31466 Ag/AgCl REFERENCE ELECTRODE

This reference electrode has a small fill hole near the cap that is covered with an elastic.

It is recommended that the electrode be renewed via the procedure included (Document #31434).

If you decide to refill via the elastic covered hole, **DO NOT USE A METAL SYRINGE NEEDLE.** A plastic tip must be used. If a metal other than the one in the electrode is used, it may shift the potential of your electrode.

CARE OF WORKING ELECTRODES

Introduction

The following information should be used to clean the working electrode in your Model 464 Pulsed Electrochemical Detector. The mechanical polishing procedures are used to clean any metal electrode material and sometimes used for cleaning glassy carbon electrodes. Metal electrodes can also be cleaned with pulse amperometric techniques. Glassy carbon, unlike metal electrodes, is a porous material and chemicals can penetrate a few microns into the electrode surface. Therefore, the more aggressive cleaning techniques, such as mechanical polishing, will significantly alter the electrode surface and contribute to a longer equilibration period before the electrode can be used for analytical work. Some special techniques have been developed to clean glassy carbon electrodes. One should use the procedure which causes the least amount of physical damage to the surface of the working electrode.

Note: The working electrodes available for the Model 464 Pulsed Electrochemical Detector are precision devices that are flat to less than 0.001 inch. Thus, any unnecessary polishing of the surface may cause electrode defects. Enclosed in the electrode polishing kit (31476) are the following items which can be purchased separately:

31490 Polishing Compounds (6 micron Diamond paste and 0.05 micron Alumina slurry).

31489 Polishing Cloth (Texmet and Microcloth).

Glassy Carbon Working Electrodes (Single or Dual):

In caring for the glassy carbon electrode the following procedures have worked and are listed in order of increasing severity. The more drastic procedures require longer equilibration times before analytical work can be done. To maximize the productivity from your detector it is recommended that an extra working electrode be available for immediate use. Install a new gasket whenever you change or remove the electrode more than once.

Chemical Cleaning Procedures

A) Remove the Glassy Carbon Electrode from the cell compartment and rinse with distilled water. This will remove any encrusted buffer salts from the electrode surface. Once this is done, clean the electrodes with reagent grade methanol. Apply several drops of methanol to the surface of the electrode and dry off with laboratory or lense tissue. This simple procedure may clean the electrode enough to bring back a suitable response rapidly.

B) If the above cleaning technique does not work the next procedure is a little more aggressive. Remove the electrode from the cell compartment and rinse off with distilled water. Dry the electrode and apply one to two drops of chromic acid cleaning solution to the surface of the electrode. Let the solution stand for 30 seconds and then rinse off with RO water. Replace the electrode into the cell compartment.

Electrochemical Cleaning Procedure

Some organic compounds are deposited on the electrode surface when high potentials (over 800mV) are used. These can often be desorbed from the electrode surface using electrochemical techniques as indicated below.

1. Apply a -600mV potential to the glassy carbon electrode for 1 minute.
2. Then apply a +1200mV potential for 10 minutes.
3. Finally, apply the potential necessary for the analysis of your compound.

Mechanical Cleaning Procedures

If the above procedures do not sufficiently clean the glassy carbon working electrode then mechanical polishing of the surface is required. The following polishing procedure is suggested and can be used to polish any solid electrode (metal or glassy carbon).

A) Fine Polishing Procedure for Minor Imperfections

Note: Use the 0.05 micron white polishing alumina with the gray Microcloth disc.

1. Remove from the enclosed polishing kit a piece of Microcloth (this is the soft feeling polishing pad) and place this on the glass plate supplied. Note that the polishing discs have an adhesive backing. Remove the adhesive backing and place the polishing disc on the glass plate.

2. Mark the glass plate on the side with the Microcloth to describe the polishing material used. This will prevent you from mixing different polishing materials on that pad. Wet the Microcloth with several drops of distilled water and then add 2-4 drops of the alumina suspension (note: shake before application). The alumina suspension is the large bottle containing the white solution

3. Remove the working electrode from the cell compartment and rinse with distilled water to remove any traces of buffer.

4. Apply the working electrode face down directly on the Microcloth impregnated with alumina. Use a smooth medium pressure to polish the electrode. The electrode should be moved in a circular pattern to ensure homogeneous grinding action. Do this for 2 - 3 minutes.

5. Once polishing is complete, remove the electrode and clean with copious amounts of distilled water. Place the electrode face up in distilled water in a sonic cleaner for 3 minutes to ensure the removal of any alumina grit. Rinse the electrode with RO water and replace into the cell compartment.

B) Coarse Polishing Procedure for Major Imperfections

Note: Use the 6 micron yellow diamond paste with the white Texmet disc.

1. Apply to the other side of the glass plate a Texmet disc (the white material that feels like paper). For application remove the adhesive backing.

2. Place several drops of distilled water onto the Texmet disc and then place one 1/4 inch dab of the yellow diamond paste onto the disc (this is supplied in a small syringe).

3. Again, remove the electrode from the cell compartment and rinse with distilled water prior to applying it to the polishing pad.

4. Apply the working electrode face down directly on the polishing grit on the Texmet. Use a smooth medium pressure to polish the electrode. The electrode should be moved in a circular pattern to ensure homogeneous grinding action. Do this for 2 - 3 minutes.

5. Once polishing is complete remove the electrode and clean with copious amounts of distilled water. Place the electrode face up in distilled water in a sonic cleaner for 3 minutes to ensure the removal of any diamond grit. Rinse the electrode with RO water and then continue with a fine polish using the procedure described above in Section A.

Waters
Division of MILLIPORE

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WATERS 464

PULSED ELECTROCHEMICAL DETECTOR

Operator's Manual

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Waters Publications

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APPENDIXES

LIST OF FIGURES

HOW TO USE THIS BOOK

Using this book This book provides all the procedures required for installing, operating, maintaining, and troubleshooting the Waters 464 Pulsed Electrochemical Detector.

The book is conveniently divided into seven chapters:

Chapter 1, **Theory of Operation**, discusses the theory of electrochemical detection and the principle of operation for the Waters 464.

Chapter 2, **Hardware Overview**, covers flow cell options, and front and rear panel controls and indicators.

Chapter 3, **Installation**, covers unpacking the instrument, flow cell preparation and installation, fluid connections, plus power-up and initial testing.

Chapter 4, **Operating and Utility Modes**, describes accessing/ exiting a mode, entering mode values, the DC, Scan and Pulse operating modes, and the System and Calibration utility modes.

Chapter 5, **Basic Operation**, outlines a basic procedure for making a run.

Chapter 6, **Maintenance**, provides information for fuse replacement, voltage selection, reference electrode renewal, and polishing the working electrode.

Chapter 7, **Troubleshooting,** contains a guide for pinpointing routine problems and the recommended actions necessary for their correction.

Additional Information Appendix A lists Waters 464 specifications.

Appendix B provides product ordering information.

Appendix C lists line cycle/second conversion information.

Appendix D covers warranty/service information.

Special print type This book uses bold type to make text easier to understand:

Display message **M464 SOFTWARE REV X.XX**
SELECT MODE (≠ Ø): DC

1

THEORY OF OPERATION

This chapter describes the:

- Theory of electrochemical detection
- Principle of operation: Waters 464 Pulsed Electrochemical Detector

1.1 THEORY OF ELECTROCHEMICAL DETECTION

Electrochemical detection theory consists of two main topics:

- Electrolysis reactions
- Current potential curves

1.1.1 Electrolysis Reactions

Electrochemical detection differs from other methods of detection in that the analyte undergoes an electrochemical reaction while being detected. Upon elution from the column, the analyte passes through the electrochemical cell where it undergoes either an oxidation or a reduction at the working electrode. The controller (potentiostat) maintains the potential of the working electrode (relative to a reference electrode) at a value which causes the analyte to be electrolyzed. It simultaneously measures the electrolysis current resulting from the oxidation (or reduction) of the analyte.

An electrical current is the rate of flow of electricity, for example: coulombs/second. The electrolysis current is a measure of the rate of the reaction taking place at the working electrode. It should be recognized that in order for a molecule to undergo an oxidation (reduction) at an electrode, a three step process (Figure 1-1) must be completed as follows:

1. Mass transport of the analyte molecule from the bulk of solution to the electrode surface. Even in rapidly flowing streams, there is a stagnant layer of fluid at the electrode surface through which diffusion is the mode of mass transport. Diffusion in liquids at room temperature is relatively slow (typical liquid phase diffusion coefficients are 1-10 X 10^{-6} cm/sec.[2]). The flow rate and design of the flow cell are the primary factors which determine the rate of mass transport.

2. Electron transfer at the electrode surface. The rate of this step is determined primarily by the applied potential. Generally, it will be chosen so that this step is very rapid relative to the rate of mass transport. It is at this stage that the electrochemical process takes place.

3. Mass transport of the electrolytic product(s) from the electrode surface. This is a diffusion-limited step. Products formed by electrochemical reaction are removed from the electrode surface by a diffusion-limited step as the mobile phase passes over the electrode.

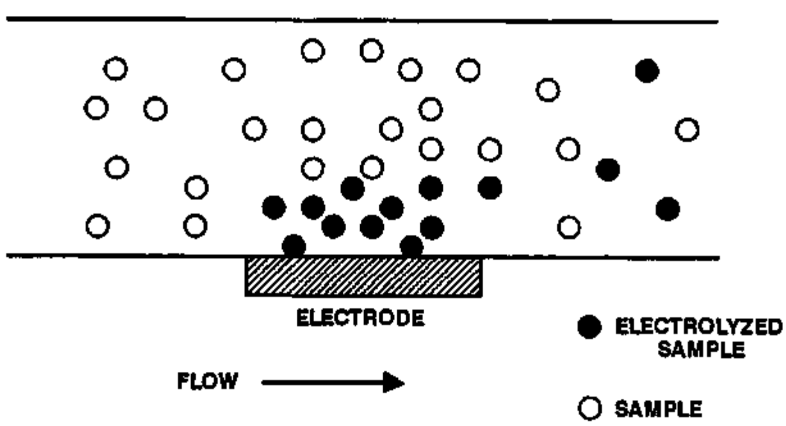

Figure 1-1 Stages of an Electrochemical Reaction

In any multistage process, the slowest step determines the overall rate. For an electrolytic reaction, diffusional mass transport is almost always the slowest step for analytically useful reactions. The mass transport limited current in a flow cell is given by this equation:

$$i = n\,FA(D/\delta)C$$, where

- i = mass transport limited current in a given flow cell
- n = number of electrons transferred
- F = Faraday's constant, 96,500 coulombs/equivalent
- A = electrode area
- D = diffusion coefficient
- δ = diffusion layer thickness
- C = concentration of the analyte in the flow cell

When the column efficiency (N, plate number) and capacity factor (k') are constant, the instantaneous concentration of the analyte eluted from the column is directly proportional to the mass injected.

1.1.2 Current-Potential Curves

The selection of the appropriate applied potential should be based on an understanding of the current-potential curve(s) of the analyte(s). The current-potential curve should be obtained under identical conditions. A current-potential curve for a flowing solution at constant flow rate is called a hydrodynamic voltammogram (Figure 1-2). The illustration shows an idealized hydrodynamic voltammogram for an oxidation.

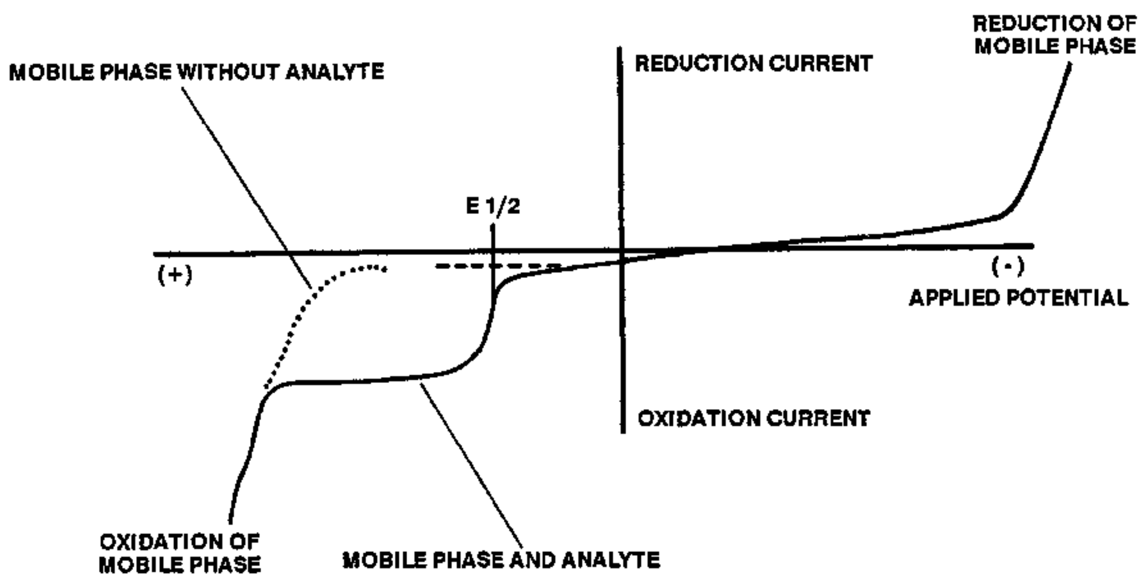

Figure 1-2 Current-Potential Curve

The significant characteristics of a current-potential curve are the $E_{1/2}$ and the limiting current plateau. The $E_{1/2}$ is very nearly equal to the standard reduction potential of the analyte. The limiting current plateau corresponds to those potentials which result in nearly instantaneous electrolysis of the analyte upon reaching the electrode surface. In general, the best compromise between signal and noise is observed for potentials which are at the very beginning of the limiting current plateau. Operating at greater potential does not increase the signal and is likely to increase noise.

When there are multiple oxidizable species present, their current-potential curves are additive. For example, there will be multiple limiting current plateaus each with its characteristic $E_{1/2}$. Selective detection with an electrochemical detector is accomplished by using the smallest value of applied potential that will electrolyze the sample and give a mass transport limited current. Increasing the potential can result in the oxidation of additional components.

1.2 PRINCIPLE OF OPERATION: WATERS 464

The principle of operation focuses on the analytical flow cell and the electronics.

Analytical flow cell

The Waters 464 analytical flow cell contains three electrodes: a reference electrode, a working electrode, and a counter electrode. There are two types of analytical flow cells—nonmetallic or stainless steel. In the nonmetallic cell (Figure 1-3),the working electrode is the first pin (electrical connection), and the counter electrode is the second pin on the dual working electrode block. In the stainless steel cell (Figure 1-4), the stainless steel block serves as the counter electrode.

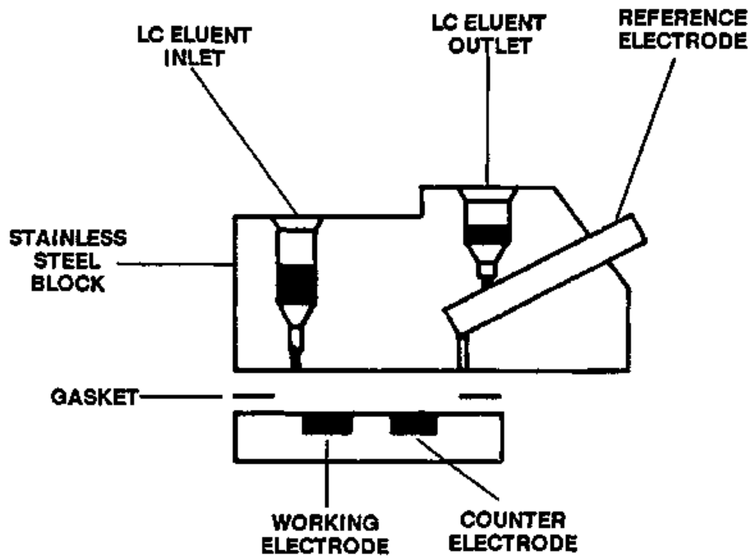

Figure 1-3 Nonmetallic Analytical Flow Cell

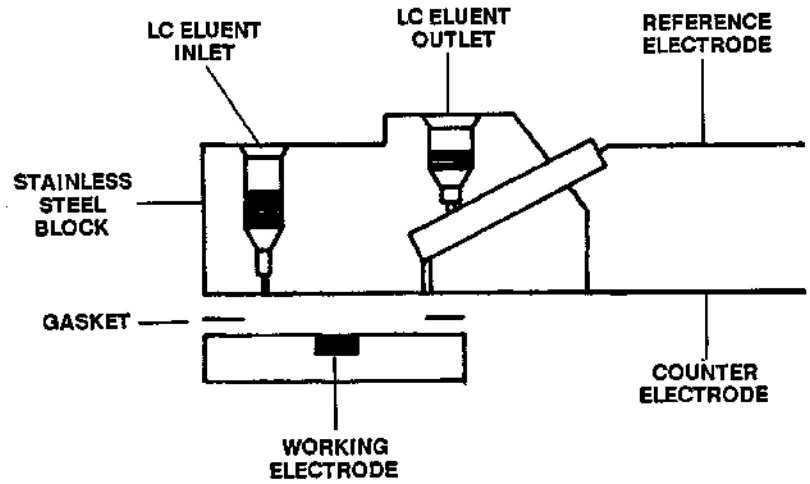

Figure 1-4 Stainless Steel Analytical Flow Cell

When the CELL ON/OFF key is ON, the flow cell is connected to the potentiostat. The potentiostat applies the desired potential between the working and reference electrodes. The counter electrode completes the current path.

