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LC-4C

March, 2000

MF-9081 INSTRUCTION MANUAL

Electrochemical Detector

Bioanalytical
Systems, Inc

2701 Kent Avenue
West Lafayette
Indiana 47906

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DO NOT touch the cell unless the cell switch is in the “standby” position.

在电化学检测器的电极池开关切换到“ＳＴＡＮＤＢＹ”的位置之前，
切勿触及电极池

Never touch the electrochemical cell, the cell wires, or any part of the cell while the detector is operating. Before touching the cell, make sure that the cell switch on the front panel of the LC-4C amperometric controller is in the “STANDBY” or “OFF” positions. Do not operate cell while dry, or without connection to the reference electrode. Failure to obey these warnings may result in damage to both the controller and the electrochemical cell. Such damage is not covered by warranty.

在电化学检测器工作时，切勿触及电极池、电极池连线或电极
池的任何部件。必须确认ＬＣ－４Ｃ电化学检测器的控制器的前部
显示屏上的电极池开关已经切换到“ＳＴＡＮＤＢＹ”或〞ＯＦＦ〞的位
置，才能触及电极池。在电极表面仍然干燥或尚未连接参比电
极之前，不得将电化学检测器切换到〞ＯＮ〞的位置。不按照此警
告使用，将可能同时损坏检测器的控制器和电极池。这样的损
坏不属保修范围，一切后果均由使用者自负。

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. Preface

This manual covers operation and service information for the LC-4C AMPEROMETRIC CONTROLLER. The LC-4C is a general purpose potentiostat which can be used for many purposes. In this manual, we assume that a CC-5 cell compartment has also been purchased with the LC-4C. There are other options that are described in BAS product literature and companion manuals.

To use this detector, you will require a liquid chromatograph, a data recording device, and appropriate end-fittings and tubing for the connection between column and detector. The LC-4C is a component of the LC-44 detector and the modular BAS 480 series of liquid chromatographs.

The information in this manual represents the combined experience of both the chemists and engineers associated with LCEC from its inception. Background information can be found in the “Principles of EC Detection and Troubleshooting Guide” (P/N MF-9083). Standard operating procedures are described in this manual; however, we have also sought to include the not-so-obvious experimental details which often make or break the user’s chances of maintaining an extra edge of performance.

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Several accessories may be added by the user or by service personnel at a later date:

1. The LC-4C Dual CELL LEADS (EW-8114) provide the additional cable to transform an existing single-electrode detector into true dual-electrode operation. A second LC-4C controller (or an LC-3C/D/E Petit Ampère) must also be purchased. Installation instructions for dual-electrode operation are provided in Section 5.

2. A Preheater Module (EW-4055) may be added for better baseline stability at high detector gain or in adverse environments. Its installation and use are described in Section 6.

3. An analytical Cartridge Column Heater (EF-1041) may also be added to the CC-5 compartment. A removable panel on the back of the instrument is replaced by the LC-23C heating block, which holds up to 2 cartridges. This option is described in a separate manual and product brochure. A variety of cartridge columns and guard columns are also available from BAS.

4. A Rheodyne or UniJet microbore injection valve may be added by the user when the small cover plate on the front of the CC-5 is removed.

5. ChromGraph™ software with the DA-5 interface makes it possible to control the functions of the LC-4C and also to implement techniques such as pulsed electrochemical detection.

The addition of options (3) and (4) to your electrochemical detector will convert it into the basis of a complete liquid chromatograph.

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Section 2. Support Policy

2.1 User Updates

To activate your warranty and receive product update information news and valuable information related to this and other BAS products, fill out and return the Warranty Enrollment Card which was shipped with the instrument. We would like to know who you are, and what more you want to know about electrochemical detection.

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 box and contents for inspection by the freight handler. BAS will replace any new instrument damaged in shipping with an identical product as soon as possible after the claim filing date. Claims not filed within 30 days after 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. Bioanalytical Systems refuses all unauthorized return shipments.

2.3 Product Warranty

BAS products are fully warranted against defects in material and workmanship. The LC-4C amperometric controller is unconditionally warranted for one year from date of shipment, except when failure is due to obvious abuse or neglect, unauthorized tampering, procedures not described in manuals, or improper connection of electronic units to other components. Electrochemical cells are warranted for 60 days from date of shipment under the same exclusions. Chromatographic columns and injection valves are warranted for 30 days. The following items are not covered under any warranty: carbon paste, activated aluminum oxide, lamps, panel lights, fuses, pump seals, valve seals, and reference electrodes.

For any product expressly covered under this warranty, BAS is liable only to the extent of replacement of defective items. Bioanalytical Systems, Inc. shall not be liable for any personal injury, property damage, or consequential damages of any kind whatsoever. The foregoing warranty is in lieu of all other warranties of merchantability and fitness for a particular purpose.

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2.4 Service Information

Bioanalytical Systems provides a skilled service staff available to solve your technical problems if an equipment-oriented problem should arise. For further details, call customer service personnel (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 the problem you are having in full detail. Turnaround time on service can be quoted to you at the time your RA# is issued, although we can not determine the actual amount of service required until we have received your unit and diagnosed the problem. All correspondence and shipments should be sent to:

RA # ___ , Service Department
Bioanalytical Systems, Inc.
2701 Kent Avenue
West Lafayette, IN 47906

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Section 3. Installation

3.1 Inspection of Your Shipment

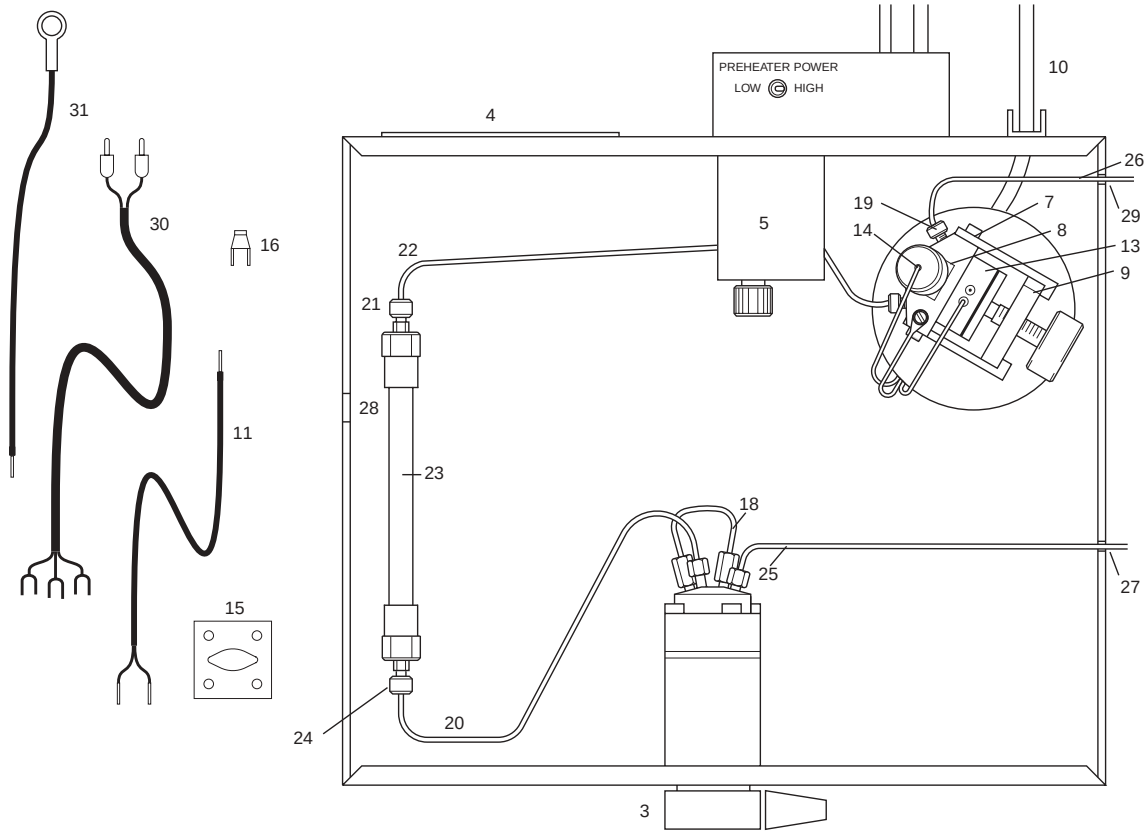
After carefully unpacking the instrument, check the contents of the packages and inspect for breakage (see Section 2). Electrodes and flow cell components (if purchased) are packaged in a gray box. Assembly of these various parts will be outlined in the following chapters. Another gray box, containing the PK-4 polishing kit, is provided with the flow cell. The polishing kit includes an Electrode Manual, which describes electrode polishing, use of gaskets, and reference electrode assembly.

Please retain the shipping box and packing material until you have fully tested the unit to be certain that no damage was incurred during shipping.

If there are any discrepancies, retain the packing slip and contact BAS Customer Service for assistance (see Section 2).

Figures 3.1–3.4 show the various components of the complete system.

Figure 3.1. Top view of CC-5 flowcell configured as part of a BAS 480 system. Refer to Table 3.1 for descriptions.



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Table 3.1 Parts of the assembled LC-44 Amperometric Detector (refer to Figures 3.1–3.4).

Ref. #	Description
1	LC-4C Amperometric Detector
2	CC-5 Flowcell Compartment
3	Injection Valve (installed only in the BAS 480 liquid chromatographs, but available from BAS for installation by customers who wish to upgrade their detectors and use them as the basis for an LC system)
4	Utility Panel (this is removed when the unit is upgraded for cartridge column temperature control, model LC-22C/23C)
5	Optional Preheater Module (120 V/60 Hz or 240 V/50 Hz)
6	Optional LC-22C Temperature Controller for Detector Preheater Module or Cartridge Column Heater Temperature Control (120 V/60 Hz or 240 V/50 Hz)
7	Stainless Steel Auxiliary Electrode
8	Reference Electrode Retainer (packaged in detector accessories box)
9	Working Electrode Clamp and backing plate (packaged in detector accessories box)
10	Single Electrode Cable (dual electrode cable has two working electrode leads)
11	Generator Electrode Lead
12	Power Cord/Fuse location on LC-4C Controller
13	Dual Working Electrode Block (glassy carbon is standard)
14	Reference Electrode (pkg. of 3 RE-4 Ag/AgCl/gel reference electrodes is provided in detector accessories box)
15	TG-2M Electrode Gasket (4 of these translucent gaskets are in the same small box with the electrode jumper and foam sheets)
16	Dual Electrode Jumper (use is optional) (packaged with the electrode gaskets)
17	PTFE Inlet Tubing, 0.25-mm i.d. (not shown; packaged with detector accessories) (not used with cell preheater)
18	20-µL Loop (not provided with detector purchases, BAS 480 ONLY)
19	PEEK Fingertight Nut (cell outlet)
20	Valve to column inlet tube (already prepared with correct end fittings, not provided with detector purchases, BAS 480 ONLY)
21	PEEK Fingertight Nut for column to cell inlet tubing
22	Inlet Tubing, Stainless Steel (for preheater) or Teflon (without preheater, #17)
23	Analytical Column, 100 × 3.0 mm, 3 µm ODS (BAS P/N MF-8954) (not provided if only detector is purchased)
24	Column End Fittings (not provided with detector purchases, BAS 480 ONLY)
25	Connecting Tube from PM-80 Pump to Injection Valve (when installed)
26	PTFE Outlet Tubing (0.5-mm i.d.) (note the small piece of larger tubing located midway on the tube, used to secure the tubing in a notch on the right side of the cell compartment; unless the tubing is secured in the notch, it may be pinched closed when top is lowered; see #30)
27	Notch in cabinet for tubing connection from pump
28	Notch in cabinet to allow use of longer columns (e.g., conventional 25 cm columns) which will extend outside the CC-5 cabinet
29	Notch in cabinet for cell outlet tube (be sure to press the portion of tube with a thicker, Tygon sheath into this slot)
30	Output cable to chart recorder
31	Optional ground connector

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Figure 3.2. Front view of LC-44 Detector with temperature control. Refer to Table 3.1 for descriptions of numbered components.

