User's Manual

Electrochemical Detectors

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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.

TABLE OF CONTENTS

APPENDICES

LIST OF FIGURES

LIST OF TABLES

1. Preface

This manual covers operation and service information for the following Bioanalytical Systems products:

Part No.	Description
MF-9094	LC-4B/17A Amperometric Detector
MF-9100	LC-4B/17AT Amperometric Detector
MF-9101	LC-4B/17AT Amperometric Detector (50 Hz)
MF-9098	Dual 4B/17A Amperometric Detector
MF-9102	Dual 4B/17AT Amperometric Detector
MF-9104	Dual 4B/17AT Amperometric Detector (50 Hz)
MF-2034	Dual Electrode Upgrade Kit
EW-4055	Cell Preheater Module
MF-2033	LC-17A Accessories Kit
MF-2035	LC-17AT Accessories Kit
MF-2036	LC-17AT Accessories Kit (50 Hz)

Common to all BAS electrochemical detectors are the LC-4B AMPEROMETRIC CONTROLLER and the LC17A FLOWCELL. The LC-17A was introduced in March 1986 as an improved thin-layer flow cell and Faraday cage. It replaced all previous BAS thin-layer cell designs.

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

1. The LC-4B Dual Upgrade Kit provides the additional electronics, cables and transducer components to transform an existing single electrode detector into dual electrode operation. The rationale and installation instructions for this upgrade is provided in Chapter 9.

2. An optional Preheater Module may be added for better baseline stability at high detector gain or in adverse environments. Its installation and use is described in Chapter 10.

3. An analytical Cartridge Column Heater may also be added to the LC-17A compartment. A removable panel on the back of the instrument is replaced by the LC-23B compartment 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 injection valve may be added by the user when the small cover plate on the front of the LC-17A is removed.

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

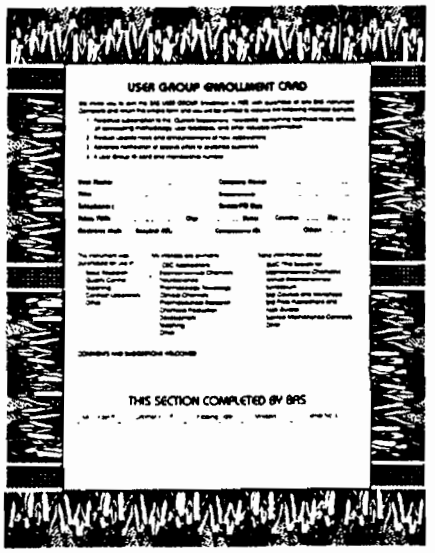
To use this detector, you will require a liquid chromatograph, a data recording device, and appropriate end fittings and tubing for the connection between column and detector. The LC-4B/17A is

a component of the modular BAS 400 series of liquid chromatographs.

The information compiled in this manual represents the combined experience of both the chemists and engineers associated with LCEC from its inception. Background information is provided for both the new and experienced users whose curiosity is piqued by "just how this thing works." Standard operating procedures are described; 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.

2. Support Policy

2.1. User Updates


USER GROUP ENROLLMENT CARD

THIS SECTION COMPLETED BY BAS

To receive product update information news, and valuable information related to this and other BAS products fill out and return the BAS USER'S GROUP application which was shipped with the instrument. We would like to know who you are, and what more you want to know about electrochemical detection!

The B.U.G. form is located in the front of this manual and may be torn away from the binding.

2.2. Damaged Shipments

Breakage of any part of this instrument during shipping should be reported immediately to BAS Customer Service. It will be necessary to retain the original packing box and contents for inspection by the freight handler. BAS will replace any new instrument damaged in shipping with an identical product as expediently as possible after the claim filing date. Claims not filed within 30 days after shipping date will be invalid.

Do not return damaged goods to Bioanalytical Systems without first contacting Customer Service for a Return Authorization Number (RA#). When a defective part is returned to BAS, the RA# immediately identifies you as the sender and describes the item being returned. Bioanalytical Systems refuses all unauthorized return shipments.

2.3. Product Warranty

Bioanalytical Systems, Inc. products are fully warranted against defects in material and workmanship. The LC-4B electronic controller is unconditionally guaranteed 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 electronic units to other components. Electrochemical cells are warranted for 60 days from date of shipment under the same exclusions. Chromatographic columns and injection valves are warranted for 30 days. The following items are not covered under any warranty: carbon paste, activated

aluminum oxide, lamps, panel lights, fuses, pump seals, valve seals, reference electrodes.

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

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 (317/463-4527) who may choose to route your problem to the correct individual. Following discussion of your specific difficulties, an appropriate course of action will be described and the problem resolved accordingly. Do not return any products for service until a RETURN AUTHORIZATION NUMBER (RA#) has been obtained. The RA# identifies you as the sender and describes to us the problem you are having in full detail. Turnaround time on service can be quoted to you at the time your RA# is issued, although we can not determine the actual amount of service required until we have received your unit and verified the problem. All correspondence and shipments should be sent to:

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

3. Installation

3.1. Inspection of Your Shipment

After carefully unpacking the instrument, check the contents of the packages and inspect for breakage. Electrodes and flow cell components are packaged in a gray box. Assembly of these various parts will be outlined in the following chapters. Another gray box contains the components of the PK-3 polishing kit which will be used for maintenance of the glassy carbon working electrode shipped with the instrument.

Both gray boxes also contain a folded poster which illustrates electrode polishing instructions, use of gaskets, and reference electrode assembly.

If there are any discrepancies, retain the packing slip and contact BAS Customer Service for assistance.

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.

Table 3.1 Parts of the Assembled LC-4B/17A Amperometric Detector (refer to Figures 3-1 through 3-4)

Diagram Ref.	Quantity	Description
1	1	LC-4B Amperometric Controller
2	1	LC-17AT Flowcell Compartment (in LC-17A version the preheater (#5) is missing)
3	1	Injection Valve (installed only in the BAS 400 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	1	Utility Panel (this is removed when the unit is upgraded for cartridge column temperature control, model LC22A/23B)
5	1	Preheater Module (120V/60 Hz, or 240V/50 Hz)
6	1	LC-22A Controller for Detector Preheater Module or Cartridge Column Heater Temperature Control (120V/60 Hz or 240V/50 Hz)
7	1	Stainless Steel Auxiliary Electrode

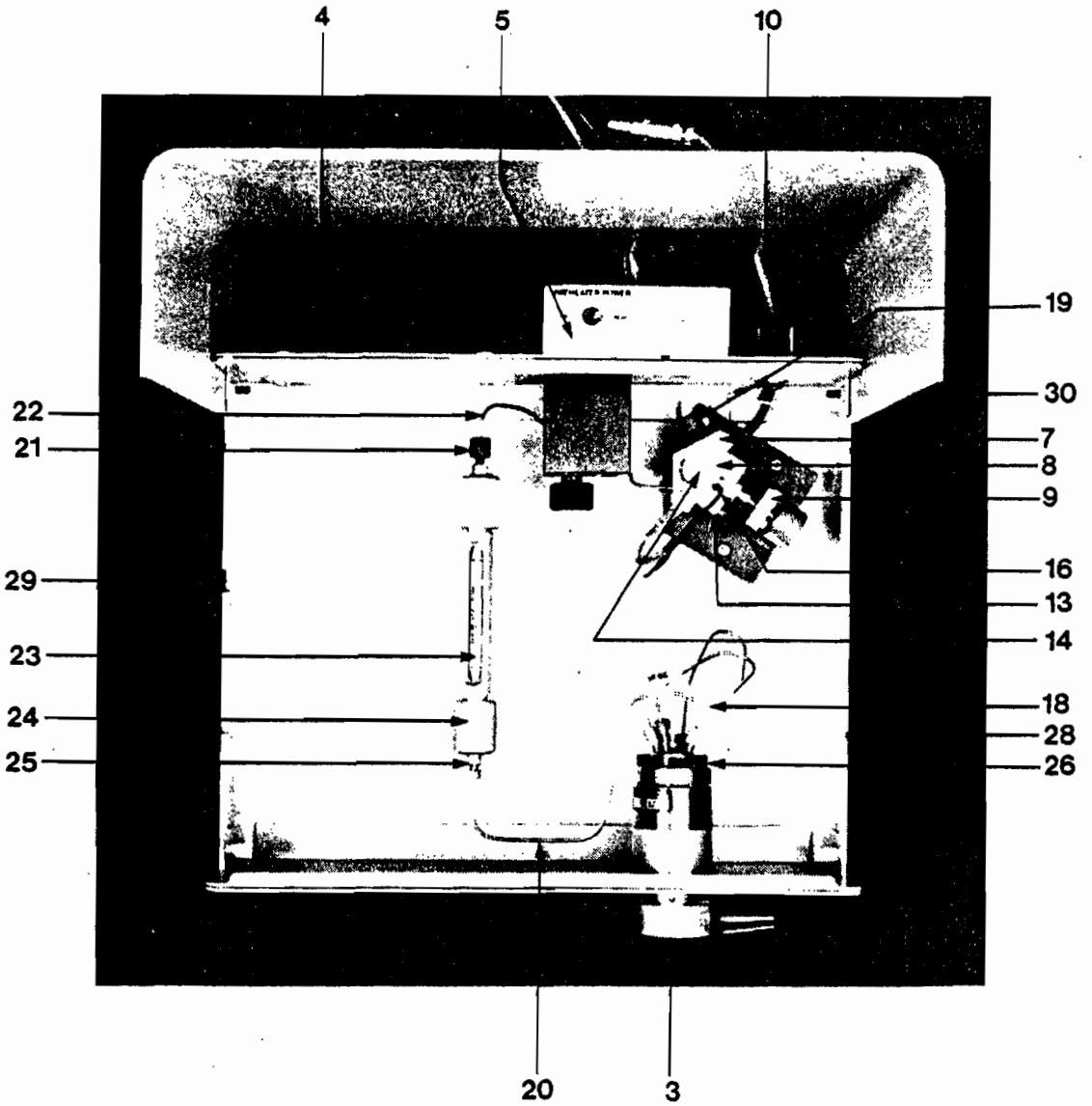


Figure 3-1. Top view of LC-17AT module, configured as part of a BAS 400 system. Refer to Table 3-1 for descriptions and part numbers of components.

8	1	Reference Electrode Retainer (packaged in detector accessories box)
9	1	Working Electrode Clamp (packaged in detector accessories box)
10	1	Single Electrode Cable (dual electrode cable has 2 black working electrode leads and a longer shield)
11	1	Single Electrode Cable Shield
12	1	Power Cord/Fuse Location on LC-4B Controller
13	1	Dual Working Electrode Block (glassy carbon is standard)
14	1	Reference Electrode Connection (pkg of 3 RE-4 Ag/AgCl/gel reference electrodes are provided in detector accessories box)
15 (not shown)	1	TG-2M Electrode Gasket (4 of these green gaskets are in the same small box with the electrode jumper and foam sheets)
16	1	Dual Electrode Jumper Position (use is optional)
17 (not shown)	30 cm	PTFE Inlet Tubing, 0.25 mm i.d. (packaged with detector accessories) This is not used with cell preheater.
18	1	200 microliter loop (NOT PROVIDED WITH DETECTOR PURCHASES, BAS 400 ONLY)
19	1	Red Plastic Fitting (cell outlet)
20	1	Valve to Column Inlet tube (already prepared with correct endfittings, NOT PROVIDED WITH DETECTOR PURCHASES, BAS 400 ONLY)
21	2	Plastic Fingertight Fitting for column to cell inlet tubing
22	1	Inlet Tubing (Stainless Steel for LC-17AT or teflon for LC-17A)
23	1	Analytical Column, Cartridge Type, 100 x 3 mm, Phase II, 3 um ODS (BAS P/N MF-6213) (NOT PROVIDED WITH DETECTOR PURCHASES, BAS 400 ONLY)

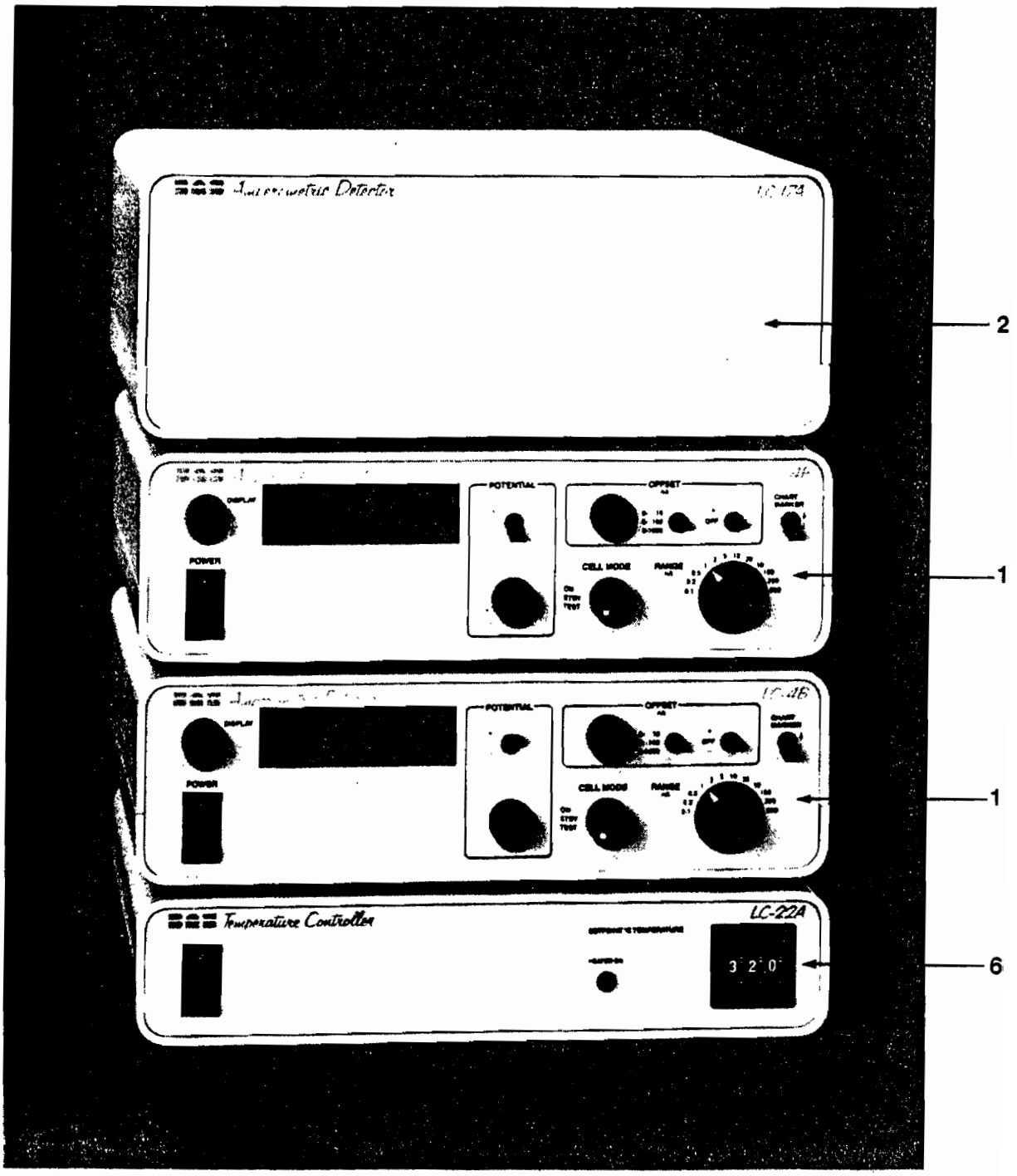


Figure 3-2. Dual LC-4B/17AT Detector (MF 9102).

24	1	*Cartridge Column Holder (NOT PROVIDED WITH DETECTOR PURCHASES, BAS 400 ONLY)
25	2	*Cartridge Column Endfittings (NOT PROVIDED WITH DETECTOR PURCHASES, BAS 400 ONLY)
26	1	*Connecting Port from PM-48 Pump to Injection Valve (when installed)
27	100 cm	PTFE Outlet Tubing (0.5 mm i.d.) (note the small piece of larger tubing located midway on the tube. This is 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.)
28		Notch in cabinet for tubing connection from pump
29		Notch in cabinet to allow use of longer columns (e.g. conventional 25 cm columns) which will extend outside the LC-17A cabinet
30		Notch in cabinet for cell outlet tube. BE sure to press the portion of tube with a thicker, tygon sheath into this slot.

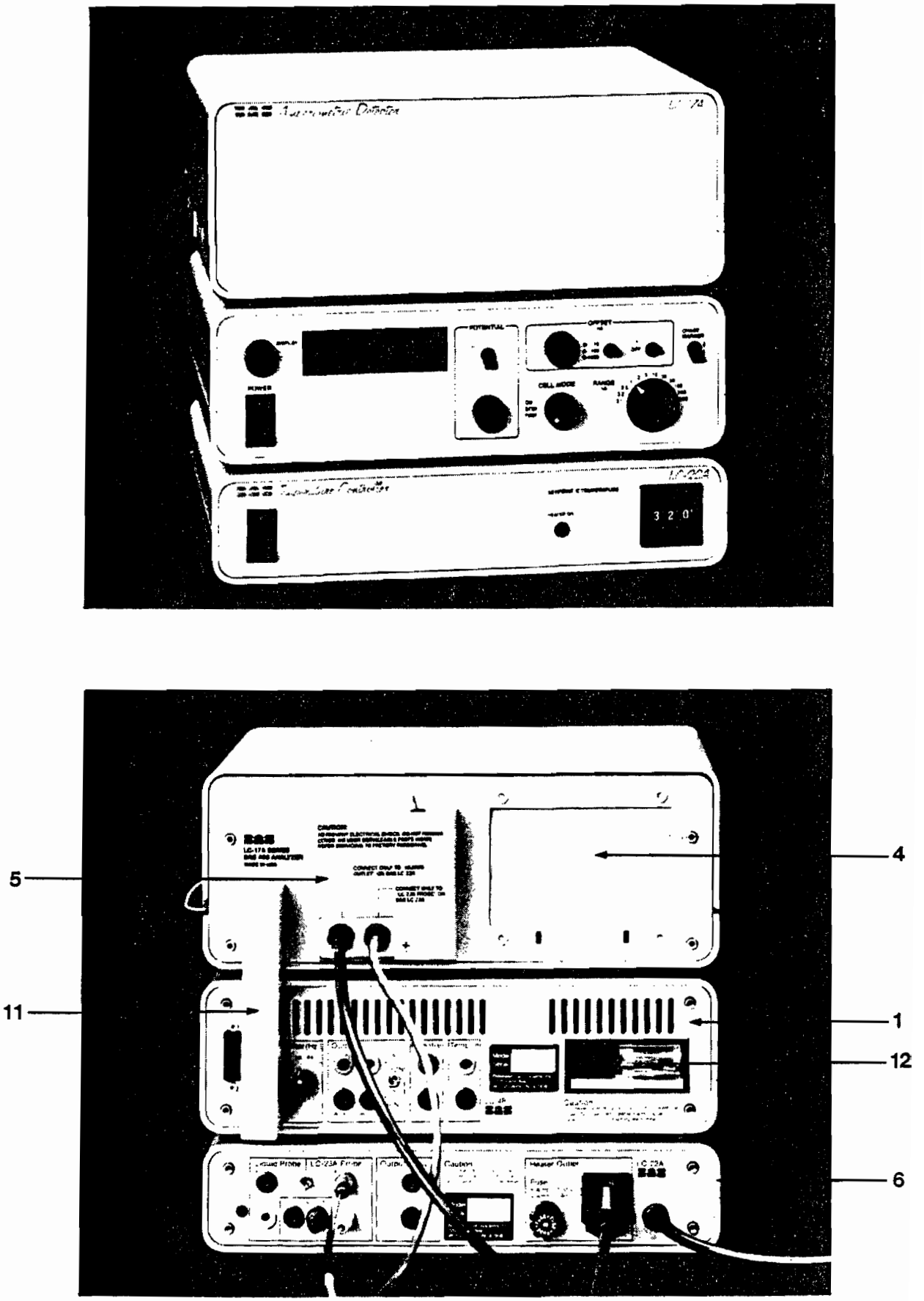


Figure 3-3. Front and rear views of LC-4B/17AT Detector. An additional cable (not shown) is used between the LC-4B and LC-22A for temperature readout on LC-4B.

Figure 3-4. BAS 400 Liquid Chromatograph with PM-48 Pump at bottom level. The column and injection valve are mounted in the detector compartment at the top level.

Table 3.2 Parts List for LC-4B Dual Electrode Upgrade Package (refer to Figure 3-10 for placement of cables and connectors)

Quantity	Description
1	LC-4B Electronic Controller
1	Interconnect Cable
2	Connecting Brackets
1	Dual Electrode Cable
1	Dual Electrode Shield
1	Power Cord

3.2. Power Requirements

The LC-4B and (optional) LC-22A instruments are operated with either 100, 120, 220, 240 VAC and 50 or 60 Hz power, but the correct voltage must be selected before use at the rear panel line cord connector. Maximum power consumption is less than 50 watts. The units should be shipped with the voltage option already matched to the power requirements of the destination.

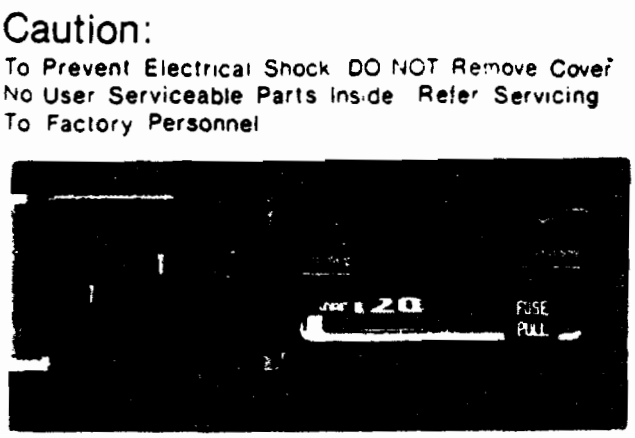


Figure 3-5. Power Selection on the LC-4B

Should the power option need to be changed, unplug the line cord and slide the plastic window to the left. The orientation of the small circuit board now exposed in this socket determines the voltage option. If the voltage labelled on the outer edge of this board is not that required, pull out the board and turn it (either by rotation or inversion) such that the desired voltage is readable. Reinsert the board and push the fuse holder back into the cavity. As illustrated in Figure 3-5, the board is oriented for 120 V, 60 Hz operation. Also check to see the fuse is the proper rating.

Table 3.3. Correct Fuse Selection

Voltage	Fuse
110V/60 Hz	175 mA/SB
100V/50 Hz	175 mA/SB
220-240V/50 Hz	100 mA/SB

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-4B/17A(T) Amperometric Detector decreases the influence of these problems. It offers significant advantages when compared with earlier models. The LC-17AT **Preheater Module**, when controlled by the LC-22A 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. The connecting cables to the LC-4B are kept short and shielded by a special conduit.

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 insures adequate ground to all components of the system and eliminates the possibility of a ground loop. 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.

6. Avoid areas where radio frequency interference is likely.

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 400 Liquid Chromatograph. The BAS PM-48 Pump in this system provides excellent flow rate precision and minimal pump pulsation. The PM-48 Pump may be connected directly to the injection valve port (e.g., #26 in Figure 3-1).

Nearly all pumps require pulse damping to reduce short term flow rate fluctuations. Since short term flow fluctuations are difficult to measure, use the system pressure as an indication of flow rate. If system pressure consistently varies by more than 100 psi, the flow variations may be too extreme for optimum detector performance. In this case, extra pulse damping is desirable.

BAS offers a rugged pulse damper (P/N MF-4000) that is suitable for use with any LCEC system. 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. It terminates in 0.062" tubing at both ends. BAS modular LC pumps (e.g. PM-48 in the BAS 400 Liquid Chromatograph) are already provided a pulse damper. This effectively steadies the flow rate over time.

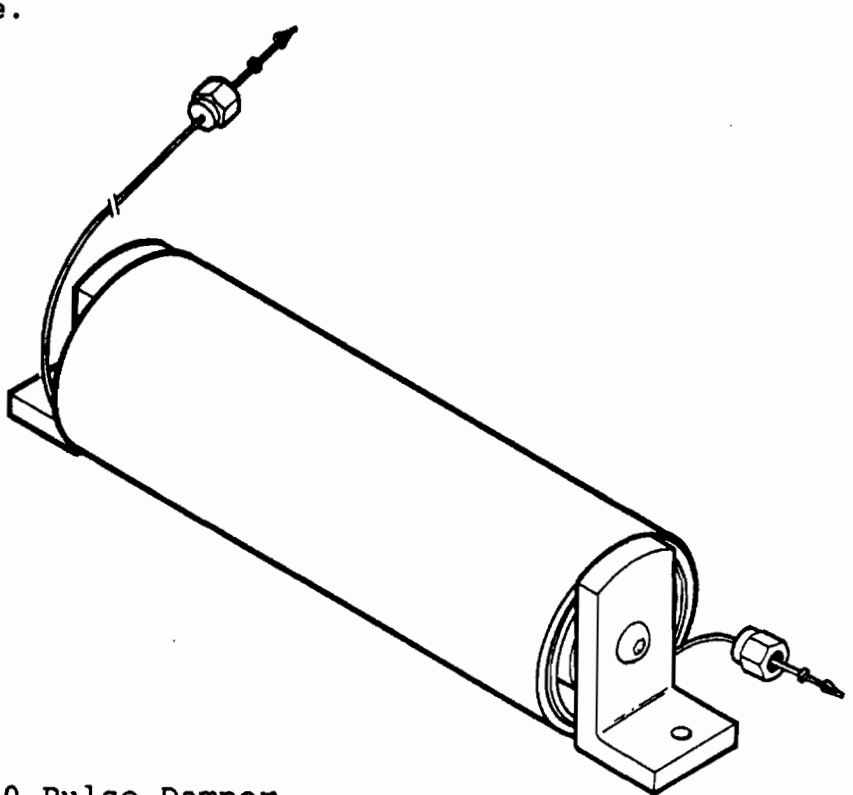


Figure 3-6. MF-4000 Pulse Damper

3.5. Arrangement of Components and Connections

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NOTE: Should you be installing as retrofits either the LC-4B Dual Upgrade Kit or the LC-17A Preheater Module please refer to the installation instructions in Section 9.3 or Section 10.2 respectively. The instructions below are for start-up in a new installation.

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1. Arrange the various components per Figure 3-7. The LC-4B controller(s) must always be placed immediately under the LC-17A(T) compartment. This arrangement is necessary for the correct placement of the cable shield. The LC-17A(T) has been sealed along all joints. If an accidental leak should occur, the compartment could hold over 500 mL of liquid before overflowing to components below.

2. If you opted to purchase the LC-17AT detector with Preheater Module, connect the 2 cables on the preheater as directed on the back panel to the LC-22A Temperature Controller (Figure 3-8). If you wish to see temperature readout on the front panel of the LC-4B, you must make an additional connection between the LC-4B and LC-22A. Locate the cable with 2 banana plugs on each end (orange and black going to green and black). Make the connection with the corresponding jacks on the back panels of the LC-4B and LC22A. In a dual electrode detector, make sure that you make the connection to the LC-4B you plan to use for readout of electrode "W1".

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NOTE: EARLY MODELS OF THE PREHEATER HAD A 3-PRONG PLUG. IF YOU HAVE SUCH A UNIT, DO NOT PLUG THE THREE PRONG CABLE PLUG ON THE PREHEATER MODULE INTO A NORMAL WALL SOCKET. IT MUST ONLY BE PLUGGED INTO THE LC-22A.

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3. Connect the electrode cable (single or dual) from the LC-17A(T) to the LC-4B(s) per Figure 3-9. Clip the cable shield into the locking notch on the detector cable bracket, per Figure 3-10. Tilting the LC-17A(T) backwards slightly helps. Push down on the cable shield to lock it in place.

4. If you are installing dual LC-4B's, attach the interconnect cable per Figure 3-9. Screw it down into both connectors securely.

5. For additional support, two brackets and 4 screws are provided for every additional component in the "stack" of instruments. Brackets are attached at the back panels in the positions noted in Figure 3-10. The LC-4B, LC-17A and LC-22A instruments can all be bracketed together.

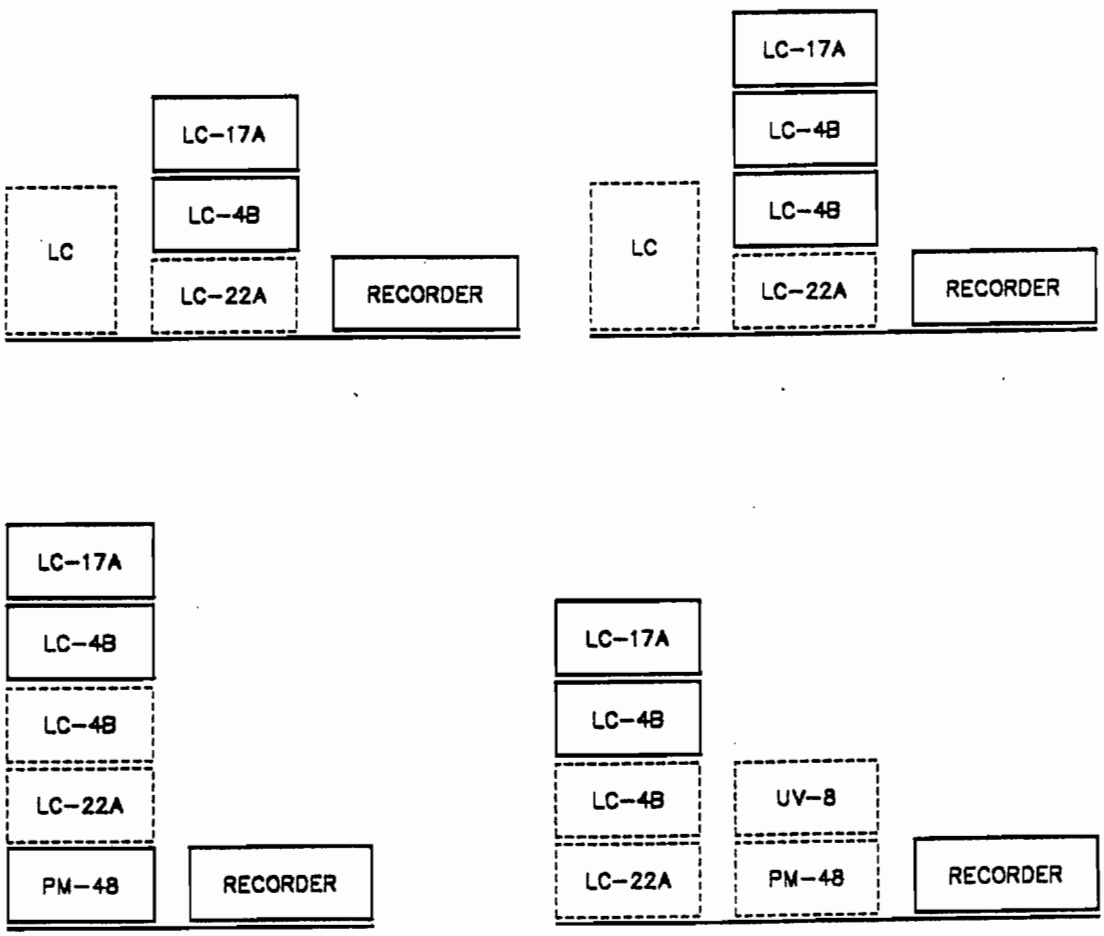

Figure 3-7. Schematic for stand-alone electrochemical detectors and BAS 400 components

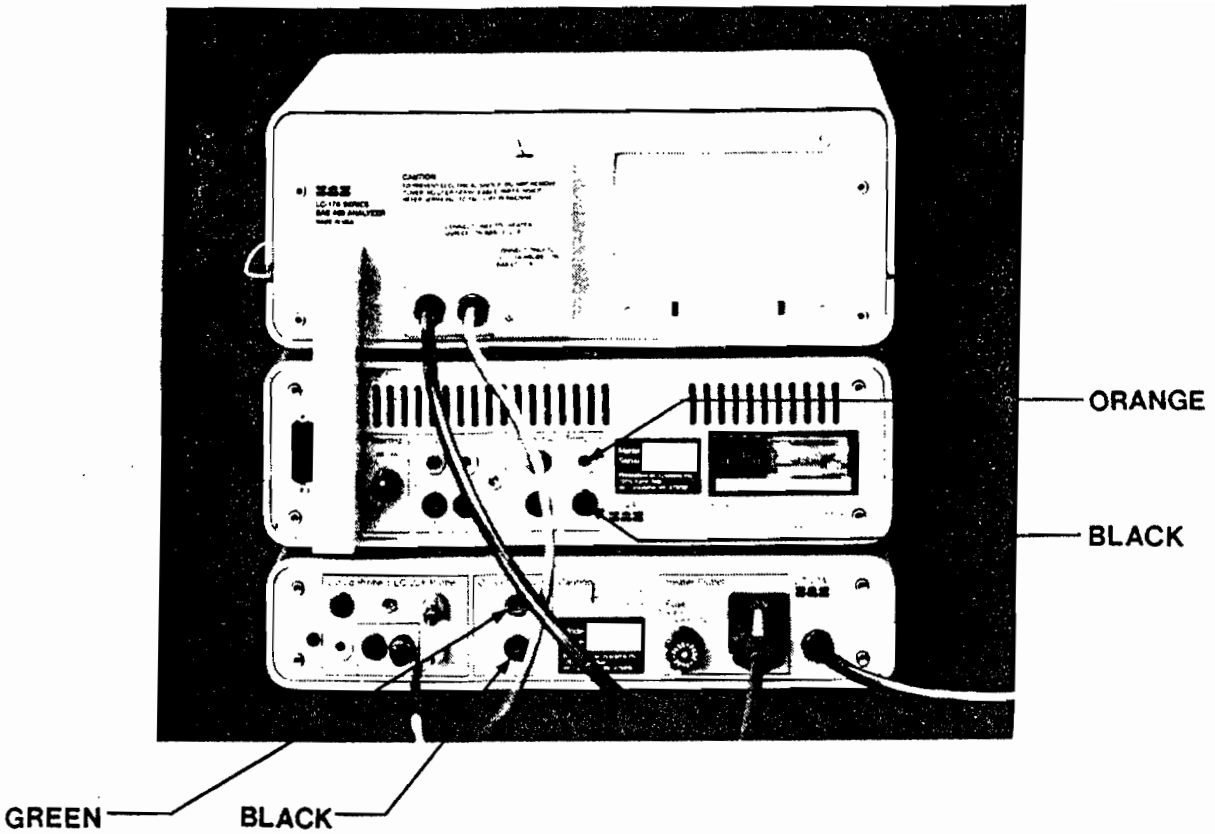

Figure 3-8. Connection of cables from LC-17AT Preheater Module to LC-22A Temperature Controller. For Dual Electrode detectors, route the cable from the LC-22A controller to the LC-4B which will control electrode "W1".

6. Connect the output leads to the strip-chart recorder, integrator, or data station.

   a. On each LC-4B, there exist 2 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 full scale recording device.

   b. Flip the POS FOR OXDN/POS FOR 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 can find more information in section 4-3. Correct positions of this switch ensures a positive output signal.

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

      White: "+" or "HI" recorder input
      Black: "-" or "LO" recorder input
      Black jumper: recorder ground terminal.

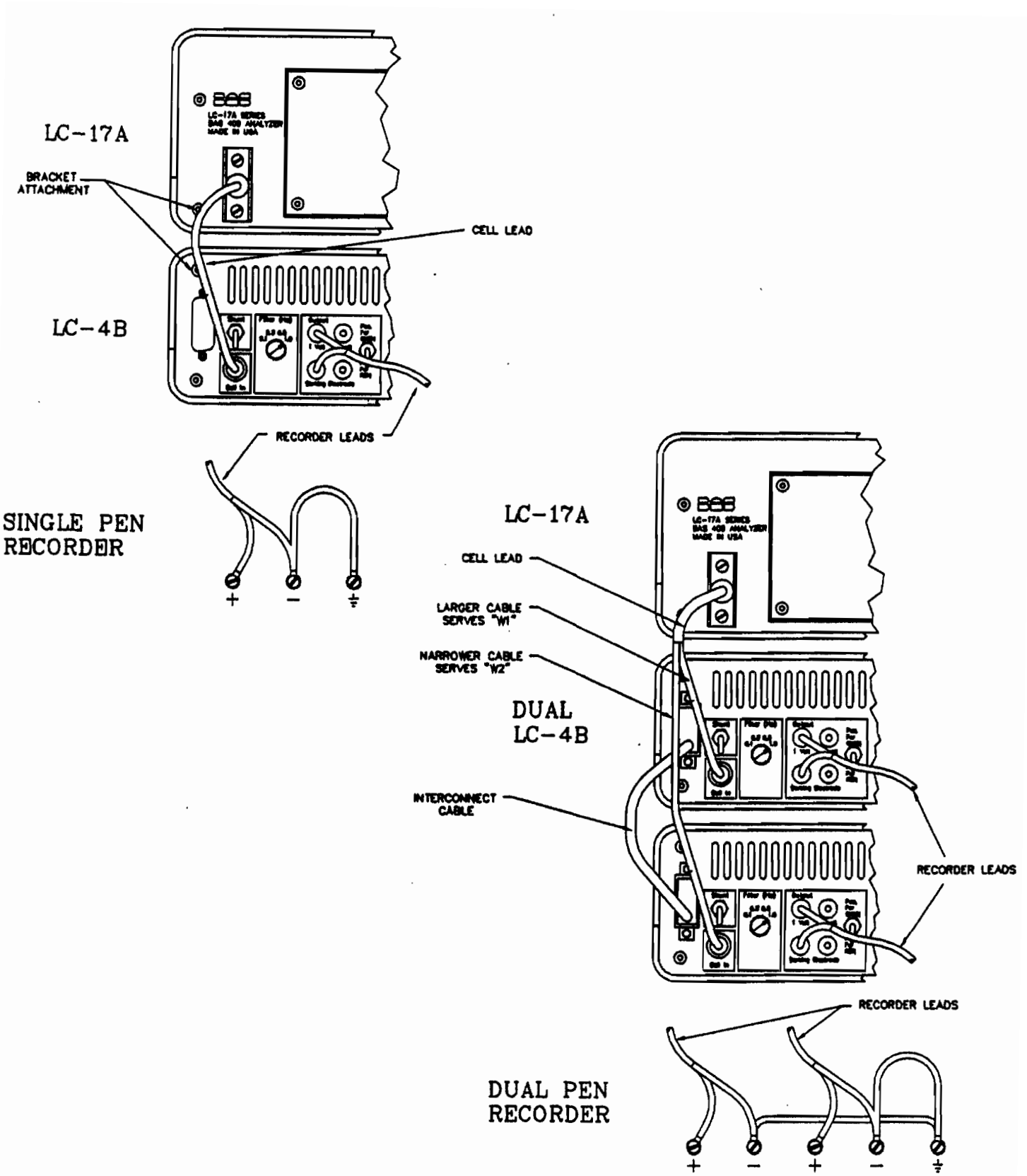

Figure 3-9. Connection of leads to LC-4B(s) for single (left) and dual (right) operation.

d. Using the ZERO ADJUST or ZERO CHECK on your recorder, zero the recorder at a convenient point on the right side of the chart paper.

