



BAS 200B

August 1995

MF-9082

INSTRUCTION MANUAL

Liquid Chromatograph

Bioanalytical
Systems, Inc
2701 Kent Avenue
West Lafayette
Indiana 47906

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MANUFACTURER'S NOTE

This instrument, either wholly or in part, is manufactured for research purposes only.
Use for medical diagnosis is not intended, implied or recommended by the manufacturer.
Use for this purpose and accountability for the same rests entirely with the user.

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Section 1. INTRODUCTION

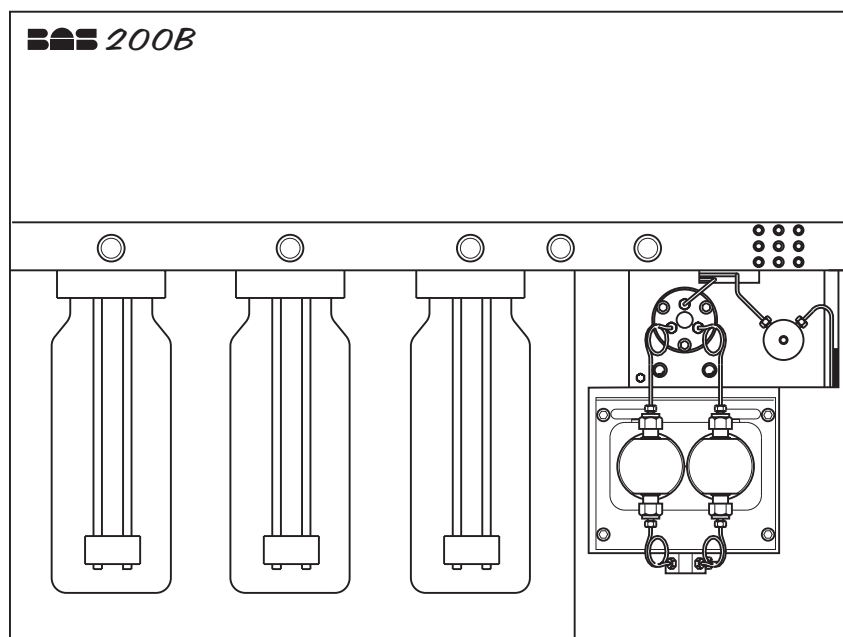
1.1 About this Manual

This manual explains installation, operation, and service procedures for the BAS 200B Liquid Chromatograph. It also includes a short tutorial exercise; perform this to become acquainted with the operational control logic of the BAS 200B.

The BAS 200B comprises of several *subsystems* that are integrated within a single instrument. This BAS 200B Instruction Manual is likewise organized into separate reference sections for each of these modules. Some subsystems (e.g., electrodes) have their own manuals, which will be cited where appropriate. This manual describes controls, detailed operating procedures, troubleshooting tips, and routine maintenance for each subsystem.

See the ChromGraph[®] Instruction Manuals (P/N MF-9070 and MF-9071) for information about optional software used with this instrument.

Figure 1.1 Front view of the BAS 200B Liquid Chromatograph.



1.2 Description of the BAS 200B

The BAS 200B is a state-of-the-art liquid chromatography (LC) system for microbore, standard analytical, and preparative separations.

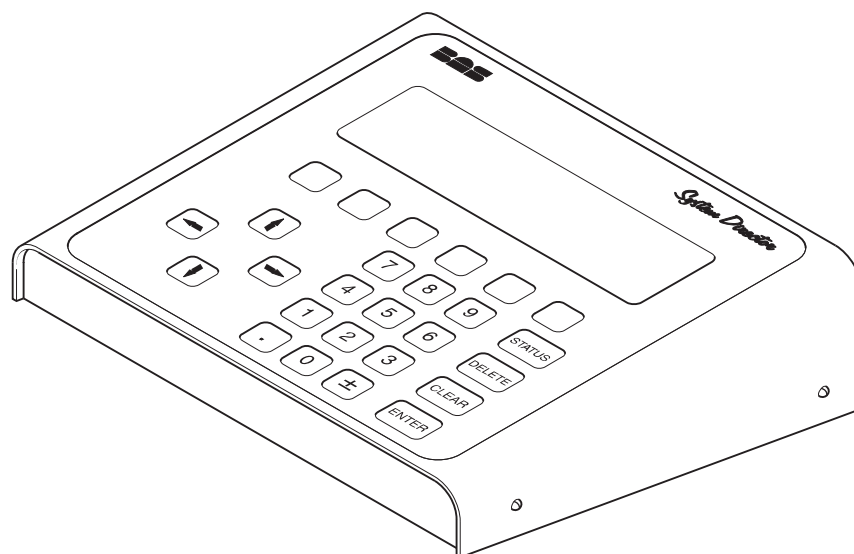
The front face (Figure 1.1) and sides of the chassis comprise the “wet” portion of the liquid chromatograph. All points of operator use are arranged here; these include the three mobile phase bottles and their associated utilities, the ternary gradient solvent delivery system, the sample injection valve, the convection air oven, and the optional detectors.

Generally, user access to the interior will be localized to the convection air oven. The compartment is insulated on all outside walls with a sandwich of foam to improve thermal stability, and the oven door is gasketed to prevent air leaks. When opened, the door is completely clear of the operator's work area.

From an engineering standpoint, the BAS 200B is divided into several electromechanical subsystems. The modules can be easily removed for diagnosis of system malfunction.

The BAS 200B can be controlled in two ways. The first option, the **BAS 200B System Director** (Figure 1.2), provides a sophisticated, user-friendly means to enter all the required parameters. Up to seven method files may be stored in RAM, even when system power is off. The second option, the PC-based **ChromGraph System** (optional), uses similar but more sophisticated control software, and provides for four-channel data processing. One or two external detector outputs may be plugged into the BAS 200B; these analog signals are conveniently digitized on the internal CPU detector board and sent to ChromGraph for data processing. Timed events, autosampling control signals, and standard recorder/integrator analog outputs are also provided.

Figure 1.2 BAS 200B System Director. This membrane keypad unit controls all subsystems, either as independent modules or in unison as part of a larger method file. Up to seven method files may be stored in RAM, even when system power is off.



1.3 Instrument Specifications

The design criteria for the BAS 200B are outlined below. These capabilities provide the necessary hardware to execute nearly all common LC applications.

- Ternary solvent gradient delivery, including gradients for microbore columns to reduce sample size and improve detectability. Gradients from 0 to 100% are formed from up to nine contiguous line segments, with 0.5% relative accuracy and 0.1% precision. "Unit body" check valve cartridges contain double balls and seats in series for reliability and fast repair. Each pump head is a self-contained unit that includes piston, check valves, and seal, for changeovers in seconds. The purge valve and solid-state low dead volume pressure transducer are accessible at the top of the pump panel. Standard, optional pulse damper for highest sensitivity amperometric detection.
- Rigorous deoxygenation and thermostatic controls for up to three solvents. Temperature setpoints: ambient, 35 °C, and 50 °C, under microprocessor control with immersion-style solid-state sensor. Sophisticated error and liquid-level sensing for safety shutdown. Sparging via integral gas dispersion frit and precision all-stainless needle valves. Safety-coated flasks may be sealed against 5 psi for headspace pressurization and passive priming of the low pressure side of solvent delivery system. Recycling capability for unattended operation.
- Convection air oven for immunity from thermal and radio frequency interference at high gain. Accommodations for columns of all sizes and combinations. Optical and amperometric cells are mounted within the compartment for stability at high gain. Setpoint is variable from ambient to 70 °C, in 0.1 °C increments. Control to ± 0.15 °C, using actively controlled software modulation of duty cycles. Fan control.
- Keypad controller (BAS System Director) to operate modules separately or in unison under a single method file. Smart keys geared to the chromatographer's needs. Software designed by practicing chemists. Automatic battery backup of method files in memory for one year. Up to seven files resident at any time.
- ChromGraph data reduction and control package capable of four-channel integration. Internal A/D converter (voltage to frequency, 16 bit). Raw data storage; reintegration options; real-time display; complete printout of reports, data, and methods for archival storage, data exchange, multitasking. Two A/D channels may be used for analog signals from external detectors.

- Time-programmable control of all internal detector parameters, including gain, electronic filter, wavelength, and potential. Programmable baseline autozero for long runs at user-selected times or on demand in real time.
- Amperometric detector requires no tools. Optional active control of incoming mobile phase temperature with self-directed duty cycle to reduce environmental influences. Thermodynamically defined reference electrode is provided as a disposable unit and is capable of use under pressures to 6 bar. Single or dual electrode operation via software control; autozero via time program or on demand; gain and potential are time-programmable. Glassy carbon, gold, mercury/gold, platinum, and silver electrode materials.
- Small footprint; only 21-inch (53-cm) width for ternary gradient solvent delivery system and ancillary utilities, temperature-stabilized separation and EC detection, keypad, and sample injector.

Section 2. SUPPORT POLICY

2.1 User Updates

To activate 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 that was shipped with the instrument.

2.2 Damaged Shipments

Breakage of any part of this instrument during shipping should be reported immediately to BAS Customer Service. You must retain the original packing containers 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 of the shipping date will be invalid.

Do not return damaged goods to BAS 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. BAS *refuses* all unauthorized return shipments.

2.3 Product Warranty

Bioanalytical Systems, Inc. (BAS) products are fully warranted against defects in material and workmanship. The BAS 200B Liquid Chromatograph is warranted to be free of such defects for 90 days 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 components. BAS agrees to either repair or replace at its sole option and free of part charges to the buyer any parts of such instrumentation, which, under proper and normal conditions of use, prove to be defective within three (3) months from date of shipment.

Some parts of the BAS 200B system are inherently “disposable” by virtue of their limited lifetimes. These components are expressly excluded from the foregoing warranty. Examples of parts of this nature include, but are not limited to:

energy sources	fuses
detector electrodes	reference electrodes
pump seals	gaskets
pump pistons	separation columns
pump check valves	mobile phase flasks
o-rings of any type	lamps

Under such circumstances, any repair or replacement services, providing they do not relate to a proven defect in materials or workmanship, shall be performed only at BAS' then current rates for parts and services.

This warranty specifically does not cover loss or damage to instrumentation which might be sustained in transit to the buyer's facility, as to which BAS disclaims any responsibility whatsoever.

BAS shall use its best efforts to perform all warranty service hereunder at the buyer's facility, and as soon after notification by the buyer of a possible defect as is reasonably practicable, provided, however, that BAS reserves the right to require that the buyer return the instrumentation to BAS' production facility, transportation charges prepaid, when necessary to provide proper warranty service.

In lieu of the foregoing, BAS may at any time elect, in its sole discretion, to discharge its warranty by accepting the return of such instrumentation and refunding the purchase price to the buyer.

The foregoing warranty and remedy are exclusive and expressly in lieu of all other warranties express or implied either in fact or by operation of law, statutory or otherwise, including warranties of merchantability and fitness for use. BAS neither assumes nor authorizes any other person to assume for it any other liability in connection with the sale, installation, service, or use of its instrumentation. BAS shall have no liability whatsoever for special, consequential, or punitive damages of any kind from any cause arising out of the sale, installation, service, or use of its instrumentation.

2.4 Service

BAS provides a skilled service staff to solve your technical equipment problems. For further details, call customer service personnel (1-800-845-4246) who will 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 your problem in full detail. Turnaround time on service can be quoted to you at the time your RA# is issued, although we cannot 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# [insert your assigned number], Service Department
Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

Section 3. INSTALLATION

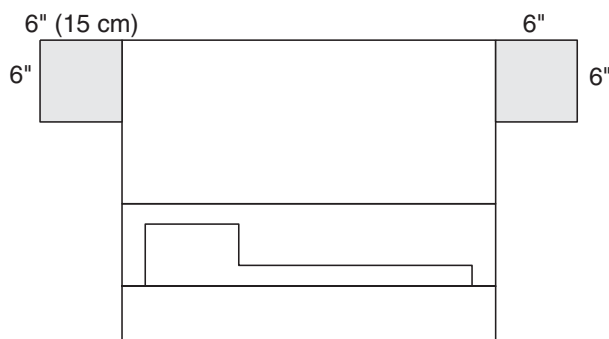
3.1 Local Environment

When selecting a location for the BAS 200B, follow these guidelines:

1. 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.
2. Ensure that all system components share a ground circuit. This is best accomplished by plugging all components into the same multi-outlet power strip. Plugging the components into independent outlets can produce ground loops (current flowing between ground circuits at slightly different potentials) which produces baseline noise.
3. Locate the unit on a stable bench. Vibrations can hamper the performance of any sensitive instrument.
4. Select a room where the temperature remains stable throughout the day. Avoid installing the unit near drafty areas, windows, air ducts, ovens, or refrigerators.
5. Place the chromatograph away from busy, congested areas. Remote, isolated areas are best for high-sensitivity work.
6. Avoid carpeted or very dry areas; static electricity can affect instrument performance. Anti-static floormats and benchmats guard against spiking caused by static charge.
7. Avoid areas where radio frequency interferences are likely. Beeper-type paging devices can be a problem in some installations.

NOTE: It is very important that at least six inches (15 cm) of free air space be provided in front of all ventilation grilles on the sides of the BAS 200B. Also, nothing must be placed under the BAS 200B that might block exhaust vents on the bottom. See Figure 3.1.

Figure 3.1 Minimum spatial clearance around the BAS 200B. Shaded space is reserved for ventilation.



3.2 Electrical Connections

Voltage

The BAS 200B operates on 120 V/60 Hz, which is provided by the main power supply. Your laboratory voltage may not match this requirement. In such cases, incoming power can be converted to 120 V by proper installation of the line cord connector found on the left side panel. The unit should be shipped with the voltage option already matched to the power requirements of the destination, but this should be verified by the user.

Unplug the line cord and slide the plastic window down. 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 does not match your laboratory voltage, pull out the board and turn it until the desired voltage is readable. Reinsert the board and push the fuse holder back into the cavity. Also check the table below to see that the fuse is the proper rating.

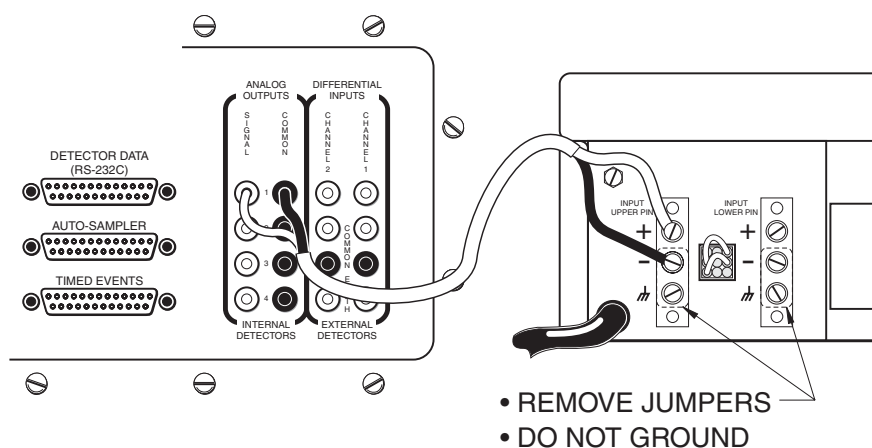
VOLTAGE	FUSE
120 V	6A, slowblow, 120 V
100 V	6A, slowblow, 120 V
220–240 V	3A, slowblow, 240 V

Analog Strip-Chart Recorders

Analog output voltages from the BAS 200B range from -0.2 to 10 V. The small negative range provides some recognition of negative detector voltages. To make connections:

- Using the analog recorder cable(s) provided, plug the green male plug into the blue female jack (on BAS 200B) and the black plug into the brown female jack. The corresponding spade lugs should be attached to the “+” and “-” terminals (or “high” and “low,” respectively) on your recorder (see Figure 3.2). Do not attach any additional grounds.

Figure 3.2 Recorder connections from BAS 200B.



2. Set the chart recorder to the “zero” (or equivalent) check position. Use its “zero adjust” control to establish a convenient place on the paper for zero volts. Usually this is at the 0 or 10% point on the left side of the chart paper.
3. Set the input range of the chart recorder to 1.0 V full scale. The scale will be calibrated in “nanoamps full scale” (for electrochemical detector) as designated by the detector file. For example, if you put “zero” at the 0% position on the chart paper and program the electrochemical detector to show a gain of 5 nanoamps, then a full-scale deflection corresponds to 5 nA, a half-scale deflection to 2.5 nA, etc.

Stand-Alone Workstations and Integrators

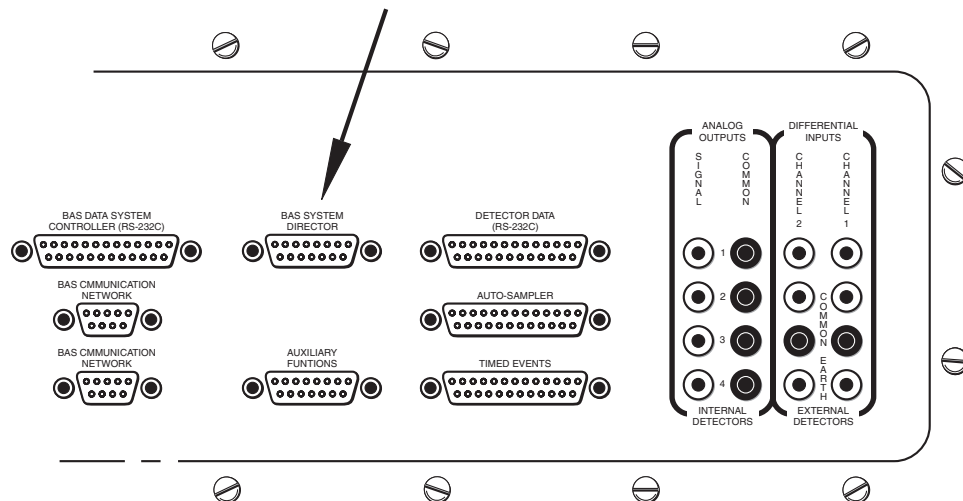
Use the analog recorder cable(s) provided. Plug the green banana plug into the blue output jack on the BAS 200B rear panel; plug the black banana plug into the brown output jack on the BAS 200B. Attach the corresponding spade lugs to the “+” and “-” terminals (or “high” and “low,” respectively) of your integrator or data station. Do not attach any additional grounds. The output voltage from the BAS 200B will be -0.2 V to 10 V. Typically, all data will be collected from 0 to 1.0 V.

Consult your integrator or data station’s documentation for operational guidance.

The BAS System Director

To install the BAS System Director keypad controller, attach its cable connector to the back-panel socket labeled “BAS System Director” (see Figure 3.3). Screw the cable connectors (at both ends) securely to their mating parts.

Figure 3.3 Back panel board, BAS 200B. The keypad cable screws onto the top center connector.



3.3 Liquid Connections

Mobile Phase

Three 1-liter bottles are provided as reservoirs for mobile phase. They can be used in any combination desired: you can pump mobile phase from any one, or from all three (either sequentially or simultaneously), or vary the proportion with time (gradient operation). Only bottle A (the leftmost) can be used to recycle mobile phase from the column back into its reservoir.

Wash the bottles thoroughly before installing them on the BAS 200B. Use a nonalkaline laboratory detergent, such as RBS-35™ from Pierce. Rinse the bottles thoroughly with deionized water.

All mobile phases and solvents used in the BAS 200B should be vacuum-filtered through a 0.2- μ m membrane filter. This both degases and removes dust and grit that could damage the pump seals and clog the column. CLEAN, PARTICLE-FREE MOBILE PHASES ARE CRITICAL TO ALL LC METHODS!

All three bottles should be at least half filled with mobile phase or solvent. If you will only be using one liter of mobile phase, we recommend that it be put in bottle A. Bottle B can be filled with 40% acetonitrile (60% water), and bottle C with neat acetonitrile. These are convenient for flushing the system and the column. Alternatively, deionized water may be put in the unused bottles.

Attach the bottles by turning the screw-ring caps rather than the bottles; this keeps the solvent uptake line from wrapping around the heater/sensor tubes that extend into the bottle. Tighten the bottles firmly, but not excessively.

Column

The BAS 200B system is shipped with a cartridge-style reverse-phase C₁₈ column *already installed* in a cartridge holder. The packing material is a spherical, 3 μ m-diameter silica, bonded with a polyfunctional octadecyl hydrocarbon chain. This material is highly hydrophobic. The column is flushed with 40:60 acetonitrile:water (v:v) prior to shipping.

For future reference, installation instructions for these columns are as follows:

1. Connect the cartridge in its holder to the chromatograph, using the plastic fingertight fitting provided. Use a second fingertight fitting to connect the outlet of the column to the detector. IMPORTANT: Liquid flow through the column should be in the same direction that you read the label.
2. To install a cartridge, first remove the entire assembly from the chromatograph. Then unscrew the holder, remove the old cartridge, and carefully insert the new cartridge. For best results, tighten both caps equally so the cartridge is centered in the holder.

Reseal the holder to finger tightness. *Further tightening is unnecessary* and may damage the seal or holder.

Never use tools to tighten the holder! If the cartridge leaks at less than its rated pressure limit, the cartridge or seal surface must be dirty, scratched, or deformed. Replace the cartridge and check again for leaks. If the new cartridge leaks, the high-pressure seals must be replaced (see Section 8.7).

3.4 Degassing Utility

Up to three solvent mixtures can be accommodated in the BAS 200B. Each mixture may be heated to 35 °C or 50 °C under software control. Needle valves control the flow of an inert sparging gas to the mobile phase bottles. Each bottle is sealed by an inert polytetrafluoroethylene (PTFE) o-ring. (Wetted surfaces are PTFE, passivated 316 stainless steel, and borosilicate glass.) A continuous blanket of inert gas can be maintained in the bottles, both to prevent atmospheric gases from dissolving in the mobile phase, and to provide a positive prime to the pump.

To install the gas utility, proceed as follows:

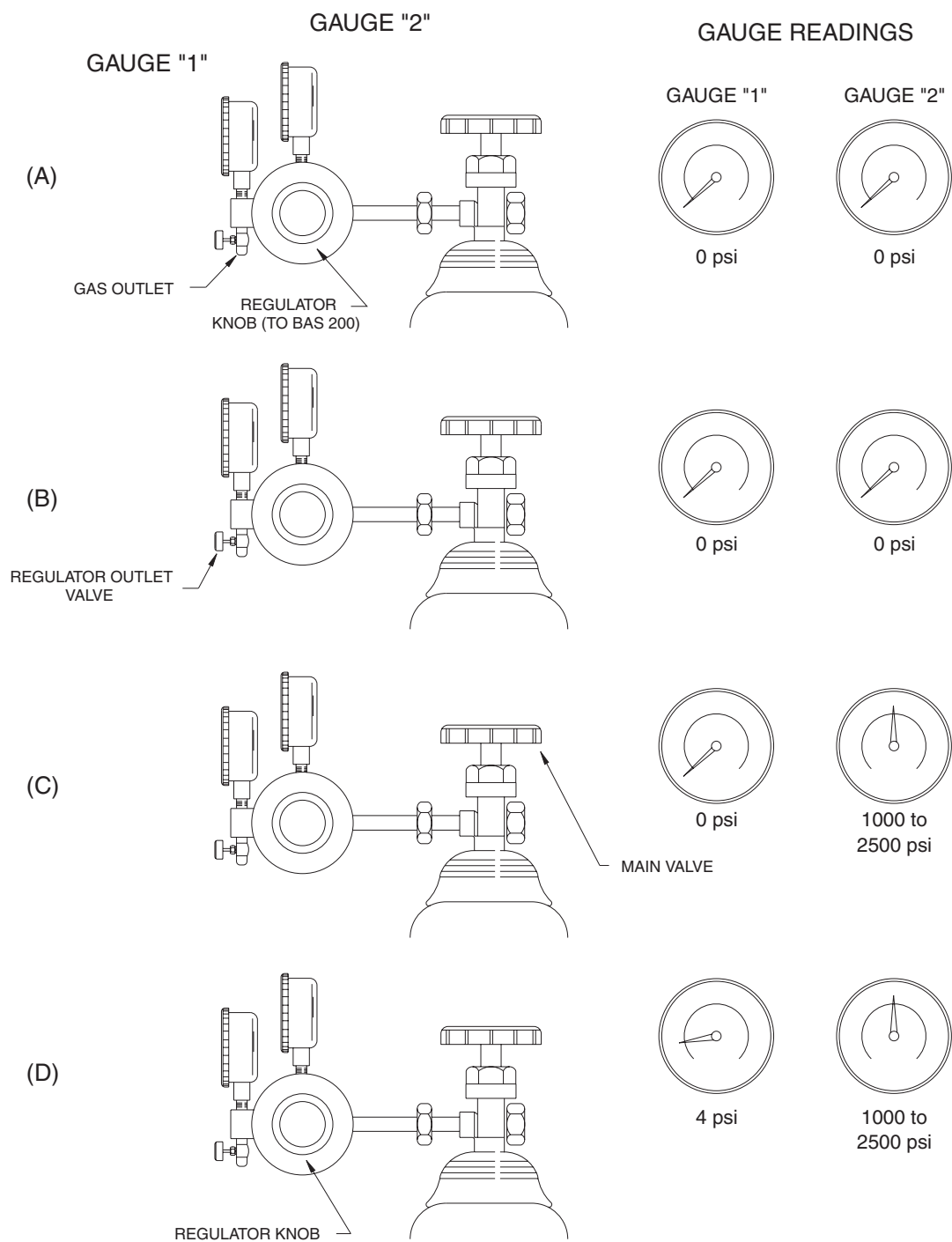
1. Install a dual stage regulator (P/N MF-9302 or equivalent) on a tank of inert gas. The lower stage of the regulator should be graduated in 1-psi increments.

NOTE: Ensure that the gas tank is securely anchored to the lab bench by standard clamp fixtures for safety.

2. Refer to Figure 3.4. Turn the regulator knob fully counterclockwise (CCW). Shut off the regulator outlet valve (fully clockwise, CW). Open the main tank valve. Confirm tank pressure at the first stage gauge.
3. Slowly turn the regulator knob CW until the second stage gauge reads 4 ± 1 psi.
4. Attach the 1/8"-diameter inlet helium line to the BAS 200B and to the regulator outlet valve. The connection to the BAS 200B is made at the 1/8" union under and behind the pump trim panel. Finger-tighten, then snug with a wrench.
5. Close the inlet front panel gas control knob (rotate fully CW). Open the regulator outlet valve (rotate CCW).
6. Slide the remaining plastic tube (for the helium exhaust vent) onto the remaining 1/8" steel tube (under pump). Route the tube under the chassis to the rear of the instrument.

This tube may be attached to a user-supplied cold trap, bubbler, or cup sink to capture solvent vapors. If toxic solvents are used as mobile phase components, BAS strongly recommends that you study the possible hazards outlined by the solvent manufacturers. Use an appropriate trapping procedure if necessary.

Figure 3.4 Adjustment of helium inlet pressure prior to connection to BAS 200B. (A) Shut off regulator knob (fully CCW). (B) Shut off regulator outlet valve (fully CW). (C) Open main valve. (D) Slowly open regulator knob until Gauge 1 reads 4 ± 1 psi.



7. Open the regulator outlet valve. Gas is now supplied up to the inlet valve on the BAS 200B.
8. Make sure that the exhaust valve on the BAS 200B is open (fully CCW). Now open the inlet valve on the BAS 200B (fully CCW). Gas should start bubbling through the mobile phase bottles.
9. Allow the gas to bubble for a few minutes for routine degassing. For methods that require rigorous deoxygenation, much more bubbling, combined with heating the mobile phase, may be required. The valves above the bottles are inlets, and may be used to adjust the relative gas flow into the bottles. The bottles share a common exhaust.
10. Close the exhaust valve (fully CW) on the BAS 200B. Bubbling will stop within a few minutes (if not, check that all the bottles are tight, and that the recycle line is plugged with a dummy fitting). The bottles are now under 4 psi pressure; verify that your regulator is holding at 4 psi, and adjust regulator knob slightly if not. (This knob works opposite to all the others; turn it CW to increase pressure.)
11. Test all fittings for leakage using soap bubbles or a commercial product designed for this.

IMPORTANT: NEVER SHUT OFF THE PRESSURE AT THE REGULATOR WITHOUT FIRST OPENING THE EXHAUST VALVE ON THE BAS 200B. Any drop in pressure in the gas supply will cause mobile phase to move into the gas lines.

To remove and replace a bottle, proceed as follows:

1. ALWAYS open the exhaust valve on the BAS 200B *first*.
2. Close the inlet valve on the BAS 200B.
3. Remove the bottle.
4. Replace the bottle.
5. Open the inlet valve on the BAS 200B to allow bubbling.
6. Finally, close the exhaust valve on the BAS 200B.

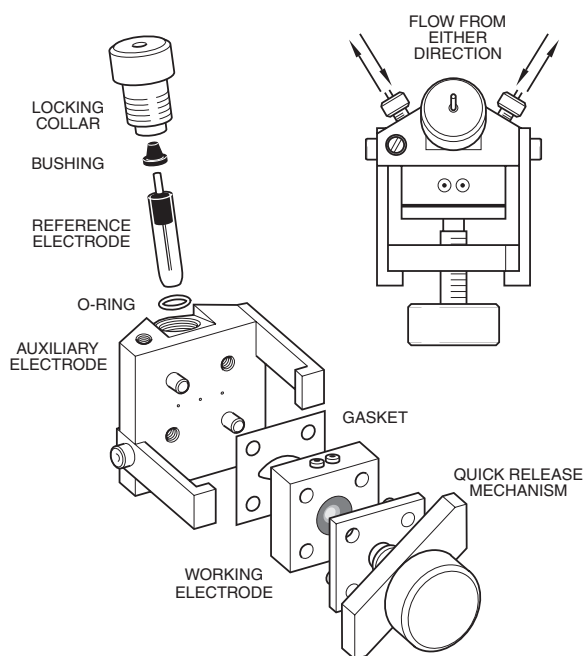
3.5 Electrochemical Detector

The electrochemical detector is typically arranged first in line following the column. It is mounted to a plastic base. Several different detectors are available with the BAS 200B, so only a general description of detector connection can be presented here. Connect the detector after the column has been equilibrated with mobile phase.

Installation is as follows:

1. Attach the working electrode and gasket to the auxiliary electrode, using the clamp and backing plate as shown in Figure 3.5. Tighten securely.

Figure 3.5 Cross-flow thin-layer amperometric cell design.



2. Connect the column outlet to the detector inlet tube. A reusable plastic fitting is provided. *Avoid using metal ferrules because they become permanently attached.*

Pull the plastic fitting back so that at least 5 mm of steel tubing is exposed at the tip. Insert the tubing into the column as far as it will go. Hold the tube in this position and finger-tighten the plastic fitting.

3. Start the pump. The reference electrode well in the auxiliary electrode will fill up. Siphon off this fluid several times, until there are no bubbles visible in the well.
4. Stop the pump. Insert the reference electrode o-ring into the well, and push it down so it sits flush on the ledge inside the well.

5. Siphon off excess fluid to the level of the o-ring.
6. Carefully insert the reference electrode through the o-ring, so no bubbles are trapped under it.
7. Dry the area on top of the o-ring, and any fluid that squirted out behind the auxiliary electrode. Place the bushing on top of the reference electrode, and screw down the locking collar, finger tight.
8. Route the detector outlet either to the UV-Vis inlet (if used) or to waste. Then start the pump and check for leaks.
9. Route the plastic outlet tube to the outside through the column compartment outlet port. This tube may be connected to (a) a separate detector positioned outside the BAS 200B (see Section 3.7), (b) a waste receptacle, or (c) the recycle port of the solvent delivery tray (see Section 3.8); this option allows for the closed-loop operation of the BAS 200B under isocratic mobile phase conditions, *using the A bottle only*.
10. The electronic cable provides for either one- or two-electrode operation. Should your system be a single-electrode BAS 200B, or if you intend to use only one of the two electrodes, connect the black lead labeled "W1" to the electrode. The cable labeled "W2" will be inactive. If you have the dual-electrode option installed, connect "W2" to the second electrode. Connect the white female lead to the reference electrode and the red female lead to the auxiliary electrode.

3.6 External Detectors

An external detector should be positioned to minimize the length of tubing from the column outlet to the detector inlet. The detector *must not block* the fan vents located on the left side panel of the BAS 200B (see Figure 3.1).