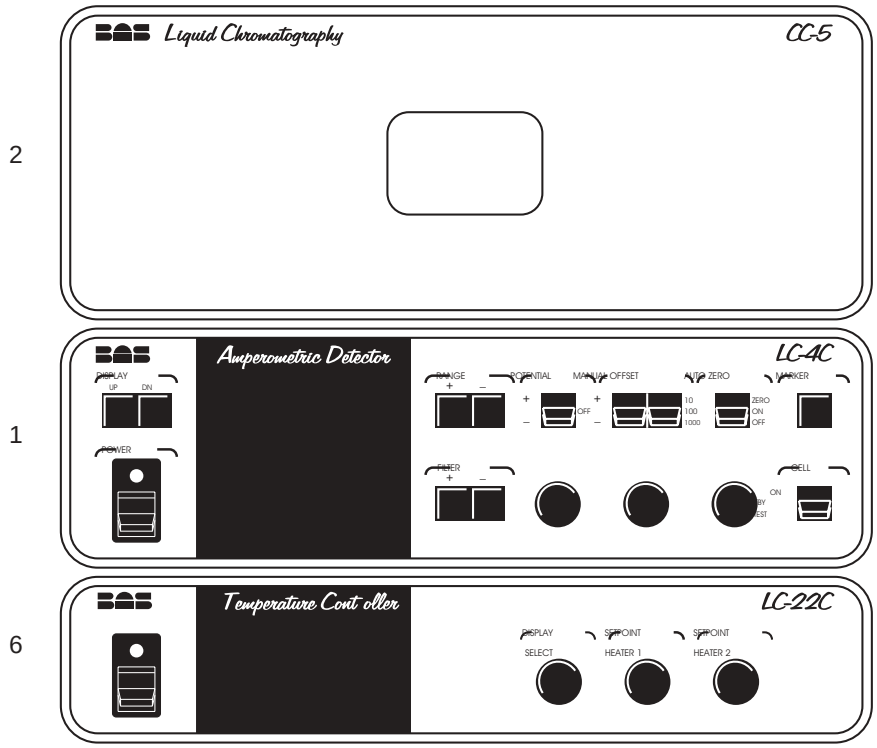
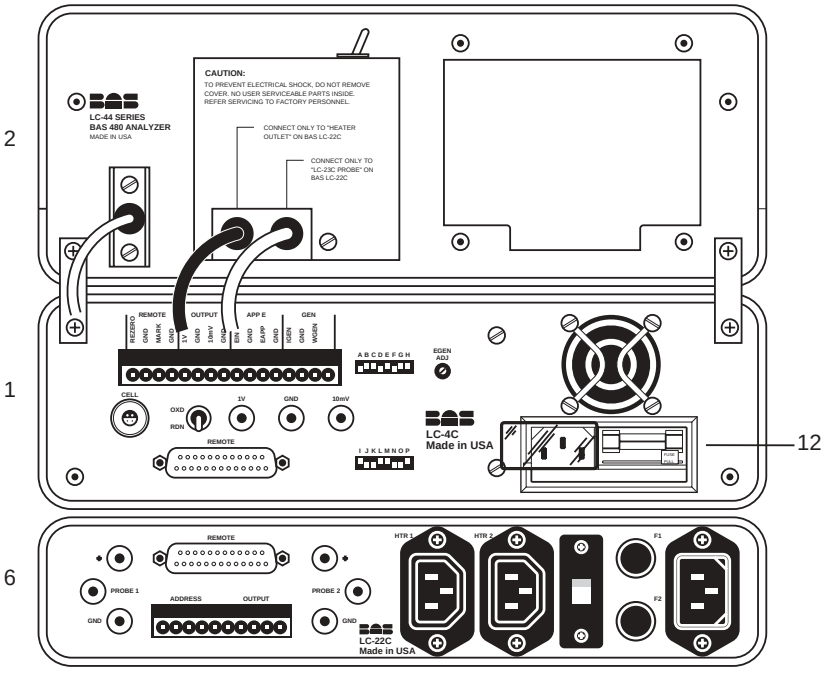
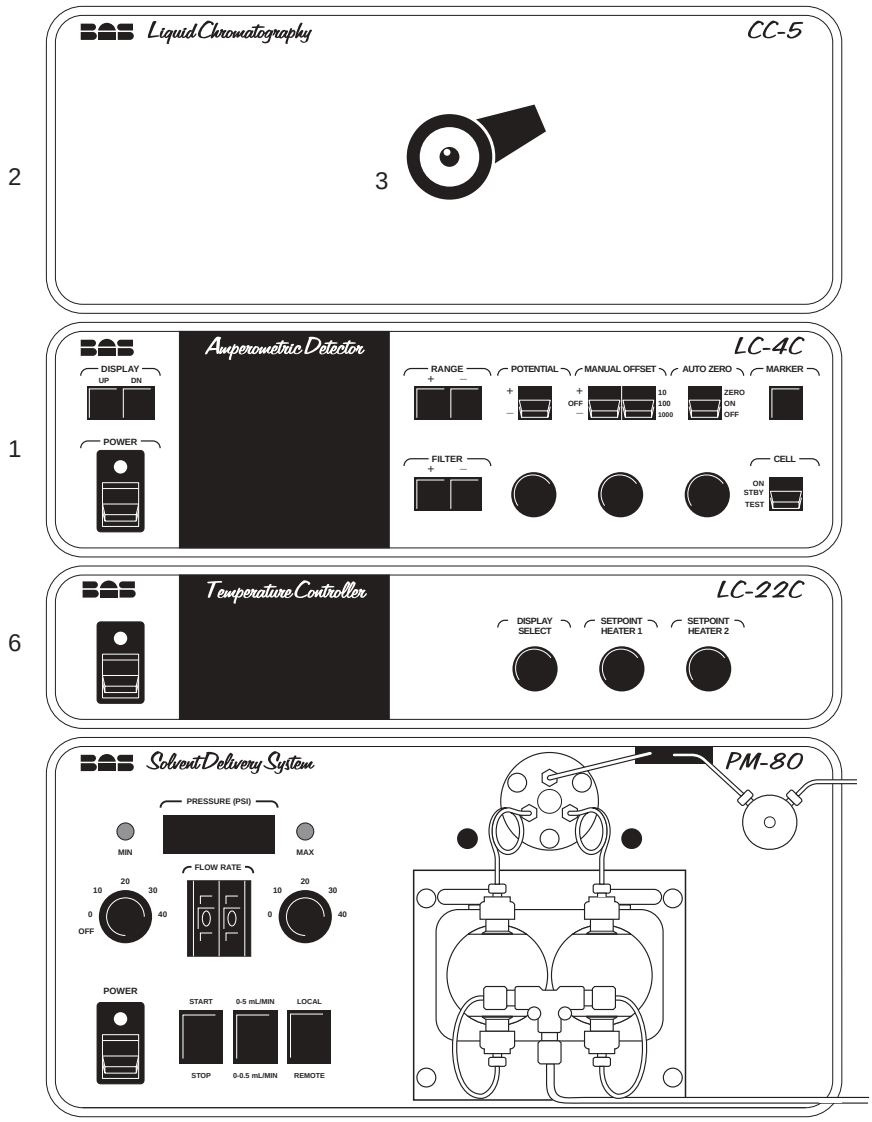


Figure 3.3. Rear view of LC-44 Detector with temperature control. Refer to Table 3.1 for descriptions of numbered components.



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Figure 3.4. BAS 480 Liquid Chromatograph with PM-80 Pump at bottom level. The column and injection valve are mounted in the detector compartment at the top level. Refer to Table 3.1 for descriptions of numbered components.

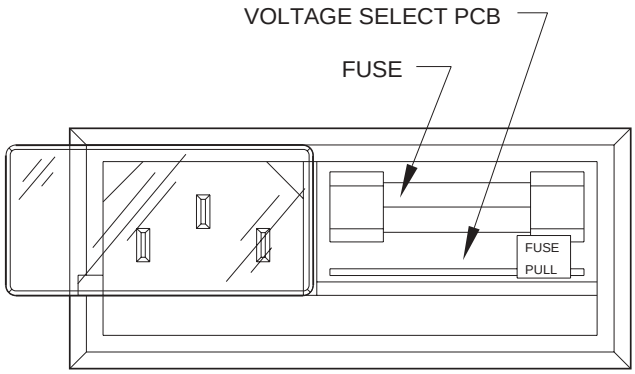


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3.2 Power Requirements

The LC-4C and (optional) LC-22C instruments are operated with either 100, 120, 220, or 240 V AC and 50 or 60 Hz power, but the correct voltage must be selected at the cord connector before use. Maximum power consumption is less than 120 watts. The units should be shipped with the voltage option already matched to the destination, but double check the settings to be sure they match the power requirements of your laboratory.

Figure 3.5. Power selection on the LC-4C.



Should the power option need to be changed, unplug the line cord and slide the plastic window to the left (as shown in Figure 3.5). The orientation of the small circuit board now exposed in this socket determines the voltage option. If the voltage labeled on the outer edge of this board is incorrect, pull out the board and turn it (either by rotation or inversion) so that the desired voltage is readable. Reinsert the board and push the fuse holder back into the cavity. Also check Table 3.2 to see that the fuse is the proper rating.

Table 3.2. Correct fuse selection.

Voltage	Fuse
110 V/60 Hz	1 A/SB
100 V/50 Hz	1 A/SB
220–240 V/50 Hz	500 mA/SB

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3.3 Local Environment

Amperometric detection is a highly sensitive technique. The currents measured typically fall in the picoampere or nanoampere range. Hence, smooth operation can easily be influenced by electrical disturbances in the environment. Also, since detection is due to a chemical reaction, its response (and baseline drift) is temperature-dependent; this is very noticeable at high detector gain.

The new design of the LC-44 Amperometric Detector decreases the influence of these problems. It offers significant advantages over earlier models. The Preheater Module, when controlled by the LC-22C Temperature Controller, eliminates long-term drift due to room temperature fluctuations. The new flowcell design is well shielded from stray capacitance problems; moreover, it is wholly contained within an easily opened, grounded compartment.

When selecting a location for the detector, follow these guidelines:

1. Connect the entire chromatograph (pumps, detector, recorder, temperature controller, etc.) to the same grounded power line. This ensures adequate ground to all components of the system and eliminates the possibility of a ground loop, which could produce baseline noise. Also, select a power line that is lightly used. Other laboratory instruments such as ovens, vortex mixers, centrifuges, and large motors may cause spikes in the power line.

2. Locate the chromatograph on a stable bench. Vibrations can hamper the performance of any sensitive instrument.

3. Select a room where the temperature remains stable throughout the day. Avoid installing the chromatograph near windows, air ducts, ovens, or refrigerators.

4. Place the chromatograph away from busy, congested areas. Remote, isolated areas are best for high-sensitivity work.

5. Avoid very dry areas and areas that are carpeted. Static electricity can affect instrument performance. Antistatic floormats and benchmats are useful if spiking caused by static charge is a problem.

6. Avoid areas where radio frequency interference is likely. Beeper-type paging devices can be a problem in some installations.

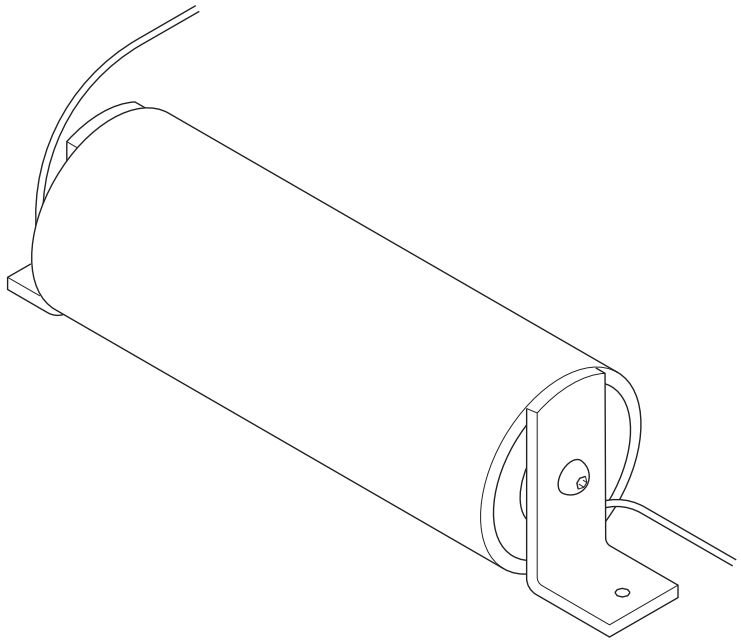
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3.4 Solvent Delivery Requirements

Electrochemical detection requires a solvent delivery system that delivers a constant rate of flow. The detector will respond to long-term and short-term changes in flow rate. A gradual increase in flow rate will produce a gradual increase in baseline current response. Likewise, a rapid cycling in flow rate will cause a sinusoidal baseline response. Both types of flow rate changes will severely limit detector performance. Ideally, this instrument will be part of a BAS 480 Liquid Chromatograph. The BAS PM-80 Pump in this system provides excellent flow-rate precision and minimal pump pulsation. The PM-80 Pump may be connected directly to the injection valve port (see #26 in Figure 3.1).

