terminate the pump's run, press ABORT to terminate the run. ABORT cancels all timed events and returns the DA-5 and pump 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 window. In addition to the pushbuttons described above, the RUN STATUS window 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 (Figure 8.4). Graph colors are selected in the GRAPHICS MENU (Section 10, page 36).

Figure 8.4. Selecting data display for a run.

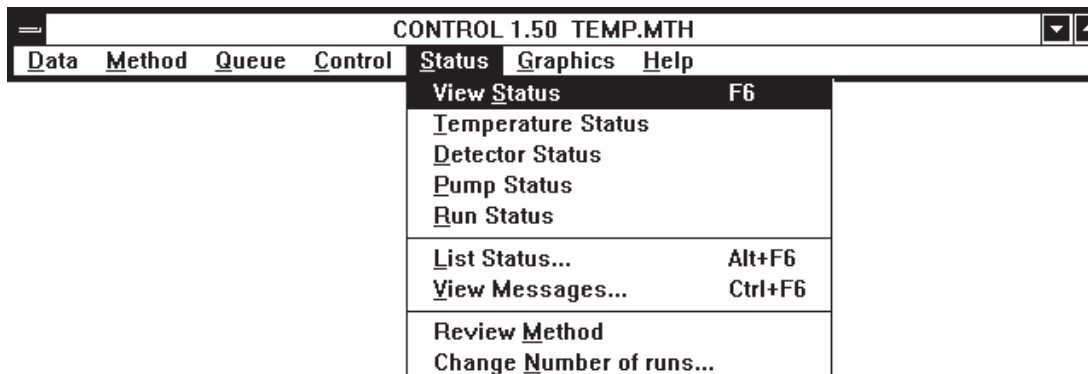


When the first run is complete, the system prepares for the next run in the series. The DA-5 returns to time 0.0 conditions. The data are saved, the RUN NUMBER is incremented by one, the NUMBER OF RUNS is decremented by one, and the new run is either started or waits for a trigger.

8.3 Adding Runs

Additional runs may be scheduled while a batch of runs is in progress. Simply open the STATUS menu (Figure 8.5) and take the CHANGE NUMBER OF RUNS option. Then enter the updated LAST RUN NUMBER.

Figure 8.5. The status menu.



8.4 Method Review

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 (Figure 8.5). 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 (Figure 8.4).

8.5 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, up to four characters, ending in a letter. ### is the run number from 1-255. 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.

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 viewed with any word processor.

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 never 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 in the serial interface screen (Figure 4.1, page 8).

SECTION 9. 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 255 runs in a series (the maximum for any data name) the data name can be changed with a second Method that allows another 255 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).

9.1 Defining A Queue

To chain Methods together, take the QUEUE option from the main menu (Figure 9.1). Choose DEFINE OR EDIT QUEUE. A dialog box will appear (Figure 9.2). Up to 20 methods can be chained together in the QUEUE listbox, each one occupying a line.

Figure 9.1. The Queue Menu.

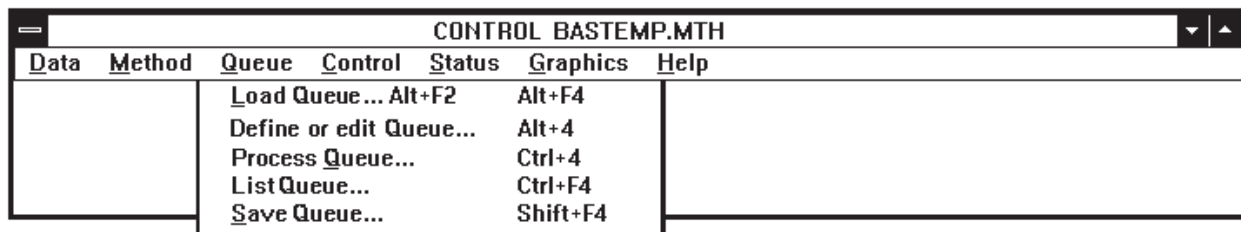


Figure 9.2. Chaining Methods together with a Queue.

#	Method file	Data	First	Last	tEquil
1	MCATS	MCAT	1	20	0.0
2	HEMO	HEMO	10	20	30.0
3	SHUTDOWN	NULL	1	1	0.0

Method file Data name First run Last run tEquil,min

File Path: Erase .LOG

Buttons: Cancel, OK, Save, Process Queue >>

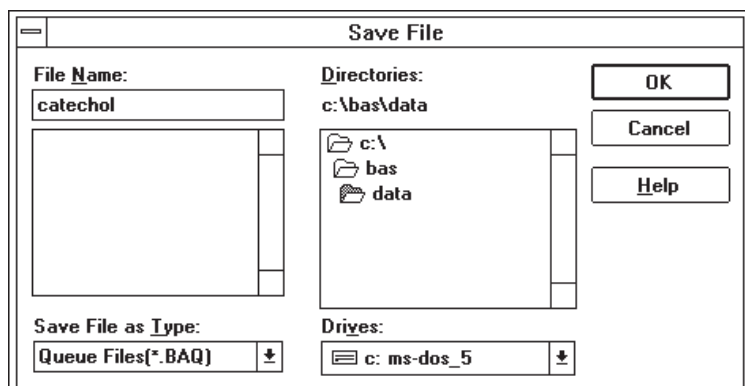
For each line in the listbox, enter a line number, the name of the Method to be run, the name of the data files to be saved, and the first and last run numbers of the sequence to be performed. The data filenames and run numbers in the listbox supersede those in the Method.

