Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Waters 3465 Electrochemical Detector

Overview and Maintenance Guide

715007395
Version 01

General information

Audience and purpose

This guide is intended for use only by professionally trained and qualified laboratory personnel who operate and maintain Waters products.

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Contact Waters with technical questions regarding the use, transportation, removal, or disposal of any Waters product. You can reach us through the Internet, telephone, fax, or conventional mail.

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iRequest	iRequest is a secure Web service form that allows you to request support and service for Waters instruments and software or to schedule a planned service activity. These types of support and services may be included as part of your maintenance plan or support plan. You may be charged for the requested service if you do not have appropriate plan coverage for your product. **Note:** In areas managed by authorized distributors, iRequest may not be available. Contact your local distributor for more information.
Local office contact information	For worldwide locations, telephone, fax, and conventional mail information is available at the Local Offices website.
Corporate contact information	Waters Corporation Global Support Services 34 Maple Street Milford, MA 01757 USA From the USA or Canada, phone 800-252-4752 or fax 508-872-1990.

Intended use

The 3465 Electrochemical Detector (ECD) is used in combination with Ultra Performance Liquid Chromatography for the electrochemical detection and quantification of suitable analytes in liquid samples. You can use the instrument for the chromatographic analysis of a wide range of electroactive analytes in the following fields:

- Bioanalytical analyses
- Food analyses
- Environmental analyses

Note: For research purposes only. While clinical applications are shown, this instrument is not tested by the manufacturer to comply with IVD regulations or standards.

Operation of an electrochemical detector can involve the use of hazardous materials, including corrosive fluids and flammable liquids. Only users with the following expertise should operate the instrument:

- Chemical laboratory technician degree or comparable vocational training.
- Fundamental knowledge of liquid chromatography and equipment.
- Participation in an installation of the system performed by the manufacturer or a company authorized by the manufacturer, and suitable training on the system and chromatography software.
- Knowledge and experience in the safe handling of toxic and corrosive chemicals and knowledge of the application of fire prevention measures prescribed for laboratories.

Information on safety practices is provided with your instrument. Before using your instrument or accessories, you must thoroughly read these safety practices.

EMC considerations

FCC radiation emissions notice

Changes or modifications not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment. This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

Canada spectrum management emissions notice

This class B digital product apparatus complies with Canadian ICES-001.

Cet appareil numérique de la classe B est conforme à la norme NMB-001.

ISM classification: ISM group 1 class B

This classification was assigned in accordance with CISPR 11 Industrial Scientific and Medical (ISM) instrument requirements.

Group 1 products apply to intentionally generated and/or used conductively coupled radio-frequency energy that is necessary for the internal functioning of the equipment.

Class B products are suitable for use in both commercial and residential locations and can be directly connected to a low-voltage, power-supply network.

This equipment complies with the emission and immunity requirements described in the relevant parts of IEC/EN 61326: Electrical equipment for measurement, control, and laboratory use — EMC requirements.

EMC emissions

Do not use the equipment in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). The radiation can interfere with the equipment's proper operation.

Legal manufacturer

	Waters Corporation 34 Maple Street Milford, MA 01757 USA

Safety considerations

Some reagents and samples used with Waters instruments and devices can pose chemical, biological, or radiological hazards (or any combination thereof). You must know the potentially hazardous effects of all substances you work with. Always follow good laboratory practices and consult your organization's standard operating procedures as well as your local requirements for safety.

Applicable symbols

The following symbols can be present on the device, system, or packaging.

Symbol	Definition
	Manufacturer
	Date of manufacture
	Confirms that a manufactured product complies with all applicable European Community directives
	UK Conformity Assessed marking confirms that a manufactured product is in conformity with the applicable requirements for products sold within Great Britain
	Australia EMC compliant
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Confirms that a manufactured product complies with all applicable United States and Canadian safety requirements
	Environmentally friendly use period (China RoHS): indicates the number of years from the date of manufacture until the product, or components within the product, are likely to be discarded or degrade into the environment
	Consult instructions for use
	Alternating current
	Electrical and electronic equipment with this symbol may contain hazardous substances and should not be disposed of as general waste For compliance with Waste Electrical and Electronic Equipment legislation, contact Waters Corporation for the correct disposal and recycling instructions
	For indoor use only

Symbol	Definition
	No pushing
	Do not connect to an LC system
	Indicates the maximum load you can place on that item (for example, 10kg)
	Serial number
	Part number, catalog number

Safety hazard symbol notice

The  symbol indicates a potential hazard. Consult the documentation for important information about the hazard and the appropriate measures to prevent and control the hazard.

Electrical power safety notice

Do not position the device so that it is difficult to disconnect the power cord.

Equipment misuse notice

If equipment is used in a manner not specified by its manufacturer, the protection provided by the equipment may be impaired.

Considerations specific to the device

Working environment and safety

This instrument's intended use is to detect electroactive substances in liquid samples in combination with a UPLC or HPLC system in a GLP-approved environment. Operators using

the system require experience, the appropriate education, and an extensive understanding of GLP rules. To avoid unsafe situations, use this system only for its intended use.

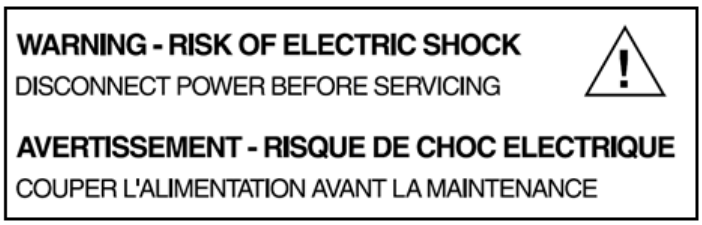
System operation

To ensure optimal performance, Waters recommends that you inspect the detector and carry out maintenance procedures regularly. Preventive maintenance contracts are available for that purpose. Contact your local dealer or the nearest sales office for more information.

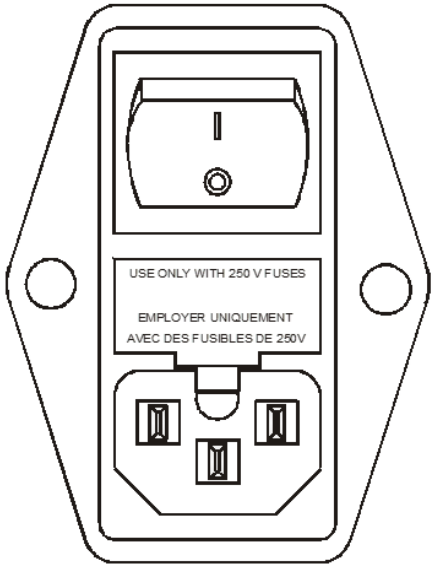
Electrical safety

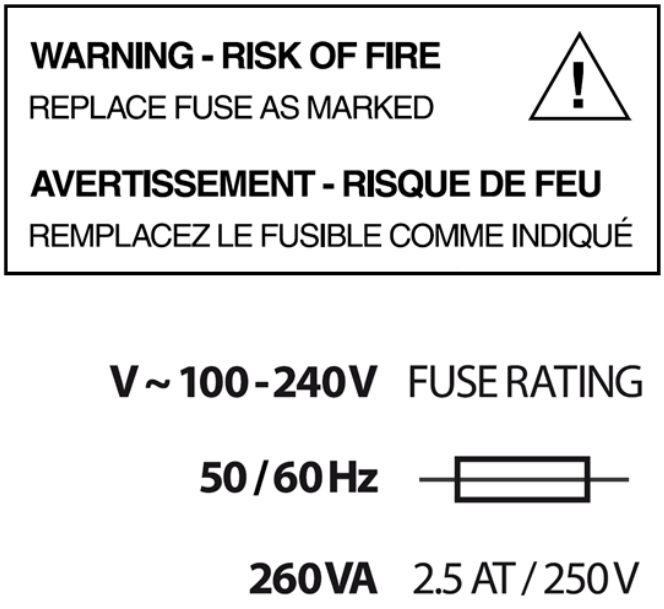
Warning: To avoid electric shock, do not remove protective panels from the device. The components within are not user-serviceable.

The removal of protective panels on the instrument can result in exposure to potentially dangerous voltages. Disconnect the device from all power sources before disassembly.



Untrained personnel should not open the instrument. This may only be done by authorized service engineers. Replace or repair faulty insulation on power cords immediately after discovery of the fault. Confirm that the actual power voltage is the same as the voltage for which the instruments are wired. Ensure that power cords are connected to correct voltage sources: grounded AC power source, line voltage 100 – 240 VAC. Connect the instrument to a protective earth via a ground socket. Only use the 3465 ECD with appliances and power sources with proper protective grounding to prevent damage through build-up of static electricity. The power source should exhibit minimal power transients and fluctuations. If necessary, connect to a filtered mains socket.





Replace blown fuses with fuses of the proper type and rating as indicated on the rear panel and as noted in the list of accessories and spares. The fuse holder is integrated in the mains connector. Ensure that the instrument is never put in operation with fuses of a different type. This could cause fire.

Note: Only use manufacturer-supplied I/O cables to connect with other devices.

Thoroughly connect shielding to common. Manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices and with cables that do not meet relevant safety standards. Place the detector on a flat, smooth surface. Do not block the ventilation holes located at the bottom and lower rear panel of the detector. Blocking these holes may impair the cooling capability of the power supply.

Solvents

The solvents used may be flammable, toxic, or corrosive. To prevent injury:

- Do not use an open flame near the device.
- Do not install the system in the same room with other equipment that emits or could potentially emit sparks.
- Provide protective equipment near the instrument.
- When solvent gets into the eyes or on the skin, flush it away immediately.
- Provide equipment, such eye wash stations and safety showers, as close to the system as possible.
- Use proper eye and skin protection when working with solvents.
- Seal sample containers (vials) to minimize any risks related to solvent vapor.

Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Ensure that you understand the hazards associated with the chemicals and take appropriate measures with regards to safety and protection.

Biological hazard

When you analyze biological fluids, you must take all possible precautions and treat all specimens as potentially infectious. To avoid personal contamination with biologically hazardous, toxic, or corrosive materials, and to avoid spreading contamination to uncontaminated surfaces, wear clean, chemical-resistant, powder-free gloves when performing procedures with the 3465 ECD.

Ventilation hazard

Warning: To avoid personal contamination with toxins and biologically hazardous materials when using the system, follow appropriate safety precautions, and ensure adequate ventilation and air exchange in the laboratory.

Waste disposal

Perform periodic leak checks on LC tubing and connections. Do not close or block the drain in the oven compartment. Do not allow flammable or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable or toxic solvents through the municipal sewage system.

Safe disposal

Contact recycling@waters.com with any questions or concerns regarding the proper handling or disposal.

Dispose of Waters instrumentation products in accordance with applicable requirements and best practices as described below.

- Follow appropriate procedures for flushing the instrument's fluid paths of any hazardous samples or solvents.
- Waters instruments are subject to European Union's Waste Electrical and Electronic Equipment (WEEE) and Restriction of Hazardous Substances (RoHS) Directives. According to these directives, do not dispose of instruments in the general waste stream. Similar "e-

waste” laws also apply in other jurisdictions. In all cases, ensure that a certified electronics recycler processes end-of-life instruments.

- Some Waters instruments use batteries, mercury-containing lamps, or other replaceable components during the life span of the instrument. Handle such materials in accordance with local laws governing their processing and safe disposal.

Applications: quality control

Routinely run three QC samples that represent subnormal, normal, and above-normal levels of a compound. If sample trays are the same or very similar, vary the location of the QC samples in the trays. Ensure that QC sample results fall within an acceptable range and evaluate precision from day to day and run to run. Data collected when QC samples are out of range might not be valid. Do not report this data until you are certain that the instrument performs satisfactorily.

Equipment misuse notice

If equipment is used in a manner not specified by its manufacturer, the protection provided by the equipment may be impaired.

Safety advisories

Consult the "Safety advisories" appendix in this publication for a comprehensive list of warning advisories and notices.

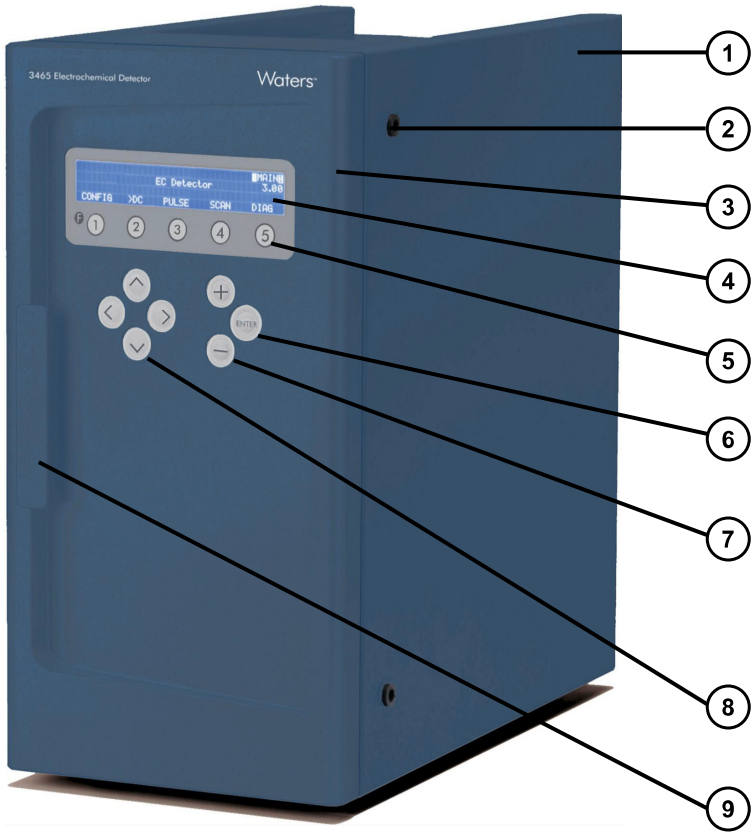
Table of contents

1 Introduction

Congratulations on your purchase of the 3465 Detector. This detector enables you to perform all LC/UPLC applications using electrochemical detection. The 3465 Detector includes a highly stable Faraday-shielded oven compartment accommodating column and flow cell. The flow cell provides an unsurpassed S/N ratio for extremely sensitive electrochemical analyses. The 3465 Detector has three operational measurement modes: DC, SCAN, and PULSE. For a few specific applications, the 3465 Detector can support up to two flow cells (optional).

1.1 Instrument description

Figure 1–1: 3465 Detector - Front side



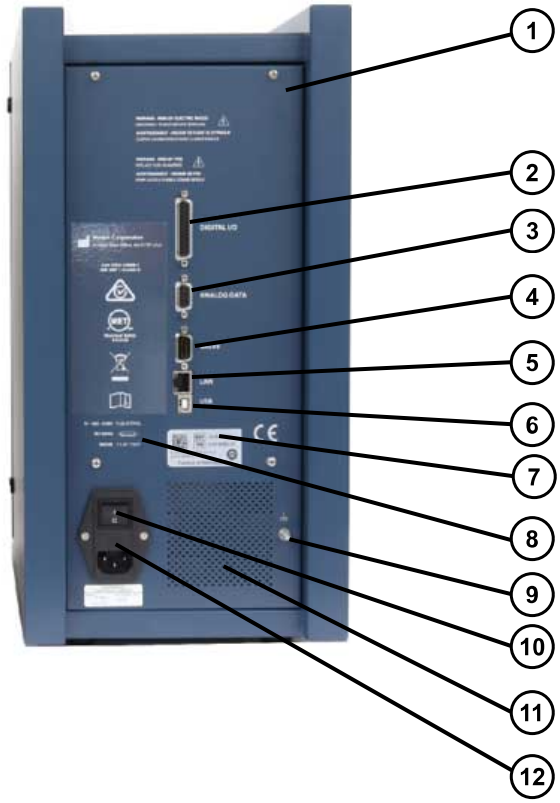
1. Instrument housing
2. LC tubing inlet/outlet
3. Instrument door panel
4. 4 x 40 Ch LCD display
5. Function keys
6. <Enter> key

7 "+" and "-" value keys

8 Cursor keys

9 Door handle (for opening door)

Figure 1–2: 3465 Detector - Back side



1 Instrument rear panel

2 Digital I/O connector (25-p sub-D fem)

3 Analog data (9-pin sub-D fem)

4 Valve connector (9-pin sub-D male)

5 LAN connector (RJ45 jack)

6 USB connector (USB B)

7 Type label (PN, SN, etc.)

8 Fuse and power rating

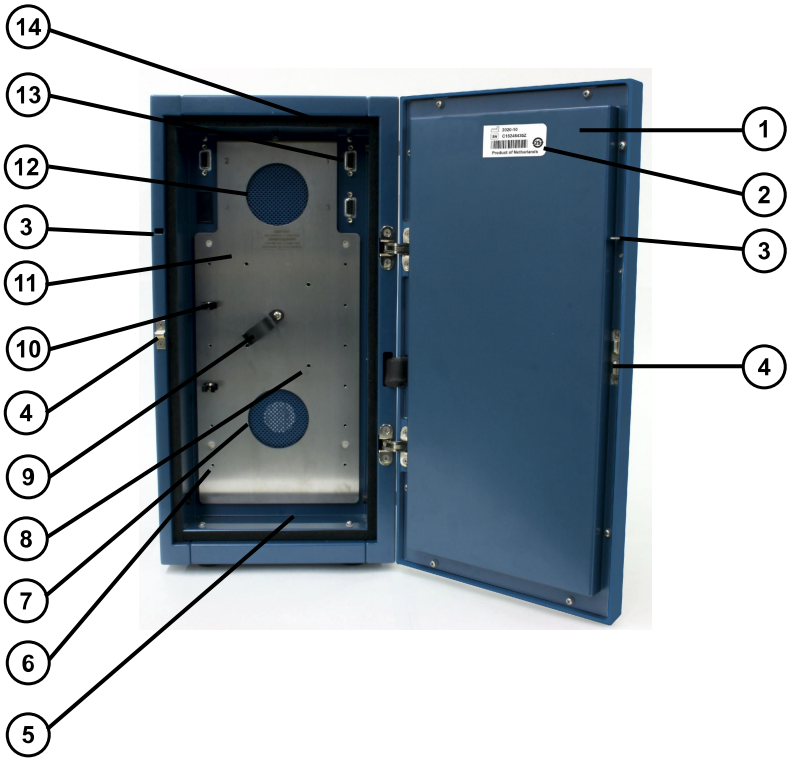
9 Grounding stud

10 Mains switch/inlet

11 Ventilation holes

12 Fuse compartment

Figure 1–3: 3465 Detector - Oven compartment



1 Door panel, rear

2 Type label

3 Door sensor

4 Door lock

5 Drain

6 Mounting hole (column clamp)

7 Bottom fan heater (exhaust)

8 Mounting hole (cell clamp)

9 Flow cell clamp

10 Column clamp

11 Mounting plate

12 Top fan heater (intake)

13 Cell connector (9-pin sub-D)

14 Cell cabinet

2 Installation

2.1 Storage requirements

The 3465 Detector is shipped to your facility in one box with the following dimensions:

Parameter	Requirement
Height	44.0 cm (17.3 inches)
Width	22.0 cm (8.7 inches)
Depth	43.0 cm (16.9 inches)

Ensure that you have sufficient space to store the packed instrument under the following storage conditions:

Parameter	Requirement
Storage temperature range	-25 °C to 50 °C (-14 °F to 122 °F)
Storage humidity range	20% to 80%, non-condensing

2.2 Site preparation requirements

Note: Although not detailed in this document, the installation site must comply with applicable local laws and regulations with regard to electrical and mechanical installations, building safety, the use and disposal of potentially hazardous materials and chemicals.

For a successful onsite installation of the instrument, complete the personal computer, laboratory, electrical and power, and chemicals requirements at your location in advance.

2.2.1 Acquisition computer

The computer used for instrument control requires the following specifications:

- Free LAN port (onboard, PCI, PCI express, or PCI-X)
- Free USB port (required for FW updates)
- Windows 10

For further information about software packages and requirements, visit the Waters website www.waters.com.

Note: Installing software requires a computer with administrator access. Ensure that the PC and its USB ports are authorized/able to accept third-party software. To avoid unnecessary delays during the installation, inform your IT department well in advance to arrange authorization.

Notice: For uninterrupted operation of the system, Waters advises that you turn off or disable the following:

- Screensavers
- (USB, LAN) hibernate mode
- Auto hard disk shutdown (energy saving)
- Automatic Windows updates
- Exhaustive scanning by virus scanners. (In your antivirus software, turn off the option **Check Files at Change** for the relevant data storage directory.)

The computer should be placed in the vicinity of the 3465 Detector, within a maximum distance of 2.5 meters.

2.2.2 Laboratory requirements

Your instrument is intended for indoor use only in an industrial or commercial environment (EN55011 group 1 class B ISM equipment). It is suitable for the following categories: installation category II, pollution degree 2, and equipment class I.

Table 2–1: Table 2-1: Environmental specifications

Parameter	Requirement
Operating temperature range	10 °C to 35 °C (50 °F to 104 °F)
Maximum altitude	2000 m
Operating humidity range	20% to 80%, non-condensing

Notice: For optimum analytical performance, it is recommended that the ambient temperature of the laboratory be between 20 °C and 25 °C and be held constant to within ± 2 °C throughout the entire working day.

Notice: For optimal temperature stability of the cell cabinet, the oven temperature should be set to at least 7 °C higher than ambient temperature.

Note: Do not place the system next to heating or cooling pipes or expose the instrument to direct sunlight or air drafts (AC system or open windows).

Requirements for the laboratory bench on which the instrument will be installed:

- Stable, clean, flat, and smooth surface.
- Enough mechanical strength to hold at least the weight of the detector. A 3465 Detector without cell weighs 14.4 kg (31.7 lbs). A fully dressed detector with flow cells, peripherals, columns, and valves may weigh 20 kg or more.
- A 3465 Detector has the following dimensions: Depth 44.0 cm (17.3 inches), Width 22.0 cm (8.7 inches), and Height 43.0 cm (16.9 inches). Take into account that additional space is necessary on all sides to prevent obstruction of ventilation holes and allow sufficient heat dissipation. Keep at least 15 cm free at the back. Keep at least 5 cm distance if there is another device on one side. Keep at least 10 cm distance if there are devices on both sides.

2.2.3 Electrical and power requirements

The customer is responsible for providing appropriate electrical power and power outlets in the laboratory.

1. The installation of electrical supplies and fixtures in the laboratory must comply with all local regulations and safety standards.
2. The power source should exhibit minimal power transients and fluctuations. The AC main supply voltage source should not fluctuate more than +/- 10% from nominal voltage. If your main voltage is unstable (>10% of nominal voltage), use an Uninterruptible Power Supply (UPS). The main supply must include a correctly installed protective earth conductor.

   Notice: To protect against power transients (voltage spikes and power surges), it is recommended that you connect the equipment over an electrical surge protector.

3. The 3465 Detector is equipped with a universal AC/DC switched-mode power adapter rated for 100–240 V AC and 50/60 Hz. Every detector is delivered with a set of two power cords for the following regions:
   - EUR (CEE7/7 plug to IEC60320 C13 plug)
   - US (NEMA 5-15 plug to IEC60320 C13 plug)

   Notice: For regions with other main plugs/sockets (for example, UK, Switzerland, Brazil), ensure that you have the appropriate power cords available at the time of installation and that these power cables are properly grounded and meet the safety standards that apply in your country. Contact your local distributor with any questions.

4. The maximum power consumption of the 3465 Detector on full power is < 200 W. The typical power consumption is < 50 W.
5. Connect the detector to a grounded AC wall socket with a line voltage of 100–240 V AC (as specified in the sections above) using the supplied power cables. The instrument should be connected to a protective earth via the socket. Ensure that the detector is placed in such a way that the main power connection can be reached easily to disconnect it from the main power by removing the power cable.

Warning: Use only manufacturer-supplied cable to connect devices. Thoroughly connect shielding to common ground. The manufacturer accepts no liability for damage, direct or indirect, caused by connecting this instrument to devices with cables that do not meet relevant safety standards.

2.2.4 Chemicals

Mobile phase and flush/storage solutions must be clean because they are in direct contact with the working electrode of the electrochemical cell. High-purity chemicals, including water, are prerequisites. All chemicals should be electrochemically clean, HPLC-grade or better. For water used in the preparation of mobile phases, a purification apparatus is recommended that is able to supply high-purity deionized water with resistivity of >18 Mohm*cm and low (<10 ppb) TOC level.

2.3 Unpacking

Inspect the transport box for possible damage when it arrives. Immediately inform the transport company of any damage, otherwise it may not accept responsibility. Keep the transport box; it is designed for optimum protection during transport and may be needed again. Carefully unpack the system and inspect it for completeness and possible damage. Contact your supplier about damage or if not all marked items on the checklist are included. Prior to shipment, your detector was thoroughly inspected and tested to meet the highest possible demands. The results of all tests are included.

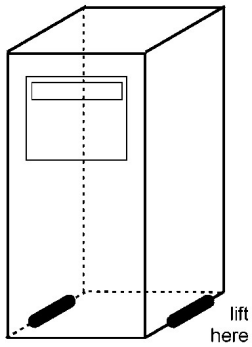
See the checklist below for reference:

1. Delivery is in accordance with the order.
2. Delivery is undamaged.
3. All items on Start Up Kit list are included.
4. Certificates of performance are included:
    a. Detector
    b. Flow cells[1]
5. See startup kit list for weblink to user manual.

To unpack the detector, lift it from its box by both hands. Never lift the detector by its front door; lift it by the sides.

[1] [1]Flow cells are not part of the 3465 Detector and must be ordered separately.

Figure 2–1: Lift instructions for 3465 Detector



With both hands under the instrument, lift the detector and bring it to its operation location. Install the detector in an area that meets the environmental conditions.

Figure 2–2: Location of ventilation holes in the 3465 Detector (rear)

Remove the protective tape from the detector LCD screen. Leave the instrument to adopt ambient temperature for at least half an hour in the place of installation.

Notice: If the instrument was stored cold (<10 °C) and switched on immediately, the oven might not work. This is by design (at temperatures below 10 °C, the heater is switched off in the embedded software). Allow the detector (electronics) to warm up a little longer to reach ambient temperature before switching on the oven.

Notice: Use the detector indoors only. Place the detector upright (on its instrument feet) on a stable, flat, and smooth surface. Do not place the instrument in an area subject to excessive dust or shocks. Do not place it near a source of heat or in direct sunlight, because this may influence the heating capabilities of the instrument. Ensure that the detector is placed in such a way that the main power connection can be reached easily to disconnect it by removing the power cable. Do not block the ventilation holes at the back and bottom of the instrument. Blocking might impair the cooling capability of the power supply.

Do not place heavy objects/instruments on top of the detector. Objects can be placed on any side of the detector; however, make sure these objects are placed at a distance of 5 cm from the detector (if objects are placed on only one side of the instrument) or 10 cm from the instrument (if objects are placed on both sides of the instrument).

2.4 Main power connection

Verify that the fuses and voltage range on the rear side of the instrument are appropriate for the power outlet to be used.

Warning: To avoid electric shock, connect the system to a suitable main power supply with a correctly installed protective ground conductor. Never use the system without a properly connected ground conductor.

Leave the instrument powered-off until specifically mentioned in the procedure.

2.5 Connecting the Ethernet cable

The Waters instrument communicates with the acquisition computer through the dedicated local area network (LAN). At the acquisition computer, the instrument network card provides the communications interface.

You must install the instrument control software driver (ICS) in the acquisition computer so that the computer can control the instrument. (Consult the software installation instructions that accompany the instrument control software for details). To communicate over LAN, the computer must have a free (PCI, PCI Express, or PCI-X) LAN port.

The 3465 Detector has a fixed IP address: 192.168.0.65, with subnet mask 255.255.255.0. Gateway and DNS are not filled in.

The instrument is delivered with a special crossover LAN (UTP) cable (Waters PN: 700013076), which is part of the 3465 Detector Startup Kit, part number 200000485 for a single flow cell unit and 200000492 for a dual flow cell unit (Waters PN 200000492).

Notice: To ensure stable and error-free communication, use only the manufacturer-supplied LAN cable to connect the 3465 Detector to LAN. Create a small, dedicated local area network (Instrument LAN) to connect the 3465 Detector to the PC. Do not connect the 3465 Detector over a company LAN. If needed, a second network adapter with a different (unique) IP address range can be applied.

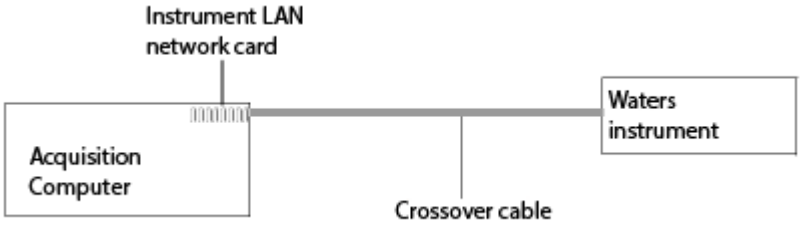
The next section describes the procedure to connect the instrument to the PC using the crossover UTP cable.

Configure the IP address of the Instrument LAN network card by executing the following steps:

1. Right-click on the **Network** icon in the bottom-right of the **Windows** taskbar and open the menu **Network & Internet settings**. Alternative: Open the **Windows** start menu, open the **Setting** menu, and open the **Network & Internet** menu.
2. From the right-hand panel of the **Network & Internet** window, open the **Change adapter settings** menu.

3. Right-click on the **Local Area Connection** icon of the LAN card in your personal computer and click on **Properties** to open the Network card setting.
4. Open the **Internet Protocol Version 4 (TCP/IPv4)** menu (double-click).
5. Configure the network IP address and subnet mask (for example, IP 192.168.0.1, Subnet mask 255.255.255.0). Gateway and DNS fields are not filled.
6. Close the menus by clicking the **OK** buttons. The network IP address of the LAN network card is now set up for communication with the 3465 Detector.
7. Connect the crossover (UTP) cable to the **RJ45 Jack** of the LAN card of your PC (typically located on the backside of a desktop PC).
8. Connect the other end of the crossover LAN (UTP) cable to the LAN port on the rear panel of the 3465 Detector.
9. Power-on the 3465 Detector. Set the detector temperature to 35 °C if an Operational Qualification (OQ) will be executed, or set it to the temperature at which your ECD application is running. Allow the instrument to stabilize for at least half an hour before starting experimental work or performing an OQ.

Figure 2–3: Single Waters instrument connection



A direct connection requires the special crossover UTP cable delivered in the detector startup kit. With an Ethernet switch, any regular UTP cable can be used.

2.6 Software

The 3465 Detector can be used in combination with PC control software. The following software packages are available for control of the 3465 Detector:

Vendor	Software	Version number[a]
Antec	Dialogue Elite[b]	2.20.2.1
Waters	Empower 3 FR4 and later	

a. Version compatibility: compatible with the listed version (minimum).

b. Used for Firmware loading only.

Only 3465 Detectors with FW version 1.09 and later can be controlled with Empower software.

The Antec Dialogue Elite software is required for the upload of firmware (embedded software controlling the 3465 Detector electronics). In this section, the installation of Dialogue Elite and the 3465 Detector is briefly described.