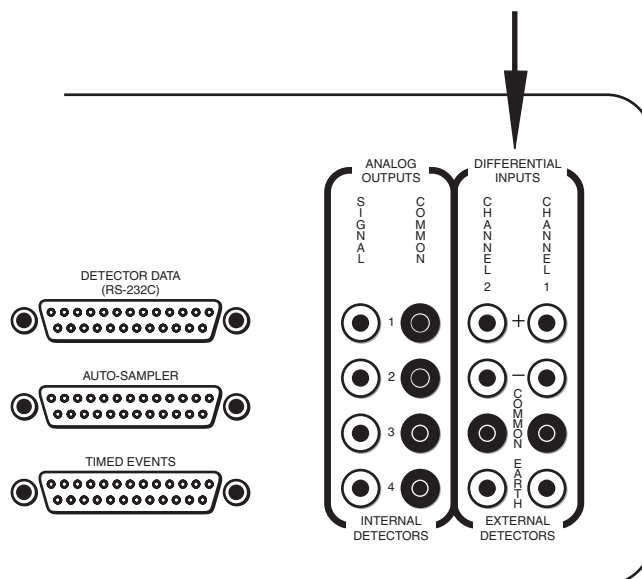
Connect the outlet tube from the BAS 200B column compartment to the inlet port or tubing of the external detector. A low-dead-volume stainless union (BAS P/N MR-4063) is recommended. You may wish to use plastic fittings, to avoid permanently mounting ferrules on the tubing.

The outlet tube from the external detector should be routed to (a) another external detector, (b) a waste receptacle, or (c) the recycle port of the solvent delivery tray (see Section 3.8); this option allows for the closed-loop recycling of mobile phase back into bottle A, and must *only* be used with isocratic, 100% A pump files.

The electrical output of the external detector may be connected to an ordinary strip-chart recorder, a computing integrator or outside data station, or the BAS ChromGraph Data System. In the last case, the analog voltage output from your external detector must be plugged into one of two channels in the back of the BAS 200B (see Figure 3.6). Your external detector's output signal should be in the range of 0 to +1.0 V.

Connect a single external detector to Differential Inputs Channel 1, and a second (if any) detector to Channel 2. In most cases, connect only to the (+) and (-) inputs, and ignore the (common) and (earth) connectors.

Figure 3.6 Back panel connections for external detector signal to be processed by the BAS System Controller.



3.7 Recycling a Mobile Phase

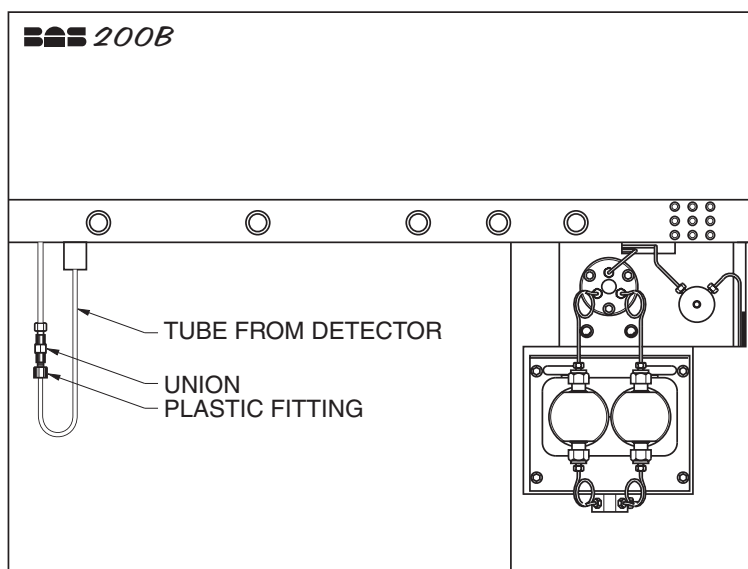
In certain situations, recycling of the mobile phase from the detector(s) back to the mobile phase reservoir is advisable. Recycling provides a means to operate the system continuously without the need for periodically making up new mobile phase. The system is always stabilized and ready to receive samples. Recycling is warranted *when all of the following are true*:

1. The system is being operated with a pump file of “100% A” only.
2. The column is already equilibrated with the mobile phase.
3. The volume of the mobile phase is large relative to the total volume of samples injected (10,000 to 1 ratio is typical).
4. The mobile phase is stable to chemical decomposition and biological contamination during the anticipated period of use.

NOTE: Before initiating recycling, make sure that the column is equilibrated. Until stable retention times are achieved, all mobile phase should be passed to waste. This is especially true with any ion-pair separation.

To initiate recycling, connect the detector outlet tube to the recycling union port behind the “A” mobile phase bottle (see Figure 3.7). *All mobile phase will be returned to bottle A*, so ensure that your pump file does not instruct the system to pump from bottles B or C. Otherwise, contamination of bottle A and its eventual overfilling will result.

Figure 3.7 Connection of the detector outlet tube to the recycle port, which is located behind the “A” mobile phase bottle.



Section 4. RUNNING THE BAS 200B SYSTEM

This section contains a practical, step-by-step guide to running your chromatograph. Information from throughout this manual will be pulled together into a tutorial session so you can learn the correct operating procedure.

This tutorial is meant to be as general as possible, so you can learn to operate the chromatograph with your own analytes and your own conditions. However, some applications are extremely complex (e.g., reductions, thiols, and disulfides). If you plan such an application, we suggest you begin with something simpler, such as the separation of acetaminophen (Section 4.8).

The BAS 200B is available in several formats depending on the size of the column, type of detector, and type of controller. This tutorial will be applicable to all configurations, but will be biased towards a standard-bore gradient system with an LCD System Director. Consult the following manuals for specific directions for other configurations:

Electrode Polishing and Care	A-1302
UniJet Microbore Columns	MR-9231
ChromGraph Control Software	MF-9070
Principles of EC Detection	MF-9083

4.1 Configuring the System

You must tell the system which options you wish to use before proceeding. These options include which onboard and external detectors to use, whether to synchronize the pumps, and how to coordinate the start of runs with an autosampler.

The easiest way to reach the hardware configuration screen on the LCD System Director is from the sequence of screens that appears during startup. To begin the sequence, either turn on the main power, or if it is already on, press the reset button next to the main power switch on the left side of the chromatograph.

The first screen to appear is the self-test screen (Figure 4.1). From this screen press any key and the software versions screen will appear (Figure 4.2). Now press any key and the edit file screen will appear (Figure 4.3). From this screen press the CONFIG function to reach the configuration screen (Figure 4.4).

Figure 4.1 The self-test screen.

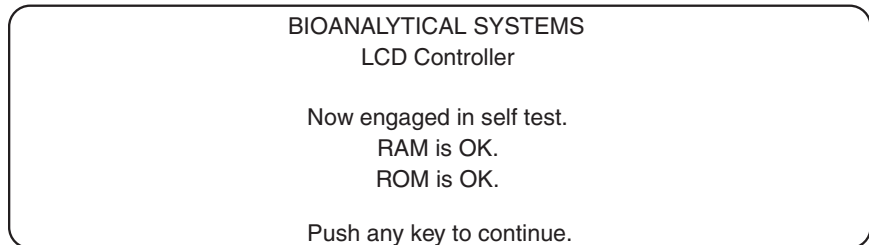


Figure 4.2 This screen indicates the ROM versions.

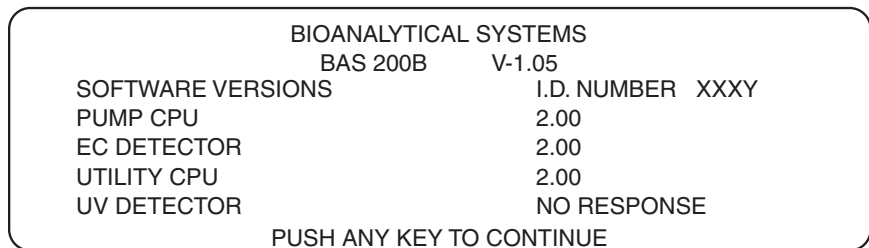


Figure 4.3 The edit file screen. Press the CONFIG function to shift to the hardware configuration screen.

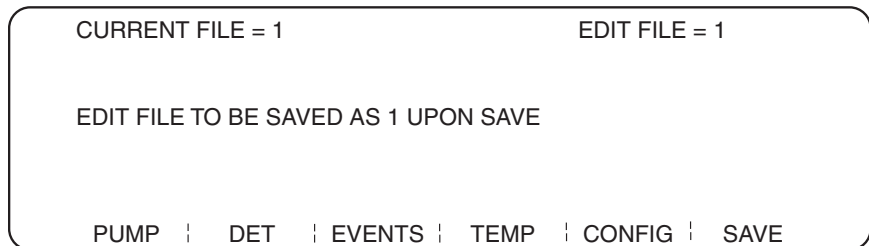
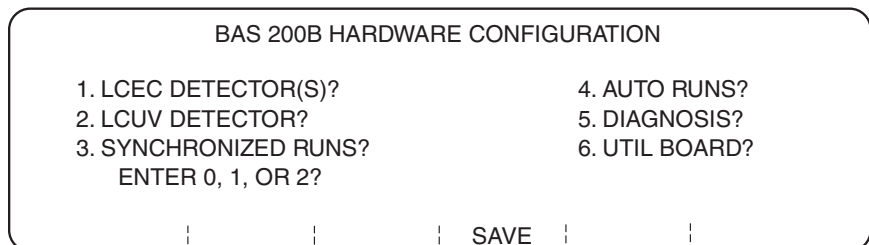


Figure 4.4 Configure the system from this screen.



For each item in the configuration screen, enter a number, then press the ENTER key. For all options except LCEC detector, enter a "1" if you want the option, a "0" if you do not. The following options are available:

LCEC DETECTOR(S)	Select 0, 1, or 2 detectors.
LCUV DETECTOR	This option is no longer available. Select 0.
SYNCHRONIZED RUNS	Select whether pump synchronization is to be used. Synchronization coordinates the start of each run with the pump cycle, and should be used with all gradient applications.
AUTO RUNS	Select whether a sequence of runs is to proceed automatically without an external trigger. Used only with certain types of autosampler (Section 7.2).
DIAGNOSIS	Select whether certain diagnostic numbers should appear in the status screens. Used only by BAS technicians.
UTIL BOARD	This item must be selected for temperature control and external communications to work.

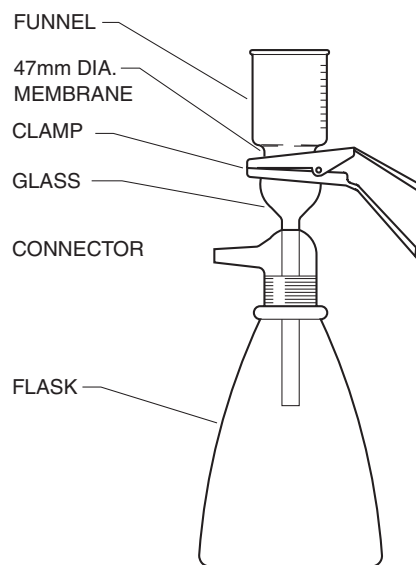
Press the SAVE function to put these options into effect and return to the edit file screen.

4.2 Wetting (Cleaning) the Column

Reverse-phase columns are very hydrophobic, and should be thoroughly wetted before their first use. Columns that have been stored for a long time may have dried out, and also should be wetted before use. The wetting procedure also will clean a dirty column.

Begin by putting appropriate solvents in the mobile phase bottles. All solvents and mobile phases should be made from the highest quality (LC-grade) ingredients available, and should be vacuum-filtered through a 0.2- μm membrane (Figure 4.5).

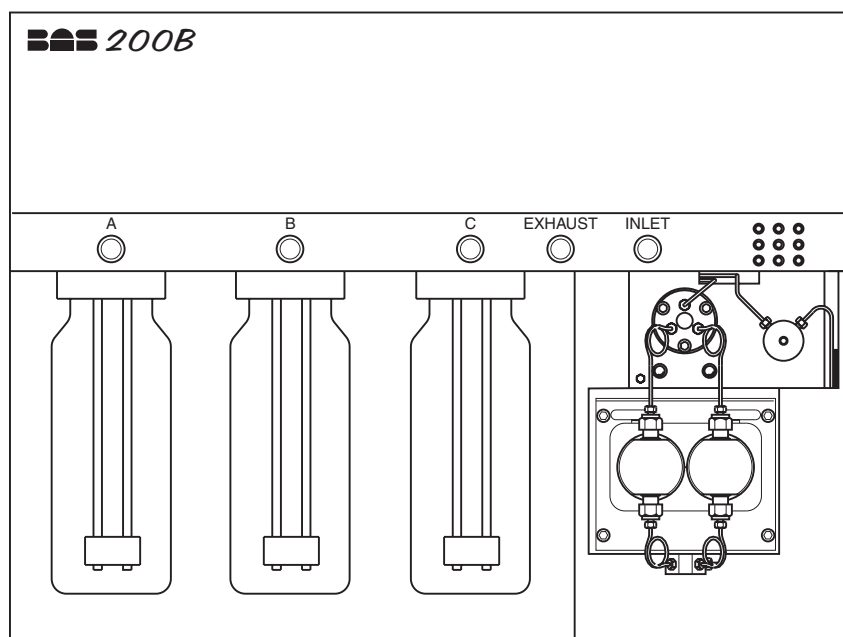
Figure 4.5 Mobile-phase filtration unit, P/N MF-6126.



Wash all the bottles and rinse them with LC-grade water. Assuming that this is an isocratic separation, put your mobile phase in bottle A, 40:60 (v:v) acetonitrile:water in bottle B, and neat acetonitrile in bottle C.

Install the bottles to hand tightness (it's better to turn the cap than the bottle, so the solvent-uptake line doesn't get twisted). With the gas inlet valve (Figure 4.6) closed (clockwise) and the exhaust valve open (counterclockwise), slowly open the regulator knob (CLOCKWISE!) until the gauge reads 4 psi. Now open the gas inlet valve so gas bubbles through all three bottles. If necessary, adjust the bottle inlet valves A, B, C (Figure 4.6) to balance the bubbling in the three bottles. Allow the gas to bubble for 10 minutes, then close the exhaust valve. All bubbling should stop within a few minutes. If it doesn't, check that the solvent recycle line is firmly plugged (Figure 3.7), and that the bottles are tight.

Figure 4.6 Front panel of BAS 200B showing gas control.



If this will be an isocratic separation, be sure that the pulse damper is in place. If it is to be a gradient separation, install the bypass tubing in place of the pulse damper (Section 6.5).

Wetting/Cleaning the Column

1. Install the column (Section 3.3). Route the outlet from the column to a waste bottle for later disposal as per local regulations. If this is a new column, you may skip to step 5.
2. Purge the system with 40:60 acetonitrile. Follow the purge instructions on page 25.
3. Assuming that bottle B contains 40:60 acetonitrile:water, set the pump gradient screen as in Figure 4.7. Then press EXEC. This sends the instructions for line 0.0 to the pump, which begins pumping at the stated flow rate. Press the STATUS key until the pump status screen is reached (Figure 4.8). Pump pressure should be between 2500 and 3500 psi. You can adjust this by changing the flow rate (return to the pump gradient screen by pressing the STATUS key twice; then make the change and press EXEC). Pump 30 mL through the column.

Figure 4.7 Pump gradient screen.

EDIT FILE = 1	NORMAL = 1	uBORE = 2	STBY = 3? 1
PRESSURE LIMITS:	MAX 4000	MIN 200	PSI
TIME	FLOW	%A	%B
0.0	1.0	0.0	100.0
			%C
			0.0
EXEC PURGE STOP SAVE ESCAPE			

Figure 4.8 Pump status screen.

CURRENT FILE = 1				TIME = --.--
PUMP CONDITIONS				
FLOW	%A	%B	%C	PRESSURE
1.0	0.0	100.0	0.0	2875
FOR MORE CONDITIONS OR TO EXIT PUSH 'STATUS' KEY				

4. Stop the pump by pressing the STOP function. Press the STATUS key until you reach the pump status screen, then wait until the pressure drops to 30 psi or below. Press the STATUS key twice to reach the pump gradient screen.
5. Purge the system with neat acetonitrile. Follow the purge instructions on page 25.
6. Modify the pump gradient screen to pump from the bottle containing acetonitrile, then press EXEC. Press the STATUS key until the pump status screen is reached, then observe the pressure. Since acetonitrile is less viscous than 40:60 acetonitrile:water, the pressure will begin to drop. You can increase flow rate to maintain a pressure near 3500 psi. Pump 300 mL through the column.

7. Stop the pump and observe the pressure until it reaches 30 psi or below.
8. Purge the system with 40:60 acetonitrile:water. Follow the purge instructions below.
9. Pump 30 mL 40:60 acetonitrile:water through the column to remove all the neat acetonitrile. The column is now clean and wetted, and ready to receive mobile phase (or for storage).

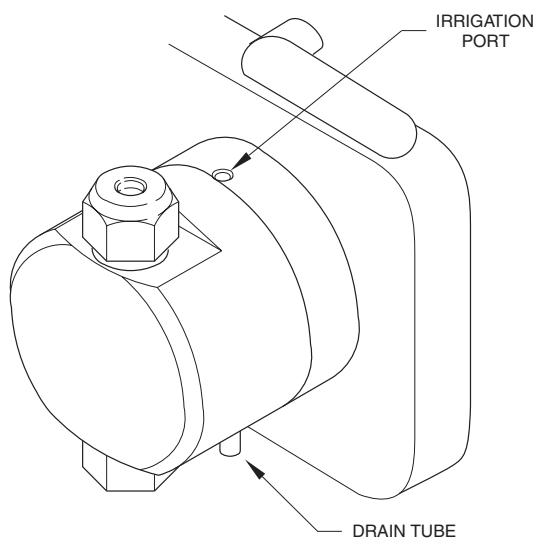
Purging the Lines

A purge brings fresh solvent from the bottle of your choice through the system up to the prime/purge valve. It washes out old mobile phase and helps clear bubbles from the system. Purge for 5 minutes if the pulse damper is installed, or for 2 minutes if the bypass is installed. Proceed as follows:

1. Squirt a few drops of water into the pump heads to wet the pistons and seals (Figure 4.9).

NEVER START A DRY PUMP — IT CAUSES SEAL WEAR!

Figure 4.9 Irrigating the pump heads.



2. Place a 50-mL syringe on the prime/purge valve and open the valve one turn counter-clockwise. If the column is not installed, turn the injection valve halfway between its inject and load positions, to prevent flow from coming this way.

- From the edit file screen (Figure 4.3), press the PUMP function key, then the PURGE function key. You are now in the purge screen (Figure 4.10).

Figure 4.10 Conditions for a purge.

PURGE FILE = 1			
PURGE CONDITIONS			
%A	%B	%C	MAX PRES
100.0	0.0	0.0	3000
DURATION OF PURGE = 5.0 MINUTES			
PURGE			ESCAPE

- Enter the percentage flow for each bottle, a maximum pressure of 3000 psi, and a purge duration of 2 or 5 minutes. Press PURGE to begin.
- The pump will begin slowly and then speed up, trying to achieve 3000 psi. Since the prime/purge valve is open, the system will never reach this pressure; pump speed will increase to the maximum 5 mL/min.
- When the specified purge duration is reached, the pump will stop and the pump gradient screen will appear (Figure 4.7).
- Close the prime/purge valve. If you have the injection valve in a midway position, turn it to the inject position.

4.3 Starting the Mobile Phase

- Purge the system with mobile phase following the instructions in Section 4.2. If this is to be a gradient separation, you must purge each bottle that contains mobile phase: enter equal percentages for each bottle to be purged (Figure 4.11).

Figure 4.11 Purge screen for a binary gradient that will use bottles B and C.

PURGE FILE = 1			
PURGE CONDITIONS			
%A	%B	%C	MAX PRES
0.0	50.0	50.0	3000
DURATION OF PURGE = 2.0 MINUTES			
PURGE		SAVE	ESCAPE

- Modify the pump gradient screen for conditions appropriate for this separation (Figure 4.12):

Figure 4.12 Pump gradient screen for an isocratic run.

EDIT FILE = 1	NORMAL = 1	uBORE = 2	STBY = 3?	1
PRESSURE LIMITS: MAX 4000		MIN 200 PSI		
TIME	FLOW	%A	%B	%C
0.0	1.0	100.0	0.0	0.0
EXEC PURGE STOP SAVE ESCAPE				

In the upper right corner, enter a "1" for flow rates between 0.1 and 5.0 mL/min, or a "2" for flow rates between 0.01 and 0.50 mL/min.

On the next line, enter the upper and lower pressure cutoffs; we suggest 200 and 4000 psi for most applications. If the pressure moves outside of these boundaries during operation, the pump will stop.

Next enter the flow rate and bottle percentages for line 0.0. These are the initial conditions that will be sent to the pump when the EXEC button is pressed. Press ENTER after each value is keyed in, or press ENTER to accept the existing value. Bottle C will be calculated automatically. Note that you must finish a line before you can perform any other functions.

A single 0.0 line is sufficient for an isocratic run, but any run in which timing is required (gradients, data collection, coordination with an autosampler) must have a final time line as well. Saving a method

3. Press EXEC to start the pump under the conditions specified in line 0.0 of the pump gradient screen. Take the SAVE option to permanently save the information to a method file. The first SAVE brings up the edit file screen (Figure 4.13), which allows you to save the information permanently to one of seven method files.

Figure 4.13 The edit file screen appears during the SAVE operation.

CURRENT FILE = 1	EDIT FILE = 1
EDIT FILE TO BE SAVED AS 1 UPON SAVE	
PUMP DET EVENTS TEMP CONFIG SAVE	

4.4 Operating the EC Detectors

CAUTION: ALWAYS CHECK DETECTOR STATUS (Figure 4.14) TO MAKE SURE THE DETECTORS ARE OFF BEFORE TOUCHING THE CELL LEADS. STATIC ELECTRICITY IN YOUR FINGERS CAN DAMAGE THE AMPLIFIERS.

Figure 4.14 The EC detector status screen. Note that the detectors are off.

CURRENT FILE = 1		LCEC			TIME = 0.0
DETECTOR CONDITIONS					
LCEC	POT	GAIN	OFFSET	OUTPUT	
1 OFF	±700	±500 nA	0.000 nA	0.000 uA	
2 OFF	±700	±500 nA	0.000 nA	0.000 uA	
FOR MORE CONDITIONS OR TO EXIT					
PUSH 'STATUS' KEY					REZERO

Once the column has been equilibrated with the mobile phase, connect the flow through the EC detector according to the instructions in Section 3.5. Connect the cell leads to the electrodes as follows:

W1 (working electrode 1):	black wire, male connector
W2 (working electrode 2):	white wire, male connector
Reference electrode:	white wire, female connector
Auxiliary electrode:	red wire, female connector

The EC detectors can be turned on once flow is established and the cell leads are connected. If you are already at a screen that has a detector option, press DET. From any other control screen, press ESCAPE or METHOD to reach a screen with DET. You will eventually reach the EC detector screen (Figure 4.15). Enter the number of detectors in the upper right corner, then fill the time lines (for dual detectors there are two lines for each time). Note that W2 cannot be used by itself — if you are using a single EC detector it must be W1.

Figure 4.15 The EC detector screen.

LCEC FILE = 1		0, 1 OR 2 DETECTORS? 1				
TIME	LCEC	POT	GAIN	FLT	SIGN	RCDR
0.0	1	+ 800	+ 500 nA	0.1	+	10%
EXEC ON OFF SAVE REZERO ESCAPE						

As with the pump gradient file, isocratic runs can have a single (0.0) line, but any runs requiring timing must have at least a 0.0 and a final time line. Additional time lines can be added to change parameters during the run. There will be an automatic rezero at each time line, so lines can be added to force a rezero at any particular time. For each line, enter:

POTENTIAL	Key in a number between 0 and 2000 mV, and press the +/- button to change the sign if necessary.
GAIN	Scroll through the available choices (0.1 nAfs – 100 μ Afs, + or –) using the right and left arrow keys. Scroll through the available choices using the right and left arrow keys. 0.05 Hz gives the most filtering, and 0.2 Hz the least. 0.1 Hz is a typical value.
SIGN	Use the +/- key to determine the polarity of the analog output (pen direction).
RCDR	Enter a positive pen displacement as a percentage of full scale (0–100%). This is used mostly with integrators, to give a slight positive value to the baseline. It takes effect when a rezero command is issued.

For each column, use the ENTER key to accept either a newly selected value or the existing value. You must complete a line before proceeding to other commands.

Press the ON key to turn on the detector when it has not been recently used. ON sets the gain at the relatively insensitive range of 1 μ Afs, which is sufficient to handle the charging current produced by the electrode. You may press the STATUS key to see the EC status screen (Figure 4.14) to observe the initial current surge and its decay.

When the detector output has stabilized, which may take several hours, press EXEC to put the 0.0 time line conditions into effect. You also should press REZERO, to bring the output to zero.

To permanently save the detector settings to the method, press SAVE at the EC detector screen. This brings up the edit file screen (Figure 4.13), which allows you to save the information to one of seven methods.

4.5 Temperature Control

Three types of temperature control are mediated by the temperature control screens: mobile-phase bottles, oven, and optional cell heater (Figure 4.16). To reach these screens, take the TEMP option from any screen on which it appears. From any control screen, press ESCAPE or METHOD to reach a screen with TEMP.

Figure 4.16 Oven and cell temperature control.

```

TEMPERATURE FILE = 1

OVEN:      TEMP SETPOINT = 20.0°C
CELL:      TEMP SETPOINT = 20.0°C

EXEC | FAN | OFF | SAVE |           | ESCAPE

```

Set the oven temperature to the nearest tenth of a degree by keying in the desired temperature (20–70 °C). Temperatures of 30–35 °C are routinely used to remove the influence of fluctuations in room temperature. Key in the desired temperature and press ENTER, or press ENTER to accept the previous value. For no temperature control, use 20 °C.

The cursor will next move down to the cell line. If the instrument has a cell temperature option, and you wish to use it, enter the temperature here and press ENTER. Otherwise, accept the 20 °C value and press ENTER.

The System Director now shifts to the bottle temperature screen (Figure 4.17). Bottle temperatures are used to aid degassing, since warm fluids hold less dissolved gas than do cool fluids. It is common to warm the mobile phase to 35 °C. Enter a “2” for each bottle to be warmed to 35 °C, then press the ENTER key to reach the bottom of the screen.

Figure 4.17 Bottle temperature control.

```

TEMPERATURE FILE = 1
MOBILE PHASE:  OFF    35°C   50°C   SELECT
  BOTTLE A      1      2      3      3
  BOTTLE B      1      2      3      2
  BOTTLE C      1      2      3      2

SELECT 1 FOR OFF, 2 FOR 35°C, 3 FOR 50°C
EXEC | FAN | OFF | SAVE |           | ESCAPE

```

To turn on the temperatures, press EXEC. To toggle the oven fan between on and off, press FAN. To save the temperature instructions press SAVE, which returns you to the edit file screen (Figure 4.13). From here press SAVE to permanently save the instructions to one of seven methods.

4.6 Manual Operation

There are many instances where fully automatic chromatography is not needed. When developing a separation, for example, you may wish to set the chromatograph for an 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 control screens provide a convenient way to control the BAS 200B. In general, the EXEC function turns components on with the conditions found in the screen (if there are several time lines, the conditions in the 0.0 time line are executed). The STOP function key turns components off.

To inject a sample in manual mode, first turn on and equilibrate all components. Start the mobile phase as above. Turn on the appropriate detector(s) and let them equilibrate. EXECute the temperature controls. Turn on the chart recorder and monitor the baseline. When the unit is equilibrated, EXECute the EC detector to establish the time 0.0 conditions. Rezero the detectors from the appropriate status screens.

Now do an injection. Turn the injection valve rapidly to the load position. (Always turn the injector valve rapidly with a snap of the wrist. You will not hurt the injector, and the snap will disturb the baseline less than will a slow turn.)

Fill the injector with your sample, according to one of the methods in Section 7. Without removing the filling syringe, snap the injector to the inject position. Mark the chart and wait for the peak to elute. If peak height is unsatisfactory, return to the detector control screen and change the gain to an appropriate value.

4.7 Programmed Operation

Programmed operation is needed to coordinate runs with peripheral equipment such as an autosampler or data analyzer, and when timed events such as gradients or gain and wavelength changes are needed.

Before beginning a programmed run, make sure that all the modules that require time lines are complete. These are the pump gradient screen (Figure 4.18), detector screen (Figure 4.19), and if needed, the events screen (Section 7.2). The run will end when the longest time line of any of these screens is reached.

Figure 4.18 Pump gradient screen for a gradient run.

EDIT FILE = 1	NORMAL = 1	uBORE = 2	STBY = 3? 1
PRESSURE LIMITS:	MAX 4000	MIN 200 PSI	
TIME	FLOW	%A	%B %C
0.0	1.0	100.0	0.0 0.0
5.0		50.0	50.0 0.0
7.0		50.0	50.0 0.0
7.1		100.0	0.0 0.0
EXEC	PURGE	STOP	SAVE ESCAPE

Figure 4.19 EC detector screen for the gradient run.

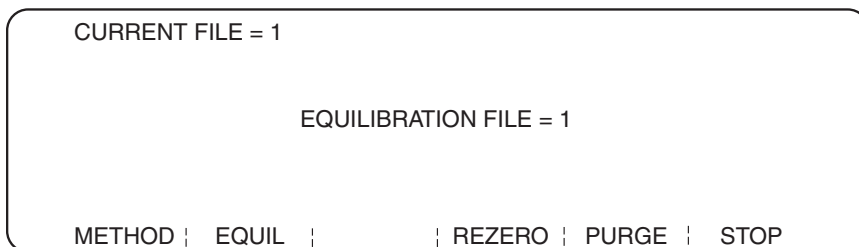
LCEC FILE = 1	0, 1 OR 2 DETECTORS? 1					
TIME	LCEC	POT	GAIN	FLT	SIGN	RCDR
0.0	1	+ 800	+ 20 nA	0.1	+	0%
7.1	1	+ 800	+ 20 nA	0.1	+	0%
EXEC	ON	OFF	SAVE	REZERO	ESCAPE	

Next make sure that all relevant screens have been EXECuted. These can be done individually, or all at once through the following procedure: from any control screen, press ESCAPE or METHOD to reach the edit file screen (Figure 4.20). Take the SAVE option, which brings up the equilibration screen (Figure 4.21). Now press the EQUIL function, which sends instructions for the entire method to the BAS 200B.

Figure 4.20 The edit file screen.

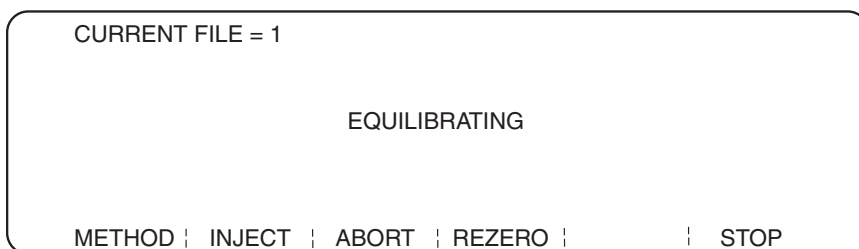
CURRENT FILE = 1	EDIT FILE = 1				
EDIT FILE TO BE SAVED AS 1 UPON SAVE					
PUMP	DET	EVENTS	TEMP	CONFIG	SAVE

Figure 4.21 The equilibration screen. Press EQUIL to send all the instructions to the BAS 200B.



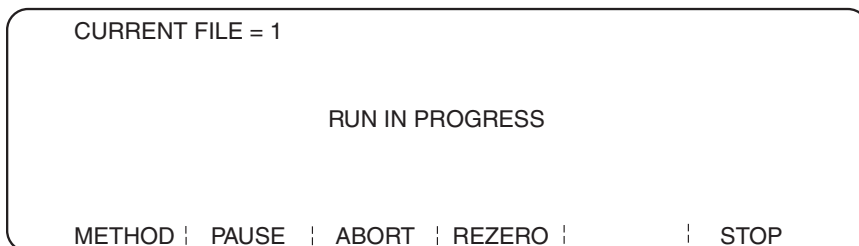
When the EQUIL command is sent, the System Director changes to the equilibrating screen (Figure 4.22). This screen must be visible for communication with intelligent autosamplers (like the BAS Sample Sentinel) to occur (Section 7.2). You may use the STATUS key to get status information, but must return to the equilibrating screen before the autosampler sends its inject signal.

Figure 4.22 The BAS 200B now has the instructions and is equilibrating. The run can be started manually by pressing the INJECT function key.



Once an inject signal is received from the autosampler or the keyboard, the run-in-progress screen (Figure 4.23) will appear. You can use the STATUS key from this screen also, but again, you must return to the run-in-progress screen before the next injection. When the run ends (when the last time line is reached) the screen reverts to the equilibrating screen.