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

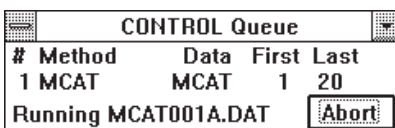
Enter a FILE PATH only to send data to a location different from the default data location. For most purposes, leave this section blank.

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 (Figure 9.3). A Queue name can have up to eight characters, and will automatically be assigned a .BAQ extension.

Figure 9.3. Save Queue dialog.

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 (Figure 9.4) will indicate the current Method and the scheduled runs.

Figure 9.4. Status of the Queue.

9.2 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 are 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.

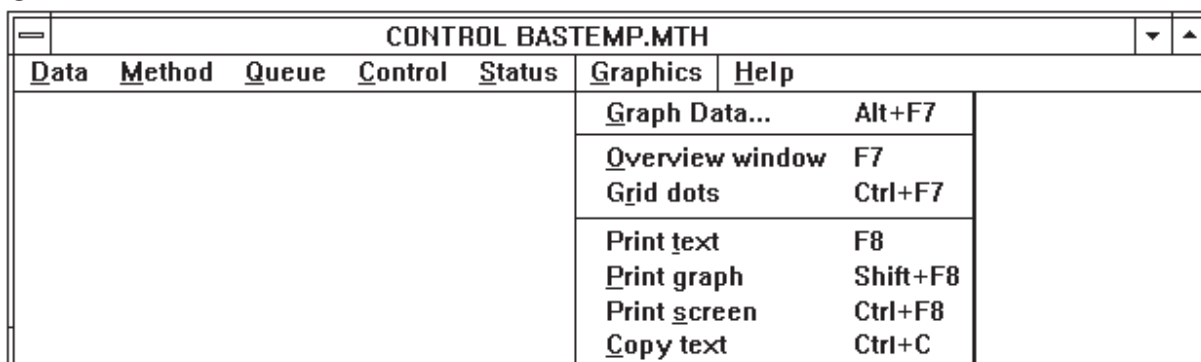
To create a SHUTDOWN Method to slow the pump, create a method with a lower flow rate (PUMP GRADIENT SCHEDULE). Save this Method under the name SHUTDOWN, and add this name at the end of the Queue (Figure 9.2).

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.

SECTION 10. USING THE GRAPHICS MENU

The GRAPHICS MENU (Figure 10.1) allows you to control the appearance of the graph, including which items are graphed and all colors. In addition, options for printing both graphs and text are included in this menu.

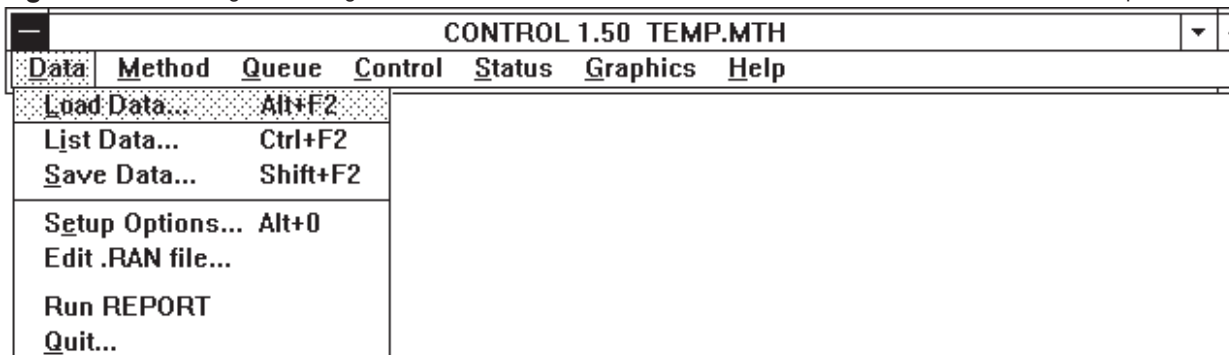
Figure 10.1. The drop-down GRAPHICS MENU.



10.1 Formatting The Graph

Before formatting the graph, you must have a data set in RAM. Either collect new data with a chromatographic run (Section 8, page 29) or switch to the SIMULATE mode (Figure 4.1, page 8) and load an old data set using the LOAD option in the DATA MENU (Figure 10.2).

Figure 10.2. Loading data through the DATA MENU. You must be in SIMULATE mode to use the LOAD DATA option.



Select the GRAPH DATA option in the GRAPHICS MENU (Figure 10.1). A GRAPH OPTIONS screen opens (Figure 10.3). Each data set in RAM will have a section where it can be selected for graphing. Check the box for each data set to be graphed, and select the line color using the drop-down listbox of colors.

Figure 10.3. Options for drawing the graph.