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NOTE: If you have an integrator or data station with wide dynamic range, match the output of the LC4B to the input range of your data device. The LC4B has both a 1 V and 10 mV output jack, making it compatible with the majority of popular. There is a built-in overange on each output that is 10 times the value (e.g. the range on the 10 mV output is from 10 mV to 100 mV). Use the gain switch on the LC-4B Detector to keep the signal you record within the range of your recording device.

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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 Ohm. If it is not, ground the component with a jumper wire.

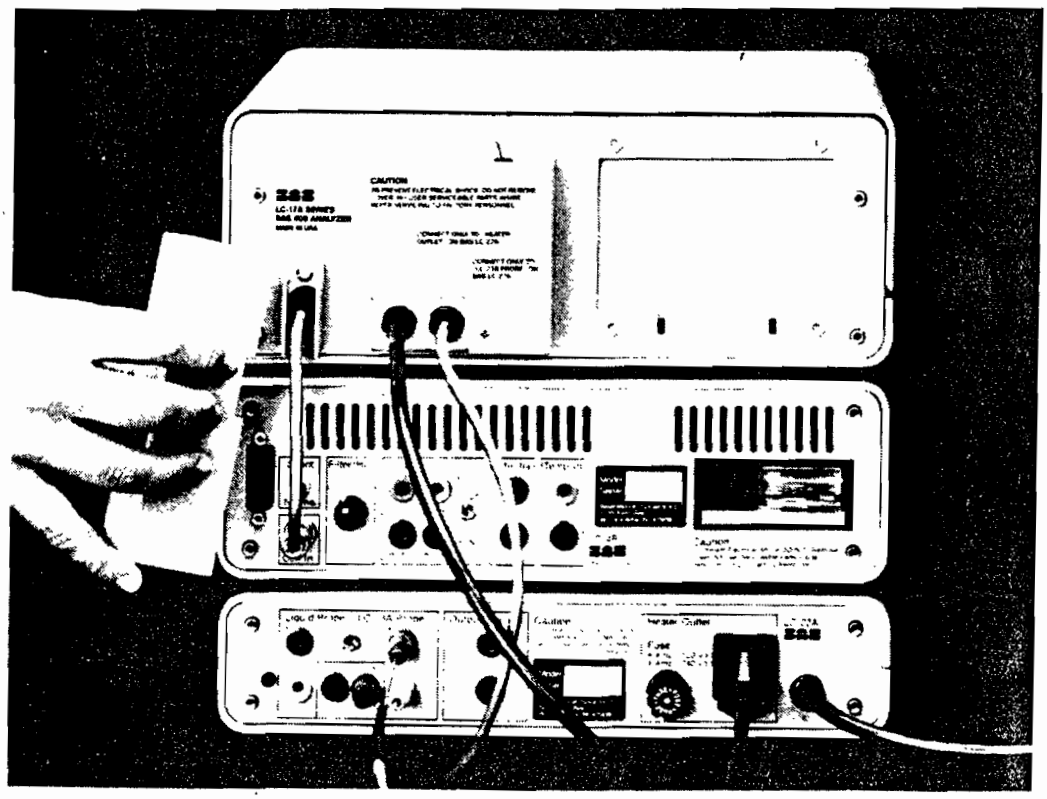

Figure 3-10. Attaching single electrode cable shield over detector cable. Keep the shield attached at all times. It is an integral part of the LC-17A transducer design.

3.6. Chromatography Connections

1. Place the interconnected detector components next to the column outlet from the chromatograph, or install your column inside the LC-17A (Figure 3-1). Several slots in the LC-17A chassis along the sides and back provide tubing access, no matter what the orientation. On a BAS 400 system, the detector components may sit directly on top of the PM-48 Pump. See Figures 3-2, 3-3, and 3-7 for ideas.

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

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

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2. Flush the system with 200 milliliters of acetonitrile/water (50:50, v:v) and divert all column effluent to a waste receptacle. This flushing step cleans any residual chemicals from the column remaining from previous injections and also "wets" the hydrophobic stationary phase for reproducible separations.

   If you are using a BAS PHASE II, or other BAS column, please review and follow the wetting procedure desribed in BAS Application Note No. 80 which is shipped with each column. Failure to follow this procedure will result in unacceptable column performance.

   Mobile phase should be pumped through the system to equilibrate the column. If you are using an ION-PAIRING REAGENT in your mobile phase composition (e.g. Sodium Octyl Sulfate), you should allow up to 2 hours for equilibration (e.g. at a flow rate of 2 mL/min.).

3. While the column is equilibrating, remove the 3 reference electrodes from the detector accessories package. Follow diagrams on the enclosed instruction sheet 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. A suggestion for storage is offered on the instruction sheet packed with the accessories.

4. Assemble the remaining parts of the thin-layer electrode and reference electrode retainer. See the instruction sheet in the accessories package for identification of parts.

   Remove the clamp assembly from the packing box. If the

plate and clamp screw are not already assembled, wet the O-ring on the screw and twist the plate into place (Figure 3-13).

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 while the flow continues 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.

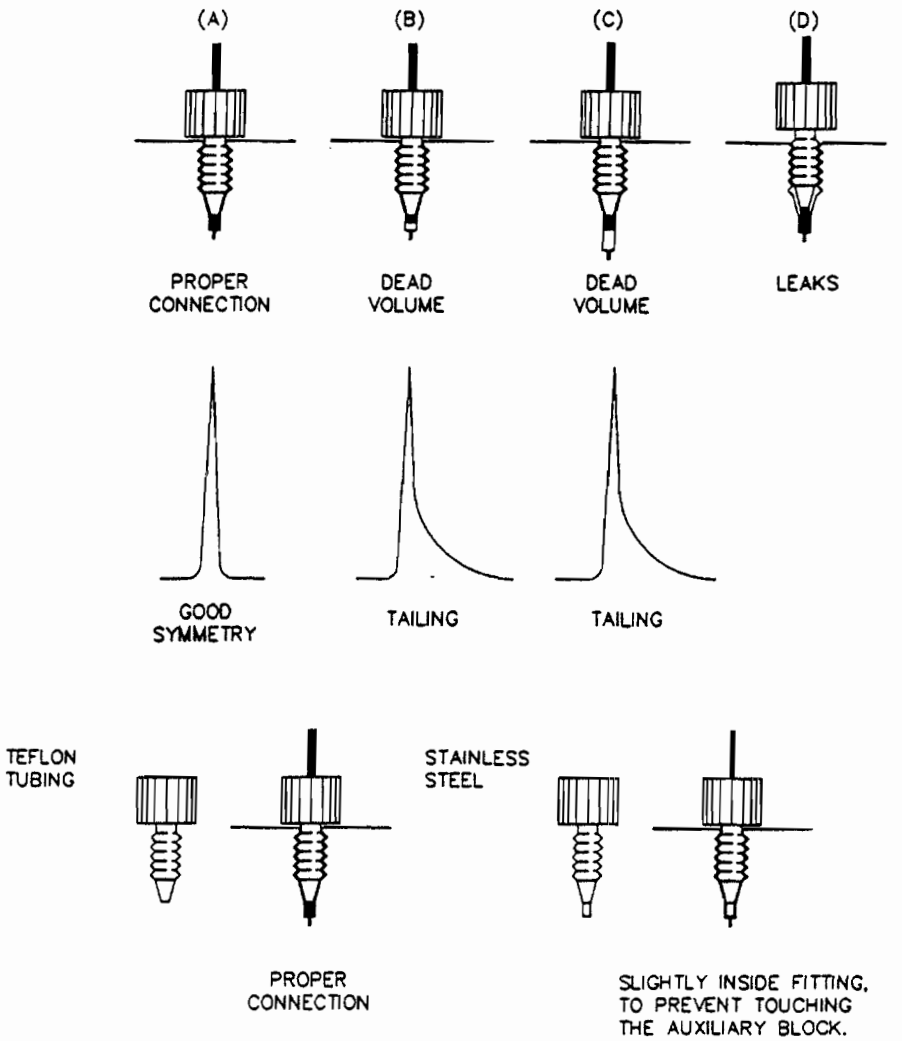

Figure 3-11. Correct alignment of Teflon Tubing and Stainless Steel Tubing in Fingertight Fittings.

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NOTE: For units with cell temperature control (Preheater), you should NOT allow the thin metal tubing on the inlet to touch the stainless steel auxiliary block. The plastic fittings on the capillary steel inlet tube act 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.

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6. Systems WITHOUT 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 the other end (waste) through the groove in the LC-17A cabinet (see Figure 3-1, #30).is routed through any available slot to a waste receptacle.

7. Systems WITH LC-17A PREHEATER Module: connect the column to the thin STAINLESS STEEL tube of the preheater block (Figure 3-1, #22). The Preheater is generally 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 LC-17A, 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.

8. Place a green TG-2M cell gasket (MF-1046) over the four registration pins on the steel auxiliary electrode block. Align the WORKING ELECTRODE BLOCK in the configuration desired. Please note the orientation of the dual electrodes in relation to the flow through the thin-layer cell. (Figure 3-12).

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NOTE: The gaskets determine the dead volume in your thin-layer cell. You can add additional gaskets on top of each other to increase the dead volume of the cell. Additional gasket thicknesses are also available for separate purchase (e.g. TG-5M = 0.005 inches or 127 microns thick, TG-15M = 0.015 inches or 881 microns thick, TG-2M = 0.002 inches or 51 microns 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. You can't simply increase/decrease flow from your pump since the chromatographic separation will be affected. In some cases, e.g. microbore, a very small gasket may be desirable. In other cases, e.g. amalgam electrodes, a thicker channel is preferred.

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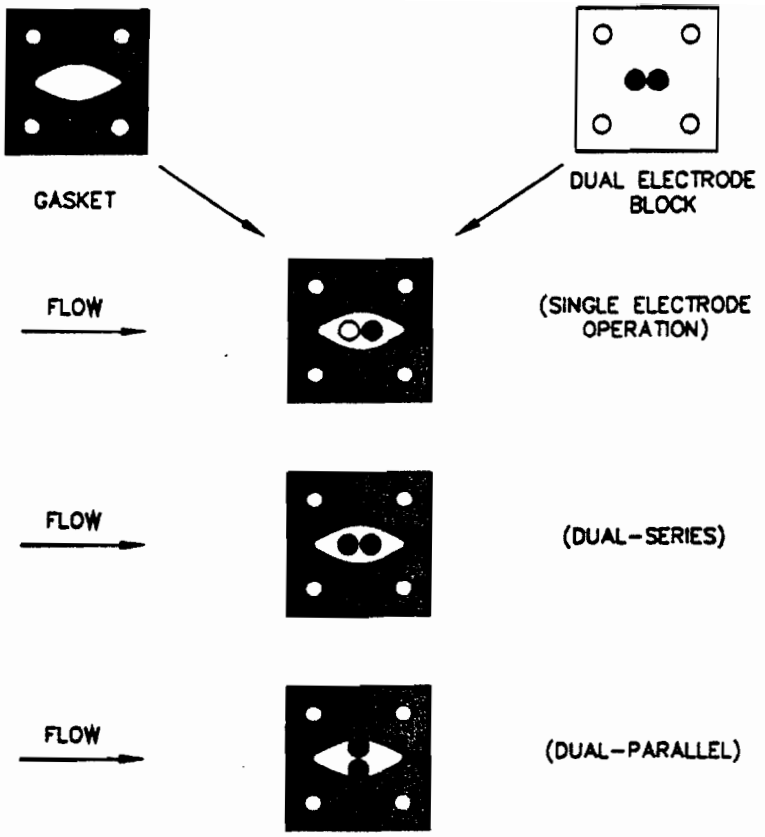


Figure 3-12. Placement of Thin-Layer Gasket and Orientation of Working Electrode for Single Electrode, Dual Series or Dual Parallel Operation.

9. Slip the clamp into the guide slot, push up and forward, and screw until tight. Don't be excessively forceful when tightening.

10. Remove one of the reference electrodes from the Sodium Chloride solution and assemble with the retainer, O-Ring and Bushing as shown on the instructions in the shipping box. The Bushing should be all the way through the top of the retainer and will act as an insulator between the connecting cell lead and the retainer.

11. At this point, fluid should be rising into the reference electrode well. Let it keep rising until it is almost at the vent hole, then PUSH the assembled reference electrode into the well and twist to lock it in place. The O-ring will wipe the inside of the well dry as you push the retainer down.

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

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.

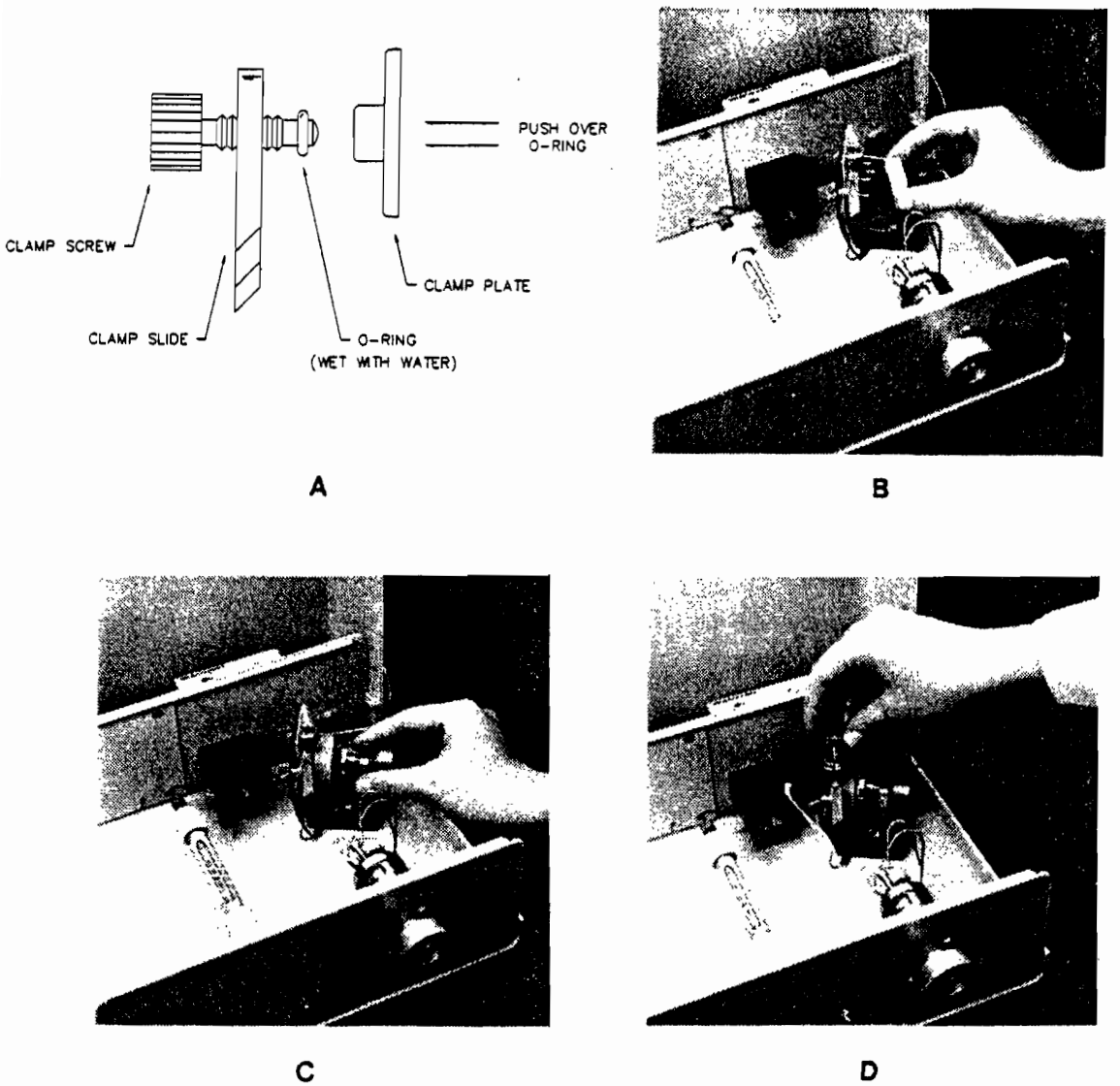


Figure 3-13. Final Assembly of Cell Components.
A. Clamp Slide and Clamp Screw Assembly.
B. Alignment of Electrode Block over Registration Pins AFTER Gasket has been added.
C. Clamp Slide is inserted into groove in base of cell, and tightened with the Clamp Screw.
D. Assembled reference electrode retainer is pushed into receptacle after fluid starts rising to top of the cell.

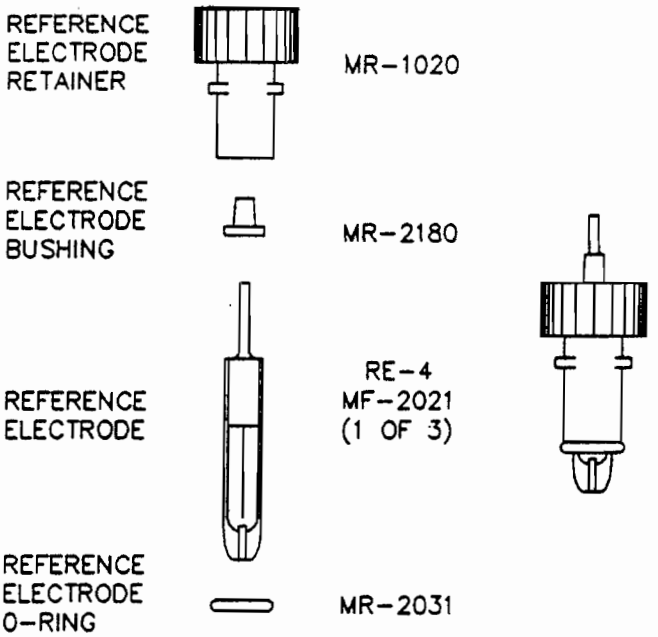


Figure 3-14. Exploded and assembled view of reference electrode components.

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

White connector (socket): to reference electrode pin
Black connector (pin): to working electrode socket (side socket)
Red lug connector: to auxiliary electrode (screw on top surface).

- -

NOTE: Two working electrodes are provided. You may use either, and reserve the remaining one as a "back-up" sensor. Before changing over from one electrode to the other, please be certain that the power to the LC4B is OFF. Always turn power to OFF before removing electrode leads on the thin-layer cell.

For best results, orient the electrode block so that the pin connections are on top for easy access.

- -

15. To use the ELECTRODE JUMPER (Figure 3-15), 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 (see Figure 4-12).

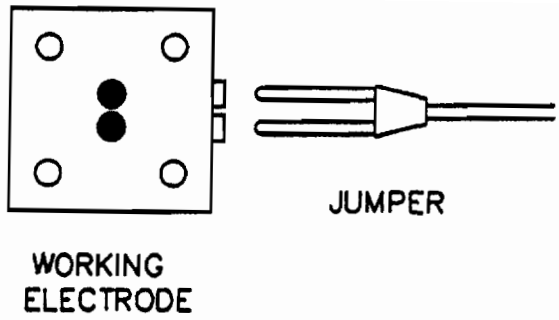


Figure 3-15. Dual Electrode Jumper (packaged with other accessories in the gray box).

16. Customers with DUAL electrode detectors can operate each electrode at an independent applied potential. Each electrode is controlled by a single LC-4B 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. Connect each black wire to the pin connectors on the electrode block as follows:

Black wire with "W1" label: to working electrode #1 (your choice, this will be W1 on LC-4B display).

Black wire with "W2" label: to working electrode #2 (your choice, this will be W2 on LC-4B display)

The red and white wires are connected as for single electrode detectors (see above).

- -

NOTE: For dual electrode operation, the electrodes may be oriented in two ways. For series experiments (1 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. However, if you intend to use the HIGH CURRENT SHUNT feature on the detector, "W2" MUST BE UPSTREAM (see Section 9). The High Current Shunt is most frequently used when you anticipate generation of a large amount of current as in the cleavage of a disulfide bond. You will not be measuring this current so you route it away from the detection circuitry. Again, W2 must be the electrode selected for HIGH CURRENT SHUNT applications.

- -

4. Principles of Electrochemical Detection

This section includes basic background information on the theory of electrochemical detection. It is not mathematically oriented. Concepts important to the LCEC technique are explained.

4.1. Electrochemical Fundamentals

LCEC may be considered in terms of electrolysis at a fixed point along a flowing stream. The stream in this case is the eluate from the analytical LC column, a sequence of solute ("analyte") zones separated with varying degrees of resolution. These zones pass into a very low volume thin-layer cell, where the flow is constrained to a thin film passing over a planar electrode held at a fixed potential. Figure 4-1 illustrates an exploded view of this region. If the potential is greater (more positive for oxidations, more negative for reductions) than that required for the electrolysis of the analyte, a measurable charge passes from electrode to analyte (or vice versa). The resulting current is directly proportional to the concentration of solute passing through the channel.

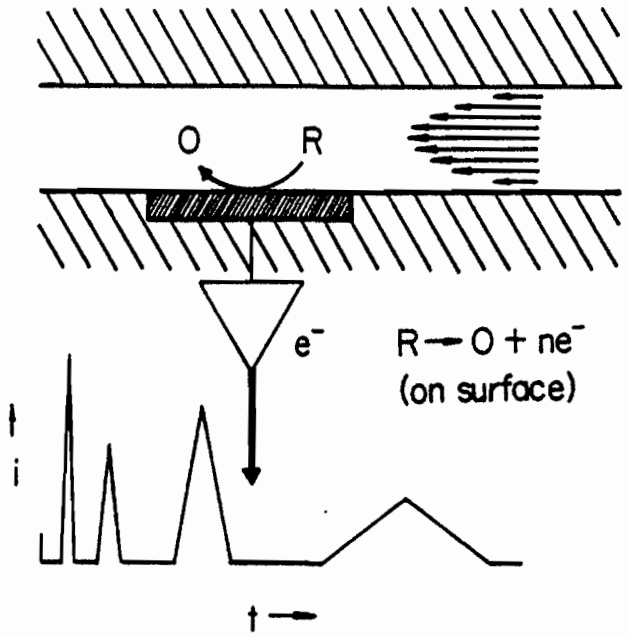


Figure 4-1. Exploded view of electron transfer at the surface of a thin-layer electrode. A laminar flow profile passes over the electrode in the thin-layer zone containing reduced analyte R. Oxidation to O at the electrode surface releases electron(s) to the surface. This current is subsequently converted to a voltage which drives the recorder and produces the chromatogram.

The electrode may be thought of as a chemical reagent. The more positive its potential, the stronger an oxidizing agent it becomes; when the potential is made more negative, it becomes a stronger reducing agent. In either case, as the concentration of solute rises and falls in passing through the thin-layer cell, the electrolysis current proportionately follows these changes. This current, as a function of time, is amplified and sent to a recorder to yield a chromatogram.

LCEC is an AMPEROMETRIC determination. Unlike measurements (such as pH) of a potential difference under zero current conditions, LCEC measures current at a fixed potential. It must always be remembered that this experiment involves heterogeneous electron transfer (from one phase to another, different phase) and the

success of the experiment depends in large part upon the care by which these components are chosen and operated.

Figure 4-2. MICHAEL FARADAY (1791-1867). Don't miss seeing his laboratory when you visit the Royal Institution in London (U.K.).

Crucial to all amperometric determinations is FARADAY'S LAW, which states that:

$$Q = nFN \quad (1)$$

Q is the number of coulombs (a unit of charge) used in converting N moles of material, n is the number of moles of electrons lost or gained in the transfer process per mole of material, and F is Faraday's constant, 96,500 coulombs/mole of electrons). Differentiation of (1) with respect to time yields the current, which is the measure of the rate at which material is converted:

$$\frac{dQ}{dt} = i = nF\frac{dN}{dt} \quad (2)$$

Equation (2) therefore relates a measurable quantity, the current to the fundamental redox process occurring in the cell.

Beginning in 1986, Bioanalytical Systems established a new standard for its electrochemical transducers in the BAS 200 and BAS 400 liquid chromatography systems (Figure 4-3). As from the beginning, the proven thin-layer concept remains central to this design. In it, two blocks form a sandwich around a thin fluoropolymer gasket which defines a microliter flowcell. Two working electrodes (glassy carbon or other material) are embedded along one wall of the channel, whereas the reference and auxiliary electrodes are directly opposite, typically only 50 micrometers distant.

The nature of the working electrode, particularly its bulk composition and surface treatment, is critical to detector performance. Many organic compounds react at significantly different rates depending on the electrode used. It is normally desirable to carry out the electrode reactions at the greatest possible rate in order that the current be limited only by mass transport of molecules to the surface and not by their reaction rate at the surface. This situation affords the greatest sensitivity and stability <u>without</u> sacrificing selectivity.

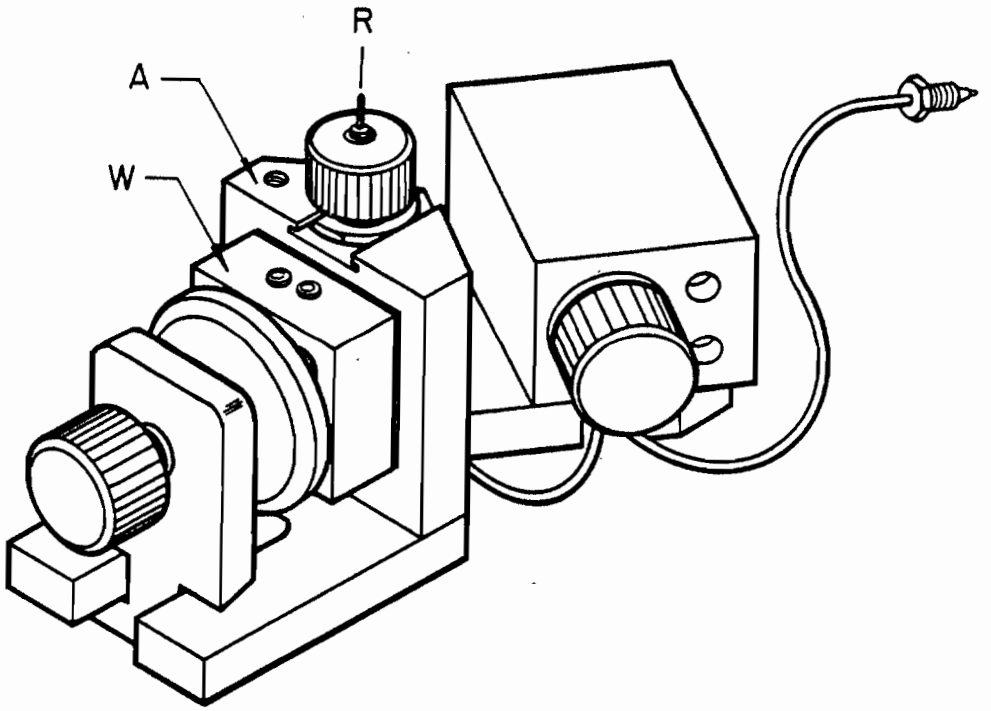


Figure 4-3. Schematic representation of thin-layer cell design for LC-17A and BAS 200 systems.

Glassy carbon, carbon paste, mercury amalgamated gold, platinum, nickel and silver have all been used in electrochemical cells similar in design to that shown in Figure 4-3. For chromatographic purposes we are most concerned about: (1) range of available potential, (2) chemical and physical compatibility with the mobile phase, and (3) long-term stability. In the usual case, all three considerations are closely related.

Carbon paste (a mixture of spectroscopic graphite powder and a dielectric material such as mineral oil, silicone oil, and paraffin wax) was used for initial LCEC experiments (ca. 1974) due to its excellent properties for organic electrochemical reactions. For most published applications of carbon paste electrodes, the useful lifetime of the electrode surface can extend to many months. However, if extremes of potential are used ($\geq$ 1 Volt vs. Ag/AgCl) and/or if the mobile phase contains high concentrations of organic solvent, one can anticipate the need for more frequent renewal of the electrode surface.

Carbon paste is rarely used since most users prefer the convenience of glassy carbon. However, it is still hard to match the performance of a well packed carbon paste electrode. Glassy carbon falls more closely in line with our desire for a universal electrode material. Glassy carbon is a hard, amorphous carbon material capable of being polished to a mirror-like finish. When housed in a Kel-F block, a glassy carbon cell offers good solvent resistance, a feature particularly useful with mobile phases

containing acetonitrile or large percentages of methanol. It has been used in entirely non-aqueous systems with good success.

All BAS detectors feature twin glassy carbon electrodes as the standard. The thin-layer design offers the most flexibility of any in terms of electrode materials; it also permits 1 or 2 electrodes to be contained in virtually the same cell volume. Even if only 1 electrode is to be used, the second sensor provides good diagnostic information should trouble arise.

- -

Table 4-1. Working Electrodes for LC-17A Flowcell

Electrode Material	BAS Part Number
Dual Glassy Carbon	MF-1000
Dual Gold	MF-1002
Dual Glassy Carbon/Gold	MF-1006
Dual Platinum	MF-1012
Dual Nickel *	MF-1009
Dual Silver	MF-1008
Dual Carbon Paste **	MF-1004

* Not a stocked item. Please allow 6-8 week lead time. Other materials are available by special order. Please contact your BAS representative for a quotation.
**Carbon paste not included. Please order separately.

- -

It is widely believed that sample molecules, lipids, or proteins will "poison" the electrode surface after a few samples have been injected. This notion undoubtedly arises from classical voltammetry (including polarography) where the presence of molecules of high molecular weight leads to serious problems. Voltammetric measurements quite often involve sample concentrations over the range 10^{-5} to 10^{-3} moles/liter. In some cases the electrode reaction itself leads to polymer formation which "passivates" the surface causing unreliable results.

In the case of amperometric detection, the column isolates the electrode from many contaminants, the mobile phase continuously cleanses the surface, and the usual sample concentrations are in the range of 10^{-8} to 10^{-6} moles/liter. Furthermore, the electrode is only exposed to individual compounds for a minute or less due to the narrow elution zones encountered in liquid chromatography. All of these factors heavily favor the electrode in comparison with direct (i.e. no chromatography) electrochemical measurements. Hence, with LCEC experiments, electrode "life" is extended much longer than with traditional voltammetric experiments.

It is almost universal practice to use a working electrode which is fixed at ground potential and to vary the potential of the neighboring solution with respect to a particular reference electrode. This situation often baffles those who have not been

introduced to the simple fact that it is the **POTENTIAL DIFFERENCE BETWEEN AN ELECTRODE AND THE SOLUTION which is of importance in electrochemistry** and NOT the potential of the electrode material itself. Normally this potential difference extends across an interphase region (the "electrical double layer") which is quite thin. It is the purpose of the electronics to control this potential difference while at the same time converting the current (resulting from the electrode reaction) into a voltage which is easily recorded or processed by computer.

Figure 4-4 illustrates a recorder tracing encountered following initial application of a predetermined potential difference to a thin-layer cell containing a freshly prepared carbon paste electrode. The mobile phase is passing through the detector cell but no sample has been injected into the chromatograph. Why then is so much current measured? Initially some charge is required for the electrode solution interface to achieve the applied potential difference. Additional charge will pass if the oxidation states of functional groups on the graphite are altered to a new state of equilibrium. Finally, impurities in the mobile phase and the mobile phase itself will oxidize (or reduce) resulting in a steady-state background current. Water will oxidize (or reduce) at all potentials although the rate of the reaction is quite slow except at very positive (or negative) extremes.

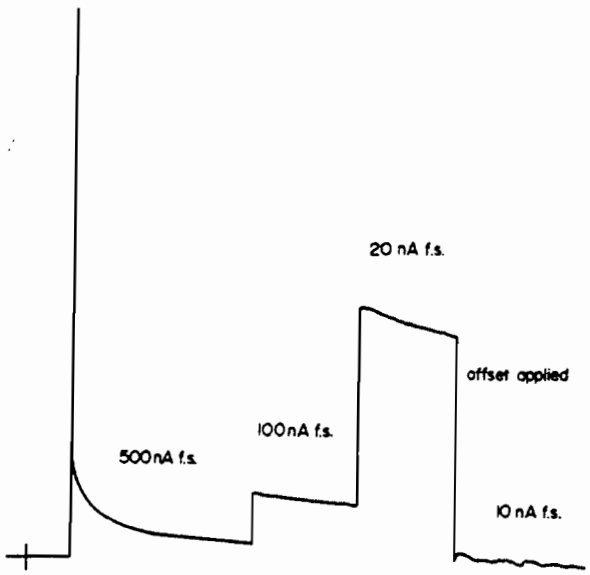


Figure 4-4. Typical equilibration response of electrode when it is first turned on.

The background current is electronically "bucked out" by the offset current which is applied by the user. This results in a steady baseline at recorder zero (Figure 4-4). It is always desirable to operate with the minimum absolute background current to optimize sensitivity. The chromatogram is recorded "on top" of a current which often exceeds the peak heights for eluted compounds. High background currents increase the susceptibility of the instrument to flow variation noise (e.g. pump pulsations) and can result in serious nonlinear (negative) deviations in calibration curves.

It is useful to evaluate the peak-to-peak baseline noise, i_p, over a time period about ten times the width of the chromatographic peak (Figure 4-5). The frequency content of the noise relative to that of the chromatographic peak is very important since frequencies much higher or lower can usually be removed. The high frequencies are easily filtered out and the very low frequencies are, in effect, baseline drift which is eliminated when the peak is quantitated. The amount injected which would give a peak height equal to the background current noise is clearly below the limit of usefulness. An amount that would give a peak height 5 times the background current noise is useful under many circumstances.

Figure 4-5 summarizes in graphic form the various parameters useful in evaluating detector performance. These are often confused in real practice. **Sensitivity** is the slope of the response versus amount (Figure 4-5). **Detection limits** refer to the amount of analyte required to give a signal X times greater than the noise (usually X = 2). The two terms are not synonymous. The reader should be able to foresee situations where two detectors demonstrate equal sensitivity but unequal detection limits. The only difference necessary is the noise. We recommend that LCEC users report sensitivity for each peak of interest as well as the noise. With this information it is possible for the reader to predict a reasonable detection limit for each substance being determined.

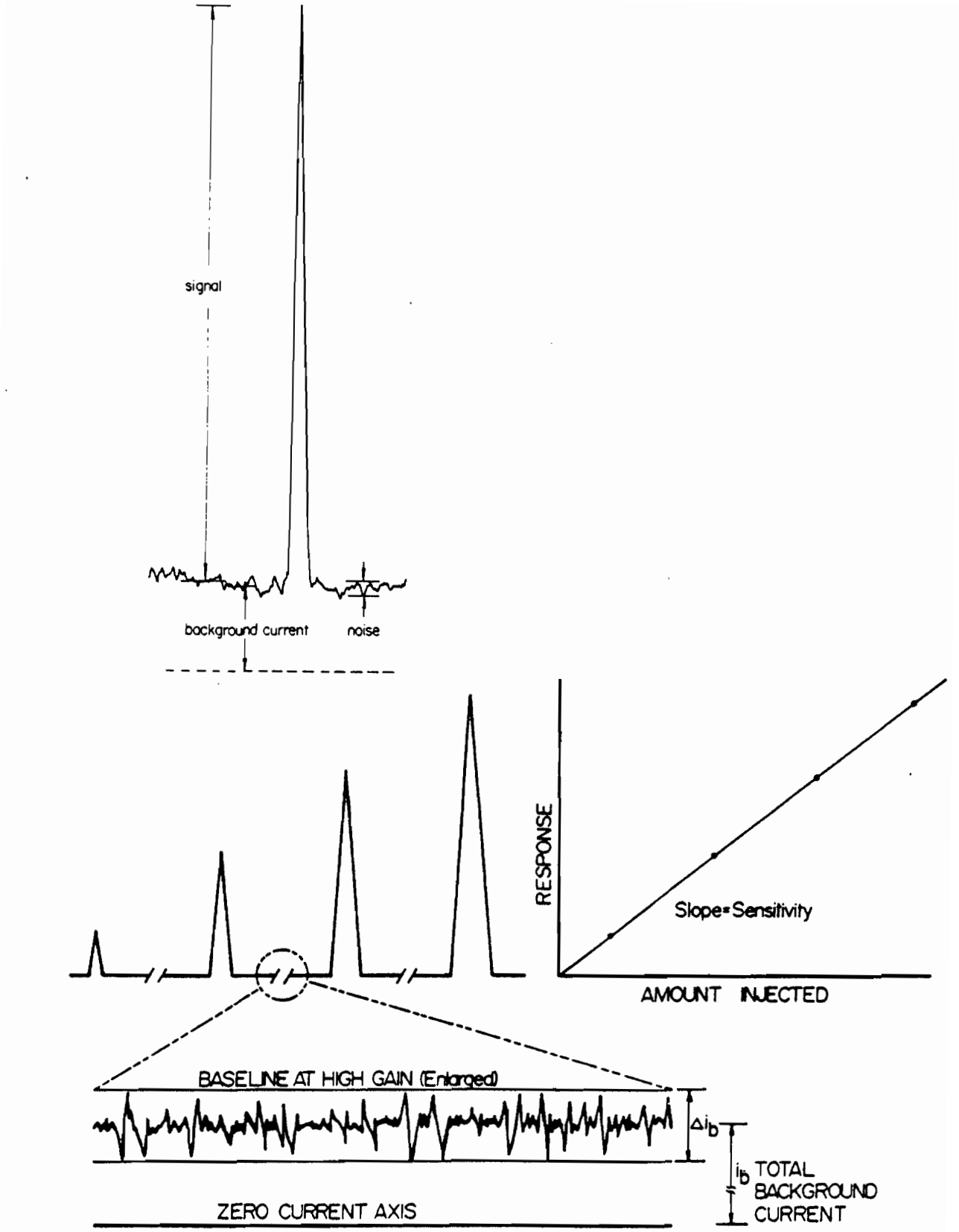


Figure 4-5: Performance parameters for detector evaluation.

4.2. Hydrodynamic Voltammograms

Let's begin our discussion by using a hypothetical electrochemical detector. Suppose we are operating the unit in an oxidative mode with a carbon electrode and have initially chosen a potential low enough so that no detection of our test analyte should be possible. An injection of the test analyte is made; as predicted, no response is seen at the expected time. The potential is raised another 100 mV, the injection repeated, and the current response noted. This process is repeated until the potential becomes so high that the background current is prohibitive (usually +1.2 V). Suppose our test analyte were electroactive in the range examined. Then a plot of peak height versus applied potential would look similar to the solid line in Figure 4-6B. Three zones along this curve would become apparent.