Detector electronics

The control amplifier C applies the cell voltage required to maintain the potential difference between the working and reference electrodes, equal to the signal potential, E_{sig}. The cell current resulting from the electrolysis of the analyte is measured by amplifier I/E and presented as a voltage for signal processing by the rest of the detector. In DC Mode, E_{sig} is a fixed voltage. In Pulse Mode, E_{sig} is a three-step program (the duration and values of which are user specified). In Scan Mode, E_{sig} is a voltage ramp that is swept over a desired range of applied potentials. The DC Mode is generally used for high sensitivity analyses. The Pulse Mode is used for analytes which cannot be analyzed in the DC Mode. The Scan Mode is used for method development.

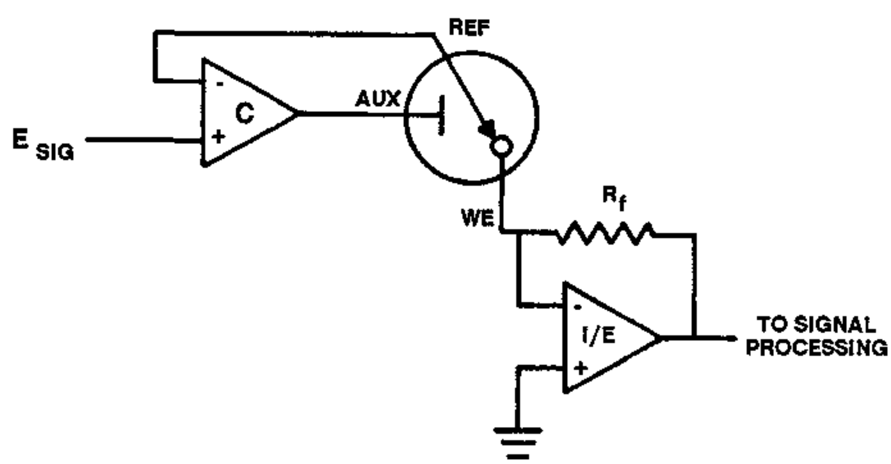

Figure 1-5 Detector Electronics

2

HARDWARE OVERVIEW

The chapter contains descriptions of:

- The Waters 464
- Front panel controls and components
- Rear panel controls and components

2.1 WATERS 464 DESCRIPTION

The Waters 464 Pulsed Electrochemical Detector (Figure 2-1) is a low noise, high sensitivity detector designed for High Performance Liquid Chromatography (HPLC). Its sensitivity and flexibility (in Pulse, DC, or Scan modes) allows the Model 464 to be used in a wide range of chromatographic applications, including catecholamines in plasma, sulfite analysis in foods, iodide, CN^- and other ions.

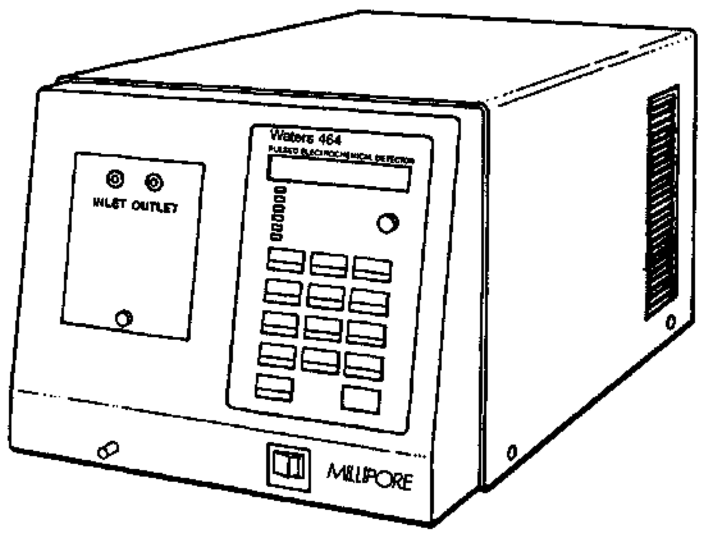

Figure 2-1 Waters 464 Pulsed Electrochemical Detector

2.1.1 Hardware

High sensitivity analyses are a result of thin-layer flow cell designed to maximize signal-to-noise ratio and prevent bubble entrapment. The signal-to-noise ratio is further enhanced by very low noise circuitry and advanced noise rejection electronics. The result of this design is a usable sensitivity at the 100 pico-amp full-scale current range.

2.1.2 Hardware Options

Options include a choice of either a stainless steel or nonmetallic flow cell and a second potentiostat board to allow dual potentiostat operation (available with stainless steel flow cell only). A wide selection of electrode materials and configurations are available to support applications in the biomedical, pharmaceutical, and environmental areas.

2.2 FRONT PANEL CONTROLS AND COMPONENTS

The front panel controls and components (Figure 2-2) consist of the:

- Display
- Brightness control
- Indicators
- Keypad
- POWER switch
- Flow cell drawer assembly (after installation)
- Overflow

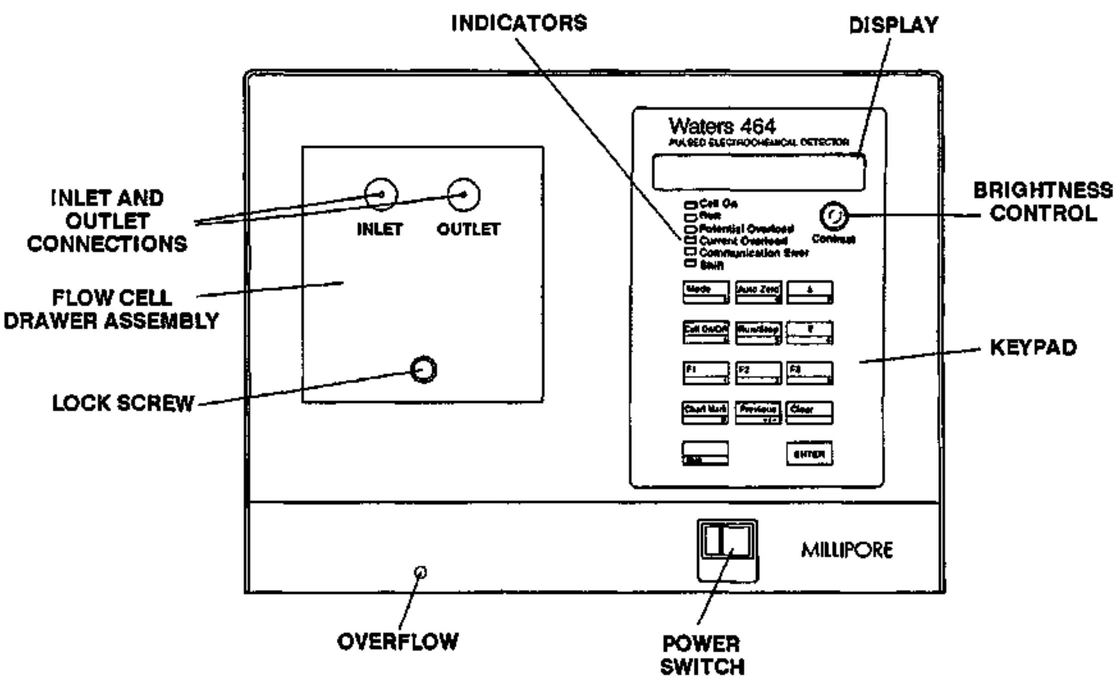

Figure 2-2 Front Panel Controls and Components

2.2.1 POWER Switch

Pushing the POWER switch to ON supplies power to the Waters 464 and initiates a two-minute warm-up delay. Pushing the POWER switch to OFF turns off the Waters 464.

2.2.2 Indicators

Cell On The Cell On indicator is a green LED that lights when the Waters 464 is in an operating mode (DC, Scan, or Pulse). The green light indicates that the flow cell is active and the programmed potential is being applied between the working and reference electrodes.

Run The green Run indicator lights when the Waters 464 is in an operating mode (DC, Scan, Pulse). This LED indicates that the detector is measuring cell current and making updated information available to a recording device from the rear panel.

Potential Overload The yellow Potential Overload LED lights when the detector is unable to maintain the desired potential difference between the working and reference electrodes. This condition generally indicates a problem in the flow cell, the flow cell connections, or the reference electrode. A trapped bubble in the flow cell is an example of a condition that can cause a potential overload.

Current Overload The yellow Current Overload LED lights when the current exceeds the full-scale current range.

Communication Error This indicator is for future detector options.

Shift Shift status allows the entry of numbers on the keypad. Press the SHIFT key, and then enter any number from 0 through 9.

2.2.3 Keypad

Numbers The keypad in Shift status contains the standard numbers 0 through 9. The numbers are active only when ENTER and a parameter name are displayed on the bottom line of the screen.

+/- The +/- key allows you to change the sign of the entered value. Press the key once to reverse the sign of a displayed number. The key has no effect if you have not entered a number.

CLEAR The CLEAR key permits you to re-enter a desired value. If you have entered a value and have not yet pressed ENTER, press CLEAR to clear the numbers from the screen, allowing you to re-enter the desired value. Pressing CLEAR and then ENTER, PREVIOUS, or MODE—without entering in a new value—enters the cleared value.

MODE Pressing the MODE key at any time except during a calibration or a self test causes the Select Mode screen to appear. If a run is in progress, data acquisition stops, but the flow cell remains on.

During a setup, the MODE key can function as an ENTER key. If you press MODE instead of ENTER, the parameter value that you keyed in is entered, and the Select Mode screen appears. When you go back to the same setup, the parameter for the value you entered appears on the display. (See ENTER and PREVIOUS keys.)

UP/DOWN ARROWS (↑ ↓) The UP and DOWN ARROWs allow you to scroll through the operating modes or toggle between parameter selections. The arrows are active when their symbols are displayed within parentheses on the bottom line of the screen.

ENTER Pressing the ENTER key enters the keyed values or selections into memory and causes the next screen of the selected mode to appear on the display. (See MODE and PREVIOUS keys.)

F1, F2, and F3 These keys are currently inactive and reserved for future use.

PREVIOUS Pressing the PREVIOUS key enters the keyed values or selections into memory and causes the previous screen of the selected mode to appear on the display. (See MODE and ENTER keys.)

AUTO ZERO Pressing the AUTO ZERO key automatically sets the background current baseline to zero. The flow cell must be on and the 464 running (Cell On and Run indicators lit) for AUTO ZERO to operate. AUTO ZERO affects both recorder and integrator outputs.

If you press AUTO ZERO when the Current Overload indicator is not lit, the detector is auto zeroed immediately. However, if the Current Overload indicator is lit, the auto zero is delayed for about 10 seconds since the software must reset the overload condition first. (If an overload condition still exists after you press AUTO ZERO, you may have selected an incorrect current range.)

CELL ON/OFF When the flow cell is off, pressing CELL ON/OFF electrically connects the current measurement circuitry to the flow cell. When the flow cell is on, pressing CELL ON/OFF disconnects the circuitry from the flow cell. When the flow cell is off, the programmed initial potential is not applied to the cell and no scanning, pulsing, or monitoring can be done.

RUN/STOP When a run is not in progress (Run indicator not lit), pressing the RUN/STOP key starts the programmed measurement. When a run is in progress (Run indicator lit), pressing the RUN/STOP key stops the measurement, although the programmed initial potential continues to be applied to the flow cell.

CHART MARK Pressing the CHART MARK key causes a chart mark to appear on the recorder output. The direction of the spike is determined by the +OUTPUT parameter on the System Mode screen. If you select ANODIC, the mark appears as a positive spike; if you select CATHODIC, it appears as a negative spike.

2.2.4 Display Components

Liquid crystal multi-line display (LCD) All Waters 464 menus are displayed on the 5/8-inch by 3 3/4-inch Liquid Crystal Display (LCD) at the top center of the front panel. The display is divided into two lines.

The top line displays the selected mode of operation and, whenever the Run indicator is lit, a real-time current reading.

The bottom line displays the selected parameter, units, toggle selections, and the current value of the selected parameter.

Brightness control The knob under the display adjusts the brightness and viewing angle of the screen display.

2.2.5 External Flow Cell Drawer Assembly

The flow cell drawer assembly contains the flow cell through which the samples pass.

Detector inlet line connection Tubing connector used to connect a column to the detector. Recommended inlet tubing is .009-inch ID with 25 inches of tefzel or 12 inches of stainless steel.

Detector outlet line connection Tubing connector used to connect the detector to waste. Recommended outlet tubing is .020-inch ID with the same lengths as the inlet tubing.

Lock screw Screw used to loosen/tighten the flow cell drawer assembly.

2.3 REAR PANEL CONTROLS AND COMPONENTS

The rear panel controls and components consist of the:

- Recorder output connectors
- Analog output connectors
- External events connectors
- IEEE interface connector
- Grounding jack connectors
- Power input assembly

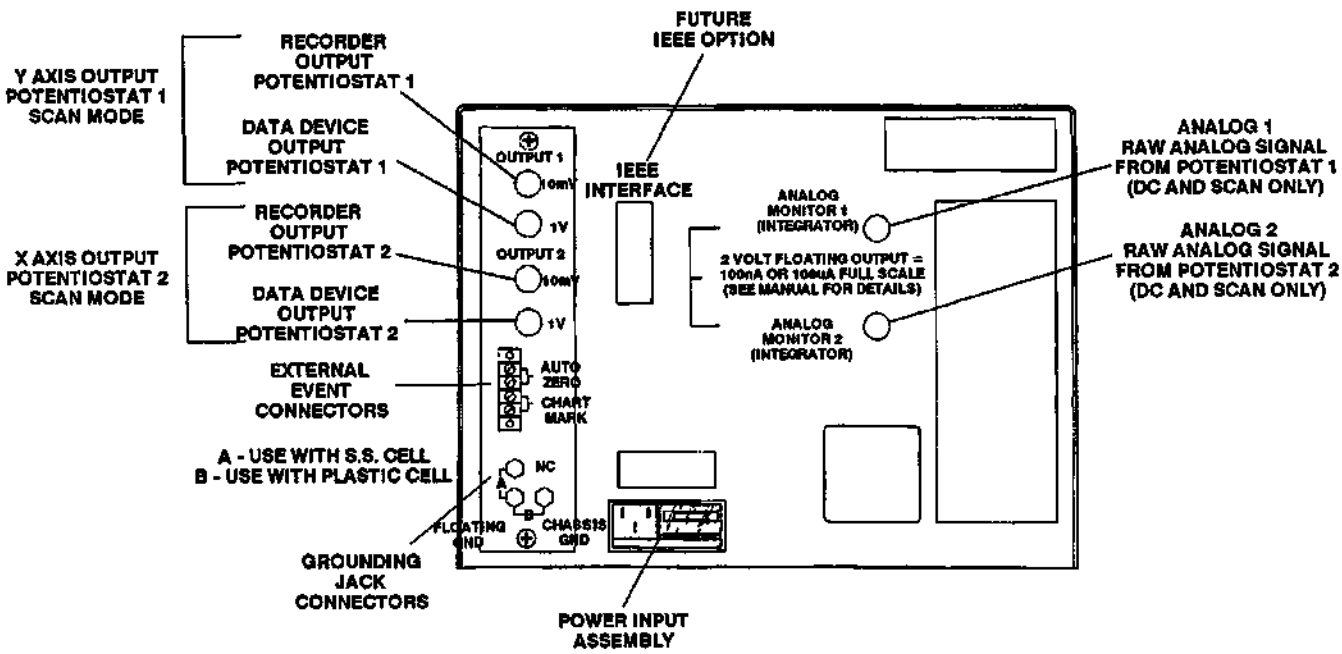

Figure 2-3 Rear Panel Controls and Components

2.3.1 Recorder Output Connectors

OUTPUT 1 The 10 mV and 1V OUTPUT 1 BNC connectors are used to transmit the current response signal (I_1) from the flow cell to a recorder or data device. The 10 mV connector is typically used for a strip chart recorder; the 1 V connector is typically used for output to a 1 Volt Full Scale (VFS) data device or to record a cyclic voltammogram on an XY recorder. Select the connector compatible with your recording device.

OUTPUT 2 If the optional second potentiostat is installed and the detector is in Dual Potentiostat Mode, the 10 mV and 1V OUTPUT 2 BNC connectors are used to transmit either the second current response signal (I_2) or the difference current (ΔI: the difference between I_1 and I_2) from the flow cell to a recorder or data device. You select the signal to be recorded with the OUTPUT 2 parameter on the 2PSTAT screen.

In Scan Mode, the OUTPUT 2 connector is automatically reconfigured to transmit a voltage proportional to the applied potential. Select the 10 mV or 1 V output (whichever is correct for your recording device) and send to the x-axis of an XY recorder.

You can connect OUTPUT 2 to any device that records potential. Select the connector (10mV or 1V) compatible with your recording device.

2.3.2 Analog Output Connectors

ANALOG MONITOR 1 (INTEGRATOR) The ANALOG 1 BNC connector allows you to send Waters 464 raw analog output from a DC or Scan run to a data device.

CAUTION

The Analog Monitor output should not be used in Pulse Mode. It bypasses the digital circuitry designed to smooth pulsed output data. Without this smoothing, pulsed output is not usable.

The 2 V full scale output is directly proportional to the 100 nA or 100 µA full scale current range that you selected from the System Mode screen.

