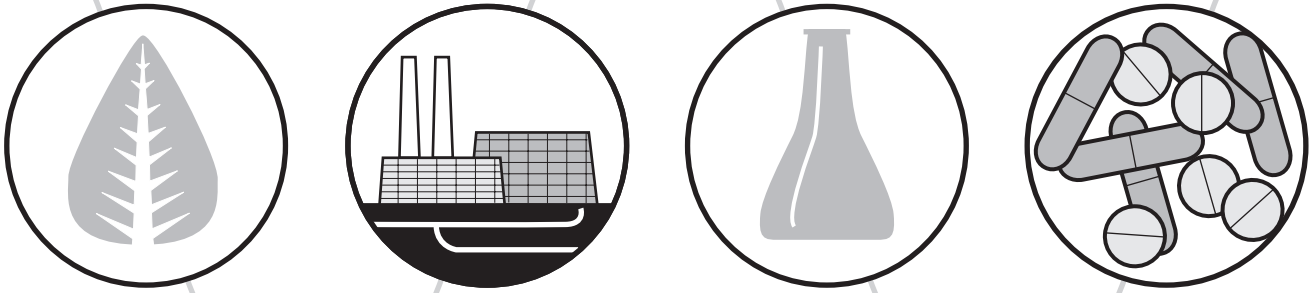
Waters 2465 Electrochemical Detector

Operator's Guide



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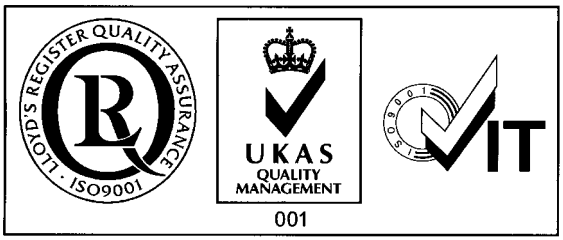


2465 Detector Safety Precautions

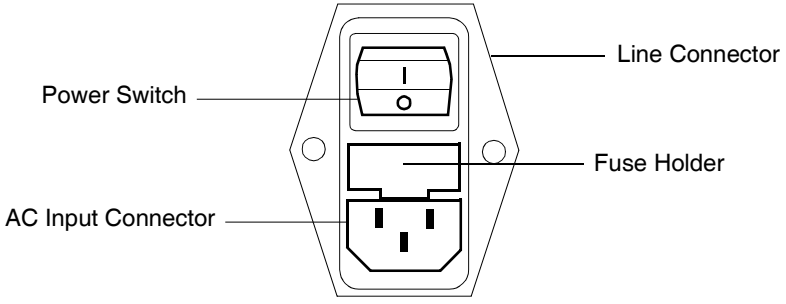
Caution: *Untrained personnel should not open the instrument. Removing protective panels on the instrument can result in exposure to potentially dangerous voltages. Disconnect the instrument from all power sources before disassembly.*

Caution: *To avoid electrical shock, power off the 2465 Detector and unplug the power cord before maintaining or servicing the instrument. The I/O connectors on the rear of the instrument have a risk of electrical shock.*

Caution: *Use the correct fuses and power setting for your location (U.S.A. provides 110 V; your location may provide 240 V).*

Caution: *Replace blown fuses (see figure below) with fuses of proper type and rating as stipulated on the rear panel and specified in Section 4.8.1, Replacing Fuses. The fuse holder is integrated in the line connector.*

To prevent the risk of fire, never operate the instrument with the incorrect type of fuses.



Caution: *Be sure that power cords are plugged into the correct voltage sources. The instrument should be connected to a protective earth via a ground socket. Replace faulty or frayed power cords.*

Note: *When you use the instrument, follow generally accepted procedures for quality control and methods development.*

If you observe a change in the retention of a particular compound, in the resolution between two compounds, or in peak shape, immediately determine the reason for the changes. Until you determine the cause of a change, do not rely on the separation results.

Note: *The Installation Category (Overvoltage Category) for this instrument is Level II. The Level II Category pertains to equipment that receives its electrical power from a local level, such as an electrical wall outlet.*

Attention: *Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.*

Important : *Toute modification sur cette unité n'ayant pas été expressément approuvée par l'autorité responsable de la conformité à la réglementation peut annuler le droit de l'utilisateur à exploiter l'équipement.*

Achtung: *Jedwede Änderungen oder Modifikationen an dem Gerät ohne die ausdrückliche Genehmigung der für die ordnungsgemäße Funktionstüchtigkeit verantwortlichen Personen kann zum Entzug der Bedienungsbefugnis des Systems führen.*

Avvertenza: *eventuali modifiche o alterazioni apportate a questa unità e non espressamente approvate da un ente responsabile per la conformità annulleranno l'autorità dell'utente ad operare l'apparecchiatura.*

Atención: *cualquier cambio o modificación efectuado en esta unidad que no haya sido expresamente aprobado por la parte responsable del cumplimiento puede anular la autorización del usuario para utilizar el equipo.*

注意：未經有關法規認證部門允許對本設備進行的改變或修改，可能會使使用者喪失操作該設備的權利。

注意：未经有关法规认证部门明确允许对本设备进行的改变或改装，可能会使使用者丧失操作该设备的合法性。

주의 : 기기 검교정 담당자의 승인 없이 무단으로 기기를 변경 또는 수정하는 경우에는, 그 기기 운영에 대한 허가가 취소될 수 있습니다.

注意 : 規制機関から明確な承認を受けずに本装置の変更や改造を行うと、本装置のユーザとしての承認が無効になる可能性があります。

Caution: *Use caution when working with any polymer tubing under pressure:*

- *Always wear eye protection when near pressurized polymer tubing.*
- *Extinguish all nearby flames.*
- *Do not use Tefzel tubing that has been severely stressed or kinked.*
- *Do not use Tefzel tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.*
- *Be aware that methylene chloride and dimethyl sulfoxide cause Tefzel tubing to swell, which greatly reduces the rupture pressure of the tubing.*

Attention : *Soyez très prudent en travaillant avec des tuyaux de polymères sous pression :*

- *Portez toujours des lunettes de protection quand vous vous trouvez à proximité de tuyaux de polymères.*
- *Eteignez toutes les flammes se trouvant à proximité.*
- *N'utilisez pas de tuyau de Tefzel fortement abîmé ou déformé.*
- *N'utilisez pas de tuyau de Tefzel avec de l'acide sulfurique ou nitrique, ou du tétrahydrofurane (THF).*
- *Sachez que le chlorure de méthylène et le sulfoxyde de diméthyle peuvent provoquer le gonflement des tuyaux de Tefzel, diminuant ainsi fortement leur pression de rupture.*

Vorsicht: *Bei der Arbeit mit Polymerschläuchen unter Druck ist besondere Vorsicht angebracht:*

- *In der Nähe von unter Druck stehenden Polymerschläuchen stets Schutzbrille tragen.*
- *Alle offenen Flammen in der Nähe löschen.*
- *Keine Tefzel-Schläuche verwenden, die stark geknickt oder überbeansprucht sind.*
- *Tefzel-Schläuche nicht für Tetrahydrofuran (THF) oder konzentrierte Salpeter- oder Schwefelsäure verwenden.*
- *Durch Methylenchlorid und Dimethylsulfoxid können Tefzel-Schläuche quellen; dadurch wird der Berstdruck des Schlauches erheblich reduziert.*

Precauzione: *prestare attenzione durante le operazioni con i tubi di polimero sotto pressione:*

- *Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.*
- *Estinguere ogni fonte di ignizione circostante.*
- *Non utilizzare tubi Tefzel soggetti a sollecitazioni eccessive o incurvati.*
- *Non utilizzare tubi Tefzel contenenti tetraidrofurano (THF) o acido solforico o nitrico concentrato.*
- *Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi Tefzel, che riducono notevolmente il limite di pressione di rottura dei tubi stessi.*

Advertencia: *manipular con precaución los tubos de polímero bajo presión:*

- *Protegerse siempre los ojos en las proximidades de tubos de polímero bajo presión.*
- *Apagar todas las llamas que estén a proximidad.*
- *No utilizar tubos Tefzel que hayan sufrido tensiones extremas o hayan sido doblados.*
- *No utilizar tubos Tefzel con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.*
- *No olvidar que el cloruro de metileno y el óxido de azufre dimetilo dilatan los tubos Tefzel, lo que reduce en gran medida la presión de ruptura de los tubos.*

警告：當在有壓力的情況下使用聚合物管線時，小心注意以下几點：

- 當接近有壓力的聚合物管線時一定要戴防護眼鏡。
- 熄滅附近所有的火焰。
- 不要使用已經被壓癟或嚴重彎曲的特氟隆(Tefzel)管線。
- 不要在特氟隆(Tefzel)管線中使用四氫呋喃(THF)或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞楓會導致特氟隆(Tefzel)管線膨脹，大大降低管線的耐壓能力。

警告: 当在有压力的情况下使用聚合物管线时，小心注意以下几点:

- 当接近有压力的聚合物管线时一定要戴防护眼镜
- 熄灭附近所有的火焰。
- 不要使用已经被压瘪或严重弯曲的特氟隆(Tefzel)管线。
- 不要在特氟隆(Tefzel)管线中使用四氢呋喃(THF)或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砜会导致 特氟隆(Tefzel)管线膨胀，大大降低管线的耐压能力。

경고 : 폴리머재질의 튜빙을 압력하에서 사용할 때는 다음 사항에 유의하십시오.

- 압력을 받은 폴리머 튜빙 부근에서는 반드시 보호안경을 착용할 것
- 모든 화기의 접근을 금함
- 눌리거나 뒤틀린 Tefzel 튜빙은 사용하지 말 것
- Tefzel 튜빙을 테트라히드로퓨란 (THF)이나 염산 및 황산과 함께 사용하지 말 것
- 디클로로메탄(methylene chloride)와 디메틸설폭시드(dimethyl sulfoxide)는 Tefzel 튜빙을 팽창시켜 쉽게 파열되므로 주의할 것

警告: ポリマーチューブに圧力をかけて取り扱う場合は、次のように注意してください。

- 加圧したポリマーチューブの付近では、常に保護めがねを着用してください。
- 付近の火はすべて消してください。
- 激しい応力やねじれを受けたTefzelチューブは使用しないでください。
- テトラヒドロフラン（THF）、濃硝酸、あるいは濃硫酸には、Tefzelチューブを使用しないでください。
- メチレン-クロライドやジメチルスルホキシドはTefzelチューブを膨張させ、チューブの破断圧力を大幅に低下させますので、注意してください。

Caution: *The user shall be made aware that if the equipment is used in a manner not specified by the manufacturer, the protection provided by the equipment may be impaired.*

Attention : *L'utilisateur doit être informé que si le matériel est utilisé d'une façon non spécifiée par le fabricant, la protection assurée par le matériel risque d'être défectueuses.*

Vorsicht: *Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwenddung des Gerätes unter Umständen nicht ordnungsgemäß funktionieren.*

Precauzione: *l'utente deve essere al corrente del fatto che, se l'apparecchiatura viene usta in un modo specificato dal produttore, la protezione fornita dall'apparecchiatura potrà essere invalidata.*

Advertencia: *el usuario deberá saber que si el equipo se utiliza de forma distinta a la especificada por el fabricante, las medidas de protección del equipo podrían ser insuficientes.*

警告：使用者必須非常清楚如果設備不是按照制造廠商指定的方式使用，那么該設備所提供的保護將被消弱。

警告: **使用者必须非常清楚如果设备不是按照制造厂商指定的方式使用, 那么该设备所提供的保护将被消弱**

경고 : 제조사가 지정한 것 이외의 방법으로 기기를 사용하는 경우에는, 사용자가 위험으로부터 보호될 수 없는 경우가 발생할 수 있음에 유념하십시오.

警告 : ユーザは製造業者が指定していない方法で装置を使用した場合は装置が提供する保護が損なわれることがあるということを承知しているものとします。

Caution: *To protect against fire hazard, replace fuses with those of the same type and rating.*

Attention : *Remplacez toujours les fusibles par d'autres du même type et de la même puissance afin d'éviter tout risque d'incendie.*

Vorsicht: *Zum Schutz gegen Feuergefahr die Sicherungen nur mit Sicherungen des gleichen Typs und Nennwertes ersetzen.*

Precauzione: *per una buona protezione contro i rischi di incendio, sostituire i fusibili con altri dello stesso tipo e amperaggio.*

Advertencia: *sustituya los fusibles por otros del mismo tipo y características para evitar el riesgo de incendio.*

警告：為了避免火災的危險，應更換同种類型及規格的保險絲。

警告: **为了避免火灾的危险, 应更换同种类型及规格的保险丝**。

경고 : 화재를 방지하기 위해서는 퓨즈 교체 시 같은 종류, 같은 등급의 것을 사용하십시오.

警告 : 火災の危険防止のために、ヒューズの交換は同一タイプおよび定格のもので行なってください。

Caution: *To avoid possible electrical shock, disconnect the power cord before servicing the instrument.*

Attention : *Afin d'éviter toute possibilité de commotion électrique, débranchez le cordon d'alimentation de la prise avant d'effectuer la maintenance de l'instrument.*

Vorsicht: *Zur Vermeidung von Stromschlägen sollte das Gerät vor der Wartung vom Netz getrennt werden.*

Precauzione: *per evitare il rischio di scossa elettrica, scollegare il cavo di alimentazione prima di svolgere la manutenzione dello strumento.*

Precaución: *para evitar descargas eléctricas, desenchufe el cable de alimentación del instrumento antes de realizar cualquier reparación.*

警告：要避免觸電，請在修理或保養器材前把電源線拔出。

警告：为避免可能引起得触电危险，在修理前请切断电源连接。

경고: 전기 충격의 가능성을 피하기 위해서는, 기기를 수리하기 이전에 전원 코드를 차단하십시오.

警告：感電の危険性を避けるために、装置の保守を行う前には装置の電源コードを引き抜いてください。

Commonly Used Symbols

⎓	Direct current Courant continu Gleichstrom Corrente continua Corriente continua 直流電 直流电 직류 直流
~	Alternating current Courant alternatif Wechselstrom Corrente alternata Corriente alterna 交流電 交流电 교류 交流
	Protective conductor terminal Borne du conducteur de protection Schutzleiteranschluss Terminale di conduttore con protezione Borne del conductor de tierra 保護的導線端子 保护性的接地端 보호 도체 단자 接地

Commonly Used Symbols *(Continued)*

	Frame or chassis terminal Borne du cadre ou du châssis Rahmen- oder Chassisanschluss Terminale di struttura o telaio Borne de la estructura o del chasis 結構或底盤端子 机架或底盘接地端 프레임 또는 틀 단자 フレームまたはシャーシアース
	Caution or refer to manual Attention ou reportez-vous au guide Vorsicht, oder lesen Sie das Handbuch Prestare attenzione o fare riferimento alla guida Actúe con precaución o consulte la guía 小心或查閱手冊 小心或查阅手册 경고 또는 사용설명서 참조 警告またはマニュアルを参照
	Caution, hot surface or high temperature Attention, surface chaude ou température élevée Vorsicht, heiße Oberfläche oder hohe Temperatur Precauzione, superficie calda o elevata temperatura Precaución, superficie caliente o temperatura elevada 警告，熱表面或高溫 警告,热表面或高温 경고, 뜨거운 표면 또는 고온 警告、熱くなっている面、あるいは高温

Commonly Used Symbols *(Continued)*

	Caution, risk of electric shock (high voltage) Attention, risque de commotion électrique (haute tension) Vorsicht, Elektroschockgefahr (Hochspannung) Precauzione, rischio di scossa elettrica (alta tensione) Precaución, peligro de descarga eléctrica (alta tensión) 警告，小心触電（高壓電） 警告,小心触电（高压电） 경고, 전기충격의 위험 (고압) 警告、電気ショックの危険性（高電圧）
	Caution, risk of needle-stick puncture Attention, risques de perforation de la taille d’une aiguille Vorsicht, Gefahr einer Spritzenpunktierung Precauzione, rischio di puntura con ago Precaución, riesgo de punción con aguja 警告，小心尖狀物刺傷 警告,小心尖状物刺伤 경고, 뾰족한 것으로부터의 상해 위험 警告、ニードルで穴をあける危険性
	Caution, ultraviolet light Attention, rayonnement ultrviolet Vorsicht, Ultraviolettes Licht Precauzione, luce ultravioletta Precaución, emisiones de luz ultravioleta 警告，紫外光 警告,紫外光 경고, 자외선 警告、紫外線

Commonly Used Symbols *(Continued)*

	Fuse Fusible Sicherung Fusibile Fusible 保險絲 保险丝 퓨즈 ヒューズ
1	Electrical power on Sous tension Netzschalter ein Alimentazione elettrica attivata Alimentación eléctrica conectada 開啓電源 接通电源 전원 켜기 電源オン
0	Electrical power off Hors tension Netzschalter aus Alimentazione elettrica disattivata Alimentación eléctrica desconectada 關閉電源 切断电源 전원 끄기 電源オフ

2465 Electrochemical Detector Information

Intended Use

The Waters® 2465 Electrochemical Detector is designed for HPLC applications.

Biological Hazard

When you analyze physiological fluids, take all necessary precautions and treat all specimens as potentially infectious. Precautions are outlined in “CDC Guidelines on Specimen Handling,” *CDC – NIH Manual*, 1984.

Calibration

Follow acceptable methods of calibration with pure standards to calibrate methods. Use a minimum of five standards to generate a standard curve. The concentration range should cover the entire range of quality-control samples, typical specimens, and atypical specimens.

Quality Control

Routinely run three quality-control samples. Quality-control samples should represent subnormal, normal, and above-normal levels of a compound. Ensure that quality-control sample results are within an acceptable range, and evaluate precision from day to day and run to run. Data collected when quality-control samples are out of range may not be valid. Do not report this data until you ensure that chromatographic system performance is acceptable.

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Preface

The *Waters 2465 Electrochemical Detector Operator's Guide* describes the procedures for unpacking, installing, operating, verifying, maintaining, and troubleshooting the Waters® 2465 Electrochemical Detector (ECD). It also includes appendixes containing instrument specifications, spare parts, and a glossary.

Anyone who installs, maintains, operates, or troubleshoots the Waters 2465 Electrochemical Detector can use this guide. All personnel who use this guide should be familiar with HPLC terms and practices and be capable of performing basic HPLC system operations such as making fluidic connections.

Organization

This guide contains the following:

Chapter 1 summarizes the features of the 2465 Detector and describes the theory and principles of operation.

Chapter 2 describes how to install the 2465 Detector, make power, fluidic, and signal connections, and connect the detector to other devices.

Chapter 3 describes how to set up and operate the 2465 Detector in remote and stand-alone modes.

Chapter 4 describes how to clean and replace various parts of the 2465 Detector.

Chapter 5 describes the error messages, diagnostics, and recommended actions to solve problems with the 2465 Detector.

Appendix A contains operational, environmental, optical, and voltage specifications for the 2465 Detector.

Appendix B lists recommended and optional spare parts for the 2465 Detector.

Appendix C provides sample methods and application notes for the 2465 Detector.

Appendix D provides a glossary of terms for the 2465 Detector.

Related Documentation

***Waters Licenses, Warranties, and Support*:** Provides software license and warranty information, describes training and extended support, and tells how Waters handles shipments, damages, claims, and returns.

Online Documentation

***Empower Help*:** Describes all Empower windows, menus, menu selections, and dialog boxes for the base software and software options. Also includes reference information and procedures for performing all tasks required to use Empower software. Included as part of the Empower software.

Printed Documentation

***Waters Bus SAT/IN Module Installation Guide*:** Describes installation of the Waters Bus SAT/IN™ Module.

***Millennium^{32} System Installation and Configuration Guide*:** Describes Millennium^{32} software installation. Discusses how to configure the computer and chromatographic instruments as part of the Millennium^{32} System. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E^{32} module, the busLAC/E™ card, and interface cards used to communicate with serial instruments.

***Empower System Installation and Configuration Guide*:** Describes Empower software installation, including the stand-alone Personal workstation, Workgroup configuration, and the Enterprise client/server system. Discusses how to configure the computer and chromatographic instruments as part of the Empower System. Also covers the installation, configuration, and use of acquisition servers such as the LAC/E^{32} module, the busLAC/E card, and interface cards used to communicate with serial instruments.

Documentation Conventions

The following conventions can be used in this guide:

Convention	Usage
Bold	Bold indicates user action such as keys to press, menu selections, and commands. For example, “Click **Next** to go to the next page.”
Italic	Italic indicates information that you supply such as variables. It also indicates emphasis and document titles. For example, “Replace *file_name* with the actual name of your file.”
`Courier`	Courier indicates examples of source code and system output. For example, “The `SVRMGR>` prompt appears.”
`Courier Bold`	Courier bold indicates characters that you type or keys you press in examples of source code. For example, “At the `LSNRCTL>` prompt, enter **`set password oracle`** to access Oracle.”

Convention	Usage
Keys	The word *key* refers to a computer key on the keypad or keyboard. *Screen keys* refer to the keys on the instrument located immediately below the screen. For example, “The A/B screen key on the 2414 Detector displays the selected channel.”
…	Three periods indicate that more of the same type of item can optionally follow. For example, “You can store *filename1*, *filename2*, … in each folder.”
>	A right arrow between menu options indicates you should choose each option in sequence. For example, “Select **File** > **Exit**” means you should select **File** from the menu bar, then select **Exit** from the File menu.

Notes

Notes call out information that is helpful to the operator. For example:

Note: *Record your result before you proceed to the next step.*

Attentions

Attentions provide information about preventing damage to the system or equipment. For example:

Attention: *Never lift the 2465 Detector by the door at the front, but only by its sides, or you may damage the detector.*

Cautions

Cautions provide information essential to the safety of the operator. For example:

Caution: *To avoid burns, turn off the lamp at least 30 minutes before removing it for replacement or adjustment.*

Caution: *To avoid electrical shock and injury, turn off the detector and unplug the power cord before performing maintenance procedures.*

Caution: *To avoid chemical or electrical hazards, observe safe laboratory practices when operating the system.*

Chapter 1
2465 Detector Theory of Operation

This chapter introduces the Waters® 2465 Electrochemical Detector (2465 Detector). It summarizes the 2465 Detector's features and major components, and describes the theory and principles of operation. To use the detector effectively, you should understand its principles of operation and design.

1.1 Detector Introduction

Electrochemical detection theory involves the understanding of:

- Electrolysis reactions
- Current-potential curves

The 2465 Detector is amperometric, because the detector's response is measured in amperes, or current. Coulometry measures the quantity of charge and computes the absolute mass of analyte from Faraday's law:

$$Q = n \times F \times N$$

where:

Q = Mass of analyte

n = Number of moles (M) of electrons lost or gained

F = Faraday's constant (96,500 coulombs/mole of electrons)

N = Number of moles of analyte

1.1.1 Electrolysis Reactions

Electrochemical detection differs from other detection methods in that the analyte undergoes an electrochemical reaction while being detected (Figure 1-1). Upon elution from the column, the analyte passes through the electrochemical cell, where it undergoes either oxidation or reduction at the working electrode (WE). The controller (potentiostat)

maintains the potential of the working electrode (relative to a reference electrode) at a value that causes the analyte to electrolyze. It simultaneously measures the electrolysis current resulting from the oxidation (or reduction) of the analyte.

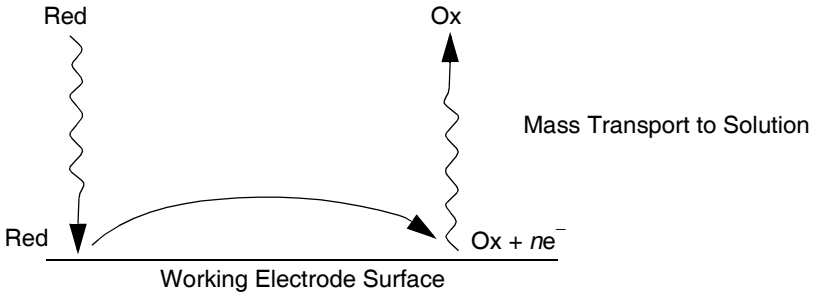


Figure 1-1 Electrolysis at Working Electrode

An electrical current is the rate of electricity flow. It is also a measure of the rate of the reaction taking place at the working electrode. The more positive the working electrode potential, the more strongly it can oxidize passing species.

In order for a molecule in solution to undergo an oxidation or reduction at an electrode, it must complete a three-step process: diffusion to the electrode surface, oxidation or reduction, then diffusion away from the vicinity of the working electrode (to allow acceptance or release of electrons from another molecule). The details of the process are as follows:

1. **Mass transport of the analyte from the bulk of solution to the electrode surface**

   Even in rapid LC flow rates, there is a stagnant layer of fluid at the electrode surface through which diffusion is the mode of mass transport. Diffusion in liquids at room temperature is relatively slow. Typical liquid phase diffusion coefficients are 1 to 10×10^{-6} cm/sec^2. Small molecule diffusion rates do not differ widely for molecular weights around 100. Therefore, the flow rate, geometry of the flow cell, diffusion coefficient, and viscosity of the fluid are in effect the primary factors that determine the rate of mass transport.

2. **Electron transfer at the electrode surface**

   The rate of this step is determined primarily by the applied potential. Generally, the potential selected is high so that this step is very rapid relative to the rate of mass transport. The Nernst equation describes this behavior:

   $E = E^0 + (RT/nF) \ln [Ox]/[Red]$

where [Ox] and [Red] are the surface concentrations of the oxidized and reduced forms of the analyte, or:

$\Delta E = (E - E^0) = RT/nF \ln [Ox]/[Red]$

where:

E = Potential

E^0 = Standard potential of the analyte

R = Gas constant

T = Temperature

n = Number of electrons

[Ox] = Concentration of oxidized form

[Red] = Concentration of reduced form

If ΔE is greater than zero, the concentration of the oxidized form is greater and oxidation results. If ΔE is less than zero, the concentration of the reduced form is greater and reduction results.

3. Mass transport of the electrolytic product(s) from the electrode surface

In any multiple-stage process, the slowest or the rate-limiting step determines the overall rate. Because the oxidized or reduced forms of a molecule have to reach the electrode surface, the growth of the current is limited by the rate of mass transport. There are generally three modes of mass transport: convection, migration, and diffusion. Diffusion is the slowest. Thus mass transport is a diffusion-limited step. The limiting current in a flow cell, when ΔE is large, is given by the following equation, which defines a linear relationship:

$$i_{LIMITING} = nFA\left(\frac{D}{\delta}\right)C$$

where:

i = Mass transport limited current in a given flow cell

n = Number of electrons transferred

F = Faraday's constant

A = Electrode area

D = Diffusion coefficient

δ = Diffusion layer thickness

C = Concentration of the analyte in the flow cell

Therefore, when the flow rate is constant, the diffusion layer thickness is constant, and the current is proportional to the concentration of the analyte. As with any concentration-dependent detector in which the column efficiency and capacity factor are constant, the peak height is directly proportional to the mass injected on the column.

1.1.2 Current-Potential Curves

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

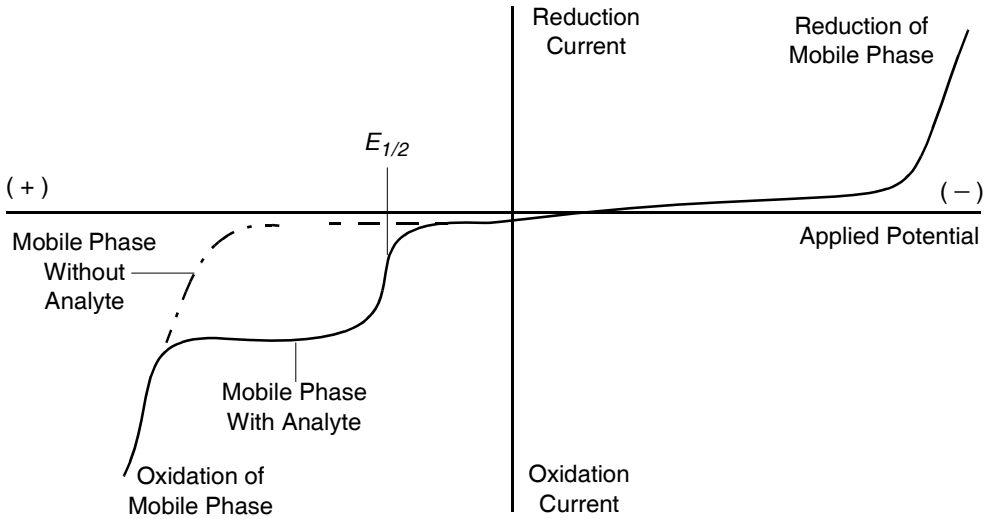


Figure 1-2 Current-Potential Curve

The significant characteristics of a current-potential curve are the $E_{1/2}$ and the limiting current plateau. The $E_{1/2}$ is very nearly equal to the standard reduction potential of the analyte. The limiting current plateau corresponds to those potentials that result in nearly instantaneous electrolysis of the analyte upon reaching the electrode surface. In general, select the smallest potential at the plateau that can oxidize all peaks of interest. Operating at greater potential does not increase the signal and is likely to increase noise.

When multiple oxidizable species are in solution, the resulting current-potential curve is the sum of the individual current-voltage curves. This resulting current-potential curve has multiple limiting current plateaus, each with its characteristic $E_{1/2}$. Selective detection with an electrochemical detector is accomplished by using the smallest value of applied potential that electrolyzes the sample and gives a mass transport limited current. Increasing the potential can result in the oxidation of additional components with an increase in noise.

Figure 1-3 shows the hydrodynamic voltammogram of norepinephrine at a glassy carbon working electrode (A) and the current of the baseline (B).

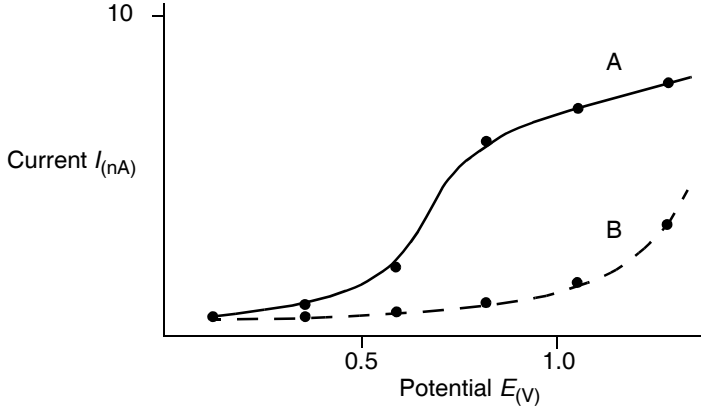


Figure 1-3 Hydrodynamic Voltammogram of Norepinephrine

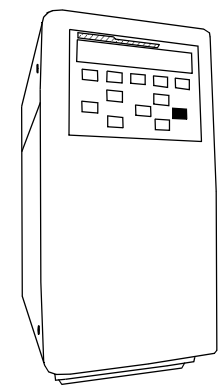
1.2 Detector Features

The 2465 Detector is a single-channel electrochemical detector designed for high-performance liquid chromatography (HPLC) applications and is capable of operation either in stand-alone mode or in remote mode.

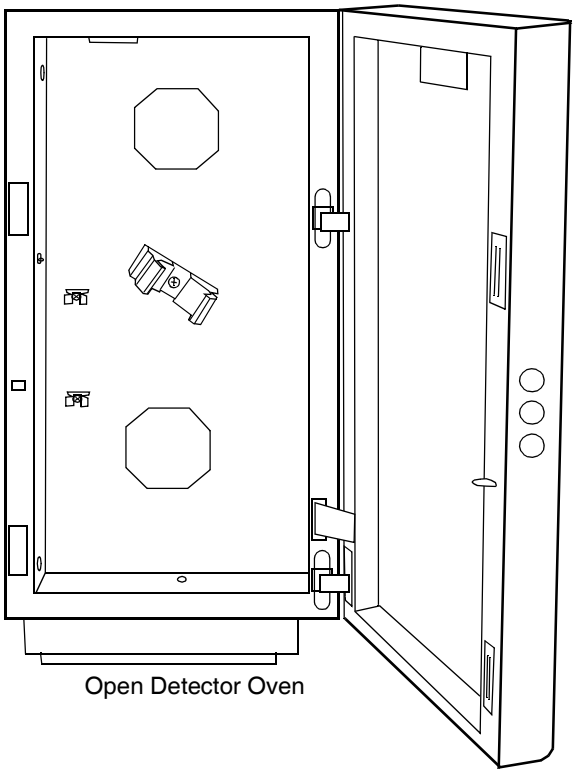
The 2465 Detector is designed as either a modular stand-alone detector using a high-resolution analog output, for use with a chart recorder, integrator, or other data station, or as an integral part of a Waters HPLC System and Empower software.

The 2465 Detector is configurable with a variety of flow cells, variable volumes, reference electrodes (REFs), materials for working electrodes (WEs), and working electrode diameters. The 2465 Detector supports a number of column diameters from capillary LC up to standard LC. The 2465 Detector includes a stable Faraday-shielded detector oven, which accommodates both the flow cell and column (Figure 1-4). The 2465 Detector uses

newly designed electronics with a noise filtering system for improved performance, which results in an overall increase in signal-to-noise ratios.



2465 Detector with Closed Door



Open Detector Oven

Figure 1-4 2465 Detector Oven

The 2465 Detector has the following capabilities:

- **Stand-alone programmability** – Stores up to nine user-defined programs (also called time files or methods), each consisting of up to 50 programmable timed events and two threshold events.
- **Three modes of operation** – Supports direct current (DC), pulsed amperometric detection (PAD), and scan modes.
- **Multiple flow cell dimensions** – Offers a variety of flow cells including the standard flow cell (2-mm, 3-mm) and the micro flow cell (0.7-mm); both configurations use the wall-jet flow cell design and are available with a variety of spacers.
- **Multiple working electrodes (WE) for the standard flow cell** – Offers four types of working electrodes: glassy carbon (GC), gold (Au), platinum (Pt), and silver (Ag).

- **Multiple reference electrodes (REF) for the standard flow cell** – Offers three reference electrodes: salt-bridge silver/silver chloride (sb REF), in-situ silver/silver chloride (ISAAC), and hydrogen reference (Hy-REF).
- **Dummy cell** – Facilitates troubleshooting by enabling you to isolate and test the electronics and control without the variability introduced by the presence of a real flow cell.
- **Detector oven (column and flow cell heater compartment)** – Faraday-shielded oven; provides thermal operating stability and reduces noise and drift characteristics.
- **Method editing and storage** – Supports basic method programming, storage, and retrieval from the front panel.
- **Diagnostic capability** – Supports built-in diagnostic tools to optimize functionality and performance.
- **Rear panel I/O connectors** – Include main power, chassis ground connector, two contact closure outputs, two relays, six external event inputs, and an RS-232C connector for full instrument control (optional).

1.3 Detector Design

1.3.1 Electronics and Data Acquisition

In the electrochemical flow cell, the electron transfer takes place at the working electrode during an oxidation or reduction reaction. The resulting electrical current is amplified by the current-potential (*I/E*) converter (Figure 1-5).

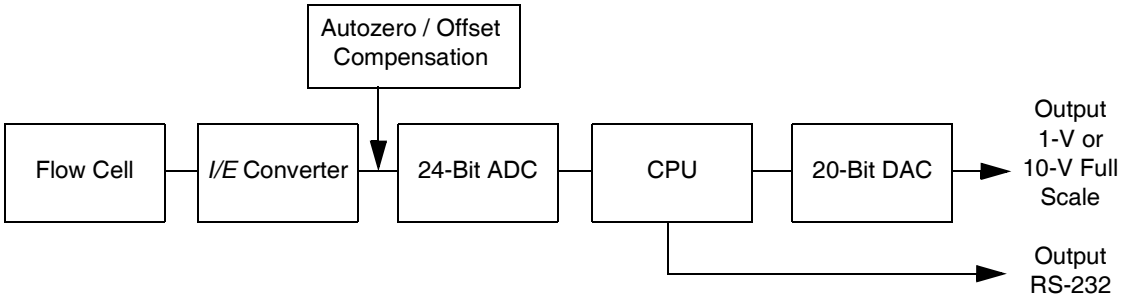


Figure 1-5 Signal Processing from Flow Cell to Output

The signal or current from the *I/E* converter can be compensated with autozero or offset, and is digitized using a 24-bit ADC (analog-to-digital converter). The signal is processed

in the CPU using noise filtering or more complex data processing in PAD. Finally the signal is sent:

- Through a 20-bit DAC (digital-to-analog converter), then as a 1- or 10-V full scale output (which you can select from the Configuration menu)
- As an RS-232 output

1.3.2 Electronics

I/E Converter

The *I/E* converter selects the resistor appropriate to the current range at the selected applied cell potential.

Sensor Board

Description

The sensor board receives inputs from the flow cell. The current is digitized and any offset settings and compensation are applied for the range selected. The processor receives this modified signal and passes it on to the flash memory and other circuitry. The sensor board reacts to the state of various signals on the DB-25 connector on the back of the unit, and passes signal information on to the control board. The sensor board generates an analog 1- to 10-V output on the BNC connector analogous to the input signal received from the flow cell.

Interconnections

The sensor board has two connectors that extend through the back of the unit. One is a 15-pin connector for event signals (labeled A), and the other is a standard BNC connector for the analog output (labeled Output). Internally, the board also has a connector interfacing the board to the flow cell by way of the internal cell cable assembly. Power, ground, and data are received from the control board through the sensor cable assembly.

Control Board

Description

The control board receives control signals using the RS-232 port as well as temperature information from the sensor. All voltages used by the sensor board, heaters, and fans are developed on the control board. The control board receives inputs from the keypad and displays information on the LCD display. The control board takes the signal from the sensor board's processor and generates the visual information for the LCD display and

also passes information to a controlling computer using the RS-232 port on the back of the unit. The control board has a connector (labeled B) on the rear panel of the unit. This connector provides and accepts signals for injector control.

Interconnections

The control board is central to all operations of the 2465 Detector. As such, most subassemblies connect directly to it or through intermediate cables. The power supply subassembly generates a filtered +24 V to the control board, which then generates operating voltages of +5 V and +13.5 V for use by other subassemblies.

The LCD display and keypad components of the front door connect to the control board using a door cable assembly. The detector oven's heater subassembly, fans, and temperature sensor assembly connect directly to the control board.

The optical door sensor connects to the control board using an optical cable assembly, and the sensor board connects using a sensor cable assembly.

1.3.3 Rear Panel

The following input/output (I/O) and digital signal connectors are available on the 2465 Detector rear panel:

- **Connector A (15-pin Sub D)**
  - *Inputs*: Cell on/off, start, autozero, and reset
  - *Outputs*: Two programmable relays and two TTL auxiliary outputs
- **Connector B (15-pin Sub D)** – Electronically actuated injection, inject start (input), and mark (output)
- **Connector C (phone jack)** – For use with a manual injector (optional)
- **Analog Output (BNC)** – User selectable 1-V or 10-V full scale, 20 bit
- **RS-232C (9-pin Sub D)** – Full control and digital data output, 24 bit

The rear panel also has a ground stud (instrument ground connector), power connector, and fuses.

1.3.4 Filtering

The detector applies a digital filter. Filter performance depends on the filter time constant you select. The filter time constant adjusts the filter response time to achieve an optimal signal-to-noise ratio.

Lower time constant settings:

- Remove less baseline noise
- Produce narrow peaks with minimal peak distortion and time delay
- Make very small peaks harder to discriminate from baseline noise

Higher time constant settings:

- Greatly decrease baseline noise
- Shorten and broaden peaks

The default time constant, 0.1 second, may be too small to suit all applications. Use the following equation to calculate an appropriate time (filter) constant for special applications:

$$TC = 0.2 \times PW$$

where:

TC = Time constant (filter) setting

PW = Peak width at half height of the narrowest peak

Figure 1-6 shows the relationship between the time constant and response times.

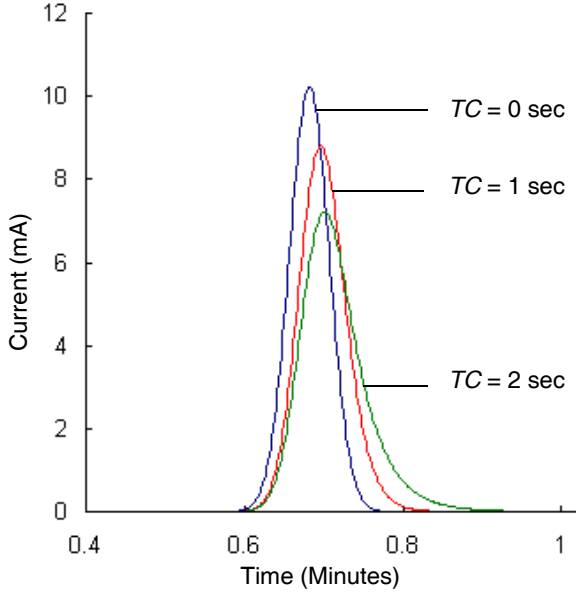


Figure 1-6 Time Constant (Filter Setting) Comparison

1.3.5 Autozero and Maximum Compensation

Autozero (AZERO or Azero) is a function that compensates the signal or current from the *I/E* converter and sets the output voltage to 0 V or to the offset voltage (see Section 3.1.7, Function Key Commands). When Azero is selected, the status of Comp in the DC and Pulse Stat screens changes from `Comp=off` to `Comp=on`.

Maximum compensation (MaxComp) is the maximum cell current that can be compensated in a particular measurement range (Table 1-1). The 2465 Detector autozero range or maximum compensation depends on the range of the current setting.

Table 1-1 Ranges and Maximum Compensation

Range (Full Scale, FS)	MaxComp (Maximum Compensation)	Mode
5 to 200 µA	±2.5 mA	DC or PAD (Pulse)
100 nA to 2 µA	±25 µA	DC or PAD (Pulse)
10 to 50 nA	±2.5 µA	DC or PAD (Pulse)
200 pA to 5 nA[a]	±250 nA	DC only
10 to 100 pA[a]	±25 nA	DC only

a. Current is much higher in pulse and scan modes than in DC mode. Therefore, it is not possible to select pA ranges in pulse or scan mode.

To use Azero when you see an Out of Range message, you may need to change measurement ranges. For example, in the 100 pA range in DC mode (see Section 3.4, Using DC Mode), the maximum compensation is ±25 nA. If your system has a background cell current (I_c) of 24 nA, it is still possible to measure in this range, but you must compensate the current by autozeroing, because $I_c > I_{range}$ (24 nA versus 100 pA). After autozeroing, the Out of Range message disappears and the actual cell current appears in the screen (for example, `Ic=24 nA`).

However, if your system has a background cell current of 26 nA, selecting Autozero does not compensate the current, because 26 nA is greater than the MaxComp allowed in Table 1-1. The screen has an Out of Range message in either case. To measure this current, first change to the 200 pA range (see Section 3.4.1, Setting Initial Conditions in DC Mode, and Section 3.5.1, Setting Initial Conditions in Pulse Mode), because the MaxComp is 250 nA for the 200 pA range.

1.3.6 Startup Diagnostics

The 2465 Detector is equipped with a checksum verification and calculation diagnostic that is automatically invoked at startup. An eight-digit checksum value appears on the LCD display once the checksum is calculated.

Note: *The correct checksum for your firmware version is in the Release Notes.*

The 2465 Bootloader program is a special type of program that resides permanently in memory. It is responsible for initializing application-independent communication. It is also responsible for starting up the application software for the 2465 Detector.

1.3.7 Temperature Control

The detector oven, a heated flow cell and column compartment at the front of the 2465 Detector, is used to stabilize the detector's performance. It can also be effective in aiding the chromatographic separation at the column. The heater stabilizes background current. The increase in temperature can increase noise, but reduces the rate of baseline drift.

Attention: *Because an electrochemical detector is sensitive to environmental changes, do not operate it close to vents or a window.*

A clear, level, smooth surface is required to allow the ventilation system under the 2465 Detector to work properly.

1.4 Flow Cell Design

1.4.1 Flow Cell Operation

Before the flow cell can operate, it must be properly prepared, then switched on. The procedure to switch on the flow cell differs slightly for each mode. There are three ways to switch on the flow cell:

- From the front panel.
- Using the Cell On timed event. This input command can be used to switch on and stabilize the flow cell by means of a timer.
- Using a data system such as Empower™ software to control the 2465 Detector in remote mode.

Attention: *To avoid damaging the flow cell, do not turn it on unless the fluid lines are connected and mobile phase is flowing.*

The standard flow cell is available with a glassy carbon (GC), platinum (Pt), gold (Au), or silver (Ag) working electrode (Figure 1-7). In combination with the spacer size set (25, 50, and 120 µm), a variety of detection volumes down to 11 nL can be attained. The inlet block is separated from the working electrode block by means of a spacer (not shown in Figure 1-7).