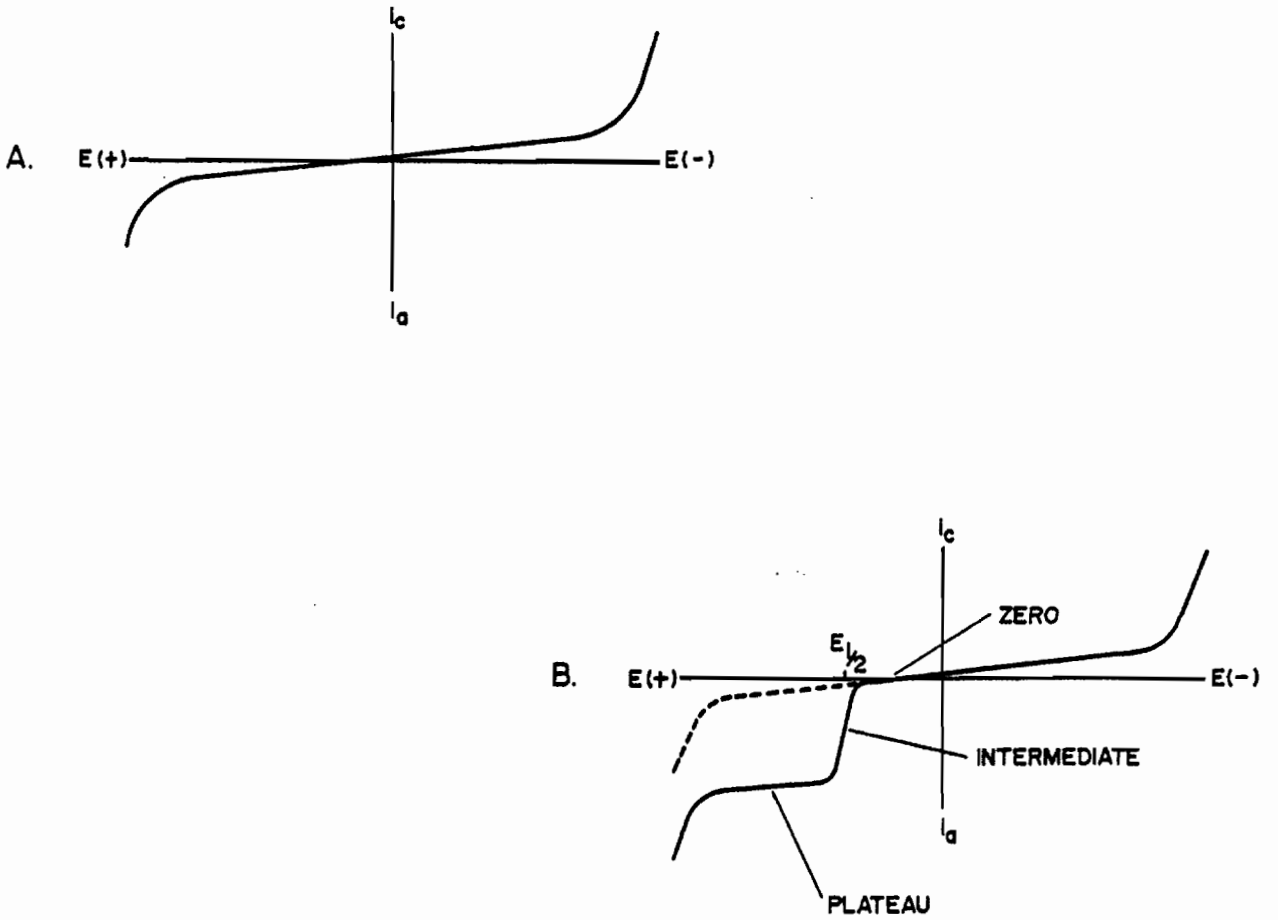


Figure 4-6. Hydrodynamic voltammogram for mobile phase alone (A) and mobile phase in solute (B). The waveform for the solute is characterized by the half-wave potential $E_{1/2}$.

1: Zero current region. The potential was not sufficient to force to oxidation to occur.

2. Intermediate region. The peak height is rising with increasing potential. Here the potential is controlling the kinetics of the heterogeneous electron transfer from the solute to the electrode surface.

3. Plateau region. In this zone, the peak height is independent of the potential. Diffusion to the electrode surface is the rate determining factor; i.e., the current is proportional to the rate of transport of molecules per unit surface area and per unit time.

In most situations it is advantageous to operate the detector at a potential in the plateau region within 50-100 mV of the final break in the curve. Such a choice will offer maximum selectivity. The background current will be minimal and fewer interferences are likely. These advantages diminish when high potentials (beyond ± 1 volt) are required. This point is illustrated in Figure 4-7 where the voltammograms are shown for equimolar solutions of four oxidizable compounds (A-D). The curves have different oxidation potentials, as exemplified by the location of the waves. Each compound also has its own particular limiting current, depending on such factors as the number of electrons transferred, the rate of that transfer from bulk solution to the surface, and the type of electrode surface used. Substance A, for example, is easier to oxidize than C, but yields a greater current response due to some combination of the above factors. Detecting A is very simple via LCEC. By setting the potential at 2, A may be clearly measured with no response from B, C, or D. In order to detect C, however, the potential must be set at a value which is sufficient for oxidation of B and A as well. Selectivity in this case is also therefore dependent on the chromatography. Figure 4-7 also illustrates the chromatograms that would be obtained for potentials 1 through 5. Note that once in a plateau region, the current response for that compound is the same for any higher potential. Also, the farther one moves out in potential, the greater the number of compounds that will show up on a complex chromatogram. At potential 5, the selectivity (both electrochemically and chromatographically) is inadequate to permit separation of C and D.

Dual electrode LCEC can overcome some of the limitations described above. Since BAS dual electrodes are interchangeable between parallel and series arrangements, and since different electrode materials can be used together in one cell, several alternatives not available in 1 electrode cells become possible. In the previous example, it might be possible to distinguish between C and D based on a difference measurement in a dual series configura-tion. Detailed examples of the use of dual electrodes for complex problems are available in BAS application notes.

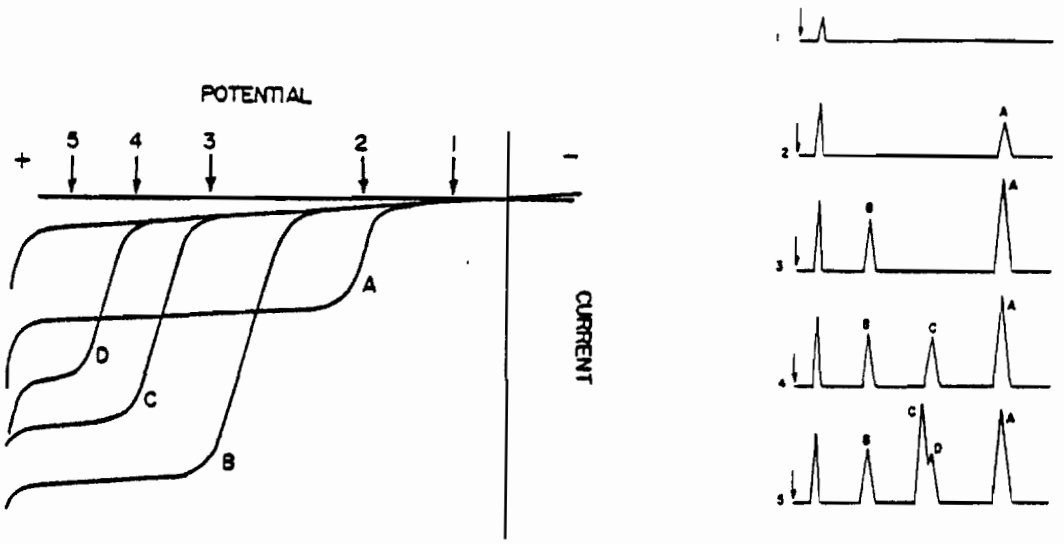


Figure 4-7. Left: hypothetical hydrodynamic voltammograms for four oxidizable solutes A,B,C,D. Right: hypothetical chromatograms for EC detector operated at potentials 1-5.

4.3. Oxidation or Reduction?

Most of the discussion thus far assumes that you have some idea of whether you will be looking at either an oxidative or reductive electrochemical reaction. What if you don't know? Don't panic! It is not always that obvious. Hydrodynamic voltammograms can give you some information, but they require several injections onto your LCEC analyzer, use mobile phase, and put wear on the column. There is an easier way.

Cyclic voltammetry (CV) is a quick and inexpensive means of learning the electrochemical behavior of the analyte in the mobile phase you plan to use. Mobile phase changes can cause shifts in applied potential. To keep the assay optimized, you should either do an HDV as described, or run a cyclic voltammogram on the sample in your new mobile phase. A sample cyclic voltammogram is illustrated. Appendix 4 also lists CV data for a wide range of compounds. If you are continually faced with new analytical problems, you will find a CV instrument an invaluable accessory for LCEC.

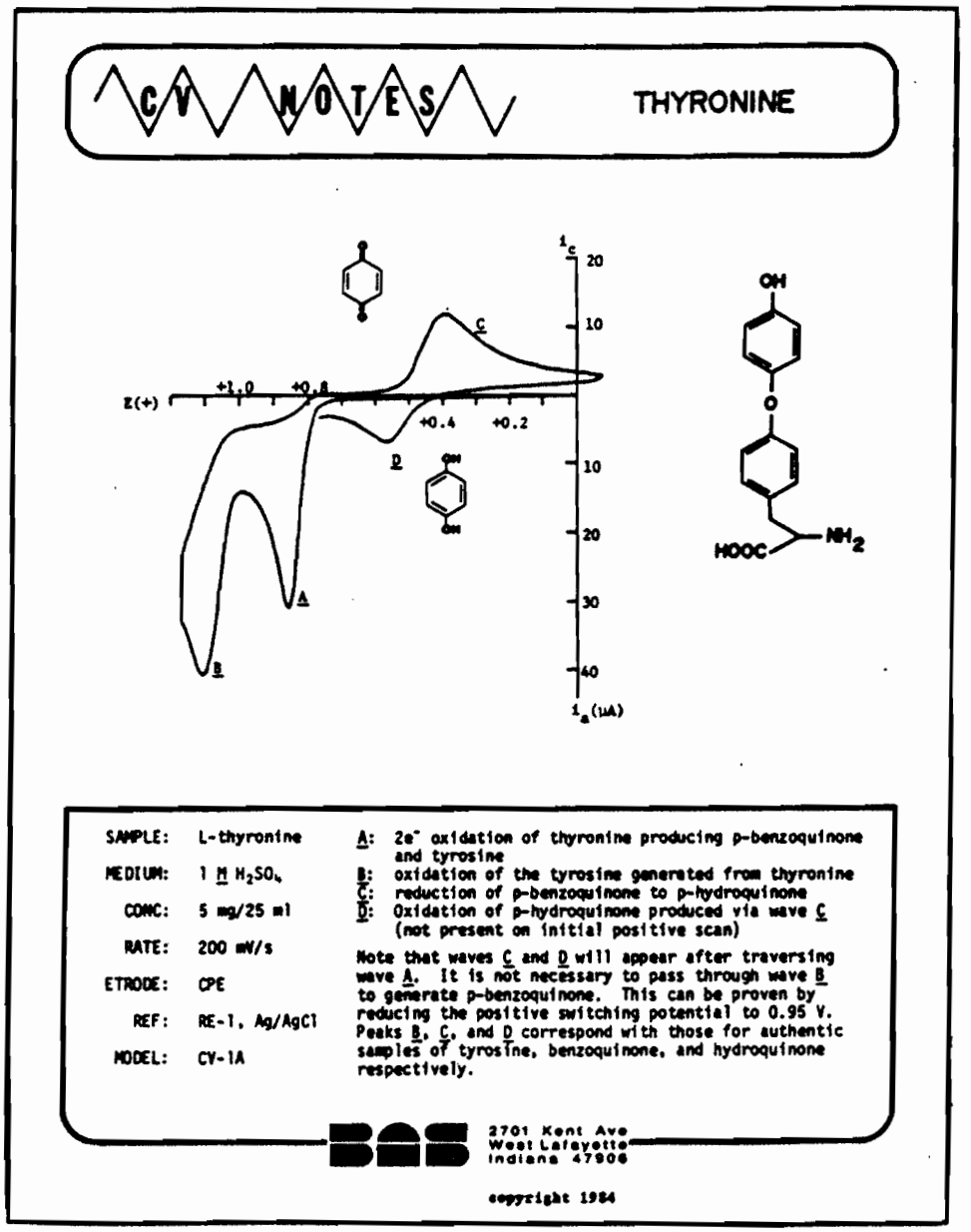


Figure 4-8. This cyclic voltammogram of thyronine tells us that a potential of 0.85-0.90 V is necessary to detect it. It also undergoes an interesting follow-up reaction which could be detected by dual electrode LCEC.

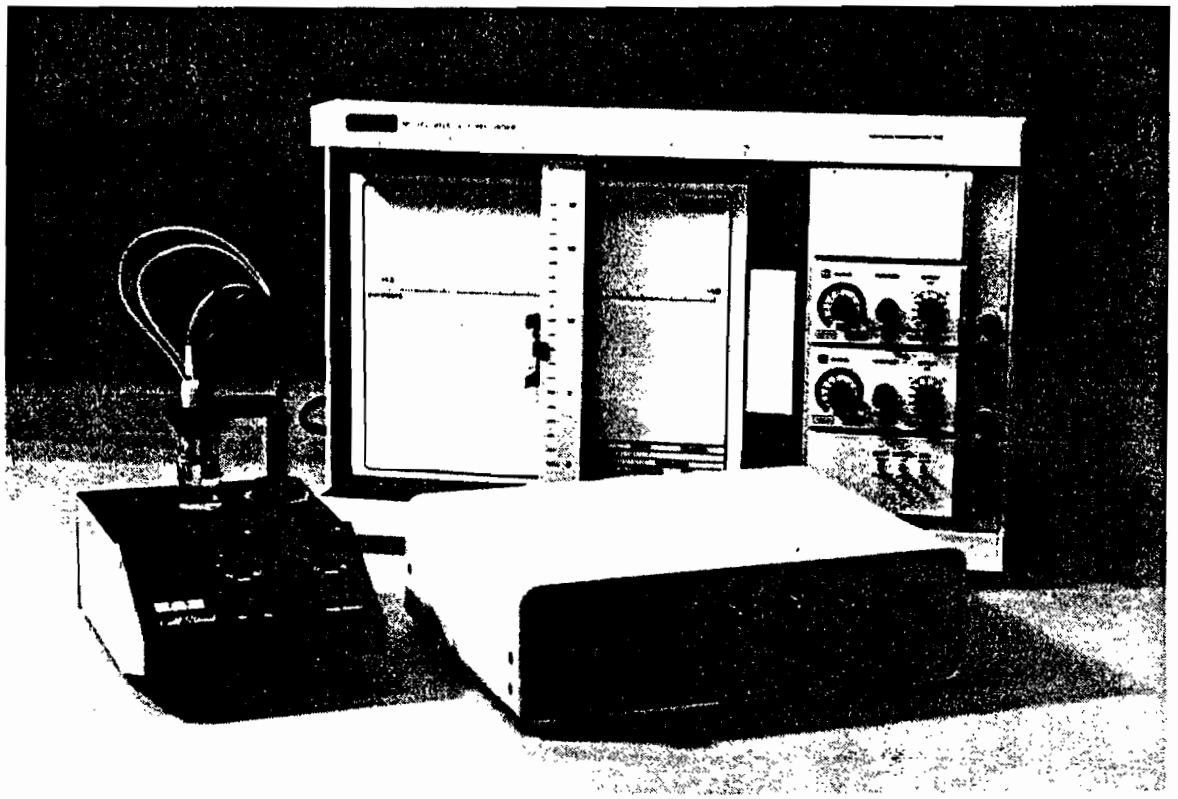


Figure 4-9. BAS Cyclic Voltammetry Instruments

4.4. Mobile Phase Limitations

Since amperometric detection depends on the transfer of electrons between a solute and the electrode surface, it is important to choose a solvent (mobile phase) that effectively permits the electrode reaction to occur. The primary limitations on the mobile phase are:

1. ELECTROLYTE MUST BE PRESENT, usually at 0.01 M to 0.1 M concentrations, to convey charge through the electrochemical cell,

2. The solvent must have a sufficiently high dielectric constant to freely permit IONIZATION OF THE ELECTROLYTE, and,

3. The mobile phase (electrolyte + solvent) must be ELECTROCHEMICALLY INERT at the electrode surface; i.e., the background current at the applied potential should be negligible, with no chemical deterioration of the surface.

Even under the above restrictions, the scope of LCEC is very wide,

since all ion exchange and most reverse phase separations employ these types of mobile phases. Initially all reports on LCEC dealt exclusively with ion-exchange columns using aqueous buffers. A majority of the separations performed with LCEC are now done with reverse phase packing materials (e.g. BAS Biophase ODS or Phase II bonded phases). By bonding silyl hydrocarbon chains to a particle of silica, one fashions a packing capable of retaining nonpolar and weakly polar solutes from polar mobile phases. The versatility of reverse phase materials can be further extended to ionic solutes by adding small amounts of ion pairing reagents to the mobile phase (e.g. BAS P/N CF-1090 sodium octyl sulfate). Polar bonded phases (diol or nitrile) have also been used. Few applications with silica gel columns have been reported, since the mobile phases needed (non-polar solvents with low dielectric constants) to effect separations on these materials are incompatible with the requirements of LCEC. There are some ways to get around this problem with non-aqueous solvents (see section 6.1).

4.5. Uncompensated Resistance

The simplest electronic equivalent of a three electrode electrochemical cell would resemble Figure 4-10. The goal of the instrumentation is to impress all of the potential applied by the potentiostat across the interface between the electrode surface and the solution. This provides the sharpest potential gradient to the molecules.

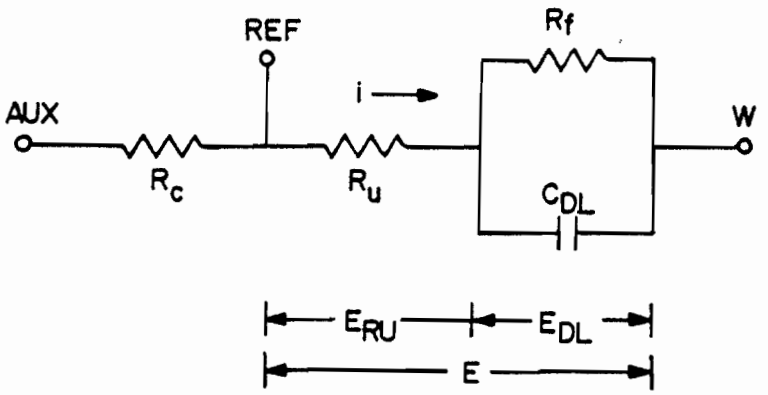


Figure 4-10. Simple electronic equivalent of a detector cell.

Since the potential is applied between the reference and working electrodes, the user's control of the distribution of this potential between R_u and the interfacial double-layer impedance is impossible. The reference electrode may be moved closer to the working electrode (thereby minimizing R_u), but the term R_u will always be present to some degree. For this reason, R_u is termed the uncompensated resistance. C_{DL} is the double-layer capacitance (this capacitance is charged up when the detector is turned on, and is the cause of the initial off-scale transient) while R_F is the Faradaic resistance. The latter term represents the resistance to charge transfer across the interfacial region.

Since uncompensated resistance is always present, the simplest expression possible for the applied potential, E, is

$$E = E_{Ru} + E_{DL}$$

Figure 4-10 describes this distribution. The difference between E and real applied potential E_{DL} is the term E_{Ru}. Since the electrical equivalent in this region is a simple resistor,

$$E_{Ru} = iR_u$$

where i is the current passing through R_u (and the double layer) at any time. This factor, dependent on both the current and the resistance in the cell, is referred to as "iR drop."

The cell described in Figure 4-3 possesses very low uncompensated resistance. The electrodes face each other directly across the thin-layer channel. Hence, even if cell currents reach hundreds of microamperes, E_{Ru} remains negligible and the interfacial potential remains constant.

The "curse" of high uncompensated resistance is narrow dynamic range. Spatial relationships within electrochemical cells are critical, and the compact nature of the updated thin-layer design in the LC-17A is optimal in this regard.

4.6. The Current Response

Principally two contributions to the current response of a thin-layer electrochemical cell are encountered. These are:

1. The FARADAIC RESPONSE, due to redox processes either from analyte or solvent impurities, and

2. The CHARGING CURRENT, required to charge the double-layer capacitance at the solution/ electrode interface.

Normally the charging current is not an issue since the detector is operated at fixed potential. Once the potential is supplied to the cell and the background decays to an acceptably flat level, only Faradaic contributions exist.

The fundamental relation for LCEC operation,

$$i = nFAJ_{x=0}$$

describes the current in terms of the flux J and various constants. The flux is dependent on the flow rate, cell dimensions, and the concentration and diffusion coefficient of the analyte being oxidized or reduced. A rigorous derivation incorporating a detailed expression for the flux permits the following equation:

$$i_{lim} = 1.467\ nFAC_o\ (D/h)^{2/3}\ (U_v/d)^{1/3}$$

where n is the number of electron equivalents/mole, F is Faraday's constant, A is the area of the electrode (cm^2), C_o is the concentration of reactant in the bulk eluent ($mole/cm^3$), D is the diffusion coefficient (cm^2/s), h is thickness of the channel, U is the volume flow rate (cm^3/sec), and d is the width of the channel. From this expression, it is evident that the diffusion limited current should be proportional to the concentration of the analyte, the area of the electrode, to the cube root of the velocity through the cell and inversely related to the cube root of the channel thickness.

Figure 4-11 illustrates the plot of the current response versus linear velocity (cm/s) through the cell for a number of channel thicknesses. The current response was normalized by dividing the peak current by the concentration of the injected analytes. Faster flow rates favored greater peak heights. A similar plot was made of the normalized coulometric (area) response vs. the linear velocity. The coulometric response decreased with increasing velocities. Remem-ber that the current response describes the rate of conversion while the coulometric response relates to the amount converted. The rate can be high in spite of low amounts converted and vice versa.

4.7. The Background Current

The background current is principally Faradaic current arising from the oxidation (or reduction) of electroactive impurities in the mobile phase. It is entirely analogous to the background absorbance in ultraviolet detector systems. Common sources of background current are oxidation or reduction of the mobile phase solvent or buffer salts, oxygen (reductive), ferrous iron (oxidative), metal ions (reductive), etc. For the simple and usual case where the background current is caused mainly by the mobile phase, a plot of background current versus applied potential would resemble Figure 4-12. Note that the current is exponentially related to the applied potential at both a positive and negative limit. Within these limits, however, a fairly flat low background response is typical.

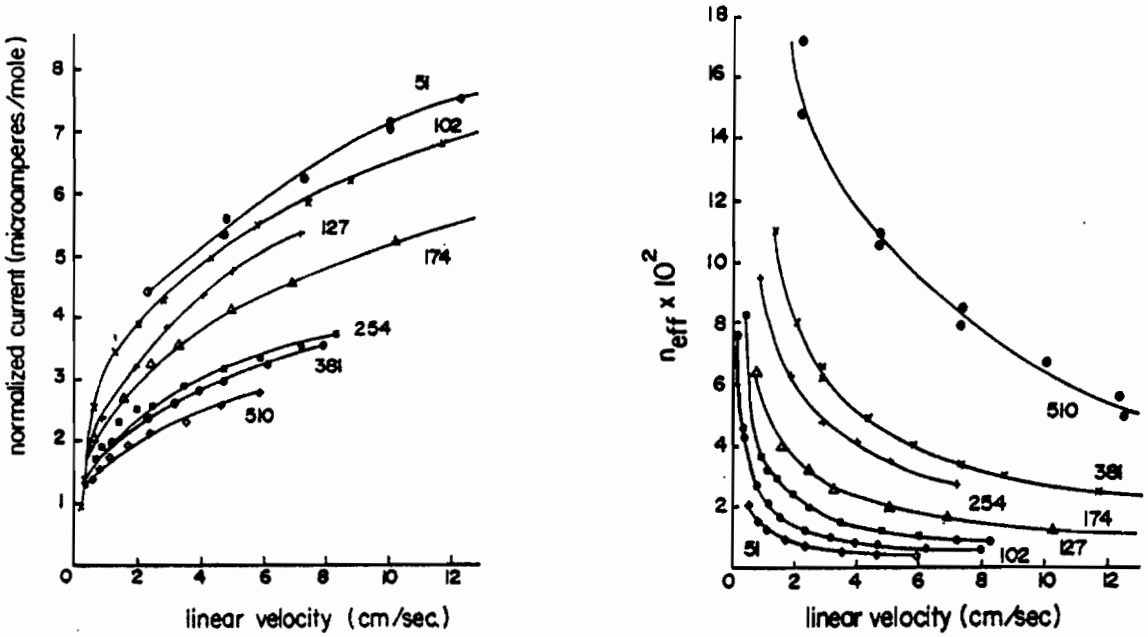


Figure 4-11. Peak height (left) and peak area (right) versus linear velocity for various channel thickness (indicated on plot in micrometers).

4.8. Noise

Noise is the random or periodic pattern superimposed on the steady-state background signal. Usually measured from peak to peak, the noise represents the summation of spurious contributions from pump pulsation, flow cell hydrodynamics, surface reactions, static electricity, power line noise, and electronic amplification. Obviously it is desirable to minimize noise. Specific guidelines are given below; detailed advice is outlined in Appendix II.

1. Select a pump capable of delivering pulseless flow relative to the detection limits you desire. Use pulse dampers. Use a complete BAS LCEC Analyzer system.

2. Passivate your liquid chromatograph frequently. Check with the manufacturer for their recommendations on this. Usually, an acid washing (e.g. 6 N HNO_3) is employed. Be sure to disconnect your column and cell before attempting this procedure.

3. Follow good laboratory practice and maintain the pump seals and check valves in top working order. Lubricate the pump or maintain as specified by the manufacturer. Be fastidious about flushing the system thoroughly when you don't plan to use it for a long period. This helps eliminate random flow fluctuations.

4. Connect all components in your system to the same circuit. In this manner you will avoid ground loops.

5. Avoid solvents capable of destroying the electrode surface. This is of concern primarily for carbon paste or chemically modified electrode surfaces.

How does the background current and noise affect performance? As with most other quantitative measure-ments, the noise with an electrochemical detector is dependent on the magnitude of the background signal. Generally, the greater the background, the greater the noise, and the ratio of the noise to the background current stays approximately the same. This trend is true for the amperometric detector glassy carbon materials. The noise will follow the same trend as the background current (Figure 4-12). It is impossible to operate at extremely high gain with high background currents if ultratrace measurements are to be achieved.

4.9. Attaining Good Signal-to-Noise Ratios

The parameter most useful for analytical comparison is the signal-to-noise ratio (SNR). An extremely responsive electrode may be equally noisy, just as the apparent baseline quiescence of another electrode may be due to passivation, thus rendering it worthless. Neither situation may be evaluated by comparing just the signal (the response from injection of analyte) or just the noise. For this reason the SNR is most pertinent.

Response should be directly proportional to surface area. Therefore, if we were to use an electrode of 10 times the area, it should give 10 times the response. Such improvement would be highly significant, if the cell noise remained at its old value. However, if the noise increased proportionately, the use of a larger surface would be questionable.

In actual practice, larger surface area electrodes are less useful. Figures 4-13 and 4-14 illustrate this trend. Both the analyte signal (peak height) and peak-to-peak noise are plotted. Each increment of added area adds proportionately less to the output signal but adds linearly to the output noise. The smaller the electrode, the greater the signal to noise ratio. For this reason, large plate electrodes, porous flow-through cells, or other large surface electrodes, demonstrate no advantages in the SNR. If you intend to use the "Jumper" to couple the dual electrodes together, you should expect to see a less favorable SNR. The experiment might still be useful if you are trying to differentiate a particularly small peak and need a larger signal.

Since noise is dependent on potential, it follows that the smallest minimum detectable quantities will occur in cases where the substances of interest are easily oxidized or reduced. Figure 4-15 demonstrates the value of judicious potential selection.

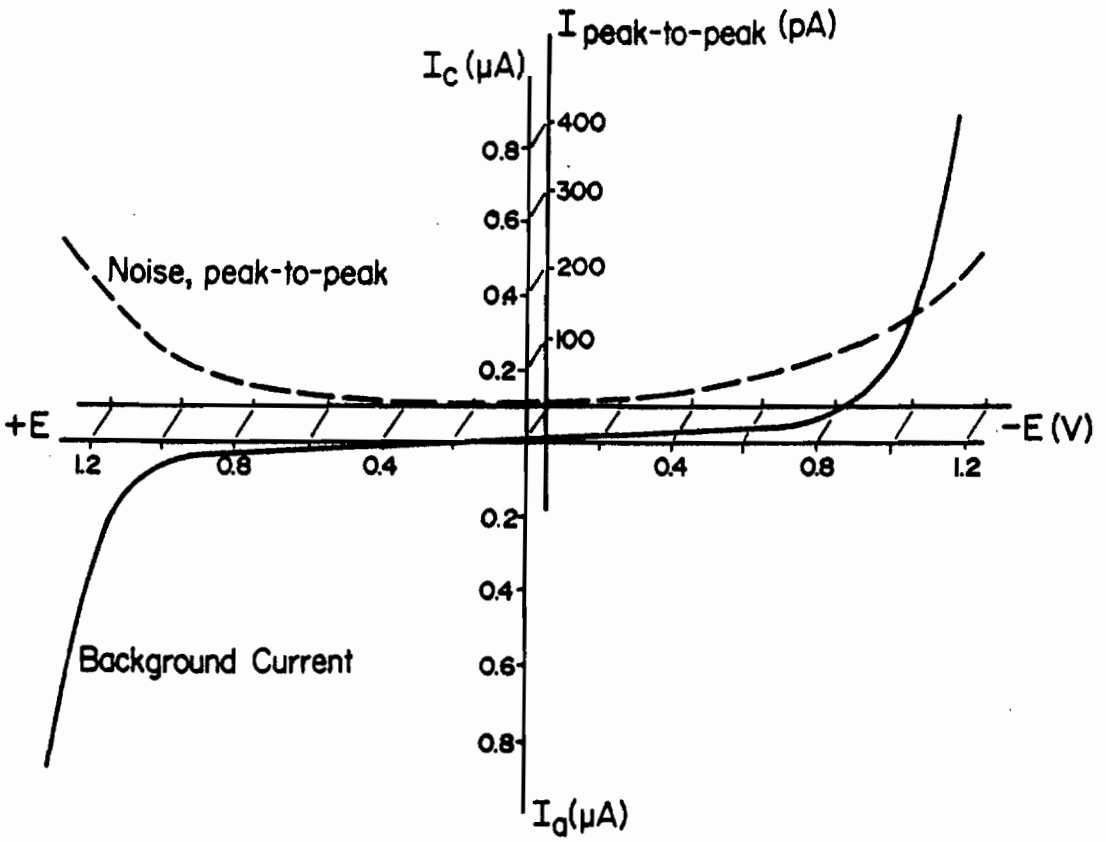


Figure 4-12. Generally, the greater the background, the greater the noise. Both follow the same trend.

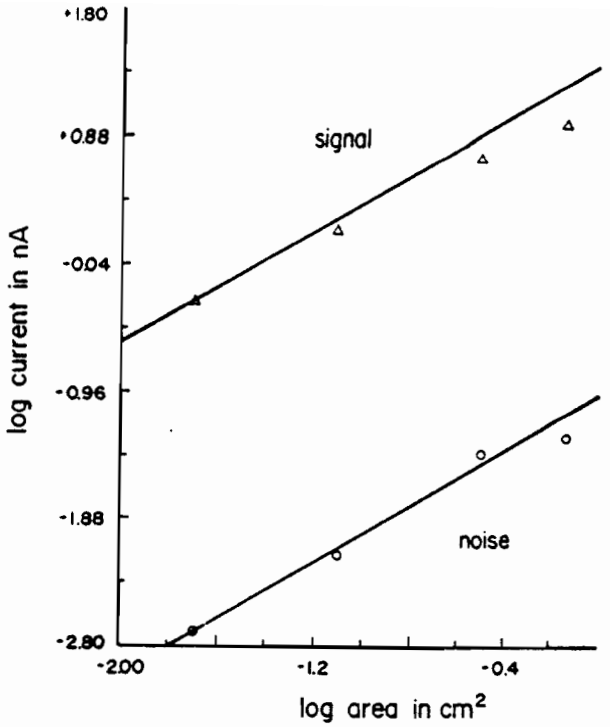


Figure 4-13. Signal and noise for electrodes of different surface area. The solid lines indicate the increase in current expected for a linear response in area.

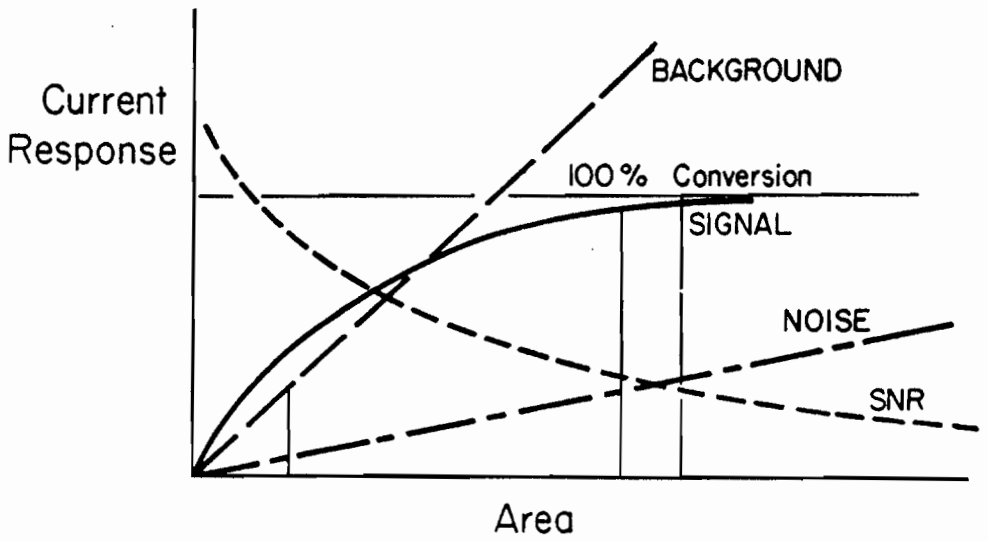


Figure 4-14. As the electrode surface area increases, the signal-to-noise ratio becomes unfavorable.

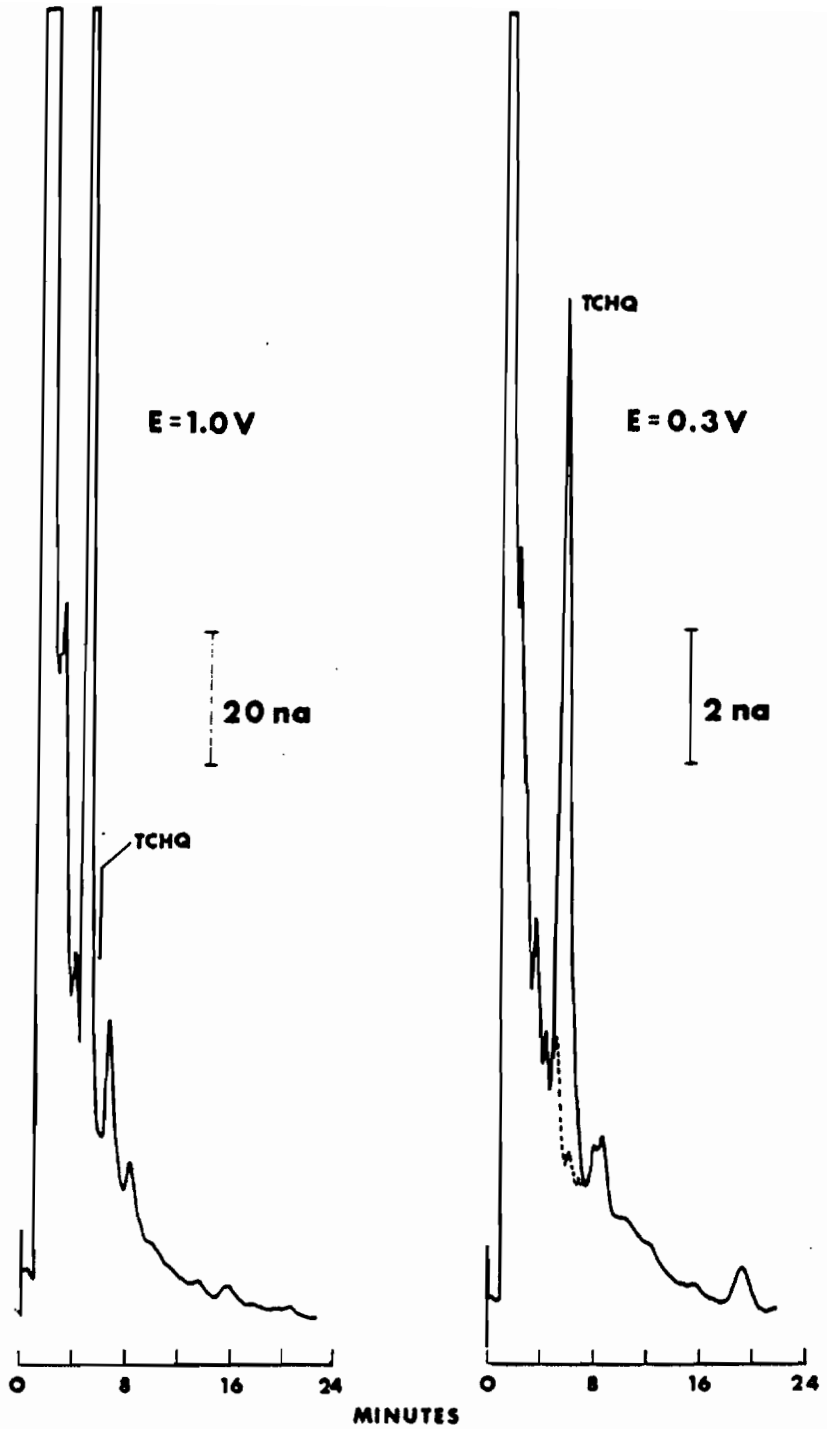


Figure 4-15. Detection of tetrachlorohydroquinone (TCHQ) at two different applied potentials. This illustrates the specificity of electrochemical detection which can in turn influence the sensitivity. Determination of the optimum applied potential is not unlike determination of the optimum wavelength used in UV detection methods. See Sections 4.2 and 4.3.

4.10. Dual Electrodes

The thin-layer format in BAS electrochemical transducers permits two (or more) electrodes of the same or different composition to be placed in the flowcell cavity. From an analytical perspective, the two simplest orientations of twin sensors are designated "series" and "parallel" depending on their spatial relationship with respect to the flow. In the BAS transducer described in Figure 4-3, these may be interconverted in seconds by unclamping the working electrode, rotating 90 degrees, and retightening. What are the analytical possibilities?

In the dual PARALLEL amperometric mode, two electrodes are placed adjacent to each other on one side of the flowstream across the channel from the auxiliary electrode. (Figure 3-12). This arrangement permits the simultaneous recording of chromatograms at two individual potentials. There are two major applications: (1) simultaneous determination of substances with significantly different oxidation (reduction) potentials, and (2) simultaneous measurement of peak current ratios for the purpose of confirming the identity of eluted compounds. Both applications save valuable time by permitting a single chromatographic experiment where two would have been required with a single electrode. Perfectly analogous experiments have been carried out with "dual wavelength" UV detectors.

