NOTE FOR COULARRAY USERS

CAUTION:

The CoulArray detector may be susceptible to errant behavior such as a lock up upon power up. Under some conditions this could result in damage to the CoulArray detector module and cells. To avoid this situation, the following procedure should be used every time the CoulArray system is turned on:

1. Before turning on the CoulArray detector module make sure the computer and pump(s) are turned off.

2. Turn on the CoulArray Detector Module first and wait 10 seconds.

3. Next, turn on your pump(s).

4. Finally, turn on your computer and then start the CoulArray program.

5. You may now use the CoulArray as needed.

NOTE: Any damage sustained to the CoulArray detector and cells resulting from the failure to follow the above procedure will not be covered under ESA's warranty.

Model 5600A
CoulArray® Detector

OPERATING MANUAL

ESA, Inc.
22 Alpha Road
Chelmsford, MA 01824-4171 U.S.A.

Tel: (978) 250-7000
Fax: (978) 250-7090

P/N 70-4685 Rev. E

NOTICES:

This system is covered by a limited warranty. A copy of the warranty is included with this manual. The analyst is required to perform routine maintenance as described herein on a periodic basis to keep the warranty in effect. For routine maintenance procedures, refer to Chapter 5.

All information in this manual is subject to change without notice and does not represent a commitment on the part of ESA, Inc.

The CoulArray Detector and various components in the system are covered by the following patents: US: 4,233,031; RE32,920; 4,404,065; 4,497,199; 4,511,659; 4,552,013; 4,753,714; 4,804,455; 4,863,873; 4,976,994; 5,104,639. Canada: 1138043 (1982); 1139841 (1983); 1167277 (1984); 1167526 (1984); 1195383 (1985); 1238362 (1988); 1251515 (1989); 1271811 (1990). Japan: 1536120; 1827931; 2018280; 2059320; 2072937. France: 2422948; 0223532; 0227281; 0033188; 0567564. Germany: P3681691.4; P3686030.1; 3174440; 0567564. Italy: 0223532; 0227281; 0033188. EPO: 0122009. UK: 2012435B; 0223532; 0227281; 0033188; 0567564.

All software provided by ESA, Inc. is furnished under a license agreement as described on its package. The software may be used on a single CoulArray system and only in accordance with the license agreement. The user may make one copy of each program disk for archival purposes.

Printed in the United States of America.

WARNINGS AND SAFETY PRECAUTIONS

The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the instrument.

1) Make certain that you are familiar with the contents of this manual before using the system.

2) Maintain a well-ventilated laboratory. If the mobile phase contains a volatile organic solvent, ensure that the laboratory is well ventilated so that a buildup of organic solvent cannot occur.

3) Avoid open flames and sparks. Do not use an open flame and do not use any equipment that can cause sparks in the laboratory.

4) If a leak occurs, turn off power to the module and remedy the situation immediately.

5) All components of the HPLC system must be plugged into a grounded power line. Make certain that all parts of the system are properly connected to a common ground.

   Do not attempt to bypass the earth ground connection, a serious shock hazard could result.

6) Static charges may build-up due to use of organic solvents. These charges can lead to fire or explosion. Take appropriate precautions to eliminate the buildup of static charges (e.g., ground the waste bottle).

7) Wear protective goggles when handling solvents. Install a sink as close as possible to the module. If any solvents splash on the skin or eyes, immediately rinse the affected parts in the sink.

8) Make certain that you understand the toxicological properties of all solvents that are used with the CoulArray detector module. If you are analyzing biological/clinical samples, treat them in accordance with the Infectious Disease Control Program of your institution.

9) Install the unit in a location where ambient temperature variations are minimized. Avoid placing the unit in direct sunlight, near a heating or an air conditioning duct.

10) The maximum recommended pressure rating of tubing and devices used in the CoulArray thermally controlled system organizers (CoulArray Thermal Organizer, CoulArray Thermostatic Chamber and CoulArray Temperature Module) is 275 bar using PEEK™ tubing. The maximum pressure limit used also depends on the specific device(s) used in the organizer. Refer to the devices' manufactures for their maximum pressure rating.

11) The HPLC pump(s) or solvent delivery device(s) used in conjunction with any of the CoulArray thermally controlled system organizers must be CE approved and contain a properly installed and maintained pressure safety device and/or pressure sensor that conforms to the requirements of ISO 4126-1.

12) PEEK tubing is used in a variety of locations. While it has excellent chemical resistance to most common organic solvents, it is attacked by concentrated nitric acid and sulfuric acid, and tends to swell in solutions with high concentrations of chloroform, dimethylsulfoxide and tetrahydrofuran.

13) Do not use the CoulArray detector and its accessories in a manner not specified by ESA. Otherwise the safety protection provided by the equipment may be impaired.

HINTS FOR TROUBLE-FREE OPERATION

The suggestions below serve to maximize system performance and minimize down time.

- Always filter the sample and mobile phase using a 0.22 µm filter. If the sample contains protein and/or lipids, use a 10,000 MW cut-off filter to ensure that high molecular weight compounds do not enter the system.

- When a new mobile phase or column is used, allow sufficient time for system equilibration. This equilibration may take an hour or so if a buffer is used. However, if a mobile phase containing an ion-pairing reagent (e.g., SDS) is used, the required time may be extended. The equilibration time is dependent on the degree of sensitivity that is required.

- Always run a standard when a column or the mobile phase is replaced.

- Ensure that the cells have mobile phase flowing through them at all times when a potential is applied (a reduced flow rate is acceptable during short term shutdown).

- Do not use metal filters in the solvent bottles.

- Monitor the system pressure. Any significant change (e.g., more than +/- 4 bar) is an indication that something may have changed.

- When you make a fitting, do not overtighten. Initially, the fitting should be made hand tight. Pump mobile phase through the system; if a leak is observed, then tighten the fitting gradually until the leak stops. Overtightening can irreversibly damage fittings.

- The use of a guard column to clean up samples is recommended. The guard column should be changed on a periodic basis.

- A change in the shape of a peak (e.g., tailing) or a change in the retention times suggests that the column is deteriorating.

- Periodically transfer data to an external storage device. The CoulArray Detector generates a significant amount of data during a run. If data is stored on the hard disk, you may quickly fill it up.

- Make sure that the pump seal flushing solution is installed and is working properly. Change the wash solution on a weekly basis.

- If an abnormal or "funny" noise (e.g., a change in the intensity of pitch) is observed, it is likely that a change in the system has occurred. This statement is clearly not scientific; however, if you observe a change in the noise from the system, it is worthwhile to track down the cause.

- Ensure that all drain tubing and ground wires are firmly attached.

- When using a manual injector to trigger each cycle of the CoulArray Detector (WAIT EXTERNAL), make sure to select the Manual Contact Closure Option (by first accessing the hardware configuration button, then options, then the instrument tab and setting the external start field to Manual Contact Closure).

- Before turning on the CoulArray detector module, make sure the computer and pump(s) are turned off. Turn on the CoulArray detector module first. Next, turn on your pumps. Finally, turn on your computer and start the CoulArray program.

- Use PEEK filter elements (Part Number 70-3824) in the in-line prefilter holders located after the injector. Use graphite filter elements (Part Number 70-0898) before the injector.

- The CoulArray software application program is incompatible with various power management programs such as Microsoft® Windows power management. To avoid interruption and loss of data make sure that any power management programs are disabled.

WARRANTY

ESA, Inc., warrants that each of the products manufactured and sold by ESA to be free from defects in material and workmanship in normal use and service from the date of shipment to you as the original purchaser for the following periods: ESA Instruments - one year; Cells, Sensors and Electrodes - 90 days. This warranty does not cover, and no warranty is provided for, parts which by their nature are normally required to be replaced periodically consistent with normal maintenance, including, without limitation, fuses, tubing, pump piston seals, injector rotors, check valves, filters, and any software included in any product. If any product covered by this warranty is returned to the original shipping point, transportation charges prepaid, within the applicable warranty period set forth above and upon examination ESA determines to ESA's satisfaction that such product was defective in material or workmanship at the time of delivery to you, ESA will, at ESA's option, repair or replace the product or defective part thereof or refund the original purchase price of the product. The foregoing not withstanding, ESA will not be responsible for damage to any product resulting from misuse, negligence or accident or resulting from repairs, alterations, or installation made by any person or firm not duly authorized by ESA in writing or for any damage to any cell assembly resulting from the flow being impeded. If any cell assembly is used with control modules or potentiostats other than those manufactured by ESA, this warranty shall be void. ESA shall not be liable for failure to comply with statutes relating to safety and health, including, without limitation, standards promulgated under the Occupational Safety and Health Act (OSHA) of 1970, as amended, and regulations issued pursuant thereto.

If, at any time during the period ending ninety (90) days after delivery of any product to you, you report and document any error in any software provided with such product and developed by ESA or any failure of any such software substantially to conform to ESA's software description that limits or prevents use of the software by you, ESA, at ESA's option, will use reasonable efforts to correct any such error or failure, will replace such software or will terminate your license to use the software and refund the price of the related product. In connection with any such termination and refund, you will return the related product to ESA forthwith upon request. The warranty will apply only to those portions of the software that were developed by ESA and that incorporated all program corrections and modifications, if any delivered to you. It will not apply to any error or failure due to machine error or to the misuse by or negligence of any person or entity other than ESA or to any software which is modified by any person or entity other than ESA.

With respect to equipment sold to you but not manufactured by ESA, ESA MAKES NO WARRANTY OF ANY KIND, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, but will make available to you, to the extent permitted, the warranties of the manufacturer of the relevant equipment following your timely written request.

Under no circumstances shall ESA be liable for damage to persons or property. All replacement shall be made FOB factory and without liability to ESA for drayage, freight or labor costs.

If you fail to pay when due any portion of the purchase price of any product or any other payment required from you to ESA, whether hereunder or otherwise, all warranties and remedies granted in this Section and all of ESA's warranties and obligations to service, repair, replace, correct or otherwise remedy defects, errors or failures under any other contract between you and ESA may, at ESA's option be terminated.

THIS WARRANTY IS THE ONLY WARRANTY GIVEN BY ESA WITH RESPECT TO PRODUCTS AND THE SOFTWARE PROVIDED WITH THE PRODUCTS AND IS GIVEN IN LIEU OF ALL OTHER WARRANTIES, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. YOUR EXCLUSIVE REMEDIES AND ESA's SOLE LIABILITY FOR ANY NON-CONFORMITY OR DEFECT IN THE PRODUCTS AND SUCH SOFTWARE WILL BE THOSE EXPRESSED HEREIN. UNDER NO CIRCUMSTANCES WILL ESA's LIABILITY ARISING FROM THE PERFORMANCE OR FAILURE TO PERFORM OF ANY PRODUCT OR SOFTWARE, IN CONTRACT, IN TORT (INCLUDING NEGLIGENCE), OR OTHERWISE, EXCEED THE PURCHASE PRICE OF THE PRODUCT AND SOFTWARE. IN NO EVENT WILL ESA BE LIABLE, IN CONTRACT, IN TORT (INCLUDING NEGLIGENCE), OR OTHERWISE FOR SPECIAL, INCIDENTAL, CONSEQUENTIAL OR ANALOGOUS DAMAGES, INCLUDING, WITHOUT LIMITATION, DAMAGES RESULTING FROM LOSS OF USE, LOSS OF PROFITS, LOSS OF BUSINESS OR LOSS OF GOODWILL, EVEN IF ESA HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

This warranty shall be governed by, and construed and enforced in accordance with, the substantive laws of the Commonwealth of Massachusetts.

This warranty shall be non-transferable and shall run to the benefit of the original purchaser only.

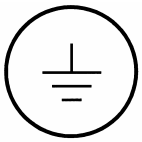
SAFETY/OPERATING SYMBOLS

The following symbols appearing on the unit or in the manual are defined as follows:

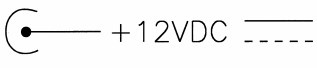
This symbol on the instrument indicates that the user should refer to the operating manual before attempting to connect the power/interface cables and operate the instrument.

This symbol on the instrument states that high voltage may be present when panels/covers are removed. Any adjustment, maintenance and repair of the opened apparatus under voltage should be avoided as far as possible and, if inevitable, must be carried out only by a skilled individual who is aware of the hazards involved.

This symbol on the instrument states that elevated temperatures may be present in the system. The user should take care that the internal components are not touched when the temperature is elevated.

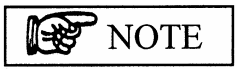


This symbol in the unit indicates a protective ground terminal.



This symbol on the rear panel indicates a D.C. input voltage connection.

The blocked statement is used throughout the manual to indicate potential dangers that might result in personal injury or damage to property.



The blocked statement is used throughout the manual to highlight important information about the system.

The blocked statement is used throughout the manual to indicate conditions that could cause component failure or invalid data.

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Table of Contents

Table of Contents

1 Introduction

1.1 The Role of the CoulArray Detector

The *ESA CoulArray® Detector* is a coulometric multi-electrode electrochemical detector for high performance liquid chromatography (HPLC).

The instrument is designed to detect and quantitate trace levels of electroactive compounds in complex matrices. A typical application is the qualitative and quantitative analysis of a broad range of biogenic amines in biological tissues.

The CoulArray system includes:

- An electrochemical detector (a control module and electrochemical cells).
- An organizer module to house the column, cells and related components. A variety of thermostated and non-thermostated organizer modules are available (Section 3.3).
- Application software for instrument control, data acquisition, data processing and data reporting.
- A personal computer.

1.2 Introduction to HPLC with Multi-Electrode Detection

In a high performance liquid chromatograph, a detector monitors some physical parameter of the mobile phase. When a compound elutes from the column, the signal from the detector changes. As an example, an absorbtion detector is commonly used to monitor the absorbance of light at 254 nm. When the detector observes a change in the signal at the appropriate retention time, it is likely that the "compound of interest" has been eluted from the column. A plot of the detector response as a function of time is called a chromatogram (Figure 1-1).

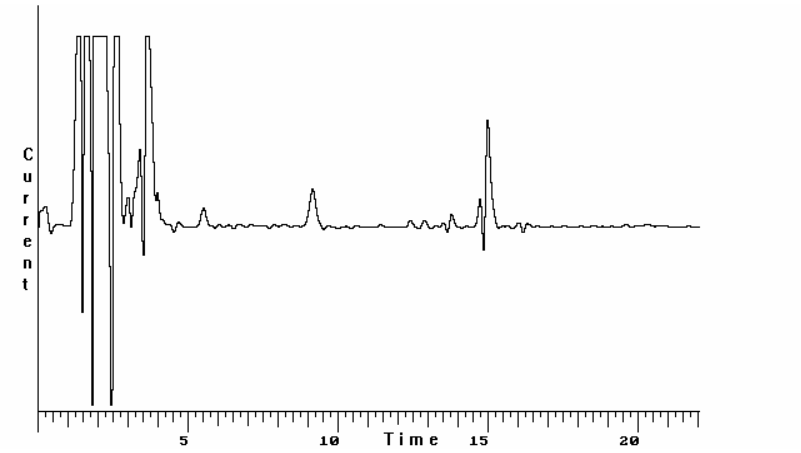

Figure 1-1: A Typical Chromatogram

It should be noted that it is very possible that the observed signal might be due to some other compound in the sample that happened to have the same retention time as the compound of interest. In this case, the reported identification and concentration of the compound of interest would be incorrect.

In contrast, the CoulArray uses a multi-electrode detector system in which a series of electrochemical cells (up to 16) are set at different potentials to oxidize (or reduce) the compounds that elute from the column. This approach allows for the collection of a number of chromatograms rather than a single chromatogram, and thus allows for the identification of the compound of interest based on the retention time and its oxidation (reduction) characteristics on several traces. It is very unlikely that two compounds that elute at the same time have a similar oxidation (reduction) profile. A typical multi-electrode chromatogram is shown in Figure 1-2.

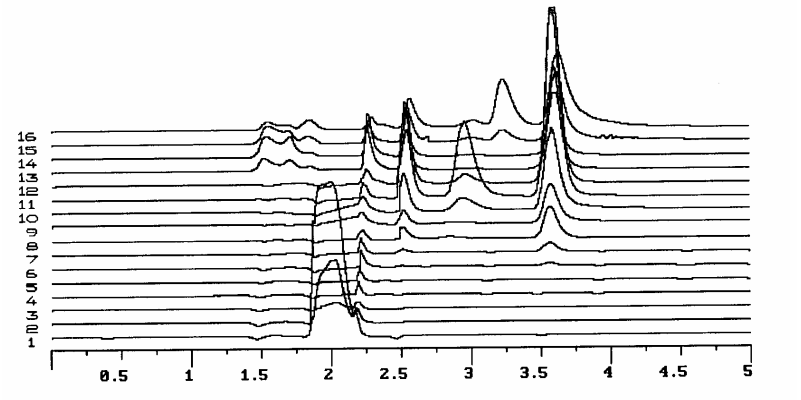

Figure 1-2: A Typical Chromatogram From A Multi-Electrode Detector System

To demonstrate the utility of the CoulArray detector system, consider an example in which the compound of interest has a retention time of 14.2 minutes and an oxidation potential of 650 mV. The presence of a signal on the appropriate chromatographic trace (and the absence of a signal on traces that were obtained from detectors set at lower potentials) provides a greater degree of certainty than if only a single parameter was measured (e.g., absorbtion at 254 nm).

A discussion of the fundamentals of multi-electrode detection for HPLC is presented in Appendix 3.

1.3 How Does the CoulArray Detector Fit Into an HPLC System

An HPLC system is modular in design (Figure 1-3) and includes the following devices:

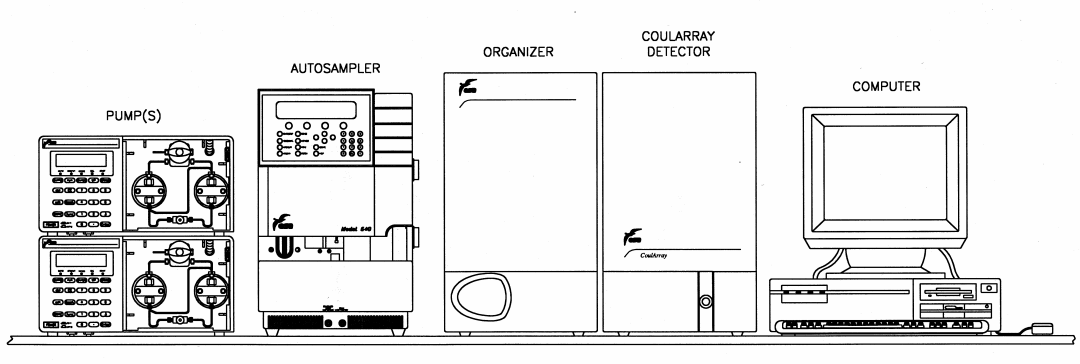

Figure 1-3: A Typical Configuration of an HPLC System with a CoulArray Detector, Autosampler and Thermal Organizer

- *The Solvent Delivery System* - The solvent delivery system provides the mobile phase to the column.

- *Injector* - A manual or automated injector with a sample rack can be used to place the sample into the mobile phase.

- *HPLC Column* - The HPLC column is used to effect the separation of the components of the sample. The column that is used is dependent on the nature of the separation (typically, a reverse phase column is used).

- *Detector* - The array detector may include 4, 8, 12 or 16 cells in series. The potential of each electrode can be independently controlled from - 1000 mV to + 2000 mV.

- *Organizer Module* - The injector, column and electrochemical cells are installed in a chamber. In most applications, the chamber is thermostated; leading to a significantly higher degree of reproducibility in the chromatogram. A non-thermostated module (the *CoulArray Organizer*) can be supplied if temperature control of the column and detector is not required for the application.

- *Personal Computer and Software* - Instrument control, data acquisition, processing, storage and reporting is effected via the personal computer and application program. The program provides for:

  a) Setting the analytical conditions (e.g., flow rate, detector potentials, etc.)
  b) Controlling the autosampler (ESA Model 540, 542 Autosamplers)
  c) Acquiring and presenting analytical data
  d) Filtering and smoothing of the raw data
  e) Peak detection and integration
  f) Peak identification
  g) Quantitation of compounds that are identified
  h) Report generation
  i) Data storage and export

A detailed discussion of the application program is provided in the *CoulArray Applications Software Manual* (Part Number 70-5673).

1.4 Documentation for the System

Three manuals are provided with the instrument:

- The *Getting Started Manual*, which is an overview of the use of the software.

- The *Operating Manual* (this manual), which discusses the instrumentation.

- The *Applications Software Manual*, which describes the software.

An on-line Help program is provided via the application software.

Manuals for the mobile phase delivery system, autosampler, thermostatic chamber (if supplied), temperature module (if supplied) and computer are provided with these devices.

1.5 Contents of this Manual

This manual is designed to describe the installation, operation, maintenance and troubleshooting of the CoulArray Detector. It includes:

- *Chapter 2* - **Setting up the Detector**, describes suitable laboratory conditions for the detector and includes information about interfacing the unit to other devices.
- *Chapter 3* - **Fluidics Installation**, describes step-by-step instructions on how the fluidics are installed in an HPLC system.
- *Chapter 4* - **Installing the Computer and Software,** explains how the software is configured for the individual system.
- *Chapter 5* - **Starting the System,** describes how to operate the CoulArray Detector to collect analytical data and includes information about routine operating procedures.
- *Chapter 6* - **Maintenance and Operating Information**, describes periodic activities that should be performed to ensure the maximum performance.
- *Chapter 7* - **Troubleshooting**, discusses a protocol that can be used to determine the cause of problems that are observed with the instrument.
- *Chapter 8* - **Long-Term Shutdown**, describes the steps that should be taken when the system will not be used for an extended period of time.
- A series of appendices are provided, which include product specifications, a list of spare parts and a discussion of the theory of operation of the system.

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2 Setting up the Detector

2.1 Overview

This chapter describes how the laboratory should be prepared to optimize the performance of the *CoulArray*® Detector and its system components indicates how the unit is interfaced to other devices such as the pumps and the computer. A detailed discussion of the installation procedure is provided in Chapter 3.

2.2 Unpacking the CoulArray Detector

The basic CoulArray Detector system includes a control module, an organizer module, cells, a computer and an accessory kit. Other items may be included (e.g., an autosampler) in the shipment as indicated on the shipping documentation. Carefully unpack your shipment and inspect the contents to verify receipt of all components as listed on the Parts List found in the inside pocket of this manual.

Each Cell Assembly provides four channels. The appropriate number of Cell Assembly kits (Part Number 55-0685E) is provided to meet the requirements of the system.

Carefully inspect the shipping cartons and all components. If there is any damage to a carton or any components, contact both the shipping agent and ESA (or its representative) immediately. If there is any evidence that the control module an AC powered organizer has been damaged in shipping, do not plug the units into the power line. Contact ESA or your authorized agent for advice.

If any parts are missing, call ESA's customer service department and indicate the missing part numbers.

It is recommended that the shipping cartons be retained as they should be used if it becomes necessary to transport the system.

Please complete the warranty card found in the inside pocket of this manual and return to ESA, Inc.

2.3 Power Requirements

⚠ WARNING: The control module of the *CoulArray Detector* and the system organizers use three-prong power cords that include a ground wire. These units must be connected to a properly grounded three-prong plug to ensure proper safety and operation of the detector. If there is any doubt concerning the intended power supply, a qualified electrician should be contacted to ensure a properly operating and grounded power outlet.

It is recommended that all the components of the HPLC, including the detector, autosampler and oven are connected to a surge suppressor.

The *CoulArray Detector* is configured for the voltage that meets the power requirements of the location to which it is shipped. The four voltage options that are available at the power input receptacle on the back of the unit are 100/120/230 and 240V. The indicator on the power entry module should be checked to verify the proper setting. If it is necessary to change the setting, contact ESA at 1-800-275-0102 or its Representative for assistance.

The *Thermal Organizer* has two voltage options, 115V and 230V. The 115V setting is for 100/200 VAC operation. The 230V setting is for 220/240 VAC operation.

⚠ CAUTION: Before connecting the power check that the power setting is correct for the location it is to be used in. If it is incorrect call ESA Service at 1-800-275-0102 or your local ESA representative for assistance.

The 100/120 VAC power cable consists of a three-slot receptacle for attachment to the power inlet on the control module and a three-prong plug for connection to a standard grounded output of 100/120V.

The 230/240 VAC power cable consists of a three-prong receptacle for attachment to the power inlet on the back of the control module. The other end of the cable has three color-coded wires that are used to attach to the appropriate plug.

The color-coding of the wires meets ISO and VDE conventions as follows:

Earth Ground:	Green with Yellow Stripes
Neutral:	Blue
Line:	Brown

The power consumption of the control module only is approximately 40 watts.

The *CoulArray Control Module* contains a built-in line filter to reduce RFI at any line input voltage.

2.4 Locating the Unit in The Laboratory

All components of the system including pumps, autosampler, organizer module, etc. should be located on a sturdy table. A typical system configuration is shown in Figure 2-1. Refer to Appendix 1 for dimensions, weights and power consumption.

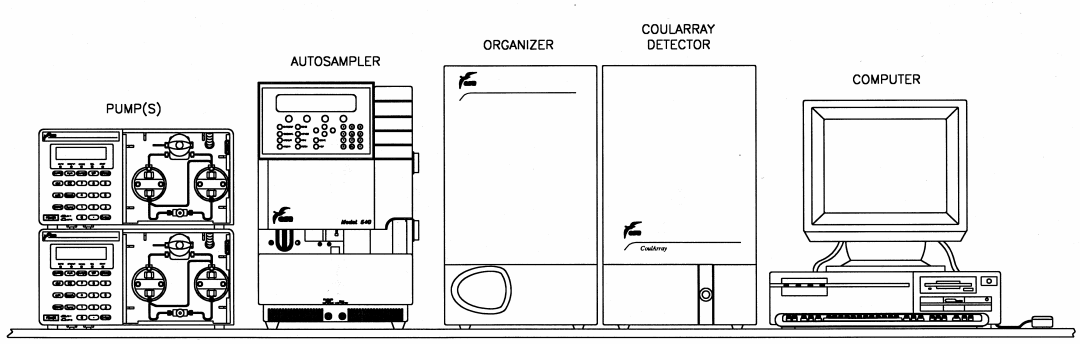

Figure 2-1: A Typical System Configuration

The system should be placed in an area that is free from drafts or other significant temperature changes. Avoid placing it near air conditioning vents, windows, ovens, etc.

The control module should not be connected to an electrical line which also serves units with a large power drain or which may be subject to power surges. Typically systems of this type include centrifuges, ovens, refrigerators and fume hoods.

2.5 Electrical Connections

NOTE: Chapter 3 and 4 includes a complete protocol for the installation of the system. Follow the steps provided in those chapters to connect all devices to the Control Module.

A variety of devices are connected to the CoulArray Control Module via the front panel of the control module (Figure 2-2) as described below.