The output is independent of the Sensitivity Setting and Time Constant parameters, but is affected by the Offset parameter.

CAUTION

This output is referenced to the floating ground of the potentiostat board—not chassis ground. Any device connected to the output must have a differential input with good common mode rejection (we recommend >100 dB). If you connect the output to an input BNC connector shell at chassis ground (as is the case on an oscilloscope), your results will be unreliable.

ANALOG MONITOR 2 (INTEGRATOR) The ANALOG 2 BNC connector works exactly like the ANALOG 1 connector, except that the output comes from the optional second potentiostat board.

2.3.3 External Events Connectors

There are two external events connectors on the rear panel:

- CHART MARK
- AUTO ZERO

CHART MARK Same function as manual CHART MARK key. Contact closure automatically assures acceptance of a marker signal from an external device such as an automatic injector. Causes CHART MARK contact closure signal output to be activated.

AUTO ZERO Same function as manual AUTO ZERO key. Contact closure automatically assures that each chromatogram starts at the same baseline. Sets both recorder and integrator output to zero.

2.3.4 IEEE Interface Connector

IEEE INTERFACE The IEEE INTERFACE connector is only used when the Communications option, a future option, is installed.

2.3.5 Grounding Jack Connectors

N.C. This jack is unconnected. To maintain the floating ground condition in the Waters 464, simply insert the plug into the A position, as shown on the rear panel.

FLOATING GROUND This jack is connected to the Waters 464 floating ground. It must be connected to the N.C. jack to maintain the floating ground, or to the CHASSIS GROUND jack to change the Waters 464 flow cell and output reference to chassis ground.

CHASSIS GROUND To operate the Waters 464 as a grounded instrument, you must connect the CHASSIS GROUND and FLOATING GROUND jacks.

NOTE: In operation with a nonmetallic cell, the jack should be in the B position. In this configuration, the working electrode is at chassis ground.

If a 316 stainless steel flow cell is used, the jack should be in the A position. The working electrode here is at virtual ground.

2.3.6 Power Input Assembly

Plug-in line voltage card The plug-in line voltage card, located at the lower edge of the fuse compartment, allows you to conform to the line voltage at your location.

Line fuse The line fuse is located in the fuse compartment on the right side of the power input assembly. Make sure you use a 3 amp slow-blow fuse for 120 V operation and a 2 amp slow-blow fuse for 240 V operation.

Line cord jack The line cord jack is designed to connect to 120 V or 240 V (48 to 62 Hz) wall power through a female plug. Slide the window on the power input assembly fully to the right to insert the line cord. This prevents the fuse and plug-in line voltage card from being exposed while power is applied to the system.

3

INSTALLATION

This chapter describes:

- Unpacking and inspection
- Flow cell preparation
- Fluid connections
- Environmental considerations
- Power-up
- Initial testing procedures

3.1 UNPACKING AND INSPECTION

The Waters 464 is shipped in two cartons.

The first carton contains:

- The Pulsed Electrochemical Detector (without the flow cell and flow cell drawer assembly)
- A line cord, BNC cables, and adaptors
- Grounding jack and fuses
- An Operator's Manual

The second carton contains:

- One flow cell drawer assembly (including cell top)
- The working electrode block, gaskets, reference electrode, reference electrode bushing (nylon), and O-ring
- Additional frits and filling solution
- Fittings and tubing
- Polishing discs, polishing compounds, and a glass plate for cleaning the working electrode

Unpacking To unpack the Waters 464:

1. Locate the packing list.
2. Unpack the contents of the shipping cartons. As you unpack, check carton contents against the packing list for completeness.
3. Save the shipping cartons for future transport or shipment.

Inspection If you see any damage or discrepancy when you inspect the components, immediately contact the shipping agent and Waters. The toll-free phone number at Waters is 1-800-252-HPLC.

For further information, refer to Appendix D, Warranty/Service Information.

3.2 PREPARING THE FLOW CELL

Before using the detector, complete the assembly of the flow cell. If you are using a nonmetallic flow cell, refer to Figure 3-1 for an exploded view. For a stainless steel flow cell, refer to Figure 3-2 for an exploded view. Flow cell preparation includes:

- Installation of gasket and working electrode block
- Installation of the reference electrode (including nylon bushing and O-ring)
- Partial insertion of the flow cell drawer assembly
- Electrical connections
- Installation of the flow cell drawer assembly

All flow cell components are to be installed on the flow cell drawer assembly (Figure 3-1 or 3-2). Lay the drawer on its side—with components up—for the installation procedures that follow.

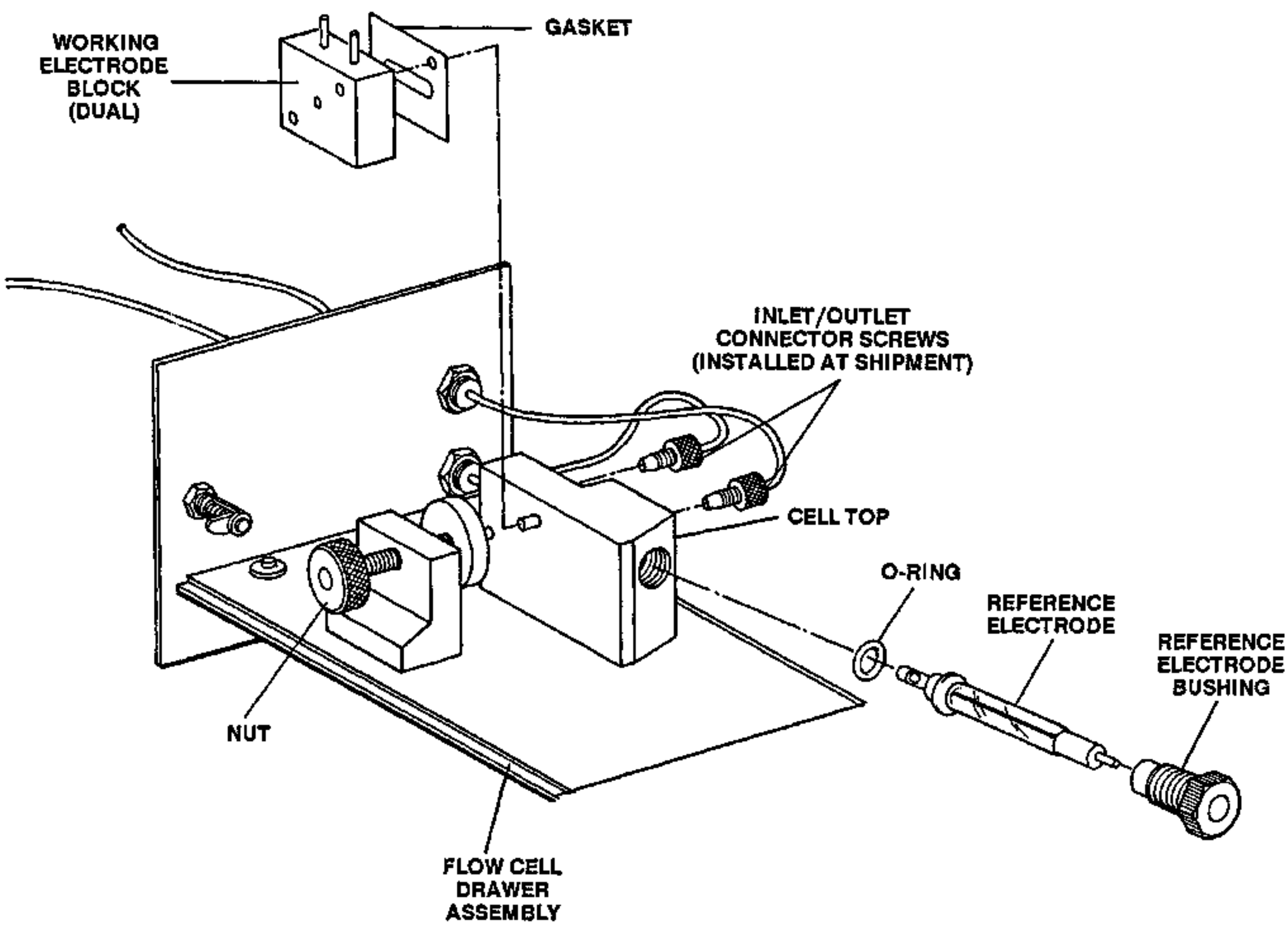

Figure 3-1 Exploded View of Nonmetallic Flow Cell

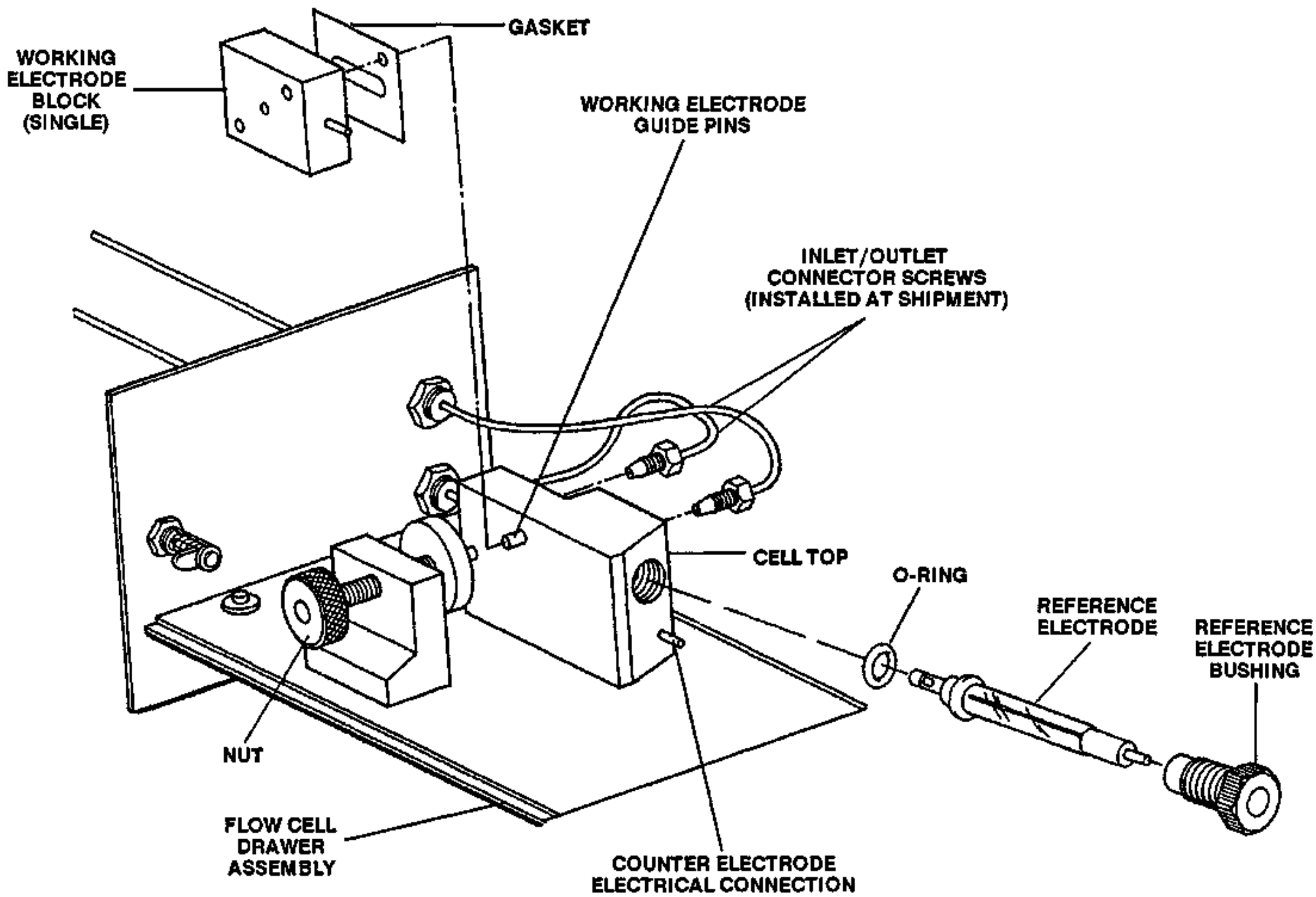

Figure 3-2 Exploded View of Stainless Steel Flow Cell

3.2.1 Installing the Gasket and Working Electrode Block

The working electrode block contains the working electrode. For the nonmetallic flow cell, there is a dual working electrode. For the stainless steel flow cell, there is a single working electrode. To install the gasket and working electrode block (Figure 3-3):

1. Fit the Teflon™ gasket onto the bottom of the cell top.

2. Fit the working electrode block onto the bottom of the cell top.

NOTE: If you are using a nonmetallic flow cell, make sure the two electrical connections point upward—toward you. If you are using a stainless steel flow cell, make sure the single electrical connection points to the rear of the flow cell drawer assembly.

3. Turn the nut to secure the gasket and the working electrode block.

NOTE: Do not overtighten the nut when using a nonmetallic flow cell. Overtightening can cause cell leakage.

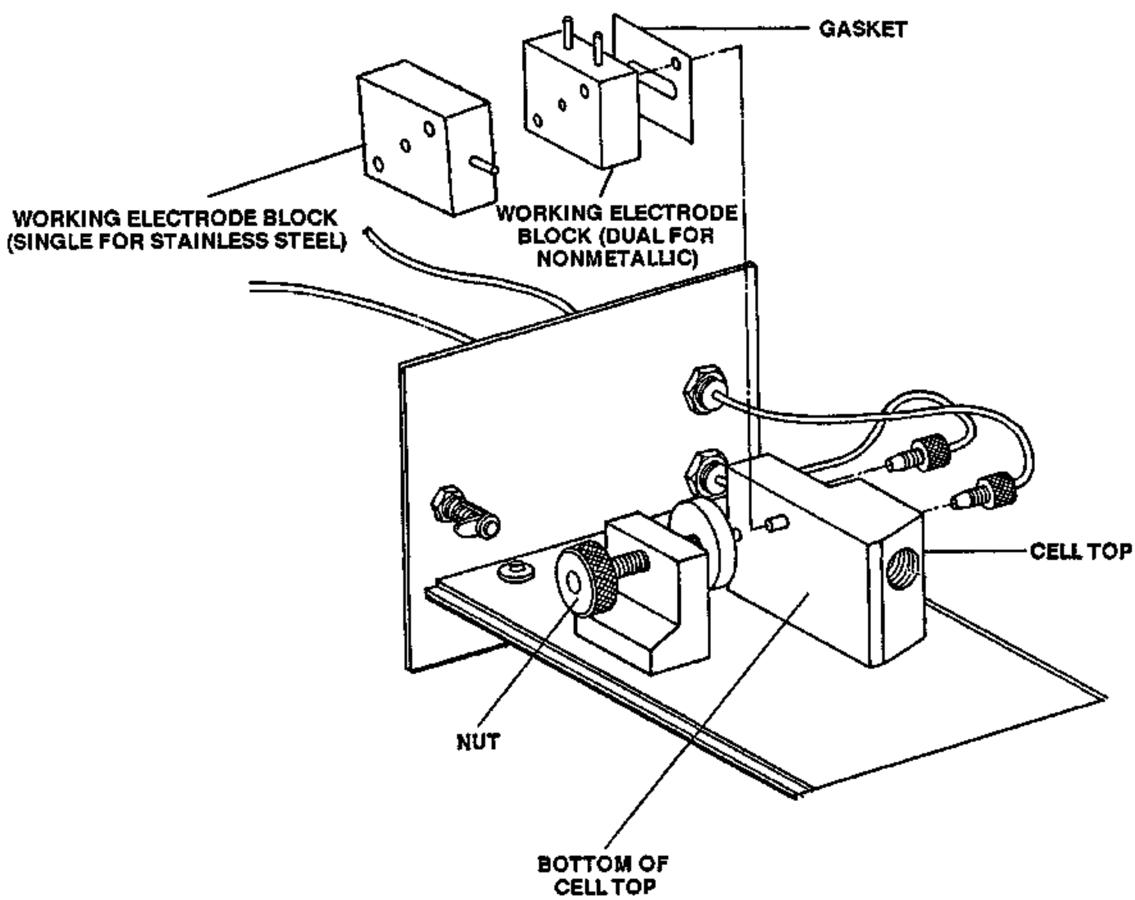

Figure 3-3 Installing the Gasket and Working Electrode Block

3.2.2 Installing the Reference Electrode

This procedure includes installation of the O-ring, reference electrode, and nylon reference electrode bushing. To install the reference electrode (Figures 3-4 and 3-5):

1. Gently remove the blank protective cap from the front end of the reference electrode.

2. Place the KALREZ® O-ring over the frit end of the reference electrode, and slide it into place against the glass lip of the electrode.

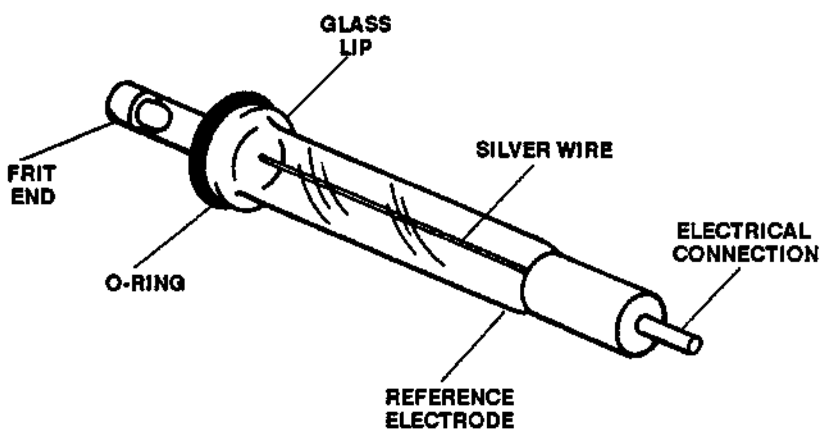

Figure 3-4 Installing the O-ring

3. Tap out all the air bubbles from the reference electrode. You may need to place the electrode with frit end down to make sure the tip is saturated.