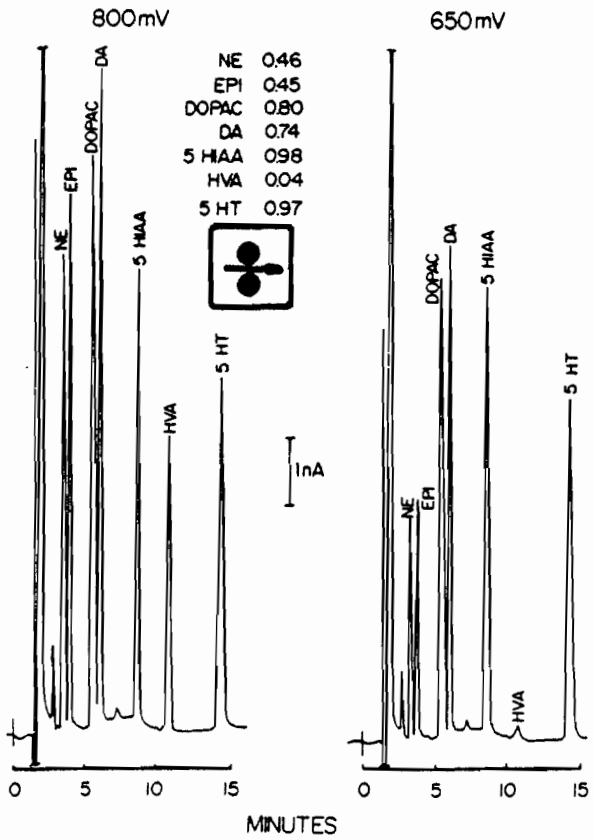


Figure 4-16. Dual-Parallel LCEC: Detection of biogenic amines in brain (from BAS Application Note 59). In the SERIES

In the SERIES dual electrode amperometric mode, the two electrodes are positioned with one behind the other. The downstream electrode can be used to study the product(s) created at the upstream electrode. There are many applications of this scheme but the major use is to improve the detection of compounds which have electrochemically reactive products. This is accomplished by detecting the products at a potential where interferences are minimized. For example, the detection limit for compounds which react at potentials where background processes interfere can be improved by detecting the product(s) in a more favorable part of the available "potential window." This is successful because most background (media) reactions are chemically irreversible e.g. reduction of oxygen in aqueous media, reduction of hydrogen ion, and/or oxidation of water. Many "ring-disk" electrochemical experiments have used these same ideas.

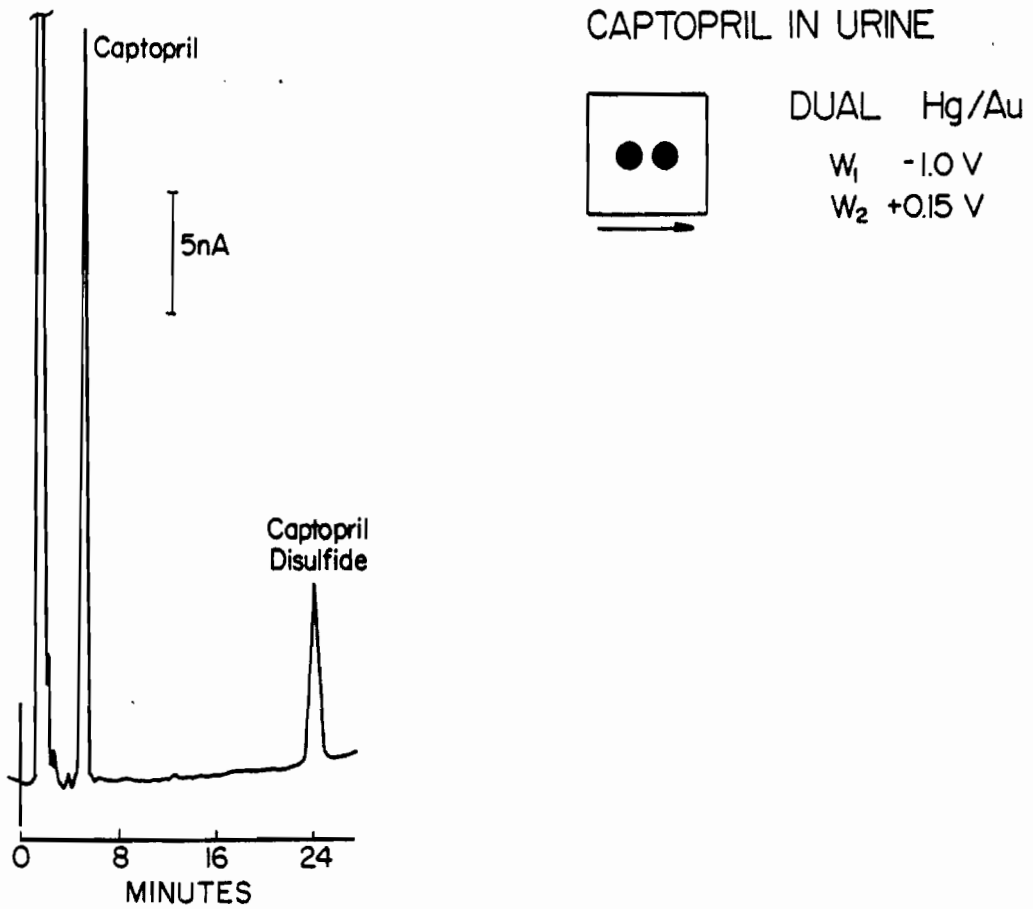


Figure 4-16. Dual-Series LCEC: detection of captopril in urine (from BAS Application Note 56).

5. Operating the Electrochemical Detector

5.1. Front Panel (Figure 5-1)

POWER

Applies main electrical power to controller circuitry; the red indicator light directly above switch will be illuminated when power is on.

NOTE: When turning main power on or off, the working electrode (CELL MODE) should be placed in the Standby position (STBY).

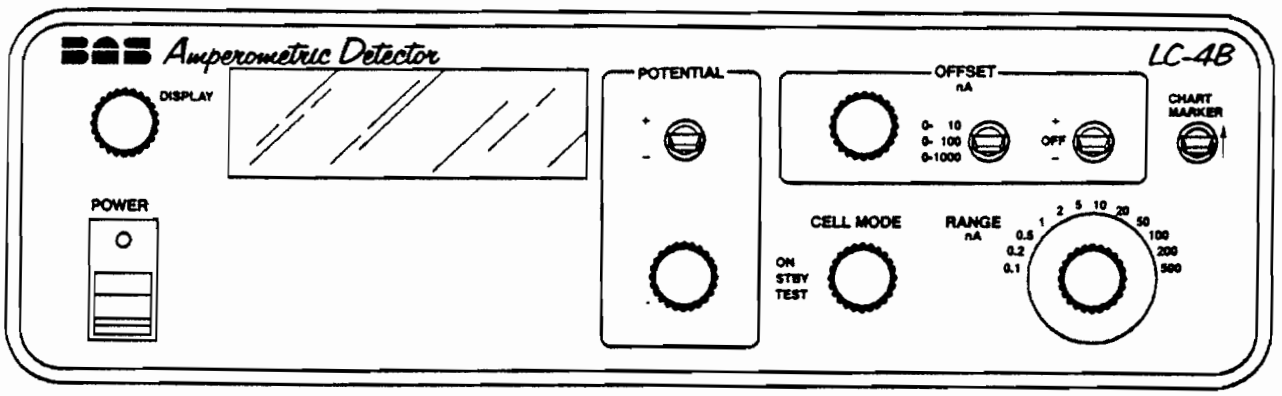


Figure 5-1. Front panel of enhanced LC-4B detector controller.

DISPLAY

This switch selects the function to be monitored by the internal digital voltmeter (DVM) and displayed on the front panel window. Applied potential (volts), output current (nanoamperes), offset current (nanoamperes) and temperature (degrees C) are chosen by turning the DISPLAY knob (rotary switch) until the desired function is displayed on the left side of 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 of the lighted panel. The ranges for each of the individual functions are: applied potential, 0 ± 1.99 volts; offset current, 0 ± 1000 nAmps; and column temperature, ambient to 99.9 degrees C.

NOTE: Temperature is displayed only when the LC-4B controller is used with a BAS Temperature Controlling device. These include the LC22A/23A Column Heater for 15, 25 or 30 cm analytical columns, the LC22A/23B Cartridge Column Heater, and the Cell Preheater in the LC-17AT accessory package. In all cases, a cable must connect the LC-4B to the LC-22A. This cable is provided with the

temperature controlling instrument. Only one device can be joined to the LC-4B for temperature readout.

- -

POTENTIAL

Any applied potential value between 0 and 1.99 volts can be selected by turning the DISPLAY switch until the APPL E window is illuminated and then turning the POTENTIAL knob to the required value as monitored on the front panel display. Selection of (+) or (-) indicates positive or negative potentials respectively.

OFFSET

The OFFSET controls are used to null out the steady-state background current and thereby establish the operating baseline. The magnitude of the OFFSET current is shown on the front panel display when the DISPLAY selector switch has been turned to the OFFSET function. The OFFSET knob can be turned to null out background currents between 0 - 10 nA, 0 -100 nA, or 0 -1000 nA depending on the range chosen. The range is determined by the three position switch just to the right of the OFFSET knob. The remaining toggle switch located in the OFFSET area selects the polarity of the zeroing current. When set on +, the circuit will null anodic (oxidative) currents; on -, it will null cath-odic (reductive) currents. When OFF is selected, the OFFSET circuitry is deactivated, although the detector continues to function normally in all other respects.

CELL MODE

This 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 of this manual).

RANGE

The RANGE switch determines the controller output. Range is expressed in nA/V. Selections from 0.1 to 500 nA/full scale deflection are provided. For example, a setting of 10 indicates 10 nanoamps full scale sensitivity; therefore an output signal of 5 nanoamps would produce a pen deflection of one-half full scale. Note that full scale deflection is either 1 volt or 10 mV depending on the Output range chosen (see Sect. 5.2, and installation Section 3.5, #6 for discussion of Output). Also note that the maximum output voltage is approximately 10 volts at the 1 volt jacks or 100 mV on the 10 mV jacks.

CHART MARKER

This front panel toggle switch will route a small voltage to the recording device for the purpose of marking a start point on the chromatogram at the time of injection. It is manually actuated.

5.2. Rear Panel

MULTI-PIN CONNECTOR

This connector is used to couple two LC-4B controllers together. It is only employed in dual electrode detectors. The short interconnect cable is provided with the dual electrode detector, or dual electrode upgrade packages. See Installation Section 3.5, #4).

SHUNT

The high current shunt can only be operated in dual mode LCEC (most likely the SERIES arrangement). The working electrode chosen as "W2" should be controlled by the LC-4B in which SHUNT is actuated. The other LC-4B will control the downstream "W1" electrode and should have the shunt switch in the NORMAL position.

For single working electrode LCEC, always flip this switch to the "normal" position.

If you plan to use this option for dual LCEC, the upstream electrode (W2) must be the one shunted, and the Range control must be set at 100, 200, or 500 nA. When the shunt is actuated, the "output" and "nA" will flash. This indicates the actual units of the displayed current are microamperes and not nanoamperes.

CELL IN

The cell lead cable is attached to this connector. To make this connection, insert the cell lead cable connector into this socket, until the two components "snap" into place. Do not force this connection. Make certain the two components are properly aligned before making the connection. When the LC-17A(T) detector compartment is used, a steel shroud will shield the short cable from spurious Radio Frequency Interferences (see Installation Section 3.5, #3).

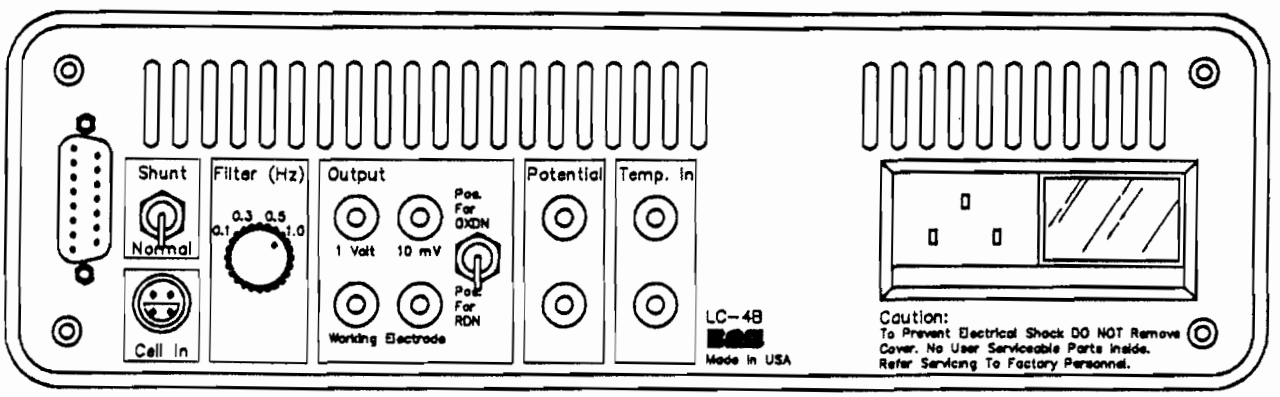


Figure 5-2. Rear panel of enhanced LC-4B detector.

FILTER

This control adjusts the cutoff frequency of the active filter to 0.1 Hz, 0.3 Hz, 0.5 Hz, or 1.0 Hz. The optimum filter setting depends upon peak width. For very high speed separations, such as those typical with 3 um or microbore columns, the 0.5 Hz or 1.0 Hz setting will be suitable. For slower separations, the 0.3 Hz or 0.1 Hz settings will be best. Basically, the optimum filter setting is dependent upon the type of chromatography. Try 0.1 Hz and then 0.3 Hz on your standard run. If the runs do not appreciably differ in the heights of early eluting peaks, stay with the 0.1 Hz filter.

OUTPUT

These terminals feed the analog output from the LC-4B to a recording device. The output signal is a voltage corresponding to the amount of current produced at the working electrode. Two terminals are provided, depending on the recording device employed. The blue and black jacks are for recorders requiring a 10 mV input; the green and black jacks are for those requiring a 1 V input. BE SURE TO CHECK THE INPUT REQUIREMENTS FOR YOUR RECORDER! The toggle switch on the right should be up for oxidative work and down for reductive work. This will provide a positive going output voltage for oxidation or reduction reactions respectively.

POTENTIAL

This terminal monitors the applied potential via an external voltmeter. It is only used for troubleshooting purposes.

TEMP IN

This terminal is used to connect the output of the **LC-22A Temperature Controller** to the LC-4B monitor. When the units are connected, the actual temperature (degrees C) of the **LC-23A** or **LC-23B Heating Compartment** or **LC-17A Cell Preheater** can be read directly on the front panel display.

5.3 Rear Panel, LC-17AT Flow Cell Compartment

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 LC-17A(T) when shipped.

If you wish to convert from a single electrode cable to a dual electrode cable, it will be necessary to remove the cable screws on the rear panel, and slide the old cable from the groove underneath the flow cell. When installing the new cable, slide the leads under the same groove. Do not attempt to remove the

black base under the flow cell since it is sealed into position with a waterproof adhesive, and also has set screws. Don't forget to reinstall the ground lug on the cable in the same position on the back panel as the previous cable.

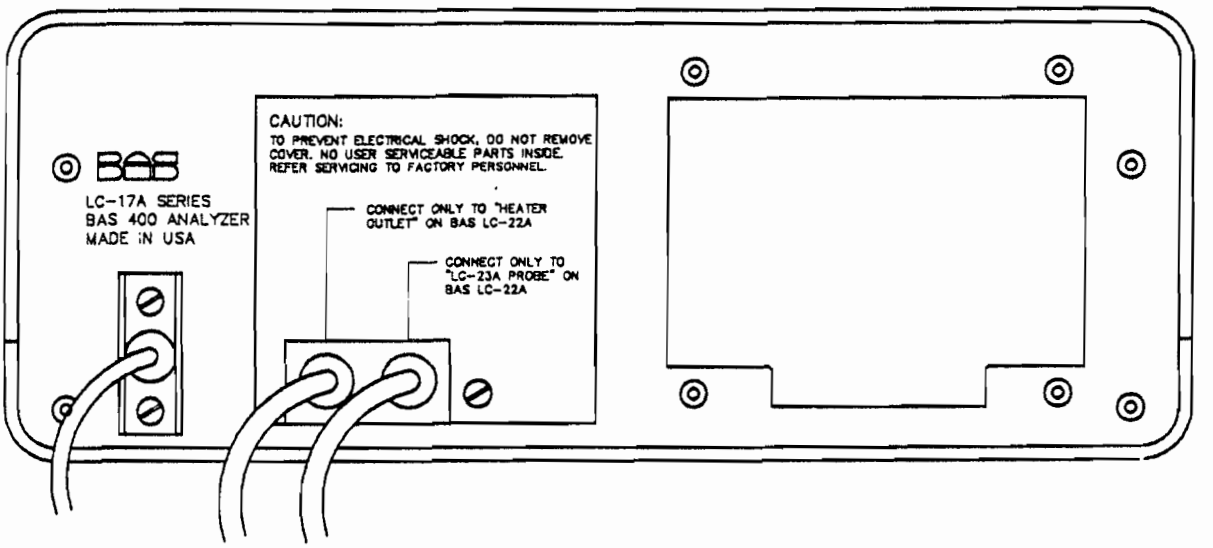


Figure 5-3. Rear Panel View of LC-17AT Detector Compartment (LC-17A Compartment does not have the Preheater)

Detector Preheater Module

The **Preheater Power** switch 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).

The thermal characteristics of the preheater can be tuned by proper switch position. From a practical viewpoint, the user is concerned with accuracy ("is the block at the chosen setpoint?") and precision ("what is the peak-to-peak temperature oscillation around the setpoint?"). To reach temperatures from 40-65 degrees 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 - 45 degrees C, flip the switch to **Low**. Overshoot will be negligible since heater power is cut back.

Utility Panel

Occasionally it may be necessary to use oversize columns in the LC-17A. The large side slots provide some access, but if the user desires a larger opening, the rear "Utility Panel" may be removed. The four screws should be put back into the LC-17A and retightened to maintain the strength of the rear panel. This panel is also removed when an LC-23B cartridge column heater upgrade is installed.

We advise retaining the "Utility Panel" in position whenever possible since it provides continuity of shielding against RFI.

5.4. Routine Operating Procedure

5.4.1. Mobile Phase Preparation

Use only high purity solvents and buffer salts for preparing mobile phases. Glass distilled solvents are best. Water should be deionized first and then glass distilled.

Filter all mobile phases through a 0.2 um membrane filter. Figure 5-4 illustrates the filtering device recommended by BAS for doing this. It is borosilicate glass throughout and also uses ground glass joints to avoid rubber contamination. The filtration step will reduce problems with column degradation significantly. CLEAN, PARTICLE FREE MOBILE PHASES ARE CRITICAL TO ANY LC METHOD.

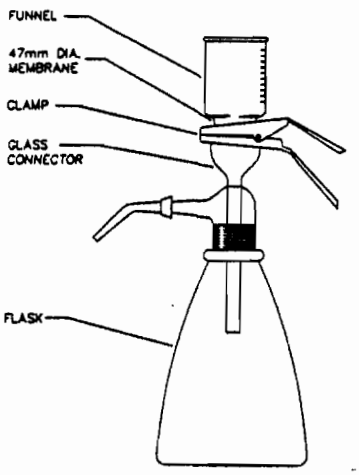


Figure 5-4.
MF 6126 Mobile Phase Filtration kit (membranes not shown).

Mobile phases should be DEGASSED prior to use. Allowing the solution to sit under the vacuum generated by an aspirator for 5-15 minutes should be sufficient. The MF-6126 kit can also be used for degassing when the filtration apparatus on the top of the unit is replaced by a stopper and the side arm is attached to an aspirator. Small amounts of volatile organic modifiers may be lost, but not enough to cause any difficulty. For reproducible chromatography consistency in mobile phase preparation is required.

If it is necessary to DEOXYGENATE the mobile phase, a considerably more elaborate scheme will be required. See Section 8.3.

5.4.2. System Preparation

Be sure that the system is clean and well equilibrated with fresh mobile phase. Don't forget to rinse out glassware used to hold the mobile phase if it has been gathering dust for some time. It is particularly important that new columns be well flushed prior to connecting the detector cell. It is recommended that new BAS columns be washed in the sequence described in BAS Application Note 80, which is shipped with each column. This will not only remove residual packing solvents, but will also give sufficient time for elimination of bubbles from the system. For C_{18} and C_8

stationary phases, this step also reproducibly "wets" the mobile phase surface.

5.4.3. Cell Preparation

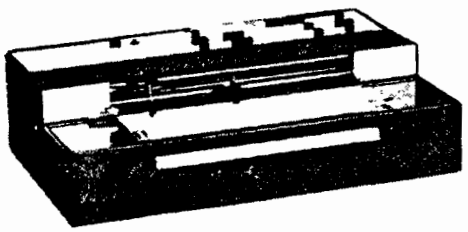
New glassy carbon electrodes need only be lightly rinsed with methanol and lightly wiped dry before use. Make sure the working electrode contacts are dry. Detailed instructions for restoring neglected cells may be found in Chapter 6, and the poster shipped in the accessories box. Gold/mercury amalgam electrodes must be freshly prepared; see Chapter 6, and the instructions poster.

If a new electrode is to be installed, do the following or refer to "Chromatography Connections", Section 3.6. In the event that bubbles occur, loosen the clamp slightly <u>with mobile phase flowing</u> and open the cell enough for the bubbles to escape. Retighten the clamp and wipe the cell dry. Twist the Reference Electrode Retainer 1/4 turn and remove the RE-4 Reference Electrode. Allow the cavity to fill to nearly the drain port. Reinsert the RE-4 with its Retainer and twist into the locked position. If the Retainer is impossible to lock into place, replace the o-rings and try again. Temporary swelling of the o-rings by solvent may be the problem.

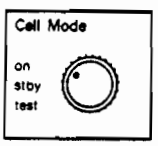
5.4.4. Normal Start-up

1. Be sure no bubbles are present in the thin-layer region or reference electrode cavity. Recheck electrical and chromatography connections (see Chapter 3).

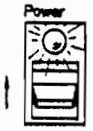
2. If using the LC-17AT Cell Preheater to maximize baseline stability, turn it on via the LC-22A controller. Use the HIGH setting on the Preheater for 40-65 degrees C and the LOW setting for 30-45 degrees C, and set the LC-22A accordingly.



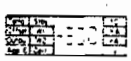
3. Zero the recorder with the recorder zero control. Baseline should be on the right side of the paper.

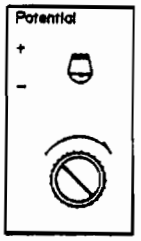


4. Turn the CELL MODE switch to STANDBY.

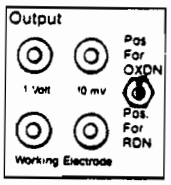


5. Turn **POWER** switch to ON.



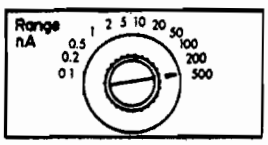


6. Select desired potential. Use the **DISPLAY** switch to adjust the LED display to "App E." Use **POTENTIAL** switch to change the sign (+ or -) and magnitude of this applied potential.

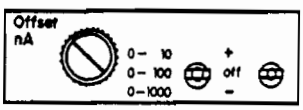


7. Set **OUTPUT** switch on rear panel output for correct mode (oxidation or reduction).

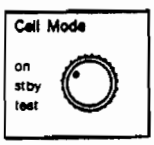
8. Set the output **FILTER** switch on the rear panel to 0.1 Hz, or whatever is appropriate for the type of chromatography you are doing. (see Section 5.2)



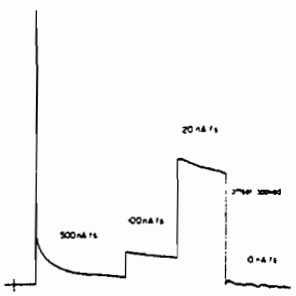
9. Set **RANGE** to 500 nA.



10. Turn the **OFFSET** to the **OFF** position.

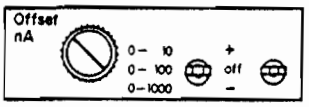


11. Turn the **CELL MODE** switch to **ON**.



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, as shown in the figure. 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 necessary.



13. When the background stabilizes, turn the **OFFSET** on and use the offset controls to rezero to the baseline position selected earlier on the recorder.

    The system is now ready for standards and samples.

5.4.5 Shutdown and Storage

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 the recommended procedures, considerable extension of electrode lifetime will be gained.

1. Turn the working electrode switch to **STANDBY** position.
2. Turn off the pumping system.
3. Turn off the main **POWER** to controller.
4. Disconnect electrodes (thin-layer cell and reference). Store reference as recommended in the Instructions poster shipped with the instrument (Figure 5-5). Rinse off the working electrode block with deionized water, air dry, and store it in the plastic box used in shipping.

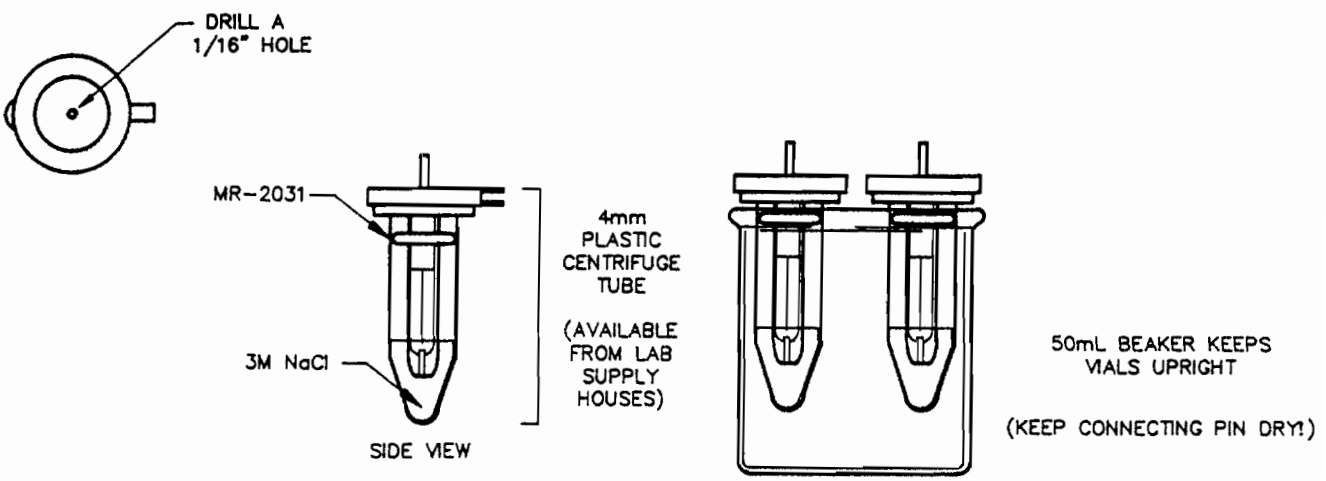


Figure 5-5. Storage of the RE-4 Reference Electrode

6. Detector Electrodes: Preparation and Servicing

6.1 Electrodes As Active Surfaces

In electrochemical detection, the signal being monitored is the direct response to an ACTUAL CHEMICAL REACTION, as compared to the physical measurement occurring in other LC detectors (e.g. refractive index, absorbance, fluorescence). Electrochemical detectors sometimes get the reputation of being "finicky" when compared to optical detectors, but it is important to realize that they behave in a very different manner, and this difference is responsible for the higher degree of sensitivity you can expect from EC methods. You will learn to handle electrochemical detection the same way you would handle any chemical reaction, by considering several variables which can influence the outcome of the reaction. In LCEC, the reaction product that concerns us is the current (i.e. the response, or signal).

The response is dependent on the chemical (electrochemical) reaction variables. These include the electrode surface where the reaction is taking place, the mobile phase (reaction medium), and the compound undergoing the reaction. The fundamental electrochemical relationships for this mode of detection were discussed in chapter 4. This section will go into greater detail on some of the practical aspects of the electrodes, including electrode materials, detector cell design, solvent considerations, and the maintenance, service, and performance of each.

The general requirements for electrochemical detection are that the mobile phase be conducting, the working (indicating) electrode be chemically inert, and the analyte be electrochemically oxidizable or reducible at the electrode surface in the chosen solution. Solution conductance is met by having an electrochemically inert salt (an ionic conductor) dissolved in the mobile phase. This places some restrictions on the mobile phase composition. Usually aqueous or partially nonaqueous solutions are used, though totally nonaqueous solutions can be used as long as an appropriate salt is dissolved in them. Since the majority of the liquid chromatographic separations being performed today use reverse phase packing materials, this requirement is easily met. It is also advisable that the mobile phase be a buffer solution for both electrochemical and chromatographic reasons.

Ideally the working electrode should be inert to the electrolytic solution and only respond to the analyte in a thermodynamically defined, potential-dependent fashion. Many times this is not the case. The kinetics of heterogeneous charge transfer between the electrode and the analyte, in addition to the reactivity of the electrode itself, enter into the situation. For example, Figure 6-1 shows actual current-voltage curves (normalized hydrodynamic voltammograms, see Chapter 4 for further discussion of these waveforms) for the oxidation of a substituted o-hydroquinone on four carbon-based electrodes. The sharpest break with potential occurs with the CP-O while the broadest voltammogram occurs on the CP-W, indicating that faster electron

transfer kinetics occur on CP-O relative to CP-W. The CP-S and glassy carbon electrode materials exhibit kinetics similar to CP-O. Fast electron transfer kinetics characterized by sharply rising voltammograms improve the selectivity of the overall determination. Some carbon paste formulations (graphite mixed with hydrocarbon and fluorocarbon polymers) show slower kinetics, similar to the CP-W described above. The sensitivity or response for a given amount injected is approximately the same in the diffusion limited region, but for these materials, a greater potential is usually required for equivalent response.

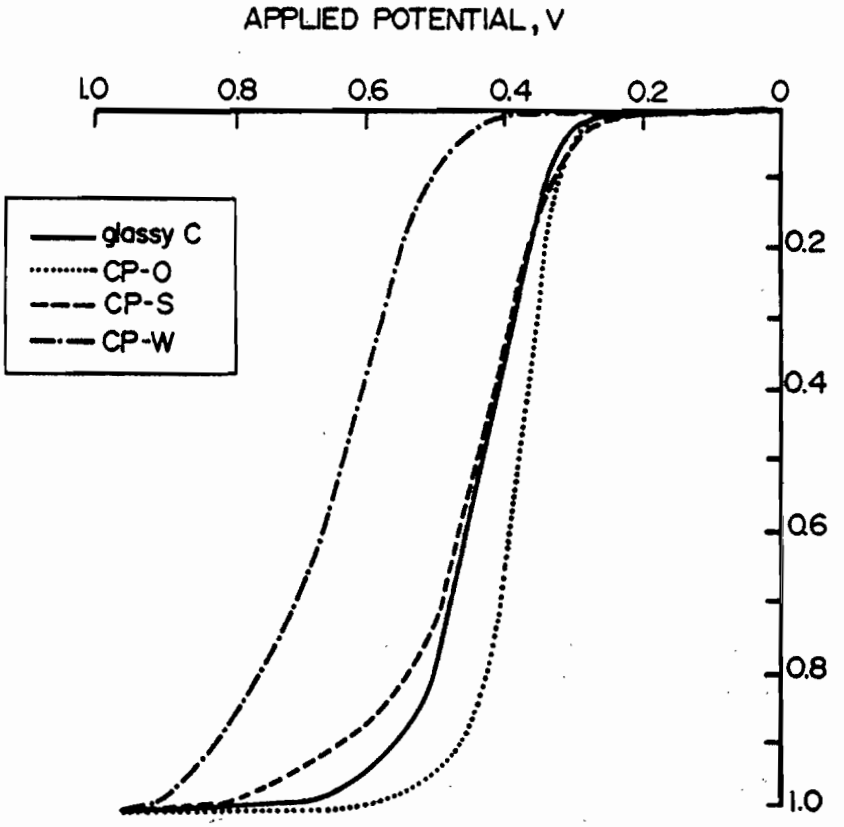


Figure 6-1. Hydrodynamic voltammograms for various carbon pastes and glassy carbon.

Electrochemical reactivity can be altered considerably by changing the electrode material. In many cases this can be highly advantageous. The large hydrogen overpotential characteristic of mercury electrodes in protic solutions extends the attainable negative potential range (past carbon) and makes difficult reduction reactions possible. For this reason mercury remains the material of choice in these potential regions. However, the reduction of dissolved oxygen, a very facile reaction on mercury over a wide potential range, does not occur until well into the negative potential region on a glassy carbon electrode. Thus, the oxygen overpotential of glassy carbon is much better than mercury (Figure 6-2) and precludes the need for oxygen purging at moderately negative potentials.

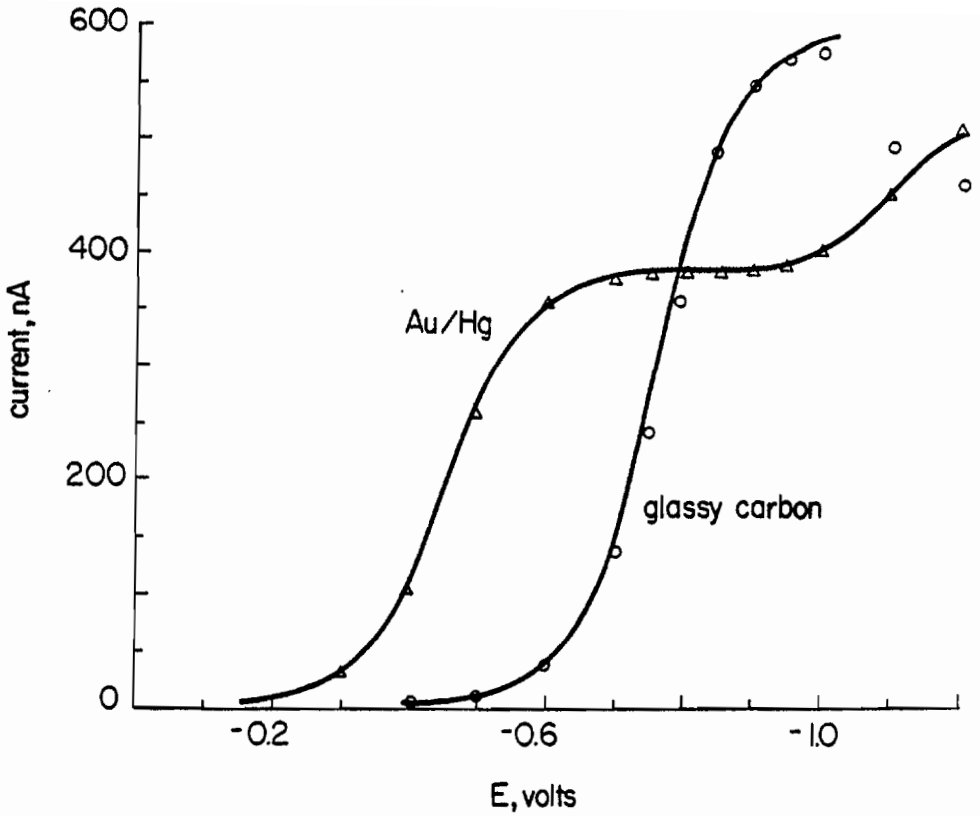


Figure 6-2. Hydrodynamic voltammograms for oxygen on glassy carbon and mercury electrode.

Not all electrode materials will withstand solvents. All carbon paste formulations are limited to varying degrees (usually not more than 10%), depending on the graphite binder. Glassy (vitreous) carbon, platinum and mercury (amalgamated gold) are far more resistant to organic solvents.

All electrode materials require some surface conditioning or modification before they stabilize to a constant background current level. The conditioning process is observed as a slowly decaying current output from the detector after the electrode is turned on (see Section 5.4). This may take only a few minutes for an electrode that has been switched off momentarily to as long as a few hours for a freshly prepared glassy carbon electrode at high negative or positive potentials. Longer stabilization times will be required for high sensitivity operation.

6.2. Cell Design

Previous to March 1986, BAS electrochemical cells could be structured quite differently, by the nature of the reference

electrode, location of the auxiliary electrode, inlet/outlet port positions, working electrode materials, etc. Except for the latter feature, these permutations have been replaced by a simplified, universal thin-layer cell which provides all the capabilities of the past and also meets future expectations. As discussed earlier in Chapter 4, this cell (Figure 6-3):

1. eliminates iR problems,
2. eliminates "antenna effects" at high detector gain,
3. provides for microbore and fraction collection capability by an improved "zero dead volume" flow channel,
4. reduces the access time for all serviceable cell components to just a few seconds,
5. preserves dual series and parallel electrode configurations, and adds a new "jumper" feature
6. permits several different types of working electrode shapes and materials.
7. reduces drift effects at high amplification (low settings on RANGE) in adverse laboratory environments.

The only consumable components in the design are the new RE-4 reference electrode and the modified dual glassy carbon working electrode. Both are described in detail in the next section.

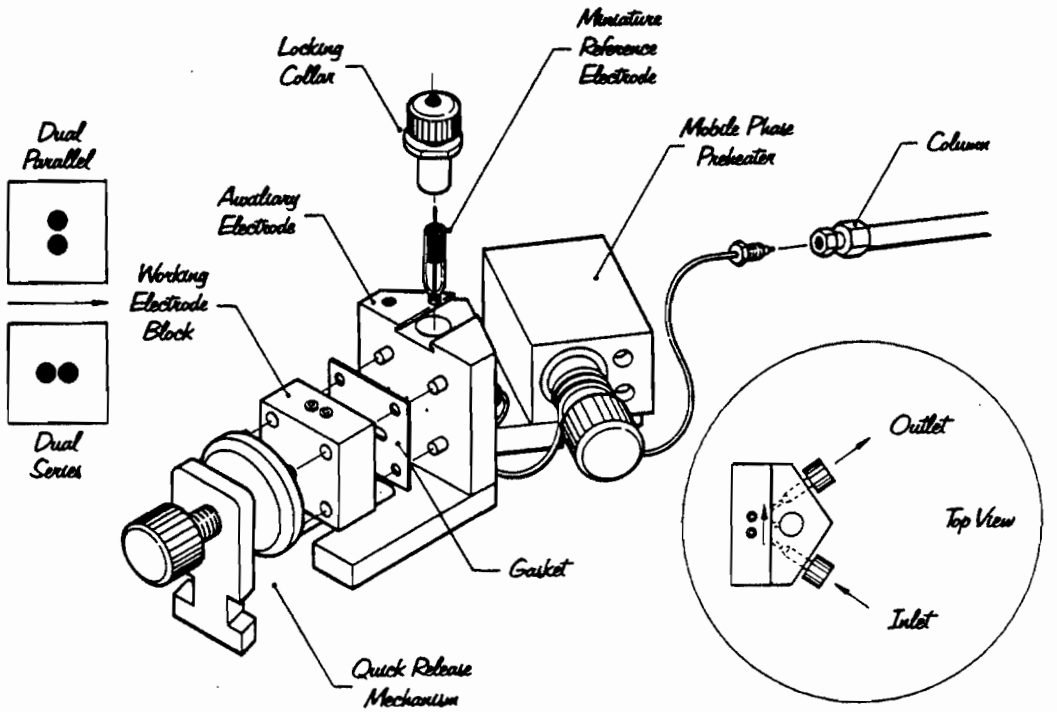


Figure 6-3. Thin-layer cell with optional preheater module for LC-17AT and BAS 200 systems.