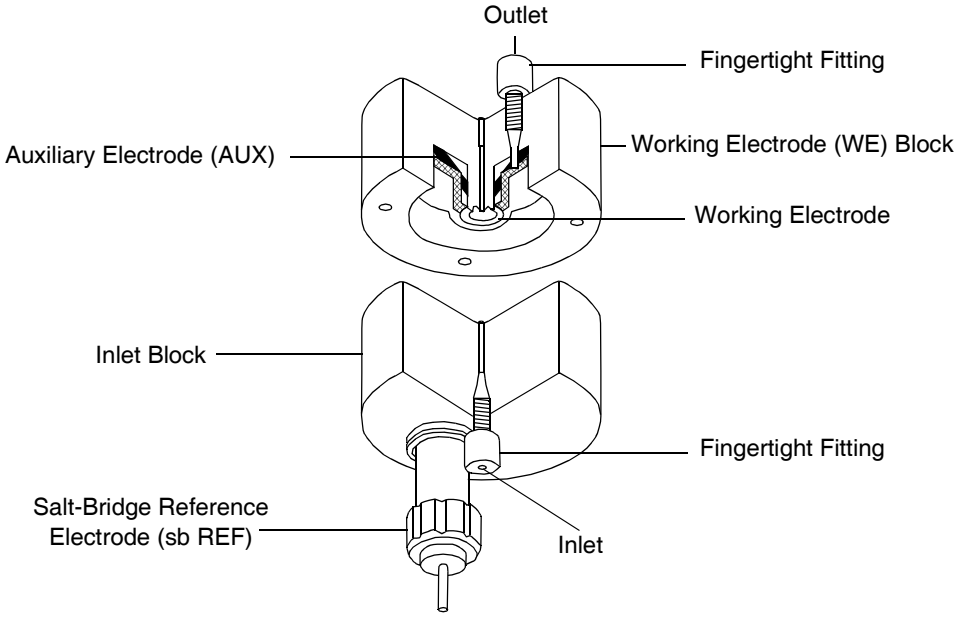


Figure 1-7 Flow Cell with a Salt-Bridge Reference Electrode

The in situ Ag/AgCl (ISAAC) reference electrode is recommended for standard applications. For special applications, the Hy-REF electrode is available. A third traditional reference electrode is the salt-bridge Ag/AgCl. This guide refers to the GC ISAAC reference electrode design as the most likely configuration for your use.

The 2465 Detector electrochemical flow cell has been developed for ultra-trace analysis in standard, microbore, and capillary LC-EC. The 2465 flow cell confined wall-jet configuration has proven to yield stable, reliable results. Considerable care is taken in the electrode material quality and finishing of the electrodes in the flow cell, which

contributes to overall performance of the detector. Additionally, this flow cell is simple to use and maintain.

The glassy carbon flow cell is tested before shipment. However, flow cells with a metal WE cannot be tested because the electrode surface changes chemically during use in an HPLC system with mobile phase. Performance of an electrochemical flow cell is best represented as the signal-to-noise ratio for a particular analyte selected by the user application.

1.4.2 Three-Electrode Potentiostat

The standard flow cell uses a three-electrode configuration (Figure 1-8). The working potential is set between the working electrode and the auxiliary electrode. The auxiliary electrode is kept at a precisely defined reference electrode potential by means of a potentiostat (an electronic feedback circuit that compensates for polarization effects at the electrodes).

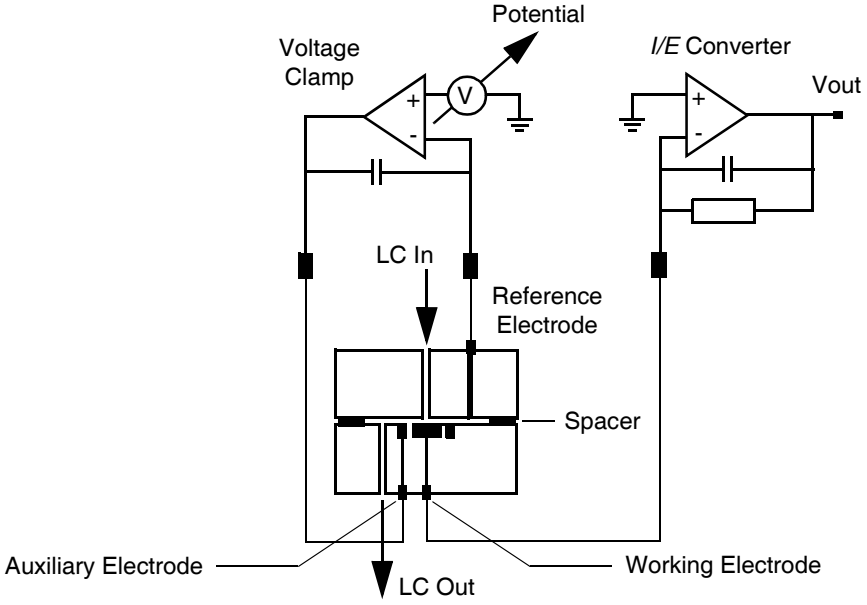


Figure 1-8 Three-Electrode Electrochemical Cell

At the working electrode, which is kept at virtual ground, the electrochemical reaction takes place as electrons are transferred. This results in an electrical current to the I/E converter. An integrator can monitor the output voltage. The oxidation or reduction

reaction requires only two electrodes. However, the three-electrode configuration of the 2465 Detector has the following advantages over a two-electrode configuration:

- If the working potential is applied only over the auxiliary electrode versus the working electrode (without the reference electrode), the working potential would continuously change due to polarization effects at the electrodes, resulting in highly unstable working conditions.
- If the working potential is applied only over the reference electrode versus the working electrode (without the auxiliary electrode), the working potential would be very well defined. However, the potential of the reference electrode is only well defined if the current drawn is extremely low (pico-amperes), making it difficult to miniaturize the reference electrode.
- A three-electrode configuration combines the best of both electrodes. The reference electrode stabilizes the working potential and the auxiliary electrode can supply high currents. This results in the large dynamic range of a three-electrode system.

1.5 Electrodes

This section describes the selection of:

- Working electrode materials and diameters
- Spacer thickness
- Reference electrodes

1.5.1 Materials for Working Electrodes

Electrochemical detection puts high demands on the working electrode material, so the working electrode should be made of an electrochemically inert material. To avoid an irregular flow profile over the electrode and keep a constant diffusion layer, the electrode requires a very smooth surface. The 2465 electrodes are manufactured and tested to ensure that the fabrication process attains this requirement. Finally, the analyte of interest must be oxidized (or reduced) with favorable *I/E* characteristics, and a high signal must be obtained at a low working potential. For most applications, glassy carbon is the working electrode material of choice.

Other materials are favorable under certain circumstances. For example, you can use a silver working electrode for the analysis of iodide. The following oxidation reaction occurs for iodide at the silver working electrode:

$$Ag + I^- \rightarrow AgI + e^-$$

where:

Ag = Silver

I = Iodide

e^- = Electron

This reaction already takes place at a very low working potential (1 mV), which results in extremely high selectivity (Table 1-2). This allows the determination of iodide in urine samples with minimal sample pretreatment.

Table 1-2 Potential Limits and Applications for Working Electrodes

WE Material	Alkaline Potential Limits (V)	Acidic Potential Limits (V)	Major Application
Glassy carbon (GC)	–1.50 to +0.60	–0.80 to +1.3	Catecholamines, neurotransmitters
Gold (Au)	–1.25 to +0.75	–0.35 to +1.1	Carbohydrates
Platinum (Pt)	–0.90 to +0.65	–0.20 to +1.3	Alcohols, glycols
Silver (Ag)	–1.20 to +0.1	–0.55 to +0.4	Halides, cyanide
Copper (Cu)	0 to +0.6	–	Amino acids, carbohydrates

Another consideration in choosing a working electrode is the oxidation or reduction of mobile phase constituents or working electrode material, which occurs when the potential exceeds the limits in Table 1-2. At high positive working potentials, the water in the mobile phase electrolyzes and results in a strong increase of background current and noise. Formation of metal oxides, which increase background current, is a limiting factor for metal electrodes. Glassy carbon and platinum have the highest positive potential limits and are therefore often used in oxidative electrochemical detection. The use of platinum electrodes for negative potentials is limited by the ease of reducing hydrogen ions to hydrogen gas.

1.5.2 Working Electrode Diameter

The size of the working electrode is an important factor in LC-EC because it affects both the signal and the noise. Several working electrode diameters (nominally 0.7, 2, and 3 mm) are available for the standard flow cell. In a standard LC system the signal and the noise increase linearly with the working electrode diameter, so the S/N ratio remains approximately the same.

In a micro-LC system, an increase in the working electrode diameter increases the noise more than the signal. Therefore, in micro-LC, a decrease of the working electrode diameter results in a better S/N ratio.

The choice for a flow cell is primarily based on the HPLC column diameter to obtain the best possible detection limit for a standard, microbore, or capillary column. The recommended combinations (Table 1-3) usually give the best S/N ratios. Other combinations that result in acceptable sensitivities are possible for many applications.

Table 1-3 Recommended Flow Cells with Different Columns

Column Diameter	Recommended Flow Cell
1 mm and larger	2-mm GC
Smaller than 1 mm	0.7-mm micro-GC

1.5.3 Spacer Thickness

The thickness of the spacer affects the linear flow velocity in the flow cell. The thinner the spacer, the smaller the cell volume, resulting in a higher linear flow velocity (Table 1-4).

Table 1-4 Flow Cell Volume with Spacers

Spacer (µm)	Cell Volume (µL) with 3-mm WE	Cell Volume (µL) with 2-mm WE	Cell Volume (µL) with 0.7-mm WE
25	0.15	0.08	0.011
50	0.29	0.16	0.022
120	0.71	0.38	0.053

The signal increases when using a thinner spacer, while the noise remains approximately constant, improving the signal-to-noise ratio (Figure 1-9).

Note: *Figure 1-9 applies only under diffusion limiting current conditions (where the signal is limited only by diffusion).*

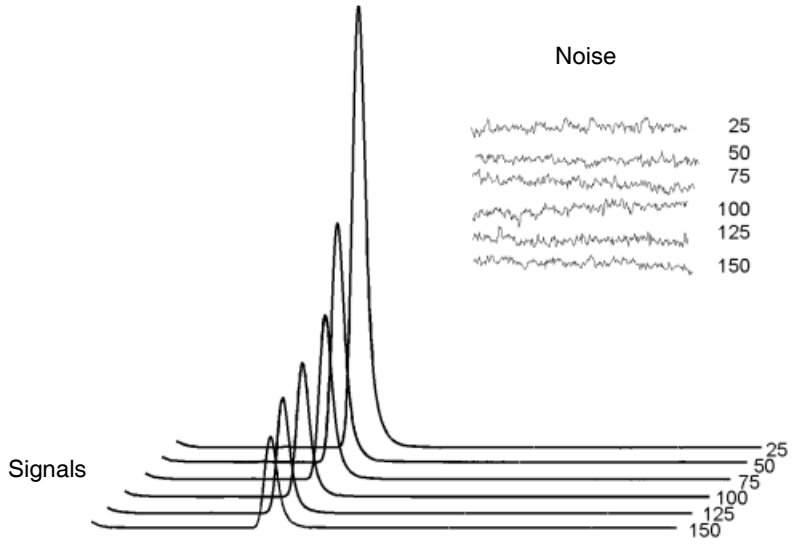


Figure 1-9 Signal and Noise for Norepinephrine with Different Spacers

For any given flow rate, decreasing the spacer thickness increases the pressure drop over the flow cell, and eventually obstructs the flow. The minimum spacer thickness available is 25 µm.

Note: *The flow cell requires one spacer. Apply the spacer with care. Overtightening the bolts can cause excessive pressure in the flow cell and increase the noise considerably.*

1.5.4 ISAAC Reference Electrode

The ISAAC reference electrode is a simpler-to-use substitute for the classic salt-bridge type of reference electrode (see Section 1.5.6). The ISAAC reference electrode is in direct contact with the mobile phase. Installation is simple and there are no air bubbles to check.

Note: *Waters recommends 2 mM chloride ions for the ISAAC reference electrode.*

Either potassium or sodium chloride may be used. In many cases sodium chloride is preferred, to avoid solubility issues in the presence of other substances, such as perchlorate, which is often used in the sample preparation stage of some samples.

Note: *The chloride concentration determines the potential. Therefore, each time a fresh mobile phase is prepared, it should contain the identical concentration of chloride ions.*

The standard electrode potential of the Ag/AgCl electrode (in a 1.0 M chloride ion solution) for the following half-reaction is defined as E^0:

$$AgCl(s) + e^- <=> Ag(s) + Cl^-$$

where $E^0 = 0.222$ V.

The potential of the reference electrode is dependent on the chloride concentration, as described by the following equation:

$$E_{ref} = E^0_{AgCl} - \frac{RT}{F} \bullet \ln[Cl^-]$$

where:

E^0 = Standard electrode potential

E_{ref} = Potential of reference electrode

R = Gas constant (8.314 J M^{-1} K^{-1})

T = Absolute temperature (293 K)

F = Faraday's constant

The potential (E) of the Ag/AgCl reference electrode at 2 mM KCl is 379 mV (Table 1-5). The potential difference (ΔE) with the salt-bridge reference electrode in saturated KCl is 189 mV. Therefore, if an application is running at 800 mV (versus Ag/AgCl with saturated KCl), the potential setting using the ISAAC reference electrode (versus Ag/AgCl in 2 mM KCl) is:

800 – 189 = 611 mV

Table 1-5 Potential of the Ag/AgCl Reference Electrode

Cl^- (mM)	$E_{Ag/AgCl}$ (mV)	ΔE (mV)
3500 (saturated KCl)	190	0
2500	199	9
1500	212	22
500	240	50
100	280	90
20	321	131
10	338	148
8.0	344	154
6.0	351	161

Table 1-5 Potential of the Ag/AgCl Reference Electrode *(Continued)*

Cl^- (mM)	$E_{Ag/AgCl}$ (mV)	ΔE (mV)
4.0	361	171
2.0	379	189
1.0	396	206
0.5	414	224

The use of chloride in the mobile phase dictates the following restrictions:

- The ISAAC reference electrode is not recommended at a high working potential (greater than 1 V versus Ag/AgCl in 2 mM KCl) because Cl^- is oxidized and contributes to the background current.
- In ion chromatography the addition of Cl^- may lead to undesired chromatographic changes when dilute buffers are used.
- Using a silver working electrode is not recommended with the ISAAC reference electrode because the addition of Cl^- to the mobile phase causes formation of an AgCl coating on the working electrode, leading to inactivation.

At high pH (above pH 10) or high modifier concentrations, the ISAAC reference electrode is less suitable and a Hy-REF electrode is recommended.

In addition, the ISAAC reference electrode is not usable with NH_4^+ buffers. The ISAAC reference electrode should not be used with buffers that contain ligands which can form Ag complexes such as NH_4^+.

Note: *Waters recommends 2 mM chloride ions for the ISAAC reference electrode.*

Maintaining a constant chloride concentration is required for the stable operation of the ISAAC reference electrode. The actual concentration of chloride can be affected not only by the presence of the potential applied, but also by the solubility equilibrium of all the species present in the mobile phase. The cases above are examples where the actual chloride concentration is changed, either due to chelation and/or precipitation, which results in lowering the actual concentration of chloride available to the reference electrode. This destabilizes the operation of the 2465 Detector ISAAC flow cell and is often manifested as a decreased response of analytes and increased noise during the analysis.

Table 1-6 shows the concentration of sodium chloride or potassium chloride needed to obtain various concentrations of chloride ions.

Table 1-6 Mass of Anhydrous Sodium and Potassium Chloride per Liter for Various Molar Concentrations

Cl^- Concentration (mMol/L)	Cl^- Concentration (Mol/L)	NaCl (g/L)	KCl (g/L)
0.1	0.0001	0.01	0.01
0.2	0.0002	0.01	0.01
0.5	0.0005	0.03	0.04
1	0.001	0.06	0.07
1.5	0.0015	0.09	0.11
2	0.002	0.12	0.15
3	0.003	0.18	0.22
4	0.004	0.23	0.30
5	0.005	0.29	0.37
10	0.010	0.58	0.75
15	0.015	0.88	1.12
20	0.020	1.17	1.49
25	0.025	1.46	1.86
30	0.03	1.75	2.24
50	0.05	2.92	3.73
100	0.1	5.84	7.46
500	0.5	29.22	37.28
1500	1.5	87.66	111.83
2500	2.5	146.10	186.38
3500	3.5	204.54	260.93

1.5.5 Hy-REF Reference Electrode

The Hy-REF is a hydrogen reference electrode, and its potential depends on the pH of the mobile phase. The Hy-REF electrode is fully comparable with the standard Ag/AgCl reference electrode for baseline stability and S/N ratio. The Hy-REF electrode is easier to use and virtually maintenance free because, like the ISAAC reference electrode, it does

not require a salt bridge. Air bubbles cannot be trapped (as when a salt-bridge Ag/AgCl is used). No filling solution or addition of Cl^- to the mobile phase is required, and the Hy-REF is compatible with high percentages of common organic modifiers such as methanol and acetonitrile.

Depending on the pH of the mobile phase, the potential setting of the working electrode compared to the Hy-REF electrode may differ significantly from that of the Ag/AgCl reference electrode. Current-potential (*I/E*) curves for the typical Ag/AgCl reference electrode show a shift of more than 200 mV at pH 3.1 (when working with catecholamines); no shift appears at pH 12 (such as with PAD of carbohydrates). Therefore, when using the Hy-REF electrode, first construct a hydrodynamic (or scanning) voltammogram. Table 1-7 shows the potential of the Hy-REF electrode and the Ag/AgCl electrodes at different pH values.

Table 1-7 Cell Potential (Hy-REF and Ag/AgCl Electrodes) Versus pH

pH	$E_{Hy\text{-}REF\ -\ Ag/AgCl}$ (mV)
3.3	232
6.2	130
7.5	90
11.8	0

If an Ag/AgCl REF is replaced by a Hy-REF electrode, the pH effect must be taken into account. The relationship is described by:

$$E_{Hy\text{-}REF} = E_{Ag/AgCl} - 328 + 29.9\ \text{pH}$$

where E = Potential.

For example, a working potential of 800 mV (versus an Ag/AgCl electrode with saturated KCl) at pH 3 has to be changed to:

$$E_{Hy\text{-}REF} = 800 - 328 + 29.9 \times 3 = 561.7\ \text{mV (versus a Hy-REF electrode)}$$

1.5.6 Salt-Bridge Ag/AgCl Reference Electrode

The reference electrode of the salt-bridge Ag/AgCl type consists of a silver rod coated with solid AgCl, immersed in a solution of saturated KCl containing KCl crystals (see Figure 4-8 for a schematic representation of the Ag/AgCl reference electrode). Electrical contact with the other electrodes in the flow cell is made through a salt bridge consisting of a wetted cotton wool frit, which is electrically conducting and slows down leakage of

KCl. The closed reservoir prevents contamination. This reference electrode for the standard flow cell is factory-filled with KCl.

For certain applications another chloride salt is required. For example:

- Sodium chloride must be used instead of potassium chloride in mobile phases containing perchlorate because potassium perchlorate precipitates and clogs the cotton wool frit.
- At high modifier percentages, the reference electrode must be filled with lithium chloride because the potassium salt is prone to precipitation.

1.6 Principles of Detector Operation

To use the 2465 Detector effectively, you should be familiar with the operational modes, the fluidics including the configured flow cell and electrodes, the electronic design of the detector, and the theory and principles of operation. The operational modes of the 2465 Detector are:

- Section 1.6.1, DC Mode
- Section 1.6.2, Pulse (PAD) Mode
- Section 1.6.3, Scan Mode

1.6.1 DC Mode

Direct current (DC) mode measures the current of a sample at a fixed potential. A *hydrodynamic voltammogram* is constructed using DC mode by running several chromatograms at different working potentials (see Section 3.7.2, Constructing a Scanning Voltammogram). Both peak height and background current are plotted against the working potential as part of method optimization.

DC Mode Primary Parameters

The primary parameters used in DC mode are as follows:

- **Potential** – Cell potential (E_c), measured in volts.
- **Range/Sensitivity** – Parameter that sets the current range so that the largest peaks are on scale; similar to attenuation in an absorbance detector.
- **Filter Time Constant** – A filter that is applied to smooth the data. High frequency noise is efficiently removed and chromatographic peaks can be detected with a better signal-to-noise ratio.

- **Max Compensation** – The largest background current that can be autozeroed. It is determined by the Range/Sensitivity setting. This is the maximum range attainable when the detector is autozeroed. The cell potential reading on the display shows the actual current, while the analog output voltage is set to zero. When the autozero is executed, the autozero range varies with output analog potential range.
- **Offset** – Compensation for background current, expressed as a percentage of the range setting. You can set a maximum offset of +50% and –50% in 5% steps. For example, 20% is a 200-mV offset when the maximum output is 1.0 V (2 V at maximum 10.0 V).
- **Polarity** – Setting of the data signal as positive or negative; applies to the digital and analog outputs. The polarity of the output can be reversed. Oxidative and reductive analysis have opposite currents. For data acquisition, chromatographic peaks traditionally have a positive amplitude.
- **Temperature** – Temperature control of the detector oven (with mobile phase, flow cell, and column) to improve performance. Electrochemistry is susceptible to temperature fluctuations because the mobile phase oxidation or reduction contributes significantly to the background noise, and these reactions are influenced by changes in room temperature. Elevation of the temperature above ambient can reduce the sensitivity of electrochemistry to environmental changes.
- **Methods Programmability** – Ability to program the 2465 Detector (using timed events to change the parameters) and run using the Events feature. Method time files 1 to 5 are reserved for DC mode. The parameters are as follows:
  - Potential (cell potential E_c)
  - Filter Time Constant
  - Range
  - Offset
  - Auto Zero Enable
  - Output Events
  - End Cycle or Run Time

1.6.2 Pulse (PAD) Mode

Pulsed amperometric detection (PAD) or pulse mode regenerates the working electrode at a frequency of 0.5 to 3 Hz by applying a series of potential changes. This is particularly useful for certain applications where the working electrode is rapidly fouled due to adsorption of insoluble reaction products. A well-known application area of PAD is analysis of carbohydrates.

Pulse mode differs from DC mode as follows:

- The output signal is sampled during only a part of the total pulse cycle. During the sampling time (t_s) the signal generated at the working electrode is collected, and this value is sent to the detector output. The output is refreshed each pulse cycle. Therefore, the frequency of data output is determined by the pulse duration.
- When the data acquisition rate of the data system or integrator is higher than the pulse cycle frequency, a typical stepwise pattern may appear in the chromatogram. Ideally, the data acquisition rate should be matched to the detector output. The data system's sampling rate should be no less than twice the signal frequency, expressed as points per second.
- The background or cell current is usually higher in pulse mode (100 to 1000 nA) than in DC mode. Only the nano- and microampere ranges are available in the pulse mode.
- After prolonged use of the flow cell with a gold working electrode in pulse mode, a brown precipitate forms on the auxiliary electrode. This coating may electrically isolate the auxiliary electrode and increase the noise. Cleaning the auxiliary electrode approximately every six months with steel wool prevents this.

Caution: *To prevent damage to the working electrode, do not touch the working electrode with steel wool.*

- Hy-REF electrodes are maintenance free and are particularly appropriate for carbohydrate analysis. Ag/AgCl reference electrodes are less suitable due to silver oxide formation, and they require regular (monthly) maintenance.
- For carbohydrate analysis, only CO_2-free sodium hydroxide should be used since carbonate anions may disturb the ion exchange chromatography. The CO_2-free sodium hydroxide is available from several suppliers as a 50% solution (19.2 M). NaOH pellets are not recommended because of their high CO_2 content.

  Carbonate-free solutions are best prepared by adding the appropriate volume of 50% (wgt:wgt) sodium hydroxide solution to CO_2-free HPLC-grade water. Boiling and vacuum sonication remove CO_2 from water before the addition of NaOH.
- The accuracy of certain pH electrodes is poor at high pH. For applications at high pH it is sometimes better to calculate the pH from the OH^- concentration.
- Organic modifiers (acetonitrile) are not recommended.

Pulse Settings

In PAD of carbohydrates, the working potential is applied as a series of three potential steps during t_1, t_2, and t_3 (Figure 1-10).

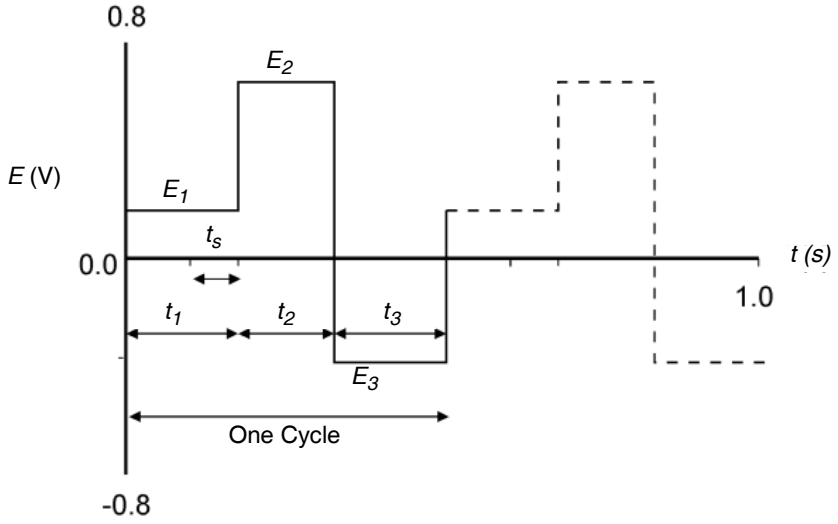


Figure 1-10 Potential Steps in Pulsed Amperometric Detection

The sequence of events during one cycle of PAD is as follows:

1. ***E_1, t_1,* Measurement** – The detection potential is applied. Detection and data collection occur during time interval t_s (sampling time). The time difference $t_1 - t_s$ is the stabilization time.
2. ***E_2, t_2,* Cleaning** – A monolayer of metal oxide forms at the working electrode due to the high positive potential.
3. ***E_3, t_3,* Conditioning** – The metal oxide monolayer that is formed during t_2 is electrochemically reduced by applying a negative potential, renewing the electrode surface.

Optimizing Wave Forms

LaCourse and Johnson published several papers on the optimization of wave forms in PAD. Several considerations are important for the choice of the pulse duration. Optimization depends on the working electrode material, the sample constituents, and the required detection frequency. The impression may arise that the number of variables (three potential steps and four time settings) may lead to a time-consuming optimization procedure, but in practice, pulse mode is straightforward.

The WE material determines the potential for the cleaning steps, E_2 and E_3. At alkaline pH, gold oxide is already formed at E_2 > +200 mV (versus Ag/AgCl). At a higher potential, the formation of a metal oxide layer is accelerated and you may choose a shorter time setting. In practice, an E_2 value of +650 to +750 mV during 200 ms (t_2) gives good results.

The choice of t_3 depends on the potential E_3 and the t_2 and E_2 setting. The duration of t_3 and the magnitude of E_3 must be large enough to completely remove the metal oxide. Reductive dissolution already occurs at E_3 < 0 mV, but a more negative voltage speeds up this process (Table 1-8). For example, an E_3 value of –800 mV during 200 ms or –300 mV during 360 ms can be used.

Table 1-8 Selection of Pulse Parameters

Pulse	Parameter	Desired Value[a]	Duration	Sampling
1	E_1	0	400 (t_1)	100 (t_s)
2	E_2	+650	200 (t_2)	
3	E_3	–650	200 (t_3)	

a. mV relative to the peak potential for the reduction of gold oxide

The measured potential is compound dependent, and literature data can be used as a starting point for further optimization. Andrews and King describe the optimization of waveforms and the selection of pulse potentials.

A sampling time (t_s) can be chosen between 20 and 100 ms in 20-ms steps. These are multiples of 50 Hz to prevent noise due to oscillations of the AC power supply. Increasing t_s results in an increased signal up to a certain limit. A limiting factor is the accumulation of adsorbed species at the working electrode, attenuating the signal. Another consideration for all time settings is that increasing the time decreases the detection frequency.

Stabilization Time

Before sampling, a stabilization time is applied (t_1), which determines the level of the background current. For example, if t_1 = 100 ms and t_s = 100 ms, the current is not stabilized before sampling ($t_1 - t_s$ = 0 ms). Depending on the potential settings of E_2 and E_3, a large positive or negative background current (microamperes) may be detected, seriously limiting the detection. A stabilization time of 100 to 400 ms is often used.

Working Electrode Materials for Pulse Mode

Gold and platinum are used as working electrodes for pulse mode. The change in cell current during pulse mode is illustrated in Figure 1-11 for gold (Au), platinum (Pt), and glassy carbon (GC) working electrodes. When the potential is changed, a large charging current is detected (Figure 1-11, parts 1, 3, and 5), followed by stabilization of the current (parts 2, 4, and 6). The output signal is sampled during a fraction of part 2, depending on the pulse settings.

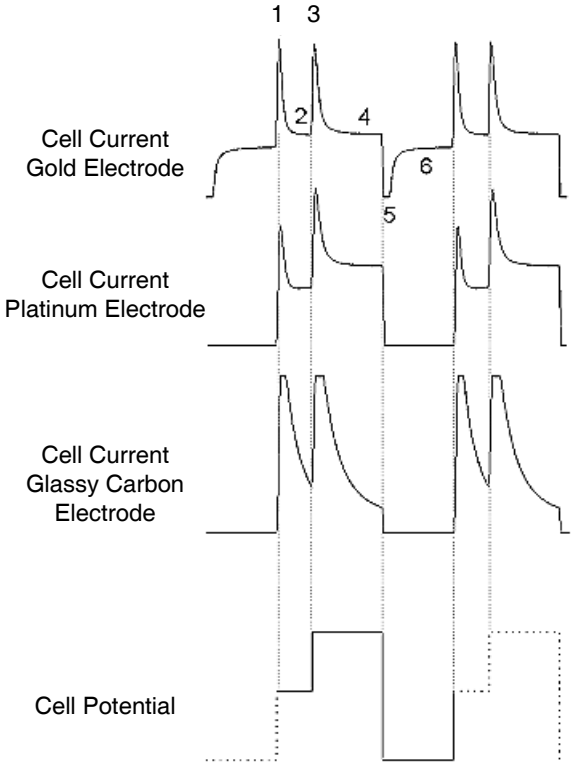


Figure 1-11 Change in Cell Current During PAD

The cell current of the noble metals gold and platinum is stabilized faster than the cell current of glassy carbon due to the much lower capacitance of the noble metals. For platinum and glassy carbon, the negative peaks run far off-scale. However, the profile is similar to the mirror image of the positive peaks.

Data Acquisition in Pulse Mode

An important difference between DC mode and pulse mode is the frequency of the output signal. In DC mode the signal is produced continuously up to 10 Hz, but in pulse mode the sum of t_1, t_2, and t_3 determines the observed data acquisition rate. A new data point is generated during each measurement pulse, and once every cycle, the t_s signal is sent to the output. This can be visualized by magnification of a peak in the chromatogram (Figure 1-12).

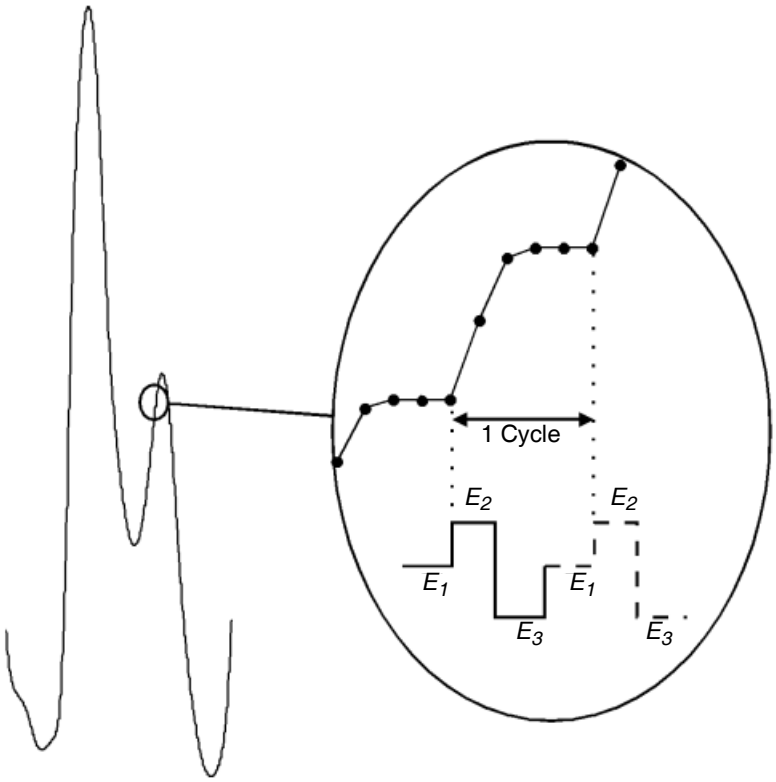


Figure 1-12 Magnified View of a Chromatogram Obtained with PAD

In pulse mode, the data sampling time occurs only at t_1 in the cycle (where cycle = $t_1 + t_2 + t_3$). For example, if $t_1 = t_2 = t_3 = 100$ ms, then $t_{cycle} = 300$ ms or approximately 3 points per second. Data acquisition at a higher rate is unnecessary because this results in acquisition of multiple data points containing the same output value (Figure 1-13, A and B). Matching the pulse cycle to the data rate keeps the peak shape unchanged (C). Decreasing the data rate to less than half the pulse frequency changes the peak shape (E).

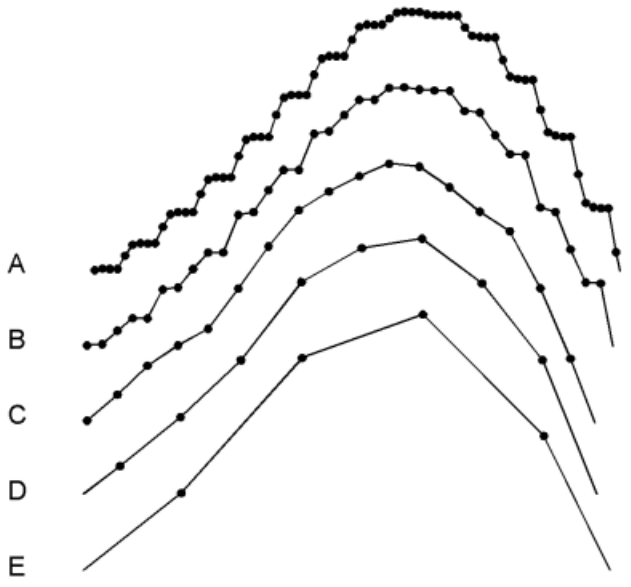


Figure 1-13 Chromatograms Acquired at Different Data Rates

Scan in Figure 1-13	Pulse Cycle Time Factor
A	5.0
B	2.5
C	1.2
D	0.6
E	0.3

Pulse Mode Primary Parameters

In PAD for carbohydrate detection, the working potential is applied as a series of three potentials: t_1, t_2, and t_3 (see Figure 1-12). These timed events during one cycle of PAD correspond to changes in potential applied across the flow cell electrodes. The following primary parameters are available for the timed events:

- t_1 – Time when the detection potential is applied. Detection and data collection occur during time interval or sampling total time.
- t_2 – Time when a monolayer of metal oxide forms at the working electrode due to the high positive potential.

- t_3 – Time when the metal oxide monolayer that is formed during t_2 is electrochemically removed by applying a negative potential, renewing the electrode surface.
- t_s – Sampling time when the current is monitored.
- **Potential E_1, E_2, and E_3** – Applied cell potentials at times t_1, t_2, and t_3, respectively.
- **Range/Sensitivity** – User setting for the current range so that the largest peaks are on scale; equivalent to attenuation in an absorbance detector. The effective working range for pulse mode is lower than for DC mode.
- **Max Compensation** – The largest background current that can be autozeroed. It is determined by the Range/Sensitivity setting. This is the maximum current that can be autozeroed from the analog signal. The potential range for pulse mode is limited as the current is higher so a small range is available.
- **Offset** – Compensation for background current, expressed as a percentage of the range setting.
- **Polarity** – Applies to the digital and analog outputs; allows you to set the data signal as positive or negative.
- **Temperature** – Control of the temperature of the mobile phase, flow cell, and column to significantly improve performance. By elevating the temperature above ambient, the sensitivity of electrochemistry to environmental changes can be reduced. Electrochemistry is susceptible to temperature because the mobile phase oxidation or reduction contributes significantly to the background noise, and these reactions are influenced by changes in room temperature.
- **Methods Programmability** – Allows you to program the 2465 Detector using timed events to change the parameters. Method time files 6 to 9 are reserved for pulse mode. You can change the following parameters:
  - Potential (cell potentials E_1, E_2, and E_3)
  - Filter Time Constant
  - Range
  - Offset
  - Auto Zero Enable
  - Output Events
  - End Cycle or Run Time

1.6.3 Scan Mode

Scanning Voltammograms

Scan mode of the 2465 Detector optimizes working potential by performing a *scanning voltammogram* (Figure 1-14), which plots *E* (potential) in volts versus *I* (current) in nanoamps. A scanning voltammogram is a forward scan of a substance and an eluent under continuous flow with no column. The pure standard, dissolved in mobile phase, must be available for each peak to be optimized. The voltage runs between two preset values and the current is measured. Scans of the substance in eluent are compared with eluent alone, and the greatest area of difference between the two curves is determined. A high *E* gives a maximum signal and more noise, but a low *E* gives less interference, so the best signal-to-noise ratio (S/N) is a compromise.

A scanning voltammogram can be obtained when pure analyte is available for all compounds of interest. A *half scan* sweeps the potential from low to high voltage setting *E*. A *full scan* includes the forward and reverse scans, from low to high and back to low. In *continuous mode,* the voltage sweeps up and down between both potentials.

Figure 1-14 shows the scanning voltammograms of 2,4-dimethylphenol (1 – DMP), phenol (2 – P), 2-chlorophenol (3 – CP), 4-nitrophenol (4 – NP), and buffer (5 – blank).

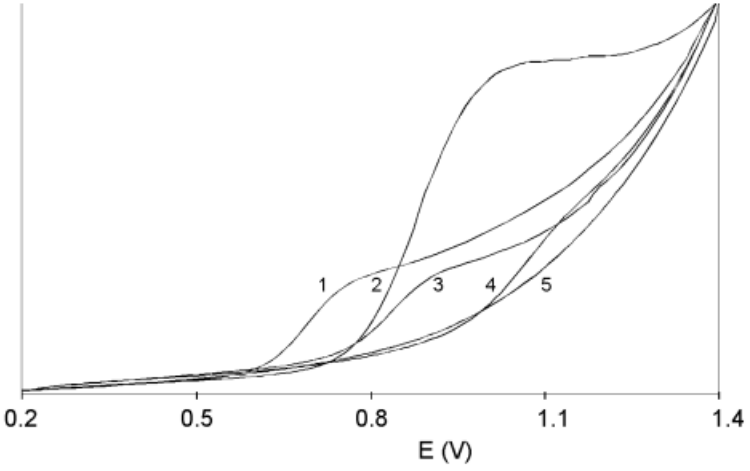


Figure 1-14 Examples of Scanning Voltammograms

Scan Mode Primary Parameters

- ***E_1* Potential** – Potential applied at the beginning of the scan cycle.
- ***E_2* Potential** – Potential reached at the end of the scan cycle.
- **Scan Cycle** – Length of the cycle, which can be half, full, or continuous. *Half* indicates a single ramp between E_1 and E_2. *Full* indicates a ramp from E_1 to E_2, then from E_2 to E_1. *Continuous* indicates that the full ramp is repeatedly cycled.
- **Range/Sensitivity** – Setting for the current range so that the scan current remains on scale; equivalent to attenuation in an absorbance detector.
- **Offset** – Compensation for background current, expressed as a percentage of the range setting.
- **Temperature** – Control of the temperature of the mobile phase, flow cell, and column to significantly improve performance. By elevating the temperature above ambient, the sensitivity of electrochemistry to environmental changes can be reduced. Electrochemistry is susceptible to temperature because the mobile phase oxidation or reduction contributes significantly to the background noise, and these reactions are influenced by changes in room temperature.

Note: *The Auto Zero Enable key is available during the scan.*

1.7 References

The following references contain additional information:

- R.W. Andrews and R.M. King, “Selection of potentials for pulsed amperometric detection of carbohydrates at gold electrodes,” *Anal. Chem.*, 62: 1990, pp. 2130–2134.
- D.C. Johnson, D. Dobberpuhl, R. Roberts, and P. Vandeberg, “Review. Pulsed amperometric detection of carbohydrates, amines and sulphur species in ion chromatography - the current state of research,” *J. Chromatogr.*, Vol. 640: 1993, pp. 79–96.
- D.C. Johnson and W.R. LaCourse, “LC with pulsed ECD at gold and platinum electrodes,” *Anal. Chem.*, Vol. 62: 1990, pp. 589A–597A.
- W.R. LaCourse and D.C. Johnson, “Optimization of waveforms for pulsed amperometric detection of carbohydrates following separation by LC,” *Carbohydrate Research*, Vol. 215: 1991, pp. 159–178.
- W.R. LaCourse and D.C. Johnson, “Optimization of waveforms for pulsed amperometric detection of carbohydrates based on pulsed voltammetry,” *Anal. Chem.* Vol. 65: 1993, pp. 50–55.

1

Chapter 2
Installing the 2465 Detector

Use this chapter to install the 2465 Detector (Figure 2-1).

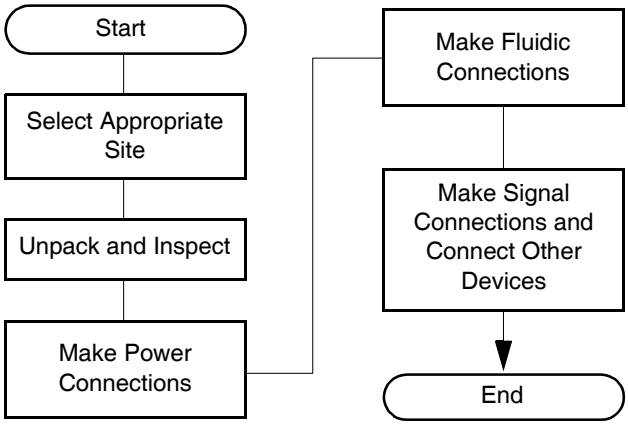


Figure 2-1 Major Steps for Installing the 2465 Detector

2.1 Site Selection and Power Requirements

2.1.1 Site Selection

Figure 2-2 shows the dimensions of the 2465 Detector. Install the 2465 Detector in an area that meets the requirements in Table 2-1.

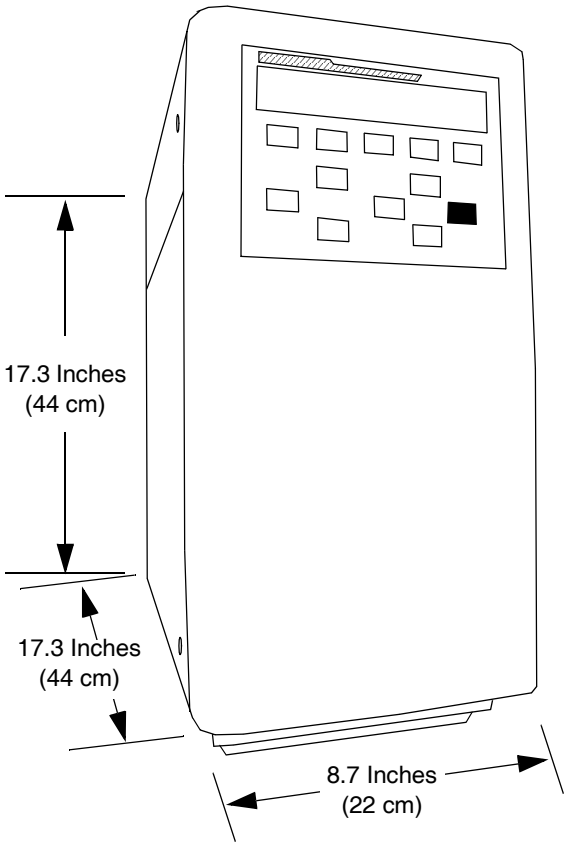


Figure 2-2 Dimensions of the 2465 Detector

Table 2-1 Installation Site Requirements

Parameter	Requirement
Operating temperature range	39 to 104 °F (4 to 40 °C)
Storage temperature range	–104 to 158 °F (–40 to 70 °C)
Relative humidity range	20 to 80%, noncondensing
Storage humidity range	0 to 90%, noncondensing
Bench space	6 inches (15.25 cm) clearance at rear; access to power switch and power cord; clean, level, smooth surface
Vibration	Negligible
Static electricity	Negligible

Table 2-1 Installation Site Requirements *(Continued)*

Parameter	Requirement
Power	Grounded AC, 100 to 240 VAC (auto-selecting), 50/60 Hz
Weight	30.9 lb (14 kg) without flow cell or column

Attention: *You must mount the 2465 Detector on a level surface to allow the drip management system (drain tube) to work properly. You can connect a drain tube to a waste container to collect solvent leaks from the detector oven.*

A clear, level, smooth surface is required to allow the ventilation system under the 2465 Detector to work properly.