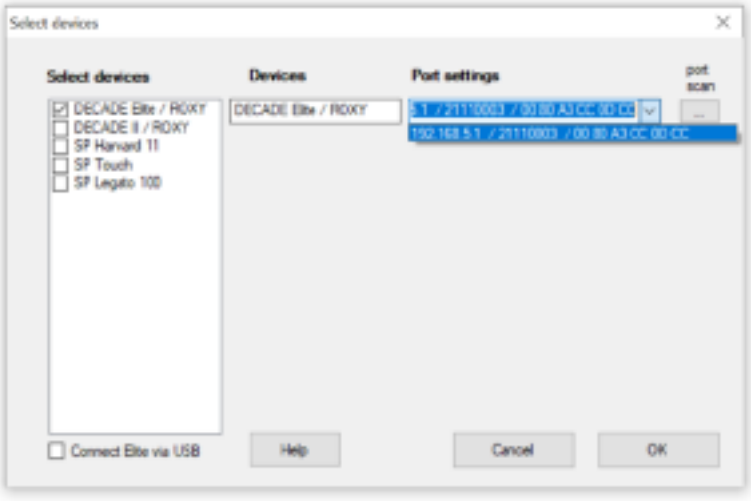
This is by no means a replacement of the installation documentation available for the software packages. Refer to the software documentation for details.

Note: Ensure that you have Administrator access rights in your system before you start installation of the software packages. Dialogue Elite users must have read/write access to all software folders and sub-folders.

2.6.1 Dialogue Elite

To install the software:

- Request software by submitting an iRequest.
- Double-click on the **setup.exe** file to start the installation wizard.
- Follow the instructions of the installation wizard for successful installation of the software.
- Insert the Dialogue Elite license dongle to obtain full access to the software (without dongle, it will operate in demo mode).
- Ensure that the LAN connection is configured and that the LAN cable is connected.
- Power-up the detector by means of the main switch on the rear panel.
- Start Dialogue Elite from the Windows start menu.
- During startup, the **Select devices** menu appears as shown here.
  Figure 2–4: Select devices menu



- When a 3465 Detector is available, it is automatically detected, and the IP address appears in the port settings box. If it does not, press port scan or type in the default IP address 192.168.0.65.

- The pull-down field shows all responding devices and their IP and MAC addresses. If in doubt, confirm the unique MAC address on the rear panel IO connector.
- Type `OK`. The instrument connects and is ready for use.

2.7 Fluidics connections

This section describes the installation and priming of all fluidics connections needed to use the 3465 Detector for analysis of substances with UPLC-ECD. When working with HPLC solutions and mobile phases, take the following precautions:

Warning: Use proper eye and skin protection when working with solvents. The solvents used may be flammable, toxic, or corrosive. Organic solvents are toxic above a certain concentration. Ensure that work areas are always well-ventilated. Use of open fire in the vicinity of this system must be strictly prohibited. Do not install the system in a room with any other equipment that emits or could emit sparks. Wear protective gloves, safety glasses, and other relevant protective clothing when working on the device.

With respect to third-party UPLC equipment, such as LC pumps, auto samplers, injection valves, and column heaters used in combination with the 3465 Detector, the equipment connected to the system should be designed specifically for use in Ultra High Performance Liquid Chromatography and capable of delivering flow rates in the range of 1 µL/min to 2 mL/min.

The manufacturer accepts no liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.

2.7.1 Tubing connectors

For optimal operation and to minimize peak broadening, carryover, and other detrimental factors, it is important that all tubing connections on the injection valve, columns, and flow cells be made without introducing dead volumes.

Notice: Use only the original polymeric finger-tight fittings supplied by Waters to make LC connections on the flow cell inlet and outlet. Do not use metal tubing on the flow cell because it may lead to damage or incorrect operation. Use PEEK, PEEKsil, or fused silica tubing (with FEP sleeves).

For columns and injection valves, use only nuts, ferrules, or finger-tight fittings recommended by the parts manufacturers. For Vici Valco valves, use Valco nuts and ferrules; for Rheodyne valves, use Rheodyne hardware, and so on. The use of unsuitable connectors may lead to damage of the parts or the introduction of dead volumes.

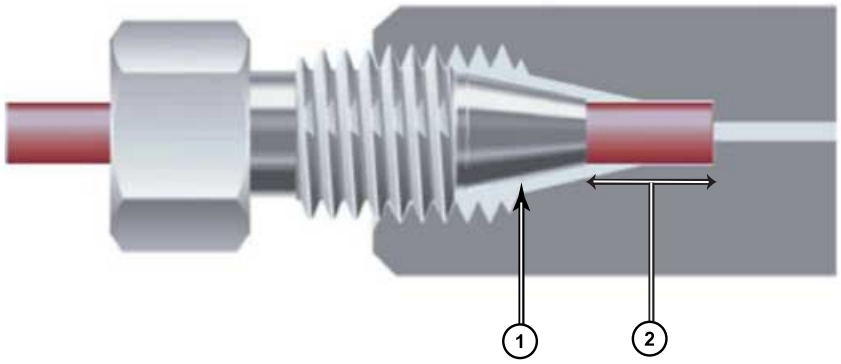
Note that the tubing length (length L below) required to make a good connection differs for each brand of connection. If length L is not correct, it could result in faulty peaks and carryover.

Essentially, when you create a connection, the ferrule on the tubing is compressed into the valve to create a leak-tight seal. Take the following into account when creating the connection:

If L is too long, the ferrule cannot form a seal in the connection. This may cause irreparable damage to the port of the valve, column, or other part:

- Part of the tubing may end up in the connection port internals.
- Internals of the port may be damaged.

Figure 2–5: Length is too long



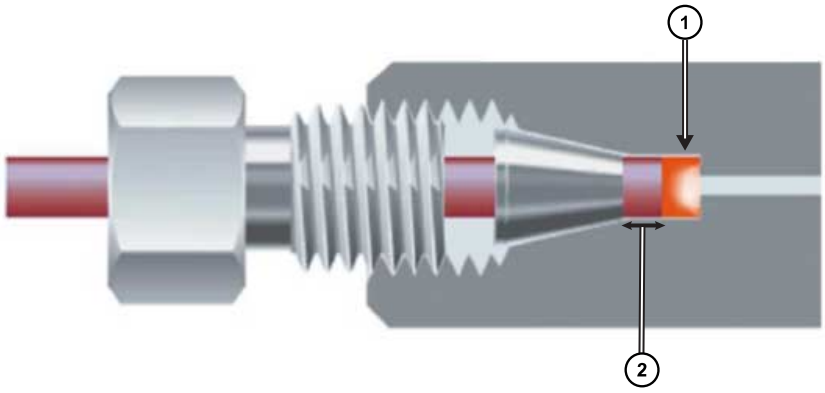
1 Ferrule does not seal the connection

2 L = too long

If L is too short, this may result in:

- Leakage
- Dead volume at the end of the ferrule (a “mixing chamber”)

Figure 2–6: Length is too short



1 Tubing does not touch end of the connection, resulting in dead volume

2 L = too short

Every ferrule type needs an appropriate length of tubing for connecting it to the connection port, depending on the depth of the connection port. Refer to the information provided by the manufacturer.

2.7.2 Mobile phase

Electrochemical detection is a sensitive detection technique characterized by extremely low detection limits. A typical detection limit of 100 pmol/L or lower for catecholamines is no exception. Improving detection limits is always limited by the weakest link in an LC-EC system. In daily practice, a few rules must be followed to fully exploit the linear dynamic range and low detection limits of an EC detector. These rules are related not only to hardware but also to mobile phase composition, degassing, temperature and pH stability, and several other factors.

Mobile phase requirements:

- Electrochemically clean, HPLC-grade or better
- Ion strength 20 - 200 mmol/L
- Buffer pH near pKa
- In-line 0.2-µm filter and degassing of mobile phase
- EDTA for trapping of metal ions

Mobile phase must be clean because it is in direct contact with the working electrode in EC detection. High-purity chemicals, including water, are prerequisites. In some applications, EDTA is added to the mobile phase to electrochemically trap metals such as Fe^{2+} by forming an inactive complex. However, at higher working potentials (typically > 1.2 V vs. salt bridge AgCl REF), EDTA can become electrochemically active and is not recommended. In that case only a passivation step with 30% H_3PO_4 is recommended.

Electrolytes ensure contact between the three electrodes in an electrochemical flow cell. Low ion strength destabilizes an EC system and noise increases. Extremely high buffer concentrations cause problems of salt formation. Therefore, concentrations between 20 and 200 mmol/L are recommended.

Also, constant pH is important for baseline stability and reproducible results. Stability of pH is best when close to pK_a of a buffer ion. Frequently used buffers include phosphate, carbonate, acetate, and citrate. Modifiers such as methanol, propanol, and acetonitrile can be used without problems in DC amperometry, but not in pulsed amperometric detection because peaks are strongly attenuated. In our experience, the quality and useful life of organic modifiers can be problems that result in increased noise levels. Metal inlet frits in mobile phase bottles may increase baseline noise. If so, alternative filters are available.

Vacuum degassing: Considerable amounts of the gases N_2, O_2, and CO_2 may be dissolved in HPLC mobile phases. Whenever the temperature changes, solvents mix, or a pressure reduction occurs, these gases may show up as very small air bubbles. To avoid noise baselines, use the inline degasser incorporated in the Waters instruments.

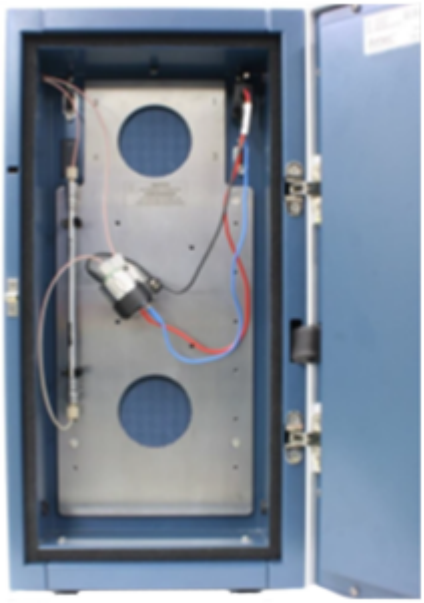
Helium degassing: Degassing using helium is an effective and universally applicable method, but it is recommended only when working in reductive electrochemical detection and pulsed

electrochemical detection (analysis of carbohydrates using anion-exchange chromatography with NaOH as mobile phase). All gases except helium are removed completely. Helium is not EC active and does not significantly change the mobile phase properties. To prevent mobile phase contamination, use only high-purity helium.

2.7.3 Installation and startup

For a successful installation and startup, follow the next steps carefully:

1. See the Installation of flow cell and column in the 3465 Detector figure.
   Figure 2–7: Installation of flow cell and column in the 3465 Detector



2. Before connecting the HPLC system to the detector, you should passivate all metal parts with 30% phosphoric acid over 20 minutes. The acid is flushed through the pump, the pump tubing, the dampener, and the injector (in load and inject positions), and then routed to waste.

   Notice: Ensure that all parts that are not acid-resistant (such as nylon inlet filters, column, and flow cell) are not connected during this step.

3. After flushing with the acid, you must thoroughly flush the system with HPLC-grade water. Ensure that no traces of acid are left in the tubing or pulse dampener (confirm with pH paper). Flush the system with HPLC buffer.

   Notice: If an ISAAC reference electrode is used, ensure that the mobile phase contains at least 2 mmol/L chloride (KCl or NaCl) ions.

4. Before connecting a new column, read the manufacturer's instructions. Thorough pre-conditioning of a column is always required. Only a pre-conditioned column is electrochemically clean. If not, the background current may be unacceptably high and substantial fouling of the working electrode occurs. For reverse-phase columns, flushing

with 50% methanol in water for three days at a low flow rate is highly recommended. Before switching to mobile phase, flush with water (10 column volumes) to prevent precipitation of buffer salts.

5. Passage of air bubbles through the flow cell leads to unacceptable noise levels and spikes. Therefore, the use of an in-line degasser is strongly recommended. A one-time degassing step of the HPLC buffer is almost never sufficient. If the 3465 Detector is used for reductive ECD (at a negative working potential), additional steps should be taken to remove oxygen from the mobile phase. These include degassing with helium and the use of stainless steel tubing.
6. Consult the flow cell chapter for detailed information about the installation of the flow cell. Although the oven of the detector can be operated in a temperature range of 7 °C above ambient to 60 °C, do not operate the flow cells above 50 °C.

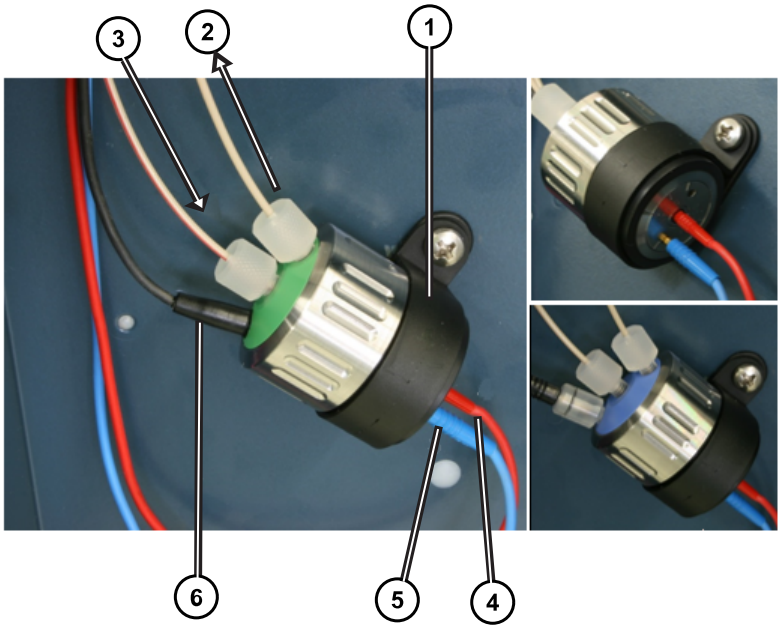
   Notice: Do not use the flow cells at temperatures above 50 °C; higher temperatures might lead to damage of the cell.

7. See the Figure 2–8: SenCell mounted at an angle of approximately 45° in the detector (Page 37) figure to help make the electrical and fluidics connections. Connect the flow cell to the corresponding cell connector in the oven compartment. Cell connectors are marked with a label for identification. In the case of a 3465 Detector SCC, connect the flow cell to the cell connector on the left-hand side marked “Cell 1”.

   Notice: The cell connector inside the oven compartment is electrostatic sensitive. Ensure that the flow cell is OFF when removing or connecting the cell cable. Never switch ON the flow cell when:

   - The cell cable is not correctly connected.
   - The cell is only partly (or not at all) filled with mobile phase/buffer/electrolyte.
   - The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection, because substantial damage to the working electrode or electronics may occur.

Figure 2–8: SenCell mounted at an angle of approximately 45° in the detector



1. Cell clamp
2. Cell outlet (tubing connection from cell to waste); ensure that the outlet is positioned on the top side to prevent entrapped air bubbles
3. Cell Inlet (tubing connection from column to cell)
4. WE contact (red)
5. AUX contact (blue)
6. REF contact (black)

Left: SenCell with ISAAC reference mounted. Top-right: electrical connections of WE (red connector) and AUX electrode (blue connector), for SenCell type flow cells this electrode is stainless steel. Bottom-right: SenCell with salt bridge reference electrode.

8. Before switching ON the flow cell, ensure that the mobile phase contains sufficient electrolyte (buffer ions). A stable baseline cannot be obtained if the cell is switched ON with only water or another nonconducting mobile phase. Also, ensure that no air bubbles are trapped in the flow cell.
9. The outlet tubing from the flow cell should lead to a reservoir that is at a higher level than the flow cell. This ensures a small back pressure, which prevents air-bubble entrapment. The outlet tubing should be under the liquid level, to avoid electrical noise from “dripping” of mobile phase.

10. Set the cell potential, switch ON the flow cell, and allow the system to stabilize for approximately 30 minutes. A good stabilization curve shows a mono-exponential decline without jumps or spikes.
11. Connect the 3465 Detector to the PC control software (Empower 3).

Your system is now ready for use. The 3465 Detector has been developed for continuous operation. For maximum stability, it is recommended that you leave the system ON continuously. If preferred, the flow cell may be switched OFF when not in use.

3 Maintenance and shutdown

3.1 Maintenance

This section describes all maintenance that can be performed by the end user. All other maintenance and service procedures must be performed by authorized service engineers.

3.1.1 Periodic check for leakage

- Perform leak checks on LC tubing, flow cells, and connections daily, and verify that the drain on the bottom of the oven compartment is not blocked or closed.
- Do not allow solvents to accumulate.
- Follow a regulated, approved waste disposal program.
- Empty and clean the waste container regularly. Never dispose of such products through the municipal sewage system.
- Check every day that the mobile phase bottles contain enough mobile phase for the number of analyses planned.

3.1.2 Periodic check of the oven temperature

The operator should perform regular checks to verify if the actual oven temperature is in accordance with the set temperature of the 3465 Detector.

Warning: If the actual temperature exceeds 70 °C, switch off the detector immediately and contact Waters or the local representatives for service. Do not touch the metal parts inside the oven compartment because they could be hot. Do not use or switch on the instrument before it is serviced at the factory.

If the heater is defective and the temperature exceeds 70 °C, the instrument raises an error message (Err 23).

3.1.3 Flow cell

Check the performance of the detector and flow cell on a daily basis by evaluating background current, noise, and signal. An increase in background current, noise, and/or a loss of sensitivity may be a sign of contamination of the working electrode (WE) and/or a sign that maintenance is required on the reference electrode (REF) of the flow cell. If necessary, perform maintenance on the flow cell.

3.1.4 Cleaning

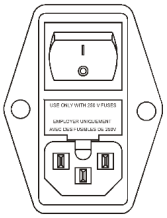
In general, the 3465 Detector needs very little maintenance. The outside of the detector may be cleaned with a non-aggressive cleaning liquid.

Notice: Do not use organic solvents to clean the exterior of the detector, because this may damage the paint layer.

In the case of leakage in the cell cabinet (tubing, connectors, cell, column, or other components), remove the spilled mobile phase or other solutions as soon as possible because they could damage the paint layer or result in the deposition of salt crystals (from buffered mobile phases), which could block the drain in the bottom of the cell cabinet. Remove any dust on the protective screens that cover the fans in the oven compartment.

3.1.5 Replacement of fuses

Warning: To avoid electrical shock, before examining or removing fuses, power-off an instrument or device, and then remove its power cord from the receptacle on the back of the unit. For continued protection against fire, replace fuses with those of the same type and rating only. As a general practice, even when only one out of two fuses is open or otherwise defective, replace both fuses.



If the fuses blow out repetitively, contact Waters or its representatives for instructions and/or service of the instrument.

3.2 Shutting down the system

A few steps are necessary to switch off an LC system with electrochemical detector for a longer period of time. Shutting down is no different than the process for most other HPLC systems.

Perform the following procedure:

1. Switch off the flow cell using the keyboard (standalone) or via the software (Empower).
2. Consult the column documentation for the appropriate storage liquid; apply this and ensure that the column is properly flushed. A reverse-phase C18 column is usually stored in 50:50 acetonitrile/water.
3. Take out the column, mount the corresponding end caps, and store the column in an appropriate place.

4. Flush and store the system with 50:50 water/acetonitrile (or methanol). Switch the injector valve between load and inject a few times. Ensure that all tubing and filters are flushed so no traces of salt are left that could precipitate and clog the system.
5. Remove the flow cell from the system by disconnecting the inlet and outlet capillary.
6. Open the cell, flush with water, and use some tissues to carefully dry the cell. Be careful not to damage the spacer in a VT-03 or FlexCell (the SenCell does not have a spacer).
7. Close the cell and store dry. In the case of a salt bridge REF, cap it and store it separately to prevent drying out. Alternatively, put the sb-REF in a 10-mL vial under a KCl solution and close the vial with a cap.
8. Switch off the detector (and other LC equipment) via the main switch (switch to position "0") on the rear panel.

> **Notice:** Avoid precipitation of high salt concentrations in organic solvent. Wash out salts with water if necessary.

Note: If the electrochemical detector will be idle for less than a week, provided the flow cell is switched off, the detector can be left idle or powered-down, with the solvent flowing at a low flow rate. When ready for the next analysis, you can switch on the detector with the flow cell and fresh mobile phase and re-equilibrate.

4 Detector controller

4.1 Introduction

The 3465 Detector has been designed for maximum functionality and ease of use. The control of ECD parameters via the keyboard and LCD display (3465 Detector only) is such that, without reading this chapter, it should be possible to operate the detector. This chapter is intended as a reference guide in case questions arise during operation.

The information in the various screens is presented in alphabetical order. For each item, an explanation is given, together with the item's nature and the screen or screens that appear. The nature of an item can be:

- Control: parameters with a cursor box (□) can be attained via cursor buttons and changed by the **value** button.
- Status: without a cursor box, a parameter reflects the current status.
- Functions: parameters in CAPITALS are commands accessible via function buttons F1 to F5.
- The **Enter** button is used to accept changes in cell potential and range.

In the top-right corner of each screen, the name of the present screen is displayed. If available, the bottom-left function button displays the previous screen, and the bottom-right one the next screen.

Figure 4–1: 3465 Detector keyboard. The cursor is on Range" which allows changes using the value buttons + and -. The Enter button is used only to confirm changes in potential (Ec) and range.

4.2 Overview of 3465 Detector screens

Figure 4–2: DC mode

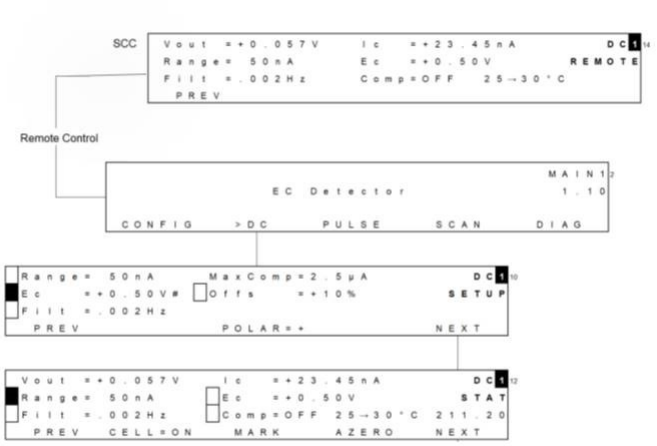


Figure 4–3: Pulse mode

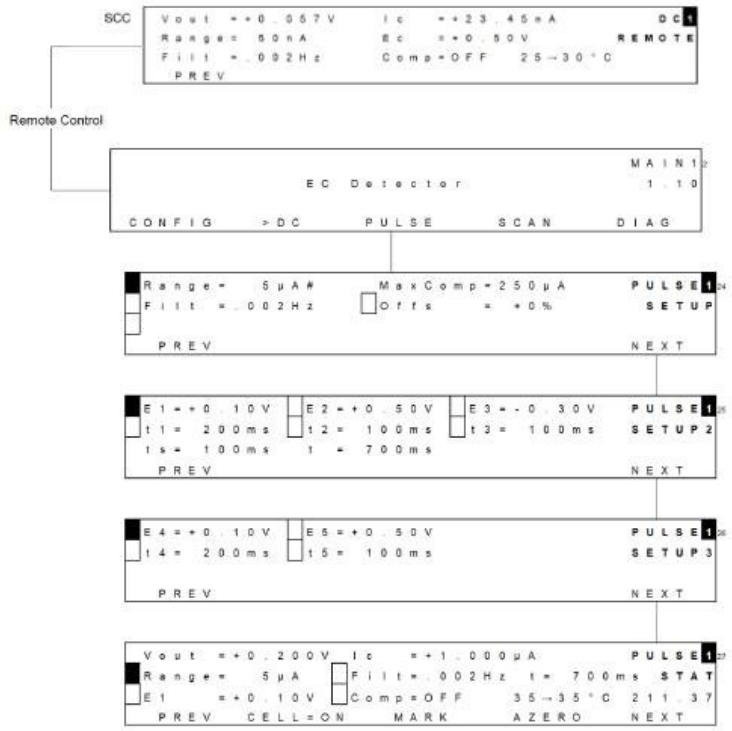


Figure 4–4: Scan mode

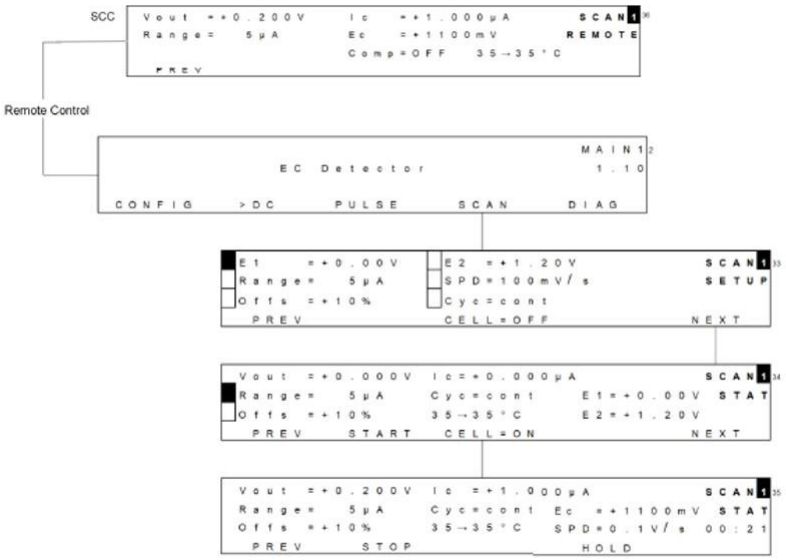


Figure 4–5: CONFIG menu

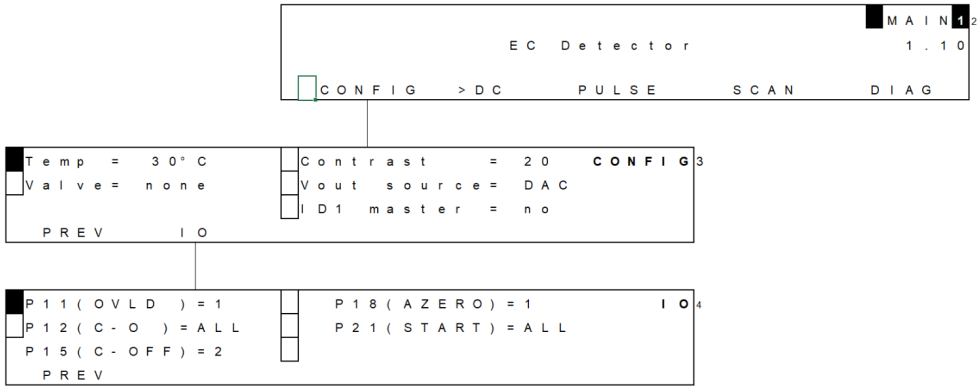
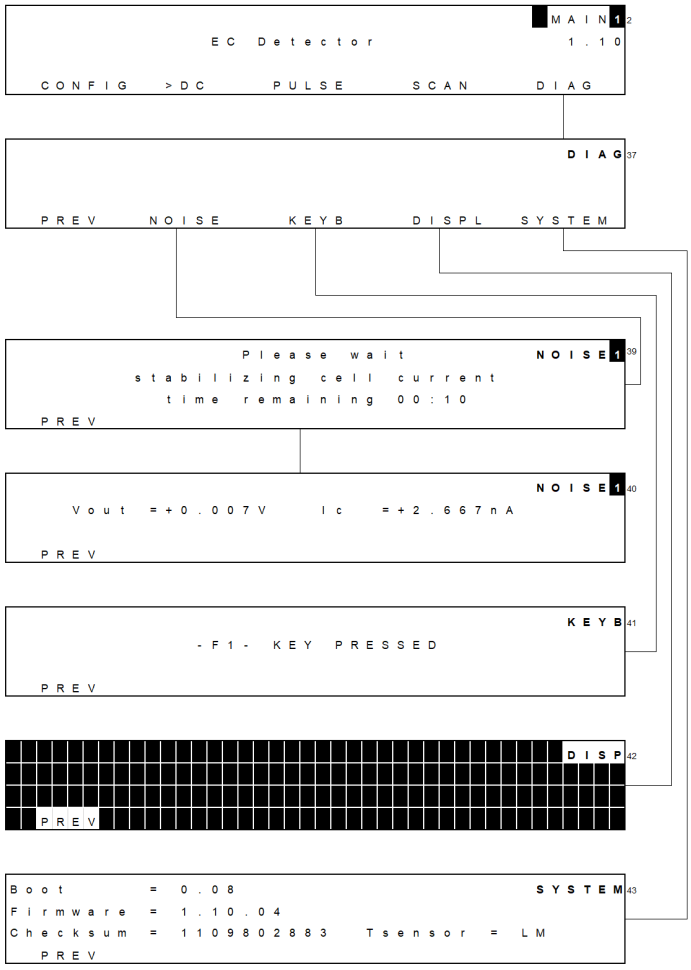


Figure 4–6: DIAG menu



4.3 Parameters

Explanation: Type S is status, F is function, and C is control.