Figure 4.23 The run-in-progress screen appears when the run starts.



Manual injections also can be performed in conjunction with programmed operation. If, for example, you have no autosampler but are using gradient elution, you will need to inject the sample precisely at the start of the gradient. To do this, first put the BAS 200 into the equilibrating mode (Figure 4.22). Then turn the injection valve to the load position, fill it with sample, and leave the syringe in place. Next press the INJECT function key and watch the screen

while your hand is on the injection lever. When the System Director changes to the run-in-progress screen (Figure 4.23), snap the lever to the inject position. If these are gradient runs, you should have pump synchronization turned on, and will get a synchronizing message before the run starts.

4.8 Separation of Acetaminophen

The following separation of acetaminophen may be performed as a tutorial or as a system check. This is a simple isocratic separation, and acetaminophen can be detected by both EC and UV detectors.

MOBILE PHASE:	75 mM monochloroacetic acid, 0.7 mM EDTA, pH 3.0. To one liter of this buffer add 25.6 mL acetonitrile.
COLUMN:	C ₁₈ reversed phase, 100 x 3.2 mm, 3 μm particles (P/N MF-6213).
FLOW RATE:	1 mL/min
EC DETECTOR:	WORKING ELECTRODE: Cross-flow glassy carbon (P/N MF-1000) POTENTIAL: +0.8 V vs. Ag/AgCl GAIN: 200 nAfs FILTER: 0.1 Hz
UV DETECTOR: (External)	WAVELENGTH: 254 nm GAIN: 0.01 AUFS FILTER: 0.1 Hz
AMOUNT INJECTED:	10 ng

Preparation of Mobile Phase

To 800 mL LC-grade water add 250 mg EDTA (ethylenediaminetetraacetic acid-disodium salt) and 5 NaOH pellets. Stir until dissolved. Add 7.1 g monochloroacetic acid and stir until dissolved. Adjust pH to 3.0 with sodium hydroxide solution. Bring to 1 L with LC-grade water, then add 25.6 mL acetonitrile. Filter through a 0.2-μm regenerated cellulose membrane.

Preparation of Acetaminophen Solution

STOCK:	Dissolve 12.5 mg acetaminophen in 12.5 mL methanol in a 25-mL volumetric flask. Bring to 25 mL with LC-grade water.
--------	---

STANDARD:

Dilute the stock solution 1:100 with LC-grade water (10 μL stock to 990 μL water). Dilute this intermediate solution 1:10 with water (100 μL intermediate solution to 900 μL water). The resulting standard contains 10 ng acetaminophen per 20 μL injection.

Figure 4.24 shows a typical separation of acetaminophen using the EC detector. Figure 4.25 shows the same separation as recorded by a UV/Vis detector.

Figure 4.24 Separation of acetaminophen with EC detector.

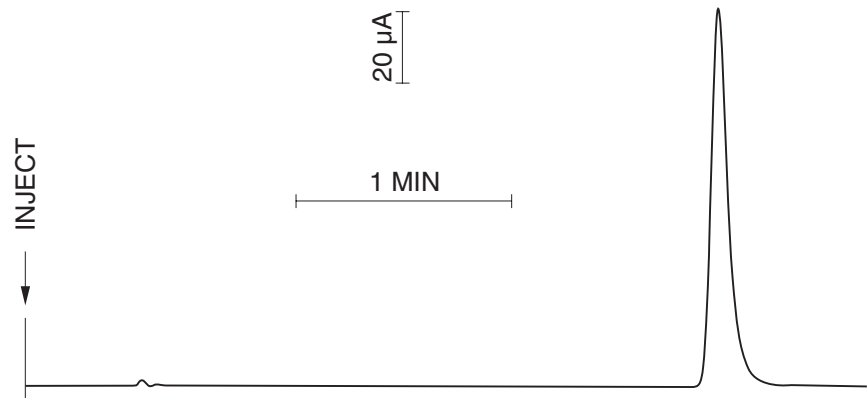
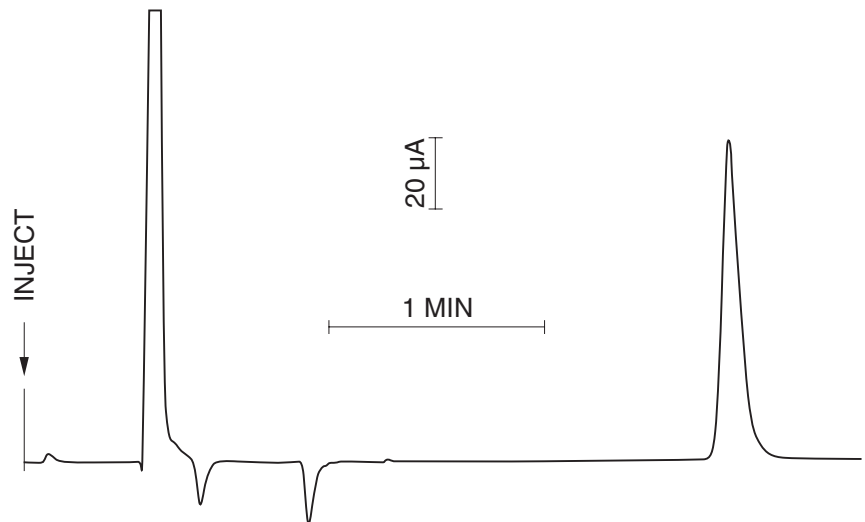


Figure 4.25 Separation of acetaminophen with UV detector.



4.9 Shutdown

The BAS 200B is designed to run continuously. When you consider the equilibration time of the various components, it is more efficient to keep the system running at normal flow rates even if you are using it sporadically every few days. The only components that should be turned off during these inactive periods are the lamps of any external UV/Vis detectors.

For longer periods of inactivity, the BAS 200B should be shut down completely. The most important principle to remember when shutting down the system 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 off the detectors and temperature control.
2. Stop the pump and observe system pressure until it drops below 30 psi.
3. Open the exhaust gas valve (Figure 4.6), then shut the main valve on the gas cylinder.
4. Remove each bottle that had mobile phase in it, and rinse off the utility tubes with a squeeze bottle of water. Wash the bottles, put about 250 mL 40:60 acetonitrile in them, and reinstall. It will not be necessary to pressurize the system with gas.
5. Follow the purge procedure (Section 4.2) to bring 40:60 acetonitrile:water through the system to the prime/purge valve. Purge whichever bottles held mobile phase.
6. Using the usual flow rate for the column, pump 100 mL 40:60 acetonitrile:water through the entire system, including the injection valve (in the inject position), column, and detectors.
7. Towards the end of cleaning, flush the irrigation ports on the pump heads with a few drops of water (Figure 4.9).
8. Turn off the power. Disassemble the EC electrode and rinse and dry its parts. Working electrodes should be stored clean and dry. Gel-filled reference electrodes should be stored with their tips immersed in 3 M NaCl.
9. Remove the column and cap it for storage.
10. Wash the bottles, rinse them with methanol, and allow them to dry before installing them loosely on the system.

Section 5. BAS SYSTEM DIRECTOR

5.1 Hardware and Software Overview

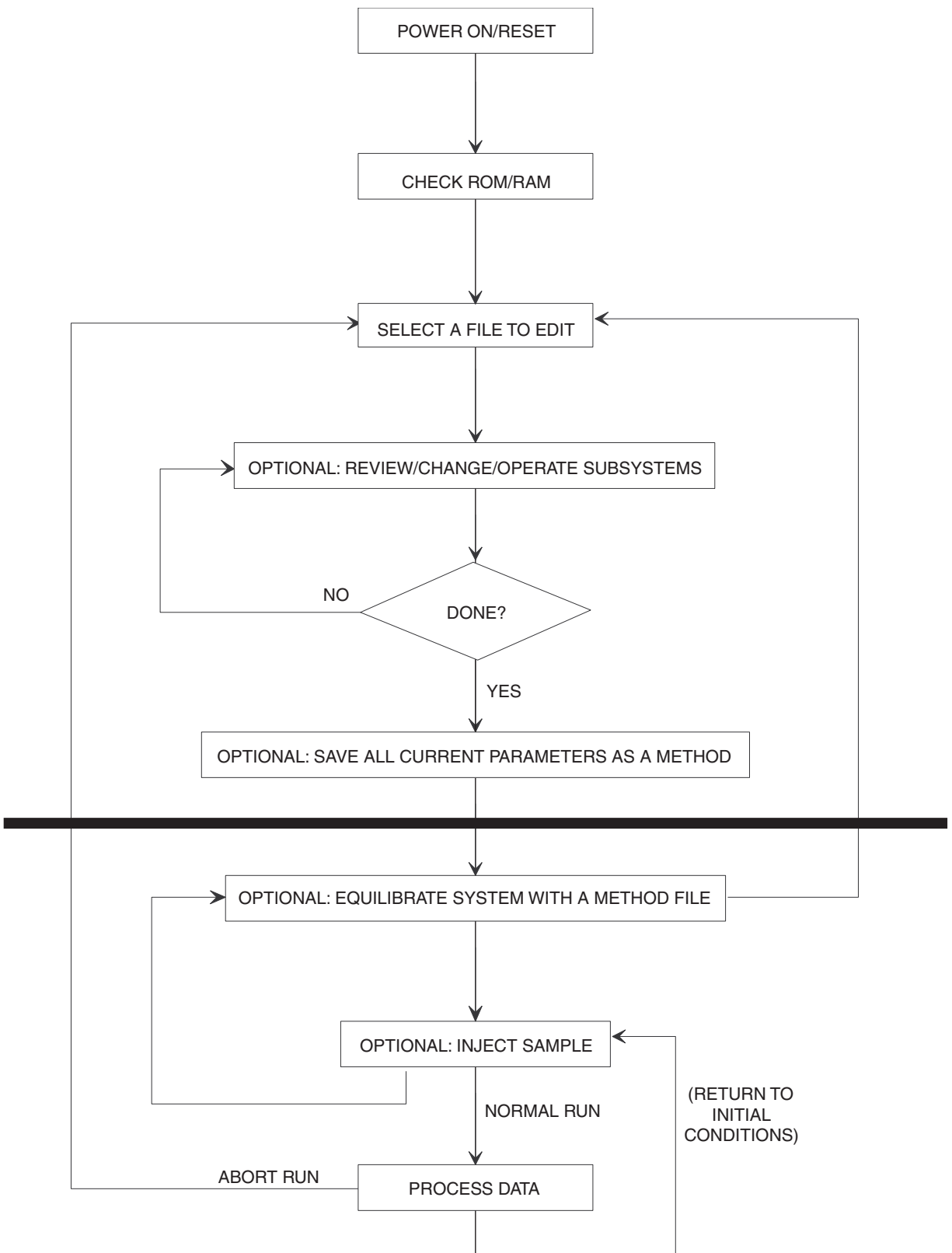
The BAS System Director provides control of the various BAS 200B subsystems. It is strictly an input device; no data processing capabilities are provided. Visual feedback is provided by a 40-character by 8-line liquid crystal display. All parameters are entered using a membrane keypad which includes 6 soft keys, 12 numeric keys, and 10 fixed command keys (Figure 5.1).

Figure 5.1 BAS 200B System Director.



The organization of the keypad software is diagrammed in Figure 5.2. Upon power-up or reset, ROM and RAM are checked. An edit file is selected; this file may be modified or used directly to run samples. The files (seven are available in battery memory) consist of pump control, temperature control, timed events, and detector operating conditions. Each of these "subfiles" also contains immediate execution capability for direct control of the particular module.

Figure 5.2 Flowchart showing major branches of BAS System Director software.



Immediate Execution Mode

In many cases, you may wish to operate the BAS 200B as an independent set of modules, all under time-invariant conditions. Here the logic *above* the thick line of Figure 5.2 will be utilized. Each module is individually accessed, operated, and deactivated. The real-time operating conditions and outputs may be inspected via the STATUS utility.

To review or modify pump parameters, use screen 11. (See Section 5.3 for individual screen descriptions.) Also included on screen 11 are the essential immediate execution commands: EXEC, STOP, PURGE, SAVE, and ESCAPE. Use the STATUS utility (screen S3) to review the system's current back pressure and programmed solvent mixture. Make changes to parameters by returning to screen 11. When finished making adjustments, return to screen 3 by pressing SAVE; the pump subfile may be saved permanently as part of a larger method file.

Similar routines exist for the detector, timed events, and temperature utility files, using the other soft keys on screen 3.

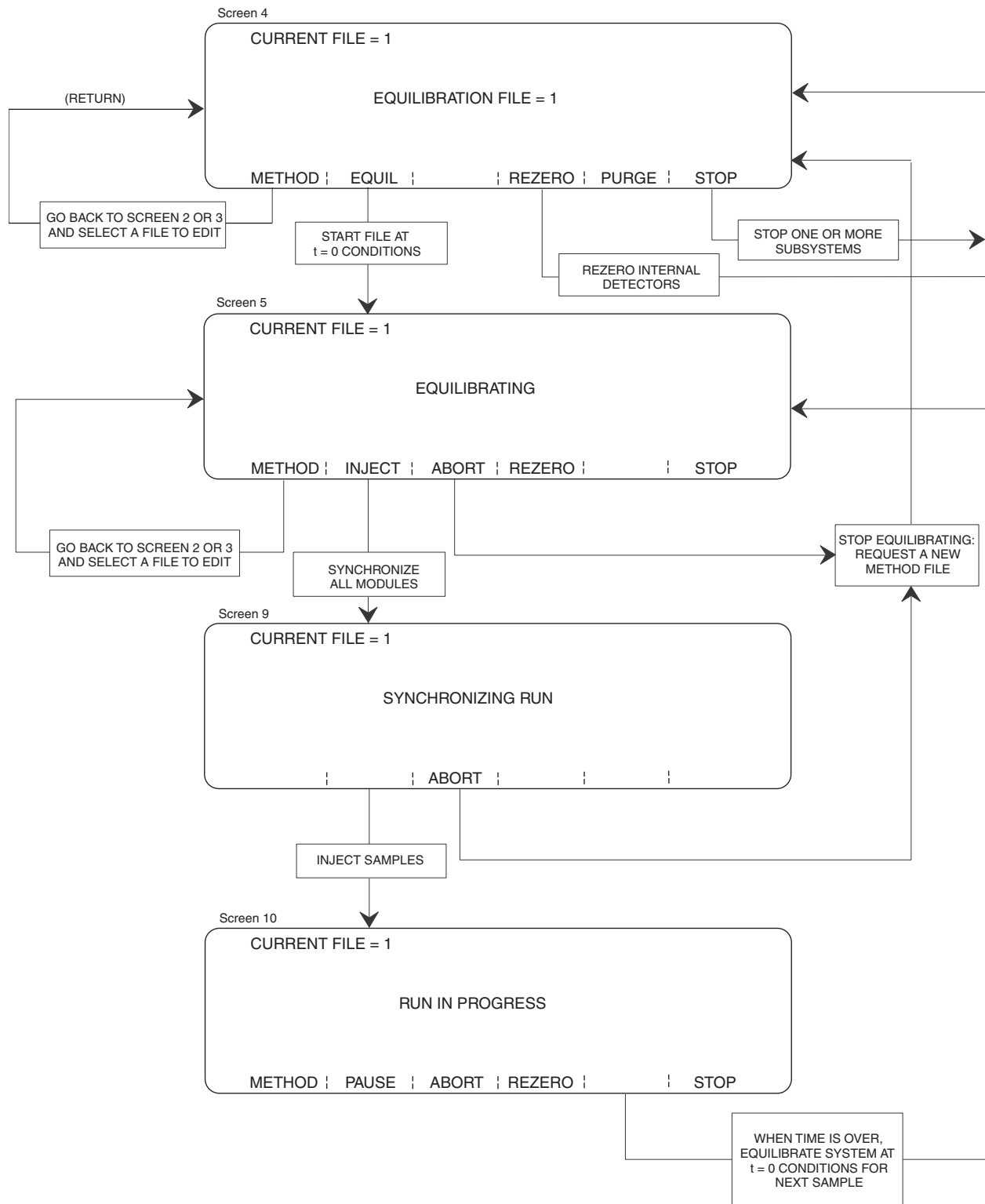
Method Files

Below the thick line of Figure 5.2, you may operate the BAS 200B in a programmed run mode. This mode of operation is required when performing any time-dependent changes (gradient elution, programmable detector gain changes, rezero, etc.). It is also required for cycling through a method several times in a row, as would be done when using autosamplers. This software is represented by the screens of Figure 5.3.

Select a method file in the first screen. Press the EQUIL soft key to begin operating the file under $t = 0$ conditions. Once the detector baselines, system temperatures, and column pressure have stabilized, the detector background current may be nulled out by pressing REZERO as often as desired.

When all subsystems are equilibrated and stabilized, press INJECT. At this time, the BAS 200B works through a synchronization routine before actually triggering any autosamplers, etc. This routine assures run-to-run retention time reproducibility and is explained further below.

Figure 5.3 Running the BAS 200B under a method file.



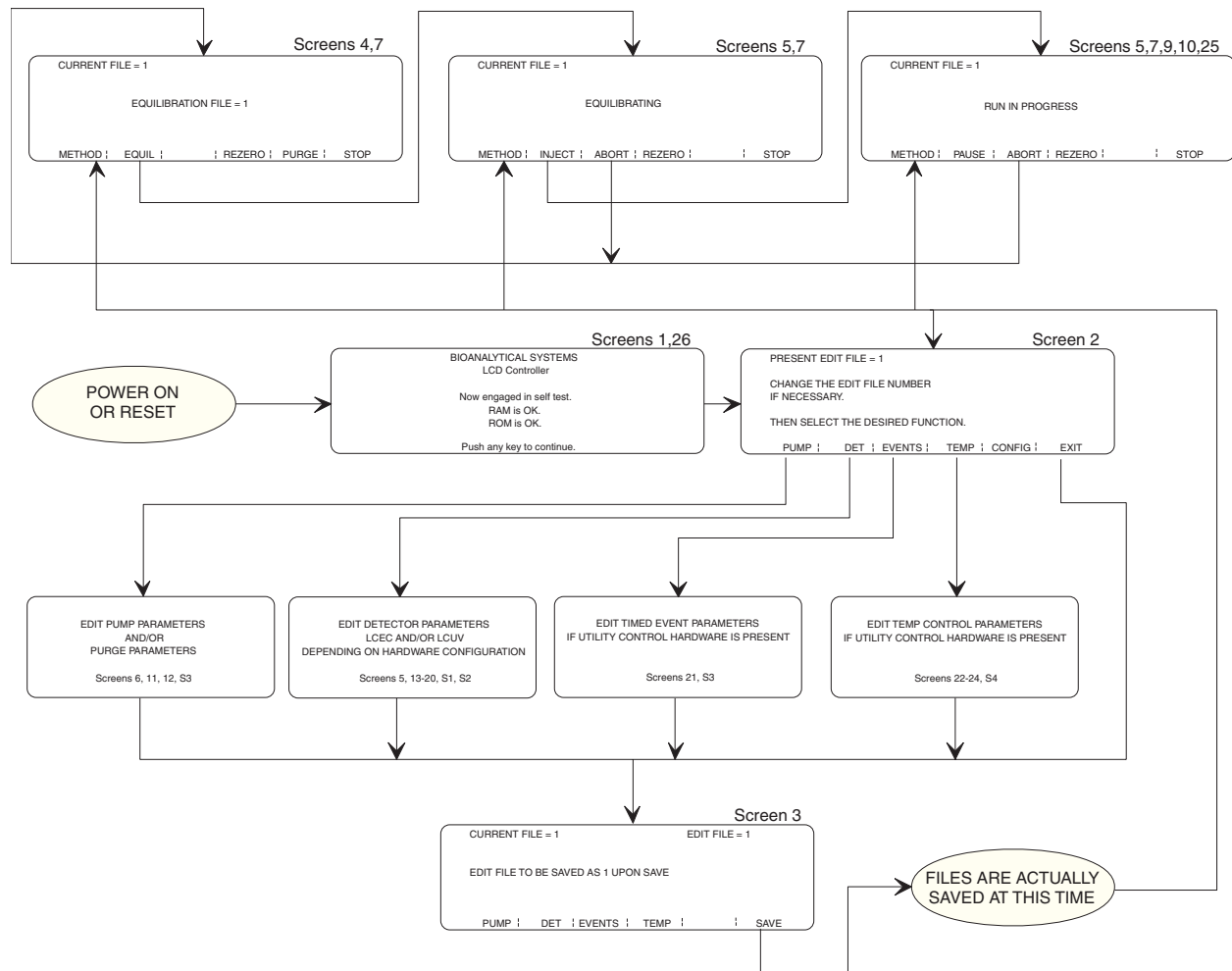
5.2 Explanation of Special Keys

+/-	Changes sign of parameter.
CLEAR	Changes value of parameter to zero.
DELETE	Removes entire timeline in pump or detector file. Cursor must be at start of timeline to be effective.
STATUS	Accesses current run parameters and outputs for all modules, in a closed loop.
ENTER	Accepts an existing parameter, or marks the end of a new numerical entry.
↑ ↓	Scrolls through timelines of a pump or detector file when cursor is at the start of a timeline.
← →	Scrolls through the choices for detector gain or filtering. Active at limited points in the software.
. →	Must be done in immediate sequence on screen 2 only. Asks questions concerning hardware configuration of BAS 200B (screen 26).
. ←	Must be done in immediate sequence on screen 2. Provides identity of software modules resident in BAS 200B.
. CLEAR	Must be done in immediate sequence on screen 2. Deletes all files in memory and replaces them with default conditions. Overrides battery backup feature of file preservation. Returns to System Configuration file (screen 26) after a slight pause.

5.3 Detailed Explanations of Screens

The screens shown in Figure 5.4 are individually discussed in this section. The functions of the six unlabeled soft keys in the top row will vary, depending on which screen is displayed. The following descriptions provide detailed explanations of every available function on every screen (screens 1–27, S1–S4).

Figure 5.4 Complete flowchart of BAS System Director operation.



Screen 1

```

BIOANALYTICAL SYSTEMS
LCD Controller

Now engaged in self test.
RAM is OK.
ROM is OK.

Push any key to continue.

```

This is the first screen displayed when the BAS 200B is turned on or when the reset button on the left side of the cabinet is pushed.

If an error is detected, it will be displayed with instructions. Information about error screens is found in Section 5.4.

When you press any key, screen 27 will appear, showing software versions.

Screen 2

```

PRESENT EDIT FILE = 1

CHANGE THE EDIT FILE NUMBER
IF NECESSARY.

THEN SELECT THE DESIRED FUNCTION.

PUMP | DET | EVENTS | TEMP | CONFIG | EXIT

```

EDIT FILE = # This screen allows you to select an edit file and begin editing the operating parameters. Select one of seven method files by pressing the appropriate number (default = 1).

PUMP Screen 11 will appear, allowing you to edit the operating conditions for the BAS 200B solvent delivery system.

DET Allows you to edit the various detector parameters. If both EC and UV detectors are installed, screen 13 gives you a choice between editing EC and UV detector conditions. If only an EC detector is installed, screen 14 will appear immediately. If only a UV detector is installed, screen 16 will appear immediately.

EVENTS Screen 21 appears, allowing you to edit the timed events file for the method.

TEMP Screen 22 appears, allowing you to edit operating temperatures of the oven, EC cell (if installed), and mobile phase reservoirs.

CONFIG Screen 26 appears, where the system hardware configuration options are entered.

EXIT If no editing is to be done for the method, press EXIT. Screen 3 will appear so the method can be saved or used.

STATUS Pressing the STATUS key calls the first of four screens that display the current operating conditions of the BAS 200B. There is no effect on the file being edited or on the operating conditions. STATUS may be called at any time. After you have viewed the status screens (S1, S2, S3, S4), the system returns to the screen that had been displayed before the interruption.

(Screen 2 continued next page.)

(Screen 2, continued)

The following operations are reserved for special functions, which are only available from screen 2. Push the two keys separately, in succession.

- . → Pressing these keys will advance control to screen 26, where the system hardware control options are configured.
- . ← Pressing these keys will cause screen 27 to appear. This screen describes the present software versions (ROM) installed in the system.
- . CLR Pressing these keys in succession will clear all parameters in all seven files to the default values. Control will pass to screen 26 for reconfiguration of the system.

Screen 3

CURRENT FILE = 1 EDIT FILE = 1

EDIT FILE TO BE SAVED AS 1 UPON SAVE

PUMP | DET | EVENTS | TEMP | CONFIG | SAVE

CURRENT FILE = # Displays which of the seven method files is currently defining the operating conditions for the BAS 200B.

EDIT FILE = # Displays which file is currently being edited.

EDIT FILE WILL BE SAVED AS # UPON SAVE Allows you to rename and store the current method in a different file, so that the old method is not lost.

PUMP Screen 11 will appear, allowing you to edit the operating conditions for the BAS 200B solvent delivery system in the given method.

DET Allows you to edit detector parameters. If both EC and UV detectors are installed, screen 13 appears, giving you a choice between editing EC and UV detector conditions. If only an EC detector is installed, screen 14 will appear immediately. If only a UV detector is installed, screen 16 will appear immediately.

EVENTS Screen 21 will appear, allowing you to edit the timed events file for the method.

TEMP Screen 22 will appear, allowing you to edit operating temperatures of the oven, EC cell (if installed), and mobile phase reservoirs.

SAVE Stores the method under the chosen file number, with all the updates that had been saved in the pump, detector, events, and temperature screens. If the BAS 200B is not currently operating, the display will advance to screen 4, to allow selection of a method for equilibration. If the BAS 200B is equilibrating a file, the display will return to screen 5. If the BAS 200B is in the middle of a run, control will revert to screen 11.

(Screen 3 continued next page.)

(Screen 3, continued)

STATUS Pressing the STATUS key calls the first of four screens that display the current operating conditions of the BAS 200B. There is no effect on the file being edited or on the operating conditions. STATUS may be called at any time. After you have viewed the status screens (S1, S2, S3, S4), the system returns to the screen that had been displayed before the interruption.

Screen 4

```

CURRENT FILE = 1

EQUILIBRATION FILE = 1

METHOD | EQUIL |           | REZERO | PURGE | STOP

```

CURRENT FILE = # Displays which of the seven method files is currently defining the operating conditions for the BAS 200B.

EQUILIBRATION FILE = # The new method file to be equilibrated is selected here. Select one of seven method files by pressing the desired number (default = 1).

METHOD Returns the display to screen 2 for further editing of method files. The BAS 200B will continue to operate according to the method listed as CURRENT FILE = #.

EQUIL The BAS 200B will begin equilibrating according to the initial conditions of the method listed on the screen as EQUILIBRATION FILE = #. The equilibration file then becomes the current file. Screen 5 will then appear.

PURGE Screen 6 will appear, allowing you to purge the system according to the conditions established in the PUMP portion of the method file.

STOP Screen 7 will appear, allowing you to turn off some or all of the BAS 200B components.

STATUS STATUS has the usual significance (see screen 2).

Screen 5

```

CURRENT FILE = 1

                                EQUILIBRATING

METHOD | INJECT | ABORT | REZERO |           | STOP

```

CURRENT FILE = # Displays which of the seven method files is currently defining the operating conditions for the BAS 200B.

METHOD Returns the display to screen 2 for further editing of method files. The BAS 200B will continue to operate according to the method listed as CURRENT FILE = #.

INJECT If sample is poised in the manual injector or autosampler sampler loop and ready for injection, pressing INJECT begins the injection synchronization process. Control is transferred to screen 9.

If the system is configured for AUTORUNS, "HOW MANY RUNS?" will be asked on this screen. If you wish to cycle the BAS 200B through a designated number of runs (as for autosampling), reply with the number of cycles desired and then press ENTER. The system will go into its synchronization routine (screen 9).

ABORT Screen 4 will appear. The BAS 200B will continue to operate under the conditions of CURRENT FILE. This allows you to change conditions without turning off any components.

STOP Screen 7 will appear, allowing you to turn off some or all of the BAS 200B components.

STATUS STATUS has the usual significance (see screen 2).

Screen 6

```

PURGE FILE = 1

                                PURGE CONDITIONS
                                %A          %B          %C          MAX PRES
                                100.0       0.0         0.0         2000

DURATION OF PURGE = 2.0 MINUTES

PURGE |           |           |           |           | ESCAPE

```

PURGE FILE = # The current conditions for purging the numbered pump file are listed. The conditions may be permanently changed only by leaving this screen and changing the pump portion of the method file.

PURGE Pressing this key will cause the system to attain the maximum flow rate (not exceeding the selected MAX PRES). The duration of the purge will be updated on the display. At the end of purging, control returns to the previous screen (4 or 12).

ESCAPE Control returns to the previous screen (4 or 12). If a purge is in progress it will be stopped.

STATUS STATUS has the usual significance (see screen 2).

Screen 7

```

CURRENT FILE = 1

OFF?
PUMP | DET | TEMP | ALL |      | NONE

```

This screen allows you to turn off components of the BAS 200B. It can be called up from screens 4, 5, and 10 with the STOP key.

PUMP The pump will be turned off and the previous screen (4, 5, or 10) will return. If detectors or the oven are on, they will remain on.

DET If only one EC detector is installed, it will be turned off. The previous screen (4, 5, or 10) will return. Note that there are no changes in the operating conditions of the pump, the oven, or the UV detector (if installed).

If there is a dual EC detector and both working electrodes are on, screen 8 will allow you to select which to turn off.

If a UV-Vis detector is installed, the lamps are not turned off here. They are turned off by accessing the method file, followed by screens 13, 16, and 17.

TEMP The oven, fan, mobile phase heaters, and EC cell heater (if installed) will be turned off and the last screen (4, 5, or 10) will reappear.

ALL The BAS 200B pump, oven, fan, mobile phase heaters, and EC detectors will be turned off and a request for a new equilibration file (screen 4) will be displayed. Note that the UV-Vis lamps are not turned off here. They are turned off (or on) by accessing the method file, followed by screens 13, 16, and 17.

NONE No components are turned off and the previous screen (4, 5, or 10) returns.

STATUS STATUS has the usual significance (see screen 2).

Screen 8

```

TURN OFF WHICH DETECTORS?

W1 | W2 | BOTH |      |      | EXIT

```

This screen can be accessed from screens 4, 5, 10, and 14. After an entry is made, the previous screen is displayed.

W1 W1/W2 causes the corresponding working electrode to be turned off. Both operate independently. Screen 4, 5, 10, or 14 will then reappear.

BOTH Both working electrodes are turned off. Screen 4, 5, 10, or 14 will then reappear.

EXIT Screen 4, 5, 10 or 14 will immediately reappear and there will be no change in operation of the BAS 200B.

STATUS STATUS has the usual significance (see screen 2).

Screen 9

```

CURRENT FILE = 1

                SYNCHRONIZING RUN

|               | ABORT |               |

```

During this time, the BAS 200B is waiting for the pump to reach the correct point in its cycle to begin a gradient or other time-based procedure. At flow rates of 1–2 mL/min, this will be less than 20 seconds. When the correct point in the cycle is reached, the BAS 200B advances to screen 10, and you should immediately turn the handle from “load” to “inject.” This is also the time at which an autosampler will inject.

At least one module must contain a time file of at least two lines for synchronization to be meaningful. If it is not necessary to change either the pump or detector parameters during the run, circumvent this requirement by creating a second timeline which duplicates the $t = 0$ parameters for the pump at a time equal to the run time per sample.

ABORT Pressing this key during the synchronization waiting period stops the synchronization routine and returns control to screen 5.

NOTE: A minimum of 5 piston strokes must be carried out during the EQUILIBRATING panel (screen 5) before an INJECT command is effective. Otherwise, the system may remain stalled on screen 9. If this occurs, press ABORT, then EQUIL, and wait for 5 strokes to pass. (Watch the green pump head LEDs to count strokes.) Now press INJECT again, and when screen 10 appears, inject the sample.

Screen 10

```

CURRENT FILE = 1

                RUN IN PROGRESS

METHOD | PAUSE | ABORT | REZERO |           | STOP

```

CURRENT FILE = # When this screen is displayed, the clock will be running and the BAS 200B will follow the method listed as CURRENT FILE = #. While in this mode, you will usually have one of the STATUS screens displayed.

METHOD By pressing METHOD, you can call up another method for editing. The current file remains in control of the BAS 200B.

PAUSE Stops the clock. The gradient mixture and detector operating parameters will hold at the values they had reached at the time of the pause. Screen 25 will appear. The CONT (continue) command of screen 25 will revert control to this screen, and the clock will resume.

ABORT Screen 4 will appear, allowing selection of a new method for equilibration. The BAS 200B will continue to operate under the instructions listed in CURRENT FILE = #.