6.3. Reference Electrode Maintenance

The purpose of the reference electrode is to provide a stable, reproducible voltage to which the working (or indicator) electrode potential may be referenced. A reference electrode may be considered a small battery whose voltage (potential) is determined by the chemistry taking place between a solid conductor (usually a metal salt) and the electrolytic solution around it. Ideally, if a small current is passed through the electrode, the potential change is negligible and, in any case, returns to the initial value when the current ceases. In addition, the potential value should not vary with time and should be reproducible from electrode to electrode. The most common reference electrodes meeting these criteria are the silver/silver chloride (Ag/AgCl) and the mercury/mercurous chloride (calomel) electrodes. Introduced in March 1986, the Bioanalytical Systems RE-4 Ag/AgCl reference electrode is illustrated in Figure 6-4.

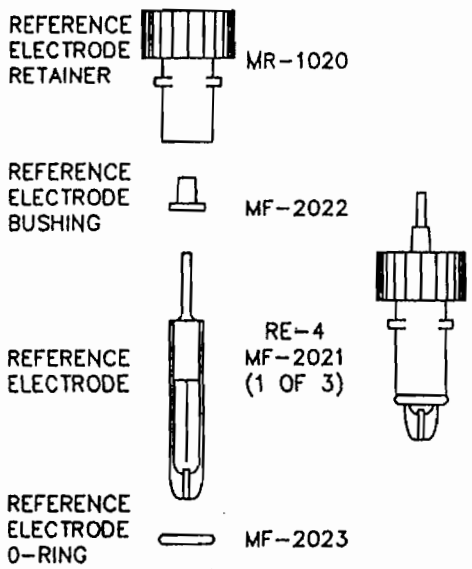


Figure 6-4. Components of RE-4 Reference Electrode system.

The reference potential is dependent on the chloride concentration in the electrolyte as well as the standard redox potential of the electrochemical reaction. In the case of the Ag/AgCl electrode the electrolyte is 3M sodium chloride. Any change in this salt concentration will cause a change in the reference potential. In order to maintain a constant concentration of chloride ion in the surrounding electrolyte, a barrier is placed between this internal filling solution and the external solution (e.g. mobile phase). The barrier is a porous material that allows contact between the solutions yet minimizes their mixing. The filling solution is also gelled to further impede diffusion. In this manner the chloride ion concentration is held virtually constant.

The major cause of failure of the RE-4 Reference Electrode is dehydration of the gel. To avoid dehydration, keep the electrode frit immersed in 3 M NaCl when not in use on the detector. In an emergency, a small bit of wet lab tissue placed over the tip and secured by Parafilm will suffice. In any case, once dehydration occurs, the loss is irreversible.

If drift in the reference potential is suspected, it may be checked against a second reference electrode's potential. The test is done by simply dipping the electrode to be tested and a new reference electrode into a beaker containing any salt solution (e.g. 3 M NaCl) and measuring the potential difference between them with a voltmeter. If the two electrodes are similar (e.g. Ag/AgCl versus another Ag/AgCl or calomel versus another calomel), the meter should read 0 $\pm$ 20 mV. If one of the electrodes is Ag/AgCl and the other calomel, make the calomel the black (or negative) input. The meter should read -60 $\pm$ 20 mV. Although it is always advisable to have a second RE-4 Reference Electrode on hand at all times for this test, any standard Ag/AgCl or calomel electrode can be used (e.g. the reference electrode of a two electrode pH meter). A combination pH electrode is not suitable for these tests.

While many used electrodes could be salvaged by simply refilling with fresh internal solution, BAS remained with a sealed design for several reasons. For example, if any backpressure is placed on the cell, a tendency for the mobile phase to flow through the reference electrode frit and out the refilling port is common. The loss of potential control is dramatic.

Please keep in mind that reference electrodes do not last forever, and this is to be expected based on their mode of action. The rate at which their performance degrades is dependent upon how much the detector is used, and how they are stored. You may want to "rotate" electrodes from among the 3 received with your instrument. You should anticipate purchasing spares every 2-3 months with heavy usage, and storing new electrodes in fresh 3 M NaCl solution upon receipt.

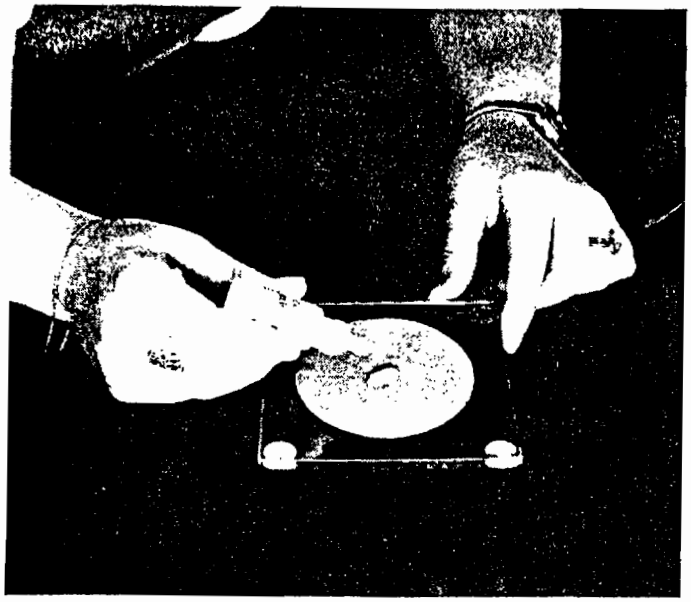
Don't forget to check on the electrodes periodically. Partially evaporated solutions will no longer be 3 M solutions.

6.4. Glassy Carbon Electrodes

Included in all LC-17A Amperometric Detectors is a BAS dual glassy carbon working electrode (MF-1000). In single electrode operation, this transducer allows for a "back-up" sensor so that the user can easily isolate the cause of performance malfunctions. In dual mode operation, the twin sensors may be controlled by tandem LC-4B's in sequential or parallel orientation with respect to flow.

Glassy carbon is a chemically resistant, highly conductive, mechanically rigid, glass-like material that is firmly imbedded in

a fluorocarbon block to form the detector electrode. It can be brought to a high luster by metallographic polishing procedures. Because of their mechanical ruggedness and chemical resistance, the glassy carbon cells can be used under high fluid velocity and/or totally nonaqueous solvent conditions.



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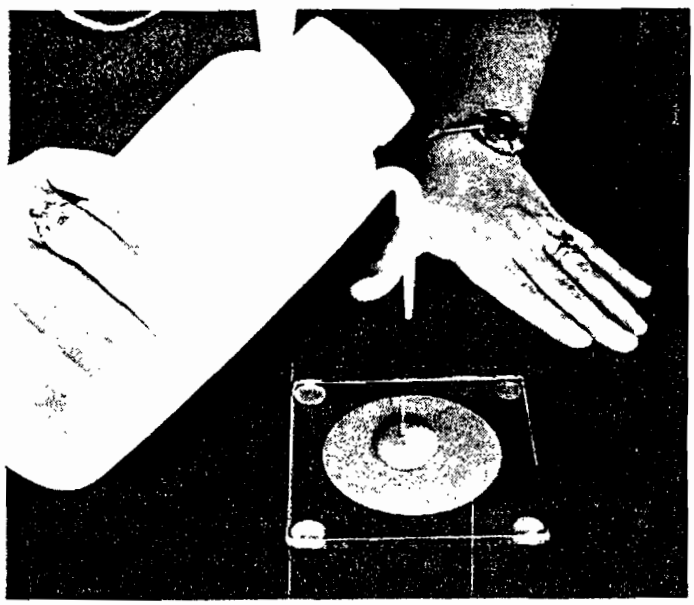

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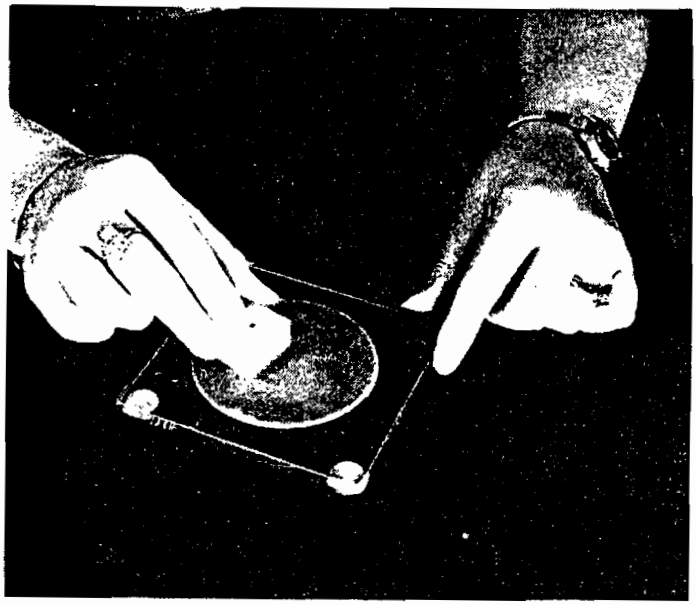

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Figure 6-5. Polishing the Glassy Carbon Electrode. See Instruction Poster packaged with detector accessories.

The LC-17A(T) Amperometric Detector is provided with a polishing kit (Model PK-3, MF 2056) for resurfacing all types of BAS working electrode surfaces. It consists of 3 types of polishing pads, 3 types of polish in small vials, and a glass plate with rubber feet. Each polishing pad has a pressure sensitive adhesive backing. To polish the surface, affix a pad to the glass plate provided. Specific instructions are provided on the Poster for the various types of working electrode materials available from BAS.

Avoid scratching the electrode or thin-layer cell surfaces. Rough handling (e.g. dropping the cell) may break the seal between the electrode and the plastic block or disrupt electrical contact points within the body of the cell.

6.5 Gold/Mercury Electrodes

The advantage of using mercury as an electrode material is the large hydrogen overpotential. In this design the mercury is deposited as a thin film on a gold substrate in place of the conventional mercury drop. Thus, deleterious effects associated with the HMDE or DME such as vibration, poor mechanical stability, or edge effects are eliminated in the thin-film design.

Since dissolved oxygen is easily reduced on mercury, it must be completely removed from the system. Removal of oxygen and general chromatographic precautions and procedures for reductive LCEC are discussed further in Chapter 8.

Maintenance of the Gold/Mercury Electrode

When you first use this electrode and periodically during its lifetime, a new mercury film must be placed on the gold disk. This is accomplished by first removing the old amalgam surface, cleaning and polishing the gold surface, and then by preparation of the new amalgam.

The LC-17A(T) Amperometric Detector is provided with a polishing kit (Model PK-3, MF 2056) for resurfacing all types of BAS working electrode surfaces. It consists of 3 types of polishing pads, 3 types of polish in small vials, and a glass plate with rubber feet. Each polishing pad has a pressure sensitive adhesive backing. To polish the surface, affix a pad to the glass plate provided. Specific instructions are provided on the Poster for the various types of working electrode materials available from BAS.

It is not always necessary to complete re-polish the mercury amalgam electrode. Sometimes, the mercury can be reapplied to the old surface. See the Instructions Poster for additional information.

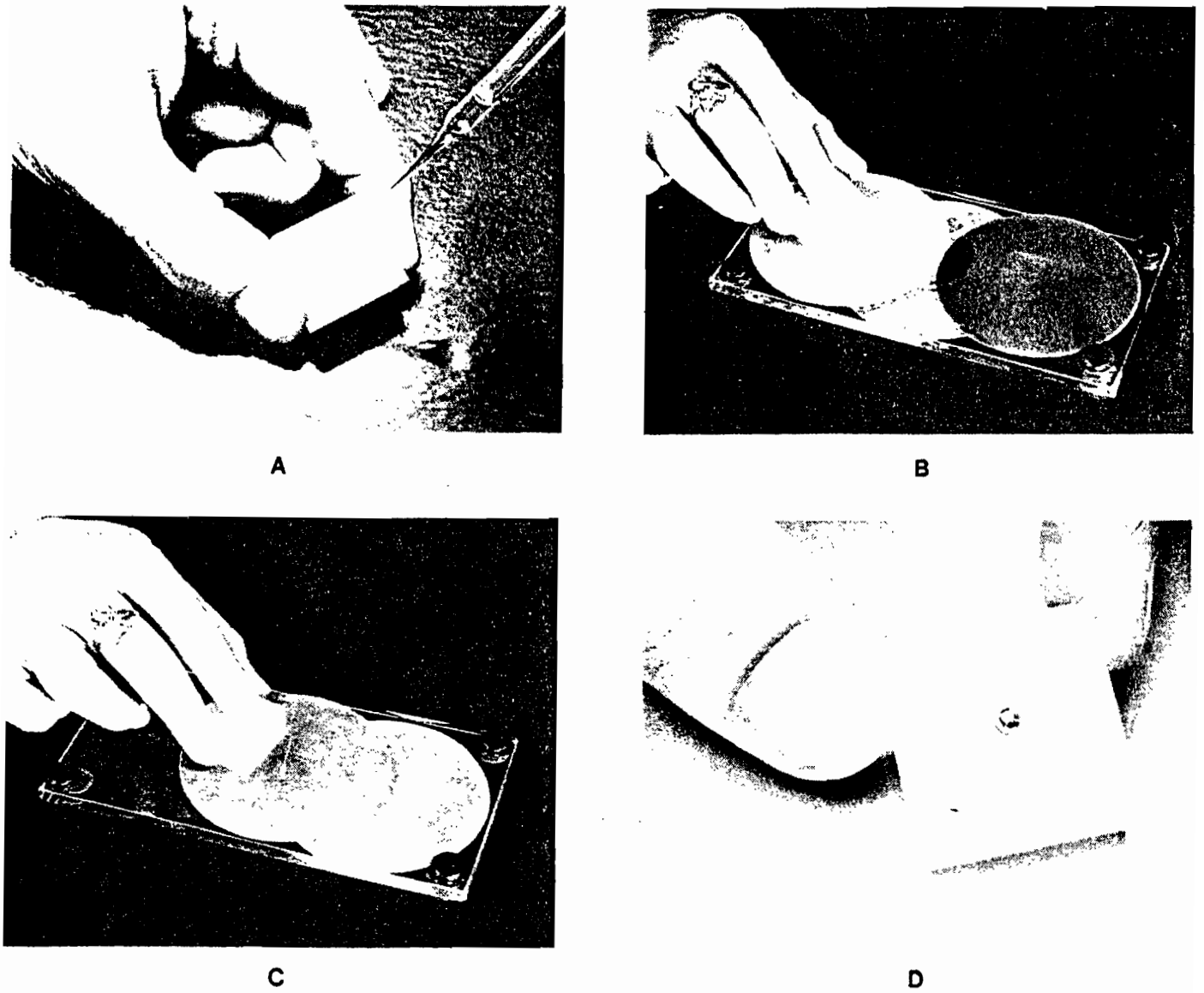


Figure 6-6. Preparing the gold surface for a new layer of mercury. See Instructions Poster for Details.

There is usually a period of equilibration following the formation of the gold amalgam. Allowing the amalgam to equilibrate overnight is usually a good idea. During this time, the response to a given compound changes and is not suitable for use. The equilibration time varies with the application and the specific conditions used. Make certain your response is reproducible before attempting any real samples. BAS LCEC Application Note No. 45 discusses this in greater detail for the oxidative applications of the gold/mercury electrode.

6.6. Carbon Paste Electrodes

Carbon paste is a mixture of graphite with an inert binding material. The common binders are paraffin oil, ceresin wax, and

silicon grease. Currently, BAS only offers one variety (type CP-O) which has an oil base. These electrode materials exhibit high reproducibility and have good anodic potential range with small background (residual) currents. The disadvantage of paste electrodes is that they generally cannot be used in nonaqueous solvents. Solutions containing up to 10% (v/v) methanol or 5% (v/v) acetonitrile are tolerable, but these will decrease useable electrode lifetimes.

The choice of which materials to use in a given situation is a matter of personal preference rather than any hard and fast rules. CP-O is the most widely used but this may be more from BAS recommendations and initial applications work rather than any functional differences.

Repacking Carbon Paste Electrodes

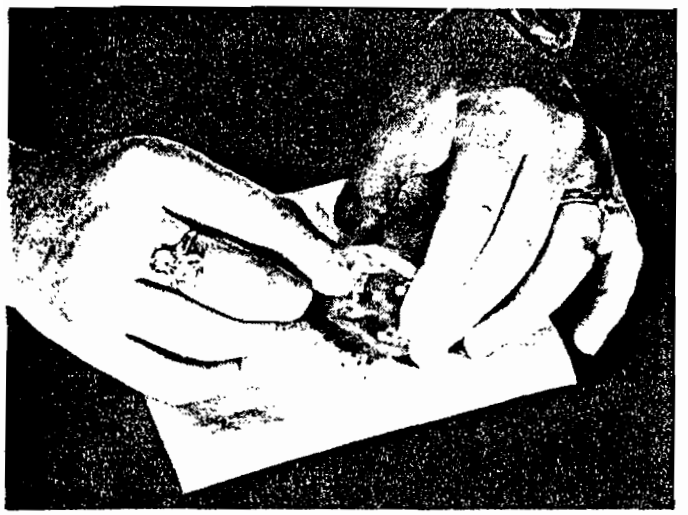
Repacking the electrode is a simple task for experienced operators and only requires a few minutes. The repacking procedure is depicted in Figure 6-7. Consult the Instructions Poster packed with your detector for more details.

A newly repacked electrode will usually require 20-90 minutes for the background to stabilize before quantitative determinations may be attempted. With a properly packed cell, the background current will decay to a value of 2-50 nanoamperes, depending on mobile phase, applied potential, etc.

It is advisable to have a second electrode on hand to facilitate a quick change-over when an electrode is no longer useable. Bulk carbon paste should not be exposed to air for a long length of time. Like other forms of graphite, it tends to absorb impurities from the air (e.g. cigarette smoke). As one electrode is waning in performance, the second electrode can be packed and set aside, ready to replace the defective electrode when necessary.

Recycling the Mobile Phase

If the LC system is being used on a daily basis, the electrode can be left "on" continuously. Simply turn the flow rate down to 0.1 - 0.2 mL/min, drop the applied potential to approx. 500 mV or less, and leave the pump running overnight. Make certain you have plenty of mobile phase in the reservoir. Alternately, route the outlet from the LCEC cell to the reservoir so you "recycle" the mobile phase until you return. The start up time the following morning is substantially reduced.



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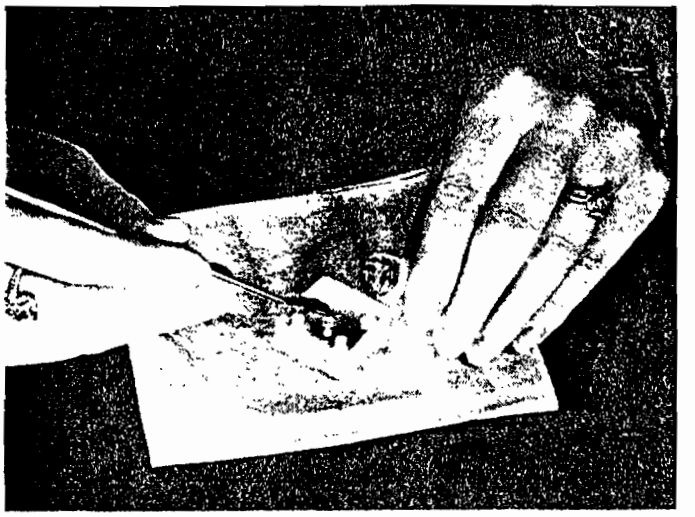

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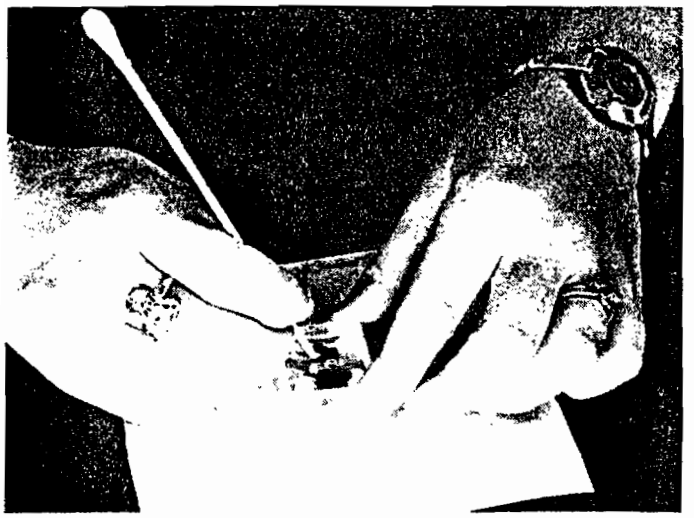

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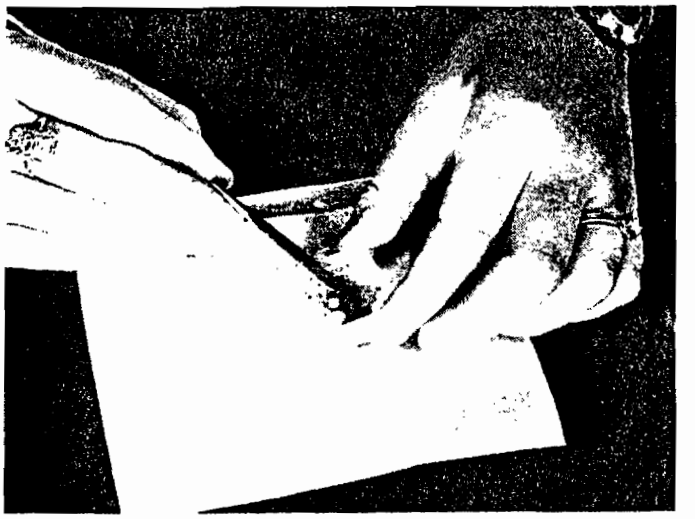

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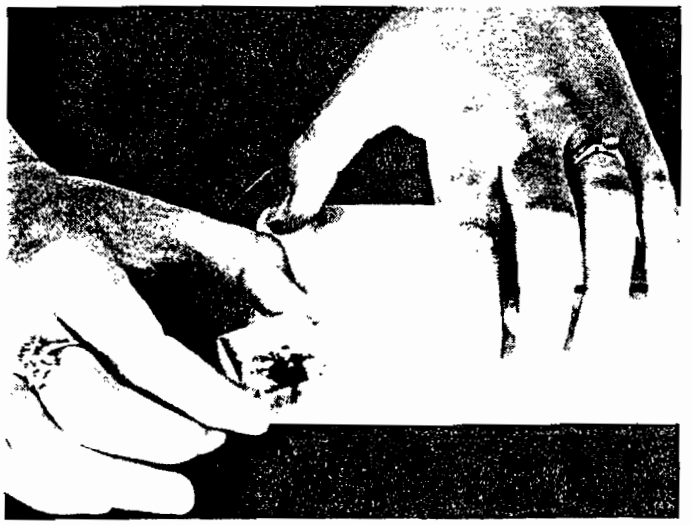

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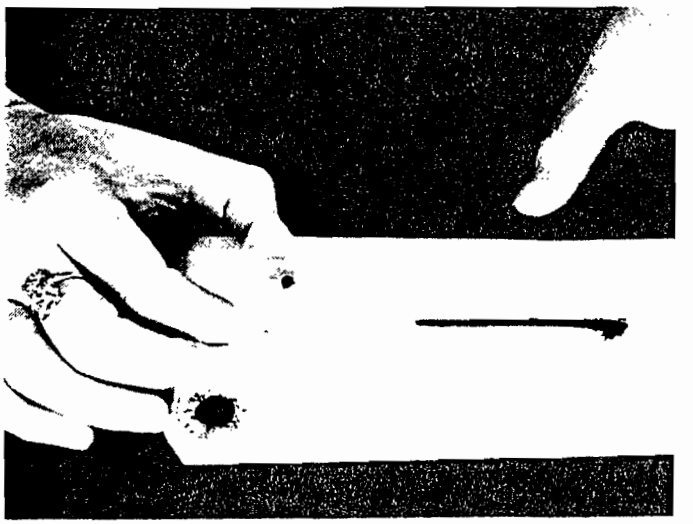

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Figure 6-8. Preparation of a Carbon Paste Electrode
See Instructions Poster for more details.

7. Oxidative Mode LCEC

The oxidation of a compound involves the electron transfer from a molecule TO the electrode surface. The two major advantages of most oxidative applications are that (1) oxygen is not electrochemically active and (2) solid electrodes can be used. Of course, both of these are definite advantages in the LCEC experiment as well. The importance in removing oxygen for reductive electrochemistry was discussed in Chapter 4, and the LC system modifications required for these determinations will be discussed in Chapter 8.

The working electrode materials most commonly used are based on a carbon matrix, typically an anisotropic solid such as glassy carbon. Obviously, other materials exist and can be used for the LCEC determination. For example, gold, nickel, silver, platinum, and a thin mercury amalgam can all be used for specific measurements.

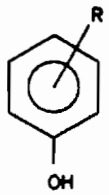
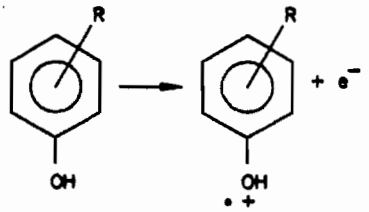
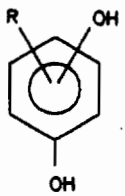
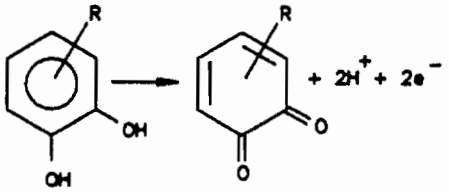
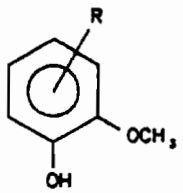
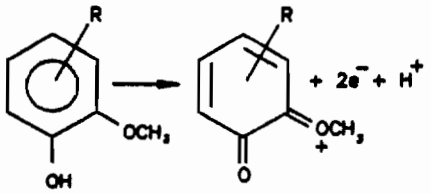
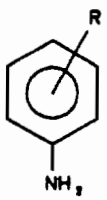
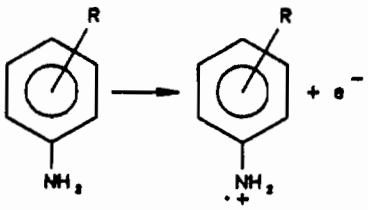
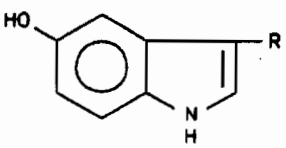
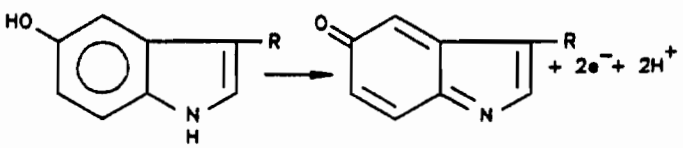
This section will primarily deal with typical functional groups that can be oxidized, but the list generated below is by no means exhaustive. The only conclusive way of knowing if a compound is electrochemically active is by a technique such as cyclic voltammetry. Even with our experience, we can not always "guess" at how a specific compound may behave. A voltammetric assessment can provide information about the reversibility of the reaction as well, which can influence your decision on which mode of LCEC to use (e.g. Dual Series). In general, for electrochemical oxidation to take place, one can look for the same features and reactive centers as one would for a homogeneous oxidation reaction, e.g. delocalized electrons, stability of product, etc. This does not mean that if a homogeneous chemical reaction does take place then this is directly applicable to the heterogeneous electrochemical reaction. This correlation is many times thought to occur and is many times an incorrect assumption.

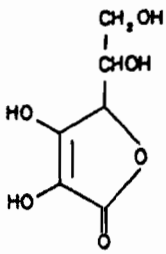
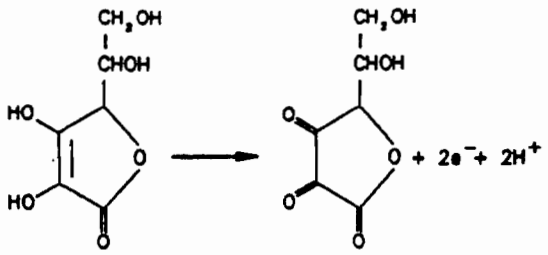
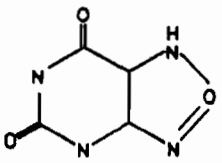
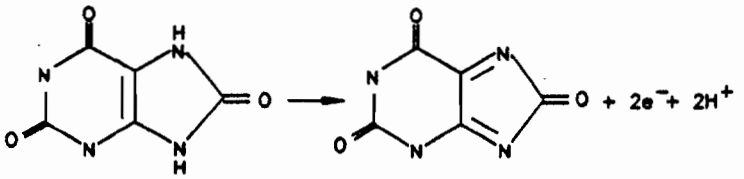
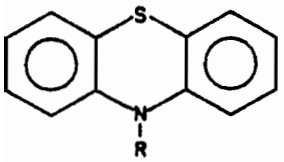
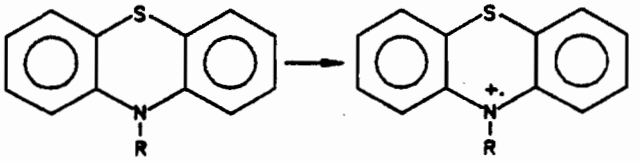
7.1. Typical Functional Groups

The listing in Table 7.1 is not all inclusive but only indicates some typical functional groups and compounds that are known to be electrochemically active. The Appendix to this manual contains additional information based on cyclic voltammetry studies. If you have any questions regarding the electrochemical activity of your particular compound under a given set of conditions, it is best to actually do the voltammetry experiment.

A BAS cyclic voltammetry instrument or BAS 100A Electrochemical Analyzer is a worthwhile investment for laboratories which are continually investigating new compounds.

Table 7-1. Functional Groups Suitable for Oxidative Electrochemical Detection

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	PHENOLS		PHENOL PENTACHLOROPHENOL PARABENS MORPHINE TYROSINE
	HYDROQUINONES		CATECHOLAMINES
	VANILLYL COMPOUNDS		HOMOVANILLIC ACID VANILMANDELIC ACID FERULIC ACID
	AROMATIC AMINES		ANILINE BENZIDINE
	INDOLES		TRYPTOPHAN SEROTONIN 5-HIAA

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	ASCORBIC ACID		ASCORBIC ACID (VITAMIN C)
	XANTHINES		URIC ACID THEOPHYLLINE
R–SH	THIOLS	$2R\text{–}SH \longrightarrow R\text{–}S\text{–}S\text{–}R + 2e^- + 2H^+$	CYSTEINE GLUTATHIONE
	PHENOTHIAZINES		CHLORPROMAZINE

8. Reductive Mode LCEC

This section details specific liquid chromatographic procedures for reductive mode LCEC.

The section is divided into four parts:

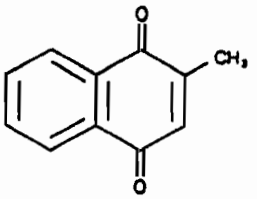
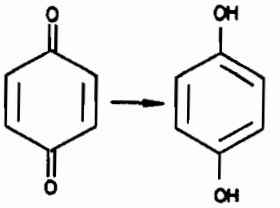
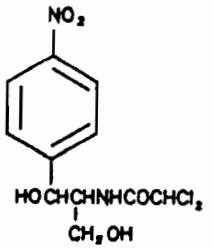
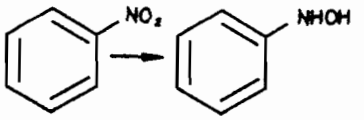
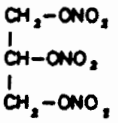
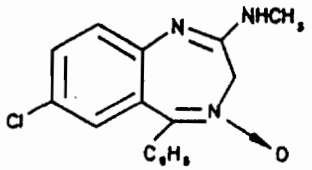
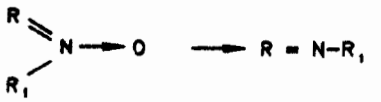
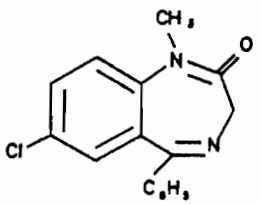
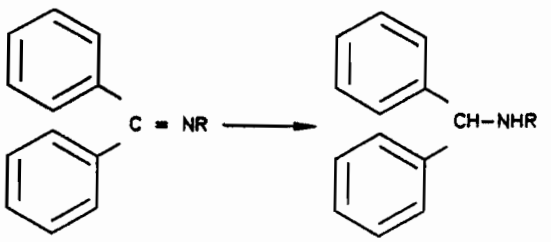
1. typical functional groups detectable by reductive LCEC,
2. system modifications required for reductive LCEC,
3. degassing procedures for both mobile phase and samples, and
4. relative merits of glassy carbon and mercury/gold electrodes for reductive LCEC.

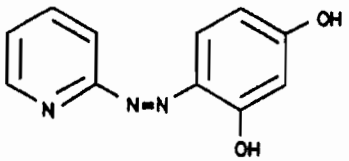
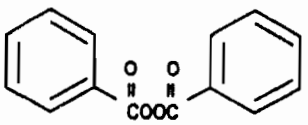
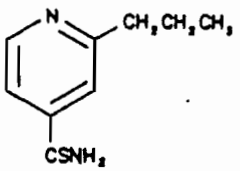
The preparation of both glassy carbon and mercury/gold electrodes for reductive LCEC is mentioned in Chapter 6, and described in detail in the Instructions Poster shipped with the detector accessories. Users with questions concerning the cell itself should read this material first.

8.1. Typical Functional Groups Detectable by Reductive LCEC

By far the majority of the LCEC literature deals with oxidative mode electrochemical detection, but considering strictly the electrochemical literature of organic compounds, reduction processes have been examined in much greater detail. Table 8-1 describes some functional group candidates capable of being analyzed by reductive LCEC. Note that within a given functional group class, a broad range of reduction potentials may exist due to the effects of substituent groups. Generally the more delocalized the electrons become, the more easily reducible the substance. In addition, electron withdrawing groups on an aromatic ring will enhance the reduction reaction.

Table 8-2. Functional Groups Suitable for Reductive Electrochemical Detection.

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	QUINONES		VITAMIN K3
	AROMATIC NITRO		CHLORAMPHENICOL
	ALIPHATIC NITRO		NITROETHANE NITROGLYCERIN
	ORGANOMETALLICS		TRIPHENYLLEAD CIS–PLATINUM
	N–OXIDES		CHLORDIAZEPOXIDE
	AZOMETHINE		DIAZEPAM NITRAZEPAM

ACTIVE FUNCTIONAL GROUP	CLASS	TYPICAL ELECTROCHEMICAL REACTION	EXAMPLES
	AZO COMPOUNDS	$R-N=N-R \longrightarrow RNHNHR$	(4-(2-PYRIDYLAZO)RESORCI
	PEROXIDES	$ROOR \longrightarrow 2ROH$	BENZOYL PEROXIDE
$(CH_3)_2NNO$	NITROSAMINES	$RNNO \longrightarrow RNNH_2$	DIMETHYLNITROSAMINE
	THIOAMIDES	$RNHCR_1 \longrightarrow RNHCHR_1$ (S, SH)	PROTHIONAMIDE

8.2. System Modifications for Reductive LCEC

Reductive mode LCEC requires some mechanical modifications to remove dissolved oxygen from both the mobile phase and sample. It is equally necessary to make sure it does not reenter the system. Oxygen is removed from the mobile phase and sample by bubbling an inert gas (e.g. nitrogen, helium, argon) through the solutions. Refluxing the mobile phase at the same time will thoroughly deoxygenate the system. A mechanical arrangement such as shown in Figure 8-1 can be set up and dedicated to a specific LC system for this purpose. Table 8-2 details specific parts needed for this modification to modular BAS LCEC Systems. We emphasize that all plastic tubing in the system must be replaced with stainless steel. A one or two liter roundbottom flask with reflux condenser and heating mantle serves as the mobile phase reservoir. One neck is used for the condenser and the other two necks for the nitrogen inlet and pump outlet respectively. Ordinary laboratory grade nitrogen supplied at ca. 20 psi is tapped at two separate needle valves for independent control of both solvent and sample degassing. An 1/8" o.d. stainless steel outlet line runs to the inlet check valve of the pump. Large rubber septa seal off the mobile phase from the outside environment. Temperature is maintained by an LC-22A Temperature Controller set at 30-35 degrees C. The stainless steel Temperature Probe (BAS p/n EW 8116) measures the temperature of the liquid and regulates electrical power to the heating mantle.

Samples are degassed with the second needle valve. An optional nitrogen saturation chamber filled with water wets the gas thoroughly before proceeding to the sample container.

The outlet tube from the column to the detector must be replaced with a special steel connector (BAS P/N MF 1029). No plastic tubing may be used! This connector uses special plastic endfittings to electrically isolate the cell from the rest of the LC system (which is grounded). The steel tubing prevents gas intrusion. If you are using a preheater module, the steel tubing in this is appropriate.

Modifications are now completed for reductive LCEC.

If you think this seems like a lot of preparation, you are correct. After the initial setup, a system like this can be fairly reliable for reductive LCEC work, but it has limitations. For example, no gradient elutions can be run under these conditions. For customers requiring a full-featured liquid chromatograph with solvent deoxygenation capabilities, the BAS 200 Analyzer is the instrument of choice.

8.3. Degassing Procedures for the Mobile Phase

The following chromatographic start-up procedure is recommended for reductive LCEC applications. Before initiating flow through the LC system, heat the mobile phase to 40-45 degrees C and bubble the inert gas rather vigorously through it for 1-2 hours. Start

pumping the mobile phase through the LCEC system during the last 15-30 minutes of vigorous bubbling. Even though the mobile phase is degassed, the entire system is not. Oxygen penetrates the stationary phase pores and it must be flushed out of these pores by initiating the flow of degassed mobile phase through the LC system.