The PM-80 pump includes a pulse damper to reduce short-term flow rate fluctuations. For other pumps, however, an external pulse damper may improve performance. BAS offers a rugged pulse damper (P/N MF-4000) that is suitable for use with any LCEC system (see Figure 3.6). It is connected between the pump and injection valve. The damper contains a flattened coil of 1/4" tubing which expands at high pressures and flattens at low pressures. This effectively steadies the flow rate over time.

Figure 3.6. MF-4000 Pulse Damper.



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3.5 Arrangement of Components and Connections

NOTE: Should you be installing as retrofits either the LC-4C Dual Upgrade Kit or the CC-5 Preheater Module, refer to the installation instructions in Section 5 or Section 6.2, respectively. The instructions below are for startup in a new installation.

1. Arrange the various components per Figure 3.7. The LC-4C controller(s) must always be placed immediately under the CC-5 compartment. The CC-5 has been sealed along all joints. If an accidental leak should occur, the compartment could hold over 500 mL of liquid before overflowing to the components below.

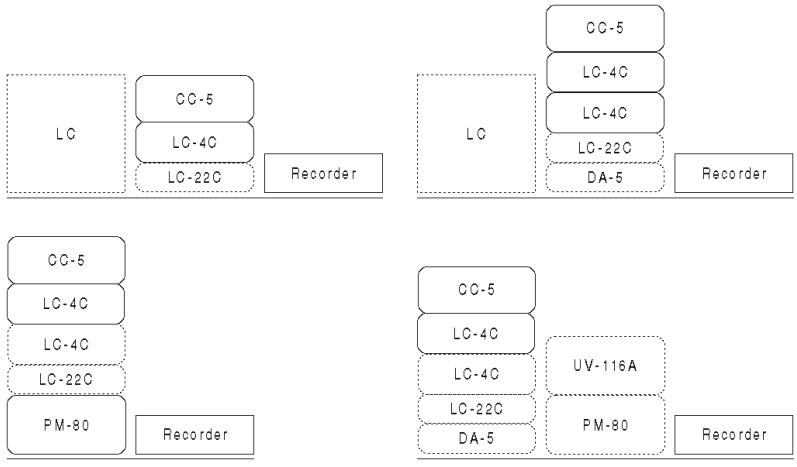
2. If you purchased the CC-5 compartment with Preheater Module, connect the two cables on the preheater to the LC-22C Temperature Controller (see Figure 3.8). The large power cable from the Preheater Module plugs into either of the two matching connectors on the LC-22C rear panel. The control lead with color coded banana plugs connects to the corresponding set of banana jacks. If you plugged the power cable into the HEATER1 connector, plug the banana plugs into the PROBE1 jacks. If you used HEATER2 for the power cord, connect the banana plugs to the PROBE2 jacks.

3. Connect the electrode cable (single or dual) from the CC-5 to the LC-4C(s) per Figure 3.9.

4. If you will be using the generator electrode, connect the insulated lead of the GEN detector cable to the WGEN connector on the 16-position terminal strip of the LC-4C back panel. Connect the noninsulated lead to the GND connector. If you will be monitoring the output of the GEN electrode, connect leads to IGEN and GND. (CAUTION: Refer to page 28 under GEN for more details.)

5. For additional support, two brackets and four screws are provided for every additional component in the “stack” of instruments. Brackets are attached at the back panels in the positions shown in Figure 3.9.

Figure 3.7. Schematic for stand-alone electrochemical detectors and BAS 480 components.



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Figure 3.8. Connection of cables from CC-5 Preheater Module to LC-22C Temperature Controller.

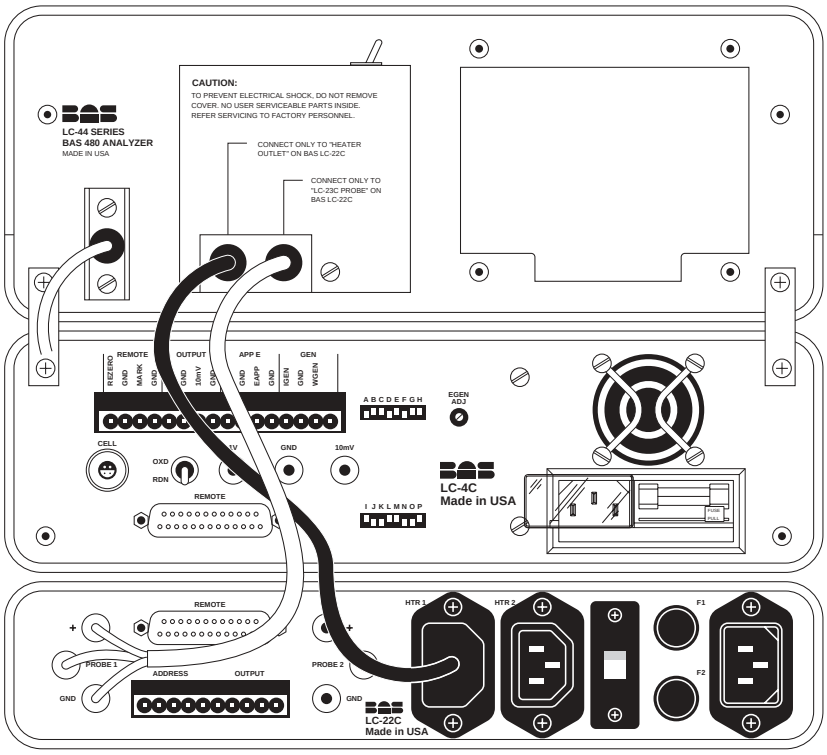
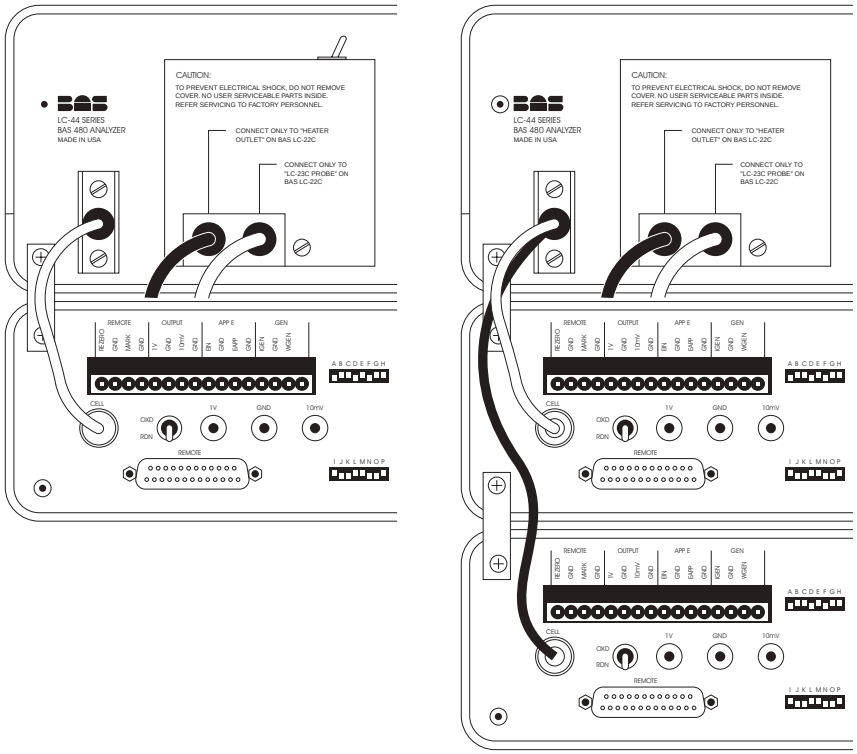


Figure 3.9. Connection of leads to LC-4C(s) for single (left) and dual (right) operation.



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6. Connect the output leads to the strip-chart recorder, integrator, or data station. See Section 4.2 for more information on the LC-4C's rear panel connections.

   a. On each LC-4C, there are four sets of output jacks (see Figure 3.9). Use the green and black sockets for a 1.0 V recording device. Use the blue and black sockets for a 10 mV recording device. Alternatively, use the bare-wire connectors at the OUTPUT section of the 16-position terminal strip on the LC-4C back panel.

   b. Flip the OXD/RDN switch on the rear panel to the mode you are using (oxidation or reduction). If you are unfamiliar with the meaning of these terms, you will find more information in BAS' "Principles of EC Detection and Troubleshooting Guide" (P/N MF-9083). Correct positioning of this switch ensures a positive output signal. This switch can be used to reverse the direction (polarity, see Section 4.2, page 27) of the pen travel (output signal) on the chart recorder.

   c. At the recorder, connect the leads as follows:

      White lead (red plug): "+" or "HI" recorder input
      Black lead (black plug): "–" or "LO" recorder input
      Black jumper (green plug): recorder ground terminal
      (LO is thus jumpered to GND)

      GROUNDING NOTE: It is important that the third plug on the recorder leads be connected to ground. If your data system does not have a ground, connect a wire (included: part #32, Figure 3.1) from the OUTPUT GND of the terminal strip to the threaded hole at the upper left corner of the rear panel. If this is a dual-detector system, also connect a wire between the OUTPUT GNDs of the two LC-4Cs.

   d. Using the ZERO ADJUST or ZERO CHECK on your recorder, zero the recorder at the appropriate side of the chart paper.

NOTE: If you have an integrator or data station with wide dynamic range, match the output of the LC-4C to the input range of your data device. The LC-4C has both 1 V and 10 mV outputs, making it compatible with the majority of popular chart recorders, integrators and workstations. There is a built-in overange on each output that is 10 times the nominal value (e.g., the range on the 10 mV output is from 0 to 100 mV). Use the RANGE switch on the LC-4C Detector to keep the signal you record within the range of your recording device.

7. The entire LC system should be connected to the same ground point. This point should be the safety ground (third wire) of the power circuit you are using.

   To ensure a properly grounded system, have a qualified electrician measure the resistance between each component chassis and circuit ground. The resistance should be less than 1 Ω. If it is not, ground the component chassis together with a jumper wire.

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3.6 Chromatography Connections

1. Place the interconnected detector components next to the column outlet from the chromatograph, or install your column inside the CC-5 (Figure 3.1). Several slots in the CC-5 chassis along the sides and back provide tubing access, no matter what the orientation. On a BAS 480 system, the detector components may sit directly on top of the PM-80 Pump. See Figures 3.2–3.4 and 3.7 for ideas.

   MICROBORE INSTALLATION: A microbore column should be connected directly between the injection valve and the flow cell. You must use a special UniJet™ injection valve (MF-4161) that uses nonconducting fittings. Connect the microbore column to the correct output port of this valve with a high-pressure PEEK fingertight fitting (MF-4165). Release the flow cell from the CC-5 cabinet by undoing the hex screw. Slide the flow cell toward the column until the column bottoms in the entry port, and secure with another fingertight fitting.

   NOTE: It will be necessary to thoroughly degas the mobile phase prior to use. This is particularly important when the cell preheater or column heater is set at 35 °C or higher. The warmth reduces dissolved gas solubility to the point where bubbles may appear in the tubing to and from the electrochemical cell.

   If you are using a flow restrictor to provide back pressure, be sure not to exceed approximately 100 psi (6 atm).

2. Flush the system with 200 mL of acetonitrile/water (40:60, v:v) and divert all column effluent to a waste receptacle. This flushing step cleans any chemicals remaining from previous injections from the column, and also "wets" the hydrophobic stationary phase for reproducible separations. For new columns see below.

   NOTE: Always connect a BAS column so fluid flows in the direction you read the label (because the inlet and outlet frits have different pore sizes).

   Most new BAS columns must be washed before use.* Washing will not only remove residual packing solvents, but will give sufficient time for elimination of bubbles from the system. For C_{18} and C_8 stationary phases, this step also "wets" the stationary phase surface. To wash the column, pump at least 200 mL neat acetonitrile through it. Then pump 50 mL 40:60 acetonitrile:water through it to remove the neat acetonitrile. (It is important not to mix neat solvents and mobile phase in the system or column, since buffer salts may precipitate.) Then pump mobile phase.

   The column should be thoroughly equilibrated with mobile phase before connecting the detector. If you are using an ION-PAIRING REAGENT in your mobile phase (e.g. Sodium Octyl Sulfate), you should allow up to 8 hours for equilibration at a flow rate of 1 mL/min.

   * Exceptions include enzyme columns, acetylcholine columns, and preloaded catecholamine columns, which must never be washed with solvents. SepStik microbore columns should be washed with acetonitrile for 15 minutes at microbore flow rates.