Ensure that the power (on/off) switch and power cord are always accessible.

2.1.2 Power Requirements

The 2465 Detector requires:

- Grounded alternating current (AC) power source
- Minimal power transients and fluctuations
- Line voltage of 100 to 240 VAC; power is auto-selecting (100 to 240 V)
- Power consumption of 260 Volt Amps (VA)
- Two 2.5-AT fuses

Caution: *To avoid electrical shock, power off the 2465 Detector and unplug the power cord before you make connections or replace a fuse.*

Attention: *Use shielded cable(s) for all I/O connections with other devices. Thoroughly connect the shielding to common.*

The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.

2.2 Unpacking and Inspecting the 2465 Detector

The 2465 Detector is packed and shipped in one carton that contains the following items:

- Waters 2465 Electrochemical Detector
- Power cord (110 V or 240 V, as appropriate for your location)
- *Certificate of Validation*
- *Declaration of Conformity*
- *Waters 2465 Electrochemical Detector Operator's Guide*
- Startup Kit (Table B-8)
- Flow cell (not shipped in the 2465 Detector carton; at least one flow cell must be purchased separately). Table B-1 lists the flow cells that are available. Table B-2 through Table B-7 list the components in each flow cell kit.

To unpack the 2465 Detector:

1. Inspect the carton for possible damage when it arrives. Immediately inform the transport company if you see any damage.
2. Remove the packing material, accessory kit, envelope, and power cord from the carton.

   Note: *Keep the carton and packing material for future transport and/or storage as they are designed for optimum protection.*

3. With both hands on the sides of the 2465 Detector, lift it from the carton (Figure 2-3).

   Attention: *To prevent damage to the door, never lift the 2465 Detector by its door, but only by its sides.*

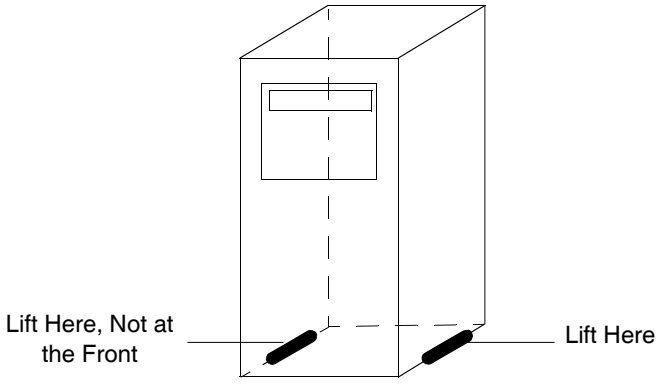


Figure 2-3 Unpacking the 2465 Detector

4. Remove the plastic wrap.
5. Inspect the detector carefully for possible damage and make sure that all ordered items are included.

   Note: *Contact your supplier if you see any damage or if any items are not included.*

6. Ensure that the instrument serial number, located on the left side panel, corresponds to the number on the *Certificate of Structural Validation*.
7. If you see any damage or discrepancy when you inspect the contents of the carton, immediately contact the shipping agent and Waters Technical Service at 800 252-4752, for *U.S. and Canadian customers*. Other customers, call your local Waters subsidiary or your local Waters Technical Service Representative, or call Waters corporate headquarters in Milford, Massachusetts 01757 (U.S.A.).

2.3 Making Electrical Power Connections

To connect the 2465 Detector to the AC power supply:

1. Make sure that the power switch is in the 0 (off) position (Figure 2-4).
2. Plug the receptacle end of the power cord into the AC input connector on the rear panel of the detector.
3. Plug the other end of the power cord into a properly grounded AC power source.

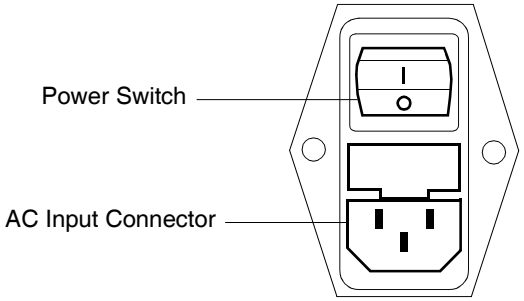


Figure 2-4 Connecting the Power Cord

4. Ensure the detector is placed on a flat, smooth, and level surface. Do not block the fan located at the bottom of the detector (Figure 2-5). Blocking the fan impairs the cooling capability of the power supply.

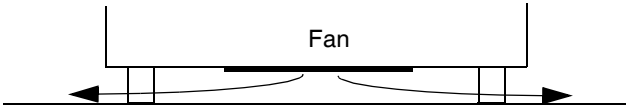


Figure 2-5 Venting the Detector

2.4 Making Fluidic Connections

The controlled detector oven, which optimizes the detection stability in the 2465 Detector, contains the flow cell and the HPLC column. The detector oven has an integrated Faraday cage and is accurately thermostatted to ensure stable working conditions. Installing the flow cell and column within a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

The installation procedures for the fluidic components are listed in Figure 2-6.

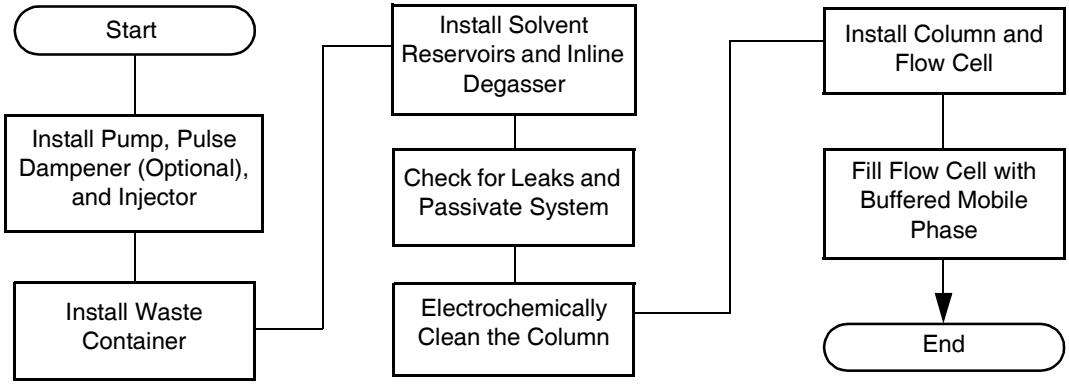


Figure 2-6 Making the HPLC Connections

Required Materials

- Flow cell (standard or micro)
- Tubing

Materials Required But Not Supplied

STOP ***Attention:*** *The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.*

STOP ***Attention:*** *The pump must be able to deliver flow rates between the following flow cell specifications:*

- *Flow rate for standard flow cell: 25 µL/min to 2.0 mL/min*
- *Flow rate for micro flow cell: 1 µL/min to 2.0 mL/min*
- *Maximum pressure, standard flow rate: 40 psi (2.76 bar, 276 kPa)*

STOP ***Attention:*** *Always position the 2465 Detector as the last detector in the series (if the system includes more than one detector).*

- HPLC pump
- Mobile phase(s) in reservoir(s)
  - HPLC-grade water

– HPLC buffer; for ISAAC reference electrode only, with 2 mM chloride ions (KCl or NaCl)

– 50% methanol in HPLC-grade water for reversed-phase HPLC

- HPLC column, preconditioned
- Drip management system, tubing, and waste container

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Caution: *Wear protective clothing when handling 15% nitric acid.*

- 15% nitric acid for passivation

Attention: *Passivation should include only the metal parts of the HPLC system. Disconnect all parts that are not acid-resistant (such as nylon inlet filters, degasser, pulse dampener, column, and flow cell) during passivation. Do not expose columns or flow cells to 15% nitric acid.*

- Helium (certain applications)
- Stainless steel tubing (certain applications)
- PEEK® tubing

Attention: *Do not use a metal frit to filter the mobile phase.*

Optional Materials

- Solvent inlet filters (recommended)
- Solvent tray, up to 14.25 inches (36.2 cm) × 8.25 inches (21.0 cm)
- Inline degasser (either vacuum degassing or sparging with helium)
- Pulse dampener
- Injector
- Inline filters and/or guard column

Attention: *The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.*

Attention: *The 2465 Detector uses standard analytical flow cells that are pressure-rated at 40 psi (2.76 bar, 276 kPa). The micro flow cells are also pressure-rated at 40 psi (2.76 bar, 276 kPa). To prevent damage, do not connect any tubing or device that might cause backpressure to exceed the pressure rating of the tubing or flow cell.*

Attention: *Do not operate the 2465 Detector with the standard flow cell above a flow rate of 2.0 mL/min or 40 psi (2.76 bar, 276 kPa) backpressure. With a micro flow cell, do not exceed 25 µL/min or 40 psi (2.76 bar, 276 kPa) backpressure. This can cause the pressure to exceed the rating of the tubing or flow cell.*

2.4.1 Installing the 2465 Detector

To install the 2465 Detector:

1. Remove any tape or protective straps or foam packing from the detector and open the door from the left side (Figure 2-7).

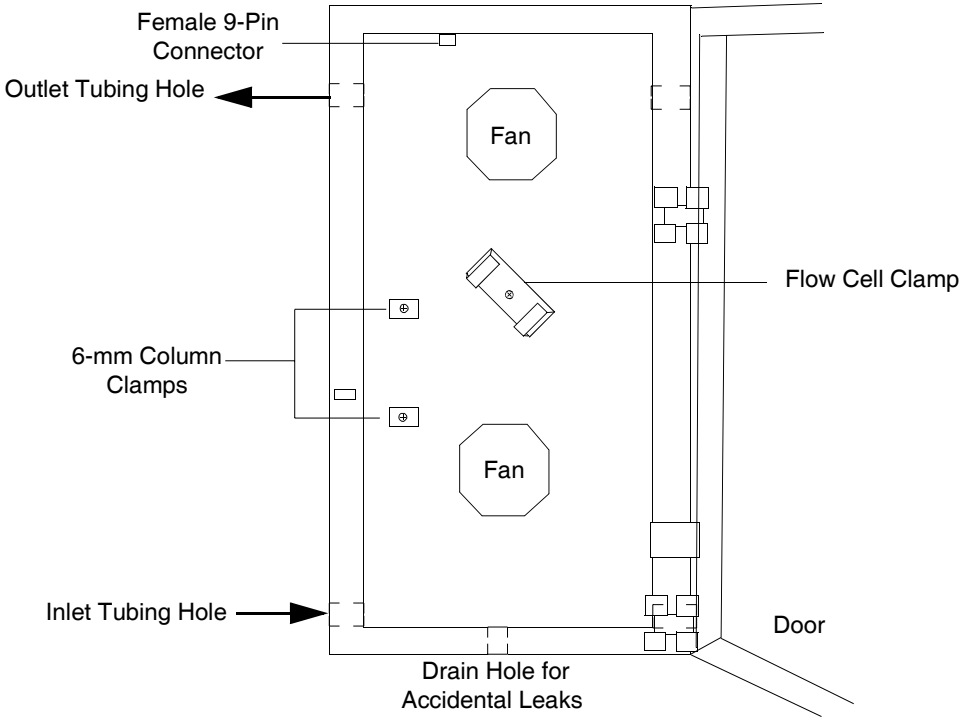


Figure 2-7 2465 Detector Oven

2. If your column requires column clamps larger than the 6-mm clamps already installed, remove the 6-mm clamps and install two 12-mm column clamps.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

3. Install a pump (see the manufacturer's instructions), pulse dampener (optional), waste container, solvent tray (optional), and mobile phase reservoir (Figure 2-8):
   a. Connect tubing from the pump to the pulse dampener.
   b. Connect tubing from the pulse dampener to the waste container, temporarily bypassing the detector.

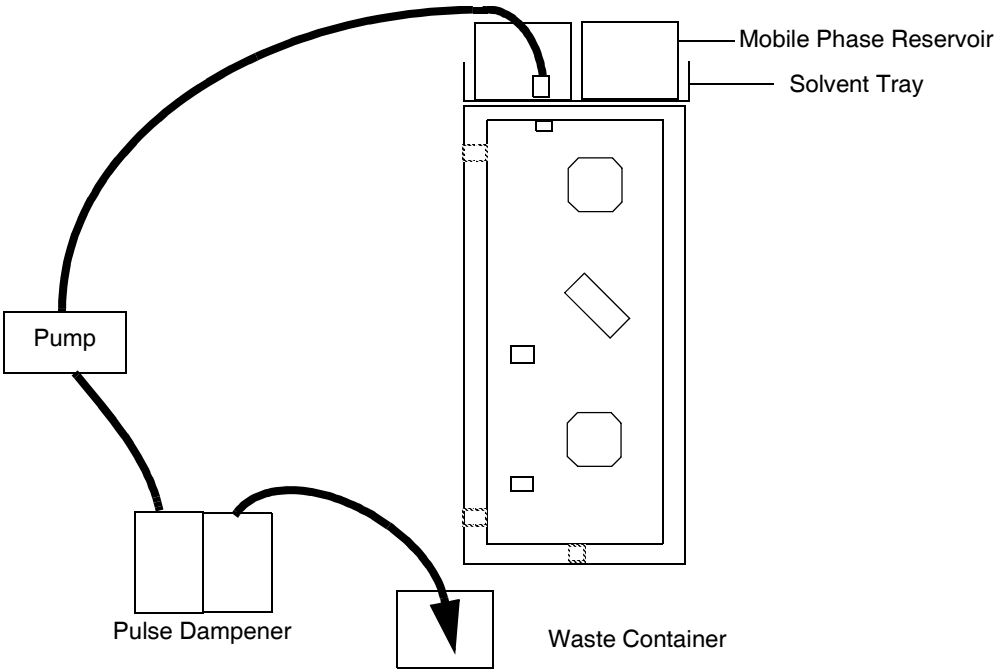


Figure 2-8 Installing the External Pump and Pulse Dampener

Note: *Waters recommends that the mobile phase contain 2 mM chloride (KCl or NaCl) ions when using the ISAAC reference electrode.*

   c. Fill a mobile phase reservoir with mobile phase. Place inlet filters on the tubing. Place a solvent tray on top of the detector, then place the reservoirs in the solvent tray.

Attention: *Do not use a metal frit to filter the mobile phase.*

d. Connect the tubing from the mobile phase reservoir to the pump.

4. You can install an inline degasser (optional) to prevent passage of air bubbles through the flow cell.

   Note: *Air bubbles passing through the flow cell can create unacceptable noise levels and spikes.*

5. Power on the pump and ensure that the system has no leaks, then stop the pump.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Caution: *Wear protective clothing when handling 15% nitric acid.*

Attention: *Ensure that all parts that are not acid-resistant (such as the nylon inlet filters, column, and flow cell) are disconnected during passivation. Do not passivate the 2465 Detector flow cell.*

6. Passivate all metal parts of the HPLC system (except parts that are not acid-resistant) as follows:

   a. Make sure that all parts that are not acid-resistant (such as nylon inlet filters, column, and flow cell) are *not* connected during this step.

   b. Disconnect the mobile phase reservoir and connect a reservoir containing 15% nitric acid.

   c. Flush nitric acid for 20 minutes through the pump, pump tubing, and dampener, then to the waste container.

   d. Thoroughly flush the HPLC system with HPLC-grade water. Check the pH of the effluent until it is neutral to make sure that all traces of nitric acid are flushed from the tubing and pulse dampener.

Attention: *If you plan to use an ISAAC reference electrode, flush the system with mobile phase containing 2 mM chloride (KCl or NaCl) ions.*

e. Remove the temporary tubing between the pulse dampener and the waste container.

f. Place tubing through the upper hole in the chassis and connect it to the waste container. Secure the tubing in the waste container with the tubing touching the side.

Note: *Set a waste tray under the detector to collect any leaks from the detector oven. Do not block the fan located at the bottom of the detector (Figure 2-5). Blocking the fan impairs the cooling capability of the power supply.*

g. Place tubing through the lower hole and connect the mobile phase reservoir to the pump. Place the reservoir higher than the flow cell, such as in the solvent tray on top of the detector.

2.4.2 Connecting a Column

The column must be electrochemically clean for use in the 2465 Detector. A new column is not considered electrochemically clean. See the manufacturer's instructions for more information.

Select the appropriate size flow cell and spacer for your column ID (see Table 1-3).

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

To connect the tubing to the column:

1. Attach a compression fitting and ferrule.
2. Connect the inlet tubing from the pump to the pulse dampener (optional) and autosampler (optional), then to the flow cell. Ensure the tubing is seated firmly, then tighten the compression screw.
3. Connect waste tubing to the column outlet and route the tubing through the top hole in the side of the chassis to a waste container. To provide constant backpressure, place the waste container above the 2465 Detector in a solvent tray, and ensure the tubing touches the side of the container.
4. Install the column in the column clamps.
5. Thoroughly precondition the column to ensure that it is electrochemically clean.

Attention: *To prevent unacceptably high background current and substantial fouling of the working electrode, use a column that is electrochemically clean. A new column is* ***not*** *electrochemically clean.*

6. For reversed-phase (RP-HPLC) columns:
   a. Flush with 50% methanol in water for 3 days at a low flow rate. Drain to a covered waste container.
   b. Flush with 10 column volumes of HPLC-grade water before switching to the mobile phase to prevent precipitation of buffer salts.
7. Equilibrate the column by running 10 column volumes of buffered mobile phase through the column.
8. Once the column is clean, place an inline filter after the injector and before the column to protect both the column and the flow cell from particulates.
9. If the 2465 Detector is used for reductive ECD (at a negative working potential), take the following additional actions to remove oxygen from the mobile phase:
   - Degas with helium.
   - Use stainless steel tubing everywhere (because it is impermeable to oxygen), except when it is in direct contact with the flow cell (due to possible electrical interference).

Attention: *Waters strongly recommends using an inline filter (0.2 µm) and/or guard column before the column to protect the flow cell and column from sample and solvent particles.*

Attention: *If you place mobile phase reservoirs on top of the 2465 Detector, a solvent tray is required. Maximum size for a solvent tray on top of the detector is 14.25 inches (36.2 cm) deep × 8.25 inches (21.0 cm) wide.*

2.4.3 Installing the Flow Cell

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Note: *Familiarize yourself with the force on the flow cell bolts, since overtightening of the bolts markedly deteriorates the S/N ratio and eventually the cell itself. The flow cell is assembled properly when it arrives and the force on the bolts is preset to 30 Ncm. To*

ensure the best performance, the black marks on both blocks should line up. To prevent overtightening, loosen the bolts, tighten them until fingertight, then tighten them 1/4-turn more.

Note: *The ISAAC reference electrode requires chloride ions in the mobile phase. Waters recommends 2 mM chloride ions (KCl or NaCl). Add and equilibrate before installing the ISAAC reference electrode.*

To install the flow cell:

1. Connect the column outlet to the flow cell inlet using small-bore tubing (0.3 mm ID). Use one fingertight fitting to install tubing in the flow cell inlet (Figure 2-9).

Attention: *Use only factory-supplied fingertight fittings in the flow cell, because others may cause serious damage. Let the tubing protrude approximately 0.6 inch (1.5 cm) from the fitting and tighten it such that the tubing is not or slightly indented by the fitting. Do not overtighten the fitting because overtightening affects the flow pattern through the tubing (turbulence) and may strongly decrease the flow cell performance.*

Note: *To prevent entrapment of bubbles, ensure that LC Out is on top.*

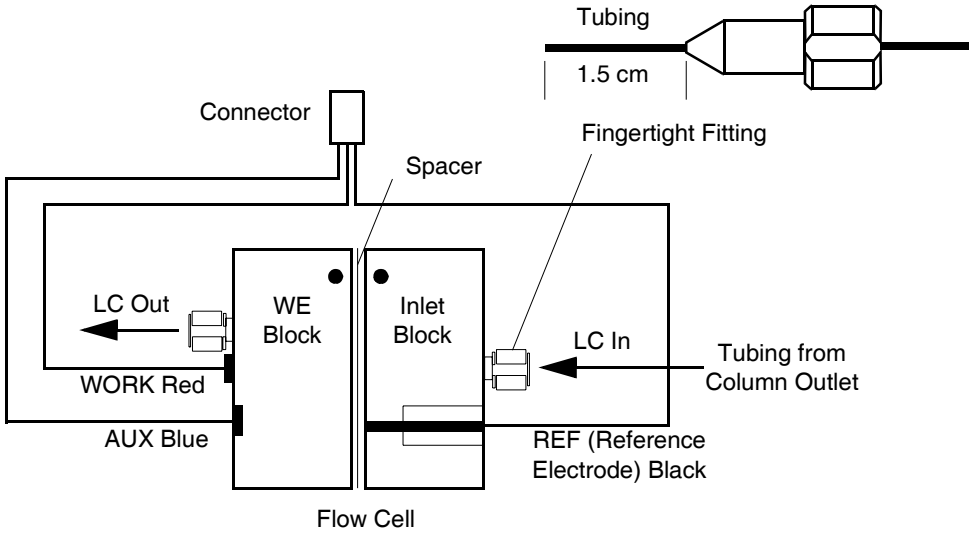


Figure 2-9 Connecting Tubing to the Flow Cell

2. Connect one end of the medium-bore tubing (0.5 mm ID) to the outlet of the flow cell using a fingertight fitting; do not overtighten. Push the other end of the tubing

through the upper-left hole in the chassis and out to the waste container (Figure 2-10).

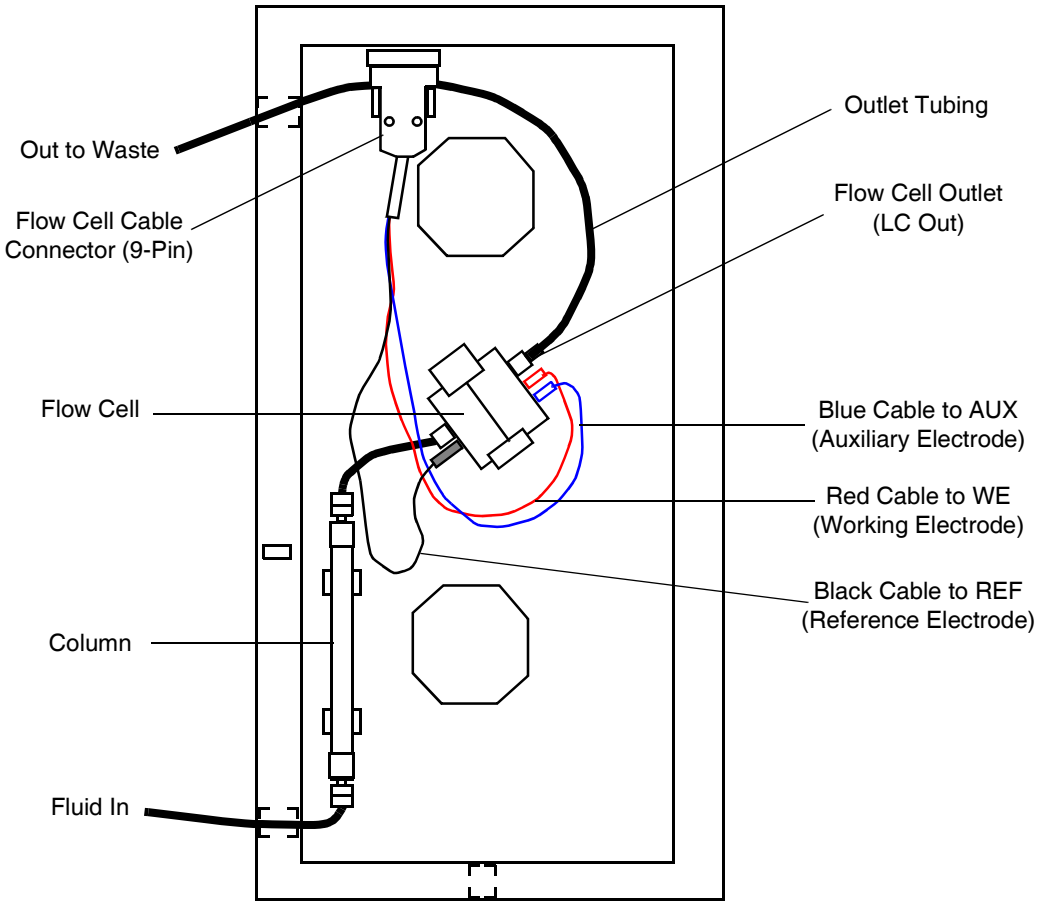


Figure 2-10 Installing the Flow Cell

3. Fill the flow cell with mobile phase and install the flow cell in the clamp as follows:
   a. Power on the HPLC pump. Keep some tissues nearby in case some mobile phase is spilled.
   b. Allow the pump to fill the flow cell by keeping the flow cell at an angle of about 45° with the outlet (LC Out) on top to force the air through the outlet.
   c. To prevent trapping of air bubbles, position the flow cell with the reference electrode at the lower side and the outlet at the upper side.

d. Slide the flow cell into the flow cell clamp from the top.

Attention: *Avoid using excessive force when installing the flow cell in the clamp by sliding the flow cell downward from above the clamp. Do not press the flow cell into the clamp from the front.*

4. Connect the flow cell cable to the flow cell as shown in Figure 2-10. Connect the WE, AUX, and REF on the flow cell using the red, blue, and black cell cable (WE – red, AUX – blue, REF – black).

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minute backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Attention: *When connecting or disconnecting the flow cell cable, ensure the flow cell power is off (see Section 3.8.1, Turning Off the Flow Cell).*

Note: *For electrochemical detection, always use the highest purity buffer salts. Segregate reagents used for electrochemical detection to reduce the likelihood of contamination.*

Dedicate glassware to preparation of buffers and mobile phases used for electrochemical detection. Filter the buffer through a 0.22-µm filter before use.

Note: *Do not leave the buffer stored in the system after use. Flush all fluidic pathways with HPLC-quality water before shutting the system down and leave HPLC-quality water in the system. Flush with 90% HPLC-quality water:10% methanol for shutdowns scheduled to be more than one day. Use a minimum of 15 mL for sparge-equipped units, and a minimum of 45 mL for inline vacuum degasser-equipped units. Some systems such as Waters Alliance might require volumes lower than this, depending on inline degasser volumes and slow-rate operation limits.*

2.5 Making I/O Signal Connections

Caution: *To avoid electrical shock, power off the 2465 Detector and unplug the power cord from the rear panel receptacle before you make signal connections or replace a fuse.*

Always replace a fuse with the same type and rating, as described on the rear panel.

Attention: *Connect the shield to the chassis ground stud. The ground stud is for shielding only, not for safety grounding. To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only.*

Attention: *Use shielded cable(s) to connect all I/Os with other devices. Thoroughly connect the shielding to common. Manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.*

To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables (including RS-232) longer than 9.8 feet (3 meters) when you make connections.

2.5.1 Rear Panel Connections

The signal connections you need to make to your 2465 Detector (Figure 2-11) depend on:

- The operating mode (stand-alone or remote control)
- The types of instruments in your HPLC system

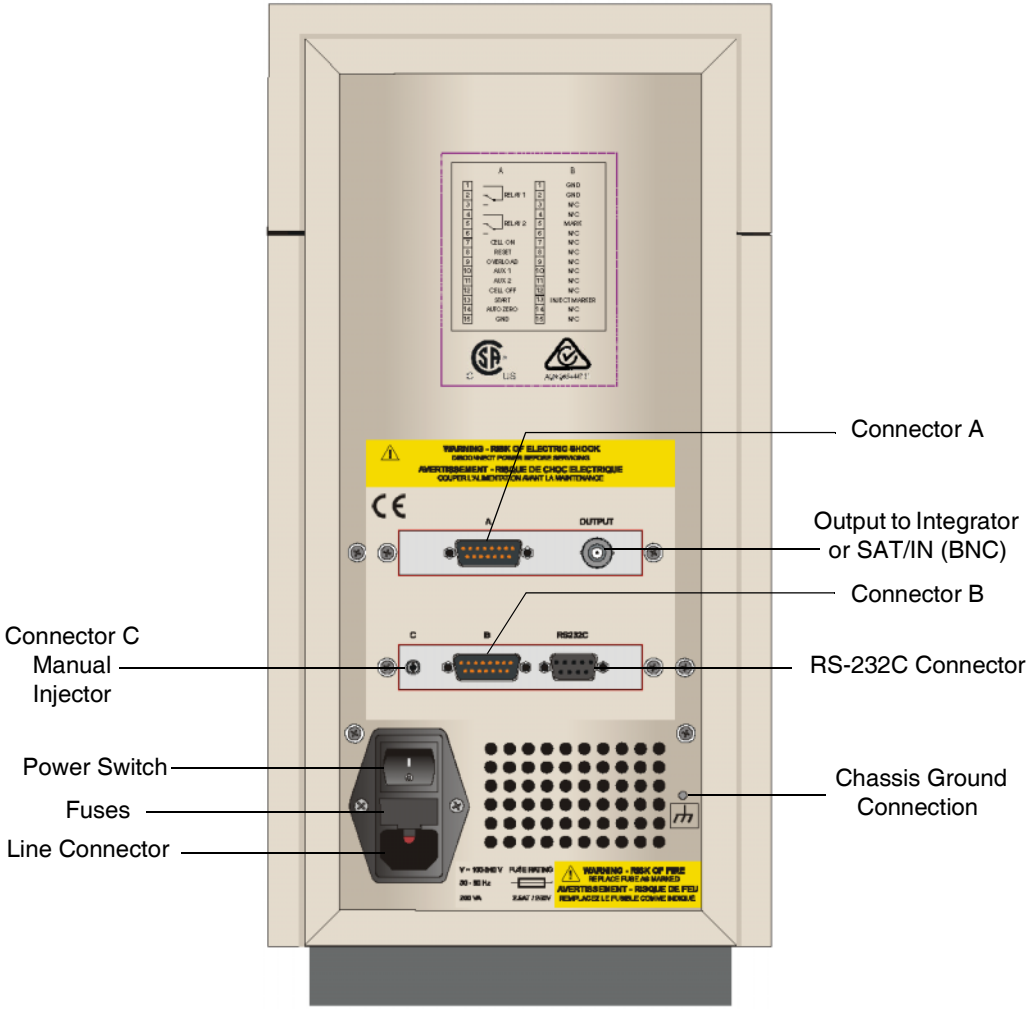


Figure 2-11 Rear Panel Connections on 2465 Detector

The 2465 Detector has several types of event inputs and outputs, relays, and TTL event switches. The 2465 power supply drives the relays. The TTL switches require a minimum pulse of 100-ms duration, low (where low is < 0.8 V). If repetitive activation is required, the next pulse should occur after 100 ms, high (where high is > 2.4 V).

The rear panel includes two 15-pin connectors, A and B, for the I/O signals (Figure 2-12).

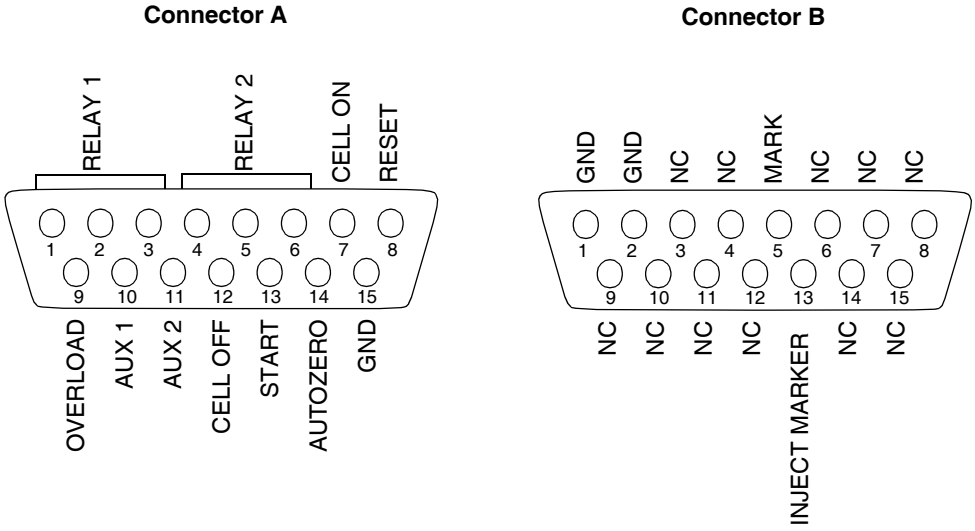


Figure 2-12 I/O Signal Inputs and Outputs

Table 2-2 and Table 2-3 describe the I/O signals available on I/O connectors A and B (see Appendix A, 2465 Detector Specifications, for the electrical specifications for the signals). The initial switch setting for relays 1 and 2 is open and the initial switch setting for the output and input events is high.

Table 2-2 Connector A

Name	No.	I/O	Description
Relay 1	1, 2, 3	Out	Contact between 1 (common) and 2 (default) or 3; activated by time file output 0100. Programmable Relay output switch. Can trigger a switch such as a column selector.
Relay 2	4, 5, 6	Out	Contact between 4 (common) and 5 (default) or 6; activated by time file output 1000. Programmable Relay output switch. Can trigger a switch such as a column selector.
Cell On	7	In	Event input using the Events feature that accepts a trigger to turn on flow cell. Level triggered.
Reset	8	In	Event input using the Events feature that resets a time file to initial conditions. Level triggered.

Table 2-2 Connector A *(Continued)*

Name	No.	I/O	Description
Overload	9	Out	Event output switch. Event is triggered when an Out of Range condition (overload) exists. Can trigger a change in flow conditions. When active, output status is low (default is high).
AUX 1	10	Out	Free programmable TTL output contact closure. Can activate an external switch, such as a column select valve. Can be triggered in a row in a time file by setting the output parameter to 0001. When active, output status is low (default is high).
AUX 2	11	Out	Free programmable TTL output contact closure. Can activate an external switch, such as a column select valve. Can be triggered in a row in a time file by setting the output parameter to 0010. When active, output status is low (default is high).
Cell Off	12	In	TTL input contact closure, which turns off the flow cell. Can be connected to a fault switch to shut down flow cell if pumping system loses prime. Level triggered.
Start	13	In	TTL input contact closure, which starts a programmed time file. Can be connected to an inject start switch from an autosampler. Level triggered.
Auto Zero	14	In	TTL input contact closure, which autozeros the detector. Can be connected to an inject start switch from an autosampler. Always accessible when Ic is on screen. Level triggered
GND or Common	15		Ground

Table 2-3 Connector B

Name	No.	I/O	Description
GND or Common	1, 2		Ground
N/C	3, 4, 6 – 12, 14, 15		Not connected

Table 2-3 Connector B *(Continued)*

Name	No.	I/O	Description
MARK (Input)	5	In	Level triggered, TTL contact closure. Accepts input after 100 ms. When activated, makes a baseline spike of 10% FS, 100 ms (0.1 s) duration.
INJECT MARKER	13	Out	Use with manual injector. Load = high, Inject = low.

The 2465 Detector can send output signals to an integrator or a Waters SAT/IN Module using a standard BNC connector for the analog output (see Output, Figure 2-11). Table 2-4 shows the signals for the Output connector.

Table 2-4 Output (±1 V or ±10 V)

No.	Polarity	Description
1	Positive	To integrator or A/D device.
2	Negative	

2.5.2 Connecting to a 2695 Separations Module (Stand-Alone)

You can connect the 2465 Detector to the Waters 2695 Separations Module, when it is not under the control of the Empower software, to perform the following:

- Autozero on inject start
- Chart mark on inject start
- Start a method on inject start
- Turn flow cell off

Generating Autozero on Inject Start

To autozero the 2465 Detector at the start of an injection, make the following connections (see Figure 2-13):

2695 Separations Module (B Inputs and Outputs)	2465 Detector (Connector A Inputs)
Pin 1 Inject Start	Pin 14 Auto Zero +
Pin 2 Inject Start	Pin 15 Ground –

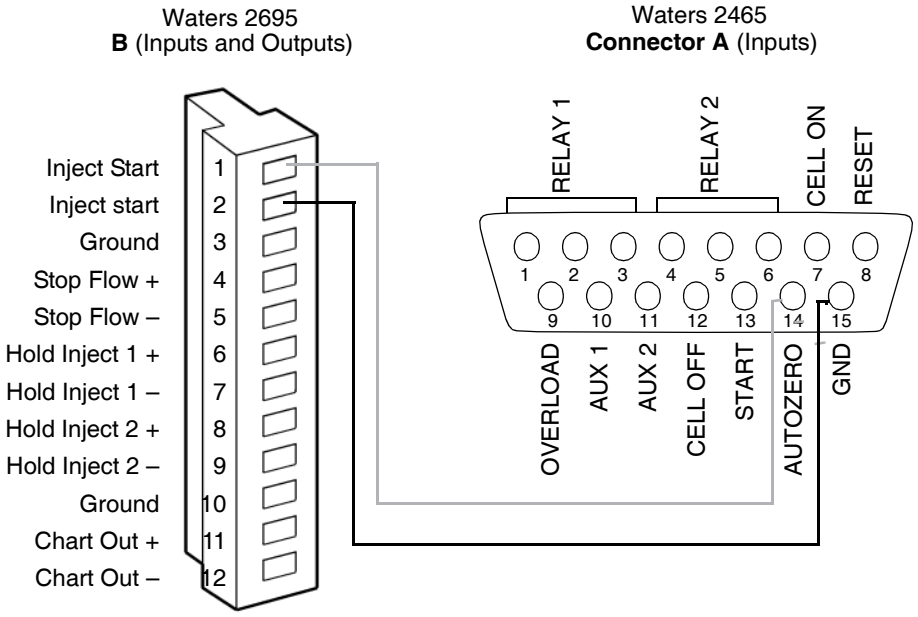


Figure 2-13 2695 Separations Module Connections to the 2465 Detector

Generating a Chart Mark on Inject Start

To generate the chart mark function on the 2465 Detector at the start of an injection, make the following connections:

2695 Separations Module (B Inputs and Outputs)	2465 Detector (Connector B Inputs)
Pin 1 Inject Start	Pin 5 Chart Mark +
Pin 2 Inject Start	Pin 1 or 2 Ground –

Starting a Method on Inject Start

To start a method on the 2465 Detector at the start of an injection from a 2695 Separations Module, make the following connections:

2695 Separations Module (B Inputs and Outputs)	2465 Detector (Connector B Inputs)
Pin 1 Inject Start	Pin 13 Method Start +
Pin 2 Inject Start	Pin 15 Ground –

Turning Off the Flow Cell

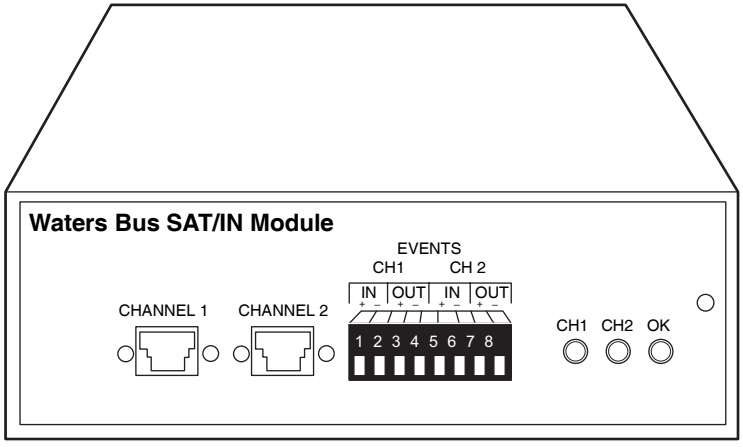
To turn off the flow cell when the flow has stopped, make the following connections:

2695 Separations Module (B Inputs and Outputs)	2465 Detector (Connector A Inputs)
Pin 4	Pin 12 Cell Off +
Pin 5	Pin 15 Ground –

2.5.3 Connecting to a busSAT/IN Module

You can perform data acquisition from the 2465 Detector with Millennium®[32] 4.0 software using the busSAT/IN Module. The following hardware must be connected:

- Laboratory acquisition and control environment (LAC/E™) module (LAC/E[32] Acquisition Server or busLAC/E card)
- Satellite interface (SAT/IN) module (Figure 2-14)



TP01138

Figure 2-14 busSAT/IN Module (Front Panel)

The Waters busSAT/IN Module translates analog signals from devices such as the 2465 Detector into digital form, then transmits the digital signals to the busLAC/E or LAC/E[32] card installed in the Millennium[32] workstation.

To connect the 2465 Detector to the Millennium[32] workstation:

1. Connect the busSAT/IN Module to the busLAC/E or LAC/E[32] card in the Millennium[32] computer, according to the instructions in the *Waters Bus SAT/IN Module Installation Guide*.
2. Connect the Waters 2465 Detector to the busSAT/IN Module using the SAT/IN cable from the 2465 Startup Kit (part number 441000333):
   a. The busSAT/IN Module connects to the 2465 Detector through the Output (BNC) terminal on the rear panel of the detector, as shown in Figure 2-15.

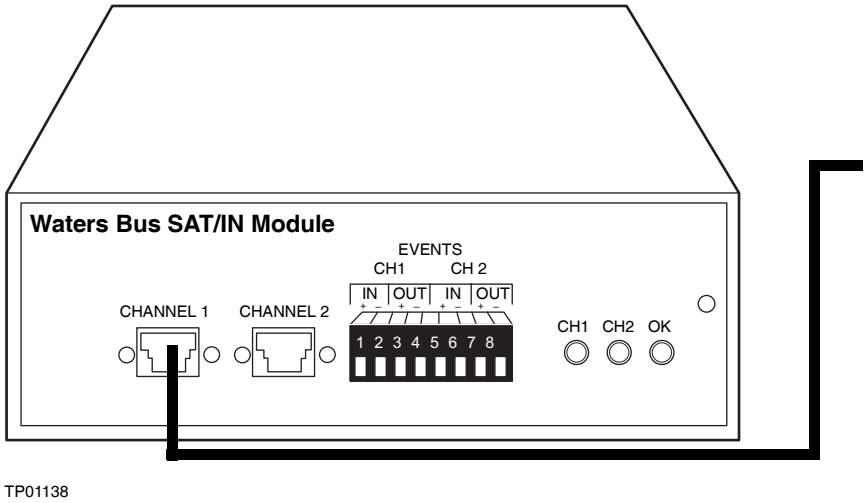


Figure 2-15 Connecting a busSAT/IN Module Channel 1 to the 2465 Detector

   b. Connect the SAT/IN cable to either the Channel 1 or Channel 2 connector on the front panel of the busSAT/IN Module.
   c. To obtain an inject start signal, connect one external I/O cable from the 2465 Startup Kit, using pins 13 and 15 on the 2465 Connector A to the Inject In signal on the module.
   d. Connect one external I/O cable from the 2465 Startup Kit using pins 13 and 15 on connector A of the 2465 to connector 1 of the busSAT/IN Module connectors.
   e. Configure the serial port for the busSAT/IN Module as described in the *Millennium[32] System Installation and Configuration Guide*.

3. Ensure that the Config Output of the 2465 Detector is set to 1 V as follows:
   a. From the 2465 Detector Main screen, select **F1 CONFIG**. The Config screen appears (Figure 2-16).

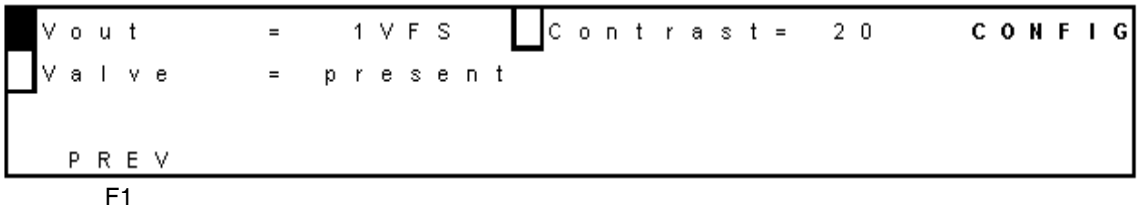


Figure 2-16 Config Screen

   b. If the V_{out} setting is 10 VFS, use the value keys to change it to 1 VFS.
   c. Select **F1 PREV**. The Main screen appears.