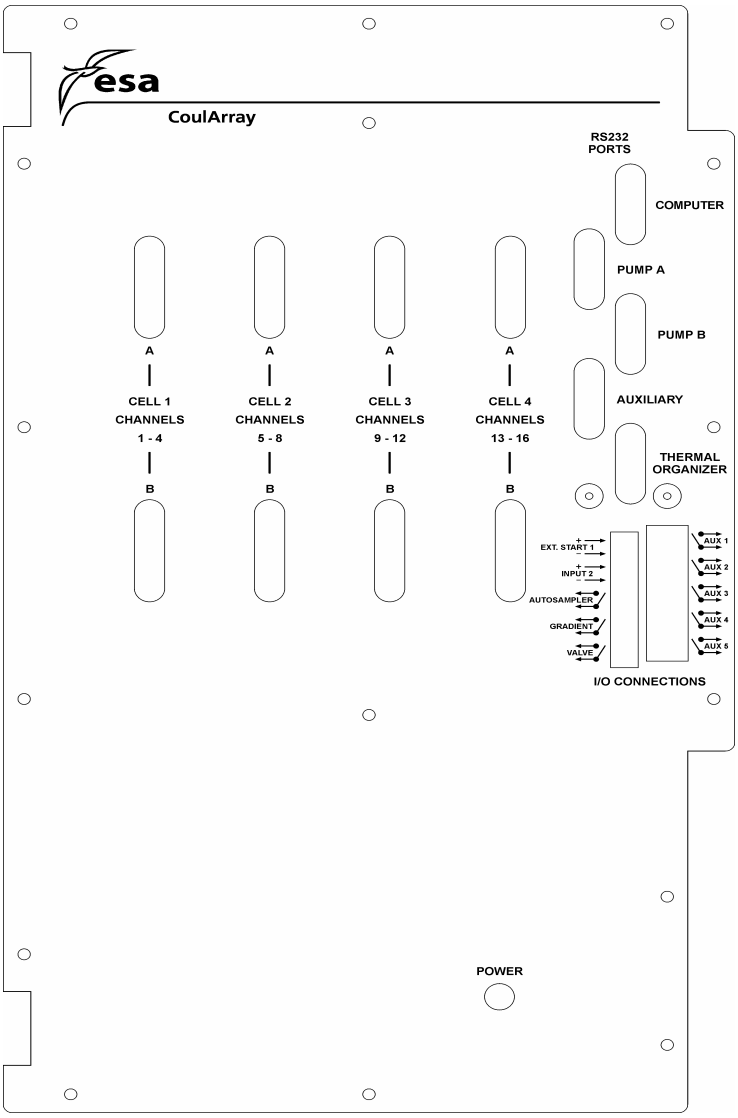

Figure 2-2: Front Panel of the Control Module

2.5.1 Analytical Cells

Analytical Cells - The analytical cells are connected to potentiostat modules in the control module. On a given potentiostat, Port A refers to the first two channels and Port B refers to the remaining channels. For example, Port A for channels 1-4 is used for channels 1-2 and Port B is used for channels 3-4.

2.5.2 RS-232 Ports

Computer - The computer is connected to the system via the RS-232 port in the upper right-hand corner using an RS-232 cable (Part Number 70-1743).

Pumps - If the system includes an ESA solvent delivery system, the pumps are connected to the system via the Pump A and Pump B ports. These pumps will be controlled via the application program.

NOTE: The pumps should be operated on a local basis during the installation of the instrument.

If the system does not contain an ESA solvent delivery system, the pumps are controlled on a local basis (see I/O Connectors, Section 2.5.3).

Auxiliary - Inactive at this time.

Thermal Organizer - Used to communicate with the CoulArray Thermal Organizer. The analyst enters the desired temperature via the *Control* dialog box of the *CoulArray for Windows* application program.

2.5.3 I/O Connectors

There are two input and eight output connectors. The inputs are TTL and the outputs are true relays.

CAUTION: When connecting TTL inputs, ensure that the potential across the terminals is 5V DC (or less). When connecting outputs, ensure that the potential across the terminals is less than 30V DC and the current is less than 0.5A.

Inputs:

- **External Start 1 or Input 2** - is used if the analyst wants to initiate operation of the time-based program via an external device. In this situation, the CoulArray detector will act as a slave. A typical application might be when a programmable autosampler is used to control the operation of the instrument.

Outputs: Normally Open (Closed when active)

- **Autosampler** - provides a signal to the autosampler that a sample should be injected onto the column. The signal can be generated manually or via the automated operation of the system as described in the Applications Software Manual. In this format, the CoulArray Detector operates as the master and the autosampler is the slave. All autosampler operations, except for inject, are set on the autosampler control panel. This port is not used if the Model 540 or 542 Autosampler is included in the system.

- **Valve** - provides a signal to an external valve to change position. The signal can be generated manually or via the automated operation of the system as described in the Applications Software Manual.

- **Gradient Start** - provides a signal to start the mobile phase flow (gradient) when a run is initiated. This output is used when an ESA solvent delivery system is not used. The gradient program must be set on the pump(s).

- **AUX (1-5)** - provides a contact closure signal to an external device. The signal can be generated manually or via the automated operation of the system as described in the Applications Software Manual.

2.5.4 Power Cord Connection

The power cord (Part Number 70-1164 [100/120V] or 70-1165 [220/240V]) is plugged into the receptacle on the back of the control module and the thermal chamber or thermal organizer. To power up the system, depress the power switch on the back of each unit.

⚠ WARNING: Connect only to a properly grounded outlet. Do not attempt to modify or bypass the ground. A serious shock hazard may result.

⚠ CAUTION: Before turning on the CoulArray detector module make sure the computer and pump(s) and Thermal Organizer are turned off. Turn on the detector module first and wait 10 seconds. Next, turn on the pump(s) and the Thermal Organizer. Finally, turn on your computer and then start the CoulArray Program.

2.6 Fluid Connections

The column and the cells are located in the organizer. A detailed discussion of the installation of the various fluidics components is presented in Chapter 3.

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3 Fluidics Installation

3.1 Overview

This chapter is designed to provide step-by-step instructions for the installation of the fluidics components of the CoulArray system. Information about unpacking of the system and initial inspection is presented in Chapter 2, while electrical connections, software installation, selecting initial operating parameters and initial checkout of the system is presented in Chapter 4.

The HPLC system that incorporates the CoulArray detector can be configured in a variety of ways, including:

- Using ESA solvent delivery modules or pumps/gradient controller from another manufacturer. If the ESA solvent delivery modules are employed, they can be controlled via the software program.

- Using a manual injector or an autosampler.

- Using the CoulArray Thermal Organizer, the CoulArray Organizer, the CoulArray Thermostatic Chamber or a CoulArray Temperature Module to house the column, cells and related components.

The installation of the system is generally similar for the various configurations and any variations due to the difference in configuration will be indicated.

3.2 Installing Fluidics Components

3.2.1 General Guidelines for Installation of the Fluidics Components of the CoulArray Detector

A typical system configuration is presented in Figure 3-1. If other components are employed, the configuration should maintain a minimum distance between:

- the injector and the column to reduce the diffusion of sample.
- the system organizer and the CoulArray control module (the cell cables are kept short to minimize noise in the signal).

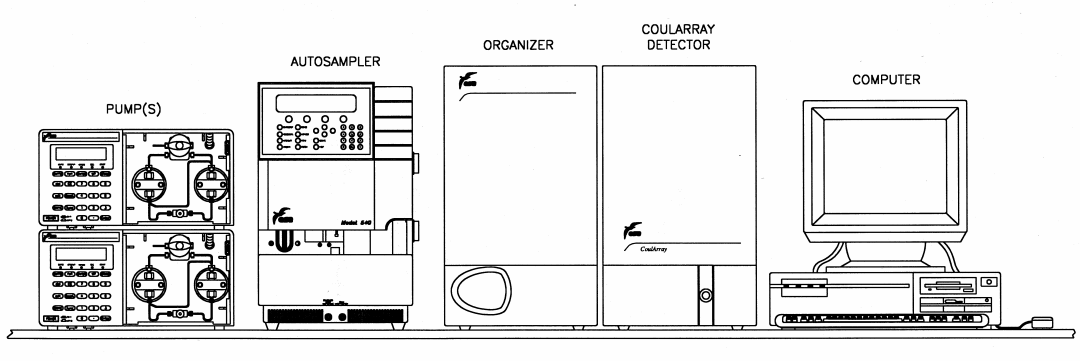

Figure 3-1: A Typical Fluidics System Configuration

The basic principle underlying the installation of the fluidics components is that the system is checked after each component is added. This sequence of adding a component and checking the system for leaks has been found to be very useful in isolating any problems and minimizing the overall effort for installation of the system.

It is suggested that the installer check out the various components of the system, including the computer, the solvent delivery system, autosampler (if present), etc. on a local basis as described in the manuals supplied with these units before starting the installation procedure described below.

A mixture of methanol/water (20/80) can be used as the mobile phase during the installation procedure (in place of the mobile phase that will be used for the operation of the system). This "installation" mobile phase should be degassed and filtered through a 0.22 μm filter.

Maintain a clean working environment and ensure that particulate matter does not enter the system. The presence of particulate matter in the system will reduce the effectiveness of the cells and may lead to premature replacement of various components.

The coulometric cells contain liquid and should not be allowed to dry out. In addition, ensure that mobile phase is flowing through the cells before the potential is applied.

An overview of components of a typical system is shown in Figure 3-2.

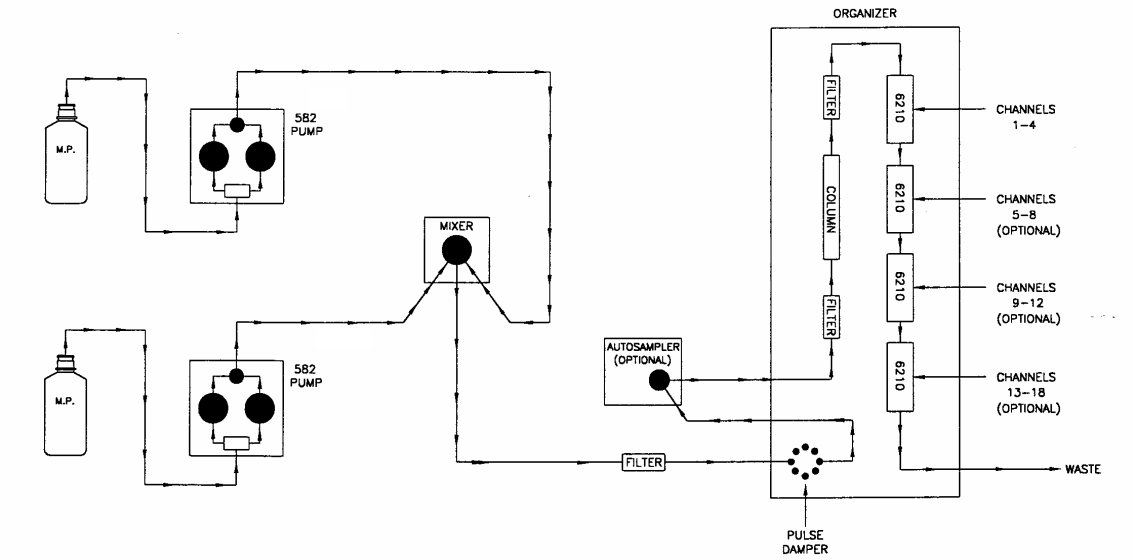

Figure 3-2: Components of the Fluidics System

3.2.2 Tubing/Fitting Considerations

a) PEEK™ tubing is provided for the various connections in the system and is included with some components (e.g., the cell kit contains pre-cut PEEK tubing to connect the cell to the next cell in the series).

- 0.010 x 1/16" PEEK tubing (Natural) is used after the sample injector and is employed to minimize band-broadening effects.

- 0.020 x 1/16" PEEK tubing (Orange) is used before the sample injector.

b) Cut the appropriate length of tubing using the Tubing Cutter (Part Number 70-1307).

c) To cut tubing:

   i) Insert the tubing in the appropriate guide hole in the cutter and position to the desired length.

   ii) Release the tabs on the cutter.

   iii) Spin the cutter a few times; remove the tubing and snap the desired piece off. As an alternative, you can continue to rotate the cutter around the tubing to finish the cut.

   iv) Inspect the tubing to ensure that the inner channel of both ends is round (if the inner channel is not properly cut, it is likely that the flow through the tube will be restricted).

d) When connecting a component to the next component in the series, use a piece of tubing that is long enough to connect the two components with a small amount of slack. The resolution of the chromatogram will be reduced if excessively long pieces of tubing are employed.

Information about making a fitting is provided with the components for the fitting.

NOTE: Turn off the flow of mobile phase while making a fitting.

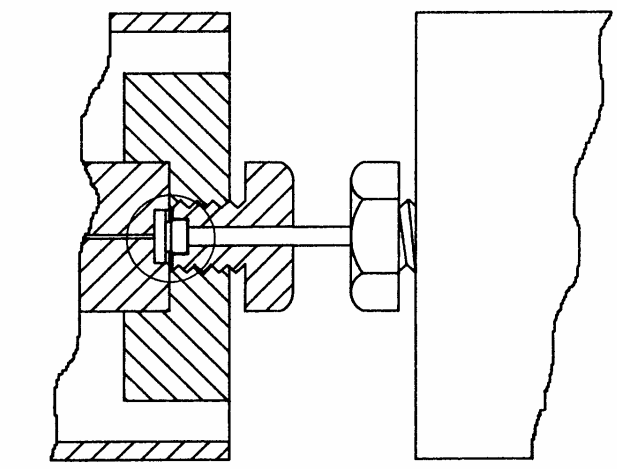

Figure 3-3: Seating the Tube Assembly

NOTE: Do not overtighten the fitting, as that may lead to permanent deformation of the ferrule.

After a fitting is made, allow the mobile phase to flow through the fitting and monitor the joint. If a leak is observed, tighten the fitting to stop any leaks. To ensure that a good fit is made, gently tug on the tubing after the connection has been made.

3.2.3 Preparing the Solvent Delivery Module

The solvent delivery module may consist of one or two pumps and a mixer. An in-line filter and a pulse damper must be installed to prepare the solvent delivery module for use with the CoulArray Detector. In this discussion, we will use the terms "solvent delivery system" and "pump" interchangeably.

If an ESA solvent delivery system is employed, the in-line filter kit (Part Number 70-0893) and the PEEK pulse damper (Part Number 70-0894) described below are provided as part of the accessory kit. If a non-ESA pump is used, these components are supplied as separate line items. The pre-filter is used to ensure that particulate matter from the solvent delivery module does not enter the system, while the pulse damper serves to reduce pulsations in the mobile phase due to the action of the pump. The pulse damper consists of a thin membrane and a reservoir of methanol.

a) Allow the solvent delivery module to deliver mobile phase at the rate of 1.0 mL/min for a few minutes to purge any particulate matter from the system. The maximum pressure for the pump should be set at 250 bar.

b) Turn off the pump and connect the pre-filter holder to the outlet of the solvent delivery module using the tubing supplied. In addition, attach tubing to the down-stream side of the filter holder. The filter holder should be installed with direction of the arrow pointing down.

c) Open the holder and insert a filter. (Use PEEK filter elements (Part Number 70-3824) after the injector and graphite filter elements (Part Number 70-0898) before the injector. See Section 6.5 for a more detailed description of how to insert the filter.

d) Start the pump again and allow the mobile phase to flow through the system for a few minutes (at a typical flow rate of 1 mL/min) to expel the air in the system. Monitor the fittings and tighten as appropriate.

e) Turn off the pump and connect the downstream end of the in-line filter to the pulse damper (Part Number 70-0894). In addition, attach tubing to the down stream end of the pulse damper.

f) Start the pump again and allow the mobile phase to flow through the system for a few minutes (at a flow rate of 1 mL/min) to expel the air in the system. Monitor the fittings and tighten as appropriate. After the fittings are secure, turn off the pump.

3.2.4 Interfacing the Fluidics Components

While the physical location of the various components (e.g., the pre-filter, the injector pulse damper, etc.) differs with the four system organizers, approach to install the various components is identical.

After each fluidics connection has been made (e.g., the down-stream tube from the pulse damper is connected to the inlet of the injector and a tube is connected to the outlet of the injector), start the pump and allow mobile phase to flow for a minute or two to check the integrity of the fittings. If leaks are observed, tighten the leaking component slowly until the leak stops, but do not overtighten.

3.2.4.1 Injector Fluidics

The tubing from the stirrer or pulse damper (if no mixer is present) should be connected to the proper inlet port of the injection valve and the downstream tubing should be connected to the proper outlet port of the injection valve.

After the fluidics connections have been made (the down-stream tube from the pulse damper is connected to the inlet of the injector and a tube is connected to the outlet of the injector), start the pump and allow mobile phase to flow for a minute or two to check the integrity of the fittings.

NOTE: If an autosampler is used, refer to the manual supplied with the autosampler for the appropriate ports for the tubing from the solvent delivery system and the tubing to the pre-filter and column.

3.2.4.2 In-line Filter

An in-line filter (Part Number 70-4093) is placed in the system between the injector and the column (pre-column). The filter is installed in the same fashion as described in Section 3.2.3. After the injector has been placed in the system, pump mobile phase through the system for a few minutes and check for leaks.

3.2.4.3 Pre-column and Column

The column is installed inside one of the Organizers and should be clamped in place. The down-stream line from the in-line filter is connected to the inlet of the column. (See the appropriate section for further details on installing columns.)

In some applications, a pre-column is employed to remove components of the sample that may be deleterious to the analytical column. If a pre-column is employed, it should be installed in the same manner as the column.

NOTE: The direction of flow of the mobile phase is indicated on the pre-column and column. Check to ensure that the flow through the column is in the correct direction before pumping mobile phase through these components.

After the column has been installed, re-check the connections before the column and tighten the connections as necessary (the system pressure will increase when the column is connected).

After the column has been placed in the system, pump mobile phase through the system for a few minutes and check for leaks. New columns should be flushed with 20 column volumes (30 mL) before connecting downstream components. The pressure required to deliver mobile phase through the system will be dramatically increased. All previous fittings in the system should be re-checked for leaks and re-tightened if necessary.

The maximum pressure setting on the pumps was set at 250 bar at the beginning of the installation procedure (Section 3.2.3). The actual pressure that is required to deliver the mobile phase through a specific column at a flow rate of 1.0 mL/min is dependent on a variety of factors, including the length and inner diameter of the column, the size of the column packing and the nature of the mobile phase. As an example, if a 4.6 mm (ID) x 150 mm long column containing 5 µm particles is used at a flow rate of 1 mL/min methanol/water (20/80), the expected pressure would be in the order of 150-200 bar.

If the pressure required to deliver the flow is greater than 250 bar, the solvent delivery system will not be capable of delivering the mobile phase when the column is installed and will shut down.

NOTE: If the solvent delivery system shuts down due to a pressure overload, the maximum pressure setting should be raised slightly (e.g., to 275 bar). If the pump cannot deliver the mobile phase at a maximum pressure setting of 275 bar, it is likely that there is a blockage in the system. Check the system (i.e., poorly cut tubing, a deformed fitting, a clogged frit in the column, a defective column, etc.) and remedy the situation.
DO NOT CONTINUE TO INCREASE THE MAXIMUM PRESSURE SETTING.

3.2.4.4 In-line Filter

An in-line filter is placed between the column and the cells. The filter is installed in the same fashion as described in Section 3.2.3. After the filter has been placed in the system, pump mobile phase through the system for a few minutes and check for leaks.

NOTE: Use PEEK filter elements (Part Number 70-3824) in the in-line prefilter holders located after the injector. Use graphite filter elements (Part Number 70-0898) before the injector.

3.2.4.5 Cells

Use the tubing and fittings provided with the cells. The cells are mounted in the Thermostatic Chamber as shown in Figure 3-3 or inside the temperature module or the Organizer and are clamped in place. Connect the downstream line from the in-line filter to the inlet of the first cell and connect a piece of tubing to the outflow of the first cell.

NOTE: The direction of flow is indicated on the cells. Check to ensure that the flow through the cells is in the correct direction before pumping mobile phase through the cells.

Turn the pump on and check for leaks. Repeat this process until all cells have been installed. The downstream line from the last cell should be placed into a suitable waste container.

NOTE: There may be a small increase in the pressure required to deliver the mobile phase when a cell is installed. If the solvent delivery system shuts down when mobile phase is pumped through the system after the installation of a cell, it is likely that there is a blockage in the system. Check the system and remedy the cause of the blockage.
DO NOT CONTINUE TO INCREASE THE MAXIMUM PRESSURE SETTING.

The cables from the cells should be led outside the thermostatic chamber (Organizer) through the hole(s) provided for the cables.

3.3 The System Organizer

3.3.1 Types of System Organizers

The System Organizer is a generic term that applies to several modules that are designed to house the fluidics components of the system. The components that can be housed in the organizer include the column, the electrochemical cells, the mixer, the pulse damper and manual injection valve. If a manual injector is included in the system, it can be housed in the CoulArray Thermal Organizer or the CoulArray Organizer

Four different system organizers are available:

- The *CoulArray Thermal Organizer* (Part Number 70-4470T) allows the user to set the temperature of the components via the CoulArray application program. The temperature is displayed on the front of the organizer and in the *Control* dialog box (Section 4.3). The Organizer should be placed on the left side of the control module. Installation of the CoulArray Thermal Organizer is described in Section 3.3.2.

- The *CoulArray Organizer* (Part Number 70-4340) is similar to the Thermal Organizer but does not include the ability to control the temperature. Installation of the CoulArray Organizer is described in Section 3.3.3.

- The *CoulArray Thermostatic Chamber* (Part Number 70-1760) houses the column and cells (but not the injector) and allows the user to set the temperature on a local basis. Installation of the CoulArray Thermostatic Chamber is described in Section 3.3.4.

- The *CoulArray Temperature Module* (Part Number 70-1832) is a thermostated chamber that contains the column and cells. The user must set the temperature on a local basis using the control box supplied with the module. Installation of the CoulArray Temperature Module is described in Section 3.3.4.

If a manual injector is included in the system, it can be housed in the CoulArray Thermal Organizer or the CoulArray Organizer, and installation instructions are added in the appropriate sections.

When another autosampler is employed, the injector is housed within the autosampler.

3.3.2 The CoulArray Thermal Organizer

The *CoulArray Thermal Organizer* (Part Number 70-4470T) is a thermostated chamber in which the temperature is set via the application software. A typical system, which includes an autosampler is shown in Figure 3-4. If a manual injector were present in the system, the injector would be present in the lower left corner of the unit.

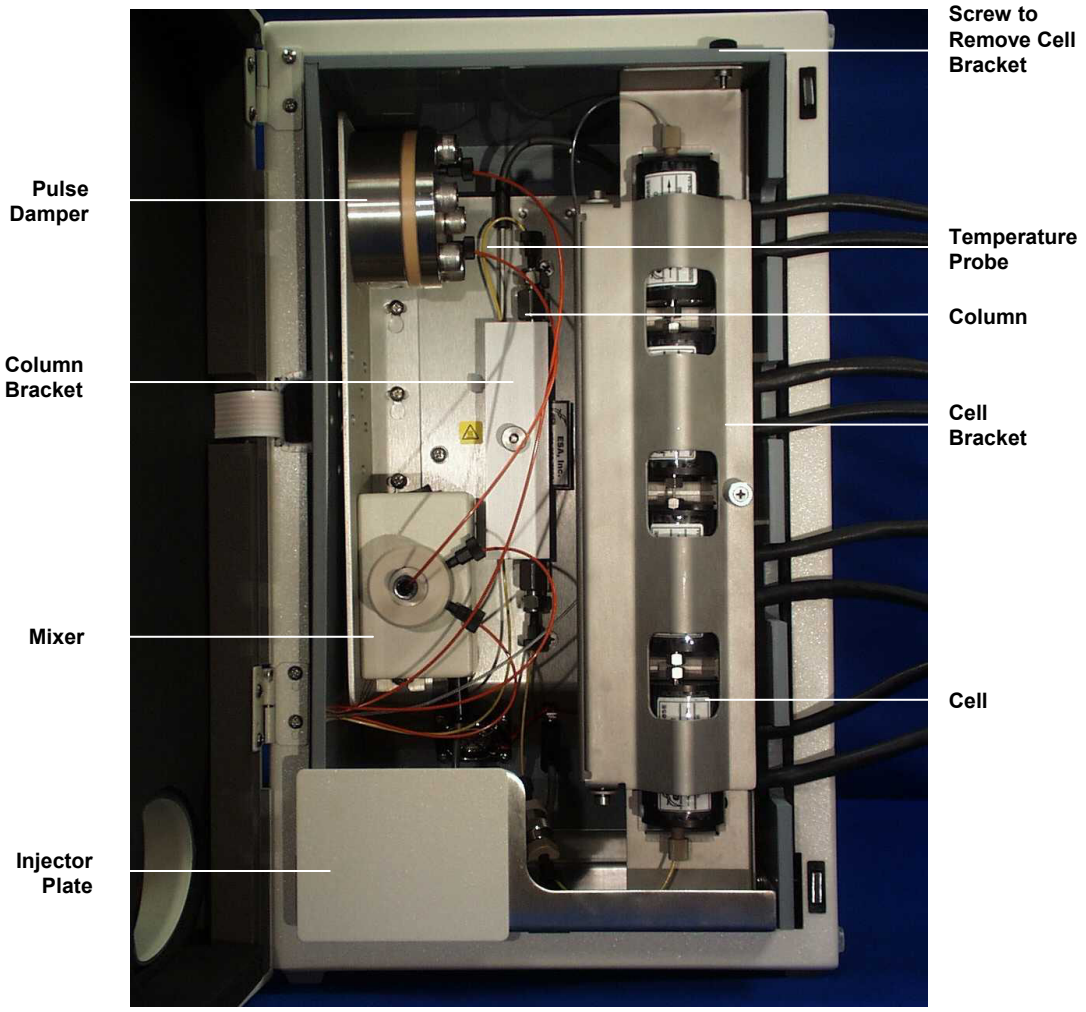

Figure 3-4: CoulArray Thermal Organizer

To open the door of the CoulArray Thermal Organizer, pull on the indents on the top right-hand side of the door or pull open from cable holes on right-hand side of unit.

In this section, we will describe how to install the various components in the CoulArray Thermal Organizer system. A general discussion of the installation of the tubing between the various components is described in Section 3.2.4.

3.3.2.1 The Injector

If an ESA Autosampler is used, the normal mounting position of the injector valve inside the autosampler is employed. The outlet tubing from the injector valve is led into the Thermal Organizer via the hole on the left panel.

If a manual injector is to be used:

a) Remove the plate in the lower left corner by unscrewing the nut that holds it in place (the nut is accessed from the rear).

b) Place the injector valve so that the sample loop is inside the chamber and the handle is to the right.

c) Secure the injector to the module using the two screws that are provided to secure the injector to the module. Make sure the injection handle is located on the right and that the door can be closed properly over the handle in both positions.

d) The drain ports of the injection valve should be directed to a small beaker or weigh boat (not supplied) so that any fluids used to flush the valve are contained. The fluid will evaporate over time.

3.3.2.2 The Dynamic Gradient Mixer

The biocompatible Dynamic Gradient Mixer (if employed) is mounted on a support bracket as shown in (Figure 3-5) which is attached on the left side of the unit. The bracket should be removed from the unit by loosening the two screws and replaced after the mixer attached (unless the pulse damper is to be attached).

Figure 3-5: The Mixer and Pulse Damper Mounted on Support Bracket

Power for the mixer is supplied via a short cable that is connected to the jack in the center of the backplane of the cabinet.

This jack is connected to a jack on the rear panel (Figure 3-6). Power is supplied to the mixer via an adapter (similar to one for a hand calculator) which is plugged into the jack on the rear panel.