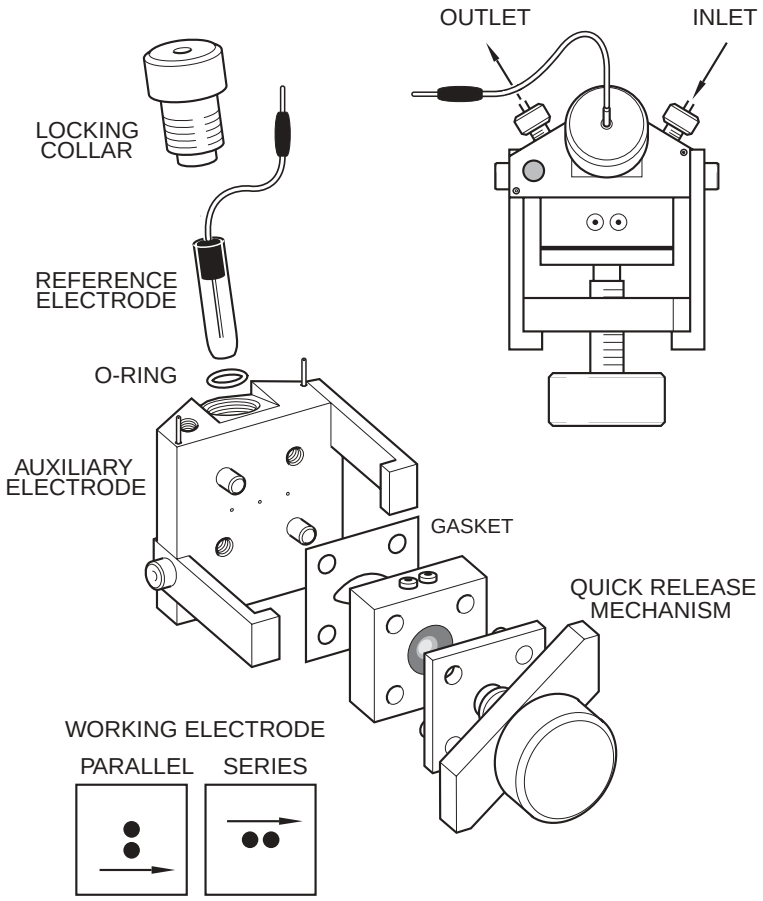
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3. While the column is equilibrating, remove the three reference electrodes from the detector accessories package. Follow diagrams in the enclosed Electrode Manual for removal of the protective sheath used in packaging and shipping. Soak all reference electrodes in a 3 M sodium chloride solution, in a setup which KEEPS THE CONNECTING PIN DRY.

4. Assemble the remaining parts of the thin-layer electrode and reference electrode retainer. See Figure 3.10 for identification of parts.

   Remove the clamp and backing plate from the packing box.

Figure 3.10. Final assembly of cell components.

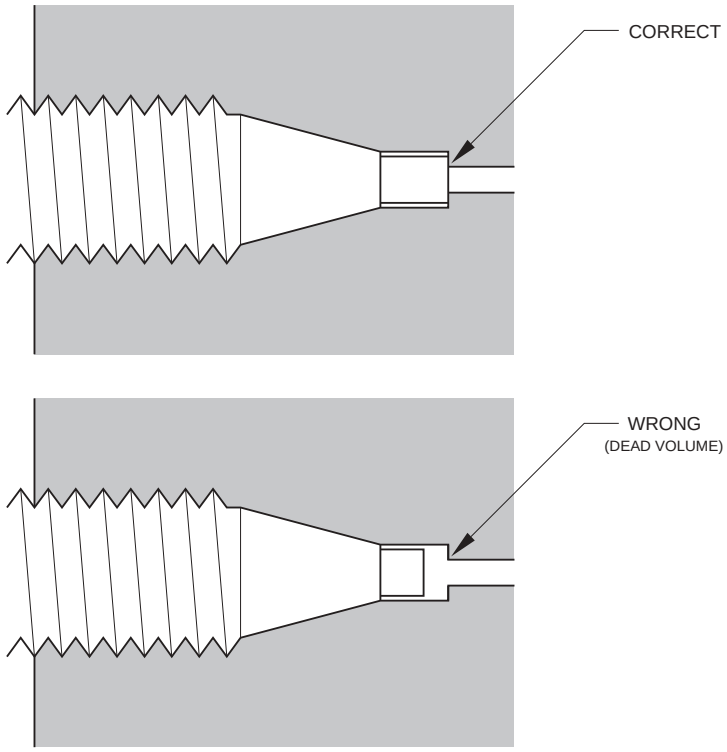


5. Turn the pump to a lower flow rate (e.g., 0.5 mL/min) and make connections to the flow cell as described in (6) or (7) below. Place a lab tissue in the vicinity of the cell to help soak up the leakage while you complete attachments. We recommend that you make the connections with the flow on, to help prevent bubbles in the connecting tubing.

   Refer to Figure 3.11 for correct assembly of the fittings and tubing prior to placing them in the cell inlet port. This is important when you are trying to maintain a zero-dead-volume connection and protect the integrity of your chromatographic peaks.

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Figure 3.11. Correct alignment of Teflon tubing in fingertight fittings.



6. Systems WITHOUT CC-5 Preheater Module: Connect the TEFLON detector inlet tube directly to the column outlet. Then connect the detector outlet tube to the auxiliary electrode block. Route outlet from the auxiliary electrode to waste through the groove in the CC-5 cabinet (see Figure 3.1, #30).

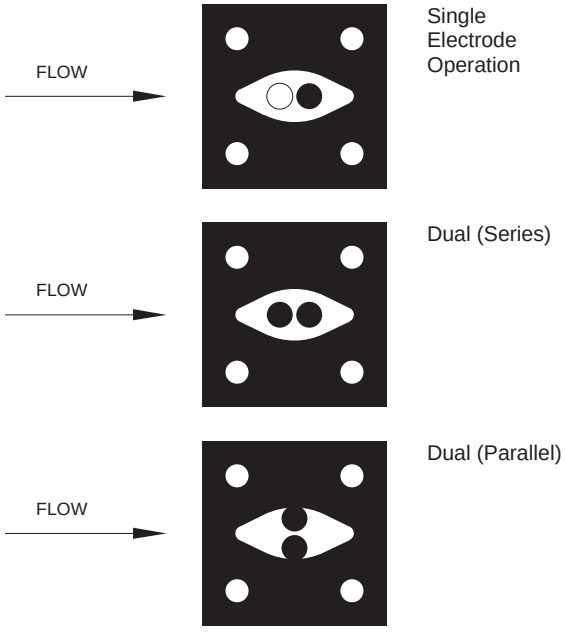
7. Systems WITH CC-5 Preheater Module: Connect the column to the thin STAINLESS STEEL tube of the preheater block (Figure 3.1, #22). The CC-5 Preheater is shipped with the tubing already wrapped in place. Remove the tape which is used to keep the tubing secure during shipping. If for some reason you can't place your column inside the CC-5, it may be necessary to obtain extra tubing (MR-4015) and a zero-dead-volume union (MR-4063) to extend the tubing outside the compartment. Note that the large side slots allow access for the column body even with the top down.

   NOTE: For units with cell temperature control (preheater), DO NOT allow the thin metal tubing on the inlet to touch the stainless steel auxiliary block. The plastic fitting on the capillary steel inlet tube acts as an insulator between the tubing (at ground potential) and the auxiliary electrode (at its own potential). If you allow the steel tubing to protrude beyond the end of the fitting (at the cell end) you will short-circuit the cell. After making this connection, use an ohmmeter to see if there is a continuous circuit between the stainless steel tubing and the auxiliary electrode (#22 and #7 of Figure 3.1). If there is, loosen the plastic nut, back the tubing out slightly, and retighten the nut. Use the ohmmeter again to make sure there is no electrical connection between the two components.

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8. Place a 0.002"-thick TG-2M cell gasket (MF-1046) over the two registration pins on the steel auxiliary electrode block, keeping the long axis of the cut-out in a horizontal orientation. Align the WORKING ELECTRODE BLOCK in the configuration desired. Note the orientation of the dual electrodes in relation to the flow through the thin-layer cell (Figure 3.12). The steel backing plate fits behind the working electrode. The two registration pins of the backing plate should occupy the two opposing holes of the working electrode block not occupied by the auxiliary electrode block. You may also use this plate as a convenient handle when polishing the working electrode.

Figure 3.12. Placement of thin-layer gasket and orientation of working electrode for Single Electrode, Dual Series, and Dual Parallel Operation.



NOTE: The gaskets determine the dead volume in your thin-layer cell. You can stack gaskets on top of each other to increase the dead volume of the cell. Additional gasket thicknesses are available for separate purchase [e.g., TG-5M = 0.005" (127 µm) thick, TG-15M = 0.015" (381 µm) thick, TG-2M = 0.002" (51 µm) thick]. Dead volume determines how FAST the sample flows through the cell. Changing gaskets allows you to influence this rate of flow independently of the flow through the rest of your LC system. The TG-2M gasket is recommended for most applications. In some cases (e.g., microbore), a very thin gasket (MF-1044) may be desirable. In other cases (e.g., amalgam electrodes), a thicker channel is preferred.

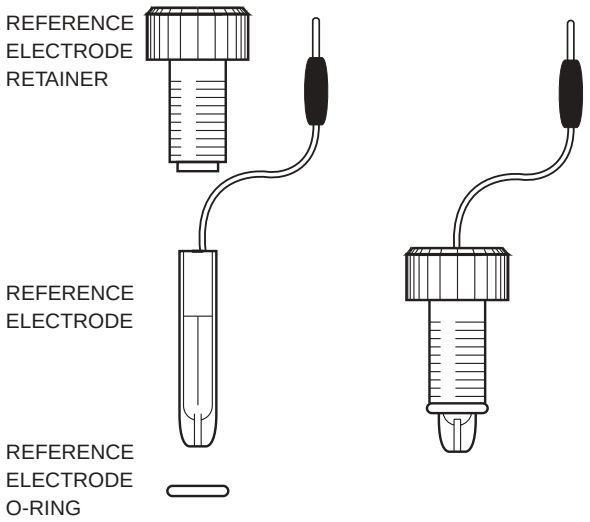
9. Slip the clamp into place with the crossbar under the projecting arms of the auxiliary electrode, and screw until tight. Don't be excessively forceful when tightening.

NOTE: Some users prefer to mount the working electrode directly to the auxiliary electrode with screws rather than the clamp. This mounting is available as an option by purchasing a stainless steel backing plate (P/N MR-1006) and stainless steel screws with a hex wrench (P/N MF-1049). To mount the working electrode, put the backing plate behind the working electrode and insert two screws through the entire sandwich into the threaded holes. Tighten securely.

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10. Remove one of the reference electrodes from the sodium chloride solution and assemble with the retainer and O-ring as shown in Figure 3.13.

Figure 3.13. Exploded and assembled views of reference electrode components.



11. Wear safety glasses for this step. At this point, fluid should be rising into the reference electrode well. Let it keep rising until it is almost to the top, then screw the assembled reference electrode into the well until it is snug. DO NOT USE EXCESSIVE FORCE! The O-ring will wipe the inside of the well dry as you screw the retainer down. It's a good idea to hold a lab tissue behind the vent hole to absorb the excess fluid as it squirts out.

12. Wipe up all spills with lab tissues before proceeding.

NOTE: Removal and reinsertion of the reference electrode as in steps 10–12 is a useful technique to remove bubbles that have formed in the reference well. Such bubbles produce a regular pattern of baseline oscillation with a period that matches the pump stroke (because the bubbles are alternately compressed and allowed to expand).

13. Make sure that flow is continuing through the entire cell (it should be dripping through the waste outlet tube into your waste receptacle). There should be no leaks from the thin-layer gasket area, connecting tubes, fittings, or reference-electrode retainer. Increase the flow rate to the level you anticipate using for your assay and check the flow through the cell again.

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14. While the power to the LC-4C controller is still OFF, make electrical connections to the working electrode controlled by a SINGLE LC-4C as follows:

    White connector (socket): to reference electrode pin
    Black connector (W1 pin): to working electrode socket
    Black connector (GEN or WG pin): to generator electrode (if used)
    Red connector: to auxiliary electrode (pin on top surface)

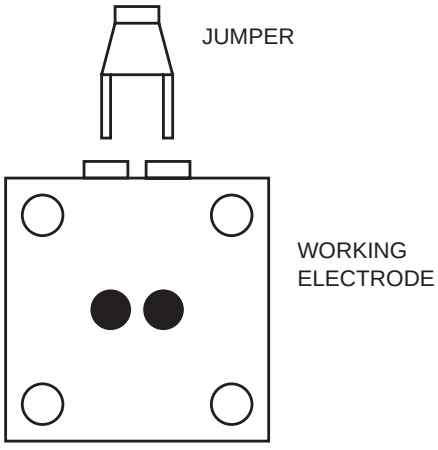
    At the other end of the single-electrode cable is a special connector that plugs into the CELL socket on the back of the LC-4C detector. Make sure that the internal pins of this connector are aligned before plugging in.