4. Insert the reference electrode (frit end first) into the hole in the side of the cell top (Figure 3-5).

5. Place the nylon bushing over the end of the reference electrode, and screw it in until finger-tight.

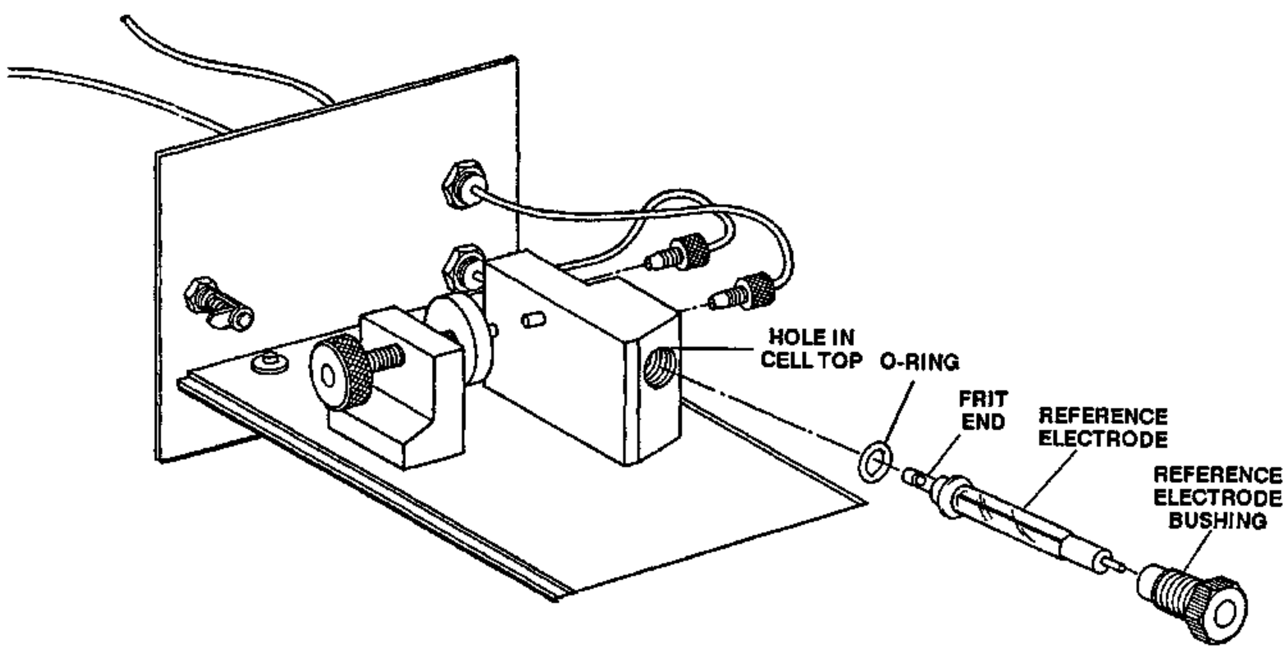

Figure 3-5 Installing the Reference Electrode

3.2.3 Partial Insertion of Flow Cell Drawer Assembly

Before you complete electrical connections, you must partially insert the flow cell drawer assembly into the detector.

Align the flow cell drawer assembly in the detector's insertion tracks, and slide the drawer part way into the detector. Make sure you have access to the three electrode leads labeled Counter, Reference, and Working I.

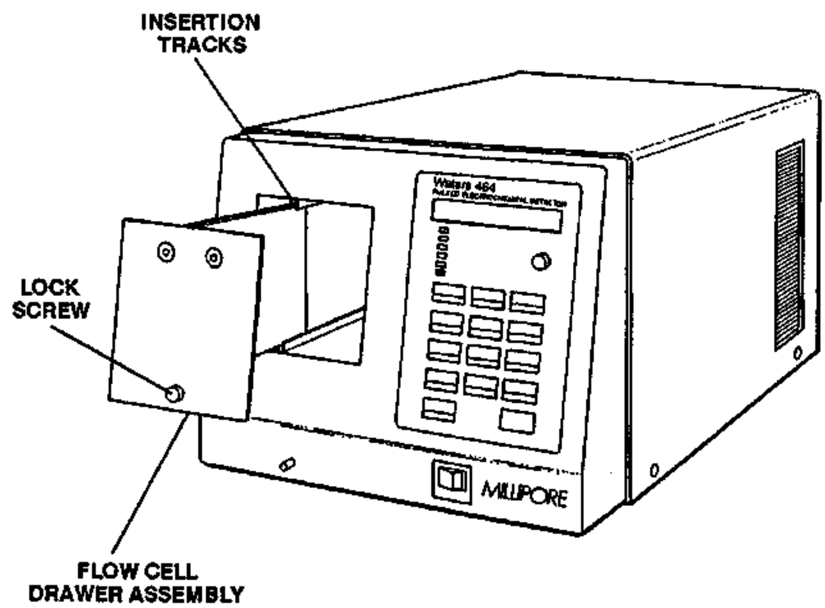

Figure 3-6 Inserting the Flow Cell Drawer Assembly

3.2.4 Electrical Connections

Electrical connections vary depending on which flow cell design is used.

Nonmetallic flow cell

To complete the electrical connections for a nonmetallic flow cell (Figure 3-7):

1. Connect the counter electrode lead (labeled COUNTER) to the right-hand pin projecting from the front of the dual working electrode.
2. Connect the reference electrode lead (labeled REF) to the pin projecting from the reference electrode.
3. Connect the working electrode lead (labeled WORKING I) to the left-hand pin projecting from the front of the dual working electrode.

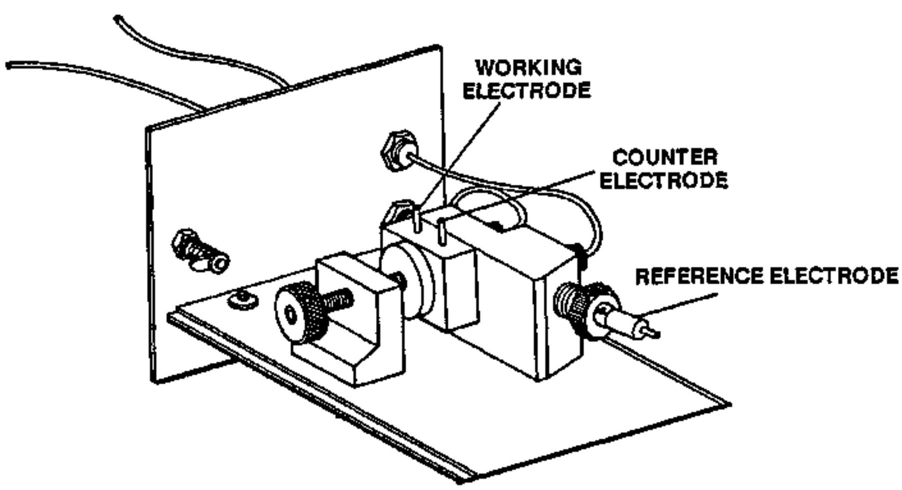

Figure 3-7 Electrodes for Nonmetallic Flow Cell

Stainless steel flow cell

To complete the electrical connections for a stainless steel flow cell (Figure 3-8):

1. Connect the counter electrode lead (labeled COUNTER) to the counter electrode pin at the cell top (the pin projecting from the cell top, adjacent to the reference electrode).

2. Connect the reference electrode lead (labeled REF) to the pin projecting from the reference electrode.

3. Connect the working electrode lead (labeled WORKING I) to the pin projecting from the rear of the working electrode block.

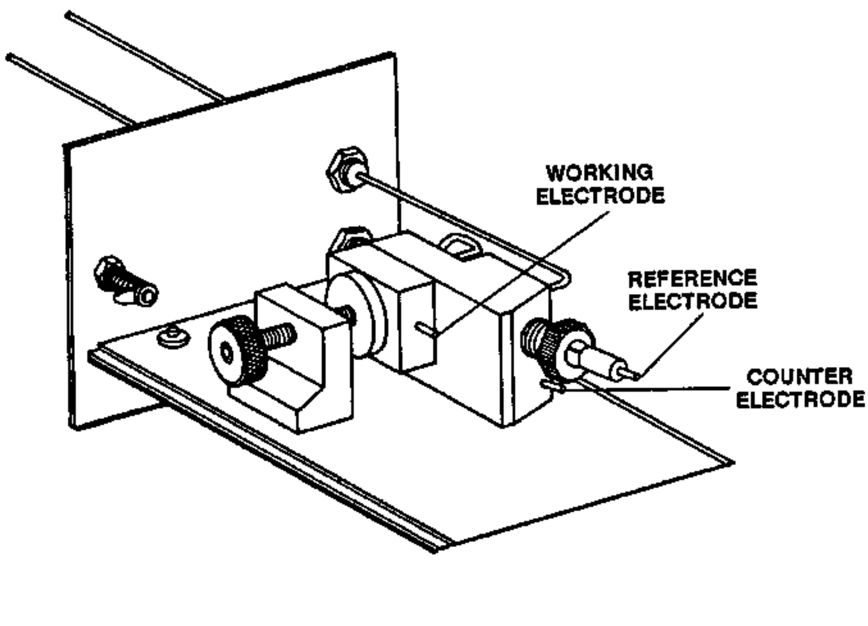

Figure 3-8 Electrodes for Stainless Steel Flow Cell

3.2.5 Installing the Flow Cell Drawer Assembly

To install the flow cell drawer assembly (Figure 3-9):

1. Align the flow cell drawer assembly in the insertion tracks, and slide the drawer into the detector. Do not crimp the three electrode leads.

2. Tighten the flow cell drawer assembly lock screw to secure the drawer in the detector.

NOTE: Failure to screw the cell in tightly can result in a poor chassis ground connection and excessive noise.

NOTE: If a nonmetallic cell is being used or if the cell is electrically isolated from the rest of the system via a plastic union to the red plastic connector, insert the black plug into the connector at the rear of the detector marked CHASSIS GROUND. If the cell is not isolated, do not use the black plug.

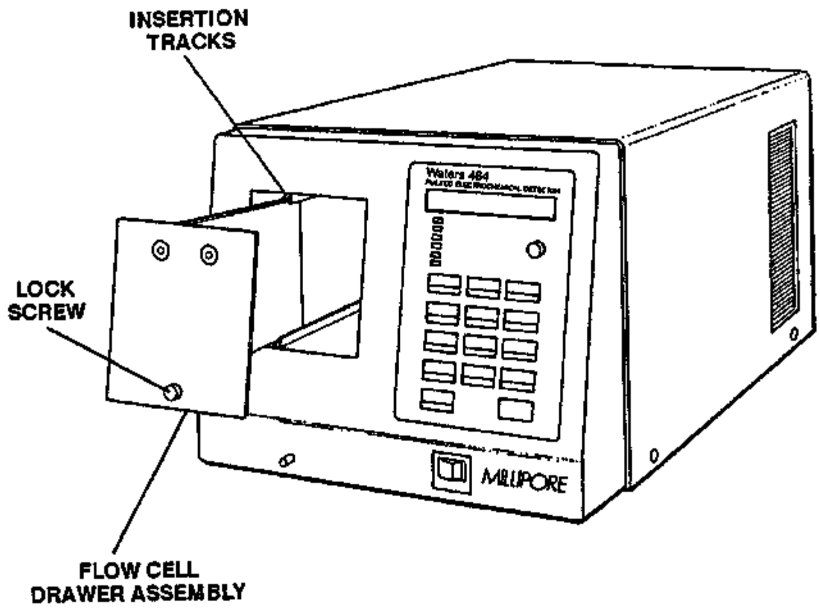

Figure 3-9 Installing the Flow Cell Drawer Assembly

3.3 FLUID CONNECTIONS

After flow cell installation is complete, make the fluid connections. To complete the fluid connections (Figure 3-10):

1. Connect the detector's inlet tube (the one terminated in the red plastic compression screw) to the HPLC column. Use .010-inch I.D. or less tubing.

2. Connect the detector's outlet tube to a waste container. Use .020-inch I.D. or greater tubing. If bubble formation is a problem, a short length (3") of .009-inch I.D. tubing may be added.

3. Make sure that all system plumbing connections are leak-free.

NOTE: When making fluid connections from a nonmetallic flow cell, use plastic (Tefzel® or PEEK) tubing and fittings as supplied with the cell drawer assembly. When making fluid connections from a stainless steel flow cell, use stainless steel tubing and fittings as supplied with the stainless cell drawer assembly.

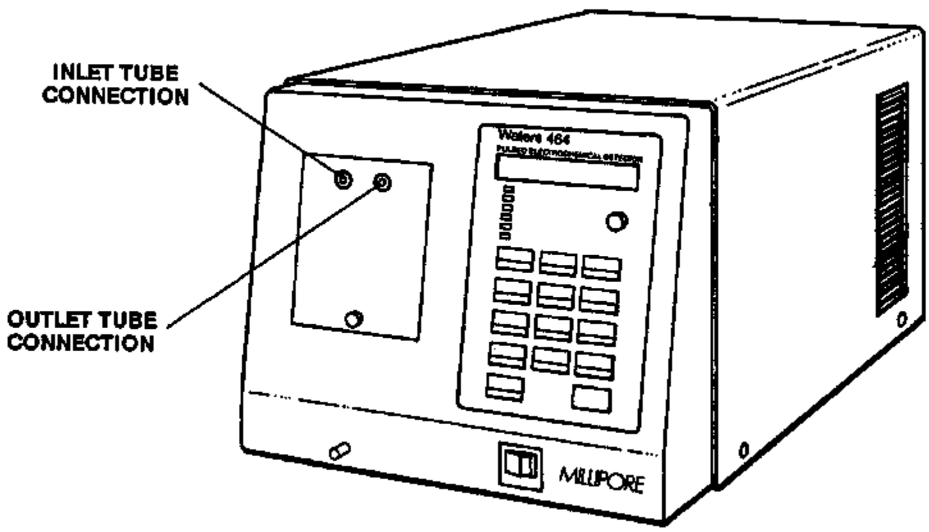

Figure 3-10 Inlet and Outlet Tubing

3.4 ENVIRONMENTAL CONSIDERATIONS

If excessive electrical noise from large laboratory equipment (centrifuge, oven, or vortex mixer) interferes with your detector, you can do one of the following:

- Use a plastic column union to isolate the column's stainless steel tubing from the detector's stainless steel tubing.
- Use plastic tubing to connect the column to the detector. You may need to remove the stainless steel tubing from the inlet of the detector if you are using a stainless steel flow cell.

In either case, install the black grounding jack, located in the lower left corner of the rear panel (Figure 3-11), in the B position, CHASSIS GROUND. In this position, the jack puts the working electrode at virtual ground for quieter operation.

NOTE: Do not remove FLOATING GROUND (A position) unless the detector is isolated from the rest of the liquid chromatography system. The nonmetallic flow cell needs to be in the B position or CHASSIS GROUND.

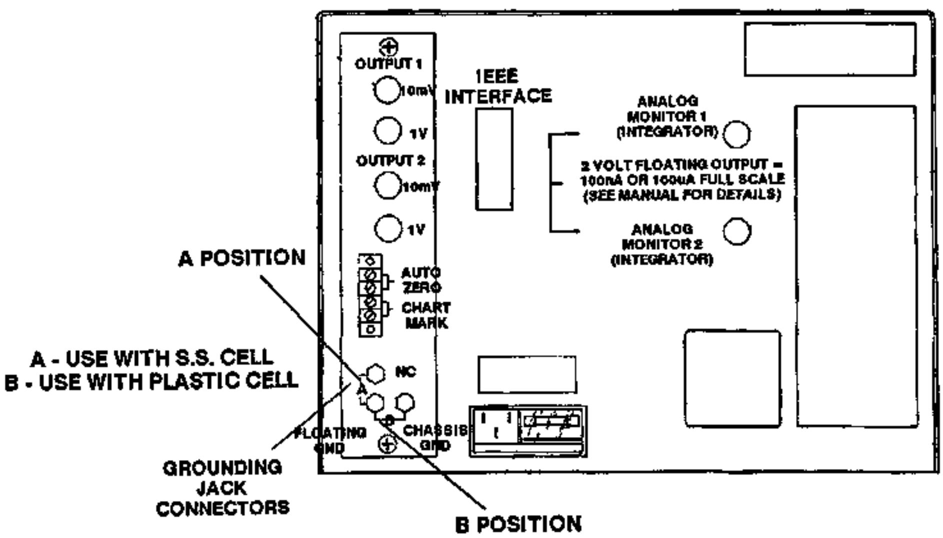

Figure 3-11 Installing Jumper in B Position

3.5 POWER-UP

To power up the Waters 464 (Figure 3-12):

1. Look through the clear plastic window next to the line cord connector. Make sure the detector is set to the proper line voltage.