GRAPH OPTIONS					
		Color			
<input type="checkbox"/> Data Set #1	Black	<input type="checkbox"/> Pump %A	Cyan		
<input type="checkbox"/> Data Set #2	Red	<input type="checkbox"/> Pump %B	Magenta		
<input type="checkbox"/> Data Set #3	Black	<input type="checkbox"/> Pump %C	Yellow		
<input type="checkbox"/> Data Set #4	Black	<input type="checkbox"/> Pressure	Black		
Background	White	Border	White		
Data Set ==>	#1	#2	#3	#4	
Number of Points	0	0	0	0	
Start time, min.					
End time, min.					
Baseline Offset Y					
Full Scale Y					
Max. Raw Y Value					
Label Y Units					
<input type="checkbox"/> % Scale for all data sets		<input checked="" type="checkbox"/> Neg. limit -10% of max.			
OK		Cancel			

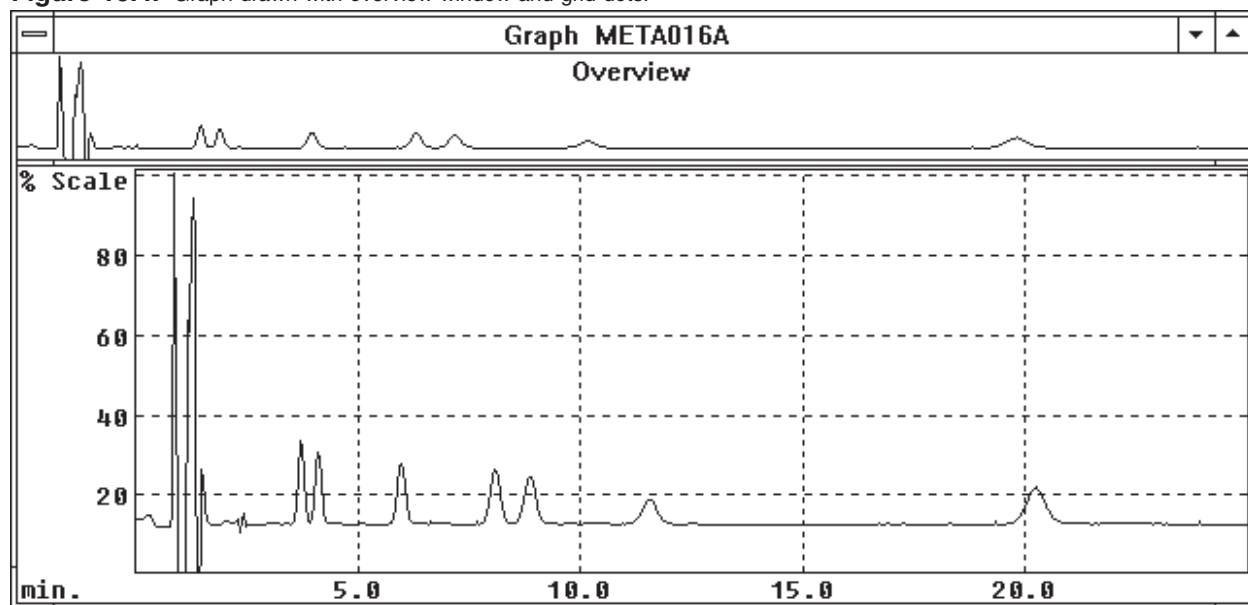
Several aspects of pump performance also can be selected for graphing, but these must be selected before the run begins. For gradient runs, the percentage of each mobile-phase bottle can be plotted against time. A continuous graph of pump pressure also can be plotted. Each of these options has its own selection for line color.

The GRAPH OPTIONS screen also contains options for controlling the overall appearance of the graph. Both background color and border color can be selected with drop-down listboxes.

The graph (Figure 10.4) will be drawn when the OK button is clicked. The graph can be made larger or smaller by dragging the borders with the mouse. All the default settings for graph colors and size will be made permanent if you exit the system and save the CONTROL.INI file (Section 13, page 48).

