CAUTION:
USING HP 1100 WITH ESA COULARRAY OR COULOCHEM III ELECTROCHEMICAL DETECTORS

It has recently been reported that several CoulArray users have observed abnormal background currents while using the HP 1100 HPLC Pump. According to HP Technical Support, the issue is due to metals leaching from the pump. The pump is not entirely made from 316 stainless and that other grades are used in the construction. Metal leaching from the pump is a significant problem when used with electrochemical detectors. HP is aware of this situation and strongly recommends that the HP 1100 pump must be "broken in" overnight using mobile phase and then actively passivated before use with any ECD.

ESA recommends that HP 1100 users contact Mr. Terry Whitt at HP (1-800-424-9759) for the appropriate passivation procedure.

ESA will not warranty any cells that are damaged by use with other manufacturers HPLC systems that have not been properly prepared for use with ECD.

If you have any questions, please contact the ESA Instrument Service Department at 1-800-275-0102 or its representative.

Coulochem® III
User's Guide Manual

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P/N 70-6502 Rev. A

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.

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

The Coulochem III 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.

Printed in the United States of America

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 delivery 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.

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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.

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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.

WARNINGS AND SAFETY PRECAUTIONS

The ESA Coulochem® III is an electrochemical detector system for high performance liquid chromatography. It can be used to determine the level of a large number of electroactive compounds in a broad variety of samples including those of biological origin. The following precautions should be followed to minimize the possibility of personal injury and/or damage to property while using the instrument.

---

1) Maintain a Well Ventilated Laboratory.

The mobile phase typically contains a volatile organic solvent. Ensure that the laboratory is well ventilated so that a buildup of vaporized solvent cannot occur. The eluent should be collected in a container that minimizes the escape of waste solvent into the atmosphere.

2) Avoid Open Flames and Sparks.

The mobile phase typically contains a flammable organic solvent. Do not use an open flame in the laboratory and do not install any equipment that can cause sparks in the same room as the instrument.

3) The Instrument must be Plugged into a Grounded Power Line.

Ensure that all parts of the system are properly grounded. It is strongly recommended that all parts of the system be connected to a common ground.

Do **not** attempt to bypass the earth ground connection. A serious shock hazard could result.

4) Treat all Samples and Mobile Phases as if they are Capable of Containing Hazardous Substances or Transmitting Disease.

The sample and/or mobile phase may contain compounds that may present a hazard to the operator of the system. Take all precautions to ensure that the mobile phase does not come into contact with the skin or eyes. A sink and eyewash should be located in the laboratory. In the event of an accident wash the affected part with copious quantities of water and seek medical assistance. If you are analyzing biological/clinical samples, treat them in accordance with the infectious disease control program of your institution.

5) Ensure that the Unit has been Disconnected from the Line before Removing the Cover.

Potentially hazardous currents and voltages may be present inside the power module.

6) Wear Protective Eyewear.

Solvents and other chemicals can damage eyes. Install a sink as close as possible to the module. If any solvents or chemicals splash on the skin or eyes, immediately rinse the affected parts in the sink. Obtain medical help as needed.

7) Monitor and Maintain Proper Pressure in Tubing and Devices used in the Coulochem Organizer.

The maximum recommended pressure rating of tubing and devices used in the Coulochem III Organizers 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 device's manufacturers for their maximum rating.

8) Use CE Approved HPLC Pump(s) or Solvent Delivery Device(s).

The pump(s) used with the Coulochem III Organizers must be CE approved and contain a properly installed and maintained pressure safety device and/or pressure sensor that conforms to the requirement of ISO 4126-1.

9) Avoid use of some Solvents with PEEK Tubing.

While PEEK tubing has excellent chemical resistance to most common organic solvents, it is attacked by concentrated nitric acid and sulfuric acid, and tends to swell (and weaken) in solutions with high concentrations of chloroform, dimethylsulfoxide, tetrahydrofuran and similar solvents. If these solvents must be used in high concentrations, the use of stainless steel may be appropriate.

10) Use Instrument in Proper Manner.

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

WARNING: Before connecting this detector to any MS Unit, the ESA Hi-Voltage Decoupling Union Kit (P/N 70-5974) must be properly installed (See Appendix C).

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 system.

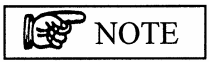
This symbol on the instrument states that high voltage may be present when panels/covers are removed. Any adjustment, maintenance, and repair of the opened apparatus under voltage should be avoided as far as possible and, if inevitable, must be carried out only by a skilled person who is aware of the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

The blocked WARNING statement used throughout the manual presents dangers that might result in personal injury.

The blocked CAUTION statement used throughout the manual presents hazards on conditions that could cause damage to the instrument or the reporting of erroneous results.



The blocked NOTE statement used throughout the manual highlights important information about the detector and its use.

Failure to follow these statements may invalidate the warranty.

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IMPORTANT OPERATING CONSIDERATIONS

A detailed discussion of the operation of the detector is presented in Chapters 1-3 and the *Coulochem® III (50W) Reference Manual*. This section describes some considerations that are especially important for optimizing performance of the system.

1. **Review and follow all information that is provided with cells, the organizer and other components of the system.**

2. **Ensure that In-Line Filters are used before each Electrochemical Cell in the System.**

   ESA cells with model number 501X (e.g., Model 5010A Standard Analytical Cell) and Model Number 502X (e.g., Model 5020 Guard Cell) contain porous graphite electrodes. These electrodes have a very large surface area to ensure a coulometric response. The graphite electrode can act as an efficient filter for small particles, such as silica fines from the column and particulate matter in the sample. These particles tend to clog the electrode and impede the flow of the mobile phase. To avoid clogging the electrode, **an in-line filter containing a filter element MUST precede each electrochemical cell in the system.** If the system uses a guard cell and an analytical cell, it will be necessary to use two in-line filters.

   In addition, it is strongly recommended that the aqueous portion of the mobile phase and all samples are filtered through a 0.22 micron filter.

3. **Users Must be Well Trained in the Use of HPLC Techniques.**

   It is imperative that the operator of the Coulochem III detector be adequately trained in HPLC techniques. Furthermore, there are a number of safeguards that must be observed in any method that is used in a clinical manner. For example, controls must be run on a regular basis and the results of the HPLC system that includes the Coulochem III must be calibrated on a regular basis to insure valid results.

   The operator must review every chromatogram to insure that system failure has not occurred. In addition the user must guard against misidentified samples, wrong amount of samples, improper sample and improperly stored samples. In the USA, CLIA covers various good laboratory procedures and practices for those performing clinical assays.

4. **Maintain a Stable and Proper Temperature for the Coulochem III Detector, Cells and the HPLC System.**

   Although the Coulochem III is designed to operate under typical laboratory conditions, it is still important to maintain a stable temperature environment when running samples. Significant temperature changes of the Coulochem III and the other HPLC system components could possibly cause errors in the results. Avoid placing any of the HPLC system components in direct sunlight. If the Thermal Organizer Module is employed to control the temperature, allow sufficient time for thermal equilibrium.

5. **Avoid Static Shocks to Coulochem III Detector and Cells.**

   Take adequate precautions to prevent static electricity discharges to the Coulochem III and cells. Static shocks could damage the detector and possibly cause errors in the results. Avoid using the detector and cells in low humidity environments and ensure that the detector and other HPLC components are properly grounded. The use of antistatic measures such as an antistatic mat or grounding oneself prior to touching any HPLC component may be required.

6. **Avoid Line Voltage Fluctuations.**

   The detector may be susceptible to fluctuations in the line voltage from the power source. Power line fluctuations such as spikes, sags, dropouts, brownouts, etc. may cause interruptions in the use of the detector and possibly cause errors in the results. If line voltage fluctuations are common in your area or if you would like to ensure that power fluctuations do not cause detector interruptions, the use of a line voltage conditioner or power surge protector with power sag protection is recommended.

7. **Avoid Placing Coulochem III and Cells Near Large RFI Sources.**

   The detector and cells should not be located near any large sources of RFI (Radio Frequency Interference). Typical large sources of RFI include refrigerators, fume hoods, centrifuges, MRI systems, NMR instruments and radio/TV station antennas. Large amount of RFI could cause baseline disturbances (constant or intermittent) and thus could result in errors in the results.

Table of Contents

1 Introduction

1.1 Overview of the Detector

The ESA Coulochem® III Multi-Electrode Detector is designed for the detection of electroactive species in the eluent from a high performance liquid chromatographic (HPLC) system. The Coulochem III detector combines superb sensitivity with a high degree of selectivity. The detector, with the Thermal Organizer Module (Part Number 70-5499TA) option is presented in Figure 1-1.

Figure 1-1: ESA Coulochem® III Multi-Electrode Detector with Organizer Module Option

There are three different modes of operation of the Coulochem III detector:

- ***DC Mode:*** The applied potential is held constant during the analytical measurement (requires the *DC Potentiostat Board*).

- ***Pulse Mode:*** The potential is changed at predetermined times during the analysis to recondition the electrode. This mode is frequently used for the analysis of alcohols and carbohydrates (requires the *Pulse/Scan Potentiostat Board*).

- ***Scan Mode:*** The potential is continuously varied to obtain a cyclic voltammogram. This mode is sometimes used to determine the optimum potential for detection (requires the *Pulse/Scan Potentiostat Board*).

In addition, *Timeline Mode* is provided to allow the user to program gain changes, potential changes, contact closures, autozeroing of the detector and the placement of an event mark on the chromatogram during a chromatographic run.

1.2 Electrochemical Detection in HPLC

☞ NOTE: This section presents a concise discussion of the use of electrochemical detection with HPLC. A detailed description of the principles and benefits of electrochemical detection is presented in Chapter 4 of the Coulochem III Reference Manual.

Electrochemistry involves the use of an applied potential to effect a chemical reaction; this potential is characteristic of the compound of interest and its environment. The current that is measured in an electrochemical reaction is proportional to the concentration of the species (compound, ion or element) that is being oxidized (or reduced). In an electrochemical detector, the eluent passes through a flow cell that provides the appropriate potentials and monitors the current.

Electrochemistry can provide a number of significant benefits:

- ***Sensitivity:*** Typically electrochemical detectors can provide limits of detection (LODs) at the picogram to femtogram level. The detection limit is dependent on the compound itself and the analytical conditions used. This limit is significantly better than that for many other commonly used modes of detection for HPLC (e.g., UV absorption).

- ***Broad Linear Range:*** The linear response range for an electrochemical detector can be as wide as 10^8. This range is larger than many other modes of detection (the linear response range for UV absorption is approximately 10^4).

- ***Selectivity:*** If two or more species elute at the same time and they have different oxidation (or reduction) potentials, it may be possible to select the operating potential so that only the compound of interest is detected at the working electrode.

ESA provides a broad range of cells (see Section 1.3.2) so that amperometric or coulometric detection can be employed with the detector.

- ***Amperometric Detection:*** When amperometric detection is employed, some fraction (usually 5-15%) of the species of interest is oxidized (reduced). The observed signal is proportional to the total amount of the species of interest present in the sample.

- ***Coulometric Detection:*** When coulometric detection is used, up to 100% of the species of interest is oxidized (or reduced). In this mode, the observed signal is directly proportional to the concentration of the species of interest.

Since a coulometric detector has very high conversion efficiency, it can provide an enhanced signal (compared to an amperometric detector). Another benefit of a coulometric detector is that an increase in selectivity can be obtained by using an additional electrochemical cell before the analytical cell to oxidize (or reduce) compounds that may co-elute with the compound of interest. This increase in selectivity is obtained if the oxidation (or reduction) potential of the co-eluting species is sufficiently different than that of the species of interest.

NOTE: In this manual, it is assumed that the reader has knowledge about sample pretreatment and the chromatographic separation of the compound(s) of interest.

1.3 Description of the ESA Coulochem III Electrochemical Detector

The ESA Coulochem III Electrochemical Detector consists of a control module, appropriate electrochemical cells and an Organizer Module (optional). The organizer module is available in two formats, an organizer for use at ambient temperature and a thermal organizer for use at elevated temperatures.

1.3.1 Control Module

The control module includes:

The User Interface — The Coulochem III user interface includes a display panel and a keypad.

- *The display* transmits information from the system to the user. It provides the operational status of the system, assists the user in setting up and editing analytical methods and displays stored methods.
- *The keypad* is used to enter information from the user to the system. It is used for establishing and editing methods as well as to select the desired data display during an analysis.

Logic Module — The Logic Module contains the microprocessor and associated electronics. The input/output connections and the remote ports are present on the Logic Module.

Memory — Analytical methods can be stored in memory (i.e., are saved if the power is turned off) and can be modified or deleted as desired.

DC Potentiostat — The DC Potentiostat provides the desired potential to the electrode for DC operation. In addition it sends the resulting current signal to a recording device via the Signal Output BNC connectors. One or two electrochemical cells can be connected to the potentiostat.

Pulse/Scan Potentiostat Board — The Pulse/Scan Potentiostat board is used to provide scan mode and pulse mode operation of the detector.

The Logic Module, DC Potentiostat Boards and Pulse/Scan Potentiostat Board are accessed via the rear panel of the unit. The various cells are connected to the detector via the potentiostats. A detailed description of the rear panel is presented in Section 2.5 of the *Coulochem III (50W) Reference Manual*.

1.3.2 Electrochemical Cells

The Coulochem III Electrochemical Detector can be provided with a variety of cells to meet the general requirements of the user application, including the Model 501X cells (which are coulometric cells) and Model 504X (which are amperometric cells). In addition, the Model 5020 Guard Cell or Model 5021 Conditioning Cell can be supplied.