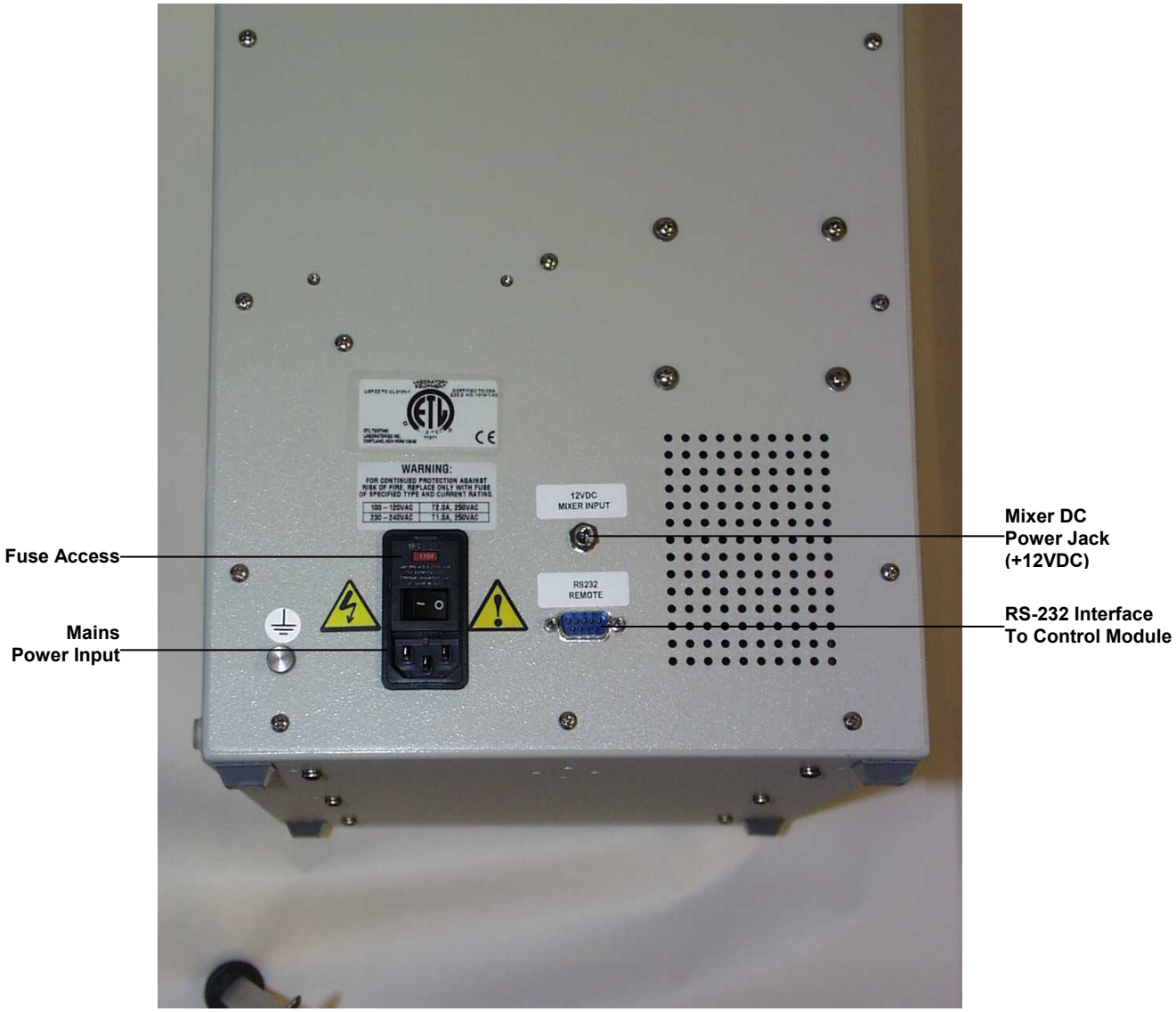

Figure 3-7: The Rear Panel

3.3.2.3 The Pulse Damper

The pulse damper is mounted on the same bracket as the mixer. If the mixer is not present, the pulse damper can be mounted in the lower position.

3.3.2.4 The Column

The column is mounted in a bracket in the center of the chamber as shown in Figure 3-8. A probe is inserted in the bracket to monitor the temperature. The standard bracket included in the organizer is designed for 15 cm columns, other column brackets are available (see Appendix 2). The knurled screw is removed to access the column.

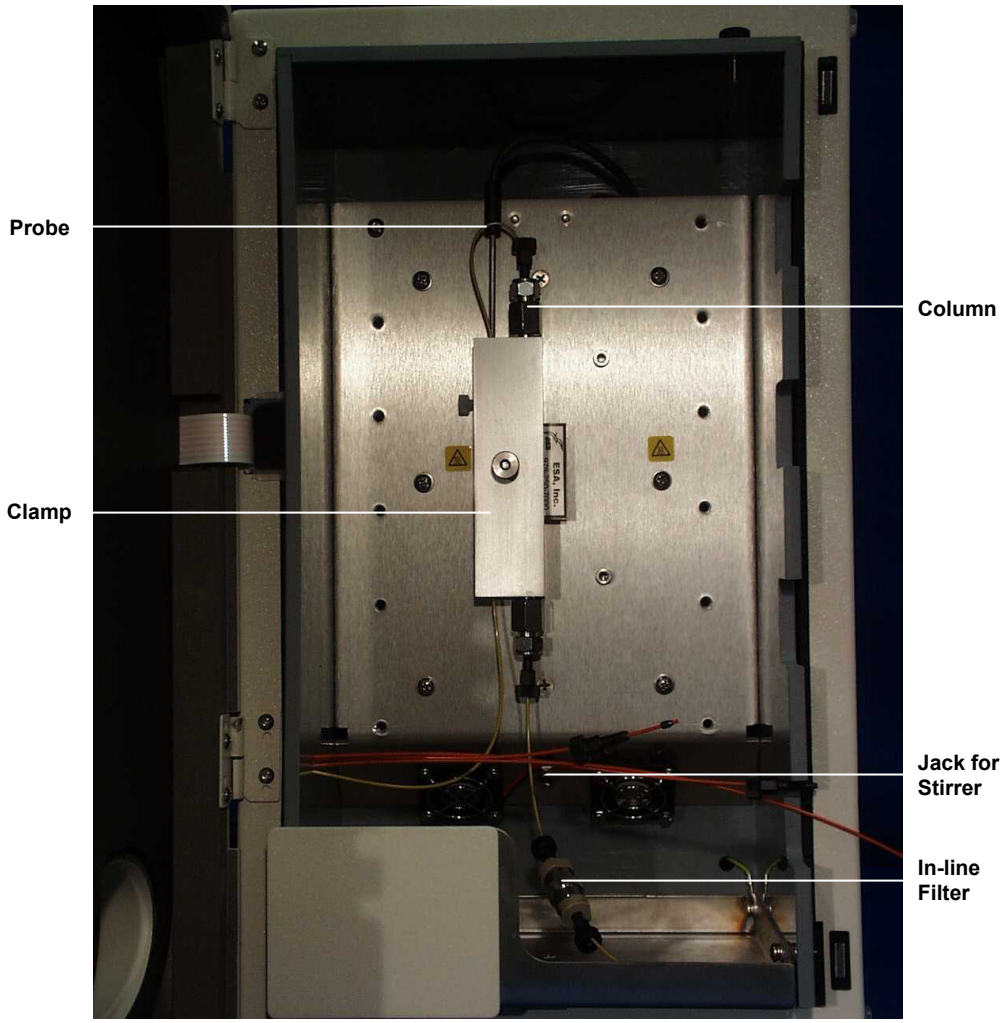

Figure 3-8: Column in Column Bracket

CAUTION: Probe must be inserted properly in the Column Bracket or the temperature will not be maintained properly. Remove screw completely, then insert probe all the way. Tighten screw before assembling clamp.

3.3.2.5 Cells

The bracket in which the cells are mounted is located in the right side of the organizer and is removed by unscrewing the knurled screw at the top, then lifting it off the positioning pins. Once the bracket has been removed from the organizer, open the knurled screw in the center to open the bracket.

Cells are mounted in the cell bracket as shown in Figure 3-9. The tubing between the column and the cells and between the cells should be as short as possible to minimize band broadening.

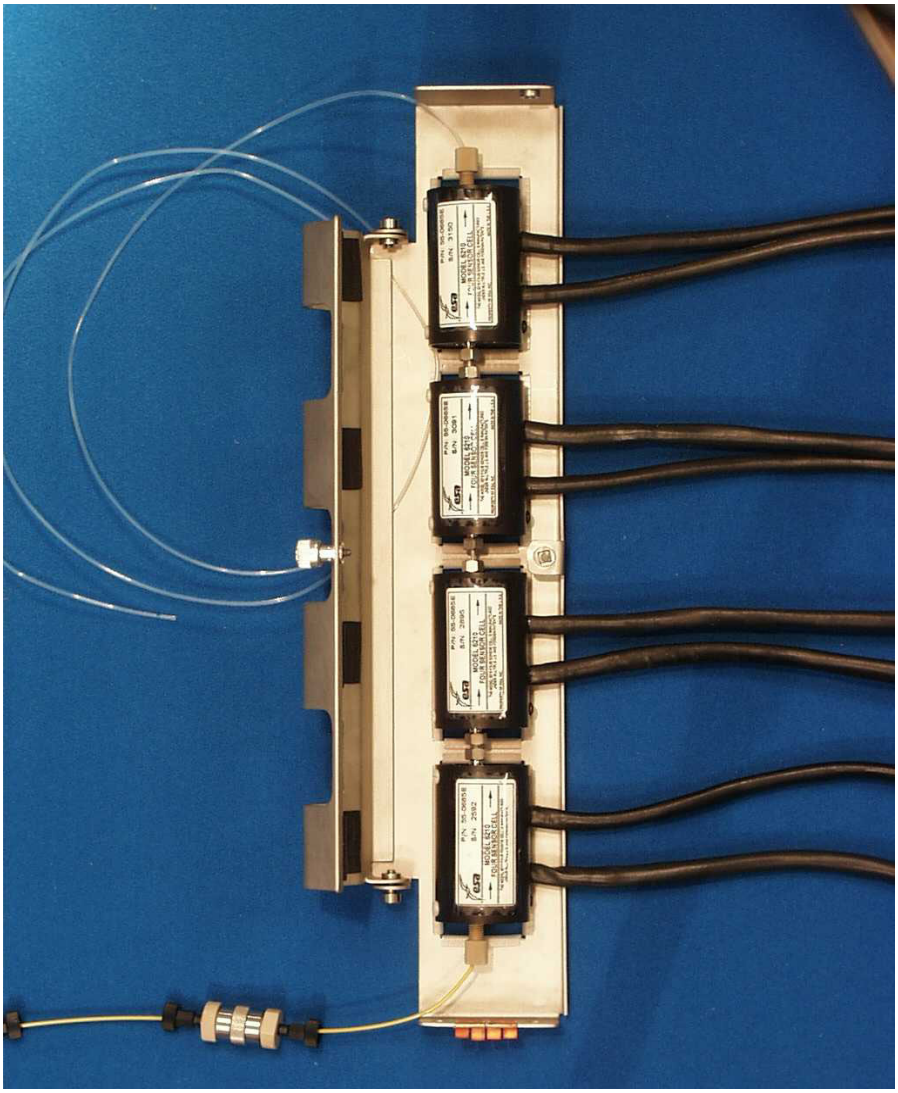

Figure 3-9: Cells in Bracket

The bracket is then closed via the knurled nut (Figure 3-10) and placed in the system.

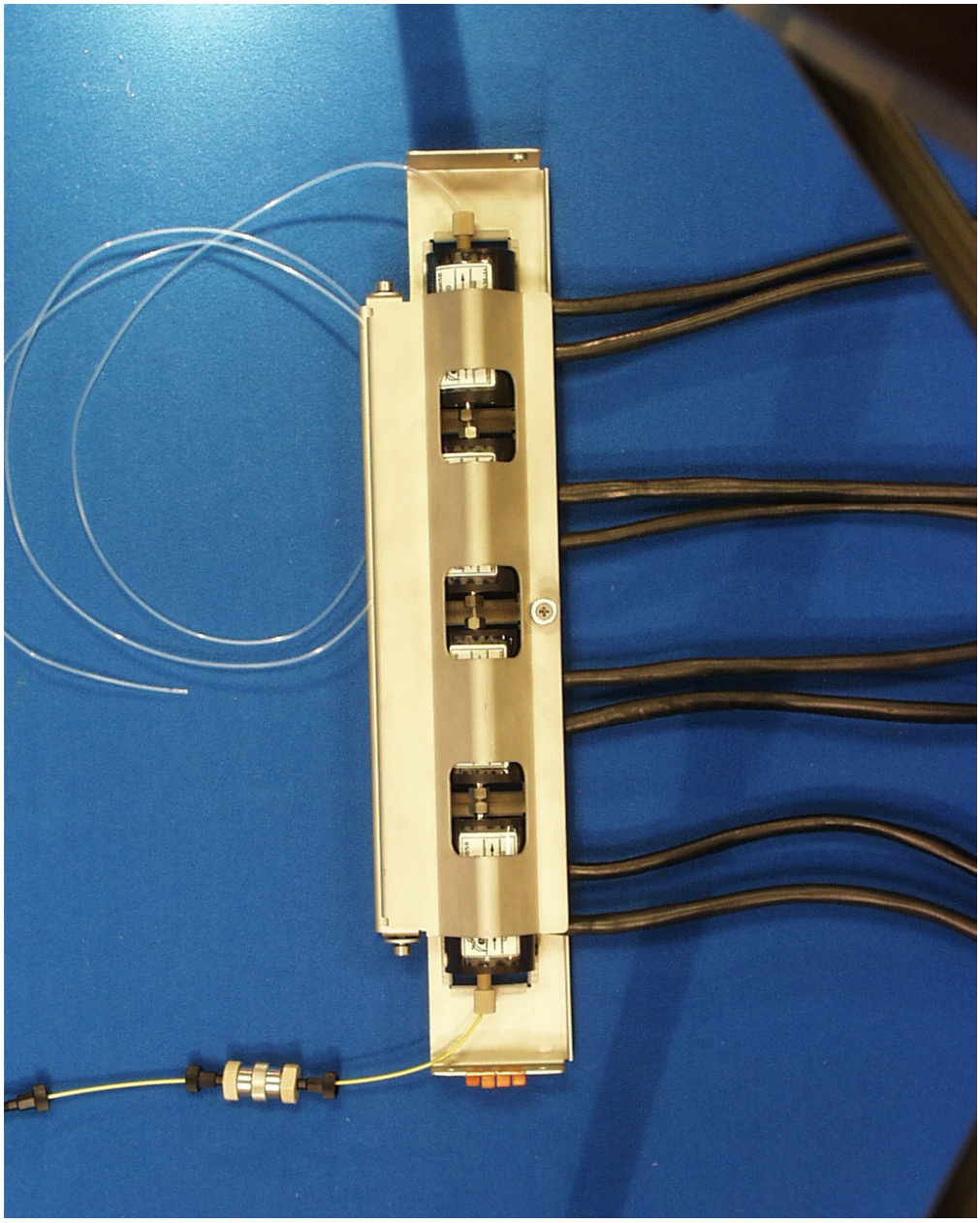

Figure 3-10: The Cell Bracket with Four Cells

The Thermal Organizer should be connected to the line voltage using the included power cord. Connect the Thermal Organizer to the CoulArray detector module (RS-232 port labeled Thermal Organizer) with the proper RS-232 cable.

3.3.3 The CoulArray Organizer

The *CoulArray Organizer* (Part Number 70-4340) contains the column, the cells, the mixer, pulse damper and injection valve and is designed to be operated at ambient temperature. To open the door of the CoulArray Organizer, pull on the indents on the top right-hand side of the door. If desired, you can remove the door by lifting it up to disconnect it from the hinges. A typical configuration is shown in Figure 3-11.

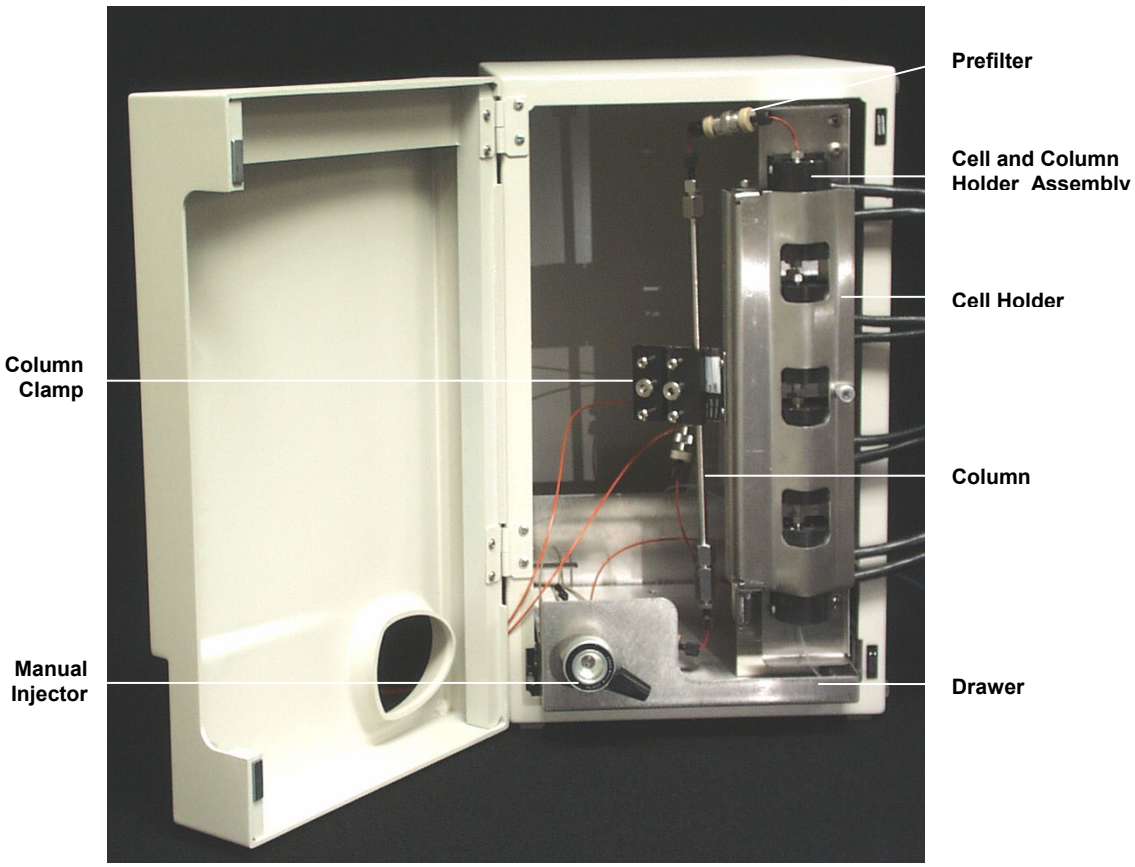

Figure 3-11: Internal Layout of Components in the CoulArray Organizer

To simplify the installing of various components, the drawer of the Organizer can be removed by firmly pulling the drawer out (Figure 3-12).

Figure 3-12: Removing/Inserting Organizer Drawer

If desired, you can fasten the Organizer to the control module using the screw on the right-hand side of the Organizer using either your fingers or a screwdriver inside the Organizer to fasten the two units together.

The ground wire on the back of the Organizer should be connected to the control module by plugging it into the receptacle on the back of the control module.

While the drawer is out connect the drain line (a piece of Tygon® tubing) to the tubing connector located on the bottom of the Organizer (Figure 3-13). The tubing exits the Organizer through the hole on the left side near the bottom.

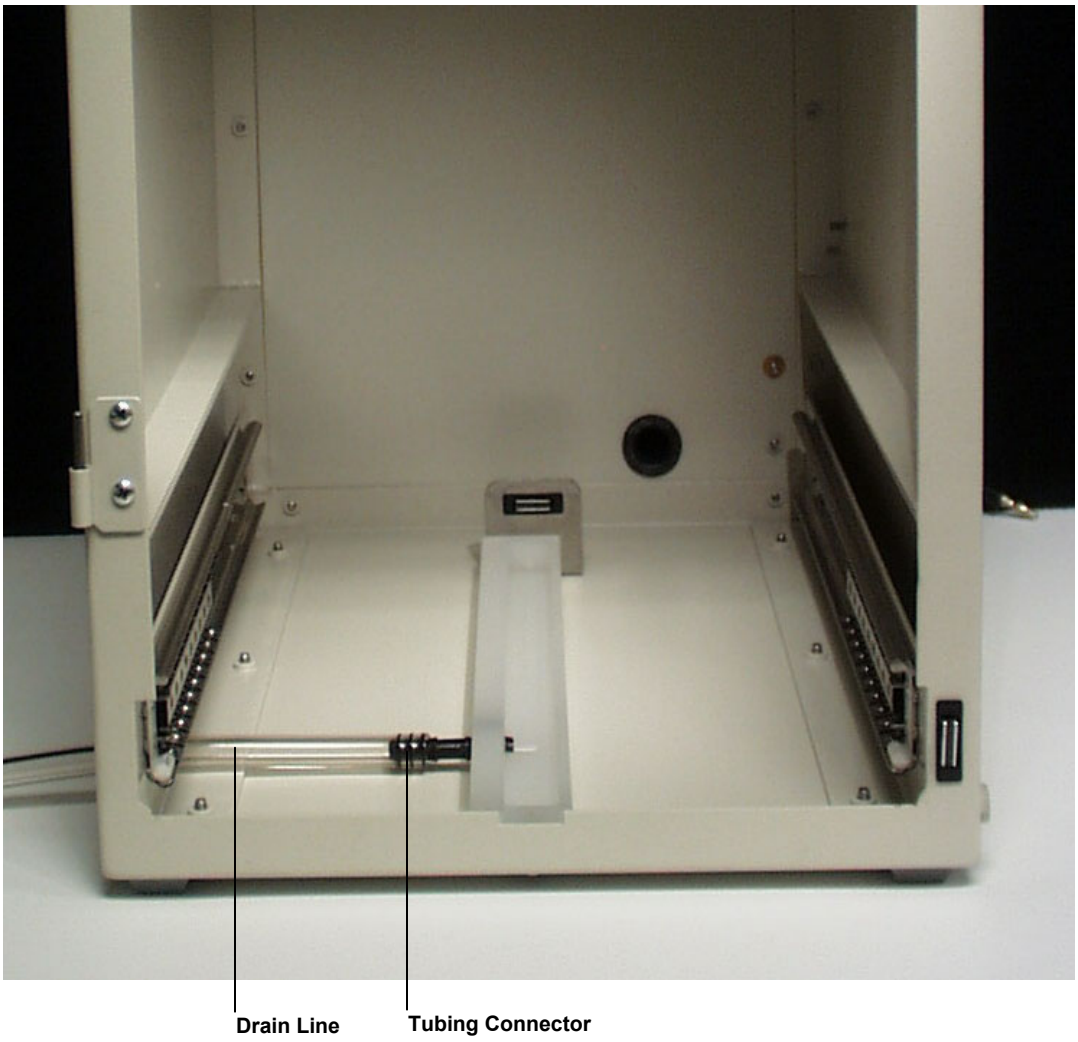

Figure 3-13: Organizer Drain Line Connection

NOTE: The drawer must be pulled out before the drain line can be connected and the two units can be fastened together.

If the unit is installed with a manual injector remove the cover plate from the lower left corner and place the injector valve so that the sample loop is inside the chamber.

Two screws are provided to secure the injector to the module. Make sure the injection handle is located on the right and that the door can be closed properly over the handle in both positions. Place the outlets of the vent tubes through the holes in the Vent Tube Bracket located on the left side of the drawer (see Figure 3-8). Keep the ends of the vent tubes at the same height as the needle port, so that liquid does not siphon out. Place a small container (e.g., a weigh boat) under the vent tube outlets to collect any excess fluid. The Pulse Damper(s) and the Gradient Mixer can be mounted on the right side of the drawer using the 8-32 x 3/8" mounting screws (provided with the component) placed through the precut slots on the side of the drawer (Figure 3-14).

The power cord to the mixer is inserted through the hole in the back of the Organizer. It is recommended that the pulse damper(s) be mounted towards the bottom of the drawer for better support. In addition, other ESA cells (e.g., Model 5020 Guard Cell, Model 5011 cell, Model 5041 cell, etc.) can be mounted to the side of the drawer using the Cell Mounting Plate Assembly (Part Number 70-1694). Alternatively, the above items can be placed unfastened on the bottom of the drawer.

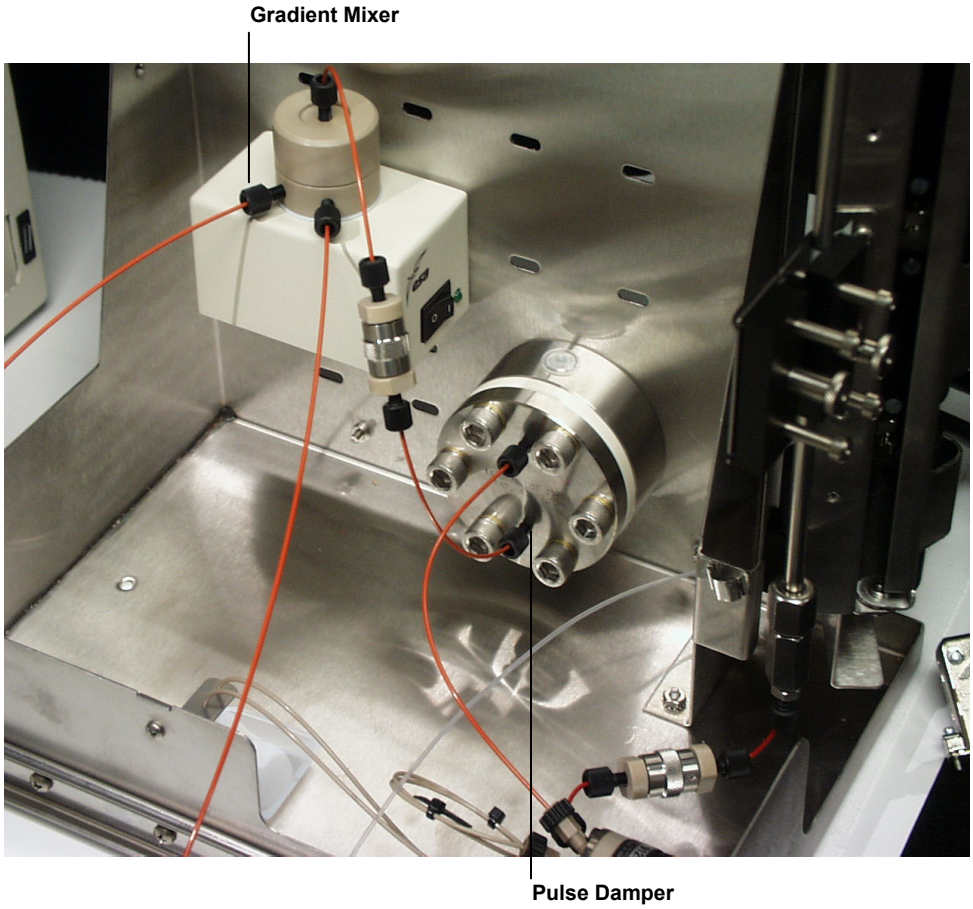

Figure 3-14: Side Mounting Gradient Mixer and Pulse Damper

The Cell and Column Holder Assembly can be removed from the drawer (lift up to remove it from the hinges) to make installation of the column(s), cell(s), pre-filter(s) and their tubing connections easier. The Cell and Column Holder Assembly is hinged to provide some flexibility when the cell cables are connected to the control module and the drawer is pulled out part way. To place the cell(s) in the cell holder, unscrew the finger-tight screw that holds the cell clamp together and open the clamp (Figure 3-15).