    Connect the other end of the GEN (WG) cable (if used) to the 16-position terminal strip on the LC-4C rear panel. The insulated lead goes to the WGEN terminal, and the bare lead goes to the GND terminal. The GEN lead MUST be disconnected at the rear panel when not in use. Otherwise, the GEN pin is LIVE when the cell is ON.

    NOTE: If you are using a dual working electrode, you may use either sensor, and reserve the remaining one as a "backup." If using one as a generator electrode, it must be upstream of the detector electrode. Before connecting or disconnecting leads to the electrodes, be certain that the CELL switch is in the STBY position. Never remove a working-electrode lead when the CELL switch is ON; static electricity in your hand can damage the detector's amplifiers.

15. To use the optional ELECTRODE JUMPER (MW-2010, Figure 3.14), insert the working electrode lead into the bottom of the jumper and then place the adjacent pins into the connectors. With the jumper, you double the size of the working electrode. This will increase the signal, but you should also expect an increase in noise. In most cases the electrodes should be oriented parallel to the flow stream. USE ONLY THE W1 LEAD AND A SINGLE LC-4C CONTROLLER WHEN USING THE ELECTRODE JUMPER!!

Figure 3.14. Dual Electrode Jumper (packaged with other accessories in the gray box).



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16. Dual-Electrode Operation:
    Customers with a single LC-4C detector can operate the generator and working electrodes at independent applied potentials (QUASI-DUAL mode). Only the working electrode produces a high-quality filtered output.

    Customers with two LC-4C detectors can operate two working electrodes, each producing high-quality filtered output (DUAL mode). Each electrode is controlled by a single LC-4C controller.

    Again, the orientation of the electrode should be considered (see Figure 3.12). See BAS applications literature for explanations of the role of the dual-series vs. the dual-parallel arrangements.

    Locate the DUAL electrode cable, which has 2 BLACK working electrode wires. The black GEN wire is separate from, but runs alongside, the DUAL electrode cable. Connect each black working electrode wire to the pin connectors on the electrode block as follows:

    Black wire with “W1" label: to working electrode #1
    Black wire with ”W2" label: to working electrode #2
    The red and white wires are connected as for single electrode detectors (see step 14). In most cases the generator-electrode lead will not be used during 2-controller operation. The GEN lead MUST be disconnected at the rear panel when not in use. Otherwise, the GEN pin is LIVE when the cell is ON.

NOTE: For dual-detector operation, it is best to ensure that both detectors are grounded together. The most reliable way to do this is to connect a jumper wire between the OUTPUT GNDs of the two LC-4Cs. Use either the bare-wire connectors or the banana jacks.

For dual electrode operation, the electrodes may be oriented in two ways (Figure 3.12). For series experiments (one electrode upstream of the other), mount the block so the pin connections are on top. For parallel experiments, mount the block so the pin connections are vertical. Either electrode may be connected to the “W1" or the ”W2" cell leads.

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At the other end of the DUAL electrode cable are two special connectors that plug into the CELL socket on the back of the LC-4C detectors. The longer of these leads controls W2, and the shorter controls W1. Make sure that the internal pins of these connectors are properly aligned before plugging in. The W1 lead can be used for SINGLE electrode applications, but the W2 lead CANNOT.

At the recorder, connect the leads as follows (see #6 above):

from W1 detector:
white lead (red plug): "+" or "HI" recorder input
black lead (black plug): "–" or "LO" recorder input
black jumper (green plug): recorder ground terminal (LO is thus jumpered to GND)

from W2 detector:
white lead (red plug): "+" or "HI" recorder input
black lead (black plug): "–" or "LO" recorder input

DO NOT use black jumper (green plug) to jumper LO to GND since this has already been accomplished through the OUTPUT GNDs of the two LC-4Cs, as under NOTE above. This prevents the occurrence of a ground loop (i.e., two grounds instead of one).

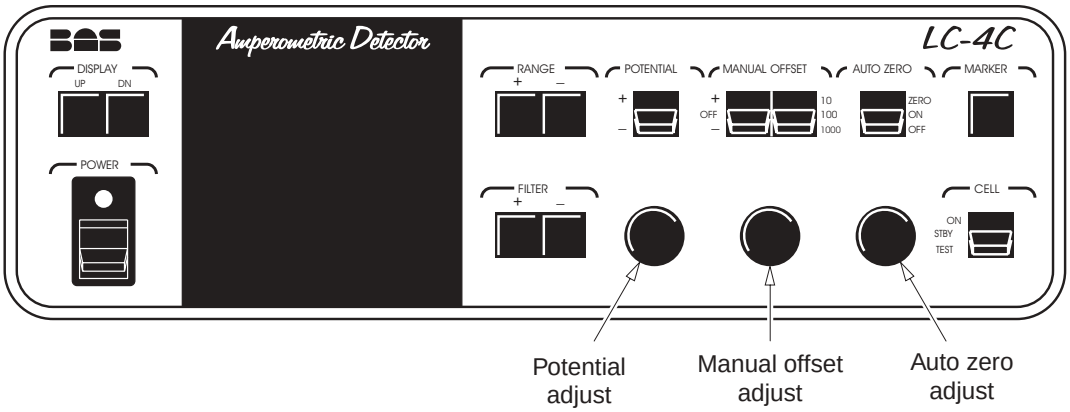
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Section 4. Operating the Electrochemical Detector

4.1 LC-4C Front Panel

This section describes each feature of the LC-4C's front panel (Figure 4.1).

Figure 4.1. Front panel of LC-4C detector controller.



POWER

Applies main electrical power to controller circuitry; the red indicator light directly above the switch will be illuminated when power is on. NOTE: When turning main power on or off, the working electrode (CELL switch) should be placed in the Standby position (STBY).

DISPLAY

This switch selects the function to be monitored by the internal digital voltmeter (DVM) and displayed on the front panel window. Current at the generator electrode (Igen, in μA or nA), potential applied to the generator electrode (Egen, in V), potential applied to the working electrode (App E, in V), output current (Output, in nA or μA), auto-offset current ($Offset_A$, in nA or μA) and manual offset current ($Offset_M$, in nA) are chosen by pressing the up or down DISPLAY keys until the desired function is displayed on the lighted panel. The digital value of the selected function will be shown in the middle of the display and the units on the right side. The nominal ranges for each of the individual functions are: generator-electrode current, 0 ± 10 μA; generator-electrode applied potential, 0 ± 1.900 V; working-electrode applied potential, 0 ± 1.999 V; output current 0 ± 50 μA; auto-offset current, 0 ± 1000 μA; and manual-offset current, 0 ± 1000 nA.

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RANGE

The RANGE keys set the controller output (gain) range. Preprogrammed ranges are selected by pressing the [+] and [–] keys and observing the display. Ranges from 0.1 nAfs (nanoamps per full-scale pen deflection) to 50 μAfs are provided in 18 steps. For example, a setting of 10 nAfs indicates that an output signal of 5 nanoamps would produce a pen deflection of one-half full scale. Note that full-scale deflection output is either 1 V or 10 mV, depending on which back-panel output jacks have been chosen (see Section 4.2, and Section 3.5, #6 for discussion of Output). Also note that we have built in a 10-times overvoltage capacity, so that the maximum output voltage is approximately 10 V at the 1 V jacks and 100 mV at the 10 mV jacks.

GENERATOR-ELECTRODE POTENTIAL

For certain applications, the upstream electrode of a dual working-electrode cell can perform an electrochemical derivatization. Products of this upstream derivatization are then detected at the downstream electrode. Set the generator potential by first keying the DISPLAY to read Egen. Then use a small screwdriver or alignment tool to turn the EGEN ADJ potentiometer on the back panel (see Figure 4.2) until the desired voltage (0 ± 1.900 V) is displayed.

WORKING-ELECTRODE POTENTIAL

Any applied potential between 0 and 1.999 V can be selected by keying the DISPLAY to read App E, then turning the POTENTIAL knob until the desired value is displayed. Selection of [+] or [–] indicates positive or negative potentials, respectively.

AUTO- AND MANUAL OFFSET, AUTO ZERO

The OFFSET controls are used to null out the steady-state background current and thereby establish the operating baseline at zero output. Two kinds of offset are provided: manual and automatic. **In general, the auto-offset is simpler and easier to operate, and we recommend using it exclusively.** In rare instances, the auto-offset may not have enough dynamic range to zero the instrument. If this occurs, use just enough manual offset to lower the background current to a value the auto-offset can handle. The AUTO-OFFSET circuitry can offset the following amounts of current in each given range:

RANGE	CURRENT
0.1 – 0.5 nAfs	0 ± 10 nA
1 – 5 nAfs	0 ± 100 nA
10 – 50 nAfs	0 ± 1000 nA
100 – 500 nAfs	0 ± 10 μA
1 – 5 μAfs	0 ± 100 μA
10 – 50 μAfs	0 ± 1000 μA

The magnitudes of the offset currents are shown on the front panel in the $Offset_A$ and $Offset_M$ displays. The AUTO-OFFSET circuitry is enabled by switching the AUTO ZERO toggle

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switch to the ON position. An auto-zero event is initiated by moving the toggle switch momentarily to the ZERO position. The Output display (and the output to the chart recorder) will be set to zero, and whatever current there was will be added to the $Offset_A$ display. A remote-control connector to AUTO ZERO is provided on the terminal strip of the back panel (see Figure 4.2).

A control knob is provided below the AUTO ZERO toggle switch on the front panel to enable a small amount of voltage to be added to the output. This may be necessary for integrators that work best with a slightly positive "zero" baseline. The effect of this control knob is not apparent on the LC-4C display: it only affects output to the recording device. An adjustment is needed if 1) the baseline on your integrator is zero or negative, or 2) your chart-recorder pen does not go to the "zero" position when the LC-4C output display reads zero. To use this control, zero both the LC-4C and the recording device independently, then turn the control knob until the baseline on the recording device is at the desired location. This control is only active when AUTO ZERO is on.

The MANUAL OFFSET knob can be turned to null out background currents from 0–10 nA, 0–100 nA, or 0–1000 nA, depending on the range chosen. The range and sign of the offset are determined by two toggle switches located above the adjusting knob. When set on [+], the circuit will null anodic (oxidative) currents; on [–], it will null cathodic (reductive) currents. As with auto-offset, the circuitry is disabled when OFF is selected.

When both AUTO- and MANUAL OFFSETS are off, the detector continues to function normally, and the OUTPUT display shows the true current output of the detector. When both offsets are on, the true current output of the detector is the sum of the Output, $Offset_A$, and $Offset_M$ displays.

MARKER

This front-panel push button will produce a momentary pen deflection on your chart recorder to mark events. A remote-control connection to MARKER is provided on the terminal strip on the back panel (see Figure 4.2).

CELL

The CELL switch controls the function of the working electrode. In the STBY mode, the working electrode is off. In the ON mode, the working electrode is on and the selected potential is applied. The TEST mode is used only for troubleshooting the controller for electronic problems (see Appendix I, page 41).

WARNING: The CELL switch must be in the STBY position when the LC-4C is turned on or off, and for any electrode servicing. Disassembling a "live" cell can permanently damage the working electrode and the electronic circuits.

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FILTER

This control adjusts the cutoff frequency of the active 4-pole Bessel filter. Filter settings between 0.02 and 1.0 Hz (in 14 steps) may be selected by pressing the [+] and [–] keys. The filter setting selected is shown on the display. A filter value of 0.1 Hz is a good general-purpose setting. The optimum filter setting depends on peak width. A filter setting of 0.02 Hz provides the greatest amount of filtering, and 1.0 Hz provides the least amount.

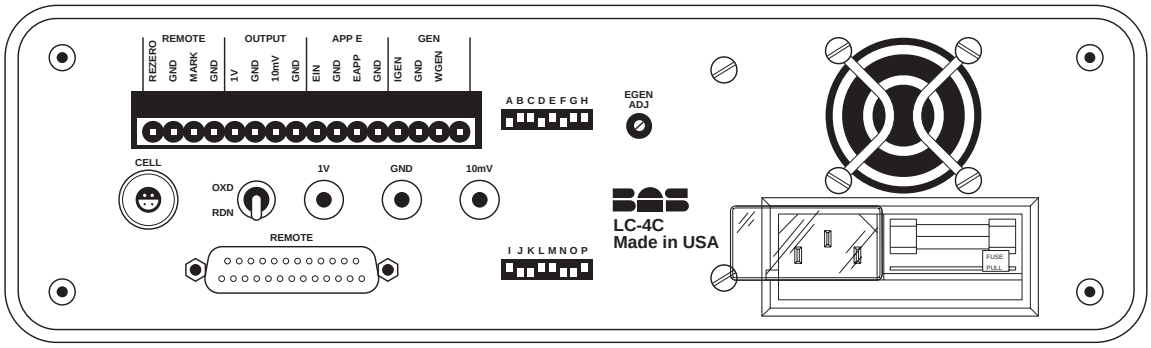
If you wish to optimize the filter setting for your separation, try several runs with different filter settings. Both peak height and noise will increase as filter frequency increases, and decrease as filter frequency decreases. Choose the range that gives the highest signal-to-noise ratio (peak height divided by baseline noise).