REZERO The EC detector baselines will be reset to the percentage of full scale defined in screen 14. If the output has drifted, you will see a change in the output and offset values displayed in the status screen S1. For the UV detector, the absorbance will be nulled to zero; this will be seen in status screen S2.

STOP Screen 7 will appear, allowing you to turn off some or all of the BAS 200B components.

STATUS STATUS has the usual significance (see screen 2).

Screen 11

EDIT FILE = 1	NORMAL = 1	uBORE = 2	STBY = 3? 1
PRESSURE LIMITS: MAX 4000		MIN 200 PSI	
TIME	FLOW	%A	%B
0.0	1.0	100.0	0.0
EXEC PURGE STOP SAVE ESCAPE			

This screen is used to establish operating conditions for the pump. On the first line, you select the range of flow rates.

NORMAL By entering "1" (NORMAL), you will be able to set the BAS 200B flow rate between 0.1 and 5.0 mL/min. The ENTER key must be pressed to advance the cursor to the next line.

uBORE By entering a "2" (followed by ENTER), the BAS 200B flow rate can be set between 0.01 and 0.5 mL/min, for use with microbore columns or other applications for which a low flow rate is needed.

STBY By entering a "3" (followed by ENTER), the pump will be inoperative when the method is used. This has been included so that the BAS 200B detectors and oven can be used with an external pump. After ENTER is pressed, the cursor advances to the second line of the screen.

PRESSURE LIMITS The maximum pressure limit can be set up to 6000 psi. It must be at least 500 psi above the minimum pressure. The default values are 5000 psi (max) and 200 psi (min). The ENTER key must be pressed after entering each limit. After the minimum value is entered, the cursor advances to set the flow rate and define gradient or isocratic conditions. If the operating pressure exceeds the maximum or falls below the minimum, the pump will be stopped. To fill the parameter file for time, flow rate, %A, %B, and %C, use ENTER to advance the cursor one step at a time.

TIME The first time displayed is always "0.0." Times may be entered in any order, and up to ten lines may be entered to define a gradient. Only three timelines of the gradient parameters will be displayed on this screen at a given time. To view other lines, press the ↑ and ↓ keys to scroll through the entire file. When the run time exceeds the longest time specified in the file, the pump reverts to the t = 0 conditions. Thus, if the final composition is to be main-

tained for any length of time, you should enter an extra line in the file. Simply create the new timeline by specifying the new time, then repeat ENTER to copy the previous %A, %B, and %C values.

Even if your system is running under isocratic conditions, it is recommended that you enter a second timeline. The time should be the sample run time. %A, %B, and %C can be copied by repetitive ENTER key strokes.

FLOW	The flow rate is fixed for the entire method.
%A	Select the desired percentage of solvents A and B.
%B	%C will be calculated as 100% - %A - %B. The
%C	cursor advances to the next line and requests a new time value.
DELETE	Deletes a complete line of instructions. The line for TIME = 0.0 cannot be deleted.
CLEAR	Deletes a single entry on a line of instructions without deleting the other values on that line.
EXEC	Begins executing the pump file shown on the screen under the TIME = 0.0 conditions. This allows you to run or alter pump conditions without having to reenter operating conditions for detectors or the oven. This command should not be used while a gradient run is in progress; ill-defined operation may result.
PURGE	Calls up screen 12 to allow editing or operation of the purge file.
STOP	Stops the pump and gradient.
SAVE	Pump parameters and gradient information are stored. Screen 3 is displayed again. You may change other parameters or store the complete method from this screen.
ESCAPE	Display returns to screen 3 <u>without</u> saving any current changes made to the pump file.
STATUS	STATUS has the usual significance (see screen 2).

Screen 12

PURGE FILE = 1			
PURGE CONDITIONS			
%A	%B	%C	MAX PRES
100.0	0.0	0.0	2000
DURATION OF PURGE = 5.0 MINUTES			
PURGE		SAVE	ESCAPE

This screen allows you to establish separate purge conditions for a given method. You can select any combination of the three solvents. Press the ENTER key after each entry.

MAX PRES The MAX PRES (maximum pressure) can be set up to 6000 psi. When the purge file is run, the pump will run as fast as required in order to maintain MAX PRES. This feature can be used for quickly purging the mixer and pump, or a column.

PURGE Screen 6 will appear, allowing you to start the purge.

SAVE The new conditions are saved and screen 11 will appear for further editing of the pump conditions.

ESCAPE Screen 11 appears, and the changes made to the purge conditions in the method are ignored. ESCAPE also terminates the purge cycle if pressed prior to its completion.

STATUS STATUS has the usual significance (see screen 2).

Screen 13

WHICH DETECTOR?			
ENTER THE DESIRED FUNCTION.			
LCEC	LCUV		ESCAPE

If both EC and UV detectors are installed, this screen will allow you to select which detector conditions are to be edited.

LCEC Screen 14 will appear.

LCUV Screen 16 will appear.

ESCAPE Recalls screen 3.

STATUS STATUS has the usual significance (see screen 2).

Screen 14

LCEC FILE = 1				0, 1 OR 2 DETECTORS? 1		
TIME	LCEC	POT	GAIN	FLT	SIGN	RCDR
0.0	1	+ 800	+ 500 nA	0.1	+	10%
EXEC ON OFF SAVE REZERO ESCAPE						

0, 1, OR 2 DETECTORS? This screen allows editing of EC detector parameters. On the first line, enter the number of electrodes to be used. If the 200B has been configured for two EC detectors but only single electrode operation is desired, enter "1" (then ENTER). To place EC detection in standby, press "0" and ENTER. If the system is configured for single electrode detection, do not press "2." Spurious results may occur.

TIME For a given method file, up to 10 lines of instructions for the EC detectors may be entered in addition to the $t = 0.0$ values. Whenever a timeline is executed during the course of a run, a rezero command is also executed. You can therefore automatically force a rezero at repeatable times in the run. Even if the detector parameters remain fixed, duplicate lines for each desired rezero time may be entered.

POT The applied potential can be set between -2048 and $+2048$ mV with respect to the reference electrode.

GAIN The gain can be set from 0.1 nA/V output to 100 μ A/V. Select the value by pressing the left arrow (to increase gain) or right arrow (to decrease gain). The gain value is preceded by a plus or minus sign. A gain setting preceded by a plus sign should be used for oxidations (minus for reductions). After the gain has been selected, press ENTER. The sign of the GAIN partly controls the direction of the peak on the strip chart recorder, in conjunction with the values for SIGN and RCDR (see Section 9).

FLT Select from four values for the output filter (0.05, 0.1, 0.15, and 0.2 Hz). The default (0.1 Hz) will provide the most noise rejection. The 0.5 Hz setting provides the least filtering. Use the right or left arrows to select, then press ENTER.

SIGN By selecting values for the GAIN, SIGN, and RCDR, you controls how the output will be displayed on the recorder. SIGN is selected by the +/- key. A plus sign indicates that an oxidation will give a positive peak on the recorder. A negative sign indicates that an oxidation will give a negative peak. See Section 9 for further details if a multielectrode run is anticipated.

RCDR Press RCDR to set the baseline rezeroing point at some percentage of full scale. For example, if the gain were set at 50 nA full scale, a 10% value would mean that the offset would be set so the baseline is at 5 nA. This would insure that slight dips in the baseline would continue to provide a positive signal for the A/D converter of your data system. When a rezero command is entered, the baseline will be brought to this position on the chart. The value can be different for each line during a run, if desired.

The RCDR value affects the detector output as seen in STATUS. In the above example with 50 nA full scale and 10% RCDR, the output would be 5.0 nA, even if no such real background current existed.

CLEAR Use CLEAR to rekey an erroneous entry. The entry changes to zero and the cursor remains in place.

DELETE When the cursor is at the beginning of a line of instructions, the line can be removed by pressing DELETE. The TIME = 0.0 line cannot be deleted.

EXEC The first line of the detector file is executed immediately. Note that if this is done while a run is in progress, the detector conditions will no longer be controlled by the method file that was selected for the run. EXECute does not, however, replace the parameters stored in memory.

ON Pressing the ON key will apply the potential(s) selected for the first line of the file, but the gain will default to 1 μ A full scale. If two electrodes have been selected in the top line of the screen, screen 15 will appear first, providing a choice between the electrodes. The ON command essentially provides a built-in warm-up file for the EC detector, at very low gain. When equilibration is nearly complete, the real operating file may be switched in by pressing EXEC.

(Screen 14 continued next page.)

(Screen 14, continued)

	ON differs from EXEC and EQUIL only in defaulting to a gain of 1 μ A full scale.
OFF	If one EC detector is on, pressing OFF will turn it off. If both EC detectors are on, this command will call up screen 8, prompting you to choose which detector to turn off. If the REZERO command was issued while the detector was on, the most recent off-set value is saved until the file is inactive.
SAVE	Stores the updated conditions for the detector and returns control to screen 3, where other modules may be edited or the whole method saved.
REZERO	The EC detector baselines will be reset to the percentage of full scale defined in this screen (RCDR). If the output has drifted, you will see a change in output and offset values in the status screen S1. REZERO is triggered automatically <u>only</u> during the start of a run (after synchronization from INJECT) or by timelines in the detector file. You must REZERO manually when operating solely from this screen.
ESCAPE	Screen 3 returns without saving any changes you have made to the detector file.
STATUS	STATUS has the usual significance (see screen 2).

Screen 15

TURN ON WHICH DETECTORS?

W1 | W2 | BOTH | | | EXIT

This screen follows screen 14 if the method specifies two EC detectors. It allows you to turn on one or both of the detectors. Both detectors are equivalent and independent.

W1	Turns on one of the EC detectors. The W1 or W2 notation corresponds to the label on the detector cable, at the flowcell.
W2	
BOTH	Applies a potential to both working electrodes. After any one of these three soft keys is pressed (W1, W2, or BOTH), screen 14 will reappear.
EXIT	Screen 14 will return with no changes to the EC detector.
STATUS	STATUS has the usual significance (see screen 2).

Screen 16

LCUV FILE = 1				
TIME	SPECTRA	WAVELENGTH	AUFS	FLT
0.0	--	200 nm	2.000	0.1
EXEC OPTIONS SAVE REZERO ESCAPE				

This screen controls parameters for the UV-Vis absorbance detector. Various submenus are also accessed through this screen. Screens 16–20 will only appear if the system hardware is configured for optical detection.

TIME For a given LCUV file, up to nine lines of instructions may be entered in addition to the $t = 0$ values. An autozero command is executed whenever time passes to the next timeline in the file. Even if detector parameters remain fixed, duplicate lines may be entered to force a rezero.

SPECTRA Pressing the +/- key toggles the value of this column. If you select -, no spectrum will be taken at the designated time. If the value is +, a spectrum will be taken, beginning at the time listed for that line. The spectrum will be assigned a reference number for later playback. The scan parameters for all spectra taken during the run are the same, and are selected on screen 18.

WAVELENGTH The wavelength can be set from 190 to 800 nm. The monochromator will select the designated wavelength for this timeline until the run time elapsed equals the time of some subsequent timeline.

AUFS The detector gain is selectable in 11 steps, from 0.0005 to 2.00 AUFS (absorbance units full scale). The values are selected by pressing the left and right arrows, to increase and decrease the gain, respectively. After the gain has been selected, press ENTER.

FLT Select a baseline noise filter using the right and left arrows, then press ENTER. Acceptable values are 0.1, 0.5, 0.7, and 1.1 Hz.

CLEAR Use CLEAR to rekey an erroneous entry. The entry changes to zero and the cursor remains in place.

DELETE When the cursor is at the beginning of a line of instructions, the line can be removed by pressing DELETE. The TIME = 0.0 line cannot be deleted.

EXEC The first line of the detector file is executed immediately. If the lamps are off, they are turned on, as long as the UV-Vis detector is not in "standby." Note that if EXEC is pressed while a run is in progress, the detector conditions will no longer be controlled by the method file that was selected for the run.

OPTIONS Pressing this key passes control to screen 17 where various choices relating to UV-Vis detection are made. These include spectrum parameters, spectra playback, chromatogram playback, and turning lamps off and on.

SAVE Pressing SAVE stores the updated conditions for the detector and returns control to screen 3, where other modules may be edited or the whole method saved.

REZERO The UV-Vis detector baseline will be reset to the recorder zero position. If the output has drifted, you will see a change in the output value in status screen S2. REZERO is triggered automatically only during the start of a run (after synchronization from INJECT) or by timelines in the UV detector file. You must REZERO manually when operating solely from this screen.

ESCAPE Screen 3 returns without saving any changes you have made to the detector file.

STATUS STATUS has the usual significance (see screen 2).

(Screen 18, continued)

- REZERO** The UV-Vis detector baseline will be reset to the recorder zero position. If the output has drifted, you will see a change in the output value in status screen S2. REZERO is triggered automatically only during the start of a run (after synchronization from INJECT) or by timelines in the UV detector file. You must REZERO manually when operating solely from this screen.
- ESCAPE** Screen 17 returns without saving any changes you have made to the detector file.
- STATUS** STATUS has the usual significance (see screen 2).

Screen 19

```

LCUV FILE = 1
CHROMATOGRAM PLAYBACK
  RATE      AUFS      FLT
10 x REAL TIME  2.000    0.1

EXEC |         |         | SAVE |         | ESCAPE

```

The most recent chromatogram may be played back at faster speeds than the initial data run. The gain is variable, to enlarge small peaks or reduce large peaks. The output is sent to the strip chart (analog) recorder jacks.

- RATE** The playback speed may be an integer multiple of the original data acquisition rate. Permissible range: 1–30.
- AUFS** The detector gain is selectable in 11 steps, from 0.0005 to 2.00 AUFS (absorbance units full scale). Select a value by pressing the left and right arrows to increase and decrease the gain, respectively. After the gain has been selected, press ENTER.
- FLT** Select a baseline noise filter using the right and left arrows, then press ENTER. Acceptable values are 0.1, 0.5, 0.7, and 1.1 Hz.
- CLEAR** Use CLEAR to rekey an erroneous entry. The entry changes to zero and the cursor remains in place.
- EXEC** The most recently acquired chromatogram will be played back at the designated rate and full scale gain. The output is available at the strip chart recorder jacks for the UV-Vis detector.
- SAVE** Stores the updated conditions for the detector and returns control to screen 17.
- ESCAPE** Returns control to screen 17. No changes made during the visit to this screen are saved.
- STATUS** STATUS has the usual significance (see screen 2).

Screen 20

LCUV FILE = 1	SPECTRA NUMBER? 1
SPECTRA PLAYBACK	
RATE	AUFS
20 nm/SECOND	2.000
EXEC SAVE REZERO ESCAPE	

Spectra taken during the most recently acquired chromatogram may be played back using the analog (strip chart) recorder jacks on the back of the BAS 200B. These are called up "by the number," using the numeral assigned in screen 16.

SPECTRUM NUMBER # You must select a number which has already been assigned to a spectrum, as requested in screen 16. If no spectra have been requested, or if you select a number larger than the number of spectra requested in screen 16, an error message will be displayed. A spectrum must be in memory from the most immediate run in order to use this screen.

RATE The acquired spectra can be played back at scan rates from 1 to 100 nm/sec. To find the location (in nanometers) of peak maxima on the strip chart paper, you must also account for the speed of the chart paper.

The resolution on the chart paper (nm/cm) is calculated by dividing the scan rate by the chart speed. For example, a 5 nm/sec playback RATE and a chart speed of 20 cm/min would yield a resolution of 15 nm/cm on the chart paper. To obtain the best resolution, use the slowest playback RATE and fastest chart speed.

AUFS The detector gain is selectable in 11 steps, from 0.0005 to 2.00 AUFS (absorbance units full scale). Select a value by pressing the left and right arrows to increase and decrease the gain, respectively. After the gain has been selected, press ENTER.

EXEC Plots the designated spectrum immediately. Make sure the strip chart recorder is running at the desired chart speed before pressing EXEC.

SAVE Stores the updated conditions for the detector and returns control to screen 17.

REZERO The UV-Vis detector baseline will be reset to the recorder zero position. If the output has drifted, you

will see a change in the output value in status screen S2. REZERO is triggered automatically only during the start of a run (after synchronization from INJECT) or by timelines in the UV detector file. You must REZERO manually when operating solely from this screen.

ESCAPE Screen 17 returns without saving any changes you have made to the detector file.

STATUS STATUS has the usual significance (see screen 2).

Screen 21

TIMED EVENTS FILE = 1				
TIME	EVENT1	EVENT2	EVENT3	EVENT4
DEFAULT	0	0	0	0
0.0	0	0	0	0

EXEC | | OFF | SAVE | | ESCAPE

The timed events screen provides four switches for controlling external detectors, integrators, or other accessories. Switches can be normally open or normally closed. A "0" indicates the switch will be in the normal position; a "1" indicates the switch is actuated.

TIME When this screen is called up, the cursor will be on the default line. A default line is included (in addition to the TIME = 0 line) so that the switches can be actuated to change states exactly at the beginning of a run (i.e., when RUN IN PROGRESS appears on the screen). If you wish to access or edit additional lines in the file, press 1 or 0, then ENTER. Up to nine additional lines may be entered. The last timeline must be complete before you exit this screen.

CLEAR Use CLEAR to rekey an erroneous entry. The entry changes to zero and the cursor remains in place.

DELETE When the cursor is at the beginning of a timeline, the line can be removed by pressing DELETE. The TIME = 0.0 line cannot be deleted.

EXEC The default line is executed immediately. NOTE: If this is done while a run is in progress, the timed events may not be controlled by the method file that was selected for the run.

OFF All switches are returned to their normal ("0") positions.

SAVE Stores the updated conditions for the detector and returns control to screen 3 for editing other module parameters or for saving the entire method.

ESCAPE Screen 3 returns without saving any changes you have made during access to this screen.

STATUS STATUS has the usual significance (see screen 2).

Screen 22

TEMPERATURE FILE = 1	
OVEN:	TEMP SETPOINT = 20.0°C
CELL:	TEMP SETPOINT = 20.0°C

EXEC | FAN | OFF | SAVE | | ESCAPE

OVEN: With this screen, the temperature of the oven and preheater for the electrochemical cell are set for a method. The oven temperature can be set between 20 °C and 70 °C. It is recommended that the EC preheater temperature be set at approximately 3 degrees above the oven temperature. Both devices may only be heated to temperatures above ambient (current ambient temperature may be requested by pressing STATUS). If ambient temperature is above the desired setpoint, active temperature control is not possible.

After the oven and cell values are entered, screen 23 will appear, allowing you to control the temperatures of the three mobile phase flasks.

CLEAR Use CLEAR to rekey an erroneous entry. The entry changes to zero and the cursor remains in place.

EXEC Starts the oven, cell heater, and mobile phase heaters (see screen 24), and brings the temperatures to the designated values.

FAN Toggles the oven fan on and off.

OFF The oven heater, oven fan, cell preheater, and mobile phase heaters are turned off.

SAVE Temporarily stores the updated conditions for the oven, cell, and mobile phase heaters. The display will return to screen 3, where these conditions may be saved as part of a method file.

ESCAPE Screen 3 returns without saving any changes you have made to the temperature file.

STATUS STATUS has the usual significance (see screen 2).

Screen 23

TEMPERATURE FILE = 1				
MOBILE PHASE:	OFF	35°C	50°C	SELECT
BOTTLE A	1	2	3	3
BOTTLE B	1	2	3	2
BOTTLE C	1	2	3	2

SELECT 1 FOR OFF, 2 FOR 35°C, 3 FOR 50°C
EXEC | FAN | OFF | SAVE | | ESCAPE

SELECT The heating of the three mobile phase flasks is controlled from this screen. The default values are "1" (mobile phase heaters off). The entry "2" codes a setpoint of 35 °C, and "3" codes a setpoint of 50 °C. Press ENTER after each numeric value (1, 2, or 3) to accept the setpoint and advance the cursor. After the three values are entered, screen 24 will appear.

EXEC The EXEC, FAN, OFF, SAVE, and ESCAPE commands have exactly the same functions as those of screen 22. After pressing EXEC, FAN, or OFF, screen 22 will appear again. After SAVE or ESCAPE, display will return to screen 3.

STATUS STATUS has the usual significance (see screen 2).

NOTE: Please consider the volatility, flashpoint, and toxicity of your mobile phase solvents before heating them. The flashpoint of tetrahydrofuran is -14 °C, for example, and ambient temperature is recommended in this or similar circumstances.

Screen 24

TEMPERATURE FILE = 1				
ENTER THE DESIRED FUNCTION.				
EXEC	FAN	OFF	SAVE	ESCAPE

EXEC The EXEC, FAN, OFF, SAVE, and ESCAPE commands have exactly the same functions as those of screens 22 and 23. After pressing EXEC, FAN, or OFF, screen 22 will appear again. After SAVE or ESCAPE, display will return to screen 3.

STATUS STATUS has the usual significance (see screen 2).

Screen 25

```

CURRENT FILE = 1

                                RUN IN PROGRESS

METHOD | CONT | ABORT | REZERO |           | STOP

```

This screen is displayed following a PAUSE command in screen 10. The 200B clock will stop and hold its value. The gradient composition will hold at the current values of %A, %B, and %C. The detectors will continue to operate, but no additional instructions for them or for the timed events will be executed.

METHOD The METHOD, ABORT, REZERO, STOP, and
ABORT STATUS keys have the same functions as those of
REZERO screen 10.
STOP
STATUS

CONT The BAS 200B clock will resume (CONTInue) from
the point at which it was stopped. Screen 10 will be
displayed again.

Screen 26

```

BAS 200B HARDWARE CONFIGURATION

1. LCEC DETECTOR(S)?           4. AUTO RUNS?
2. LCUV DETECTOR?             5. DIAGNOSIS?
3. SYNCHRONIZED RUNS?        6. UTIL BOARD?
ENTER 0, 1, OR 2?

|           |           | SAVE |           |

```

This screen is accessed by pressing "." then "→" from screen 2, or by using the CONFIG function from screen 2. The answers to questions asked on this screen determine the software version to be used in setting up system parameters. The BAS 200B *does know* certain characteristics of your system: whether you have UV detection or EC detection, the version numbers for your software, etc. It *does not know* how many electrodes are used in EC detection or whether you wish to synchronize runs.

This screen is configured once by BAS personnel. It may be accessed in your laboratory if your hardware changes or if you want to override certain features. Otherwise, it is not routinely viewed.

LCEC DETECTOR(S)? Your choices are 0, 1, or 2 (then ENTER). If the EC detector is not to be used, select 0; if only one electrode is active, choose 1, and if both electrodes are active, choose 2. If 0 is selected, no questions about EC detection will appear, and the status screen S1 will not appear.

LCUV DETECTOR? This option has been discontinued. Select 0.

SYNCHRONIZED RUNS? See Section 6.6 for an explanation of this option. Press 1 for reproducible gradient elution chromatography. If the gradient elution feature will not be used, press either 0 or 1.

AUTORUNS? Press 0 or 1, then ENTER. If 0 is selected, the system must be manually prompted to begin the analysis of each sample (using the INJECT soft key on screen 5). If 1 is selected, the 200B will automatically cycle through the method and begin a new cycle (started at INJECT) as soon as the previous run is completed. (The previous run is defined as the end of the longest time required for any module to complete its individual program.) See Section 7.1.

(Screen 26 continued next page.)

(Screen 26, continued)

DIAGNOSIS? Pressing 1, then ENTER, displays diagnostic messages and parameters. These appear in the pump and temperature control subsystems for the use of BAS service personnel. Press 0 to clear the screen.

UTIL BOARD? Press 1 for all BAS 200B versions. This board controls temperature and external communications.

Screen 27

```

          BIOANALYTICAL SYSTEMS
          BAS 200B      V-1.05
SOFTWARE VERSIONS          I.D. NUMBER  XXXY
PUMP CPU                   2.00
EC DETECTOR                2.00
UTILITY CPU                2.00
UV DETECTOR                NO RESPONSE
          PUSH ANY KEY TO CONTINUE

```

This screen lists the serial number of the BAS 200B and the version numbers for the software modules.

The second line refers to the keypad controller software. The next line is the hardware serial number for the unit as configured from the factory. The serial number is composed of three or four numerals followed by a letter. This number is saved electronically and used as a cross-reference for the use of BAS System Controller software. Do not worry if only blanks appear here.

The following lines indicate version numbers for software ROM chips on individual subsystem printed circuits. If a module is not installed, NO RESPONSE appears next to that module's name. Control reverts to screen 2 when any key is pressed.

Screen S1

```

CURRENT FILE = 1                                TIME = 0.0
                                LCEC
                                DETECTOR CONDITIONS
LCEC      POT      GAIN      OFFSET      OUTPUT
1 ON      ±XXX    ±XXX nA   ±X.XXX nA ± X.X nA
2 ON      ±XXX    ±XXX nA   ± XXX nA  ±X.XXX uA
FOR MORE CONDITIONS OR TO EXIT
PUSH 'STATUS' KEY                                | REZERO

```

This screen displays the current operating conditions of the electrochemical detector(s). If none is installed, this screen will not appear. If a detector is off, a line of dashes will appear on the display.

TIME If the electrochemical detector file consists of only the t = 0 timeline, the TIME parameter will remain at 0.0, even though the pump, UV detector, or timed events panels indicate otherwise.

If the detector module completes its time program before the pump or timed events schedules, the displayed time will return to 0.0, even though other devices may still have nonzero time showing.

REZERO The EC detector baselines will be reset to the percentage of full scale defined in screen 14. If the output has drifted, you will see a change in the output and offset values.

STATUS The only way to leave this screen is to press STATUS, which calls up the next STATUS screen.

Screen S2

```

CURRENT FILE = 1                                TIME = -----
                                LCUV
                                DETECTOR CONDITIONS
ABSORBANCE WAVELENGTH      AUFS
----- AU      ----- nm      -----
FOR MORE CONDITIONS OR TO EXIT
PUSH 'STATUS' KEY                                | REZERO

```

This screen displays the net absorbance, operating wavelength, and full scale gain of the UV-Vis absorbance detector. If this detector is not installed, this screen does not appear. If the detector is off, a line of dashes will appear on the display.

TIME If the UV-Vis detector file consists of only the t = 0 timeline, the TIME parameter will remain at 0.0, even though the pump, EC detector, or timed events panels indicate otherwise.

REZERO The absorbance will be reset to zero.

STATUS The only way to leave this screen is to press STATUS, which calls up the next STATUS screen.

Screen S3

CURRENT FILE = 1		TIME = --:--		
PUMP CONDITIONS				
FLOW	%A	%B	%C	PRESSURE
1.0	0.0	100.0	0.0	0
FOR MORE CONDITIONS OR TO EXIT PUSH 'STATUS' KEY				

This screen displays the current conditions of the pump, including the mobile phase composition and back pressure. The only key that is active when this screen is displayed is the STATUS key. When it is pressed, the next STATUS screen will appear.

Screen S4

CURRENT FILE = 1	FAN = XX
OVEN TEMPERATURE = XX.X °C	
CELL TEMPERATURE = XX.X °C	
BOTTLE A TEMPERATURE = XX.X °C	
BOTTLE B TEMPERATURE = XX.X °C	
BOTTLE C TEMPERATURE = XX.X °C	
FOR MORE CONDITIONS OR TO EXIT PUSH 'STATUS' KEY	

This screen displays the current temperatures of the oven, the EC cell preheater (if installed), and the three mobile phase flasks. It also shows whether or not the oven fan is operating.

The only key that is active when this screen is displayed is the STATUS key. When it is pressed, the BAS 200B will return to the last screen displayed before the STATUS loop was started.

5.4 Error Descriptions

A. Pump Errors

ERROR: PUMP FILE REJECTED. EDIT CURRENT PUMP FILE FOR ERRORS.

Pump CPU has rejected the most recent file sent to it. Solution: Look at the current pump file for errors. If there are errors in the file you can try to edit the file to fix the errors, or use the clear file utility: press "." then CLR from screen 2.

ERROR: PUMP CPU RAM ERROR

At least one byte of your pump CPU RAM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: PUMP CPU ROM ERROR

At least one byte of your pump CPU ROM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: PUMP CPU 8253 IS INOPERATIVE

Your pump CPU 8253 counter has failed self-test. Solution: Consult BAS for service.

ERROR: PUMP CPU μ BORE CIRCUIT FAILURE

A hardware "divide by ten" circuit in the pump CPU has failed self-test. Solution: Consult BAS for service. You may be able to continue using your pump in the normal mode.

ERROR: PUMP SYNCHRONIZATION ERROR

There is a hardware mark on the pump that is used to determine which head is pumping and that the pump is operating. If this mark is missed, a pump synchronization error will occur. Solution: Be sure that the heads of your pump are operating properly. If this error occurs repeatedly, consult BAS for service.

ERROR: SYSTEM PRESSURE < MINIMUM LIMIT

This error will be reported when the system pressure goes from greater than to less than the minimum pressure selected. Solution: Be sure that there are no leaks in your system and that both heads of your pump are delivering equal volumes of mobile phase. Opening the purge valve after the pump begins operating can cause this error. To avoid this, set the minimum pressure to 0 psi.

ERROR: SYSTEM PRESSURE > MAXIMUM LIMIT

Pressure in your system has exceeded the maximum limit you specified in the pump method file. Solution: Check the limit specified in the pump method file. If the limit set seems high for the flow rate, there may be a blockage in the system. Be sure the injection valve is not left in the halfway position, because this will block all flow and cause the pressure to exceed the limit.

ERROR: CANNOT PURGE SYSTEM ... MAXIMUM SYSTEM PRESSURE LIMIT WAS EXCEEDED AT MINIMUM FLOW RATE

The pump is being run at its minimum flow rate and the system pressure has exceeded the limit in the purge method file. Solution: Check all fittings and tubing for blockages and make sure that the injection valve is not left in the halfway position.

ERROR: PUMP OPTO-SENSOR IS INOPERATIVE

The BAS 200B pump contains a sensor that determines what head is pumping and that the pump is running. If the signal from this sensor is not sensed by the pump CPU, this error will appear. Solution: If this error occurs repeatedly, your pump needs servicing. If this error occurs infrequently, the pump is probably operating properly.

ERROR: SYNCHRONIZING ... (system hangs at this panel)

The BAS 200B pump is waiting for the opto-sensor to determine when the next left piston delivery stroke begins. The pump file is anticipated to be a multiline pump file, but only a single line of conditions (at t = 0) exists. This occurs only in isocratic methods. Solution: Go back to the pump file and enter a second line. For convenience, make the time for this line equal to the chromatography run time, and copy the t = 0 line for the % entries.

B. EC Detector Errors

ERROR: DETECTOR FILE REJECTED. EDIT CURRENT DETECTOR FILE FOR ERRORS.

Detector CPU has rejected the most recent file sent to it. Solution: Look at the current detector file for errors. If there are errors in the file you can try to edit the file to fix the errors, or use the clear file utility: press "." then CLR from screen 2.

ERROR: DETECTOR CPU RAM ERROR

At least one byte of your detector CPU RAM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: DETECTOR CPU ROM ERROR

At least one byte of your detector CPU ROM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: DETECTOR 9513A (or 9513B) IS INOPERATIVE

A 9513 counter on the detector CPU has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: DETECTOR 8256 IS INOPERATIVE

The 8256 on the detector CPU has failed self-test. The 8256 is responsible for communicating with the detector analog circuitry as well as other important circuitry in the analog system. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service. Your EC detector(s) will not operate correctly with this error.

C. Utility Control Errors

ERROR: UTILITY FILE REJECTED. EDIT CURRENT TIMED EVENTS & TEMPERATURE FILES FOR ERRORS.

Utility CPU has rejected the most recent file sent to it. Solution: Look at the current detector file for errors. If there are errors in the file you can try to edit the file to fix the errors, or use the clear file utility: press "." then CLR from screen 2.

ERROR: UTILITY CPU RAM ERROR

At least one byte of your utility CPU RAM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: UTILITY CPU ROM ERROR

At least one byte of your utility CPU ROM has failed self-test. Solution: Try resetting your system several times. If the error occurs repeatedly, consult BAS for service.

ERROR: UTILITY CPU A/D IS INOPERATIVE

The utility CPU A/D converter has failed self-test. This will render temperature control inoperative. Solution: Consult BAS for service.

ERROR: OVEN (or CELL) TEMPERATURE SENSOR ERROR

The utility CPU has determined that the oven (or cell) temperature sensor is inoperative. Either there is an invalid temperature reading or the temperature sensor is not sending temperature readings. An open circuit is a possible cause. Solution: Consult BAS for service.