- ***Standard Analytical Cell*** (Model 5010A, Part Number 70-5560): The Standard Analytical Cell provides the potential for the oxidation (or reduction) of the species of interest. The cell contains two chambers in series; each chamber includes a porous graphite coulometric electrode, a double counter electrode and a double reference electrode that is capable of withstanding pressures up to 600 psi (42 bar). Wettable surfaces include PEEK™, Kel-F®, Teflon®, palladium, graphite and Stainless Steel (type 316) at the outlet.

- ***High Sensitivity Analytical Cell*** (Model 5011A, Part Number 70-5561): The High Sensitivity Analytical Cell is used to provide the potential for the oxidation (or reduction) of the species of interest. An enhanced response coulometric electrode is coupled with a standard coulometric electrode. This cell provides very high sensitivity for electrochemical detection of HPLC eluents. The operating pressure of this cell should not exceed 600 psi (42 bar). Wettable surfaces include PEEK, Kel-F, Teflon, palladium, graphite and Stainless Steel (type 316) at the outlet.

- ***Microdialysis Cell*** (Model 5014B, Part Number 70-0520B): This cell is similar to the Model 5011A High Sensitivity Analytical Cell. It contains an enhanced response coulometric electrode coupled with a standard coulometric electrode. In addition, the microdialysis cell has been designed with the microdialysis user in mind to give very high sensitivity for electrochemical detection. The Model 5014B is specifically designed for microdialysis studies, as the reference electrode for channel 2 is isolated from the flow path to help minimize the void disturbance which is observed when artificial CSF is analyzed. The operating pressure of this cell should not exceed 600 psi (42 bar). Wettable surfaces include Stainless Steel (type 316), Kel-F, Teflon, PEEK, palladium and graphite.

- ***Model 5020 Guard Cell*** (Part Number 55-0417): The Guard Cell is used to oxidize (or reduce) electroactive species that may be present as trace impurities in the mobile phase. The guard cell is usually placed between the pump and the injector. The operating pressure of this cell should not exceed 6000 psi (422 bar). Wettable surfaces include Stainless Steel (type 316), palladium and graphite.

- ***Model 5021 Conditioning Cell*** (Part Number 55-0450): The conditioning cell is used as a third electrode to add extra selectivity to the analysis. It is placed after the analytical column. The operating pressure of this cell should not exceed 600 psi (42 bar). Wettable surfaces include Stainless Steel (type 316), Kel-F, Teflon, PEEK, palladium and graphite. This cell has a much smaller cell volume than a Model 5020 Guard Cell and is designed to operate at lower operating pressures.

- ***Analytical Cell*** (Model 5040 and Model 5041 (various Part Numbers depending on configuration)): The amperometric analytical cells include a thin layer amperometric cell which can be used with a variety of targets:

	Part Number
Gold/Ceramic Target	70-2134
Platinum/Ceramic Target	70-2135
Silver Target	70-0096
Glassy Carbon/Ceramic Target	70-2000

The Model 5040 Analytical Cell also includes a counter electrode made of stainless steel and a pH sensitive solid-state reference electrode that maintains an exceptionally stable output even as the pH is changed. The reference electrode assists in the reduction of baseline drift when the pH of the mobile phase is altered during gradient elution. It can be used over a broad pH range and has proven to be very successful in high pH mobile phases. Since the reference electrode is pH sensitive, it is especially useful when a gradient in which the pH is changed is used. This unique reference electrode is found only in ESA electrochemical sensors. The operating pressure of this cell should not exceed 200 psi (14 bar).

The Model 5041 Analytical Cell is similar to the Model 5040 cell, but is designed for microdialysis, as it has an isolated reference electrode. Both Model 504X cells are designed for narrow bore/wide bore chromatography as well as normal bore.

Wettable surfaces include:

- Model 5040 - Stainless Steel (type 316), PEEK, Kel-F, Viton®, Teflon, palladium, ceramic and the target electrode material.

- Model 5041 - Stainless Steel (type 316), PEEK, Kel-F, Viton, Teflon, Mylar, palladium, ceramic and the target electrode material.

1.3.3 Coulochem Organizer Modules

ESA provides an Organizer Module (optional) and a Thermal Organizer Module (optional) to maintain the column, cells, pulse damper and related components. These modules are fitted on top of the detector as shown in Figure 1-1. The temperature of the Thermal Organizer Module can be set as described in Section 2.7.9. A discussion of the use of these modules is presented in Chapter 14 of the *Coulochem III (50W)Reference Manual*.

1.4 Contents of the User's Guide Manual

This manual includes the following material:

- ***The User Interaction Program*** (Chapter 2) - discuses the general layout of the user interaction program and shows how the various operating modes are accessed. In addition, this chapter describes how a DC Mode method is established and executed.

- ***Operation of the Detector in an HPLC System*** (Chapter 3) - considers a broad variety of topics such as the selection of operating parameters, mobile phase considerations, start up procedures, shutdown procedures and other topics that relate to the routine use of the detector.

- ***Mobile Phase Considerations*** (Chapter 4) - describes how the mobile phase for electrochemical detection should be generated and stored.

1.5 For Additional Information

The *ESA Coulochem III (50W) Reference Manual* (Part Number 70-6501) describes installation of the detector, the role of the cell in electrochemical detection, maintenance, troubleshooting and the theory of operation. In addition, it describes programming the detector for Timeline, Scan Mode, Pulse Mode, Screen Mode and Redox operations.

The *Coulochem III (50W) Reference Manual* includes the following material:

- ***Setting Up the Detector in the Laboratory*** (Chapter 2) - describes the required laboratory environment, installing the detector in the laboratory, making electrical connections, and performing a test protocol to determine if the detector is functioning properly.

- ***Installing the Detector in an HPLC System*** (Chapter 3) - describes how the detector is connected to the HPLC system and includes a step by step procedure to assist the installer. In addition, a number of experiments are included to check overall system operation.

- ***Theory of Operation*** (Chapter 4) - discusses the principles of electrochemical detection in HPLC and includes a discussion of coulometric and amperometric detection modes. In addition, this chapter describes how electrochemical detection provides superb sensitivity and selectivity.

- ***The Coulometric Cell in Electrochemical Detection*** (Chapter 5) - describes the characteristics of the various coulometric cells that are used with the Coulochem III detector and explains the benefits of coulometric detection. In addition, this chapter describes maintenance and cleaning of coulometric cells.

- ***The Amperometric Cell in Electrochemical Detection*** (Chapter 6) - describes the characteristics of the amperometric cells that are used with the Coulochem III detector and explains the benefits of amperometric detection. In addition, this chapter describes maintenance and cleaning of amperometric cells.

- ***Maintenance Activities and Replacing Components*** (Chapter 7) - presents a discussion about a variety of activities that should be performed on a routine basis to optimize system performance.

- ***Troubleshooting*** (Chapter 8) - includes a protocol that the user should follow to determine the cause of difficulties.

- ***Programming the Detector for DC Timeline Operation*** (Chapter 9) - describes how the Timeline feature can be used to schedule a variety of activities at the start of the run or at user specified times during a run.

- ***Screen Mode Operation*** (Chapter 10) - describes the use of screen mode to eliminate possible interferents in an analytical procedure via a screening electrode.

- ***Redox Mode Operation*** (Chapter 11) - explains how the concentration of an analyte can be determined by electrochemically reducing/oxidizing a compound of interest.

- ***Pulse Mode Operation*** (Chapter 12) - discusses the use of Pulse mode to condition the surface of the electrode during the analysis. This mode of operation is commonly used when the electrochemical process generates materials that foul the cell surface during analysis.

- ***Scan Mode Operation*** (Chapter 13) - describes how the potential can be changed during a run and how a cyclic voltammogram can be obtained.

- ***Coulochem III Organizer Modules*** (Chapter 14) **-** discusses the characteristics of the organizer and describes how the user can install various fluidics components in the organizer.

To install and operate the detector, it may be necessary to obtain information from the manuals of other devices that are used in conjunction with the detector. These include (but are not limited to) the solvent delivery system, the analytical column, injector and the data recording/processing device (recorder, integrator, personal computer, etc.).

ESA maintains a complete service department to assist in the event of any difficulty with the installation or operation of the instrument. The detector includes a number of diagnostic features so that potential problems can be traced to the appropriate sub-system. When contacting the ESA service department, please provide the serial number of your unit and the software version number (which is displayed after the system has initialized, and can also be obtained by pressing the HELP button followed by the ESCAPE button at any time). If a diagnostic message is presented on the display, please provide that information to the service representative as well.

An on-line help system is included with the operating program, which can be accessed at any time by pressing the HELP button.

ESA provides instrument qualification services including Installation Qualification (IQ), Operational Qualification (OQ) and Performance Qualification (PQ). For details, please contact ESA or your local representative.

A broad variety of instrumentation and supplies for electrochemical detection, including pumps, autosamplers, columns, and mobile phases is available from ESA. These components have been specifically designed or selected to optimize electrochemical detection.

For additional information, please contact ESA or your local representative.
The ESA website (www.esainc.com) presents a broad range of information about the use of electrochemical detection.

A detailed discussion of the use of electrochemical detection in HPLC can be found in the following reference:

- Acworth, I.N. and Bowers, M (1996). An introduction to HPLC-based electrochemical detection: from single electrode to multi-electrode arrays. In Progress in HPLC, Vol. 6, Ackworth, I.N., Naoi, M., Parvez, S., and Parvez, S. (Eds.) VS Press, The Netherlands (available from ESA, Part Number 70-3419).

2 The User Interaction Program

2.1 Introduction

All commands for the control and operation of the Coulochem® III are entered via the keypad on the front panel of the detector (Figure 2-1), which includes 4 lines by 40-character display panel. In addition, a number of indicator lights (LED's) adjacent to certain keys on the front panel are used to indicate that the corresponding feature is active (e.g., the CELLS ON/OFF LED is lit when the potential is being applied to the cell(s)). If desired, an ESA Data Station can be used to control the operation of the Coulochem III via RS232 remote communication.

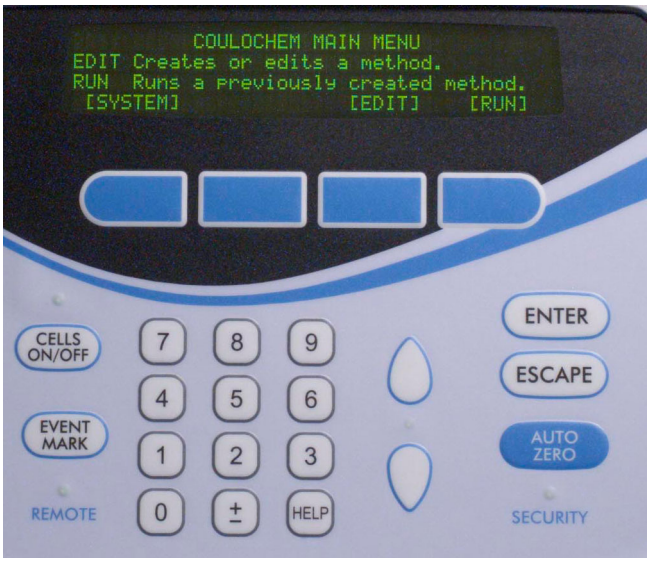

Figure 2-1: Front Panel of Control Module of ESA Coulochem III Electrochemical Detector

There are three modes of operation of the Coulochem detector, DC, Pulse, and Scan. (DC mode requires the *DC Potentiostat Board*. Pulse and Scan modes require the *Pulse/Scan Potentiostat Board*.) In DC mode, the potential is held constant and the output current is presented on the display and the recording device. In this chapter, we describe the general operation of the system and describe how the desired mode of operation is chosen. In addition, this chapter describes how a DC method is generated and executed.

DC mode with Timeline as well as Scan and Pulse modes are described in the *Coulochem III (50W) Reference Manual* (Part Number 70-6501).

2.2 Initialization of the System

When the Coulochem III detector is powered up, the system undergoes an electronic selftest. During the selftest, a variety of system components are checked and the firmware version is indicated on the display. If an electronic fault is found during the selftest, a message will be presented on the display.

Upon successful completion of the selftest, the initial display will appear as shown in Figure 2-2.

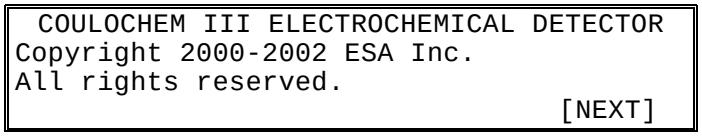

Figure 2-2: The Initialization Screen

Press the key immediately below [NEXT] to present the COULOCHEM MAIN MENU (Figure 2-3) [The temperature will not be indicated if the Thermal Organizer is not connected to the detector module].

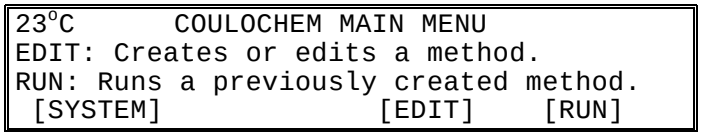

Figure 2-3: The Coulochem Main Menu Screen

NOTE: The role of the four keys immediately below the screen is dependent on the screen that is presented. These keys are termed *soft keys*, since the definition of these keys is context sensitive.

The soft keys presented in Figure 2-3 are used as follows:

- **SYSTEM** accesses a series of screens that are used to select a variety of general operating parameters such as defining default values, setting time/date, defining the communication protocol to a personal computer, etc. These screens are discussed in Section 2.7.

- **EDIT** accesses a series of screens to select the desired mode of operation and establish analytical parameters for data acquisition. A set of analytical parameters is termed a METHOD, which can be stored and recalled as desired. These screens are discussed in Section 2.4. Please note that you cannot "run" a method until you have used "edit" to create and store a method.