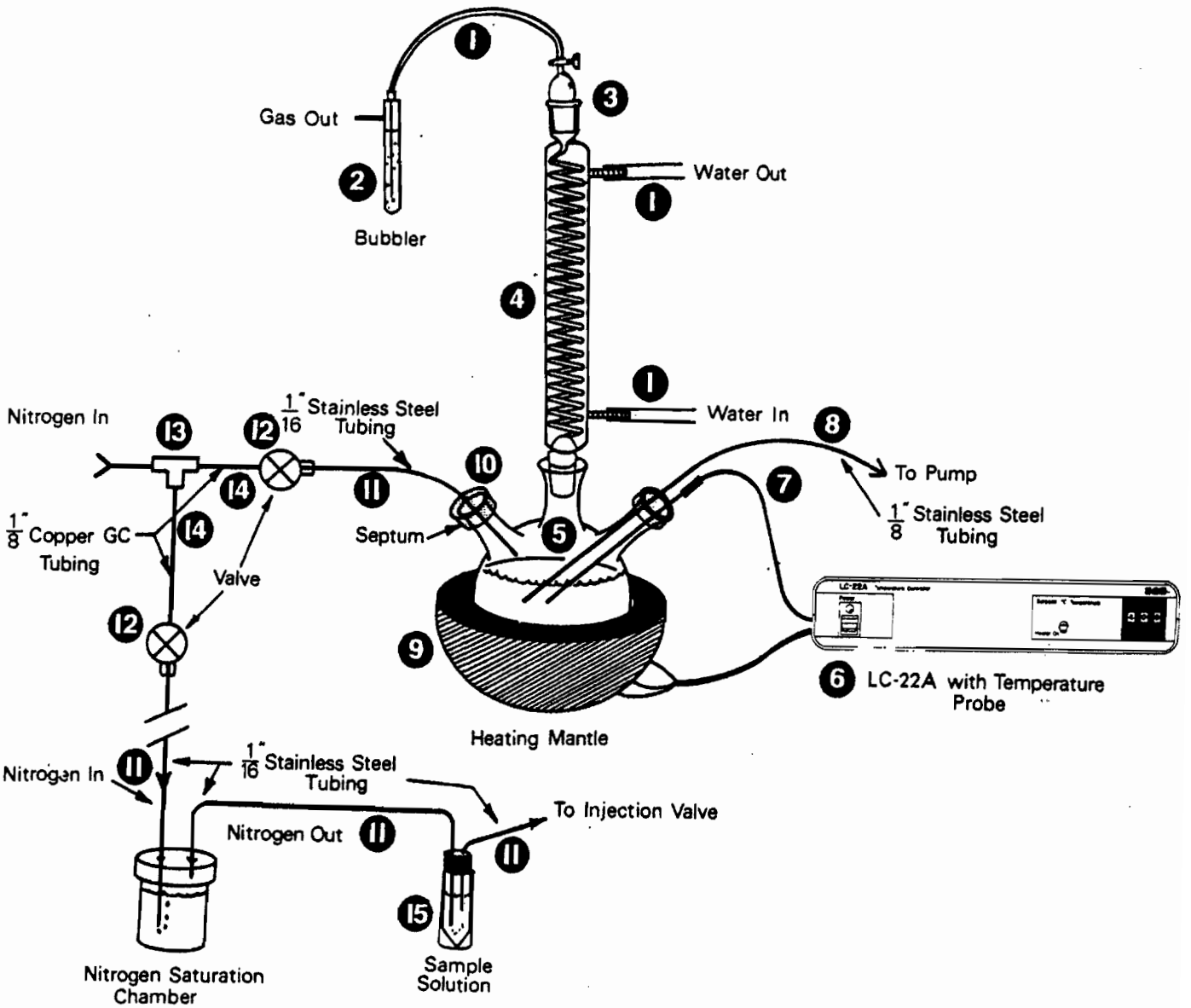


Figure 8-1. Deoxygenation apparatus for LCEC.

Table 8-2. Parts list for reductive LCEC.

Ref.	Description	Source
1	Connecting tygon tube	Common Lab Supplies
2	Gas bubbler	Reliance Glass
3	Condenser adapter	Reliance Glass
4	Condenser	Reliance Glass
5	3-neck, 1 liter, round bottom flask	Kimble
6	LC-22A temperature controller	BAS
7	Temperature probe	BAS
8	Stainless steel inlet tube for pump Model PM-48	BAS
9	Heating mantle	Scientific Products
10	Rubber septum	Common Lab Supplies
11	1/16" O.D. stainless steel tubing (when attaching to valve you may need extra fittings)	BAS
12	Needle Valve	Swagelok
13	Tee to N_2 line	Swagelok
14	1/8" O.D. copper tubing	Common Lab Supplies
15	Sample vial with rubber septum	BAS

Degassing of the system requires at least 100-200 milliliters of preheated mobile phase. Be liberal and flush thoroughly. Turn on the working electrode after flushing and keep the detector at the lowest gain (highest RANGE setting) until the background current has stabilized. Reduce the flow of inert gas through the mobile phase. Some flow must be maintained to keep the oxygen out, but this need not be as vigorous as during the initial deoxygenating. If the baseline response (background current) begins to gradually increase, the rate of bubbling inert gas through the mobile phase is insufficient to keep the oxygen out of the system and must be increased until the background stabilizes. Note: valuable time can be saved if degassing of the system is performed overnight at a minimal flow rate (0.2 - 0.3 ml/min). The working electrode can be turned on before leaving the lab in the evening to provide you with a stable system in the morning.

8.4. Degassing Samples

Sample degassing is necessary when working at potentials more negative than 0 to -0.1 V for gold/mercury and -0.3 to -0.4 V for glassy carbon electrodes as illustrated by hydrodynamic voltammograms in Figure 6-2. The potentials at which the electrodes will be insensitive to dissolved oxygen may vary, depending on the pH, concentration and nonaqueous solvent, and the previous history of the electrode surface.

Care must be taken while degassing a sample in order to preserve its original composition. This is extremely important when handling volumes smaller than 500 microliters. Presaturation of nitrogen gas with mobile phase and maintaining a gentle flow of nitrogen gas through a sample will minimize evaporation of a sample. A presaturation device is illustrated in Figure 8-1.

To degas, pass nitrogen into the sample for about 3-5 minutes. This should be regulated at a flow rate as vigorous as allowed by the sample volume (smaller volumes will have to be degassed more gently than larger ones).

To inject, it is best to slowly draw the sample into the injection loop by gentle suction. Exposure to the atmosphere is avoided, and the integrity of the closed system, particularly at the needle seal, is preserved. On the BAS 400 chromatograph we would tend to accomplish this by using the syringe mounted in the front port of the injection valve, and loading the sample loop via the waste port until fluid was seen in the syringe.

8.5. Glassy Carbon Versus Mercury/Gold Electrodes for Reductive LCEC

Both electrode surfaces have proven useful in reductive LCEC. Although mercury is usually the surface of choice with electrochemists, there are fewer reasons for using it in LCEC. In polarography, mercury provides a repeatable, fresh electrode surface, a high hydrogen overvoltage, and fast settling times. In liquid chromatography, however, dead volume must be minimized, thereby obviating the use of the usual dropping mercury electrode. For reductive LCEC, the optimal approach is to employ a thin-layer cell. The dropping mercury electrode is replaced by a glassy carbon surface or a mercury film.

To select the proper electrode, please follow these guidelines:

1. For reductions requiring potentials between +1.00 and -0.90 V (vs. Ag/AgCl), try glassy carbon prepared as described in Chapter 6 first. Glassy carbon offers better longterm stability than mercury/gold. Background currents should be less than 100 nanoamperes throughout this range. Useable performance may be obtained at more negative potentials, depending on the conditions.

2. For reductions at potentials between -0.90 and -1.1 V, a freshly prepared mercury/gold surface will probably be necessary for sufficient hydrogen overvoltage protection. Don't expect subpicomole detection limits, however! Remember, at these applied potentials you are dealing with a high energy situation (not unlike low wavelength UV detection). Everything will be noisier and your detection limits will be hindered according to this increase in noise.

3. Some applications will intimately involve the surface chemistry of one electrode material, thereby favoring that material. For example, although this is not a an electrochemical reduction, the detection of sulfhydryls on mercury occurs at a potential about 600 mV less positive than on carbon due to the following mechanism:

$$Hg + RSH \rightarrow HgSR + e^- + H^+ \quad E_p = +0.2 \text{ to } +0.4 \text{ V}$$

$$2\ HgSR \rightarrow Hg + Hg(SR)_2$$

It is the complex of mercury and the thiol that is actually undergoing the oxidation.

On carbon, it has been shown that disulfides are the usual product, with the reaction taking place at a much higher applied potential:

$$2\ RSH \rightarrow RSSR + 2H^+ + 2e^- \quad E_p = +1.0 \text{ V}$$

4. If permitted under the constraints of guidelines 1-3 above, use glassy carbon for longer service lifetimes. A gold/mercury amalgam is a solid solution at the interface between the gold substrate and the thin mercury film; eventually the gold will diffuse through the film to the surface. The advantageous hydrogen overvoltage will eventually vanish.

9. Dual Mode LCEC

9.1. Principles

The electrochemistry for dual electrode LCEC is governed by the same variables described in Chapter 4. With this instrumentation, the electrochemistry can be manipulated to improve sensitivity and extend the applications of electrochemical detection.

To understand these options the following discussion will make reference to common spectroscopic techniques which are familiar to the reader. Analogies can be made on a number of points. In spectroscopy the response of a compound to light energy is recorded as an absorbance spectrum or, in the case of fluorescence, as an excitation/emission spectrum. In electrochemistry the current response is plotted against the applied potential. When the potential scan is reversed and plotted, the electrochemical equivalent to a fluorometric emission spectrum is obtained. How does this apply to dual electrode LCEC? The analogies are shown in Figure 9-1 and 9-2. In the DUAL-PARALLEL (adjacent) configuration, the detection options available are similar to those available when using a dual wavelength photometric detector. The eluent from the LC can be monitored at two, independent, applied potentials. A simultaneous profile of reducible and oxidizable analytes can be obtained. Also, response ratios can be calculated to provide qualitative information on a particular chromatographic peak. Going back to our spectrophotometric analogue, these current ratios (i_1/i_2) parallel the absorbance ratio A_1/A_2. Both are constants which can be used for peak identification.

Simultaneous measurement in the DUAL-PARALLEL amperometric detection mode can be used to determine more than one compound in a chromatogram. For example, the detector potential at one electrode may be set sufficiently positive to oxidize all compounds of interest and the second electrode may be set at a substantially lower oxidizing potential to only react with those compounds that are electrochemically active at these lower potentials. Thus, the resultant dual tracing will allow quantitation at both applied potentials, and the lower potential chromatogram will be more discriminating, improving the selectivity of the measurement.

Another application of the DUAL-PARALLEL mode is to measure the difference chromatogram. This would be similar to choosing a particular potential window to monitor. In this mode the working electrodes are poised in a potential region where the response of the analyte of interest is dramatically different i.e. where the slope of the current-potential curve is large. The difference chromatogram enhances specificity by only displaying those compounds that are electrochemically active in the region between the two set potentials; all other responses, whether noise or analyte signal, are subtracted out. Of course, one electrode can be measuring an oxidation while the second can be monitoring a reductive reaction.

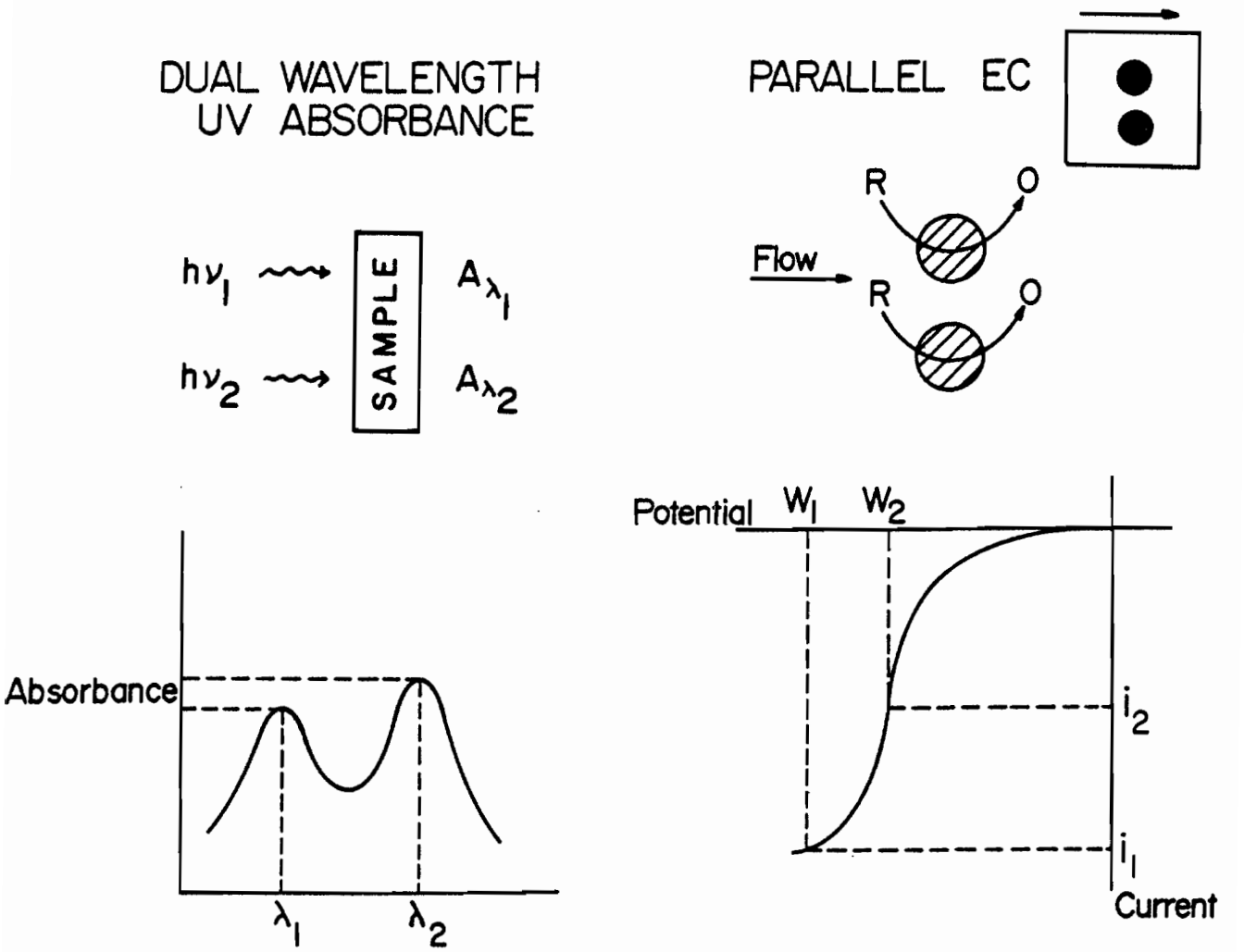


Figure 9-1. An analogy can be made between DUAL-PARALLEL amperometric detection and spectroscopic measurements. Enhanced selectivity is often the end result.

The DUAL-SERIES electrode configuration most closely resembles fluorometry. This analogy is shown in Figure 9-2. As in fluorescence, a reactive "intermediate" is produced by some excitation function which yields a product that generates some measurable response. In the case of fluorescence, it is the emission signal; in the case of the electrochemical detector it is the redox couple's follow-up reduction (oxidation) reaction. In fluorescence, the quantity that is a measure of the maximum amount of fluorescence available from a given input intensity is the "quantum efficiency." A similar term in series dual electrode electrochemical detection is the collection efficiency, the ratio of the current at the downstream electrode to the current at the upstream electrode, i.e.

$$\text{collection efficiency} = \frac{\text{current}_{\text{downstream}}}{\text{current}_{\text{upstream}}}$$

As the quantum efficiency is a statement of the expected response for a given compound under a specified set of conditions, so is the collection efficiency. A range of collection efficiencies can be obtained depending on cell dimensions (distance between electrodes and the ratio of lengths of the electrodes along the flow axis) and the homogeneous chemistry (hydrolysis, coupling etc.) which might take place prior to arrival at the downstream electrode. The maximum range of values is between 0.37 and 0.42 for reversible compounds at equal surface area planar electrodes in a thin-layer cell.

DUAL-SERIES electrochemical detection can, in certain cases, improve selectivity and detection limits. Compounds with higher collection efficiencies will dominate the response at the downstream electrode and can be measured with improved selectivity. Not all compounds have a reversible redox couple; these will not react at the downstream electrode. This selectivity may be advantageous.

The detection limits can be improved in cases where the upstream electrode is operating at a potential where noise is high or where there is an electrochemically active product formed that is measurable at the downstream electrode. The downstream electrode is operated at a potential where the noise is much more favorable. At the downstream electrode typically only 40% of the upstream electrode response is available, so the noise must drop by a factor greater than the inverse of the collection efficiency. The less than 100% collection efficiency can, in other cases, be used to obtain better detection limits. When the same two series electrodes are operated at the same potential and the difference response is recorded, the result will be the approximately 40% of the difference between the upstream and downstream electrode responses. Responses that would be equal at the same potentials such as background currents and other factors contributing to noise are nulled out.

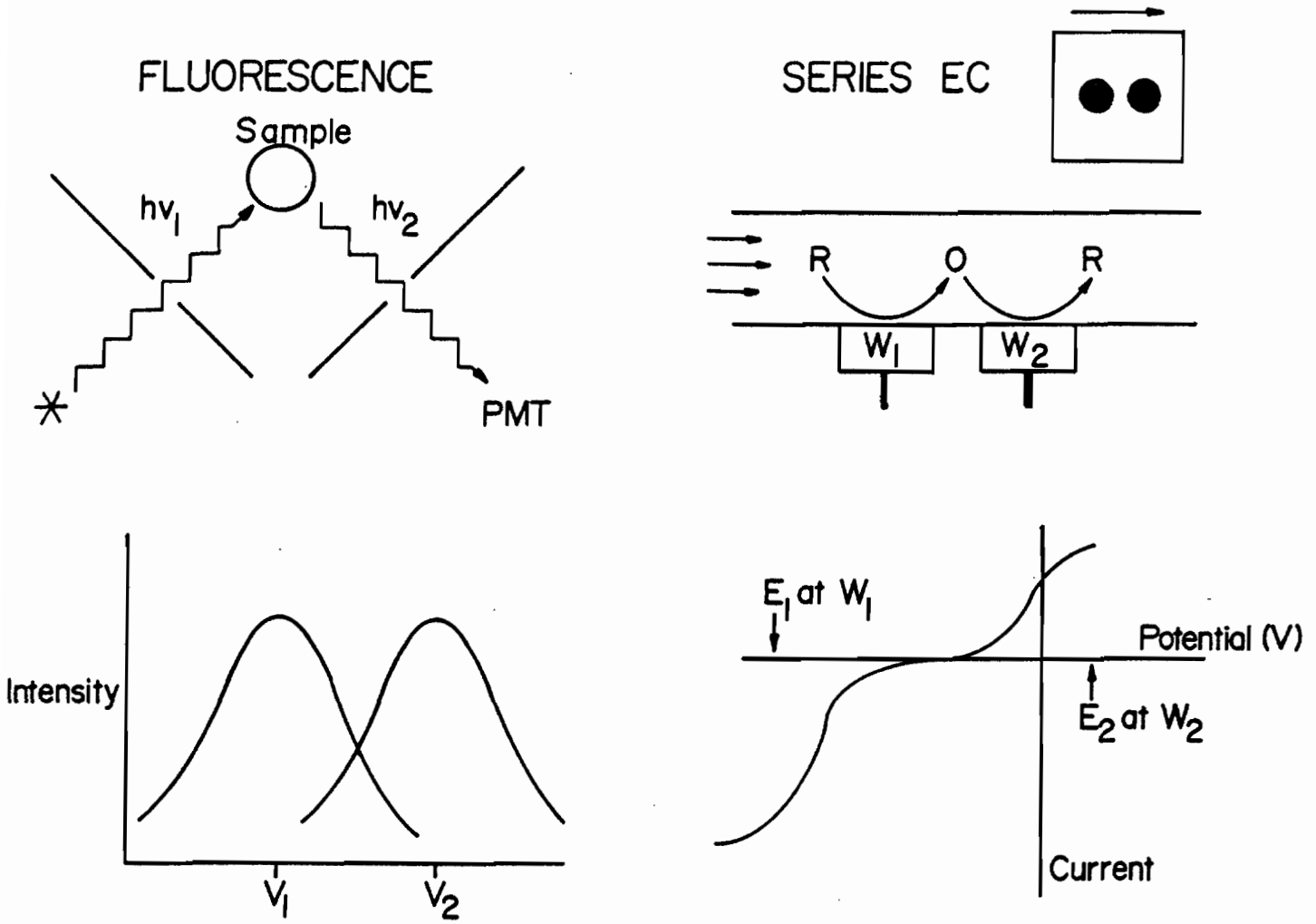


Figure 9-2. DUAL-SERIES amperometric detection methods can also enhance selectivity and thus improve detection limits. An analogy can be made with fluorescence measurements for applications which require a reaction at the upstream electrode to create a product detectable at the downstream electrode.

The reader may find the following references more detailed and informative:

1. Roston, Shoup, Kissinger. Anal. Chem., 54(1982) 1417A.
2. Goto, Sakurai, and Ishii. J. Chromatogr., 238(1982) 357.
3. MacCrehan and Durst. Anal. Chem., 53(1981) 1700.
4. Roston and Kissinger. Anal. Chem., 54(1982) 429.
5. Shoup and Mayer. Anal. Chem., 54(1982) 1164.
6. Mayer and Shoup. J. Chromatogr. 255(1983) 533.

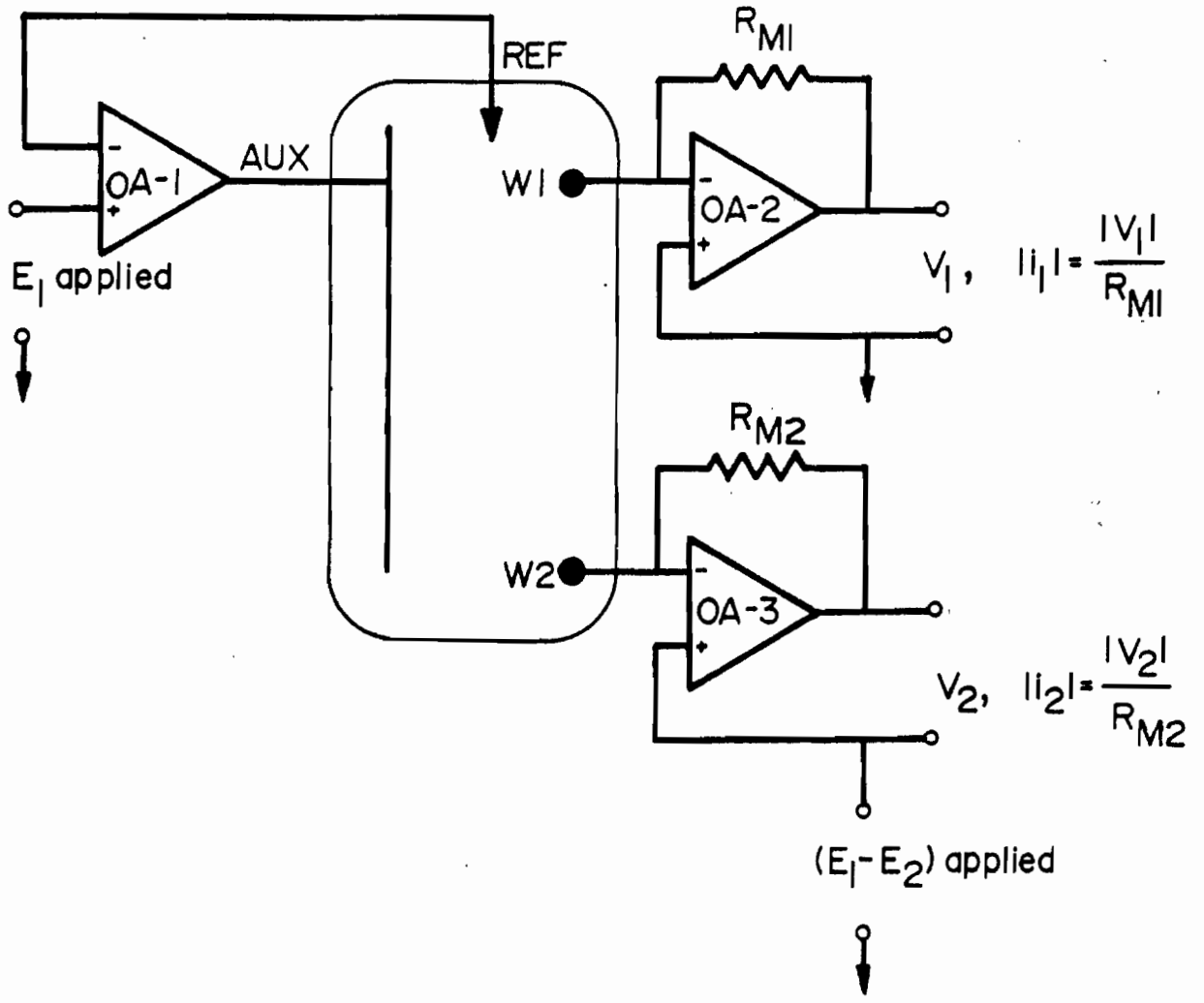


Figure 9-3. Schematic representation of the circuit for dual electrode operation of the LC-4B.

9.2. Electronics

The electronic configuration for the dual electrode system is shown in Figure 9-3. Using a common design, the potential, E_1, is applied between the reference and working electrode one, W1, via the voltage follower, OA-1. Note that W1 is held at circuit common and the auxiliary electrode is moved with respect to it. Since the solution potential, (i.e. the electrode solution interfacial potential) can not be changed to accommodate the second working electrode, the second working electrode, W2, must be changed with respect to the solution potential, i.e. W2 allowed to float with respect to W1. A potential is applied to the second electrode equal to the difference between E1 and the desired potential for W2, E2. The resultant potential is the required E2 with respect to the reference electrode. Both working electrodes utilize current-to-voltage converters followed by appropriate gain and specific output conditioning circuitry (not shown) for display on the recorder.

9.3. Installation of the Dual Upgrade Kit for an LC-4B/17A(T) Amperometric Detector

The standard LC-4B/17A(T) detector may be converted to a twin potentiostat system by adding a second LC-4B detector. Each LC-4B controls its assigned working electrode independently, in terms of both potential control and current amplification.

The upgrade kit is listed in Table 3-2. Check your inventory against this listing. To install this kit:

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

2. Remove the single electrode cable shield by simultaneously sliding it up and tilting the LC-17A(T) back. Unscrew the 2 screws holding the detector cable in place. Unplug the single electrode cell lead from the LC-4B.

3. Turn the LC-17A chassis over and loosen the 4 bottom screws into the cell. Pull the cable out of the LC-17A.

4. Reverse the above order with the dual electrode lead cable.

5. Set the detector components as shown in Figure 3-4 or 3-7.

6. The cell lead cable terminates in 2 connectors. Plug into the two LC-4B's. Attach per Figure 9-4. The connector with the larger diameter cable serves "W1" and the smaller cable feeds "W2." These designations will

appear on the respective displays of the twin LC-4B's after you have completely finished this installation and turned on the power.

7. The same labels appear on the connectors at the cell. The red and white cables terminate at the auxiliary and reference electrodes, as before.

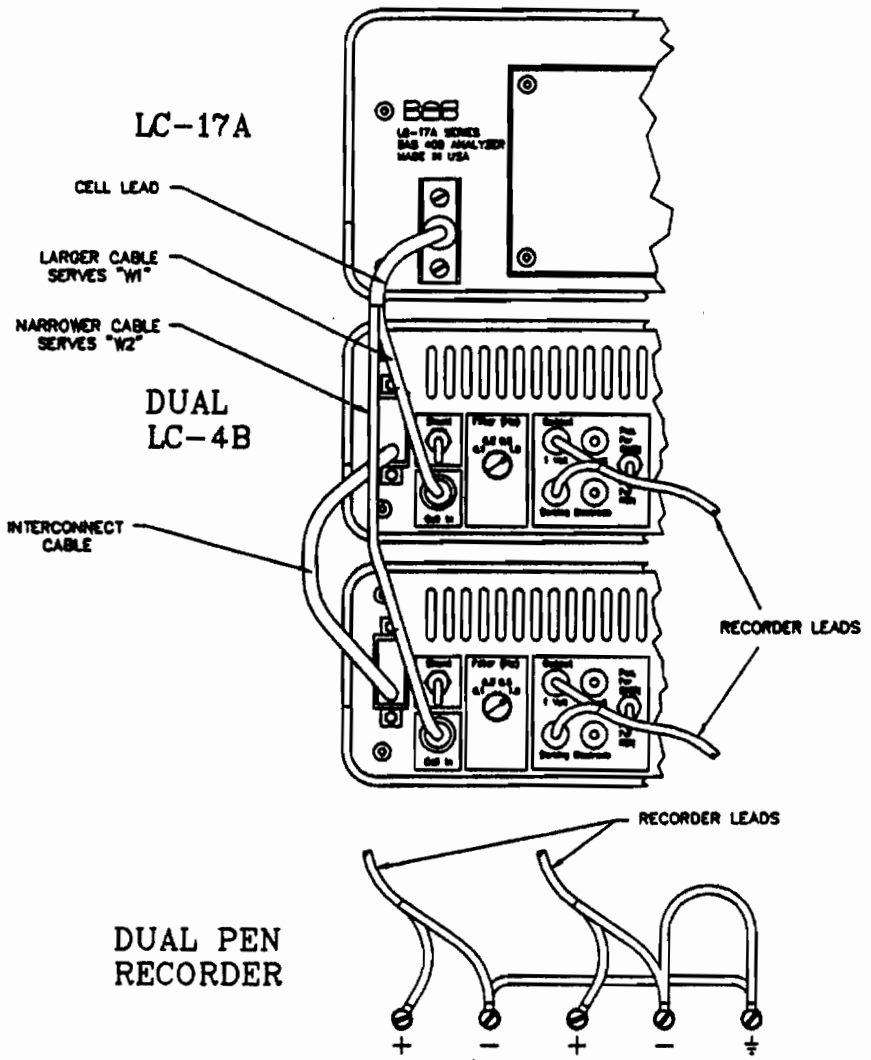


Figure 9-4. Connection of electrode cable and interconnect cable to tandem LC-4B's.

8. On the back panel, mount the extended dual cable shield by a clip and slide motion.

9. Attach the interconnect cable per Figure 9-4. Make sure the connector on this cable marked "W1" is attached to the same LC-4B as the one plugged into the thicker cable in step (6), above.

10. Attach recorder leads and zero your recorder on the right for both channels.

11. Turn on detector power. The displays will now be different; one LC-4B will show "W1" and the other "W2." These will control the respective electrodes connected to cell leads "W1" and "W2."

NOTE: Correct operation is only possible if the LC-4B controlling W1 is ON and the potential applied. 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-4B. That is permissible; the extra cabling does not interfere with this choice and the second LC-4B need not be powered. The reverse mode is not possible, however.

9.4. Installation of Single Electrode Cable or Dual Electrode Cable in the LC-17A and LC-17AT Accessories Kits

1. Identify the parts needed for the complete cable and shield as follows:

   a) SINGLE ELECTRODE CABLE KIT (EW-8136)

      1 - Single Cable Shield
      1 - Single Electrode Cable
      1 - 6-32 x 3/8 screw
      1 - 6-32 x 5/8 screw
      1 - 6-32 nut

   b) DUAL ELECTRODE CABLE KIT (EW-8129)

      1 - Dual Electrode Cable Shield
      1 - Dual Electrode Cable
      1 - Interconnect Cable
      1 - 6-32 x 3/8 screw
      1 - 6-32 x 5/8 screw
      1 - 6-32 nut

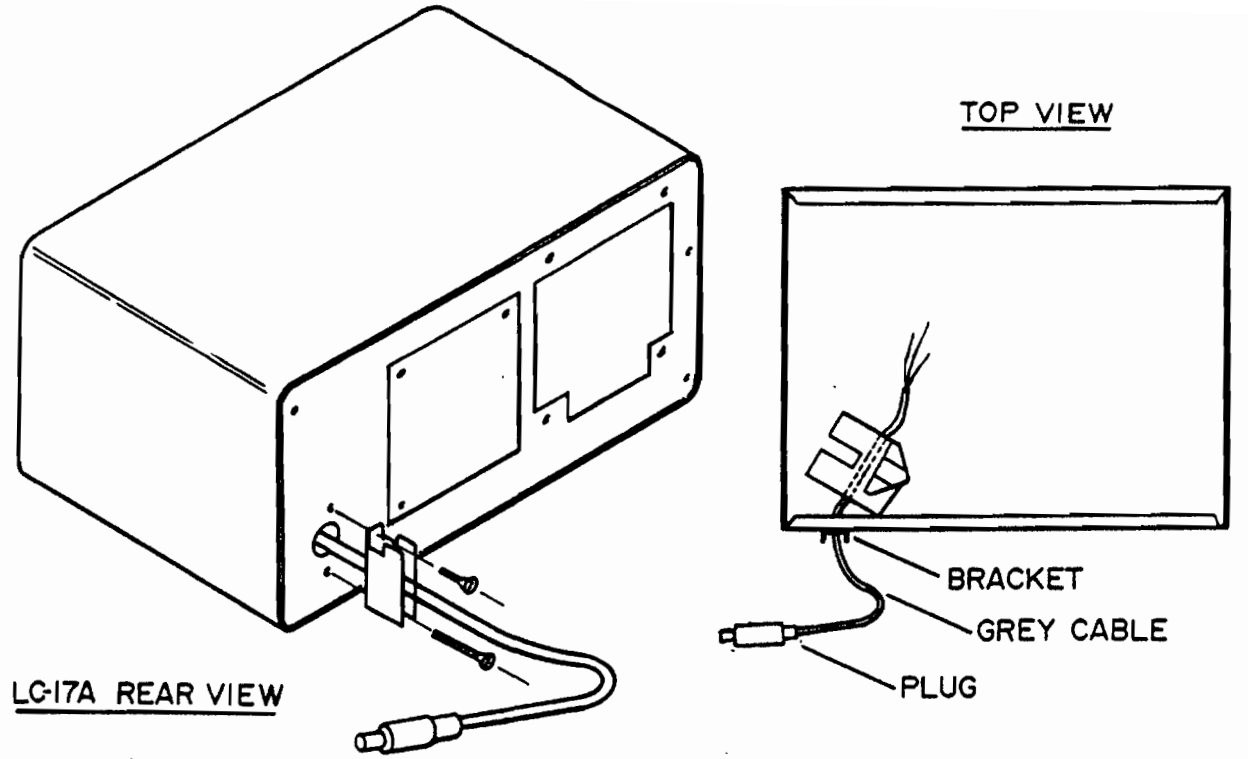
2. Find the cable in your kit with the attached metal bracket. At one end of the cable will be red, white, and black wires, and a green grounding lug. Take these and route them through the rear panel of the LC-17A chassis and through the groove under the cell support. See Figure 9-5.

3. Attach the metal bracket to the back of the LC-17A chassis using the shorter (3/8") screw in the top hole of the bracket, and the longer one at the bottom. Tighten both screws securely.

4. From the inside of the LC-17A, connect the grounding lug (green wire) to the threaded stud protruding from the bottom. This stud should be bare metal. If covered with paint, use a scalpel or other tool to scrape away enough paint to make good connect with the lug once it is in place. Secure the lug with the 6-32 nut.

5. Affix the red wire to the screw at the top of the stainless steel auxiliary electrode block.

6. Make the connection of the cable, and cable shield to the LC-4B controller as described in Section 3. This section will also provide instructions on completing the cell assembly and liquid chromatograph connections.



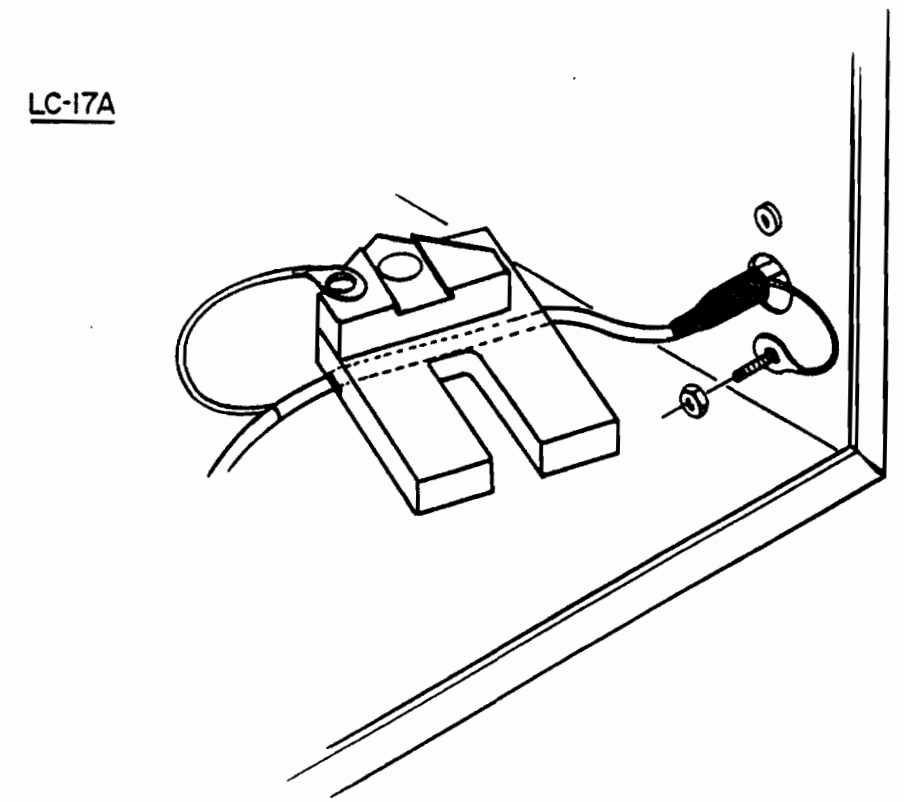


Figure 9-5. Installation of Cables in the LC-17A

10. Temperature Stabilized Detector Operation

10.1. Rationale for Use

Thermostatted control of the chromatographic column reduces the effects of ambient temperature change on electrochemical detection. When the LC23B cartridge column heater is used with the LC-17A 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, drafts) or at high detector gain, small fluctuations in temperature at the detector cell can still produce marked deviations in the detector baseline. In Figure 10-1 are shown plots of EC detector background current vs. temperature. The slope of the curve (di/dT) is significant, typically 0.5 - 1.5 nA per degree C. Hence it is easy to see how a small change in eluent temperature (e.g. 0.1 degree C) could still cause appreciable shifts in the baseline.

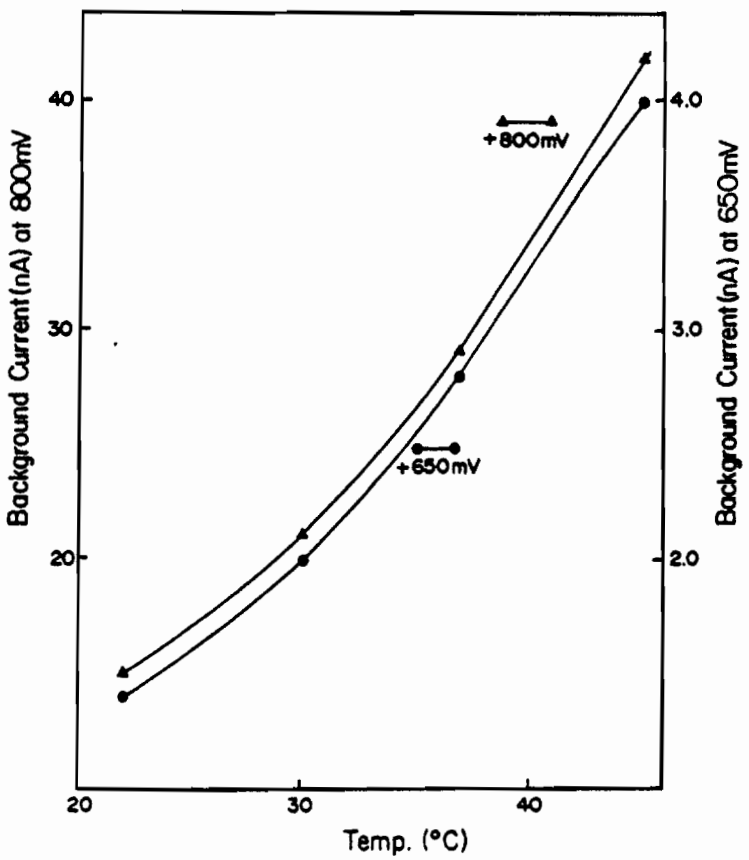


Figure 10-1. Plot of electrochemical background current versus mobile phase temperature on a glassy carbon electrode operated at 650 and 800 mV applied potential.