Parameter	Screen	Description	Type
28 > 30 ˚C	DC STAT, PULSE STAT, SCAN STAT, RUN	Displays the actual (left-hand value) and the preset oven temperature (right-hand value).	S
AZERO	PULSE STAT, DC STAT, RUN, PULSE STAT, SCAN STAT	Sets the output voltage to 0 V or to the offset voltage. Control Comp = off changes to Comp = on. If cell current exceeds the max. compensation, the message “cell current exceeds max. compensation” appears. In that case, max.	F

Parameter	Screen	Description	Type
		compensation will be applied, which may be higher than the 0 Volt level.	
Boot	SYSTEM	Displays boot firmware version.	F
CELL=ON/ OFF	DC STAT, PULSE STAT, SCAN SETUP, SCAN STAT	Toggles between cell ON and OFF. Confirmation is required—"switch cell on (off)?" Switching on resets the clock to 0.00. Pulse mode: pulsation occurs as long as the cell is on, irrespective of the screen selected. Scan mode: potential E1 is applied.	F
Checksum	SYSTEM	Displays checksum	S
Comp	DC STAT, PULSE STAT	Toggles between ON and OFF, releases auto zero offset. Switches ON if AZERO is pressed. Affects auto zero compensation only, not the % offset.	C
CONFIG	MAIN	Enters config screen.	F
Contrast	CONFIG	Sets the contrast of display.	C
Cyc	SCAN SETUP	Controls the nature of the cycle: half, full, and continuous. “Half” means that the cell potential runs from E1 to E2 and stops at E2 (/|). “Full” means that the cell potential runs from E1 to E2, back to E1, and then stops (/\). “Cont” means that the cell potential runs from E1 to E2 and back to E1 continuously (/\/\/\/\……..). Pressing “STOP” or finishing the cycle sets the potential to E1.	C
DIAG	MAIN	Enters Diag screen.	F
DISPL	TEST	Enters DISP screen for display test.	F
E1, E2, E3, E4, E5	PULSE SETUP2, PULSE SETUP3	Controls the cell potential settings of the pulse. Can be set between +2.50 and –2.50 V in 10-mV steps. Can only be set or changed after confirmation with the **Enter** button.	C
Ec	DC SETUP	Controls the cell potential; can be set between +2.50 and –2.50 V in 10-mV steps. Can only be set or changed after confirmation with the **Enter** button.	C
Ec	RUN (DC only), SCAN STAT (during scanning)	Reflects the set cell potential. Displays the actual cell potential in the scan mode.	S

Parameter	Screen	Description	Type
Filt (DC mode)	DC SETUP, DC STAT	Filter settings: RAW (100 Hz), Off (10 Hz), and 1 Hz to 0.001 Hz cutoff frequency, in 1, 2, 5 steps.	C
Filt (PULSE mode)	PULSE SETUP, PULSE STAT	Filter settings: Off and 0.5 Hz to 0.001 Hz cutoff frequency, in 1, 2, 5 steps. (Fcut-off / filter coefficients based on 1 Hz input frequency in pulse mode).	C
Filt	RUN	Reflects the actual filter setting.	S
Firmware	SYSTEM	Displays firmware version.	S
Hold resume	RUN, SCAN STAT	Toggle—holds or resumes execution of scan.	S
HOLD=0,1	RUN, SCAN STAT	Holds or continues execution of scan. Toggles between 1 and 0. Pressing hold again continues scan where it was held.	S
Ic	STAT (dc, pulse, scan), RUN, NOISE	Displays the true, non-compensated cell current, unaffected by auto zero or offset.	F
ID1 master	CONFIG	Sets sensor board 1 as master. When this setting is set to ‘yes’ all parameter settings from sensor board 1 are automatically copied/transferred to all other sensor boards present.	F
IO	CONFIG	Enter IO menu.	S
INJ=I/L	DC STAT, PULSE STAT	Displays or switches the position of the injection valve, toggles between inject (I) and load (L). If a manual injector with position sensor is applied, it echoes the position of the injector. If an electrically actuated injector is used (optional), it is possible to switch the injector with this function button.	C
KEYB	TEST	Enters “KEYB” screen, for keyboard test. Press 2x F1 to leave.	F
MARK	DC STAT, PULSE STAT	Triggers a marker signal on output.	F
MaxComp	DC SETUP, PULSE SETUP1	Maximum cell current that can be compensated for using auto zero.	S
Next	SEVERAL SCREENS	Enter next screen.	F
NOISE	TEST	Enters NOISE screen for performance test.	F

Parameter	Screen	Description	Type
Offs	DCS SETUP, DC STAT, PROG, PULSE SETUP1 ,PULSE STAT, SCAN SETUP, SCAN STAT	Percentage offset; can be set between -50 and +50%.	C
POLAR	DC SETUP, PULSE SETUP2	Inverts output polarity; toggle between + and -. Requires confirmation.	F
PREV	SEVERAL SCREENS	Return to previous screen.	F
P11(OVLD)	IO	Programmable output: Can be configured so that the overload (OVLD) signal of cell 1, 2, or 3 only is present on pin 11 when active or ALL cells.	C
P12(C-ON)	IO	Programmable input: Can be configured so that only cell 1, 2, or 3 is switched ON when active, or ALL cells.	C
P12(C-OFF)	IO	Programmable input: Can be configured so that only cell 1, 2, or 3 is switched OFF when active, or ALL cells.	C
P18(AZERO)	IO	Programmable input: Can be configured so that the signal of cell 1, 2, or 3 is zeroed when active, or ALL cells.	C
P21(START)	IO	Programmable input: Can be configured so that the data-acquisition on sensor board 1, 2, or 3 is started when active, or on ALL sensor boards.	C
Range	DC SETUP, DC STAT, PROG, PULSE SETUP1, PULSE STAT, SCAN SETUP, SCAN STAT	Range setting, varying from 10 pA to 200 µA full scale, in 1, 2, and 5 steps. In the pulse and scan modes 10 nA to 200 µA full scale can be used.	C
S	SCAN SETUP	Scan speed; can be set from 1 to 100 mV/s in 1, 2, 5 steps.	C
SPD	SCAN STAT	Scan speed; can be set from 1 to 100 mV/s in 1, 2, 5 steps	C
START	RUN, SCAN STAT	Starts a scan in scan mode.	F
STOP	RUN, SCAN STAT	Scan mode: STOP aborts scan and resets cell potential to E1.DC.	F
SYSTEM	DIAG	Enter SYSTEM menu	F
t	PULSE SETUP2, PULSE STAT	Displays the total duration of one pulse (t1 + t2 + t3 + t4 + t5).	S

Parameter	Screen	Description	Type
t1, t2, t3, t4, t5	PULSE SETUP2, PULSE SETUP3	Duration of potential step E1, E2, E3, E4, and E5. Time can be set between 0 (t2 – t5) or 100 (t1) and 2000 ms in 10-ms increments. Maximum pulse duration is 9999 ms.	C
Temp	CONFIG	Controls the temperature of the oven. Range: off, 15 – 60 ˚C, selectable in 1 ˚C steps. The oven is stable from 7 ˚C above ambient oven temperature.	C
ts	PULSE SETUP2	Controls the duration of the sampling time in pulse mode. The time can be set between 20 ms and t1-60ms in 20-ms increments.	C
Tsensor	SYSTEM	Displays active temperature sensor.	S
Valve	CONFIG	User confirmation whether a manual valve is connected to phone jack C on rear panel. If present: INJ=I or INJ=L appears in DC/Pulse Status screen.	S
Vout	STAT (DC, PULSE, SCAN), RUN, NOISE	Displays output signal.	S
Vout source	CONFIG	Sets the output source from the analog data output: DAC (processed digital signal after 16-bit AD conversion) or I/E (true analog signal from the I/E converter).	S

5 Detection and parameters

5.1 Introduction

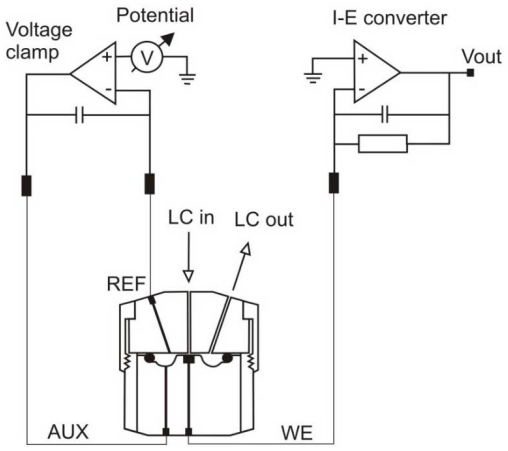
One of the characteristics of electrochemical detection is its tremendous dynamic range. In amperometric detection, peak heights may vary from micro-amperes down to the pico-ampere range. The 3465 Detector covers a wide range—from 200 µA down to 10 pA full scale—without being limited by electronic noise. For this reason, the 3465 Detector is equipped with a 24-bit ADC and a 16-bit DAC for analog data output. One of the key features is that data can be sampled with data collection rates up to 100 Hz (100 points/sec) in DC mode, which ensures that the fast peak responses typical in UHPLC can be detected with sufficient resolution.

5.2 Three-electrode configuration

The circuitry of the 3465 Detector is designed for operation with electrochemical flow cells with a three-electrode configuration (Fig. 5-1). The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The AUX is kept at a precisely defined reference electrode (REF) potential by means of a "voltage clamp". This is an electronic feedback circuit that compensates for polarization effects at the electrodes.

At the WE, which is kept at virtual ground, the electrochemical reaction takes place; that is, electrons are transferred at the WE. This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage of the I/E converter is digitized in the instrument by means of a 24-bit A/D converter and processed. The resulting output current can be acquired digitally by PC control software (Empower) or in analog using the analog data output on the rear panel, connected to a recorder or an external A/D converter.

Figure 5–1: Electrochemical cell with a three-electrode configuration



Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration. If the working potential would be applied only over an AUX versus the WE (without REF), the working potential would continuously change due to polarization effects at the electrodes, resulting in highly unstable working conditions.

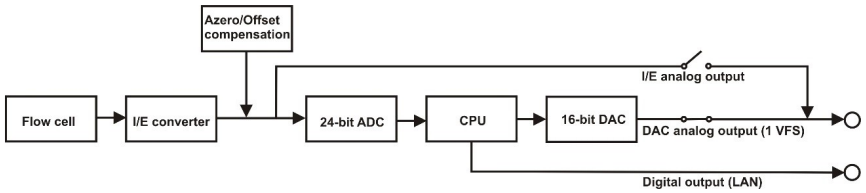
If the working potential would be applied only over the REF versus the WE (without AUX), the working potential would be very well defined. However, the potential of an REF is well defined only if the current drawn is extremely low (pico-amperes), resulting in a very limited dynamic range.

A three-electrode configuration combines the best of both electrodes. The REF stabilizes the working potential and the AUX can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

5.3 Internal organization

At the working electrode (WE) in the electrochemical flow cell, the electron transfer takes place via an oxidation or reduction reaction. The resulting electrical current is amplified by the current-potential (I/E) converter.

Figure 5–2: 3465 Detector signal processing from electrochemical flow cell to output



The signal from the I/E converter can be compensated with auto zero or offset and is digitized using a 24-bit ADC. The CPU processes the signal (with noise filtering, for example); more complex data is processed in the PAD. Finally, after the 16-bit DAC, the signal is set to a 1 V full-scale analog data output (by default, Output = ADC). Also, the true analog signal from the I/E converter (before AD conversion) is available via the analog data output connector. This output can be selected in the CONFIG menu by setting the parameter Output = I/E.

5.4 Dual flow cell control (optional)

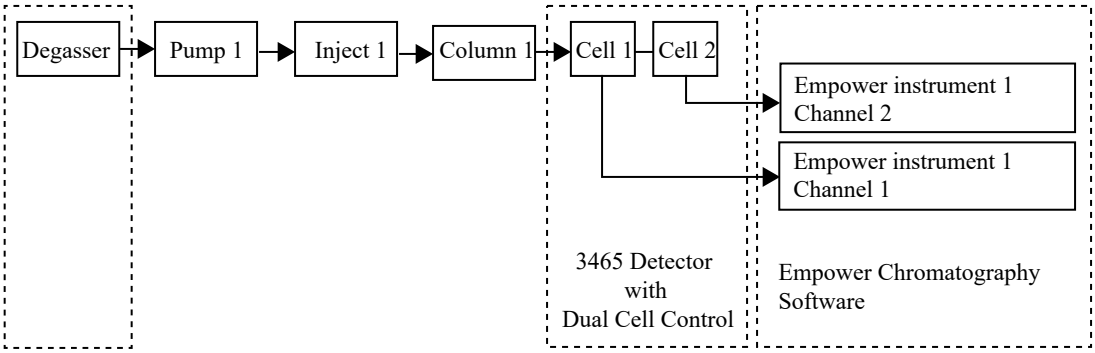
The 3465 Detector electronics are located on two different PCBs (printed circuit boards)—the control board and the sensor board. The control board is dedicated to communication with the PC (LAN), keyboard, and display. It has a processor with an “event handler” that takes care of all user commands and hardware interrupts. The sensor board is fully dedicated to data acquisition and flow cell control. This architecture makes it possible to extend the functionality of the detector to more than one flow cell by simply adding a sensor board. The control board and

other hardware are prepared for more than one sensor board. Typically, a two-cell configuration can be used for detection in serial or parallel mode.

5.4.1 Serial mode detection

In serial mode, one LC system is used with two flow cells in series. For data acquisition, two data channels are applied with the same time base. Serial mode detection is especially suitable for OX-RED or RED-OX applications—examples are analysis of vitamin K and nitrotyrosine—using micro HPLC. The first flow cell is a FlexCell that converts the analyte of interest in a detectable substance. The second flow cell is a SenCell or VT-03 cell that is used for detection. Note that it is necessary to work with micro HPLC because the conversion rate of the reactor cell is too low when using standard HPLC.

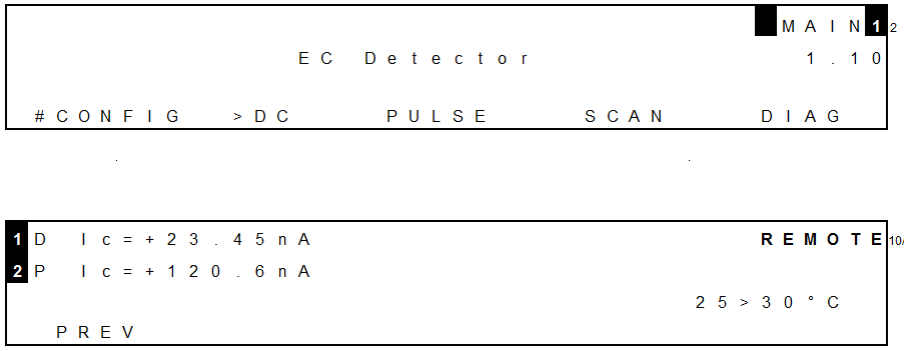
Figure 5–3: Typical configuration for serial mode detection: Cell 1 is a FlexCell; cell 2 is a SenCell for detection; Empower channel 1 and 2 use the same time base as system 1



5.4.2 Navigation in dual cell menu

All menus for a dual flow cell system are similar to those for a single cell system, with two exceptions. First, in the top-right corner, a number indicates the active cell. Toggle with the “+” and “-“ buttons between sensor boards. If the board number does not change, it means that the second sensor board is not installed or not properly recognized. Second, a new status screen is available in dual cell systems that indicates the status of both cells in a single screen. However, for convenience, it is best to use PC control from the Empower software.

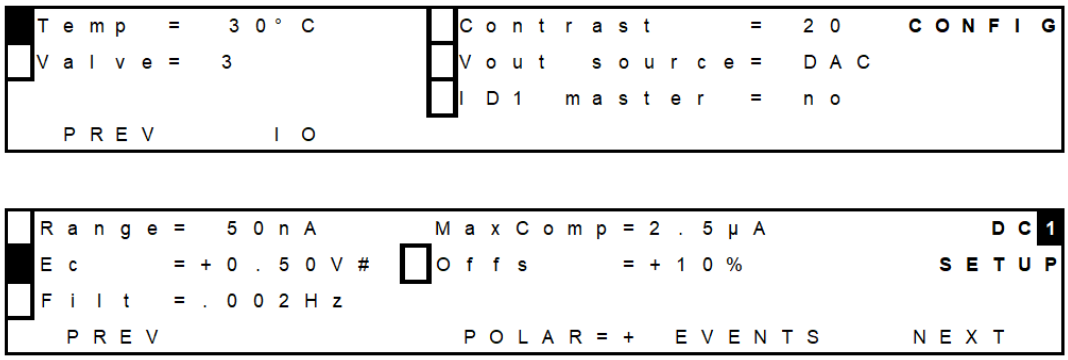
Figure 5–4: 3465 Detector main menu (top) with active cell indicator in top-right corner; Multi-STAT screen showing cell 1 (DC mode) and cell 2 (PULSE mode)



5.5 Parameters

Operational parameters are controlled from the SETUP screens in the 3465 Detector. The parameters are filter, cell potential, and offset. Temperature is set in the **CONFIG** menu.

Figure 5–5: Selection of parameters in the DC SETUP screen



5.5.1 Range

Range selection is done in the SETUP or STAT screen in DC, PULSE, and SCAN modes. A number of ranges can be selected; the maximum current that can be compensated for using auto zero and offset differs among ranges. The high sensitivity ranges (10 pA - 5 nA) have the best noise specifications. In fact, there is a tradeoff between best noise specification at sensitive ranges and maximum compensation at the less sensitive ranges. This is an inevitable consequence of the tremendous dynamic range that is covered by electrochemical detection. The information listed in the following tables is valid for 3465 Detectors with FW version 1.09 or higher.

Table 5–1: Table 2. DC ranges and maximum compensation

Range	Max comp	Range	Max comp
200 µA	2.5 mA	20 nA	2.5 µA
100 µA	2.5 mA	10 nA	2.5 µA
50 µA	2.5 mA	5 nA	250 nA
20 µA	250 µA	2 nA	250 nA
10 µA	250 µA	1 nA	250 nA
5 µA	250 µA	500 pA	250 nA
2 µA	25 µA	200 pA	250 nA
1 µA	25 µA	100 pA	25 nA
500 nA	25 µA	50 pA	25 nA
200 nA	25 µA	20 pA	25 nA
100 nA	25 µA	10 pA	25 nA
50 nA	2.5 µA		

In the PULSE and SCAN modes, current is much higher than in DC mode. Therefore, it is not possible to select pA ranges.

Table 5–2: Table 3. PAD ranges and maximum compensation

Range	Max comp	Range	Max comp
200 µA	2.5 mA	500 nA	25 µA
100 µA	2.5 mA	200 nA	25 µA
50 µA	2.5 mA	100 nA	25 µA
20 µA	250 µA	50 nA	2.5 µA
10 µA	250 µA	20 nA	2.5 µA
5 µA	250 µA	10 nA	2.5 µA
2 µA	25 µA		
1 µA	25 µA		

5.5.2 Offset

A maximum offset of +50% and -50% can be set in 5% steps. For example, 20% is a 200 mV offset when the maximum output is 1.0 volt.

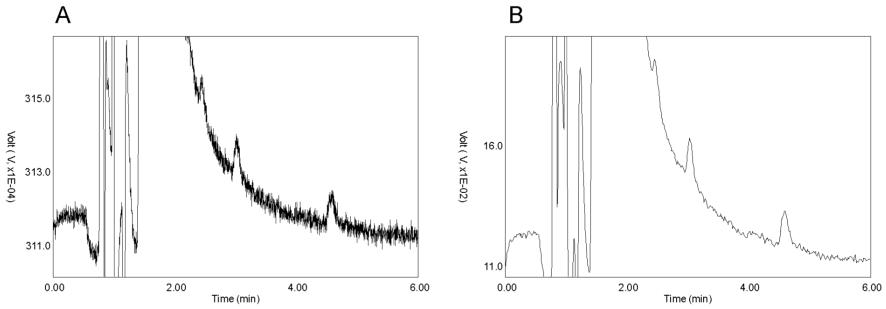
5.5.3 Polarity

The polarity of the output can be reversed. Oxidative and reductive analyses generate opposite currents. In data acquisition, traditional chromatographic peaks have a positive amplitude. Therefore, selection of polarity is useful.

5.6 Filter

High frequency noise is efficiently removed, and chromatographic peaks can be detected with a better signal-to-noise ratio.

Figure 5–6: Signal to noise ratio is improved using a filter (A vs. B)



The 3465 Detector is equipped with ADF (Advanced Digital Filter) as a tool to filter the acquired signal and improve the sensitivity (signal-to-noise ratio) of the analysis. The next chapter explains the filter setting, including detailed background information about filtering.

5.6.1 DC mode

In the following tables, the available filter settings for DC mode are listed with the corresponding data rate of the output. Data rate is expressed as the number of data points per second (Hz). In DC mode, the data rate is not an adjustable parameter but is coupled to the filter setting, except for RAW. RAW is special; the incoming data are not filtered, and you can select a data rate between 1 and 100 Hz.

Table 5–3: Table 4. DC mode filter setting and corresponding data rate

Filter setting DC mode (Hz)	Data rate (Hz)
RAW	100 (default), 50, 20, 10, 5, 2, 1
10	100
5	50
2, 1	20
OFF	**10**
0,5 0.2, 01	10

Table 5–3: Table 4. DC mode filter setting and corresponding data rate (continued)

Filter setting DC mode (Hz)	Data rate (Hz)
0.05	5
0.02	2
0.01, 0.005, 0.002, 0.001	1

Filter OFF is also a special case. The data rate is fixed at 10 Hz, and the data is not filtered. Setting OFF is therefore the same as RAW at 10 Hz.

5.6.2 Pulse mode

In pulse mode, the working electrode is dynamically and continuously regenerated by a series of potential steps in a cyclic manner. Data is processed differently, and the data rate is defined by the total duration of the five potential steps in a pulse: t1 + t2 + t3 + t4 + t5. The typical pulse duration is between 0.5 and 2 s (data rate between 2 and 0.5 Hz). Filter settings in pulse mode are selectable, between 0.5 and 0.001 Hz, and OFF.

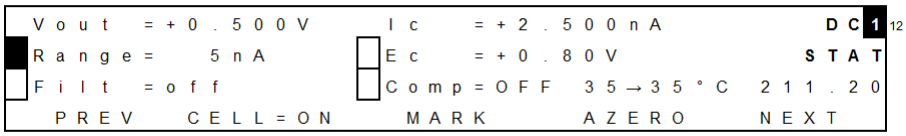
5.6.3 Pulse 2 mode (available in remote control only)

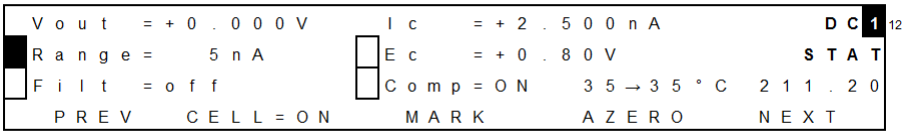
In pulse 2 mode, filter settings are selectable, between 0.5 and 0.001 Hz, and OFF.

5.7 Autozero

The Autozero function (F4) is available in the STAT screen. The screen for DC mode is shown here.

Figure 5–7: Stat screen in DC mode - Top: compensation off. Bottom: after executing an Autozero





The Autozero function can be used to set the output voltage of the I/E converter (signal) to 0 Volt. This is done by means of a compensation amplifier in the analog measurement circuitry.

This compensation circuitry makes it possible to measure small current signals (analyte peaks) in a sensitive range even if the background cell current is high. For example, with a background current of 20 nA, you can still do measurements in the 1 nA range, because the maximum compensation for that range is 25 nA.

Applying Autozero will set the baseline in CDS (close to) zero. The ICell reading will not change, it is the uncompensated current.

Note: Do not execute an autozero, offset, or range change when the cell current is not stable or the baseline did not stabilize.

If an Autozero, offset, or change of range is initiated on an unstable or changing current signal (baseline), it may lead to the display of an erratic current value or erratic offset in current. This is expected behavior. An Autozero, offset, or change of measurement range will trigger an internal calculation procedure in the detector based on the actual cell current. This is an iterative process; the cell current is measured several times during the calculation process. If the cell current is not stable during the calculation process, it can lead to errors.

Note: Solution: If it does happen, the issue can be solved by setting the Compensation to OFF (Comp = OFF) in the STAT screen or CDS control software. Wait until the background current is stable/stabilized and execute an Autozero again. The detector should now display the correct current.

If event tables are programmed in LC methods in the CDS software with an Autozero action, ensure that the autozero is programmed in a part of the chromatogram where the signal is stable —at the beginning of the run, during the hold-up time (before the unretained/solvent peak elutes), or at the end of the run.

6 Measurement modes

6.1 DC mode

In Direct Current (DC) mode, a static potential is constantly applied to the EC flow cell to establish an electrochemical oxidation or reduction reaction. The resulting current signal is continuously measured and sent to the detector output.

Figure 6–1: Plot of cell potential versus time

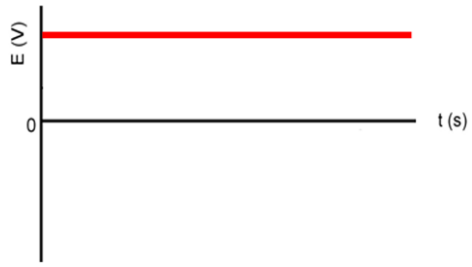
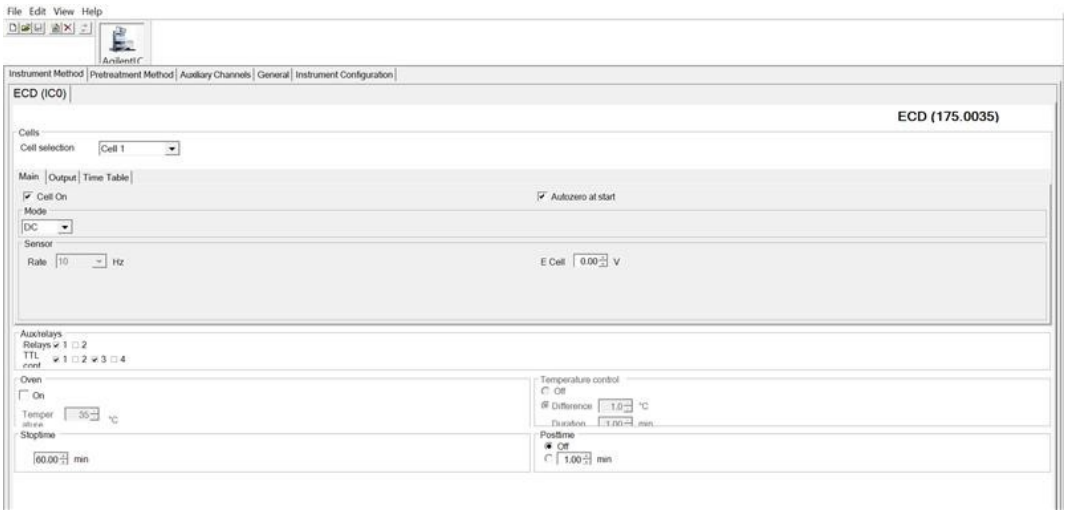


Figure 6–2: Empower Instrument Method window: DC mode settings

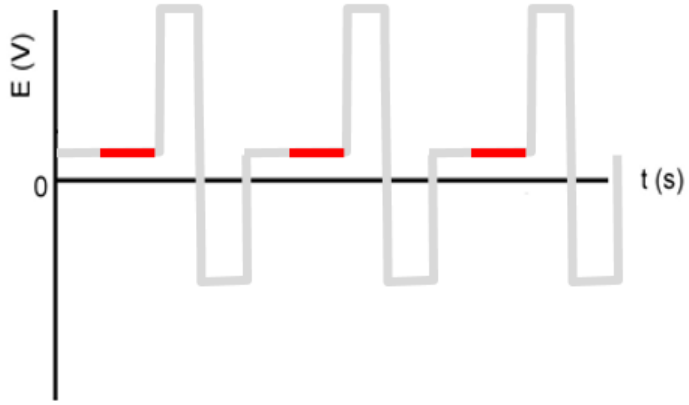


DC mode can be used for detection using relatively inert working electrode (WE) materials such as glassy carbon (GC) or boron-doped diamond. Suitable analytes in this case are typically aromatic compounds with functional groups that can be easily oxidized at relatively low potentials. One other requirement for successful operation in DC mode is that the target compounds and reaction products should not easily absorb or contaminate the electrode surface, which can lead to inactivation of WE electrode activity and loss of response.

6.2 Pulse mode

The 3465 Detector can also be operated in Pulse mode. Pulse mode is different from DC mode. Instead of a constant potential, a series of potential steps is applied in a cyclic manner. The signal is sampled during a fraction of the total pulse cycle. During the sampling time (ts) the signal is collected, and this value is sent to detector output. The frequency of data output is determined by the pulse duration: t1 + t2 + t3 + t4 + t5. The duration is typically between 0.5 and 2 s (data rate between 2 and 0.5 Hz).

Figure 6–3: Pulse mode example



In the pulse mode example, plot of a three-step potential waveform (gray curve) versus time and the time during which the current signal is measured (red part).

This mode is particularly useful for certain applications where the working electrode is rapidly fouled due to adsorption of reactant or reaction products or/and when using metal electrodes like gold, platinum, or titanium. Metal electrodes are the electrodes of choice for oxidation/reduction of aliphatic compounds with functional groups. Such compounds are hard to oxidize on inert electrode materials. Due to surface adsorption of the target compound on metal electrodes, the activation energy barrier for the reaction is lowered, allowing oxidation/reduction at much lower potential. Due to the fact that either the reactant remains at the WE surface or a metal oxide layer is formed, the electrode is deactivated and needs a pulsed potential to continuously clean and regenerate a metal surface for the reaction.

Figure 6–4: Empower Instrument Method window: Pulse mode settings

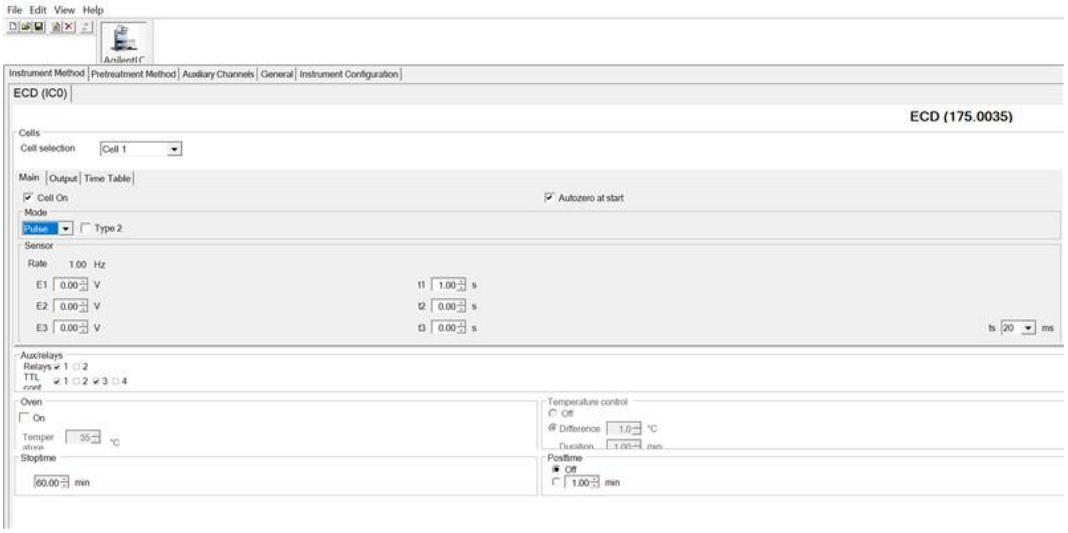
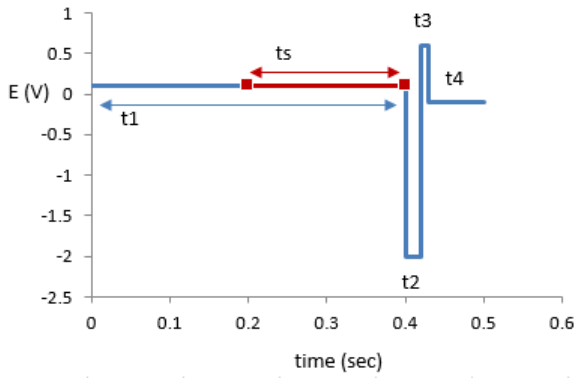


Figure 6–5: Potential steps in pulse mode



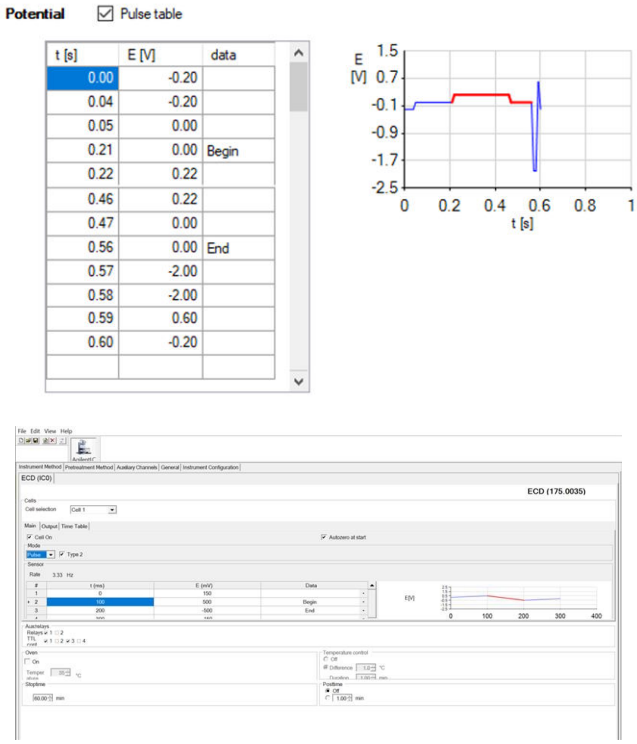
The measurement potential is applied during t1; the actual current measurement occurs during ts. Steps t2, t3, and t4 are for regenerating the electrode. This process repeats itself continuously when the cell is on.

Up to five potential steps can be defined in pulse mode. The sampling interval (period at which the current is measured) is applied at the end of E1. More detailed information about pulsed amperometric detection is available in Introduction (Page 72).

6.3 Pulse mode 2

The 3465 Detector (FW 1.09 and up) offers an extended pulse mode, pulse mode 2. In pulse mode 2 it is possible to program a multi-step waveform with up to 30 time-potential (t,E) coordinates and a maximum pulse duration of 4 seconds (see the following figure).