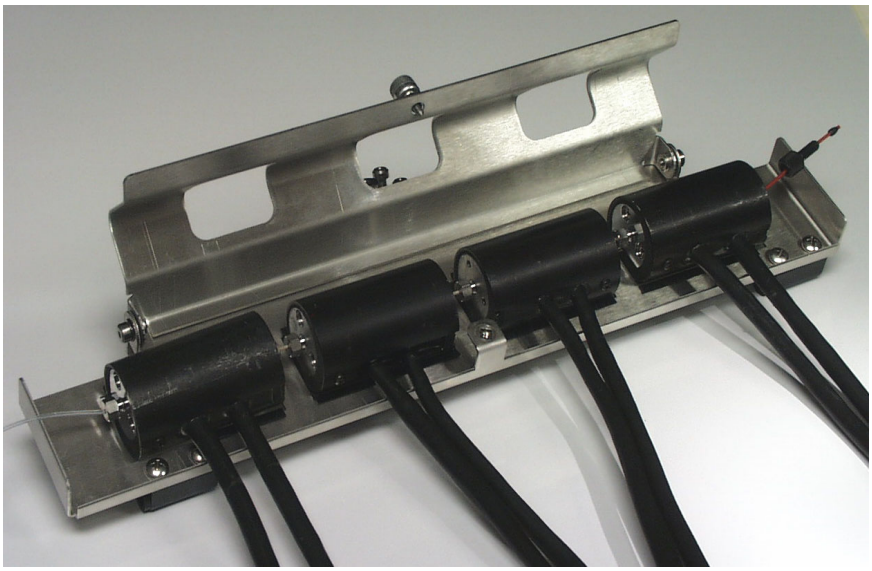

Figure 3-15: Model 6210 Cell Installation

Place the cell(s) in the rectangular cutouts, install the proper tubing (see Section 3.2.2), and close and fasten the clamp (Figure 3-16).

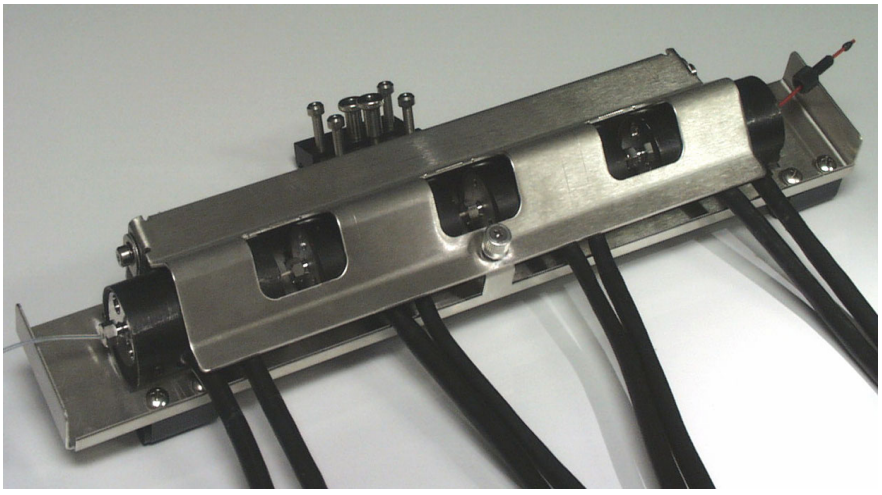

Figure 3-16: Closed Cell Holder

To place a column in the Column Clamp (Figure 3-17), loosen one of the thumbscrews that hold the black Column Holding Block in position. Place the column in the groove between the black block and the rest of the Column Clamp. Tighten the thumbscrew until the column is held firmly in place. A second column can be mounted in the other holder using the above procedure.

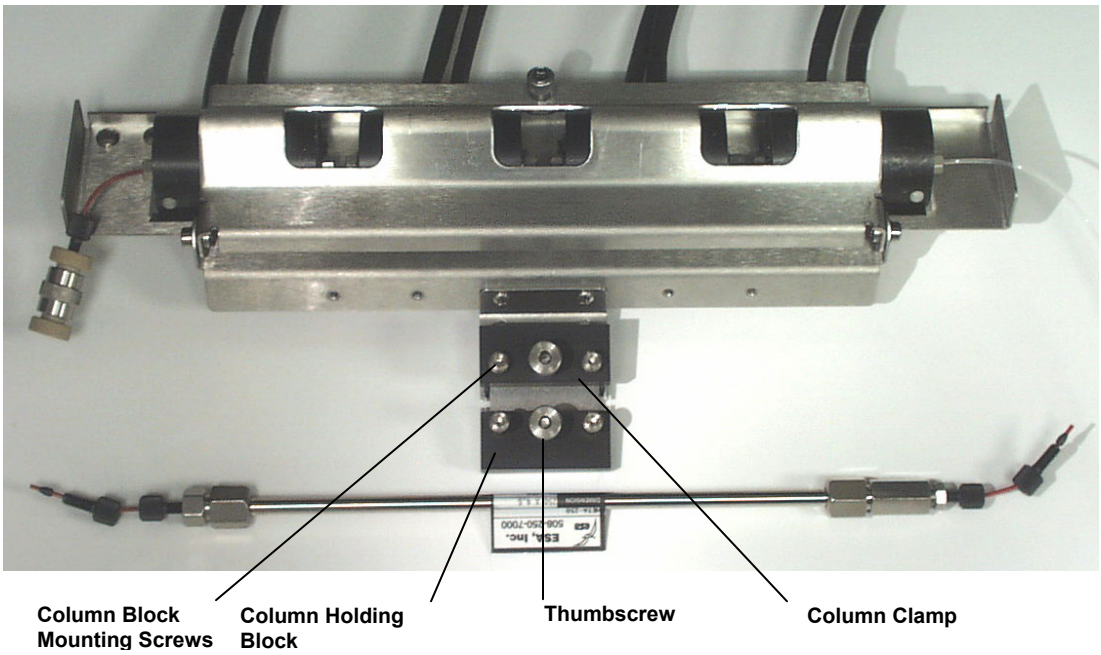

Figure 3-17: Column Installation

NOTE: There are several different positions and angles in which the Column Clamp can be mounted onto the Cell Holder using the Column Clamp Mounting Screws (Figure 3-17). The Column Clamp can be placed at a right angle to the Cell Holder using the two holes nearest the black column holding block on the Column Holder. To place the Column Clamp straight out from the cells, use the two holes furthest away from the columns. The Column Clamp can also be positioned at different vertical heights on the Cell Holder using the mounting holes on the side. Choose whichever position and angle fits and is most convenient for your setup.

The columns can then be connected to cell(s) and pre-filter(s) (Figure 3-18). After placing the Cell and Column Holder Assembly back in the Organizer, it can be connected to other HPLC components (e.g., injectors and waste line) with the proper tubing and connectors.

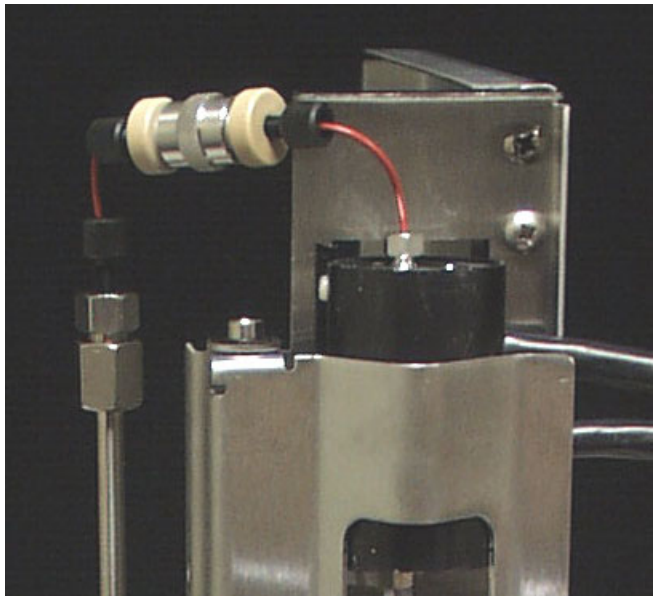

Figure 3-18: Detail of Column, Pre-filter and Cell Connection

The Temperature Module and the Thermostatic Chamber are controlled on a local basis. The manual supplied with each unit provides instructions for setting the desired temperature. The Thermal Organizer is controlled via the CoulArray for Windows application program and setting the desired temperature is described in the manual provided with that program.

3.3.4 The CoulArray Thermostatic Chamber

When the CoulArray Thermostatic Chamber is employed, the column and cells are mounted on the door of the chamber (which is removable). The cells are mounted using the metal clamp and the column is mounted via the springs on the right side. The left side of each spring can be removed from the bracket to mount the column. It is recommended that the cells are installed with the door removed. A typical configuration is shown in Figure 3-19.

If an ESA Model 540 autosampler is used with the CoulArray Thermostatic Chamber, the normal mounting position of the injector valve inside the autosampler is employed. The outlet tubing from the injector valve is led into the chamber.

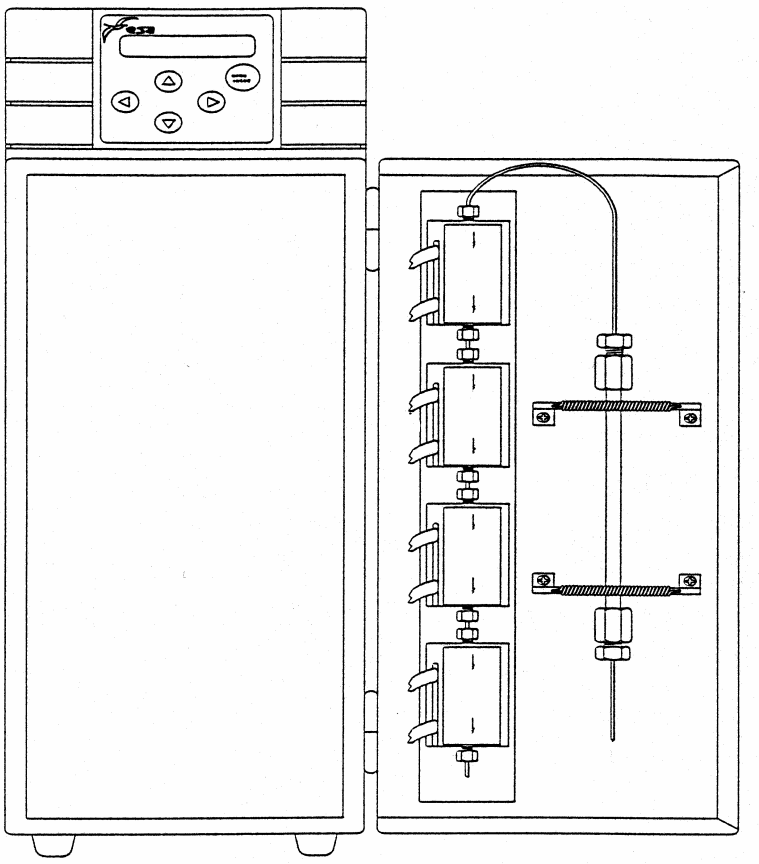

Figure 3-19: Internal Layout of Components in the Thermostatic Chamber

⚠ WARNING: There are heating coils inside the thermostatic chamber. The temperature of the walls of the chamber may be high enough to cause personal injury.

⚠ CAUTION: A space of approximately 1 cm (½") should be maintained between the CoulArray Thermostatic Chamber and the Control Module.

When the CoulArray Thermostatic Chamber is installed with an ESA Model 540 Autosampler, the injection valve is mounted on the right side of the autosampler so that the side of the valve with the tubing is inside the Thermostatic Chamber. Refer to the manual provided with the autosampler for directions on moving the valve inside the autosampler.

3.4 Electrical Connections

The I/O terminal strips must be placed into the I/O Connector socket on the front of the control module. Place the right terminal block (Part Number 70-1634) in the socket so that the holes for the wires face the right edge of the control module, then place the left terminal block (Part Number 70-1656) into the socket so that the holes for the wires face the left edge of the control module. The I/O strips should be pressed in place to ensure good electrical contact.

Connect the various devices to the control module as follows:

- *Computer* - Connect the computer to the control module using the RS-232 Cable (Part Number 70-1743). Connect the end labeled "To Computer" to either COM1 or COM2 on the computer. (Make a note of which COM port is employed.) Connect the other end of the cable to the control module. Ensure that the jack screws on the sides of the connectors are fastened to the control module.
- *Pumps* - If an ESA solvent delivery module is used, connect the pumps to the control module using the RS-232 cable (Part Number 70-4054) and the Fiber Optic RS-232 converter and cable (Part Number 70-4053). Ensure that the jack screws on the sides of the connectors are fastened to the control module.

  If non-ESA pumps are used, connect the pump (or gradient controller) to the Gradient Start contacts on the I/O connector block (if applicable).
- *Autosampler* - The autosampler should be connected to the control module using the Autosampler contacts on the I/O connector block and the proper connection on the back panel of the autosampler using the screwdriver supplied with the CoulArray detector accessory kit. (The cable is included with ESA autosamplers.) Make sure that the ground wire is attached to the control module by the fingertight screw near the I/O connector.
- *Organizer* - A ground wire is located on the back of the module. Insert the connector on the end of the wire to its receptacle on the back of the control module.
- *Thermal Organizer* - The Thermal Organizer should be connected to the control module using the RS-232 cable provided with the module to the Thermal Organizer port.
- *Cells* - The cables from each cell should be connected to the appropriate port on the control module. Make certain that the A cable for a given cell is connected to the A port and the B cable is connected to the B port. Ensure that the jack screws on the sides of the connectors are fastened to the control module.

CAUTION: Before turning on the CoulArray control module, make sure the computer, pump(s) and Thermal Organizer are turned off. Turn on the CoulArray module first. Next, turn on your pump(s) and Thermal Organizer. Finally, turn on your computer and start the CoulArray program.

NOTE: The RS-232 and the I/O cables can be placed in the cable chase to reduce the clutter of wiring. The cable chase is located on the bottom right side of the control module. To remove the cover, firmly grasp the cover and slide it towards the rear of the control module and then lift it out. After the cables have been routed through the cable chase replace the cover by making sure that the metal tabs on the cover are properly inserted into the slots on the control module and sliding the cover towards the front of the unit. To make routing the cables easier, remove the door to the control module first.

Plug the power cord into the back of the control module and the other end into a properly grounded electrical outlet. Turn the power on by pressing the switch on the back of the control module.

If external start devices, valves or auxiliary devices are included in the system, attach them to the appropriate contacts on the I/O connector block at this time. Make sure to attach the ground wire to the control module by the fingertight screw near the I/O connector.

The Temperature Module and the Thermostatic Chamber are controlled on a local basis. The manual supplied with each unit provides instructions for setting the desired temperature. The Thermal Organizer is controlled via the CoulArray for Windows application program and setting the desired temperature is described in the manual provided with that program.

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4 Installing the Control Module and Software

4.1 Electrical Connections

Two I/O terminal strips are provided with the CoulArray Control Module that are placed into the I/O Connector socket on the front of the control module (Figure 4-1). Place the right terminal block (Part Number 70-1634) in the socket so that the holes for the wires face the right edge of the control module, then place the left terminal block (Part Number 70-1656) into the socket so that the holes for the wires face the left edge of the control module. The I/O strips should be pressed in place to ensure good electrical contact.

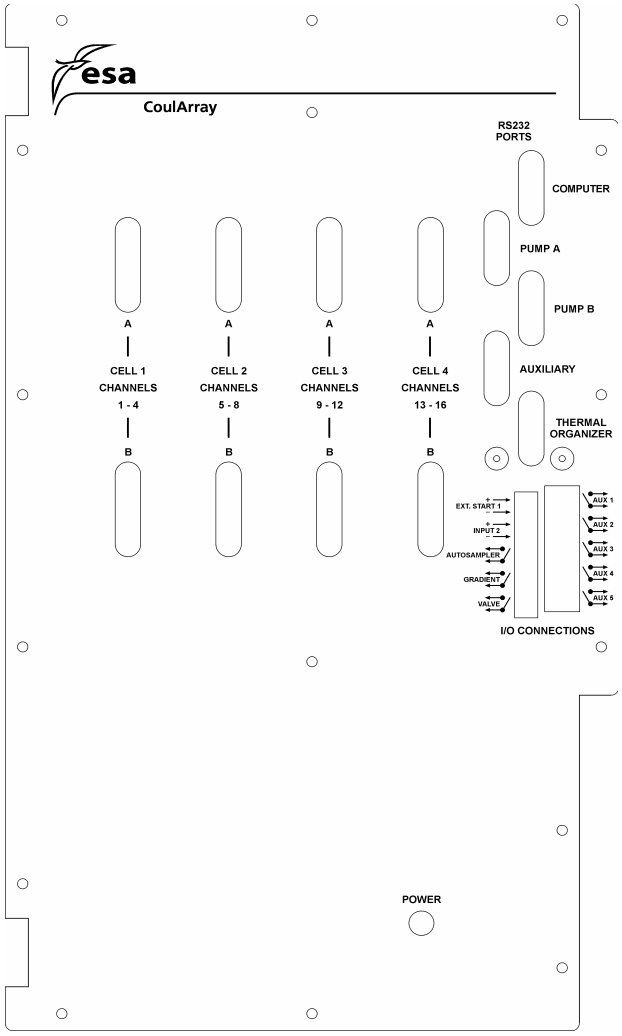

Figure 4-1: Front Panel of the Control Module

Connect the various devices to the control module as follows:

- *Computer* - Connect the computer to the control module using the RS-232 Cable (Part Number 70-1743). Connect the end labeled "To Computer" to either COM1 or COM2 on the computer. (Make a note of which COM port is employed.) Connect the other end of the cable to the control module. Ensure that the jack screws on the sides of the connectors are fastened to the control module.

- *Pumps* - If an ESA solvent delivery module is used, connect the pumps to the control module using the RS-232 cable (Part Number 70-4054) and the Fiber Optic RS-232 converter and cable (Part Number 70-4053). Ensure that the jack screws on the sides of the connectors are fastened to the control module.

  If non-ESA pumps are used, connect the pump (or gradient controller) to the Gradient Start contacts on the I/O connector block (if applicable).

- *Autosampler* - If an ESA Model 540 or 542 Autosampler is used, it should be directly connected to the computer via an RS-232 cable (Part Number 70-1743). All other autosamplers should be connected to the control module using the Autosampler contacts on the I/O connector block and the proper connection on the back panel of the autosampler using the screwdriver supplied with the CoulArray detector accessory kit. (The cable is included with ESA autosamplers.) Make sure that the ground wire is attached to the control module by the fingertight screw near the I/O connector.

- *Organizer* - A ground wire is located on the back of the module. Insert the connector on the end of the wire to its receptacle on the back of the control module.

- *Thermal Organizer* - The Thermal Organizer should be connected to the control module using the cable provided with the module to the Thermal Organizer port.

- *Cells* - The cables from each cell should be connected to the appropriate port on the control module. Make certain that the A cable for a given cell is connected to the A port and the B cable is connected to the B port. Ensure that the jack screws on the sides of the connectors are fastened to the control module.

⚠ CAUTION: Before turning on the CoulArray control module, make sure the computer, pump(s) and Thermal Organizer are turned off. Turn on the CoulArray module first. Next, turn on your pump(s) and Thermal Organizer. Finally, turn on your computer and start the CoulArray program.

NOTE: The RS-232 and the I/O cables can be placed in the cable chase to reduce the clutter of wiring. The cable chase is located on the bottom right side of the control module. To remove the cover, firmly grasp the cover and slide it towards the rear of the control module and then lift it out. After the cables have been routed through the cable chase replace the cover by making sure that the metal tabs on the cover are properly inserted into the slots on the control module and sliding the cover towards the front of the unit. To make routing the cables easier, remove the door to the control module first.

Plug the power cord into the back of the control module and the other end into a properly grounded electrical outlet. Turn the power on by pressing the switch on the back of the control module.

If external start devices, valves or auxiliary devices are included in the system, attach them to the appropriate contacts on the I/O connector block at this time. Make sure to attach the ground wire to the control module by the fingertight screw near the I/O connector.

The Temperature Module and the Thermostatic Chamber are controlled on a local basis. The manual supplied with each unit provides instructions for setting the desired temperature. The Thermal Organizer is controlled via the CoulArray for Windows application program and setting the desired temperature is described in the manual provided with that program.

4.2 Loading Software

The computer should be installed as described in the documentation provided with the computer. (The software should be used with Windows XP and higher. It may work satisfactorily on Windows 95, 98 and NT 4.0, but it is not supported on these operating systems.)

The *CoulArray® for Windows®* Application Software is provided on a CD-ROM disk, which should be installed as indicated in Appendix A of the CoulArray Applications Software Manual. Version 1.10 or higher is required if the CoulArray Thermal Organizer is included in the system. If this is an upgrade installation, the software will be included.

4.3 Configuring the System

Once the software has been installed, run the *CoulArray* program. Access the *Configuration* dialog box by selecting the **Hardware Configuration** button. Next, select the *Instrument* page tab. Select the appropriate entries for Pump Number, Cell Options, Autosampler and COM Port. (Also, select the External Start and Cell Inversion options as required.) A detailed discussion of the software installation protocol is included in the *CoulArray Applications Software Manual*.

> ☞ **NOTE: Upon startup of the CoulArray program, it will attempt to connect to the control module using the default port of COM1. Wait until the error message is displayed, then select OK. At this point, you can choose a different COM port setting (if desired).**

Access the *Launch* Window (Figure 4-2) by selecting the ESA icon. From the *Launch* Window, select the **Hardware Control** button to present the *Control* dialog box (Figure 4-3).

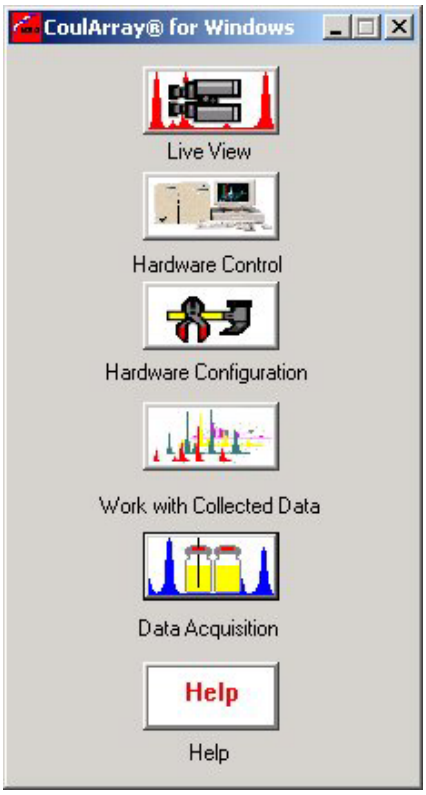

Figure 4-2: The CoulArray for Windows Launch Dialog Box

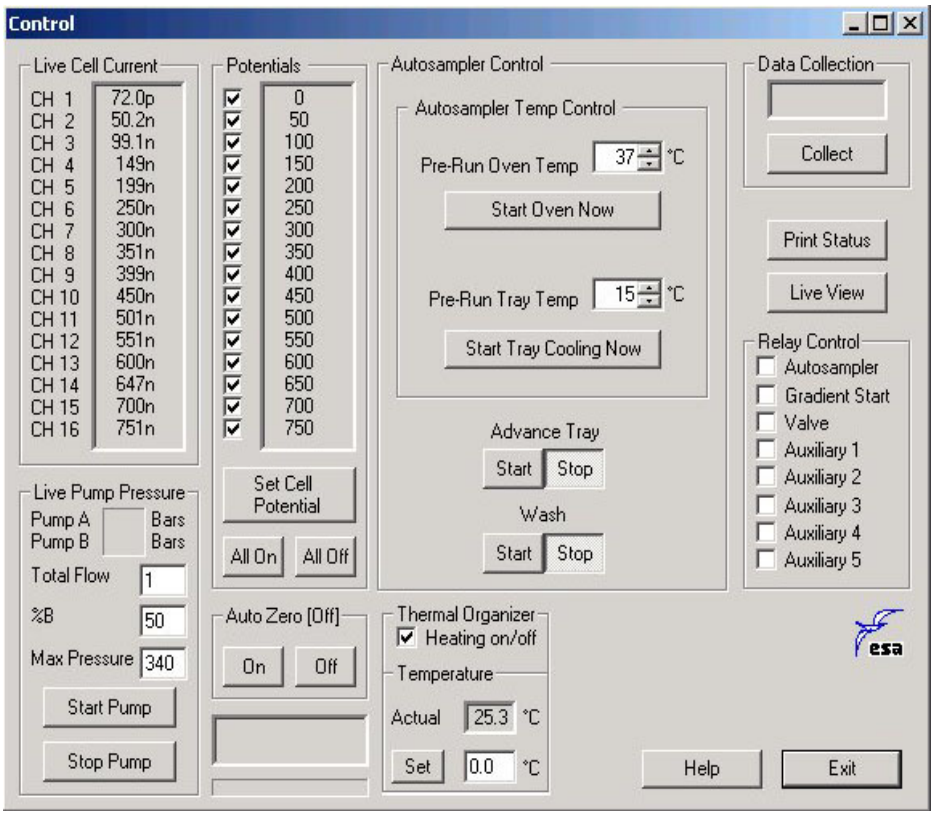

Figure 4-3: The Control Dialog Box

The format of the Control dialog box is dependent on the system configuration. The Control dialog box in Figure 4-3 corresponds to a system with two ESA Model 582 Pumps, 16 channels, a Model 542 Autosampler and a CoulArray Thermal Organizer.

- If the system does not include ESA Model 582 Pumps, the field in the lower left will be empty.
- If the system does not include the Model 540 or 542 Autosampler, the *Autosampler Control* fields will include only the *Wash* buttons.
- If the system does not include the Thermal Organizer; the field in the center right will be absent.

4.4 Setting Instrumental Parameters and Checking the System

4.4.1 Introduction to the Control Window

The *Control* window is used when the analyst wants to operate the system on a manual basis. This approach is more useful for installation of the system, as the analyst can readily control a variety of devices that are used during the installation (e.g., pumps and detector) and rapidly change parameters as desired.

In control mode, the following operations can be performed:

- Setting the Mobile Phase flow rate and composition (only if ESA pumps are employed using the RS-232 port).
- Setting the Maximum Pressure for the System (only if ESA pumps are employed using the RS-232 port).
- Setting the Temperature (if the Thermal Organizer is installed).
- Starting a Gradient (non-ESA pumps).
- Setting the Potential for Each Cell.
- Autozeroing the Detector.
- Opening/Closing of Valves (if applicable).
- Injecting Samples.
- Collecting/Recording/Saving Data.