   The power voltage setting should match the line voltage at your location (nominally, 120 V or 240 V).

   CAUTION

   If the line voltage is improperly set, do not operate the Waters 464 until the proper voltage is set.

2. Plug the female end of the line power cord into the power cord connector on the detector's rear panel.

3. Plug the male end of the line power cord into a grounded wall socket with the proper line voltage.

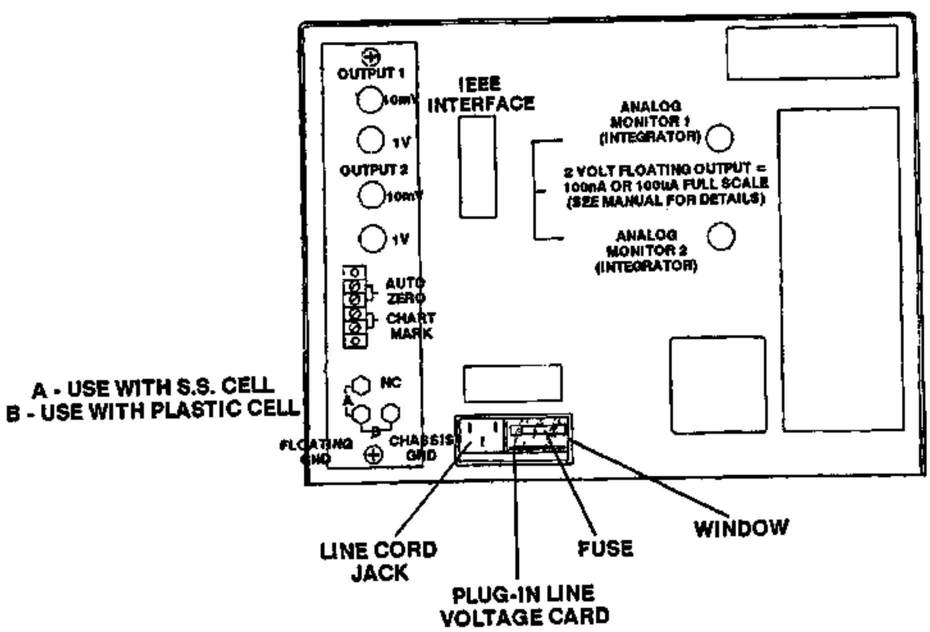

Figure 3-12 Power Input Assembly

4. Push the detector's POWER switch (Figure 3-13) into the ON position. The detector has a two-minute warm-up period. At the beginning of the warm-up period, the screen first displays:

   PERFORMING STARTUP TESTS

   The screen then immediately displays:

   2 MINUTE WARM UP DELAY
   TIME REMAINING 120 SEC

   The displayed screen counts down to 0 seconds, and the system beeps at the end of the warm-up period. The screen displays:

   M464 SOFTWARE REV X.X
   SELECT MODE (↑ ↓): DC

The Waters 464 is ready for the initial testing procedure.

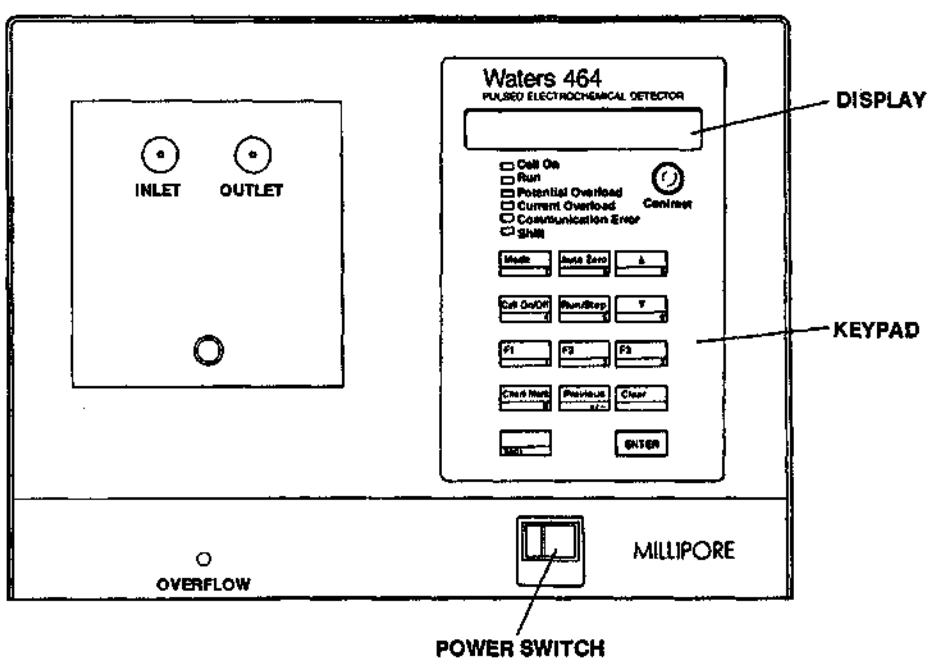

Figure 3-13 Front Panel POWER Switch and Keypad

3.6 INITIAL TESTING PROCEDURE

NOTE: Before running the initial testing procedure, make sure that all components of the HPLC system are leak-free.

The initial testing procedure is a run using the dummy cell to quickly test detector electronics. To run the initial testing procedure:

1. Select the System Mode with the UP or DOWN ARROW key. Then press ENTER. The screen displays:

   MODE: SYSTEM
   I UNITS: (↑ ↓) nA

2. Use ENTER to scroll to the Cell Type screen. Then toggle to DUMMY using the UP or DOWN ARROW key. The screen displays:

   MODE: SYSTEM
   CELL TYPE: (↑ ↓) DUMMY

3. Use the MODE key to select the dummy cell and exit System Mode. The screen displays:

   M464 SOFTWARE REV X.X
   SELECT MODE: (↑ ↓) DC

4. Press ENTER to select DC Mode. Using the numeric keypad for the E value and the UP or DOWN ARROW for the others, set the following values:

   - E: 500 mV
   - I RANGE: 100 nA
   - TIME CONSTANT: 1 sec
   - OFFSET: 0.00 nA

5. To start the experiment, press CELL ON/OFF and then RUN/STOP. The Cell On and Run indicators light, and a current value of -50 nA is displayed on the screen (the tolerance is +.5 nA, so any value between 49.5 nA and 50.5 nA is acceptable). A typical result is:

   MODE: DC I= -49.93 nA
   OFFSET: (↑ ↓) 0.00 nA

6. If the value is outside the acceptable range, perform the calibration procedure described in Chapter 4, Operating and Utility Modes. After calibration, repeat the initial testing procedures.

If the results are acceptable, press RUN/STOP. Then press CELL ON/OFF and return to the Select Mode screen by pressing MODE. The screen displays:

M464 SOFTWARE REV X.X
SELECT MODE (↑ ↓) : DC

If the results are still unacceptable, call 1-800-252-HPLC in the United States for assistance. International customers should call their local Waters subsidiary or representative.

4

OPERATING AND UTILITY MODES

This chapter covers:

- An overview of operating and utility modes
- Accessing/Exiting a mode
- Entering mode values
- DC Mode
- Scan Mode
- Pulse Mode
- System Mode
- Calibration Mode

4.1 OVERVIEW

The Waters 464 provides two types of modes:

- The DC, Pulse, and Scan modes are operating modes, used to make a run.

- The System and Calibration modes are utility modes, used for activities such as set up, calibration, and testing.

4.2 ACCESSING/EXITING A MODE

After the initial warm-up period, the Waters 464 displays:

M464 SOFTWARE REV X.XX
SELECT MODE (↑ ↓): DC

You can access any one of the five modes (DC, Scan, Pulse, System, or Calibration) from this Select Mode screen.

To access the default DC Mode, press ENTER. To access any other mode, use the UP or DOWN ARROW to select the desired mode, and press ENTER (Figure 4-1). To exit a given mode, press MODE.

Press MODE to return to the initial screen.

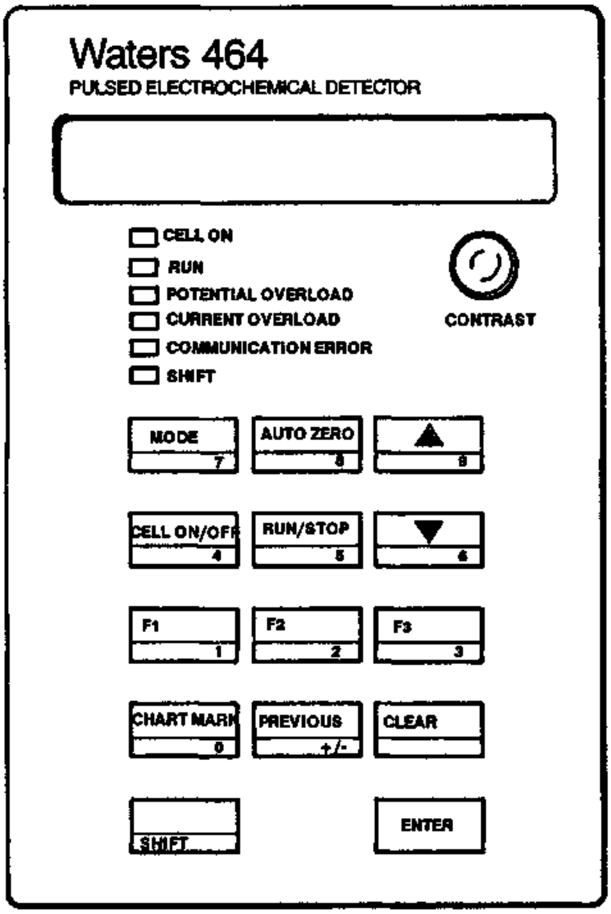

Figure 4-1 Keypad on Front Panel

4.3 ENTERING MODE VALUES

After you enter the DC, Scan, Pulse, or System mode, you can enter parameter values.

NOTE: Detailed procedures for the Calibration Mode are outlined in Section 4.8.

DC, Scan, or Pulse Mode

There are three ways to enter parameters in the DC, Scan, or Pulse modes.

- If the screen displays the Up and Down Arrows, use the UP or DOWN ARROW to scroll to the desired value, and press ENTER.
- If the screen does not display the arrows, press SHIFT, enter the value on the numeric keypad, and press ENTER. Pressing ENTER deactivates Shift status.
- Press ENTER without entering a value on the numeric keypad to accept the default parameter.

System Mode There are two ways to enter parameters in the System Mode.

- Use the UP or DOWN ARROW to scroll to the desired value, and press ENTER.
- Press ENTER without changing a value to accept the default parameter.

4.4 DC MODE

In a DC Mode run, the Waters 464 applies a steady DC potential to the flow cell and measures the resulting current versus time. DC is the most widely used mode.

There are four DC Mode parameters:

- E (potential)
- I Range (current)
- Time Constant
- Offset

E E is the potential applied between the working and reference electrodes whenever the flow cell is on. Press SHIFT, and enter the desired potential on the numeric keypad. The default value is 0 mV. Press ENTER after you select the value.

I Range I Range is the maximum current that can be measured during an experiment. Use the UP or DOWN ARROW to select a range. The default value is 100 nA. The two current ranges extend from 0.1 nA to 100 nA and from 0.1 µA to 100 µA in a 1-2-5 sequence (µA = microamps; nA = nanoamps).

Time Constant Time Constant allows you to control the degree of smoothing of high frequency noise. The value you enter determines the number of data points averaged for each point taken.

A low value (short time constant) allows you to measure more rapidly changing flow cell currents. However, noise filtering is not as effective as with a higher value.

For a system without rapidly changing flow cell currents, a higher value (longer time constant) produces a smoother signal than a lower value. More points are averaged for each point taken, resulting in a smoother curve.

NOTE: Specifying a time constant too high relative to the peak width can distort the peak amplitude.

Use the UP or DOWN ARROW to select a time constant. The range of values is from .1 sec. to 5 sec. in a 1-2-5 sequence. The default value is 1.0 sec.

NOTE: 1-2-5 sequence = .1, .2, .5, 1, 2, 5, 10, 20, 50, 100

Offset Offset is the value of the offset current. The offset current is added to the flow cell current to produce the baseline. The current offset range is -160.00 nA to +160.00 nA (or -160 µA to +160 µA on the µA scale). Use the UP or DOWN ARROW to select desired offset, or press AUTO ZERO on the front panel keypad to automatically set the baseline to zero. The default value is 0.00 nA.

NOTE: AUTO ZERO overrides manual offset.

4.5 SCAN MODE

In a Scan Mode run, the Waters 464 scans from one potential to another (and back again, if desired) and measures the resulting current versus potential. You can use Scan Mode to perform cyclic staircase or linear sweep voltammetry experiments. You can also use it with your choice of working electrodes to pre-screen the sample response in the mobile phase.

4.5.1 Scan Mode Parameters

There are seven Scan Mode parameters:

- E1 (initial potential)
- E2 (final potential)
- I Range
- Scan Cycles
- Scan Rate
- Offset
- I=/E=

E1 E1 is the potential where the scan starts—the initial potential. It is also the potential applied to the electrode when RUN/STOP is not active and a scan is not in progress. The range is -2000 mV to +2000 mV. Press SHIFT, and enter the desired potential on the numeric keypad. The default value is 0 mV.

E2 E2 is the potential where the scan ends—the final potential. The range is -2000 mV to +2000 mV. Press SHIFT and enter the desired potential on the numeric keypad. The default value is 1000 mV.

I Range I Range is the maximum current that can be measured during a run. Select the desired range using the UP or DOWN ARROW. The current ranges extend from 0.1 µA to 100 µA and 0.1 nA to 100 nA in a 1-2-5 sequence. The default value is 100 nA. A range between 1 µA and 10 µA using a g/ml standard sample solution is often a good starting point. (µA = microamps; nA = nanoamps).

NOTE: 1-2-5 sequence = .1, .2, .5, 1, 2, 5, 10, 20, 50, 100

Scan Cycles Scan Cycles indicates the type of scan used during the measurement—half, full, or continuous. A linear sweep begins at E1, scans to E2, and stops. A cyclic scan begins at E1, scans to E2, reverses and scans back to E1, and stops. A continuous scan is an indefinitely repeated full scan. Use the UP or DOWN ARROW to select the desired scan type. The default setting is CONT (continuous).

Scan Rate Scan Rate is the rate at which the scan advances in millivolts per second. Use the UP or DOWN ARROW to select the desired scan rate. The default value is 10 mV/sec. The range extends from 1 mV/sec. to 100 mV/sec. in a 1-2-5 sequence.

Offset Offset is the value of the offset current. The offset current is added to the flow cell current to produce the current baseline. Use the UP or DOWN ARROW to select the desired offset value, or press AUTO ZERO to automatically set the current baseline to 0. The default value is 0.00 nA. The ranges extend from -160 µA to +160 µA and from -160.00 nA to +160.00 nA .

I=/E= At the beginning of a scan, the value next to I equals the real-time current value. The value next to E is the potential.

4.5.2 XY Recorder Connections

In Scan Mode, you can record the I (current) and E (potential) signals on an XY recorder. Both signals are normally transmitted to an XY recorder through the 10 mV BNC connectors on the rear panel—the I signal through OUTPUT 1 and the E signal through OUTPUT 2. To connect the detector to an XY recorder (Figure 4-2):

1. Connect the detector's 10 mV OUTPUT 1 connector (I) to the y-axis of the recorder.
2. Connect the detector's 10 mV OUTPUT 2 connector (E) to the x-axis of the recorder.

NOTE: You would also use OUTPUT 2 if the second potentiostat option is installed in the Waters 464.

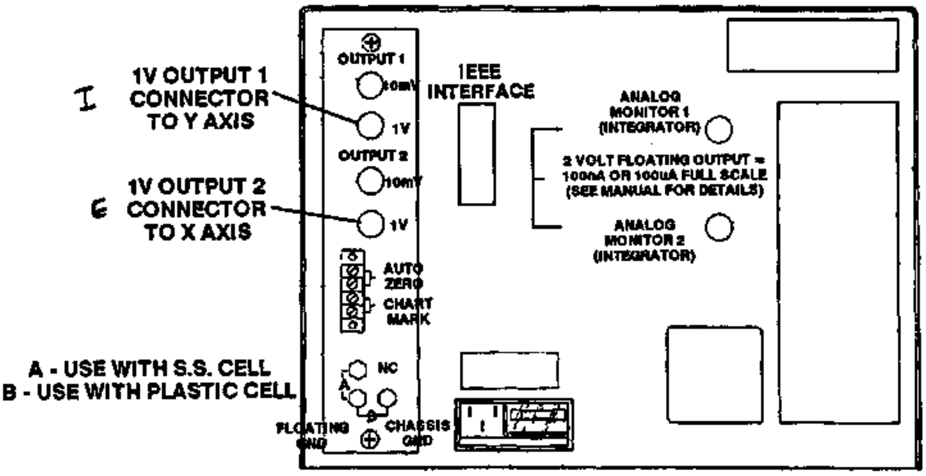

Figure 4-2 Output Connectors on Rear Panel for Cyclic Voltammetry with an XY Recorder

4.6 PULSE MODE

In a Pulse Mode run, the Waters 464 applies a repeating series of three stepped potentials to the flow cell and measures the current resulting from the first potential versus time. Since it can condition an electrode surface during the measurement, you can use Pulse Mode as an alternate technique for runs in which DC Mode does not provide an optimum response due to fouling of the working electrode. You can also use Pulse Mode to clean an electrode. Pulse Mode is generally not as sensitive as DC Mode.