Attention: *To ensure proper startup of the busSAT/IN Module, do not turn power on to the module until you perform all procedures described in the* Waters Bus SAT/IN Module Installation Guide*. Improper startup can damage the unit and void the warranty.*

Attention: *The busSAT/IN Module does not have a power switch. To prevent damage to the module, always disconnect the power cord at either the wall outlet or the power supply before attaching or removing the power connection to the busSAT/IN Module.*

2.5.4 Connecting to a 746 Data Module

You can connect the 2465 Detector to a Waters 746 Data Module using the analog output connector on the rear panel of the detector. The analog output signal provides both ±1 and ±10 V outputs that are scaled to the range sensitivity setting and the voltage offset setting.

Attention: *To minimize the chance of creating a ground loop that can adversely affect measurement, connect the shield of the cable to the chassis ground at one end only.*

To send the integrator output signal from the 2465 Detector to the 746 Data Module:

1. Using one integrator cable from the 2465 Detector Startup Kit, connect the 746 Data Module to the 2465 Detector through the Output (BNC) terminal on the rear panel of the detector.

2. Ensure that the Config Output of the 2465 Detector is set to 1 V as follows:
   a. From the 2465 Detector Main screen, select **F1 CONFIG**. The Config screen appears (Figure 2-16).
   b. If the V_{out} setting is 10 VFS, use the value keys to change it to 1 VFS.
   c. Select **F1 PREV**. The Main screen appears.

Attention: *To prevent oversaturation of the signal from the 2465 Detector to the 746 Data Module, do not exceed the input voltage rating of the 746 Data Module. At the 2465 Detector, either change the range or maximize the compensation as offset.*

2.5.5 Making RS-232 Connections

The rear panel includes one RS-232 interface connector for digital signal communications. Use the RS-232 interface connector to connect the 2465 Detector to RS-232 devices, such as an RS-232 communications port in an Empower Personal, Workgroup PC, or Enterprise client configuration. The RS-232 connector uses a standard RS-232 cable, which is provided in the 2465 Detector Startup Kit.

Attention: *To avoid possible damage to components, power off all instruments on the RS-232 control bus before you connect an RS-232 interface cable to an instrument.*

Note: *When you connect the RS-232 cable and establish communications, the 2465 Detector operates in remote mode (see Section 3.2.4, Remote Mode).*

To connect the 2465 Detector to a Waters data system:

1. Connect the single receptacle end of the RS-232 cable to an RS-232 device, such as an RS-232 communications port or Equinox card in an Empower system.
2. Connect the other end of the cable to the RS-232 connector on the 2465 Detector rear panel.

   Attention: *The maximum total cable length between RS-232 devices in a system is 65 feet (20 meters). The maximum recommended cable length between two RS-232 devices is 10 feet (3 meters). Longer total cable lengths can cause intermittent RS-232 communication failures.*

3. Ensure all RS-232 cable screws are fastened tightly.
4. Ensure all input/output connections are correct, as in the example diagram in Figure 2-17.

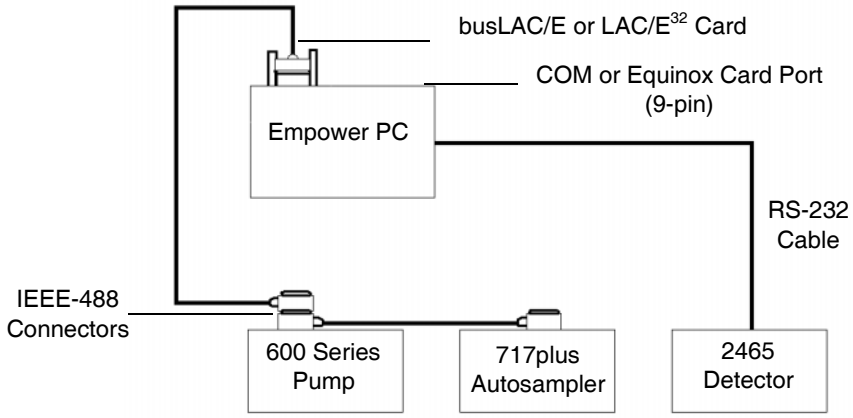


Figure 2-17 IEEE-488 and RS-232 Connections in a Waters Empower System

To configure the 2465 Detector for RS-232 communication (and operate in remote mode), see Section 3.3.1, Preparing the Detector for Remote Control from Empower.

2.6 Verifying COM Port Settings

You do not need to manually set the COM port to the settings in this procedure, because they are internally set when communication with the detector starts through the COM interface. Settings will be done automatically, but you may want to verify the COM port settings.

To verify the COM port settings:

1. On Windows® XP, right-click **My Computer** on the desktop, then select **Properties**. The System Properties dialog box appears.
2. Click the **Hardware** tab, then click **Device Manager**. The Device Manager dialog box appears.
3. Expand **Ports** in the tree, right-click the appropriate COM port, then select **Properties**. The Communications Port (COM1 or COM2) Properties dialog box appears.
4. Click the **Port Settings** tab. The correct settings for the 2465 Detector are:
   - Bits per Second = 38,400
   - Stop Bits = 1
   - Parity = None

- Data Length = 8
- Flow Control = None

5. Click **OK**.
6. Close the dialog boxes (Device Manager, System Properties, and other dialog boxes).

Chapter 3
Operating the 2465 Detector

After you have installed the Waters 2465 Electrochemical Detector (see Chapter 2, Installing the 2465 Detector), it is ready to set up and operate either:

- As a stand-alone electrochemical detector with a system such as the Alliance® system or with a pump, autosampler, chart recorder, or integrator
- In remote mode using Empower software or other data system software

Note: *To ensure accurate operation, and before pumping any mobile phase, solvent, buffer, or electrolyte through the flow cell, be sure to perform the procedures in Section 3.1, Starting Up the Detector.*

3.1 Starting Up the Detector

3.1.1 Powering On the Detector

Before you power on the 2465 Detector, ensure the power cord is properly installed from the detector rear panel to the power source (see Section 2.3, Making Electrical Power Connections).

Attention: *Do not turn on the flow cell until it is prepared properly (see Section 3.4.2, Section 3.5.5, or Section 3.6.2).*

Note: *The 2465 Detector does not have any audible alarms, bells, or beeps.*

To start up the 2465 Detector:

1. Power on the 2465 Detector by pressing the power switch on the rear panel of the detector (see Figure 2-11). The detector runs several diagnostic tests and displays a message (Figure 3-1).

Figure 3-1 Calculating Checksum Screen

2. If an error message appears, contact Waters Technical Service (see Section 4.1.4).

   If the detector passes the diagnostic tests, an eight-digit checksum appears (Figure 3-2).

   Note: *The correct checksum for your firmware version is in the Release Notes.*

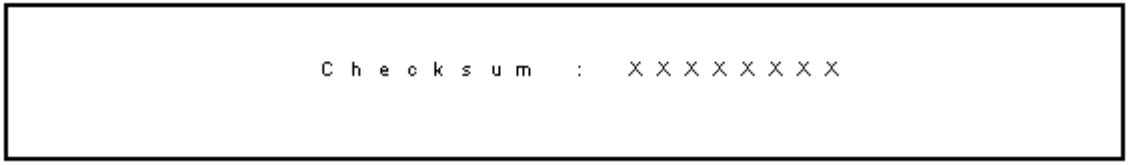


Figure 3-2 Checksum Screen

After a few seconds, the Main screen appears (Figure 3-3).

- The name of the detector is in row 1.
- The firmware version is in row 3.
- The five function keys are in row 4 (see Section 3.1.5, Using the Function Keys).

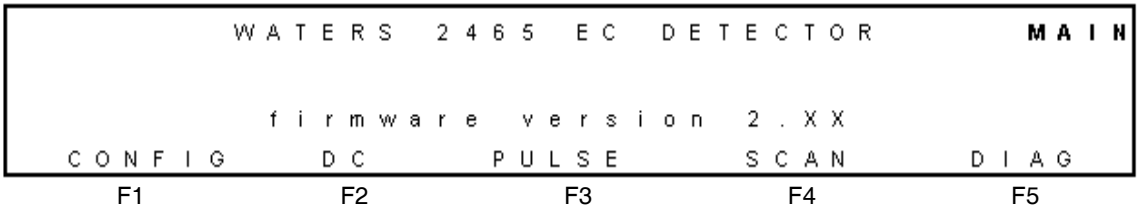


Figure 3-3 Main Screen

3.1.2 Using the Display

This section describes how to use the display and the keypad on the front panel of the 2465 Detector. All messages and screens appear on a 4 × 40 liquid crystal display (LCD).

You use the 12-key membrane keypad, located below the display (Figure 3-4), to select screens, parameters, and commands.

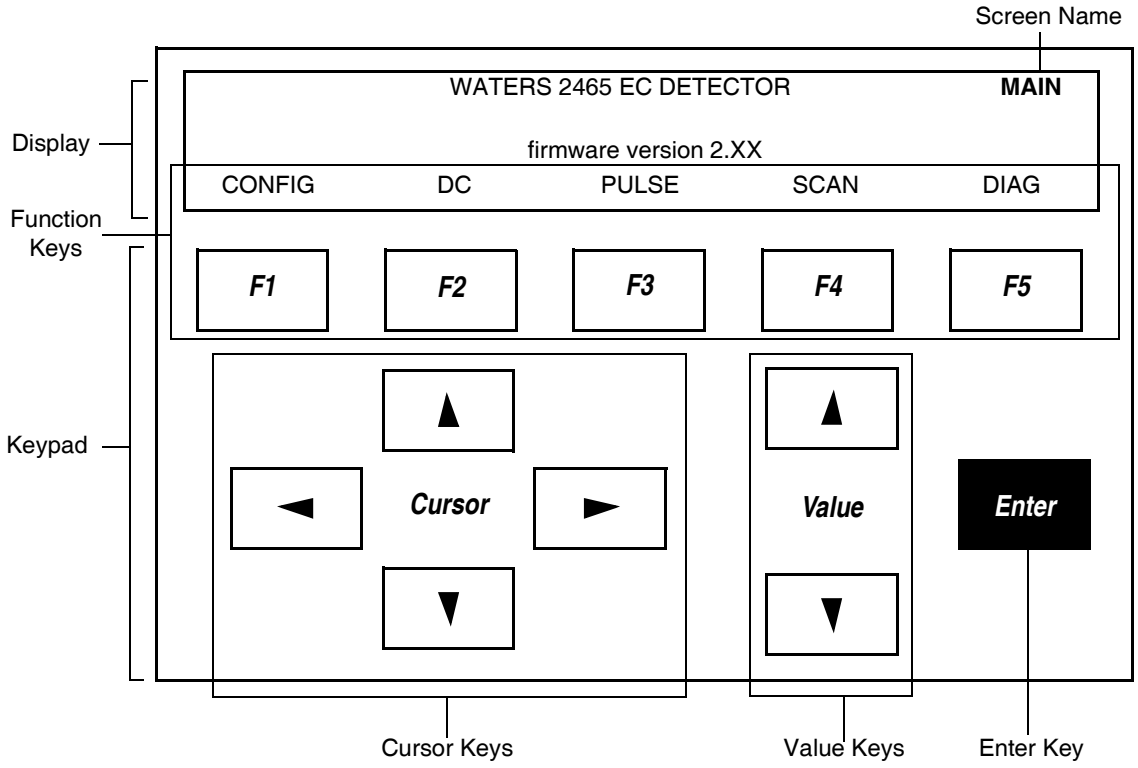


Figure 3-4 2465 Detector Display and Keypad

3.1.3 Using the Keypad

The keypad consists of 12 keys:

- **Function keys F1 through F5 (5)** – Allow you to select the screens that are named in the bottom row of the display.
- **Cursor keys (4)** – Allow you to move the cursor (using the up, down, left, and right arrows) to the parameters that can be edited or entered (parameters that have a cursor box, ☐ or ■, to the left of the name).
- **Value keys (2)** – Allow you to scroll up and down (using the arrows) through a preset range of allowed values and select one value.
- **Enter key (1)** – Confirms changes to the cell potential setting E_C.

3.1.4 Finding the Parameters

Three types of parameters can appear on each screen:

- **Function** – Parameters on the bottom row of the display and in capital letters are commands that you can access using function keys F1 through F5. Table 3-2 describes the commands in alphabetical order with screen location(s) and descriptions (see Section 3.1.7, Function Key Commands).
- **Control** – You can move to any parameter with a cursor box using the four cursor keys. Table 3-5 describes the control parameters in alphabetical order with screen location(s), descriptions, and type.
  - ▮ Cursor box is selected. When the cursor box is solid, you can change the parameter using the plus and minus value keys.
  - ▯ Cursor box is not selected.
- **Status** – A parameter without a cursor box displays the current status or value of the parameter. You cannot edit a status parameter. Table 3-5 describes the status parameters in alphabetical order with screen location(s), descriptions, and type.

3.1.5 Using the Function Keys

The top row of keys on the keypad, F1 through F5, correspond to the bottom row of the display. These are function keys, and they have variable commands that change with the screen. The commands for the function keys are capitalized. For example, in Figure 3-4, pressing function key F4 SCAN accesses the Scan Setup screen.

The function keys on the Main screen access the following:

- **F1** – Config (Configuration) screen
- **F2** – DC Setup screen (see Section 3.2.1, DC Mode)
- **F3** – Pulse Setup1 screen (see Section 3.2.2, Pulse (PAD) Mode)
- **F4** – Scan Setup screen (see Section 3.2.3, Scan Mode)
- **F5** – Diag (Diagnostics) screen for the diagnostic tests (see Chapter 5, Diagnostics and Troubleshooting)

3.1.6 Using the Keypad to Change Parameters

When you use the keypad, keep in mind that:

- The Enter key is used only to accept changes in cell potential (E_c). The suffix # appears if you change the value. Pressing Enter removes the # and saves the entered value.
- The screen name appears in the top-right corner of the display (rows 1 and 2) in capital letters.
- In general, F1 PREV and F1 QUIT access the previous screen, and F5 NEXT accesses the next screen in the current mode. To return to the Main screen, select F1 repeatedly.

3.1.7 Function Key Commands

Table 3-1 explains the commands that can appear in the bottom row of the 2465 Detector display for the function keys F1 through F5. Command names are capitalized.

Table 3-1 Function Key Commands

Command Name	Function Key	Screens	Description
ADD	F2	PROG	Adds the line (active data row) to the time file. Confirmation is requested if an existing time is overwritten. Time 000.00 always exists, so changing this time results in an overwrite warning.
AZERO (Autozero)	F4	DC STAT, PULSE STAT, RUN, SCAN STAT	Sets the digital flow cell current (I_c), which is the remote data stream, and the output voltage to 0 or to the offset level. Control parameter Comp = Off changes to Comp = On.
CELL=ON/ OFF	F2 or F3	DC STAT, PULSE STAT, SCAN SETUP, SCAN STAT	Toggles the flow cell On and Off. Confirmation is required – “Switch cell on/off?” **Pulse mode** – Pulsation occurs as long as the cell is on, regardless of the selected screen. **Scan mode** – Potential E_1 is applied.

Table 3-1 Function Key Commands *(Continued)*

Command Name	Function Key	Screens	Description
CONFIG	F1	MAIN	Accesses the Config screen: • To change the display contrast • To change the voltage output
DC	F2	MAIN	Accesses the DC Setup screen in DC mode.
DEL (Delete)	F3	PROG	Deletes the current line from the time file. Deleting time 000.00 deletes all entries in the time file, and confirmation is required.
DIAG (Diagnostics)	F5	MAIN	Accesses the Diag screen for performing the diagnostic tests (see Section 5.2, Diagnostics).
DISPL (Display)	F4	DIAG	Accesses the Disp (Display) screen for testing the display (see Section 5.2.4, Display Test).
ENDCYCLE	F5	PROG	Enters a screen for setting the end cycle time, which controls duration of the time file. When this time is reached, execution of the time file stops. If programmed, the next run is started. Allowed values: 0.01 to 999.99 minutes; cannot be smaller than smallest time in the time file plus 0.01 minute. Default value: 0.01 minute.
EVENTS	F4	DC SETUP, PULSE SETUP2	Accesses the Events Setup screen.
HOLD/ RESUME	F2 or F5	RUN, SCAN STAT	Toggles between holding and resuming the execution of a time file or scan.
KEYB (Keyboard)	F3	DIAG	Accesses the Keyb (Keyboard) screen for testing the keypad.

Table 3-1 Function Key Commands *(Continued)*

Command Name	Function Key	Screens	Description
MARK	F3	DC STAT, PULSE STAT	Triggers a marker signal (chart mark) on output (baseline spike of 10% FS, 100 ms duration).
NEXT	F1 or F5	Several screens	Accesses a related screen in the current mode.
NO	F4	Several screens	Negates a requested change to a parameter, then returns to the previous screen. Select No if you first make a change to a parameter, then decide not to change it.
NOISE	F2	DIAG	Accesses the Noise screen and starts the noise performance test (see Section 5.2.4, Display Test).
POLAR= +/– (Polarity)	F3	DC SETUP, PULSE SETUP2, PROG	Inverts output polarity; toggles between + and –. Change requires confirmation. Affects both digital and analog data.
PREV (Previous)	F1	Many screens	Returns to the previous screen.
PULSE	F3	MAIN	Accesses the Pulse Setup1 screen in pulse mode.
QUIT	F1	RUN	Stops the time file, returns to the Events Setup screen, and resets the cycle counter (Cyc) to 1. Also resets Outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000).
RUN	F3	EVENTS SETUP	Accesses the Run screen, and waits for the Start input trigger (external or keypad) to start a run.
SCAN	F4	MAIN	Accesses the Scan Setup screen in scan mode.
SCROLL	F4	PROG	Scrolls through a time file, one line at a time.

Table 3-1 Function Key Commands *(Continued)*

Command Name	Function Key	Screens	Description
START	F2 or F3	RUN, SCAN STAT	**DC and pulse modes** – Toggles between Stop and Start to control execution of a time file. **Scan mode** – Starts a scan.
STOP	F2 or F3	RUN, SCAN STAT	**DC and pulse modes** – Toggles between Stop and Start to control execution of a time file. Selecting Stop aborts the run, resets the cycle counter (Cyc) to 1, and deactivates the outputs Aux 1 and 2 and Relays 1 and 2 (status: 0000). **Scan mode** – Stops the scan and resets the cell potential to E_1.
YES	F2	Several screens	Confirms that you want to change a parameter, then returns to the previous screen.

3.1.8 Status and Control Parameters

You can change active control parameters using the cursor and value keys (see Section 3.1.3, Using the Keypad). Table 3-2 explains the status and control parameters that appear on the 2465 Detector screens, primarily in the top three rows of the display.

Table 3-2 Status and Control Parameters

Parameter Name	Screens	Description	Type
28 > 30°C	DC STAT, PULSE STAT, RUN, SCAN STAT, REMOTE (DC, PULSE, SCAN)	Displays the actual temperature (left value) and the temperature setting (right value) of the detector oven.	Status
Azero	PROG	Controls Autozero, which can be programmed in a time file. Toggles between Set and Not.	Control

Table 3-2 Status and Control Parameters *(Continued)*

Parameter Name	Screens	Description	Type
COMP (Compen-sation)	DC STAT, PULSE STAT, REMOTE (DC, PULSE, SCAN)	Toggles between on and off, and releases the Azero offset. Switches on if Azero is selected. Affects Azero compensation only, not the % offset.	Control
Contrast	CONFIG	Changes the contrast of the display. Allowed values: 1 (lightest) to 20 (darkest). Default: 20.	Control
Cyc (Cycle)	RUN	Displays the cycle counter. If a time file has to be executed more than once (Cycles > 1), this parameter counts the number of times a time file has been started. Reset (external) or F1 QUIT sets Cyc to 1 and the Events Setup screen appears. F3 STOP aborts the cycle.	Status
	SCAN SETUP (control), SCAN STAT (status)	Controls the length of each cycle: • **Cyc=half** – The cell potential runs from E_1 to E_2 and jumps back to E_1. • **Cyc=full** – The cell potential runs from E_1 to E_2, back to E_1, then stops. • **Cyc=cont** – The cell potential runs continuously from E_1 to E_2 and back to E_1. Selecting F2 STOP or finishing the cycle (for half or full) sets the potential to E_1.	Control
Cycles	EVENTS SETUP	Controls the number of repeats of a time file. Allowed values: 1 to 999 or cont (continuous).	Control
E_1, E_2, E_3	PULSE SETUP2, REMOTE PULSE	Controls the cell potential settings of the pulse.	Control

Table 3-2 Status and Control Parameters *(Continued)*

Parameter Name	Screens	Description	Type
E_c (Cell Potential)	DC SETUP, EVENTS SETUP (DC only), PROG (DC only), REMOTE DC, REMOTE SCAN	Controls the cell potential in 0.01-V steps between +2.00 and –2.00 V. Controls the cell potential in a time file (without confirmation). The suffix # appears when you change the value. You must confirm a change by selecting Enter.	Control
	DC STAT, RUN (DC only), SCAN STAT (during scanning)	Displays the set cell potential. During scanning, displays the actual cell potential.	Status
File	EVENTS SETUP	Displays the selected time file number: • **DC mode** – File numbers 1 to 5 • **Pulse mode** – File numbers 6 to 9 The time files remain stored in RAM even after switching off the 2465 Detector.	Control
Filt (Filter)	DC SETUP, DC STAT, PROG, REMOTE (DC, PULSE, SCAN)	Displays filter time constant settings. Allowed values: 0.1, 0.2, 0.5, 1, 2, or 5 seconds.	Control
	RUN	Displays the actual filter time constant.	Status
I_c (Cell Current)	EVENTS SETUP, NOISE, RUN, STAT (DC, PULSE, SCAN), REMOTE (DC, PULSE, SCAN)	Displays the true, noncompensated cell current, unaffected by autozero or offset.	Status
MaxComp (Maximum Compen-sation)	DC SETUP, PULSE SETUP1	Displays the maximum cell current that can be compensated for using autozero.	Status

Table 3-2 Status and Control Parameters *(Continued)*

Parameter Name	Screens	Description	Type
Offs (Offset)	DC SETUP, DC STAT, PROG, PULSE SETUP1, PULSE STAT, SCAN SETUP, SCAN STAT	Controls or displays the percentage offset. Allowed values: –50% to +50% in 5% steps.	Control
	RUN	Displays the percentage offset during execution of a time file.	Status
Outp (Output)	PROG	Controls four output functions in time files: • **0** – Open/high • **1** – Closed/low AUX1: 0001, AUX2: 0010, RELAY 1: 0100, RELAY 2: 1000. Combinations are possible (for example, enter 0101 to change the status of AUX1 to Low and RELAY1 to Closed at the same time).	Control
Range	DC SETUP, DC STAT, PROG, PULSE SETUP1, PULSE STAT, SCAN SETUP, SCAN STAT, REMOTE (DC, PULSE, SCAN)	Displays the range setting. Allowed values in DC mode: 10 pA to 200 µA full scale, in 1, 2, or 5 steps. Allowed values in pulse and scan modes: 10 nA to 200 µA full scale, in 1, 2, or 5 steps.	Control
SPD (Speed)	SCAN SETUP	Controls the scan speed. Allowed values: 1 to 50 mV/s in 1, 2, or 5 steps.	Control
t (Time)	PULSE SETUP2, PULSE STAT	Displays the total duration of one pulse ($t_1 + t_2 + t_3$) in ms.	Status
t_1, t_2, t_3	PULSE SETUP2	Controls the duration of potential steps E_1, E_2, or E_3. Allowed values: 0 (t_2, t_3) or 100 (t_1) and 2000 ms in 10-ms steps.	Control

Table 3-2 Status and Control Parameters *(Continued)*

Parameter Name	Screens	Description	Type
Temp (Temperature)	DC SETUP, PULSE SETUP1	Controls the temperature of the detector oven. Allowed values: off, or 15 to 45 °C in 1-°C steps. The detector oven is stable from 7 °C above ambient (see Table 3-5) after warming up.	Control
Time	PROG	Controls the time to execute a row in a time file. Allowed values: 0 to 999.99 minutes with 0.01-minute resolution. End cycle time programs the time to stop the execution of a time file. Default: 0.01 minute.	Control
t_s (Sampling Time)	PULSE SETUP2	Controls the duration of sampling time in pulse mode. Allowed values: 20 to 100 ms in 20-ms steps.	Control
Vout (Voltage Out)	CONFIG, EVENTS SETUP, NOISE, RUN, STAT (DC, PULSE, SCAN), REMOTE (DC, PULSE, SCAN)	Displays the output signal. Allowed values: 1 or 10 VFS (volts full scale).	Status
Valve	CONFIG	Informs the detector whether or not a valve is installed. Allowed values: None or Present.	Status

3.2 Overview of the 2465 Detector Modes

The 2465 Detector can operate as a stand-alone detector or controlled by a data system such as Empower software (remote mode). As a stand-alone detector, it can operate in three modes: DC mode (Section 3.2.1), pulse or PAD mode (Section 3.2.2), and scan mode (Section 3.2.3). You can program and run time files in DC mode and pulse mode.

3.2.1 DC Mode

You can use DC mode (as a stand-alone detector) to:

- Stabilize the detector.
- Determine if a compound is electrochemically active.
- Optimize sample detection by constructing a voltammogram.
- Determine the optimum start and stop times of a sample.

You can test samples directly, or program and run a time file in DC mode (see Section 3.4, Using DC Mode). Figure 3-5 shows a typical navigation flow in DC mode.

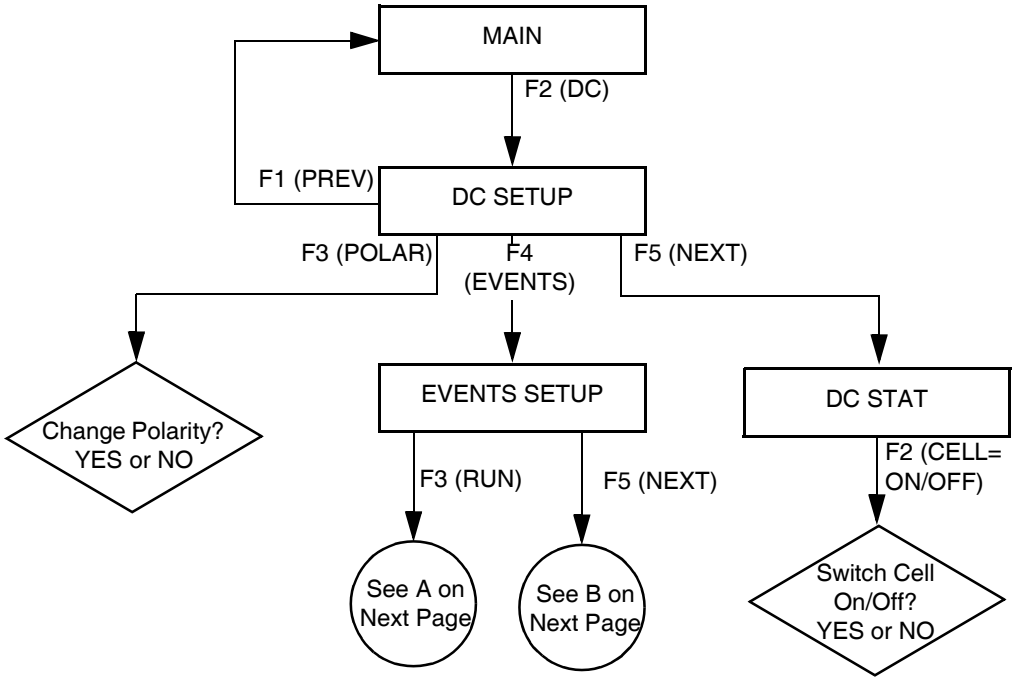


Figure 3-5 DC Mode Navigation

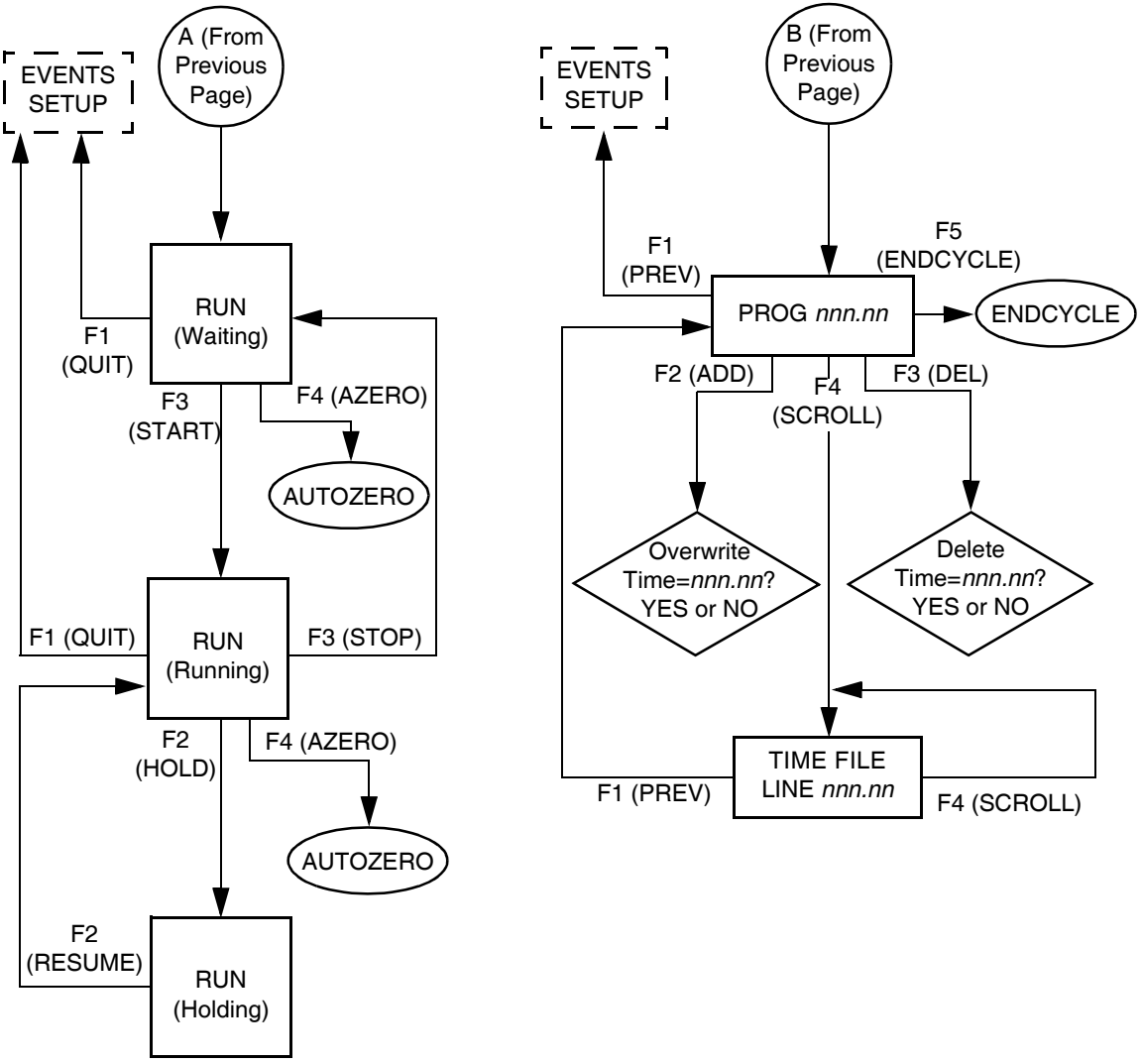


Figure 3-5 DC Mode Navigation *(Continued)*

3.2.2 Pulse (PAD) Mode

Use pulse mode (as a stand-alone detector) for pulsed amperometric detection (PAD) of large molecules such as carbohydrates. You can test samples directly, or program and run a time file in pulse mode (see Section 3.5, Using Pulse (PAD) Mode). Figure 3-6 shows a typical navigation flow in pulse mode.

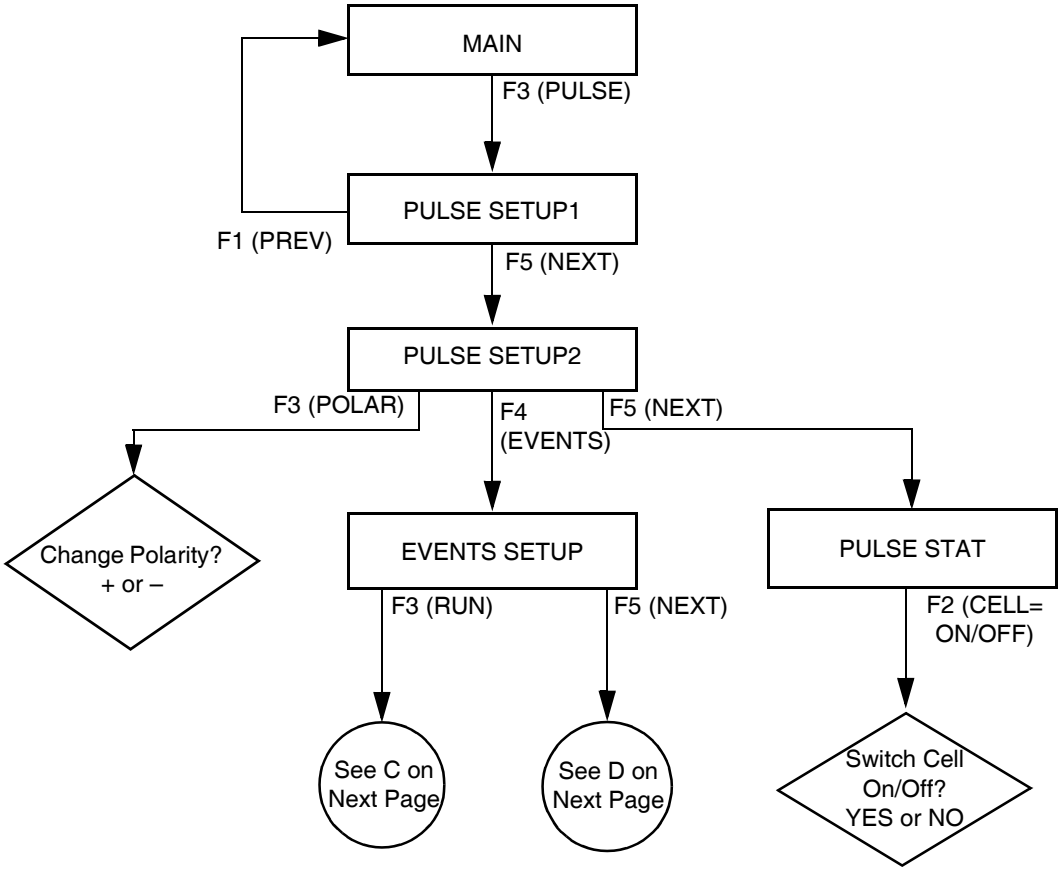


Figure 3-6 Pulse Mode Navigation

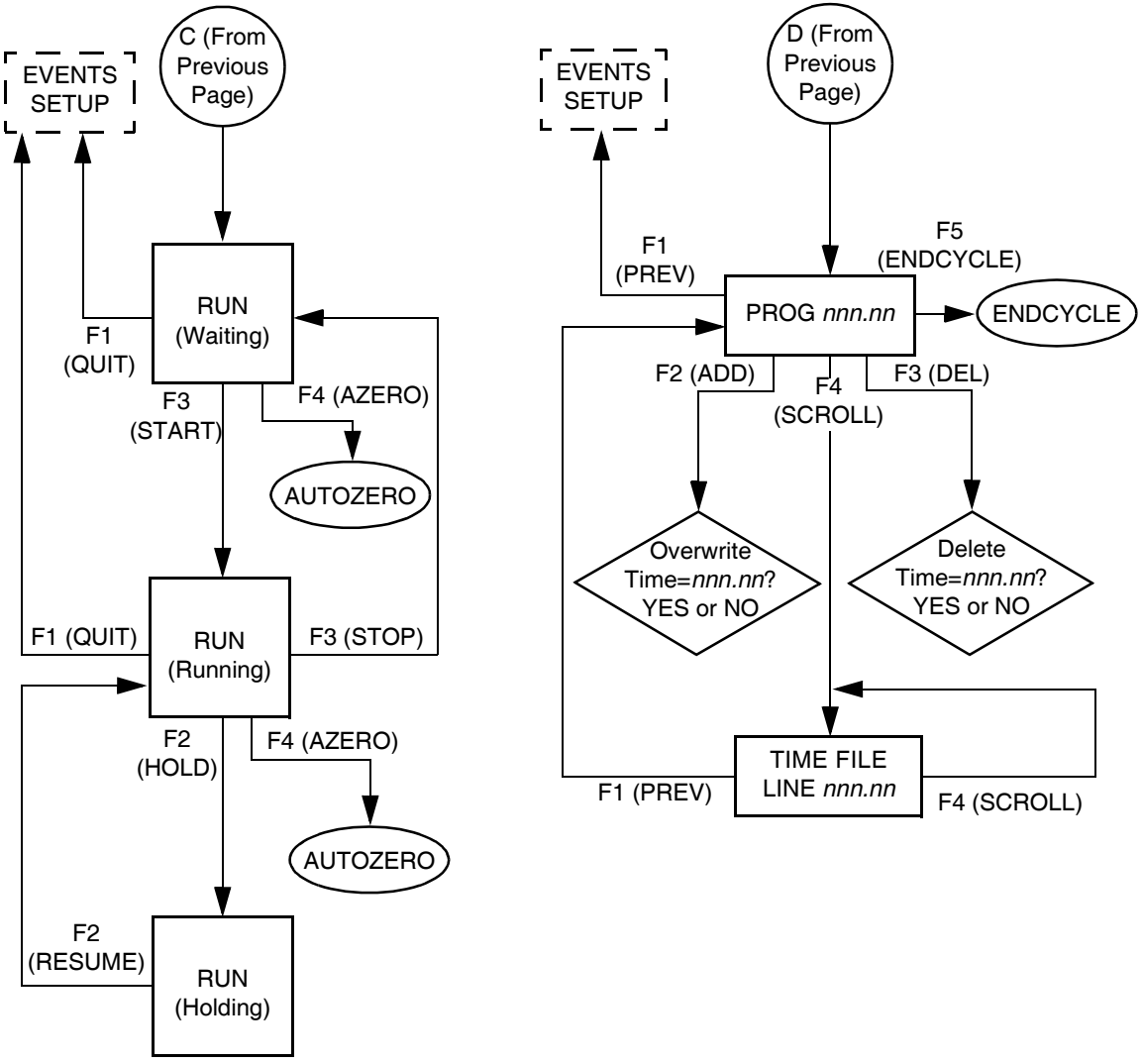


Figure 3-6 Pulse Mode Navigation *(Continued)*

3.2.3 Scan Mode

You can use scan mode (as a stand-alone detector) for developing a method. You can test samples directly in scan mode (see Section 3.6, Using Scan Mode). However, you cannot program and run time files in scan mode. Figure 3-7 shows a typical navigation flow in scan mode.

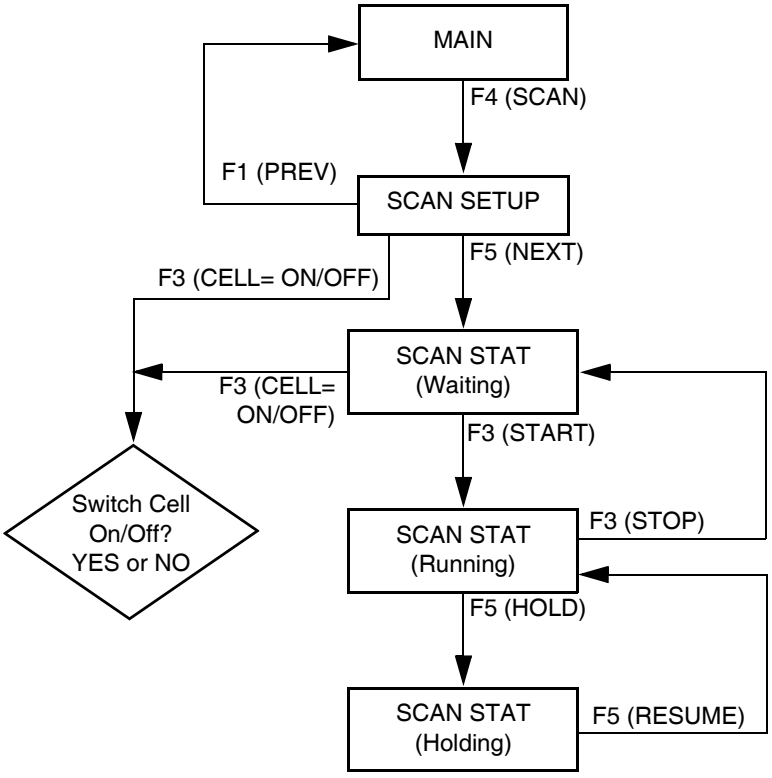


Figure 3-7 Scan Mode Navigation

3.2.4 Remote Mode

The 2465 Detector operates in remote mode when samples are run under the control of an external data system such as Empower software, and an Empower method is setting up and/or running. The 2465 Detector operates in remote mode from the instant that the setup method starts until the time a run ends or is aborted. Remote mode occurs when Empower is setting up a run or actively collecting data from the 2465 Detector. During remote mode, the word “Remote” appears as the title in the upper-right corner of the 2465 display (Figure 3-8 through Figure 3-10).

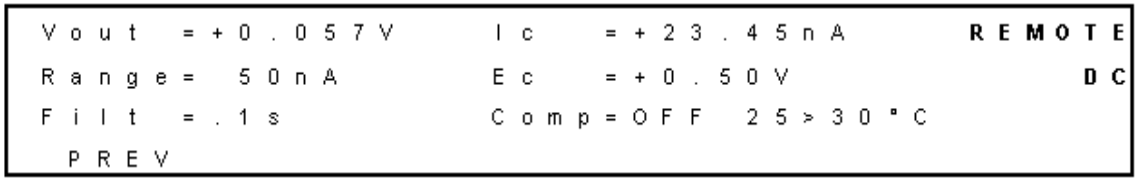


Figure 3-8 Remote DC Mode

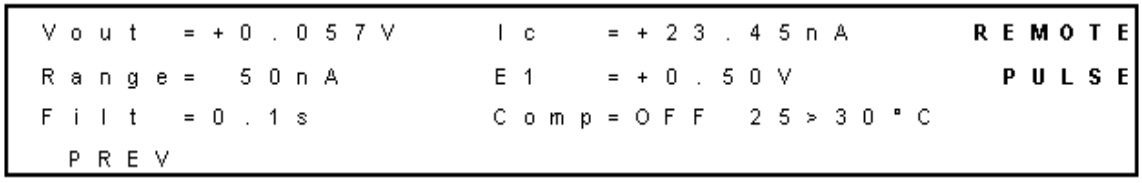


Figure 3-9 Remote Pulse Mode

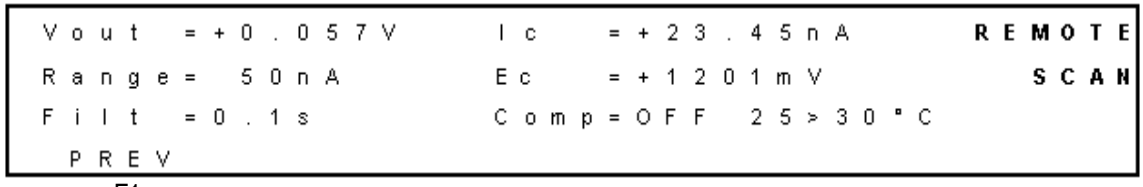


Figure 3-10 Remote Scan Mode

When an Empower run is in process, remote mode prevents any use of the front panel keypad of the 2465 Detector. The status parameters are updated during the run.

The front panel keypad of the 2465 Detector is available whenever Empower is not setting up a method or performing a run.

Under Empower control, the 2465 Detector uses the RS-232 connector (Section 2.5.5, Making RS-232 Connections). To connect the 2465 Detector to your HPLC system, see Section 2.4, Making Fluidic Connections. To make signal connections to an external system, see Section 2.5, Making I/O Signal Connections.

Note: *Parameter settings and some time programmable functions are different in the stand-alone mode and the remote mode (using Empower software).*

3.2.5 Introduction to Time Files

The Events feature of the 2465 Detector enables a time-based, automated control of electrochemical detection. Time files can be programmed and saved in stand-alone mode (not remotely controlled by Empower software; see Section 3.4.3, Creating a Time File in DC Mode, and Section 3.5.6, Creating a Time File in Pulse Mode). Time files are particularly useful for changing settings (such as sensitivity or autozero), for controlling external equipment (for example, a trigger to start integration software), or for changing the potential to optimize each peak in a chromatogram.