4.4.2 Initializing Pump Flow

4.4.2.1 Using ESA Pumps

If the system includes an ESA mobile phase delivery system, the flow rate, maximum pressure and %B, the composition of the mobile phase (if a gradient system is employed), can be set via the *Control* window.

☞ NOTE: If the system contains a single ESA pump, it may be configured so that the above parameters cannot be controlled via the control window and the pump is connected to the I/O block instead of the RS-232 port. In this event, the directions in Section 4.2.2.2 should be used.

To edit a value, click on the numerical value for that field and type the desired value.

Use a *Total Flow* of 1 mL/min and % B = 50 (if two pumps are used). The *Max Pressure* value should be the same as was set on the pump after the column was installed.

At this point, change the mobile phase from methanol:water (20:80) to the mobile phase that you intend to use for your analytical work. Make certain that the mobile phase is filtered through a 0.22 µm filter.

To initiate the flow, press ***Start Pump*** (the Gradient Start field is not active in this mode). The *PUMP A* and *PUMP B* (if used) entries in the lower left corner will indicate the present pressure. If the pumps stop due to an increase in the back pressure, raise the *Max Pressure* value by 10 bar.

It is suggested that you pump mobile phase through the system for an hour or two and monitor for leaks before proceeding.

4.4.2.2 Using Non-ESA Pumps

The composition of the mobile phase and the flow rate of the solvent delivery system are set on a local basis. The *Gradient Start* field is active in this mode, but the *Total Flow*, % B, and the pressure indicators for the pump are not active.

At this point, change the mobile phase from methanol:water (20:80) to the mobile phase that you intend to use for your analytical work. Make certain that the mobile phase is filtered through a 0.22 µm filter. If the pump(s) stop due to an increase in the pressure, raise the *Max Pressure* value by 10 bar.

It is suggested that you pump mobile phase through the system for an hour or two and monitor for leaks before proceeding.

4.4.3 Setting Electrode Potentials

The potential of each channel can be set using the *Cell Setup* dialog box accessed by selecting the **Set Cell Potential** button.

⚠ CAUTION: Do not apply potentials to electrodes without mobile phase flowing through the cells.

There are two ways to set the cell potentials:

a) Enter the desired value in each field; or

b) Use the *Increment Potentials* field in the upper right corner.

In most cases, the cell potentials are set at a fixed increment (e.g., Cell 1 is at 400 mV, Cell 2 is at 440 mV, Cell 3 is at 480 mV, Cell 4 is at 520 mV, etc.).

The *Increment Potentials* field in the upper right corner can be used to set the desired series using the following entries:

Start with Cell:	**1**
Start Potential:	**400**
Last Cell:	**16**
Increment:	**40**

After the desired values have been entered, click on **Increment**. The potential for each cell will be indicated in the appropriate field. If desired, you may edit any individual channel. To set the potential for cell 10 to be 635 mV, move the cursor to that field, click the mouse and enter the value via the keypad. When editing the potentials is complete, click OK to return to the *Control* dialog box (Figure 3-14).

To activate the potentials, click the **All On** button. The *Control* dialog box will appear as shown in Figure 4-4. The *Live Cell Current* field indicates the present current for each cell in the system and will be updated every second.

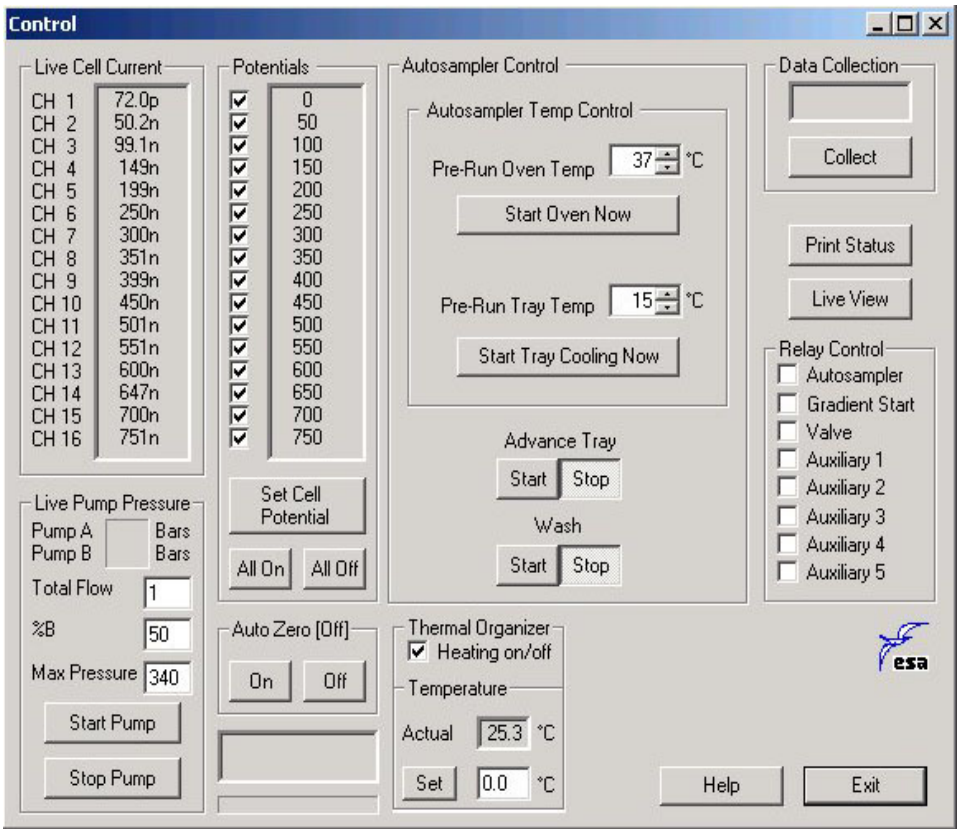

Figure 4-4: The *Control* Dialog Box after the Potentials have been Entered

Autozero the detector, using the **Autozero ON** button. The *Live Cell Current* column displays the currents after autozeroing; these will typically be non-zero but in the pA range. If the cells have not yet stabilized, monitor the currents for a period of time.

NOTE: If you want to turn off a specific cell, click on the check box adjacent to that cell.

The current for each channel will be updated every 0.5 seconds. To view the currents in a graphic format, press **Collect**. The display will appear as shown in Figure 4-5.

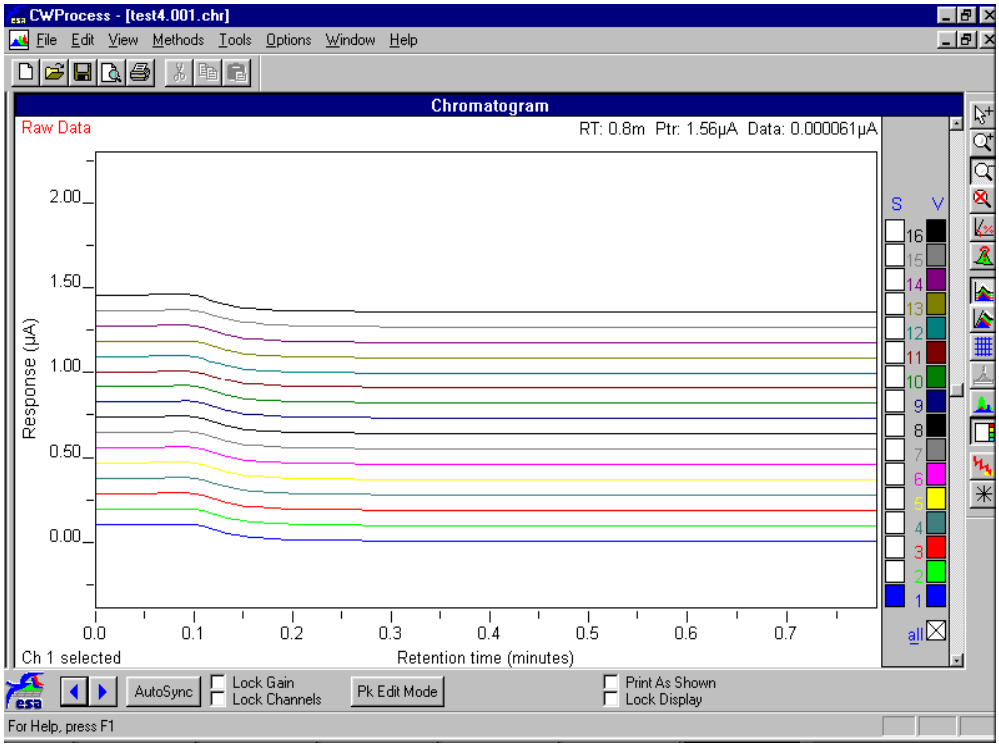

Figure 4-5: The Chromatogram Display

When the graphic display is being presented, the *Control* window can be viewed again by selecting the **CWAcquire** button at the bottom of the screen on the task bar.

4.4.4 Monitoring System Performance

The observed current for each channel is dependent on a variety of factors including the nature of the mobile phase that is used and the cell potential; thus it is very difficult to determine a "satisfactory response" to indicate that the system is performing on an acceptable basis. The information below is presented as a "rough rule of thumb" using a mobile phase that contains methanol:water (100 mM buffer) (20/80) at a flow rate of 1 mL/min and a cell potential less than 500 mV.

The user should be concerned only if significant deviations are observed.

- When the cells are initially powered up, the background current should be less than 10 μA. After a few minutes, the current should stabilize to less than 200 nA.

- The drift in the signal for a given cell should be less than 2% over a 10 minute period after the stabilization period indicated above.

If you suspect that the cell current is excessively noisy or has excessive drift, refer to Chapter 7, which describes a series of troubleshooting protocols. A cell simulator test load is provided with the system, which can be used to test potentiostats and the control module, as described in Section 7.10.

The amount of time needed to allow the cell to equilibrate to a steady baseline is dependent on a number of factors, such as the nature of the mobile phase, the potential applied to the cell and the level of sensitivity needed for the analysis. The equilibration could take as little as a few minutes or several hours for very sensitive analyses.

4.4.5 Checking Ancillary Devices on the System

If ancillary devices, such as an autosampler or additional valves, are present on the system ensure that the desired action occurs when the position of the device is changed via the appropriate field in the center of the fourth column on the *Control* window.

Typically, an audible sound will be heard when the position of the valve is changed or the autosampler is operated.

If the device does not operate when the signal is issued, check that the cabling is secure on the control module and the device. If the cable is satisfactory, perform any internal diagnostic procedures suggested by the manufacturer of the other device to ensure that it is working correctly on a local basis. The output of the detector module can be checked by applying an ohm-meter to the appropriate I/O connection.

To check that the Thermal Organizer is operating, check the Heating on/off box, enter a temperature that is above ambient (e.g., 45°C) and press the **Set** button. Monitor that the reading on the monitor and the reading on the Thermal Organizer increases.

4.4.6 Injecting a Standard

Once the baselines are at an appropriate level, a standard can be injected to determine that the CoulArray detector is capable of analyzing the components of interest at the appropriate concentrations.

It is very difficult to define precise settings and an "acceptable" response, due to the tremendous diversity in analytical conditions that can be used by the system. The approach employed in this section is designed to provide a framework for the operator to determine if the system is operating in a normal fashion.

Set the cell potentials at the range for the compounds of interest. The cell potentials can be changed by the process indicated in Section 3.5.3. If only one or two potentials are to be changed, click on the appropriate field and enter the desired value and wait for the baseline to stabilize.

Inject the standard and click **AUTOZERO ON** to zero the baselines.

- If you are using an autosampler, press the ON indicator adjacent to *Autosampler* under *Relay Control*, then press **Collect** to view the chromatogram.

- If you are using a manual injector, press **Collect** to view the chromatogram.

If an expected peak is not observed, the system cannot be autozeroed or the chromatogram appears excessively noisy, refer to Chapter 6 (Troubleshooting) for assistance.

5 Starting the System

5.1 Overview

When the system is operating, several chromatographic and electrochemical equilibria are established. If the unit is turned off and then on again, it is necessary to wait until these equilibria have been re-established to obtain maximum performance. For routine operation, it is recommended that the power to the electrodes and mobile phase flow be maintained at all times. This is especially important if the system is used to detect very low concentrations of the compounds of interest.

In this section, we assume that the instrument has been installed as described in Chapter 3. A short term shutdown protocol is discussed in Section 5.5.

⚠ CAUTION: If the column and/or the cells are allowed to become dry, irreversible damage can occur to these components. Chapter 8 describes the procedure that should be used to prepare these components for long-term shutdown.

5.2 A Starting Protocol

This section describes the activities that should be performed before collecting analytical data. It assumes that the system has been installed as described in Chapter 3 and that the flow of mobile phase and power to the electrodes were maintained during the shut-down procedure. If the system was fully shut-down (i.e., the flow was stopped and power to the electrodes was shut off) refer to Section 8.3.

5.2.1 Starting Checklist

The following items should be checked on a routine basis before equilibrating the system:

- There is sufficient mobile phase for the expected analyses.
- The temperature that is set on the Thermal Organizer (Thermal Chamber, Thermostatic Chamber) is correct for the separation.
- There is sufficient pump seal flush solution.
- The seal flush system is primed and is flowing properly.
- All connections are leak free. Check for the presence of salt on joints and at the base of all components. In addition, check for liquid at the base of all components. If a salt deposit and/or liquid are observed, tighten the offending joint (but do not overtighten). In a few cases, a new fitting may be required.
- The autosampler temperature(s) is correct (if applicable).
- The autosampler syringe is bubble free.
- The autosampler wash syringe has sufficient solution for the expected analyses.
- The filters in the mobile phase storage bottles are clean and free of particulate matter.
- There is sufficient room on the disk for storage of the desired data to be collected. It is suggested that data be backed up on a daily basis.
- The background currents have not changed significantly from the levels that were observed on the previous day.
- The pump(s) pressure level has not changed significantly from the level that was observed on the previous day.

WARNING: The temperature of the column organizer may be set above the ambient temperature. To avoid injury, if the temperature is elevated, allow the compartment to cool before handling components in the organizer.

5.2.2 Equilibration of the System

Set the desired flow rate and allow the system to equilibrate (approximately 20 system volumes). This equilibration can be done via the *Control* window for ESA pumps controlled via the RS-232 port. If the system employs non-ESA pumps, the mobile phase flow rate is set on the pump. To employ a gradient for equilibration, a method (see Chapters 5 and 6 of the *CoulArray Applications Software Manual*) should be established.

Before samples are analyzed, it is suggested that a reference sample is analyzed to ensure that the system is working in a satisfactory manner.

5.3 Normal Operation

The application program provided with the CoulArray® detector allows for two different modes of operation of the system:

- *Control Mode* - which is used to manually control all operations of the system. This mode is normally employed during method development, troubleshooting and related activities, when the operator wants to rapidly determine the effect of small changes in the system or monitor system performance.

  Operation of the system in Control Mode is described in Chapter 3 of the *CoulArray Applications Software Manual*.

- *Automated Mode* - in which a programmed method is used to collect data, process data and report data. The method includes a time-based data acquisition program to control all aspects of the operation of the instruments.

  Establishing a method in Automated Mode is described in Chapter 6 of the *CoulArray Applications Software Manual*.

  Operation of the system in Automated Mode is described in Chapter 5 of the *CoulArray Applications Software Manual*.

5.4 Hints for Successful Operation

In this section we present a series of practical suggestions that have been found to maximize the utility of the overall HPLC system and minimize down time.

- Always filter the sample and the mobile phase using a 0.22 μm filter. If the sample contains protein and/or lipids use a 10,000 MW cut-off filter to ensure that high molecular weight compounds do not enter the system.
- When relieving pressure, do it gradually. Do not open a fitting on the high pressure side of the system to relieve pressure.
- When a new mobile phase or column is used, allow sufficient time for system equilibration. Typically it may take an hour if a buffer is used; however if a mobile phase containing an ion-pairing reagent (e.g., SDS) is used, the required time may be extended.
- Run at least one standard with every batch of samples. Typically 10% of the samples should be standards.
- Ensure that the cells have mobile phase flowing through them at all times when a potential is applied.
- Do not use metal filters in the solvent bottles.
- Monitor the system pressure. Any significant change (e.g., more than +/- 4 bar) is an indication that something may have changed.
- When you make a fitting, do not overtighten. Initially, the fitting should be made hand tight. Pump mobile phase through the system; if a leak is observed, then tighten the fitting gradually until the leak stops. Overtightening can irreversibly damage fittings.
- The use of a guard column to protect the analytical column is recommended. The guard column should be changed on a periodic basis.
- A change in the shape of a peak (e.g., tailing) or a change in the retention times may suggest that the column is deteriorating.
- Periodically transfer data to an external storage device. The CoulArray detector generates a significant amount of data during a run. If data is stored on the hard disk, you may quickly fill it up.

- Adhere to all maintenance procedures and schedules. See Chapter 6 of this manual for CoulArray maintenance procedures.

- If a change in the audible intensity or pitch is heard, it is likely that a change in the system has occurred. While this is clearly not a scientific observation, it may be worthwhile to track down the cause.

- Use PEEK™ filter elements (Part Number 70-3824) in the in-line prefilter holders located after the injector. Use graphite filter elements (Part Number 70-0898) in holders before the injector.

5.5 Short-Term Shutdown

5.5.1 Shutting the System Down for a Short Period of Time

A short-term shutdown should be employed when the instrument will be used again in a short period of time (e.g., the next morning or after a weekend). It is designed to minimize the period of time required to prepare the system for a new analysis.

To shut the system down for a short period of time:

a) Ensure that the mobile phase reservoirs contain sufficient solvent for the shut down period.

b) Ensure that the upper pressure limit is set to approximately 35 bar above the pressure observed with the normal flow rate (to protect the system in case of a blockage).

c) Set the cell potentials to the normal operating conditions or less than 700 mV, whichever is less.

5.5.2 Reducing Solvent Consumption During Short-Term Shutdowns

It is recognized that maintaining the analytical conditions for lengthy periods may lead to significant waste of mobile phase. In addition, maintaining the analytical conditions will increase the wear and tear on the solvent delivery system. There are two options that are commonly used to minimize the consumption of mobile phase:

a) Recycle the mobile phase by placing the outlet tube from the detector into the solvent reservoir. This option can be used only if an isocratic mobile phase is employed.

b) Use a lower flow rate during the maintenance period. A flow rate of 0.1 to 0.2 mL/min is sufficient. This option is typically used when a gradient mobile phase is employed.

6 Maintenance and Operating Information

6.1 Overview

The maintenance activities indicated below should be performed on a routine basis. The intervals are based on a "typical" usage of the instrument and are dependent on the nature of the sample and the mobile phase. As the user gets a better understanding of the system, it is likely that the frequency of each of these procedures may be altered to meet the needs of the laboratory. The maintenance activities will depend on the configuration of your system.

A critical element in maintaining optimum performance of the system is insuring that particulate matter does not enter the system. A series of guidelines for sample and mobile phase preparation is presented in Section 6.3.

From time to time, it may be necessary to clean or replace various components in the system such as the electrochemical cells (Section 6.4), the in-line filter elements (Section 6.5) and the column (Section 6.6).

☞ NOTE: The operator should refer to Section 5.4 for additional hints for successful operation.

6.2 Scheduled Maintenance

6.2.1 Daily Maintenance

On a daily basis, ensure that:

- The pump is working and there is sufficient mobile phase for the expected analyses.
- The temperature on the Thermal Organizer (Thermostatic Chamber, Temperature Module) is correct.
- There is sufficient pump seal flush solution.
- The seal flush system is primed and is flowing properly.

- All connections are leak free. Check for the presence of salt on joints and at the base of all components. In addition, check for liquid at the base of all components. If a salt deposit and/or liquid are observed, tighten the offending joint (but do not overtighten). In a few cases, a new fitting may be required.

- The organizer module temperature setpoints are correct.

- The autosampler syringe is bubble free.

- The autosampler wash syringe has sufficient washing solution for the expected analyses.

- The filters in the mobile phase storage bottles are clean and free of particulate matter.

- There is sufficient room on the disk for storage of the desired data to be collected. It is suggested that data be backed up on a daily basis.

- The background currents have not changed significantly from the levels that were observed on the previous day.

- The pump(s) pressure level has not changed significantly from the level that was observed on the previous day.

6.2.2 Weekly Maintenance

On a weekly basis:

- Replace the filter element between the pump and the column (Section 5.5).

- Replace the pump flushing solution.

- Perform a flow rate check on the pump(s).

- Check all electrical connections to ensure that they are properly seated.

6.2.3 Monthly Maintenance

On a monthly basis, inspect the condition of the PEEK™ tubing to detect potential problems (and replace if necessary).

6.2.4 Quarterly Maintenance

On a quarterly basis:

- Inspect (and change, if necessary) the seals and pistons in the pump (*).
- Inspect the pump check valve operation (*).
- Replace the 20 µm mobile phase filters.
- Replace the filter element between the column and the cells.
- Refer to the manual provided with the pump for detailed information on these items.

6.2.5 Cleaning the System

Particulate matter (e.g., dust) in the system should be removed using a dry tissue. From time to time, it is possible that fittings may leak (most likely in the organizer module). If a fitting is observed to leak, remove the spill with a dry tissue. If the mobile phase being delivered by the fitting contains a buffer; take it apart, rinse with water to remove the buffer and reassemble.

6.3 Mobile Phase and Sample Considerations

To optimize the overall performance of the system, it is critical to ensure that particulate matter is not allowed to enter the system or form inside. Particulate matter will rapidly degrade the column, cells and filters. The following activities should minimize the formation of particulate matter:

- Use reagents of the highest level of purity to generate the mobile phase (contact ESA for details on sources of suitable reagents).
- Water used to prepare the mobile phase should be obtained from a good reverse osmosis system (or equivalent) and should have a resistance of 18.3 megohm-cm.
- Filter the sample before injection through a 0.2 µm filter that is compatible with its constituents.
- Filter the mobile phase before use through a 0.2 µm filter that is compatible with its constituents.

- If particulate matter is observed in the mobile phase (e.g., microbiological growth from the buffer), it should be discarded.

- Ensure that the stationary phase is stable with respect to the mobile phase. Avoid the use of mobile phases that slowly dissolve the stationary phase.

- If a gradient is used, ensure that any salts, buffers, etc., remain soluble when the fraction of the organic component in the mobile phase is increased.

- As an example, if you are running a pH 5.5 acetonitrile buffer from 50/50 to 20/80, ensure that it is soluble in the 20/80 mobile phase. It is suggested that this test be performed in a beaker or test tube (e.g., by mixing one part of buffer and four parts of acetonitrile), rather than in the instrument.

- If a gradient is used, ensure that the sample remains soluble when the fraction of the organic component in the mobile phase is increased. This test should also be performed in a beaker or test tube.

- Ensure that the system is kept clean at all times.

- If a component is replaced, flush the system with 20 volumes (25-50 mL) of the mobile phase before re-installing any downstream components to ensure that all particulate matter is removed from the system.

6.4 The Electrochemical Cells

6.4.1 Routine Operation of the Cells

The electrochemical cells are used to oxidize (reduce) the compounds of interest. Up to 16 channels can be used (each cell includes four channels). If the instrument includes a thermally controlled chamber, the cells will be physically located in the compartment. For other instruments, the cells will be found in the CoulArray Organizer.

WARNING: The temperature of the column organizer may be set above the ambient temperature. To avoid injury, if the temperature is elevated, allow the compartment to cool before handling components in the organizer.

NOTE: Refer to the Coulometric Sensor Technical Note entitled "Maintaining Cell Performance for Coulometric Sensors" for a complete listing of cell maintenance steps. (A copy is included with each cell).

Steps to maximize performance and longevity of the electrochemical cells include:

a) Minimize the possibility of particulate matter in the system by use of the considerations in Section 6.3.

b) Use the CLEAN CELL command as a step in the method. The CLEAN CELL command allows you to place an elevated potential on the cell for a short period of time. It is suggested that you use a potential that is approximately 100 mV more positive than the highest working potential (or 100 mV more negative than the lowest working potential) for a minute or two.

c) Whenever possible, use a maximum potential of 700 mV.

CAUTION: Use of a cleaning potential above 1000 mV or a cleaning potential for more than 10 minutes should be avoided as it may damage the cells. Also, the use of some mobile phases with CLEAN CELL may damage the cells.

6.4.2 Cell Cleaning Procedure

If the CLEAN CELL command does not restore the electrode to an acceptable level, it may be necessary to clean the cells as described below. The procedures should not be performed on a routine basis but used only if the results from the CLEAN CELL operation is not satisfactory.

CAUTION: Do not passivate the cells.

The general procedure to clean the cells is as follows:

a) Disconnect the power to the cells. This is done by selecting *All Off* in the Set Cell Potential column of the *Control* window of the operating program.

b) Disconnect the cell cables from the potentiostat boards in the CoulArray® control module.

c) Remove the cells and in-line filter from the outlet end of the column.

d) Using a syringe (Part Number 50-6020), flush the cell with the appropriate solutions as described below in the indicated order (it is advisable to place a finger tight ferrule and nut on the drain end of the cells and back flush the cells as well). Use 5-10 mL of each flush solution.

If necessary, remove the cells from each other by loosening the fittings on the connecting tubing.

⚠ WARNING: Sodium hydroxide and the organic solvents used in this procedure can cause personal injury. Wear protective clothing and use appropriate eye protection.

☞ NOTE 1: Always use an in-line prefilter when flushing the cells.

☞ NOTE 2: Some solvents are not compatible with PEEK (e.g., DMSO). The use of a stainless steel in-line prefilter (Part Number 55-0448) is recommended.

General Cleaning	Deionized water Methanol Deionized water
Lipophilic materials	Deionized water Methanol Acetonitrile Tetrahydrofuran Acetonitrile Methanol Deionized water
Organics	Deionized Water Dimethyl Sulfoxide Deionized Water
Silica (from Column Packings)	Deionized Water 2 M Sodium Hydroxide (leave in cell for 10-30 minutes) Deionized water

e) Replace the in-line prefilter before the cell module and attach the system to the end of the HPLC column. Pump approximately 5 mL of the mobile phase through the cell while flushing to waste before taking analytical measurements.

6.4.3 Changing Cells

If you are experiencing significant problems, which cannot be remedied by the troubleshooting procedures, discussed in Chapter 6 (or by the above cell cleaning exercises), it may be necessary to replace a cell.

To change cells:

a) Disconnect the power to the detector cells. This is done by setting the Set Cell Potential entries on the *Control* window to **All Off**.

b) Remove the cell cable from the CoulArray control module.

c) Remove the defective cell.

d) Install the new cell and allow approximately 20 mL of mobile phase to flow through it.

e) Attach the cell cable to the control module and set the Set Cell Potential entries on the *Control* window to **All On**.

f) Monitor the background current and baseline noise for a few minutes.

6.5 The In-line Filter Elements

6.5.1 Routine Use of the In-line Prefilters

The role of an in-line prefilter is to trap particulate matter that could be generated by the device immediately before it, so that small particles do not affect the next device in the system. The CoulArray accessory kit includes two filters: one is used between the injector and the column, and the other between the column and the detector. In addition, an in-line prefilter (supplied with the pump module) should be placed between the pump and the injector.

☞ NOTE: Use PEEK filter elements (Part Number 70-3824) in the in-line prefilter holders located after the injector. Use graphite filter elements (Part Number 70-0898) before the injector.