There are nine Pulse Mode parameters:

- E1
- T1
- E2
- T2
- E3
- T3
- Total Pulse T
- I Range
- Offset

E1 E1 is the first potential applied to the flow cell and the only potential applied to measure the current response. The other two potentials applied to the flow cell are used for cleaning and conditioning. It is also the potential applied to the electrode when a run is not taking place. Press SHIFT, and enter the desired potential on the numeric keypad. The default value is 500 mV.

T1 T1 is the time that the E1 potential is applied to the electrode. Since the current is measured near the end of T1, the minimum value is 10 cycles. Select the desired value using the UP or DOWN ARROW. The default value is 30 cycles (0.499 sec.) at 60 Hz and 30 cycles (.60 sec.) at 50 Hz.

NOTE: See Appendix C for line cycle/millisecond conversions.

E2 E2 is the potential applied to the flow cell immediately after E1. The current cannot be measured while this potential is applied. Press SHIFT, and enter the desired potential on the numeric keypad. The default value is 1000 mV.

T2 T2 is the time that the E2 potential is applied to the electrode. The minimum value is 10 cycles. Select the desired value using the UP or DOWN ARROW. The default value is 20 cycles (0.33 sec.).

E3 E3 is the potential applied to the flow cell immediately after E2. Press SHIFT, and enter the desired potential on the numeric keypad. If 0 cycles is specified in the T3 parameter, the E3 step of the run is eliminated, regardless of the value you enter for E3. The default value is 0 mV.

T3 T3 is the time that the E3 potential is applied to the electrode. Select the desired value using the UP or DOWN ARROW. There is no minimum value for this parameter; a value of 0 eliminates the E3 potential step. The default value is 10 cycles (0.166 sec.).

Total Pulse T Total Pulse T is the sum of the T1, T2, and T3 pulse times. The total pulse time determines the number of points on a peak. Select the desired value using the UP or DOWN ARROW. If this time is 1 sec. (60 cycles) and the peak is 10 sec. wide (600 cycles), there will be 10 data points on the peak (10 sec. peak width divided by 1 sec. pulse time). Do not specify a total pulse time that results in one data point per cycle. The default value is 0.999 sec. (effectively 1 sec. or 60 cycles).

I Range I Range is the maximum current that can be measured during a run. Select the desired value using the UP or DOWN ARROW. The default value of 100 nA is a moderately sensitive starting point that gives the curve a reasonable chance of being on scale for many runs. The current ranges extend from 0.1 µA to 100 µA and from 0.1 nA to 100 nA in a 1-2-5 sequence (µA = microamps; nA = nanoamps).

NOTE: 1-2-5 sequence = .1, .2, .5, 1, 2, 5, 10, 20, 50, 100

Offset Offset is the value of the current that is added to the flow cell current to produce the current baseline. Select the desired value using the UP or DOWN ARROW, or press AUTO ZERO to automatically set the current baseline to 0. The ranges are from -160.00 µA to +160.00 µA and -160.00 nA to +160.00 nA. The default value is 0.00 nA.

4.7 SYSTEM MODE

In System Mode, you can specify parameter values and settings that will be in effect for all modes of operation.

There are four System Mode parameters:

- I Units
- Output
- Cell Type
- Beep on I Overload
- Preset Auto Zero

I Units I Units are the current units in effect whenever a current or current range is specified or displayed. Use the UP or DOWN ARROW to toggle beetween µA (microamps) and nA (nanoamps). The default is nA.

Output Output is a simple polarity switch that sets the recorder movement polarity. Use the UP or DOWN ARROW to toggle between ANODIC and CATHODIC. When set to ANODIC, output voltage is positive for oxidation current and negative for reduction current. When set to CATHODIC, the opposite occurs. The default setting is ANODIC.

Cell Type Cell Type indicates whether a real cell or the Waters 464 internal dummy cell is in use. The dummy cell consists of a 10 MΩ resistor in parallel with a 2 µfd capacitor. Use the UP or DOWN ARROW to toggle between YES and NO. The default setting is NO.

Beep on I Overload Beep on I Overload indicates whether the Waters 464 will beep when a current overload condition occurs. Use the UP or DOWN ARROW to toggle between YES and NO. The default setting is NO.

Preset Auto Zero Preset Auto Zero allows the baseline to be set at some percent of full scale. Allowable settings are ±25, ±10, and ZERO. Use this parameter only when the data integration device in use cannot integrate negative voltages (usually due to baseline drift.) Use the UP or DOWN ARROW to toggle to the desired auto zero percentage. The default setting is ZERO.

NOTE: When you select a value other than ZERO, the dynamic range of the Waters 464 is reduced by the percentage selected.

4.8 CALIBRATION MODE

In Calibration Mode you can restore the Waters 464 original default values, calibrate analog (current measurement) circuitry, and test the digital circuitry for a suspected malfunction.

There are three Calibration Mode functions:

- Default Values
- Calibrate
- Self Test

4.8.1 Default Values

The Default Values function allows you to restore the default parameter and calibration values when necessary. Use the Default Values function when:

- The lithium memory-backup battery fails. When the battery fails, the Waters 464 may power up with random analog default values, giving you unreliable run results. If your results appear unreliable (unexpected or confusing data), restore the default values and recalibrate the detector before you resume operations.

- You suspect a setup error and receive unexpected or confusing results from a run. Restore the default values, recalibrate the detector, and restart the run.

CAUTION

Selecting the default values nullifies the most recent calibration. Recalibrate the Waters 464 before making a run to ensure that subsequent results are reliable.

To restore the default values:

1. From the Select Mode screen, press the UP or DOWN ARROW until CALIBRATION appears on the screen. Then press ENTER.

2. Use ENTER to scroll to the Default Values function.

3. Use the UP or DOWN ARROW to select YES. Then press ENTER. The default parameter and calibration values are entered. The screen displays:

   RECALIBRATION REQUIRED

4. At this point, use the Calibrate function to recalibrate the Waters 464 to make sure that the data from subsequent runs is reliable.

4.8.2 Calibrate

The Calibrate function allows you to check and calibrate most of the current measurement circuitry (analog) and some of the digital circuitry. You should calibrate the detector, for example, after restoring the default values, since using the Default Values function nullifies any previous calibration.

To calibrate the Waters 464:

1. From the Select Mode screen, press the UP or DOWN ARROW until CALIBRATION appears on the screen. Then press ENTER.

2. Use the UP or DOWN ARROW to select YES or NO on the Default Values screen and then press ENTER. The Calibrate screen is displayed.

3. Using the UP and DOWN ARROWs, select YES. Then press ENTER. The screen displays:

   MODE: CALIBRATION
   CALIBRATING PSTAT1 . . .

This indicates that calibration is in progress. It takes about 90 seconds to calibrate the current measurement circuitry for both zero and full scale values.

When calibration is successfully completed, the screen displays:

CALIBRATION COMPLETE
HIT ANY KEY TO CONTINUE

At this point, you can examine the calibration values. The screen displays:

MODE: CALIBRATION
READ CALIB VALUES (↑ ↓) NO

Use the UP or DOWN ARROW to select YES or NO. Then press ENTER.

If you select NO, the Waters 464 automatically calibrates the second potentiostat, if the option is installed. The calibration procedure for the second potentiostat is identical to the one for the first potentiostat. If the second potentiostat is not installed, the Self Tests begin.

If you select YES, the screen displays five different values, in sequence.

NOTE: If you press ENTER at any time during this process, the Self Tests begin.

The first four values correspond to the zero readings in four different current ranges: .1, 1, 10, and 100. The current range for the displayed zero reading is listed in parentheses next to PSTAT 1 ZERO.

The screen displays:

VALUES: (↑ ↓) CONT. (ENTER)
PSTAT 1 ZERO (.1) XXXXX

The XXXXX zero value changes four times, in relation to the changing current range value.

XXXX for the first four zero readings (at .1, 1, 10, and 100 current ranges) must range from -1000 to +1000. If the values are not within this range, an error condition exists.

The final value is for the full scale range. The screen displays:

VALUES: (↑ ↓) CONT. (ENTER)
PSTAT 1 FULL SCALE XXXXX

The XXXXX value must range from 9000 to 11000. If the value is not within this range, an error condition exists.

The Waters 464 now calibrates the second potentiostat, if installed, and then begins the Self Tests.

NOTE: Press MODE at this point to access the Self Test function, if necessary.

4.8.3 Self Test

The Self Test function allows you to test the analog and digital circuitry in the Waters 464. It is a much more comprehensive test of the detector's operating condition than the Calibrate function, since it tests much more of the analog circuitry and virtually all of the digital circuitry.

The self test consists of ten tests:

- RAM Test
- LC Display Test
- Beeper Test
- Keypad Test
- Front Panel Indicator Test
- Auto-Injector Interrupt Test
- Recorder DAC Test (digital)
- Analog Tests (three)
  - Calibration
  - Bias DAC Zero Test
  - Combined Bias and Offset DAC Test

NOTE: To perform the Self Test completely, you will need a digital voltmeter with at least a 4 1/2 digit readout and a DC accuracy of 0.05 percent.

If you detect an error condition during any of the tests, call 1-800-252-HLPC for service information. International customers call their local Waters subsidiary or representative.

To begin the tests:

1. From the Select Mode screen, select the Calibrate Mode. Use the UP or DOWN ARROW to select the Self Test function.

2. Use the UP or DOWN ARROW to select YES on the Self Test screen. Press ENTER. The first test in the Self Test sequence is the RAM Test. The test begins after you press ENTER.

RAM Test

This first self test checks and verifies that RAM is present and functional.

The screen displays:

RAM TEST:
PERFORM TEST? (↑ ↓)　　NO

Press the UP or DOWN ARROW key to read either YES or NO in the lower right corner of the screen. To perform the test, press ENTER when YES appears on the screen. The screen displays:

RAM TEST:
TEST IN PROGRESS

If RAM passes the test and the 464 is the standard configuration (no second potentiostat), the screen displays:

AUX RAM FAIL AT 0000HEX
HIT ENTER

The screen acknowledges that auxiliary memory (potentiostat #2) is not installed. If a second potentiostat is installed, the LC Display Test continues.

A primary RAM failure indicates an error condition.

LC Display Test

Next in the self test sequence is the LC Display Test. The first part of the self test checks the LC display. The test displays the matrix for each character of the lower line of the display, with all pixels filled in and each matrix underlined.

The upper line of the screen displays:

PRESS ENTER IF LINE 2 OK

Check the lower line of the display for missing pixels.

Next, the test displays the matrix for each character of the upper line of the display, with all pixels filled in and each matrix underlined.

The lower line of the screen displays:

PRESS ENTER IF LINE 1 OK

Check the upper line of the display for missing pixels.

Missing pixels indicate an error condition. Note their display positions.

Beeper Test

Next in the self test sequence is the Beeper Test. When the LC Display Test is complete, the screen displays:

BEEPER TEST:
PRESS ENTER TO STOP BEEP

If you hear a steady beep, press ENTER. The tone should be continuous and fairly loud. A soft or intermittent beep indicates an error condition.

Keypad Test

The next test in the self test sequence is the Keypad Test. When the Beeper Test is complete, the screen displays:

HIT ALL KEYS, ENTER LAST

This message displays throughout the Keypad Test.

Press each of the keys on the front panel keypad, both the commands and the shifted numbers, ending with ENTER. (Pressing ENTER ends the test.)

As you press each key, the symbol or label corresponding to the key appears on the lower left part of the screen. An incorrectly displayed label indicates an error condition.

Front Panel Indicator (LED) Test

The next test in the self test sequence is the Front Panel Indicator Test. This test checks five of the six front panel indicators. It does not test the Potential Overload indicator because the indicator is not driven by the digital circuitry. When the Keypad Test is complete, the screen displays:

CELL ON LIGHT LIT
PRESS ENTER TO CONTINUE

Press ENTER if the Cell On indicator is the only indicator lit. Then repeat the procedure for the Run, Communication Error, Shift, and Current Overload indicators. If any of the five indicators do not light or more than one indicator lights at the same time, an error condition is indicated.

Auto-Injector Interrupt Test

The next test in the self test sequence is the Auto-Injector Interrupt Test. This test checks for proper handling of an interrupt transmitted via the rear panel (normally a signal from an auto-injector). You may want to perform this test if you have just installed an auto-injector and cannot get it to operate correctly. The test indicates whether the problem is with the Waters 464.

NOTE: If you have no use for this test, press ENTER in response to each prompt, without sending an interrupt to the rear panel. You can ignore the failure indication and go on to the next test.

Unwanted interrupts

The first part of the Auto-Injector Interrupt Test checks for spurious (unwanted) interrupts. As soon as you press ENTER for the last indicator in the Front Panel Indicator Test, the screen displays:

SPURIOUS AUTO ZERO TEST
HIT ENTER TO START

The bottom line of the screen then displays:

TESTING PLEASE WAIT

If no unwanted interrupt occurs, the screen displays:

SPURIOUS CHART MARK TEST
HIT ENTER TO START

The bottom line of the screen then displays:

TESTING PLEASE WAIT

If no unwanted interrupt occurs, the screen moves on to Normal Interrupts and displays:

MOMENTARILY SHORT AUTO-
ZERO THEN PRESS ENTER

An unwanted interrupt indicates an error condition.

Normal interrupts

The next portion of the Auto-injector interrupt Test checks for proper handling of interrupts. After the spurious Chart Mark test, the screen displays:

MOMENTARILY SHORT AUTO-
ZERO THEN PRESS ENTER

Use a short wire (jumper) to short the AUTO ZERO and the associated ground screw on the rear panel terminal strip. Then press ENTER. If auto zero interrupt is detected, the bottom line of the screen momentarily displays:

EXT AUTO ZERO OK

The screen then displays:

MOMENTARILY SHORT CHART
MARK THEN PRESS ENTER

Short the CHART MARK and the associated ground screw on the rear panel terminal strip. Then press ENTER. If chart mark interrupt is detected, the bottom line of the screen momentarily displays:

EXT CHART MARK OK

The inability to detect a normal interrupt indicates an error condition.

Recorder DAC Test (Digital)

The Recorder DAC (Digital to Analog Converter) Test checks the accuracy of the digital DAC output voltages from the detector's rear panel. When the interrupt test is complete, the screen displays:

> **OBTAIN A 4 1/2 DIGIT DVM**
> **(OR BETTER) & HIT ENTER**

Locate a digital voltmeter with at least a 4 1/2 digit readout and a DC accuracy of 0.05 percent. Then press ENTER. At this point, follow the screen instructions. You will need to:

1. Connect the digital voltmeter to the four BNC connectors on the detector's rear panel (Figure 4-4).

2. Measure the voltages. The detector passes the test if the readings are within the limits listed on the screen. A reading outside of the limits indicates an error condition.

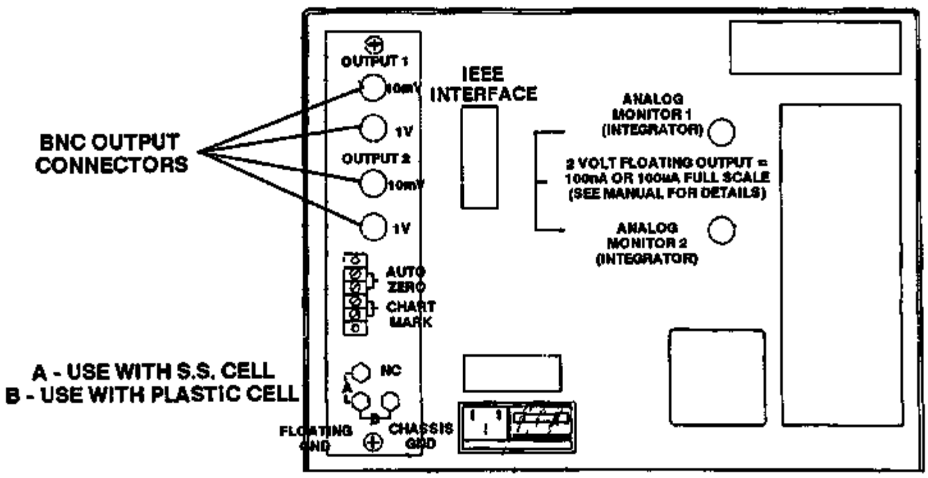

Figure 4-3 Digital Voltmeter Connection for Recorder DAC Test

Analog Tests

There are three Analog Tests:

- Calibration
- Bias DAC Zero Test
- Combined Bias and Offset DAC Test

When the Recorder DAC Test is complete, the screen displays:

DIGITAL TESTS COMPLETE
DO ANALOG TESTS?(↑ ↓) NO

Calibration

Use the UP or DOWN ARROW to select YES, and press ENTER. The screen displays:

PERFORMING CALIBRATION

The Run indicator lights, and the Calibration Test runs for a few minutes. If the calibration is successful, the screen displays:

CALIBRATION OK
PRESS ENTER TO CONTINUE

The calibration values for a zero reading on the 0.1 nA (nanoamp) scale for the first potentiostat are displayed:

VALUES:(↑ ↓) CONT: (ENTER)
PSTAT1 ZERO (.1) XXXXX

If you want to continue, press ENTER. If you want to read the values, use the arrows to read values at .1, 1, 10, 100, and Full Scale nA on Pstat 1. Values for the .1, 1, 10 and 100 nA readings should be between -1000 and +10000. The Full Scale value should be between 9000 and 11000.