- **RUN** accesses the RUN METHOD SELECTION screen, which is used to choose a stored method to be run and then execute the selected method. Selection and execution of a method is discussed in Section 2.5.

2.3 Introducing the Keypad

The keypad is used to enter data (Table 2-1) or initiate an action (Table 2-2).

Table 2-1: Data Entry Keys

Key	Function
Numeric Keys	Used to enter the appropriate value for various parameters (e.g., the cell potential). When you have entered the desired value, press ENTER.
+ -	Used to change the sign of a numeric entry. The sign should be changed before the numeric entry is made.
▲ (∗)	Scroll to the next value in a sequence (e.g., the Current Range value). When the desired value is presented, press ENTER.
▼ (∗)	Scroll to the previous value in a sequence (e.g., the Current Range value). When the desired value is presented, press ENTER.
HELP	Presents context sensitive information about the active screen.
ENTER	Used to indicate that the value presented in the display for a given parameter is the desired value for the parameter. If the value that has been entered is outside the range for the parameter, the value will not be accepted and a message with the appropriate limits will be presented.
ESCAPE	Clears partial entries so you may re-enter the correct values.

(*): Active only when its activity indicator LED is lit.

Table 2-2: Action Keys

Key	Function
EVENT MARK	Used to place a spike on the recorder trace with a deflection of -10 to +10% full scale.
AUTOZERO	Sets the output signal to zero at the current range in use. A double click of the Autozero key will set the DC signal outputs to zero for all current ranges.
CELLS ON/OFF	This key is used to indicate if the working potential is to be applied to the cells (including the guard cell). It is a toggle switch; when the potential is applied, the LED adjacent to it is illuminated.

The **REMOTE** LED on the keypad is illuminated when the detector is under the control of an external device. All keys on the keypad are deactivated when the LED is lit except the AUTOZERO key, the soft keys and the CELLS ON/OFF key.

The **SECURITY** LED on the keypad is illuminated when the system is operating in local or remote modes and the security feature is active to prevent accidental changes in the operating parameters. In "remote secure" mode none of the front panel keys are active.

2.4 Generating/Editing a DC Method

2.4.1 Overview

To generate/edit a DC method, the following steps are performed:

a) Selection of the method number (Section 2.4.2).

b) Editing of the Mode Selection Screen (Section 2.4.3).

c) Editing of the DC Mode Screen to set the Guard Cell Potential and Security (Section 2.4.4).

d) Editing of the Potential, Current Range, Full Scale Output, Filter and Baseline Offset for each channel (Section 2.4.5).

e) Saving the method (Section 2.4.6).

f) Method Number Zero (Section 2.4.7)

2.4.2 The Edit Method Selection Screen

When you select [EDIT], the EDIT METHOD SELECTION screen (Figure 2-4) is used to access a series of screens that are used to generate a method or change an existing method. This screen is accessed by pressing [EDIT] on the COULOCHEM MAIN MENU screen (Figure 2-3).

```
          EDIT METHOD SELECTION
Method Number: (1)  ·CATS·
Mode: DC       Date: Jan 3,2001  7:40
 [CANCEL]                         [EDIT]
```

Figure 2-4: The Edit Method Selection Screen #1

The Coulochem III Electrochemical Detector can store 25 analytical methods, which are sequentially numbered from 1-25.

When the EDIT METHOD SELECTION screen is opened, Method Number 1 is presented. The date/time fields refer to when the method was created or last edited.

If a different method is to be edited, press the ▲ arrow key until the desired method is presented. As an example, if method 2 is selected, the screen might appear as shown in Figure 2-5. The method numbers are assigned in numerical order. After you have scrolled past the last defined method, the Mode entry will indicate Undefined and the date/time fields will be blank.

```
          EDIT METHOD SELECTION
Method Number: (2)  ·DOGS·
Mode: SCAN     Date: Jan 3,2001  15:40
 [CANCEL]                         [EDIT]
```

Figure 2-5: The Edit Method Selection Screen #2

The [CANCEL] key returns the COULOCHEM MAIN MENU screen (Figure 2-3).

2.4.3 The Mode Selection Screen

☞ NOTE: The protocol for editing a method is the same as that for generating a new method.

To generate or edit a DC method:

a) When you have accessed the method to be edited (or the empty method), on the EDIT METHOD SELECTION screen (Figure 2-4), press [EDIT] to present the MODE SELECTION screen (Figure 2-6). The cursor will be on the first character of the method (default mode is DC). Parameters indicated in parentheses are edited via the ▲ or ▼ keys.

```
              MODE SELECTION
This method's Mode is DC
New Mode is (DC) with (2) Channel(s)
[CANCEL]          [PREVIOUS]      [NEXT]
```

Figure 2-6: The Mode Selection Screen

NOTE: If a different mode of operation is desired, use the ▲ or ▼ key to select it. Pulse and Scan modes will be presented as options if the Pulse/Scan Potentiostat board is installed. Operation of the system in Scan mode, Pulse mode, DC Timeline mode or Pulse Timeline mode is discussed in the *Coulochem III (50W) Reference Manual*.

b) After the mode has been selected, press the ENTER key to move the cursor to the value for the number of channels (cells), edit that value using the ▲ or ▼ key. In DC mode, the options are 1 or 2 (channels). After you have indicated the number of channels, press NEXT to present the DC MODE screen.

2.4.4 The DC Mode Screen

The DC MODE screen is presented in Figure 2-7. If a Guard Cell is not installed, the message *Guard Cell not detected* is presented and you should skip step (a).

```
                    DC MODE
Guard Potential E {0} mV
Run time security is {on} code {0}
[CANCEL]                          [NEXT]
```

Figure 2-7: The DC Mode Screen

a) Enter the desired potential for the Guard Cell (the range is between -2000 mV and +2000 mV) via the numerical keypad and press ENTER. The value will be accepted and the cursor will move to the *Run time security* field. Parameter fields indicated in brackets are edited via the numeric keypad.

b) If an invalid value is entered, the ENTRY LIMITS EXCEEDED screen (Figure 2-8) is presented. Press NEXT to return to the active screen.

```
          ENTRY LIMITS EXCEEDED
Value Minimum: -2000,   maximum: 2000
Select Value to be returned
                                  [NEXT]
```

Figure 2-8: The Entry Limits Exceeded Screen

c) If run time security is desired, the field should read **on**. The field can be toggled to off or on using the ▲ or ▼ key. If run time security is selected, the cursor will be moved to the first character in the code field and you can enter a security code for this method. Use of the security code is described in the Reference Manual. After you have entered the code, press ENTER and then press [NEXT] to access the DC MODE CHANNEL 1 Potential/Current Range Screen.

2.4.5 The DC Mode Channel 1/2 Screens

The CH1 Potential/Current Range screen (Figure 2-9) is used to set the potential and current range for Channel 1. When the screen is accessed, the cursor will appear to the right of the last character of the Potential field.

```
DC MODE                      CHANNEL 1
Potential E:{50}mV
Current Range R: (100uA)
[CANCEL]          [PREVIOUS]     [NEXT]
```

Figure 2-9: The DC Mode Channel 1 Screen #1

a) Enter the desired potential using the numeric keypad (Range -2000 mV to +2000 mV). If an invalid entry is made, the ENTRY LIMITS EXCEEDED SCREEN (Figure 2-8) will be presented. After the potential has been set, press ENTER to move the cursor to the *Current Range* field.

b) Select the desired current range via the ▲ or ▼ keys (the LED between the two keys will be illuminated). The range is from 10 pA to 1 mA in a series of 1-2-5 steps (10 pA, 20 pA, 50 pA, 100 pA, etc.). After the current range has been set, press [NEXT] to present an additional screen of parameters for DC Channel 1 (Figure 2-10). The cursor will appear on the *Full Scale Output* field.

```
DC MODE                      CHANNEL 1
Full scale output: (1.0 Volts)
Filter:(5.0)Sec. Baseline Offset:{0}%
[CANCEL]          [PREVIOUS]     [NEXT]
```

Figure 2-10: The DC Mode Channel 1 Screen #2

c) Select the desired Full-scale output via the ▲ or ▼ keys (the LED between the two keys will be illuminated). The choices are -1 V, -0.1 V, 0.1 V and 1.0 V. Press ENTER when the desired choice is made. The cursor will appear on the *Filter* field.

d) Select the desired filter via the ▲ or ▼ keys (the LED between the two keys will be illuminated). The choices are 0.2 sec to 10 sec in a series of 1-2-5 steps. Press ENTER when the desired choice is made. The cursor will appear on the *Baseline Offset* field.

e) Select the desired baseline offset via the numeric keypad and press the [ENTER] key. The choices are any value within ±50% of full scale.

f) Press the [NEXT] key to present the DC MODE CHANNEL 2 Potential/Current Range screen, which is identical to Figure 2-9, but describes the second DC channel. Complete the two screens for DC Channel 2 in the same manner as for channel 1. After you have completed the editing of the two screens, press [NEXT].

2.4.6 The Save Method Screen

The SAVE METHOD screen (Figure 2-11) is used to store the method in memory. When the screen is opened, the cursor will be placed on the method number for the next available unused method even if you began by editing a method previously created. Use the▼ key to return to the method you wish to overwrite or chose to store the newly edited method as a new method at the unused method number presented.

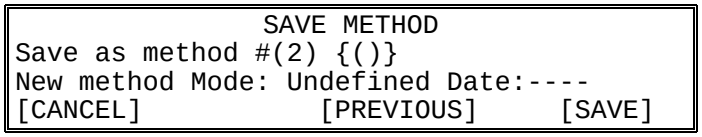

Figure 2-11: The Save Method Screen

After the method number has been selected, press ENTER. The cursor will move to the {()} field and you can enter additional information about the method (i.e., name of the method, your initials, etc.) using the numerical keypad. Alphabetic characters can be accessed by pressing 9 and then using the ▲ key. (e.g., pressing 9 then the arrow key two times will display the letter "B". Pressing the 9 key again will allow for the next alphanumeric character, etc. Names may be 16 characters long.). Two examples of naming a method are given in Sections 9.4 and 9.5 of the *Coulochem III (50W) Reference Manual*. When you have entered all the alphanumeric characters press ENTER to return the cursor to the method number field.

Press [SAVE] to save the method. The method will be stored in memory. The date and time will be automatically entered when you save the method. A prompt will be presented to press [NEXT] to continue. Press [NEXT] to return to the Main Menu.

2.4.7 Method Number Zero

Method Number: (1) is always displayed first when you press [EDIT] while in the Main Menu. Normally you would use the ▲ key to scroll to higher method numbers looking for a method to edit or an unused method number. If, however, you use the▼ key you will reach Method Number: (0).

If, after editing a method you choose to OVERWRITE a previously created method that previously created method will be stored temporarily as Method Number Zero (0).

You may use the▼ key to access Method 0 to review what you have overwritten or to recover what you have overwritten. We recommend that you do not deliberately store a method using method number zero because it will be lost the next time you (or another operator) overwrites a method.

2.5 Running a DC Method

To Run a DC Method:

a) Press Run on the COULOCHEM MAIN MENU (Figure 2-3) to present the RUN METHOD SELECTION screen (Figure 2-12). The last method SAVED will be presented as your first option.

```
          RUN METHOD SELECTION
Method Number: (2)"ABC"
Type: DC     Date: Jan 5,2002 8:22
[CANCEL]                      [RUN]
```

Figure 2-12: The Run Method Selection Screen

b) Use the ▲ or ▼ keys to select the desired method. When the desired method is presented, press [RUN] to present the DC METHOD RUNNING screen (Figure 2-13). This is the first of three screens that describe the operation of the DC Method (Figure 2-13 through Figure 2-15). The parameters indicated on the three running screens are described in Table 2-3, and can be edited as described below.

```
          DC METHOD 2 RUNNING
E1{500}mV R(1 mA) i  -0.00mA   0%FS
E2{650}mV R(1 mA) i  -0.00mA   0%FS
[STOP]        [EDIT]       [SETTINGS]
```

Figure 2-13: The DC Method 2 Running Screen #1

c) The [SETTINGS] key accesses the DC METHOD Screen #2 (Figure 2-14), which displays the remainder of the method parameters.

```
          DC METHOD 2 RUNNING
Filt1(2.0) Sec  Output 1.0V  0%Offset
Filt2(2.0) Sec  Output 1.0V  0%Offset
[STOP]  [EDIT]     [CELL]     [GUARD]
```

Figure 2-14: The DC Method 2 Running Screen #2

DC Running Screen #2 displays the filter, recorder output, and baseline offset values chosen when the method being run was created. The filter values may be edited while the method is running by pressing the [EDIT] key but again the change will not be incorporated into the method. You may not change the values for recorder output or baseline offset while in RUN mode.

d) The [CELL] key will return you to Running Screen #1 where electrode potentials and currents are displayed.

e) DC METHOD RUNNING SCREEN #2 will identify the furthest right softkey as [GUARD] if you have a Guard Cell installed on the Coulochem III detector. If you want to change the Guard Potential or view the present Guard Current, press [GUARD], which presents the DC METHOD RUNNING SCREEN #3 (Figure 2-15).

```
            DC METHOD 2 RUNNING
Guard Potential: {0} mV
Guard Current: 1.23 mA
[STOP]   [EDIT]      [SETTINGS]  [CELL]
```

Figure 2-15: The DC Method 2 Running Screen #3

You may edit the Guard Potential while in RUN mode by pressing the [EDIT] key but again the new value will not be saved in your method. The [CELL] key presents the DC METHOD RUNNING Screen #1 (Figure 2-13) again. The [SETTINGS] key will return you to DC METHOD RUNNING Screen #2 (Figure 2-14) again.