A time file contains a series of lines (or rows) in which you can change the events (settings of the 2465 Detector) with a time resolution of 0.01 minute (0.6 second). Time files 1 through 5 are reserved for DC mode, and time files 6 through 9 are reserved for pulse mode.

You create (or program) a time file using the Prog screen, then run a time file using the Events Setup screen. Time files are saved even if the 2465 Detector is powered off.

Note: *You cannot create or run time files in scan mode.*

Programmable Parameters in Time Files

The following programmable parameters can control the status of external equipment using the 2465 Detector output contacts (see Table 3-2, Status and Control Parameters, and Section 2.5, Making I/O Signal Connections):

- Cell potential (E_c in DC mode, and E_1, E_2, and E_3 in pulse mode)
- Range
- Autozero (Azero)
- Offset (Offs)
- Filter (Filt)
- Output (Outp)
- Polarity (Polar)

You can use a table format such as in Table 3-3 to plan your time file before programming it. Each default time file has a starting time line consisting of the initial conditions (at 000.00 min) and the end cycle time (at 000.01 min). You can change the first line and the end cycle time, and each file can have a total of 50 lines. You cannot delete the end cycle time.

Table 3-3 Default Time File in DC Mode

Time (min)	Line No.	Range	Filt	Auto Zero	Output	Polar	E_{Cell}	Offset
000.00	00	1 nA	1 s	not	0000	+	0.80 V	0%
Plan your events here								
000.01	End cycle time (end of run)							

The following commands are available at the Prog screen when you program a run in DC or pulse mode (see Table 3-1, 2465 Detector Commands):

- **Previous** – To return to the Events Setup screen after the timed events are programmed, select **F1 PREV**.
- **Add** – To add a line (containing the parameter values or events in the current screen) to the current time file, select **F2 ADD**.
- **Del** (Delete) – To delete a line from the current time file, select **F3 DEL**. A message asks if you want to `Delete time nnn.nn?` To delete, select **F2 YES**. To keep the line and return to the Prog screen, select **F4 NO**.
- **Scroll** – To scroll to the next line in the time file, select **F4 SCROLL**. If you select **F4 SCROLL** from the end cycle time, line 01 in the time file appears.
- **EndCycle** – To review the final line in the time file, which contains the stop time for the run, select **F5 ENDCYCLE**. The end cycle time defaults to 0.01 minute after the final programmed line. You can increase the end cycle time.

Run Commands (Stand-Alone Detector)

When the 2465 Detector is operated as a stand-alone detector (not remotely controlled by Empower), several commands are available in DC, pulse, and scan modes (see Table 3-1, 2465 Detector Commands).

To program and run a time file, select **F5 NEXT** from the Events Setup screen (see Section 3.4.3, Creating a Time File in DC Mode).

The following commands are available from the Events Setup screen:

- **Run** – The initial Run (Waiting) screen (Figure 3-11) allows you to:
  - Autozero the detector by selecting **F4 AZERO**.
  - Start a programmed run (time file) by selecting **F3 START**.
  - Quit by selecting **F1 QUIT**. The Events Setup screen appears.

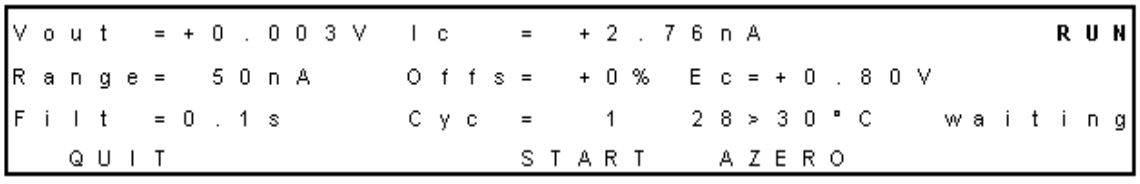


Figure 3-11 Run (Waiting) Screen Before Starting a Run

- **Start** – To start a run, select **F3 START** from the Run (Waiting) screen. The Run screen appears (Figure 3-12).

  Note: *You can also start a run by programming a time file (see Section 3.4.3, Creating a Time File in DC Mode) or by an external signal (for example, from Empower software; see Section 3.2.4, Remote Mode).*

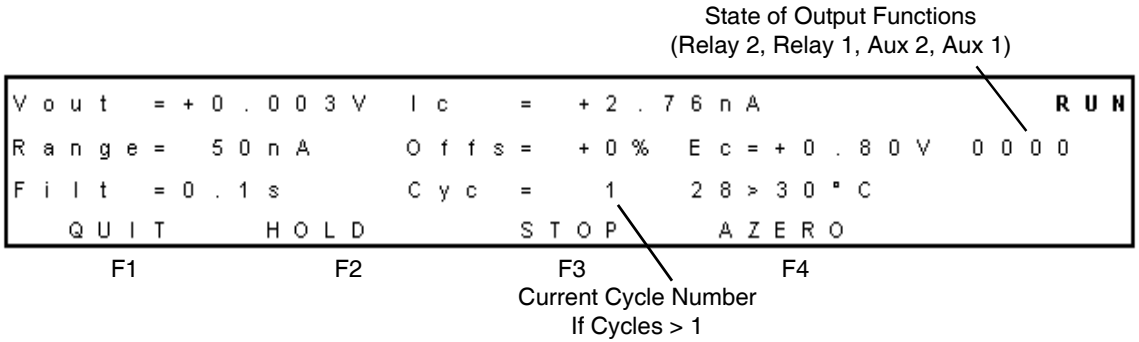


Figure 3-12 Run Screen After Starting a Run

- **Quit** – To stop a run and return to the Events Setup screen, select **F1 QUIT**.
- **Hold** – To hold or pause the run, select **F2 HOLD**. The run is suspended with the conditions specified in the current timed events file. F2 HOLD changes to F2 RESUME.
- **Stop** – To stop the run, select **F3 STOP**. F3 STOP changes to F3 START.
- **AZero** – To autozero the detector, select **F4 AZERO**. Comp. changes from OFF to ON.
- **Resume** – To restart the run from the time of suspension (after placing the run on hold), select **F2 RESUME**. When the run resumes, the cycle counter increments until it reaches the number selected in the timed events file. Once the run and all cycles are completed, the Events Setup screen appears.

- **Run Continuously** – To run continuously, select **CONT** (continuous) for cycles in the Setup screen (**Cyc = CONT**). The Run screen appears (Figure 3-12) and the detector runs under the current conditions until you select F1 QUIT.

End Cycle Time

Select **F5 ENDCYCLE** to see the final line for the file and to check whether the time file is empty or not.

- If the number under PROG is 01 or if the end cycle time is 000.01 (Figure 3-13), the time file is empty and ready to edit. Select **F1 PREV**.

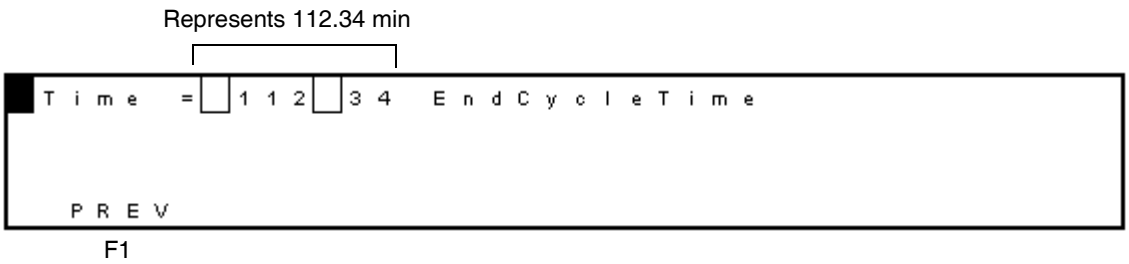


Figure 3-13 End Cycle Time Screen for an Empty Time File

- If the number under PROG (in the upper-right corner) is greater than 1 or if the end cycle time is greater than 000.01, the time file is already programmed.
- If the file contains timed events that you want to save for later use, select another file in the Events Setup screen by moving the cursor to **File =** and pressing the plus or minus value arrow.
- If the file contains timed events that are no longer used, you can erase one or more lines or the entire time file as follows:
  - **To delete one line** – Select **F4 SCROLL** until the line that you want to delete appears, then select **F3 DEL**. At the question `Delete time` *`nnn.nn`* `?`, select **F2 YES**. Repeat if you want to delete more lines in the time file.
  - **To delete the entire file** – Select **F4 SCROLL** repeatedly until Time = 000.00 min, then select **F3 DEL**. At the question `Delete timefile ?`, select **F2 YES**.

3.2.6 Programming Output Event Functions in Time Files

Four output event functions can be programmed at each time. The notation of the output 0000 corresponds to the four output controls at the back panel of the controller. These are relay 1, relay 2, AUX1, and AUX2 (see Section 2.5, Making I/O Signal Connections).

For example, to activate AUX1, the output is set to 0001. To make a contact closure using relay 1, the output is set to 0100. The contact is made between pins 1, 2, and 3 at 12-pin connector A of the upper I/O connector. At the same time the contact between 4, 5, and 6 is interrupted.

Table 3-4 lists the output commands and the corresponding external contacts. Combinations of commands enable control of multiple external contacts at the same time. For example, the command 0101 activates AUX1 and relay 1 at the same time.

Table 3-4 Outputs and Commands

Output	Command
AUX1	0001
AUX2	0010
Relay 1	0100
Relay 2	1000

3.3 Preparing the 2465 Detector for Operation

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Caution: *Wear protective clothing when handling 15% nitric acid.*

When you prepare the 2465 Detector for operation, observe the following precautions:

- Use the highest purity reagents for mobile phases (see page 41).

Attention: *If you plan to use an ISAAC reference electrode, flush the system with HPLC buffer containing chloride ions. Waters recommends 2 mM chloride (KCl or NaCl) ions with the ISAAC reference electrode.*

Note: *For electrochemical detection, always use the highest purity buffer salts. Segregate reagents used for electrochemical detection to reduce possible contamination. Dedicate glassware to preparation of buffers and mobile phases used for electrochemical detection. Filter the buffer through a 0.22-µm filter before use.*

- Degassing is recommended (see “Optional Materials” on page 42).

Note: *Air bubbles passing through the flow cell can create unacceptable noise levels and spikes.*

- A pulse dampener is recommended (see "Optional Materials" on page 42).
- Column pretreatment is required because a new column is not electrochemically clean and will most likely bleed, causing problems (see Section 2.4.2, Connecting a Column).

Attention: *To prevent unacceptably high background current and substantial fouling of the working electrode, use a column that is electrochemically clean. A new column is* not *electrochemically clean.*

- Do not overtighten the fingertight fittings (see Section 2.4.3, Installing the Flow Cell).

Attention: *Use only factory-supplied fingertight fittings in the flow cell, because others may cause serious damage. Let the tubing protrude approximately 0.6 inch (1.5 cm) from the fitting and tighten it such that the tubing is not or slightly indented by the fitting. Do not overtighten the fitting because overtightening affects the flow pattern through the tubing (turbulence) and may strongly decrease the flow cell performance.*

- Passivation with nitric acid is required for the metal parts of the HPLC system (see Section 2.4.1, Installing the 2465 Detector).

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Caution: *Wear protective clothing when handling 15% nitric acid.*

Attention: *Passivation should include only the metal parts of the HPLC system. Disconnect all parts that are not acid-resistant (such as nylon inlet filters, degasser, pulse dampener, column, and flow cell) during passivation. Do not expose columns or flow cells to 15% nitric acid.*

Ensure that all parts that are not acid-resistant (such as the nylon inlet filters, column, and flow cell) are disconnected during passivation. Do not passivate the 2465 Detector.

- Follow special precautions for mobile phase preparation for pulse mode (see "Materials Required But Not Supplied" on page 41 and "Optional Materials" on page 42).

Attention: *If you plan to use an ISAAC reference electrode, flush the system with HPLC buffer containing 2 mM chloride (KCl or NaCl) ions.*

- Special precautions for reductive mode measurement (see Section 2.4.2, Connecting a Column, on page 47).

3.3.1 Preparing the Detector for Remote Control from Empower

To prepare the 2465 Detector for operation under remote control, first set up the detector, then install the Empower Instrument Control Options Pack on the Empower PC.

Setting Up the Detector

To set up the detector:

1. Connect the 2465 Detector to any available COM port on the Empower PC using a standard RS-232 cable. The COM port configuration is set up automatically by the 2465 Empower Instrument Interface software (see Section 2.6, Verifying COM Port Settings).
2. Log in to Empower Pro or QuickStart.
3. If you have the privileges to do so, click **Configure System** to access Configuration Manager.

   Note: *The acquisition server PC serial ports must be configured before you can configure the 2465 Detector in a system.*

   Note: *You must have the security privileges that allow you to access Configure System (Configuration Manager) functions in Empower software.*

4. Configure the PC serial ports for the 2465 Detector:
   a. Click **Acquisition Servers**, then right-click the acquisition server and select **Properties**. The Acquisition Server dialog box appears.
   b. Click the **Serial Ports** tab (Figure 3-14).
   c. Select **W2465** for COM1 or COM2 under PC Serial Ports.
   d. Click the **Instruments** tab. The software configures the serial ports, then scans the busLAC/E.
   e. Verify that the 2465 Detector appears at the address you specified, then click **OK**. The acquisition server configuration is updated.

   Note: *If a serial card is used, then configure the 8-port serial card instead of the PC ports.*

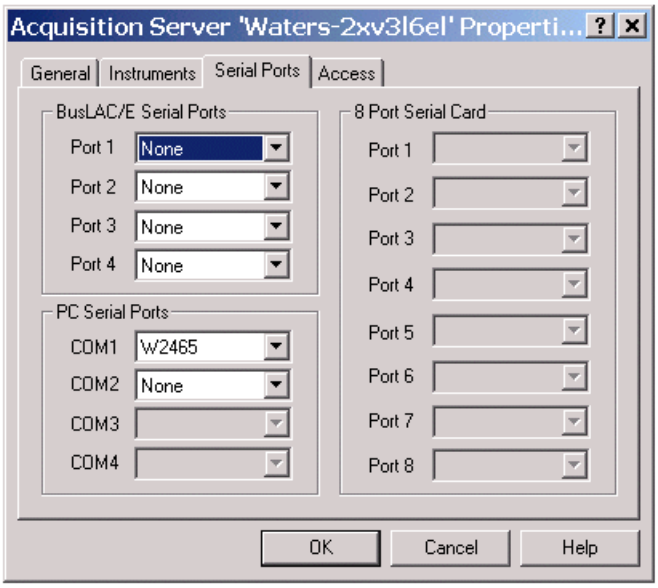


Figure 3-14 Acquisition Server Dialog Box

Installing the Empower Instrument Control Options Pack

The Empower Instrument Control Options Pack is installed on a Personal, Workgroup, or Client PC that already has Empower software. You must have the security privileges that allow you to access Configure System (Configuration Manager) functions in Empower software.

Note: *As a prerequisite, Empower software must be installed on the Personal, Workgroup, or Client PC before installing the Empower Instrument Control Options Pack.*

To install the Empower Instrument Control Options Pack on the Empower Personal, Workgroup, or Client PC:

1. Log out and close Empower software, then reboot the acquisition PC to which the 2465 Detector will be connected.
2. Insert the Empower Instrument Control Options Pack CD into the CD-ROM drive. The installation starts automatically and the Welcome page appears.
3. Follow the instructions in the InstallShield Wizard to install the Waters 2465 Software.
4. When the installation is finished, remove the CD from the Empower PC and store it in a protected location.

5. In Empower software, configure an acquisition system that includes the 2465 Detector. For details, see the *Empower Help*.

   Note: *For more information about RS-232 communications, see Section 2.5.5, Making RS-232 Connections.*

6. In Empower software, configure a new instrument method that includes the 2465 Detector. For details, see the *Empower Help*. For information about using the 2465 Detector with Empower software, see Section 3.2.4, Remote Mode.

3.3.2 Changing from Remote Mode to Stand-Alone Mode

If you want to use the 2465 Detector in stand-alone mode after control by Empower software (remote mode), Waters strongly recommends that you restart the 2465 Detector (turn the 2465 Detector off, wait 10 seconds, then turn it on).

Note: *The parameter settings may differ between remote mode and stand-alone mode.*

3.3.3 Equilibrating the Detector

Use this procedure to equilibrate the detector in stand-alone mode, which helps to ensure a stable temperature and operating environment. You can also program an event in a time file to equilibrate the detector (see Section 3.4.3, Creating a Time File in DC Mode).

Note: *If operating in remote mode, use Empower software to equilibrate the detector.*

To equilibrate the 2465 Detector:

1. From the Main screen, select **F2 DC**. The DC Setup screen appears (Figure 3-15).

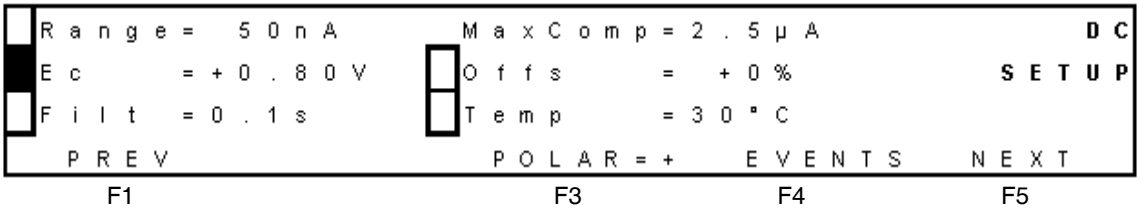


Figure 3-15 DC Setup Screen

Note: *You can also select **F3 PULSE**, then set the temperature in the Pulse Setup1 screen, or select **F4 SCAN**, then set the temperature in the Scan Setup screen.*

2. To set the temperature of the detector oven:

   a. Using the cursor keys, move the cursor to **Temp**. The current setting is displayed (not the actual temperature of the detector oven).

b. Using the value keys, increase or decrease the temperature in 1-°C steps. The allowed values for the temperature range are off, or 15 to 45 °C. After warming up, the detector oven is stable from 7 °C above ambient temperature. Table 3-5 shows the practical temperature range at different ambient (room) temperatures.

Table 3-5 Detector Oven Temperature Settings

Ambient Temperature (° C)	Minimum Temperature (° C)	Maximum Temperature (° C)
4	11	40
20	27	45
30	37	45

Figure 3-16 shows the temperature derating curve for the detector oven.

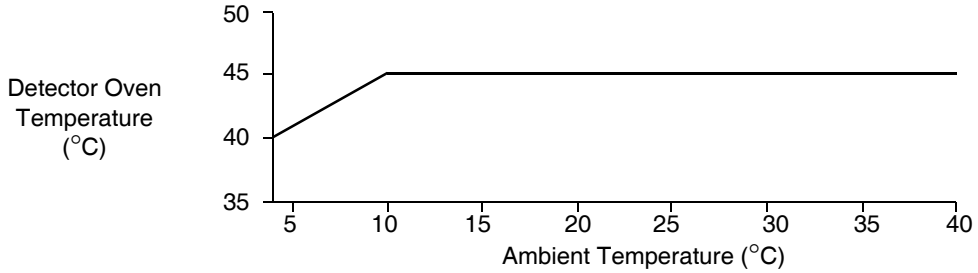


Figure 3-16 Derating Curve for Detector Oven Temperature

3. Select **F5 NEXT**. The DC Stat screen appears (Figure 3-17).

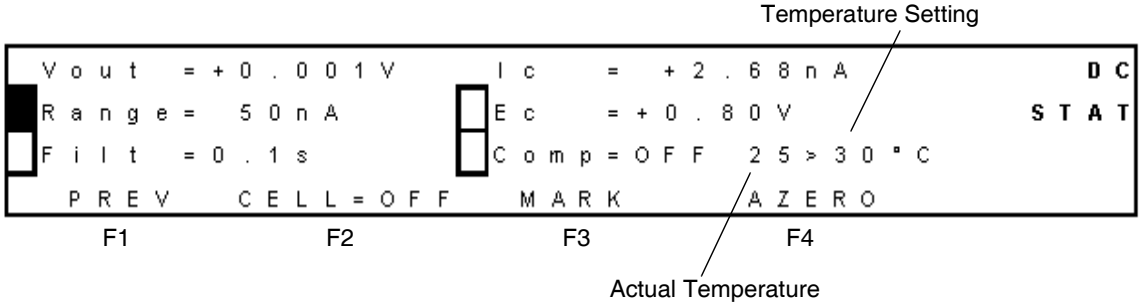


Figure 3-17 DC Stat Screen

Note: *Allow the detector to warm up for approximately 30 minutes.*

4. While the detector is warming up, turn on the pump and allow the mobile phase to flow at 0.5 to 1.0 mL/min.

   Attention: *To prevent damage to the flow cell, ensure that the pressure does not increase above 40 psi (2.76 bar, 276 kPa).*

5. Monitor the temperature and the baseline until they are stable.
6. Switch on the flow cell (see Section 3.4.2, Section 3.5.5, or Section 3.6.2).
7. With mobile phase flowing, allow the detector to equilibrate at least 60 minutes before beginning to acquire data.

Your system is now ready for use. The 2465 Detector has been developed for continuous operation. If preferred, the flow cell can be turned off at night (see Section 3.8.1, Turning Off the Flow Cell).

Attention: *For maximum stability, keep the system powered on continuously.*

3.4 Using DC Mode

You can use DC mode to perform direct current (DC) analyses. After you are comfortable with the 2465 Detector, use Table 3-6 for a quick reference list of tasks in DC mode.

Table 3-6 DC Mode Quick Reference List

Task in DC Mode	Starting Screen	Command Sequence
Check settings	MAIN	F2 DC > F5 NEXT
Make a chart mark	DC SETUP	F5 NEXT > F3 MARK
Turn the flow cell on or off	DC SETUP	F5 NEXT > F2 CELL=.... > F2 YES
Change polarity	DC SETUP	F3 POLAR=... > F2 YES
Autozero the detector	DC SETUP	F5 NEXT > F4 AZERO
Run without a time file	DC SETUP	F5 NEXT
Create and run a time file	DC SETUP	F4 EVENTS > F5 NEXT > [change values as needed] > F2 ADD > [repeat last 2 steps for each line] > F1 PREV > F3 RUN > F3 START

Table 3-6 DC Mode Quick Reference List *(Continued)*

Task in DC Mode	Starting Screen	Command Sequence
Run a time file	DC SETUP	F4 EVENTS > F3 RUN > F3 START
Find and run a time file	DC SETUP	F4 EVENTS > F5 NEXT > F4 SCROLL > F1 PREV > F3 RUN > F3 START
Stop a (running) time file	RUN	F3 STOP
Hold, then resume a (running) time file	RUN	F2 HOLD > F2 RESUME

3.4.1 Setting Initial Conditions in DC Mode

To set initial conditions in DC mode:

1. From the Main screen, select **F2 DC**. The DC Setup screen appears (Figure 3-18).

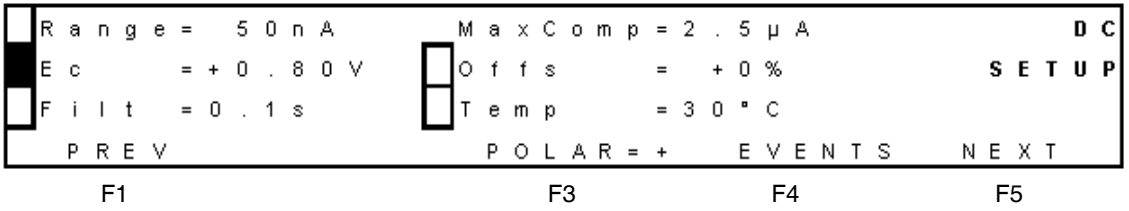


Figure 3-18 DC Setup Screen

Note: *You can change the Range, E_c (cell voltage), Filt (filter time constant), Offs (offset), and Temp (detector oven temperature), if needed (see Table 3-2, Status and Control Parameters).*

2. To turn on the column heater in the detector oven:
   a. Move the cursor to **Temp**.
   b. Using the value keys, increase the temperature setting to a value at least 7 °C above ambient temperature (allowed values: off, or 15 to 45 °C).
3. To check the polarity:
   - If `F3 POLAR=+`, the polarity is positive, and an oxidation current gives a positive signal.
   - If `F3 POLAR=-`, the polarity is negative, and a reduction current gives a positive signal.

4. If you want to change the polarity setting, select **F3 POLAR**.
   - If the polarity is positive, the Change Polarity to Negative screen appears (Figure 3-19). To change it to negative, select **F2 YES**. The DC Setup screen appears.

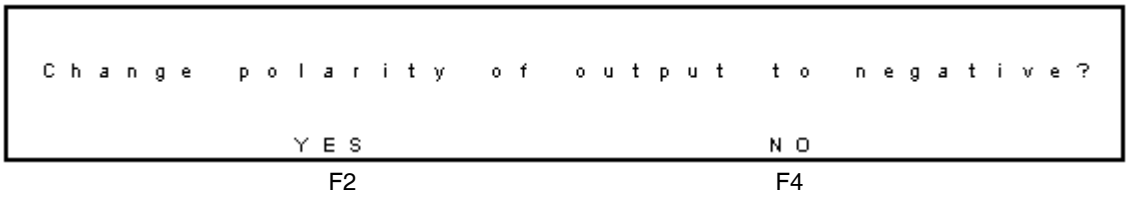


Figure 3-19 Change Polarity to Negative Screen

   - If the polarity is negative, the Change Polarity to Positive screen appears (Figure 3-20). To change it to positive, select **F2 YES**. The DC Setup screen appears.

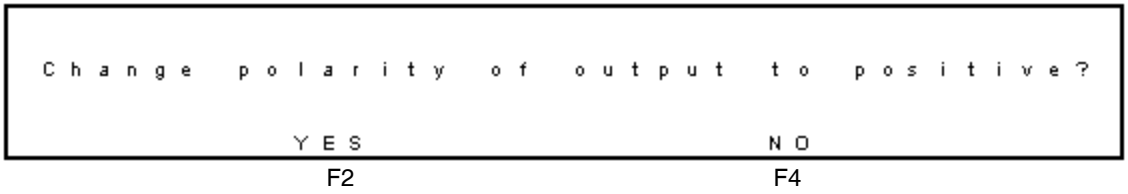


Figure 3-20 Change Polarity to Positive Screen

   - If you do not want to change the polarity, select **F4 NO**. The DC Setup screen appears.
5. To turn on maximum compensation (Comp):
   a. Select **F5 NEXT**. The DC Stat screen appears (Figure 3-21).

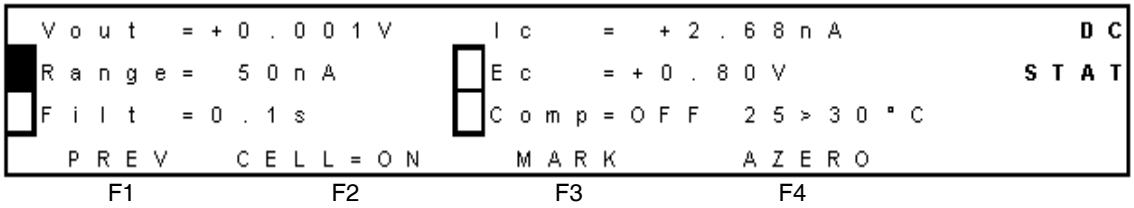


Figure 3-21 DC Stat Screen

b. Using the cursor keys, select **Comp**.

c. Using either value key, change OFF to **ON**.

Note: *You can also change the Range, Filt (filter time constant), and E_c (cell voltage), if needed (see Table 3-2, Status and Control Parameters).*

6. Following all precautions, turn on the flow cell (see Section 3.4.2, Turning the Flow Cell On and Off in DC Mode), and allow the 2465 Detector to equilibrate.

3.4.2 Turning the Flow Cell On and Off in DC Mode

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minimum amount of backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Note: *In electrochemical detection, switching the flow cell on or off is like switching a lamp in a UV detector on or off: both events destabilize the system. It takes some time before conditions are stabilized. Stabilization time depends on the range setting, and gets longer at more sensitive range settings.*

To turn the flow cell on in DC mode:

1. From the Main screen, select **F2 DC**. The DC Setup screen appears.
2. Select **F5 NEXT**. The DC Stat screen appears (Figure 3-22) and the value for F2 CELL is OFF.

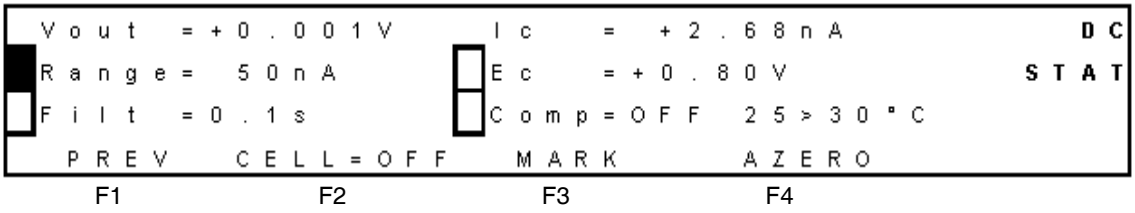


Figure 3-22 DC Stat Screen with Cell Off

3. Select **F2 CELL=OFF**. The Switch Cell On screen appears (Figure 3-23).

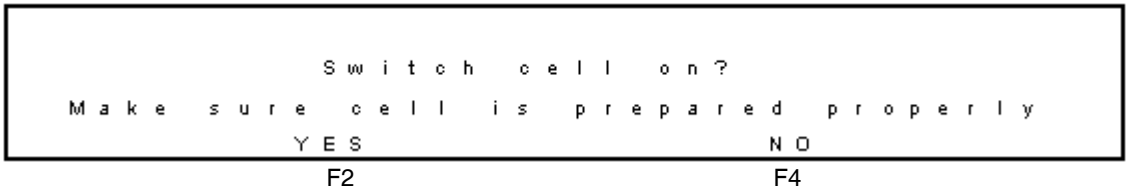


Figure 3-23 Switch Cell On Screen

4. Select **F2 YES**. The DC Stat screen appears and the value for F2 CELL is ON.

To turn the flow cell off in DC mode:

1. From the Main screen, select **F2 DC**. The DC Setup screen appears (Figure 3-18).
2. Select **F5 NEXT**. The DC Stat screen appears (Figure 3-22) and the value for F2 CELL is ON.
3. Select **F2 CELL=ON**. The Switch Cell Off screen appears (Figure 3-24).

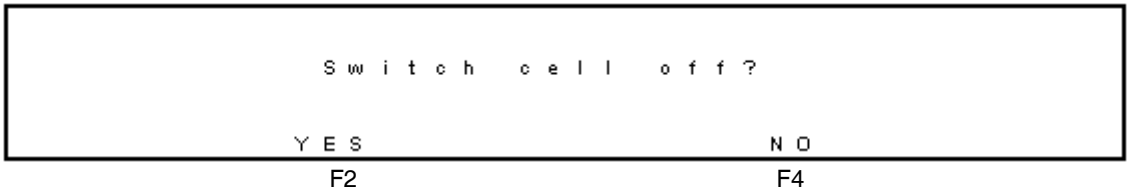


Figure 3-24 Switch Cell Off Screen

4. Select **F2 YES**. The DC Stat screen appears and the value for F2 CELL is OFF.

3.4.3 Creating a Time File in DC Mode

The following example describes how to program a simple time file in DC mode. Each row in Table 3-7 represents one line to be programmed, and each parameter change represents one event. The entire table represents one time file.

In this example, an autozero is performed at 0.00 minute (initial conditions), the cell potential (E_c) changes at 4.80 minutes, and two events occur at 4.90 minutes (a 10% offset and another autozero). The end cycle time is 5.00 minutes.

Table 3-7 Programming a Sample Time File in DC Mode

Time (min)	Line No.	Range	Filt	Auto Zero	Output	Polar	E_{cell}	Offset
000.00	1	50 nA	1 s	set	0000	+	0.80 V	0%
004.80	2	50 nA	1 s	not	0000	+	0.65 V	0%
004.90	3	50 nA	1 s	set	0000	+	0.65 V	10%
005.00	End cycle time (end of run)							

To create timed events in DC mode:

1. From the Main screen, select **F2 DC**. (Alternatively, from the DC Stat screen, select **F1 PREV**). The DC Setup Screen appears (Figure 3-25).

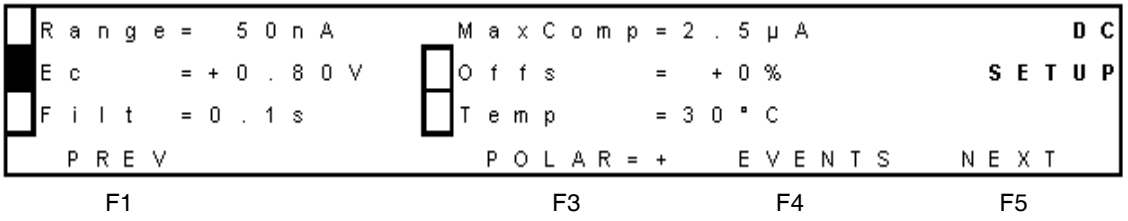


Figure 3-25 DC Setup Screen

2. Select **F4 EVENTS**. The Events Setup screen appears (Figure 3-26).

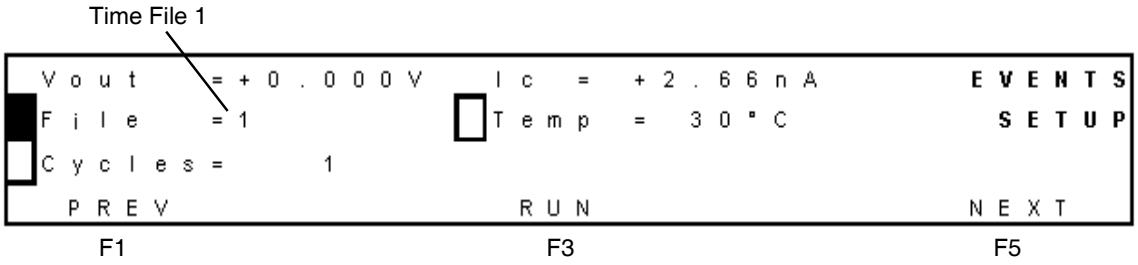


Figure 3-26 Events Setup Screen

3. Ensure that the initial conditions are acceptable for your purpose. At a minimum, select the following initial conditions:

   a. Time file 1 (**File = 1**) is the default time file. To program time file 2, select **File**, then use the value keys to select **File = 2**.

   b. Ensure that the value for the Temp is the same as on the DC Setup screen.

   Note: *If you want to program a temperature change, allow sufficient time after the change for equilibration.*

   c. If needed, change the number of cycles to **Cycles = 1** using the down cursor key and the value key.

   Note: *Select a cycle value of 1 when an "inject start event in" is connected to pins 13 and 15 of the 2465 Detector (see Section 2.5, Making I/O Signal Connections).*

   d. Select **F5 NEXT**. The Prog screen appears (Figure 3-27). The number under PROG shows that this time file currently has one line. Time = 000.00 establishes the initial conditions.

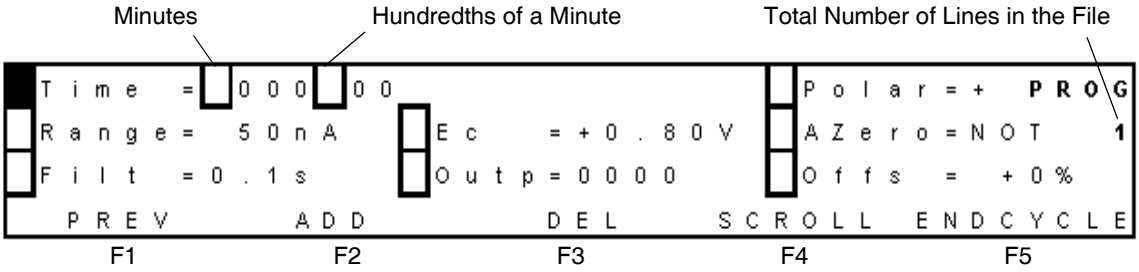


Figure 3-27 Prog Screen with Initial Conditions

4. If the total number of lines in the file is greater than one:
   a. Select **F3 DEL**. The Delete Timefile screen appears (Figure 3-28).

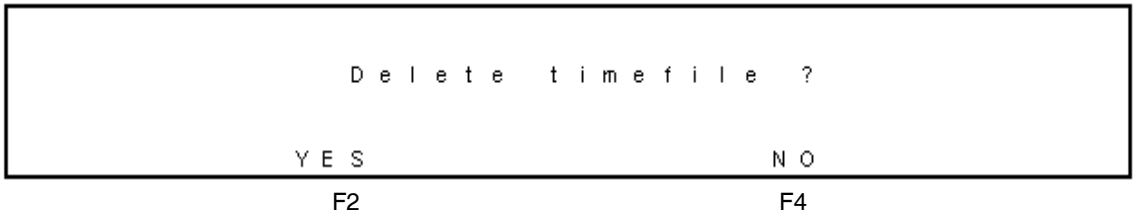


Figure 3-28 Delete Timefile Screen

   b. Select **F2 YES** to delete the contents of time file 2 (except the first and last lines of file 2). The Prog 1 screen appears.
   c. To verify that time file 2 is empty, select **F1 PREV**. The End Cycle Time screen appears. Verify that **Time = 000.01**, indicating that the file is empty.
   d. Select **F1 PREV**. The Events Setup screen appears. Verify that **File = 2**, indicating that file 2 is the active file.
   e. Select **F5 NEXT**. The Prog screen appears.
5. Change line 1 in time file 2:
   a. Verify that **Time = 000.00**.
   b. Select **AZero**, then use either value key to select **SET**.
   c. Select **F2 ADD**. The Overwrite Time 000.00 screen appears (Figure 3-29).

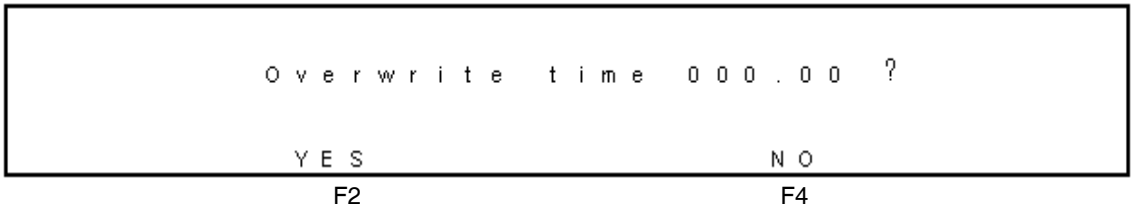


Figure 3-29 Overwrite Time Screen

d. Select **F2 YES**. The Prog screen appears (Figure 3-30).

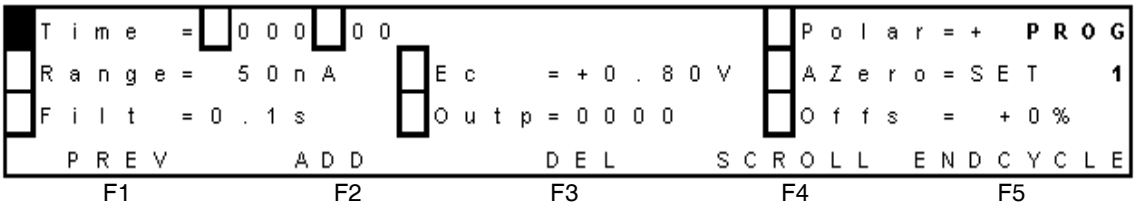


Figure 3-30 Prog Screen After Changing Line 1

6. Create line 2:
   a. Select **Time**.
   b. Move the cursor to minutes, then change the value to **4**.
   c. Move the cursor to hundredths of a minute, then change the value to **80**.
   d. Select **Ec**, then change the value to **+0.65V**. The suffix # appears when the cell potential changes (Figure 3-31).

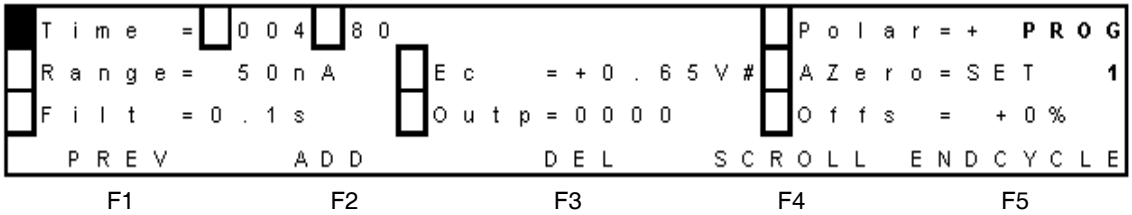


Figure 3-31 Prog Screen After Changing Cell Potential

   e. Press the **Enter** key to confirm the change to E_c. The suffix # disappears.
   f. Select **AZero**, then change the value to **NOT**.
   g. Select **F2 ADD**. The Prog screen appears (Figure 3-32).

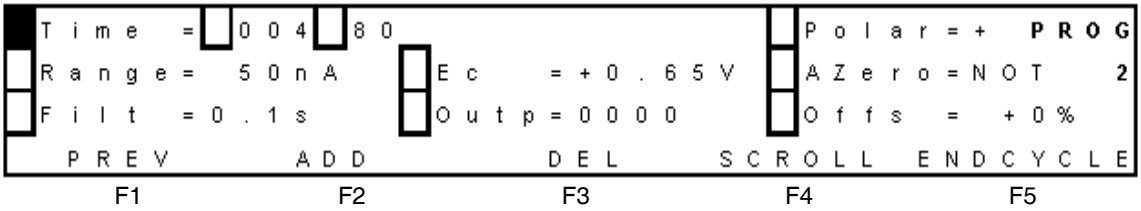


Figure 3-32 Prog Screen After Adding Line 2

7. Create line 3:
   a. For Time, select hundredths of a minute, then increase the value to **90**.
   b. Select **AZero**, then change the value to **SET**.
   c. Select **Offs**, then change the value to **+10%**.
   d. Select **F2 ADD**. The Prog screen appears (Figure 3-33). The number under PROG, 3, shows that this time file currently has a total of three lines. The time that this file will execute is 4.90 minutes after the start.

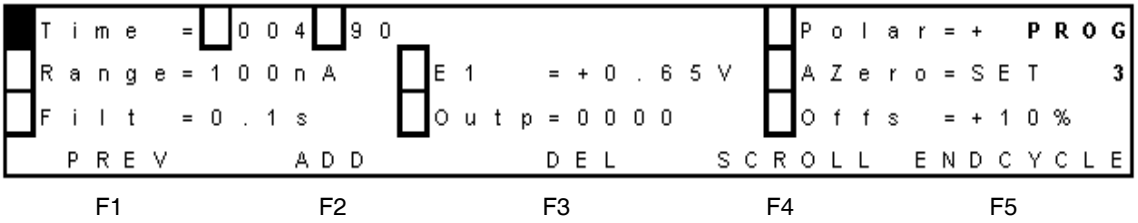


Figure 3-33 Prog Screen After Adding Line 3

Note: *The default end cycle time is 0.01 minute later than the time of the last programmed line. The end cycle time must be at least 0.01 minute later than the last programmed line.*

8. Change the last line of the time file:
   a. Select **F5 ENDCYCLE**. The End Cycle Time screen appears (Figure 3-34).

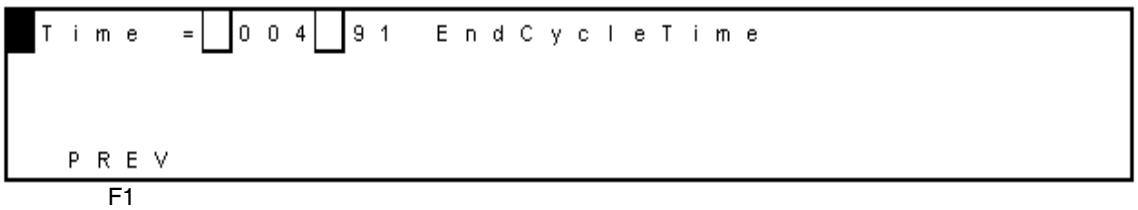


Figure 3-34 End Cycle Time Screen

   b. Increase the end cycle time to 005.00 minutes.
   c. Select **F1 PREV**. The Events Setup screen appears (Figure 3-35).