6.5.2 How Often Should Filter Elements be Changed

Under ideal chromatographic conditions (see Section 6.3), changing of the filter elements should not be necessary. In typical use, however, filters **do** get clogged and must be replaced on a periodic basis. The frequency of replacement is dependent on the level of particulate matter present in the mobile phase and the sample, as well as the production of fine particles from the stationary phase.

As a general rule of thumb, the filters after the pump and after the injector should be changed on a weekly basis and the filter after the column should be changed every three months. The frequency will depend on the nature of the sample, mobile phase and number of samples that are analyzed. As you gain experience with the system, it is likely that you will determine the optimum period of use for a filter.

If a filter must be changed more frequently than once a week, it may be worthwhile to determine the cause of the excessive particulate matter and modify the mode of preparation and/or filtration of the mobile phase or the sample.

If the post-column filter must be changed frequently, it may be worthwhile to switch to a different column or change the composition of the mobile phase.

It is valuable to maintain a log of system operation as described in Section 6.8. The pressure required to deliver a specific flow rate should be indicated in the log; when the pressure increases, it may be time to change a filter.

6.5.3 Checking the Pressure Drop Across a Filter

⚠ CAUTION: When you are checking the filter elements, do not relieve the pressure by opening a fitting on the high pressure side of the instrument (e.g., between the pump and the column). To relieve the pressure, reduce the flow rate to zero and let the pressure fall slowly.

To measure the pressure drop across a filter element:

a) Disconnect the power to the detector cells. This is done by setting the Set Cell Potential entries on the *Control* window to **All Off**.

b) Disconnect the fitting at the outlet of the suspect filter.

c) Run the pump at a flow rate of 1 mL/min, and record the pressure reading.

d) Disconnect the suspect filter and again record the pressure drop.

e) Determine the filter pressure drop by subtracting the second reading from the first reading. If the pressure drop is significant (10 bar or more), change the filter.

6.5.4 Changing a Filter Element

⚠ CAUTION: When you are changing the filter elements, do not relieve the pressure by opening any fitting on the high pressure side of the instrument (e.g., between the pump and the column). To relieve the pressure, reduce the flow rate to zero and let the pressure fall slowly.

a) Disconnect the power to the detector cells. This is done using the Set Cell Potential entry on the *Control* window.

b) Turn off the mobile phase flow and allow the pressure to fall slowly.

c) Disconnect the filter assembly by removing the gland nuts on either end of the assembly.

d) Remove both end nuts from the filter assembly.

e) Remove the filter element. If necessary, carefully insert a small wooden dowel or plastic rod to displace the filter.

⚠ CAUTION: Be careful not to scratch the filter housing.

f) Rinse the filter housing with deionized water.

g) Replace one end nut. Insert a new ESA filter element in the filter housing. Ensure that the element is properly centered and seated against the surface of the end nut.

h) Replace the second end nut and tighten carefully until contact between the cap and the filter is felt. The filter is properly installed if both end nuts are approximately equidistant from the center of the filter housing.

⚠ CAUTION: Do not overtighten. Overtightening can crush the filter and render it useless, possibly damaging other components of the system.

i) Re-install the filter housing in the system. Ensure that the direction of flow is as indicated on the filter housing.

☞ NOTE 1: Initially only the upstream end of the filter should be attached to the system. Pump approximately 5 mL of the mobile phase through the filter to waste before attaching the downstream end of the filter to the system, this step will serve to wash the filter and ensure that particulate matter does not enter the cells.

☞ NOTE 2: Use PEEK filter elements (Part Number 70-3824) in filters located after the injector. Use graphite filter elements (Part Number 70-0898) before the injector.

j) Check for leaks after reconnection of all fittings. Retighten (but do not overtighten) fittings if necessary.

6.6 Replacing the Column (Guard Column)

6.6.1 How Often Should the Column be Changed?

The frequency of changing the column (guard column) is dependent on a variety of factors including the nature of the sample, the resolution that is required, the effectiveness of the filtering and the nature of the mobile phase.

Under normal conditions, an analytical column should last for several hundred analyses. The column should be changed when it is no longer able to provide the desired resolution, or when it develops high back pressure. The guard column, which protects the analytical column, should be changed more frequently.

6.6.2 Changing the Column (Guard Column)

To change the column (guard column):

a) Disconnect the power to the detector cells.

b) Turn off the mobile phase flow and allow the pressure to fall slowly.

⚠ CAUTION: Do not relieve pressure by opening a fitting upstream of the column. This may lead to rupture of the membrane in the pulse damper.

c) Remove the column (guard column) from the system by removing the gland nuts from either side. If the column (guard column) is a cartridge system, follow the manufacturer's instructions.

d) Connect the inlet side of the new column (guard column) to the gland nut. Ensure that the direction of flow is as indicated on the column housing. If a guard column is used, place it between the in-line prefilter and the new column. It is recommended that new ferrules be used when a new column is installed.

☞ NOTE: Initially only the upstream end of the column should be attached to the system. Pump approximately 20 column volumes of the mobile phase through the column to waste before attaching the downstream components to the system. This step will serve to wash the column and ensures that particulate matter does not enter the cells.

e) Attach the downstream end of the column and allow the system to equilibrate. The period of time required for equilibration is dependent on the nature of the mobile and stationary phases. Typically 30 minutes to an hour is sufficient; however, up to 12 hours may be required if ion-pairing reagents are used.

6.7 Making a Fitting

☞ NOTE: The handbook "HPLC Fittings" by Paul Upchurch (Upchurch Scientific, Box 1029, Oak Harbor WA 98277) provides a discussion of the use of fittings and is recommended reading.

6.7.1 Cutting PEEK Tubing

From time to time, it may be necessary to replace a piece of PEEK tubing in the system. PEEK can be readily cut using a tubing cutter (Part Number 70-1307). If this cutter is not available, a sharp razor blade can be used.

After you have cut the tubing, check that:

- The inner channel of the tube end is round. If the inner channel is not round, it is likely that the flow will be restricted.

- The tubing end is flat and perpendicular to the tubing. If the cut is at an angle or there are burrs on the end, it is likely that a proper seal will not be made.

It is suggested that you monitor the flow from the tube for a few seconds at a flow rate of greater than 2 mL/min. If the tube is cut properly, the mobile phase will be delivered in a steady stream from the center of the tube. If the tube is not cut properly, the mobile phase will be delivered in droplets from the side of the tube.

6.7.2 Fittings

There are many types of fittings available for use in HPLC. When using a fitting, always follow the manufacturer's instructions.

In many cases, the tube is terminated with a SealTight® ferrule and a nut. The tubing is inserted into the center hole of the nut and the ferrule is placed on the tube. The nut is then tightened into the desired component (e.g., the injector) to make the fitting. When tightening the nut, make certain that the tubing is pushed all the way into the fitting that is being mated throughout the entire procedure.

When a fitting is made, it is suggested that initially the fitting be made finger-tight. When the mobile phase flow is established, the fitting can be tightened ¼ turn at a time until the leak stops. DO NOT OVERTIGHTEN. To ensure that a good fit is made, gently tug on the tubing after the connection has been made.

6.8 Maintaining a Log of Instrument Usage

It is suggested that a log be maintained that includes information about the use of the system. This information will be very useful in monitoring system performance and can be used to develop a schedule for replacement of filters, columns, etc. In addition, this information will be very useful for troubleshooting when problems are observed.

The following information should be recorded:

User's Name
Analytical Conditions
Time Started/Time Completed
Number of Samples Run
Baseline Current
Back Pressure
Serial Numbers of all Components (especially columns, cells, etc.)
Mobile Phase Lot Number (if applicable)
Mobile Phase Component Lot Numbers (if applicable)
Maintenance Performed

In addition, any abnormal events and the replacement of any component should be entered into the log.

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7 Troubleshooting

7.1 Overview

Although the instrument consists of several components, troubleshooting can be simplified by a consideration of the following guidelines:

- In almost all cases, there is one proximate cause for the problem. As an example, an increase in the noise in a chromatogram may be due to:
  - The pump (e.g., the pump is not properly primed).
  - The mobile phase (e.g., the mobile phase is not properly degassed).
  - The column (e.g., the column is contaminated).
  - The detector (e.g., an electronic problem).
  - A cell (e.g., a contaminated cell).
  - The pulse damper (e.g., the membrane may be ruptured).
  - A fitting (e.g., there may be a leak at a fitting).
- A fundamental knowledge of the role of each component of the system is extremely useful in diagnosing the problem.
- The availability of critical spare parts to substitute into the system is extremely useful. A listing of spare parts and supplies is provided as Appendix 2.
- If any aspect of the analytical conditions are to be changed, run a "before and after" to ensure that the effect of the change is understood. Do not consider any change as "trivial". As an example, if you change the supplier of the buffer salt, verify that the change has no effect on the analysis.
- Run standards as part of the troubleshooting process.

A series of diagnostic procedures is presented below that will assist in pinpointing the cause of the problem. Since some problems from the pump or column are observed via the detector, we include a detailed discussion of potential problems for all components in the system. In addition, each section provides information that should be useful in the solution of the problem.

To isolate the source of the problem, it may be valuable to perform independent checks of each of the components. These tests are located in the manual for the individual components (to check the detector electronics, refer to Section 7.10).

⚠ CAUTION 1: When you are troubleshooting the system, do not relieve the pressure by opening any fitting on the high pressure side of the instrument (e.g., do not open the fitting between the pump and the in-line fitting). To reduce the pressure, reduce the flow rate to zero and let the pressure fall slowly. Failure to do so could result in rupturing the membrane in the pulse damper.

⚠ CAUTION 2: If it is necessary to remove the cells from the system, make certain that the potentials applied to the cells are set to *All Off* first. This is done via the Potentials column on the *Control* window.

☞ NOTE: Ensure that all drain lines and grounding wires are securely attached.

7.2 Erratic Baseline

Cause	Comments	Solution
Dissolved gases in pumphead	If dissolved gases come out of solution in the pumphead, the flow rate will vary and cyclic noise will be observed. The frequency of the pattern will increase as the flow rate is increased.	Degas the mobile phase (preferably using a vacuum degassing technique) and reprime the pump.
Dissolved gases in detector cell	If gas bubbles enter the detector, sharp noise spikes or random noise will be observed. These are usually fairly random and may be a periodic.	Increase the pressure on the system by approximately 20 bar by placing a piece of 0.005" ID tubing on the waste end of the cells for a few minutes. Degas the mobile phase with a vacuum degasser. Increase the flow rate for 30 minutes to remove trapped gases. If the above solutions do not solve the problem, remove the cells from the system. Flush them with water, then with degassed methanol and again with water. Reinstall the cells.
Pumphead problem	Check pump seals. Check the check valves.	If worn or leaking, replace. If defective, clean or replace.
Pulse damper membrane rupture.	Check for leaks or use a pulse damper that is known to be good.	Replace membrane or pulse damper
Mobile phase not properly mixed		Stir mobile phase and pump 50 ml through the system.
Mobile phase not degassed		Degas the mobile phase and re-prime pump.
Contaminants eluting from column		Remove column from system and see if problem persists.
Cell problem		Clean cell. See coulometric sensor technical note (P/N 70-1989). Replace or remove cell to a less critical position in the array.
System not grounded	All components in the system should be connected to a common ground.	Check to ensure that all ground connections are secure and that the power source is well grounded.

7.2 Erratic Baseline (Cont.)

Cause	Comments	Solution
Electronics problems		Determine system noise using the Cell Simulator Test Load (see Section 6.10).
Temperature changes	Check thermally controlled chamber. Check HVAC.	Make sure probe of Thermal Organizer is placed in column bracket. Make sure room temperature is constant, keep CoulArray away from drafts and direct sunlight. Use a Thermal Organizer.

7.3 High Background Currents on Most or All Channels

This section considers the situation when the background current has noticeably increased in a short period of time.

Cause	Comments	Solution
Electroactive impurities in the mobile phase	Avoid using organic amines (such as triethylamine) as an organic modifier. Organic amines tend to contain electroactive impurities. The presence of high background currents occurs most frequently when a new bottle of reagent is used, the mobile phase has been stored for some time or some change in the analytical protocol (frequently unintentional) has been made.	Use a different source of each component in the mobile phase on a sequential basis until the problem goes away. As an alternative, reduce the potentials (if possible).
Electroactive species being eluted from the column	This frequently occurs when a new mobile phase is being used or when a new column is installed.	Allow the system to equilibrate for an hour with the new mobile phase (or until the baseline is stable). If ion-pairing reagents are used, it may take up to 12 hours to obtain a stable baseline. It may be necessary to use a new column. If a new column is installed, it may take up to 24 hours for the background to stabilize.

7.4 Erratic (Noisy) Baseline or High Background Current on One or a Few Channels

Cause	Comments	Solution
Cell problems		Replace or move cell to a less critical position in the cell array. See also coulometric sensor technical note (P/N 70-1989).
Defective potentiostat	Connect the cell to a different potentiostat to determine if this is the cause of the problem. Use the Cell Simulator Test Load (see section 6.10).	If a potentiostat is defective, contact ESA
Adsorption on the electrode	Some electrochemical reactions lead to products that are adsorbed on the surface of the electrode. This may produce a decreased current (and an increase in noise).	Clean the cell using the clean cell command. (Software Manual, Section 6.4.3). See also coulometric sensor technical note (P/N 70-1989).
Leaking cell connection		Check for wetness about the cell and tighten fittings if necessary.
Contaminants "leaching" from system components		Check all mobile phase reservoir filters, the column, and in-line filters. Replace the offending part.
Loose ground wire		Ensure that the ground wire is firmly attached.

7.5 Increase in Back Pressure

Cause	Comments	Solution
Accumulation of particulates from the mobile phase or injected samples	Typically this can be avoided by using freshly prepared and filtered mobile phase and samples.	Replace the in-line filter elements (Section 5.5.3).
Accumulation of particulates from the column	Silica based columns will slowly degrade if the pH is above 7.5 or below 2.5.	Replace in-line filter elements after the column. Consider changing the mobile phase composition or column type.
Clogged injector or column	Ensure that mobile phase and sample are filtered before use.	Flush injector or column to remove particulate matter. Isolate suspect component. Refer to manufacturer's cleaning directions or replace.
Plugged tubing		Isolate plugged tubing and replace.
Clogged cell		Remove cell from system and check back pressure. Clean cell as described in coulometric sensor technical note (P/N 70-1989).

7.6 Decrease in Flow Rate

Cause	Comments	Solution
Dissolved gases in pumphead	If dissolved gases come out of solution in the pumphead, the flow rate will be variable.	Loosen fittings on pumpheads to dislodge bubbles (relieve pressure slowly). It may be necessary to degas the mobile phase. Use a bubble trap in the mobile phase flow line to the pump.
Leakage in pumphead		Replace pump seals and if necessary pistons. Refer to pump manual for assistance.

7.7 Loss of Response/Shift of Peaks

Loss of Response is the abrupt loss of a peak or peaks from the chromatogram or shift of peaks to another channel when using a set of analytical conditions which are known to provide a useful chromatogram.

Cause	Comments	Solution
Accidental change of a system parameter		Check to ensure that all settings are correct (e.g., cell potentials, flow rate, temperature of thermal chamber, etc.). Check to ensure that the appropriate potentials are being applied to the cells.
Compounds of interest not sufficiently stable	Check response from a standard as a function of time.	Use a different set of analytical conditions. Use proper storage conditions (e.g., preservative, cooling).
Composition of mobile phase has changed		Prepare new mobile phase.
Cells not properly connected		Check cell cable connections to potentiostats. Check proper flow direction of cells.
Injector malfunction		Refer to manufacturer's manual.
Leak after injector		Check fittings.
Cell deactivation	Fouled or dirty cells.	Use clean cell (software manual, section 6.4.3). See coulometric sensor technical note (P/N 70-1989).
Absorption on graphite filter elements	Some compounds can absorb on the surface of graphite filter elements. This can cause a decrease in or the elimination of the compounds' peak heights.	Use PEEK filter elements after (downstream of) the injector. Replace cell(s).
Damaged cell(s)	Caused by improper use, such as potentials on and no mobile phase flow.	Replace cell(s).
Temperature changes	Check thermally controlled chamber. Check HVAC.	Make sure room temperature is constant, keep CoulArray away from drafts and direct sunlight. Use a Thermal Organizer.

7.8 Inability to Autozero the System

Cause	Comments	Solution
Autozero performed on a noisy channel		Check the noise of the channel. Remedy the noise problem and then autozero the system again.
Autozero not included in the method		Add the autozero command to the method.
Autozero performed on a large peak or a void disturbance		Autozero the system on a flat region of the chromatogram.

7.9 The System Does Not Respond to Commands or the Method is Interrupted

Cause	Comments	Solution
Loose cable		Ensure that all cables are firmly in place.
Fault in the computer or communications link		Reboot the computer.
Blown fuse		Replace the fuse. See section 7.11.
Check sum error	Error in transmitting data from control module to computer. Due to computer's resources used by another program or process.	Find program using resources and disable. Find Fast and Power Managers are especially resource intensive. Disable these.

7.10 Testing the Control Module and the Cells

If you believe that you have a noisy or otherwise defective cell (i.e., the system and other cells and/or electrodes are performing in a normal fashion), or that the control module is not functioning properly, the following procedure should be performed:

a) Set Cell Potential to **All Off** for the cells.

b) Remove the cell cable from the appropriate potentiostat board in the control module and place the cell simulator test load on the potentiostat where the suspected problem is. The cell simulator test load has two channels.

   To test channels 1 and 2 place the cell simulator test load on the A socket of the CHANNEL 1-4 potentiostat, to test channels 3 and 4 place the cell simulator test load on the B socket of the CHANNEL 1-4 potentiostat, etc.

c) Set the potentials to the two Cell Simulator Test Load channels to + 500 mV.

d) Place the Set Cell Potential ON for the two channels by selecting the boxes next to the two channels. Wait a few minutes for the currents to stabilize. The currents on these two channels should be between 40 and 60 nA.

e) Autozero the system. The currents should now be between -100 and +100 pA.

f) Select **Collect** on the *Control* window. Collect data for 3-5 minutes.

g) Select **Stop** and then save this data to a file such as TEST.

h) In Peak Edit mode, select the **LOW** filter option in the study and display the channels of interest. Display the noise trace using 100 pA full scale. Select the largest peak and determine the peak height. The peak height should be less than 10 pA (0.000010 µA).

NOTE: It may be desirable to establish a short method to collect and store the data. This method could be used to obtain a permanent record of the noise for reference.

If the cell simulator test load results indicate that the potentiostat is functioning properly, it is likely that the cell is defective.

7.11 Fuse Replacement

7.11.1 CoulArray Detector Module

Turn off the power and remove the power cord. Remove the cover from the power input module using a small bladed screwdriver and remove the fuse block assembly. Remove all the fuses and replace with new ones. See fuse ratings below. To return the assembly to the fuse holder, hold it on the sides slowly slide the assembly in and press the front until seated properly.

CoulArray Detector Module Fuses
100/120 VAC = T1A 250V (Part Number 70-0751)
230/240 VAC = T0.5A 250V (Part Number 70-1867)

Check that the proper voltage setting is selected on the right hand side and then reapply power. If the unit fails to function, call ESA Service at 1-800-275-0102 or contact your local ESA representative.

WARNING: Replace only with the same type and size of fuse.

7.11.2 Thermal Organizer

Turn off the power, remove the power cord. Using a small flat bladed screwdriver carefully pry open the fuse housing cover using a flat bladed a screwdriver.

Insert the screwdriver in the notch on the top of the red fuse holder section and remove the fuse block assembly. Remove all the fuses and replace with new ones. See fuse ratings below. Fuses are placed in the rear most section of the holder to return the assembly to the fuse holder, hold it on the sides slowly slide the assembly in and press the front until seated properly.

Thermal Organizer Fuses
115 VAC = T2A 250V (Part Number 70-0750)
230 VAC = T1A 250V (Part Number 70-0751)

Check that the proper voltage setting is selected on the right hand side and then reapply power. If the unit fails to function, call ESA Service at 1-800-275-0102 or contact your local ESA representative.

⚠ WARNING: Replace only with the same type and size of fuse.

8 Long-Term Shutdown

8.1 Overview

When the system is operating, several chromatographic and electrochemical equilibria are established. If the unit is turned off and on again, it will be necessary to wait until these equilibria have been re-established to obtain maximum performance; these processes can take a considerable period of time. For routine usage, maintain power to the cells and the flow of mobile phase at all times. This is especially important if the system is used to detect very low concentrations.

The conditions described below are to be used when the system will be shutdown for a lengthy period or if you cannot ensure that the flow of mobile phase during the shutdown period can be maintained. See Section 5.6 for short-term shutdown conditions.

8.2 Shutting Down the System for an Extended Period of Time

⚠ CAUTION: When you are shutting the system down, do not relieve pressure by opening any fitting on the high pressure side of the instrument (e.g., between the pump and the column). To relieve the pressure, reduce the flow rate to zero and let the pressure fall gradually. Failure to do this could result in the rupture of the membrane in the pulse damper.

To shut the system down:

a) Disconnect the power to the detector cells by selecting **All Off** in the Set Cell Potential column in the Control window.

b) Flush the system with 10-20 void volumes (25-50 mL) of water to remove all buffers, salts, etc.

c) Flush the system with 10-20 void volumes (25-50 mL) of methanol:water (50:50).

☞ NOTE 1: If the system includes an autosampler, flush the injection system with water to remove trace levels of buffer, salts, etc., which may be present. When flushing the system, always use filtered, high purity water. Flushing the system can be done by injecting a few "water" samples during the shut down procedure.

☞ NOTE 2: If the column manufacturer recommends the use of some other solvent to flush the column, disconnect the cells before flushing the system.

d) Disconnect the flow cells and associated in-line filters from the end of the column and cap the ends.

8.3 Restoring the System After a Long-Term Shutdown

☞ NOTE: During the procedure described below, the operator should monitor the system pressure, check for leaking fittings and ensure that all components of the system appear to be operating in a normal fashion.

If the system has been shut down for a long period of time:

a) Flush the storage solvent from the system using 25-50 mL of water. During this time, check the tubing and fittings for leaks.

☞ NOTE: It may be necessary to purge air from the system as described in the pump manual.

b) Reattach the flow cells to the system. Pump an additional 25-50 mL of the water through the system to flush the storage solvent from the cells.

c) Flush the system with 50 mL of mobile phase before applying potentials to the cells.

d) Apply the potentials to the cells by entering **All On** in the Set Cell Potential column in the *Control* window.

e) Flush the system with another 25-50 mL of mobile phase. During this period, monitor the cell currents using the *Control* window as described in Chapter 3 of the *Applications Software Manual*.

Before samples are analyzed, it is suggested that a standard is analyzed to ensure that the system is working in a satisfactory manner.

Appendix 1 Specifications

A1.1 System Specifications

The CoulArray® detector is available in a variety of configurations to meet the specific needs of the laboratory.

A1.2 Operating Principle

The CoulArray detector is used to:

a) Analyze electroactive compounds in the eluent via a multichannel electrochemical detector using DC mode.

b) Identify and quantitate the compounds of interest by comparing the peak area (height) to that obtained using standards.

c) Generate data reports and export the analytical data to external devices.

A1.3 Major Components

A1.3.1 Overview

The ESA CoulArray Detector is a modular system that includes:

- The CoulArray Thermal Organizer, the CoulArray Organizer, the CoulArray Thermostatic Chamber and CoulArray Temperature Module can be used with the system. Specifications for the CoulArray Thermal Organizer and the CoulArray Organizer are presented in Section A1.3.4 and Section A1.3.5 respectively, specifications for the CoulArray Thermostatic Chamber and CoulArray Temperature Module are provided in the documentation supplied with these units.
- Electrochemical detector cells (see Section A1.3.2).
- A control module (see Section A1.3.3 for details).
- An application program (see Section A1.3.4 for details).
- A personal computer (see Section A1.3.5 for details).

A1.3.2 Detector Cells

Operating Mode:	DC
Coulometric Electrodes:	4, 8, 12 or 16 channels Four channels per cell module The cells are housed in a thermal chamber (or Organizer)
Potential Range:	From -1000mV to +2000mV in 1mV increments
Current Ranges:	0 ± 50 nA, 0 ± 5 µA, 0 ± 100 µA autoranged Full-scale current for each electrode can be displayed from 100 pA to 100 µA Acquisition rate of 2 points per second on each channel Autozero, up to 5 µA on 50 nA and 5 µA scale and 100 µA on 100 µA scale Electrochemical data transmitted to application program via potentiostats in control module
Output Resolution:	30 fA on 50 nA range 3 pA on 5 µA range 47 pA on 100 µA range
Noise Specification:	< 5 pA peak-to-peak with a 10 MΩ, 2 µF load using the low filter setting
Operating Temperature Range: (Instrument or Control Module)	10-35°C
Operating Temperature Range: (Cells)	-10 to 45°C
Operating Humidity Range:	5 to 95% Relative Humidity (non-condensing)
Storage Temperature: (Instrument or Control Module)	-10 to 60°C

A1.3.3 Control Module

The control module houses the potentiostats and the logic board. It provides two-way communication between various components and the personal computer.

Potentiostats:	1 quad potentiostat board for each detector cell (four channels)
Logic Board:	
RS-232 Ports:	RS-232 communication ports for pumps, auxiliary (not presently used), Thermal Organizer, computer
I/O Connections	2 External inputs Maximum voltage: 5 VDC 8 Contact closure outputs (Autosampler, Gradient for non-ESA pumps, Valve, 5 Auxiliary functions) All output contact closures are true relay contact closures (normally open) and can be used interchangeably Maximum voltage: 30V Maximum current: 0.5A
Power:	100/120/230/240 VAC, 50/60 Hz, 36 VA
Dimensions:	Height: 44.5 cm (17.5 in) Width: 27.0 cm (10.7 in) Depth: 46.5 cm (18.3 in) Weight: 12 kg (26 lb)

A1.3.4 CoulArray Thermal Organizer

The *CoulArray Thermal Organizer* is used to house the column and cells and to provide a temperature controlled environment for columns, cells, gradient mixer, pulse damper and injection valve.

Temperature Control:	Via RS-232 Interface to CoulArray Detector Module
Settable Temperature Range:	(Ambient +5) to 45°C max.
Temperature Accuracy: (Measured near column)	≤1.5°C
Temperature Accuracy: (Control loop)	<1°C
Temperature Stability: (Measured near column)	<0.75°C across column
Temperature Reproducibility:	<0.2°C
Warm up time:	< 30 min (from 20 to 45°C within 2°C to stabilized temperature for empty Thermal Organizer)
LED Indicators:	LED's for temperature, Actual Temp, Set Point, Fault and Heating
Display:	3 digit readout of set and actual temperature, fault codes
Temperature Resolution:	Selectable in 1°C units
Dimensions:	Height: 44.5 cm (17.5 in) Width: 27.0 cm (10.7 in) Depth: 44.5 cm (17.5 in) Weight: 13.6 kg (30 lb)
Power Requirements:	100-120 VAC 50/60Hz 120 VA 230-240 VAC 50/60Hz 140 VA

A1.3.5 Organizer

Dimensions:	Height: 44.5 cm (17.5 in) Width: 27.0 cm (10.7 in) Depth: 44.5 cm (17.5 in) Weight: 6.6 kg (14.5 lb)
Operating Temperature:	10 to 40°C
Humidity:	5 to 95% (non-condensing)
Storage Temperature:	-10 to 60°C

A1.3.6 Application Software

The Application Software provides the following functions:

Instrument Control
Autosampler Control (ESA Model 540, ESA 542 Autosampler only)
Data Acquisition
Raw Data Filtering
Baseline Subtraction
Peak Detection and Integration
Manual Peak Detection and Integration
User Editable Peak Matching Parameters
Quantitative Analysis
2 Dimensional and 3 Dimensional Plotting of Data
Generation of Sample Reports
File Conversion (Lotus, Oracle, etc.)