Figure 6–6: The new pulse mode 2 with freely programmable t,E table; in red, the sampling of data for acquisition



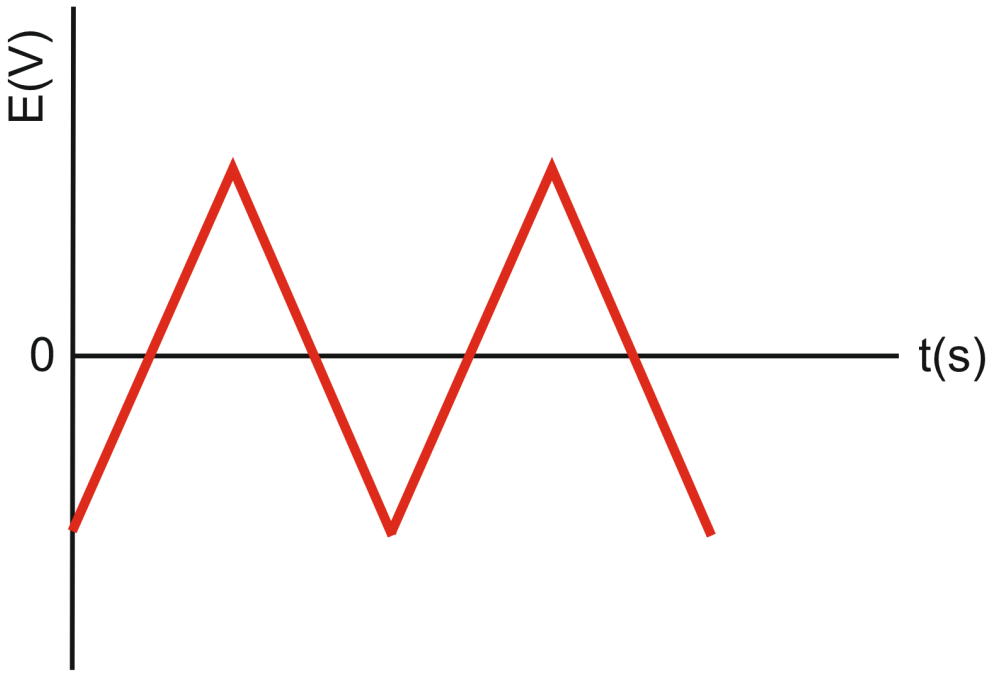
The measurement time interval in which the current is measured, marked by Begin and End markers, is freely programmable in the pulse table. This new pulse mode extends the application areas of the detector to analysis using PAD detection with more sophisticated potential waveforms, as in amino acid analysis.

Pulse mode 2 is not available in manual operation using keyboard/display. The new pulse mode can only be used through software control (Empower). When disconnecting the device from the CDS, it automatically switches back to the standard pulse mode, and a warning message appears.

6.4 Scan mode

For method development, the scan mode is available. In scan mode, the cell potential is swept between two preset values (E1 and E2) using a certain scan speed (in mV/s), and the current is measured during the sweep. This method can be used to study REDOX behavior and to determine the optimum detection potential of a pure analyte dissolved in mobile phase.

Figure 6–7: Scan mode example: plot of cell potential versus time (two full scans)



Note: Scan mode can be used in method development, but it is not a measurement mode used in HPLC-ECD analysis itself. It is available only in stand-alone operation. Scan mode is not available in any CDS.

See Optimization of the working potential (Page 76) for details on how to optimize the working potential for detection using scanning voltammetry.

7 Noise suppression - ADF

7.1 Introduction

In addition to its tremendous linear dynamic range and selectivity, electrochemical detection is well-known for its very low limits of detection. To further improve these detection limits, the 3465 Detector is equipped with ADF (Advanced Digital Filter). The improvement factor in signal-to-noise (S/N) ratio depends on the frequency relation of signal and baseline noise. S/N improvements from a factor 5 up to more than 100 are possible.

To understand how a digital filter works, the importance of frequencies in chromatographic analysis will be explained. Then we will look at peak width, filter settings, cutoff frequency, amplitude response plots, and a few chromatograms before and after application of ADF.

7.2 Frequency

The scientific definition of frequency is “the number of completed alterations per unit of time”. It has two dimensions: count and time. Frequency is usually expressed in Hz, which is counts per second.

The counts themselves can run in a regular, evenly spaced manner, as with sine waves whose curve shapes do not change. Alternatively, the counts can run in an irregular manner within the specified unit of time. If the latter happens, frequencies vary if broken down into smaller units of time.

In the example of Figure 7–1: Example of a signal with regular, evenly spaced alterations: a sine (Page 64), a signal is shown with a frequency of 12 alterations in 5 minutes. To express its frequency in a more scientific way, a full period is precisely determined and expressed in Hertz (or s^{-1}). It is a sine wave with a frequency of 0.04 Hz (Figure 7–2: A full period is 0.41 min (25 s), which corresponds to a frequency of 1/25 = 0.04 Hz. (Page 65)).

Figure 7–1: Example of a signal with regular, evenly spaced alterations: a sine

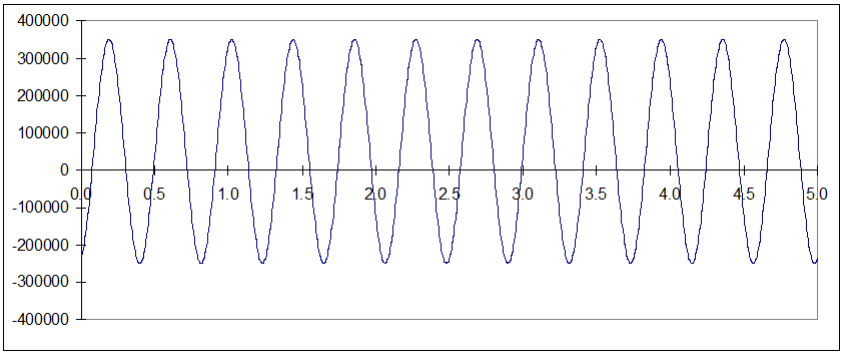
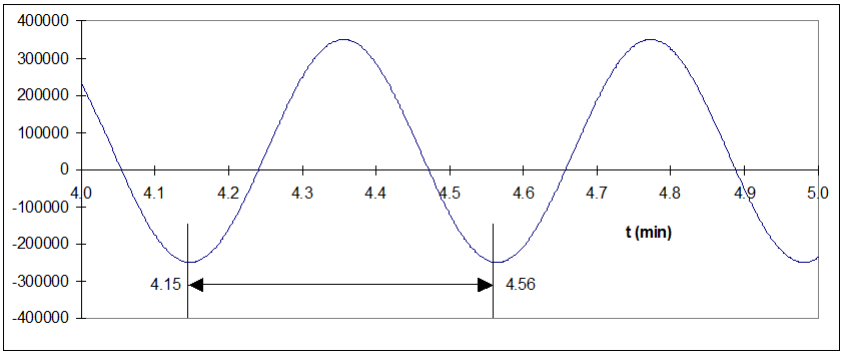


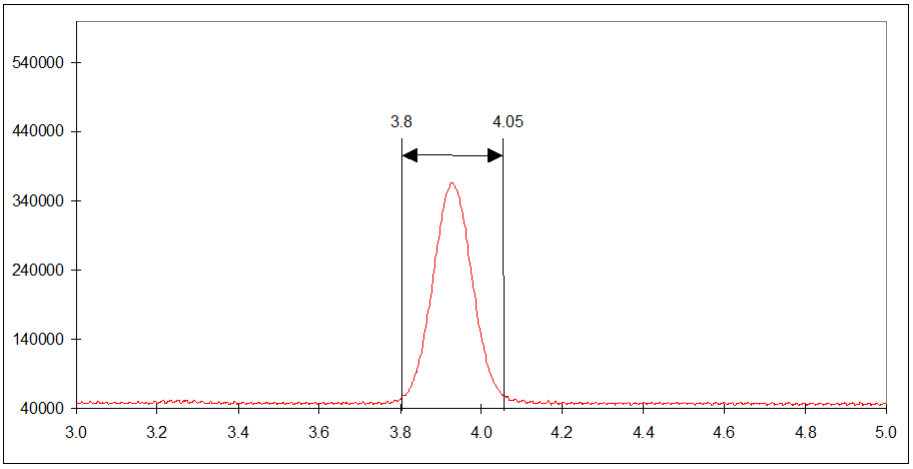
Figure 7–2: A full period is 0.41 min (25 s), which corresponds to a frequency of 1/25 = 0.04 Hz.



7.2.1 Frequency of signal and noise

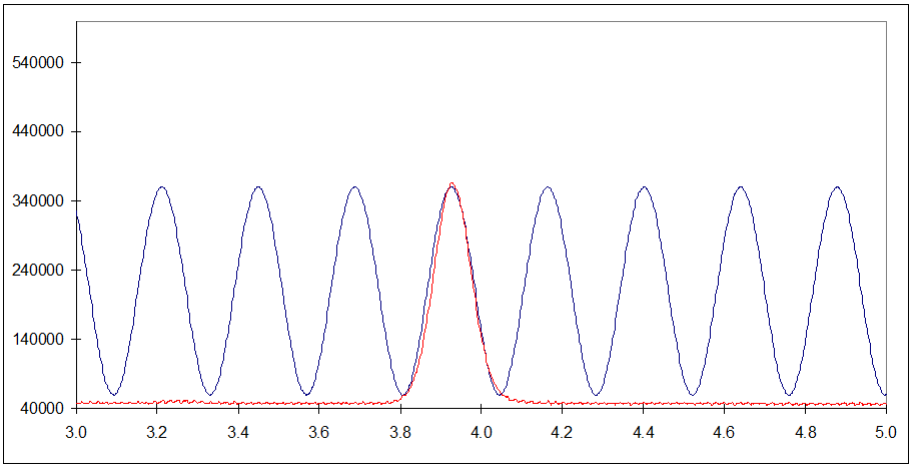
A chromatographic peak can also be expressed in terms of frequencies. The way to determine this frequency is the same. The duration of the full peak is measured and expressed in Hz.

Figure 7–3: Frequency tells how often something happens: 1 peak in about 0.25 min (15 s), f = 1/15 = 0.07 Hz



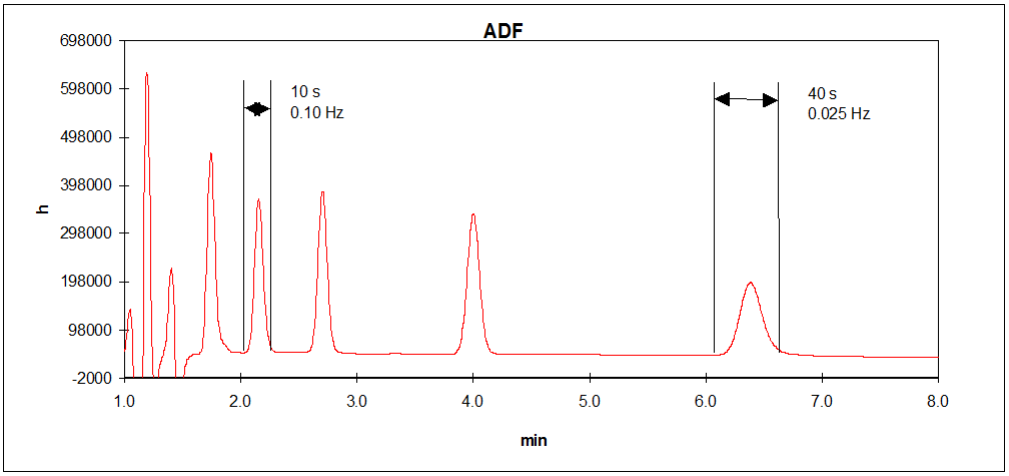
This is further illustrated by an overlay of the same chromatographic peak with a sine of 0.07 Hz (Figure 7–4: Overlay of a chromatographic peak with 0.07 Hz sine (Page 66)).

Figure 7–4: Overlay of a chromatographic peak with 0.07 Hz sine



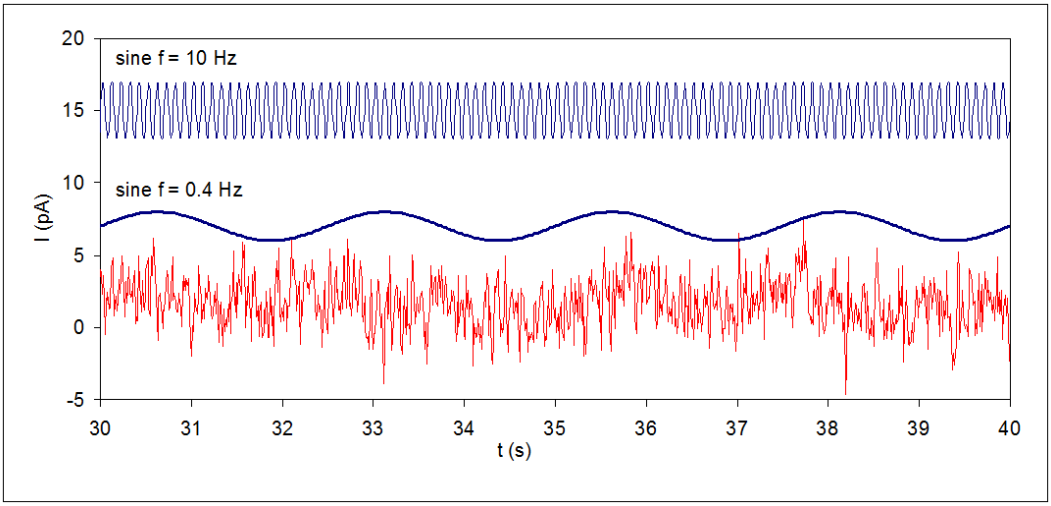
Narrow chromatographic peaks are typically in front of a chromatogram, while peaks with longer retention times get wider. As a consequence, frequencies are not constant but vary between 0.1 and 0.01 Hz, which corresponds to 10 – 100 s peak width

Figure 7–5: Typical chromatogram with peak widths between 10 and 100 s



Noise in chromatography can come from various sources. Pump pulsations are typically shown as a very regular noise pattern, while electronic noise has a more random character. This is illustrated in Figure 7–6: Typical random noise in chromatography (lower trace) (Page 67), where a noise trace is shown with an overlay of 10 and 0.4 Hz sines.

Figure 7–6: Typical random noise in chromatography (lower trace)



Looking closely at the lower noise trace, you can recognize both frequencies (and others). This is typical of noise in chromatography: a collection of more or less random frequencies.

7.2.2 Low pass noise filters

Noise filters work by suppressing certain frequencies in the acquired signal. Low pass filters typically allow chromatographic peaks (low frequency) to pass, while higher frequency noise is attenuated. No matter how advanced, it is impossible to use a low pass filter successfully if there is no difference in frequency between signal and noise.

Analog filters are made of capacitors, resistors, and amplifiers (opamps). Digital filters are mathematical routines to process an acquired signal. Traditionally, in many detectors for chromatography an analog low-pass filter (rise time filter) is applied. A “passive” RC filter consists of resistors and capacitors. An active, higher order filter can be considered as a series of these RC filters. In a fourth-order filter the signal coming from the first filter is filtered again in a second, a third, and a fourth. During these steps, loss of signal occurs simply because of all the resistors that are applied. Operational amplifiers, which are “active” components, are applied in each stage to restore the signal to its original value.

With the availability of powerful processors, digital signal processing has become an excellent alternative to hardware filters. In its most simple form, a running average filter takes the average of n data points to create a new data point. For example, in a 5-point running average filter, output data point y[80] is calculated from measured data points x[80] – x[84] as:

$$y[80] = \frac{x[80] + x[81] + x[82] + x[83] + x[84]}{5}$$

Each input data point has the same weighting factor of 1/5. In more advanced digital signal processing, a more complicated equation is used to calculate the output data point y[n]:

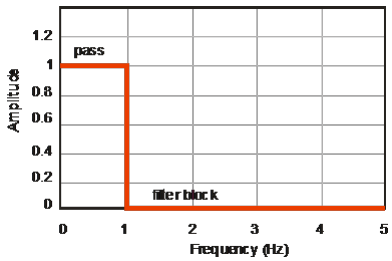
$$y[n] = a_0 x[n] + a_1 x[n-1] + a_2 x[n-2] + a_3 x[n-3] + \cdots$$

In contrast to the previous equation, each data point has a different weighting factor (a). The sum of these weighting factors (a0…n) will always be 1.

Characteristic of noise filters is that processing the signal results in a delay. This is inevitable; the mathematics of digital signal processing requires a number of previous data points to process a new data point. A filter characteristic in DSP is often named after the scientist who “invented” the mathematics behind the signal processing routine. Well-known names in this field include Bessel, Chebychev, Savitsky, Golay, Hamming, and many others.

7.2.3 Amplitude response plot

Figure 7–7: Amplitude response plot of an ideal low pass filter with a cutoff frequency of 1 Hz



There are several ways to describe filter characteristics. An amplitude response plot gives important information on filter behavior. Suppose our signal of interest has a frequency between 0 Hz and 1 Hz, and all higher frequencies are noise. An ideal filter is shown in Figure 7–7: Amplitude response plot of an ideal low pass filter with a cutoff frequency of 1 Hz (Page 68), where signal frequencies between 0 and 1 Hz completely pass, while frequencies higher than 1 Hz are completely blocked.

In practice, filters behave a bit differently than the ideal. An amplitude response plot shows a more gradual attenuation profile at higher frequency. The cutoff frequency is where the output signal amplitude is 70% of the input signal, also known as the 3 dB point.

Figure 7–8: An amplitude response plot of a low pass filter with a cutoff frequency of 1 Hz; it is a 2 (A), 4 (B), and 8 (C) pole Bessel filter

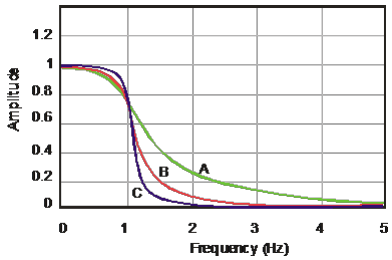
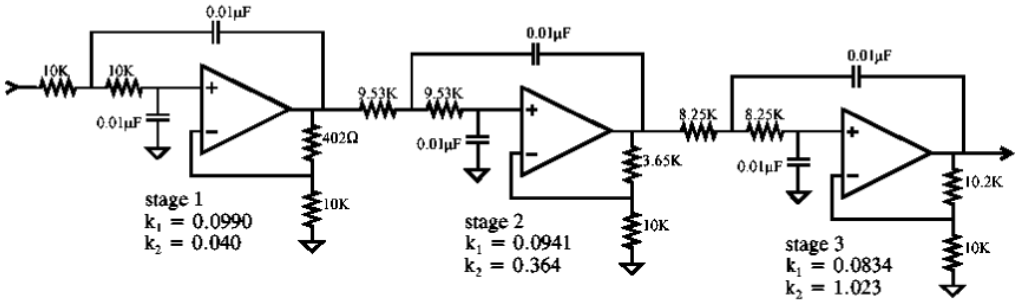


Figure 7–8: An amplitude response plot of a low pass filter with a cutoff frequency of 1 Hz; it is a 2 (A), 4 (B), and 8 (C) pole Bessel filter (Page 68) shows that the number of poles is important; a filter behaves more ideally as the number of poles increases. In a hardware filter the number of poles is the number of filter circuits that are placed in series.

Figure 7–9: Analog 6-pole Bessel filter



A digital filter does not have poles, but it is characterized by the number of input data points used to calculate a new output data point. For example, a 9-point digital filter (Savitzky-Golay) is given as:

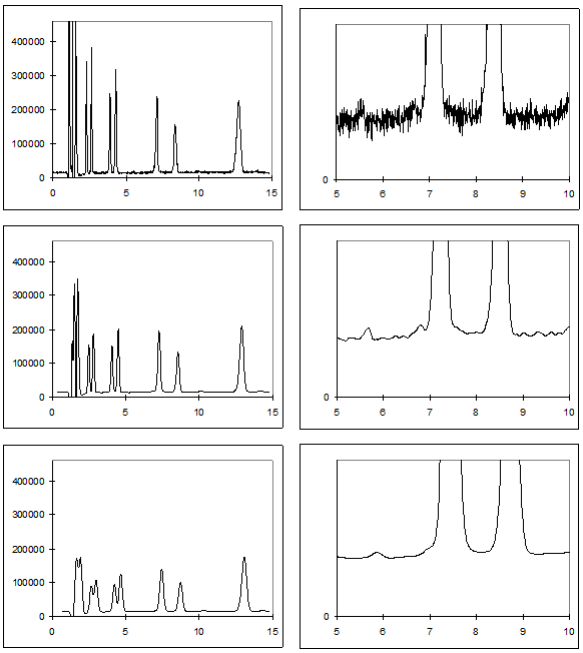
$$Y[1] = -0.090909091\,X[1] + 0.060606061\,X[2] + 0.168831169\,X[3] + 0.233766234\,X[4] + 0.255411255\,X[5] + 0.233766234\,X[6] + 0.168831169\,X[7] + 0.060606061\,X[8] + -0.090909091\,X[9]$$

Note that the sum of coefficients is exactly 1. Y [n] is the output data point and X [n] are input data points. Generally speaking, the performance of a digital filter improves with more input data points, but greater processor capacity is required for the large number of calculations.

7.3 Applying ADF in chromatography

If noise frequencies in LC-EC differ from the frequency of the signal, noise can be suppressed. Using the right filter setting (cutoff frequency) will specifically attenuate noise and improve the signal-to-noise (S/N) ratio. No matter how advanced a filter is, it is only possible to apply low pass filtering if noise frequencies are higher than the frequency of the signal.

Figure 7–10: From top to bottom, filter settings of 0.5, 0.02 and 0.002 Hz; narrow peaks in front of the chromatogram are deformed at 0.005 Hz, whereas wider peaks show hardly any deformation (see peak at t~ 13 min)



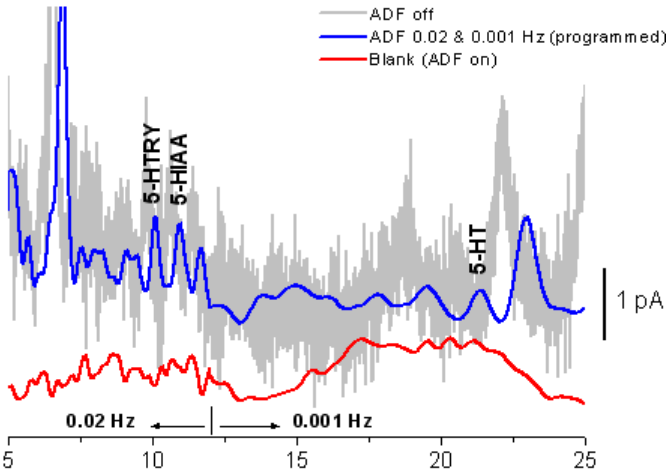
A prerequisite for a “good” noise filter for data acquisition in liquid chromatography is that it improves the S/N ratio without significant distortion of the signal of interest. This is particularly difficult if the frequency of the signal is close to the frequency of the noise.

The 3465 Detector has a number of filter settings to optimize for best possible signal-to-noise ratio. The width of the peaks of interest is important because wider peaks allow stronger filter settings simply because of the lower frequency of such peaks. A recommended filter setting to start further optimization is given as Filter setting = 1 / [2 * (peak width)].

So at a 10-s peak width a 0.05 Hz filter setting is recommended. If peaks are 50 s, a 0.01 Hz filter is recommended to start. Note that if a chromatogram has interesting peaks of 10 s as well as 50 s, it may not be possible to work with one filter setting. In that case it is advisable to switch to a stronger filter setting for the second half of the chromatogram using a timed event. To optimize for the best S/N ratio, use the lowest acceptable cutoff frequency.

After optimization, do not change the cutoff frequency setting during analysis of a calibration sequence. Use the same settings for analysis of samples and calibration standards.

Figure 7–11: Analysis of 20 pmol/L 5-hydroxytryptophan, 5-HIAA, and 5-HT using ADF to improve detection limits



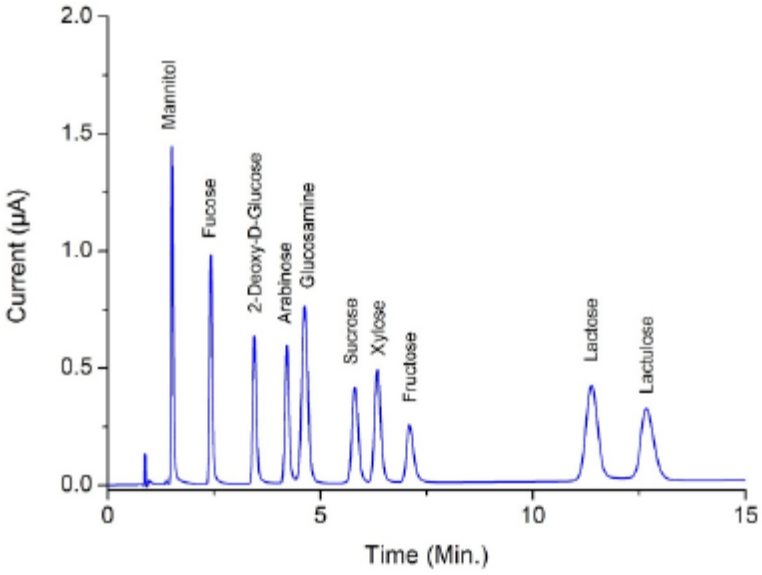
The S/N improvement depends on the composition of the frequency spectrum. Improvement up to a factor 100 (compared with an unfiltered signal) may be obtained. As high frequency noise is suppressed, remaining noise components will be in the same frequency range as chromatographic peaks. Because suppressing noise will always result in some suppression of signal, it is best to switch the 3465 Detector to the highest acceptable sensitivity.

8 Pulsed amperometric detection (PAD)

8.1 Introduction

The 3465 Detector can operate in pulsed amperometric detection (PAD) mode, in which the working electrode (WE) is regenerated at a frequency of 0.5 to 3 Hz by the application of a series of potential changes. This is particularly useful in applications where the working electrode is rapidly fouled due to adsorption of insoluble reaction products. A well-known application area for PAD is the analysis of carbohydrates.

Figure 8–1: Carbohydrate analysis in pulse mode



8.1.1 Pulse mode versus DC mode

Pulse mode is quite different than DC mode. Instead of a constant potential, a series of potential steps is applied in a cyclic manner. The signal is sampled during a fraction of the total pulse cycle. During the sampling time (ts) the signal is collected and the value is sent to detector output. The frequency of data output is determined by the pulse duration: t1 + t2 + t3 + t4 + t5. The duration is usually between 0.5 s and 2 s (data rate between 2 Hz and 0.5 Hz). The background or cell current is usually considerably higher (100 nA to 1000 nA) than in DC mode. Only nano- and micro-ampere ranges are available in pulse mode. The background current is typically between 0.1 µA and 2 µA.

When the frequency of the data acquisition system (integrator) is higher than the pulse frequency, a stepwise pattern may appear in the chromatogram. This is called “oversampling”, and the steps

are usually only visible after considerable magnification of the chromatogram. If an AD converter is used to record the analog signal, it is recommended that you keep the sampling rate of the AD converter in pace with the data rate of the detector. For example: for a waveform with a pulse duration of 0.5 seconds (2 Hz data rate), use a sampling rate of 2 Hz on your AD converter. Or, if this is not selectable, use the closest possible higher sampling rate.

In FW1.09, an extended pulse mode, “pulse mode 2”, was introduced, in addition to the existing pulse mode. In pulse mode 2 it is possible to program a multi-step waveform with up to 30 time-potential (t,E) coordinates and a maximum pulse duration of 4 seconds. The time interval in which the current is measured (marked by “Begin” and “End” markers) is freely programmable in a pulse table. This new pulse mode extends the application areas of the 3465 Detector to analysis using PAD detection with more sophisticated potential waveforms, such as that used in amino acid analysis. See Measurement modes (Page 59) for more details.

8.1.2 High pH of mobile phase

In carbohydrate analysis, another special consideration must be taken into account. Pulse mode detection uses a flow cell with a gold electrode. Separation employs an anion exchange column and a mobile phase of 20 to 100 mmol/L sodium hydroxide. The pH is between 12 and 14. This puts some demands on the HPLC system and flow cells. After prolonged use of a flow cell with a gold working electrode (WE) in pulse mode, the gold oxide that is generated at the WE precipitates on the auxiliary electrode (AUX). This gold oxide coating may electrically isolate the AUX and result in an increase in noise. Cleaning the AUX electrode with metal wool can remove this coating.

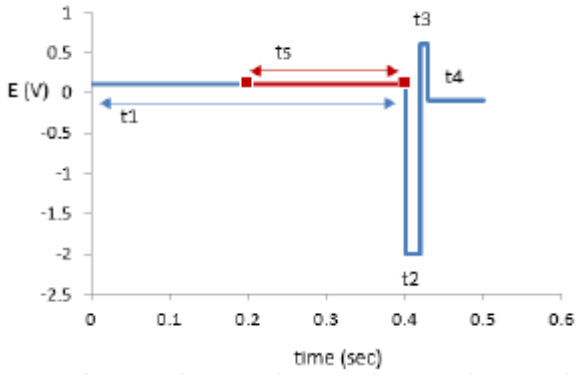
Reference electrodes of the Ag/AgCl type are not suitable for carbohydrate analysis. Due to silver oxide formation, they require regular (monthly) maintenance. HyREF reference electrodes are maintenance-free under these conditions and are therefore well-suited.

If a mobile phase with a high pH (pH >10 in carbohydrate analysis) is used, the standard Vespel rotors from the injection valve should be replaced by Tefzel rotors, which are pH-resistant. For carbohydrate analysis, only CO2-free sodium hydroxide should be used because carbonate anions may disturb the ion exchange chromatography. The CO2-free sodium hydroxide is available from several suppliers as a 50% solution (19.2 mol/L). NaOH pellets are not recommended because of their high CO2 content. Organic modifiers (including acetonitrile) strongly attenuate the signal of most carbohydrates in PAD and are therefore not recommended.

8.1.3 Pulse settings

In PAD of carbohydrates, a series of potentials is applied in a continuous, cyclic manner. The detection potential is applied during time interval t1. Data collection occurs within t1, during time interval ts (sampling time). The time difference t1-ts is the stabilization time.

Figure 8–2: Potential steps in pulsed amperometric detection



The detection potential is applied during t1, and detection occurs during ts. Steps t2, t3, and t4 are for regenerating the electrode. This process repeats itself continuously when the cell is on.

During the next time intervals (t2 through t4), the electrode is “cleaned” by reductive and oxidative potential steps.

8.1.4 Optimization of wave forms

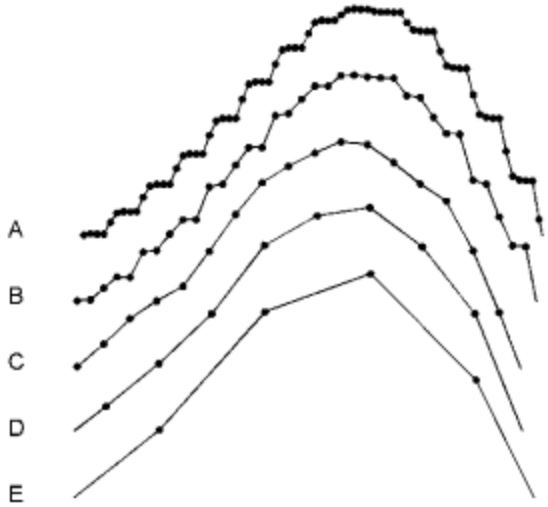
LaCourse and Johnson have published several papers on optimization of wave forms in PAD. [2-4] Several considerations are important in the choice of pulse duration. Optimization is dependent on the working electrode material, the sample constituents, and the required detection frequency. It might seem that the number of variables (five potential steps and six time settings) would lead to a time-consuming optimization procedure. In practice, pulse mode is more straightforward, and several excellent review papers and application notes have been published.