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4.2 LC-4C Rear Panel

This section describes features of the LC-4C's rear panel (Figure 4.2).

Figure 4.2. Rear panel of LC-4C detector.



CELL

The cell-lead cable is attached to this connector. Make certain the two components are properly aligned before making the connection. To make the connection, insert the cell-lead cable connector into this socket, until it snaps into place. Do not force this connection. When two LC-4C's are connected in DUAL mode, the detector connected to the longer cell lead controls the W2 electrode. The W1 lead (of a dual cable) can be used for SINGLE electrode applications, but the W2 lead CANNOT.

OXD/RDN SWITCH

The OXD/RDN toggle switch (to the right of the CELL connector) will reverse the polarity of the output signal, thus changing the direction the pen moves on the chart recorder. DO NOT reverse polarity by reversing the leads coming from the LC-4C. In general, the switch should be up for oxidative work and down for reductive work. This will provide a positive-going output voltage for oxidation or reduction reactions (a positive-going output is needed for most integrators and computer workstations). Chart recorders can usually take negative-going signals; when using two detectors, you may reverse one of the switches to have the pens coming from different sides of the chart paper.

OUTPUT JACKS

These three banana-jack terminals (to the right of the OXD/RDN switch) feed the analog output from the LC-4C to a recording device. The output signal is a voltage corresponding to the amount of current produced at the working electrode. Two output voltages are provided. Use the blue and black jacks for recorders requiring a 10 mV input, and the green and black jacks for those requiring a 1 V input. BE SURE TO CHECK THE INPUT REQUIREMENTS FOR YOUR RECORDER! To use both outputs simultaneously, you must join their ground wires into a single connection to the black jack, or make a "Y" connector to split the black jack into two leads. Alternatively, attach bare-wire leads to the appropriate OUTPUT connectors on the terminal strip (see below).

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TERMINAL STRIP

A 16-connector terminal strip allows connections for the following functions:

REMOTE

Connections here allow remote REZERO (AUTO-ZERO) and MARK (CHART MARK). The functions operate identically to those on the front panel, but are initiated by a switch closure to ground, or a low level TTL signal, from a remote instrument such as an autosampler or integrator.

OUTPUT

Both 1 V and 10 mV outputs are provided. These outputs are identical to those from the banana jacks described above, and merely provide a bare-wire alternative for connections.

APP E

Connections are provided for both control and monitoring of working-electrode applied potential. The EIN connector takes an externally applied voltage and adds it to the working electrode. Thus, if you apply 0.6 V to this connector, and 0.2 V via the front-panel controls, the working electrode will be at 0.8 V relative to the reference electrode. The correct value of 0.8 V will be displayed in the App E section of the front-panel display.

The EAPP connector can be used to monitor the applied potential with an external voltmeter, and may be used in the self-test procedure (see Appendix I, page 41).

GEN

This section of the terminal strip concerns the generator electrode. The IGEN connector provides an output for monitoring the current produced at the generator. Ranges are selected at the rear-panel dip switches G and H (see below). Connect leads to IGEN and GND to monitor this output. This is a 1 V signal.

Potential is applied to the generator electrode via a connection to WGEN. The WGEN lead has a male pin connector at the electrode end and two wires at the other end. Connect the male pin to the generator electrode, the insulated wire to the WGEN connector on the terminal strip, and the bare wire to the GND connector of the terminal strip. WGEN is LIVE whenever the CELL switch is ON. Therefore, disconnect the GEN lead from WGEN when not using the generator. Alternatively, cover the live end of the lead with an insulator (a 200 µL pipette tip works well). Otherwise, DAMAGE TO THE INSTRUMENT MAY RESULT!

EGEN ADJ

See GENERATOR-ELECTRODE POTENTIAL (Section 4.1, page 24) for a description of this feature.

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DIP SWITCHES

The LC-4C has two eight-position dip switches for setting certain gain ranges and defaults. Switches A to E set the default gain range of the working electrode. Dip switch F is not used. The default gain range is automatically set at power-up, and will be in effect until a change is made with the RANGE control. The factory-set default gain range is 0.5 µAfs. You may want to reset this default to the gain at which you typically work. Then the LC-4C will automatically re-equilibrate after a power outage. Dip switch positions are as follows:

Power-up	Dip switch position				
gain	A	B	C	D	E
0.1 nAfs	DN	DN	DN	DN	DN
0.2 nAfs	UP	DN	DN	DN	DN
0.5 nAfs	DN	UP	DN	DN	DN
1.0 nAfs	UP	UP	DN	DN	DN
2.0 nAfs	DN	DN	UP	DN	DN
5.0 nAfs	UP	DN	UP	DN	DN
10 nAfs	DN	UP	UP	DN	DN
20 nAfs	UP	UP	UP	DN	DN
50 nAfs	DN	DN	DN	UP	DN
0.1 µAfs	UP	DN	DN	UP	DN
0.2 µAfs	DN	UP	DN	UP	DN
0.5 µAfs	UP	UP	DN	UP	DN
1.0 µAfs	DN	DN	UP	UP	DN
2.0 µAfs	UP	DN	UP	UP	DN
5.0 µAfs	DN	UP	UP	UP	DN
10 µAfs	UP	UP	UP	UP	DN
20 µAfs	DN	DN	DN	DN	UP
50 µAfs	UP	DN	DN	DN	UP

All other settings of A to E are invalid and will cause the gain display to go blank.

The factory-set default gain range for monitoring the output of the generator electrode (Igen) is 10 µAfs. The default can be reset with the following positions of dip switches G and H:

Gain	Dip switch position	
range	G	H
100 nAfs	UP	UP
1.0 µAfs	UP	DN
10 µAfs	DN	UP
Invalid	DN	DN

The invalid switch setting will cause the decimal point and units to be suppressed on the Igen display.

The bottom set of dip switches (marked I to P) allows the user to choose the power-up default filter setting for the working electrode, the PC remote channel number, and a remote enable.

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Switch positions I to L select the power-up default filter setting of the main working electrode (see table below). The selected filter frequency will be adopted at power-up, and will remain in effect until changed via the front-panel filter controls. The factory-set default range is 0.1 Hz. As with the power-up gain range, set the default filter to the setting you typically use.

Power up	Dip switch position			
filter	I	J	K	L
0.02 Hz	UP	UP	UP	UP
0.03 Hz	DN	UP	UP	UP
0.04 Hz	UP	DN	UP	UP
0.05 Hz	DN	DN	UP	UP
0.06 Hz	UP	UP	DN	UP
0.08 Hz	DN	UP	DN	UP
0.10 Hz	UP	DN	DN	UP
0.15 Hz	DN	DN	DN	UP
0.20 Hz	UP	UP	UP	DN
0.30 Hz	DN	UP	UP	DN
0.40 Hz	UP	DN	UP	DN
0.50 Hz	DN	DN	UP	DN
0.80 Hz	UP	UP	DN	DN
1.00 Hz	DN	UP	DN	DN

Switch positions M, N, and O select the PC-REMOTE selection (see table below). These settings will allow up to 8 detectors to be independently addressed by a remote computer. Each independent detector must be given a unique identification by setting dip switches M, N and O. The default setting is W1.

Remote	Dip switch position		
address	M	N	O
W1	UP	UP	UP
W2	DN	UP	UP
W3	UP	DN	UP
W4	DN	DN	UP
W5	UP	UP	DN
W6	DN	UP	DN
W7	UP	DN	DN
W8	DN	DN	DN

Switch position P enables/disables PC remote control. The default setting is Remote Enabled.

PC remote	P
Remote enabled	UP
Remote disabled	DN

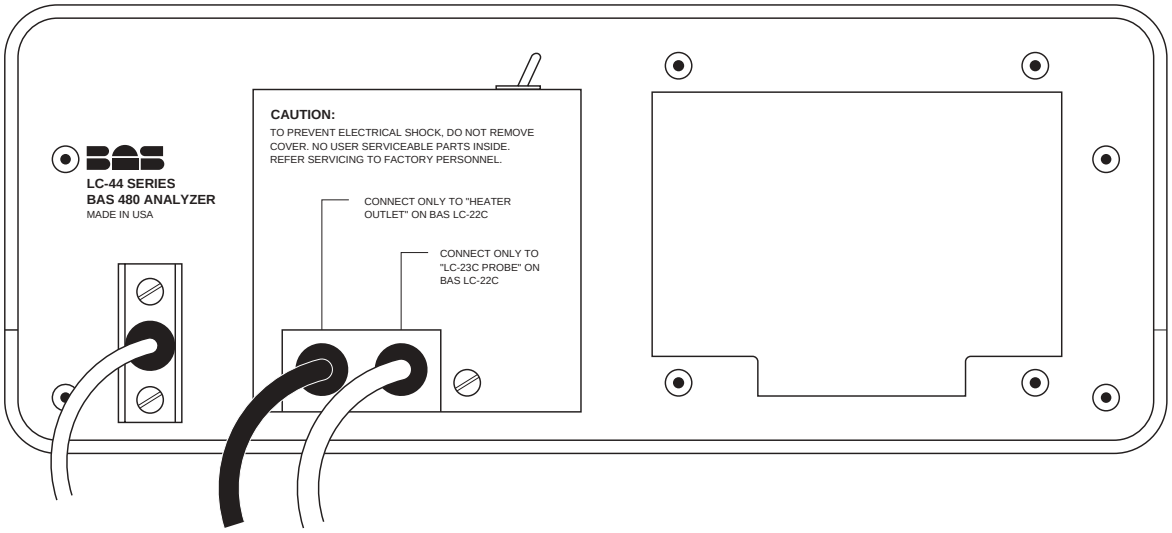
NOTE: Enabling PC remote does not put the LC-4C in REMOTE, but allows the PC interface to control REMOTE/LOCAL operation.

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4.3 CC-5 Rear Panel

This section describes features of the CC-5 Detector's rear panel (Figure 4.3).

Figure 4.3. Rear Panel view of CC-5 Detector Compartment (Preheater option installed).



CELL LEAD

The mounting bracket on either the single- or dual-electrode cable mounts to the rear panel with two 6-32 screws. The cable is grounded to one of these screws from the inside. The cable is generally installed in the CC-5 when shipped.

To convert from a single-electrode cable to a dual-electrode cable, remove the cable screws on the rear panel, and slide the old cable through the back of the CC-5. Install the new cable by reversing this procedure. Don't forget to reinstall the cable's ground lug on the back panel.

DETECTOR PREHEATER MODULE (optional)

The Preheater Power switch (see Figure 3.1 and Section 6) attenuates the wattage entering the preheater to about 60% in the Low setting. The High setting transmits full AC power to the heater (10 W).

Selection of the High or Low setting depends on the desired operating temperature. To reach temperatures from 40–65 °C, flip the switch to High. The output power will be sufficient to attain the setpoint temperature and remain in control. For operation from ambient to 45 °C, flip the switch to Low. Overshoot will be negligible since heater power is cut back.

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UTILITY PANEL

Occasionally it may be necessary to use oversize columns in the CC-5 compartment. The large side slots provide some access, but if you need a larger opening, the rear Utility Panel may be removed. The four screws should be put back into the CC-5 and retightened to maintain the strength of the rear panel. This panel is also removed when an LC-23C cartridge-column heater is installed. We advise leaving the Utility Panel in position whenever possible since it provides continuity of shielding against radio-frequency interference.

4.4 Routine Operating Procedure

Normal Startup

WARNING: The CELL switch must be in the STBY position when the LC-4C is turned on or off, and for any electrode servicing. Disassembling a "live" cell can permanently damage the working electrode and the electronic circuits.