ERROR: BOTTLE A (or BOTTLE B or BOTTLE C) TEMPERATURE SENSOR ERROR

The utility CPU has determined that the bottle A (or B or C) temperature sensor is inoperative. Either there is an invalid temperature reading or the temperature sensor is not sending temperature readings. An open circuit is a possible cause. Solution: Consult BAS for service.

ERROR: INTERNAL OVER-TEMPERATURE ERROR. FILTERS NEED SERVICING.

There is a temperature sensor mounted on the utility CPU printed circuit board that measures the mean temperature of the electronics compartment inside the BAS 200B. If this temperature exceeds a maximum limit, this error message will appear. Solution: Follow the instructions on how to service the filters on the BAS 200B (Section 9.3). If this error message still appears after the filters have been cleaned, there may be a defective fan in the system. Consult BAS for service.

ERROR: TEMPERATURE CONTROL IS INOPERATIVE; FAIL-SAFE MECHANISM HAS BEEN TRIPPED. CONSULT MANUAL FOR DETAILS.

There is a hardware fail-safe mechanism that protects the BAS 200B heater elements from overheating in the event that the utility CPU does not turn them off. If the oven compartment or any of the mobile phase bottles is extremely hot when this error appears, temperature control may not be operating properly. Fail-safe activation will be indicated by the three green LEDs flashing on the front of the BAS 200B. Solution: If this mechanism is tripped and the oven compartment or any of the mobile phase bottles is extremely hot, consult BAS for service. This mechanism can be tripped when there actually is no error; the cause could be abnormal transients in AC power. If this happens, reset your system to override the mechanism. The warning mechanism will remain tripped as long as the temperatures are above maximum allowable setpoints. You must allow the system to cool down before resetting it.

ERROR: CHECK BOTTLE A (or BOTTLE B or BOTTLE C) MOBILE PHASE LEVEL

As a safety feature, the utility CPU detects the heating of a mobile phase bottle with little or no liquid in it. Solution: Check the level of mobile phase in bottle A (or B or C). If the level is empty or low, you must add more solution or turn temperature control off. This commonly occurs when you remove a bottle while still applying heat to it.

Section 6. THE SOLVENT DELIVERY SYSTEM

6.1 Overview

The BAS 200B Liquid Chromatograph features a sophisticated combination of hardware and software controls to accurately and precisely meter the mobile phase to the separations zone of the instrument. The subsystem includes:

- a. a mobile phase manifold that provides three mobile phase bottles with thermostatic temperature control, gas sparging, and gastight seals. Convenient front panel needle valves are provided to control sparging rates, pressurization, etc.
- b. type 316 stainless steel tubing to prevent oxygen permeation back into the system after degassing or more rigorous deoxygenation.
- c. a stainless steel ternary proportioning valve to blend the three mobile phases for either isocratic or gradient elution.
- d. a dynamic (magnetic) mixing chamber.
- e. a microprocessor-controlled, computer-designed dual piston pump. Each head is removable as a single, precision-engineered assembly that can be interchanged in seconds. The construction of the pump is solid (cast aluminum and machined 316 stainless steel); no sheet metal is included. The result is greater durability, mechanical reliability, and serviceability.
- f. a solid-state pressure transducer to monitor system back pressure in real time and dynamically compensate compressibility during gradient elution.
- g. an optional pulse damper as a standard feature for use at high sensitivity (if desired).
- h. a purge valve for bypassing the column, etc., while flushing the solvent delivery system.

Figure 6.1 shows the essential components of the subsystem.

Figure 6.1 Front view of the BAS 200B showing mobile phase manifold, dual-piston pump, pressure transducer, and purge valve. The low pressure proportioning valve and dynamic mixer are not visible in this figure.

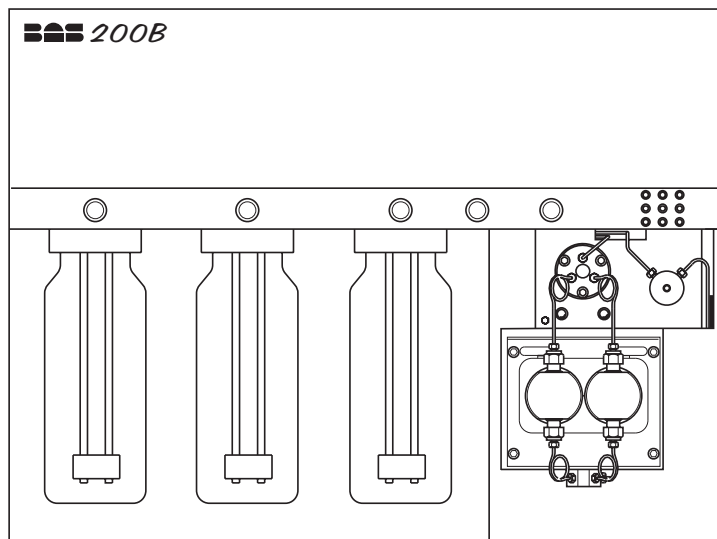
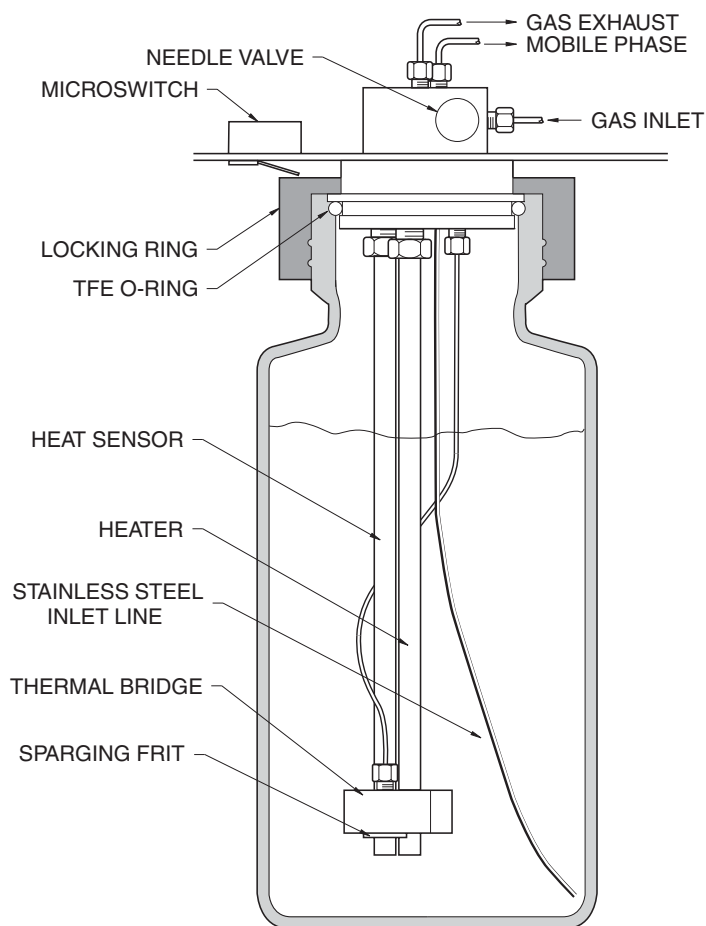


Figure 6.2 Detailed view of one of the three BAS 200B mobile phase reservoirs.



6.2 Understanding the Solvent Bottle Utilities

Each of the three mobile phase reservoirs provides sophisticated conditioning facilities for optimal liquid chromatography (see Figure 6.2).

Each bottle is a male-threaded, borosilicate glass 1200-mL container that has been pressure-tested to 10 psi (0.7 bar). It is coated with a thick polymer film for safety. The bottle is mounted to the mobile phase manifold using a captive screw ring. When screwed in, a large polytetrafluoroethylene (PTFE) o-ring seals the type 316 stainless steel “cap” (attached to the manifold) and the bottle.

Within each bottle are a heater, a solid-state temperature sensor, a gas supply and dispersion frit, and a mobile phase inlet line. The wetted materials are 316 stainless steel, borosilicate glass, and PTFE. The sparging gas is vented through an exhaust port in the stainless steel cap.

The lower end of the heater tube, sensor tube, and gas dispersion frit are collectively organized in a stainless steel block. The block also provides a heat sink; this safety measure allows the system to shut down when the liquid level in the bottle is too low. The algorithm for temperature control in the BAS 200B is as follows:

1. Whenever the sensor temperature is below the setpoint, the utility microprocessor instructs the heater to turn on.
2. The duty cycle (% of time on during an AC cycle) is dynamically determined by the magnitude of the difference between the actual sensor temperature and the derived setpoint.
3. Typically, the sensor sees a modest rate of temperature change, since the mobile phase absorbs most of the heat energy. However, as the mobile phase is depleted, the stainless heat sink eventually is exposed to mostly headspace gas, and the rate of temperature change at the sensor accelerates. When this occurs, the heater is shut down by the microprocessor, and an error message is displayed.

For mobile phase recycling, the “A” position is provided with a return tube from the exterior of the bottle back into the headspace. This tube is terminated on the exterior with a plugged union. When the plug is removed, the open union port may be connected to the exit tube from the detector. In this manner the mobile phase may be recycled indefinitely. Since the sample volumes are typically 10,000-fold less than the quantity of mobile phase, contamination of the mobile phase is not a problem.

NOTE: If you elect to recycle, be sure your pump file specifies only 100% A for all times during the method file; otherwise your bottle volume will not remain at a steady state.

6.3 Understanding the Mobile Phase Manifold

The mobile phase manifold can be seen upon removal of the oven module and the outer cover. A schematic diagram of the gas and liquid connections within the manifold appears in Figure 6.3.

The stainless steel cap of each bottle extends up inside the tray. Within each cap is the gas inlet control needle valve for each bottle. The heater and temperature sensor leads are routed to appropriate electrical connectors. An inlet manifold accepts gas delivered from the underside of the shelf (see Figure 6.4) and safety-tests it for unsafe pressure. A pop-off check valve dissipates the excess incoming gas pressure if it exceeds about 8 psi. The remaining gas flow may be regulated with the INLET knob, which controls the flow to all bottles simultaneously.

After the INLET needle valve, gas flow is proportioned among the A, B, and C bottles according to their respective back pressures. The flow into the individual bottles may be independently adjusted by the A, B, and C needle valves above each bottle.

The EXHAUST control is capable of sealing off the common exhaust lines in order to pressurize the bottles. Should excess pressure develop, a second pop-off valve is provided to bleed the exhaust system. The system cannot be pressurized if any bottle is missing or not tightly sealed against the PTFE o-ring, or if the recycle line is unplugged. The gaseous exhaust (from either normal or safety venting) is directed out the underside of the mobile phase manifold (Figure 6.4) and can ultimately be passed to the rear of the BAS 200B by use of the tubing provided.

In many cases, you are advised to route this exhaust to a fume hood or, in some other way, to trap fumes from toxic solvents without blocking the exhaust port. Strict attention should be paid to the Material Safety Data Sheets (MSDS) supplied by the solvent manufacturer.

The mobile phase path in the manifold is shown in black in Figure 6.3. A continuous 316 stainless steel tube extends from the bottom of the reservoir to the respective inlet port on the solvent proportioning valve. The blended mobile phase exits the valve assembly on the top center port, and is routed to the pump.

Figure 6.3 Gas and liquid connections within the mobile phase manifold.

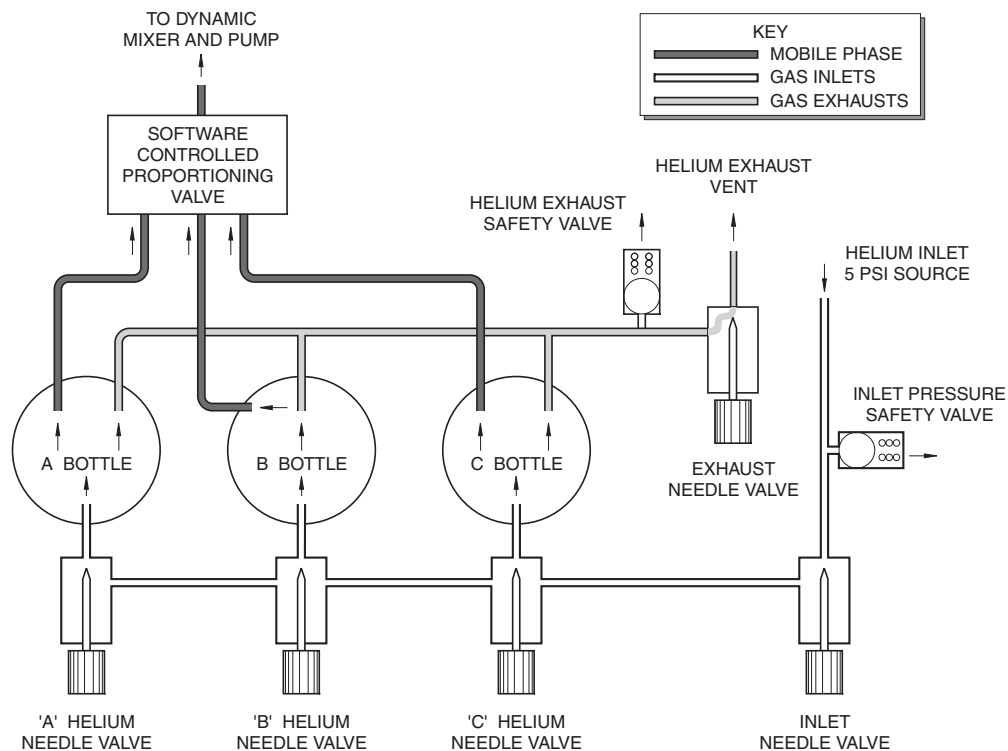
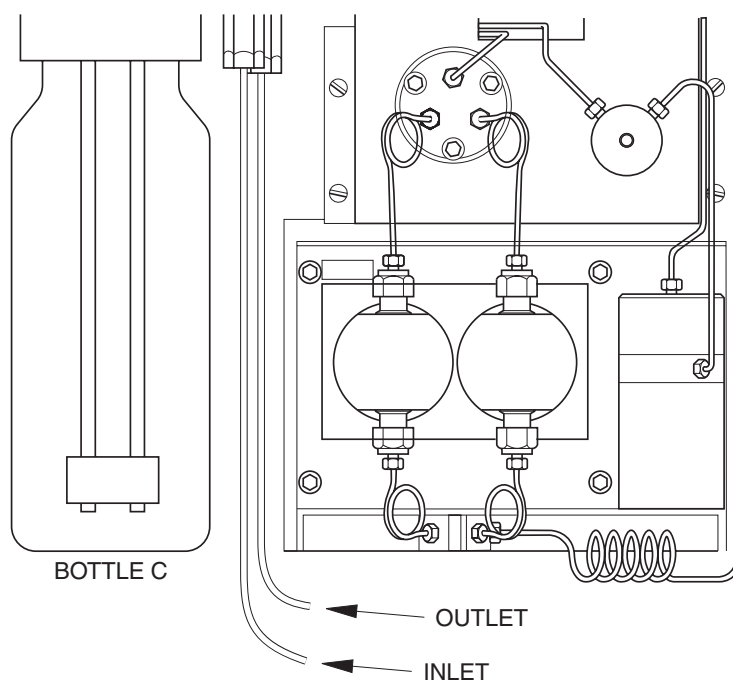


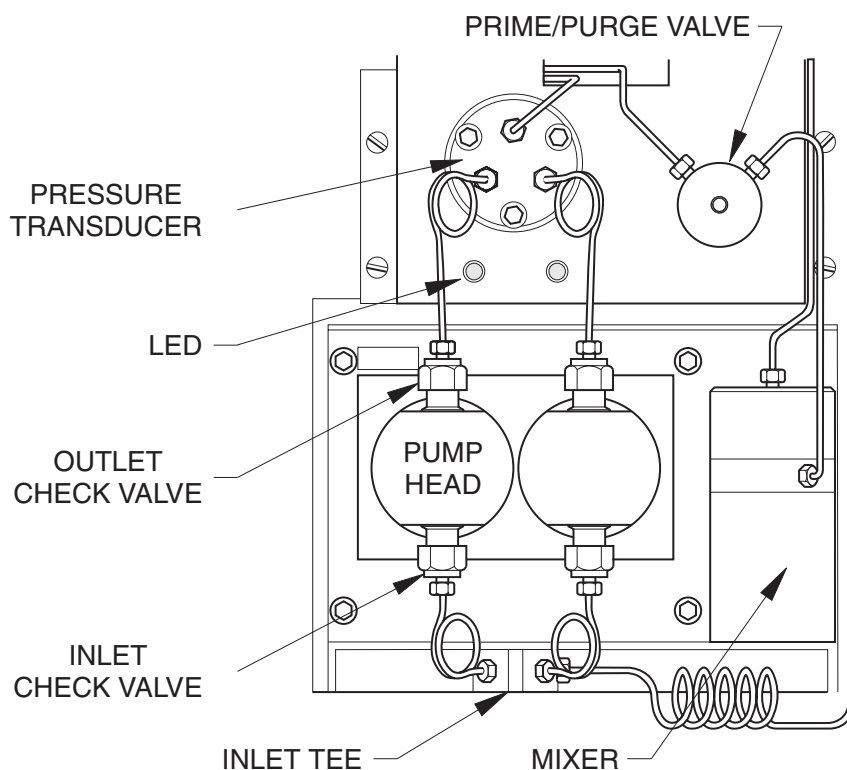
Figure 6.4 Location of the inlet (forward port) and exhaust (rear port) sparging gas lines on the BAS 200B mobile phase manifold. This view is only visible if the outer pump trim panel is removed.



6.4 Understanding the Pump

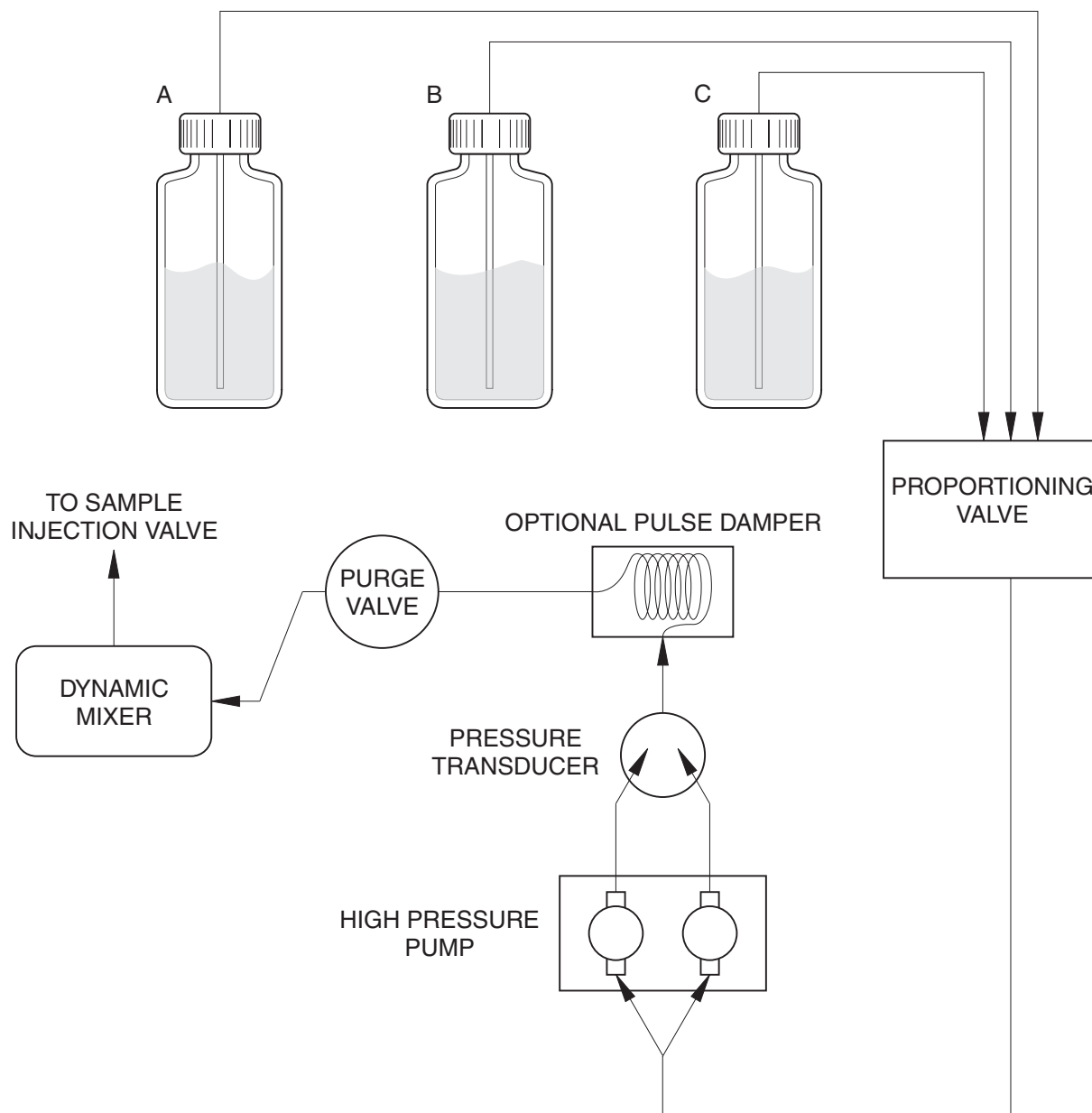
The pump section of the solvent delivery system is located in the lower right corner of the BAS 200B (see Figure 6.5). From the solvent proportioning valve, a single line containing the programmed A/B/C mixture is split and routed to the two pump heads (Figure 6.6). The high-pressure output from the two heads is recombined at the pressure transducer. An optional pulse damper (or bypass tube) may be installed between the pressure transducer and the purge valve. From the purge valve, the mobile phase travels down to the high-pressure mixer, and from there into the oven compartment.

Figure 6.5 Components associated with high-pressure pumping module. These include dual piston pump, pressure transducer, optional pulse damper (located behind the purge valve bracket, not visible here), and purge valve. A trim cover has been removed to expose the high pressure magnetic mixer.



Two diagnostic LED indicators are provided above the pump. These light when a particular pump head is undergoing the discharge (high-pressure) portion of its cycle. Combined with pump pressure information (available using the STATUS key), these LEDs can help diagnose pump problems.

Figure 6.6 Solvent flow path associated with high pressure pump.



6.5 Operating the Solvent Delivery System

This section assumes that the instrument has been installed per Section 3. These instructions include the use of all utilities, which may not be necessary in every application. The solvent delivery system should be operated in the following manner.

Loading, Sparging, and Pressurizing the Mobile Phase Manifold

In this procedure, you will adjust the sparging gas pressure, seal the bottles on the manifold, sparge the bottles with inert gas, begin warming them to optional setpoints, and purge all air from the system.

1. Open the EXHAUST valve two full turns counterclockwise (CCW). Close the INLET, A, B, and C valves fully clockwise (CW).
2. Adjust gas regulator pressure to 4 ± 1 psi. Use a dual-stage regulator (P/N MF-9302) or equivalent. Use only inert gas.

NOTE: Nitrogen does not reduce dissolved gas concentrations appreciably; it merely displaces other equally soluble gases such as oxygen!

3. Fill all three bottles with mobile phase(s). If you are not using bottles B and C, it is convenient to fill them with 40% acetonitrile (in water) and 100% acetonitrile. Alternatively, fill them with LC-grade deionized water. The mobile phase and solvents should have been prepared in accordance with Section 4.2 and filtered through a 0.2- μ m membrane.
4. Screw each bottle in place so that it is snug but not overtight.
5. Open the INLET valve fully CCW.
6. Adjust gas flow through the A, B, and C bottles using the individual controls above each bottle. Sparge for at least 10 minutes.
7. Access and execute a temperature file if the mobile phases are to be heated to 35 °C or 50 °C.

CAUTION! Do not use a setpoint that is higher than should be used for your mobile phase. Consider its flashpoint, volatility, etc., before heating.

8. Close the EXHAUST valve (fully CW). The bubbles should slow down and ultimately stop or slowly trickle out. The mobile phase bottles are now pressurized.

9. Follow this procedure to purge the system with fresh mobile phase and remove any trapped air:
 - a. Turn the POWER on.
 - b. Squirt a few drops of water into the plunger-irrigation ports (Figure 6.7).
 - c. Attach a 50-mL disposable syringe to the prime/purge valve and open the valve one turn (system pressure must be below 30 psi before opening the valve).
 - d. Purge the system at 5 mL/min for 5 minutes. Use the PURGE option found in the PUMP section on the System Director. Set the conditions as in Figure 6.8 (assuming a 5 minute purge from bottle A). Press PURGE from this screen and the pump will slowly increase flow rate to reach to maximum pressure. Since the prime/purge valve is open, this pressure will not be reached, and the pump will attain its maximum flow rate of 5 mL/min.

During this purge you may alternately draw back and release the syringe plunger to help dislodge air bubbles from the system. (If there is no column attached, turn the injector valve halfway through its arc to prevent sucking air from this direction into the syringe.)

- e. The pump will automatically stop after 5 minutes and the Pump Control screen will appear on the system director. Set the pump flow to 1 mL/min. Close the prime/purge valve and turn the injector valve to the INJECT position.
- f. 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 and collect the waste solvent at the outlet of the injection valve.
- g. To change solvents, repeat steps d–f as appropriate.

The system is now well flushed and ready to operate.

Figure 6.7 The plunger-irrigation port.

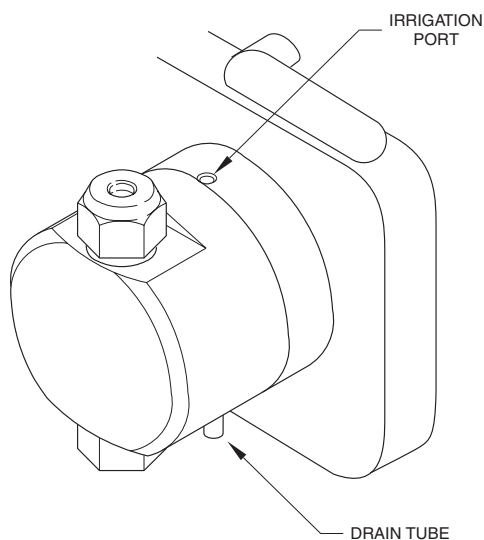


Figure 6.8 Purge conditions.

PURGE FILE = 1			
PURGE CONDITIONS			MAX PRES
%A	%B	%C	
100.0	0.0	0.0	2000
DURATION OF PURGE = 5.0 MINUTES			
PURGE			SAVE ESCAPE

Opening the Purge Valve or Other High-Pressure Connections

Allow the system back pressure to decrease to less than 30 psi before cracking open the purge valve. This protects the lifetimes of both the column and the pulse damper.

Irregular Noise in the Pump

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. If you hear noise during regular operation, there is a good chance that gas is trapped in the head. Correlate the noise with the green pump head LED indicators. If the problem is due to check valves, the faulty check valve is either the inlet check valve of that head or the outlet check valve of the opposite one (see Section 6.7.3).

Bypassing the In-Line Pulse Damper

The dead volume of the pulse damper is about 10 mL, and it creates an unacceptable amount of dead volume for gradient elution operation. A bypass tube is provided.

To install the bypass, first flush the pulse damper with 40% acetonitrile to remove any mobile phase, which is corrosive. Then disconnect the tubing (one end on the pressure transducer and the other at the purge valve); connect the bypass tube to the vacated fittings. Leave the pulse damper otherwise in place.

Leaks in the Sparging System

The sparging system should be gastight at 4 psi when the EXHAUST valve is closed and all bottles are attached. A tiny amount of residual gas still bubbling out is acceptable. However, if the flow rate of gas looks unchanged after closing EXHAUST, there is probably a poor seal somewhere in the manifold.

Remember that all exhaust lines are common to all three bottles. Therefore, if bottle B visibly shows the highest gas flow rate, the leak *is not necessarily in B*; the path through the B sparging frit is just the path of least resistance.

Tighten the bottle rings a little more to make a better seal. Wait a few minutes. If this does not effect a seal, the leak can be pinpointed by removing two of the bottles, closing off their respective inlet valves (fully CW), and screwing in 1/4-28 threaded solid plastic plugs in the exhaust port of the bottle cap. If the system still leaks, the remaining bottle, or the exhaust safety check valve, is the problem. The latter can be checked by submerging the gas outlet vent tube; no bubbles should appear. Also make sure the system is set for 4 psi regulator pressure.

If the system is now gastight, test the other bottles by returning them to the system one at a time. Be sure that you remove the diagnostic plug before reinstalling the bottle. If a leak develops when you add a bottle, the o-ring for that bottle should be replaced.

Bubble Formation (Outgassing)

The rationale for using an inert gas for sparging is based on its very low solubility in aqueous and nonaqueous mobile phases. In an LC experiment the appearance of gas bubbles in the tubing, column, or detector will produce rhythmic spiking in the baseline. Sparging with an inert gas reduces bubble formation due to:

- a. thermal effects. If the oven is set hotter than the mobile phase reservoirs, outgassing is possible when the mobile phase passes through the oven, because dissolved gas is less soluble at higher temperatures.
- b. mixing effects. Gas solubility is reduced when solvents of differing polarities are mixed together.

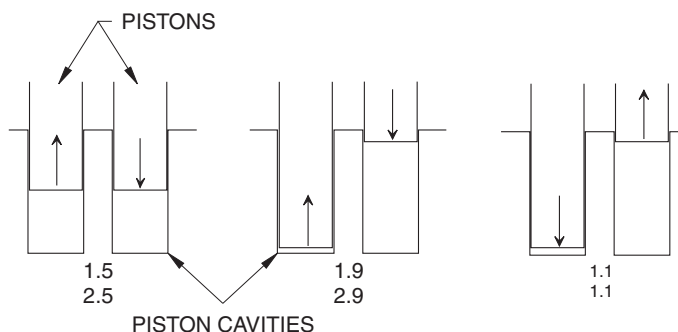
By displacing soluble gases with a less-soluble inert gas, these effects are minimized. However, if outgassing is still obvious, you may wish to:

- a. elevate the temperatures of the mobile phase reservoirs to at least that of the column compartment, or conversely lower the column compartment temperature and detector preheater temperature to a value no greater than the mobile phase reservoir temperature.
- b. avoid using 100% nonaqueous mobile phase in one bottle and pure solvents in the others. Blending these "off-line" in a minor % range is often beneficial. For example, instead of placing 100% acetate buffer in A and 100% methanol in B, make up 95% acetate/5% methanol for A and 90% methanol/10% water for B, and vacuum filter these. Outgassing is much easier to avoid with the latter A/B combination.

6.6 Pump Synchronization

Synchronizing the time of injection during the pump cycle is very important for gradient operation, particularly when the system void volume is small or when the flow rates are low. Consider the possible “synch” errors shown in Figure 6.9. Since the BAS 200B utilizes a low pressure gradient, the designated %A/ %B/ %C composition must be formed during the intake stroke of each piston. In this unit, a piston displaces 100 μL of the mobile phase.

Figure 6.9 Schematic of piston location at three points in the dual piston pump cycle. The first row of numbers under the diagram refers to the variable number of pistonfuls of the $t = 0$ solvent mixture that the system is already committed to deliver before starting the gradient. The second row of numbers lists the variable number of pistonfuls if the left side is always used to start a gradient.



So for a flow rate of 0.5 mL/min, 12 seconds are required to expel one pistonful and 24 seconds to complete an entire cycle. If the gradient were to begin changing on the start of the very next piston, an error of up to 12 seconds could occur. If it happened to start on one side every time, the delay could be 24 seconds! This is particularly a concern for late-eluting peaks, because the variable number of pistons containing $t = 0$ mobile phase before the profile was underway would cause a variable retention time offset (absolute error).

For this reason, **the BAS 200B tells the operator when to inject**, not vice versa. In general, the correct routine is:

1. For gradient runs, turn pump synchronization on (Section 5, screen 26). For isocratic runs, synchronization can be either on or off.
2. Equilibrate the system (Section 5, screen 4).
3. Load sample into the manual injection valve, but **do not inject it yet**.
4. Press INJECT. The BAS 200B will locate the start of the next *left* pistonful. In the meantime, the screen notifies you that it is SYNCHRONIZING.
5. When the left pistonful begins its forward delivery stroke, the screen changes to RUN IN PROGRESS. At this transition, **make manual injections**.
6. At the end of the run, the system returns to the $t = 0$ conditions to re-equilibrate and awaits a new INJECT command.