Table 2-3: DC Mode Running Parameters

Parameter	Description	Range	Default	Type
Potential (E1, E2)	Voltage to be applied to the cell when cells are ON	-2000 mV to +2000 mV (integer increments)	0 mV	Input
Current Range (R)	Used to set the sensitivity level of the detector	100 pA to 1 mA (1,2,5 step increment)	1 mA	Input
i	Instantaneous current	-1 mA to +1 mA (units dependent on range)		Result
%FS	% of full scale, display of signal output sent to recording device	-100 to +100%	1 mA	Output
Filt1 and Filt2	Time constants for the filters used to smooth baselines.	0.2 to 10.0 seconds	5.0 Sec	Input
Output	Sets the maximum signal output expected at the rear panel Signal Out BNC connectors	-0.1 V, -1.0 V, 0.1 V, and 1.0 V	1.0 V	Cannot edit in run mode
%Offset	Percent offset added to baseline signal	-50% to +50%	0%	Cannot be edited in run mode
Guard Potential	Potential applied to guard cell test electrode	-2000 mV to +2000 mV	0 mV	Input
Guard Current	Instantaneous guard cell current	-2 mA to +2 mA (units in uA or mA)		Result

If desired, you can change an input parameter during a run. To edit a parameter, press the [EDIT] key, move the cursor to the desired parameter with the ENTER key, set the desired value and press ENTER again.

If you do not finish the process by pressing the ENTER key until the flashing cursor is no longer visible then the channel you began to edit will remain in stasis and no changes will occur nor will a live signal be sent to the recording device.

☞ NOTE: If you change a parameter in this manner, the change will not be incorporated into the stored method. If you want to edit the method on a permanent basis, it will be necessary to edit the method as described in Section 2.5 and then store it.

The STOP key terminates data collection.

☞ NOTE: STOP does not turn the potential(s) to the cell off, which must be done via the CELLS ON/OFF key.

2.6 The Autozero Function

2.6.1 Two Autozero Levels

The Autozero function in the Coulochem III can occur on two levels.

A Level 1 Autozero is initiated by pressing the AUTOZERO key on the front panel **once** (within 1 second). It may also be initiated from the rear panel by shorting the "+" and "-"AZ terminals on the I/O connector block **once** or from Timeline by issuing an Autozero command only **once**. In addition, the user can initiate a Level 1 Autozero in Remote Mode by sending a **single** Autozero command from the data station.

When a Level 1 Autozero event is initiated the Coulochem III captures the value of the recorder output signal at the current range in use and subtracts it from all future recorder output signals on a continuous basis. The result is that recorder output drops to zero initially and then remains at zero until a chromatographic event causes a change in the baseline. Examples of chromatographic events are an analyte peak eluting from the column, injector void volume disturbances, baseline drift and/or baseline noise.

It is important that the detector accurately display these changes to the baseline signal even after the Autozero event. Another type of baseline change can be seen if the operator changes current ranges (detector sensitivities) after a Level 1 Autozero event. These baseline changes can be very large if current range changes encompass a major step in the internal amplification protocol used by the Coulochem III to accurately display signals over nine orders of magnitude.

Thus if your chromatographic analysis method requires that the current range be changed during a chromatographic run to optimize signal display for a very wide range of analyte concentrations, you may see a significant offset in the baseline caused simply by the changes in detector sensitivity.

A Level 2 Autozero will significantly reduce the baseline offsets that may occur when a current range is changed during a chromatographic run.

A level 2 Autozero event may be initiated by pressing the AUTOZERO key **twice** within one second. It may also be initiated from the rear panel by shorting, unshorting, and shorting again the "+" and "-"AZ terminals on the I/O Connector block within one second. Within Timeline you can initiate a Level 2 Autozero by programming **two** Autozero events to occur within 0.01 minutes of each other. (See Section 9.5 of the *Coulochem III (50W) Reference Manual* for an example of Level 2 Autozero programming.) In Remote Mode the data station can cause a Level 2 Autozero to occur by sending **two** Autozero commands within one second.

When a Level 2 Autozero is initiated, the Coulochem III measures the offsets existing at every stage of signal amplification and creates a table of corrections to be applied for each current range option from10 pA to 1 mA. These corrections are valid only as long as operating temperatures and baselines do not drift significantly. The Level 2 Autozero should be re-initiated after each chromatographic run has been completed and before the next sample injection. To be most effective a Level 2 Autozero should be initiated on the most sensitive current range you expect to use. Because Level 2 Autozero events require much more intensive microprocessor utilization they take much longer to complete. You should allow enough time between a Level 2 Autozero event and the next peak so that the peak is not lost or zeroed out.

Table 2-4 shows *maximum* Autozero times for Level 1 and Level 2 Autozero events for both Single (channel 1) and Dual Channel (channel 1 and 2) operations. The detector may actually complete the Autozero Event in *less* time as it optimizes its protocol depending on current range (for Level 1), baseline noise, and magnitude of the signals to be zeroed out.

Table 2-4: Worst Case Autozero Times

Channel Mode	Single Range - Level 1	All Ranges - Level 2
Single Channel	8 seconds	18 seconds
Dual Channel	14 seconds	33 seconds

2.6.2 Selecting the Autozero Level Type

If you do not require the use of a current range change during a chromatographic run, then a Level 1 Autozero should be used. This will keep the Autozero time to a minimum (see Table 2-4). Typically, a Level 1 Autozero would best serve most users that are analyzing for just one or a few compounds that are reasonably close in concentration (usually within an order of magnitude) to one another.

On the other hand if you need to make one or more changes to the current ranges during a chromatographic run, then a Level 2 Autozero should be used at the beginning of each run. This will minimize any baseline offsets between the current range changes to allow for proper quantification of your chromatogram.

A typical researcher that would need this is a user analyzing microdialysis samples. When analyzing Microdialysis samples, one may wish to simultaneously quantitate two (or more) analytes that are present in the sample at vastly different concentrations. Therefore, to properly quantitate the analytes in a single chromatographic run, a different current range would be desired for each analyte. The current range can be changed manually by the front panel, or in an automated manner by using Timeline or via a data station such as EZChrom *Elite* from ESA that has the capability of controlling the Coulochem III remotely via RS232.

2.7 The System Screens

2.7.1 Role of the System Screens

The SYSTEM MENU screens are used to edit a variety of parameters that relate to the overall use of the detector, rather than being related to a single method, and include the following screens:

- RS232 Setup Screen (Section 2.7.2)
- Deletion and Restoring of Methods (Section 2.7.3)
- Use of Event Marks (Section 2.7.4)
- Time and Date Screen (Section 2.7.5)
- List/Remote Screen (Section 2.7.6)
- Inputs Screen (Section 2.7.7)
- Contacts Status Screen (Section 2.7.8)
- Thermal Organizer Setup Screen (Section 2.7.9)

The SYSTEM SETUP menu (Figure 2-16) is accessed by selecting [SYSTEM] on the COULOCHEM MAIN MENU (Figure 2-3). When the menu is accessed, the RS232 screen with the highlight on the R of RS232 is presented, and other screens can be accessed via the ▲ or ▼ keys.

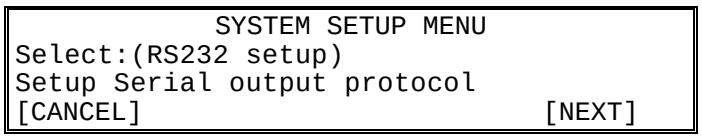

Figure 2-16: The System Setup Menu Screen

To edit the present area, press [NEXT].

2.7.2 RS232 Setup Screen

The RS232 SETUP screen (Figure 2-17) is used to indicate the parameters to be used to communicate with your personal computer. It is accessed by pressing [NEXT] on the SYSTEM SETUP MENU when the *Select area* field indicates RS232 setup. The values indicated in Figure 2-17 are the default values by ESA and should be changed to conform to the communication requirements of your computer and/or data station. You can set the default values via the [DEFAULT] key.

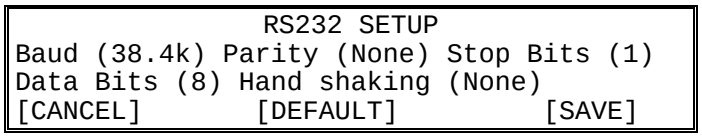

Figure 2-17: The RS232 Setup Screen

When the screen is accessed, the cursor will blink to the right of the *Baud* entry. Use the ▲ or ▼ keys to select the desired value (the system can transmit data at 4.8K, 9.6K, 19.2K, 38.4K, 56 K, 57.6K and 115.2 K baud). When the desired value is indicated, press ENTER.

The cursor will blink to the right of the *Parity* field. Use the ▲ or ▼ keys to select the desired value (None, Even or Odd). When the desired value is indicated, press ENTER.

The cursor will blink to the right of the *Stop Bits* entry. Use the ▲ or ▼ keys to select the desired value (1 or 2). When the desired value is indicated, press ENTER.

The cursor will blink to the right of the *Data Bits* entry. Use the ▲ or ▼ keys to select the desired value (7 or 8). When the desired value is indicated, press ENTER.

The cursor will blink to the right of the *Hand shaking* entry. Use the ▲ or ▼ keys to select the desired value (None or Hardware). When the desired value is indicated, press ENTER.

After you have completed entry of the RS232 parameters, press [SAVE] to save the entries.

NOTE: It is not necessary to scroll through the entire screen. Once you have edited the appropriate field, press [SAVE] to save the method.

2.7.3 Deleting and Restoring a Method

The DELETE/RESTORE METHODS screen (Figure 2-18) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
            SYSTEM SETUP MENU
Select:(Delete/Restore methods)
Del/Restore all user stored methods
[CANCEL]                          [NEXT]
```

Figure 2-18: The System Setup Menu Screen

When you press [NEXT], the DEL/RESTORE ALL USER ENTERED METHODS screen (Figure 2-19) is presented.

```
   DEL/RESTORE ALL USER ENTERED METHODS
RESTORE – RESTOREs methods from backup
DEL – DELETEs all user entered methods
[CANCEL]           [RESTORE]      [DEL]
```

Figure 2-19: The Del/Restore All User Entered Methods Screen

The [RESTORE] key will restore methods that have been deleted, provided that they have not been overwritten.

The [DEL] key deletes **all** user-entered methods except Method Zero. Method Zero contains the parameter values for the last method that was overwritten. See Section 2.4.7 for more information about Method Zero.

2.7.4 Event Marks

The EVENT MARKS screen (Figure 2-20) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
            SYSTEM SETUP MENU
Select:(Event Marks)
Sets Event Mark, Autozero & Height
[CANCEL]                          [NEXT]
```

Figure 2-20: The System Setup Menu Screen

When the [NEXT] key is pressed, the DC and PULSE EVENT MARKS screen (Figure 2-21) is presented.

```
         DC and PULSE EVENT MARKS
Mark autozero events (off)
Event width:(1.0)Sec.  Chan:(1) hgt:(5)%
[CANCEL]           [DEFAULT]    [SAVE]
```

Figure 2-21: The DC and Pulse Event Marks Screen

When the screen is accessed, the *Mark autozero events* entry will blink. Use the ▲ or ▼ keys to select the desired value (off or on). If *on* is selected, a hgt (height) field is presented adjacent to the *Mark autozero events* field. This new field is accessed via the ENTER key and is used to indicate the height of the Autozero event marker; the range is from -10 to +10%, and is selected by the keypad.

After the *Mark autozero events* field (and *hgt* field, if applicable) is edited, press ENTER to access the *Event width* field. Select the desired width of the event marker using the ▲ or ▼ keys (0.2 to 5.0 in 1-2-5 steps) and press ENTER. The *Chan* field will blink.

Edit the *Chan* field by using the ▲ or ▼ keys to select the desired value (1, 2 or 1+2), and press ENTER. The *hgt* field will blink and you can indicate the height of the event marker; the range is from -10 to +10%, and is selected by the keypad. Press ENTER to indicate that the value is desired. After completing the DC Pulse and Event Marks fields, press [SAVE] to save the entries.

2.7.5 Time & Date

The TIME & DATE SETUP screen (Figure 2-22) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
             SYSTEM SETUP MENU
Select:(Time & Date)
Set Time and Date
[CANCEL]                          [NEXT]
```

Figure 2-22: The System Setup Menu Screen

When the [NEXT] key is pressed, the TIME & DATE SETUP screen (Figure 2-23) is presented.

```
             TIME & DATE SETUP
Mo. {1} Day {7} Year {2002}
{PM}   Hour {10} Min {44}
[CANCEL]                          [SAVE]
```

Figure 2-23: The Time & Date Setup Screen

Enter the present date and time by accessing each field and pressing ENTER after the entry is made. Press the [SAVE] key to set the clock in motion at the correct time and date.

☞ NOTE: When the date is presented on the display during operation, it is presented via a 24-hour clock (e.g., 7:00 AM is presented as 7:00 and 7:00 PM is presented as 19:00).

2.7.6 List/Remote

The LIST/REMOTE SETUP screen (Figure 2-24) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
            SYSTEM SETUP MENU
Select:(Remote/List)
Set remote mode or list stored methods
[CANCEL]                        [NEXT]
```

Figure 2-24: The System Setup Menu Screen

Press the [NEXT] key to present the LIST REMOTE SCREEN (Figure 2-25).

```
                LIST/REMOTE
LIST – List stored methods to RS232
1 / 2 Select number of remote channels
[CANCEL]    [LIST]     [ONE]     [TWO]
```

Figure 2-25: The List/Remote Screen

The List command is used to send a list of the stored methods to a computer via the RS232 interface. If either of the above activities is desired, press the appropriate key.

If you pressed [LIST], you will be returned to the Main Menu after the method list is sent out through the RS232 port to your printer.