1. Be sure no bubbles are present in the thin-layer region or reference electrode cavity. Recheck electrical and chromatography connections (see Section 3).
2. If using the Cell Preheater to maximize baseline stability, turn it on via the LC-22C controller. Use the HIGH setting on the Preheater for 40–65 °C and the LOW setting for 30–45 °C, and set the LC-22C accordingly.
3. Zero the recorder with its zero control. Baseline should be on the right side of the paper. (This may vary among chart recorder models.)
4. Turn the CELL switch to STBY.
5. Turn the POWER switch to ON.
6. Select desired potential. Use the DISPLAY keys to set the LED display to App E. Use the POTENTIAL toggle switch to change the sign (+ or –), and the knob to change the magnitude, of the applied potential.
7. Set OUTPUT switch on rear panel output for correct mode (oxidation or reduction).
8. Set the output FILTER to 0.1 Hz, or whatever is appropriate for the type of chromatography you are doing. Set the filter using the [+] and [–] keys on the front panel (see Section 4.1).
9. Set RANGE to 1 μA. Use the [+] and [–] keys on the front panel, and observe the display.
10. Turn both OFFSETS to the OFF position.

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11. If you are sure all cell leads are connected, turn the CELL switch to ON.

12. When the electrode is first turned on, you should see a large current signal. This is the "charging and transient background current" and will gradually decay to a steady-state background. You can gradually increase the current amplification (go to smaller RANGE numbers) on the RANGE switch. You will see a temporary increase in signal with each change in RANGE. Go only as far down in RANGE as you need for the assay at hand. There is no benefit in placing the detector on the lowest possible setting when this is not needed.

13. When the background stabilizes, turn the AUTO ZERO on and momentarily push the toggle switch to the ZERO position to rezero the output.

The system is now ready for standards and samples.

Shutdown and Storage

If the detector will be used every day or every few days, leave it on. A bacteria-resistant mobile phase (such as monochloroacetic acid) may be recycled when the system is not in use. Mobile phases that promote bacterial growth (acetates, citrates, phosphates) should not be recycled, and should be changed every few days (generally every two days). Pump these at low flow rates when the system is not in use.

If the detector will be unused for more than 2 or 3 days, the controller should be turned off and the electrodes stored properly. By following these recommended procedures, considerable extension of electrode, column, and system lifetime will be gained.

1. Turn the CELL switch to STBY position.

2. Pump about 50 mL 40:60 acetonitrile:water through the system. This will remove corrosive mobile-phase salts from the plumbing and the column. Turn off the pumping system.

3. Turn off the main POWER to controller.

4. Disconnect electrodes (thin-layer cell and reference). Store the reference electrode as recommended in the separate electrode manual shipped with the instrument. Rinse off the working-electrode block with deionized water, air dry, and store it in the plastic box used in shipping. Clean and dry the auxiliary-electrode block, and plug the column ends.

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Section 5. Upgrading to a Dual Amperometric Detector

The standard LC-44 detector may be converted to a true dual-potentiostat system by adding a second LC-4C detector (see Section 3.6, #16). Each LC-4C controls its assigned working electrode independently, in terms of both potential control and current amplification.

The upgrade requires only an LC-4C controller (or an LC-3C/D/E Petit Ampère) and a dual-cell lead (EW-8114). To set up for dual operation:

1. Turn off power to the detector and disconnect the cell leads from the transducer.

2. Unscrew the two screws holding the detector cable in place. Notice the longer screw and nut for attachment of the ground lug. Unplug the single electrode cell lead from the LC-4C.

3. Slide the old cable out through the back of the CC-5. Insert the new (dual) cable.

4. Reverse step 2 to mount the dual-electrode lead cable. Don't forget to reinstall the cable's ground lug on the inside back panel.

5. Set the detector components as shown in Figure 3.7.

6. The cell lead cable terminates in two connectors that plug into the two LC-4Cs (see Figure 5.1). The short, larger diameter cable serves W1 and the long, smaller diameter cable feeds W2. The lengths should prohibit mixing up connections. For dual-detector operation, it is best to ensure that both detectors are grounded together. The most reliable way to do this is to connect a jumper wire between the OUTPUT GNDs of the two LC-4Cs. Use either the bare-wire connectors or the banana jacks.

7. W1 and W2 labels appear on the electrode leads at the cell. The red and white cables terminate at the auxiliary and reference electrodes, as before.

8. The existing GEN cell lead may remain in place, but will not be used in conjunction with dual detectors. DO NOT connect the GEN lead to the rear-panel WGEN connector unless you plan to use it as a generator. The WGEN terminal becomes LIVE when the CELL switch is turned ON.

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9. Attach recorder leads and zero your recorder for both channels.

   To attach recorder leads:

   from W1 detector: white lead (red plug): "+" or "HI" recorder input
   black lead (black plug): "–" or "LO" recorder input
   black jumper (green plug): recorder ground terminal (LO is thus jumpered to GND)

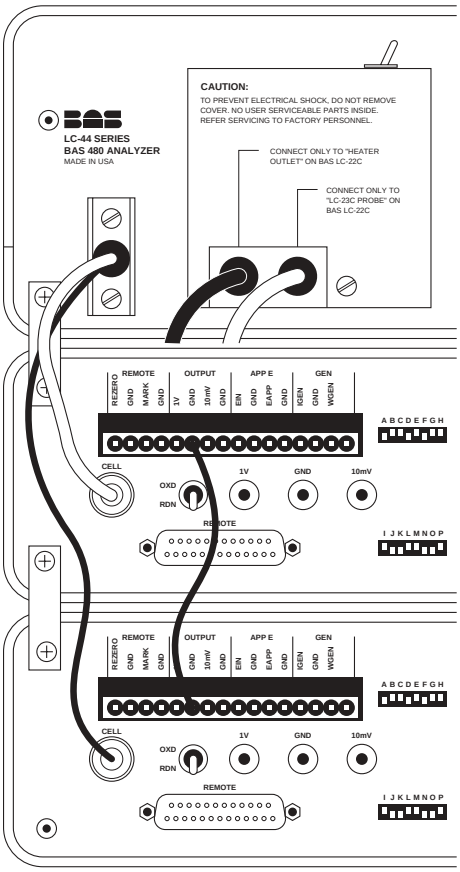
   from W2 detector: white lead (red plug): "+" or "HI" recorder input
   black lead (black plug): "–" or "LO" recorder input

   DO NOT use black jumper (green plug) to jumper LO to GND since this has already been accomplished through the OUTPUT GNDs of the two LC-4Cs. This prevents the occurrence of a ground loop (i.e., two grounds instead of only one).

10. Turn on detector power.

    NOTE: Correct operation is only possible if the LC-4C controlling W1 is ON. Think of the W1 and W2 controllers in a master/slave relationship. For W2 to operate, W1 must be active first. You may elect to operate W1 by itself, as you would with a single LC-4C. That is permissible; the second LC-4C need not be powered. The reverse mode is not possible, however.

Figure 5.1. Connection of electrode cable to tandem LC-4Cs.



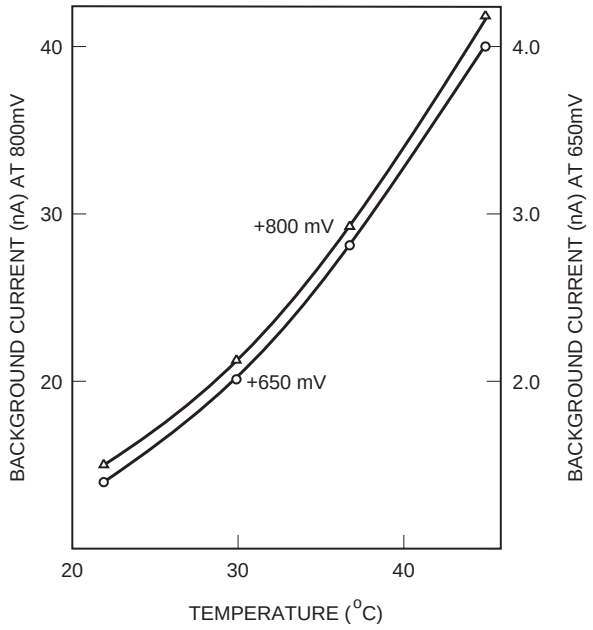
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Section 6. Temperature-Stabilized Detector Operation

6.1 Rationale for Use

Thermostatted control of the effluent temperature before it reaches the cell reduces the effects of ambient temperature change on electrochemical detection. Furthermore, temperature control of the column protects the separation from the effects of temperature change, and when the LC-23C cartridge column heater is used with the CC-5 detector package, the distance between the heated column and cell inlet is quite small and there will be sufficient temperature control to minimize effects on detection. However, in extreme environments (e.g., lab temperature changes due to automated heating/cooling shutdown after business hours, or proximity to vents, ducts, or drafts) or at high detector gain, small fluctuations in temperature at the detector cell can still produce marked deviations in the detector baseline. In these cases both a column heater and a cell preheater may be needed. In Figure 6.1 are shown plots of EC detector background current versus temperature. The slope of the curve (di/dT) is significant, typically 0.5–1.5 nA per °C. Hence it is easy to see how a small change in eluent temperature (e.g., 0.1 °C) could still cause appreciable shifts in the baseline at high gain.

Figure 6.1. Plot of electrochemical background current versus mobile phase temperature on a glassy carbon electrode operated at 650 and 800 mV applied potential.



What phenomena are responsible for this dependence? The background current in electrochemical detection derives from several contributions, the majority component being the oxidation or reduction of the solvent itself.

$$2H_20 \longrightarrow 0_2 + 4H^+ + 4e^-$$

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For water, the reaction is sluggish at moderate potentials; this is due to poor heterogeneous electron transfer kinetics at the electrode surface. Elevations in temperature increase the heterogeneous rate constants, and the background current (the measure of the rate of electron transfer) correspondingly increases. From a noise standpoint, if we must operate at elevated temperature, we must do so precisely. In many cases, only a small rise over ambient is a good compromise. In doing so, one gains control of environmentally induced baseline drift without fighting large temperature coefficients.

Elevated temperatures similarly affect the magnitude of the peak current. It is not unusual to increase peak currents 50–70% (over ambient) by elevated-temperature operation. Although the temperature dependence of diffusion coefficients alone cannot explain this, it is probable that the diffusion layer thickness decreases as the viscosity drops. The concentration gradient at the electrode surface is accentuated, and the end effect is larger peak currents.

Taken separately, the trends in both background current and peak current versus temperature are inadequate in predicting the effect, if any, on detection limits. When the pertinent data are properly expressed in terms of the signal-to-noise ratio, the improvement is not so dramatic. For example, operation at 55 °C requires more vigorous temperature precision than at 35 °C. Thus, a 1.8-times increase in peak current is counterbalanced by a 2- to 3-times increase in baseline noise. A small increase in temperature (35 °C vs. ambient) makes the most sense in terms of signal/noise (Table 6.1).

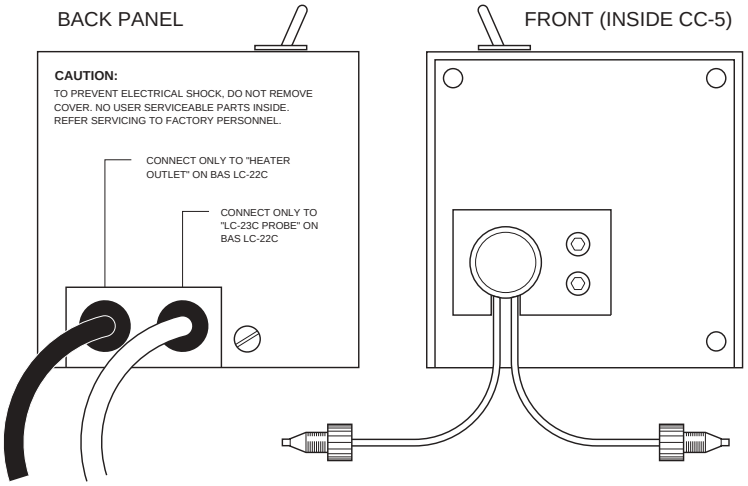
Table 6.1. Signal and noise vs. temperature setpoint on CC-5 Preheater Module.
Conditions: 2.2 mL/min, +750 mV/GC/RE-4 reference, reversed-phase ion-pair separation, norepinephrine is test solute.

Temperature	Peak Height	Noise	S/N
ambient	1.9	0.1	19
35	2.7	0.1	27
45	3.7	0.2	18
55	4.9	0.25	20

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Cell temperature control is an option on all BAS CC-5 series detectors. It may be factory-installed or placed in service by the user in just a few minutes. The Preheater Module is shown schematically in Figure 6.2. A retractable core intimately contacts 2 to 3 turns of capillary tubing for thermal equilibration; the core houses a miniature heater and a solid-state temperature sensor. Both of these devices are connected to a BAS LC-22C Temperature Controller. A switch on the Preheater Module selects the power of the heating circuit, either 10 Watts or 6–7 Watts. A protective cover on the unit isolates the user from all electrical circuits for safety.

Figure 6.2. Optional CC-5 Preheater Module ready for user installation.