6.7 Maintenance

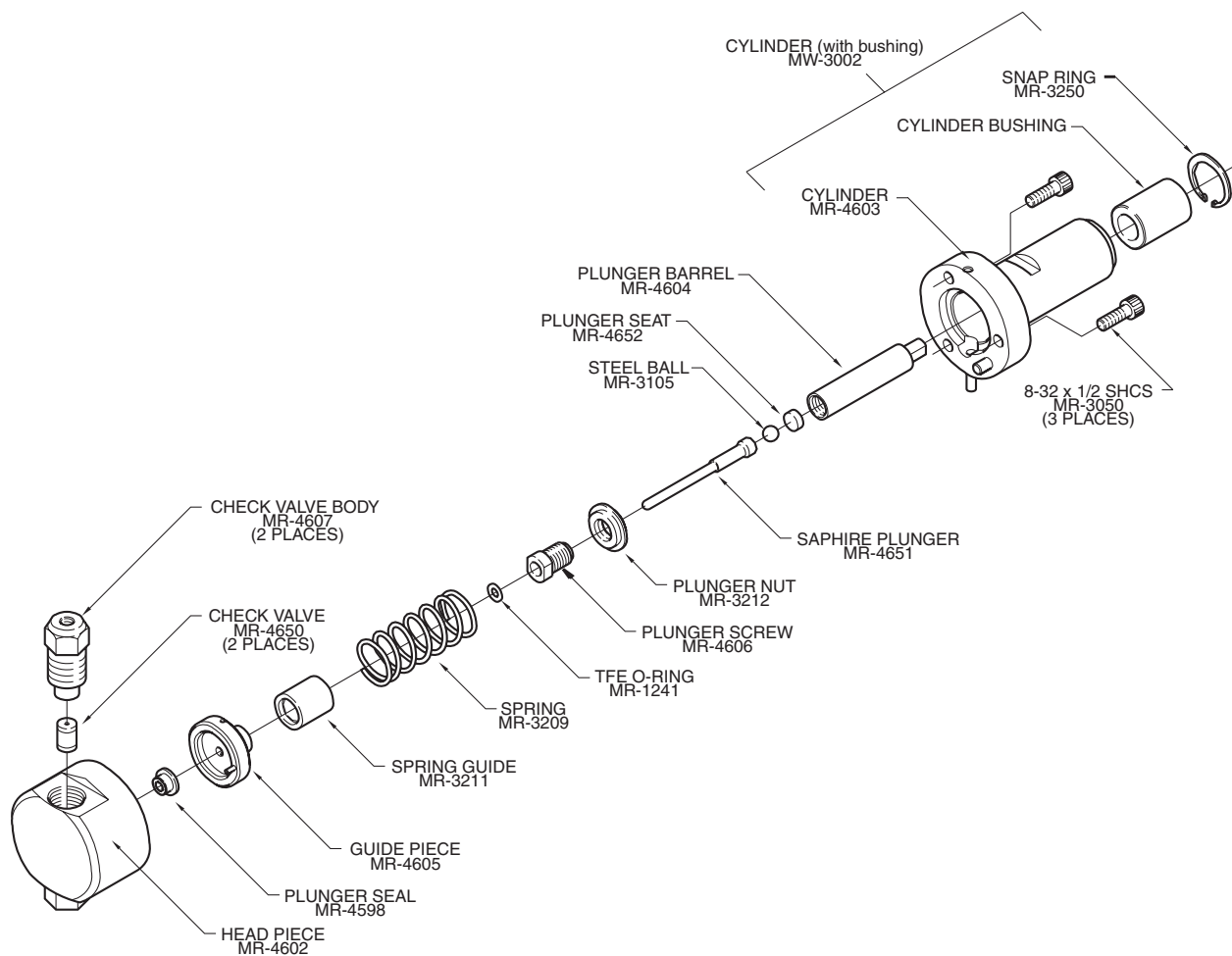
6.7.1 Pump Head Servicing

The procedures outlined below encompass all user services that require removal of the pump heads. (See Figure 6.10 for a detailed view of pump head parts.) They include:

- pump head replacement
- plunger seal replacement
- plunger spring replacement
- plunger free-play adjustment
- plunger replacement

This section will detail the entire procedure, from head removal to reinsertion. The text will indicate which sections can be skipped when performing simple maintenance.

Figure 6.10 Exploded view of pump head.



A. Pump Head Removal

An entire pump head may be 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 toward the remaining pump head.

CAUTION! The pump head will be ejected with some force!

4. To install a new pump head, proceed to section F.

B. Pump Head Disassembly

Servicing and 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. Slightly loosen all three screws 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 (Figure 6.11).
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 (Figure 6.12).

Figure 6.11 Removal of pump head rear section.

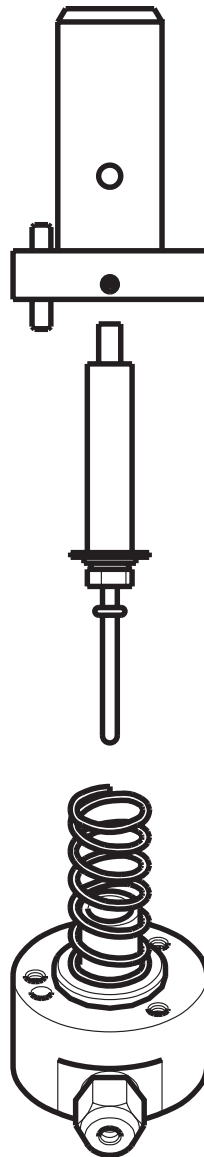
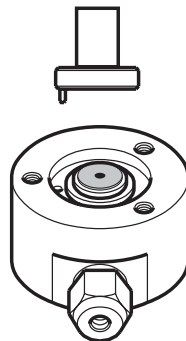


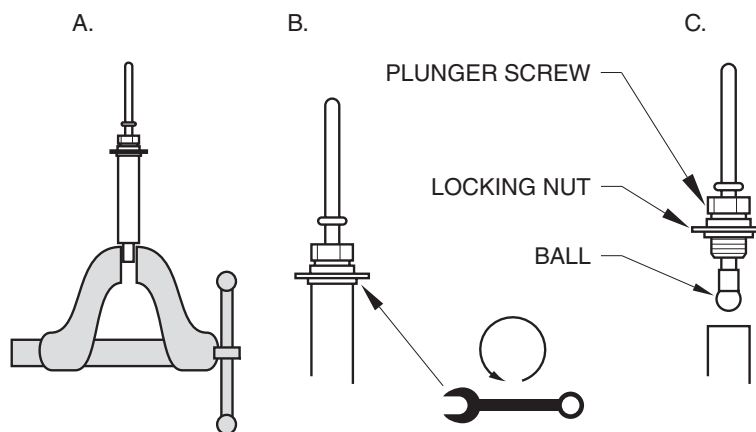
Figure 6.12 Exposing the plunger seal.



C. 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 with gentle scrubbing using a green Scotchbrite[®] scouring pad moistened with water. If the plunger is chipped, scored, or scratched, replace it and 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 section D (seal replacement) or section E (reassembly). 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 (Figure 6.13). The tip of the piston assembly has two flat surfaces that the vise can grip. Do not overtighten the vise; this will damage the assembly.
5. Loosen the plunger locking nut by turning it 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, then remove and replace the rod. A small ball bearing sits at the base of the rod, and is held in place with a dab of grease; do not lose this 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.

Figure 6.13 Replacing or adjusting the sapphire rod.

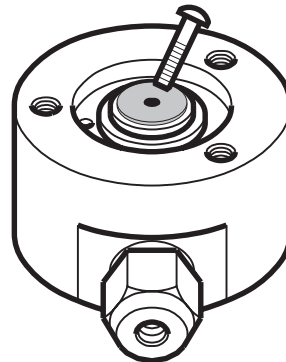


7. To adjust the new or old sapphire rod, tighten or loosen the plunger screw by hand, while testing free-play (see item 2 for free-play test procedure). When the adjustment is correct, hold the plunger screw with a wrench and tighten the plunger locking nut (clockwise) to hold the adjustment.
8. Retest the plunger free-play (see item 2). There is a tendency for the rod to bind slightly when the plunger locking nut is tightened. You may have to loosen the plunger locking nut again, and back off the plunger screw a bit for a final adjustment.
9. Proceed to section D (plunger seal replacement) or section E (reassembly).

D. 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 (Figure 6.14). Be careful not to scratch the metal of the pump head with the screw.

Figure 6.14 Removing the plunger seal.



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 (P/N MR-4598) with methanol, and push the seal fully into its seat with your thumb or the guide piece. Be careful not to scratch the seal with your fingernail.
5. Proceed with pump head reassembly (section E). Be sure to follow the break-in procedure for new plunger seals (section 6.7.2) after the pump is reassembled.

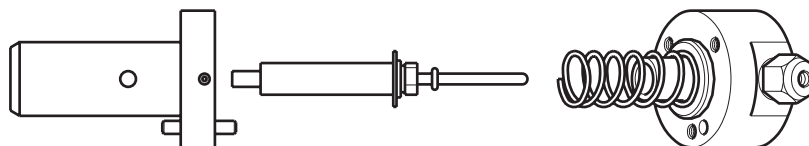
E. Pump Head Assembly

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 (Figure 6.12), with its registration pin seated into the matching hole on the inner face (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 (Figure 6.10) 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. Reinstall the o-ring on the sapphire rod.
6. Irrigate the plunger-seal area and the sapphire rod with methanol.

IMPORTANT! SEAL MUST BE MOIST BEFORE YOU INSTALL THE PLUNGER ASSEMBLY!

7. Insert the plunger assembly with sapphire rod entering the plunger seal (Figure 6.15).
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.

Figure 6.15 Reassembly of the pump head.



F. Pump Head Installation

1. Push the pump head into place in the solvent delivery system.
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 this startup procedure:
 - a. Turn the POWER on.
 - b. Flush the plunger-irrigation ports with a few drops of water (see Figure 6.7). A plastic squeeze bottle is ideal for this.
 - c. Attach a 50-mL disposable syringe to the prime/purge valve and open the valve one turn (system pressure must be below 30 psi before opening the valve).
 - d. Purge the system at 5 mL/min for 5 minutes. Use the PURGE option found in the PUMP section on the System Director. Set the conditions as in Figure 6.8 (assuming a 5 minute purge from bottle A). Press PURGE from this screen and the pump will slowly increase flow rate to reach preset pressure. Since the prime/purge valve is open, this pressure will not be reached, and the pump will attain its maximum flow rate of 5 mL/min. During this purge you may alternately draw back and release the syringe plunger to help dislodge air bubbles from the system. (If there is no column attached, turn the injector valve halfway through its arc to prevent sucking air from this direction into the syringe.)
 - e. The pump will automatically stop after 5 minutes and the Pump Control screen will appear on the System Director. Set the pump flow to 1 mL/min. Close the prime/purge valve and turn the injector valve to the INJECT position.
 - f. 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 and collect the waste solvent at the outlet of the injection valve.
 - g. To change solvents, repeat steps d–f as appropriate.
 - h. If necessary, reset the pump flow to 1 mL/min or other appropriate setting.
5. If new plunger seals were installed, follow the break-in procedure in section 6.7.2.

6.7.2 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. Squirt a few drops of water into the irrigation ports in the pump heads.
2. Purge the system with filtered 40:60 (v:v) acetonitrile:water.
3. Attach a column to the system. It need not be a good column, as its only purpose is to provide back pressure.
4. Run the pump for two hours at a pressure of 3000–4000 psi. Adjust the pump flow as necessary to achieve this pressure.
5. After two hours, you may switch to mobile phase and begin chromatography.

6.7.3 Check Valve Servicing

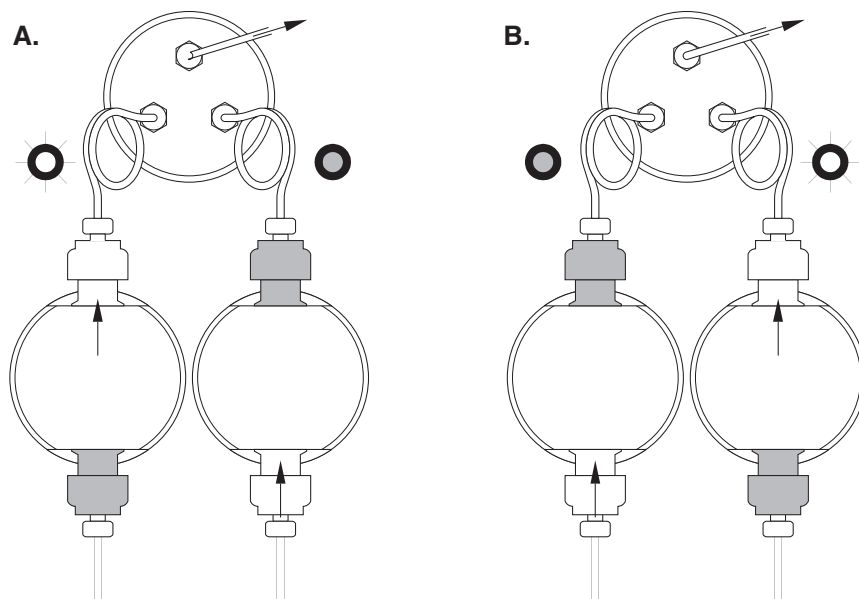
The BAS 200B solvent delivery system uses four cartridge-type check valves (P/N MR-4650) that do not contain removable parts. The same valve is used for both the inlet and outlet check valves on the pump. Each check valve cartridge contains two precision-engineered balls and seats in series, for a reliable 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 (section 6.7.1.F, item 4) with freshly degassed mobile phase or solvent. The check valves can be cleaned by sonication (section B) or 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. Figure 6.16 diagrams flow during a full cycle of two pump strokes. When the left pump head is compressing, the green LED above it lights. The inlet check valve below it closes, preventing any back flow. The outlet check valve above it opens, allowing mobile phase to proceed toward 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 back flow into the right pump head from the pressure transducer.

Figure 6.16 Solvent flow during compression of the left (A) and right (B) pump heads. Shaded check valves are closed.



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 were not to close, fluid would flow from the left pump head into the transducer, then down into the right pump head. The opposite applies to the right pump head.

Because of this relationship, there are three 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.

A. Check Valve Removal

1. Stop the pump and allow pressure to dissipate. Then turn off 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.

B. 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 with methanol.
4. Reinstall the check valve assembly following instructions in section D. If check valve problems continue, install a new cartridge (section C).

C. 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 (section B).
4. Insert the new cartridge. Be certain to install the cartridge so it allows flow in the proper direction (see Figure 6.16). 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.

D. Check Valve Installation

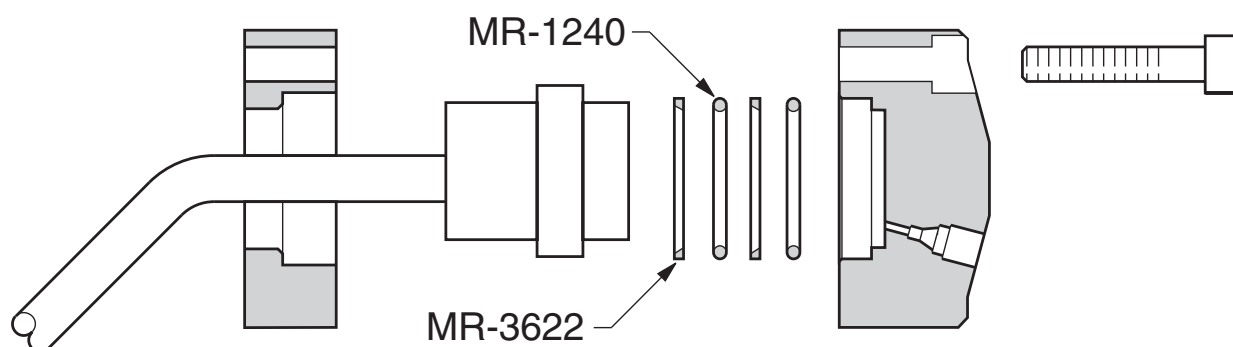
1. Squirt some methanol through the check valve. This will wet it and also allow you to observe whether the direction of flow is correct.
2. Install the check valve assembly onto the pump head with a wrench.
3. Hold the check valve assembly stationary with the wrench and connect the inlet or outlet tubing with a second wrench.
4. Follow the startup procedure in section 6.7.1.F (item 4) to prime the pump and remove all air from the check valves and lines.

6.7.4 Pressure Transducer Servicing

The pressure transducer will rarely need attention. If a leak ever develops, there are two PTFE o-rings inside that need to be replaced (P/N 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 three 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 three hex-head screws. Remove the front face of the housing and the two Teflon o-rings (Figure 6.17). Discard the o-rings. Save the steel spacers that were underneath the o-rings. Clean all parts that are to be installed.

Figure 6.17 Pressure transducer disassembly.



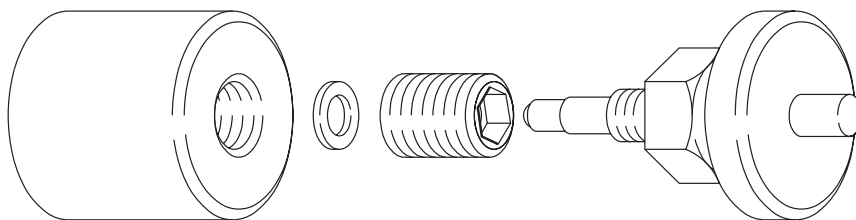
4. Stretch the two new o-rings slightly with your fingers. Do this a little at a time, until the o-rings just slide over the transducer (they should be snug, but not tight enough to get scratched on the tip of the transducer). NOTE: Avoid scratching, crimping, or tearing the o-rings. Use your fingertips, not tools. Be careful not to scratch the o-rings with your fingernails.
5. Install a steel spacer (chamfer down) over the transducer. Slide one o-ring on, then install the other steel spacer (chamfer down). Slide the last o-ring on. Be careful not to scratch the o-rings on the sharp corner of the pressure transducer.
6. Wet the o-rings with water. Install the front face of the pressure transducer onto its back face, observing the original alignment. Alternately tighten the three hex-head screws to 35 in/lbs.
7. Purge the pump (section 6.7.1.F, item 4).

6.7.5 Prime/Purge Valve Servicing

The prime/purge valve 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. Stop the pump and allow pressure to drop to zero. Turn the POWER switch off.
2. Open the valve-stem nut (Figure 6.18) all the way, and unscrew the valve stem. The entire stem assembly can now be removed.

Figure 6.18 Prime/purge valve disassembly.



3. Insert the metal hex wrench provided in the kit into the seal retainer, and turn counter-clockwise to remove. It may be necessary to grasp the tool with pliers for greater torque.
4. Straighten a paper clip and form a 1/8" right-angle bend at its tip. Reach into the valve body and carefully remove the old Teflon seal. Do not scratch the seat. Clean all parts to be reinstalled.
5. Insert the new Teflon 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.
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. Check that the valve stem turns without binding.
9. Purge the pump (section 6.7.1.F, item 4). Close the prime/purge valve firmly and check for leakage during normal operation.

6.7.6 Monitoring Pump Performance

The simplest method of monitoring pump performance is to develop a regular habit of observing the pressure display. Learn what is normal pressure and normal pressure fluctuation under your conditions, so you can recognize abnormal behavior.

Pump performance also can be monitored with a strip-chart recorder, using the analog pump-pressure output on the rear panel. First, set your chart recorder for an input of 10 V. Connect two wires from the TEST connectors to the input of the chart recorder. Above the TEST connectors is a hole labeled ADJUST. Use an electronic alignment tool to adjust the potentiometer screw within this hole until the chart recorder pen is about midscale. Now reduce the input voltage of the chart recorder in steps, adjusting the potentiometer as necessary. 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 V per 1000 psi. The chart can be calibrated with the following formula:

$$P = \frac{1000 \times D \times V}{W}$$

where:

P = pressure fluctuation (psi)

W = width of chart paper (mm)

D = magnitude of pen deflection (mm)

V = input range of chart recorder (V)

Run the chart recorder fast enough so that the shape of each pump stroke can be seen clearly. Record the input voltage, overall pressure, chart speed, and flow rate on the chart. Also indicate which direction represents a pressure increase (+) and which a decrease (-), and which strokes were made by the right pump head (R) and which by the left (L).

6.8 Pump Troubleshooting

6.8.1 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 section on pressure fluctuations if the difference between the highest and lowest pressures is greater than about 100 psi.

6.8.2 No Solvent Flow

If green LEDs DO NOT alternate:

1. Check flow rate setting. Is it a valid, nonzero range (0.1–0.5 or 0.1–5 mL/min)?
2. Is there an error message on the display? If so, call BAS.

If green LEDs DO alternate:

1. Air in the pump. Check for loose connections on the inlet side of the pump. Purge with freshly degassed mobile phase.
2. Mobile phase reservoir is empty. Purge with fresh mobile phase.
3. Solvent-uptake line is clogged.
4. Check valves malfunctioning.

6.8.3 Pump Stops: Low-Pressure Limit

1. The mobile phase reservoir is empty. Make fresh mobile phase and purge.
2. MIN PRES is set too high. Reset to 200 psi or a reasonable value for your conditions.
3. There is a leak in the system. Check all connections.

6.8.4 Pump Stops: High-Pressure Limit

1. MAX PRES is set too low. Reset to 4000 psi or a reasonable value (1000 psi above your typical operating pressure) for your conditions.
2. The injection valve is not fully in the inject or load position. Rotate it to one side.
3. There is a clog in the flow path. It could be anywhere between the pump outlet and the detector. Start opening fittings at the detector and work toward 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.)

6.8.5 Pressure Fluctuations

1. Purge the pump 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.
2. 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 check valve as described in Section 6.7.3.
3. 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 check valve as described in Section 6.7.3.
4. One or both pump heads may need an overhaul. See Section 6.7.1.
5. One of the outlet lines from the pump to the pressure transducer may be clogged. Remove and examine. Replace as necessary.

6.8.6 Fluid Leaks

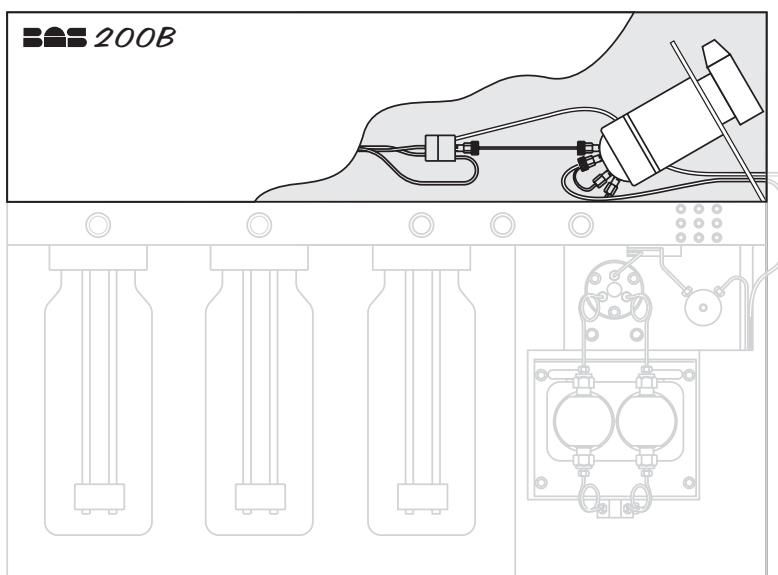
1. Fluid leaks from (or salt accumulates around) fittings. Leaks indicate that fittings need to be tightened or replaced (see Section 9.2).
2. Fluid leaks (or salt accumulates) around pump heads or from irrigation waste ports. This indicates that the plunger seals are worn and leaking. They must be replaced (see Section 6.7.1.D) to prevent internal corrosion of the pump head.
3. Fluid leaks (or salt accumulates) around the junction of the front and back halves of the pressure transducer. Replace pressure transducer o-rings (see Section 6.7.4).
4. Fluid leakage (or salt accumulation) at the prime/purge valve. Replace the seals.

Section 7. SAMPLE INJECTION

7.1 Manual Injection

The BAS 200B uses the widely accepted, readily available Rheodyne 7125 Syringe Loading Sample Injector in its standard configuration (see Figure 7.1). A modified Rheodyne 9125 injector is provided with microbore units. Both partial and full loop loading are possible with both injectors.

Figure 7.1 Location of injector on BAS 200B.



Coordinating Injections with the Start of a Run

1. Load sample into the manual injection valve or autosampler loop, but *do not inject it yet*.
2. Press INJECT. The BAS 200B will locate the start of the next *left* pistonful. In the meantime, the screen notifies you that it is SYNCHRONIZING.
3. When the left pistonful begins its forward delivery stroke, the screen changes to RUN IN PROGRESS. At this transition, *make manual injections*. Autosamplers are triggered to deliver automatically via a back panel connection.
4. At the end of the run, the system returns to the $t = 0$ conditions to re-equilibrate and awaits a new INJECT command.

The Rheodyne instructions are reproduced verbatim on the following pages, with permission from Rheodyne. BAS provides a full inventory of Rheodyne replacement parts for these valves.



Operating Instructions For Model 7125 Syringe Loading Sample Injector

1.0 INTRODUCTION

The Model 7125 Syringe Loading Sample Injector is a rotary valve designed for high performance liquid chromatography. Supplied with the valve in a separate bag are the following items (see Fig. 5B):

- Two socket wrenches;
- One open-end wrench;
- Two 22cm long tubes (0.51mm I.D.) for vents;
- Four tube fittings (threaded bushing and ferrule) for tubing connections to valve ports;
- One 7125 needle port cleaner;
- Two #8-32 screws for valve mounting.

The 7125 is supplied with a #22 ga needle with a CTFE hub (part #7215) which is shipped inserted in the needle port (underneath the red protective cap). This needle should be removed from the port before use of the valve, but it should be left fully inserted in the port during periods of idleness to keep the needle seal properly sized.

The standard sample loop supplied with the 7125 is the 20 microliter size (part #7022).

Following are the standard sample loops available. Each loop is supplied with fittings for direct connection to the valve.

Catalog number	Sample Loop size (µl)	Tubing bore	
		mm	inches
7020	5	.18	.007
7021	10	.30	.012
7022	20	.51	.020
7023	50	.51	.020
7024	100	.51	.020
7025	200	.76	.030
7026	500	.76	.030
7027	1000	.76	.030
7028	2000	1.00	.040
7029	5000	1.00	.040

2.0 WARRANTY

Rheodyne products are warranted against defects in materials and workmanship for a period of one year following date of shipment. Rheodyne will

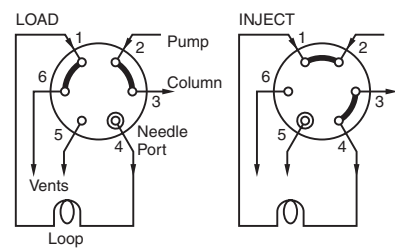


Fig. 1: Model 7125 Flow Diagram

make repairs or replacements free of charge upon return to the factory, transportation paid, of the defective item.

3.0 THEORY OF OPERATION

The Model 7125 is a six-port sample injection valve in which loading of the sample loop is accomplished with a syringe through a needle port built into the valve shaft.

Figure 1 shows the flow diagram of the valve. The six small circles represent the ports in the valve stator (rear of injector). The two heavy arcs represent the connecting passages in the rotor seal. The larger circle represents the needle port (in the rotor seal). The needle port is used to fill the sample loop in the LOAD position. In the INJECT position the loop is switched into the solvent stream and the needle port is vented through valve port 5. Rotation of the knob through 60° switches the valve from LOAD (CCW) to INJECT (CW).

Figure 2 shows the needle port geometry. When the syringe needle is fully inserted, the flat tip of the needle touches the flat face of the stator so that the entire volume of sample discharged from the syringe enters the stator passage which is part of the sample loop. Therefore, all of the sample discharged from the syringe becomes injected onto the column. There is no sample loss.

The flat stator face is polished alumina ceramic - a hard surface which cannot be damaged by the needle tip.

The maximum operating temperature of Model 7125 is 80°C.

Two methods of loading the sample can be used: The complete loop filling method and the par-

tial filling method. Following is a description of these methods.

3.0.1 FILLING THE LOOP COMPLETELY

This is the conventional method in which an excess of sample is used to insure that the sample loop is completely filled. The volume of sample is determined precisely by the loop volume and the highest degree of precision is obtained. The method is explained in Section 6.0.1.

3.0.2 PARTIALLY FILLING THE LOOP

If only small quantities of sample are available, this is the method of choice. In this method a microsyringe is used to determine the volume of sample delivered to the loop. The loop has been previously filled with solvent from the last run and the syringe delivery causes the solvent to be displaced by the sample. The displaced solvent exits from vent tube 6.

With this method it is possible to inject samples ranging from less than one microliter up to approximately 50% of the loop capacity. Sample loops can be used on the Model 7125 ranging from 5 microliter capacity up to 5 milliliters. The partial filling method of injection is described in Section 6.0.2.

4.0 GETTING STARTED

To prepare for initial use of the Model 7125, you should follow the installation and start-up instructions in Sections 5 and 6. Also be sure to read the **CAUTION** and **WARNING** notes in section 4.1. Sections 7, 8 and 9 give helpful operating suggestions, maintenance and servicing information.

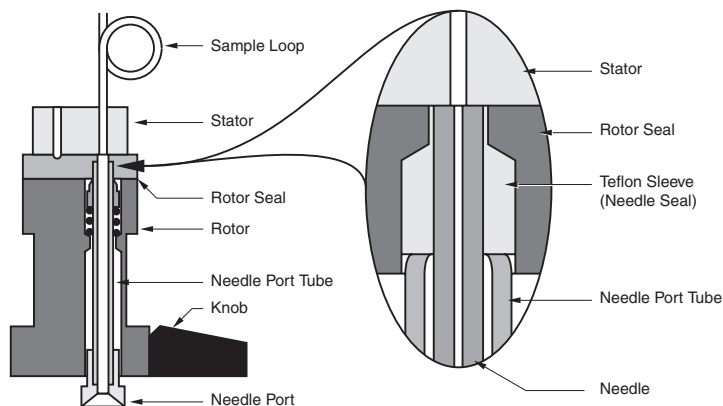


Fig. 2: Model 7125 Needle Port Geometry

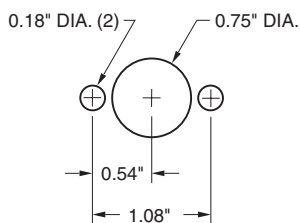


Fig. 3: Panel Holes Required

4.1 IMPORTANT SAFETY NOTICES

4.1.1 CAUTION:

Be sure to use the proper size of syringe needle to avoid damage to the valve. See Section 4.2.

4.1.2 WARNING:

When using the Needle Port Cleaner, discharge the syringe slowly to avoid squirting solvent back at yourself. See Section 5.1.

4.1.3 WARNING:

When using sample loops larger than the 100 microliter size, protect yourself from the rapid ejection of mobile phase coming out of the needle port when the valve is turned from INJECT to LOAD. See Section 9.9.

4.2 USING PROPER SYRINGES

In both methods of sample loading it is necessary to insert a syringe needle into the needle port. In the complete filling method it is possible to use any syringe with Luer tip together with the needle supplied with the 7125. In the partial filling method, conventional low pressure microsyringes can be used. These are available from several manufacturers and should have the following needle specifications:

Needle dimensions: 0.028" O.D. x 2" long, without electro taper. Point style 90 (square end).

Failure to use needle of proper size can result in damage to the injector.

Rheodyne also supplies suitable microsyringes. Following are the part numbers:

7201 10 μ L Syringe
7202 25 μ L Syringe
7205 50 μ L Syringe
7210 100 μ L Syringe
7225 250 μ L Syringe
7250 500 μ L Syringe

5.0 INSTALLATION

a) Figure 3 shows the panel holes required for mounting the Model 7125. In addition you may need a hole to run the two vent tubes through the panel. Maximum panel thickness is 0.19". To mount the valve, first remove the knob by loosening the two knob set screws. Use the two #8-32 screws supplied to fasten the valve to the panel.

If the Rheodyne #7161-020 Valve Position Sensing Switch is to be used, refer to the mounting instructions for that accessory.

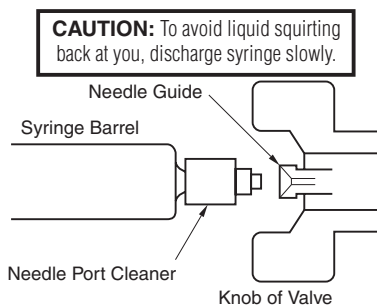


Fig. 4: Use of Needle Port Cleaner

b) When replacing the knob be sure that the two set screws are tightened securely on the two flats on the shaft. The third threaded hole in the knob is left blank. The set screws should be tightened only on flats.