8.1.5 Output frequency

An important difference between the DC and pulse modes is the frequency of the output signal. In DC mode the signal has a 1-100 Hz frequency; in pulse mode the frequency is determined by the duration of the pulse. Once every cycle, the ts signal is sent to output.

If a stepwise chromatogram pattern is seen when zooming in, it means that data acquisition has an unnecessarily high sampling frequency. This leads to large data files but not to better chromatograms. Data acquisition at 1 Hz is usually sufficient.

Figure 8–3: A detailed part of a chromatogram acquired at different data frequencies



The data rate is (A) 5x, (B) 2.5x, (C) 1.2x, (D) 0.6x, and (E) 0.3x the frequency of the pulse. C is 1 Hz data rate.

8.1.6 Working electrode material

Gold and platinum are used as working electrodes for PAD. Glassy carbon appears to be unsuitable due to the high electric capacitance of this material. Resurfacing of the noble metal working electrode is based on formation and removal of a metal oxide layer. This is impossible with glassy carbon.

8.1.7 References

1. D.C. Johnson, D. Dobberpuhl, R. Roberts, and P. Vandenberg, “Review. Pulsed amperometric detection of carbohydrates, amines and sulphur species in ion chromatography - the current state of research,” *J. Chromatogr*. 640 (1993) 79-96
2. D.C. Johnson, W.R. LaCourse, “LC with pulsed ECD at gold and platinum electrodes”, *Anal. Chem*., 62 (1990) 589A-597A
3. W.R. LaCourse and D.C. Johnson, “Optimization of waveforms for pulsed amperometric detection of carbohydrates following separation by LC”, *Carbohydrate Research*, 215 (1991) 159-178
4. W.R. LaCourse and D.C. Johnson, “Optimization of waveforms for pulsed amperometric detection of carbohydrates based on pulsed voltammetry”, *Anal. Chem*. 65 (1993) 50-55

9 Optimization of the working potential

9.1 Introduction

A current/voltage (I/E) relationship, or voltammogram, characterizes an analyte. It gives information on the optimum working potential, which can be used to improve detection sensitivity and selectivity.

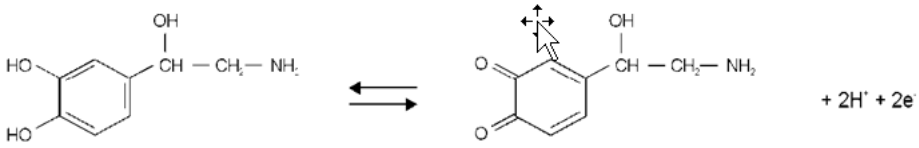
There are several ways to obtain a voltammogram:

- A hydrodynamic voltammogram is obtained in DC mode by running several chromatograms at different working potentials. Both peak height and background current are plotted against the working potential. The hydrodynamic voltammogram has an advantage in that the I/E relationship of all analytes of interest can be obtained simultaneously in one set of experiments (boundary condition: all analytes should be sufficiently separated under the applied LC conditions). Furthermore, under real chromatographic conditions, reliable information about the S/N ratio is obtained.
- A scanning voltammogram is obtained in the “scan” mode of the 3465 Detector: the voltage runs between two preset potential values (E1 and E2) and scan speed (in mV/s), and the current is measured.

9.2 Electrochemical reactions

In electrochemical detection (ECD), a reaction of the analyte at an electrode surface is monitored. This distinguishes ECD from most other detection techniques in which detection is based on the physical properties of an analyte (molecular mass in mass spectrometry; molar absorptivity in absorbance detection). For electrochemically active compounds, the potential between reference electrode (REF) and working electrode (WE) determines the reactivity of the analyte at the WE. The potential difference supplies the energy level needed to initiate or enhance the electrochemical reaction. Different analytes may have different oxidation or reduction potentials, which determines the selectivity of ECD.

Figure 9–1: Oxidation/reduction of norepinephrine



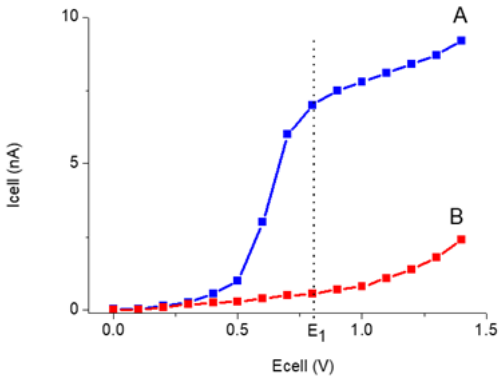
This is an example of an electrochemical reaction. Norepinephrine is converted into a quinone by oxidation at the WE. Two electrons are transferred at the WE, resulting in an electrical current that is amplified by the controller.

9.3 Hydrodynamic and scanning voltammograms

9.3.1 Hydrodynamic voltammogram

A hydrodynamic voltammogram is constructed when the pure analyte is not available and separation over an analytical column is required. Under real chromatographic conditions, reliable information about the S/N ratio is obtained. The peak heights obtained from the sequence of chromatograms are plotted against the working potential used. Also, the back-ground current (I-cell) is plotted.

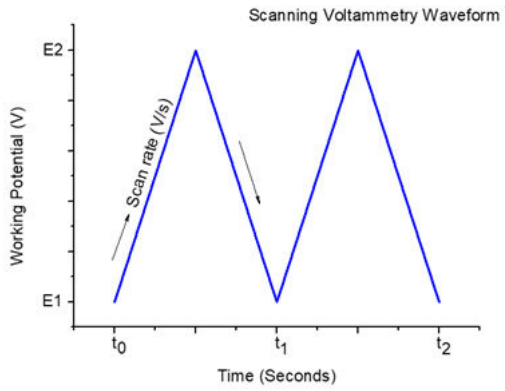
Figure 9–2: Hydrodynamic voltammogram of norepinephrine at a glassy carbon working electrode (A) and the current of the baseline (B). At E1 the electrochemical signal becomes diffusion limited.



9.3.2 Scanning voltammogram

An alternative for the chromatographic construction of an I/E relationship is the application of scanning voltammetry. In a scanning voltammetry experiment, the working electrode potential is ramped up and down between two preset potentials (E1 and E2), and the current is measured while the analyte is continuously flushed through the flow cell. This is repeated as many times as necessary. The rate of voltage change over time is defined as the scan rate (mV/s).

Figure 9–3: Scanning voltammetry potential waveform



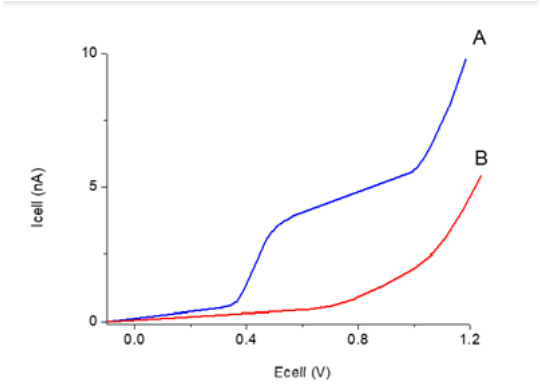
The current is plotted against the working potential to give a voltammogram (I/E curve), as shown in the scanning voltammetry potential waveform figure.

In scanning voltammetry, no HPLC separation is involved. The signal is the sum of all EC active substances. It takes only a few minutes to construct a scanning voltammogram. This is an advantage, especially when a number of analytes must be characterized. However, it is a prerequisite to have the pure analyte dissolved in buffer. A scan of the buffer (blank) should be used to distinguish between solvent peaks and analyte peaks.

Note: Any contamination in the buffer may lead to artifacts.

As seen in the previous and following figures, when the working potential is increased the electrochemical reaction is enhanced and the signal increases. At a certain potential, the I/E curve flattens. All analyte molecules that reach the working electrode are converted at such a high rate that the analyte supply becomes the limiting factor. At the working electrode surface, a stagnant double layer exists where molecular transport takes place by diffusion only. Therefore, the current at (and beyond) this potential is called the *diffusion limited current*.

Figure 9–4: Scanning voltammetry of 1.0 µmol/L norepinephrine (A) at a glassy carbon working electrode at a scan speed of 10 mV/s. Scan B is the blank solvent.



With respect to *sensitivity*, a high working potential is important. However, at higher working potentials, more analytes are detectable. With respect to *selectivity*, a low working potential is favorable.

Working at a potential on the slope of the I/E curve results in less reproducibility in HPLC. A small fluctuation in the applied potential, or any change in the system (a pH change for instance) may result in differences in measured peak height. In practice, the choice of working potential is a compromise among sensitivity, selectivity, and reproducibility. In the example of *scanning voltammetry potential waveform*, a working potential (E1) of 0.8 V was chosen.

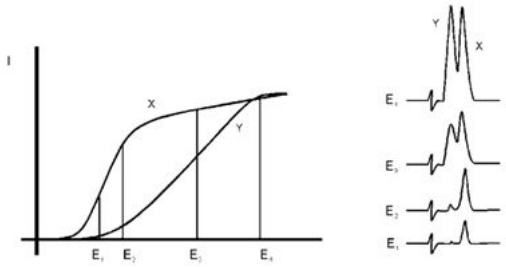
9.4 Optimization using a voltammogram

When interfering peaks appear in the chromatogram, it is sometimes possible to optimize the method with regard to selectivity. If the interfering compound has a higher oxidation potential, a working potential is chosen that gives the best selectivity (the largest difference in peak height).

In the example, the selectivity for compound X is improved considerably by decreasing the potential to E_2 or E_1. If compound Y is the compound of interest, optimization of selectivity in this way is not possible and the chromatography must be optimized.

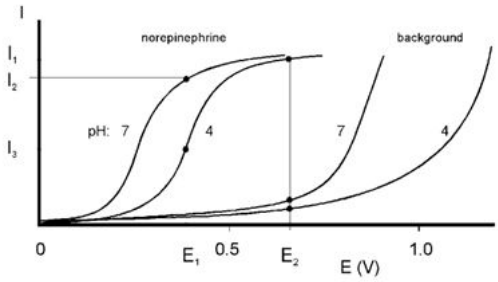
Electrochemical detection differs from most other LC detection methods in that a reaction takes place in the detection cell. Due to reaction kinetics, an increased temperature speeds up the oxidation/reduction reaction. However, this holds not only for the analyte but also for the background current and possible interferences. An elevated temperature will therefore not automatically lead to a better detection. A constant temperature is essential for a stable baseline and reproducible detection conditions.

Figure 9–5: Selectivity in LC-EC of compounds X and Y is optimized by choosing the working potential with the largest difference in peak height.



Electrochemical reactions are pH-sensitive. For norepinephrine, the I/E curve shifts to a lower potential at higher pH. When the working potential is high (E_2) and the signal is diffusion limited, an increase in pH results only in a small increase of the peak height. When the working potential is lower (E_1) and the signal is not diffusion limited, the signal strongly increases at higher pH. In both cases the background current increases at a higher pH.

Figure 9–6: At a higher pH, the I/E curve of norepinephrine shifts to the left.



Reaction kinetics predict that electrochemical detection is mass flow dependent. When the LC flow is stopped in LC-EC, the analyte is oxidized completely and the signal decreases rapidly. This means that the flow rate affects not only temporal peak width and analysis time but also peak height. Also, the background signal is sensitive to fluctuations in the flow rate. It is important to use a pulse-free solvent delivery system.

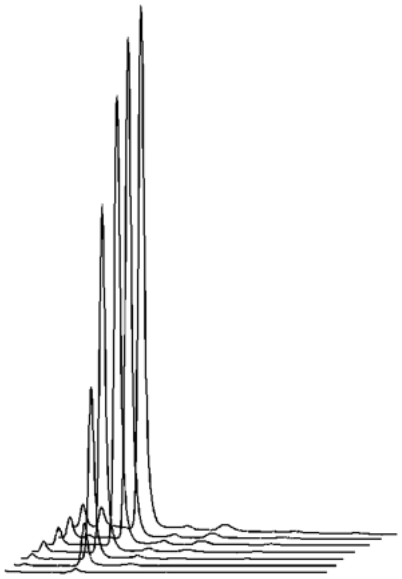
9.5 Construction of a hydrodynamic voltammogram

Before a hydrodynamic voltammogram can be obtained, the chromatographic conditions should be optimized. Then the following steps are taken:

1. A solution of the analyte at a concentration between 1 and 100 µmol/L is prepared in mobile phase.
2. The electrochemical detector is stabilized in DC mode at a high potential. After stabilization, the background current is read from the display of the detector (I-cell), and the noise is measured.
3. The run is started by injecting the compound. If no signal is obtained at the high working potential, it may be concluded that the compound is not electrochemically active. In such cases, derivatization of the compound may be an option.
4. If a peak is measured, the working potential is decreased by 50 or 100 mV, and steps 2 through 4 are repeated until the lowest potential setting.
5. The peak heights and the background currents are plotted against the working potential.

The working potential that gives the best sensitivity is obtained by plotting the signal-to-noise ratio against the working potential.

Figure 9–7: Construction of a hydrodynamic voltammogram for norepinephrine. Chromatograms are obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), in 100-mV steps.



10 3465 Detector specifications

The following pages outline the 3465 Detector specifications.

10.1 Environmental, dimensional, weight, and power requirements

Working temperature	10 to 35 °C (indoor use only)
Storage temperature	-25 to +50 °C
Humidity	20% to 80% RH
Safety and EMC	CE (CB Scheme), UL, CSA (cMETus approval)
Equipment group and class	Group 1, Class B
Installation category	II
Pollution degree	2
Dimensions	43 (D) x 22 (W) x 44 (H) cm = 16.9" (D) x 8.7" (W) x 17.3" (H)
Weight	14.4 kg (32 lbs) without flow cell and column (SSC version)
Installation	Install upright on flat and smooth surface; keep space under the detector free.
Power requirements	100-240 VAC, 50/60 Hz, 260 VA, auto-sensing
Main fuse	2.5 AT / 250 V, 5 x 20 mm, IEC 60127-2 For safety reasons, no other internal fuse or circuit breaker is operator accessible; they should be replaced only by Waters authorized personnel. Use only manufacturer-supplied fuses.

Note: For optimum analytical performance, Waters recommends that the ambient temperature of the laboratory be between 20 and 25 °C and that it be held constant to within ± 2 °C throughout the entire working day. For optimal performance of the oven, its temperature should be set at least 7 degrees higher than ambient temperature.

10.2 3465 General

Operating modes	DC, PULSE, PULSE 2, SCAN
Other modes	CONFIG, DIAG, and SERVICE
Sensors	1 flow cell (SCC), up to max. of 2 flow cells (DCC)
Autozero	Triggered by keyboard, rear panel TTL, or remote PC control (LAN)
Max. current compensation (Autozero)	25 nA - 2.5 mA in DC and PULSE modes, dependent on range setting
Offset	+50% to -50% of max. output voltage, 5% steps
PC control	Parametric control and data acquisition via LAN port (USB service port)
Embedded software	Flash technology, upgradeable via PC (USB)
Oven	7 °C above ambient to 60 °C, accuracy 0.5 °C, stability 0.1 °C; accommodates column and flow cells
Rear panel connectors	1x IEC inlet (Main), 1x USB B, 1x RJ45 LAN, 1x 9-pin sub-D Male (Valve), 1x 9-pin sub-D Female (Analog output), 1x 25-pin sub-D Female (Digital I/O)
Analog output (DAC)	-1 to +1 V full scale (via 16-bit D/A converter)
Analog output (I/E)	-2.5 to +2.5 V full scale (unprocessed I/E converter signal)
Digital I/O (HW)	2x Relay, 5x TTL outputs (CMOS 3.3V logic), 13 TTL inputs (programmable), 1x GND
Programmable I/O functionality	Cell on, Cell off, Autozero, Start, Overload, Relay, Auxiliary
Valve control	VICI Valco 2-pos electrically actuated valve (E2CA, EHCA) via serial cable, manual valve, 1x inject marker output

10.3 3465 DC Mode

Range	10 pA - 200 µA in 1, 2, 5 increments
Filter (ADF)	RAW (100 Hz), OFF (10 Hz), 10 - 0.001 Hz in 1, 2, 5 increments
Potential (Ec)	-2.50 V to + 2.50 V in 10-mV increments

Data Rate	1 - 100 Hz in 1, 2, 5 increments, dependent on filter setting
Noise	< 2 pA with dummy cell (load of 300 MΩ/470 pF) in 1 nA range, filter off, Ec +800 mV and temperature of 35 °C.

10.4 3465 PULSE mode

Range	10 nA - 200 µA in 1, 2, 5 increments
Filter (ADF)	0.5 - 0.001 Hz in 1, 2, 5 increments OFF: for unprocessed data
Potential (Ec)	-2.50 V to + 2.50 V in 10-mV increments
Data rate	1/(pulse duration) Hz
Waveform	Max 5 potential steps
Pulse times (t1-t5)	t1: 100 ms - 2000 ms; t2, t3, t4, t5: 0 - 2000 ms in 10-ms increments
Sampling times (ts)	20 ms - [t1 - 60] ms

10.5 3465 PULSE mode 2

Range	10 nA - 200 µA in 1, 2, 5 increments
Filter (ADF)	0.5 - 0.01 Hz in 1, 2, 5 increments OFF: for unprocessed data
Potential (Ec)	-4.90 V to + 4.90 V in 10-mV increments
Data rate	1/(pulse duration) Hz
Waveform	Free programmable multi-step waveform with up to 30 time-potential (t, E) coordinates and max. pulse duration of 4 s. Time points in 10-ms increments.
Sampling time	Sampling interval is freely programmable, defined by Begin and End markers.

10.6 3465 SCAN mode

Range	10 nA - 200 µA in 1, 2, 5 increments

Potential (Ec)	-2.50 V to +2.50 V in 10-mV increments
Data rate	1 Hz
Scan rate	1 - 100 mV/s in 1, 2, 5 increments
Cycle	Half, Full, Continuous

11 Rear panel I/O

This chapter describes all rear panel functionality. Besides the main power inlet, the 3465 Detector has five connectors on the rear panel for communication, data output, and I/O.

Figure 11–1: 3465 Detector rear panel

11.1 USB B connector

USB type B connector for serial instrument control over USB; for service use only:

- Based on USB-to-serial UART interface using the FT232R chip from FTDI (Future Technology Devices International Ltd)
- FT232R is fully compliant with USB 2.0 specifications
- Fixed communication baud rate: 921600 bps
- Communication over USB is used for software (FW) update of the instrument using only the boot loader FW upload utility

11.2 LAN connector

RJ-45 bus for serial instrument control over LAN:

- Network configuration of Xport via Lantronix device installer software utility
- Fixed communication baud rate: 921600 bps
- Communication over LAN is used for parametric instrument control and data acquisition
- 10Base-T or 100Base-TX (Auto-Sensing) serial-to-Ethernet connectivity

Note: To establish communication over LAN, the LAN cable must be connected when starting the detector using the main power switch on the rear panel. If no communication cable (either LAN or USB) is connected during startup, communication via USB is enabled (default).

Consult the installation section for details on configuration and setup of communication over LAN.

11.3 Digital I/O connector

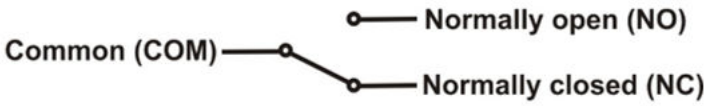
The detector has one 25-pin digital I/O connector, the XE "digital I/O connector", which enables control of (or by) external equipment. The I/O connector contains 18 TTL contacts (5 outputs and 13 inputs, 3.3 V CMOS logic), 2 RELAYS (contact closure), and 1 ground (GND connection).

11.3.1 TTL inputs and outputs

TTL inputs and outputs are default = high (3.3 Volt). The TTL inputs are level-triggered; the contacts require a minimum TTL-low pulse duration of 100 ms. If multiple activations are required, the next pulse should be given after 100 ms TTL high. When the input is kept low, only one activation occurs.

11.3.2 Relays

The 3465 Detector has two freely programmable contact closure outputs:



- Relay1: pin 1 normally closed, pin 2 normally open, pin 3 common
- Relay2: pin 4 normally closed, pin 5 normally open, pin 6 common

The maximum rating for these contact closure outputs is 24 VDC (switching voltage) and 0.25 A.

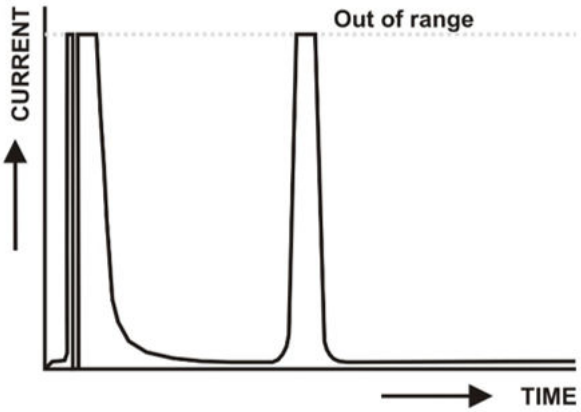
11.3.3 Aux

The 3465 Detector has four freely programmable TTL outputs, AUX1 – AUX4 (pins 7 – 10). These contacts are default “high” 3.3 V (inactive); when active, the status is “low” 0 V.

11.3.4 Overload

The overload output (pin 11) can be used to monitor if the cell current goes out of range during a chromatographic run. An “Out of range” error appears when the cell current (Icell) exceeds the limit of the current range at which the measurement is performed.

Figure 11–2: Example of a chromatogram where the cell current exceeds the maximum current level and the signal is "out of range".

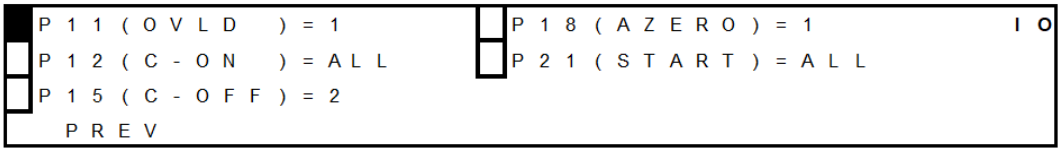


Note: It is important to recognize an “out of range” (overload) situation, because it may lead to erratic results when quantifying analyte concentrations in samples.

If, for instance, the cell current goes out of range during the recording of an analyte peak, it can in most cases be easily recognized by a flat peak top and a very abrupt transition to the flat top at the edges.

By default, the status of the overload output is “high” 3.3 V. When the cell current has the status “out of range”, the overload output changes status to “low” 0 Volt, until the cell current returns to a value within the measurement range. The overload output (pin 11) is one of the configurable I/Os.

Figure 11–3: Overload output



The configurable I/Os can be programmed in the IO menu, which is a submenu of the CONFIG menu. By default, the overload output is assigned to cell 1: “P11 (OVLD) = 1”. This means that only when the cell current of cell 1 is out of range does the status of the overload output change to “low” 0V. For all other cells present in the 3465 Detector, an “out of range” situation does not trigger a response on pin 11.

The following options can be selected for the configuration of pin 11 (with a 3465 Detector TCC):

P11(OVLD) = 1 Overload output active for cell 1 only

P11(OVLD) = 2 Overload output active for cell 2 only

P11(OVLD) = 3 Overload output active for cell 3 only

P11(OVLD) = Overload output inactive

P11(OVLD) = All* Overload output active for all cells present

*When this option is selected, the overload output will be active for all cells present in the 3465 Detector. If the cell current of any cell goes out of range, the overload input pin 11 becomes active.

11.3.5 Cell on Cell off

The 3465 Detector has three TTL inputs to switch on cells (pins 12-14) and three inputs to switch off cells (pins 15-17). This input command can be used, for example, to switch on and stabilize the flow cell early in the morning by means of a timer. Two of the inputs are configurable (pin 12 and pin 15; cell on and off, respectively) in the IO menu (see the previous chapter about overload output). The configuration settings of these inputs are: 1, 2, 3, 4, 5,' ', and all. If all is selected, all cells present in the 3465 Detector are switched on or off when the corresponding input is triggered.

11.3.6 Autozero

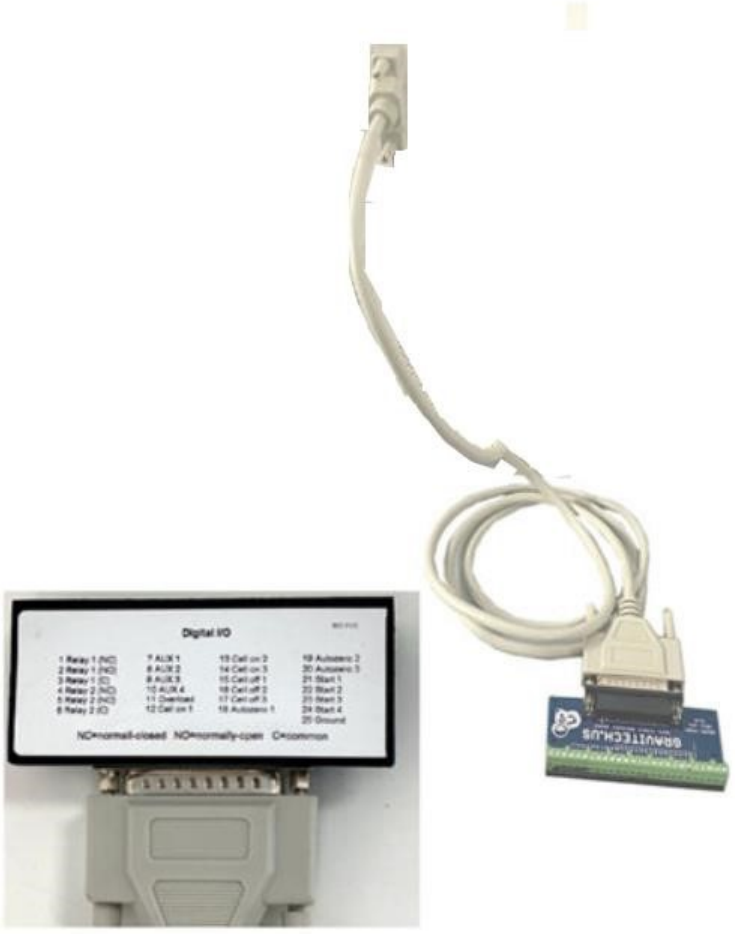
The 3465 Detector has three TTL inputs (pins 18 - 20) available to autozero cell current. Triggering these inputs enables external activation of the autozero command. This function is active only when the "I-cell" is displayed. One autozero input (pin 18) is configurable in the IO menu (see the previous chapter about overload output). The configuration settings of this input are 1, 2, 3, 4, 5, ' ', and all. If "all" is selected, the cell current of all cells present in the 3465 Detector are zeroed when the input is triggered.

11.3.7 3465 Start

The 3465 Detector has four TTL inputs (pins 21 – 24) available to start data acquisition and/or start a scan. One start input (pin 21) is configurable in the IO menu and can be used, for example, to start the data acquisition for all cells synchronously using only one trigger input when **All** is selected.

Note: The manufacturer accepts no liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.

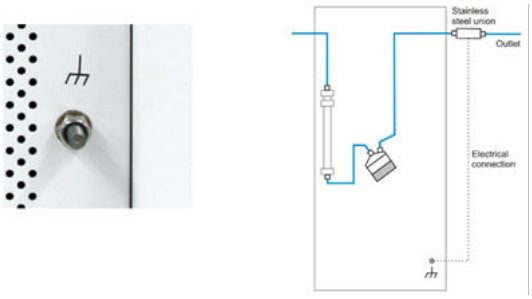
Figure 11–4: Digital I/O board and cable



11.4 Chassis grounding stud

A chassis grounding stud is available on the rear panel in the lower-right, next to the ventilation holes of the power supply compartment. This grounding stud is connected to the central grounding point of the instrument and can be used for shielding purposes; for example, to shield the flow cell outlet from external electrical interferences (troubleshooting noise issues) or to shield the flow cell from other equipment that might be coupled in series with the electrochemical detector.

Figure 11–5: Left: 3465 Detector rear panel grounding stud. Right: example of shielding the flow cell by grounding the solvent outlet tubing



Note: Use the chassis grounding stud for shielding only, not for safety grounding.

12 Troubleshooting

If problems occur during the operation of the instrument, the information in this chapter may help identify and solve the source of the issues.

Errors can be categorized in two types:

- Instrument errors
- Analytical problems

This chapter describes both types of errors. In the event that the problems cannot be solved after following the instructions in this troubleshooting section, contact your local supplier for further assistance.

12.1 Instrument errors

Incidental fault conditions may occur in any instrument. The 3465 Detector generates an error message containing an error number with a short description for several hardware fault conditions. The error messages appear in the instrument's LCD display.

Table 12–1: Error messages

Error	Message
11	Checksum error
12	Temperature sensor 1 error
13	Disconnect flow cell x
14	Control board SDRAM error
20	No sensor board detected
23	Heater defect • Turn off instrument immediately • Disconnect the power cord • Call for service

Warning: To avoid burn injuries, power-off the detector if the actual temperature exceeds 70 °C. Do not touch the metal parts inside the oven compartment because they could be hot. Contact the manufacturer or its representatives for service. Do not switch on the instrument before the instrument is serviced.

Ensure that you perform maintenance regularly. If one of the errors occurs, contact your local supplier for further instructions. If the instrument does not power-up, see the following remedies.

Table 12–2: No detector response

Possible cause	Remedy
No power	Check line voltage setting, plug in power cord
Power switch off	Turn this switch ON (at the rear panel)
Faulty fuse	Replace fuse
Divergent mains voltage	Verify line voltage

Warning: To avoid electric shock, connect the instrument to a grounded power source with a line voltage within the specified ratings.

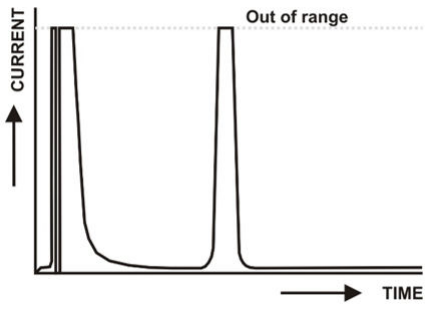
The instrument may display the following messages on the LCD screen or PC control software during a measurement:

Table 12–3: Messages

Message	Advice
01 Out of range[a]	The output is either above +1.0 V or below –1.0 V. Pressing AZERO may give an adequate readout again. If not, the autozero function cannot compensate the background cell current. Advice: Use a less sensitive range in the SETUP menu.
02 PAD overload	The charging current in pulse mode is out of range. Pressing AZERO may give an adequate readout again. If not, we recommend changing the pulse settings (increase t1) or using a less sensitive range.

a. An ‘Out of range’ error appears when the cell current Icell exceeds the limit of the current range at which the instrument performs the measurement.

Figure 12–1: Example of a chromatogram where the cell current exceeds the maximum current level and the signal is “out of range”



It is important to recognize an out-of-range (overload) situation, because it may lead to erratic results when quantifying analyte concentrations in samples.

12.1.1 Erratic cell current value or offset after current compensation

Initiating an autozero, offset, or a change of range on an unstable or changing current signal (baseline) may lead to an erratic current value or erratic offset in current. An autozero, offset, or change of measurement range triggers an internal calculation procedure in the detector based on the actual cell current. This calculation is an iterative process, and the cell current is measured several times during the calculation process. If the cell current is not stable during the calculation process, this might lead to errors.

Note: To avoid errors, do not execute an autozero, offset, or range change when the cell current is not stabile or the baseline did not stabilize.