A1.3.7 Personal Computer

The precise configuration of the computer is provided with the documentation supplied with your system. The *minimum* configuration for the system is:

Intel® Pentium® IV Microprocessor
128 MB RAM
40 GB Hard Disk
CD-ROM Drive
VGA Monitor - Minimum Display1024 x 768 Pixels)
2 - RS-232 Port (9 Pin) (1 if Model 540 or 542 Autosampler is not installed)
Mouse
Operating System - Windows® XP Professional

A1.4 System Dimensions

The precise dimensions and weight depend on the configuration of the system. The dimensions indicated below correspond to a typical system, which includes one pump, a thermal chamber, a control module, a computer and printer.

Height: 54.0 cm (21.3 in)
Width: 157.5 cm (62.0 in)
Depth: 46.5 cm (18.3 in)
Weight: 85 kg (190 lb)

Approximately 1.7 m (6.7 ft) of laboratory bench space is required for the above configuration.

A1.5 System Electrical Requirements

The current/wattage requirement depends on the precise configuration of the system. The information indicated below corresponds to a typical system, which includes a CoulArray Thermal Organizer, Control Module, a computer and printer.

100/120/230/240 VAC, 50/60 Hz, 1000 VA

All components should be connected to a common power line and the system should be grounded through an outlet with a protective earth contact.

Appendix 2 Recommended Supplies and Spare Parts

A2.1 Manual Injectors and Accessories

10 µL Injection Syringe with 22 Gauge Blunt End Needle	50-6022
100 µL Injection Syringe with 22 Gauge Blunt End Needle	50-6024
50 µL Injection Syringe with 22 Gauge Blunt End Needle	50-0872
Model 9125 Inert Rheodyne Injector (20 µL loop)	70-0021
Model 9125-M Rheodyne Manual Injector (2 µL loop)	70-1979
Needle Port Cleaner	50-0880
Sample Loop, 10 µL Rheodyne, PEEK	70-1081
Sample Loop, 100 µL Rheodyne, PEEK	70-1084
Sample Loop, 20 µL Rheodyne, PEEK	70-1082
Sample Loop, 5 µL Rheodyne, PEEK™	70-1080
Sample Loop, 50 µL Rheodyne, PEEK	70-1083
Syringe Needle, 22 Gauge Luer Taper	50-0883

A2.2 Columns and Accessories

ACH-SPR Holder	70-1026
ACH-SPR Solid Phase Reactor for Acetylcholine	70-0640
Cartridge, Acetylcholine ACH-3-G Guard	70-0639
Cartridge, Guard C-18M 4/pkg. (used with Meta-250 Column)	70-1972
Column, ACH-3 Analytical, 3.2 mm x 25 cm, with Guard Holder	70-0638
Column, ACH-3 Analytical, 3.2 mm x 25 cm, with Guard Holder and 3 Guard Cartridges	70-1042
Column, DHBA-250, 3mm x 25 cm	70-2115
Column, HR-80, 4.6 mm x 8 cm	68-0100
Column, MCM HPLC, 5 micron, 4.6 mm x 15 cm	70-0340
Column, Meta-250, 4.6 mm x 25 cm with Guard Holder	70-1956
Column, Meta-250, 4.6 mm x 25 cm with Guard Holder and 4 Guard Cartridges	70-1973
Column, Microdialysis MD-150 Analytical, 3.2 mm x 15 cm	70-0636
Column, Microdialysis MD-150 x 1 Microbore, 1 mm x 15 cm	70-1745
Fitting, Guard Column Holder, 2/pkg.	70-1394
Guard Column 20 x 4 mm, 2/pkg.	70-1393

A2.3 Filters, Pulse Dampers, Connectors, Tubing, etc.

Cell Flushing Syringe, 1/4" x 28, Threaded End	50-6020
CoulArray System Fittings Kit	70-3873
Dispensing Tips, 15 Gauge x 1/2" (50/pk)	40-0264
Ferrule for Cells (Super-Flangeless™ PEEK) 6210 & 5014B	70-1740
Ferrule, 1/16" Parker-CPI, S.S. (various columns)	50-6067
Ferrule, 1/16" SealTight, PEEK (replacement for 70-3675)	70-3677
Ferrule, 1/16" Valco, S.S.	50-6105
Filter Assembly, 10µm, Ultrahigh Molecular Weight Polyethylene, Solvent Reservoir	70-1302
Filter Element, Graphite, for PEEK Prefilter (5/pk)	70-0898
Filter Element, PEEK (5/pk) (use with 70-0893)	70-3824
In-Line Prefilter Kit, PEEK	70-0893
Membrane Filters, 0.22 µm (100/pk)	40-0179
Nut & Ferrule, 1/16" SealTight, S.S. (ESA pumps)	70-3878
Nut & Ferrule, 1/16" SealTight®, PEEK	70-3675
Nut & Ferrule, 1/8", 5/16-24, PEEK (ESA pumps)	70-3674
Nut, 1/16" Fingertight, PEEK (ESA pumps)	70-0714
Nut, 1/16" Parker-CPI, S.S. (various columns)	50-6066
Nut, 1/16", Valco, S.S.	50-6107
Nut, 1/8", Flangeless, Polypropylene	70-0315
Nut, between Cells (Super-Flangeless S.S.) 6210	70-1739
Pulse Damper, PEEK	70-0894
Replacement Filter, 10µm, Ultrahigh Molecular Weight Polyethylene	70-1303
Rheodyne Ferrule, 1/16"	50-0882
Rheodyne Long Bushing, 1/16" Male	50-0881
Syringe Filters, 0.45 µm (25/pk)	55-0807
Syringe, 1 ml Plastic, with Luer-Lok Tip	40-0190
Syringe, 30 ml Plastic, with Luer-Lok Tip	50-0873
Tubing, Cutter, for Polymeric Tubing, PEEK	70-1307
Tubing, PEEK, 0.005" ID, Red (specify Length)	70-0491
Tubing, PEEK, 0.007" ID, Yellow (specify Length)	70-0492
Tubing, PEEK, 0.010" ID, Natural (specify Length)	70-0088
Tubing, PEEK, 0.020" ID, Orange (specify Length)	70-0493
Tubing, Teflon®, 1/8" OD x 1/16" ID 36" long	50-0186B
Union, 10-32, PEEK	70-1304

A2.4 Mobile Phases

Mobile Phase “A” (4 liters)	45-0171
Mobile Phase “B” (1.8 liters)	45-0168
Mobile Phase Cat-a Phase II (1 liter)	45-0216
Mobile Phase MD-TM (2 liters)	70-1332
Mobile Phase UCAT/METS (2 liters)	70-3067
Reagent MB Microbicide	70-1025

A2.5 Sensors and Cables

Cable, 5600 to Dual Channel Cell (5010, 5011, 5014)	70-1837
Cable, 5600 to Two Single Channel Cells (5021, 5040, 5041)	70-1838
Cell Simulator Test Load	70-1790
Replacement Cell Assembly (Four Sensor Cell)	55-0685E
RS-232 Cable, CoulArray to Computer	70-1743

A2.6 Computer, Software, Manuals

CoulArray Applications Software Manual	70-5673
CoulArray Applications Software	70-4003
DeskJet Printer	70-1962
Getting Started Manual	70-5674
Operating Manual	70-4685
Personal Computer (Intel® Pentium® Microprocessor)	70-1765
Personal Computer and DeskJet Printer	70-1788
Pirouette Full Version with Pattern Recognition Transfer Utility	70-5906
Pirouette Lite Version with Pattern Recognition Transfer Utility	70-5907
Pirouette Full Version	70-5844
Pirouette Lite Version	70-5845

A2.7 Autosamplers and Accessories

Hand Crimper for Aluminum Caps	63-0201
Model 540 Autosampler	70-1448
Model 540 Autosampler with Biocompatible Stream Switching	70-1485
Model 540 Autosampler with Tray Temperature Control	70-1484
Model 540 Autosampler with Tray Temperature Control and Biocompatible Stream Switching	70-1486
Model 542 Autosampler with Sample Tray Cooling & Standard Tray	70-4151
Model 542 Autosampler with Standard Tray	70-4152
Vial Cap Septum, Silicon Teflon Backed (1000/pkg.)	50-6146
Vial Cap, Aluminum Crimp Type with Rubber/Teflon Coated Septum (1000/pkg.)	50-6143
Vial Cap, Plastic Screw Type for 1.8 mL Glass Screw Top Vial (1000/pkg.)	50-6145
Vial Kit for 0.25 mL Plastic Crimp Top Vials (1000/kit) includes: 0.25 mL Plastic Glass Crimp Top Vial (70-1681) Aluminum Cap with Rubber/Teflon Coated Septum (50-6143)	70-1695
Vial Kit for 1.8 mL Crimp Top Vials (1000/kit) includes: 1.8 mL Glass Crimp Top Vial (70-1248) Aluminum Cap with Rubber/Teflon Coated Septum (50-6143)	63-0200
Vial Kit for 1.8 mL Screw Top Vials (1000/kit) includes: 1.8 mL Glass Screw Top Vial (70-1247) Plastic Cap (50-6145) Silicone/Teflon Backed Septum (50-6146)	63-0250
Vial, 0.25 mL Plastic Crimp Top (1000/pkg.)	70-1681
Vial, 0.25 mL Plastic Crimp Top for Model 465 (1000/pkg.)	50-6270
Vial, 1.8 mL Glass Crimp Top (1000/pkg.)	70-1248
Vial, 1.8 mL Glass Screw Top (1000/pkg)	70-1247

A2.8 Pumps and Accessories

Cable, RS-232, CoulArray to Fiber Optic Converter	70-4054
ESA-LPG Low Pressure Gradient Assembly	70-0762
Fiber Optic Cable, Model 582 Solvent Delivery Module to Fiber Optic Converter	70-0685
Fiber Optic to RS-232 Converter	70-4053
Gradient Upgrade for Model 582 (includes Pump and Mixer)	70-4051
Mobile Phase Vacuum Degassing Unit, Non-metal, 2 Channel	70-1482
Mobile Phase Vacuum Degassing Unit, Non-metal, 3 Channel	70-1483
Model 582 Solvent Delivery Module for use with CoulArray Detector	70-4050
Model 582 Solvent Delivery Module with Accessory Kit	70-4049

A2.9 Tools and Replacement Parts

Fuse, 0.5 amp (for 230/240V operation), (2 fuses are required), Detector Module	70-1867
Fuse, 1 amp (for 100/120V operation), (2 fuses are required), Detector Module	70-0751
Screwdriver, #2 (4" long), Phillips	70-1049
Screwdriver, 3/32" (2" long), Slotted	50-0257
Wrench, Open End, 1/2" x 9/16"	50-0868
Wrench, Open End, 1/4" x 5/16"	50-0366
Wrench, Open End, 3/8" x 7/16"	50-0867
Wrench, Open End, 5/16" x 3/8"	50-0564

A2.10 Model 5600A CoulArray Accessories

CoulArray Analog Input Adapter	70-2050
CoulArray Temperature Module	70-1832
CoulArray Thermostatic Chamber	70-1760
Four Channel Upgrade (one 4 channel potentiostat and cell)	70-1806
GuardStat™ with Model 5020 Guard Cell and Accessories	70-2189
Terminal Block 10 Pos, Left Side	70-1656
Terminal Block 10 Pos, Right Side	70-1634

A2.11 CoulArray Organizer Accessories

Cell Holder Assembly	70-4711
Cell Mounting Plate Assembly	70-1694
Column Clamp, Organizer	70-4601
CoulArray Organizer	70-4340

A2.12 CoulArray Thermal Organizer Accessories

CoulArray Thermal Organizer Unit	70-4470T
Universal Rubber Grommet	70-5335
Cable, RS-232 CoulArray Thermal Organizer	70-5224
Cable, 6” long for Mixer	70-5336
Cell Mounting Plate Assembly	70-1694
Column Clamp Assembly, Small (5 cm columns)	70-5365
Column Clamp Assembly, Standard (15 cm columns)	70-5366
Column Clamp Assembly, Large (25 cm columns)	70-5367
Dynamic Gradient Mixer, Biocompatible	70-4000
Fuse, 1 amp (for 230/240V Operation), (2 fuses required)	70-0751
Fuse, 2 amp (for 100/120V Operation), (2 fuses required)	70-0750

A2.13 Books

Coulometric Electrode Array Detectors for HPLC. Progress in HPLC/HPCE, Vol.6, I.N. Acworth, M. Naoi, H. Parvez, S. Parvez	70-3419

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Appendix 3 Electrochemical Detection in HPLC (HPLC-ECD)

A3.1 Overview

The CoulArray® detector employs a multi-channel electrochemical detection system. This chapter briefly describes:

- The fundamentals of electrochemical detection (ECD) - Section A3.2.
- The advantages of coulometric ECD - Section A3.3.
- The benefits of multi-channel HPLC-ECD - Section A3.4.
- What types of compounds are electroactive - Section A3.5.
- Mobile phase considerations for electrochemical detection - Section A3.6.

For additional information the following references are recommended:

HPLC - Snyder, L.R. and Kirkland, J.J., Introduction to Modern Liquid Chromatography (Second Edition), John Wiley, New York, 1979.

Electrochemical detection - Horvai, G. and Pungor E., Electrochemical Detectors in HPLC and Ion Chromatography. (1989) Vol. 21, Issue 1. *Critical Reviews in Analytical Chemistry*.

Coulometric and multi-channel coulometric ECD
Matson *et al*, n-Electrode Three-Dimensional Liquid Chromatography with electrochemical Detection for Determination of Neurotransmitters. *Clin. Chem.* (1984) Vol. 30, No. 9, 1477-1488.

Svendsen, C. N., Multi-electrode Array Detectors in High-performance Liquid Chromatography: A New Dimension in Electrochemical Analysis. *Analyst*, (1993) Vol. 188, No 2, 123-129.

Acworth, I. N., Naoi, M., Parvez, H., Parvez, S., Coulometric Electrode Array Detector for HPLC. Progress in HPLC/HPCE, Vol. 6 (available from ESA; Part Number 70-3419).

A3.2 Fundamentals of HPLC-ECD

Electrochemistry involves the study of the effect of an electric potential on a chemical compound. At a specific potential a compound can be oxidized (loss of electrons) or reduced (gain of electrons) as shown below:

$\mathbf{HQ \rightarrow Q + 2\ e^- + 2\ H^+}$	**(oxidation)**	**A3-1**
$\mathbf{Q + 2\ e^- + 2\ H^+ \rightarrow HQ}$	**(reduction)**	**A3-2**

where **HQ** = hydroquinone and **Q** = quinone

The processes described in equations A3-1 and A3-2 are termed *half reactions*. A pair of species involved in these half reactions is termed a *redox couple* (e.g., **HQ** and **Q**).

In a basic electrochemical cell (Figure A3-1), a pair of inert electrodes are placed in a solution with an electrolyte containing an analyte. A potential applied across the electrodes is the driving force for oxidation or reduction of the analyte and results in current flow. The magnitude of current (measured with an ammeter) can be related to the concentration of analyte as a means of quantitation.

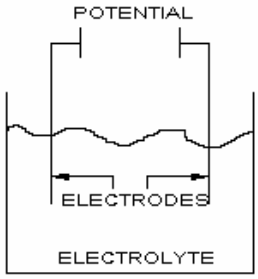

Figure A3-1: An Electrochemical Cell

The basis of operation of a typical HPLC-ECD is the measurement of current at a constant potential using a three electrode system. The electrode where the oxidation or reduction reaction occurs is termed the *working electrode*, while the electrode where a complementary electrolytic reaction takes place is termed the auxiliary or *counter electrode*. A *reference electrode* is incorporated to provide a stable potential against which the working electrode is set. A *potentiostatic* circuit maintains the potential and amplifies the current (signal) produced from the reaction of the analyte.

☞ NOTE: The CoulArray detector can be used for both oxidation and reduction. For sake of simplicity, the following discussion will consider oxidation; all comments also apply to reduction.

A3.2.1 The Potential Required to Effect an Electrochemical Reaction

Obtaining optimal sensitivity and selectivity with HPLC-ECD requires knowledge of the appropriate potential to effect the desired reaction (i.e., oxidation of the analyte). This depends on several factors including the nature of the electrode surface, pH, mobile phase composition and chemical structure of the analyte.

The optimal potential for a particular analyte can be determined by measuring the oxidative current (at a constant concentration) over a range of working electrode potentials. A plot of the signal generated (peak height or area) as a function of the applied potential is termed an *hydrodynamic voltammogram* (HDV) or current/voltage (CV) curve. A typical HDV is shown in Figure A3-2.

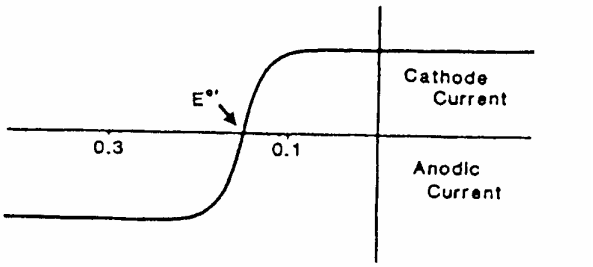

Figure A3-2: A Typical HDV

Visual inspection of an HDV indicates the potential at which the oxidative current (or signal) is maximal (E_{max}). In **Figure A3-2**, E_{max} for oxidation is approximately 200 mV. The potential used for a particular analyte is an important issue. If the potential is too low, sensitivity will be compromised. If the potential is too high, other species may also be oxidized and interfere with the analyte of interest. The optimal potential is typically 20 mV above E_{max}.

A3.2.2 Coulometric and Amperometric Detectors

Most HPLC-ECD cells are designed so that column eluent **flows by** the working electrode. A fraction (typically 5 - 15 %) of the analyte is oxidized while the remainder is unchanged. The signal is proportional to the **concentration** of the analyte. Operation of an ECD in this mode is termed *amperometry* and the detector is termed *amperometric.*

The CoulArray cells are designed so that eluent flows **through** a porous graphite working electrode. Essentially all the analyte is oxidized and the signal is proportional to the **quantity** injected. Operation of an ECD in this mode is termed *coulometry* and the detector is termed *coulometric*.

There are several advantages to the use of a coulometric detector:

- The response is very stable.

  The high surface area coulometric detector is much less susceptible to loss of response due to contamination of the working electrode than the amperometric detector.

- The peak area for an analyte is reproducible and predictable.

With a coulometrically efficient detector the signal obtained for a given quantity of analyte can be predicted using Faraday's law.

$$\mathbf{Q = nFN} \qquad \textbf{A3-3}$$

where: **Q** is the total charge transferred (coulombs)
n is the number of electrons transferred per mole (equivalents/mole)
F is Faraday's constant (96,500 coulombs/equivalent)
N is the number of moles of analyte

Since the coulometric cell oxidizes or reduces 100% of the analyte (assuming that the appropriate potential is applied) then the total integrated peak area will equal the total charge:

$$\mathbf{Q = \int i\,dt} \qquad \textbf{A3-4}$$

where: **i** is the current (amps)
t is time (sec)

The peak area will be directly proportional to the amount of analyte. If the same analyte quantity (N) is presented to the detector, then the same amount of signal (area) will be obtained. While small day-to-day variations in flow rate, temperature, and mobile phase composition significantly affect the signal (area or height) obtained using amperometric sensors, the coulometric detector response is unaffected.

An additional benefit of coulometric efficiency is that if n (number of equivalents/mole) is known, then N (moles) can be directly obtained from peak area using Faraday's law (i.e., without the use of external standards or response factors).

The most important benefit of the coulometric detector is obtained when more than one such detector is used in series. This is the basis of the CoulArray technology (Section A3.3).

A3.3 Multi-electrode Detection in HPLC

Ideally, chromatographic conditions provide well-resolved analytes to the detector. In many cases, however, samples contain a variety of species that may interfere, leading to erroneous results for the compounds of interest. With single channel electrochemical detection, all compounds that can be oxidized at or below the working potential will be detected.

In this discussion, consider a sample containing:

Compound	Retention time (min)	Emax (mV)
A	1.7	620
B	1.9	815
C	6.5	760
D	6.6	880

If compounds A and D are to be quantitated, a working potential of 900 mV is required. If this potential is used:

- Oxidation of compound B will interfere with the analysis of A.
- Oxidation of compound C will interfere with the analysis of D.

With single channel detection, it is necessary to change chromatographic conditions to resolve these analytes.

Operation of the CoulArray detector is based on the principle that compounds differ in their voltammetric behavior. The CoulArray detector uses a series of coulometric working electrodes set at a series of increasing potentials. Each electrode oxidizes essentially all of the material that can be oxidized at the potential to which it is set, thereby providing resolution of co-eluting compounds.

Analysis of the four compounds in the above table using a series of eight coulometric electrodes set at incrementally increasing potentials (i.e., 420, 480, 540, 600, 660, 720, 780, 840, and 900 mV) would result in selective detection of:

- **A** at 660 mV
- **B** at 840 mV
- **C** at 780 mV
- **D** at 900 mV

In this fashion, a series of coulometric detectors provides the necessary resolution without modification of the chromatographic conditions.

A3.4 Using Response Ratios for Qualitative Analyses

If a series of coulometric detectors is employed, the response for each compound typically occurs across more than one electrode due to oxidation along the C/V curve of the analyte. As an example, if E_{max} for a given compound is 750 mV, it is likely that some oxidation will be observed at the electrodes set at 660 mV, 720 mV and 780 mV. With a series of coulometric detectors, the proportion of the signal between adjacent electrodes for a particular analyte remains constant (e.g., the E_{720} / E_{660} signal ratio is 2.43). This provides additional information to indicate that the eluted compound is indeed the compound of interest. As an example, if the ratio is 1.66, it is likely that the compound that eluted at a given time is not the compound of interest (or two compounds co-eluted). For additional information on the qualitative nature of these ratios, please refer to the recommended readings at the beginning of this chapter.

A3.5 What Kind of Compounds are Electroactive

A wide variety of compounds are capable of being monitored via an electrochemical detector. In general electroactivity is dependent on the presence of an electroactive functional group. Many compounds that are easily oxidized contain groups with a lone pair of electrons such as the hydroxyl group of a phenol or the amino group of an aniline. Conversely, many compounds, which are easily reduced, contain groups such as a carbonyl or a nitro group.

The structure of some compounds that are easily oxidizable or reducible are shown in Figure A3-3.

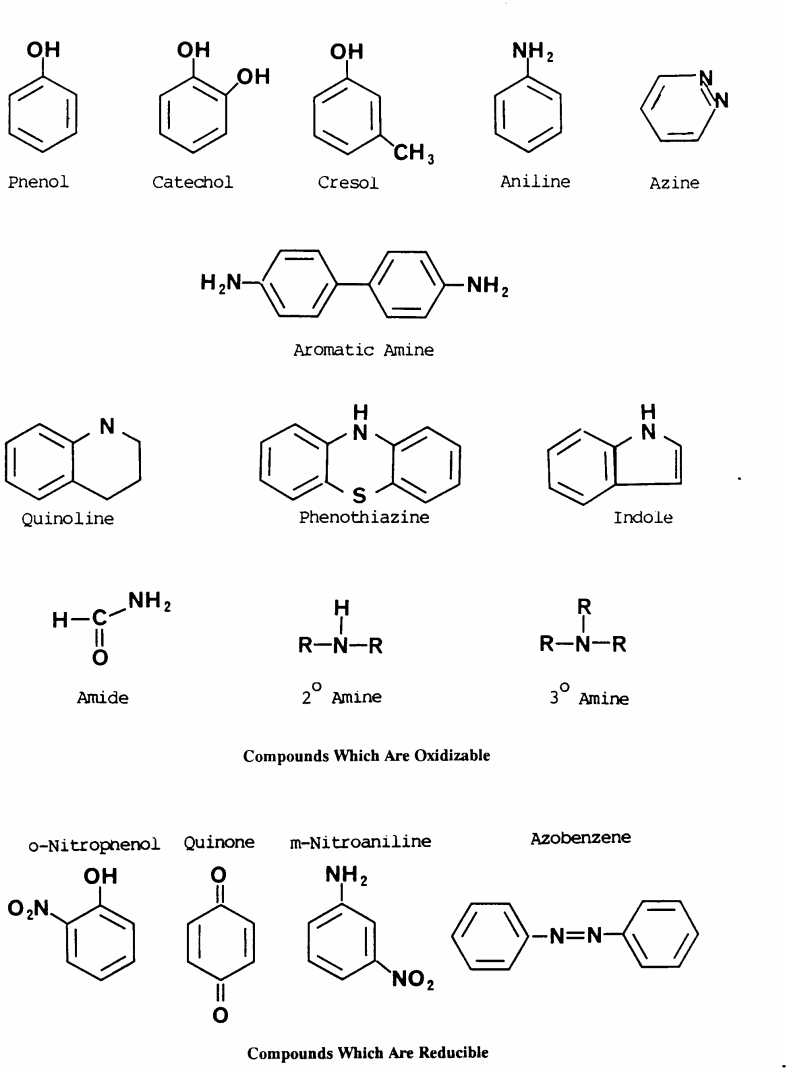

Figure A3-3: Typical Compounds which are Electroactive

There are many classes of compounds that can be detected by electrochemical methods, including:

- Antioxidants: e.g., BHT
- Carbohydrates: e.g., glucose, aligosaccharides (using pulsed amperometric detection)
- Catecholamines: e.g., DA, NE, 5-HT
- Drugs of Abuse: e.g., LSD, morphine
- Enkephalin Peptides: e.g., methionine enkephalin
- Pesticides: e.g., Aminocarb
- Pharmaceuticals: e.g., Carbamazepine, Fluorazepam

In addition, many compounds can be readily converted into derivatives which are electroactive. A few examples of these techniques and applicable compounds include:

- Primary Amino Acids (pre-column derivatization with o-phthalaldehyde)
- Phenols (post-column derivatization with bromine)
- Glycosides, Acetylcholine (enzymatic methods)
- Cocaine (photochemical method, the compound is irradiated to form an electroactive species)

There are a number of avenues available to determine if a compound is a potential candidate for electrochemical detection. These include:

- *Searching the Literature*: Computerized databases can be readily used to see if electrochemical detection has been reported for the compound of interest.
- *Exploratory Experimentation*: A compound can be placed in the flow cell (i.e., injected into the HPLC without a column) with the detector set at a relatively high positive and a relatively high negative potential. If a response is observed, the compound may be electrochemically active.
- *Manufacturer's Bibliography*: ESA maintains a large listing of compounds for which electrochemical methods have been reported. Please feel free to contact ESA for applications information.