CAUTION: To avoid liquid squirting back at you, discharge syringe slowly.

c) Connect the two 22cm long tubes (supplied) to valve ports 5 and 6. Use the longer threaded bushing on port 6, and the short one on port 5. Both of these tubes should be bent so that their ends point downward to facilitate collecting the vented liquid. To prevent siphoning effects, the outlet ends of both tubes should be at the same horizontal level as that of the needle port.

d) Connect pump to port 2 and the column tube to port 3, using the longer threaded bushing for the column tube. Leave the column disconnected from the valve during initial cleaning operations.

The use of a Rheodyne Column Inlet Filter will protect your column inlet frit from plugging from particles in the sample or from pump and valve wear particles.

5.1 USING THE NEEDLE PORT CLEANER

Connect the 7125 Needle Port Cleaner (part #71 25-054) to a syringe as shown in Figure 4. A syringe of at least 2 ml capacity with Luer tip (not Luer Lock) is recommended - such as Rheodyne part #7252. Use the cleaner to flush out the needle port with mobile phase with the valve in the INJECT position. To do this, push the tip of the cleaner against the conical opening of the Needle Guide and discharge the syringe slowly. Observe **WARNING 4.1.2**. The discharged liquid can squirt back at you if you are not careful.

5.2 INITIAL OPERATION

To clean out the valve and prepare it for connecting to the column, establish solvent flow through the valve. Turn valve to LOAD, then INJECT (to fill the sample loop). Use the 7125 Needle Port Cleaner to flush out the needle port with about 1 ml of mobile phase. This will leave the needle cavity filled with mobile phase. The excess solvent will flow out port 5 and leave vent tube 5 filled with solvent. Repeat the flushing step with the valve in LOAD position. This will fill vent tube 6.

Before connecting the valve to the column, make some practice injections using mobile phase. Follow the instructions in Section 6. After practicing, connect the column and proceed with the chromatography.

6.0 MAKING AN INJECTION

6.0.1 FILLING THE LOOP COMPLETELY

This is the conventional method in which the volume of sample injected is precisely determined by the volume of the loop plus valve passages. Use a syringe of suitable capacity together with the #22 ga needle supplied (90° point).

- Observe **WARNING 4.1.3** and turn valve to LOAD.
- Load the syringe with sample.
- Insert the syringe needle into the needle port all the way until the hub almost touches the needle guide. The needle tip will touch the stator face. Do not push hard on the syringe - just be sure it is bottomed.
- Gently discharge the syringe to completely fill the loop.
- Leave the syringe in position and turn the valve to INJECT.
- Remove the syringe.
- Alternatively, use the syringe to suck up the sample from the vial into which vent tube is dipped.
- Be sure that the loading passages have been flushed with solvent after the last injection to prevent cross-contamination between runs.
- In this method, vent tube 5 is not used. Flushing of the needle port and valve passages is done with the valve in LOAD position.

In the complete filling method, an excess of sample must be used because the fluid velocity in the sample loop tubing varies from a maximum at the tube axis to zero at the wall. As sample pushes solvent ahead of it during loading, the locus of the sample-solvent interface becomes diffuse. Solvent remains along the wall. The amount of sample in the loop approaches its maximum value asymptotically. About 2 to 3 loop volumes of sample are required to achieve 95% of maximum. This is the minimum recommended for good precision, but 5 to 10 loop volumes will provide better precision. You should determine this experimentally for yourself.

6.0.2 PARTIALLY FILLING THE LOOP

In this method, the syringe delivery determines the sample volume injected (see Section 3.0.2).

- With valve in INJECT position, use the 7125 Needle Port Cleaner (part #7125-054) to flush out the needle port with about 1 ml of eluting solvent. This will flush out residual contamination from the previous injection. The flushing liquid will exit out of vent tube 5.
NOTE: See Section 9.3 for possible elimination of this flushing step.
- Observe **WARNING 4.1.3** and turn valve to LOAD.
- Load the syringe with the desired sample volume
- Insert syringe needle into the needle port all the way until the hub or barrel almost touches the needle guide. The needle tip will touch the

stator face. Do not push hard on the syringe - just be sure it is bottomed.

- e) Gently discharge the syringe contents.
- f) Leave syringe in position and turn valve to INJECT.
- g) Remove the syringe and leave the valve in INJECT position.

In the partial filling method, no more than half a loop volume of sample should be passed into the loop in order to maintain volumetric accuracy. With larger volumes some of the sample is lost out vent tube 6. This is because of flow velocity inequalities throughout the loop tubing.

7.0 ADJUSTING FOR HIGHER PRESSURE OPERATION

The three small set screws in the stator (see Figure 3) have been factory-set so that when the three stator screws are fully tightened, the spring force between the valve rotor and stator is sufficient to hold 34 MPa (5000 psi). If leakage is to be corrected, or if operation up to 48 MPa is to be done, proceed as follows: The three set screws should be **loosened** about 1/20 turn each (18° of rotation) and the three stator screws **tightened** an equal amount. If this new setting fails to accomplish leak-free operation, repeat the procedure by an additional 1/20 turn. Avoid excessive tightening which will accomplish nothing but increased wear of the rotor seal. If it is necessary to loosen spring tension, either to lower the operating pressure or to adjust for a new rotor seal which may be thicker than the one being replaced, reverse the above procedures; i.e., first loosen the stator screws. Then tighten the set screws.

8.0 MAINTENANCE

With normal use, the Model 7125 should give thousands of injections without trouble. The main causes of premature failure are:

- a) Incorrect needle tip shape can cause the ceramic stator face to chip, which then causes deep scratching of the rotor seal surface.
- b) Abrasive particles in the sample which cause scratches on the rotor seal surface.

The rotor seal wears with use and is the only part that routinely needs replacement. It can be expected to last between 6 months and 2 years.

8.1 CHANGING ROTOR SEAL

To change the rotor seal proceed as follows:

- a) Leave the Model 7125 attached to the panel and leave knob on. If it is necessary to remove the valve from the panel before servicing, then replace the knob after removal from panel (this simplifies the disassembly).
- b) Remove the three stator screws (refer to Figure 5). Do not change the setting of the set screws in the stator at this time.
- c) Remove stator, stator face, and stator ring from valve body by pulling axially to disengage the various pins.
- d) Remove the rotor seal by prying it off of the four seal pins, using a screwdriver or knife blade.
- e) The isolation seal and bearing ring usually are left in place because they rarely need changing.

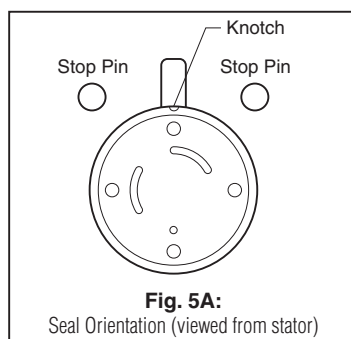


Fig. 5A:

Seal Orientation (viewed from stator)

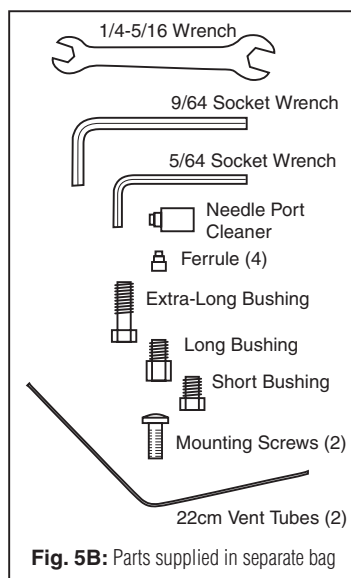


Fig. 5B: Parts supplied in separate bag

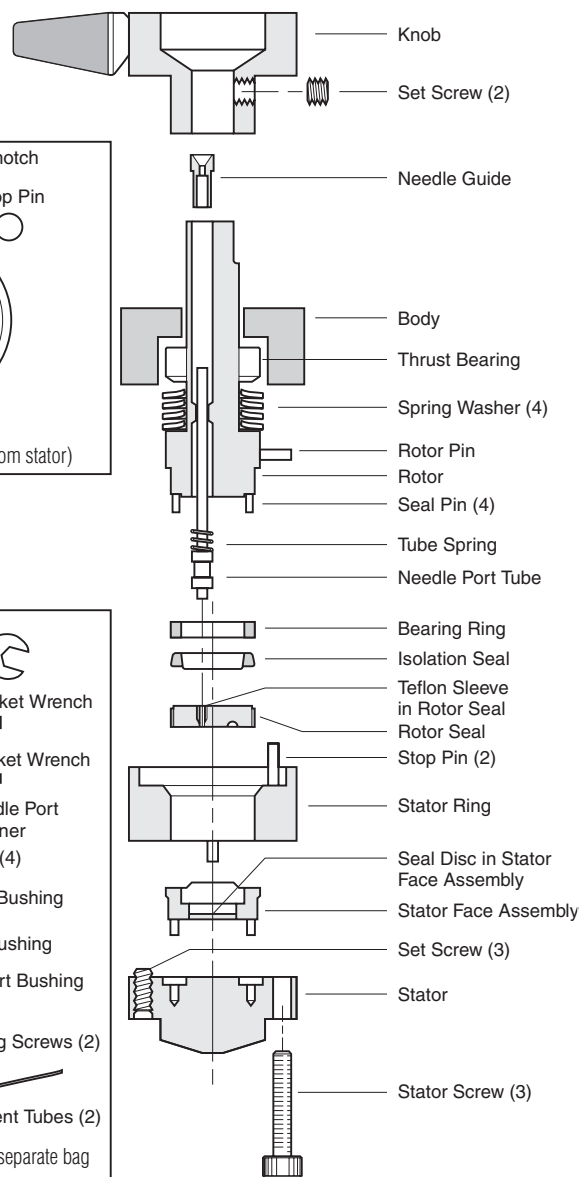


Fig. 5: Model 7125 Exploded View

- f) Install a new rotor seal (part #7125-047) on the four seal pins by following the reassembly procedure in Section 8.2.

8.2 REASSEMBLY

Reassembly of the valve requires putting all parts back together as shown in Figure 5 while observing the following:

- a) Be sure that the rotor seal is correctly oriented as shown in Figure 5A with rotor seal slots facing the stator. The needle port tube will be in line with the Teflon sleeve in the rotor seal when the rotor seal is in the correct position.
- b) In replacing the stator ring, be sure that the two stop pins are replaced in their holes in the stator ring, then push the stator ring squarely onto the rotor assembly so that the stop pins enter

the mating holes in the body (with the rotor pin between the two stop pins) and so that the isolation seal slips inside the stator ring without hanging up.

- c) Make sure that the seal disc is in place in the stator face assembly and that its holes are aligned with the six holes in the ceramic. This seal disc usually stays tightly in place inside the stator face assembly and should not be removed.
- d) In replacing the stator face assembly on the stator, the small notch in the rim of the face assembly should be in line with port 1 of the stator. Make sure that the two pins enter the holes in the stator.
- e) Mount the stator and stator face assembly on the valve by pushing it onto the two pins on the

stator ring and then adding the three stator screws. Be sure that the stator face assembly remains properly in position on the stator. It has a tendency to fall off; so, be careful. Tighten each screw a little at a time to keep the stator surface parallel to the stator ring surface. If the three set screws in the stator were left unchanged, then tighten the three stator screws until all parts are held firmly in place. The three set screws will insure that the gap between stator and stator ring is uniform and exactly as it was before disassembly.

- f) If the set screws need adjusting because a new rotor seal was installed or because leakage has to be stopped, refer to Section 7.0 and be sure that each set screw is turned an equal amount so that after the stator screws are retightened, the gap between the stator and stator ring is uniform all around.
- g) Note that there are three threaded holes in the knob but only two set screws are used. The set screws should be tightened only on the two flats milled on the shaft.
- h) After installation of a new rotor seal, it is usually necessary to form the needle seal around your syringe needle. See Section 9.2.

8.3 SUGGESTED SPARE PARTS

7125-047

Vespel Rotor Seal for 7125/7126 (standard)

7125-079

Tefzel Rotor Seal for 7125/7126

Spare fittings for new tubing connections:

7010-009 Bushing

7010-011 Long Bushing

7010-062 Extra Long Bushing

7010-010 Ferrule (one required for each make-up)

9.0 OPERATING SUGGESTIONS AND TROUBLE-SHOOTING

9.1 LEAKAGE

If liquid is observed dripping out between stator and stator ring, the stator screws should be tightened as explained in Section 7.0. Leakage out the needle port or vent tube (other than that caused by loading the loop) is caused by scratches on the rotor seal. Try tightening stator per Section 7.0, or, if this fails to stop the leakage, replace the rotor seal (part #7125-047).

NOTE: If the vent tubes from ports 5 and 6 do not have their outlet ends at the same horizontal level as the needle port, siphoning can occur which is often misinterpreted as "leakage."

9.2 NEEDLE SEAL LEAKAGE

Because the outside diameter of the syringe needle varies from syringe to syringe, the needle seal (Teflon sleeve in the rotor seal) may not immediately seal properly around a needle which is smaller than average. This will result in a loss of accuracy in loading the sample. The spring loaded needle tube will eventually reform the Teflon sleeve to make a good seal, but if you do not want to wait, do the following:

With needle removed from the needle port, push on the plastic needle guide. This will assist the spring in deforming the Teflon sleeve. Do not push so hard that the Teflon sleeve is squashed too much. A few gentle tries will produce the desired result.

To check for a proper liquid seal around the syringe needle, fill the syringe with water and slowly discharge the syringe with the injector in the LOAD position. Notice the lack of resistance to syringe discharge. Now repeat the action with the injector handle half-way between LOAD and INJECT. (The pump must be off in this position.) Now it should feel noticeably harder to discharge the syringe.

The needle seal is designed to seal around the needle only to a few psi of pressure. Since a microliter syringe can produce much higher pressure with just a small force on the plunger, do not expect the needle seal to completely prevent syringe discharge with the handle in the half-way position.

9.3 FLUSHING BETWEEN INJECTIONS

Measurements have indicated that under proper operating conditions the residual sample left in the needle cavity and on the needle seal surface after an injection varies between .001 and .01 microliter. This represents 0.1% to 1% of a 10 microliter injection. If this amount of cross-contamination between successive injections is acceptable, then you do not need to flush the needle port between injections. Eliminate step (a) in Section 6.0.2.

However, it is wise to check the magnitude of cross-contamination periodically and to use the flushing step when in doubt. Conditions that can produce excessive cross-contamination are:

- a) Needle is too short so that the needle tip does not touch the stator face. Minimum length from hub to needle tip should be 5.00 cm (1.97 inch).
- b) Syringe is not held in place with needle bottomed in needle port while turning from LOAD to INJECT.
- c) Dirt particles or needle seal shavings in the needle port are preventing the needle tip from touching the stator face.

Even when cross-contamination is not a concern, it is good practice to flush the valve (Section 5.1) about every ten injections. This prevents buildup of contamination and also keeps the needle port and vent tube 5 filled with solvent, preventing air from inadvertently entering the sample loop.

9.4 USE OF AQUEOUS BUFFERS OR SALT SOLUTIONS

To prevent the formation of salt crystals in the valve, flush out the flow passages and the needle port with water after usage of salt solutions.

9.5 USE OF HIGH pH SOLUTIONS

The standard rotor seal is Vespel, a DuPont polyimide which has exceptionally good wear resistance. However, it is susceptible to alkaline attack, deteriorating rapidly when used with solutions of pH over 10. An alternative material, Tefzel, is available for alkaline applications (see Section 8.3).

9.6 PLUGGED VALVE PASSAGES

If valve passages get plugged, they can be opened by removing the stator as described in Section 8.1 and cleaning the passages with a small wire (.015-inch maximum diameter).

9.7 KEEPING NEEDLE SEAL IN PROPER SHAPE

During periods of idleness of the chromatograph, such as overnight or during weekends, it is advisable to leave the 22 ga needle (supplied) fully inserted in the needle port. This will keep the needle seal (Teflon sleeve in the rotor seal) in proper shape and prevent it from being squeezed down too small.

9.8 CALIBRATING SAMPLE LOOPS

Sample loop sizes are designated by nominal values, which can vary from the actual value by as much as 20%. This is due to the .001" tolerance on the tubing I.D. (see table below). Since both standards and unknowns are usually analyzed with the same loop, knowledge of the absolute loop volume is rarely needed. If the actual loop volume must be known, it is best to calibrate it in place on the valve, so that the flow passages in the valve (one in the rotor and two in the stator) are also taken into account.

Tubing bore	Volume tolerance resulting from bore .001" tolerance
.012"	± 17%
.020"	± 10%
.030"	± 7%

9.9 USE OF LARGE SAMPLE LOOPS

When large sample loops are used, a few microliters of mobile phase will be expelled from the needle port and vent tube 6 when the valve is returned to LOAD. This happens because the compressed fluid in the sample loop expands when it is exposed to atmospheric pressure. Since the compressibility of most solvents is about 10^{-4} per atmosphere, the solvent in a 1-ml sample loop will expand about 20 μ l upon decompressing from 21 MPa (3000 psi). Observe WARNING 4.1.3. A small test tube or absorbent tissue can be placed at the needle port when returning the valve to LOAD, in order to safely catch the expelled liquid.

9.10 CHANGING SAMPLE LOOPS AND COLUMN CONNECTIONS

The depth of the tubing holes in the valve ports may vary slightly from port to port and from valve to valve. A fitting made up in one port may leave a dead space in another port. It is good practice, therefore, to label sample loops so that if they are removed, they will be replaced in the same orientation in the same valve. Also be sure that the column connecting tube at port 3 is made up with no dead space.

7.2 Interfacing the BAS 200B with an Autosampler

The BAS 200B can communicate with autosamplers for automatic operation. The specific procedure to be used depends on the type of autosampler that is available.

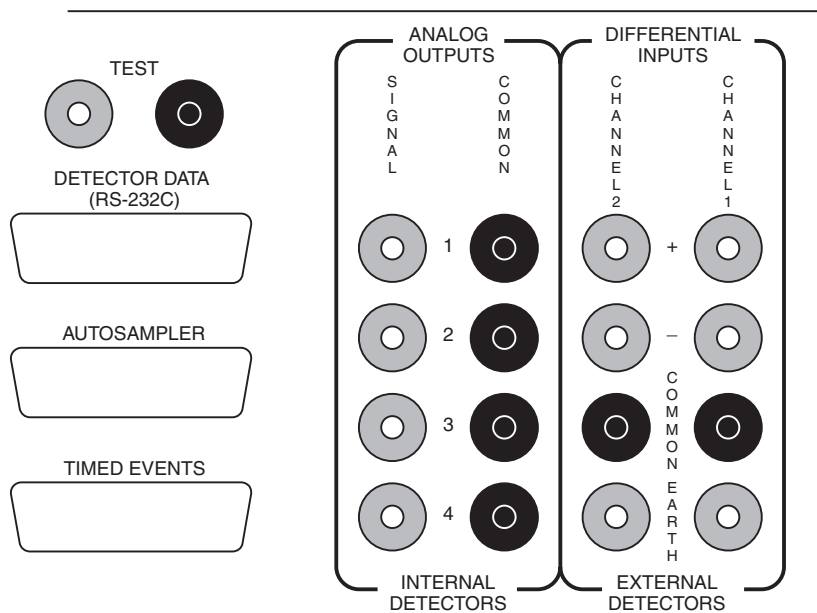
The CMA\200 Microsampler and BAS Sample Sentinel™

The CMA\200 Microsampler and BAS Sample Sentinel autosampler are capable of two-way communication with the BAS 200B. 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, both autosamplers provide the vial position for each injection to ChromGraph, BAS' computer-based control and data processing system.

Connect to the autosampler with cable ER-9510 (CMA\200) or EW-4456 (Sample Sentinel). Use the AUTOSAMPLER (not autosampler/RS-232) connection on the back of the BAS 200B (Figure 7.2). Plug the other end of the cable into the REMOTE connector on the CMA\200's power supply. The Sample Sentinel has two terminal strips on its rear panel that match its Y-shaped cable.

Once the cable is installed, you must program the chromatograph and the autosampler. In the CMA\200's System Specifications, TTL INPUT POLARITY must be set to "00000000." For the Sample Sentinel, change INJECT HOLD ACTIVE to HI in the OPTIONS section of its menu.

Figure 7.2 Rear panel of the BAS 200B. Communication cables, ER-9510 and EW-4456, should be plugged into the AUTOSAMPLER connector. Cable EW-8162 uses the TIMED EVENTS connector.

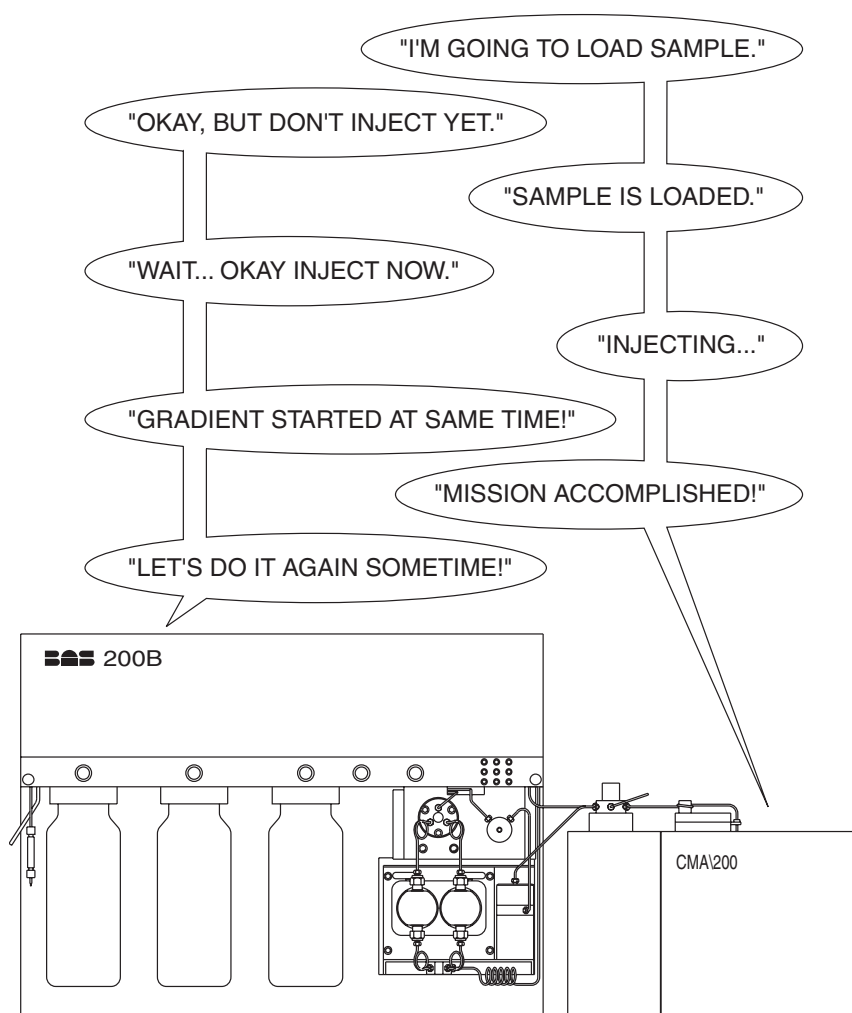


It's best to make the autosampler's run length a minute longer than that of the chromatograph. This will ensure that the BAS 200B is ready to receive the "sample loaded" message from the autosampler, and minimize the time the sample sits in the loop.

Program the BAS 200B as you normally would. AUTO RUNS should be off. If gradients are being used, turn pump SYNCHRONIZATION on. Make sure that any re-equilibration time at the end of the gradient is included in the run times for both the autosampler and the chromatograph.

A consideration of the sequence of events during a typical run (see Figure 7.3) is helpful for an understanding of the interaction between the instruments, and for troubleshooting.

Figure 7.3 Two-way communication between the BAS 200B and the CMA/200.



The sequence of events is as follows:

1. To put the BAS 200B in a ready state, set the LCD System Director to the equilibrating mode (the detectors should be equilibrated and ready to go).
2. If you are using ChromGraph CONTROL software, enter the number of samples to be injected. Set the remote trigger and start the run. The chromatograph is now ready to accept a start signal from the autosampler.
3. Now start the autosampler. It will first go through a flush, then load a sample into the loop.
4. When the sample is loaded, the autosampler will send a start signal to the BAS 200B. If this signal is not received (e.g., if the cable is unplugged, or if the BAS 200B is not in the equilibrating mode), neither unit will do anything. The chromatograph will not start its run, and the autosampler will not inject the sample. This is an important safeguard for your samples; if the unattended chromatograph runs out of mobile phase, it will shut down and no samples will be injected.
5. When the start signal from the autosampler is received, the BAS 200B starts its run. It sends two start signals; one triggers the autosampler to inject, and the other triggers a remote data-collection device (if connected). The autosampler will flush itself after injecting, then do nothing for the remainder of the run. Only when its run time has elapsed will it load the next sample.

Other Autosamplers: Who's in Charge?

Many autosamplers do not have the communications ability of the CMA200 and Sample Sentinel. To operate successfully with other autosamplers, the BAS 200B and the autosampler must operate in a master/slave relationship.

We cannot expect two instruments working together to perform integrated timed runs without some means of synchronization. No two clocks keep exactly the same time. If, for example, you were to program both the chromatograph and the autosampler to do repetitive 10-minute runs, and started them at the same time, eventually they would become out-of-sync. The start times would separate due to differences in the clocks, and injections would occur in the middle of the run. Therefore, one instrument must be in charge and tell the other instrument when to start its run. This effectively resynchronizes the clocks at the start of each run, so they never get significantly out-of-sync. The choice of which instrument is the master depends on the capabilities of the autosampler.

BAS 200B in Charge

It's best to have the BAS 200B in charge, for two reasons. First, if the chromatograph were to break down, it would not send inject signals to the autosampler, so the samples would be preserved. The second reason applies only to gradient runs. A gradient run on the BAS 200B does not actually start when you press the inject button. If pump synchronization is on (which it should be for gradients), the run starts only at a predefined position of the pistons. This ensures that the gradient is formed precisely from run to run, which reduces variation in retention times (see Table 7.1). The time it takes for the pistons to reach their predefined positions is variable, depending on pump speed and on their positions at the time the inject button is pressed. If an autosampler were in charge of a gradient run, it would inject the sample at the same time it signaled the BAS 200B to start its run. But the BAS 200B would then wait a variable amount of time until the gradient actually started. This variable delay would wreak havoc on retention times and therefore peak heights.

Table 7.1 Retention times (minutes) of six steroids with gradient elution.

	<u>Peak 1</u>	<u>Peak 2</u>	<u>Peak 3</u>	<u>Peak 4</u>	<u>Peak 5</u>	<u>Peak 6</u>
<u>Synchronized (n = 10)</u>						
mean	1.80	2.81	3.34	3.61	5.01	7.99
SD	0.01	0.01	0.01	0.01	0.02	0.03
%SD	0.70	0.50	0.40	0.30	0.50	0.40
<u>Nonsynchronized</u>						
mean	1.79	2.84	3.37	3.66	5.09	8.07
SD	0.06	0.06	0.06	0.06	0.05	0.08
%SD	3.06	2.03	1.69	1.54	0.94	0.93

Putting the BAS 200B in charge requires an autosampler that will load the sample into the loop, then wait for an inject signal. Some autosamplers provide SAMPLE ENABLE terminals, which are normally shorted together by a jumper for independent operation. Removing the jumper prevents the autosampler from injecting until a switch closure is received across these terminals. BAS has designed a cable (P/N EW-8162) to conduct start signals to these autosamplers and to other peripheral equipment.

The 25-pin end of the cable is connected to the TIMED EVENTS connector on the back of the BAS 200B (Figure 7.2). Color-coded leads carry signals from all four timed-event relays in the BAS 200B, as well as remote start and stop functions for the chromatograph. A key to pin numbers and wire colors appears in Table 7.3, and on an instruction sheet packed with the cable. Connect one timed-event lead (TE-1) and its ground to the SAMPLE ENABLE terminals, and another (TE-2) to the integrator or data station. Set TE-1 (timed event switch 1) and TE-2 to turn on at time zero, and to turn off at 0.1 minutes (a six-second closure) as in Table 7.2. For convenience, make this programming a permanent part of all your methods, so it's in place when you wish to use peripheral equipment.

Table 7.2 Timed Events file from BAS 200B control software.

Line #	Minutes	Event 1	Event 2	Event 3	Event 4
1	Default	OFF	OFF	OFF	OFF
2	0.0	ON	ON	OFF	OFF
3	0.1	OFF	OFF	OFF	OFF

Programming for the BAS 200B-in-charge mode follows one simple rule: *the autosampler's run length must be shorter than that of the chromatograph*. Otherwise you will get only one sample injection for every two chromatograph runs. The following sequence of events will make this clear:

1. To start the system, first start the autosampler. After flushing, it will load the sample and wait for a trigger from the chromatograph.
2. Equilibrate the BAS 200B with AUTORUNS on.
3. Specify the number of samples to be processed, and press inject.
4. The start of the run will trigger the autosampler to inject, and the data system to begin collecting. If properly programmed, the autosampler will end its run before the chromatographic run ends, and load the next sample. The difference in time between the two run lengths determines how long the sample waits in the loop.
5. When the chromatograph is done with its run, it automatically begins the next run, triggering the autosampler to inject again.

This sequence explains why an autosampler run that is too long will produce alternating sample runs and blank runs. After the first run, the chromatograph starts the second run. But if no sample has been loaded, no sample can be injected, so no peaks appear in this run. After the second chromatographic run starts, however, the autosampler loads its loop with the second sample. Since the second chromatographic run has already begun, no inject signal is received. The sample sits in the loop until the chromatograph triggers an injection on its third run. This pattern continues until the chromatograph has done all the runs (and the autosampler half the runs) programmed.

The sequence of communication between the BAS 200B and the autosampler also determines the performance of the system in the event of an equipment breakdown. If the BAS 200B's functions are interrupted, no inject signals will be sent to the autosampler. The currently loaded sample will remain in the loop, and all subsequent samples will be preserved. If the autosampler ceases to function, however, the chromatograph will continue to make the programmed number of runs, because it receives no signals from the autosampler. This is a minor problem, as it only involves the loss of some mobile phase.

Autosampler in Charge

If your autosampler has no provision for loading a sample and waiting for an inject signal, you must designate the autosampler as master and the BAS 200B as slave. In this mode, do not use pump synchronization, even for gradients. The system is configured so the autosampler sends a start signal to the BAS 200B. Use the EW-8162 cable to connect the START-200 lead and its ground to the autosampler's terminals for starting peripheral equipment. One of the timed-events leads and its ground can be used to start data collection.

With the autosampler in charge, *the BAS 200B's run must be shorter than that of the autosampler*. This is critical, because mistakes here will lead to loss of samples.

AUTORUNS must be off, because the chromatograph must sit and wait for a start signal from the autosampler. The sequence of events is as follows:

1. Set the BAS 200B in the equilibrating mode.
2. Start the autosampler. Each sample will be loaded and injected without regard to the status of the chromatograph. At each injection, a start signal will be sent, telling the BAS 200B to start the run. The BAS 200B must be in the equilibrating mode to receive this signal.

Following the sequence of events allows us to predict the outcomes of various errors. For example, if the BAS 200B's run is too long, it will not be in the equilibrating mode when the autosampler injects, and the run will not start. The chromatograph will wait until the next injection, thereby missing every other sample.

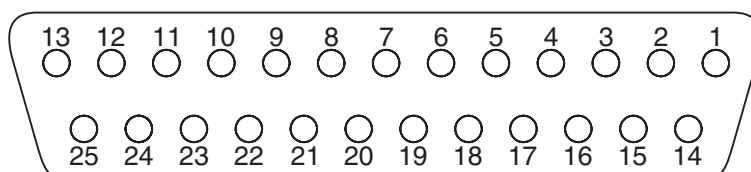
Another type of error occurs if the autosampler stops functioning. In this case, the chromatograph will merely remain in the equilibration mode, and all samples will remain in the autosampler.

The worst type of error occurs if the chromatograph malfunctions; the autosampler will continue injecting on its own and all samples will be lost.

7.3 Timed Events Connections

A 25-pin D-connector labeled TIMED EVENTS provides access to the various remote start/stop and switch closure operations of the BAS 200B. This connector is located on the back panel (Figure 7.4). The pins provide the signals shown in Table 7.3.

Figure 7.4 TIMED EVENTS connector (exterior view from rear of BAS 200B).



The BAS 200B may be remotely triggered to start and stop via pins 1, 2, and 25. Either TTL logic or switch closures may be used. Pins 11–22 provide four switch closures for control of peripheral equipment. The logic of the contact switch closures may also be inverted for your hardware, if necessary, since both normally open (NO) and normally closed (NC) contacts are provided. Almost any custom communication scheme can be implemented through these timed events.