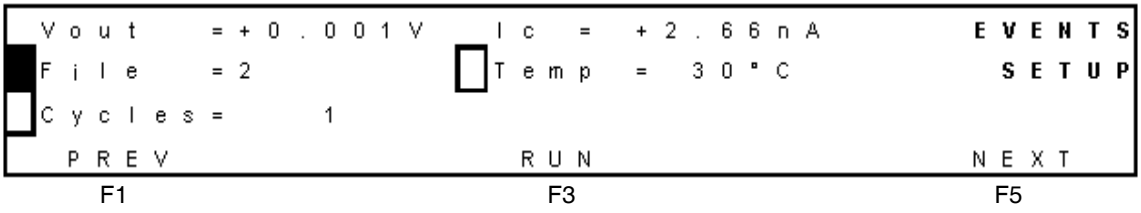


Figure 3-35 Events Setup Screen with Time File 2

9. Review all four lines in time file 2:

   a. Select **F5 NEXT**. The Prog 3 screen appears, and Time = 000.00 minutes.

   b. Review the values for the first line of time file 2.

   c. Select **F4 SCROLL**. The second line of time file 2 appears, and Time = 004.80 minutes. Review the values.

   Note: *Lines scroll according to their time (in this case, you should see the lines at times 0.00, 4.80, 4.90, then 5.00, in increasing order, then 0.00 again).*

   d. Repeat step c for all lines in the time file. Finish at the end cycle time.

   e. Select **F1 PREV**. (If you were not at the end cycle time, the End Cycle Time screen appears. Select **F1 PREV**.) The Events Setup screen appears.

Time file 2 is ready to run (see Section 3.4.4, Running a Time File in DC Mode).

3.4.4 Running a Time File in DC Mode

To run a time file in DC mode:

1. From the Events Setup screen, select **F3 RUN**. The Run (Waiting) screen appears (Figure 3-36).

   The system waits for a start command. This can be a keypad command or an external trigger from connector A on the rear panel (see Section 2.5, Making I/O Signal Connections).

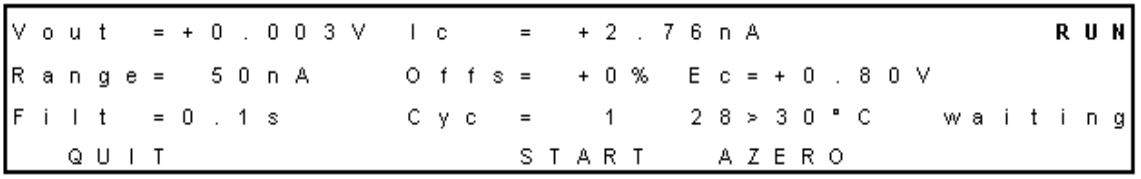


Figure 3-36 Run (Waiting) Screen

2. Select **F3 START** to start the run. The Run screen appears (Figure 3-37).

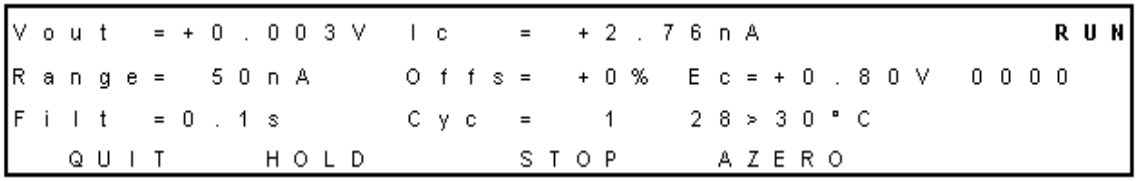


Figure 3-37 Run Screen After Starting a Run

3. You can do the following:
   - To pause or put the run on hold during the run, select **F2 HOLD**. When you are ready to resume the run, select **F2 RESUME**.
   - To stop the run, select **F3 STOP**. The Run (Waiting) screen appears.
   - If Cycles is set to Continuous, select **F3 STOP**, then select **F1 QUIT**. The run stops and the Events Setup screen appears.
   - If Cycles is set to a number from 1 to 999, the Events Setup screen appears at the end of the run.
   - When you want to return to the Main screen, select **F1 QUIT** > **F1 PREV** > **F1 PREV**.

3.5 Using Pulse (PAD) Mode

You can use pulse mode to perform pulsed amperometric detection (PAD). After you are comfortable with the 2465 Detector, use Table 3-8 as a quick reference list of tasks in pulse mode.

Table 3-8 Pulse Mode Quick Reference List

Task in Pulse Mode	Starting Screen	Command Sequence
Check settings	MAIN	F3 PULSE > F5 NEXT > F5 NEXT
Make a chart mark	PULSE SETUP1	F5 NEXT > F5 NEXT > F3 MARK
Turn the flow cell on or off	PULSE SETUP1	F5 NEXT > F5 NEXT > F2 CELL=.... > F2 YES
Change polarity	PULSE SETUP1	F5 NEXT > F3 POLAR=... > F2 YES
Autozero the detector	PULSE SETUP1	F5 NEXT > F5 NEXT > F4 AZERO
Change the range	PULSE SETUP1	F5 NEXT > F5 NEXT
Create and run a time file	PULSE SETUP1	F5 NEXT > F4 EVENTS > F5 NEXT > [change values as needed] > F2 ADD > [repeat last 2 steps for each line] > F1 PREV > F3 RUN > F3 START
Run a time file	PULSE SETUP1	F5 NEXT > F4 EVENTS > F3 RUN > F3 START
Find and run a time file	PULSE SETUP1	F5 NEXT > F4 EVENTS > F5 NEXT > [press up or down value keys to select a time file] > F3 RUN > F3 START
Stop a (running) time file	RUN	F3 STOP
Hold, then resume a (running) time file	RUN	F2 HOLD > F2 RESUME

3.5.1 Setting Initial Conditions in Pulse Mode

To set initial conditions in pulse mode:

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears (Figure 3-38).

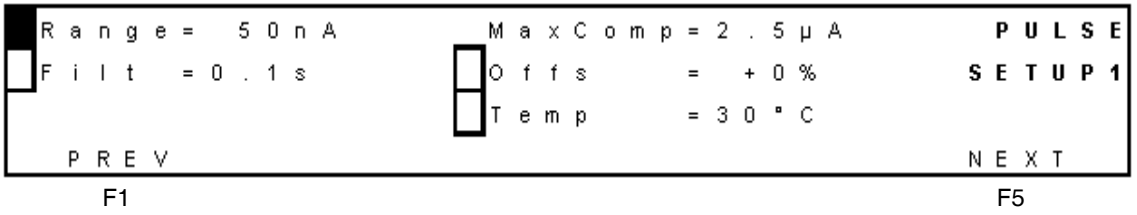


Figure 3-38 Pulse Setup1 Screen

Note: *You can change the Range, Filt (filter time constant), Offs (offset), and Temp (detector oven temperature), as needed (see Table 3-2, Status and Control Parameters).*

2. Select **F5 NEXT**. The Pulse Setup2 screen appears (Figure 3-39).

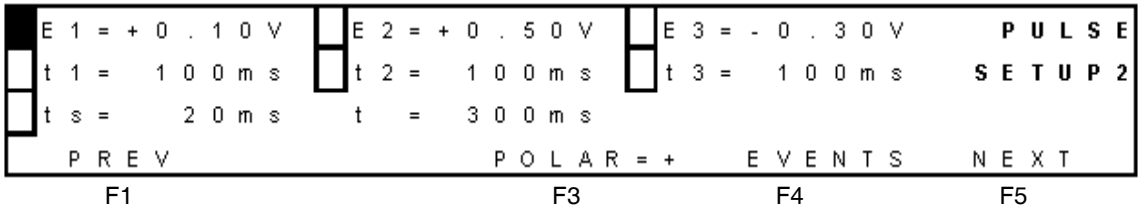


Figure 3-39 Pulse Setup2 Screen

Note: *Once a change is made to the potential and entered, it is executed immediately.*

3. You can change the following parameters, as needed (see Table 3-2, Status and Control Parameters):

   - E_1, E_2, E_3 – Cell potential settings of the pulse.
   - t_1, t_2, t_3, t_s – Duration of potential steps E_1, E_2, and E_3.

   Note: *The value of t_s will always be at least 60 msec less than t_1.*

4. You can check and change polarity in pulse mode as follows:
   - If F3 indicates `POLAR=+`, the polarity is positive, and an oxidation current gives a positive signal. If F3 indicates `POLAR=-`, the polarity is negative, and a reduction current gives a positive signal.
   - To change the polarity setting, select **F3 POLAR**.
   - If the polarity is positive, the Change Polarity to Negative screen appears (Figure 3-40). To change it to negative, select **F2 YES**. The Pulse Setup2 screen appears.

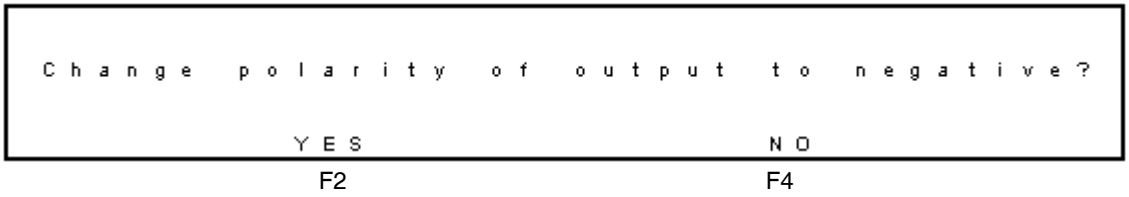


Figure 3-40 Change Polarity to Negative Screen

   - If the polarity is negative, the Change Polarity to Positive screen appears. To change it to positive, select **F2 YES**. The Pulse Setup2 screen appears.
   - If you do not want to change the polarity, select **F4 NO**. The Pulse Setup2 screen appears.
5. Select **F5 NEXT**. The Pulse Stat screen appears (Figure 3-41).

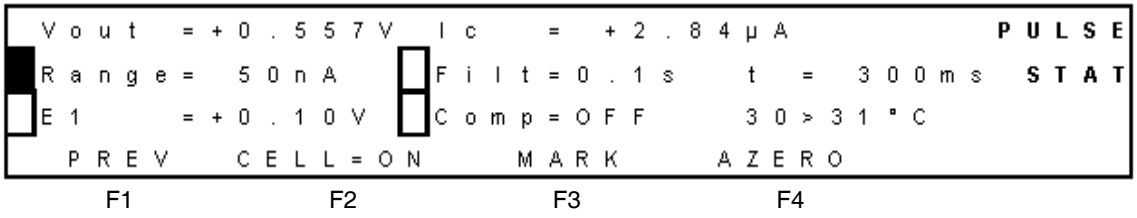


Figure 3-41 Pulse Stat Screen when Flow Cell Is On

If the flow cell is off (see F2 in Figure 3-42), see Section 3.5.5, Turning the Flow Cell On and Off in Pulse Mode.

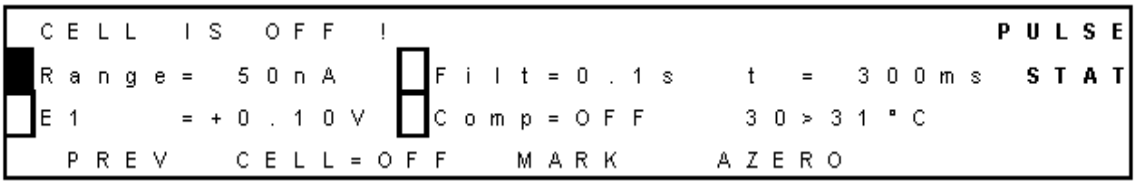


Figure 3-42 Pulse Stat Screen when Flow Cell Is Off

Note: *You can change the Range, E_1 (cell voltage), Filt (filter time constant), and Comp (maximum compensation), as needed (see Table 3-2, Status and Control Parameters).*

6. You can check the status of the following parameters (see Table 3-2, Status and Control Parameters):
   - Verify that the flow cell is on or off (F2).
   - Check the actual cell current (output signal in volts) at V_{out}.
   - Check the cell potential (I_c).
   - Check the actual detector oven temperature (to the left of the >) and the temperature setting (to the right of the >).
   - Track the time since entering the Pulse Stat screen.
7. You can also do the following:
   - Select **F3 MARK** to make a chart mark.
   - Select **F4 AZERO** to autozero the detector.
8. When you want to return to the Main screen, select **F1 PREV** > **F1 PREV** > **F1 PREV**.

3.5.2 Changing the Range in Pulse Mode

To change the range in pulse mode:

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears (Figure 3-38).
2. If Range is not selected, move the cursor to select it.
3. Use the value keys to change the range.
4. Select **F5 NEXT**. The Pulse Setup2 screen appears (Figure 3-39).
5. Select **F5 NEXT**. The Pulse Stat screen appears (Figure 3-41).

3.5.3 Making a Chart Mark in Pulse Mode

To make a chart mark in pulse mode (see Section 2.5, Making I/O Signal Connections):

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears (Figure 3-38).
2. Select **F5 NEXT**. The Pulse Setup2 screen appears (Figure 3-39).
3. Select **F5 NEXT**. The Pulse Stat screen appears (Figure 3-41).
4. Select **F3 MARK**. The 2465 Detector triggers a marker signal (chart mark) on output (a baseline spike of 10% FS, 100-ms duration).

3.5.4 Autozeroing the Detector in Pulse Mode

To autozero the detector in pulse mode (see Section 2.5, Making I/O Signal Connections):

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears (Figure 3-38).
2. Select **F5 NEXT**. The Pulse Setup2 screen appears (Figure 3-39).
3. Select **F5 NEXT**. The Pulse Stat screen appears (Figure 3-41).
4. Select **F4 AZERO**. The 2465 Detector sets the output voltage to 0 V or to the offset voltage, and control parameter `Comp = Off` changes to `Comp = On`.

3.5.5 Turning the Flow Cell On and Off in Pulse Mode

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minimum amount of backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Note: *In electrochemical detection, switching the flow cell on or off is like switching a lamp in a UV detector on or off: both events destabilize the system. It takes some time before conditions are stabilized. Stabilization time depends on the range setting, and gets longer at more sensitive range settings.*

To turn the flow cell on in pulse mode:

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears.
2. Select **F5 NEXT**. The Pulse Setup2 screen appears.
3. Select **F5 NEXT**. The Pulse Stat screen appears (Figure 3-43) and the value for F2 CELL is OFF.

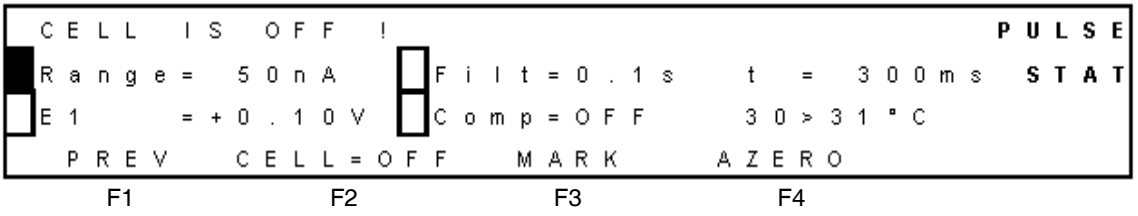


Figure 3-43 Pulse Stat Screen with Cell Off

4. Select **F2 CELL=OFF**. The Switch Cell On screen appears (Figure 3-44).

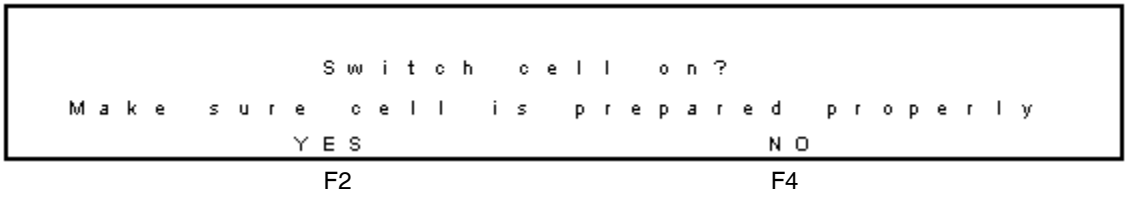


Figure 3-44 Switch Cell On Screen

5. Select **F2 YES**. The Pulse Stat screen appears and the value for F2 CELL is ON.

To turn the flow cell off in pulse mode:

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 screen appears.
2. Select **F5 NEXT**. The Pulse Setup2 screen appears.
3. Select **F5 NEXT**. The Pulse Stat screen appears and the value for F2 CELL is ON.
4. Select **F2 CELL=ON**. The Switch Cell Off screen appears (Figure 3-45).

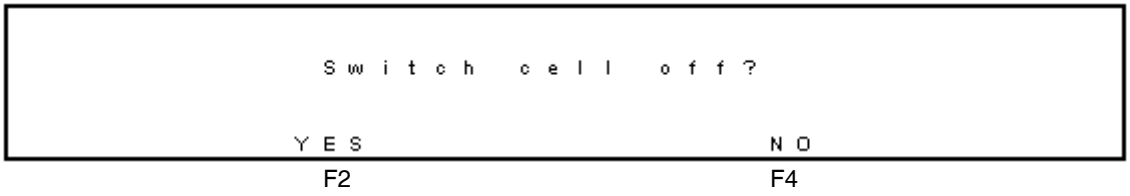


Figure 3-45 Switch Cell Off Screen

5. Select **F2 YES**. The Pulse Stat screen appears and the value for F2 CELL is OFF.

3.5.6 Creating a Time File in Pulse Mode

The following example describes how to program a simple time file in pulse mode. Each row in Table 3-9 represents one line to be programmed, and each parameter change represents one event. The entire table represents one time file.

In this example, an autozero is performed at 0.00 minute (initial conditions), the cell potential (E_c) changes at 4.80 minutes, and two events occur at 4.90 minutes (a 10% offset and another autozero). The end cycle time is 5.00 minutes.

Table 3-9 Programming a Sample Time File in Pulse Mode

Time (min)	Line No.	Range	Filt	Auto Zero	Output	Polar	E_{cell}	Offset
000.00	1	100 nA	1 s	set	0000	+	0.80 V	0%
004.80	2	100 nA	1 s	not	0000	+	0.65 V	0%
004.90	3	100 nA	1 s	set	0000	+	0.65 V	10%
005.00	End cycle time (end of run)							

To create timed events in pulse mode:

1. From the Main screen, select **F3 PULSE**. The Pulse Setup1 Screen appears (Figure 3-46).

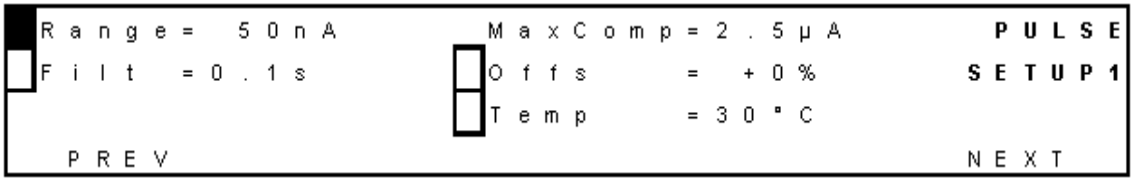


Figure 3-46 Pulse Setup1 Screen

2. Select **F5 NEXT**. The Pulse Setup2 screen appears (Figure 3-47).

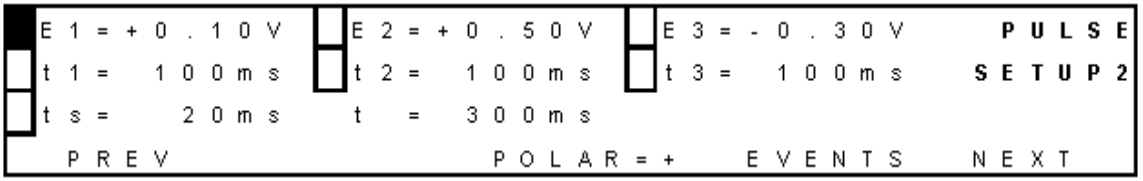


Figure 3-47 Pulse Setup2 Screen

3. Select **F4 EVENTS**. The Events Setup screen appears (Figure 3-48).

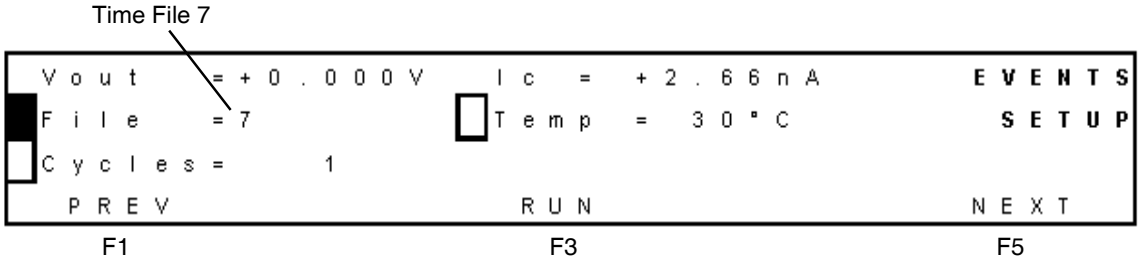


Figure 3-48 Events Setup Screen

4. Ensure that the initial conditions are acceptable for your purpose. At a minimum, select the following initial conditions:

   a. Time file 6 (**File = 6**) is the default time file. To program time file 7, select **File**, then use the value keys to select **File = 7**.

b. Ensure that the value for the Temp is the same as on the Pulse Setup1 screen.

Note: *If you want to program a temperature change, allow sufficient time after the change for equilibration.*

c. If needed, change the number of cycles to **Cycles = 1** using the down cursor key and the value key.

Note: *Select a cycle value of 1 when an "inject start event in" is connected to pins 13 and 15 of the 2465 Detector (see Section 2.5, Making I/O Signal Connections).*

d. Select **F5 NEXT**. The Prog screen appears (Figure 3-49). The number under PROG shows that this time file currently has one line. Time = 000.00 indicates the initial conditions.

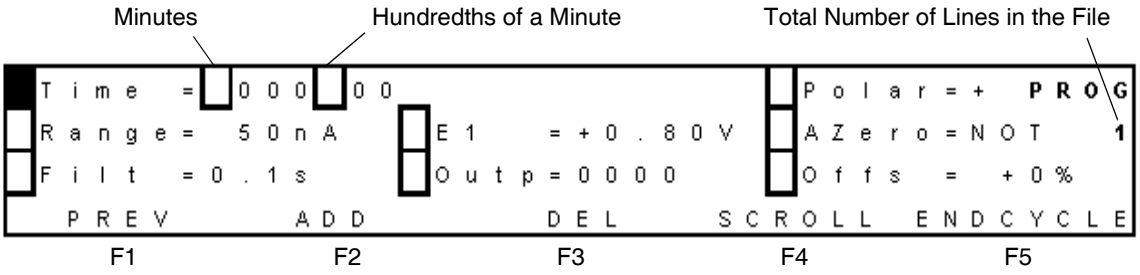


Figure 3-49 Prog Screen with Initial Conditions

5. If the total number of lines in the file is greater than one:

   a. Select **F3 DEL**. The Delete Timefile screen appears (Figure 3-50).

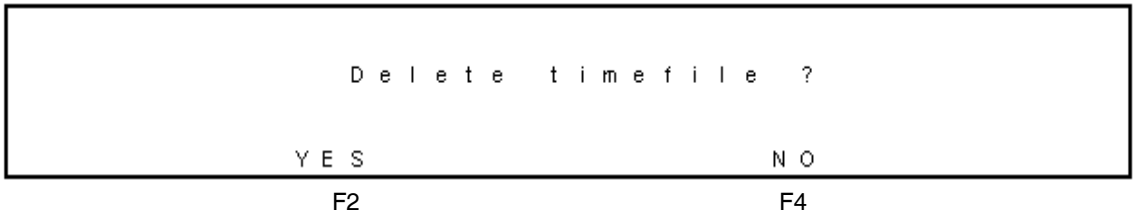


Figure 3-50 Delete Timefile Screen

   b. Select **F2 YES** to delete the contents of time file 7 (except the first and last lines of file 7). The Prog screen appears.

   c. To verify that time file 7 is empty, select **F1 PREV**. The End Cycle Time screen appears. Verify that **Time = 000.01**, indicating that the file is empty.

d. Select **F1 PREV**. The Events Setup screen appears. Verify that **File = 7**, indicating that file 7 is the active file.

e. Select **F5 NEXT**. The Prog screen appears.

6. Change line 1 in time file 7:

   a. Verify that **Time = 000.00**.

   b. Select **AZero**, then use either value key to select **SET**.

   c. Select **F2 ADD**. The Overwrite Time screen appears (Figure 3-51).

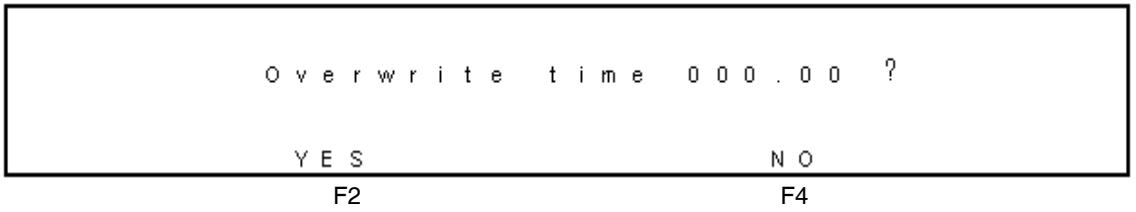


Figure 3-51 Overwrite Time Screen

d. Select **F2 YES**. The Prog screen appears (Figure 3-52).

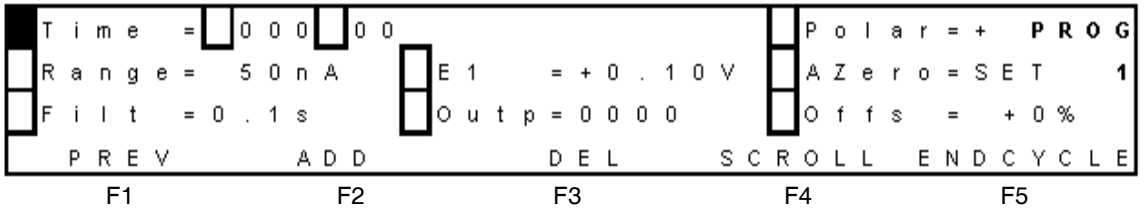


Figure 3-52 Prog Screen After Changing Line 1

7. Create line 2:

   a. Select **Time**.

   b. Move the cursor to minutes, then change the value to **4**.

   c. Move the cursor to hundredths of a minute, then change the value to **80**.

   d. Move the cursor to Range, then change the value to **100**.

   e. Select **E1**, then change the value to **+0.65V**. The suffix # appears when the cell potential changes.

   f. Press the **Enter** key to confirm the change to E_1. The suffix # disappears.

   g. Select **AZero**, then change the value to **NOT**.

h. Select **F2 ADD**. The Prog screen appears (Figure 3-53).

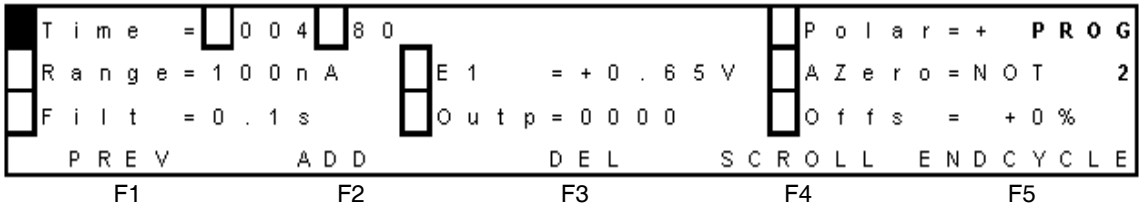


Figure 3-53 Prog Screen After Adding Line 2

8. Create line 3:
   a. For Time, select hundredths of a minute, then increase the value to **90**.
   b. Select **AZero**, then change the value to **SET**.
   c. Select **Offs**, then change the value to **+10%**.
   d. Select **F2 ADD**. The Prog screen appears (Figure 3-54). The number under PROG, 3, shows that this time file currently has a total of three lines. The time that this file will execute is 4.90 minutes after the start.

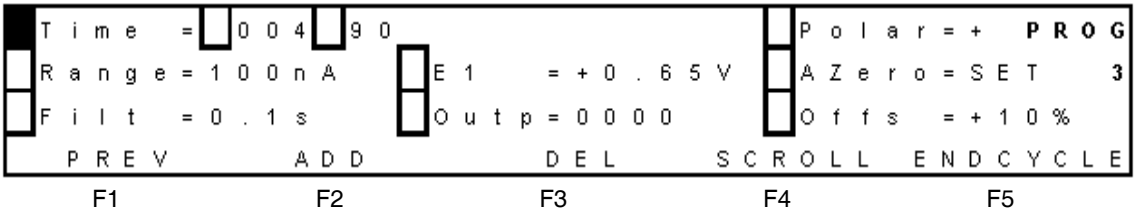


Figure 3-54 Prog Screen After Adding Line 3

Note: *The default end cycle time is 0.01 minute later than the time of the last programmed line. The end cycle time must be at least 0.01 minute later than the last programmed line.*

9. Change the last line of the time file:
   a. Select **F5 ENDCYCLE**. The End Cycle Time screen appears (Figure 3-55).

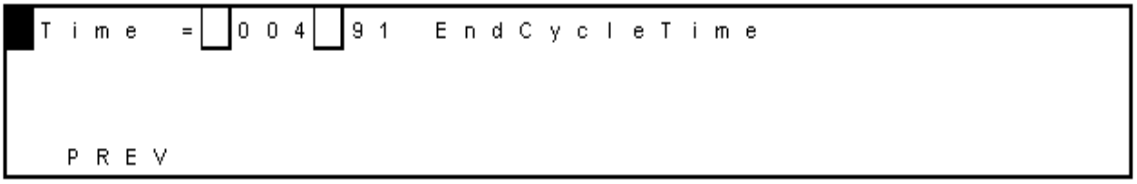


Figure 3-55 End Cycle Time Screen

b. Increase the end cycle time to 005.00 minutes.

c. Select **F1 PREV**. The Events Setup screen appears (Figure 3-56).

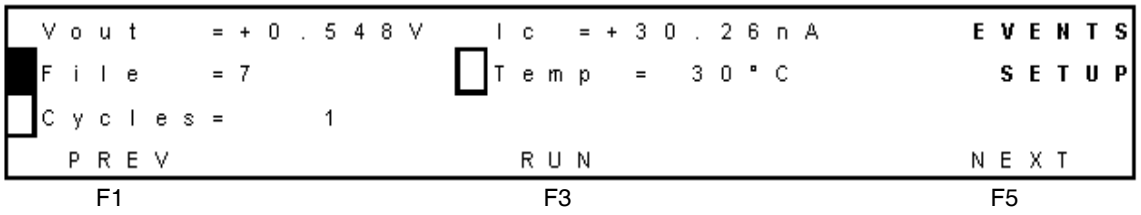


Figure 3-56 Events Setup Screen with Time File 2

10. Review all four lines in the time file:

    a. Select **F5 NEXT**. The Prog 3 screen appears, and Time = 000.00 minutes.

    b. Review the values for the first line of the time file.

    c. Select **F4 SCROLL**. The second line of the time file appears, and Time = 004.80 minutes. Review the values.

    Note: *Lines scroll according to their time (in this case, you should see the lines at times 0.00, 4.80, 4.90, then 5.00, in increasing order, then 0.00 again).*

    d. Repeat step c for all lines in the time file. Finish at the end cycle time.

    e. Select **F1 PREV**. (If you were not at the end cycle time, the End Cycle Time screen appears. Select **F1 PREV**.) The Events Setup screen appears.

Time file 7 is ready to run (see Section 3.5.7, Running a Time File in Pulse Mode).

3.5.7 Running a Time File in Pulse Mode

To run a time file in pulse mode:

1. From the Events Setup screen, select **F3 RUN**. The Run (Waiting) screen appears (Figure 3-57), and the system waits for a start command. This can be a keypad command or an external trigger from connector A on the rear panel (see Section 2.5, Making I/O Signal Connections).

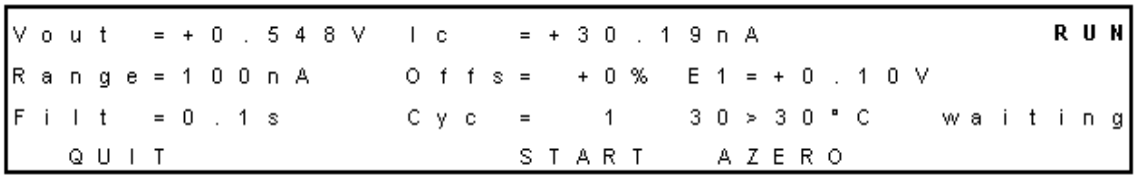


Figure 3-57 Run (Waiting) Screen

2. Select **F3 START** to start the run. The Run screen appears (Figure 3-58).

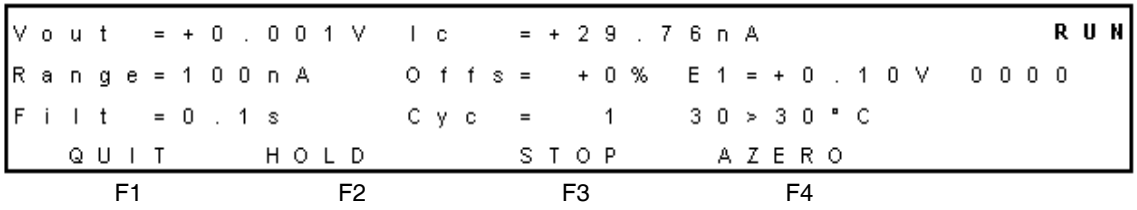


Figure 3-58 Run Screen After Starting a Run

3. You can do the following:
   - To pause or put the run on hold during the run, select **F2 HOLD**. When you are ready to resume the run, select **F2 RESUME**.
   - To stop the run, select **F3 STOP**. The Run (Waiting) screen appears.
   - If Cycles is set to Continuous, select **F3 STOP**, then select **F1 QUIT**. The run stops and the Events Setup screen appears.
   - When you want to return to the Main screen, select **F1 QUIT** > **F1 PREV** > **F1 PREV** > **F1 PREV**.

   Note: *If Cycles is set to a number from 1 to 999, the Events Setup screen appears at the end of the run.*

3.6 Using Scan Mode

You can use scan mode to perform simple scans. After you are comfortable with the 2465 Detector, use Table 3-10 as a quick reference list of tasks in scan mode.

Table 3-10 Scan Mode Quick Reference List

Task in Scan Mode	Starting Screen	Command Sequence
Check settings	MAIN	F4 SCAN
Change the temperature setting and monitor it	MAIN	F4 SCAN (to change the setting), then F5 NEXT (to monitor the temperature)
Turn the flow cell on or off	SCAN SETUP	F3 CELL=.... > F2 YES
Start a scan	SCAN SETUP	F5 NEXT > F2 START
Stop a scan	SCAN STAT	F2 STOP
Hold, then resume a scan	SCAN STAT	F5 HOLD > F5 RESUME

3.6.1 Setting Initial Conditions in Scan Mode

To set the initial conditions in scan mode:

1. From the Main screen, select **F4 SCAN**. The Scan Setup screen appears (Figure 3-59).

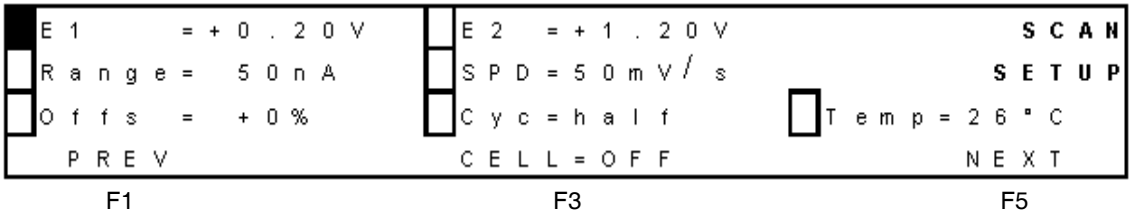


Figure 3-59 Scan Setup Screen

2. You can change E_1 and E_2 (cell voltage), Range, Offs (offset), SPD (scan speed), Cyc (cycle length), and Temp (detector oven temperature), as needed (see Table 3-2, Status and Control Parameters).
3. Select **F5 NEXT**. The Scan Stat screen appears (Figure 3-60, with 10 V full scale).

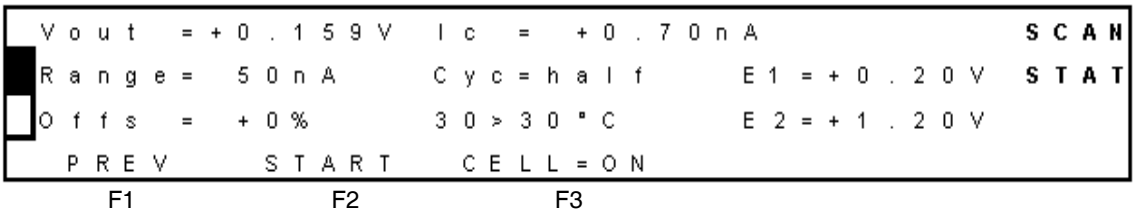


Figure 3-60 Scan Stat Screen

4. You can change the Range and Offs (offset), as needed (see Table 3-2, Status and Control Parameters).

3.6.2 Turning the Flow Cell On and Off in Scan Mode

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minimum amount of backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Note: *In electrochemical detection, switching the flow cell on or off is like switching a lamp in a UV detector on or off: both events destabilize the system. It takes some time before conditions are stabilized. Stabilization time depends on the range setting, and gets longer at more sensitive range settings.*

To turn the flow cell on in scan mode:

1. From the Main screen, select **F4 SCAN**. The Scan Setup screen appears (Figure 3-61) and the value for F3 CELL is OFF.

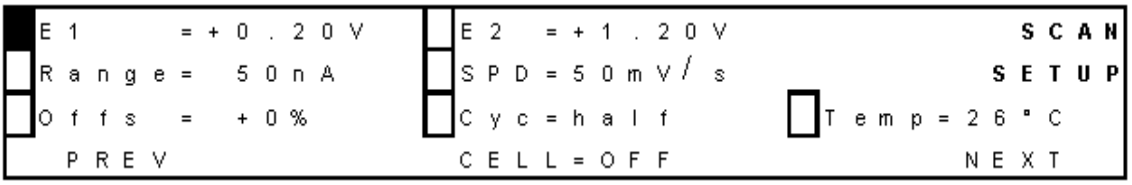


Figure 3-61 Scan Setup Screen

2. Select **F3 CELL=OFF**. The Switch Cell On screen appears (Figure 3-62).

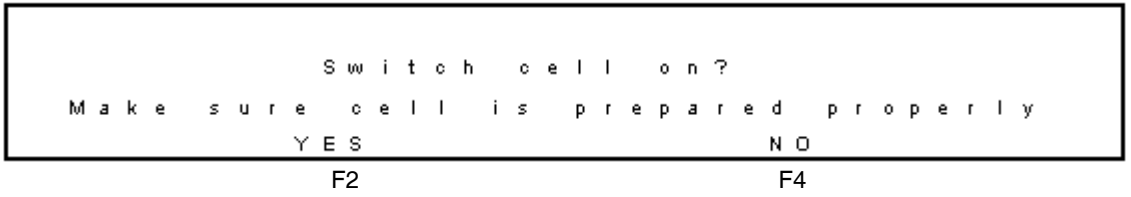


Figure 3-62 Switch Cell On Screen

3. Select **F2 YES**. The Scan Setup screen appears and the value for F3 CELL is ON.

To turn the flow cell off in scan mode:

1. From the Main screen, select **F4 SCAN**. The Scan Setup screen appears and the value for F3 CELL is ON.
2. Select **F3 CELL=ON**. The Switch Cell Off screen appears (Figure 3-63).

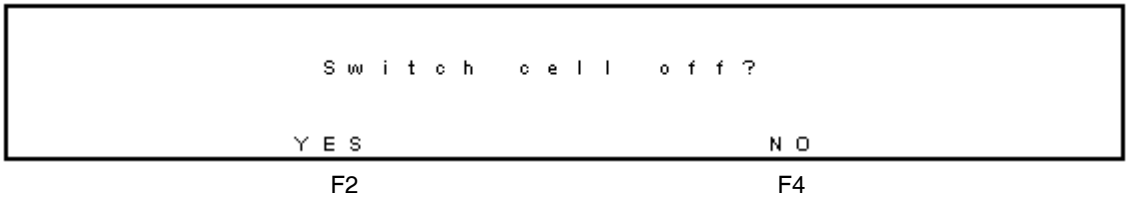


Figure 3-63 Switch Cell Off Screen

3. Select **F2 YES**. The Scan Setup screen appears and the value for F3 CELL is OFF.

3.6.3 Performing a Scan in Scan Mode

To perform a scan in scan mode:

1. From the Main screen, select **F4 SCAN**. The Scan Setup screen appears (Figure 3-64).

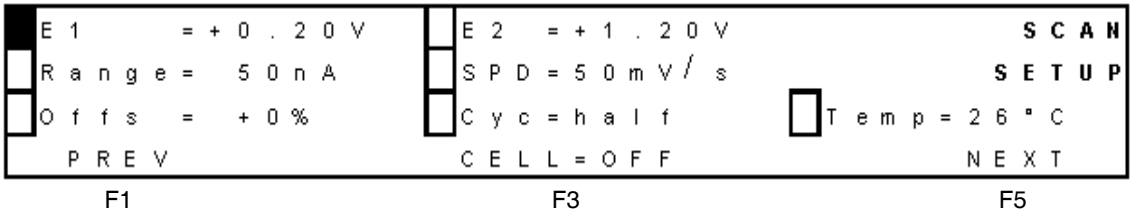


Figure 3-64 Scan Setup Screen

2. You can change E_1 and E_2 (cell voltage), Range, Offs (offset), SPD (scan speed), Cyc (cycle length), and Temp (detector oven temperature), as needed.
3. Select **F5 NEXT**. The Scan Stat screen appears (Figure 3-65).

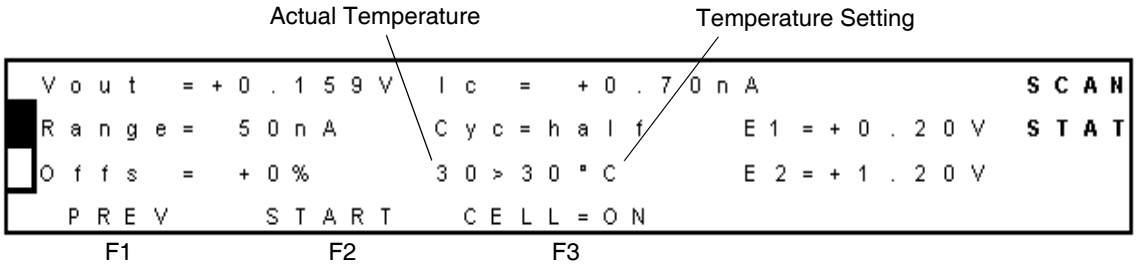


Figure 3-65 Scan Stat Screen

4. To prepare for a scan:
   a. If you see an `Out of Range` message on the top row, increase the range, then allow sufficient time for V_{out} and I_c values to appear.
   b. You can change the value for offset (Offs), as needed.
   c. Monitor the actual temperature until it stabilizes.
   d. Ensure the flow cell is on (see Section 3.6.2, Turning the Flow Cell On and Off in Scan Mode).
5. Select **F2 START**. The Scan Stat screen appears (Figure 3-66).

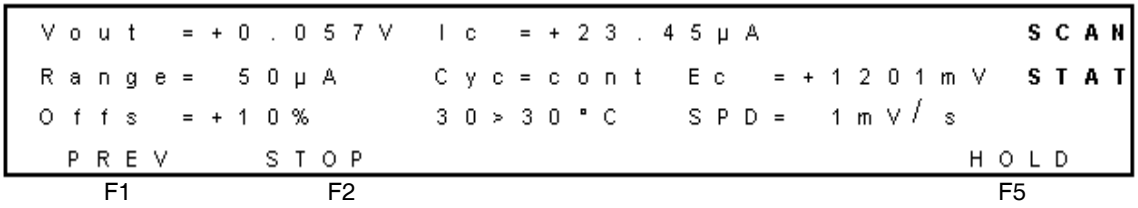


Figure 3-66 Starting a Scan

6. If you need to stop the scan, select **F2 STOP**.

   You can also select **F5 HOLD** to pause or put the scan on hold. The values for V_{out} and I_c continue to change. Select **F5 RESUME** to resume the scan.
7. When the scan is finished, select **F2 STOP**.
8. When you want to return to the Main screen, select **F1 PREV** > **F1 PREV**.

3.7 Optimizing the Working Potential

You must optimize the working potential for each different set of conditions or methods in which you plan to run samples (temperature, flow cell size, electrodes, mobile phase, flow rate, sample composition, column, and so on).