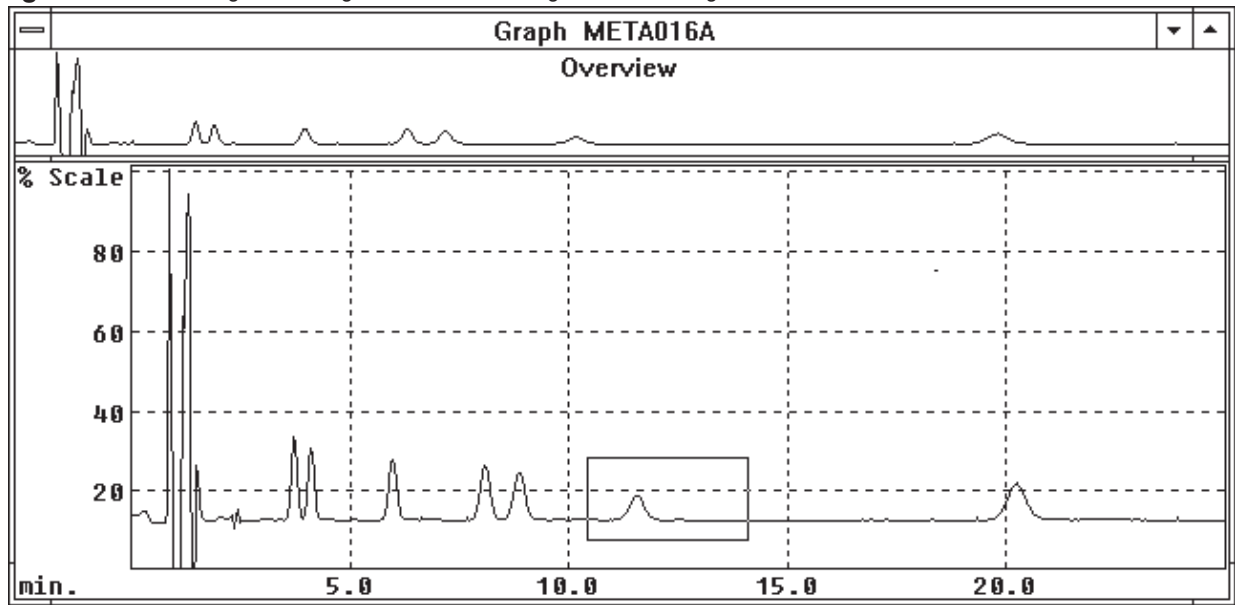
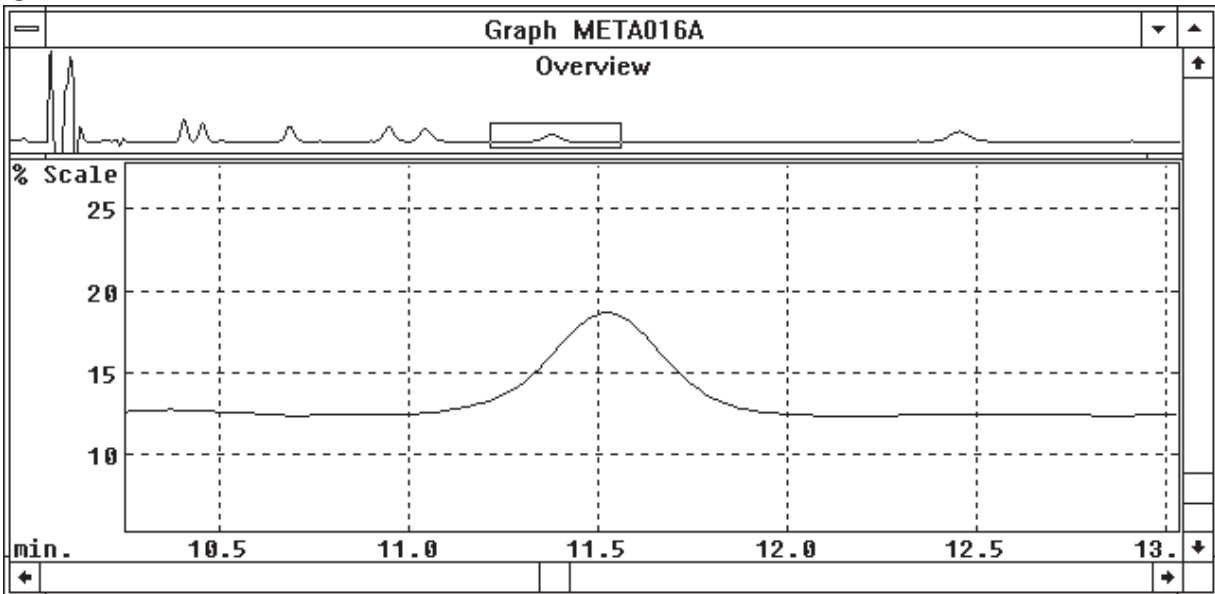
The graph can be customized so its scale is in detector units by changing LABEL Y UNITS and FULL SCALE Y as appropriate (e.g., for an EC detector at a range of 100 nAfs, use nA and 100). This change goes into effect for all subsequent runs in the same session. The change is lost when you exit.

Two additional options present in the GRAPHICS MENU (Figure 10.1) allow for customization of the graph. OVERVIEW WINDOW enables a second window, which always shows the entire graph, even when parts of the main graph have been enlarged. Turn this feature on by clicking on its name, or by pressing F7. GRID DOTS overlays a grid on the graph, to facilitate estimations of peak height and retention time. Turn it on by clicking on its name or typing CTRL-F7. Both these features are illustrated in Figure 10.4.

Figure 10.4. Graph drawn with overview window and grid dots.

10.2 Zoom

Parts of the graph may be enlarged at any time, even while data are being collected, using the zoom feature. Simply click and hold the left mouse button on a section of the graph, drag it 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. Figure 10.5 shows the outline of the rectangle, and Figure 10.6 shows the resultant graph. Notice that the overview window retains the original graph, with a rectangle delineating the enlarged area. This rectangle may be dragged along the overview window, thus enlarging new 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.

Figure 10.5. Rectangle indicating section of chromatogram to be enlarged.**Figure 10.6.** Enlarged section of chromatogram. Overview window indicates the position of the enlarged section.

10.3 Printing and Copying

The printing options are accessed through the GRAPHICS MENU (Figure 10.1). To print text from the output screen, highlight the desired lines and click on PRINT TEXT (or press F8). To print the graph, click on PRINT GRAPH or press SHIFT+F8. To print the entire screen (all text and all windows) click on PRINT SCREEN or press CTRL+F8.