6.2 Installation of Detector Preheater Module on CC-5

1. Open the CC-5 cover to the vertical position. Remove the temporary cover from the rear panel (3 screws).

2. Disconnect the tubing between the column and the detector inlet port.

   NOTE: Do not make any electrical connections until step 7. Also, the cover on the Preheater Module must remain in place for safety. Connections to line voltage are exposed when this cover is removed.

3. Slip the heat exchanger block through the permanent rear panel. Use the 3 screws from step 2 and affix the module to the rear panel.

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4. Connect the heat exchanger tubing to the column and detector. The plastic nuts supplied on the tubing act as an insulator between ground potential and the potential of the auxiliary electrode block. Do not allow the steel tubing to protrude beyond the end of the cell fitting. This may short-circuit the cell (see Section 3.6 #7 for further instructions). Make a standard zero-dead-volume connection at the column end.

   NOTE: On current models, the core is machined to diameters which will accept different types of tubing. Capillary tubing is supplied for maximum flexibility.

5. Place the LC-22C Temperature Controller directly under the LC-4C controller(s), per Figure 6.3.

6. Brackets are available to secure the temperature controller, LC-4C and CC-5 together for increased stability. See Section 3.5 #5 for details.

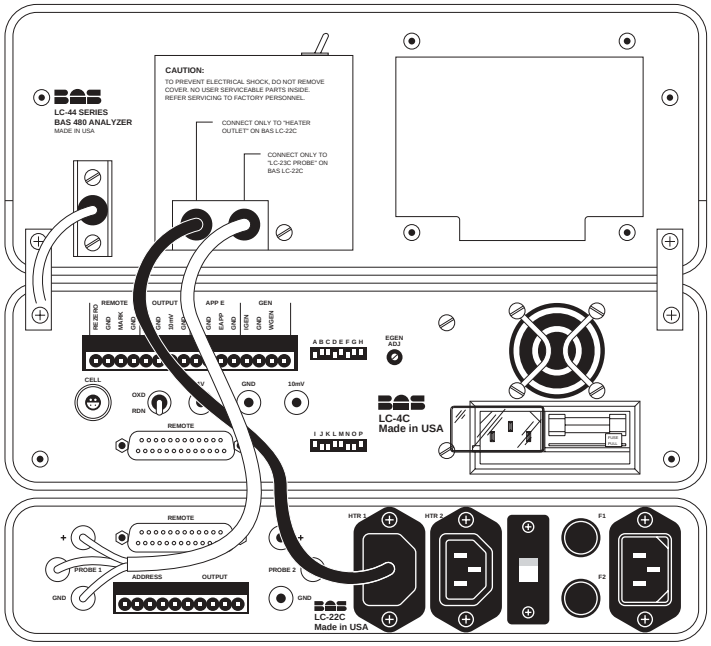
7. Make electrical connections:

   a. Insert the red, yellow and black plugs into the color-matched jacks on the LC-22C Temperature Controller labeled PROBE 1 or PROBE 2.

   b. Plug the Preheater Module power cord into the corresponding power receptacle (HEATER 1 or HEATER 2) on the LC-22C Temperature Controller back panel.

   c. Plug the AC power line cord on the LC-22C to an appropriately grounded wall outlet.

Figure 6.3. Electrical connections for CC-5 Preheater Module.



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Appendix I. Electronic Troubleshooting

Test Procedure, LC-4C

1. With the CELL switch in the STBY position, turn the POWER on. The light directly above the power switch should come on and one of the functions in the LED display should be illuminated. If the lights do not come on, check the fuse, power cord, and wall outlet.

2. Set MANUAL OFFSET polarity switch to OFF. Turn the OFFSET control knob fully counter-clockwise. Set AUTO ZERO to OFF.

3. Set the 10/100/1000 OFFSET range control switch to 10.

4. Set the RANGE to 50 μAfs.

5. Set the FILTER to 0.3 Hz.

6. Set the OUTPUT polarity switch on the rear panel to OXD (up).

7. Plug the black probe of a digital voltmeter into the GND, and the red probe into EAPP, of the APP E section on the rear 16-connector terminal strip. Key the front-panel display to App E. Arbitrarily select voltages between –1.999 and +1.999 V using the POTENTIAL toggle switch and adjusting the knob. Readings on the voltmeter and the front panel display should be within 5 mV.

8. Move the red probe of the voltmeter to the green 1 V output jack or the 1 V connector in the OUTPUT section of the terminal strip. Set the CELL switch to the TEST position. Using the first two columns of the following table as a guide, set the POTENTIAL and RANGE as indicated, and compare Output of the front-panel display and the voltmeter reading with the table. Using the first entry from the table as an example, when APP E is set to 1.0 (V) and the RANGE is 50 μAfs, the output as read on the front panel display should be about 10 μA and the corresponding output voltage should be about 0.20 V. Check the remainder of the outputs (current and potential) for the applied potentials and ranges in the table. It may take 10 to 15 seconds for the voltmeter reading to stabilize when switching between certain ranges, due to the influence of the active filter on the LC-4C. This is normal!

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Applied E (V)	Range	Display Output	Back Panel Output (V)
1.000	50 µA	9.7–10.2 µA	0.194– 0.206
1.000	20 µA	9.7–10.2 µA	0.485–0.515
1.000	10 µA	9.7–10.2 µA	0.980–1.030
1.000	5.0 µA	9.80–10.20 µA	1.960–2.040
1.000	2.0 µA	9.80–10.20 µA	4.900–5.100
1.000	1.0 µA	9.80–10.20 µA	9.800–10.20
0.100	0.5 µA	0.950–1.050 µA	1.900–2.100
0.100	0.2 µA	0.950–1.050 µA	4.750–5.250
0.100	0.1 µA	0.950–1.050 µA	9.500–10.50
1.000	50 nA	9.7–10.2 nA	0.194–0.206
1.000	20 nA	9.7–10.2 nA	0.485–0.515
1.000	10 nA	9.7–10.2 nA	0.980–1.030
1.000	5.0 nA	9.80–10.20 nA	1.960–2.040
1.000	2.0 nA	9.80–10.20 nA	4.900–5.100
1.000	1.0 nA	9.80–10.20 nA	9.800–10.20
0.100	0.5 nA	0.950–1.050 nA	1.900–2.100
0.100	0.2 nA	0.950–1.050 nA	4.750–5.250
0.100	0.1 nA	0.950–1.050 nA	9.500–10.50

9. Return to a POTENTIAL of +1.0 V and RANGE of 20 nA. The OUTPUT should be +0.485 to +0.515 V. Flip the rear panel switch to RDN. The OUTPUT should now be –0.485 to –0.515 V. Flip back to OXD.

10. Flip the MANUAL OFFSET polarity to [+]. Change the display to $Offset_M$. Turn the OFFSET control knob until a reading of 10 nA is shown on the LC-4C display. The output reading on the voltmeter should be 0 V ± 150 mV. Switch the OFFSET polarity to [–] while leaving the POTENTIAL polarity at [+]. The voltmeter should now read +1 V ± 150 mV. Again, voltmeter readings may take 10–15 seconds to stabilize. Return the OFFSET polarity switch to OFF.

11. If the instrument fails any step of this procedure, contact BAS customer service personnel (1-800-845-4246), and advise the service representative that you have completed this electronics check.

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Appendix II. Operation of the LC-4CE

The LC-4CE is a modification of the standard LC-4C. Two circuit elements have been added to better meet the requirements of capillary electrophoresis:

1. A clamp circuit, to protect the LC-4C electronics if the high voltage ground is lost.

2. A waveform generator circuit, to allow electrochemical pretreatment of the working electrode for improved response.

Clamping Circuit

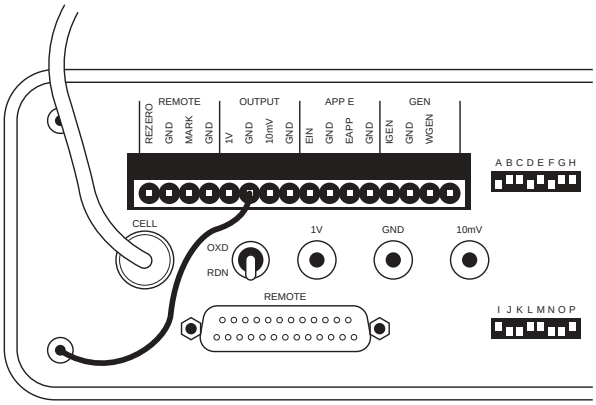
The clamping circuit has no apparent external connections, but uses the LC-4CE's ground as a return path for its high-voltage power supply current. This means that the LC-4CE AND the high-voltage power supply of the electrophoresis unit MUST BOTH be connected to Earth ground for the clamping circuit to operate and protect the LC-4CE.

The LC-4CE has an isolated power supply to avoid ground loops and the noise associated with them. It must be grounded at one point for best performance and for the clamp circuit to operate.

If a *strip chart recorder* is connected to the LC-4CE, a jumper wire between the "–" or "LO" recorder input and recorder ground will provide the Earth ground.

If an *integrator* or *data station* with a differential input is connected to the LC-4CE, the unit should be grounded by placing a jumper wire between the "GND" screw terminal and one of the cabinet PEMs (see Figure A.1). For best noise performance, use only one of the "GND" screw terminals in the OUTPUT section (pins 6 or 8) of the terminal strip to short to Earth ground. Check with the manufacturer of your electrophoresis unit to find out how to ground its high-voltage power supply (if it is not already grounded).

Figure A.1. The LC-4CE, grounded for differential input devices.



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Waveform Generator Circuit

The waveform generator provides a bipolar square wave centered at 0 V. There are four controls associated with the waveform generator:

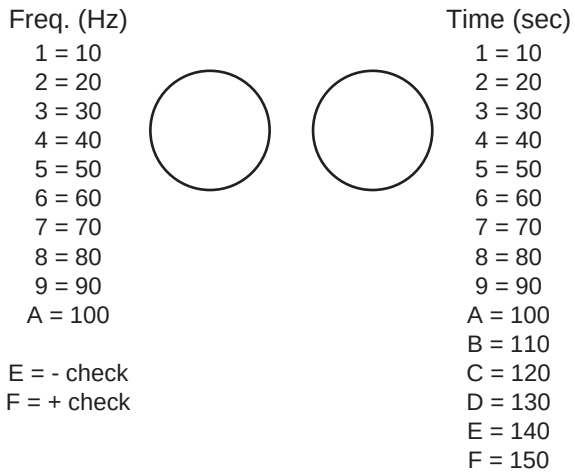
Clean	(on back panel)
Clean adj.	(on back panel)
Freq. (Hz)	(on bottom cover)
Time (sec)	(on bottom cover)

The **Clean** push button starts an electrode cleaning cycle. The **Clean adj.** button sets the amplitude of the bipolar square wave. Clockwise rotation increases the amplitude; the span of the adjustment is 0 to ±‘2.2 V.

NOTE: The waveform is symmetrical around 0 V, so if the positive amplitude is set to +1.00 V, the negative portion of the waveform will be –1.00 V.

The other two controls are located on the bottom cover of the LC-4CE (see Figure A.2). Both controls are 16-position rotary switches.

Figure A.2. LC-4CE bottom cover switches for control of frequency and duration of waveform.



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The **Freq. (Hz)** switch sets the frequency of the square wave. Only 12 positions of this switch are used:

0 = not used	8 = 80 Hz
1 = 10 Hz	9 = 90 Hz
2 = 20 Hz	A = 100 Hz
3 = 30 Hz	B = not used
4 = 40 Hz	C = not used
5 = 50 Hz	D = not used
6 = 60 Hz	E = – check
7 = 70 Hz	F = + check

All positions marked "not used" in the table above will disable the waveform generator. Switch positions "E" and "F" are used in conjunction with the back panel **Clean** push button. As long as the **Clean** button is held, the potential of the LC-4CE will be held at either the negative ("E") or positive ("F") setting. The front panel display setting of **App. E** will display the amplitude of the bipolar square wave; the back panel control **Clean adj.** can be used to adjust the amplitude.

The **Time (sec)** switch controls how long the waveform will be applied to the cell. This switch uses 15 of the 16 available positions:

0 = not used	8 = 80 sec
1 = 10 sec	9 = 90 sec
2 = 20 sec	A = 100 sec
3 = 30 sec	B = 110 sec
4 = 40 sec	C = 120 sec
5 = 50 sec	D = 130 sec
6 = 60 sec	E = 140 sec
7 = 70 sec	F = 150 sec

Position "0", marked as "not used", will disable the waveform generator.

NOTE: The time is determined by counting the pulses at the set frequency, so there will always be an integer number of pulses.

NOTE: The waveform generator circuit uses the outlet for the Remote Applied Potential option in the standard LC-4C, and so precludes the installation of this option.

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Warranty 3
Working electrode 18

Z

Zero 24

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