3.7.1 Constructing a Hydrodynamic Voltammogram

Attention: *Before attempting to obtain a hydrodynamic voltammogram, make sure you optimize the chromatographic conditions.*

To construct a hydrodynamic voltammogram:

1. Prepare a solution of the analyte at a concentration between 1 and 100 µM/L in mobile phase.
2. Allow approximately 60 minutes to stabilize the electrochemical detector in DC mode at a high potential, at the selected temperature, with the flow cell off.
3. Read the background current from the display of the detector (I_c) and measure the noise.

4. Start the run by injecting the compound, and observe the signal.
   - If no signal is obtained at a high working potential, the compound is not electrochemically active. Derivatization of the compound may be an option.
   - If a peak is measured, decrease the working potential by 50 or 100 mV, and repeat steps 2 to 4 until the lowest potential setting is obtained. Figure 3-67 shows chromatograms that were obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), with 100-mV steps.

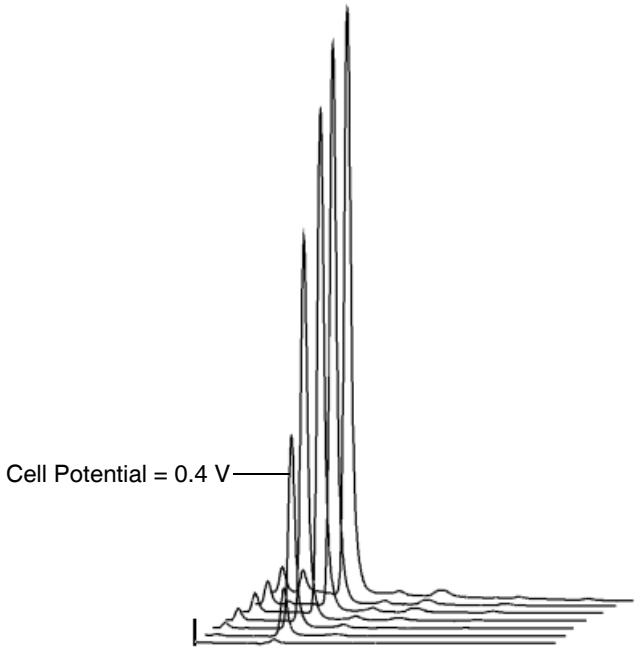


Figure 3-67 Constructing a Hydrodynamic Voltammogram for Norepinephrine

5. Plot the peak heights and the background currents against the working potential (Figure 3-68).

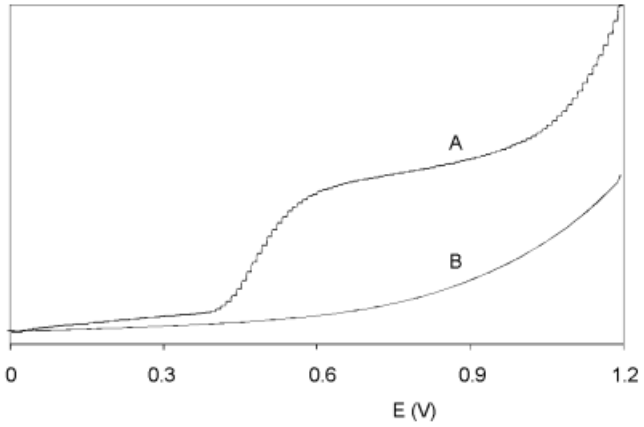


Figure 3-68 Scanning Voltammetry of Norepinephrine

Figure 3-68 shows the scanning voltammetry of 1.0 µM/L norepinephrine (scan A) at a glassy carbon WE, at a scan speed of 10 mV/s. Scan B is the blank solvent. Scan A is the sum of the analyte signal and the background signal.

6. Plot the signal-to-noise ratio against the working potential to obtain the working potential that gives the best sensitivity.

3.7.2 Constructing a Scanning Voltammogram

Use scan mode to program the Scan Setup screen (Figure 3-69).

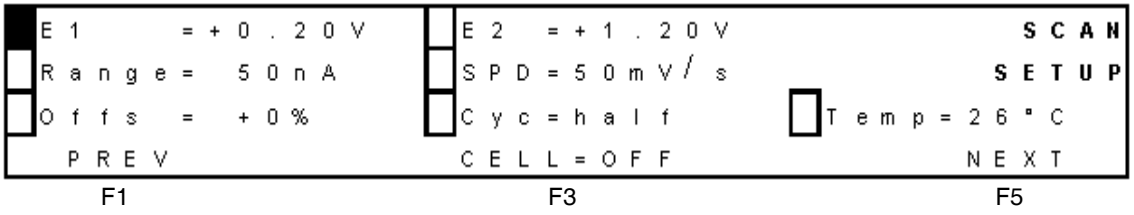


Figure 3-69 Programming Scan Mode

Depending on your data acquisition software and experimental setup, you can select a half, full, or continuous scan cycle, as follows:

- **Cyc=half** – A half scan sweeps the potential from low (E_1 = 0.20 V) to high (E_2 = 1.20 V; see Figure 3-68 and Figure 3-70).

- **Cyc=full** – A full scan includes the forward and reverse scans, i.e., from low (0.20 V) to high (1.20 V) and back to low.
- **Cyc=cont** – In the continuous scan cycle, the voltage is swept up and down continuously between the low and high potentials, E_1 and E_2.

Figure 3-70 shows the scanning voltammograms of 2,4-dimethylphenol (DMP), phenol (P), 2-chlorophenol (CP), 4-nitrophenol (NP), and a blank (buffer only).

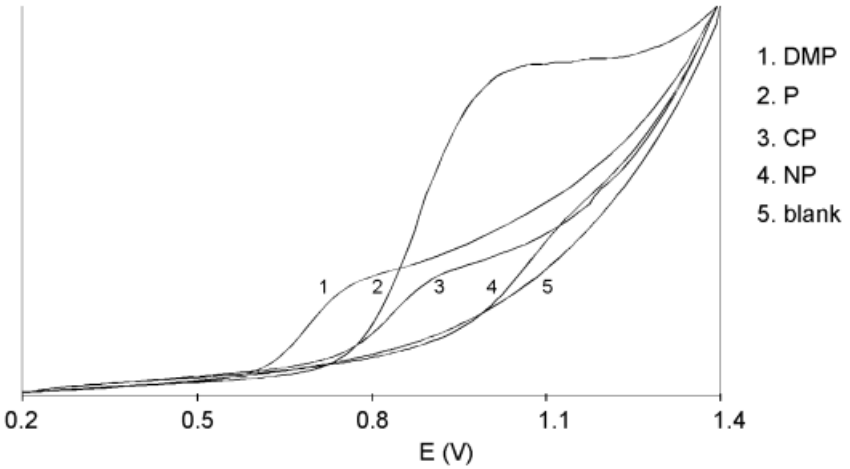


Figure 3-70 Overlay of Four "Half" Forward Scans

To obtain a scanning voltammogram such as in Figure 3-68 and Figure 3-70:

1. Remove the column from the LC system and record the voltammogram in flow injection analysis (FIA) mode.
2. Perform an initial scan to assess the optimum start and stop times of the scan:
   a. Dissolve the pure compound (preferably in the HPLC buffer) at a concentration of approximately 10 to 100 µM/L, then dilute it in HPLC buffer to the desired concentration.
   b. Install an injection loop of 100 µL.
   c. Set the LC flow rate at 40 µL/min.
   d. Detect the analyte sample for approximately 2.5 minutes. Decrease the flow rate if more scanning time is needed.
3. Start an initial run in DC mode at a high potential to estimate the required start and stop time of the scan after sample injection (Figure 3-71).

In scan mode, the scan is obtained at the plateau of the analyte sample. In this example, scanning takes place on top of the broad peak between 0.4 and 2.4 minutes after injection in FIA mode.

Note: *To prevent unreliable results, ensure that the analyte delivery is constant and without fluctuations.*

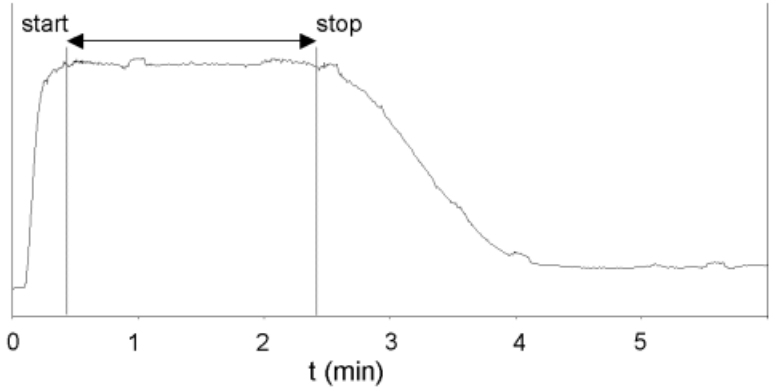


Figure 3-71 Chromatogram of a Sample in DC Mode

4. Set the data rate of the integrator at 1 Hz. This is the same rate as the voltage steps during the scan. A higher rate might induce a typical stepwise pattern.
5. To set up the scan:
   a. From the Main screen, select **F4 SCAN**. The Scan Setup screen appears (Figure 3-72).

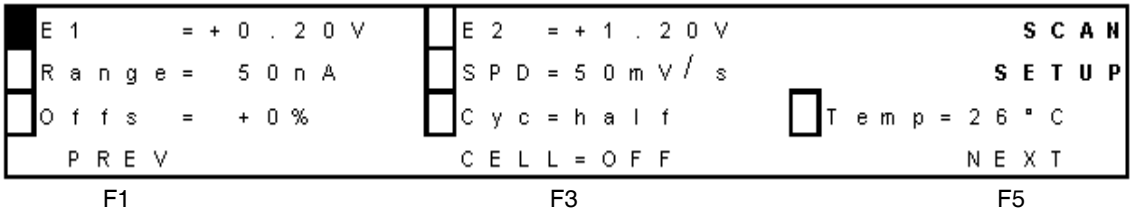


Figure 3-72 Programming Scan Mode

   b. Change the temperature if needed, and allow the detector to stabilize.
   c. Select a lower potential (E_1) and an upper potential (E_2).
   d. Set the range (Range) at 5 µA.
   e. Leave the offset (Offs) at +10%.

f. Select a scan speed (SPD) of 10 mV/s.

g. Set the cycle (Cyc) to half.

h. Ensure that the flow cell is on.

6. Scan the analyte:

   a. Inject the analyte.

   b. Select **F5 NEXT**. The Scan Stat screen appears.

   c. When the analyte enters the flow cell (see step 3 to calculate), select **F2 START**.

   d. Select **F2 STOP** when finished.

   e. Select **F1 PREV** to return to the Scan Setup screen.

7. Obtain a background scan by scanning the buffer.
8. Compare the background scan to the sample scan.
9. Repeat each scan three times for reliable results.

3.8 Shutting Down the 2465 Detector

Attention: *To prevent precipitation of salts and subsequent damage to the pump and/or detector, never switch off a pump with mobile phase in it. First turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell), then change the mobile phase to HPLC-quality water or a water/methanol mixture. For maximum stability, keep the system powered on continuously with buffer pumped through at a low flow rate.*

3.8.1 Turning Off the Flow Cell

The 2465 Detector has been developed for continuous operation. If preferred, the flow cell can be turned off at night.

To turn off the flow cell, use one of the following options from the Main screen:

- Select **F2 DC** > **F5 NEXT** and check F2. If CELL=ON, select **F2 CELL=ON** > **F2 YES**.
- Select **F3 PULSE** > **F5 NEXT** > **F5 NEXT**, and check F2. If CELL=ON, select **F2 CELL=ON** > **F2 YES**.
- Select **F4 SCAN** and check F3. If CELL=ON, select **F3 CELL=ON** > **F2 YES**.

3.8.2 Shutting Down for a Short Time

To shut down the detector on a routine basis (overnight or up to several days):

1. In the DC Stat, Pulse Stat, or Scan Stat screen, check the flow cell status. The flow cell can remain on if buffer or mobile phase is pumped continuously at a low flow rate, or you can turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Recirculate the mobile phase.
3. Decrease the pump flow rate to approximately 10% of normal flow rate.

3.8.3 Shutting Down for a Long Time

To shut down the detector for a long time (weeks):

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Flush the flow cell and column thoroughly with a 90:10 mixture of water:organic modifier (methanol or acetonitrile) to remove all traces of buffer salts. Do not leave the flow cell in water, but in a 90:10 mixture.
3. Store the column in methanol or acetonitrile, or according to the manufacturer's instructions.
4. Clean and store the flow cell dry.
5. Power off the 2465 Detector by pressing the power switch on the rear panel.

Attention: *Keep the salt-bridge Ag/AgCl reference electrode soaking in a container with a saturated KCl solution.*

Note: *If you plan to restart the 2465 Detector after a long shutdown period, and a water/methanol mixture has been in the flow cell, Waters recommends that you polish the electrode before restarting the detector (see Section 4.5, Maintaining the Salt-Bridge Reference Electrode).*

Chapter 4
Maintaining the 2465 Detector

Use this chapter to maintain the electrodes and the 2465 Detector.

Note: *For maximum stability, leave the system powered on continuously.*

4.1 Introduction

Read the maintenance instructions before starting the procedures for the tasks in this chapter. If you are uncertain about how to perform the procedures, contact Waters Technical Service (see Section 4.1.4) to have a trained service representative perform the procedure.

4.1.1 General Safety Precautions

Perform periodic leak checks on the flow cell, LC tubing, and connections.

Do not close or block the waste drain.

Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.

This instrument has a lithium battery. Only qualified service personnel should replace the battery. Dispose of the battery properly as chemical waste.

When you perform the maintenance procedures in this chapter on your 2465 Detector, follow these safety considerations.

Caution: *Untrained personnel should not open the instrument. Removing protective panels on the instrument can result in exposure to potentially dangerous voltages. Disconnect the instrument from all power sources before maintenance.*

Caution: *To prevent possible electrical shock, never disconnect an electrical assembly (including all interface cables) while power is applied to the detector.*

Power off the detector before making any electrical connections. Power off the detector and disconnect the power cord before performing maintenance procedures.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Attention: *To meet the regulatory requirements of immunity from external electrical disturbances that may affect the performance of this instrument, do not use cables longer than 9.8 feet (3 meters) when you make connections.*

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minute backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Attention: *When connecting or disconnecting the flow cell cable, ensure the flow cell power is off (see Section 3.8.1, Turning Off the Flow Cell).*

4.1.2 Frequency of Electrode Maintenance

Perform maintenance on the electrodes at the frequency in Table 4-1.

Table 4-1 Schedule of Electrode Maintenance Tasks

Electrode	Frequency	Task
Salt-bridge (Ag/AgCl) reference electrode	Monthly	Add saturated KCl or saturated NaCl solution and salt crystals to salt bridge. Make sure *no* air bubbles are present. Rarely, the cotton wool frit may need replacing.
Glassy carbon working electrode	Every 1 to 3 months	Polish the electrode and replace the spacer. Maintenance schedule depends on the application.
ISAAC reference electrode	Every 3 months	Polish, then coat with ISAAC solution. Replace the spacer.
Hydrogen reference electrode	As needed	No maintenance is needed when used for carbohydrate analysis. If not in use, disassemble the flow cell and store the components dry (see Section 4.2, Disassembling the Flow Cell).
Auxiliary electrode	As needed	No maintenance is needed, except in PAD of carbohydrates.

4.1.3 Spare Parts

Stock the recommended spare parts to minimize downtime (see Appendix B, 2465 Detector Components and Spare Parts). Appendix B lists all parts that you can replace. Parts not included in the list require replacement by a trained service representative.

4.1.4 Waters Technical Service

For problems with HPLC equipment, computer software, or hardware other than the 2465 Detector, consult the documentation or the manufacturer for the applicable instrument or program.

If you encounter a problem with the 2465 Detector that you cannot troubleshoot, contact Waters Technical Service at 800 252-4752, *U.S. and Canadian customers only.* Other customers, call your local Waters subsidiary or your local Waters Technical Service

Representative, or call Waters corporate headquarters for assistance in Milford, Massachusetts (U.S.A.).

4.2 Disassembling the Flow Cell

Use this procedure for a standard flow cell with the Hy-REF electrode or ISAAC reference electrode. The flow cell is assembled properly when it arrives. The force on the bolts is preset to 30 Ncm.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Attention: *When connecting or disconnecting the flow cell cable, ensure the flow cell power is off.*

To disassemble the flow cell:

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Stop the HPLC pump.
3. Open the door, then disconnect the following from the flow cell (Figure 4-1):
   - Inlet and outlet tubing
   - All three ends of the flow cell cable

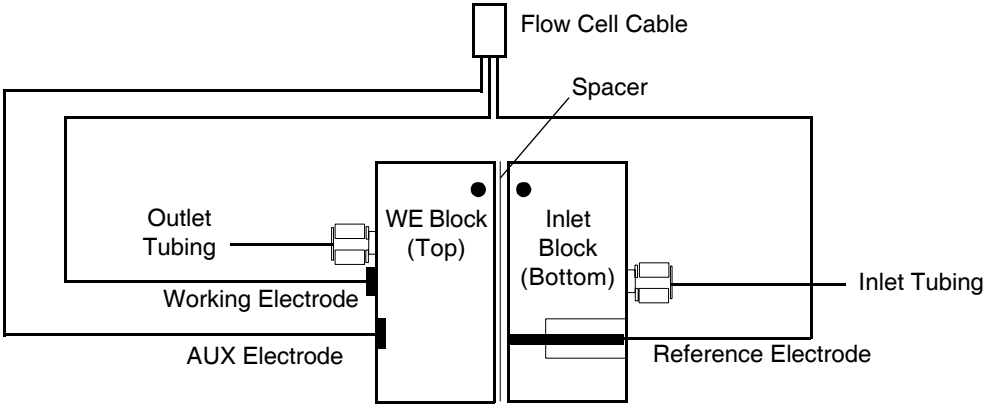


Figure 4-1 Disconnecting the Flow Cell

4. Slide the flow cell upward or downward out of the clamp.

STOP ***Attention:*** *To avoid damaging the flow cell, do not remove it by pulling it forcefully toward you.*

5. Remove the bolts from the flow cell using the hex key (Figure 4-2). The hex key is supplied in the flow cell startup kit.

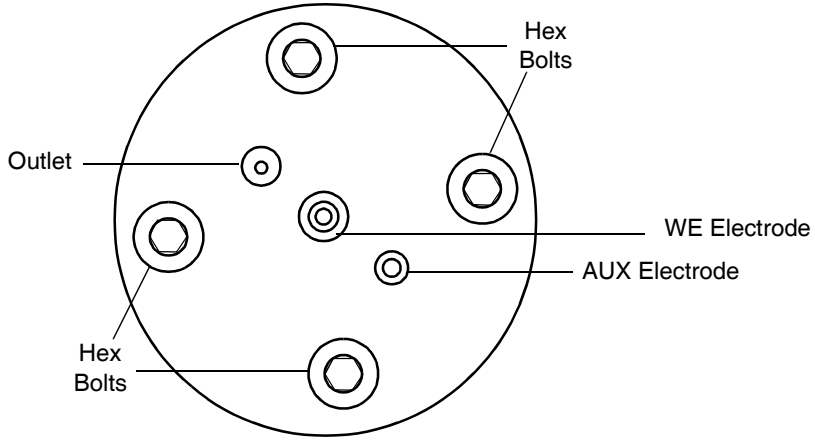


Figure 4-2 Removing the Bolts (Top View)

6. Carefully pull the blocks apart and remove the spacer from the blocks.

7. Continue with one of the following, as needed:
    - Section 4.3, Cleaning the Working Electrode
    - Section 4.4, Maintaining the ISAAC Reference Electrode
    - Section 4.5, Maintaining the Salt-Bridge Reference Electrode

4.3 Cleaning the Working Electrode

Clean the glassy carbon (GC) or metal working electrodes (WEs) after prolonged use and whenever the flow cell sensitivity or performance markedly decreases. This may be due to fouling by oxidation (reduction) reaction products that cause electrochemical changes to the electrode surface. Excessively high currents also may change the electrode surface.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Attention: *After prolonged use of the flow cell with a gold working electrode in pulse mode, the stainless steel AUX electrode acquires a yellowish sheen and the noise level increases. Polish the AUX electrode with steel wool regularly (every two months) to prevent these problems.*

Avoid unnecessary polishing. Only polish the working electrode surface if the mirror-like finish cannot be restored by wiping the electrode surface with a tissue wet with ethanol.

Attention: *Do not attempt to clean the metal (platinum, silver, or gold) working electrodes by pulsing. Instead, polish the working electrode as described in step 3 below.*

Use a separate polishing cloth for each type of working electrode and for the ISAAC reference electrode.

Note: *In pulsed amperometric detection (PAD) mode using a gold or platinum WE, polishing is only needed in exceptional circumstances because the pulsation of potential continuously regenerates the electrode surface. Furthermore, the pulsing of the potential slowly consumes the metal of the electrode, so after prolonged use, the working electrode is below the surface level of the cell and cannot be polished at all. When this happens, replace the electrode block.*

To clean the working electrode:

1. For the glassy carbon working electrode only, use electrochemical cleaning (pulsing). In pulse mode, let the potential jump between +1 and –1 V for 10 minutes. Use the following settings:
   - t_1 = 1000 msec
   - t_2 = 1000 msec
   - t_3 = 0 msec
   - E_1 = +1 V
   - E_2 = –1 V

   Note: *If the glassy carbon working electrode is not clean, continue with step 2.*

2. For platinum, silver, or gold working electrodes, clean the electrode surface:
   a. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
   b. Stop the HPLC pump.
   c. Open the door, then remove and disassemble the flow cell (see Section 4.2, Disassembling the Flow Cell).
   d. Wipe the electrode surface (in the top or WE block) with a tissue soaked with ethanol.
   e. If it still looks stained, continue with step 3.

   Attention: *To prevent damage to the electrodes, do* not *use the same polishing disc for both the ISAAC reference electrode and the working electrode.*

   To prevent scratches on the WE, which can increase the noise levels, the WE polishing disc must not have any dust or other particles. This is because the diamond slurry has 1-µm particles and must not be contaminated with larger particles.

3. Polish the electrode surface as follows:
   a. Shake the diamond slurry thoroughly before use.
   b. Rinse the WE polishing disc with HPLC-grade water.
   c. Apply a few drops of slurry on the wet polishing disc, and polish the electrode with a ‘figure 8’ motion for about 1 minute or less. Apply only gentle pressure.
   d. Check the surface visually; repeat the procedure if necessary.

4. After the WE is clean, reassemble the flow cell (see Section 4.6, Reassembling the Flow Cell). Use a new spacer.
5. Clean the WE polishing disc with HPLC-grade water and let it dry.
6. Store the dry WE polishing disc dust free in its plastic bag.

4.4 Maintaining the ISAAC Reference Electrode

The ISAAC reference electrode requires maintenance approximately once every three months. When the flow cell is opened to service the working electrode, the reference electrode should also be serviced.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

4.4.1 Cleaning the ISAAC Reference Electrode

To clean the ISAAC reference electrode:

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Stop the HPLC pump.
3. Open the door, then remove and disassemble the flow cell (see Section 4.2, Disassembling the Flow Cell). Figure 4-3 shows the location of the ISAAC reference electrode on the inlet block of the flow cell. This half of the flow cell only has a single electrical connector.

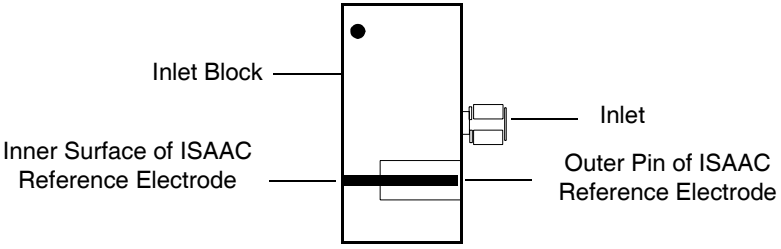


Figure 4-3 Locating the ISAAC Reference Electrode

Attention: *To prevent damage to the electrodes, do* not *use the same polishing disc for both the ISAAC reference electrode and the working electrode.*

Note: *The polishing kit contains a bottle of diamond slurry and an ISAAC polishing disc specifically for the ISAAC electrode.*

4. Gently polish the reference electrode surface as follows until the shining metal appears (Figure 4-4):
   a. Shake the diamond slurry thoroughly before use.
   b. If the bottle has not been opened, snip a small part of the top of the bottle.
   c. Rinse the ISAAC polishing disc with HPLC-grade water thoroughly to remove any dust particles. Use several milliliters of water.
   d. Apply one or two drops of slurry on the wetted polishing disc, and place the inner surface of the electrode block on the slurry.
   e. Applying only gentle pressure, polish the electrode with a ‘figure 8’ motion for a few minutes (Figure 4-4) until the shiny silver surface appears. Do not press down; allow the weight of the block to do the work. If the brown coating remains, apply more pressure and repeat the polishing. If you cannot remove the coating, the electrode is below the surface of the block; replace the inlet block.

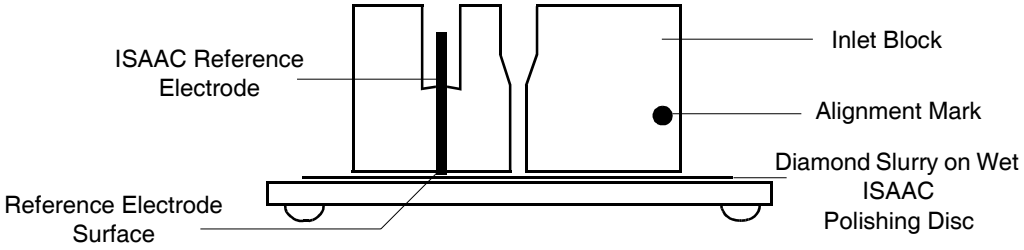


Figure 4-4 Polishing the ISAAC Reference Electrode

   f. Clean the electrode with a wet tissue and check the surface visually.
   g. If the metal surface does not shine, repeat the procedure until the shining metal surface appears.

Caution: *The ISAAC reference electrode solution is corrosive. Handle with care. Refer to the Material Safety Data Sheets for the solvents in use.*

5. Immediately after polishing, apply a few drops of the ISAAC reference electrode solution on the inner electrode surface to coat the electrode (Figure 4-5). After a few seconds, the electrode surface color changes.

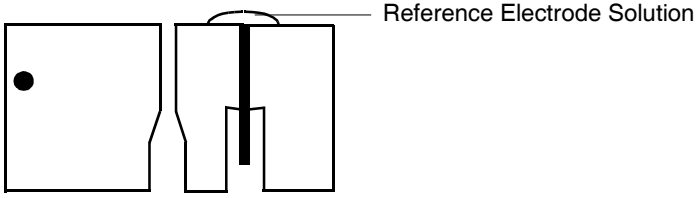


Figure 4-5 Coating the ISAAC Reference Electrode

6. Clean the polishing disc with HPLC-grade water and let it dry, then store the ISAAC polishing disc dust free in its plastic bag.
7. After 20 minutes, thoroughly flush the surface of the electrode with HPLC-grade water to remove the reference solution.
8. Reassemble the flow cell using a new spacer (see Section 4.6, Reassembling the Flow Cell).

4.4.2 Storing the ISAAC Reference Electrode

To store the ISAAC reference electrode:

1. If not in use for a long time, disassemble the flow cell (see Section 4.2, Disassembling the Flow Cell).
2. Clean the flow cell including the reference electrode with HPLC-grade water.
3. Dry the flow cell with lint-free tissues and store it dry.

4.5 Maintaining the Salt-Bridge Reference Electrode

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Three factors determine the proper functioning of the salt-bridge (SB) reference electrode:

- The chloride concentration must be kept at a strictly fixed level. This is best guaranteed by using a saturated chloride salt solution at a constant temperature.

- The salt bridge must allow proper electrical contact with the mobile phase. The higher the leakage through the frit, the better the conductivity.
- Because of their extreme compressibility, air bubbles inside or close to the salt bridge will lead to instability of the three-electrode configuration and to changes in conductivity and the ionic equilibrium of the reference electrode. This increases the noise considerably.

4.5.1 Inspecting the Salt-Bridge Reference Electrode

After prolonged use, the salt bridge in the reference electrode becomes less than fully saturated, which usually leads to poor reproducibility in electrochemical detection. The potential of the reference electrode is determined by the chloride concentration. If the salt bridge is not saturated and the KCl concentration changes:

- The noise in the system slowly but continuously increases.
- The background current increases.
- Sensitivity to movements and pump noise increases.

To inspect the salt-bridge reference electrode:

1. Check the electrode regularly for air bubbles (Figure 4-6). If an air bubble is trapped in the salt bridge or in the cotton plug that separates the salt bridge and the mobile phase, the flow cell becomes extremely sensitive to flow fluctuations and vibrations. This is caused by the high compressibility of the trapped air.

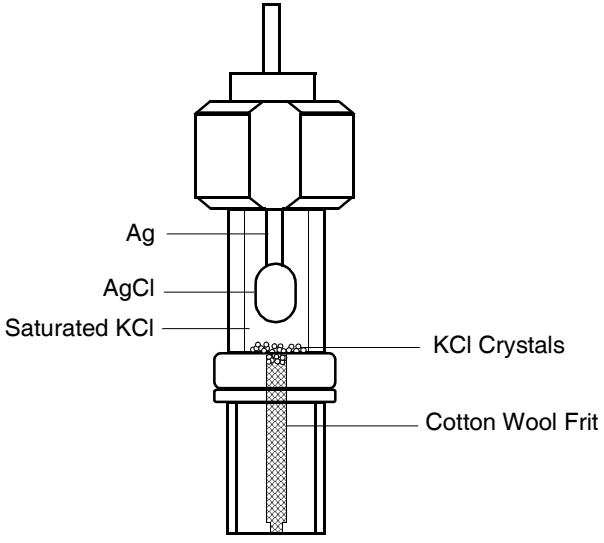


Figure 4-6 Inspecting a Reference Electrode

2. Inspect the electrode for salt crystals to ensure that some KCl crystals are present.
3. If you do not see salt crystals or if you see air bubbles, see Section 4.5.2, Cleaning the Salt-Bridge Reference Electrode.

4.5.2 Cleaning the Salt-Bridge Reference Electrode

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

To clean the salt-bridge reference electrode:

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Stop the HPLC pump.
3. Open the door, then disconnect the flow cell tubing and cable (Figure 4-7).

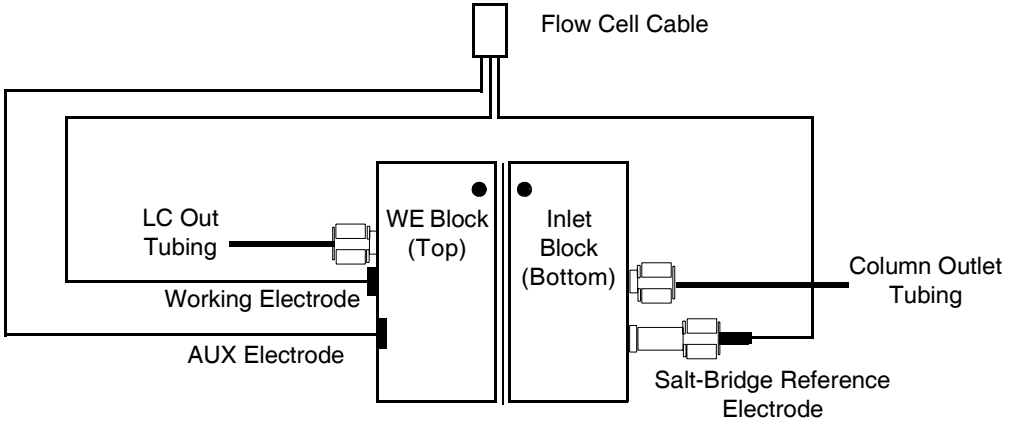


Figure 4-7 Removing the Salt-Bridge Reference Electrode

4. Remove the salt-bridge reference electrode from the inlet block.
5. Disassemble the electrode by unscrewing the swivel from the top of the electrode (Figure 4-8).

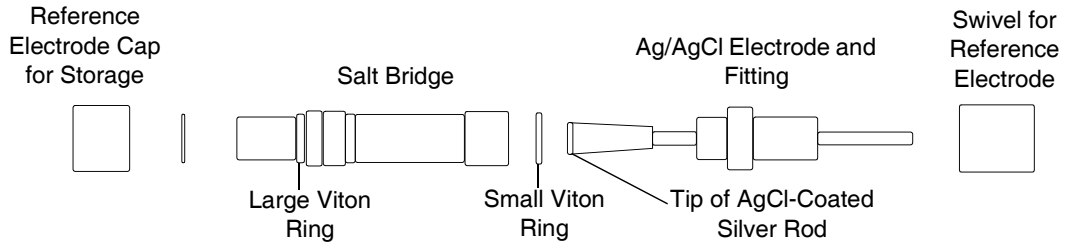


Figure 4-8 Components of the Salt-Bridge Reference Electrode

6. Remove the chloride solution from the salt bridge.
7. Clean all parts with HPLC-grade water.
8. If the silver on the tip of the Ag/AgCl electrode has a nonmetallic appearance, gently grind it on sanding paper.
9. Inspect the Viton® rings for wear and replace them if worn.
10. Examine the cotton wool frit in the salt bridge for discoloration and stains. If the frit is discolored or dried out, continue with Section 4.5.3, Replacing the Cotton Wool Frit.

    If the frit is acceptable, continue with Section 4.5.4, Installing the Salt-Bridge Reference Electrode.

4.5.3 Replacing the Cotton Wool Frit

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Required Materials

- An oversaturated and thoroughly degassed KCl solution
- A stainless steel rod of about 5-cm length and a diameter of 1 mm (a 1-mm drill); must be clean and free of contaminants such as oil or lubricant
- Clean, high-quality cotton wool

Procedure

To replace the cotton wool frit in the reference electrode:

1. If the reference electrode is new, remove the reference electrode cap.

2. Use a clean drill of 1 mm (0.039 inch) to push out the frit from the outside (Figure 4-9). Be careful not to damage the frit constriction.

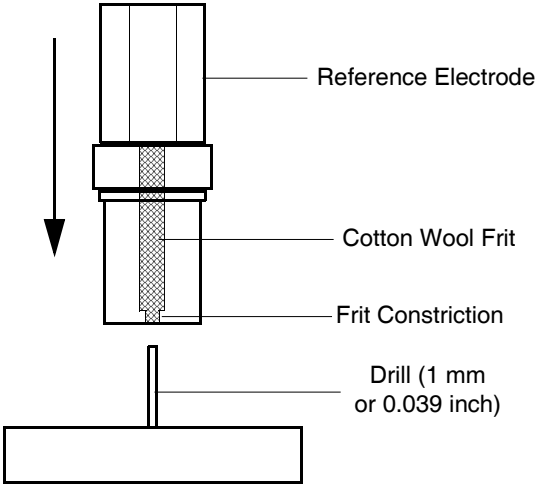


Figure 4-9 Removing the Cotton Wool Frit

3. Soak a small piece of cotton wool in saturated KCl solution. Ensure that no air is trapped in the cotton wool.
4. Clean the salt bridge thoroughly by rinsing with tap water, then HPLC-grade water.
5. Fill the salt bridge half-full with saturated KCl solution.
6. Use the drill to pack the wool from above through the KCl solution into the channel of the salt bridge. Compress it firmly, but not too much.
7. Continue with Section 4.5.4, Installing the Salt-Bridge Reference Electrode.

4.5.4 Installing the Salt-Bridge Reference Electrode

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

To install the salt-bridge reference electrode:

1. Fill the salt bridge completely with KCl solution, and add some KCl crystals from a saturated solution to ensure prolonged saturation.

2. Place the small Viton ring over the Ag/AgCl electrode and slowly insert it at an angle of 45° into the salt bridge. Make sure *not* to enclose an air bubble.
3. Tighten the black swivel of the reference electrode, but do not overtighten the swivel. A small droplet should appear at the cotton-wool frit.

   Note: *Do not remove this droplet because it ensures proper contact of the reference electrode with the mobile phase.*

4. Turn on the HPLC pump. Keep some tissues at hand as some mobile phase probably will spill during this mounting procedure.

   STOP ***Attention:*** *Use only factory-supplied fingertight fittings in the flow cell. Do not overtighten the fitting. Overtightening affects the flow pattern through the tubing (turbulence) and might markedly decrease the flow cell performance.*

5. Connect the column outlet to the flow cell inlet using small-bore tubing (0.3-mm ID) and one fingertight fitting. Let the tubing protrude approximately 0.6 in (1.5 cm) from the fitting and tighten it such that the tubing is not indented or is slightly indented by the fitting.
6. Connect the medium-bore tubing (0.5-mm ID) to the outlet of the flow cell.
7. Fill the flow cell by keeping it in an angle of 45° with the reference electrode fitting on top. Block the outlet with a finger, and let the air escape at the reference electrode fitting. Carefully inspect the thread of the fitting for trapped air bubbles.
8. When the reference electrode fitting is completely filled with mobile phase, mount the reference electrode while slowly releasing the outlet. Make sure no air bubbles are visible.
9. Align the flow cell with the flow cell clamp, keeping the reference electrode at the lower side and the outlet (LC Out) uppermost to prevent trapping of air bubbles, then slide the flow cell into the clamp.

   STOP ***Attention:*** *To prevent damage to the flow cell, do not install it by pushing it forcefully into the clamp from the front.*

10. Connect the flow cell cable to the electrodes as follows (Figure 4-10):
    - **REF** – Black wire to the reference electrode
    - **WE** – Red wire to the working electrode
    - **AUX** – Blue wire to the auxiliary electrode

    Note: *Do not power on the flow cell at this time.*

Attention: *Before powering on the flow cell, ensure that:*

- *The flow cell is filled with 20 to 100 mM electrolyte (buffer) that contains sufficient electrolyte (buffer ions). A flow cell filled with water is not adequate. A stable baseline will never be obtained if the cell is powered on with only water or another nonconducting mobile phase.*
- *No air bubbles are trapped in the flow cell.*
- *The outlet tubing from the flow cell leads to a waste container that is at a higher level than the flow cell. This ensures a minute backpressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level to prevent electrical baseline noise.*

Attention: *To prevent substantial damage to the working electrode or electronics, never power on the flow cell when:*

- *The cell cable is not correctly connected.*
- *The cell is only partly (or not at all) filled with buffer (not HPLC water).*
- *The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection.*

Attention: *When connecting or disconnecting the flow cell cable, ensure the flow cell power is off (see Section 3.8.1, Turning Off the Flow Cell).*

4.5.5 Storing the Salt-Bridge Reference Electrode

When not in use, store the salt-bridge reference electrode with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.

4.6 Reassembling the Flow Cell

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Attention: *When connecting or disconnecting the flow cell cable, ensure the flow cell power is off.*

To prevent deterioration of the S/N ratio and eventually the flow cell, do not overtighten the bolts. Also ensure that the black marks on both blocks line up before you insert and tighten the bolts. To prevent overtightening, loosen the bolts, tighten them until fingertight, then tighten them 1/4-turn more.

Note: *If you use the ISAAC reference electrode, add and equilibrate with chloride ions in the mobile phase before installing the electrode. Waters recommends 2 mM chloride ions.*

Note: *If you use the salt-bridge reference electrode, first connect the inlet and outlet, then fill the cell with mobile phase while keeping the REF hole on top (not the outlet hole). If the salt-bridge hole is filled with mobile phase, then the salt-bridge reference electrode is installed successfully without bubbles.*

Note: *Do not reuse the spacer; use a new spacer. Handle each spacer with care. Avoid wrinkles and folds, and make sure no dust or other particles are trapped. Make sure the flow cell surfaces are clean before reassembling the flow cell. Overtightening the bolts can cause excessive pressure in the flow cell and increase the noise considerably. To prevent overtightening, tighten the bolts until fingertight, then tighten them 1/4-turn more.*

To reassemble the flow cell:

1. Make sure the flow cell blocks are clean and rinsed well with HPLC grade water, and that the four holes in the spacer line up with the four bolt holes in the flow cell block. Use a new spacer.

   Note: *The flow cell must contain a spacer. Do not reuse the spacer.*

2. Align the top and bottom halves of the flow cell using the two black marker dots on the sides of the two flow cell electrode block halves.
3. Mount the two blocks together by tightening the four bolts (maximum torque 30 Ncm) using the hex key (see Figure 4-2). To prevent overtightening, tighten the bolts until fingertight, then tighten them 1/4-turn more.
4. Fill the flow cell with mobile phase, keeping it at an angle of about 45° with the outlet (LC out) on top to force the air through the outlet.
5. Connect the column outlet to the flow cell inlet using small-bore tubing (0.3-mm ID) and one fingertight fitting (Figure 4-10).

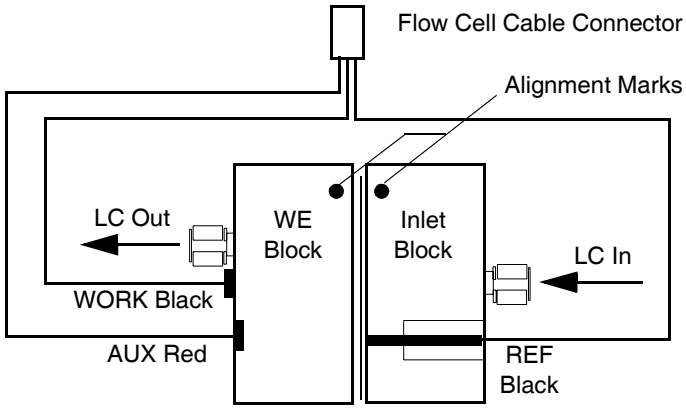


Figure 4-10 Assembling the Flow Cell

STOP ***Attention:*** *Use only factory-supplied fingertights in the flow cell. Do not overtighten the fitting. Overtightening affects the flow pattern through the tubing (turbulence) and may markedly decrease the flow cell performance.*

6. Connect the medium-bore tubing (0.5-mm ID) to the outlet of the flow cell using one fingertight fitting.
7. Turn on the HPLC pump. Keep some tissues at hand as some mobile phase probably will spill during this mounting procedure.
8. Align the flow cell with the clamp, keeping the reference electrode at the lower side and the outlet (LC Out) uppermost to prevent trapping of air bubbles, then slide the flow cell into the clamp.

STOP ***Attention:*** *To prevent damage to the flow cell, do not install it by pushing it forcefully into the clamp from the front.*

9. Reconnect the three wires of the flow cell cable to the electrodes on the flow cell as follows (Figure 4-10):
   - **REF** – Black wire to the reference electrode
   - **WE** – Red wire to the working electrode
   - **AUX** – Blue wire to the auxiliary electrode
10. Inspect the flow cell for leaks. If you detect a leak, tighten the flow cell bolts by 1/4-turn only. Overtightening the bolts will deform the spacer and leakage will persist.

4.7 Replacing the Micro Flow Cell

The micro flow cell is assembled properly when it arrives, including the capillary and fused silica connector. You do not need to install the capillary on a new micro flow cell.

Use this procedure if you need to replace the capillary in the micro flow cell.

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Required Materials

Materials that you need from the Startup Kit are:

- Fused silica capillary
- Teflon sleeve
- Fingertight fitting
- Injection block
- Glass mounting plate

Procedure

To replace the capillary in the micro flow cell:

1. Insert the fused silica capillary into the tightly fitting Teflon® sleeve (Figure 4-11).

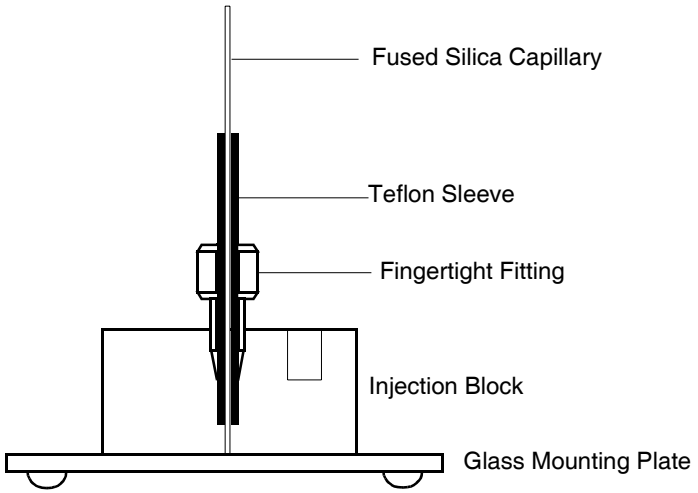


Figure 4-11 Mounting the Fused Silica Connector

2. Ensure that both the capillary and the sleeve protrude through the fingertight fitting.
3. Mount this combination carefully in the injection block. Let the fused silica protrude slightly (0.5 to 1 mm) through the injection hole.
4. Clean the glass mounting plate using ethanol or water.
5. Carefully push the block on the glass plate until the silica capillary is flush with the surface.
6. Fix the fused silica capillary firmly with the fingertight fitting while keeping a slight pressure of the block on the glass plate.