COPY TEXT allows you to copy text on the screen into Window's clipboard. Simply highlight the text with the mouse, then click on COPY TEXT or press Ctl + C

SECTION 11. USING AUTOSAMPLERS

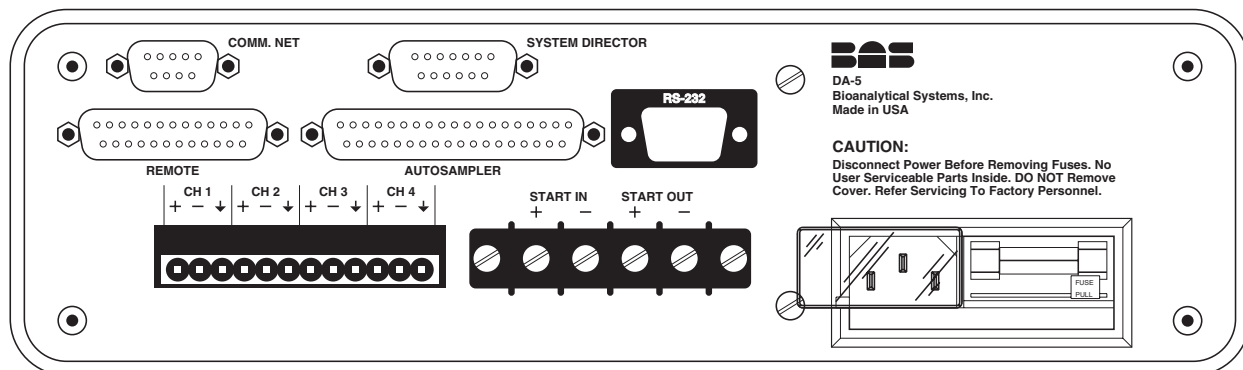
The DA-5 can communicate with autosamplers for automatic operation. The specific procedure to be used depends upon the type of autosampler that is available.

11.1 BAS Sample Sentinel Autosampler

The BAS Sample Sentinel autosampler is capable of two-way communication with the DA-5. 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. In addition to these communication signals, CONTROL reads the vial position from the autosampler* and saves this information with the data file for reports.

Connect to the autosampler with cable EW-4454. Use the AUTOSAMPLER connector on the back of the DA-5 (Figure 11.1). The Sample Sentinel has two terminal strips that match its Y-shaped cable.

Figure 11.1. Rear panel of DA-5.



When programming the autosampler and the DA-5, make sure that you've indicated an EXTERNAL TRIGGER in the DA-5 Method (Figure 11.2). For the Sample Sentinel, change INJECT HOLD ACTIVE to HI in the OPTIONS sections of its menu.

The autosampler's run length should be a minute longer than that of the DA-5. This ensures that the DA-5 is "waiting for trigger" (Figure 11.3) when the autosampler sends its "ready to inject" signal. Start the system by clicking EQUIL, then RUN, in the RUN STATUS box (or PROCESS a QUEUE). Then start the autosampler.

*Requires bcd option with autosampler

Figure 11.2. Data Acquisition Options for communicating with the BAS Sample Sentinel Autosampler.

DATA ACQUISITION OPTIONS	
Trigger type	
<input type="radio"/> Manual	<input type="radio"/> Automatic
<input checked="" type="radio"/> External	
Start data time <0.0:539.9>:	<input type="text" value="0.000"/> minutes
End data time <0.1:540.0>:	<input type="text" value="10"/> minutes
Data acquisition rate	<input type="text" value="150"/> <input type="button" value="↓"/> points per minute
Detectors enabled	
<input checked="" type="checkbox"/> DA-1	<input type="checkbox"/> DA-2
<input type="checkbox"/> DA-3	<input type="checkbox"/> DA-4
First run number	<input type="text" value="1"/> Number of runs
	<input type="text" value="20"/>
<input type="button" value="≤< OK"/>	<input type="button" value="Save"/>
<input type="button" value="OK"/>	<input type="button" value="Cancel"/>
<input type="button" value="OK ≥>"/>	

Figure 11.3. The DA-5 is waiting for a start signal from the autosampler.

CONTROL TEMP.MTH CATS001A.DAT	
Data	Method Queue Control Status Graphics Help

Method filename: TEMP	Conditions:
Operator:	Run notes:
Data filename: CATS	Detector: A=EC1
Run number: 1	Date of run: 03/23/94
Time of run: 11:52	Solvent names: A=cat's m.p. B=40% ACN C=100% ACN

Detectors enabled: A=DA1	
Waiting for external trigger...	

11.2 Other Autosamplers: DA-5 In Charge

If an autosampler is not capable of two-way communication, but will accept an inject trigger, the system can be automated with the DA-5 in charge. Program the autosampler so it will end its run and load the next sample before the DA-5 has completed its run. The autosampler must then wait for a trigger from the DA-5. Connect a two-wire cable from the START-OUT screw terminals on the rear panel of the DA-5 (Figure 11.1). The START-OUT connection provides a one second contact closure at the start of each run. Connect the other end of the cable to the SAMPLE ENABLE terminals on the autosampler.

Now program the DA-5 to run automatically. Set the TRIGGER TYPE to automatic, and indicate the number of runs (Figure 11.4). Start the Method from the RUN STATUS box, by clicking EQUIL, and then RUN (or PROCESS a QUEUE). The DA-5 will proceed automatically from one run to the next, and send a start signal to the autosampler each time.

Figure 11.4. Data Acquisition Options for communicating with an autosampler that accepts an inject signal.