If this type of error occurs, set the Compensation to OFF (Comp = OFF) in the STAT screen or CDS control software. Wait until the background current is stable/stabilized and execute an autozero again.

If you program event tables in LC methods in the CDS software with an autozero action, ensure that you program the autozero in a part of the chromatogram where the signal is stable. We recommend programming the autozero at the beginning of the run, during the hold-up time (before the unretained/solvent peak elutes) or at the end of the run.

12.2 Analytical troubleshooting

Analytical problems (such as loss of signal, an increase in noise level, high cell current, loss of sensitivity, and others) may occur in any UPLC-ECD system. It may be hard to find the cause, and several checks may be necessary to identify the source of the problem. The first step is to determine if the problem is caused by the 3465 Detector or something else in the UPLC system. For that purpose, two basic checks should be performed:

- Dummy cell test
- Stop flow test

Dummy cell test: The outcome of the dummy cell test, which is described in the next section, indicates if the problems are caused by the detector hardware (electronics).

Stop flow test: The stop flow test determines if the problems are caused by the electrochemical flow cell or something else in the UPLC system (for example, pump, autosampler, pulse damper, column, or mobile phase).

12.3 Dummy cell test

12.3.1 External dummy cell

An external dummy flow cell (700001943) is shipped with every 3465 Detector instrument for troubleshooting purposes and maintenance checks. The dummy cell test can be performed

standalone, via the LCD display in combination with an A/D converter, or Empower software. A successful dummy cell test confirms that the controller, including the cell cable, functions properly. If the result of the noise measurement with the dummy cell is within specs, the controller is excluded in a troubleshooting procedure.

Figure 12–2: Photo of the external dummy flow cell

The dummy flow cell consists of a resistor (R) of 300 MOhm and a capacitor (C) of 0.47 µF in parallel. The current is measured over the resistor according to Ohm's law (V = I x R); hence, with a working potential of 800 mV, the current drawn will be about 2.67 nA. Slight differences in this ideal value are due to the tolerance of the resistor (± 1%). The capacitor functions as a "noise generator" and in fact resembles the capacitance of a well-functioning VT-03 flow cell in an ideal UPLC setup. The noise generated via the dummy should be less than 2 pA if the filter of the controller is set to off, provided that the dummy is within the fully closed Faraday shield at the same position as the flow cell.

Table 12–4: Dummy cell test settings

Parameter	Setting
Cell potential	800 mV
Oven	35 °C, stable
Filter	Off
Range	1 nA/V

Test criteria:

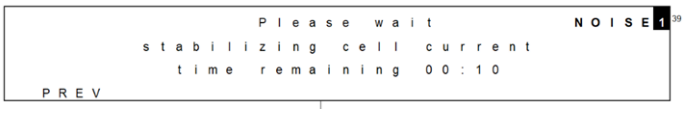
- I cell = 2.67 +/- 0.05 nA
- Noise < 2 pA

Note: The results (cell current and noise) of the dummy cell test should be within the previously noted test criteria. If the current value (Icell) and the noise are not within the criteria, it is an indication that something could be wrong with the detector hardware. Consult your local representative.

12.3.2 Internal dummy cell

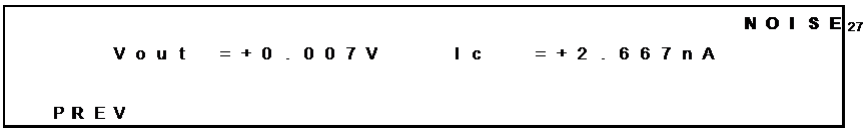
The 3465 Detector also affords the option to run an "internal dummy cell" test. This checks the performance of the electronic circuit boards (amplifier circuitry) only, so it excludes the cell cables and the external dummy flow cell. From the MAIN screen, select DIAG to enter the DIAG screen, and then select NOISE. This activates a timer in the NOISE screen, and after five minutes stabilization, autozero is activated and the dummy cell test is ready. Noise of the internal dummy cell can be measured at the output. As with the external dummy cell, the noise should be better than 2 pA. Detector settings in the NOISE screen are the same as in the external dummy cell test, with the exception of the oven temperature. Temperature is switched off.

Figure 12–3: NOISE screen 1



In the NOISE screen, the cell current and the output voltage are shown.

Figure 12–4: NOISE screen 2



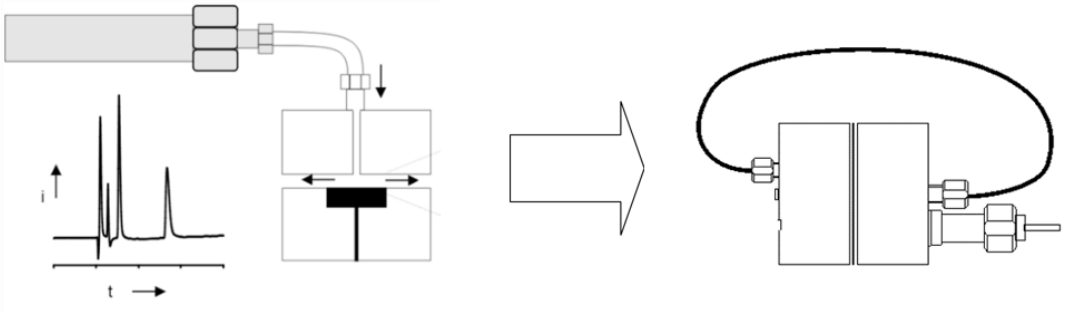
12.4 Stop flow test

The stop flow test is a basic test to determine if the problems are related to the flow cell or to something else in the UPLC system.

Perform the following steps to execute the stop flow test:

1. Switch off the UPLC pump.
2. Disconnect the tubing connection from the column outlet (see the following figure).

   Figure 12–5: Column outlet



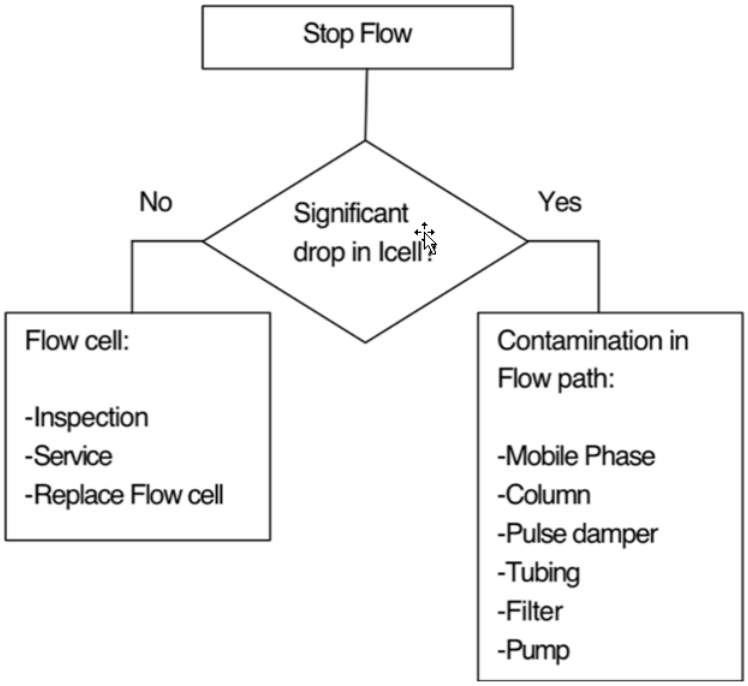
3. Disconnect the outlet tubing from the flow cell.

4. Connect the other end of the tubing (the tubing connected to the inlet of the flow cell) to the outlet of the flow cell.
5. The fluidics path of the flow cell is now completely isolated from the rest of the LC system.
6. Record a run (without injection of sample) to measure/evaluate the background cell current (Icell) and noise.
7. Compare the obtained values of the cell current and noise with the values observed before the stop flow with the pump on.

   Results:

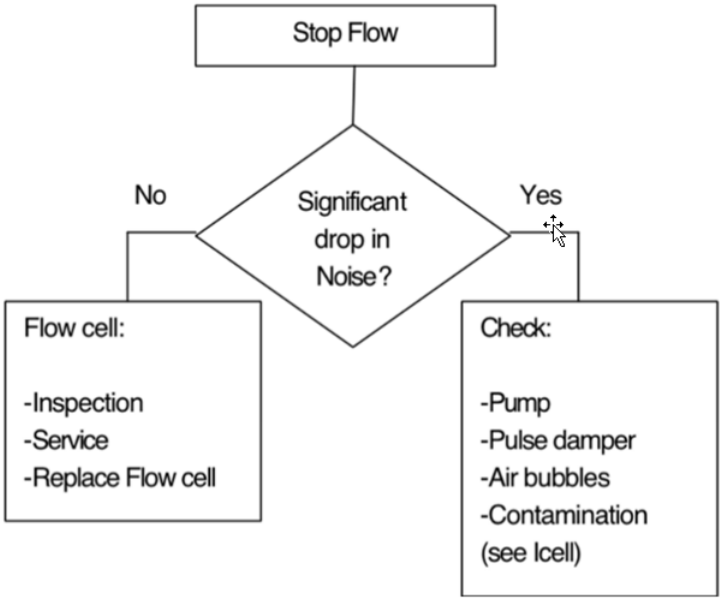
   Icell: If a significant drop in Icell is observed (for example, a drop of more than 50%), it is an indication that the problem is not flow cell related but originates from other parts of the LC system. The most obvious reasons for a high background current are electrochemically active contaminants in the mobile phase, column bleeding, and leaking pulse damper. These causes can be systematically eliminated by replacing the mobile phase or disconnecting the column or pulse damper, and so on, and then re-evaluating the cell current.

   Figure 12–6: Icell-Stop Flow



   Noise: If only a significant drop in noise is observed, it could signal, for example, a pump problem (check valves, air in pump head, compressibility issues, or leaking seals).

Figure 12–7: Noise-Stop Flow



Note: If no significant drop in noise or cell current is observed, service or replace the cell. If you still cannot solve the problem, contact your local representative.

12.4.1 Possible solutions for analytical problems

Bear in mind that analytical problems may also be caused by external influences like temperature or unstable samples. Ensure that the application was running trouble-free before and that no changes were made to the system. A number of causes and possible solutions for analytical problems are listed here. Contact your local representative if you need further help.

Table 12–5: No detector response

Possible cause	Remedy
No power	Check line voltage setting, plug in power cord
Power switch off	Turn this switch ON (at the rear panel)
Faulty fuse	Replace fuse
Divergent mains voltage	Check line voltage
Cell disconnected, or switched off	Check connection
Output disconnected	Check connection
Fouled WE	Clean WE

Table 12–6: High cell current

Possible cause	Remedy
Contaminated buffer	Replace buffer, do not recycle the buffer
High WE potential	Optimize potential, if possible: use smaller WE diameter
Salt bridge in REF not saturated	Refill with wetted KCl crystals
Retained peaks from previous runs	Wait for elution of these (very) broad peaks
Column is “bleeding”	Replace column
High amount of Fe^{2+} in buffer	Add EDTA to buffer, rinse metal parts with 15% HNO_3

Table 12–7: Noisy baseline

Possible cause	Remedy
Salt bridge in REF not saturated	Refill with saturated KCl, add wetted KCl crystals
Air bubble in REF or in cell	Remove air bubble, continuously degas the mobile phase
Slow temperature fluctuations	Isolate detector cell, set oven temperature
Fouled WE	Clean WE
Leaking REF or cell	Tighten connections with care

Table 12–8: Decreased sensitivity (low S/N ratio)

Possible cause	Remedy
Fouled WE from dirty samples	Clean WE, if possible; dilute samples
Cell potential too low	Optimize potential
Contaminated buffer (high Icell)	Replace buffer, do not recycle the buffer

Table 12–9: Saturation of output

Possible cause	Remedy
Damaged REF	Check with spare REF, replace if necessary
Damaged WE	Replace cell block
Cell incorrectly connected	Check connections (REF: black, WE: red, AUX: blue)
Cell potential too high	Optimize cell potential

Table 12–10: Base line oscillations

Possible cause	Remedy
Malfunctioning pump (regular pattern)	Check pump (seals, valves)
Over-tightened cell bolts	Adjust cell bolts, check pump pressure
Air bubbles in cell or REF	Maintenance REF
Temperature oscillations	Set oven temperature
Contaminated buffer (high Icell)	Replace buffer, do not recycle the buffer
Fouled WE	Clean WE
High concentration of Fe^{2+} in buffer	Add EDTA, passivate metal parts with H_3PO_4

13 Detector accessories

13.1 Detector accessory kit

The electrochemical detector is shipped together with a number of parts. See the delivery documentation for a complete listing of parts.

Table 13–1: Accessory Kit (Single Cell ECD - 200000485, Dual Cell ECD - 200000492) 3465 Detector

Part number	Description
700001943[a]	External dummy flow cell
700001945	Column clamp, 12 mm
700013160[a]	SenCell cell clamp kit
700001004	Fuse, 2.5 AT 250 V
700013076	LAN (UTP) cable, crossed, 3 m
700013077	USB cable, A-B, 3 m
700013074	I/O conn. board
700013075	I/O cable 25M-25M, 1.8 m
700013078[a]	Cell cable

a. For Dual Cell ECD, the quantity of 700001943, 700013160, and 700013078 is 2.

For these and other 3465 Detector parts or flow cells, see the Graphical Parts Locator at www.waters.com/wqp.

13.2 Optional AD converter cables

For several types of ADC converters in third-party (U)HLPC systems, there are dedicated output cables available. With these cables, a hassle-free direct connection can be made between the 3465 Detector data output (analog signal) and the AD converter.

Part number	Description
700013079	Assy, Cable SAT/IN Analog to DSUB, 1.8 m for SCC
700013112	Assy, Cable SAT/IN Analog to DSUB, DCC

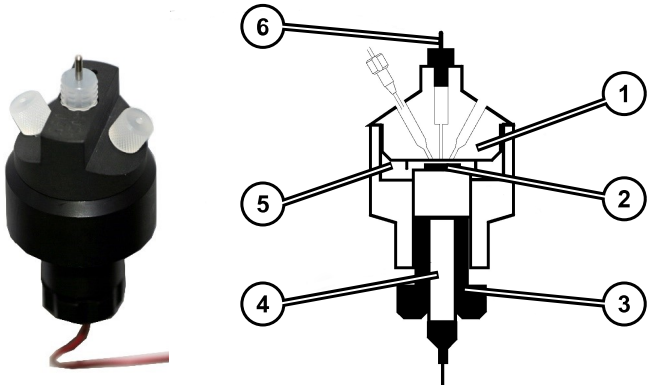
14 FlexCell

14.1 The FlexCell

14.1.1 Introduction

The FlexCell has been developed for analysis in standard and microbore LC-EC with an effective volume of only 0.5 µL. A range of different working electrode materials are available for the FlexCell and they are easily exchangeable, offering maximum flexibility for running various applications. Also, in applications where the working electrode material is electrochemically consumed, the option for an easy exchange is advantageous. Exchanging the working electrode takes only a minute.

Figure 14–1: FlexCell

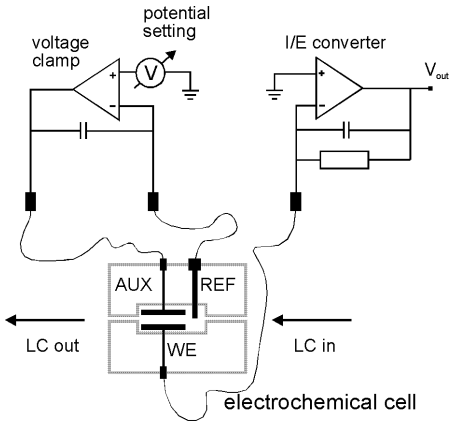


1. Auxiliary electrode inlet block
2. Working electrode disk
3. Electrode swivel nut
4. Working electrode assembly
5. 50-µm spacer
6. Reference electrode

14.1.2 The three-electrode configuration

A three-electrode configuration is used in the FlexCell (Figure 14–2: Schematic representation of an electrochemical cell with a three-electrode configuration (Page 102)). The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The auxiliary electrode is kept at the same precisely defined potential as the reference electrode (REF) by means of a “voltage clamp”, an electronic feedback circuit that compensates for polarization effects at the electrodes. At the working electrode, which is kept at virtual ground, the electrochemical reaction takes place (that is, electrons are transferred at the working electrode). This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage can be processed by an integrator, recorder, or AD convertor to generate the chromatogram.

Figure 14–2: Schematic representation of an electrochemical cell with a three-electrode configuration



Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration:

- If the working potential were applied only over an auxiliary electrode versus the working electrode (without 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 were applied only over the reference electrode versus the working electrode (without auxiliary electrode), the working potential would be very well defined. However, the potential of a reference electrode is only well defined if the current drawn is extremely low (pico-amperes), resulting in a very limited dynamic range.

A three-electrode configuration combines the best of both configurations. The reference electrode stabilizes the working potential and the auxiliary electrode can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

14.1.3 Requirements and limitations

14.1.3.1 Ions in running solution

For the three-electrode configuration to work, the solution inside the flow cell should have a low electrical resistance. This is obtained by running solution through the flow cell that contains at least 10 mM ions.

Warning: The solution running through the flow cell should have an ionic strength of at least 10 mM when the cell is ON.

Either the mobile phase contains the ions (usually the pH buffer) or the ions are added in a post-column setup. An absence of ions in the solution running through the flow cell results in destabilization of the feedback loop and potential damage to the electrodes. When the flow is not yet filled (air has high electrical resistance) or solutions without ions are being flushed through, the cell should be OFF.

14.1.3.2 Organics in running solution

The effective volume of the cell is defined by the working electrode pressing on the inlet block, with a spacer in between. Running solutions that contain more than 50% organic solvent through the flow cell increases the occurrence of leakage: the organic solvent makes the solution creep under the spacer. For such applications, a different cell design is better suited.

14.1.4 Working electrodes

14.1.4.1 Various WE materials

The surface of the working electrode is where the electrochemical reaction takes place. This puts specific demands on the working electrode material. The WE should be made of an electrochemically inert material, it should have a very well defined and flat surface, and it should have favorable I/E characteristics for the analyte of interest. Ideally, a high signal is obtained at a low working potential because noise levels increase with potential.

Different working electrode materials are available for the FlexCell: glassy carbon, gold, silver, platinum, boron-doped diamond (BDD), and copper. For most regular applications, glassy carbon is the working electrode material of choice. Some components are best detected on specific materials. For example, the analysis of iodide is best done with a silver working electrode. This reaction already occurs at a very low working potential (1 mV), which results in extremely high selectivity. This allows the identification of iodide in urine samples with virtually no sample pretreatment.

14.1.4.2 Applications

The following table gives the typical applications for various working electrode materials. Different materials also have different working potential limits. At high positive working potentials, the water in the mobile phase electrolyzes and results in a strong increase in the background current

and noise. At negative potentials, the use of platinum electrodes is severely limited by the specific ease of reducing hydrogen ions to hydrogen gas. For metal electrodes, the formation of metal oxides is a limiting factor for running oxidative measurements.

Table 14–1: Some features of different working electrode (WE) materials

WE material	Limits of working potential vs. Ag/AgCl (V)				Application example
	alkaline		acidic		
Glassy carbon	-1.50	+0.60	-0.80	+1.30	Catecholamines
Gold	-1.25	+0.75	-0.35	+1.10	Carbohydrates, thiols
BDD	-	-	-1.00	+2.00	Iodide, disulfides, phenols
Platinum	-0.90	+0.65	-0.20	+1.30	Alcohols, glycols
Silver	-1.20	+0.10	-0.55	+0.40	Halides, cyanide
Copper	-	+0.20	-	+0.60	Amino acids, carbohydrates

14.1.4.3 Boron-doped diamond (BDD) electrodes

Boron-doped diamond (BDD) is the most recent addition to the list of available FlexCell working electrodes. The BDD electrode comprises an ultra-thin film of boron-doped diamond material deposited on a Si-wafer. The electrode is anodized and is capable of detecting oxidized components at 1.5–2 V, which would otherwise require a reductive step before detection under oxidative conditions. Other special properties of BDD electrodes are inertness and excellent response stability, which make them well suited to detect phenols—an application where electrode fouling is an issue when glassy carbon is used.

Chemical compatibility: The BDD electrode operational lifetime is severely reduced when exposed to fluorinated acids, such as tri-fluoroacetic acid. Even at relatively low concentrations (2% in aqueous solution), significant damage to the diamond electrode can be seen within days of operation.

14.1.5 Reference electrode

14.1.5.1 HyREF

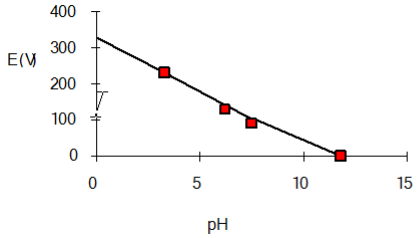
The maintenance-free HyREF is the most commonly used reference electrode for a FlexCell. This reference electrode is most suitable for use with high concentrations (>20%) of organic modifier in the mobile phase or when running alkaline mobile phase. An important characteristic of the HyREF is the pH dependence of the reference potential.

Notice: It is important to recognize that if the pH of the mobile phase is changed, the optimum working potential also changes. In such cases it is best to construct a hydrodynamic voltammogram to find the new optimum.

14.1.5.1.1 pH-dependent reference potential

When comparing the reference potential of a HyREF with that of a salt bridge (Ag/AgCl), a pH-dependent difference can be observed. It is important to work with a well buffered mobile phase when using the HyREF. Otherwise, the reference potential will not be a fixed value.

Figure 14–3: Difference in reference potential when comparing HyREF with saltbridge Ag/AgCl REF at different mobile-phase pH

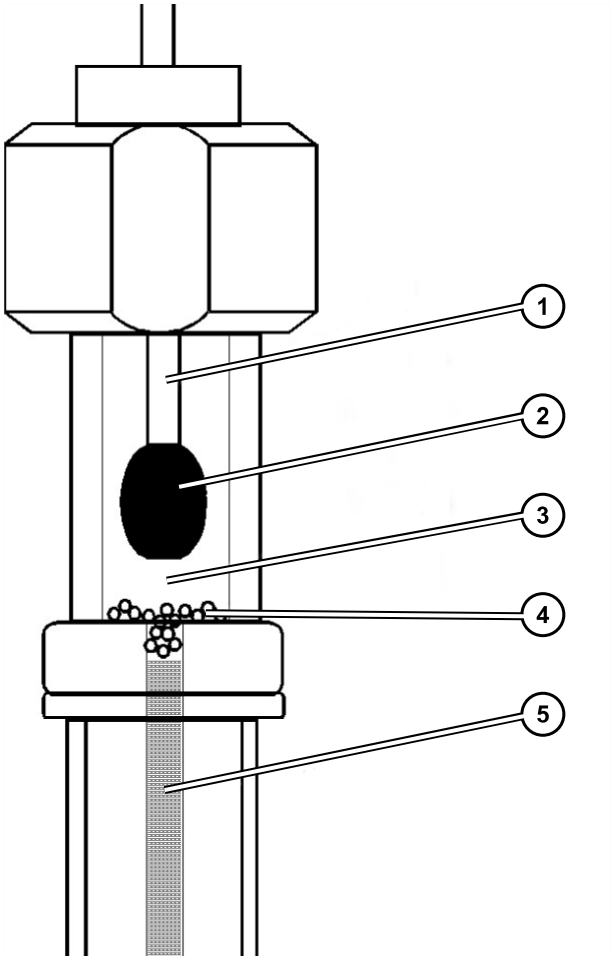


pH	E (V)
3.3	232
6.2	130
7.5	90
11.8	0

14.1.5.2 Salt bridge Ag or AgCl

The salt bridge Ag/AgCl reference electrode consists of a small container with a solid AgCl-coated silver rod immersed in a solution of saturated KCl (Figure 14–4: Magnified schematic representation of the salt bridge Ag/AgCl reference electrode (Page 106)). This type of reference electrode requires regular maintenance. Electrical contact with the other two electrodes in the flow cell is made through a wetted cotton wool frit (the salt bridge), which is electrically conductive and slows down the leakage of KCl.

Figure 14–4: Magnified schematic representation of the salt bridge Ag/AgCl reference electrode



1. Ag
2. AgCl
3. KCl saturated
4. KCl crystals
5. Cotton wool

Three aspects determine the proper function of an Ag/AgCl reference electrode.

1. 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.
2. Absence of air bubbles inside or close to the salt bridge gives the best stability of the three-electrode configuration.
3. The salt bridge must allow proper electrical contact with the mobile phase.
   For certain applications, another chloride salt is preferred:
   - For perchlorate-containing mobile phases, NaCl is used instead of KCl, because potassium perchlorate precipitates and clogs the cotton wool frit.
   - At high levels of organic modifier in the mobile phase, the chamber must be filled with lithium chloride solution instead of KCl, to prevent precipitation at the interface.

14.1.5.3 ISAAC

The in situ Ag/AgCl (ISAAC) reference electrode is the low-maintenance version of the Ag/AgCl salt bridge reference electrode.

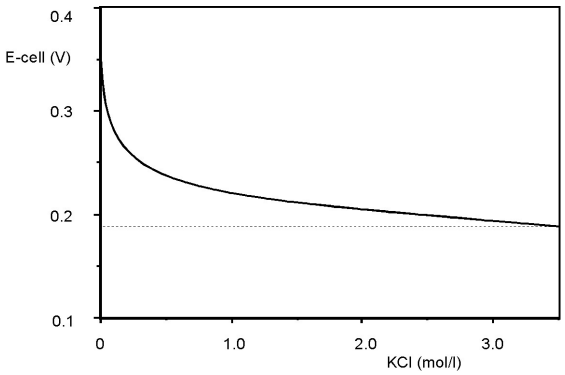
The potential of an ISAAC is defined by the concentration of chloride ions in the mobile phase. Therefore, every new batch of mobile phase should be prepared with the same concentration of chloride ions (typically 2 mM) to get reproducible results.

Important: It is important to add a fixed concentration of chloride ions to the mobile phase when using the ISAAC as a reference electrode. Without chloride ions in the mobile phase, the ISAAC gives erratic results.

14.1.5.3.1 ISAAC - Chloride-dependent reference potential

When comparing the reference potential of an ISAAC with that of a salt bridge (Ag/AgCl), a chloride-dependent difference is observed (Table 14–2: Potential of the Ag/AgCl reference electrode (Page 108)). For example, the difference in reference potential between the salt bridge Ag/AgCl reference electrode with saturated KCl and the ISAAC with 2 mM chloride ions in the mobile phase is 189 mV.

Figure 14–5: The Ag/AgCl reference potential is dependent on the chloride concentration



The relationship between potential and other chloride concentrations is described with the equation Ecell = EoAgCl - (RT/F) ln [Cl-], where R is the gas constant (8.314 J.mol-1K-1), T is the absolute temperature (293 K), and F is the Faraday constant (96485 C/mol). The EoAgCl (in 1.0 mol/L Cl- solution) for the half-reaction AgCl(s) + e- <=> Ag(s) + Cl- is 0.222 V.

Table 14–2: Potential of the Ag/AgCl reference electrode

Cl^-(mmol/L)	$E_{Ag/AgCl}$ (mV)[a]	dE (mV)[a]
3500	190	0
2500	199	8
1500	212	21
500	240	49
100	280	90
20	321	130
10	338	148
8.0	344	154
6.0	351	161
4.0	361	171
2.0	379	189
1.0	396	206
0.5	414	224

a. Rounded to the nearest whole number

A salt bridge Ag/AgCl reference is loaded with saturated KCl and the ISAAC is in contact with the concentration of chloride added to the mobile phase. dE is the potential difference compared with EAg/AgCl in saturated KCl (3500 mM Cl-).

14.1.5.3.2 Restriction using ISAAC

The restrictions using ISAAC is mentioned below:

- A high working potential (> 1.2 V vs. Ag/AgCl in 2 mmol/L KCl) will oxidize Cl-, which then contributes to background current and noise.
- In ion chromatography, the addition of Cl- may lead to undesired chromatographic changes.
- When using a silver working electrode, the addition of Cl- to the mobile phase will cause formation of an AgCl coating on the working electrode, leading to inactivation.
- At high pH or high modifier concentrations, the HyREF is more suitable.

14.2 General precautions

Note the following precautions before you assemble the flow cell:

1. The flow cell is assembled properly when it arrives. Ensure that all marked items on the checklist are included.

2. Always make sure that the surfaces of the spacer and working electrode are dry and free from particulate matter before assembling the cell.
3. Clean fingerprints from spacer and electrode surfaces with a soft tissue soaked in acetone or methanol. If the auxiliary electrode needs to be cleaned, do not apply force, which could damage the electrode surface.
4. Except for BDD electrodes, make sure that the working electrode has a mirror-like appearance before installation.

   Notice: The BDD electrode has a thin crystalline blue/greyish surface. This electrode should not be polished. Polishing this electrode damages the active surface and leads to loss of performance.

5. If the flow cell is not in use and removed from the LC system, we recommend that you disassemble the cell and clean all surfaces.

   Note: The construction of the FlexCell is such that both fluid connections can be used as either inlet or outlet.

   Notice: Never switch on the flow cell if:

   - The (black, red, and blue) cell cable is not correctly connected.
   - The cell is only partly (or not at all) filled with mobile phase.
   - The cell is filled with solutions that do not contain electrolytes.

   Damage to the working electrode or the electronics may occur.

Warning: The ISAAC reference electrode requires the presence of 2 mM chloride ions (KCl or NaCl) in the mobile phase. Add and equilibrate before installation.

Warning: Use proper eye and skin protection when working with solvents.

14.3 Installation

Maximum detection stability is attained when not only the flow cell but also the HPLC column is incorporated in the detector oven. The detector has an integrated Faraday cage and an accurately temperature-controlled oven compartment, ensuring stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

14.3.1 Connecting a FlexCell to an LC system

To connect a FlexCell to an LC system:

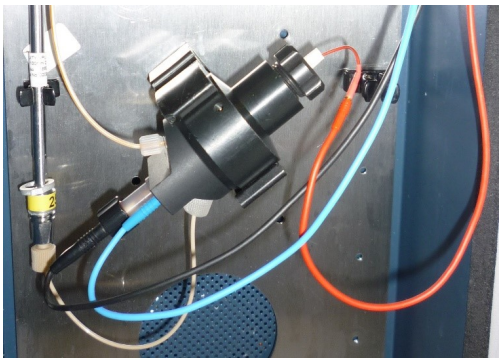
1. Install a suitable length of sharply cut 1/16-inch-OD PEEK tubing to the column outlet. Choose a tubing ID that matches the column ID:
   - 0.25-mm ID for working with normal-bore columns
   - 0.13-mm ID for working with mini-bore columns
   - 64-µm ID for narrow-bore columns
2. Keep some tissues close by because you will probably spill some mobile phase during the mounting procedure. Remove the reference electrode from the inlet block (Figure 14–1: FlexCell (Page 101)).
3. With the mobile phase running from the column, connect the column outlet to the flow cell inlet, using one of the supplied connectors, and tighten it carefully. Over-tightening affects the flow through the tubing and decreases flow cell performance.

   Notice: Use only the factory-supplied finger-tight connectors at the flow cell; other connectors may cause serious damage.
4. Install a suitable length of sharply cut 0.5-mm-ID PEEK tubing to the flow cell outlet.
5. For a salt bridge reference electrode, first make sure it is ready for use:

   Notice: When using a salt bridge reference electrode, inspect it visually before installation:
   - No air bubbles trapped in the body
   - Visual presence of salt crystals in the body
   - The cotton tip looks white and wet

   Remove the salt bridge storage cap and tighten the black swivel a little bit extra, which should result in a small droplet appearing from the frit. This droplet indicates that the frit is not dried out or clogged. Leave the droplet on the tip when inserting it into the reference chamber; it ensures proper contact of the REF with the mobile phase.
6. Close the outlet tubing (by finger) and force the running mobile phase into the reference electrode reservoir.
7. If air bubbles are visibly stuck in the reservoir, remove them with a plastic pipette.
8. Install the reference electrode in the reservoir (without trapping bubbles).
9. Place the cell at an angle of 45° in the detector oven compartment, with the outlet facing upward, and connect the cell cable as illustrated below.