If you pressed [ONE] or [TWO], the COULOCHEM III Remote Screen, Figure 2-26, will be displayed and the front panel "REMOTE" LED will illuminate.

```
       COULOCHEM III REMOTE SCREEN
EXIT exits and returns to Main menu

[EXIT]
```

Figure 2-26: The Coulochem III Remote Screen

At this point the external device connected to the Coulochem III RS232 port should send the RUN DC command (rdc;0) to take over control of the detector in DC Mode. ELITE Data Station users should simply "open" the Coulochem III instrument within the Elite software to assume RS232 control of the detector. See the User's Guide for the Elite Data Station for more information.

Or you can press the [EXIT] softkey to exit from remote mode and return to the Main Menu.

2.7.7 Inputs

The INPUTS screen (Figure 2-27) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
          INPUTS ACTIVE/INACTIVE
On - Input low same as button Pushed
Autozero(ON)   Cell off (ON)
[CANCEL]                        [SAVE]
```

Figure 2-27: The Inputs Active/Inactive Screen

This screen is referring to two of the three contact closure inputs on the Logic Module Rear Panel cover. When Autozero is in the ON state as depicted in Figure 2-27, an Autozero event will occur if the AZ ± terminals on the I/O connector block are shorted by an external device. However, it may become useful during Timeline programming to pause at two or three times during a Timeline program to await return signals from external devices indicating that they have completed their appointed tasks. External devices may be autosamplers, column switchers, manual injectors, signal relays, etc.

Normally you will need only one such input and START is reserved for that purpose. But this screen allows you the option of redefining the Autozero and Cells Off inputs so that they may be used as the START input is. To do so you must toggle them to their (OFF) states using the ▲ or ▼ keys. Then press [SAVE] to effect the changes and you will be returned to the Main Menu.

If you do turn the Autozero and Cell On/Off states to OFF, then neither can an Autozero event be triggered nor can you disconnect the electrochemical cells via a contact closure in the I/O Connector block on the rear panel. Under normal circumstances these two devices are left ON in the System menu.

2.7.8 Contacts Status

The CONTACTS STATUS screen (Figure 2-28) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
            SYSTEM SETUP MENU
Select: (Contacts)
Set contacts and monitor inputs
[CANCEL]                        [NEXT]
```

Figure 2-28: The System Setup Menu Screen

Press the [NEXT] key to present the CONTACTS & INPUTS SCREEN (Figure 2-29).

```
            CONTACTS & INPUTS
C1(off) C2(off) C3(off) C4(off) C5(off)
Cell off:on  Autozero:on  Start:off
                                [RETURN]
```

Figure 2-29: The Contacts & Inputs Screen

The C1-C5 entries describe the status of the five contact closures. The status of each contact closure (on to off or vice versa) can be changed by pressing [ENTER], moving the cursor to the desired contact closure and pressing the ▲ or ▼ keys. The *Cell off and Autozero* entries describe the present status of the system and are set via the SYSTEM menu (Section 2.7.7). They cannot be edited at this point. *Start can never be edited.*

Changing the initial status of the contact closure outputs may become necessary as you connect them to other devices you wish to control using Timeline. Being able to change them manually within the system menu allows you to test your connections to whatever device you have connected.

2.7.9 Thermal Organizer Setup

The THERMAL ORGANIZER SETUP screen (Figure 2-30) can be accessed through the SYSTEM SETUP menu, and by pressing the ▲ or ▼ keys.

```
         THERMAL ORGANIZER SETUP
Temperature Control (Off)
Thermal Organizer is Off
[CANCEL]       [RESTORE]         [SAVE]
```

Figure 2-30: The Thermal Organizer Setup Screen

This screen is used to set the desired temperature if a Thermal Organizer Module is present. When this screen is accessed, the cursor appears in the field to the right of *Temperature Control* and the ▲ or ▼ key can be used to access a field to set the desired temperature (10-60^{o}C) using the numeric keypad. After you have entered the desired value, press ENTER, then press SAVE. The set temperature is stored in non-volatile memory, and is automatically recalled when the detector is powered up.

The field after *Thermal Organizer* indicates the present status of the Thermal Organizer (e.g., On, Off, Not Installed) and is used to indicate error messages (a discussion of error messages is presented in Chapter 8 of the *Coulochem III (50W) Reference Manual*).

⚠ CAUTION: Cells provided by ESA should not be heated above 45^{o}C. Using cells above this temperature could damage them.

☞ NOTE: For proper temperature stabilization, the set temperature must be at least 5^{o}C above ambient.

3 Operation of the Detector in a HPLC System

3.1 Introduction

The Coulochem® III electrochemical detector is used as an integral part of a High Performance Liquid Chromatography (HPLC) system. This chapter discusses the routine operation of the detector in an HPLC system and includes:

- Startup and shutdown procedures,
- Techniques to establish analytical parameters and
- Sample experiments.

3.2 Startup Procedures

☞ NOTE: A review of Warnings and Safety Precautions, Safety Operating Symbols and Operating Considerations are presented in page iii-vii. The user should review this information before powering up the detector.

When the instrument is powered up, several chromatographic and electrochemical equilibria are established. If the unit is turned off and powered up again, it may be necessary to wait until these equilibria have been re-established to obtain maximum performance. At high sensitivity levels (e.g., 50 nA or below), it can take several hours or longer to re-establish these equilibria. Factors that affect system equilibria are described in the Coulochem III (50W) Reference Manual.

If you wish to immediately begin analyzing samples, we suggest that you maintain the flow of the mobile phase and the electrode potentials at all times. This will minimize the time required for electrode stabilization.

⚠ CAUTION: The mobile phase must flow through the cell whenever potentials are applied to the cell (CELLS ON/OFF indicator light is lit). Failure to do so may cause permanent damage to the cells.

An additional benefit of maintaining the power to the control module is that the life of the internal battery (which protects stored methods) will be increased.

3.2.1 Routine Startup Procedures

This section describes a protocol that should be used when turning on an installed detector to ensure maximum performance. To start the system:

a) Establish the desired mobile phase flow before turning on the detector. After the detector is powered up, access and run the desired analytical method.

b) Monitor the baseline when the desired method is run. Wait until a quiet/flat baseline has been established. The time period that is required to obtain a quiet/flat baseline depends on the sensitivity desired. As mentioned earlier, a longer time will be required for situations where maximum sensitivity is desired. If an ion-pairing reagent is present in the mobile phase, overnight column equilibration may be needed to optimize sensitivity and resolution.

c) Autozero the detector and adjust the recorder zero. The detector is now ready for analytical measurements.

3.2.2 Maintaining the Detector Between Analyses

The ideal situation is to maintain the mobile phase flow and the applied electrode potentials at the level used for analysis when the detector is not being used. It is important to ensure that the mobile phase reservoir(s) contain sufficient solvent for the maintenance period. The pump "high pressure cut-off" should be set to approximately 35 bar (500 psi) above that observed with the normal flow rate to protect the system in case of a blockage. And if possible the detector rear panel "Cell Off" contact closures should be connected to the pump rear panel output that signifies that the pump has stopped pumping. This will prolong the life of your electrochemical cell.

Maintaining the analytical conditions for lengthy periods may be expensive in terms of solvent consumption and increases the wear and tear on the pumps. Two commonly used alternatives are:

- Maintain the flow at a lower flow rate (e.g., 0.1 or 0.2 mL/min). When the flow rate is at the desired operating level, monitor the baseline signal to ensure that it is stable before making an injection.

- Recycle the mobile phase by placing the outlet tube from the detector into the solvent reservoir. If this option is selected and the solvent is recycled for a long period of time (greater than one week), check the retention times and responses of the analytes as well as system pressure and background currents to ensure that the integrity of the mobile phase has been maintained (due to possibility of evaporation/contamination of the mobile phase).

Evaporation of the more volatile components of the mobile phase may change the dynamics of the separation. If solvent recycling is used, a minimum volume of 2L should be used. Oxidation (reduction) of trace components in the mobile phase may change the baseline or create products that might react with the compound(s) of interest.

3.3 Selecting the Analytical Potential

Selecting appropriate analytical parameters for an assay is critical for maximizing the performance of the Coulochem III detector. The following are important factors in the selection of the optimum potential for an assay via HPLC with electrochemical detection:

- A search of the literature will frequently provide suitable starting conditions for your compound or compounds with similar chemical characteristics.
- Sample cleanup steps are extremely important. It is easier to separate and detect a "clean" sample than one that has undergone only a minimum of sample preparation.
- Typically the chromatographic conditions need to be finalized before the final detector settings are determined. It should be noted that factors which affect the separation (e.g., the ionic strength and the % organic modifier can alter the electrochemical characteristics of the analyte.
- If the compound of interest can be baseline separated from other components of the mixture, detection and quantitation will be far more straightforward.
- The "ideal" mobile phase for separation and the "ideal" mobile phase for detection may be very different. It may be necessary to make a compromise to obtain an appropriate level of sensitivity and selectivity.

3.4 Obtaining the Appropriate Potential for an Analysis

The appropriate potential for an analysis is the potential that provides the largest signal for the oxidation (or reduction) of an analyte while minimizing signal from interferences (e.g., electroactive compounds that co-elute with the analyte or the mobile phase itself).

NOTE: In this discussion, we assume that the compound of interest is well separated from all other electroactive species. If this is not true, it may be helpful to use Screen Mode (Chapter 10, *Coulochem III (50W) Reference Manual*).

In most cases, a reasonably good estimate of the appropriate voltage is available. By this, we mean that a similar analytical procedure has been supplied by ESA, reported in the literature or obtained from a colleague. This "reasonably good" estimate will greatly simplify the process of optimizing the potential and the sensitivity

NOTE: The working potential reported in the literature may have been obtained using a different reference electrode (e.g., Ag/AgCl) than that employed with the coulometric cells used with the Coulochem III. If this is the case, it will be necessary to convert the potential to the reference electrode used with the Coulochem III. Data obtained using the Ag/AgCl electrode can be converted to data obtained with the Coulochem III at a pH of 3 by subtracting 300 mV. ESA's reference electrode is pH sensitive and the potential will increase by approximately +60 mV per pH unit increase.

If you have a good idea of the potential, several experimental approaches can be used to find the optimum potential:

- ***Bracket the Potential*** - In this approach, determine the peak current at a variety of potentials using a sample that is injected into the system (no column need be used). A fresh sample is used for each data point and the process continually narrows down the range until the optimum potential is determined (see Section 3.3.2).

- ***Collect a Current Voltage (CV) Curve*** - A CV curve can be generated by injecting the compound or compounds of interest and measuring their electrochemical response over a range of applied potentials. One approach is to use the Timeline mode of operation in which a series of chromatographic runs are performed at incremental cell potentials. Once the chromatograms are collected, the user can quickly make a plot of peak current(peak height or peak area) vs. potential for the compound of interest (as well as the background) and determine the optimum potential. An example of a CV curve, indicating where the background potential and the analytical potential should be set is presented in Figure 3-1. A detailed discussion of the collection of a CV curve is presented in Chapter 4, *Coulochem III (50W) Reference Manual* and in Section 9.4 actually presents a Timeline Method for creating CV curves.

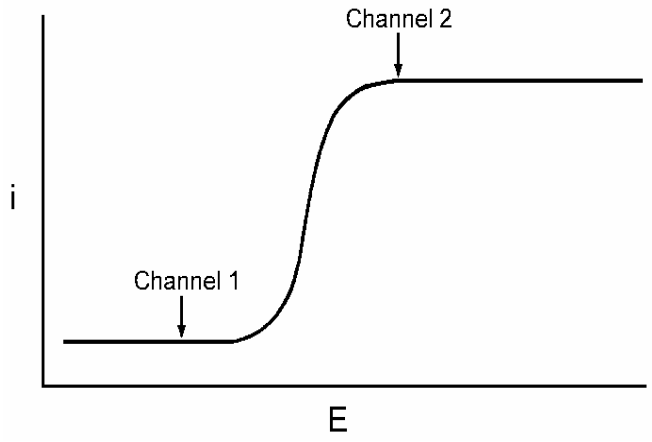

Figure 3-1: CV Curve with Selection of Background Potential (CH1) and Analytical Potential (CH2)

3.4.1 Bracketing the Potential Range

To determine the optimum potential, the following protocol can be used:

a) Remove the column and connect the injector to the in-line filter immediately preceding the flow cell using PEEK tubing.

b) Prepare a mobile phase consisting of 10% buffer (0.075 - 0.1M/ 90% Organic modifier (methanol or acetonitrile is suitable)) and pump this solvent through the system at a flow rate of 0.5 mL/min.

c) Set the potential for the first channel (or upstream electrode) to 500 mV, the potential for the second channel (downstream electrode) to 900 mV, set the current range to 10 µA and pump the mobile phase through the detector until a stable baseline is obtained (two recording channels or a data station are required to monitor both channels).

   While the Coulochem III can operate well above 900 mV, this value is suggested because most compounds can be readily oxidized below that potential. Furthermore, working at very high potentials may significantly shorten the functional lifetime of the electrodes.

NOTE: If the cell has only one electrode, set the potential to 900 mV.

d) Inject a solution which corresponds to 0.1-1.0 µg of analyte dissolved in the mobile phase. A suggested mobile phase for this purpose is ESA Test Mobile Phase (Part Number 70-3829), as this will provide a very low blank response. If the compound is oxidized under these conditions, a peak from either electrode 1 or 2 will appear very quickly (usually 1-10 seconds). If a peak is observed on either channel, inject a sample diluent blank and a mobile phase blank to ensure that the peak is due to the compound of interest and not due to any artifacts. The suggested current range for this experiment is between 100 nA and 10 µA.