DATA ACQUISITION OPTIONS	
Trigger type <input type="radio"/> Manual <input checked="" type="radio"/> Automatic <input type="radio"/> External	
Start data time <0.0:539.9>:	0.000 minutes
End data time <0.1:540.0>:	10 minutes
Data acquisition rate	150 points per minute
Detectors enabled <input checked="" type="checkbox"/> DA-1 <input type="checkbox"/> DA-2 <input type="checkbox"/> DA-3 <input type="checkbox"/> DA-4	
First run number	1
Number of runs	20
<input type="button" value="≤< OK"/> <input type="button" value="Save"/> <input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="OK >>"/>	

11.3 Other Autosamplers: 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.

To configure the system with the autosampler in charge, use a two-wire cable. Connect one end to the START-IN screw connectors on the rear panel of the DA-5 (Figure 11.1). The START-IN connector accepts either a switch closure or a negative-going (+5 ± 0.5V) TTL signal. Connect the other end of the cable to the trigger terminals on the autosampler. The autosampler's run must be longer than that of the DA-5, so the chromatograph is waiting for a trigger when the autosampler is ready to inject. Program the DA-5 to accept an external trigger, and set the number of runs (Figure 11.5). Start the system by clicking EQUIL and then RUN in the RUN STATUS box (or PROCESS a QUEUE). The message "waiting for trigger" will appear (Figure 11.3). Then start the autosampler.

Figure 11.5. Data Acquisition Options for accepting a start trigger from an autosampler.

DATA ACQUISITION OPTIONS	
Trigger type <input type="radio"/> Manual <input type="radio"/> Automatic <input checked="" type="radio"/> External	
Start data time <0.0:539.9>:	0.000 minutes
End data time <0.1:540.0>:	10 minutes
Data acquisition rate	150 points per minute
Detectors enabled <input checked="" type="checkbox"/> DA-1 <input type="checkbox"/> DA-2 <input type="checkbox"/> DA-3 <input type="checkbox"/> DA-4	
First run number	1
Number of runs	20
<input type="button" value="≤< OK"/> <input type="button" value="Save"/> <input type="button" value="OK"/> <input type="button" value="Cancel"/> <input type="button" value="OK >>"/>	

11.4 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 the sample information with the data.

The format of the .ran file is shown in Figure 11.6. Find the file TEMPLATE.RAN in the BAS directory. Copy this file into the BAS/DATA directory and use it as a template to build your own .ran file. It is important that this file be in text format, so all characters and spaces have the same width. Use a text editor to make the modifications. Control will automatically call Windows Notepad when you take the EDIT .RAN FILE option under the DATA menu (Figure 12.2). Be sure to save the file with a new name.ran.

ChromGraph ignores the first 10 lines of the file. Enter the data you wish to incorporate starting in line 11. The information **MUST** be found in the specified columns, left justified.

Run Number: (Columns 16-25) Control will examine this column at the start of each run. If a run number anywhere in the column matches the current run number, Control will incorporate the relevant sections of that line into the data file.

Vial Number: (Columns 26-35) The preferred method for saving vial numbers along with the data is to let the autosampler do it, as with the BAS Sample Sentinel equipped with the vial-position option. In this case, go to SETUP OPTIONS (Figure 11.7) and make sure that GET VIAL # in .RAN FILE is **NOT** checked and that BINARY VIAL NUMBERS is checked. Control will ignore the vial numbers in the .ran file.

If the autosampler is not equipped to send vial position data, you can specify that vial numbers in the .ran file be saved with the data. Check the box for GET VIAL # in .RAN FILE.

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, **leave this column blank**.

If volume does differ among samples, you can enter the volume for each sample. See the ChromGraph Report manual 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.

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 ACQUISITIONS (Figure 11.8). As each run is started Control will import the relevant information from the matching line in the .ran file.

Figure 11.6. A template for the .ran file.

BAS - ChromGraph REG Ver 1.0 (created:8/27/96 - 10:08:14 AM)

TITLE:Test PROJECT #:0000 0000
 ANALYST:pkw RANDOM FILE:13NE.ran
 # OF SAMPLES:9 AUTOSAMPLER CAPACITY:
 NORMAL VOLUME:1.00 NOTEBOOK #:
 PAGE(S): _____

RUN	VIAL	VOLUME	SAMPLE ID
1	1	1	Sample 110123456789012345678901
2	22	0.50	Sample 220123456789012345678901
9	90	9.9876543	Sample 990123456789012345678901
3	333	0.25	Sample 330123456789012345678901
7	70	7.77	Sample 770123456789012345678901
4	4444	4.4	Sample 440123456789012345678901
5	55555	5.5	Sample 550123456789012345678901
6	60	6.6	Sample 660123456789012345678901
8	80	8.888888	Sample 880123456789012345678901

Figure 11.7. Options for importing data from a .ran file and the autosampler.

CONTROL SETUP OPTIONS

COM Port
 Simulate COM1 COM2 COM3 COM4

Baud Rate
 9600 19200 38400

BAS ROM Versions
 DA-5 3.03e

Simulated equipment
 Pump Utility LC4C 4C/PAD DA-5

Smart Mouse positioning Auto-Backup .MTH
 Binary Vial Numbers Abort if no .RAN file
 Get vial # in .RAN file Get volume in .RAN file

OK Cancel

Figure 11.8. Entering the .ran file name in the Method.