Figure 14–6: Flow cell with cell cable connected. WORK, AUX, and REF electrodes are connected using the red, blue, and black leads of the cell cable, respectively



14.4 Maintenance

Maintenance of the working electrode is necessary if the electrode surface has been electrochemically changed. This can happen due to fouling by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is evident from strongly decreased sensitivity after prolonged use. Except for the salt bridge type, the reference electrodes are maintenance-free.

Metal working electrodes need a regular polishing/flattening step to maintain their responsiveness. If the FlexCell needs maintenance, you must disassemble the cell.

Important: Before disassembling the flow cell, read the General Precautions.

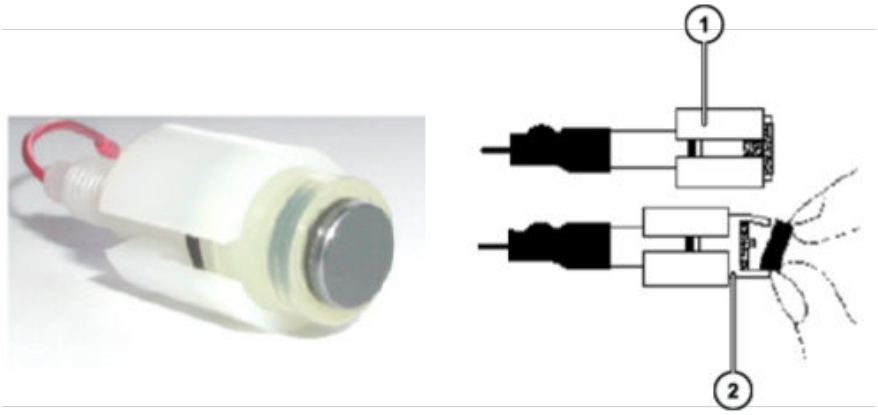
14.4.1 Disassembly of the flow cell

To disassemble the flow cell:

1. Switch the flow cell off, set the flow rate to zero, and take the FlexCell out of the system after disconnecting both finger-tight connectors from the cell.
2. Unscrew the electrode swivel nut and pull the working electrode assembly out of the cell.
3. Remove the retaining ring.

   Notice: It is important to remove the working electrode assembly before removing the retaining ring. Disassembling the FlexCell in the wrong order will damage the spacer.
4. Carefully remove the working electrode from the WE assembly (Figure 14–7: Electrode assembly with a BDD WE (Page 112)).
5. Clean and dry the spacer and the contact surface of the AUX block.
6. Remove the reference electrode. For a salt bridge reference electrode, cap it to prevent drying of the frit.

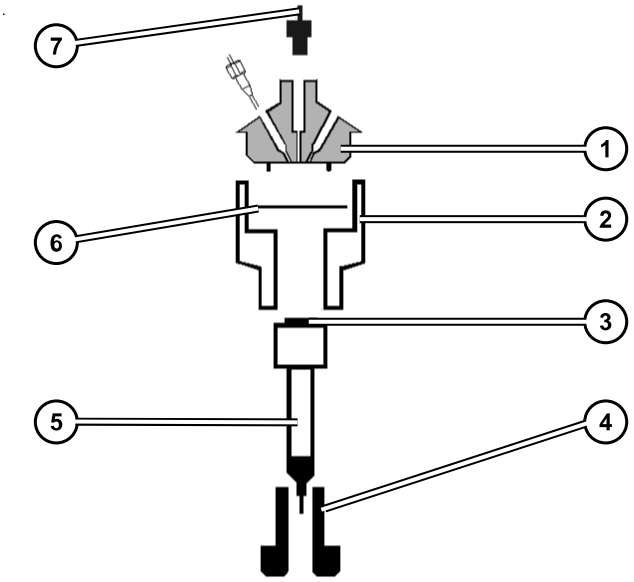
Figure 14–7: Electrode assembly with a BDD WE



1 The working electrode is fitted on the electrode shaft with electrode retaining ring

2 Held in place by a silicon electrode holder

Figure 14–8: Exploded view of FlexCell



1 Auxiliary electrode inlet block

2 Retaining ring

3 Working electrode disk

4 Electrode swivel nut

5 Working electrode assembly

6 50-µm spacer

7 Reference electrode

14.4.2 Working electrode cleaning

A typical reason to perform maintenance on the working electrode is a drop in flow cell performance. In most cases, a simple cleaning is sufficient to remove the chemical deposition that is causing the problem.

To clean the electrode surface:

1. Wipe the electrode surface with a tissue wetted with water.
2. Wipe the electrode surface with a tissue wetted with acetone.

   This is the only procedure that is allowed on BDD electrodes.

   Notice: The polishing procedure is for metal and glassy carbon working electrodes only. Do not polish BDD electrodes, because it will lead to damage of the thin reactive electrode surface layer and a loss of performance.

   If cleaning doesn't restore responsiveness, a polishing step can be applied to non-BDD electrodes. BDD electrodes must be replaced in such cases.

14.4.3 Polishing a working electrode

To polish a working electrode:

1. Shake the container with diamond slurry thoroughly before use.
2. Rinse the polishing disk with demi-water.
3. Apply a small amount of slurry on the wetted polishing disk. One drop is usually sufficient.
4. Place the working electrode face down on the polishing disk, and polish the electrode with a figure-eight motion for about one minute. Apply only gentle pressure with one finger.
5. Clean the electrode with demineralized water, and wipe it dry with an acetone-wetted tissue. Check the surface visually and repeat the procedure if necessary.
6. The electrode is ready to reinstall in the cell.

14.4.4 Flattening a metal working electrode

Metal working electrodes may require a more thorough flattening step because some conditions result in the consumption of the wetted material. For metal working electrode disks of the FlexCell

type, there is a dedicated flattening and polishing kit available (not provided with a FlexCell). Ordering information: Flattening/polishing kit for metal WE (250.1045).

The flattening/polishing kit is a tailor-made kit for a three-stage flattening-polishing procedure:

1. Flattening step on coarse plate with 30-µm coarseness
2. Flattening step on fine plate with 12-µm coarseness
3. Polishing step on polishing plate with fine diamond slurry

This procedure restores the metal WE surface to a mirror-like shine in a reproducible way. Detailed instructions are provided with the kit.

14.4.5 HyREF and ISAAC reference electrodes

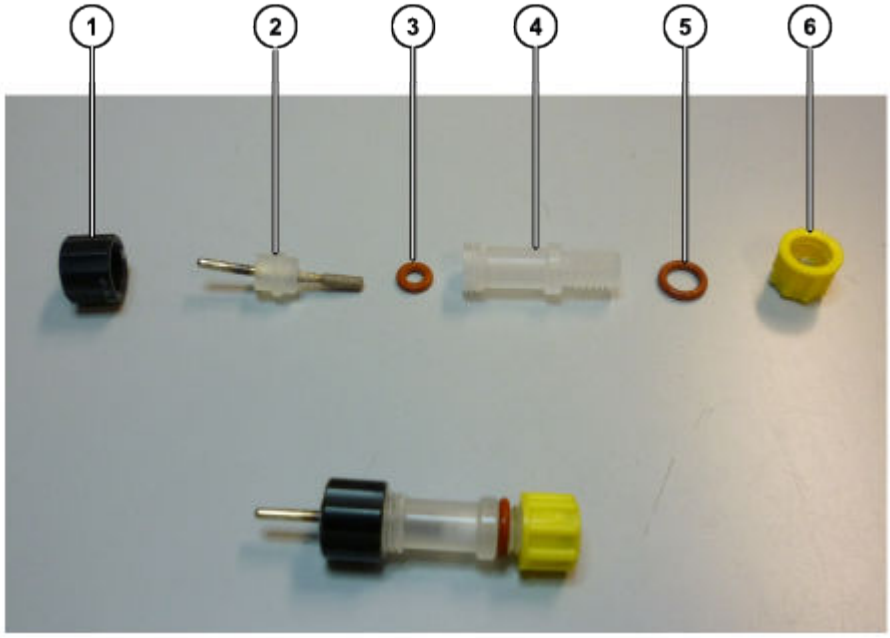
The HyREF and ISAAC reference electrodes are in principle maintenance-free. When not in use, they should be stored dry after disassembling the flow cell.

14.4.6 Salt bridge Ag/AgCl reference electrode

Note: The REF is factory-filled with saturated KCl solution. The salt bridge should be refilled with other saturated solutions of chloride salts when the mobile phase contains perchlorate (use NaCl) or a high percentage of organic modifier (use LiCl).

Important: Check the salt bridge REF regularly. If you do not see chloride salt crystals, or if you see air bubbles in the body, it must be opened and refilled.

Figure 14–9: Salt bridge Ag/AgCl reference electrode, assembled (bottom) and disassembled (top)



1. sb REF swivel (700001956)
2. sb REF Ag/AgCl electrode (700002254)
3. sb REF silicon O-ring small (700002175[a])
4. sb REF body (700001957)
5. sb REF silicon O-ring large (700002175[a])
6. sb REF sealing cap

a. 700002175 contains one small and one large O-ring.

The yellow cap prevents drying out of the cotton frit that is mounted in the tip of the body.

14.4.7 Salt bridge Ag/AgCl reference electrode check

For proper functioning, the salt bridge reference electrode should have crystals inside the body (to ensure a saturated solution), no air bubbles inside the body, and a permeable wet cotton frit in the body tip.

Visually inspect for the presence of crystals inside the REF

After prolonged use the salt bridge in the REF body will no longer be saturated, which usually leads to poor reproducibility in electrochemical detection. If the salt bridge is not saturated and the KCl concentration decreases:

1. Noise in the system will slowly but continuously increase.
2. Background current will increase.
3. Sensitivity to movements and pump noise will increase.

Visually inspect for the absence of air bubbles inside the REF

If an air bubble is trapped in the salt bridge or in the cotton plug that separates the reference electrode from 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.

Air bubbles can be visible as a transparent round deposition on the REF body walls, or trapped under the top. Shaking and holding the REF upside down makes the air bubble more visible as it moves upward through the REF.

Visually inspect for permeability of the salt bridge cotton frit

During storage, the frit can dry out despite being capped. To check if the frit is still wet, remove the cap and tighten the black swivel of the REF a little bit extra. This should result in a small droplet appearing from the frit. If the frit is dried out it must be replaced.

14.4.8 Refilling a salt bridge Ag/AgCl reference electrode

If the salt bridge Ag/AgCl electrode needs refilling (air bubble or lack of crystals inside the body), the following materials are necessary (for salt bridge filled with KCl):

- Small plastic pipette
- Potassium chloride
- 30 mL of KCl solution saturated with AgCl (700013253), which is shipped with every flow cell that contains a salt bridge

To refill a salt bridge:

1. Unscrew the black swivel from the body (Figure 14–9: Salt bridge Ag/AgCl reference electrode, assembled (bottom) and disassembled (top) (Page 115)).
2. Remove the Ag/AgCl electrode and the small O-ring.
3. If air bubble is present, remove it with the small plastic pipette.
4. Using the pipette, take some small wet crystals from the bottom of the saturated KCl solution (or add some first if they are finished or clumped), and add to the body. Do not add too many because the electrode needs space as well.
5. Completely fill up the body with saturated KCl solution.
6. Place the O-ring on top of the open body.
7. Insert the electrode, making sure not to trap air under the cap. It may be necessary to wiggle the electrode to accommodate it between the crystals.
8. Screw the black swivel back on the body, thus securing the electrode inside. Verify that a drop of solution comes out of the cotton frit when tightening the swivel.
9. Visually inspect the REF for trapped air bubbles and remove them if present (go back to step 1).

10. Cap the salt bridge until use to prevent drying of the cotton frit.

    Note: If the cotton frit is dried out or discolored, it must be replaced.

14.4.9 Refritting a salt bridge Ag/AgCl reference electrode

If the cotton frit of the salt bridge Ag/AgCl electrode needs replacement, the following materials are necessary.

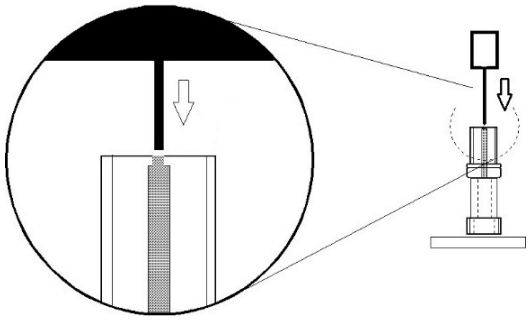
- Small glass plate
- Salt bridge REF tool (700013145), or 5-cm rod with 1-mm diameter
- Cotton wool, soaked in saturated KCl solution
- 30 mL of KCl solution saturated with AgCl (700013253), which is shipped with every flow cell that contains a salt bridge

14.4.9.1 Removing the cotton wool frit

To remove cotton wool frit:

1. Unscrew the black swivel from the body (Figure 14–9: Salt bridge Ag/AgCl reference electrode, assembled (bottom) and disassembled (top) (Page 115)).
2. Remove the Ag/AgCl electrode and small O-ring.
3. Place the open body with frit facing upward on a glass plate.
4. Use the narrow end of the salt bridge REF tool or a rod of ± 1 mm (0.039 inch) to push out the frit from the outside inward. Be careful not to damage the frit constriction in the tip of the body (Fig. 10). If the frit is dried out and giving too much resistance, soak it overnight in a beaker with demineralized water before trying again.

   Figure 14–10: Pushing the cotton wool frit out with the sb REF tool

5. Clean all parts with demi-water.

14.4.9.2 Inserting a new cotton wool frit

To insert a new cotton wool frit:

1. Place the salt bridge with the frit hole downward on a glass plate and add a few drops of saturated KCl solution.
2. Pull a small plug of wetted cotton wool into a thin string (using two tweezers, for example).
3. Place one end of the cotton string into the body and, using the sb REF tool, push the wool piece by piece from above through the KCl solution into the frit channel.
4. While holding the body on the glass plate, compress the cotton firmly. Ensure that you do not push the cotton out of the body through the narrow end.

   Result: The REF is now ready to be refilled

14.4.10 Assembly of the flow cell

Warning: The surfaces of the inlet block, working electrode, and spacer should be dry when assembling the flow cell. Unnecessary moisture inside or outside the flow cell increases the noise level considerably.

To assemble the flow cell:

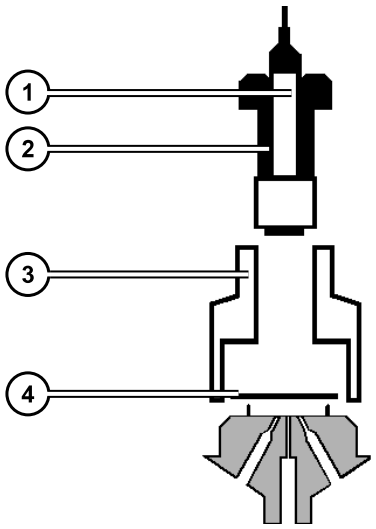
1. Take the working electrode disk and press it in its silicone holder. Try not to touch the active middle surface.
2. Clean fingerprints off the spacer, AUX, and WE surfaces using a tissue wetted with acetone.
3. Place the spacer on the inlet block, with the two holes over the two pins.
4. Screw the retaining ring on the inlet block.

   Warning: To prevent the spacer from slipping off the pins, keep the flow cell oriented as illustrated in Figure 14–11: Assembling the flow cell (Page 119), while mounting the retaining ring and WE assembly.
5. Install the working electrode assembly and verify that the groove in the assembly aligns with the internal pin (which prevents it from turning).
6. Fix the assembly in the cell body with the electrode swivel nut. Do not apply too much force; finger-tight should be enough force to make a leak-free seal between the WE, inlet block, and spacer.

   The flow cell is ready for connection to the LC system

Figure 14–11: Assembling the flow cell



1 Working electrode assembly

2 Electrode swivel nut

3 Retaining ring

4 50-µm spacer

First fit the spacer and the retaining ring, and then insert the WE assembly and secure it with the electrode swivel nut. If the flow cell is not in use and uncoupled from the LC system, we recommend that you disassemble the cell and clean and dry all surfaces.

Warning: Before flushing the LC system and uncoupling the cell from the detector, turn off the cell.

14.5 Specifications

Table 14–3: Specifications for FlexCell

Specifications	Description
Cell type	Three-electrode, thin-layer flow cell
Cell volume	Approx. 0.7 µL (50-µm spacer)
Spacers	50 µm or 130 µm
Working electrode diameter	8 mm
Working electrode area (wetted)	15 mm^2

Table 14–3: Specifications for FlexCell (continued)

Specifications	Description
Working electrodes (WE)	Glassy carbon (GC), gold (Au), boron-doped diamond (BDD), platinum (Pt), silver (Ag), copper (Cu)
Reference electrode	HyREF (Pd/H_2), Ag/AgCl
Auxiliary electrode	Carbon-loaded PTFE
Wetted materials	PCTFE, FEP, palladium, carbon-loaded PTFE, WE material (Au, Pt, GC, BDD, Ag, or Cu)
Flow rate	Typically 0.05 – 1.5 mL/min
Max. back pressure in cell	40 psi (2.8 bar)
Fluidics connections	1/16-inch o.d. PEEK tubing, with 10-32 PTCFE fingertight connectors
Electric connections	Cell cable for use with 3465 Detector

14.6 Parts list

Table 14–4: Spare parts for the FlexCell

Part number	Description
FlexCell parts	
700013126	FlexCell inlet block
700013123	WE holder assembly for FlexCell
700013128	Spacer for FlexCell cell, 50 µm
700013129	Spacer for FlexCell cell, 130 µm
Reference electrodes	
700013124	HyREF for FlexCell
700001958	Salt bridge REF
700013125	ISAAC for FlexCell
Working electrodes	
700013116	WE disk GC
700013117	WE disk Pt
700013118	WE disk Au
700013119	WE disk Ag
700013120	WE disk Cu

Table 14–4: Spare parts for the FlexCell (continued)

Part number	Description
700013121	WE disk BDD
Parts for polishing/flattening	
700001954	Polishing disk (for WE)
700001955	10 mL diamond slurry, 1 µm
700013150	Flattening/polishing kit for metal WE
Parts for reference electrode maintenance	
700013253	30 mL KCl solution saturated with AgCl
700013145	Salt bridge REF replacement tool
Connectors for LC tubing	
700013151	Finger-tight fitting PCTFE 10-32, 4 pcs

15 SenCell

15.1 The electrochemical flow cell

15.1.1 Introduction

The SenCell, a new electrochemical flow cell for (U)HPLC with ECD, has several unique features, such as a stepless adjustable working volume (spacerless concept) and toolless assembly.

The SenCell is available with a glassy carbon working electrode (WE). The SenCell design eliminates the use of plastic or metal spacers. By means of a special key, the working volume of the electrochemical cell can be steplessly adjusted without opening the cell, allowing easy optimization of the detection sensitivity for any LC application. The working volume can be adjusted between 0 and 300 nL (based on a WE of 2-mm diameter). The salt bridge Ag/AgCl reference electrode is generally recommended. For special applications, the HyREF reference electrode is available. A third reference electrode option is the in situ Ag/AgCl (ISAAC).

Figure 15–1: Assembled SenCell electrochemical flow cell with ISAAC inlet block (green). The upper part, the inlet block, is separated from the working electrode block. Right: SenCell WE block

The SenCell has been developed for ultra-trace analysis in standard, microbore, and capillary LC-EC. After extensive testing it was established that the confined wall-jet configuration gives the best results. In addition, it was found that the electrode materials quality and the finishing of the electrodes in the flow cell are decisive factors for the performance of an EC detector. While competitive designs usually deteriorate when in use, this flow cell, by design, improves in performance. The flow cell permits unusually short stabilization times; trace analysis within a few hours after startup may be expected.

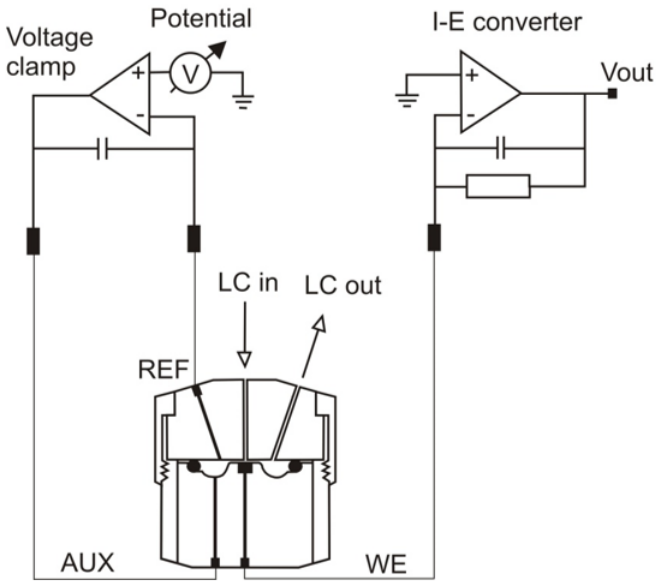
15.1.2 Three-electrode configuration

A three-electrode configuration is used in the SenCell. The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The AUX is kept at a precisely defined

reference electrode (REF) potential by means a “voltage clamp”, an electronic feedback circuit that compensates for polarization effects at the electrodes.

At the WE, which is kept at virtual ground, the electrochemical reaction takes place (electrons are transferred at the WE). This results in an electrical current to the I/E converter, which is a special type of operational amplifier. The output voltage can be measured by an integrator or recorder.

Figure 15–2: Schematic representation of an electrochemical cell with a three-electrode configuration



Essentially, for the oxidation or reduction reaction, it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration. If the working potential would be applied only over an AUX versus the WE (without REF), the working potential would continuously change due to polarization effects at the electrodes, resulting in highly unstable working conditions.

If the working potential would be applied only over the REF versus the WE (without AUX), the working potential would be very well defined. However, the potential of a REF is well defined only if the current drawn is extremely low (pico-amperes), resulting in a very limited dynamic range.

A three-electrode configuration combines the best of both electrodes. The REF stabilizes the working potential and the AUX can supply high currents. This results in the tremendous dynamic range of a three-electrode system.

15.1.3 Working electrode

Electrochemical detection puts high demands on the WE material. The WE should be made of an electrochemically inert material. Furthermore, to avoid an irregular flow profile over the electrode, it should have a very well defined surface. Finally, it is important that the analyte of interest can be oxidized (or reduced) with favorable I/E characteristics. This means that a high signal must be obtained at a low working potential. For most applications, glassy carbon is the WE material of

choice. The SenCell is currently available only with a glassy carbon electrode of 2-mm diameter. Under certain circumstances, other materials are favorable.

For example, for the analysis of iodide, a silver WE can be used. At the silver WE, the following oxidation reaction occurs for iodide: $Ag + I^- \rightarrow AgI + e^-$

This reaction already takes place at a very low working potential (1 mV), which results in extremely high selectivity. This allows the identification of iodide in urine samples with almost no sample pretreatment.

Table 15–1: Working potential limits and application area for different WE materials

WE material	Potential limits (V)				Major application
	Alkaline		Acidic		
Glassy carbon	-1.50	+0.60	-0.80	+1.30	Catecholamines
Gold	-1.25	+0.75	-0.35	+1.10	Carbohydrates
Platinum	-0.90	+0.65	-0.20	+1.30	Alcohols, glycols
Silver	-1.20	+0.10	-0.55	+0.40	Halides, cyanide
Copper	-	+0.60	-	-	Amino acids, carbohydrates

Another consideration in choosing a WE is the oxidation or reduction of mobile phase constituents or WE material that occurs when the potential exceeds the limits as given in the preceding table. At high positive working potentials, the water in the mobile phase electrolyzes and results in a strong increase in background current and noise. Formation of metal oxides, resulting in an increase in 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 ECD. For negative potentials, the use of platinum electrodes is limited by the ease of reducing hydrogen ions to hydrogen gas.

15.1.4 Detection limit

One of the most important parameters used to characterize the performance of a detection system is the signal-to-noise ratio (S/N ratio), from which the concentration detection limit is derived. It enables objective comparison not only between different electrochemical detectors but also between complete analytical methods, irrespective of the detection system used.

Table 15–2: LC-EC conditions for analysis of norepinephrine

Parameter	Description
Column	ODS-2, 3 µm, 100 x 4.6 mm
Flow rate	1.0 mL/min

Table 15–2: LC-EC conditions for analysis of norepinephrine (continued)

Parameter	Description
Mobile phase	H_3PO_4 50 mM, citric acid 50 mM, 20 mg/L EDTA, 100 mg/L octane sulphonic acid (OSA), pH=3.1 with KOH, 5% methanol
Sample	1.0 µmol/L norepinephrine, 20 µL injection
Temperature	30 °C
Cell	Flow cell with 3 mm GC WE, SB REF with 50 µm spacer
E cell	800 mV (vs. Ag/AgCl, filled with saturated KCl)
I_{cell}	approx. 3 nA

In the literature, several ways are described to determine the detection limit. In principle, it does not matter which definition of detection limit is used, as long as the definition is precise.

In this manual, the concentration detection limit (c_{LOD}) for a given compound is defined as the analyte concentration that results in a signal that is 3 times the standard deviation of the noise:

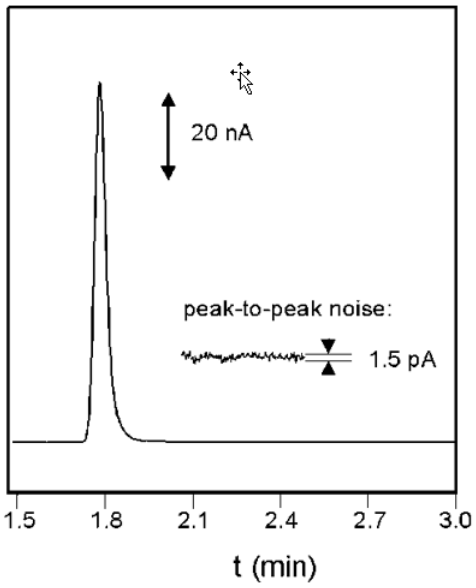
$$c_{LOD} = \frac{3 \cdot \sigma_{noise}}{signal} c_A$$

Where sigma-noise is 0.2 x peak-to-peak noise and c_A is the concentration of analyte injected.

The Example S/N ratio for norepinephrine (Page 126) image shows a typical S/N ratio for a flow cell with 2.74 mm WE. In this example, the concentration detection limit for norepinephrine based on three times the sigma-noise is 11 pmol/L (refer to the "LC-EC conditions for analysis of norepinephrine" table). Expressing the performance of a detection system by only the peak height makes no sense.

A system can easily be changed in a way that increases peak height. However, if the noise increases similarly, it has the same effect as switching a recorder to a higher sensitivity: peaks appear higher but the S/N ratio is the same. Expressing the limit of detection in an absolute amount (in picomoles) without mentioning the injection volume makes a good comparison between different systems difficult.

Figure 15–3: Example S/N ratio for norepinephrine (peak height: 80 nA, peak-to-peak noise: 1.5 pA). The amount injected is 20 pmol (1.0 µmol/L). The concentration detection limit based on three times sigma-noise in this case is 11 pmol/L.



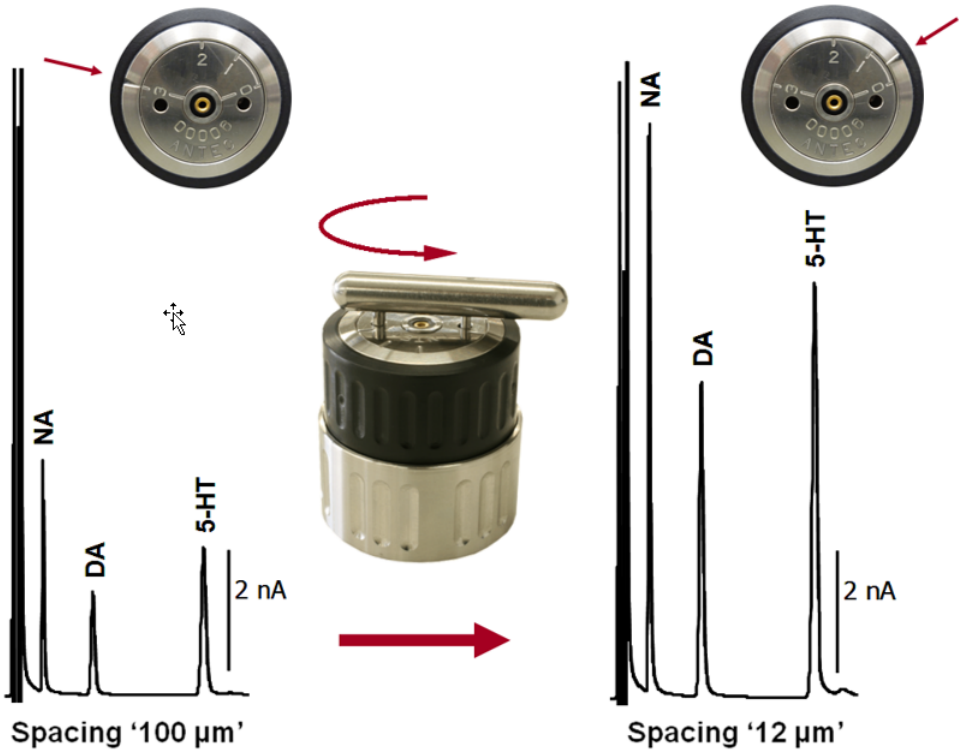
15.1.5 Cell working volume adjustment

In a traditional electrochemical flow cell that uses metal/plastic gaskets (spacers), the thickness of the gasket affects the linear flow velocity in the cell. With a thinner gasket, the cell working volume decreases, resulting in a higher linear flow velocity. For example, the working volume of a cell with a 2-mm diameter electrode with 25- or 50-µm spacer is 80 nL and 160 nL, respectively.

The signal increases with thinner spacers while the noise remains more or less constant, which can lead to improvement in detection sensitivity (signal-to-noise ratio). Several authors have described the relation between layer thickness (spacer thickness) in a thin layer flow cell and the measured current (S) as $S = k\ b^{-2/3}$, where b is the spacer thickness and k a constant.

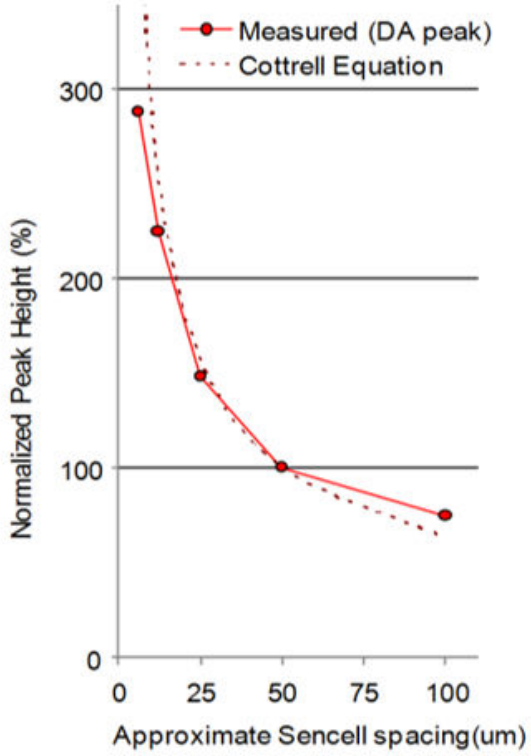
Note: The SenCell design eliminates the use of polymeric or metal gaskets. The working volume of the electrochemical cell can be steplessly adjusted using the supplied adjustment key (116.1400) without opening the cell. This allows easy optimization of the cell working volume and thus detection sensitivity (signal-to-noise ratio) for any LC application.