If the calibration fails, the screen displays:

CALIBRATION ERROR
PRESS ENTER TO CONTINUE

When you press ENTER, the calibration values for a zero reading on the 0.1 nA scale for the first potentiostat are displayed:

VALUES:(↑ ↓) CONT: (ENTER)
PSTAT1 ZERO (.1) XXXXX

In a Calibration Error condition,use the UP or DOWN ARROW to scroll through the screens and record the values (.1, 1, 10,100, and Full Scale) from each screen. Press ENTER to access the Bias DAC Zero test.

Both the CALIBRATION ERROR message and marginal values after successful calibration indicate an error condition.

Bias DAC Zero Test

The next test in the self test sequence is the Bias DAC (Digital to Analog Converter) Zero Test. This test checks the applied voltage offset for the first potentiostat.

Press ENTER after the Calibration Test to automatically start the Bias DAC Zero Test. When the test is complete, the screen displays:

PSTAT1 TEST I = X.XX nA
IF 0 +/- .5 nA PRESS ENTER

X.XX is the current reading. If the current reading is 0 +/- .5, press ENTER to go on to the next test. An out-of-tolerance reading indicates an error condition.

The last test in the self test sequence is the combined Bias and Offset DAC (Digital to Analog converter) test.

Combined Bias and Offset DAC Test

The first test first checks potentiostat 1 at a positive polarity. The screen displays:

PSTAT1 TEST I = X.XX nA
IF 40 +/- 2 nA PRESS ENTER

X.XX is the current reading. It should be 40 +/- 2 nA. If so, press ENTER.

The second test checks potentiostat 1 at a negative polarity. The screen displays:

PSTAT1 TEST I = X.XX nA
IF 40 +/- 2 nA PRESS ENTER

X.XX is the current reading. It should be -40 +/-2 nA.

5

BASIC OPERATION

This chapter outlines a basic procedure for making a run.

5.1 OVERVIEW

Use the following procedure to make a run and record the resulting peak or peaks. Before making a run, make sure the Waters 464 is properly installed. (See Chapter 3, Installation.) For detailed setup information during the experiment, refer to Chapter 4, Operating and Utility Modes.

5.2 MAKING A RUN

To make a run with recorder output:

1. Connect the recorder to the 10 mV or the 1 V OUTPUT 1 connector (Figure 5-1) on the rear panel of the Waters 464 (whichever is appropriate).

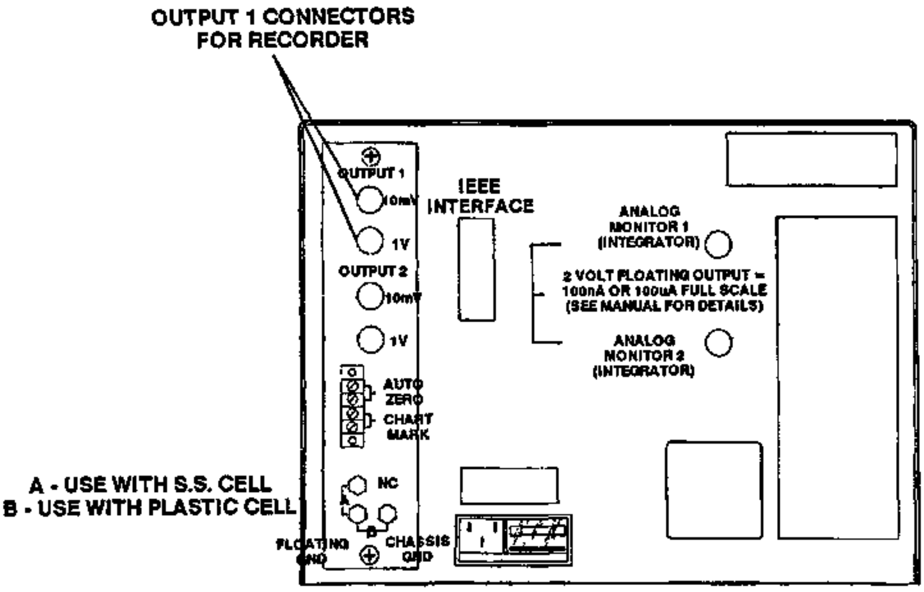

Figure 5-1 OUTPUT Connectors for Recorder

2. After the initial warm-up period, the screen displays:

   M464 SOFTWARE REV X.XX
   SELECT MODE (↑ ↓): DC

   Use the UP or DOWN ARROW to select the desired mode (DC, Scan, Pulse, System, or Calibration), and press ENTER (Figure 5-2).

3. Press SHIFT, and enter the setup values when appropriate. Refer to Chapter 4, Operating and Utility Modes.

4. Press Cell ON/OFF to turn the flow cell on. The Cell On indicator lights.

5. Press RUN/STOP to start the run. The Run indicator lights. At this point, you can monitor the display and the Current Overload indicator to keep track of the flow cell current.

6. Allow the baseline to stabilize. This can take from 15 minutes to over an hour, depending on the potential and mobile phase in use. A cold start, beginning a run immediately after applying power to the detector, also increases stabilizing time.

7. After the baseline stabilizes, install the pen, start the recorder, inject the sample, and press CHART MARK or AUTO ZERO (if desired).

8. If the setup values are appropriate for the sample, the peak(s) are recorded, the displayed current value begins to increase, and the Current Overload indicator does not light.

   NOTE: Oxidations are represented by negative current values and reductions by positive current values on the display.

9. To continue recording after the first sample, simply inject another sample without stopping the recorder or the Waters 464.

   To discontinue recording, stop the recorder. Press CELL ON/OFF to remove programmed potential from the flow cell. The Cell On indicator goes off.

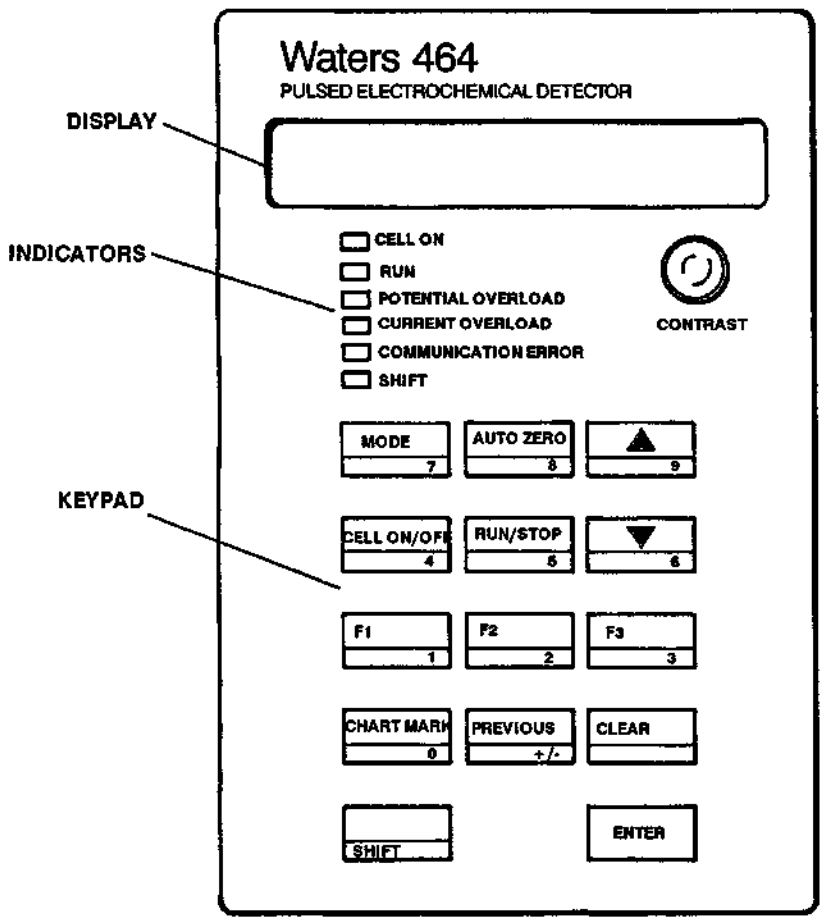

Figure 5-2 Keypad/Indicators on Front Panel

6

MAINTENANCE

This chapter covers:

- Replacing the line fuse
- Adjusting the voltage range
- Renewing the reference electrode
- Maintaining the working electrode

6.1 REPLACING THE LINE FUSE

The line fuse is located in the fuse compartment on the right side of the power input assembly on the detector's rear panel.

CAUTION

Carefully observe the following instructions when replacing the line fuse.

To remove and replace the fuse:

1. Push the POWER switch to off.

2. Unplug the line power cord.

3. Slide the window fully to the left.

4. Rotate the lever labeled FUSE PULL out and to the left, and remove the fuse.

5. Insert the new fuse, and slide the window back to the right.

NOTE: Use a 3 amp slow-blow fuse for 120 V operation and a 2 amp slow-blow fuse for 240 V operation. (Figure 6-1).

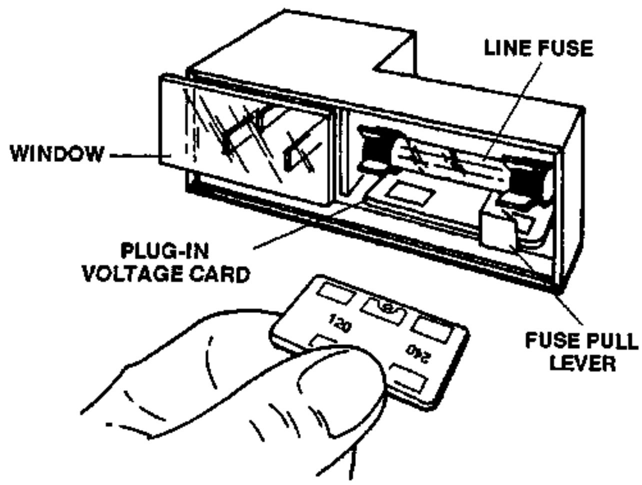

Figure 6-1 Line Fuse and Voltage Range Adjustment Card

6.2 ADJUSTING VOLTAGE RANGE

A plug-in circuit card located at the lower edge of the fuse compartment allows you to change the voltage range to conform to the line voltage at your location (Figure 6-1).

CAUTION

Carefully observe the following instructions when adjusting the voltage range.

To adjust the detector's voltage range:

1. Push the POWER switch to off.
2. Unplug the line power cord.
3. Slide the window to the left.
4. Carefully remove the plug-in voltage card.
5. Re-insert the card so that correct line voltage is visible when you slide the window back to the right.

6.3 RENEWING THE REFERENCE ELECTRODE

When the reference electrode becomes fouled (causing loss of sensitivity), you will need to renew it. Renewal of the reference electrode involves the following procedures:

- Removal/Disassembly of the reference electrode
- Renewal of the reference electrode
- Reinstallation of the reference electrode

The mechanical procedure is the same for nonmetallic and stainless steel cells.

Reference electrode removal/disassembly

To remove/disassemble the reference electrode:

1. Press CELL ON/OFF to turn the flow cell off.

2. Loosen the lock screw and remove the flow cell drawer assembly from the detector.

3. Loosen the nylon reference electrode bushing, and remove the reference electrode from the side of the flow cell top (Figure 6-2).

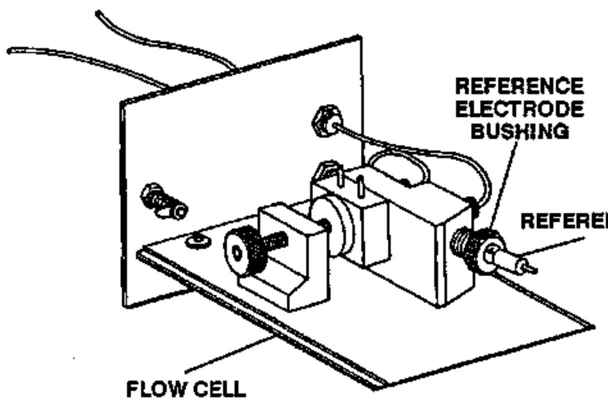

Figure 6-2 Removing Reference Electrode From Flow Cell

3. Pull or cut the old frit from the glass electrode body (Figure 6-3).

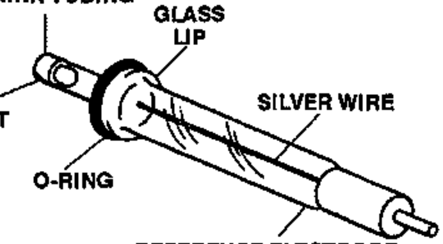

Figure 6-3 Reference Electrode

4. Shake the reference electrode to empty out all of the filling solution. A dark oxidation film may be present on the silver wire. This will not affect your chromatography.

Reference electrode renewal

Items you need to renew the reference electrode are in the cell drawer assembly start-up kit.

To renew the reference electrode (Figure 6-3):

1. Refill the electrode to about 1/4-inch from the top with the appropriate fresh filling solution for your application (either .4 M NaOH or 3 M Na Cl/Saturated AgCl).

2. With minimal handling, place a fresh frit into the teflon heat shrink tube.

   NOTE: The frit is made of a porous teflon or unfired Vycor™ (whichever is appropriate for your application) and is subject to contamination from airborne contaminants or handling.

3. Place the teflon heat shrink tubing and frit on the end of the reference electrode. Make sure there are no gaps between the electrode's glass lip and the frit.

4. While rotating the electrode, use a heat gun set at a moderate temperature to seal the frit onto the glass reference electrode.

CAUTION

Excessive heating may cause the filling solution to vaporize and push the frit away from the electrode end.

5. Use a sharp razor blade to trim the teflon heat shrink tube flush with the frit.

6. Place the O-ring over the frit end of the reference electrode, and slide it into place against the glass lip of the electrode.

   NOTE: If a leak occurs at the O-ring location, replace the O-ring.

7. Tap the frit with your finger to eliminate any air bubbles at the frit end of the electrode. Place the electrode in a vertical position for a few minutes (frit side down) to allow the entrapped air in the porous frit to escape.

Reference Electrode Reinstallation

To reinstall the reference electrode refer to Section 3.2.1, Installing the Gasket and Working Electrode Block.

6.4 MAINTAINING THE WORKING ELECTRODE

Cleaning a glassy-carbon electrode

To restore sensitivity to a glassy-carbon working electrode, clean it with a solution of 1 g chromic acid (CrO_3) in 10 ml of reagent grade sulfuric acid (H_2SO_4). Use safety glass to prevent burns from this solution.

To clean the electrode:

1. Remove the working electrode from the analytical cell, handling it only by the edges, and place it on a level surface.

2. Place one drop of the chromic acid or sodium dichromate solution on the electrode surface. Allow acid to remain for 15 minutes.

3. Rinse the electrode in Milli-Q® grade water.

NOTE: If the electrode is not cleaned by the above method, polishing with alumina (described below) is an option.

Cleaning a metal working electrode

To clean the metal electrodes (silver, gold, or platinum), you need the polishing kit supplied with your detector. The kit contains:

- Bottle of white alumina polish
- Syringe of yellow diamond paste
- Microcloth® discs (use with alumina)
- Texmet discs (use with diamond paste)
- Glass plate to use as a polishing surface

To clean the electrode:

1. Examine a microcloth disc for dust particles. Remove the backing, and adhere the disc to the glass plate.

2. Pour a drop or two of the alumina onto the center of the disc.

3. Place the electrode onto the disc, with the active surface facing downward. Polish the electrode for about 30 seconds by moving it over the alumina in a circular motion.

4. Rinse off the electrode and the disc with Milli-Q water. The electrode surface will appear cloudy from the alumina.

5. Place the electrode back on the disc and polish again without the alumina to restore the mirror finish.

6. Sonicate the electrode in Milli-Q grade water for one to two minutes to free the surface of any lodged alumina particles.

NOTE: If the electrode is too fouled to clean with alumina, use the above procedure with the Texmet® disc and the larger grit yellow-diamond paste. If you do this, be sure to repolish with the alumina to restore the best possible surface.

7

TROUBLESHOOTING

This chapter contains an overview and a troubleshooting guide for routine problems.

7.1 TROUBLESHOOTING OVERVIEW

This chapter lists possible problems and the recommended actions necessary for their correction. Problems generally fall into one of three categories:

- Electronic
- Mechanical
- Chromatographic

If you cannot correct the condition, contact the Waters Service Department at 1-800-252-HPLC. For most efficient service, have the following information available when you call:

- Name of equipment and serial number
- Symptom
- Running conditions

Electronic problems

The Waters 464 is equipped with a dummy cell, which is used to diagnose electronic problems. If the unit does not operate, first run the dummy cell test (See Chapter 4, Operating and Utility Modes). If the detector passes the test, the problem is not in the 464 detector electronics.

Mechanical and chromatographic problems

Mechanical and chromatography-related problems usually require a recorder tracing of detector output to identify the problem source.

7.2 TROUBLESHOOTING GUIDE

Symptom	Probable Cause	Recommended Action
Noisy baseline: regular frequency	Pump pulsations	Incorporate a pulse dampening system. Check to see if the pump has lost prime. Replace check valves.
	Detector waste line dripping	Place waste line below fluid level of waste container.
Noisy baseline: random noise	Electronic malfunction	Test with dummy cell.
	No electrical isolation	Place grounding plug to A (top) position.
		Install isolation union between column and detector.
	System grounded improperly	Plug all system components into single power strip (surge suppression preferred).
	Recorder voltage incorrect	Set recorder to proper output voltage.
	Output cabling incorrect	Choose proper output.
		Check wiring to recorder.
	Insufficient filter time constant	Increase time constant (DC Mode).
		Increase T1 cycles (Pulse Mode).
	Current range too sensitive	Adjust to higher current range.
	Fouling of reference electrode	Renew reference electrode filling solution and replace the frit.
	Working electrode contaminated	Clean or polish working electrode.
	Scratches on working electrode	Polish working electrode.