DATA DESCRIPTION

Method filename: TEMP

Operator:

Conditions:

Run Notes: name.ran

Time of run= 15:17 Date of run= 01/05/1998

Data name: TEST Run #: 11

Detector= A=EC1 Vial #: 2

<< OK Save OK Cancel OK >>

SECTION 12. DATA ANALYSIS WITH CHROMGRAPH REPORT

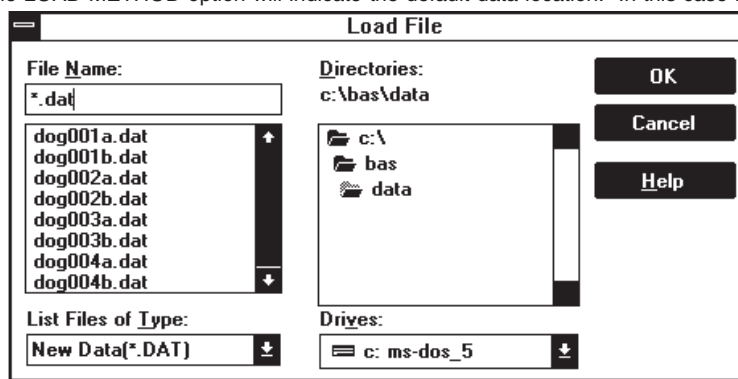
The data analysis companion to ChromGraph CONTROL is called ChromGraph REPORT. REPORT comes with its own manual, so detailed instructions for operating it are not needed here. This section will concentrate on interfacing the two modules.

12.1 REPORT In The Batch-Process Mode

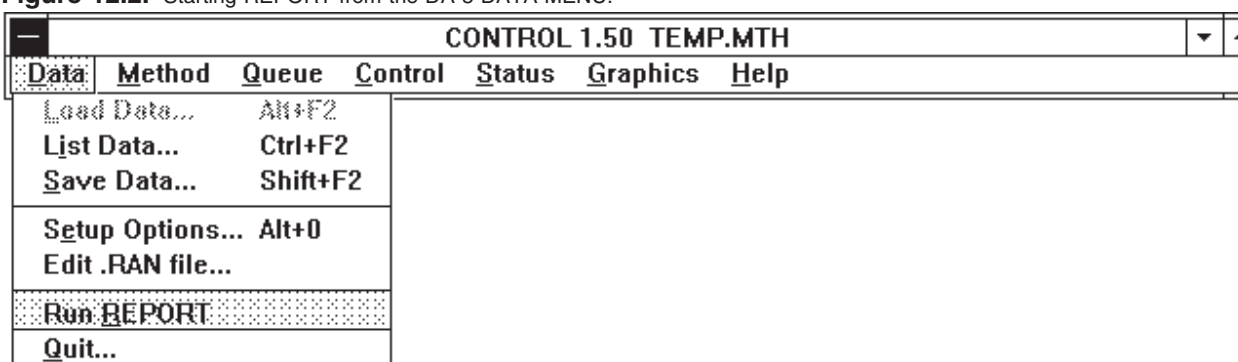
The easiest way to interface the two modules is not to interface them at all. Many users prefer to separate data collection and data analysis into two separate activities. This reduces the array of things one must do before starting a series of runs, and reduces the chance of making mistakes.

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 (Figure 12.1) to which the data will be saved. Configure the autosampler appropriately, start the system, and let the chromatograph and autosampler process the samples overnight.

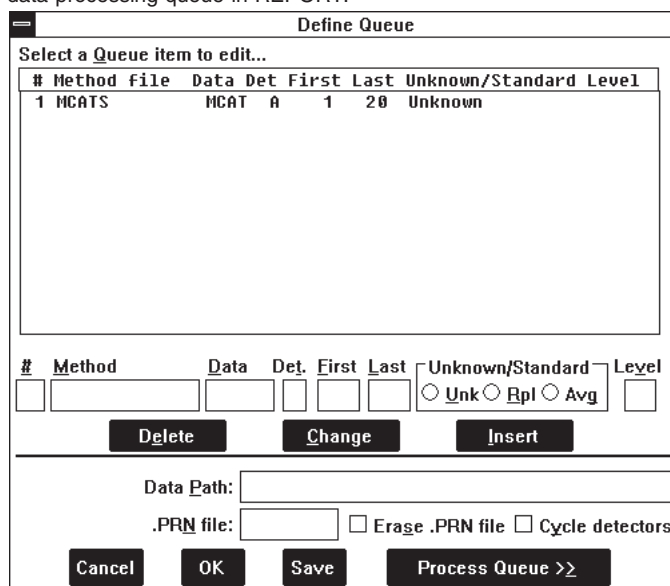
Figure 12.1. Taking the LOAD METHOD option will indicate the default data location. In this case it is C:\BAS\DATA.



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.) Now load up REPORT. This can be done by double-clicking on the REPORT icon in the BAS PROGRAMS group of Windows Program Manager, or by taking the RUN REPORT option in the DA-5 DATA MENU (Figure 12.2).

Figure 12.2. Starting REPORT from the DA-5 DATA MENU.

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 (Figure 12.3). Press the PROCESS QUEUE button, and all the runs will be analyzed.

Figure 12.3. Setting up a data-processing queue in REPORT.

12.2 REPORT In The On-line Mode

REPORT also 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 on-line mode is that printed output from early runs can be examined while later runs are still in process. We recommend that you attempt the on-line mode only after you are thoroughly familiar with both CONTROL and REPORT.

The first step in setting up the system for on-line 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 File Manager to copy these files for storage.

To ready REPORT to receive data, first load some data from the directory in which the DA-5 will save it. This sets the default data location appropriately. Next, set up a REPORT QUEUE listing the Method and run numbers to use (Figure 12.4). If more than one detector is configured you need a line for each detector. 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 (Figure 12.5). REPORT will do nothing until CONTROL writes the data files to disk.