☞ NOTE: It may be necessary to place a piece of tubing (20' x 0.005" ID) or a guard column between the injector and the detector to slightly retain the compound so that the peak can be observed.

e) If a peak from the compound of interest is observed, (i.e., it is not an artifact) reduce the potential and repeat the experiment. If a peak is still observed, reduce the potential to 250 mV and repeat the experiment. This procedure is repeated until you have bracketed the optimum potential (i.e., at 250 mV there is a signal and at 125 mV there is no signal).

The potentials that are used to bracket the optimum potential should provide peaks of essentially the same height. If the peak height from two potentials is different, compare the peak height of the higher one with the next potential that is used. For example, if the peak height is less at 250 mV than at 300 mV, compare the peak height at 350 mV. If the peak height is the same at 300 mV and 350 mV, that range should be used for further study. This range will be used to find the optimum potential as described in Section 3.4.

☞ NOTE: When the potential is changed, ensure that the baseline has restabilized before reinjecting the sample.

If a signal is not observed via the above protocol, the following steps can then be taken:

a) A negative potential could be used to determine if the compound can be reduced (rather than oxidized). Change the potential to -400 mV and repeat the protocol described above.

☞ NOTE: To change the potential to -400 mV, it is recommended that the potential be reduced by 100mV every 10 seconds or so until the desired reduction potential is reached. Allow 10-15 minutes for baseline equilibration to be reached before taking measurements.

b) A mobile phase with a different pH could be used, as it is possible that the compound exists in a form that does not exhibit electroactivity at the selected pH. As the pH is changed, ionic equilibria may be shifted so that a form of the compound that is electroactive might predominate.

NOTE: Changing the pH can have a significant impact on the separation and it is important to recognize that a compromise must be made between the optimum pH for separation and the optimum pH for detection. An acidic pH provides a reducing environment.

CAUTION: The pH should not be raised above the maximum pH recommended by the column manufacturer. Failure to do this may damage the column. If the separation requires a high pH, use of a polymer-based column may be a useful alternative.

c) Raise the potential to + 1100 mV and repeat steps c and d on the previous page. If a peak is found, reduce the potential to bracket the optimum potential.

NOTE: The use of potentials above 900 mV for prolonged periods of time may shorten the useful lifetime of the cell. At elevated potentials, the cell lifetime is a function of the potential and the generated current. If elevated potentials are used, ensure that the background current is very low (e.g., use very highly purified solvents and very low buffer concentrations).

3.4.2 If an Approximate Value for the Potential is not Known

In some cases, the analyst does not have an approximate value for the oxidation (reduction) potential for the compound of interest (e.g., the compound of interest is new or poorly characterized). In this case, the analyst may employ Cyclic Voltammetry to obtain a complete description of the electrochemical properties of the compound and determine a starting potential using various buffers, pH and electrodes.

3.5 Maximizing the Performance of the Assay

Once the appropriate chromatographic conditions and the potential for the compound of interest have been determined, a variety of steps can be used to optimize the performance of the assay.

☞ NOTE: The use of potentials above 900 mV for extended periods of time will shorten the life of the coulometric cells. If the oxidation potential for the compound of interest is significantly above 900 mV, it may be desirable to consider a different mode of detection (e.g., redox mode) or suitable derivative formation.

3.5.1 Minimizing the Background Noise

The techniques described in Section 3.3 provide the optimum potential for the oxidation (reduction) of the compound of interest. Typically, this potential is used for analysis, as the signal will be maximized. It should be noted, however, that the limit of detection for an assay (and the ability to detect the lowest possible concentration) is a function of both the signal and the noise. With coulometric electrodes the signal may already be maximized. In this case the characteristics that produce noise should be minimized so that the maximum sensitivity can be obtained.

It is important to recognize that the background noise increases as the applied potential is increased. This increase may reduce the signal-to-noise ratio and hence the sensitivity of the assay.

There are a variety of possible reasons for this:

- Water, buffers and other salts may be oxidized. The analyst should ascertain the oxidation potential for each of the components of the mobile phase (cyclic voltammetry or hydrodynamic voltammetry) and ensure that the operating potential is below this value. The practical limit is 100 mV below the oxidation potential of the most easily oxidized component of the mobile phase; this should be obtained experimentally.

- Dissolved oxygen can be reduced at potentials more negative than -400 mV. Typically, oxygen can be removed via sparging or degassing techniques. If all/most of the oxygen is removed, the background current will remain low until the reduction of a major component of the mobile phase begins.

- Impurities in the mobile phase or mobile phase modifiers may be oxidized (reduced). A detailed discussion of how to maximize the purity of the mobile phase is presented in Chapter 4. A Guard Cell located between the pump(s) and the injector may lower the contribution of these impurities.

- Materials that are strongly retained on the column may be slowly eluted (especially if a change in the composition of the mobile phase is effected).

- Corrosion sites on metallic components of the system may contribute to higher noise and loss of signal.

There are a number of steps that can be taken to minimize the background noise:

- The first electrode can be used as a screen cell to oxidize (reduce) materials in the mobile phase that have a smaller oxidation (reduction) potential. Screen mode operation is described in Chapter 10, *Coulochem III (50W) Reference Manual*.

  Typically, the working range of carbon or graphite electrodes is from +1.0V to -1.0V. This range can usually be extended by using the first electrode as a screen electrode to reduce the background noise by oxidizing (or reducing) components of the mobile phase that cause the background noise.

- Place an ESA Model 5020 Guard Cell (Part Number 55-0417) between the solvent delivery system and the injector. The potential of the Guard Cell should be set high enough to oxidize (reduce) the impurities. The Guard Cell potential can be set higher than the analytical potential (usually 25-50 mV higher).

- Use mobile phases containing water, solvents and modifying reagents of the highest purity available.

- Take special care in removal of oxygen from the mobile phase (e.g., continue to sparge the solvent container during the operation of the HPLC), if operating at extremely high/low potentials is necessary.

- Avoid drastic changes in mobile phase composition and dedicate a column to a specific analysis. This will avoid the contamination of the column by materials that are poorly eluted by one mobile phase, but better eluted by a different mobile phase.

Typically, background currents are in the range of 10-50 μA immediately after the potential is applied to the working electrode. This initially high background is a result of capacitative effects after a change in the applied potential is made. As the applied potential is increased, the background currents generated will also increase, as more and more of the materials in solution are oxidized (or reduced).

3.5.2 Selection of the Proper Output Range

Ensure that the output of the desired channel agrees with the input of the recording device being used.

Select the current range to ensure that the peak is on scale. This is usually done by experience and/or experimentation (typically, the scale is set so that the largest peak anticipated is 50-75% full scale).

3.5.3 Setting the Filter

The filter time constant is used to electronically reduce the noise in the chromatogram. A small filter value (e.g., 0.2 sec) removes little noise, while a large value (e.g., 10 sec) will perform a significant amount of smoothing. Although a large value for the filter presents a very smooth chromatogram, the analyst might be concerned about the possibility that small peaks might be eliminated in the smoothing process. On the other hand, use of a very small filter leads to a very noisy chromatogram.

As a general rule of thumb, the filter should be set to a value that is 1/4th to 1/16th the base width of the narrowest peak (in seconds) to achieve no peak reduction. Higher filter times can be used. The user should note that as long as standards and samples are all run under the same conditions, the selection of the filter value will not affect analytical results, so long as the peak is clearly observable. If the peaks of interest are very sharp and occur soon after injection, a short filter time (e.g., 2 sec) is advised. A filter of 5 sec is sufficient for most applications. A large filter time constant may attenuate the peak height, especially if the peak width is small. One can also vary the filter during a chromatographic run using Timeline - see Chapter 9 of the *Coulochem III (50W) Reference Manual*. Use smaller filter times for the first few minutes of a chromatographic run and then larger filter times later in the run.

3.6 An Experiment to Ensure that the Chromatograph and the Detector are Functioning Properly

☞ NOTE: Instrument Validation Services (Installation, Operational and Performance Qualifications) are available from ESA. Please call your local ESA representative for information.

The experiment described below, which involves the oxidation of Hydroquinone (HQ), is included so that the analyst can perform an analysis with electrochemical detection using a well-defined system. It might be useful to perform this experiment prior to developing separation/detection parameters for the specific needs of the laboratory.

1) Materials Needed:

   - a reverse phase (C_{18}) column (100 mm x 4.6 mm ID, 5 µm)
   - a mobile phase consisting of 85/15 (v/v) acetate buffer (100 mM, pH = 4.8)/methanol
   - a HQ standard which is prepared as follows:

     a) Dissolve 100 mg of HQ (Sigma H9003) in 100 mL filtered HPLC grade methanol.

     b) Take 1.0 mL of the above solution and dilute in 100 mL of the mobile phase. The resulting HQ concentration is 10 µg/mL.

2) Procedure:

   a) Set the pump flow rate to 1.0 mL/min and allow the mobile phase to equilibrate.

   b) On the detector, set the potential to 500 mV, and monitor the current using a current range of 10 µA and a filter of 5 sec. If you want to monitor both the oxidative and the reductive currents, set the potential on E2 to -300 mV and the output to -1.0 V.

   c) When the current is fairly constant, turn on the data-recording device, and press the Autozero key.

   d) Monitor the current for approximately 30 minutes. The detector current should decay to a lower steady-state value that is fairly noise-free.

   e) Inject a 10 µL sample of the test solution. With these analytical conditions, the value of k' for hydroquinone is approximately 1. The hydroquinone will elute as a sharp peak that is at least 50% full scale. The retention time will vary depending on the source of the column.

   If you are using a two-channel system, you will see two peaks. The reduction signal may be smaller than the oxidation signal (reduction signal may be as small as 60% of oxidation signal).

3) Discussion:

At 500 mV, HQ will be oxidized to form quinone (Q). If the second potentiostat is used, the cell set to -300 mV will reduce Q back to HQ. These processes are summarized below:

Hydroquinone $\rightarrow$ Quinone + $2e^-$ + $2H^+$ at 0.5 V

Quinone + $2H^+$ + $2e^-$ $\rightarrow$ Hydroquinone at -0.3 V

This experiment can also be performed using hydroquinone sulfonic acid (Sigma H7272). In this case, the peak height(s) will be smaller (because of the higher molecular weight) and the value of k' will be approximately 0.3.

Q and HQ absorb at 254 nm. If desired, an absorbance detector can be used to verify that these two compounds are indeed eluting from the column.

4 Mobile Phase Considerations

4.1 Introduction

The mobile phase used to separate the compounds of interest has a critical impact on the overall success of the analysis. A careful consideration of the selection of the components of the mobile phase and the generation of the mobile phase will be extremely useful in minimizing baseline noise and optimizing the development and execution of an analytical protocol. This chapter discusses the following:

- Generation of a mobile phase for a well defined analytical protocol (Section 4.2).
- Monitoring the baseline and reducing baseline noise (Section 4.3).
- Considerations for developing the mobile phase for a new analytical protocol (Section 4.4).

4.2 General Guidelines for the Preparation and Use of the Mobile Phase

4.2.1 Overview

In most cases, the use of HPLC with electrochemical separation typically involves the use of a separation that is well described in the literature or requires slight modification of an existing separation (e.g., neurotransmitters can be readily analyzed by the use of an ESA MD-150 Column (Part Number 70-0636) and the ESA MD-TM Mobile Phase (Part Number 70-1332). If the analyst wanted to add the analysis of a compound that was similar to the compounds that were detected by the above analytical method, it is extremely likely that little or no changes in the mobile phase would be required to allow for analysis of the neurotransmitters along with the new compound.

This section describes a series of guidelines to assist the analyst in minimizing impurities in the mobile phase in order to optimize the sensitivity and overall performance of the analytical system.

NOTE: ESA supplies several mobile phases for a variety of commonly performed applications. Please contact ESA (or your authorized distributor) for details.

4.2.2 The Impact of Impurities in the Mobile Phase

Since electrochemical detection can frequently provide significantly better sensitivity and selectivity than other methods of detection (e.g., absorbance or fluorescence techniques), it can also "detect" trace levels of impurities that may be transparent when other detectors are used. The time that is invested in ensuring that the mobile phase contains a minimum of electroactive impurities will be significantly repaid by the ability to perform more sensitive analyses. In this regard, a healthy degree of skepticism about the purity of solvents and other components of the mobile phase may be extremely useful. We note that many of the points presented in this section are good chromatographic practices that are relevant to all HPLC separations.

The following guidelines should be used to maximize the sensitivity of the system:

- Always use the highest purity reagents and solvents that are available. Water that has been passed through a properly maintained water purification system followed by a C_{18} solid phase extraction system is likely to suitable for electrochemical measurements (see Section 4.2.4).

- Carefully prepare the mobile phase in clean glassware. Do not use soap to clean glassware. Use dedicated glassware to prepare the mobile phase.

- Ensure that the chromatographic system (e.g., columns, injector, tubing, filter, pump, etc.) does not contaminate the mobile phase. Minimize the number of stainless steel components in the HPLC system. ESA has a full range of inert HPLC components available.

- Always use a guard cell and in-line filters.

- Always filter the aqueous portion of the mobile phase before use through a 0.22 μm membrane filter.

- Once a given mobile phase has been found acceptable, make as few changes as possible in its preparation and use (e.g., try to use the same brand of reagents/solvents). It may be very worthwhile to check that the level of cleanliness of each lot of the mobile phase is acceptable before it is used for analytical work.

4.2.3 Organic Solvents

The highest quality organic solvents available should be used (these are normally marketed as "HPLC Grade"). In many analyses, acetonitrile or methanol are commonly used for HPLC with electrochemical detection and alcohols such as ethanol (not denatured), propanols and hexanols are occasionally used.