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.

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

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 probably 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 is properly expressed in terms of the signal-to-noise ratio, the improvement is not so dramatic. For example, operation at 55 degrees C requires more vigorous temperature precision than at 35 degrees C. Thus, a 1.8x increase in peak current is counterbalanced by a 2-3x increase in baseline noise. A small increase in temperature (35 degrees C vs. ambient) makes the most sense in terms of signal/noise (Table 10-1).

Table 10-1. Signal and Noise vs. Temperature Setpoint on LC-17A Preheater Module

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

Conditions: 2.2 mL/min, +750 mV/GC/RE-4 reference, reverse phase ion pair separation, norepinephrine is test solute.

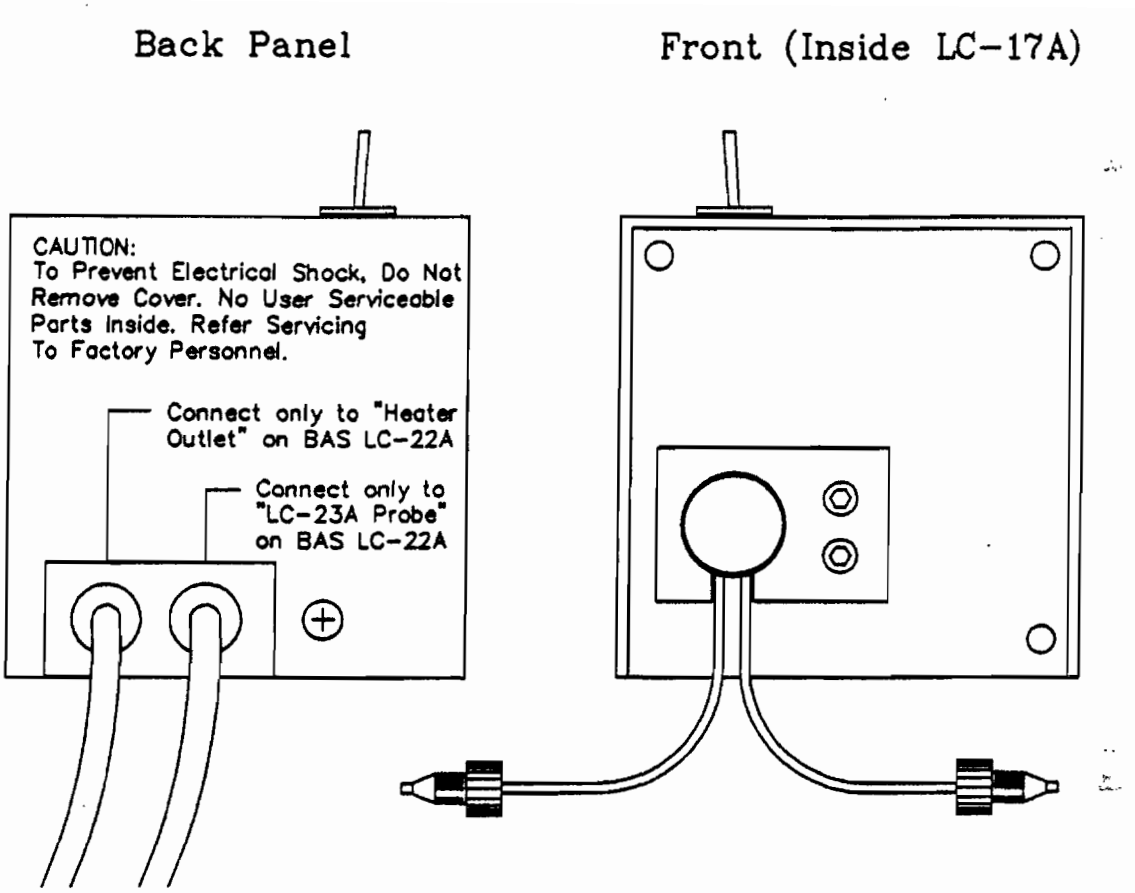


Figure 10-2. Optional LC-17AT Preheater Module ready for user installation.

Cell temperature control is an option on all BAS LC-17A 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 10-2. A retractable core intimately contacts 2-3 turns of capillary tubing for thermal equilibration; inside the core is housed a miniature heater and a solid-state temperature sensor. Both of these devices are connected to a BAS LC-22A Temperature Controller. A switch on the Preheater Module selects the power of the heating circuit between 10 Watts and 6-7 Watts. A protective cover on the unit isolates the user from all electrical circuits for safety.

10.2. Installation of Detection Preheater Module on LC-17A

- -

NOTE: Users with a factory-installed Preheater Module should skip on to section 10.3., "Optimizing Performance with the LC-17AT Detector"

- -

1. Open the LC-17A cover to the vertical position. Remove the temporary cover (3 screws).

2. Disconnect the tubing from the column, at the detector

inlet port.

- -

NOTE: Do not make any electrical connections until step 5, below. 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.

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 fitting. This may short-circuit the cell.

- -

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-22A Temperature Controller immediately under the LC-4B controller(s), per Figure 10-3.

6. Brackets are available to secure the temperature controller, LC-4B and LC-17A together for increased stability. See Section 3.5 #5 for details.

7. Make electrical connections.

   a. Insert the yellow and red plugs into the color-matched jacks on the LC-22A Temperature Controller labelled "LC-23A Column Heater." Flip the switch to the right.

   b. If you wish to have temperature readout on the front panel of the LC-4B, install the cable provided (EW-8107). This cable has 2 banana plugs on each end (orange and black going to green and black). Make connections with jacks of corresponding colors on the rear panels of the LC-22A and LC-4B. Sections of the panel are marked to identify the correct connectors. If you are using a dual electrode detector, route the cable to the LC-4B you intend to use for display of of working electrode "W1".

   c. Plug the remaining cord into the LC-22A Temperature Controller ("Heater Power" receptacle).

NOTE: Early model preheaters had a 3-prong plug. Current units have a female connector. If you have an early model, do not plug it into a wall socket! It is only intended for use with early model LC-22A controllers.

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

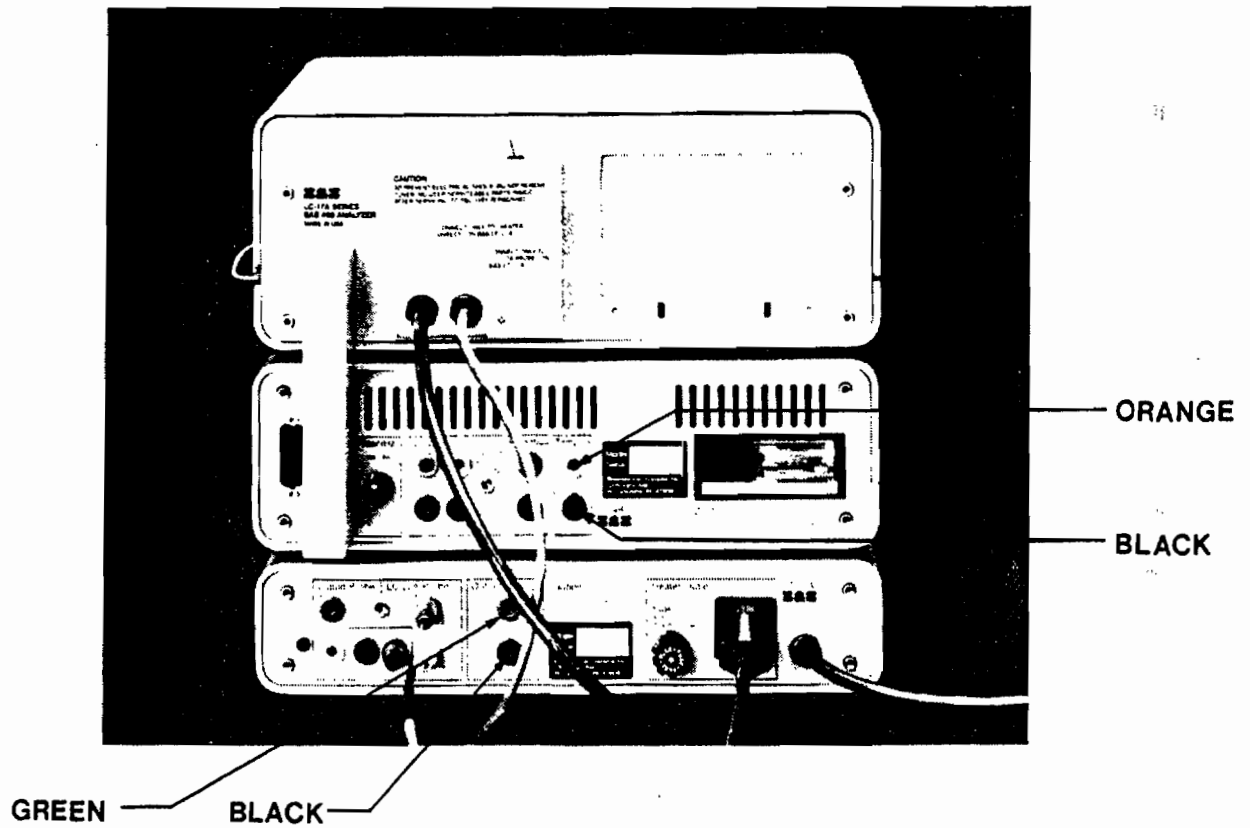


Figure 10-3. Electrical connections for LC-17AT Preheater Module.

10.3. Optimizing Performance with the LC-17AT Detector

As mentioned in section 10.1, temperature control improves baseline stability over the long-term; this can be particularly useful during extended periods of unattended operation. However, the signal/noise parameter slightly declines as the temperature set point approaches 50-60 degrees C. Thus, operation at a set point just over ambient temperature is recommended. Set up the unit as follows:

1. Turn the LC-22A Temperature Controller thumbwheel switches to 20 degrees C. The red LED for heater power (right side) should be off (unless you are working in a cold room!).

2. Plug a 10 V full scale strip chart recorder signal cable into the green/black rear panel jacks marked 0-100 degrees C. Zero the recorder at the right boundary using the recorder's controls. Switch to 10 V full scale on the recorder.

   The ambient temperature of the preheater module heat exchanger will be:

   T(degrees C) = % full scale x 100

3. Set the thumbwheel switches 5-10 degrees C over this ambient temperature. For example, if the recorder reads 22% of full scale (22 degrees C), then try a setpoint of 27-32 degrees C.

   The red LED should turn on; eventually it will "flutter" when the preheater comes up to temperature.

   The recorder pen should also rise an additional 5-10% of full scale, depending on your choice.

4. You may notice some ripple in the recorder tracing if the Preheater Module power setting is HIGH. Try switching to LOW. The ripple should be negligible and the temperature output should remain the same.

5. If you must operate at a higher setpoint, choosing low preheater power may be inappropriate. The preheater module may dissipate heat faster than it can be supplied at LOW power.

A special caution about air bubbles is in order. Your mobile phase may be poorly degassed, or even saturated from sparging with a poorly soluble gas (e.g. helium). Since gases are less soluble at higher temperature, bubbles may form while mobile phase passes through the heat exchanger. Warming the mobile phase to at least the preheater setpoint temperature is worth the savings in

frustration, particularly if you are using a setpoint above 35-40 degrees C.

When not in use for cell temperature control, the LC-22A Temperature Controller can also be used to control hot plates, heating mantles, the BAS LC-23A Column Heating Compartment, etc. For more information, refer to the LC-22A manual.

Appendix I. Electronic Troubleshooting

Test Procedure, LC-4B

1. With the function switch in the Standby 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. Initial test conditions:

   a. Set function knob to **STBY**
   b. Front Panel controls:
      1. Set **OFFSET POLARITY** switch to **OFF**. Turn the **OFFSET** control knob fully counterclockwise.

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

4. Set the **RANGE (nA)** switch to 50.

   c. Rear panel controls.
      1. Flip **FILTER** switch to 0.3 Hz
      2. Set **OUTPUT** polarity switch to "POS for OXDN".

5. Plug the black probe of the voltmeter into one of the four black jacks on the back panel. Plug the red probe of the voltmeter into the red potential jack. Turn the **MODE** select knob to "Appl E" on the display. Arbitrarily select voltages between -1.99 and +1.99V. Readings on the voltmeter and the front panel display should be within 3 mV.

6. Move the red probe of the voltmeter to the green 1 V output jack. Set the function switch to the **TEST** position. Table I gives the expected outputs for both the current on the front panel display and the voltage at the green 1 V output jack. Using the first entry from the table as an example, when the applied potential is set to 1.0 (V) and the range switch is turned to 50 nA, the output as read on the front panel display should be 10.0 nA and the corresponding output voltage should be 0.20 V. The agreement between the expected value and the actual value should be $\pm$5%. 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-4B. This is normal!

Table I

Applied E (V)	Range (nA)	Back Panel Output (V)	Display Output (nA)
1.0	50	+0.20	+10.0
0.5	5	+1.00	+5.0
0.1	1	+1.00	+1.0
0.1	0.5	+2.00	+1.0
0.1	0.2	+5.00	+1.0
0.1	0.1	+10.0	+1.0

7. Return to a **POTENTIAL** of +1.0 V and **RANGE** of 20 nA. The output should be +0.50 V, ± 5%. Flip the rear panel switch to "**POS for RDN**". The output should now be -0.50 V, ± 5%. Flip back to "**POS for OXDN**".

8. Flip the **OFFSET** polarity to "+". Change the display to **OFFSET**. Turn the **OFFSET** control knob until a reading of 10 nA is shown on the LC-4B 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**.

9. If the instrument fails any step of this procedure, contact BAS customer service personnel. Please have the information shown on the first page of Appendix II when you call, and advise the service representative that you have completed this electronics check.

Appendix II. Troubleshooting Flow Charts

The following series of charts will help you identify and correct the majority of problems you might encounter with your electrochemical detector.

If these suggestions do not help you to solve the problem, please make note of the following information before you call for service and technical assistance. It will give us a more complete picture of what you are trying to accomplish.

Detector

Type of Detector?	(Single Electrode, Dual Electrode)
Cell Preheater Installed?	(Yes, No)
Working Electrode Type?	(Glassy Carbon, Gold Amalgam, Paste, Silver, Platinum, Nickel)
Electrode Arrangement?	(Dual-Series, Dual-Parallel, Single or Jumpered?)
Range?	(nA displayed on LC-4B front panel)
Offset?	(nA)
Output?	(Volts full scale)
Applied Potential?	(Volts, + or -)
Oxidation or Reduction?	(switch on back panel of LC-4B)
SHUNT position?	(on or off for each LC-4B?)

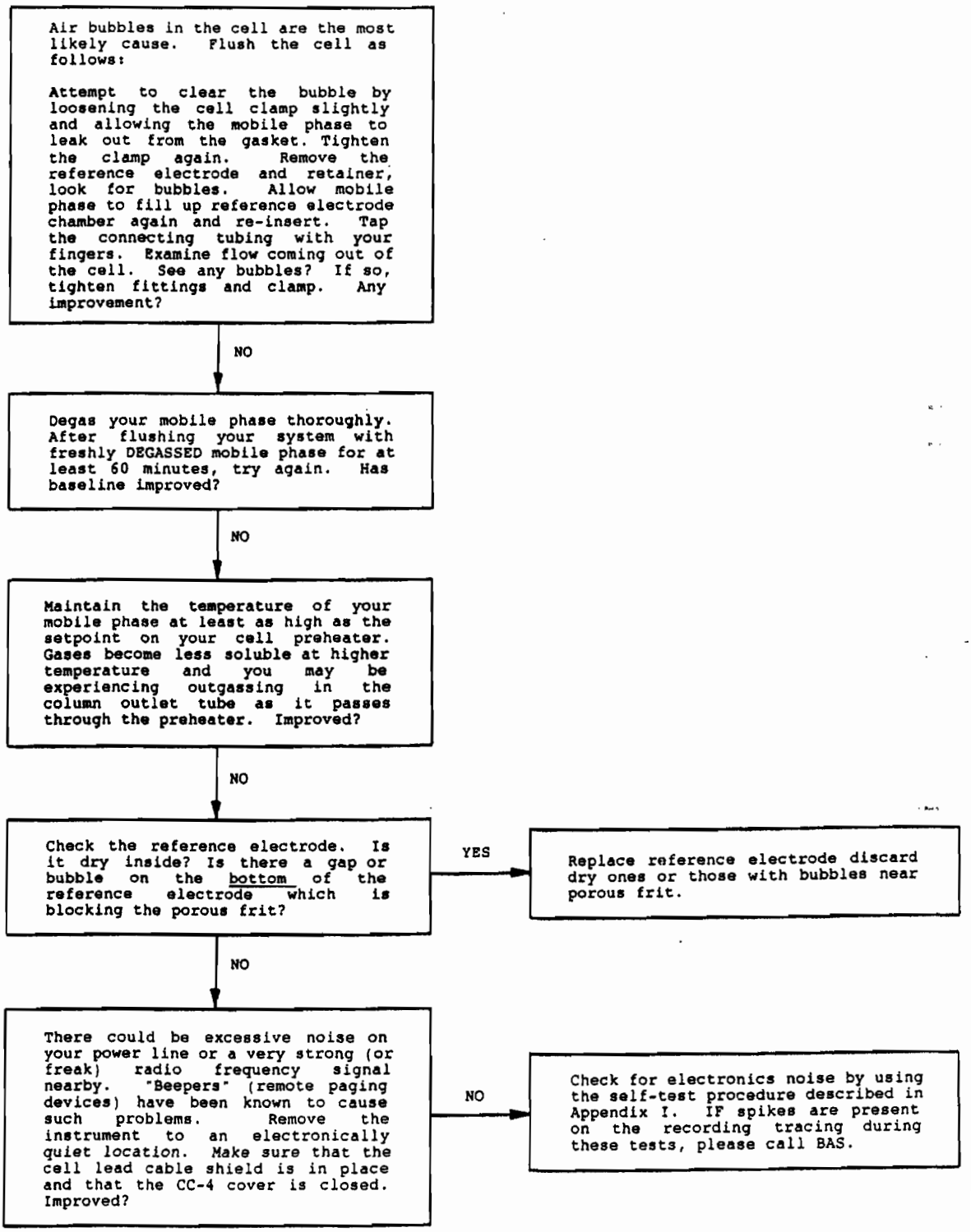
Chromatography Conditions

Mobile Phase Composition?	_____
Mobile Phase pH?	_____
Column Type and Length?	_____
Column Length?	(5, 10, 15, 25, 30 cm, or other?)
BAS Liquid Chromatograph?	(BAS 200, BAS 400, other?)
Using with other detectors?	(Yes, No)
Position of EC Detector in relation to others?	(upstream, downstream)

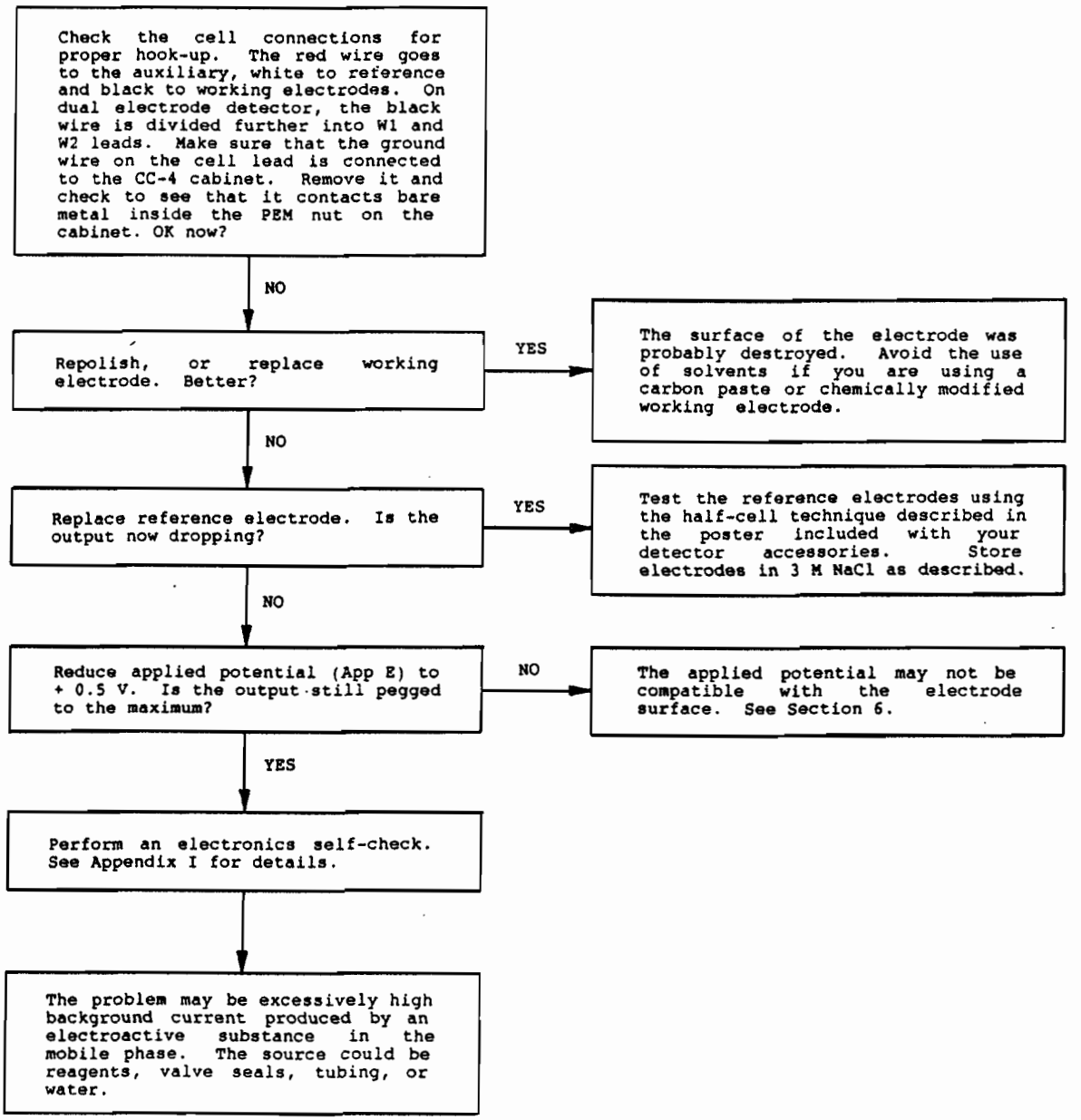
Application

Sample Type?	(biological fluid, tissue, perfusate, pharmaceutical, runoff water, cosmetic, extract, etc.)
Analyte of Interest?	_____
Concentration in Sample?	_____
Detection Limit Sought?	_____

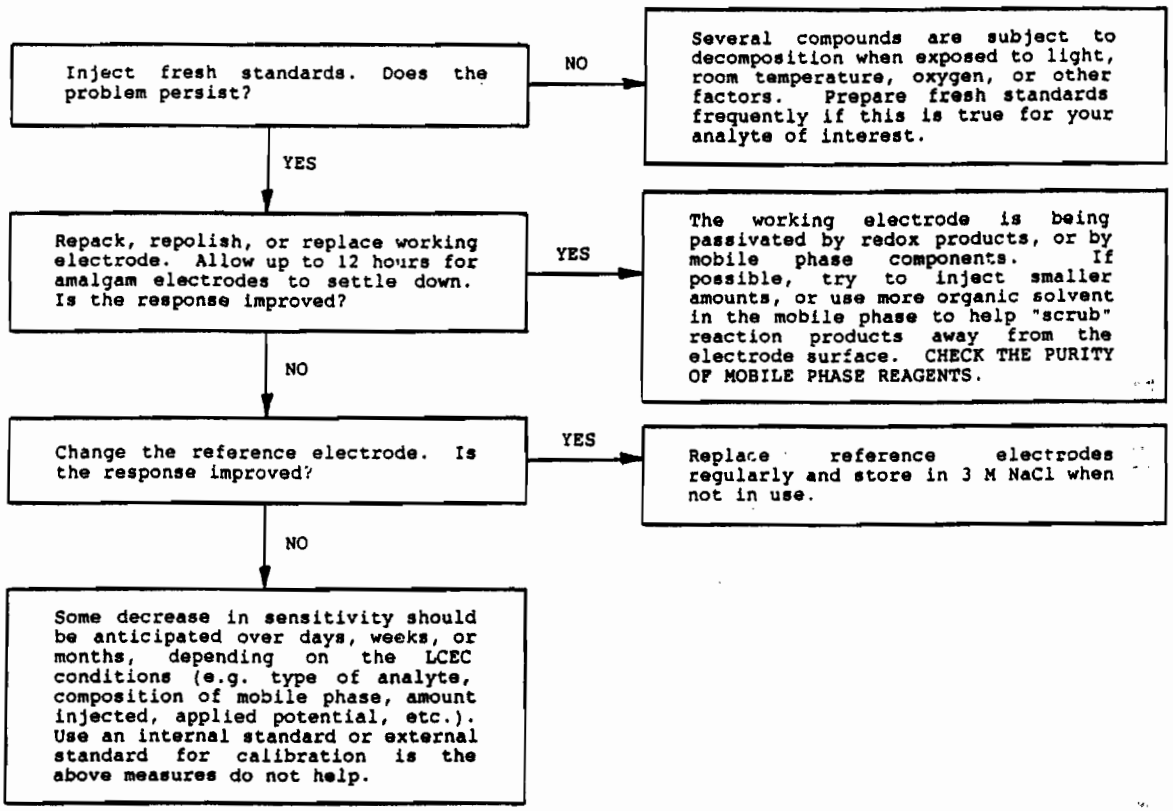
PROBLEM: VERY FAST NOISE SPIKES, PARTIAL OR FULL SCALE.



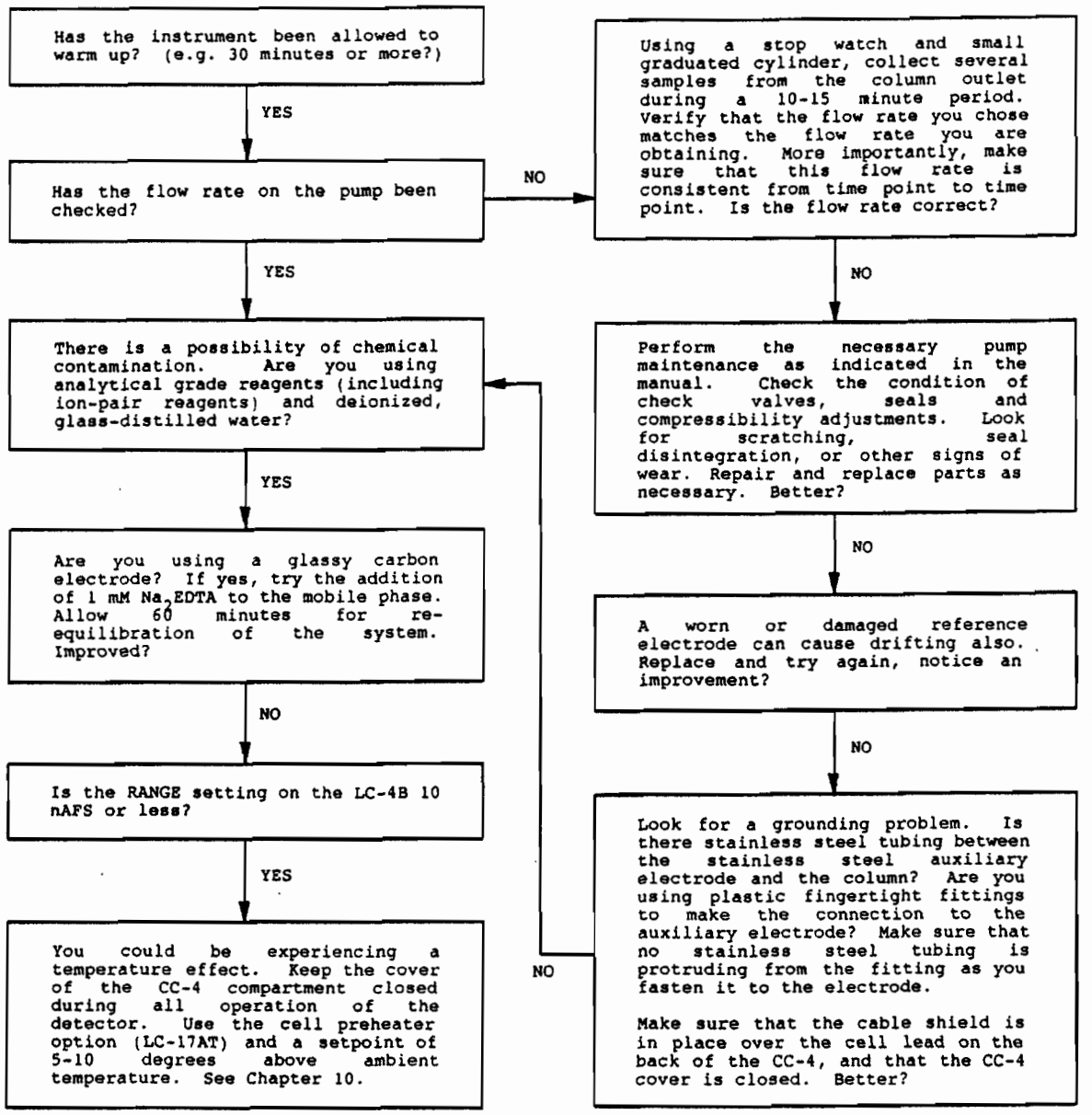
PROBLEM: DETECTOR OUTPUT IS CONSTANTLY PEGGED AT THE MAXIMUM LEVEL (120 mV or 12 V, DEPENDING ON THE OUTPUT JACKS SELECTED).



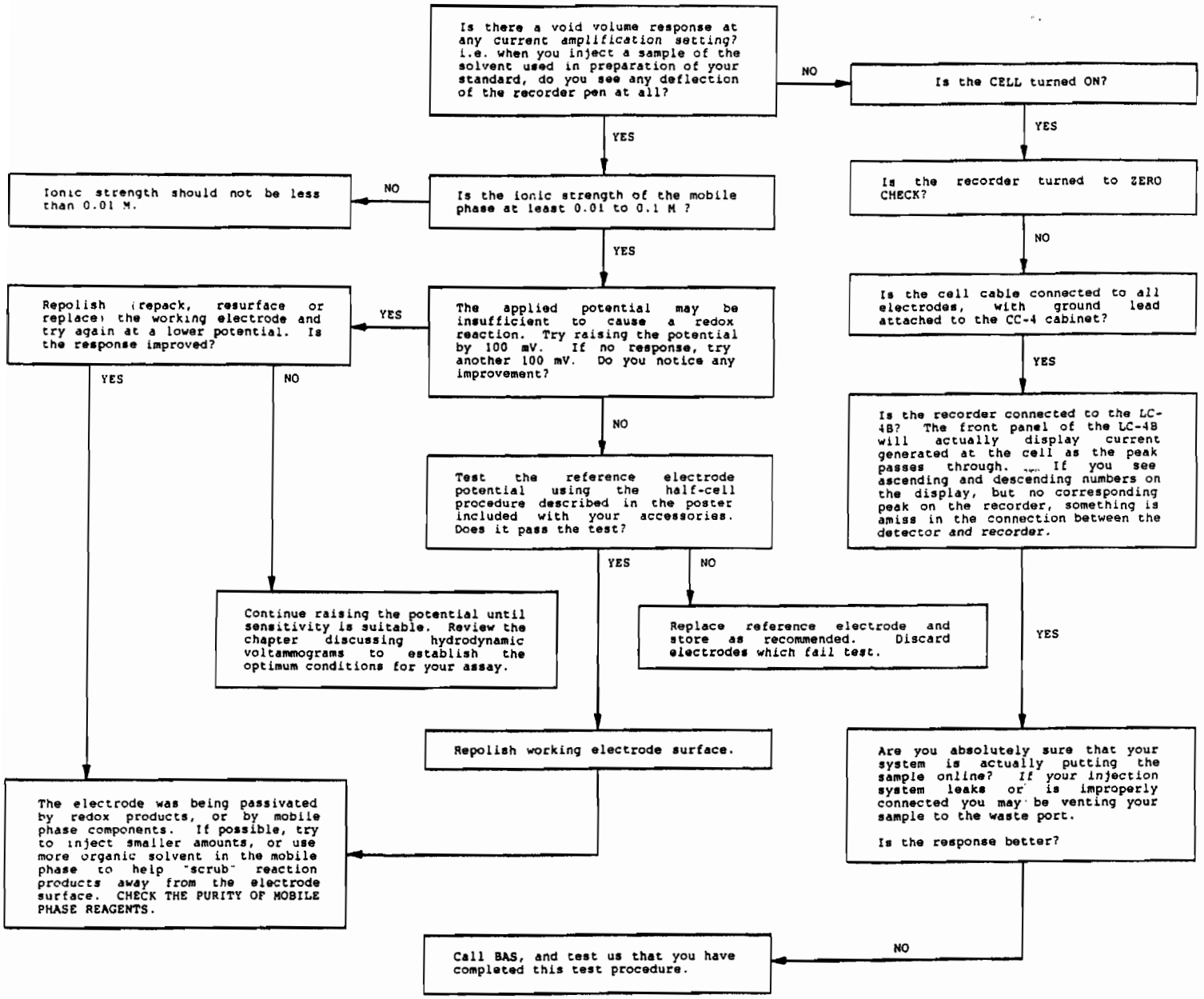
PROBLEM: ELECTRODE IS RESPONSIVE, BUT THE SENSITIVITY IS SIGNIFICANTLY LESS THAN IN PREVIOUS RUNS.



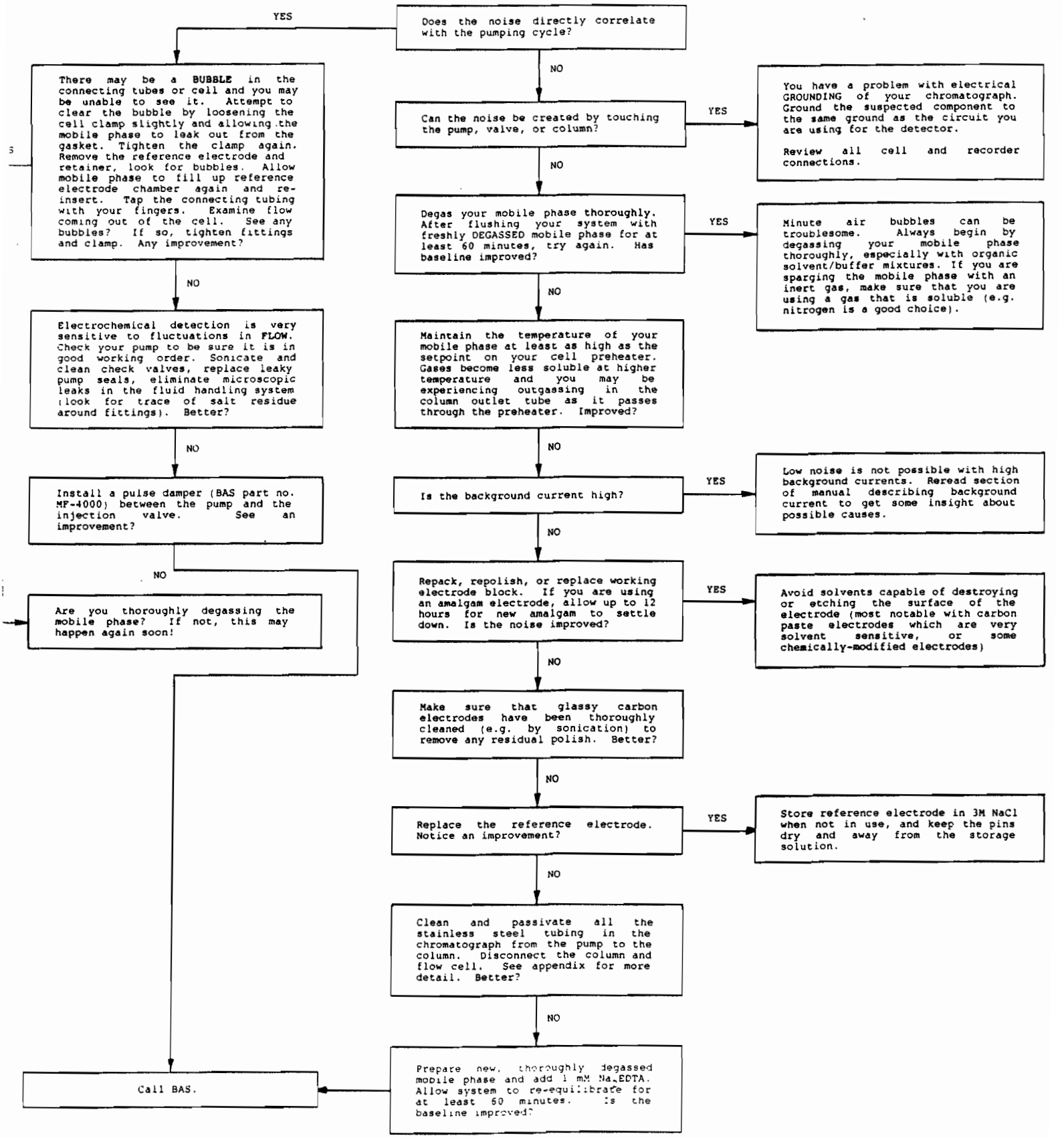
PROBLEM: IRREGULAR DRIFT IN BASELINE



PROBLEM: NO RESPONSE FROM DETECTOR!!



PROBLEM: BASELINE NOISE LIMITS SENSITIVE ANALYSIS



Appendix III

In cases where high background current is limiting detector operation, make the following checks.

1. Select the highest quality buffer salts and solvents when making the mobile phase. Use distilled, de-ionized water.

2. Filter and degas the mobile phase following recommendations in Chapter 5.

3. Operate the detector at the minimum applied potential for you compound of interest. If you're not sure what this is, do an HDV or CV experiment (see Section 4).

4. Select an electrode that is appropriate for your mobile phase. Make sure it is in good condition. Newly amalgamated gold electrode may have to "equilibrate" overnight prior to being used.

5. Check that pump seals are in good working condition.

6. If all else fails, passivate the system again following this procedure.

   Disconnect all columns and LCEC cells from the system and set aside. Cap the column to prevent drying.

   ↓

   Position a beaker near the column inlet tube to collect wastes.

   ↓

   Pump 20 mL of distilled, deionized water through the system at any flowrate.

   ↓

   Pump 300 mL of 6 M nitric acid through the system at any flowrate.

   ↓

   Pump 20 mL of distilled deionized water through the system followed by 50 mL of mobile phase.

   ↓

   Reconnect column and LCEC cell. Equilibrate with fresh mobile phase.

8. If background remains high, eliminate the column from the flow stream and connect the system directly to the LCEC cell. If background drops (despite the high pulsations), clean or replace the column.