Figure 12.4. Setting up REPORT to receive and process data passed from the DA-5.

#	Method	Data	Det.	First	Last	Unknown/Standard	Level
1	MCATS	MCAT	A	1	20	Unknown	

Method Data Det. First Last Unknown/Standard Level
 Unk Rpl Avg

Delete Change Insert

Data Path:
 .PRN file: Erase .PRN file Cycle detectors

Cancel OK Save Process Queue >>

Figure 12.5. REPORT is awaiting the data.

REPORT Queue awaits:
 MCAT001A.DAT

Cancel Retry

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.

SECTION 13. EXITING THE SYSTEM

CONTROL may be exited, and the computer turned off, whenever there are no runs in progress.

To exit the software, choose QUIT from the DATA section of the Main Menu (Figure 13.1). You will be given a choice of saving the TEMP.MTH and CONTROL.INI files (Figure 13.2). The TEMP.MTH is a backup copy of the Method currently in RAM. It is also the Method that is automatically loaded at startup. The CONTROL.INI file contains information on how you've set up the screen. Such things as the size and location of windows and status boxes, size and color of the graph, and the default data location are stored here. Check the boxes if you wish to SAVE these files. (The CONTROL.INI box will always appear unchecked. The TEMP.MTH box will appear either checked or unchecked: its setting is preserved from the previous SAVE of CONTROL.IN.)

Figure 13.1. Exiting the software.

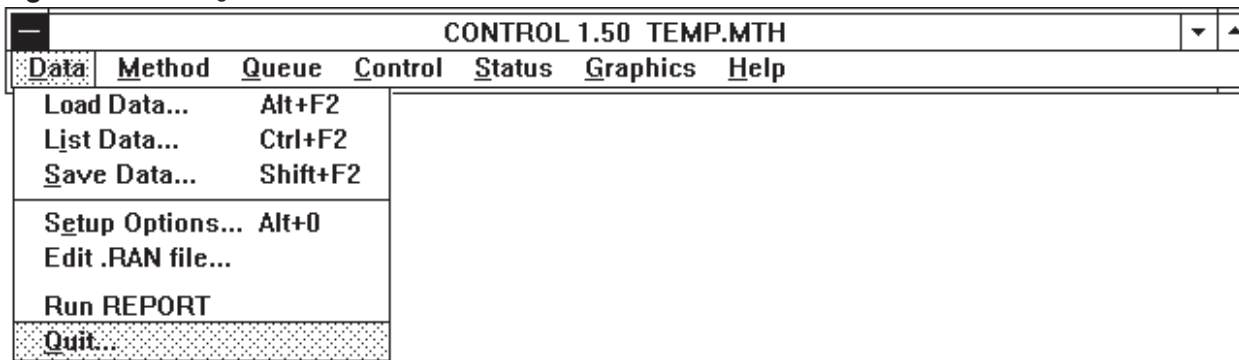
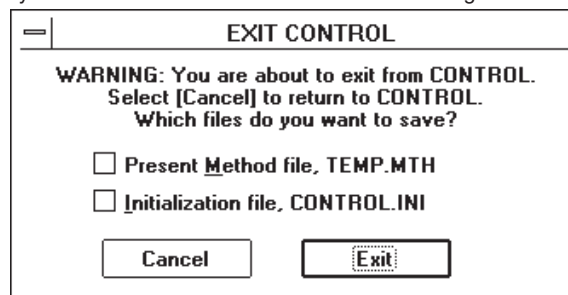


Figure 13.2. Check the boxes if you want the current method and screen settings to be recalled at your next session.



SECTION 14. SPECIFICATIONS

Number of Analog Inputs	4 detectors
Input Voltage Ranges	0.1, 1 and 10 Volts
Resolution	20 bits @ 300 ppm or less to 16 bits @ 6000 ppm
Data Acquisition Rates	10, 20, 40, 60, 75, 100, 150, 200, 300, 600, 750, 1200, 1500, 3000, 6000 ppm
Filter Constants	None, 0.05, 0.075, 0.1, 0.15, 0.25 Hz
Digital Inputs	4 digits for autosampler vial number Ready and Inject from the autosampler Run and Acknowledge from CONTROL External start screw terminals Front panel start switch
Digital Outputs	10 lines to CONTROL and Pause to the autosampler External Start screw terminals
Dimensions (L x W x H, cm)	23 x 28 x 8.5
Weight (kg)	3.9
Function Indicator	Tri-color LED
Self Test Conditions	Momentary red: self test at startup Constant red: self test failure Blank: Self test OK. Awaiting a Method Momentary orange: internal calibration Constant green: Method received, awaiting start Blinking green: acquiring data

SECTION 15. SUPPORT POLICY

15.1 User Updates

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. We would like to know who you are, and what more you want to know about electrochemical detection!

15.2 Damaged Shipments

Breakage of any part of this instrument during shipping should be reported immediately to BAS Customer Service. 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 Customer Service 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.

15.3 Product Warranty

Bioanalytical Systems, Inc. products are fully warranted against defects in material and workmanship. The DA-5 electronic controller 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.

15.4 Service Information

Bioanalytical Systems provides a skilled service staff available to solve your technical problems if an equipment-oriented problem should arise. For further details, call customer service personnel (765/463-4527) 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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