Many organic solvents are subject to slow oxidation after the bottle is opened (e.g., tetrahydrofuran will form peroxides in the presence of air). If the solvent is subject to oxidation, the background current will increase as a function of time and a freshly opened bottle of solvent should be used for the most sensitive work. As a precaution, it may be worthwhile to place a blanket of an inert gas (e.g., nitrogen or helium) over an oxidizable solvent once the bottle has been opened.

All solvents should be filtered through a 0.22 µm Nylon or PVDF membrane filter before use, unless they are labeled HPLC grade. In this case, it is likely that the manufacturer has filtered the solvents. Check with the filter manufacturer to ensure that the filter is compatible with the solvent(s) of interest.

4.2.4 Water

In many HPLC separations with electrochemical detection, water is typically the predominant component of the mobile phase. Therefore, it is especially critical to use the highest purity water available, since even ppm levels of impurities will have a dramatic impact on system noise.

Water may contain both ionic and organic impurities. The removal of trace organics for water is important because they can accumulate on a reverse phase column and result in both increased background currents and the appearance of ghost peaks eluting from the column. An additional concern is the potential presence of microorganisms, which can lead to the growth of particulate matter that may clog the column and/or the electrochemical cells.

NOTE: A detailed discussion of purification of water is presented in ESA Technical Note 70-1668P, which is included in this manual as Appendix B.

ESA strongly recommends the following procedure to purify water for use in HPLC with electrochemical detection:

a) Pass the purified water through a commercial purification system that can produce water with a resistivity of 18.2 megohm-cm. This will remove the ionic species from the water, as well as most of the organic species.

b) Pass the water through a system that includes a C_{18} solid phase extraction column (Figure 4-1). The first 100 mL that passes through the system should be discarded. Up to 2 L of water can be purified with a single disposable cartridge.

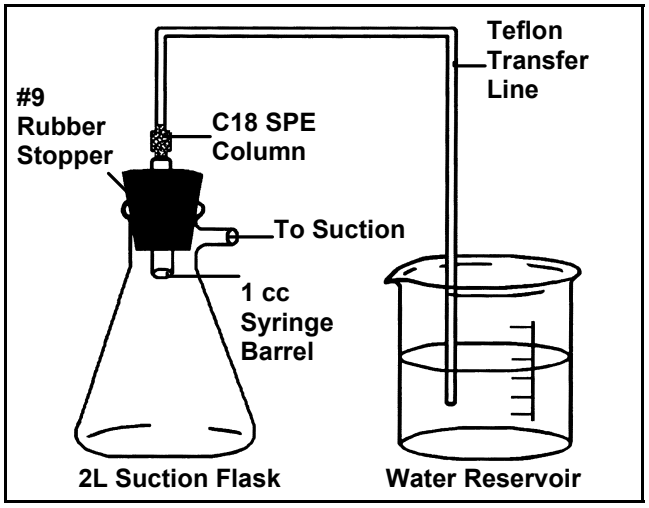

Figure 4-1: Water Polishing System

This water, which is termed "polished water" reduces the electrode background currents and provides better sensitivity.

NOTE: Commercially bottled HPLC grade water is an acceptable (albeit expensive) alternative to the use of a commercial purification system. Once a bottle of water has been opened, it should be used up within a day. The remainder should be discarded since bacterial growth, which can clog columns, filters, and electrochemical cells can occur after a day.

4.2.5 Reagents for the Preparation of Aqueous Solutions

A broad range of acids, bases, ion-pairing reagents and salts are commonly used in HPLC. Careful selection of the acids, bases, ion pairing reagents and salts used to generate the mobile phase will serve to reduce the baseline noise and maximize sensitivity. It is important to use the highest purity materials available for the preparation of the mobile phase. Normally, the concentration of buffers is kept between 50 to 100 mM to minimize the background current and baseline drift while maintaining a constant pH value.

These reagents are available from a wide variety of suppliers and the analyst should carefully review the assay information to ensure that electroactive materials are absent. It should be noted that the utility of a given reagent is a function of the applied potential that will be used for the analysis. For an example of this point, a source of sodium acetate may be perfectly acceptable for an analysis using a potential of 250 mV but may create very significant background noise at a potential of 700 mV due to an impurity that is electroactive at 600 mV. While it is very difficult to make a general set of recommendations, a few suggestions that have proven to be very effective in minimizing the background currents, thus providing the ability to perform high sensitivity analyses include:

- If a reagent from a specific manufacturer appears to provide a suitable degree of sensitivity, continue to use it.

- If a reagent has been successfully used in the past and a fresh bottle appears to create difficulties, check the lot number of the two bottles. It may be that there has been a change in the preparative and/or purification process and you should contact the manufacturer.

- If is necessary to change suppliers for a given reagent, do not assume that the two reagents are equivalent (even if they are the same grade). Run analyses with the two to verify equivalency.

4.2.6 Preparation of the Mobile Phase

To prepare the Mobile Phase:

- Always use freshly polished water to prepare the mobile phase (see ESA Technical Note 70-1668P, included as Appendix B).

- Use reagents of the highest purity to prepare the mobile phase. In many cases, impurities such as heavy metals (especially redox active transition metals such as iron) can influence the background current at the electrochemical sensor. This will create noise that will compromise the detection limit. Two criteria should be considered; the overall purity and the level of heavy metal impurities. In addition, trace metal ions can sometimes interact with the electroactive species before the species reach the electrochemical sensor.

- If a reagent is not available in a sufficiently high degree of purity, prepare the solution by other means. For example, if a solution prepared by dissolving analytical reagent grade sodium acetate trihydrate in water presents an unacceptably high background, this mobile phase can be prepared by adding aldehyde free acetic acid to water and adjusting the pH to 4.8 with concentrated NaOH using semiconductor grade NaOH (99.99+% pure).

- Whenever possible, try to use mobile phases that contain the more commonly used salts (e.g., acetate, phosphate) rather than salts with more exotic counterions (e.g., chloroacetate). The popularity of the more commonly used buffers is due to the positive experiences that have been obtained with the corresponding salts over the years. It is likely that the more popular buffer salts are more stable, are purified more thoroughly and are manufactured on a more consistent basis than more exotic buffer salts.

- A buffer system should be used at a pH near the pKa for the buffer. The maximum buffering capability takes place when the pH is within ±1 pH. Buffering ranges for commonly used salts s is presented in Table 4-1.

Table 4-1: Common Buffers for Electrochemical Detection

Buffer	pKa	Useful Buffer Range (pH)
Acetate	4.8	3.8-5.8
Citrate	3.1	2.1-4.1
	4.7	3.7-5.7
	5.4	4.4-6.4
Phosphate	2.1	1.1-3.1
	7.2	6.2-8.2
	12.3	11.3-13.3

- The electrolyte concentration should be in the order of 50-100 mM to provide a suitable level of electrolyte in solution. If the concentration is significantly higher; a more stable response and a better baseline may be obtained. In some cases, however, a higher concentration may lead to an excessive level of electroactive impurities. For some applications that require very high electrode potentials, the electrolyte concentration can be reduced to 15-25 mM.

- Phosphate buffers often produce a lower background current at high electrode potentials than other buffers (e.g., acetate). It should be noted that phosphates have a limited solubility in mobile phases with a high organic content (especially acetonitrile). If solubility issues arise, phosphate buffers generated from smaller cations (e.g., Li) are preferable to those with larger cations (e.g., Na or K).

- If very high levels of organic modifier are required in the mobile phase, Lithium Acetate, Lithium Perchlorate or Ammonium Acetate may prove useful.

- Some buffers, such as EDTA and Triethylamine are especially difficult to use at higher electrode potentials since they contribute to high background currents. As an example, EDTA will start to oxidize at 400 mV vs. Pd.

- Use a mobile phase within a short time after it has been prepared. Bacterial growth, evaporation of volatile organic solvents (e.g., methanol) and other effects can lead to changes in the composition of the mobile phase. If mobile phases are stored, they should be kept at 4-10°C in sealed containers. Always allow the mobile phase to come to room temperature and degas it before using. In general, a mobile phase should be used within one week after it has been prepared.

- Always filter the aqueous portion of the mobile phase through a 0.22 μm membrane filter before use.

- If a gradient is used, maintain the ionic strength of the mobile phase as the composition of the mobile phase is changed. It should be noted that this might lead to solubility issues at high organic buffers.

- If ion-pairing reagents are used with a gradient, ensure that a sufficient period of time for loading of the ion-pairing reagent on the stationary phase before the next injection. This should be done because the change in the composition of the mobile phase frequently will change the loading of the ion-pairing reagent on the column.

If you have difficulties with a separation, contact ESA (or its representative) for assistance. It is not unlikely that others have had similar problems. The application group will make suggestions to reduce these problems.

NOTE: ESA manufactures a number of mobile phases, including mobile phase for the analysis of catecholamines in plasma (Cat-A-Phase II, Part Number 45-0216) and microdialysis/tissue samples (MD-TM, Part Number 70-1332).

4.2.7 Removal of Dissolved Gases from the Mobile Phase

In HPLC, the mobile phase should be degassed to ensure that bubbles do not form in the system. When an electrochemical detector is used, removal of oxygen is especially important since oxygen could oxidize the compound(s) of interest. In addition, the presence of oxygen may lead to a significant background if the potential is more positive than 750 mV or more negative than -400 mV.

A variety of techniques can be used to remove dissolved gases from the mobile phase:

- Sparge with helium by continuously bubbling a *light* stream through the mobile phase with a solvent inlet filter.
- Use a mild vacuum while the flask containing the mobile phase is stirred with a magnetic stirrer.
- Use a mild vacuum while the flask containing the mobile phase is in an ultrasonic bath.
- Use a Mobile Phase Vacuum Degassing Unit (Part Numbers 70-1482 and 70-1493); this unit works as soon as it is turned on.

Typically 10 to 15 minutes of degassing using any of the above procedures is sufficient. After the mobile phase is degassed, a slow flow of helium should be passed through the mobile phase to maintain the absence of dissolved gas.

WARNING: Putting a vacuum on a glass container could result in an implosion. The flying glass from the implosion could cause personal injury.

When working at high sensitivity, degassing/sparging of the mobile phase can result in the presence of an "air peak". This is caused when a solution that is in equilibrium with the atmosphere and is thus saturated with air is injected into a mobile phase that is void of oxygen/air, outgassing at the analytical electrode can (and does) occur.

The signature of the air peak appears as a sinusoidal shaped peak that rises above and then drops below the baseline. The retention time of this peak is constant from injection to injection, and is dependent on the column length, as well as the flow rate and composition of the mobile phase. To confirm the identity of the air peak, simply inject an aliquot of air. If the suspected peak increases in size, it is an air peak. The presence of this peak is not harmful or detrimental to the analytical cell; however, it may interfere/co-elute with an analyte of interest. In this case, we suggest that the user stops degassing/sparging, prepares fresh mobile phase and verifies that the air peak is no longer present.

NOTE: If a sparging or a vacuum degassing technique is used with an isocratic system, the user should ensure that volatile components of the mobile phase do not evaporate, as that could change the chromatographic separation.

4.2.8 Additional Considerations when a Gradient Mobile Phase is Employed

When a gradient is generated, the fraction of the organic component of the mobile phase is usually increased as a function of time (in a few cases, the gradient is a pH gradient). The analyst should ensure that the increase in the organic composition does not lead to precipitation of buffer salts or the sample from the aqueous phase before using it in the system. This can be easily determined by mixing the buffer and the maximum organic phase in a beaker and observing if cloudiness appears. A similar test should be performed with the sample and the mobile phase if a pH gradient is employed (both pH extremes should be checked).

4.2.9 Minimizing Contamination of the Mobile Phase by the HPLC System

The HPLC system can be a significant cause of contamination, which can lead to background noise. Some conditions that can be traced to the HPLC system include:

- ***Column Bleed*** - If the column is used for a variety of analyses, it is possible that materials which are strongly retained with a given mobile phase will elute when the composition of the mobile phase is changed.

To determine if the column is slowly eluting retained compounds from previous analyses, deliver a mobile phase with a high organic content for several hours and monitor the current at the potential that is normally used for the analysis. The current will slowly fall if retained compounds are being eluted. A useful mobile phase for this purpose is 90/10 (v/v) methanol/water with an appropriate electrolyte such as ammonium acetate or lithium acetate. If desired, acetonitrile could be used instead of methanol for this purpose (when acetonitrile is used, take care to ensure that salts do not precipitate).

A simple way to minimize this problem is to use a dedicated column for the analysis. If this is not possible, column wash techniques should be used between different forms of analyses.

- ***Adsorbtion of Oxygen*** - If Teflon tubing is used oxygen can be adsorbed through the tubing and dissolve in the mobile phase. To avoid this phenomenon, use passivated stainless steel or PEEK tubing to connect components of the chromatographic system.

- ***Leaching of metallic ions*** - Leaching of metallic ions from the pump and stainless steel tubing can be a significant cause of background noise. This is normally caused by fairly acidic mobile phases. If this occurs, the system should be repassivated (see Chapter 3, *Coulochem III (50W) Reference Manual* for details).

4.3 Minimizing the Background Signal

4.3.1 Measuring the Background Current due to the Mobile Phase

While the steps described above should minimize the background current, the background current will typically be in the range of 10-50 µA immediately after the potential is applied to the working electrode. This current is primarily a result of the capacitive effects after a change in the applied potential. These currents will fall rapidly as the electrodes stabilize.

From a practical point of view, the purity of the mobile phase required for a given assay is dependent on the level of sensitivity that is desired for a given assay. If, for example, a minimum signal-to-noise ratio of 2/1 is desired for a given assay, the background current should be no more than 5% of the current from the most dilute sample that is to be measured.