Appendix IV

ELECTROCHEMICAL CHARACTERISTICS OF SELECTED MOLECULES OF BIOMEDICAL AND/OR ENVIRONMENTAL INTEREST.

All Cyclic Voltammograms used to obtain data on the following 9 figures used identical conditions:

Electrolyte:	0.1 M Citrate, 10% Ethanol (V/V)
Scan:	200 mV/sec.
Working Electrode:	Carbon Paste type CPO (for oxidative CV)
	Gold Mercury Amalgam (for reductive CV)
Conditions:	Deoxygenation of solution was required for Reductive CV.

Legend

Figure 1 Oxidative Cyclic Voltammetric Data for Selected Aminophenols.

Figure 2 Oxidative Cyclic Voltammetric Data for Selected Aromatic Amines.

Figure 3 Oxidative Cyclic Voltammetric Data for Selected Alkylphenols of Environmental Interest.

Figure 4 Oxidative Cyclic Voltammetric Data for Selected Chlorophenols of Environmental Interest.

Figure 5 Oxidative Cyclic Voltammetric Data for Some Vanillyl Metabolites of Tyrosine and Related Compounds.

Figure 6 Oxidative Cyclic Voltammetric Data for Some Indole Metabolites of Tryptophan and Related Compounds.

Figure 7 Oxidative Cyclic Voltammetric Data for Biologically Important Catecholamines and Other Easily Oxidized Diphenols.

Figure 8 Oxidative Cyclic Voltammetric Data for Some Natural Phenolic Acids.

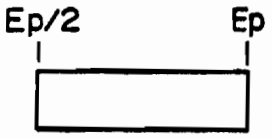
Figure 9 Reductive Cyclic Voltammetric Data for Selected Compounds of Environmental Interest.

WHAT ARE CYCLIC VOLTAMMETRY DATA CHARTS?

The following tables will help to summarize a great deal of information about the redox characteristics of a variety of molecules. This information can be used as a guideline when evaluating the feasibility of detecting these compounds using LCEC techniques. Information concerning the applied potential is not absolute: you will need to confirm the optimum using hydrodynamic voltammograms and the electrode and mobile phase necessary for your assay.

FORMAT OF THE CHARTS

The potential axis runs from left to right for reductions and from right to left for oxidations, per the traditional American convention.



NOMENCLATURE

For REDUCTIONS: the left end of the rectangle represents the potential where the current is equal to 1/2 of the peak current. The right end of the rectangle represents the potential at the peak maximum. The opposite is true for OXIDATIONS.

An empty rectangle indicates that only one forward peak is observed and no other peak is obtained on the reverse scan between limiting potentials of the medium.

A rectangle with parallel lines through it indicates that there is one reduction (or oxidation) peak and that an oxidation (or reduction) peak is observed when the scan is reversed. However, the electrochemical reaction is CHEMICALLY IRREVERSIBLE.

e.g. $ArNO_2 + 4\ e^- + 4\ H^+ \rightarrow ArNHOH + H_2O$

(Forward Scan = A Reduction)

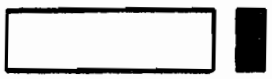
$ArNHOH \rightarrow ArNO + 2\ e^- + 2\ H^+$

(Reverse Scan = An Oxidation but it does not result in the original compound and is irreversible)

A solid rectangle means that the system is CHEMICALLY REVERSIBLE (and that the electron transfer system is sufficiently fast so that both forward and reverse processes occur on the time scale of the CV experiment).

e.g. $MV^{+2} \underset{-e}{\overset{+e}{<----->}} MV^{+1}$

A rectangle without the right (or left for an oxidation) bar indicates that a poorly defined forward peak was obtained (a shoulder on the second wave) and that the $E_{1/2}$ value was estimated by setting $E_{1/2}$ at the potential equal to 1/2 of the current at the peak shoulder.



A solid bar following a rectangle indicates the peak potential of <u>additional</u> <u>forward</u> peaks which exist before the background limit. However $E_{1/2}$ value can not be determined due to the poor resolution between adjacent peaks.

For differential pulse and other small amplitude voltammetric techniques, this symbol is used. The central vertical bar represents the peak potential and the box indicates the width of the curve at half the peak current. The same symbol is used for polarography and hydrodynamic voltammetry where the three vertical bars indicate the $E_{1/4}$, $E_{1/2}$ and $E_{3/4}$ potentials. Again, a narrow box suggests a fast heterogeneous electron transfer rate. An assymmetric box may indicate complications in the mechanism.

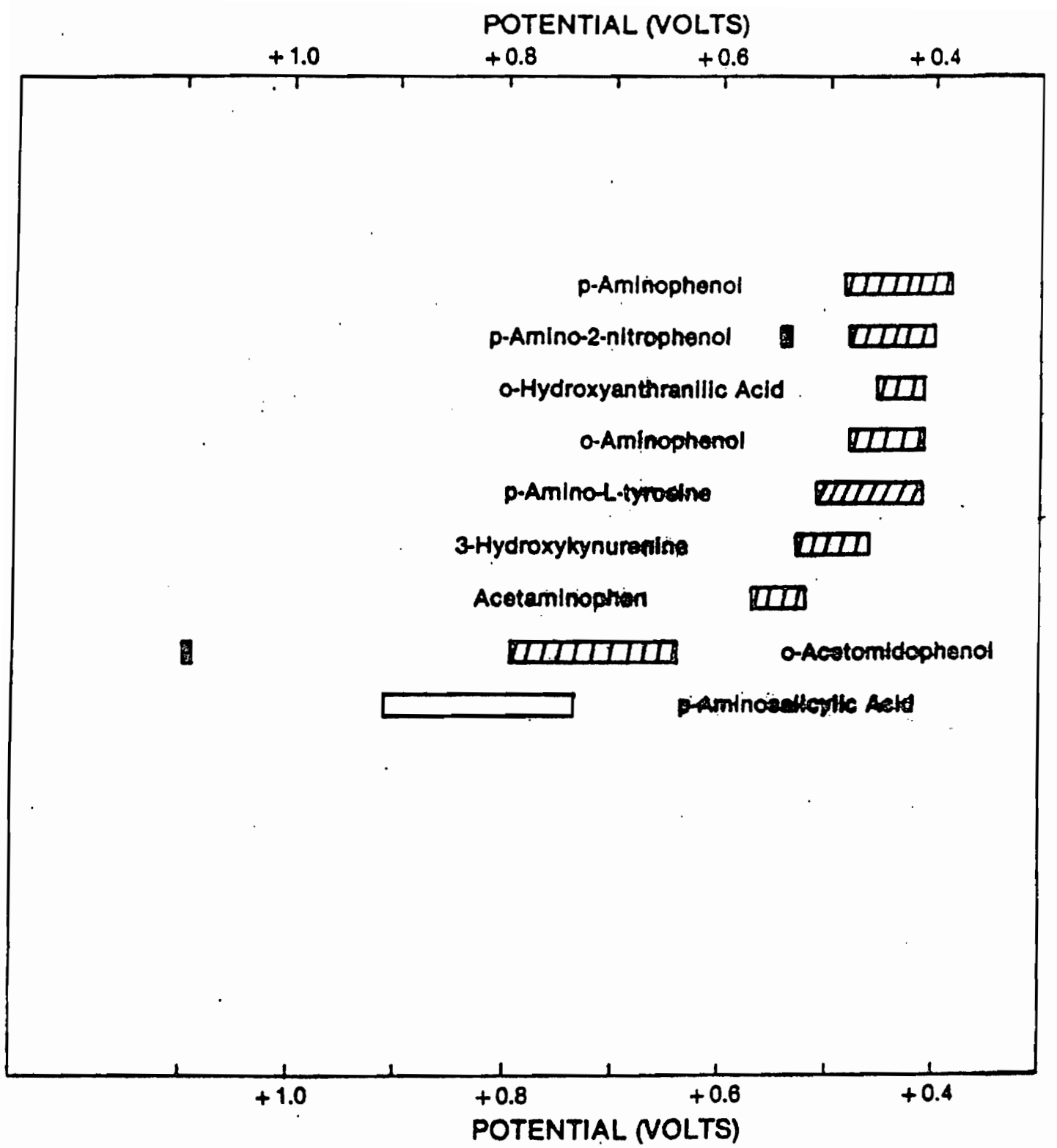


Figure 1 Oxidative Cyclic Voltammetric Data for Selected Aminophenols.

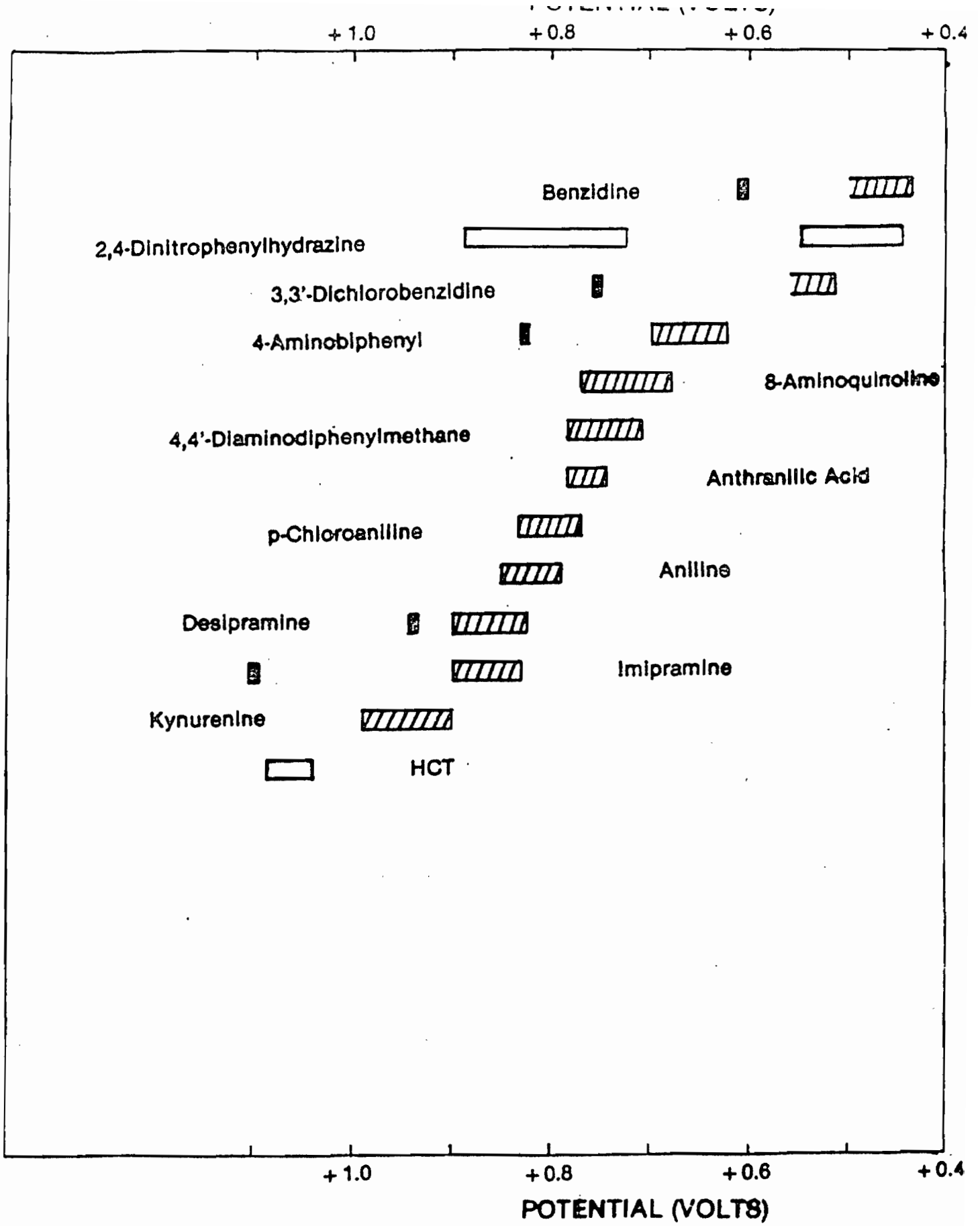


Figure 2 Oxidative Cyclic Voltammetric Data for Selected Aromatic Amines.

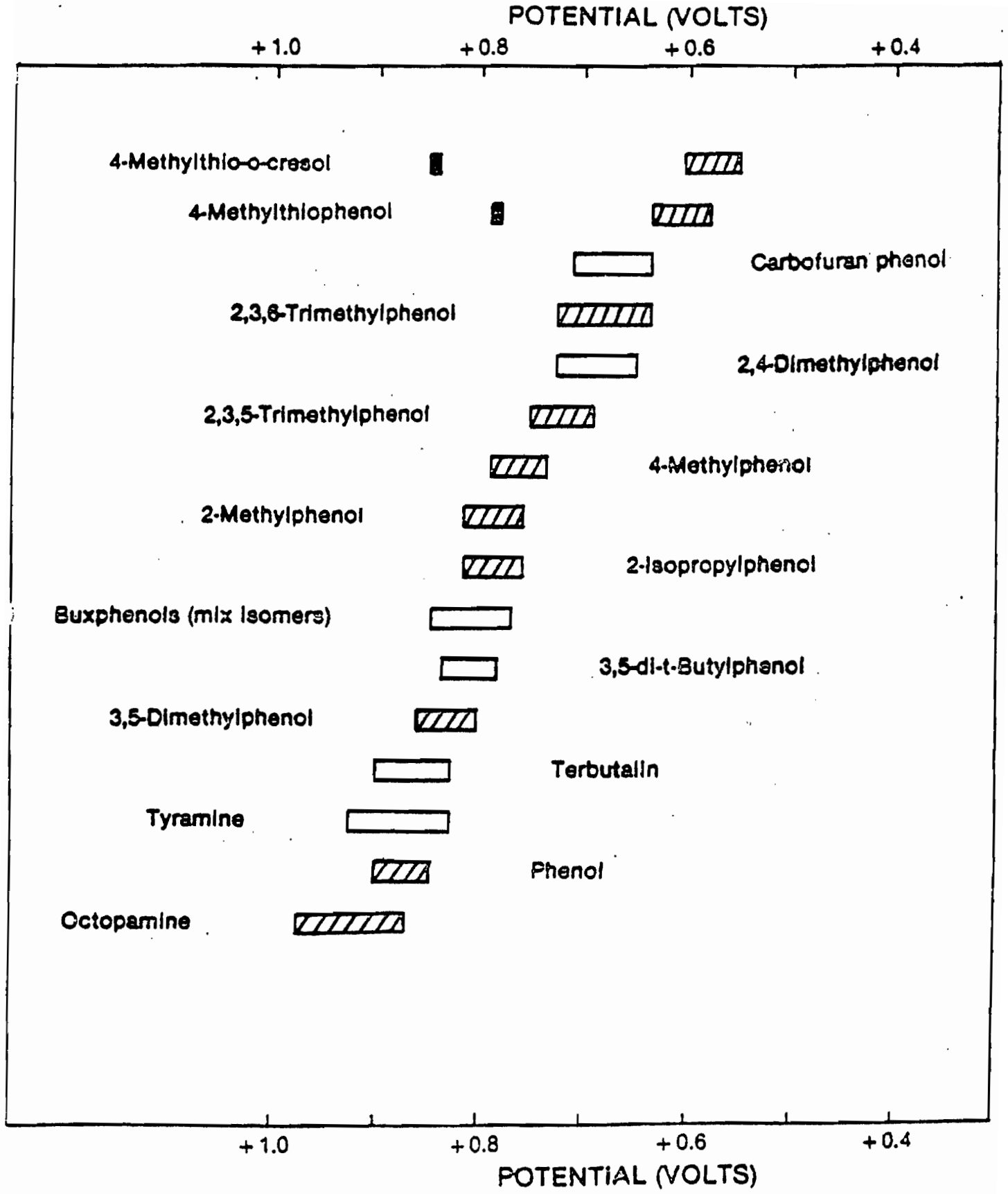


Figure 3 Oxidative Cyclic Voltammetric Data for Selected Alkylphenols of Environmental Interest.

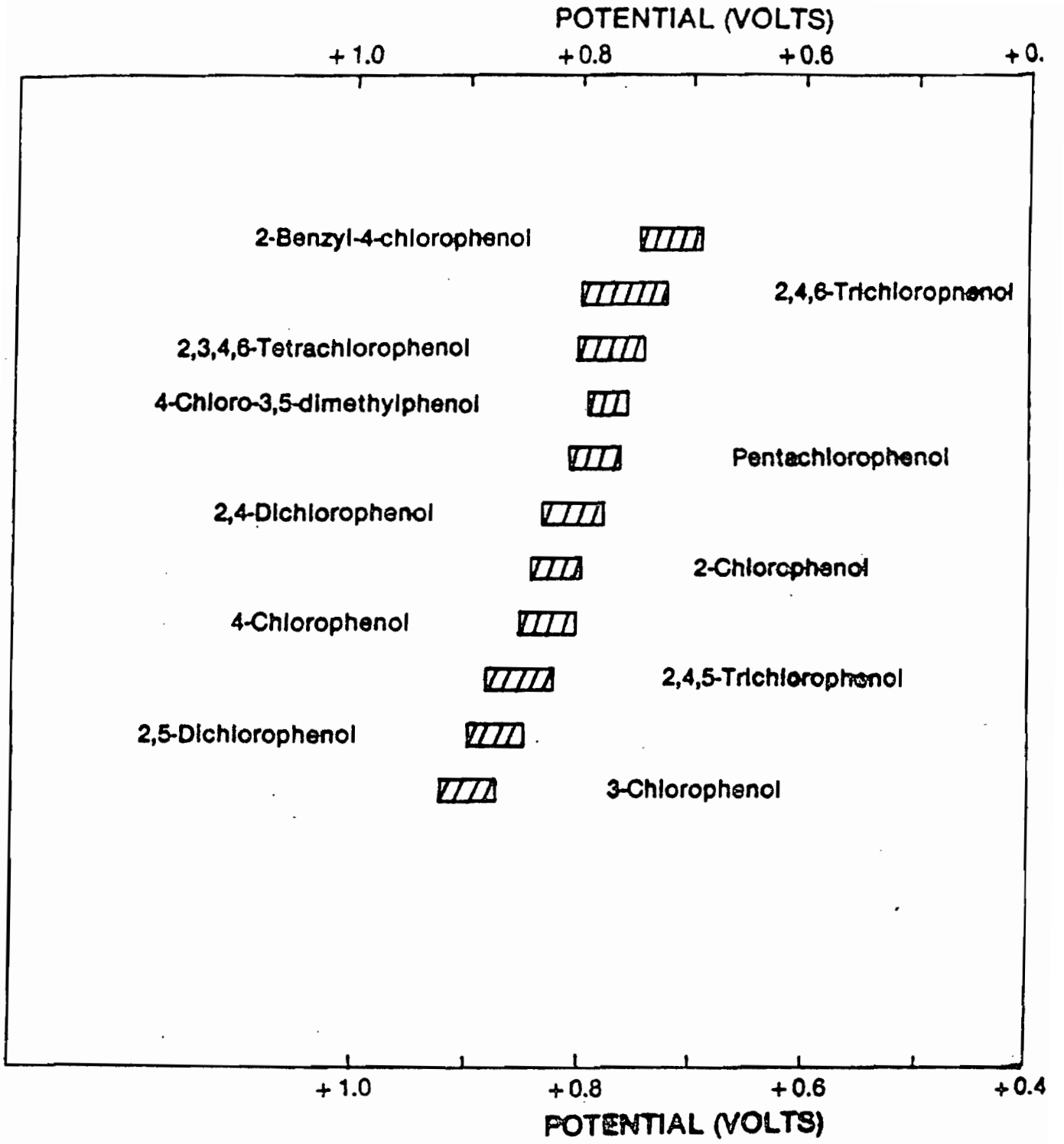


Figure 4 Oxidative Cyclic Voltammetric Data for Selected Chlorophenols of Environmental Interest.

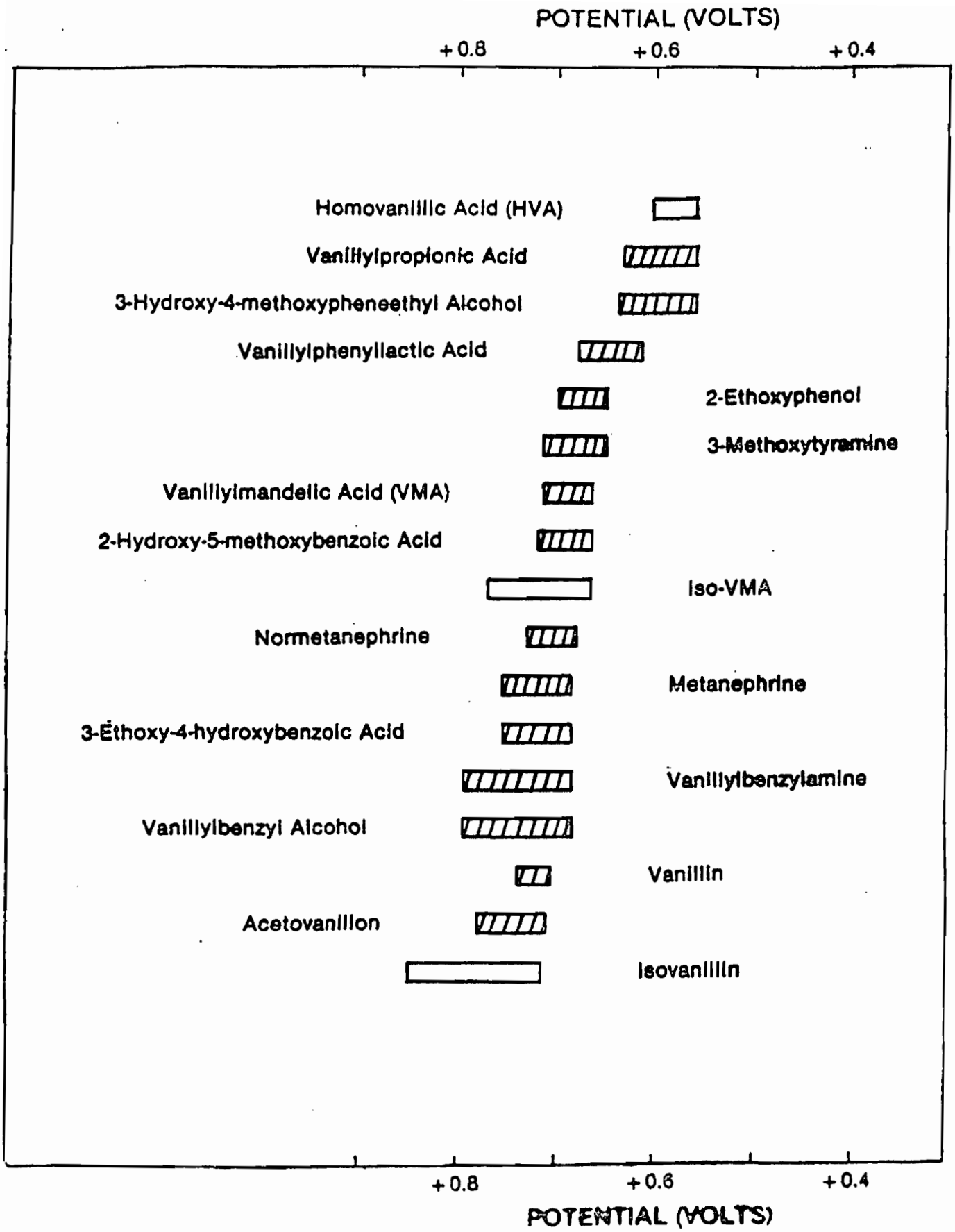


Figure 5 Oxidative Cyclic Voltammetric Data for Some Vanillyl Metabolites of Tyrosine and Related Compounds.

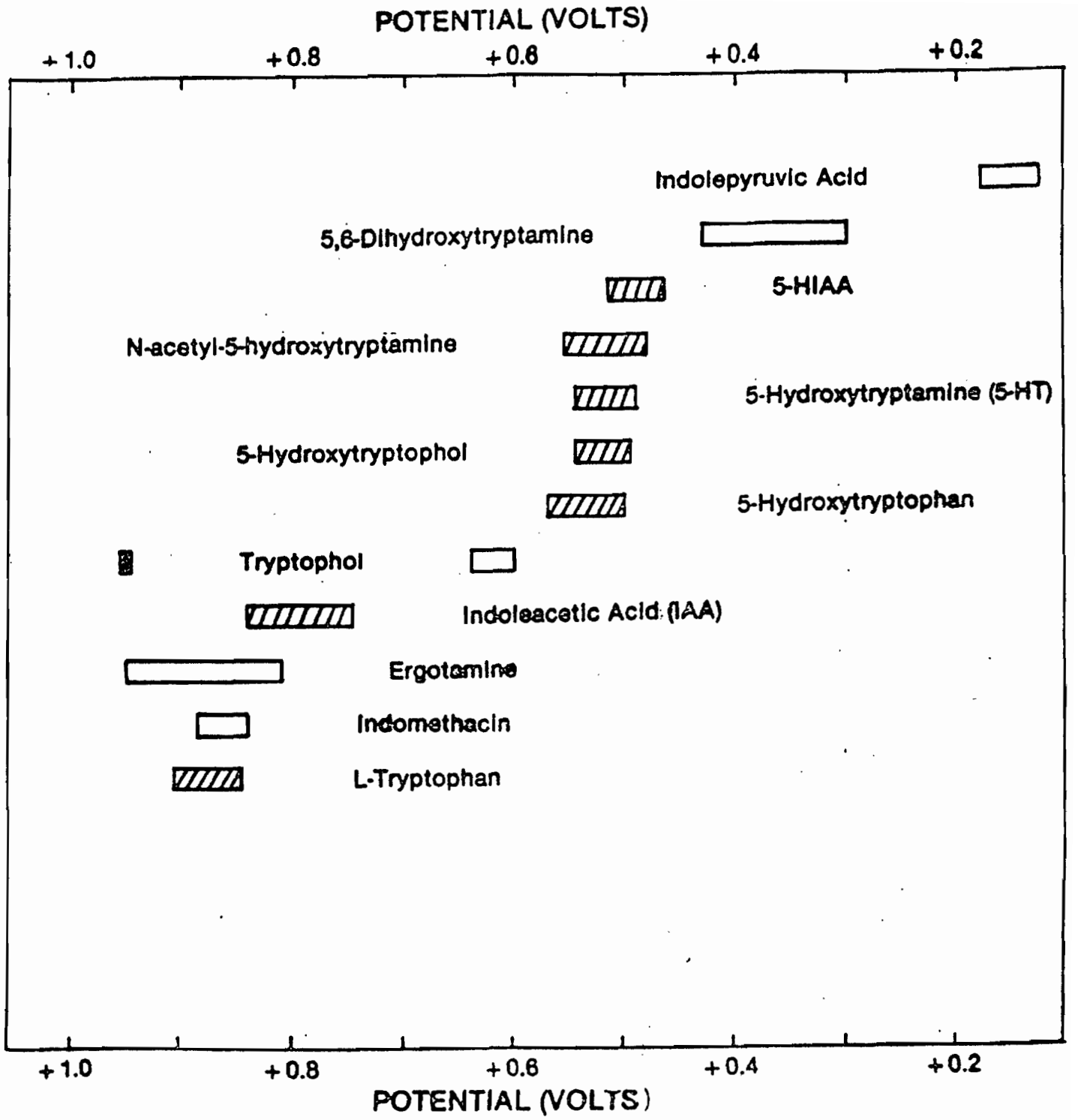


Figure 6 Oxidative Cyclic Voltammetric Data for Some Indole Metabolites of Tryptophan and Related Compounds.

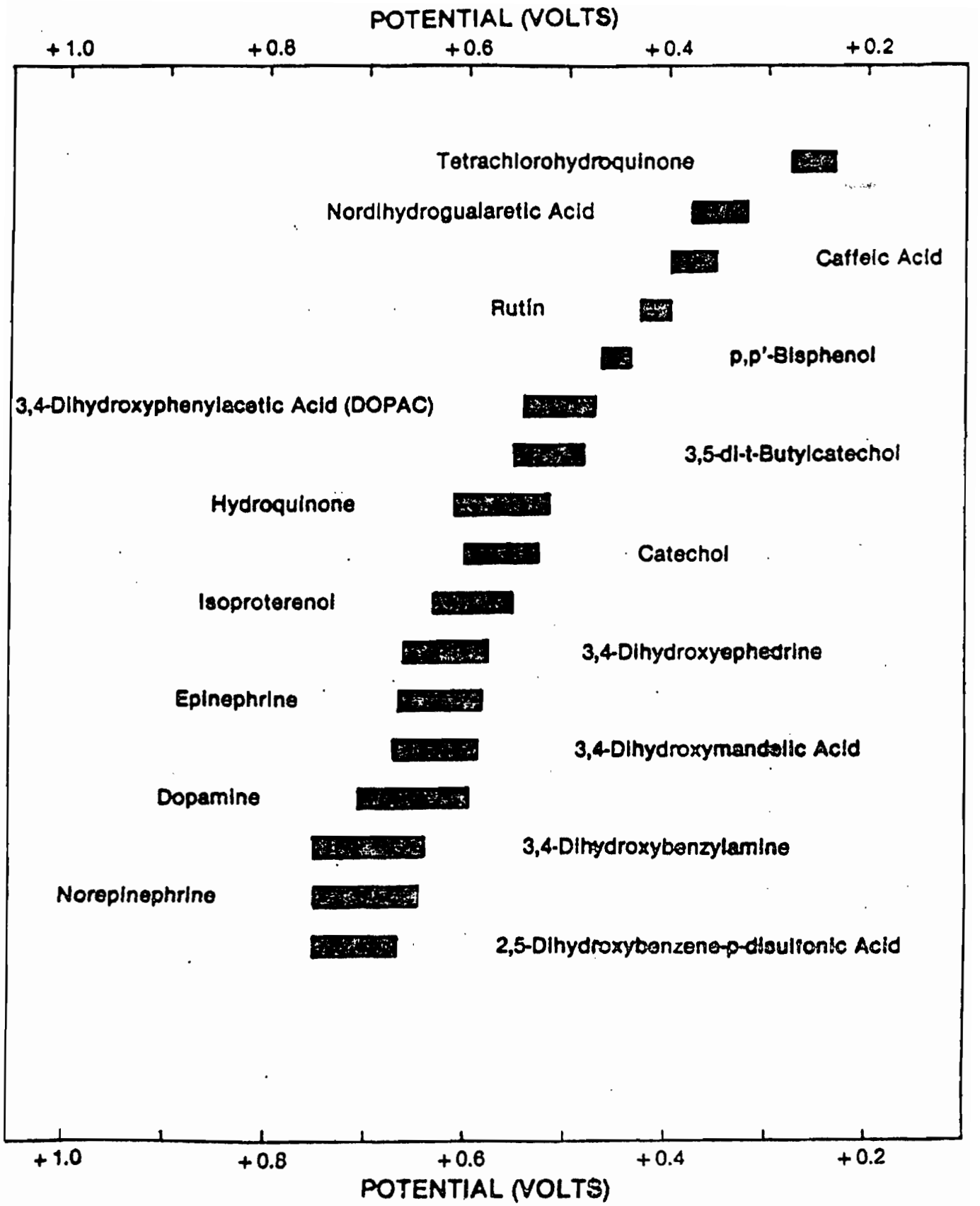


Figure 7 Oxidative Cyclic Voltammetric Data for Biologically Important Catecholamines and Other Easily Oxidized Diphenols.

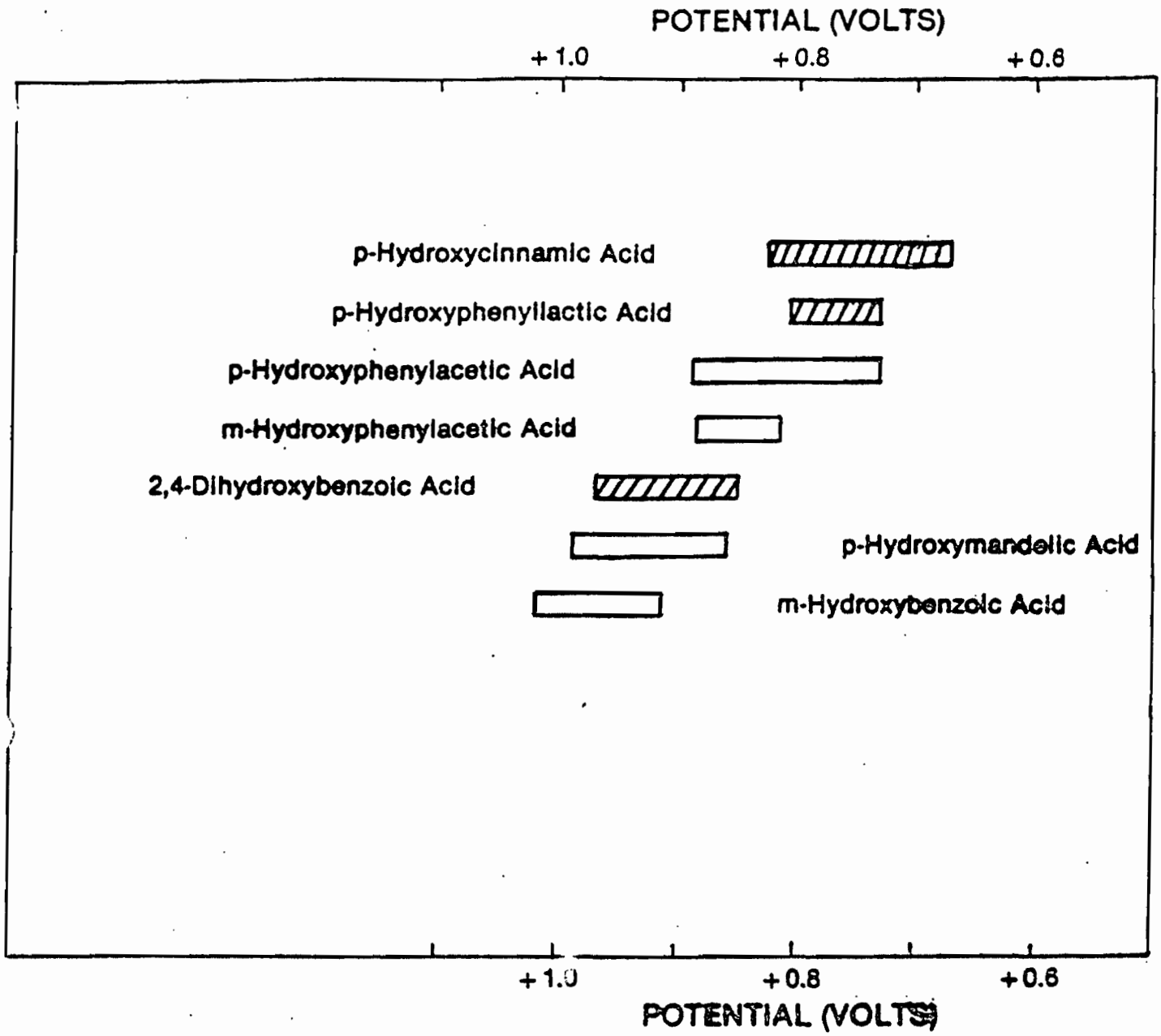


Figure 8 Oxidative Cyclic Voltammetric Data for Some Natural Phenolic Acids.

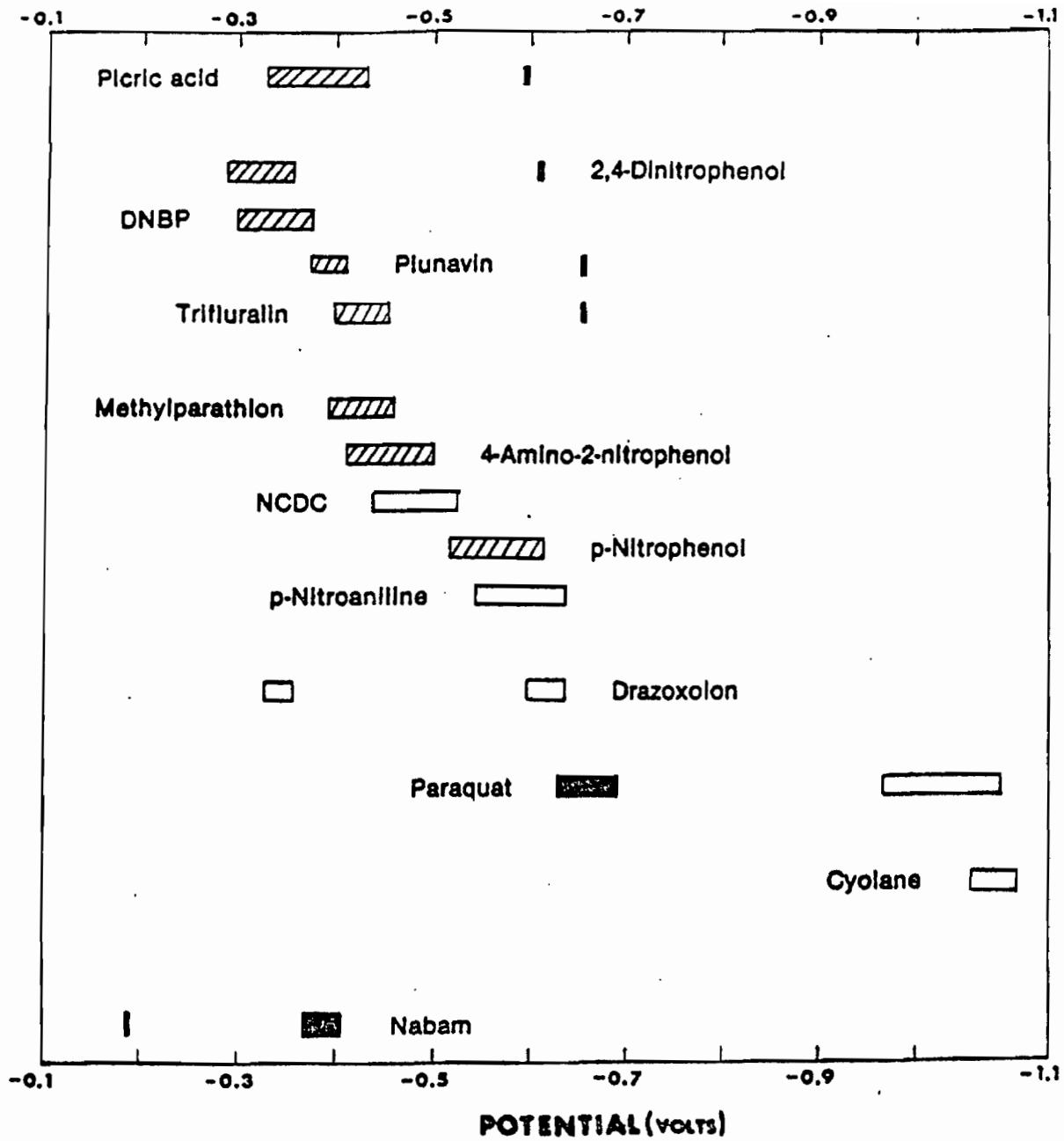


Figure 9 Reductive Cyclic Voltammetric Data for Selected Compounds of Environmental Interest.