7. Continue with Section 4.6, Reassembling the Flow Cell. You can use a syringe filled with mobile phase to fill the micro flow cell with mobile phase (Figure 4-12).

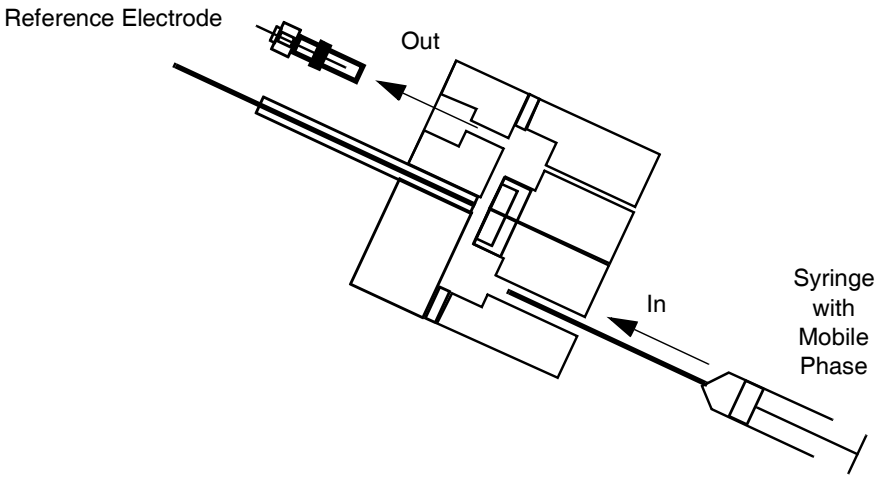


Figure 4-12 Filling the Micro Flow Cell

4.8 Other Procedures

Caution: *When you handle solvents, change tubing, or operate the 2465 Detector in general, always observe good laboratory practices. Know the physical and chemical properties of the solvents. Refer to the Material Safety Data Sheets for the solvents in use.*

Perform the following procedures as needed:

- Section 4.8.1, Replacing Fuses
- Section 4.8.2, Changing a Spacer in the Flow Cell
- Section 4.8.3, Changing a Column
- Section 4.8.4, Cleaning the Detector

4.8.1 Replacing Fuses

Caution: *To avoid electrical shock, power off the 2465 Detector and unplug the power cord before you replace the fuses. The I/O connectors on the rear of the instrument have a risk of electrical shock (Figure 4-13).*

Figure 4-13 I/O Connector Warning

Replace blown fuses with fuses of proper type and rating as stipulated on the rear panel. Use the correct fuses and power setting for your location (U.S.A. provides 110 V; your location may provide 240 V).

Caution: *Replace blown fuses with fuses of proper type and rating as stipulated on the rear panel. The fuse holder is integrated in the line connector.*

To prevent the risk of fire, ensure that the instrument is never operated with the incorrect type of fuses.

To replace the fuses:

1. Power off the 2465 Detector and remove the power cord.
2. Remove the fuse holder from the rear panel of the 2465 Detector (Figure 4-14).

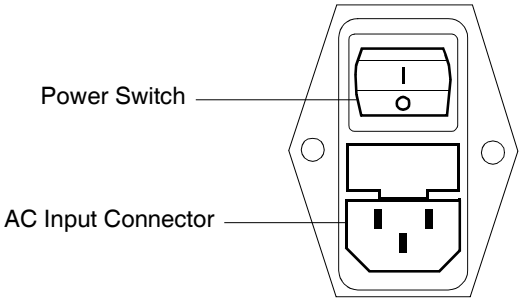


Figure 4-14 Removing the Fuse Holder

3. Remove both fuses from the fuse holder.
4. Install two new fuses in the fuse holder, then install the fuse holder. Use only 5 × 20, 2.5-AT, 250 V fuses, as described on the rear panel (Figure 4-15).

Figure 4-15 Checking the Fuse Rating

4.8.2 Changing a Spacer in the Flow Cell

Attention: *Handle the spacer with care. Avoid wrinkles and folds, and make sure no dust or other particles are trapped.*

Overtightening the bolts can cause excessive pressure in the flow cell and increase the noise considerably. Make sure the four holes in the spacer line up with the four bolt holes in the flow cell block. To prevent overtorquing, tighten the bolts until fingertight, then tighten them 1/4-turn more.

The flow cell must contain a spacer. Use a new spacer. Do not reuse the spacer.

To increase or decrease internal volume in the flow cell, you can change the spacer.

To change the spacer in the flow cell:

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Stop the HPLC pump.
3. Open the door, then remove and disassemble the flow cell.
4. Remove the spacer from the flow cell block, and rinse the flow cell blocks thoroughly with HPLC grade water.
5. Select a new spacer (Table 4-2). The minimum volume is 11 nL using a 25-µm spacer. The flow cell requires one spacer. Ensure that no particles (such as dust or hair) are trapped and that the spacer lies flat, without wrinkles. Make sure the four holes in the spacer line up with the four bolt holes in the flow cell block.

   Note: *As you use a thinner spacer, the cell volume decreases, resulting in a higher linear flow velocity in the flow cell, which eventually obstructs the flow. Conversely, a thicker spacer increases the cell volume, resulting in a lower linear flow velocity.*

Table 4-2 Changing the Flow Cell Volume

Spacer (µm)	Flow Cell Volume (µL)[a]		
	3-mm WE[b]	2-mm WE[c]	0.75-mm WE
25	0.15	0.08	0.011
50	0.29	0.16	0.022
120	0.71	0.38	0.053

a. Calculated as area above working electrode (WE area × spacer thickness). The 2465 Detector has a post-detection volume of about 1.5 mL.
b. The 3-mm WE has an actual diameter of 2.74 mm.
c. The 2-mm WE has an actual diameter of 2.00 mm.

6. Reassemble the flow cell (see Section 4.6, Reassembling the Flow Cell).

   Note: *To prevent overtightening, tighten the bolts until fingertight, then tighten them 1/4-turn more.*

4.8.3 Changing a Column

To change a column:

1. Turn off the flow cell (see Section 3.8.1, Turning Off the Flow Cell).
2. Stop the HPLC pump.
3. Open the door.
4. Disconnect the tubing from the column.
5. Remove the column from the clamps and seal the ends.
6. Install a new, electrochemically clean column (see Section 2.4.2, Connecting a Column).

4.8.4 Cleaning the Detector

Caution: *Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.*

Attention: *To prevent permanent damage to the 2465 Detector, use only water to clean the detector.*

To clean the detector:

1. Disconnect the column and flow cell to remove them from the flow path.
2. Connect the tubing to make a continuous flow path to the waste container.
3. Flush the tubing by pumping water or ethanol through the lines for 30 minutes at 0.5 mL/min.
4. Wipe the outside and inside surfaces and keypad with water and lint-free tissues.
5. Empty and rinse the waste container, then reinstall it.
6. Remove any dust on the screens that cover the fans in the detector oven.

Chapter 5 Diagnostics and Troubleshooting

Use this chapter to troubleshoot problems that you observe:

- Error messages – see Section 5.1
- Diagnostic tests – see Section 5.2
- Troubleshooting tables – see Section 5.3
  - No detector response
  - High cell current
  - Noisy baseline
  - Drifting baseline
  - Decreased sensitivity (low S/N ratio)
  - Baseline oscillations
  - Saturation of output
- Other physical symptoms such as leaks – see Section 5.4

If there is a problem, contact Waters Technical Service (see Section 4.1.4).

Note: *The current firmware version appears on the Main screen.*

5.1 Error Messages

If an error message appears, see Table 5-1.

Table 5-1 Error Messages

Message	Solution
Out of Range	May have exceeded maximum compensation, due to passthrough transient or to wrong range setting. If the wrong range was chosen, increment to the next available setting and see if the error persists. Repeat until an allowable range is reached.
Checksum Error	Contact Waters Technical Service (see Section 4.1.4).

5.2 Diagnostics

You can use the following tests to diagnose problems with the 2465 Detector:

- Section 5.2.1, Dummy Cell Test
- Section 5.2.2, Stop Flow Test
- Section 5.2.3, Keyboard Test
- Section 5.2.4, Display Test

To access the Diagnostics tests, select **F5 DIAG** from the Main screen. The Diagnostics screen displays the names of the diagnostic tests (Figure 5-1).

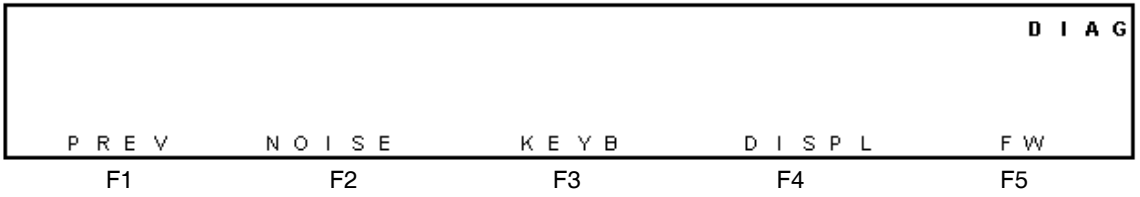


Figure 5-1 Diagnostics Screen

5.2.1 Dummy Cell Test

A successful dummy cell test confirms that the 2465 Detector functions properly. If the result of the noise measurement with the dummy cell is within specification, the 2465 Detector is excluded as a cause of the problem.

The dummy consists of a resistor (*R*) of 300 MΩ and a capacitor (*C*) of 0.47 µF in parallel. The current is measured over the resistor according to Ohm's law:

$$V = I \times R$$

where:

V = Voltage

I = Current

R = Resistance

With a working potential of 800 mV, the current drawn is 2.67 ±0.05 nA. Slight differences to this value are due to the tolerance of the resistor. The capacitor functions as a 'noise generator' and in fact resembles the capacitance of a well-functioning standard flow cell in an ideal HPLC setup.

To perform the dummy cell test:

1. Turn off the flow cell and open the door.
2. Remove each end of the flow cell cable from the flow cell.
3. Attach cable ends from the flow cell cable to the dummy cell as in Figure 5-2 (red to red, blue to blue, and black to black). Keep the flow cell in the clamp and keep the large end of the flow cell cable attached to the 2465 Detector.

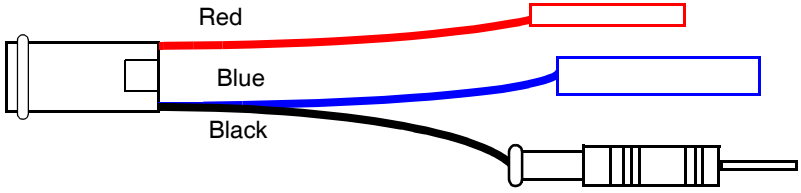


Figure 5-2 Dummy Cell

4. Ensure that the dummy cell is within the fully closed Faraday shield at the same position as the flow cell, and close the door.
5. Use the dummy cell test settings in Table 5-2.

Table 5-2 Dummy Cell Test Settings

Parameter	Setting
Cell potential	800 mV
Cell current	2.67 ±0.05 nA (read-out)
Detector oven temperature	30 °C, stable
Filter time constant	1.0 sec (or as specified)
Range	100 pA/V[a]

a. Using these settings, the results of the output voltage (highest noise peak) should be no greater than 20 mV at Integrator Out.

Note: *Keep the door closed at all times during the test.*

6. Stabilize the detector for at least 60 minutes.
7. Measure the output and cell potential values as follows:
   a. From the Main screen, select **F5 DIAG**. The Diagnostics screen appears (Figure 5-3).

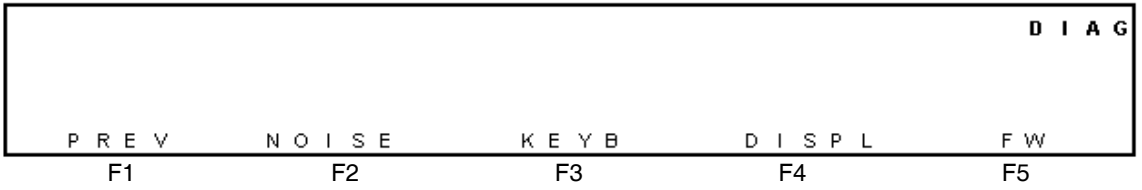


Figure 5-3 Diagnostics Screen

   b. Select **F2 NOISE**. The Noise screen appears (Figure 5-4).

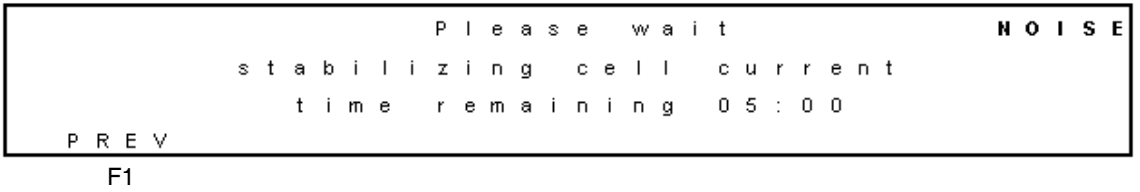


Figure 5-4 Noise Test with Dummy Cell

   c. The clock on row 3 counts down for 5 minutes while the cell current stabilizes, then the output and cell potential values (read-out of the current) appear (Figure 5-5).

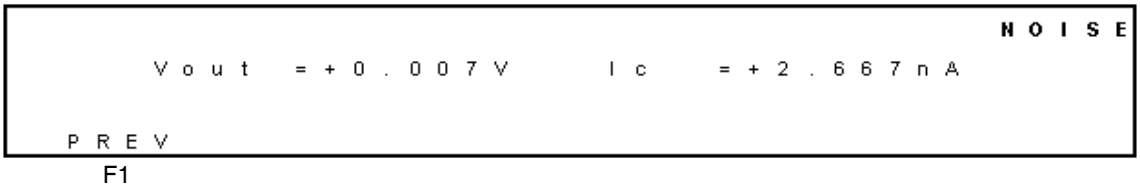


Figure 5-5 Noise Test Screen

d. The value of Ic should be 2.67 ± 0.05 nA.

e. Select **F1 PREV** > **F1 PREV** to return to the Main screen.

8. Measure the noise. The noise generated during the test should be less than 2 pA.

5.2.2 Stop Flow Test

The 2465 Detector can detect noise but often is not the cause of the noise. Use the stop flow test to isolate the origin of excessive noise, which can be caused by:

- High background current
- Improper grounding
- External instruments, pumps, and so on
- Poor conductivity mobile phase

High background is the most common problem. This can be caused by:

- Potential setting too high.
- Contaminated mobile phase from a bleeding column or a high amount of unchelated iron ions (Fe^{++}) in the mobile phase.
- Concentration of oxygen in the mobile phase too high.

In the stop flow test:

1. Read the cell current with the flow on.
2. Switch off the flow from the LC system.
3. Read the cell current with the flow off.
4. If the cell current drops significantly (for example, from 500 to 150 nA), then the mobile phase might be contaminated, or the column might be bleeding.

   Note: *Contamination can enter the mobile phase from several places, including the column. Contamination does not necessarily originate in the mobile phase bottle.*

5. Replace the mobile phase and repeat the stop flow test.
6. If the problem continues, the column may be the source. To ensure a column is electrochemically clean, allow three days of low-flow solvent.

   Alternatively, the column may need replacing. Ensure that the new column is electrochemically clean (see Section 3.3, Preparing the 2465 Detector for Operation).

5.2.3 Keyboard Test

Use the keyboard test to verify that the keys on the keypad work correctly.

To perform the keyboard test:

1. From the Main screen, select **F5 DIAG**. The Diagnostics screen appears.
2. Select **F3 KEYB**.
3. Press the F1 key on the keypad. Figure 5-6 appears if the key is working correctly, then disappears after a few seconds.

Figure 5-6 Testing a Key on the Keypad

4. Select each key until you have tested all 12 keys.
5. Select **F1 PREV** twice (rapidly) to return to the Diagnostics screen.
6. If there is a problem, contact Waters Technical Service (see Section 4.1.4).

5.2.4 Display Test

Use the display test to verify that all pixels in the 4 × 40 LCD display work correctly.

To perform the Display test:

1. From the Main screen, select **F5 DIAG**. The Diagnostics screen appears.
2. Select **F4 DISPL**. The Display screen appears (Figure 5-7). All characters on the display should be solid black except the screen name (DISP) and the F1 PREV key.

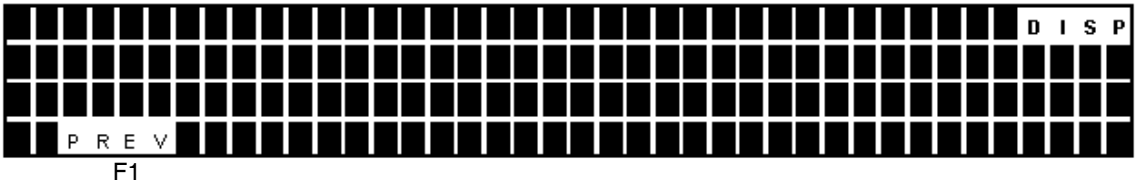


Figure 5-7 Display Test

3. Select **F1 PREV** > **F1 PREV** to return to the Main screen.
4. If you want to change the display contrast, select **F1 CONFIG**. The Configuration screen appears (Figure 5-8).

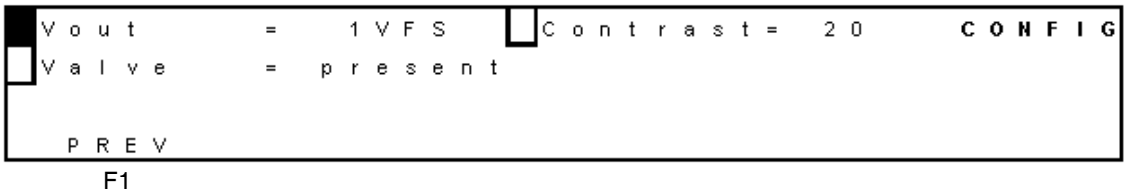


Figure 5-8 Configuration Screen

5. Use the cursor and value keys to change the contrast (allowed values: 1 to 20, where 20 is the darkest).
6. Select **F1 PREV** to return to the Main screen.
7. If there is a problem, contact Waters Technical Service (see Section 4.1.4).

5.3 Troubleshooting Tables

Use the following tables to troubleshoot the following symptoms:

- **No detector response** – see Table 5-3
- **High cell current** – see Table 5-4
- **Noisy baseline** – see Table 5-5
- **Drifting baseline** – see Table 5-6
- **Decreased sensitivity** (low S/N ratio) – see Table 5-7
- **Baseline oscillations** – see Table 5-8
- **Saturation of output** – see Table 5-9

Table 5-3 No Detector Response

Possible Cause	Remedy
No power	Check line voltage for local power outage. Ensure that power cord is plugged in correctly.
Power switch off	Turn the power switch on (at the rear panel).
Faulty or blown detector fuse	Replace detector fuse.
Flow cell disconnected or switched off	Check connections, especially flow cell cable. Check DC Stat or Pulse Stat screen to see if flow cell is on.
Recorder/integrator disconnected	Check cable connections.
Fouled working electrode	Clean electrode.
Under Empower control	Check Empower status (see Section 3.2.4, Remote Mode).

Table 5-4 High Cell Current

Possible Cause	Remedy
Contaminated buffer	Replace buffer. Do not recycle the buffer.
High working electrode potential	Optimize potential, if possible, by using a smaller working electrode diameter.
Salt bridge in reference electrode not saturated	Refill salt bridge with wet KCl crystals.
Retained peaks from previous runs	Wait for elution of these (very) broad peaks.
Column is 'bleeding'	Ensure the column is electrochemically clean (see Section 2.4.2, Connecting a Column). If column continues to bleed, replace the column with an electrochemically clean column.
High amount of Fe^{++} in buffer	Add EDTA to buffer; rinse metal parts with 15% HNO_3.

Table 5-5 Noisy Baseline

Possible Cause	Remedy
Salt bridge in reference electrode not saturated	Refill salt bridge with saturated KCl; add wet KCl crystals.
Air bubble in reference electrode or in flow cell	Remove air bubble; continuously degas the mobile phase.
Slow temperature fluctuations	Isolate detector cell; set detector oven temperature and keep door closed. Avoid setting the detector near a heating or cooling source.
Fouled working electrode	Polish the working electrode.
Leaking reference electrode or flow cell	Check the reference electrode for leaks.
	Loosen all four flow cell bolts, tighten them until fingertight, then tighten them 1/4-turn more. Check again for leaks. You can tighten the bolts a little more, but do not overtighten the bolts.
	Remove and replace the spacer.
	Check the backpressure and lower the flow rate if needed.
	Examine the fingertight connections for damage. Carefully tighten the connections 1/4-turn past fingertight.

Table 5-6 Drifting Baseline

Possible Cause	Remedy
Detector not at equilibrium	Allow sufficient time for the detector to warm up after powering on or changing the temperature.
Leaking flow cell	Loosen all four flow cell bolts, tighten them until fingertight, then tighten them 1/4-turn more. Check again for leaks. You can tighten the bolts a little more, but do not overtighten the bolts.
	Remove and replace the spacer.
	Check the backpressure and lower the flow rate if needed.
	Examine the fingertight connections for damage. Carefully tighten the connections 1/4-turn past fingertight.

Table 5-7 Decreased Sensitivity (Low S/N Ratio)

Possible Cause	Remedy
Fouled working electrode by dirty samples	Polish the working electrode. Dilute samples.
Flow cell potential too low	Optimize potential.
Contaminated buffer (high I_c)	Replace buffer; do not recycle the buffer.

Table 5-8 Baseline Oscillations

Possible Cause	Remedy
Malfunctioning pump (a regular pattern that changes with flow rate)	Check pump (seals and valves).
Overtightened flow cell bolts	Loosen flow cell bolts, tighten them until fingertight, then tighten them 1/4-turn more. Check pump pressure. May need to replace the spacer.
Air bubbles in flow cell or reference electrode	Remove air bubbles; continuously degas the mobile phase.
Temperature oscillations	Set detector oven temperature and keep door closed. Check for obstructions or deterioration of door seal.
Contaminated buffer (high I_c)	Replace buffer; do not recycle the buffer.
Fouled working electrode	Clean the working electrode.
Fe^{++} in buffer	Add EDTA; passivate metal parts with HNO_3.

Table 5-9 Saturation of Output

Possible Cause	Remedy
Damaged reference electrode	Check by comparing it to a spare reference electrode; replace if necessary.

Table 5-9 Saturation of Output *(Continued)*

Possible Cause	Remedy
Damaged working electrode	Replace flow cell block.
Flow cell incorrectly connected	Check connections of flow cell cable (REF – black, WE – red, AUX – blue).
Flow cell potential too high	Optimize flow cell potential.

5.4 Physical Symptoms

Table 5-10 lists other symptoms you may need to diagnose and repair.

Table 5-10 Physical Symptoms

Symptom	Possible Cause and Remedy
Fluid leaks	Flow cell is leaking due to: • Improper assembly (wrinkled spacer, or dirt or hair between spacer and block). • Overtightening of the flow cell bolts. Loosen all four flow cell bolts, tighten them until fingertight, then tighten them 1/4-turn more. Check again for leaks. You can tighten the bolts a little more. Do not overtighten the bolts. Remove and replace the spacer. Check the backpressure and lower the flow rate if needed. Examine the fingertight connections for damage. Carefully tighten the connections 1/4-turn past fingertight. Check the reference electrode for leaks.
	Check for and fix tubing splitting at connections, tubing loose or falling off, problems with the fingertight fittings, or bent tubing.
	Check fluid pressure to see if it exceeds recommendations (maximum 40 psi), then look for blockages in tubing or flow cell.
No LCD or Incorrect LCD	Reading wrong character or missing letter or all black due to broken crystals (see Section 5.2.4, Display Test).

Table 5-10 Physical Symptoms *(Continued)*

Symptom	Possible Cause and Remedy
No power evident (no movement, fans, or characters in the display)	Check cables and connections. Check building or lab bench power. Check for blown fuse; replace.
No communication with Empower software	Power off, wait 10 seconds, then power back on to reboot. Make sure Empower software is set up properly.
Temperature unstable, not coming up to temperature, or overheating	Make sure the door is completely shut. Remove dust from screens on the internal fans in the detector oven. Allow sufficient time for temperature to stabilize. Check the temperature setting, and make sure it is the intended value. Verify that both fans in the detector oven are working by placing a sheet of tissue over each fan. One fan should blow air out and the other fan should take in air.

Appendix A
2465 Detector Specifications

A

Table A-1 lists general specifications for the Waters® 2465 Electrochemical Detector.

Table A-1 General Specifications

Condition	Specification
Line frequency	50 to 60 Hz
Line voltage	100 to 240 VAC (self-selecting)
Operating modes	DC, Pulse, and Scan
Potential range	Between +2.00 and –2.00 V in 10-mV increments
Autozero	Triggered by keyboard, rear panel contact closure, or RS-232C control
RS-232C	Full control and data acquisition of all parameters
Detector oven	Height 37 cm, temperatures from 7 °C above ambient to 45 °C, accuracy 0.5 °C, stability 0.1 °C; accommodates HPLC column and flow cell[a]
Rear panel I/O connections	• Main power • **A (15-pin Sub D)** – *Input*: Cell on, cell off, Start, Autozero, or Reset; *Output*: Relay 1, Relay 2, AUX 1, AUX 2, Overload • **B (15-pin Sub D)** – Inject marker (output) and Baseline marker (input) • **C (phone jack)** – Not applicable • **OUTPUT (BNC)** – Analog signal • **RS-232C (9-pin Sub D)** – Full instrument control; data acquisition • **Ground stud** – Instrument ground connector

a. See Table 3-5 for maximum oven temperatures at different ambient temperatures.

Table A-2 Physical Specifications

Condition	Specification
Height	17.3 inches (44 cm)
Length (Depth)	17.3 inches (44 cm)
Width	8.7 inches (22 cm)
Weight	30.9 lb (14 kg) without flow cell and column

Table A-3 Operating and Environmental Requirements

Parameter	Requirement
Operating temperature range	39 to 104 °F (4 to 40 °C)
Storage temperature range	–104 to 158 °F (–40 to 70 °C)
Relative humidity range	20 to 80%, noncondensing
Storage humidity range	0 to 90%, noncondensing
Bench space	6 in (15.25 cm) clearance at rear; access to power switch and power cord; clean, level, smooth surface
Vibration	Negligible
Static electricity	Negligible
Power	Grounded AC, 100 to 240 VAC, 50/60 Hz

Table A-4 DC Mode

Condition	Specification
Current ranges, recorder	10 pA to 200 µA in 1, 2, or 5 steps
Filter (time constants)	0.1 to 5 sec in 1, 2, or 5 steps
Noise	< 2 pA with a dummy cell using 100 pA range (load of 300 MΩ and 0.5 µF) with 1.0 sec filter

Table A-5 Pulse Mode

Condition	Specification
Range	10 nA to 200 µA in 1, 2, 5 steps
Pulse times	t_1: 100 to 2000 ms t_2: 0 (off) to 2000 ms t_3: 0 (off) to 2000 ms in 10-ms steps
Sample times	20, 40, 60, 80, and 100 ms

Table A-6 Scan Mode

Condition	Specification
Range	10 nA to 200 µA in 1, 2, or 5 steps
Scan rate	1 to 50 mV/s in 1, 2, or 5 steps
Cycle	Half, full, or continuous Start/Stop, Hold and Autozero, Starting potential (E_1), End potential (E_2)

Table A-7 Timed Events Mode

Condition	Specification
Parameters	DC mode (5 files) and pulse mode (4 files), cycles
Control	Time-based control of 50 timed events. Controllable parameters: range, filter, cell potential (E_c), Polarity, Autozero, Offset, and Outputs (2 TTL open collector, 2 relays).

Table A-8 Flow Cell Specifications

Component	Specification
Design	Confined wall-jet design; working volume determined by spacer thickness and WE diameter
Spacers	25, 50, or 120 µm
WE diameters	2.00 and 2.74 mm (0.75 mm with micro flow cell)

Table A-8 Flow Cell Specifications *(Continued)*

Component	Specification
Cell volume	0.150 µL minimum with 3-mm flow cell 0.080 µL minimum with 2-mm (standard) flow cell 0.011 µL minimum with 0.75-mm (micro) flow cell
Working electrodes	Glassy carbon (GC), gold, platinum, and silver
Reference electrodes	Salt-bridge Ag/AgCl; ISAAC; Hy-REF
Auxiliary electrode	Stainless steel
Wetted materials	PCTFE, FEP, 316-SS (stainless steel), Viton®, silver, silver chloride, gold, and platinum
Pressure	Maximum 40 psi (2.76 bar, 276 kPa)
Flow rate	• Standard flow cell: 25 µL/min to 2.0 mL/min • Micro flow cell: 1 to 25 µL/min

Appendix B
2465 Detector Components and Spare Parts

The initial shipment of the Waters 2465 Electrochemical Detector contains:

- Waters 2465 Electrochemical Detector (without flow cell or column)
- Power cable
- Startup Kit (see Table B-8)

A flow cell must be ordered separately.

B.1 Flow Cells

You can order the flow cells listed in Table B-1 for the 2465 Detector.

Note: *A flow cell, such as the glassy carbon (GC) WE ISAAC flow cell, must be ordered separately (see Table B-2 through Table B-7).*

Table B-1 Flow Cell Kits

Waters Part Number	Component	Actual WE Diameter
205004115	Flow cell with 2-mm glassy carbon (GC) WE, salt-bridge reference electrode (see Table B-2)	2.00 mm
205004215	Flow cell with 2-mm glassy carbon (GC) WE, ISAAC reference electrode (see Table B-3)	2.00 mm
205004315	Flow cell with 2-mm glassy carbon (GC) WE, Hy-REF electrode	2.00 mm
205004320	Flow cell with 3-mm platinum (Pt) WE, Hy-REF electrode	2.74 mm
205004325	Flow cell with 3-mm gold (Au) WE, Hy-REF electrode (see Table B-5)	2.74 mm

Table B-1 Flow Cell Kits *(Continued)*

Waters Part Number	Component	Actual WE Diameter
205004330	Flow cell with 2-mm silver (Ag) WE, Hy-REF electrode (see Table B-6)	2.00 mm
205004220	Flow cell with 3-mm platinum (Pt) WE, ISAAC reference electrode (see Table B-4)	2.74 mm
205004100	Micro flow cell with 0.7-mm glassy carbon (GC) WE, salt-bridge reference electrode (see Table B-7); with 50-mL KCl solution	0.75 mm

Table B-2 Flow Cell, 2-mm GC WE, Salt-Bridge Reference Electrode

Waters Part Number	Quantity	Component
700001958	1	Salt-bridge Ag/AgCl reference electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly
700001954	1	Polishing disc for WE
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)
700001959	1	KCl solution for salt-bridge REF (50 mL) and MSDS
WAT057241	1	Connector for SAT/IN™ to 2465 analog cable

Table B-3 Flow Cell, 2-mm GC WE, ISAAC Reference Electrode

Waters Part Number	Quantity	Component
N/A	1	Glassy carbon working electrode, ISAAC reference electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly
700001954	1	Polishing disc for WE
700002069	1	Polishing disc for REF
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)
700001949	1	ISAAC solution for ISAAC reference (10 mL) and MSDS

Table B-4 Flow Cell, 3-mm Pt WE, ISAAC Reference Electrode

Waters Part Number	Quantity	Component
N/A	1	Platinum working electrode, ISAAC reference electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly
700001954	1	Polishing disc for WE
700002069	1	Polishing disc for REF
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm

Table B-4 Flow Cell, 3-mm Pt WE, ISAAC Reference Electrode *(Continued)*

Waters Part Number	Quantity	Component
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)
700001949	1	ISAAC solution (10 mL) and MSDS

Table B-5 Flow Cell, 3-mm Au WE, Hy-REF Electrode

Waters Part Number	Quantity	Component
N/A	1	Gold working electrode, Hy-REF electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly
700001954	1	Polishing disc for WE
700002069	1	Polishing disc for REF
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)

Table B-6 Flow Cell, 2-mm Ag WE, Hy-REF Electrode

Waters Part Number	Quantity	Component
N/A	1	Silver working electrode, Hy-REF electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly

Table B-6 Flow Cell, 2-mm Ag WE, Hy-REF Electrode *(Continued)*

Waters Part Number	Quantity	Component
700001954	1	Polishing disc for WE
700002069	1	Polishing disc for REF
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)

Table B-7 Micro Flow Cell, 0.7-mm GC WE, Salt-Bridge REF Electrode

Waters Part Number	Quantity	Component
N/A	1	Micro flow cell, glassy carbon working electrode, salt-bridge reference electrode
700002105	1	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	1	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)
700001950	4	Fingertight fitting
700001985	1	Hex key for flow cell assembly
700001954	1	Polishing disc for WE
700001951	2	Spacer, 120 µm
700001953	4	Spacer, 50 µm
700001952	2	Spacer, 25 µm
700001955	1	10-mL diamond slurry (1 µm) and Material Safety Data Sheet (MSDS)
700001958	1	Salt-bridge Ag/AgCl reference electrode
700001959	1	KCl solution for salt-bridge REF (50 mL) and MSDS
700002103	1	Capillary flow cell and glass plate for mounting capillary tubing connection

B.2 Startup Kit Components

Table B-8 lists the parts included in the Waters 2465 Electrochemical Detector Startup Kit (part number 200002465).

Table B-8 Startup Kit

Part Number	Quantity	Component	Description
700001004	2	Fuse 5 × 20, 2.5 AT 250 V	Detector fuses
700001942	1	RS-232 cable	For communication with Empower™ Personal, Workgroup, or Enterprise
700001943	1	Dummy cell	For troubleshooting purposes and performing noise measurement tests
700001945	2	Column clamp, 12 mm	Spare column holder to hold large diameter columns in detector oven compartment
700001948	2	External I/O cable	Detector input and output events cable
700001968	1	Cell cable	Cable making electrical connection between flow cell and detector
441000333	1	SAT/IN analog to 2465 BNC cable	Cable to connect SAT/IN connector to the 2465 Detector BNC analog output
700001994	1	Integrator cable	Detector analog signal cable
715000483	1	*2465 Electrochemical Detector Quick Install Guide*	Short user guide documenting initial setup and connections
716000885	1	*Certificate of Validation, 2465*	Waters instrument validation certificate
716000886	1	*Declaration of Conformity, 2465*	Waters instrument declaration of conformity
716000888	1	*Release Notes, 2465*	User notes with detector information for the operator, including correct checksum value

Table B-8 Startup Kit *(Continued)*

Part Number	Quantity	Component	Description
71500246502	1	*Waters 2465 Electrochemical Detector Operator's Guide*	Document on how to configure, program, operate, maintain, and troubleshoot the detector

B.3 Spare Parts

Stock the recommended spare parts to minimize downtime. Table B-9 lists all parts that you can replace. Parts not included in the list require replacement by a trained service representative.

Table B-9 Spare Parts

Waters Part Number	Component
441000333	SAT/IN Analog to 2465 BNC cable
700001941	Fuse, 5 × 20, 2.5 AT 250 V
700001942	RS-232 cable
700001943	External dummy flow cell
700001944	Column clamp, 6 mm
700001945	Column clamp, 12 mm
700001946	Flow cell clamp
700001948	External I/O cable
700001949	ISAAC solution for ISAAC reference (10 mL) and MSDS
700001950	Fingertight fitting
700001951	Spacer, 120 µm
700001952	Spacer, 25 µm
700001953	Spacer, 50 µm
700001954	Polishing disc
700001955	10 mL diamond slurry (1 µm) and MSDS
700001956	Swivel for salt-bridge REF
700001957	Body for salt-bridge REF

Table B-9 Spare Parts *(Continued)*

Waters Part Number	Component
700001958	Salt-bridge Ag/AgCl REF
700001959	KCl solution for salt-bridge REF (50 mL) and MSDS
700001960	WE block, 2.00-mm GC WE
700001961	WE block, 2.75-mm Pt WE
700001962	WE block, 2.75-mm Au WE
700001963	WE block, 2.00-mm Ag WE
700001964	Salt-bridge inlet block
700001965	Hy-REF inlet block
700001968	Cell cable
700001985	Hex key for flow cell assembly
700001994	2465 integrator cable
700002069	2465 polishing disc, REF electrode
700002003	2465 ISAAC inlet block
700002103	Capillary Connection Kit for micro flow cell
700002105	PEEK tubing, 0.02 in. (0.5 mm) ID × 3.28 ft (1 m)
700002104	PEEK tubing, 0.01 in. (0.3 mm) ID × 11.8 in. (30 cm)

Appendix C
Sample ECD Methods

You can adapt methods from extensive literature on electrochemical detection, and apply them to your own samples and mobile phases. Here are some electrochemical methods to get started (Table C-1 through Table C-6).

Table C-1 Norepinephrine

Parameter	Conditions
Column	C_{18}, 3 µm, 4.6 × 100 mm
Flow rate	1.0 mL/min
Mobile phase	50 mM H_3PO_4, 50 mM citric acid, 20 mg/L octane sulphonic acid (OSA), pH = 3.1 with KOH, 5% methanol
Sample	1.0 µmol/L norepinephrine
Injection volume	20 µL
Temperature	30 ºC
Flow cell	3 mm glassy carbon WE, salt-bridge reference electrode
Spacer	50 µm
Mode	DC
Cell potential	+800 mV

Table C-2 Catecholamines

Parameter	Conditions
Column	Atlantis column (part number 186001317), 5 µm, 3.9 × 150 mm
Flow rate	1.0 mL/min
Mobile phase	85% phosphoric acid, 10 g/L citric acid, 20 mg/L Na_2EDTA, 20 mg/L sodium octane sulphonic acid, pH = 3.0 with 50% NaOH (approximately 3.3 mL), 2 mM chloride, 5% methanol
Sample	Catecholamines

Table C-2 Catecholamines *(Continued)*

Parameter	Conditions
Injection volume	20 µL
Temperature	35 ºC
Flow cell	2-mm glassy carbon WE, ISAAC reference electrode
Spacer	50 µm
Mode	DC
Cell potential	+700 mV

Table C-3 Homocysteine

Parameter	Conditions
Column	C_{18}, 3 µm, 4.6 × 100 mm
Flow rate	1.0 mL/min
Mobile phase	1% phosphoric acid, 2 mM potassium chloride, 10 mg/L octane sulphonic acid, pH = 1.75 with 19.2 M NaOH
Sample	13.5 µg/L
Injection volume	20 µL
Temperature	Ambient
Flow cell	3-mm gold WE, ISAAC reference electrode
Spacer	50 µm
Mode	DC
Cell potential	+600 mV

Table C-4 8-Hydroxy-2'-deoxyguanosine

Parameter	Conditions
Column	C_{18}, 5 µm, 4.6 × 250 mm
Flow rate	1.0 mL/min
Mobile phase	50 mM citrate, pH = 3.5, 10% methanol
Sample	Hydrolyzed DNA
Injection volume	50 µL
Temperature	Ambient
Flow cell	3-mm glassy carbon WE, salt-bridge reference electrode
Spacer	50 µm
Mode	DC
Cell potential	+700 mV

Table C-5 Lactose, Sucrose, and Maltose

Parameter	Conditions
Column	CarboPac PA1, 4.0 × 250 mm
Flow rate	1.0 mL/min
Mobile phase	200 mM NaOH
Sample	100 nM disaccharides
Injection volume	20 µL
Temperature	30 °C
Flow cell	3-mm gold WE, ISAAC reference electrode
Spacer	50 µm
Mode	PAD (pulse)
Cell potential	E_1 = +150 mV, E_2 = +750 mV, E_3 = –800 mV t_1 = 300 ms, t_2 = 150 ms, t_3 = 150 ms, t_s = 100 ms

Table C-6 Performance Qualification

Parameter	Conditions
Column	Symmetry C_{18}
Flow rate	0.5 mL/min
Mobile phase	50 mM acetic acid, 50 mM sodium acetate, 5 mM potassium chloride
Sample	0.1 mg/L acetaminophen
Injection volume	20 µL
Temperature	35 ºC
Flow cell	2-mm glassy carbon WE, ISAAC reference electrode
Spacer	50 µm
Mode	DC
Cell potential	+650 mV

Appendix D
2465 Detector Glossary

#	A suffix after the E_c parameter to indicate that the value has been edited from the default value but not accepted. You must press Enter to store the value and apply the value.
ADC	Analog-to-digital conversion.
Ag/AgCl reference electrode	Silver/silver chloride reference electrode.
Autozero	A function that sets the output voltage to 0 V or to the offset voltage.
Aux or AUX	Auxiliary electrode.
baseline	A horizontal line drawn from each detected peak start point (liftoff) to each detected peak end point (touchdown). After individual peaks and fused peaks are identified within a chromatogram, the integration algorithm draws a baseline from the start to the end of each peak (or fused peak group). When the actual baselines are constructed, the integration algorithm calculates retention time, height, and area for each peak.
c_{LOD}	Concentration limit of detection.
continuous mode	In scan mode, the voltage is swept up and down continuously between the low and high potentials.
DAC	Digital-to-analog conversion.
DC mode	Direct current mode, where the potential of the working electrode remains constant during the analysis.
diffusion limited current	Electrolysis current that is determined by the rate of mass transfer through a thin layer of solvent adding to the electrode surface by viscous drag.
E_c	Flow cell potential, measured in volts (V).
ECD	Electrochemical detection or detector.

Empower software	Waters database software for Windows® XP or Windows 2000, which acquires, processes, reports, and manages chromatographic information.
E_{ref}	Potential of reference electrode.
event	A programmed change of a parameter in a time file; a timed event
F	Faraday's constant, 96,500 coulombs/mole of electrons
FIA	Flow injection analysis mode, where the column is deleted from the flow path (*not* fluorescence immunoassay).
fingertight	A type of fitting (connector).
FS	Full scale (units).
full scan	In scan mode, a scan where the voltage is swept up and down between the low and high potentials; includes the forward and reverse scan of a compound.
GC	Glassy carbon (*not* gas chromatography).
half scan	In scan mode, a scan where the voltage is swept in one direction between the low and high potentials; either the forward or reverse scan of a compound.
hydrodynamic voltammogram	A current-potential curve for a flowing solution at constant flow rate.
Hy-REF electrode	Hydrogen reference electrode.
I	Current, measured in amps.
I/E	Current/potential ratio.
ISAAC reference electrode	**I**n **S**itu **A**g/**A**g**C**l reference electrode.
KCl	Potassium chloride.
LC	Liquid chromatography.
LC-EC	Liquid chromatography - electrochemistry.
µA	Microamps.
mA	Milliamps.
ms	Microseconds.
nA	Nanoamps.

NaCl	Sodium chloride.
Ncm	Newton-centimeter, a unit that measures torque.
noble metals	A metal or alloy, such as gold or platinum, that is highly resistant to corrosion.
pA	Picoamps.
PAD	Pulsed amperometric detection, or pulse, mode.
potential	Cell potential (*E*), measured in volts.
pulse mode	A method of programming changes in the working electrode potential which ensures that uniform response is maintained. Generally used for carbohydrate detection with gold electrodes.
R	Gas constant.
REF	Reference electrode.
remote mode	An external data system controls the 2465 Detector. Input on the front panel of the detector is not allowed during remote mode.
reproducibility	The degree of agreement between replicate measured values; usually expressed as a standard deviation (SD) or percent relative standard deviation (%RSD).
salt-bridge REF	Reference electrode containing a device that separates an internal filling solution from the test solution, but conducts electricity.
scan mode	A method of applying a continuous linear variation in working electrode potential to obtain a current-voltage curve for an analyte; used for methods development.
scanning voltammogram	A voltammogram obtained in scan mode.
S/N ratio	Signal-to-noise ratio.
spacer	Thin transparent gasket for flow cell, thickness 25, 50, or 120 µm.
swivel	Connector on the bottom of the reference electrode.
time file	Program containing a series of data lines in which you change the settings of the 2465 Detector; created using the Prog screen; executed using the Events feature.
timed events mode	A time-based, automated and full parametric control of electrochemical detection; used for changing settings (such as

	sensitivity or autozero) during a run or between runs, or for control of external equipment.
voltage clamp	An electronic feedback circuit that compensates for polarization effects at the electrodes.
voltammogram	Graph of the measurement of a current-voltage (*I/E*) relationship.
WE	Working electrode.
working potential	Potential of the working electrode relative to that of the reference electrode.

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