☞ NOTE: If a higher potential is used, the resulting higher background current may shorten the useful lifetime of the electrode.

4.3.2 Adjusting the Potential to Reduce the Background Current

In some circumstances, the analyst may find that extreme efforts in the purification of the mobile phase are unsuccessful in obtaining the desired background level. In such cases, it may be worthwhile to reduce the electrochemical potential slightly. Reducing the potential will reduce the signal of the compound of interest as well as reducing the background current. If the background current is reduced to a greater extent than the reduction on the signal from the compound of interest, the signal-to-noise ratio (and hence the sensitivity) should increase.

NOTE: In general noise is a function of the background current. Typically, reducing the current will also reduce the noise.

4.3.3 Using a Guard Cell to Reduce the Background Current

An ESA Model 5020 Guard Cell (Part Number 55-0417) can be placed between the solvent delivery system and the injector to oxidize (reduce) any electroactive components of the mobile phase.

4.3.4 The Effect of High Electrode Potentials on the Mobile Phase

The background noise will increase at higher potentials (either oxidative or reductive) because more trace contaminants are being oxidized (or reduced). In addition, there are a number of special situations that should be considered:

- As the potential increases, water, buffers and other salts may themselves become oxidized (or reduced) and the background current will increase. The analyst should ascertain the oxidation (or reduction) potential for each of the components of the mobile phase and ensure that the operating potential is below this value. The practical limit is 50 mV below the oxidation potential of the most easily oxidized component of the mobile phase.

- At applied potentials more negative than -400 mV, reduction of dissolved oxygen can occur and as a result, the background current will increase. Typically, oxygen is removed from the mobile phase via the degassing techniques discussed in Section 4.2.7. If the degassing techniques have removed all of the oxygen, the background current will remain low until the reduction of a major component of the mobile phase begins.

The background noise will frequently be larger at high potentials (either oxidative or reductive) because more trace contaminants can be oxidized (or reduced) than at lower potentials.

4.4 Selecting the Appropriate Mobile Phase for HPLC with Electrochemical Detection

From time to time, the analyst may be required to develop a new analytical protocol and a closely related protocol is not readily available. By this we mean that it is not possible to simply adapt an assay from the literature and the analyst must select the appropriate stationary phase and mobile phase. When it is necessary to develop a separation, it is extremely important that the analyst obtain chromatographic data as well as electrochemical data about the various compounds in the sample as well as in the proposed mobile phase.

In most cases, it is better to treat the development of the assay as an integrated procedure, rather than first developing and perfecting a separation and then developing the detection procedure. If the latter approach is taken, it is quite possible that a mobile phase that provides a superb separation will not be acceptable in the detection process. As an example of this point, if the minimum potential required to detect the various compounds in the sample is, say 500 mV, the buffer cannot contain species that are electroactive at that potential. A potential difference of 100 mV between the compound of interest and components in the buffer should be maintained, if possible.

While many of the overall criteria are the same as when other detection techniques are employed, the following specific points should be considered when developing a separation.

1) The oxidation (reduction) potential of each compound in the sample (or compounds of interest that might rationally be expected to be in the sample).

2) The oxidation (reduction) potential of each component of the mobile phase.

3) The stability of the mobile phase over a period of time (i.e., will a component of the mobile phase be converted to an electroactive species over a period of time).

The electroactivity of the various compounds in the sample and the mobile phase may likely be obtained from the literature; otherwise the information can be obtained by a hydrodynamic voltammogram (see Chapter 3, *Coulochem III (50W) Reference Manual*).

Appendix A Coulochem® III Electrochemical Detector Specifications

General Operating Specifications

Detector Configuration:	DC potentiostat for 1 or 2 cells and Potentiostat for Guard Cell; and/or Scan Mode/Pulse Mode
Operating Modes:	DC, Pulse, Scan - depends on options installed (Timeline can be attached to DC and Pulse methods)
Potential Range:	± 2000 mV in 1 mV steps
Full Scale Output Range:	10 pA to 1 mA in 1-2-5 sequence (DC Mode)
Filter Time Constants (DC Only):	0.2 to 10 seconds in 1-2-5 sequence (DC mode)
Noise Specification:	< 750 fA peak to peak with a 500 MΩ, 0.47 µF test load and a 2 sec filter (DC Mode)
Signal Output:	± 100 mV, ± 1 V D.C.
Output Resolution DC Mode:	0.12 µV at 1 V full scale (24 bit bipolar)
Output Resolution Pulse/Scan Mode:	1.9 µV at 1 V full scale (20 bit bipolar)
Output Offset:	± 50% of the selected current range in 1% steps
Guard Cell Potential and Current:	± 2000 mV in 1 mV steps, ± 2 mA maximum current
Event Marker:	Triggered by keypad, timed operation or RS232 control, width, height, polarity and channel selectable
Autozero:	Triggered by front panel keypad, rear panel contact closure, timed operation or RS232 control
Function Keys:	Autozero, Event Marker, Cell On/Off, Run/Stop
RS232 Interface Capability:	Full parametric instrument control for DC and Pulse

Method Storage:	Up to 25 methods (any combination of DC, Scan and Pulse with Timeline)
Temperature Range: (Thermal Organizer Option)	Ambient +5° to 60°C
Temperature Stability: (Thermal Organizer Option)	< ± 0.5°C
Warm-up Time: (Thermal Organizer Option)	< 30 minutes (typically)
Temperature Accuracy: (Thermal Organizer Option)	≤ ± 0.5°C

Scan Mode Specifications

Scan Points:	4 selectable potentials: Initial Potential, Potl Limit 1, Potl Limit 2 and Final Potential
Current Ranges:	1 nA to 10 mA in 1-2-5 step sequence
Recorder Y-axis Outputs:	± 100 mV, ± 1 V
Scan Rate:	1-1000 mV/Sec in 1 mV increments
Scan Cycle:	1 to 9999 or continuous cycle
X-Axis Divisor:	1, 2, 5, 10

Pulse Mode Specifications

Pulse Times:	Time 1: Acquisition Delay +5 to 1000 msec Time 2: 4 to 1000 msec Time 3: 0 to 1000 msec Time 4: 0 to 1000 msec
Acquisition Delay:	50 msec to Time 1-5 msec
Pulse Voltage Range:	± 2000 mV each pulse in 1 mV increments
Charge Ranges:	10 pC to 10 mC in 1-2-5 step sequence
Recorder Outputs:	± 100 mV, ± 1 V

Timeline Specifications (DC and Pulse Mode)

Programmable Changes:	Selectable per channel at times from 0 to 9999.99 minutes in 0.01 minute increments
Programmable Events:	Current Ranges, Cell Potentials, Increment Cell Potentials, Five Output Contact Closures, Program Hold, Autozero, Event Mark, Parameter Reset, Program Loop Command, Program End Command, Filters, and Cell Off/On
Program Repeats:	1 to 9999 using Loop command
Program Start:	Via front panel keypad or rear panel contact closure
External Device Control:	Control of up to 5 external devices via contact closures

Physical Specifications

Power:	100-240 VAC, 50/60 Hz, 80 VA (with Thermal Organizer)
Dimensions:	
Coulochem III:	19.5" (L) x 9" (W) x 11.25" (H) (49.5 cm x 23 cm x 28.6 cm)
Organizer Module:	18.5" (L) x 9" (W) x 5.3" (H) (47 cm x 23 cm x 13.5 cm)
Weights:	
Coulochem III:	14.8 lbs (6.7 kg)
Organizer Module:	7.6 lbs (3.4 kg)
Thermal Organizer Module:	9.1 lbs (4.0 kg)

Environmental

Operating Temperature:	10-35°C
Humidity:	Maximum 80% RH (35°C), non-condensing
Storage Temperature:	-10 to 60°C

Specifications are subject to change without notice.

Certifications

The Coulochem III Electrochemical Detector has the following Laboratory Equipment certifications:

USA:	UL 61010A-1 1st Edition
Canada:	CSA Standard C22.2 No. 1010.1-92
European Union:	EN 61326:1997 + A1:1998 EN 61010-1 (2001-02)

Appendix B Water Quality

Water is typically the most abundant chemical in reverse phase chromatography mobile phases, therefore ultra pure water is an essential ingredient for any LCEC experiment. Commercial water systems that produce 18 megohm-cm quality water indicate that there are no ions present in the water; however, the quantity of trace organic material is often unknown.

The growth of bacteria within the water system can affect the final quality of the water due to its contamination with trace organics. These trace organics can accumulate on a reverse phase column and result in both increased background currents as well as the appearance of ghost peaks eluting from the column. Trace organics can be easily removed from the water by initially passing the water through a C_{18} solid phase extraction column (e.g., Waters® Sep-Pak® C_{18}) as shown in the illustration.

Water Polishing Technique

First pass about 100 mL of water through the cartridge to rinse the tubing, cartridge and suction flask. Then collect up to 2 L of polished water before discarding the cartridge. Use this polished water for the preparation of the mobile phase and any analytical standards or solutions. This treatment of water should help reduce the electrode background currents and thus provide greater sensitivity due to a cleaner mobile phase.

NOTE: Only water should be passed through the cartridge since other chemicals in the mobile phase may bind to the solid phase extraction cartridge and change the final composition of the mobile phase.

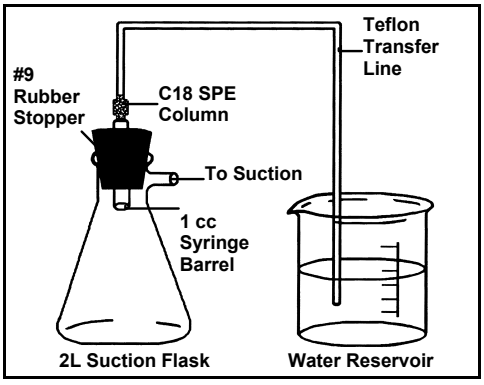

Figure B-1: Water Polishing System

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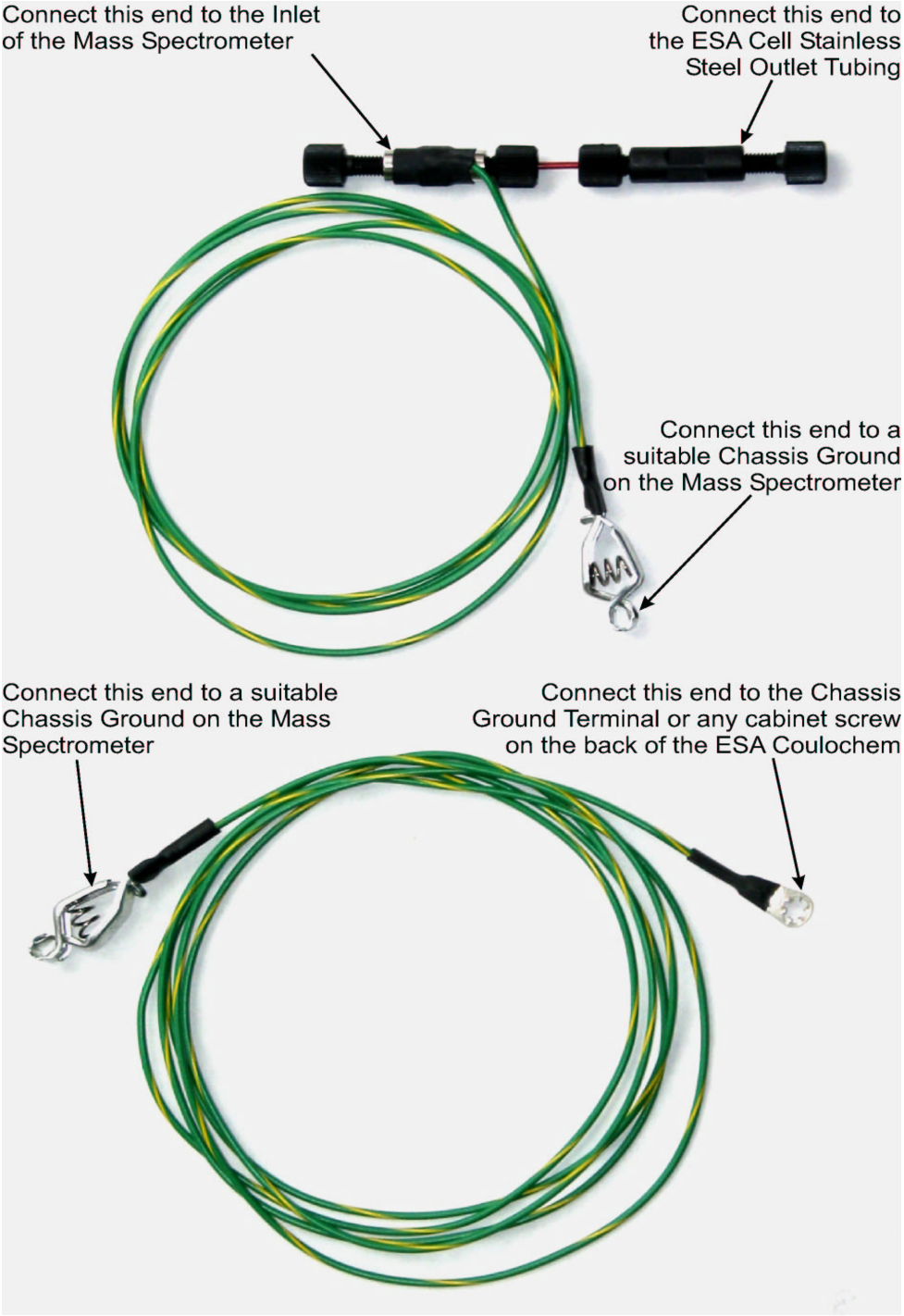
Appendix C Mass Spectrometer Decoupling Instructions



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