Table 7.3 Timed Events signals on 25-pin D-connector

Pin #	Signal	Wire Color*
1	START 200	Green
2	STOP 200	Red
11	TE 1 (NO)	Yellow
12	TE 1 (NC)	
13	TE 1 (COM)	Violet
14	TE 3 (NO)	Brown
15	TE 3 (NC)	
16	TE 3 (COM)	Orange
17	TE 2 (NO)	Blue
18	TE 2 (NC)	
19	TE 2 (COM)	Grey
20	TE 4 (NO)	White
21	TE 4 (NC)	
22	TE 4 (COM)	Black
23	N/C	
24	N/C	
25	GND	White/Black

*cable EW-8162

NO = normally open

NC = normally closed

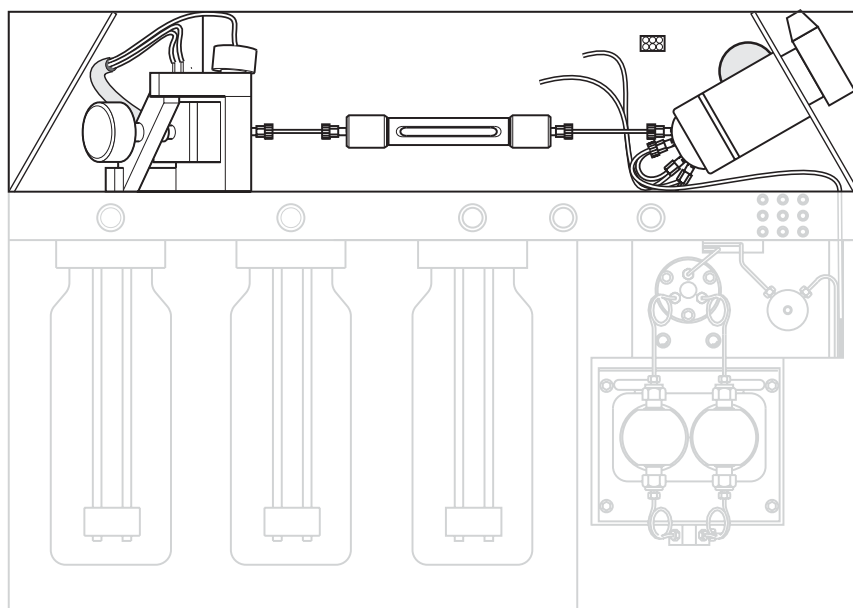
COM = common

Section 8. COLUMN OVEN

8.1 Overview

The BAS 200B Liquid Chromatograph features a forced-air column oven (see Figure 8.1). Column lengths up to about 40 cm and diameters up to 1–2 cm can be accommodated. The air oven is equally well suited for microbore separations. Both the optional electrochemical and UV-Vis detectors are within the compartment. Precise temperature control is possible only when operating at a temperature setpoint at least 3 °C over ambient oven temperatures. A high limit of 70 °C is provided.

Figure 8.1 The BAS 200B column oven.



Software control of the forced-air oven and optional electrochemical cell preheater provides two advantages:

1. Constant adjustment of the duty cycle in real time as the system comes to thermal equilibrium. This prevents overshoot of the setpoint temperature.
2. Safety shutdown of the oven system if the sensor becomes inactive.

8.2 Operating Software, BAS 200B System Director

The temperature control software is accessed as a part of a method or edit file within System Director operation.

When editing a file, press the TEMP key from screen 3. Screen 22 then appears with the cursor requesting the oven and optional detector setpoints. At any time, current temperatures may be determined by pressing STATUS to loop through the STATUS screens.

After the oven and cell preheater setpoints are entered, screen 23 is displayed. Screen 23 allows you to choose heating temperatures for the three mobile phase reservoirs A, B, and C. If the nature of the solvents permits, the temperatures of the mobile phases should be at least as high as the oven or preheater setpoints. Otherwise, outgassing (bubble formation) may occur at the low-pressure end of the column. Recall that gas solubility *decreases* with increasing temperature.

To execute the temperature file, five soft keys are available: EXECute, FAN, ON, OFF, SAVE, and ESCAPE.

EXEC	Orders the subsystem to heat until the chosen setpoints are reached. If ambient temperatures exceed any setpoints, the heaters will remain inactive until these temperatures drop below the setpoints.
FAN	The fan is <i>automatically</i> turned on whenever the oven has been started from an ambient temperature below the setpoint. It may be turned on <i>manually</i> (using the FAN soft key) at any time.
OFF	Manually turns off all temperature functions in the file. The default setting for the fan is OFF. Only the fan may be manually controlled (by toggling the FAN soft key).

Due to the proximity of the components within the BAS 200B, ambient oven temperatures are usually 22–30 °C. Since the compartment is well insulated and gasketed, even small components can raise ambient oven temperature. The oven fan motor alone is capable of increasing ambient temperatures to 33–35 °C over a half-day period; typically, oven setpoints are at least this high, so this presents no problems. However, if lower ambient temperatures are necessary, the fan should remain inactive.

In some models, venting of the oven door is available at the right and left side panels. At setpoints below 35 °C, this allows cooler ambient air to mix with current oven air, and therefore allows lower setpoints to be held in control.

8.3 Operating Comments

1. Minimum temperature overshoot is achieved when the setpoint contains a small temperature increment (+5 °C max) over ambient.
2. Electrochemical detector noise increases with temperature, but long-term drift is minimized by active control of cell temperature. The optimum electrochemical detector baseline is therefore also obtained at small temperature increments over ambient.
3. Use the fan *only* if you are running the oven at 35 °C or greater setpoints. The fan motor alone, with the oven OFF (20 °C setpoint), will slowly cause the oven air temperature to rise to approximately 35 °C, usually over 2–3 hours. The baseline drift at high EC sensitivity may be objectionable. For this reason, if a low detector temperature (e.g., 30 °C) is desired, use the preheater to maintain the detector eluent at this lower temperature, turn off the oven fan, and set the oven temperature to 20 °C.
4. Keep the mobile phase at least as warm as the oven and preheater setpoints, in order to avoid outgassing.
5. Oven temperature precision at 35 °C is typically ± 0.1 °C. This degrades to ± 0.3 °C at 70–80 °C.
6. An open connection at either solid-state temperature sensor (oven or preheater) can force an error message to appear:

OVEN or CELL TEMPERATURE SENSOR ERROR

A connector may have loosened, or the sensor is defective. Consult BAS Service.

7. Until a temperature file has been EXECuted, the values displayed during STATUS may not be valid. Horizontal bars may appear. EXECute the default file to remedy this, or use any other file of your choice.
8. TEMPERATURE CONTROL INOPERATIVE
FAIL-SAFE MECHANISM HAS BEEN TRIPPED
CONSULT MANUAL FOR DETAILS

This error message indicates that some portion of the temperature utility subsystem probably overheated. This error is also (infrequently) tripped by noisy power line conditions. If no oven component or mobile phase bottle appears overheated, line noise is probably responsible. If overheating is evident, a hardware problem exists. Consult BAS Service.

This error is only erased by pressing RESET on the left side of the BAS 200B cabinet. If any heated zone (bottle, oven, cell) is warmer than 5 °C over its maximum setpoint, the error will continue to be displayed until that zone has sufficiently cooled.

8.4 Installation

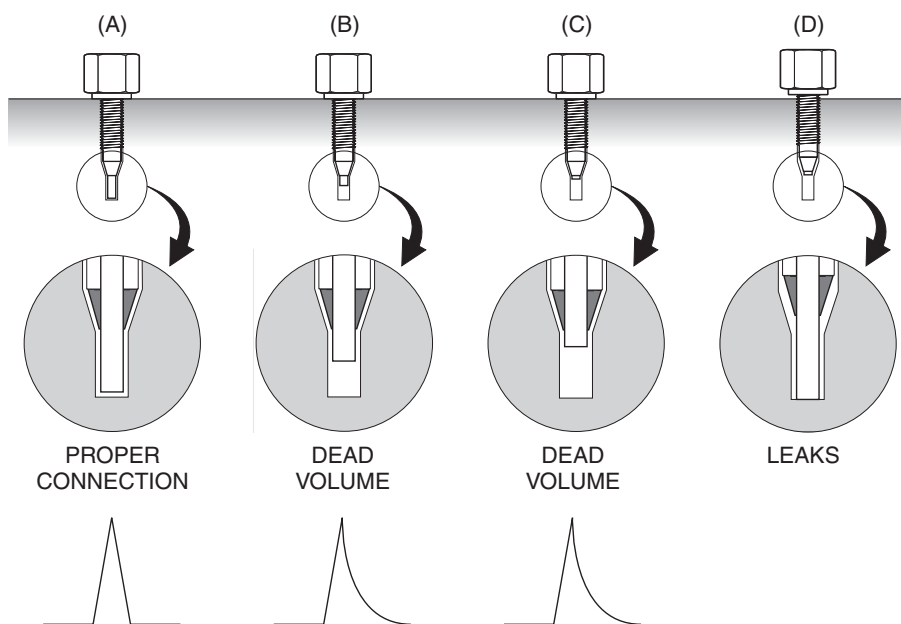
Each column is individually packed and tested before shipment, and shipped with a test report that contains the actual column testing conditions along with the calculated efficiency and skewness. Save this report for future reference.

Also provided is a test chromatogram obtained on your entire BAS 200B system. The conditions (mobile phase, test solutes) will not necessarily be identical for these two tests. In many cases (e.g., biogenic amines), the apparent efficiency and asymmetry will be considerably less on the BAS 200B test due to the nature of the solutes.

Making Connections to Avoid Unswept Dead Volume

Figure 8.2 shows how improperly made connections with fingertight fittings can contribute to system deficiencies. The connection in Figure 8.2A was properly executed; a direct passage from the tube to the fitting is evident. Sometimes, however, the tubing is inadvertently pulled back during tightening, thereby causing excessive dead space. Eddying occurs, and the peak “tails.” Always extend the tubing through the fitting before tightening, and maintain finger pressure on the tubing while the fitting is being tightened.

Figure 8.2 (A) Proper fitting; (B) short tubing; (C) longer fitting hole; (D) shorter fitting hole.



The same principles apply to permanently swaged steel fittings. When the fitting is first made, be sure to push the tube firmly into the socket while tightening. Once the fitting is made, it can be removed and reattached without worry, because the ferrule remains fixed on the tube. However, be wary of using the fitting in a different type of socket: the fitting may be too short or too long, resulting in a dead space or a leak. It is good practice not to mix swaged tubes among different columns for these reasons.

Installing Cartridge Columns

To install a cartridge in the holder, unscrew the holder and carefully insert the new cartridge. Install the cartridge in the chromatograph using two fingertight fittings (MR-4409). The preferred flow through the cartridge is from left to right as you read the label. *Avoid back flushing.*

Center the column in the holder by tightening both ends equally. Reseal the holder to finger tightness: further tightening is unnecessary and may damage the seal or holder. Never use tools to tighten this seal!

To remove the cartridge, unscrew the holder to expose the end of the cartridge. Using your thumb and forefinger, pull the cartridge out. When not in use, store the cartridge in a protective environment, such as a glass test tube with stopper. Do not add liquid to the tube. Storage in this manner will protect the cartridge's frit assemblies from dirt and scratches.

If the cartridge leaks at less than its rated pressure limit, do not try to force a seal with tools. A leak indicates that the cartridge or seal surface must be dirty, scratched, or deformed. Replace the cartridge and check again for leaks. If it still leaks, the high-pressure seals must be replaced (see Section 8.7).

8.5 Equilibration

To obtain the maximum lifetime and efficiency from BAS Biophase and Phase II columns, the mobile phase should always be prepared using high-purity buffers and fresh deionized (> 15 M Ω) water. The prepared mobile phase should always be filtered through a 0.2- μ m membrane before use. See Table 8.1 for recommended membranes and hardware. Samples should have proteins removed, when possible, by precipitation or solvent extraction, and the final solution should be filtered through a 0.2- μ m membrane before injection onto the column. BAS Microfilters are ideal for the filtration of small volume samples prior to liquid chromatography.

Table 8.1 Recommended BAS Membranes and Hardware for Mobile Phase Filtration

Source Item	Part No.	Quantity
Filter Apparatus (borosilicate glass)	MF-6125	1 ea.
Regenerated Cellulose Membranes, 0.2 μ m pore size, 47 mm	MF-5520	100/pkg
Nylon 66 Membranes, 0.2 μ m pore size, 47 mm (universal applications)	MF-5621	100/pkg

Table 8.2 Recommended BAS Microfilters and Hardware for Sample Filtration (requires desktop centrifuge, 1600 g)

Source Item	Part No.	Quantity
MF-1 Centrifugal Microfilters	MF-5500	12/pkg
0.45- μ m Regenerated Cellulose Membranes	MF-5655	100/pkg
0.2- μ m Regenerated Cellulose Membranes	MF-5658	100/pkg

Preconditioning Hydrophobic Packing Materials

Like water on Teflon membranes, polar solvents often do not “wet” hydrophobic packing materials well. The surface tension is prohibitive, and the effective surface area of the stationary phase is greatly reduced. Most of the internal pore structure is not contacted by the mobile phase.

When using your reversed-phase column for the first time or after storage, you must first flush it with 300 mL of filtered, degassed, LC-grade acetonitrile (100%) to ensure “wetting” of the packing material. This should be followed by 50 mL of acetonitrile:water (40:60, v:v). After these two steps, the analytical mobile phase can be equilibrated. This protocol is imperative for proper retention when using BAS Phase II columns.

8.6 General Care Procedures

Storage

The column should be stored when not in use for an extended period of time. Flush the column with 100 mL of 40:60 acetonitrile:water to remove buffer salts, then remove and cap the column.

Exposed Materials

Materials in contact with the mobile phase are type 316 stainless steel, Teflon, and Tefzel. Maximum pressure is 7000 psi (470 bar) for both cartridges and holders. Maximum recommended temperature is 85 °C.

More about the Cartridge Format

The cartridge format provides economy, versatility, and flexibility not afforded by a conventional column. The holder consists of two end assemblies and holder body. The end assemblies can be used with any holder body (4, 10, or 22 cm length) and with either conventional 4.6-mm i.d. cartridges or narrow-bore 3.2- or 2.1-mm i.d. cartridges. The guard cartridges can be coupled directly to cartridge columns or used with conventional LC columns. Two cartridges can be coupled end-to-end for additional resolution or selectivity, for special applications such as the determination of acetylcholine and choline using an immobilized enzyme post-column reactor, or with a guard cartridge.

The flexibility of this BAS hardware permits you to economically optimize your separation by choosing from three cartridge lengths, three internal diameters, two phases, and several particle sizes. The end result minimizes analysis time, solvent consumption, and column cost.

8.7 Maintenance

The high-pressure seals in the cartridge columns should last many years before replacement is necessary. Replacement of these seals is indicated by solvent leakage, even when different cartridges are used. *Overtightening the holders will not stop leaks; leaks can only be corrected by seal replacement.*

Before starting seal replacement, read this entire section carefully. Work on a clean, hard, flat surface. The seal is Teflon, a very soft and pliable plastic, which will be destroyed during removal of the old seal from the fitting. *Do not proceed unless you have new seals at hand.* Do not scratch the new seal.

The snap rings used in the holders and unions must be removed and replaced each time the seals are replaced. Their manipulation requires some skill and practice, so try removing and replacing the old snap rings several times before final assembly with the new snap rings.

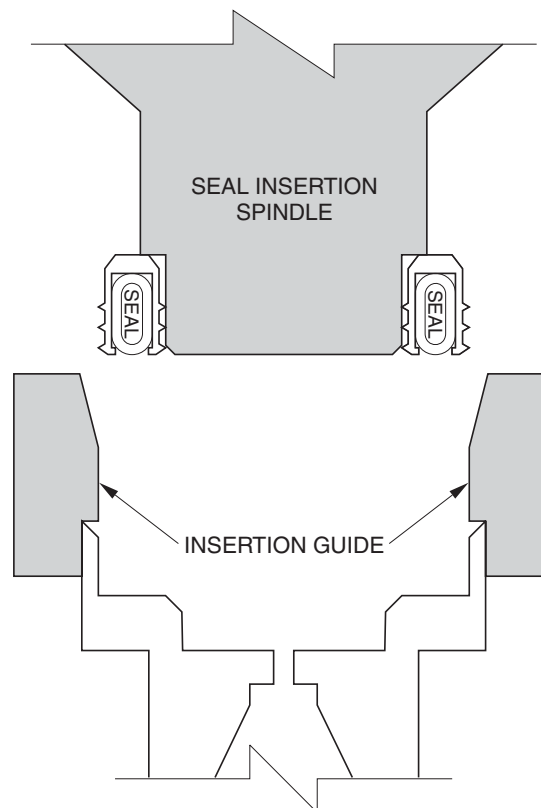
Different pliers (NO, normally open, and NC, normally closed) are used with the two types of rings supplied. Note that there is a flat side (dull) and a rounded side (shiny) to each snap ring. Be sure that the dull side is facing outward, to maintain the rated pressure limit. You will find that it is easier to maneuver the pliers into the loose snap ring if the ring is lying on a hard, flat surface.

A seal replacement kit, containing two seals, snap rings, insertion tools, and convertible (NO/NC) pliers is available as P/N MF-6028. Additional seals are available in pairs as P/N MF-6226.

End Assembly Seal Replacement

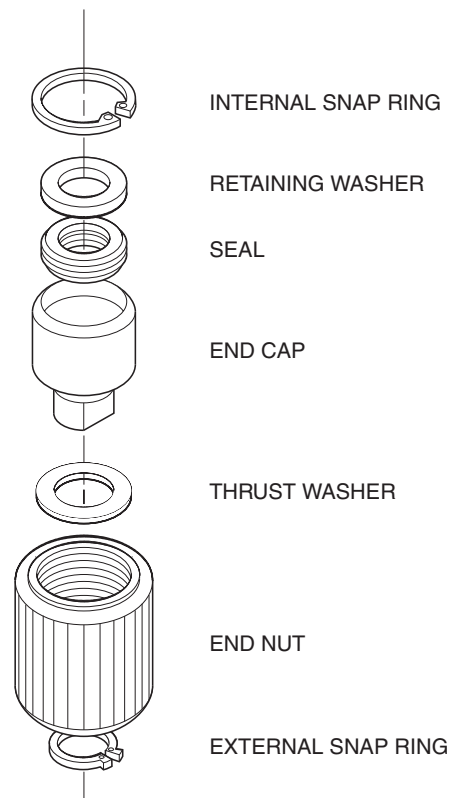
1. Remove the end nuts from the tubing end fittings.
2. Insert the tips of the NC pliers into the small openings in the external snap ring and squeeze the pliers. This will expand the ring enough so that you can slip it off the end fitting. Set aside the black thrust washer and the end nut.
3. Remove the internal snap ring from within the end fitting by using the NO pliers to compress the ring enough so that you can lift it out.
4. Remove the seal retaining washer and pry out the old seal with a small screwdriver or other tool. The old seal will be unusable after removal.
5. Use the end nut, small hole up, on the table as a tool in this step. Set the end fitting into the nut so that the seal fitting is up.
6. Place the seal insertion guide over the end fitting as shown in Figure 8.3.

Figure 8.3 End assembly seal replacement.



7. Place the new seal, spring side out, over the end of the seal insertion spindle as shown in Figure 8.3.
8. Push the seal/spindle through the guide and into the end fittings. Press down firmly. Upon removal of the spindle and guide, the new seal should be securely in place with the spring side down.
9. Replace the seal retaining washer and use the NO pliers to insert the new internal snap ring. This is the most difficult operation, so practice with the old snap ring first. Use your finger to push the compressed ring off the pliers into the recess.
10. Clamp the seal insertion spindle into a vertical position, small end up, and use it as a tool in this step. Push the newly assembled end fitting down onto the spindle. Replace the black thrust washer and the end cap as shown in Figure 8.4. Replace the external snap ring using the NC pliers.

Figure 8.4 Exploded view of end assembly.



Seal Replacement in Coupling Unions

1. Use the small Allen wrench supplied with the union to remove the union assembly from its threaded sleeve. The union is symmetrical and has two high-pressure seals.
2. Remove the internal snap ring from within one end of the union body; use the NO pliers to compress the ring enough so that it can be lifted out.
3. Remove the seal retaining washer and pry out the old seal with a small screwdriver or other tool. Repeat steps 2 and 3 on the other side.
4. With the union on a hard, flat surface, place the seal insertion guide over the union body, similar to Figure 8.3.
5. Place the new seal, spring side out, over the end of the seal insertion spindle as shown in Figure 8.3.
6. Push the seal/spindle through the guide and into the union body. Press down firmly. Upon removal of the spindle and guide, the new seal should be securely in place with the spring side down. Turn the union body over and repeat steps 4–6 on the other side.
7. Replace the seal retaining washer and use the NO pliers to insert the new internal snap ring. This is the most difficult operation, so practice with the old snap ring first. Use your finger to push the compressed ring (dull side up) off the pliers into the recess. Turn the union body over and repeat this step.
8. Reassemble and place the union body into the union sleeve. Tighten the set screw.

8.8 Cleaning

If the column has shown a gradual loss in efficiency, a possible cause is the retention of noneluted compounds on the column packing. In most cases, Biophase and Phase II columns can be sufficiently regenerated by flushing with several volumes (about 300 mL) of a strong eluting solvent such as methanol or acetonitrile. If this flushing procedure does not have the desired effect, the stationary phase may actually be "coated" with compounds of very low polarity that are present in the sample. In this case, flushing the column with a relatively nonpolar solvent such as THF or CHCl_3 may be necessary.

NOTE: In severe cases, the chemically modified silica stationary phase may be irreversibly destroyed; that is, the chemically bonded groups (octadecyl, octyl, cyano, etc.) are hydrolyzed from the silica surface. In these cases, the column must be replaced.

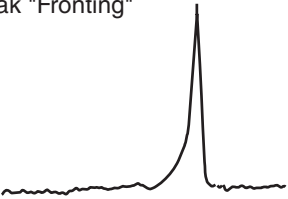
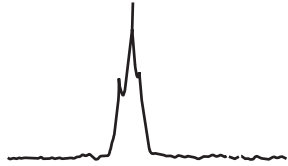
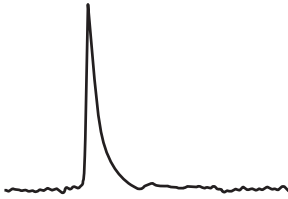
The following column cleaning procedure is recommended. Flush with:

1. 50 mL H_2O
2. 50 mL $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (40:60)
3. 300 mL CH_3CN
4. 50 mL $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (40:60)
5. 50 mL mobile phase

8.9 Troubleshooting

There are many other chromatographic problems that can be traced to the analytical column, though these problems may often have other, less obvious causes. The following troubleshooting guide (Figure 8.5) explains some of these problems and their solutions.

Figure 8.5 Troubleshooting: symptoms, causes, and remedies of common problems.

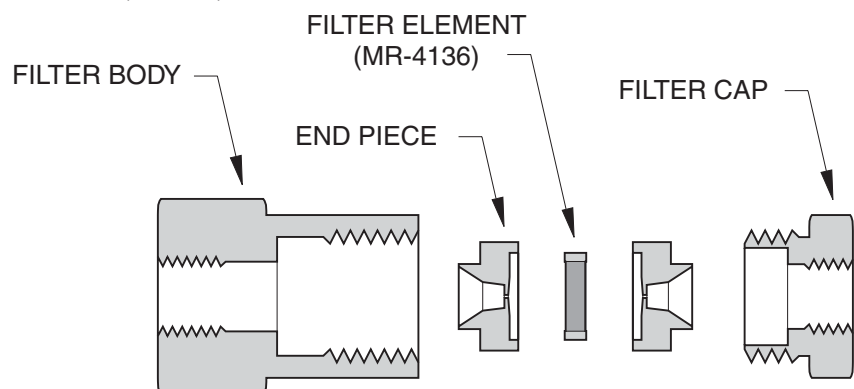
SYMPTOM	CAUSE	REMEDY
Peak "Fronting" 	A. Void in column B. "Overloading" column C. Sample in solution of significantly different composition from mobile phase	A. Refill void B. Reduce sample load C. Dilute or reconstitute sample in mobile phase
Peak "Splitting" 	A. Void in column B. Sample in solution of significantly different composition from mobile phase C. Mobile phase pH too close to p_k value	A. Refill void or turn column end for end B. Dilute or reconstitute sample in mobile phase C. Adjust mobile phase pH so sample is in non-ionic form*
Peak "Tailing" 	A. Ionic strength of mobile phase too low B. Incorrect mobile phase pH C. Old column - lost efficiency	A. Increase ionic strength B. Adjust mobile phase pH so sample is in non-ionic form* C. Flush - if no improvement, replace column
Decrease in retention time	A. Lost efficiency	A. See "Column Problems"
Increase in retention time	A. Organic modifier evaporated from mobile phase	A. Make fresh mobile phase
No retention	A. Sample ionized at mobile phase pH B. "Channeling" in column C. Silica backbone being dissolved by inappropriate mobile phase pH D. Too much organic solvent E. Packing material not wet	A. Adjust mobile phase pH so that sample is in non-ionic form* B. Replace column C. Observe pH limits of silica gel (between pH 2 and 8) D. Decrease organic solvent E. Follow recommended flush procedure

* This is assuming that the ion-suppression mode is being used. Ion-exchange and ion-pair chromatography utilize ionization. This is not strictly true for all compounds (aromatic amines, for example).

8.10 In-Line Filter

The BAS 200B includes a very low dead volume in-line filter (Figure 8.6). Its purpose is to trap microparticulate debris before it enters the injection valve or column. The filter is located between the purge valve and the sample injection valve, within the column oven.

Figure 8.6 Exploded view of BAS 200B in-line filter (MR-4135).



It is important that you use the connections already provided for this device. If you need to make alternate connections, make up the new swage in the assembled filter. **DO NOT** use tubes with fittings that have been made in a different port. In this particular component, the distance from the tube end to the ferrule differs from those in the injection valve and column. A mismatch will therefore either destroy the filter end piece or cause a loss in resolution.

Flow can be in either direction, but the etched arrow on the filter body reminds you to always use it in the same direction. The filter element is easily cleaned or replaced. Cleaning is best accomplished by sonicating in 30% HNO_3 for 20 minutes, then sonicating in distilled water for 5 minutes. Replacement filters are available from BAS (MR-4136). The porosity is 0.5 μm .

To replace the filter element, hold the filter body with a 1/2" wrench and disconnect the two 1/16" inlet/outlet lines. Unscrew the filter cap from the filter body (refer to Figure 8.6) and remove the internal parts. For best results, keep track of the end pieces so they can be reinstalled at their original locations. Sonicate all pieces. Reassemble the filter to finger tightness, then snug with two 1/2" wrenches. Make sure the 1/16" tubes are disconnected before tightening. Finally, reconnect the 1/16" inlet/outlet tubes.

Section 9. ROUTINE MAINTENANCE

9.1 Routine Maintenance

A regular maintenance schedule will keep your BAS 200B's performance up to specifications. We recommend the following:

Every Day

Inspect all fittings in the flow path for leaks.

Every Three Months

Replace plunger seals and inspect interior of pump head (Section 6.7.1).

Every Year

Clean the fan filters (Section 9.3).

9.2 Fittings

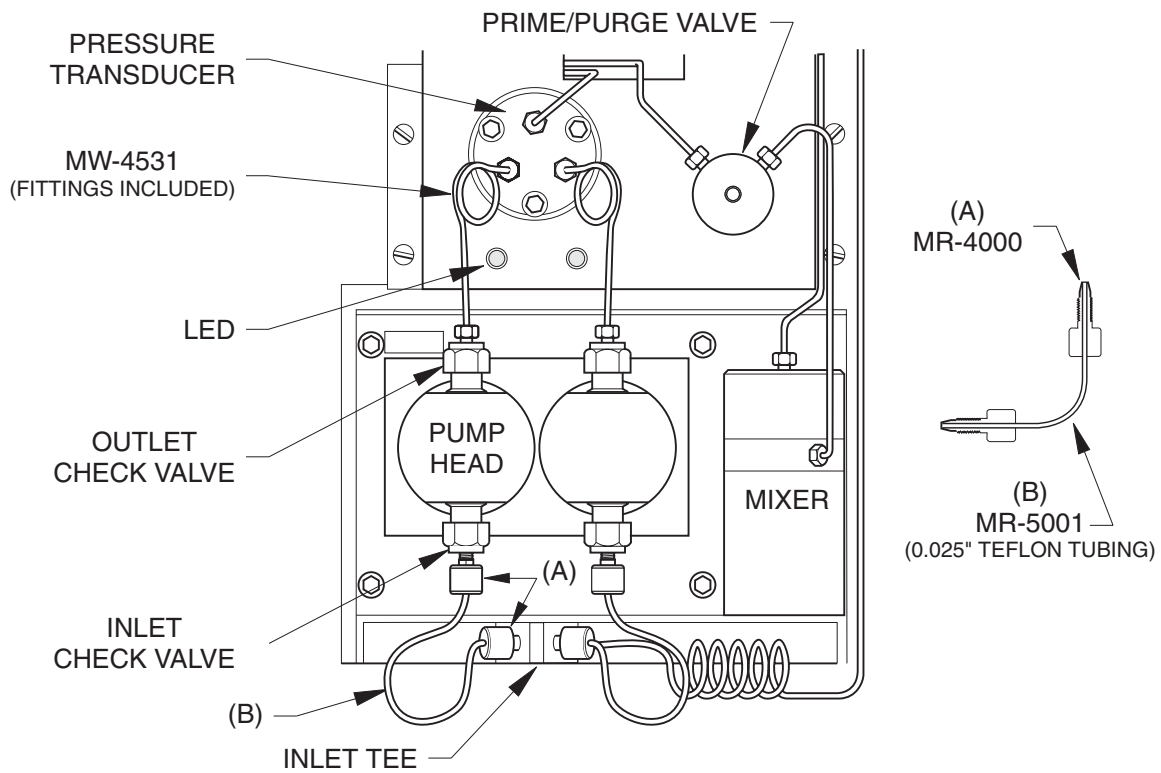
Check all fittings for leaks every day. Large leaks will be obvious. Small leaks will be apparent only by the accumulation of salt deposits around the fitting. It is not normal for fittings to have even small leaks.

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 be sufficient, unless the nut is loose. If this doesn't stop the leak, it's best to replace the fitting and line entirely. If you overtighten the nut. . .

1. The nut may break off with its threads still in the hole. A machinist will have to get it out, and the part will most likely be damaged.
2. The nut may fuse in place, and break off the next time you try to open it.
3. The tubing end may become crimped, restricting flow and causing performance problems.

Figure 9.1 gives part numbers and ferrule types for all tubing used in the solvent delivery system.

Figure 9.1 Tubing and fittings used in the solvent delivery system.

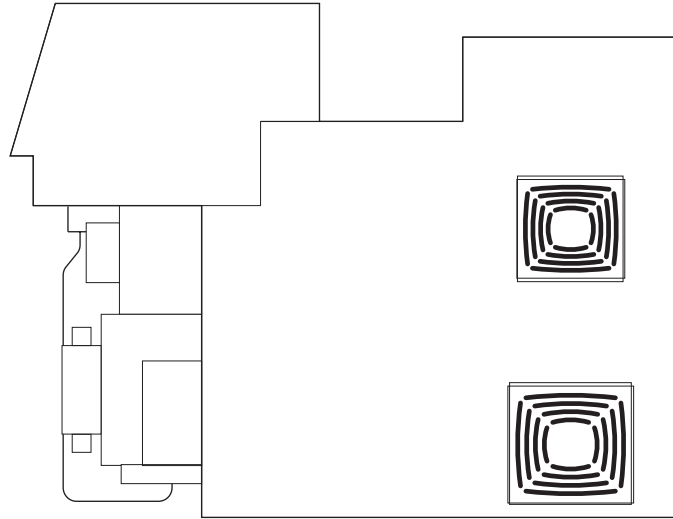


NOTE: We recommend the use of plastic fingertight fittings on any tubing that is frequently connected and disconnected. Although the fittings will wear out, they are easily replaced without the need to replace the tubing. Use P/N MR-4409 where there is enough clearance for a fitting with finger grips, and P/N MF-4166 where only a small wrench-tightenable nut will fit.

9.3 Fan Filter Servicing

The fan filters should be removed for cleaning at least once a year (more often if visibly dirty.) Proceed as follows: Remove the cover of the BAS 200B. Filter locations are shown in Figure 9.2.

Figure 9.2 Location of fan filters.



For each filter:

1. Gently pry off the retaining grid with a screwdriver.
2. Carefully pull out the filter. You may vacuum it 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.

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