A3.6 Mobile Phase and Sample Considerations

To optimize the overall performance of the system, it is critical to ensure that particulate matter is not allowed to enter the system or form inside the system. Particulate matter will rapidly degrade the column, cells and filters. The following activities should minimize the formation of particulate matter:

- Use reagents of the highest level of purity to generate the mobile phase.
- Water used to prepare the mobile phase should be obtained from a good reverse osmosis system (or equivalent) and should have a resistance of 18.3 megohm-cm.
- Filter the sample before injection through a 0.2 µm filter that is compatible with its constituents.
- Filter the mobile phase before use through a 0.2 µm filter that is compatible with its constituents.
- If particulate matter is observed in the mobile phase (e.g., microbiological growth from the buffer), it should be discarded.
- Ensure that the stationary phase is stable with respect to the mobile phase. Avoid the use of mobile phases which slowly dissolve the stationary phase.
- If a gradient is used, ensure that any salts, buffers, etc., remain soluble when the fraction of the organic component in the mobile phase is increased.

  As an example, if you are running a pH 5.5 buffer/acetonitrile mobile phase from 50/50 to 20/80, ensure that the buffer is soluble in the 20/80 mobile phase. It is suggested that this test be performed in a beaker or test tube (e.g., by mixing one part of buffer and four parts of acetonitrile), rather than in the instrument.
- If a buffer is used, ensure that the sample remains soluble when the fraction of the organic component in the mobile phase is increased. This test should also be performed in a beaker or test tube.
- Ensure that the system is kept clean at all times.
- If a component is replaced, flush the system with 20 column volumes of the mobile phase before re-installing any down-stream components to ensure that all particulate matter is removed from the system.

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Appendix 4 Return Policy

No instrument, whether in or out of warranty, may be returned without permission. Before returning the item for repair, a Return Authorization Number must be obtained orally or in writing from ESA's Service Department (telephone (800) 275-0102). This number MUST appear on the outside of the package.

All items should be carefully packed to prevent damage, insured and shipped prepaid to:

ESA, Inc.
22 Alpha Road
Chelmsford, MA 01824-4171

Attn. Service Department

Return Authorization Number _____

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GLOSSARY

This glossary provides definitions of a broad variety of terms that are commonly used in HPLC with electrochemical detection. For further information, the reader is referred to a standard text in HPLC such as:

> L.R. Snyder and J.J. Kirkland, Introduction to Modern Liquid Chromatography, second edition, Wiley Interscience, John Wiley and Sons, Inc., New York, 1979.

Or a standard text in electrochemistry such as:

> A.J. Bard and L.R. Faulkner, Electrochemical Methods, John Wiley and Sons, Inc., New York, 1980.

α (Separation factor): A measure of the difference in retention of two compounds which takes into account the void volume of a column at a given flow rate.

$$\alpha = \frac{k'_2}{k'_1} = \frac{V_2 - V_0}{V_1 - V_0}$$

where: k_1' and k_2' are the capacity factors for peaks 1 and 2 respectively.
V_1 and V_2 are the retention volumes for peaks 1 and 2 respectively.
V_0 is the void volume for the column (retention time of unretained peaks).

Ampere: The amount of current that passes through a resistance of 1 ohm when a potential of 1 volt is applied. It is equivalent to 1 coulomb/second (abbreviated A).

Amperometry: The concentration of an analyte obtained by measuring the electric current generated using a fixed potential. The current is proportional to the concentration of the compound of interest. A series of standards are used to obtain the working curve or the internal standards technique.

Analyte: The ion or compound that is being determined.

Analytical Potential: The potential that is used to effect the desired oxidation or reduction.

Anion: A negatively charged ion.

Anode: The electrode where oxidation (loss of electrons) occurs. The process is shown below for an anion:

$$A^- \rightarrow A + e^-$$

Applied Potential: The potential applied between the working and reference electrodes in an electrolytic cell.

Artifact: An unwanted peak in a chromatogram. It may be due to sample, mobile phase or system contaminants.

Autosampler: A device which automatically injects a sample into the mobile phase stream at a programmed interval. An autosampler is used for automated analysis of a large number of samples.

Autozero: An instrument function which allows the operator to establish the zero of the output so that all chromatograms start at the same baseline.

Auxiliary Electrode: The electrode where the complementary reaction to the desired process is taking place. For example, if the working electrode is performing an oxidation, the complementary reaction is a reduction that is occurring at the auxiliary reaction (also known as the counter electrode).

Background Current: The current observed when the mobile phase passing through the electrochemical cell does not contain any electroactive species of interest. The background current is the cumulative current of intentional and contaminant electroactive components of the mobile phase.

Background Noise: The signal variability that is observed when the mobile phase passing through the detector does not contain any electroactive species of interest. Variations in the background noise may arise from a number of sources such as the pump, column, detector or cells.

Band: A zone on an HPLC column that contains one (or more) component(s) of a mixture that is being separated.

Band Broadening: The phenomenon of diffusion of a band into a greater volume (e.g., post column band broadening occurs in the tubing between the end of the column and the detector).

Band Spreading: See Band Broadening.

Bar: A unit of pressure. 1 bar = 14.7 psi = 1.02 kgf/cm^2.

Baseline Resolution: Chromatographic separation of two compounds such that the peaks are totally isolated from each other. The tail from the first peak intersects the baseline before the second peak begins.

Bed: A collection of a chromatographic media (usually silica) that is used to separate a mixture. In HPLC this is usually termed the stationary phase.

Bonded Phase: A stationary phase in which a functional group is chemically bound to the stationary phase (e.g., 5 µm silica). The functional group can be polar (e.g., CN) or non-polar (e.g., C_{18}).

Calibration Curve: A plot of the detector response vs. concentration for a series of known samples (standards). It is used to determine the concentration of the analyte in unknowns.

Capacitance (C): The ratio of the charge on a pair of electric conductors to the potential between the conductors.

$$\mathbf{C} = \frac{\mathbf{Q}}{\mathbf{V}}$$

where: **Q** is the charge (coulombs).
V is the potential (volts).

When the current in an electric circuit is changed, energy is required to change the electric and magnetic fields that are associated with the flow of charge. Capacitance is the counterforce or reactance that tends to counteract the change in the electric and magnetic fields.

Capacitive Current: The current that flows in a circuit (e.g., to an electrode) due to the capacitance of the circuit (electrode) when a potential is changed.

Capacity Factor: The capacity factor is the ratio of the amount of compound adsorbed on the stationary phase to the total amount of compound in the mobile phase. The capacity factor for an adsorbed compound on an HPLC column can be determined experimentally by use of the following equation:

$$\mathbf{k'} = \frac{\mathbf{V_1 - V_0}}{\mathbf{V_0}}$$

where: $\mathbf{V_1}$ is the retention volume of the compound of interest
$\mathbf{V_0}$ is the void volume of the column

Carrier: The mobile phase.

Cation: An ion having a positive charge.

Cathode: The electrode where reduction (gain of electrons) occurs. The process is shown below for a cation:

$$M^{+2} + 2e^- \rightarrow M^0$$

Cell: See Flow Cell.

Channel (Chromatography): A longitudinal void in the column which causes band-spreading. Channeling usually occurs near the column walls.

Channel (Detector): One of the potentiostats or electrodes of the detector. The CoulArray detector can have 4, 8, 12 or 16 channels.

Chromatogram: A record of a separation which indicates the detector response as a function of time. The chromatogram indicates the elution of electroactive species from the column. For the CoulArray detector, a chromatogram is a plot of current versus time.

Chromatography: The separation of compounds in a mixture which is effected by exploiting the relative differences between their adsorption on a solid phase and their desorbtion into the mobile phase. Chromatography involves the use of a dynamic equilibrium of the compounds between the stationary phase and the mobile phase.

Clean Cell: A timeline entry that is used to change the potential on the cells for a short period. This is used to re-activate the surface of the cell or remove adsorbed contaminants.

Collapse: The settling of the packing material in a column that leads to a column void.

Column: A cylinder containing the stationary phase. The mobile phase is pumped through the column to separate the sample.

Column Void: A portion of the column which is not packed with stationary phase. A column void may be due to settling or dissolution of the stationary phase by the mobile phase. This usually results in two peaks for the same compound.

Column Volume: The volume within a column that is not occupied by stationary phase.

Contact Closure: An instrument controlled switch that can be an input or output contact closure. It is used to control an external device such as a valve.

Control Module: Component of the CoulArray detector, which includes potentiostats and interfaces to other devices such as pump, autosampler and computer.

Coulomb: The quantity of charge transferred by a current of 1 ampere for a period of one second.

Coulometric Efficiency: Operation of an electrochemical detector in a manner such that a very high percentage (asymptotically approaching 100%) of the electroactive species of interest is converted (oxidized or reduced) during the detection process.

Coulometry: The measurement of the absolute charge that is used to reduce or oxidize an electroactive species. In coulometry, the integrated current (or charge) is measured; this is proportional to the amount of the compound of interest in the sample via Faraday's law.

Counter Electrode: See Auxiliary Electrode.

Counter Ion: An ion of opposite charge than that of the ion(s) of interest. It maintains electrical neutrality of the solution and column. For example, in the separation of a number of anions, the counter ion might be Na^+.

Current: A flow of electric charge, usually measured in amperes or amps (A, mA, µA, nA, pA, fA, etc.).

Data Reduction: Conversion of the raw chromatogram to a series of electrode/time responses using operator determined parameters for minimum peak height, peak width, etc.

DC Mode: A mode of operation of the electrochemical detector. The potential across the electrodes is maintained at a constant value at all times.

Default Setting: A parameter setting that is preselected by the manufacturer (e.g., the default retention time window is ± 2%).

Degassing: The removal of dissolved gases from the mobile phase. Degassing may be effected by helium sparging or vacuum filtration.

Degree of Dissociation: Fraction of a weak acid and/or weak base that is present in the ionized form in solution.

Del: Delete

Detection Limit: The smallest amount of a compound that will produce an observable signal above the background noise. The signal-to-noise ratio selected is an integral part of the definition of the detection limit for a given situation (e.g., a system has a detection limit of 2 µg of tricyclyne with a S/N of 3).

Detector: A device that is used to determine the presence of analytes in the mobile phase. A detector will provide an electric signal that can be related to the concentration of the compound flowing through the flow cell.

Diffusion: The movement of a compound through a phase under the influence of a concentration gradient. In the column, the compounds of interest will diffuse between the mobile phase and the stationary phase as a part of the separation process. In the immediate area of the electrodes, the ions will diffuse through the eluent to and from the actual surface of the electrode. The rate of diffusion of the ions in the vicinity of the electrode is a critical issue with regard to the limiting current that is observed.

Distribution Coefficient (D): For a chromatographic band that is equilibrated, the distribution coefficient is the ratio of the concentration of the compound on the stationary phase to the concentration of the compound in the mobile phase.

$$D = \frac{[Ss]}{[Sm]}$$

where: $[S_s]$ is the concentration of the compound in the stationary phase.
$[S_m]$ is the concentration of the compound in the mobile phase.

Dominant Channel: Detector channel with the largest oxidation (reduction) current for a peak.

E: Abbreviation for potential (in V or mV).

Efficiency (Chromatographic): A measure of the "effectiveness" of a column for a given separation. Efficiency is measured by the number of theoretical plates on the column.

Efficiency (Detector): The detector efficiency is the ratio of the amount of analyte actually detected to the total amount of analyte that passes through the flow cell.

Electroactive (Species): A compound, element or ion that can be oxidized or reduced at the potential that is being employed by the detector.

Electrochemical Detection: The measurement of an electrical signal that arises from the oxidation or reduction of an analyte in order to determine the concentration of the analyte.

Electrochemistry: The study of the effect of an electrical potential on chemical entities.

Electrode: A device which can take part in an electrochemical reaction by either accepting or donating electrons and thus effecting an oxidation or a reduction, respectively.

Electrolyte: A compound that will conduct electrical current when it is dissolved in a liquid. The strength of the solution will be dependent on the degree of dissociation of the electrolyte into its component ions.

Eluent: The liquid used as a mobile phase in high performance liquid chromatography.

Eluate: The mobile phase as it leaves the column.

Elute: To remove a band from an LC column through continuous flow of the mobile phase.

Elution Volume: The volume (time x flow rate) of solvent that is required to elute a given component of the sample using a given HPLC system. The time is measured from the instant of sample injection to the time of elution (of the center of the band).

Equilibrium: The condition when a chemical system is at rest. At equilibrium, the rate of the forward reaction and the rate of the reverse reaction are equal, and as a result the concentrations of the reactants and products remain constant.

Faraday's Law: The relationship, which equates the total charge, transferred in an electrochemical reaction to the number of equivalents of the reactant.

$$Q = n F N$$

where: **Q** is the total charge transferred (coulombs).
n is number of electrons transferred in the electrochemical reaction (equivalents per mole; e.g., 1 for the Fe(II)/Fe(III) redox couple).
N is the number of moles of reactant.
F is Faraday's constant, 9.64846×10^4 coulombs/equivalent.

Femtogram: A unit of mass equal to 10^{-15} grams (abbreviated fg).

Femtomole: Amount of material equal to 10^{-15} mole (abbreviated fmole).

Filter (Chromatographic): A device which will remove particulate matter from the sample or mobile phase.

Filter (Signal): An electronic device which removes a portion of the noise in the signal output. A filter provides a smoother baseline.

Flow: Command in a time line to indicate the composition and delivery rate of the mobile phase at a given time in the timeline.

Flow Cell: The part of a detector through which the eluent or mobile phase flows. The analyte is detected by some means such as oxidation (or reduction) at a working electrode.

Flow Rate: The rate at which the mobile phase is pumped through a column (usually in mL/min).

Fronting: The phenomenon observed when the front of a peak is less steep than its tail (an asymmetric peak).

Gain: The ratio of the increase in the size of the output signal over the input signal of a detector.

Ghosting: The phenomenon observed when a late eluting peak in one chromatogram is carried over into a second chromatogram.

Gradient: Refers to a programmed change in the composition of the mobile phase during the separation.

H: Height equivalent to a theoretical plate (see theoretical plate).

Half Reaction: The description of one of the two electrochemical processes that takes place in a cell. A half reaction can be an oxidation or a reduction (e.g., hydroquinone to quinone or vice versa).

Halfwave Potential: The potential at which the current observed is half of the limiting or plateau current.

HETP: Height equivalent to a theoretical plate (see below).

Height Equivalent to a Theoretical Plate (H): A measure of the efficiency of a chromatographic column.

$$\mathbf{H} = \frac{\mathbf{L}}{\mathbf{n}}$$

where: **L** is the length of the column.
n is the number of theoretical plates.

i: Abbreviation for current (in A, mA, uA, nA, pA, etc.).

Injector: A device that is used to introduce a specified amount of sample into an LC system.

Interferent: A species that will produce an incorrectly higher signal for the compound of interest due to the fact that it elutes at the same time as the compound of interest and has a detector response that is covered by the conditions used for the compound of interest.

Integration: Calculation of the area under a chromatographic peak. This is related to the concentration of the compound in the sample.

I/O Connection: An input or output contact closure controlled by, or to control, the detector.

Ion Chromatography: A separation procedure in which a series of ions are separated (e.g., a series of cations or anions) by use of an ionic column packing material.

Ionization Constant (K): Equilibrium constant for the dissociation of a weakly ionized compound. For the ionization:

$$AB \rightleftarrows A^+ + B^-$$

the ionization constant is:

$$K = \frac{[A^+]\,[B^-]}{[AB]}$$

where: $[A^+]$, $[B^-]$ and $[AB]$ are the concentrations of the three species in solution.

Ionization constants are frequently expressed in a logarithmic basis as pK, where:

$$pK = -\log K$$

Isocratic: Refers to the use of a mobile phase with a constant composition during the chromatographic separation.

Linearity: Refers to the ability of a property of a sample (e.g., concentration) to be directly related to an experimentally measurable parameter (e.g., current) in a proportional relationship. The linearity of a calibration curve is a factor of critical interest.

Linear Velocity: Average speed of the solvent front through a column (usually measured in cm/sec).

Longitudinal Diffusion: Band-spreading in the longitudinal direction of a column as a result of randomized or eddy diffusion of the compound of interest.

Mass Transfer: Transfer of mass from the mobile phase to the stationary phase (or vice versa) or from the mobile phase to an electrode (or vice versa). Stationary phase mass transfer thus refers to the diffusion of molecules into and out of the stationary phase.

Method: A set of analytical parameters that defines the operation of the CoulArray detector. It contains data acquisition, data processing, data storage and data reporting procedures.

Microampere: A unit of current equal to 10^{-6} amps (abbreviated μA).

Microgram: A unit of mass equal to 10^{-6} gram (abbreviated μg).

Micromole: Amount of material equal to 10^{-6} mole (abbreviated μmole).

Milligram: A unit of mass equal to 10^{-3} grams (abbreviated mg).

Millimole: Amount of material equal to 10^{-3} moles (abbreviated mmole).

Mobile Phase: The liquid which is used to transport a sample mixture through a chromatographic bed (often referred to as the eluent).

n: Number of theoretical plates for a column as measured by the efficiency of a column. n can be calculated by the equation:

$$\mathbf{n = 16 \left(\frac{t_R}{t_W} \right)^2}$$

where: $\mathbf{t_R}$ is the retention time of the peak (in seconds).
$\mathbf{t_W}$ is the baseline width of the peak (in seconds).

n: Number of electrons transferred per molecule or ion in a reduction or oxidation.

N: Number of plates per meter given by: $\mathbf{N = \frac{n}{L}}$

where: **n** is the number of theoretical plates of the column.
L is the length of the column (in meters).

N: Number of moles of reactant.

Nanoampere: A unit of current equal to 10^{-9} ampere (abbreviated nA).

Nanogram: A unit of mass equal to 10^{-9} gram (abbreviated ng).

Nanomole: Amount of material equal to 10^{-9} mole (abbreviated nmole).

Negative Peak: A chromatographic peak that provides a negative deflection because the oxidation current of the elution species is less than that of the mobile phase (or the reductive current is larger than that of the mobile phase).

Nernst Equation: The relationship that describes the potential of a reversible half reaction as a function of the concentration of the oxidized and reduced form of the electroactive species.

$$E_{app} = E^{0'} + \frac{0.059}{n} \log \frac{[OX]}{[RED]}$$

where: E_{app} is the applied potential (in Volts).
$E^{0'}$ is the potential using the standard conditions (25°C, 1 atm).
[OX] and **[RED]** are the concentrations of the oxidized and reduced forms of the redox couple (in moles/liter).
n is the number of electrons transferred in the reaction.

Noise: The signal that appears on a chromatographic output due to a variety of events that are not a desired response of the detector. Noise can be random or regular (e.g., due to pump pulsation).

Organic Modifier: An organic solvent (e.g., methanol, acetonitrile) that is used to alter the elution characteristics of an aqueous solution which is used as the mobile phase.

Oxidize: The process of removing one or more electrons from an atom, ion or compound.

Oxidizing Agent: An element, ion or compound which is capable of oxidizing another species by accepting one or more electrons.

Packing: The stationary phase for HPLC.

Particle Size: The average particle diameter of the column packing material (usually in microns (µm)).

Parts Per Billion: A unit of concentration equal to nanograms/gram (10^{-9}). Usually abbreviated ppb.

Parts Per Million: A unit of concentration equal to micrograms/gram (10^{-6}). Usually abbreviated ppm.

Parts Per Trillion: A unit of concentration equal to picograms/gram (10^{-12}). Usually abbreviated ppt.

Passivation: Preparation of the HPLC system to remove and prevent electroactive materials from entering the mobile phase.

Peak: An indication on the chromatographic output that the electrochemical detector has observed the presence of an electroactive compound in the mobile phase. The signal response will be above the baseline. A peak has an approximately Gaussian shape.

Peak Area: The area underneath a recorder trace and above the extrapolated baseline. The peak area is proportional to the amount of material in the sample. For an electrochemically generated peak, the peak area can be related to the charge of the reaction which is related to the amount of the analyte by Faraday's Law. The peak area is reported in units of coulombs, and can be approximated by the equation:

$$A = bh$$

where: **b** is the width of the peak at the base.
h is the maximum peak height.

Peak Cluster: Electrochemical data for a compound that has been detected by two or more channels.

Peak Height: The measurement of the distance on a chromatogram from the baseline to its highest point of a peak. For an electrochemically generated peak, the peak height is usually given in terms of current (amps).

Peak Table: See Peak Name Table.

Peak Width: The breadth of the peak. The peak width is frequently measured at the intensity that is equivalent to 0.5 of the peak maximum. The peak width is usually expressed in minutes or seconds.

Picoamp: A unit of current equal to 10^{-12} amps (abbreviated pA).

Picogram: A unit of mass that is equal to 10^{-12} grams (abbreviated pg).

Picomole: Amount of material that is equal to 10^{-12} mole (abbreviated pmole).

pK: See Ionization Constant.

Plate: In distillation theory, a plate is the height required for one vaporization and one condensation process. It is a measure of the efficiency of the column. This concept has been transported to HPLC, where it is a measure of the efficiency of the column.

Potential: The relative voltage or electroactive force between two electrodes (usually measured or applied between the working electrode and the first reference electrode) measured in volts.

Potentiostat: An electronic assembly that interfaces the electrodes of a cell to the operating system of the CoulArray detector. Up to sixteen potentiostats can be used in the instrument. The potentiostat is responsible for applying and maintaining a given potential between the reference and working electrodes by supplying the necessary current between the counter and working electrodes.

Pulse Damper: A device that reduces changes in the flow rate of the mobile phase due to the reciprocating action of the pump(s). Changes in the flow rate can be seen as noise in the baseline.

Pump Flushing Solution: A solution which is used to clean the pumping chambers (i.e., remove deleterious salts from the pumping system) and keep the pump seals wet to reduce pump seal wear. Use of the pump flushing solution greatly extends the life of the seals and pistons.

Recycle (Mobile Phase): A process in which the eluate is returned to the mobile phase reservoir. Recycling can be used to conserve solvent during periods when the instrument is in standby mode or for some isocratic methods.

Redox Couple: The oxidized and reduced forms of a chemical species (e.g., Fe (II) and Fe(III)).

Reduce: The process of gaining one or more electrons by an ion, element or compound.

Reducing Agent: A compound, element or ion that is capable of reducing another species by donating one or more electrons.

Reference Electrode: In an electrochemical cell, the reference electrode is used to provide a stable potential from which the potential of the working electrode is measured.

Resin Based Column: A chromatographic column which employs a polymeric resin. The functional groups are chemical derivatives of the polymer itself. This is in opposition to silica columns, where the functional groups are chemically bonded to the silica.

Resistance: Opposition offered by a component to the flow of current in an electrical circuit.

Resolution: The separation of two peaks in a chromatogram. The resolution R is defined by:

$$\mathbf{R} = \frac{2(\mathbf{T_2} - \mathbf{T_1})}{\mathbf{W_2} + \mathbf{W_1}}$$

where: $\mathbf{T_1}$ and $\mathbf{T_2}$ are the retention times for peak 1 and peak 2.
$\mathbf{W_1}$ and $\mathbf{W_2}$ are the baseline width of peak 1 and peak 2.

Response Factor: The amount of a compound that was injected, divided by the area under the peak.

Retention Time: The time that has elapsed between the injection of a sample and the time when the detector response is maximized (i.e., the top of the peak) for a given compound.

Retention Volume: The volume of mobile phase that is required to elute a compound from a column.

Reverse Phase Column: A column in which the stationary phase is less polar than the mobile phase used with it. Typically a reverse phase column consists of a packing material with either an alkyl chain (e.g., C_{18}) or a somewhat polar side chain (e.g., CN).

Reversible Reaction: An electrochemical reaction in which the oxidized and reduced form of a species can be readily converted into each other. In the region of the standard electrode potential for the process, a small change in the potential can result in a change in the direction of the electrochemical reaction.

RS-232: An electrical connection by means of which two electronic devices can communicate with each other using a standard digital protocol.

RT: Abbreviation for retention time (usually in minutes).

Selectivity (Chromatographic): The ability of a chromatographic system to separate two compounds. Selectivity is measured in terms of the resolution of the chromatogram.

Selectivity (Detector): The ability of a detector to provide a distinct signal for the desired compound in a mixture, while discriminating against all other materials in the mixture.

Sensitivity: Refers to the minimum detectable quantity of an analyte with a given set of analytical conditions. The signal-to-noise ratio of the measurement should be indicated.

Separation: The elution of compounds in a sample by liquid chromatography.

Shoulder (Chromatography): A small chromatographic peak that is not baseline resolved with regard to a larger peak.

Signal-to-Noise Ratio (S/N): The signal-to-noise ratio for a data point is obtained by dividing the signal by the noise level that is associated with the measurement. A larger S/N ratio is more desirable than a small one. As the S/N ratio approaches unity, the level of certainty that a peak is real falls.

Slurry Packing: A method of packing an HPLC column whereby the particles are suspended in a solvent and forced into a column by pressure (as opposed to dry packing). Slurry packing is commonly used to pack small particles (e.g., below 10 μm diameter).

Solvation: The process of dissolving a material by a solvent.

Solute: The substance that is dissolved in a solvent.

Solvent: A liquid which is used as the mobile phase in HPLC or the liquid that dissolves the solute.

Solvent Delivery System: A device which includes one or two pumps that provides a pulse-free flow of the mobile phase to the system.

Sparge: The process of bubbling He or N_2 through a filtered solvent. Sparging is used to displace dissolved gases which have a deleterious effect on the separation and/or detection of analytes of interest. Sparging serves to displace CO_2, which could change the pH of a buffered solvent, and O_2, which could take part in electrochemical processes.

Standard: A solution containing a known amount of the compound of interest. The detector response is measured for a series of standards to obtain a standard curve.

Stationary Phase: The column packing material where adsorption and desorbtion of the components of the sample takes place. The mechanism of the interaction could be via ion exchange, adsorption or size exclusion.

Tailing: In theory, a chromatographic peak is Gaussian. If the longer retention time profile of the peak is not Gaussian, the phenomenon is termed tailing and the peak is termed asymmetric.

Temperature Coefficient: The rate of change of a phenomenon that is related to a change in the temperature.

Test Electrode: See Working Electrode.

Theoretical Plate: See Plate.

Time Constant: The period of times it takes for the signal to drop to 2% after the stimulus is removed.

Time of Flight: Period of time required for a compound to travel from one detector channel to the next.

Valve: A device which is placed in the chromatographic system to divert the flow of the mobile phase (e.g., to collect an eluting compound after separation).

Van Deempter Plot: A plot of the height equivalent to a theoretical plate as a function of the linear velocity of the mobile phase. A van Deempter plot is used to determine the linear velocity, which provides the most efficient separation (i.e., the shortest HETP).

Void: A part of an HPLC column, which does not contain packing material. A large void causes a dramatic decrease in the efficiency of a column as well as split peaks.

Void Volume: The available volume of an LC system between the injector and the detector. The void volume is the total volume of the system, less the volume occupied by the column packing. This term is approximated by the peak in the chromatogram that corresponds to the unretained analytes.

Volt: The unit of electrical potential difference.

Voltammogram: A plot of the current from an electrochemical system as a function of the potential that is impressed upon it.

W: Peak width or bandwidth.

Wait: A command in the timeline that is used to ensure that the start of data collection occurs at the same time as the injection of the sample.

Wall Effect: Band-spreading as a result of solvent flow along the column wall being different than the flow through the center of the bed.

Working Electrode: The electrode where the desired electrochemical process is occurring.

Zoom: A process whereby a selected portion of a chromatogram can be enlarged (or made smaller) to fill the entire viewing area.

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