Symptom	Probable Cause	Recommended Action
	Air in cell	With pump flow on, loosen reference electrode bushing for 10 seconds and then re-tighten.
	Cell leakage	Tighten cell seal. Replace gasket.
		Polish electrode surface to remove imperfections.
	Leak at reference electrode	Change Kel-Rez O-Ring.
	Column contamination	Clean or replace column.
	Mobile phase contamination (high background currents)	Use HPLC reagents in mobile phase preparation.
		Passivate stainless steel components of HPLC system with 6N nitric acid. (No need to passivate detector cell.)
Loss in sensitivity	Dummy cell connected	Use the System Mode to change to the real cell.
	Current range incorrect	Adjust to lower current range.
	Applied potential incorrect	Increase applied potential until current maximum is achieved.
	Leak in cell	Tighten cell seal Replace gasket.
		Polish electrode surface to remove imperfections.
	Leak in sample injector	Tighten or replace the connections or seal of sample injector.
	Fouling of reference electrode resulting in a potential shift	Renew reference electrode filling solution, and replace the frit.

Symptom	Probable Cause	Recommended Action
	Air in reference electrode	Remove reference electrode, and tap air bubbles away from frit.
	Working electrode contaminated	Clean or polish working electrode.
	Sample auto oxidation	Prepare fresh standard or sparge the mobile phase.
	Change in mobile phase composition	Correct mobile phase pH, ionic strength, or ionic composition.
	Pump seal material shredding	Replace pump piston seals.
Baseline drift	Electronic malfunction	Test with dummy cell.
	Electrode equilibration	Allow electrode to stabilize.
		Electrochemically condition electrode prior to use.
	Fouling of reference electrode	Renew reference filling solution, and replace the frit.
	Cell leakage	Tighten cell seal. Change gasket.
		Polish electrode surface to remove imperfections.
	Leaks in Liquid Chromatography System	Tighten fittings or replace ferrules.
	Working electrode contaminated	Clean or polish working electrode.
	Column bleed	Clean or replace column.
	Mobile phase contamination	Use HPLC reagents in mobile phase preparation.

Symptom	Probable Cause	Recommended Action
	Metal corrosion from stainless steel components	Passivate stainless steel components of HPLC system with 6N nitric acid.
	Temperature fluctuations	Use column heater to stabilize temperature.
	Flow fluctuations	Replace pump seals or use different pump.
Noise spikes	Air in cell	With pump flow on, loosen reference electrode bushing for 10 seconds and then retighten it.
	Air in reference electrode	Remove reference electrode, and shake out any air.
	Air in sample	Reinject without air.
	Instrument grounding problems	Plug all system components into single power strip (surge suppression preferred).
	Voltage spikes	Plug system power strip into another circuit or incorporate a voltage surge suppressor.
	Loose electrode connections	Remove connections and inspect them for damage and then reconnect them.
	Erratic pump performance causing pressure fluctuations	Inspect pump seals and check valves. Replace if necessary.
		Incorporate a pulse dampening system.
Stairstepping baseline	Highly amplified recorder or integrator signal	Adjust recorder input voltage, increase attenuation, (reduce signal amplification), or use the analog output.

Symptom	Probable Cause	Recommended Action
Flat-top peaks	Incorrect recorder input voltage	Adjust recorder input voltage, or adjust detector output cable to proper position.
	Sensitivity set too high	Select a less sensitive current range.
Current Overload indicator remains lit	Electronic malfunction	Test with dummy cell.
	Current range too low	Select higher current range.
	Insufficient offset	Press Auto Zero.
	Mobile phase contamination (high background currents)	Use superior ingredients in mobile phase preparation.
		Passivate stainless steel components of HPLC system with 6 Nitric acid.
	Sample concentration too high	Dilute sample.
	System grounded improperly	Plug all system components into into single power strip (surge suppression preferred).
Potential Overload indicator remains lit	Electronic malfunction	Test with dummy cell.
	Reference electrode malfunction	Renew reference electrode filling solution, and replace the frit.
		Remove reference electrode connection, and inspect for damage. Then reconnect.

SPECIFICATIONS

APPENDIX A

APPENDIX A
SPECIFICATIONS

Table A-1 Waters 464 Pulsed Electrochemical Detector

General	Controlled voltage	± 2 V
	Standard output	Analog 10 mV and 1 V
	Noise specifications	10 pA peak-to-peak on 5 µf/10 MΩ cell 1 sec. Time Constant @ 1 V
	Cell type	Single electrode or dual electrode thin layer flow-bycell (all cell compo nents contained in a plug-in module)
	Cell volume (standard 4 mil gasket)	4 µL
	Cell pressure	Stainless steel: 30 psi Nonmetallic: 5 psi
	Cell housing/drawer	RF shielded stainless steel and designed for mobile phase leak protection
	Base line auto zero	Front panel or remote
	Chart mark activation	Front panel or remote

	Working potential range	±2000 mV
DC Mode	Current ranges:	First Range: 0.1 nA to 100 nA Second Range: 0.1 µA to 100 µA (selectable in 1-2-5 sequence)
	Current offset	First Range: ±160 nA Second Range: ±160 µA (manual or automatic)
	Time constant	Single Potentiostat: 0.1 to 5 sec. Dual Potentiostat: 0.2 to 5 sec. (both selectable in 1-2-5 sequence)
	Working potential range	+2000 mV in 1 mV increments

Pulse Mode	Working potential range	+2000 mV in 1 mV increments (E1, E2, and E3 pulse potentials individually programmable)
	Pulse timing	3 pulse widths programmable for each measurement (programmed in line cycles, displayed in line cycles and seconds)
	Current ranges	First Range: 0.1 nA to 100 nA Second Range: 0.1 µA to 100 µA (selectable in 1-2-5 sequence)
Scan Mode	Potential range	+2000 mV (initial and final potentials individually programmable)
	Current range	First Range: 0.1 nA to 100 nA Second Range: 0.1 µA to 100 µA (selectable in 1-2-5 sequence)
	Scan rate	1 mV/sec. to 100 mV/sec. (selectable in a 1-2-5 sequence)

PRODUCT ORDERING GUIDE

APPENDIX B

APPENDIX B
PRODUCT ORDERING GUIDE

Table B-1 List of Products

	Description	Part Number
464 Standalone Detector	With Nonmetallic Cell	31440
	With 316 Stainless Steel Cell	31441
464 Accessories	Working Electrodes:	
	Glassy Carbon Single	31463
	Gold Single	31464
	Silver Single	31465
	Platinum Single	31466
	Glassy Carbon Dual	31467
	Gold Dual	31468
	Silver Dual	31469
	Platinum Dual	31470
	Reference Electrode Ag/AgCl	31446
	Reference Electrode NaOH	31487
	Reference Electrode Frits (Vycor™) (and heat shrink tubing)	31448
	Reference Electrode Frits (Teflon) (and heat shrink tubing)	31449
	Filling Solution Ag/AgCl, 4 oz.	31458
	Filling Solution NaOH, 4 oz.	31486
	Cell Gaskets, 4 mil, Single Yellow	31454
	Cell Gaskets, 4 mil, Dual Yellow	31472
	Polishing Kit	31476
	Recorder Cable Coax	31460
	Cable Adapter (Coax - Spade)	31459
	Recorder Cable (Spade - Spade)	48918
	* Nonmetallic Cell System	31481
	* 316 Stainless Steel Cell System	31482
	2nd Potentiostat Board	31475

** Cell systems include flow cell drawer, cell top, tubing, and fittings.*

LINE CYCLE/SECOND CONVERSIONS

APPENDIX C

APPENDIX C
LINE CYCLE/SECOND CONVERSIONS

Table C-1 U.S. Line Cycle/Second Conversion (60 Hz)

Line Cycles	Seconds
10	.166
20	.333
30	.499
40	.666
50	.833
60	.999
70	1.166
80	1.133
90	1.499
100	1.666
110	1.833
120	1.999
130	2.166
140	2.333
150	2.499
160	2.666
170	2.833
180	2.999
190	3.166
200	3.333

Table C-2 European Line Cycle/Second Conversion (50 Hz)

Line Cycles	Seconds
10	.200
20	.400
30	.600
40	.800
50	1.000
60	1.200
70	1.400
80	1.600
90	1.800
100	2.000
110	2.200
120	2.400
130	2.600
140	2.800
150	3.000
160	3.200
170	3.400
180	3.600
190	3.800
200	4.000

WARRANTY/SERVICE INFORMATION

APPENDIX D

APPENDIX D
WARRANTY/SERVICE INFORMATION

WARRANTY INFORMATION

Limited product warranty

Millipore Corporation, Waters Chromatography Division (**Waters**) provides this limited product warranty (the Warranty) to protect customers from non-conformity in the product workmanship or materials. The Warranty covers all new products manufactured by Waters and its subsidiaries.

The Warranty is as follows:

Waters warrants that all products sold by them will be of good quality and workmanship. The products will be fit for their intended purpose(s) when used strictly in accordance with Waters instructions for use during the applicable warranty period.

The foregoing warranty is exclusive and in lieu of all other express and implied warranties, including but not limited to fitness for any other purpose(s). In no event will Waters be liable for consequential, economic or incidental damages of any nature. Waters reserves the right not to honor this warranty if the products are abused by the customer. The Warranty will not be deemed to have failed of its essential purpose so long as Waters is able and willing to repair or replace any non-conforming part or product.

Warranty service

Warranty service will be performed at no charge and at Waters option in one of three ways:

- A service representative will be dispatched to the customer's facility.
- The product will be repaired at a Waters repair facility.
- Replacement parts with appropriate installation instructions will be sent to the customers.

Non-conforming parts or products will be repaired, replaced with new or like-new parts, or refunded in the amount of the purchase price, when the product is returned. Warranty service will be performed only if the customer notified Waters within 30 days of discovering any non-conformity.

Unless otherwise agreed at the time of sale, warranty service will not be provided by dispatching a service representative when the equipment has been removed from the initial installation location to a new location outside the home country of the selling company.

Warranty service exceptions

Warranty service will not be performed on:

- Any product or part which has been repaired by others, improperly installed, altered, or damaged in any way.
- Product or parts identified prior to sale as not manufactured by Waters. In such cases, the warranty of the original manufacturer will apply.
- Products that malfunction because the customer has failed to perform maintenance, calibration checks, or observe good operating procedure.

Repair or replacement will not be made:

- For expendable items such as filament devices, panel lights, fuses, batteries, and seals, if such items were operable at at the time of initial use.
- Because of decomposition due to chemical action.
- For used equipment.
- Because of poor facility, operating conditions, or utilities.

Warranty period

The warranty period begins when the product is installed or, in the case of a customer installation, 15 days after shipment from Waters. In no case will the warranty period extend beyond 15 months from date of shipment. If an item is replaced during its warranty period, the replacement part will be warranted for the balance of the original warranty period.

The warranty period for Waters 464 components is as follows:

1 YEAR WARRANTY
All printed circuit boards Switches
90 DAY WARRANTY
Cables Main analytical cell body
30 DAY WARRANTY
Service and workmanship Replacement parts
EXPENDABLE ITEMS
Fuses Seals Tubings/fittings Gaskets Electrodes

Where to place orders	**Mail Orders**	Millipore Corporation, Waters Chromatography Division, 34 Maple Street, Milford, MA 01757, Attention: Order Processing Department
	Telephone Orders*	1-800-252-HPLC Customer Sales Department
	Telex Orders*	94-8413
	Fax Orders*	(508) 872-1990
	International	Consult the listing of Waters Sales/Service offices at the end of this manual.

***Confirming orders mailed after a telephone, telex, or fax order has been placed must be clearly marked "CONFIRMING" to avoid duplication.**

How to place orders

Delays or errors in processing orders are frequently caused by incorrect or incomplete information. To minimize delays and errors in processing your orders, please list part numbers in ascending numerical order, and provide all of the following information:

1. Catalog numbers and descriptions.
2. Quantity desired.
3. Complete purchase order number - orders cannot be processed without it. *Requisition numbers are insufficient.*
4. Complete Ship To address and marking if applicable.
5. Complete Bill To address if other than Ship To.
6. Required delivery date.
7. Method of transportation desired.
8. Name and telephone number of person to contact if clarification is required.

Pricing Prices listed are FOB Milford, MA, unless otherwise agreed. Prices and product information contained in any catalog or price list were current at the time of printing. In a continuing effort to provide the finest products available, Waters reserves the right to change specifications, models, or prices without notice and without liability for such changes. Where price changes have occurred, prices prevailing at time of receipt of your order will apply.

Terms of payment Our terms are net 30 days from invoice date with approved credit. If your credit has not been previously established with Waters, our terms are payment in advance or COD.

SHIPMENTS-DAMAGES-CLAIMS-RETURNS

Shipments As all shipments are made FOB Milford, MA, we suggest insurance be authorized on all shipments. Instruments and major components will be packed and shipped via surface, unless otherwise requested. Supplies and/or replacement parts are packed and shipped via UPS, UPS Blue, air parcel post, or parcel post unless otherwise requested.

Damages The Interstate Commerce Commission has held that carriers are as responsible for concealed damage as for visible damage in transit. Unpack shipment promptly after receipt as there may be concealed damage even though no evidence of it is apparent. When concealed damage is discovered, cease further unpacking of the unit involved and request immediate inspection by local agent or carrier and secure written report of his finding to support claim. This request must be made within 15 days of receipt. Otherwise, claim will not be honored by the carrier. Do not return damaged goods to factory without first securing an inspection report and contacting Waters for a return authorization number.

Claims After a damage inspection report has been secured, Waters will cooperate fully in supplying replacements and handling of a claim which may be initiated by either party.

Returns No returns may be made without prior notification and authorization. If for any reason it is necessary to return material to us, please contact our Customer Service Department or your nearest Waters subsidiary/representative for a return authorization number and forwarding instructions.

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Waters
Division of MILLIPORE

U.S.A. Sales Offices
New England Waters Chromatography Division / Millipore Corporation / 34 Maple Street / Milford, MA 01757 / Tel. 508-478-2000
Chicago Waters Chromatography Division 1555 Mittel Boulevard / Wood Dale, IL 60191 / Tel. 312-766-6060
New Jersey Waters Chromatography Division 35 Airport Park / Suite 230 / Morristown, NJ 07960 / Tel. 201-285-1404
North Carolina Waters Chromatography Division / 117 Edinburgh Drive / Suite 206 Cary, NC 27511 / Tel. 919-469-2501
San Francisco Waters Chromatography Division c/o Millipore Corporation / 448 Grandview Drive / South San Francisco, CA 94080 Tel. 415-952-9200
Southwest Waters Chromatography Division 2626 South Loop West / Suite 170 Houston, TX 77054 / Tel. 713-665-7310
Washington Waters Chromatography Division 3702 Pender Drive / Fairfax, VA 22030 Tel. 1-800-526-0771

Overseas Offices
Australia Millipore Pty. Ltd. / Waters Chromatography Division / Locked Bag 18 87-89 Mars Road / Lane Cove, N.S.W. 2066 Tel. Inwats (008) 222-111 / In Sydney Area (02) 427-0511 / Telex 25934
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Finland Millipore Oy / Waters Chromatography Division / Ruukinkuja 1 / 02320 Espoo Tel. 90-801 90 77 / Telex 12-2123 milpor sf
France Millipore S.A. / Waters Chromatography Division / Boite Postale 307 / 78054 Saint Quentin / Yvelines Cédex / Tel. (1) 30.45.70.00 Telex 698 371 F
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Japan Nihon Millipore Ltd. / Waters Chromatography Division / No. 5 Koike Bldg. 3-12, Kita-Shinagawa 1-chome / Shinagawa-Ku, Tokyo 140 / Tel. 81-3-471-7191 Telex 781-02324161
Mexico Millipore S.A. de C.V. / Waters Chromatography Division / Avenida Ingenieros Militares 85 / Mexico 11230 D.F. Tel. 576-9688 / Telex 1777 442
The Netherlands Millipore B.V. / Waters Chromatography Division / Penningweg 33 Postbus 166 / 4870 AD Etten-Leur (N.B.) Tel. (0 16 08) 22000 / Telex 54 840 milwa nl
Norway Millipore AB / Waters Chromatography Division / Enebakkveien 133 / 0680 Oslo 6 Tel. (02) 67 82 53
Peoples Republic of China (Beijing) Nihon Waters Service Center Rm. 638, Xiyuan Hotel Erligou Xijiao, Beijing Tel. 8022018 / Telex 22685
Peoples Republic of China Millipore China Ltd. Waters Chromatography Division / Hope Sea Industrial Centre / 26 Lam Hing Street / Suite 105 / 1st Floor / Kowloon Bay / Hong Kong / Tel. 3-796 3736 Telex 3-7967374
Puerto Rico Millipore Corporation Puerto Rico Branch / Waters Chromatography Division KM 7.7 / State Road 172 / Cidra, Puerto Rico 00639 / Tel. 809-747-8444 Telex 385 72 76
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Waters Publications

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