Figure 15–4: Example chromatograms of 100 nM standard of catecholamines in 10 mM HAc recorded with the SenCell spacing adjustment set to position 3 and 0.5, corresponding to an approximate spacing setting of 100 µm and 12 µm, respectively



The preceding chromatogram images show an example to demonstrate the effect of cell working volume on signal. In the following image on Normalized peak height of dopamine, the peak height (normalized) as a function of spacing is shown for dopamine based on the data from the example in the preceding image.

Figure 15–5: Normalized peak height of dopamine as a function of spacing setting (red curve) based on chromatograms recorded with a 100 nM standard of catecholamines in 10 mM HAc with a SenCell



The dotted curve is a simulated curve based on the Cotrell equation (F.G. Cottrell, Z. Phys. Chem 42 (1903) 385).

Decreasing the spacing/working volume is limited by an increased pressure drop over the flow cell that will eventually lead to an obstruction of the flow. The onset is typically characterized by an increased noise level and a rise in system back pressure.

Warning: Applying low working volume settings should be done with great care because it may cause excessive pressure buildup over the flow cell, excessive baseline noise, and damage to the cell. Do not operate the cell at position 0.

It is evident that noise remains relatively constant as a function of spacing, but at a spacing of approximately 6 µm a significant increase in noise is observed, accompanied by a rise in system pressure as a restriction builds over the cell. So in this example, setting the cell spacing less than approximately 12 µm is not advisable.

Figure 15–6: ASTM noise values as a function of cell spacing

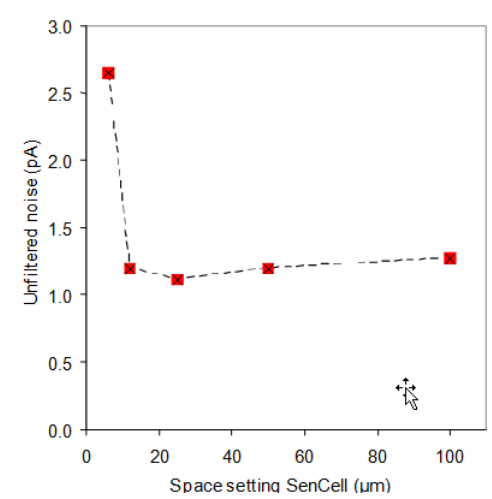
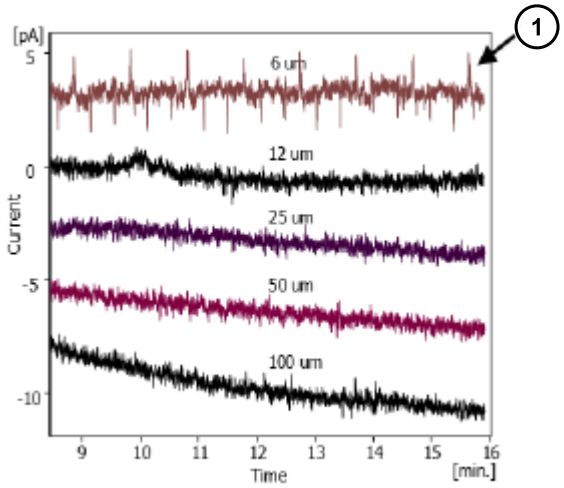


Figure 15–7: Noise traces as a function of cell spacing



1 Pump pulsations and pressure rise

SenCell spacing position 3 corresponds to approximately 100 ± 10 µm. The spacings used with the SenCell under test in this experiment were determined using a stylus profilometer.

Note: Optimization of the cell working volume focuses on finding the right balance between signal height and noise level for your SenCell, under your specific LC-EC conditions. You can achieve optimization by decreasing the cell spacing in small steps and evaluating the baseline noise and peak height of the analytes of interest until you find the optimal signal-to-noise ratio. Note that with LC applications using larger ID columns in combination with higher flow rates, the minimum spacing that can be used is larger.

Inexperienced users should use the factory-preset cell working volumes (position 1 or 2) with their SenCell.

15.2 Reference electrodes

The SenCell is available with an ISAAC (in situ Ag/AgCl) reference electrode, a salt bridge Ag/AgCl reference electrode, or a HyREF reference electrode.

15.2.1 ISAAC reference electrode

The ISAAC reference electrode is in direct contact with the mobile phase, which contains chloride ions. The chloride concentration determines the potential, so each time a fresh mobile phase is prepared it should contain exactly the same concentration of chloride ions. The standard electrode potential XE "Ag/AgCl reference electrode:standard electrode potential" of the Ag/AgCl electrode (in 1.0 mol/L Cl^- solution) for the following half-reaction is defined as E^0:

$$AgCl(s) + e^- <=> Ag(s) + Cl^- \qquad E^0 = 0.222\ V$$

The potential of the REF is dependent on the chloride concentration as described by the following equation:

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

where R is the gas constant (8.314 $Jmol^{-1}K^{-1}$), T is the absolute temperature (293 K), and F is the Faraday constant (96485 $Cmol^{-1}$).

The potential of the ISAAC at 2 mmol/L KCl is 379 mV (see the table on Potential of the Ag/AgCl reference electrode). The potential difference (dE) between the saturated KCl Ag/AgCl reference electrode and the ISAAC is 189 mV. If an application is running at 800 mV (vs. Ag/AgCl with saturated KCl), the potential setting using the ISAAC should be 611 mV (vs. Ag/AgCl in 2 mmol/L KCl).

Figure 15–8: Dependence of the Ag/AgCl REF potential on the chloride concentration

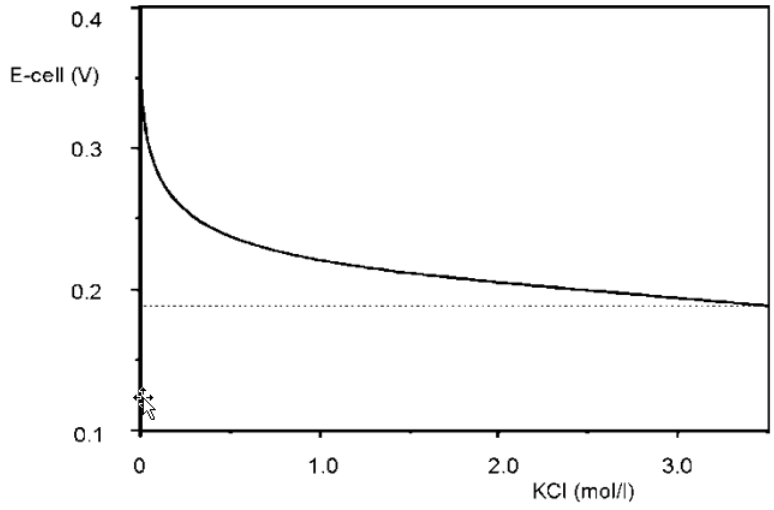


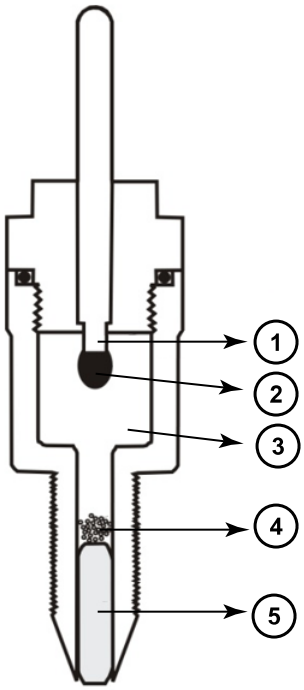
Table 15–3: Potential of the Ag/AgCl reference electrode

Cl^- (mmol/l)	E Ag/AgCl (mV)[a]	dE (mV)[a]
3500	190	0
2500	199	8
1500	212	21
500	240	49
100	280	90
20	321	130
10	338	148
8.0	344	154
6.0	351	161
4.0	361	171
2.0	379	189
1.0	396	206
0.5	414	224

a. Rounded to the nearest whole number

The addition of chloride to the mobile phase has a few restrictions. For example, the ISAAC is not recommended at a high working potential (> 1.2 V vs. Ag/AgCl in 2 mmol/L KCl) because Cl^- is oxidized and contributes to the background current. In ion chromatography, the addition of Cl^- may lead to undesired chromatographic changes. With a silver working electrode, the addition of Cl^- to the mobile phase causes formation of an AgCl coating on the working electrode, leading to inactivation. At high pH or high modifier concentrations the ISAAC is less suitable; a HyREF is recommended.

Figure 15–9: Schematic representation of the Ag/AgCl reference electrode



1 Ag

2 AgCl coating

3 KCl saturated

4 KCl crystals

5 Cotton wool

15.2.2 Salt bridge Ag/AgCl reference electrode

The reference electrode of the Ag/AgCl type with salt bridge consists of a silver rod, coated with solid AgCl and immersed in a solution of saturated KCl containing KCl crystals. 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. This REF for the SenCell is factory-filled with KCl. For certain applications, another chloride salt is preferred. With perchlorate-containing mobile phases, sodium chloride is mandatory, because potassium perchlorate precipitates and will clog the cotton wool frit. At high modifier percentages, the REF must be filled with lithium chloride, for similar reasons.

15.2.3 HyREF reference electrode

The HyREF is a hydrogen reference electrode. Its potential depends on the pH of the mobile phase. The HyREF is fully comparable to the standard Ag/AgCl REF with respect to baseline stability and S/N ratio. The HyREF is more user-friendly, and in principle this REF is completely free of maintenance requirements. Trapping of air bubbles, as is seen with the salt bridge Ag/AgCl type, is impossible because there is no salt bridge. Consequently, refilling the REF with saturated KCl is not required. Due to the absence of a salt bridge and its inertness, the HyREF is an excellent alternative to the Ag/AgCl REF, especially in cases of high modifier concentration (analysis of fat-soluble vitamins) or high pH (analysis of carbohydrates, PAD).

Depending on the pH of the mobile phase, the potential setting of the working electrode vs. the HyREF may differ significantly compared with Ag/AgCl. I/E curves, showing a shift of more than 200 mV at pH 3.1 (for example, catecholamines); no shift appears at pH 12 (for example, PAD of carbohydrates). Therefore, it is best to first construct a hydrodynamic (or scanning) voltammogram when using the HyREF. In the following "Measured cell potential (HyREF - Ag/AgCl) versus pH" table, the potential of the HyREF is measured against the Ag/AgCl (in saturated KCl) electrode at different pH values.

Table 15–4: Measured cell potential (HyREF - Ag/AgCl) versus pH

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

So, if an Ag/AgCl REF is replaced by a HyREF, the pH effect must be taken into account (refer to the "Measured cell potential (HyREF - Ag/AgCl) versus pH" table). The pH vs. voltage relation is described by: $E_{HyREF} = E_{Ag/AgCl} - 328 + 29.9\ pH$

Example: A working potential of 800 mV (vs. Ag/AgCl with saturated KCl) at pH 3 must be changed to: $E_{HyREF} = 800 - 328 + 29.9*3 = 561.7$ mV (vs. HyREF).

15.3 Installation

15.3.1 Introduction

The SenCell is delivered preassembled and ready for installation and use. For cell assembly instructions, see the Assembling/disassembling the cell (Page 139) chapter.

Figure 15–10: Left: Photo of assembled SenCell inlet block (green) with in situ Ag/AgCl (ISAAC) reference electrode (REF). Right: Bottom side of the SenCell with the cell working volume adjustment system, auxiliary (AUX) electrode contact (opening on left-hand side, next to inscription '3'), and working electrode (WE) contact (opening in the center).

The working volume is preset at the factory to position 2 (refer the preceding right-hand side image), corresponding to a spacing of approximately 50 ± 10 µm. This setting is recommended when using the SenCell in combination with standard-bore LC columns (2 mm - 4.6 mm ID) and flow rates > 200 µL/min. When using micro-bore LC columns, position 1 (25 ± 10 µm) is recommended for working volume.

15.3.2 Adjusting the SenCell working volume

Figure 15–11: Adjustment of the working volume from position 2 (left) to position 1 (right)

To adjust the working volume from position 2 to position 1:

1. Insert the pins of the adjustment key (700013143) in the two holes on the bottom side of the SenCell. Note that the diameters of the two pins differ. The larger-diameter pin should be inserted in the AUX contact.
2. Turn the adjustment key counterclockwise until the marker on the outer metal ring aligns exactly with the marker indicating position 1 (red arrow).
3. Remove the adjustment key.

Warning: Applying low working volume settings should be done with great care because it may cause excessive pressure buildup over the flow cell, excessive baseline noise, and damage to the cell. Do not operate the cell at position 0.

15.3.3 Installation in LC system

Prior to installation of the SenCell, ensure that the following precautions are followed.

1. For optimal performance, all metal parts in your HPLC system should be passivated with 30% Phosphoric Acid.
2. Before connecting a new column, read the manufacturer's instructions. Our experience is that thorough preconditioning of a column is always required. Only a preconditioned column is electrochemically clean. Otherwise, the background current may be unacceptably high and substantial fouling of the working electrode may occur. For reverse-phase columns, flushing with 50% methanol in water for three days at a low flow rate is highly recommended.
3. Before connecting the flow cell, ensure that the LC system with column is well equilibrated with mobile phase prepared using high-purity chemicals.

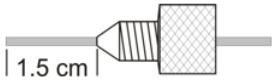
   Notice: If an ISAAC reference electrode is used, ensure that the mobile phase contains at least 2 mmol/L chloride (KCl or NaCl) ions.
4. Passage of air bubbles through the flow cell will lead to unacceptable noise levels and spikes. The use of an in-line degasser is therefore strongly recommended. A one-time degassing step for the HPLC buffer is almost never sufficient.

15.3.4 Installing the SenCell

Follow this procedure to install the SenCell.

To install the SenCell:

1. If applicable, install the SenCell clamp (700013160) from the startup kit in the center position of the 3465 Detector with a Phillips screwdriver.
2. Connect the column to the flow cell inlet with 1/16-inch OD small-bore PEEK tubing (0.3-mm ID or smaller depending on the column bore size) using the PCTFE 10-32 finger-tight fitting (700013151). Use only factory-supplied finger-tights in the flow cell; others may cause serious damage.



   Let the tubing protrude about 1.5 cm from the finger-tight fitting and tighten it such that the tubing is not or only slightly indented by the fitting. Do not over-tighten the finger-tight fitting. Over-tightening affects the flow pattern through the tubing and may strongly decrease flow cell performance.

3. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only factory-supplied finger-tight fittings in the flow cell; others may cause serious damage. Do not over-tighten the fitting.
4. Turn on the HPLC pump. Keep some tissues at hand because you will probably spill some mobile phase during this mounting procedure.
5. For a SenCell with HyREF (black inlet block) or ISAAC reference (green inlet block): Fill the flow cell while keeping it in an angle of about 45° with the outlet (LC out) to force air through the outlet.

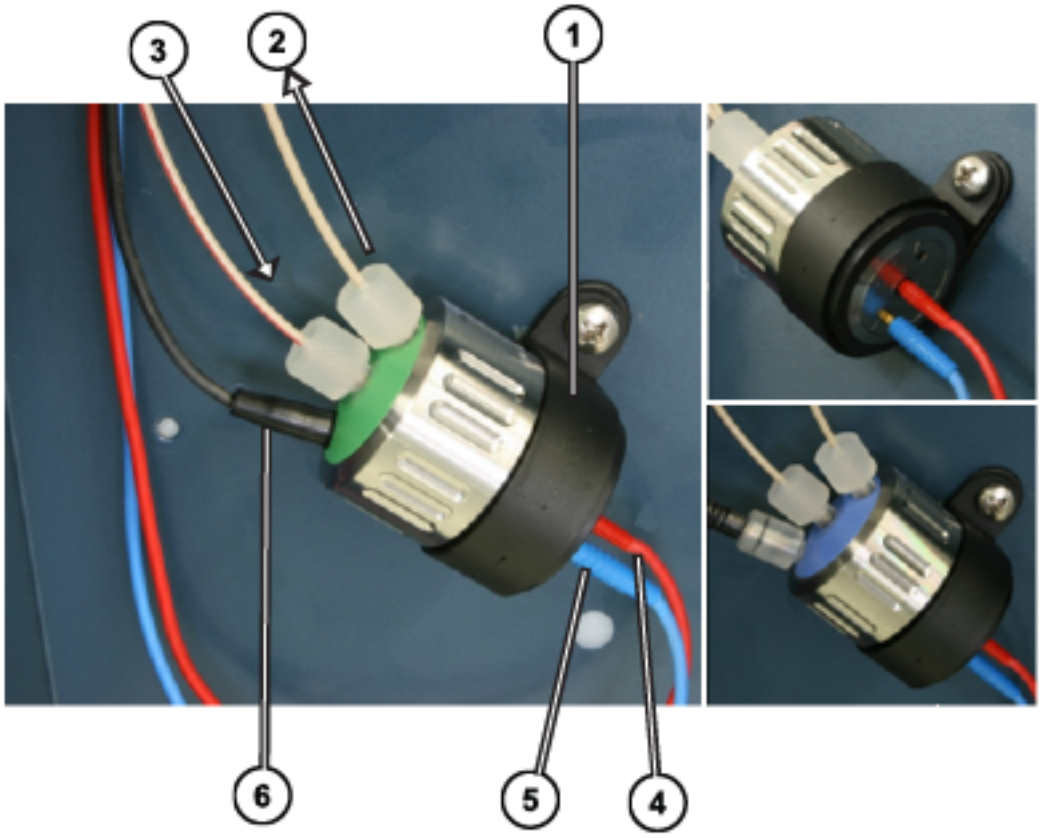
   Note: For a SenCell with salt bridge reference (blue inlet block): Fill the flow cell while keeping it in an angle of 45° with the REF fitting on top; this is best done by blocking the outlet with a finger and letting the air escape via the REF fitting. Carefully check the thread of the fitting for trapped air bubbles.

   When the REF fitting is completely filled with mobile phase, mount the REF while slowly releasing the outlet. Make sure not to entrap an air bubble.
6. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This prevents trapping of air bubbles.
7. Connect the cell cable as shown in the next figure. Red: WE contact, blue: AUX contact, and black: REF contact.
8. Switch ON the SenCell and let the cell backcurrent stabilize before starting your (U)HPLC-ECD analysis.

   Notice: The cell connector inside the oven compartment is electrostatic sensitive. Ensure that the flow cell is OFF when removing or connecting the cell cable. Never switch ON the flow cell when:

   - The cell cable is not correctly connected.
   - The cell is only partly (or not at all) filled with mobile phase/buffer/electrolyte.
   - The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection, because substantial damage to the working electrode or electronics may occur.

Figure 15–12: SenCell mounted at an angle of approximately 45° in the 3465 Detector



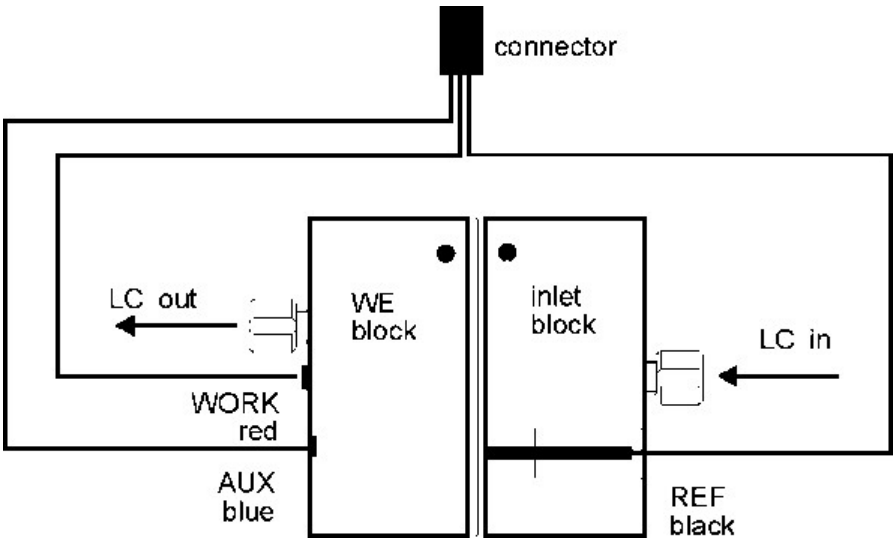
1. Cell clamp
2. Cell outlet (tubing connection from cell to waste); make sure that the outlet is positioned on the top side to prevent entrapped air bubbles
3. Cell inlet (tubing connection from column to cell)
4. WE contact (red)
5. AUX contact (blue)
6. REF contact (black)

Top Left: SenCell with ISAAC reference electrode (green inlet block). Top-right: Electrical connections of WE (red connector) and AUX electrode (blue connector). Bottom-right: SenCell with salt bridge reference electrode (blue inlet bock).

Figure 15–13: Installation of SenCell in 3465 Detector: oven compartment with column and SenCell installed

Make the electrical connections as depicted in the following figure.

Figure 15–14: Installation of flow cell



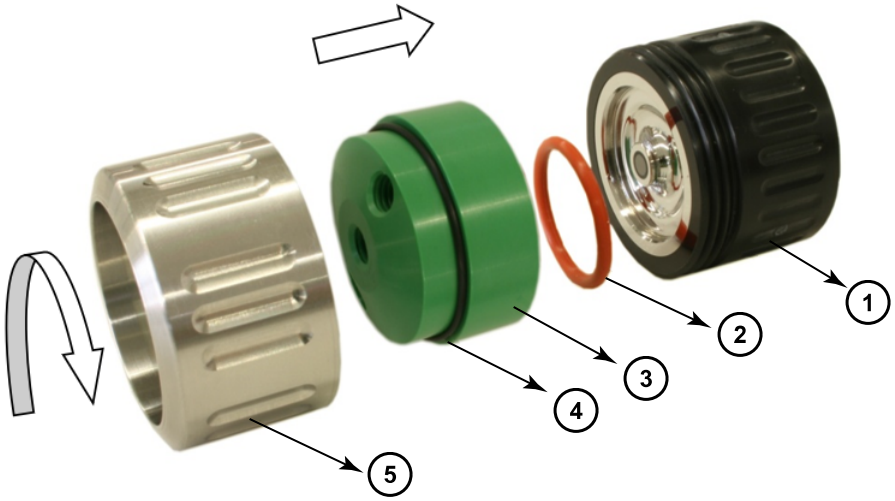
WORK, AUX, and REF are connected using the red, blue, and black cell cables.

Note: LC out should be on top to prevent entrapment of bubbles.

15.4 Maintenance

15.4.1 Assembling/disassembling the cell

Figure 15–15: Exploded view of SenCell



1. WE block
2. O-ring
3. Inlet block
4. Inlet block O-ring
5. Closing ring

The arrows indicate how to assemble the cell.

Figure 15–16: Left: unscrewing the salt bridge electrode. Right: WE block with O-ring placed in O-ring groove.

15.4.1.1 Disassembling the cell

To disassemble the cell:

1. Hold the cell in the upward position (with the metal closing ring on the top side).
2. For an SB inlet block (blue), first remove/unscrew the salt bridge REF from the inlet block. For other inlet blocks, skip this step. The salt bridge REF can be removed by turning the REF body counterclockwise.
3. Open the cell by turning the metal closing ring counterclockwise by hand.
4. Remove the inlet block.
5. Remove the silicon O-ring (orange/brown colored) from the WE block.

   Note: The cell is now fully disassembled.

15.4.1.2 Assembling the cell

To assemble the cell:

1. Ensure that all SenCell parts to be assembled are dry.
2. Check the back side of the WE block and verify that the cell working volume adjustment is set to position 1 or 2.

   Warning: Ensure that the SenCell is not set to position 0; this could cause damage to the WE or cell during assembly.
3. Hold the WE block in the upward position and insert the silicon O-ring into the groove. Before inserting the O-ring, verify that it is not damaged or swollen; replace it if necessary.

4. Confirm that the black inlet block O-ring is undamaged and properly mounted on the inlet block.
5. Place the inlet block on top of the WE block.
6. Place the metal closing ring over the inlet block and close the cell by turning the closing ring clockwise. Don’t over-tighten the closing ring.

   Note: The cell is now assembled and ready for installation/use.

15.4.2 HyREF

The HyREF reference electrode is in principle maintenance-free. When not in use, it should be stored dry.

15.4.3 ISAAC

The ISAAC reference electrode requires maintenance, but usually not more than once every three months. In practice this means that when the flow cell is opened to service the working electrode, the reference electrode should be serviced as well.

Servicing the reference electrode is done by polishing its surface, until the shining metal appears, and coating the surface with ISAAC solution. After coating, the ISAAC electrode surface should show a brownish/reddish matt finish (AgCl layer). If not used for a longer period of time, disassemble the flow cell. Clean the flow cell, including the reference electrode, with distilled water, dry it with a tissue, and store it dry.

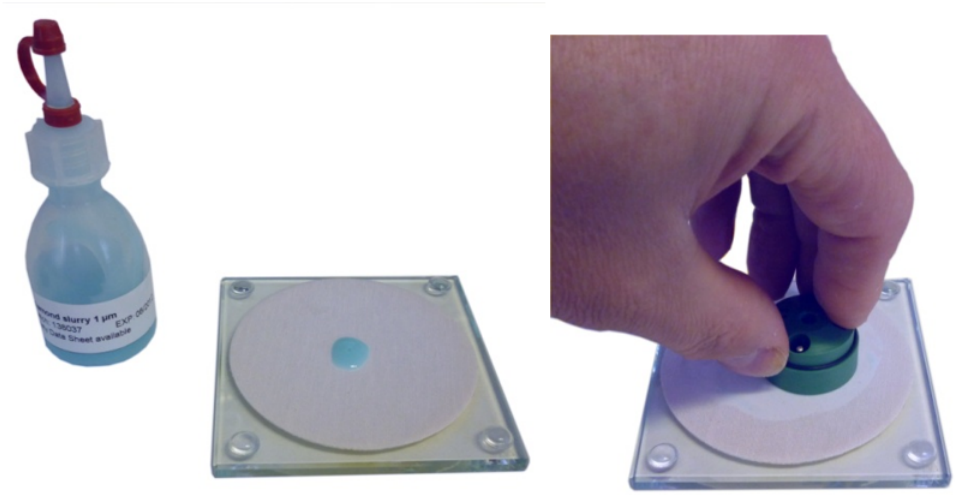
15.4.3.1 Polishing

Polishing the reference electrode is done using the factory-supplied polishing kit, which contains diamond slurry (700001955) and a polishing disc (700002069).

To initiate polishing:

1. Shake the diamond slurry thoroughly before use.
2. Rinse the polishing disc with demi-water before applying the diamond slurry.
3. Apply a few drops of slurry to the wetted polishing disc and polish the electrode with a figure-8 motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with a wetted tissue and inspect the surface visually. Repeat the procedure if necessary, until the shining metal REF surface appears.
5. Clean the polishing disc with demi-water.
6. Store the polishing disc in its plastic bag.

Figure 15–17: Apply a few drops on the polishing disc (left), and polish the electrode (right)



15.4.3.2 Coating with ISAAC solution

Warning: Take the necessary precautions (gloves, lab coat, and glasses) because the ISAAC solution is corrosive.

1. After polishing, coat the ISAAC REF with the factory-supplied ISAAC solution (700001949).
2. Apply the coating for 20 minutes.
3. Flush away the solution with distilled water. The ISAAC electrode surface should show a brownish/reddish matt finish (AgCl layer).

Figure 15–18: Left: ISAAC solution. ISAAC inlet block with a droplet of ISAAC solution applied on the freshly polished reference electrode

15.4.4 Ag/AgCl salt bridge

Three aspects determine the proper function of an Ag/AgCl 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 conduction.
- Air bubbles inside or close to the salt bridge will lead to instability of the three-electrode configuration. Because of their extreme compressibility, changes in conductivity and the ionic equilibrium of the REF occur. This increases the noise considerably.
- The REF is factory-filled with KCl unless specified otherwise. Other chloride salts should be used when the mobile phase contains perchlorate (use NaCl) or a high percentage of organic modifier (use LiCl).

15.4.4.1 Saturation and air bubbles

After prolonged use, the salt bridge in the REF will no longer be saturated, which usually leads to poor reproducibility in electrochemical detection. The potential of the REF is determined by the chloride concentration. If the salt bridge is not saturated and the KCl concentration changes:

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

Check your REF regularly. If you do not see chloride salt crystals, or if you see air bubbles, your REF needs maintenance.

15.4.4.2 Materials

- An over-saturated and thoroughly degassed KCl solution (700013253)
- SB ref tool (700013145)—not included in the SenCell ship kit; should be purchased separately
- Ordinary cotton wool

15.4.4.3 Procedure

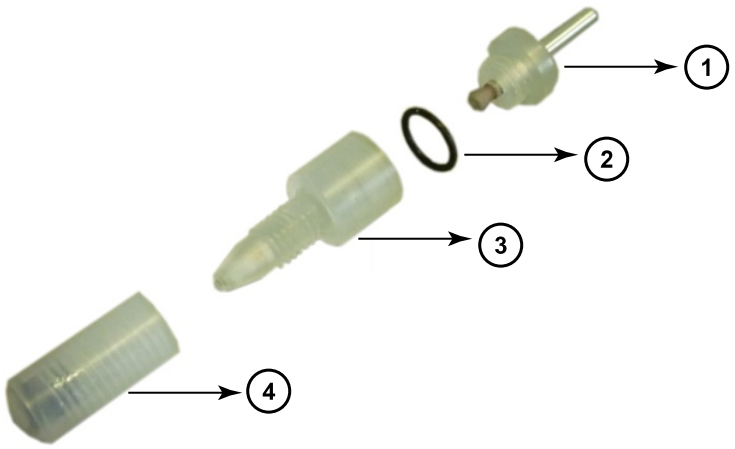
Follow the procedure to perform maintenance on Salt Bridge Reference.

Note: Use proper eye and skin protection when working with solvents.

Maintenance on Salt Bridge Reference:

1. Turn the cell OFF on the controller.
2. Stop the HPLC pump.
3. Disconnect the cell from the controller.
4. Remove the REF from the inlet block.
5. Disassemble the REF by unscrewing the black fitting.
6. Inspect the O-ring for wear, and especially the cotton wool frit; replace if necessary.

Figure 15–19: Exploded view of the reference electrode



1. Ag/AgCl electrode + fitting
2. O-ring
3. Salt bridge body
4. REF cap for storage and shipment

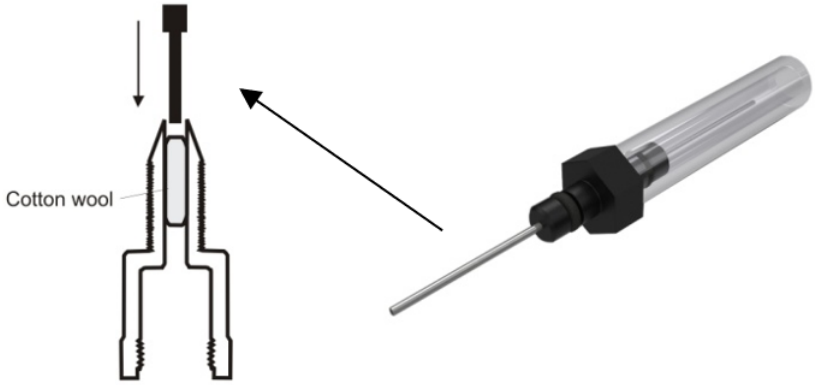
7. Remove the remaining KCl from the salt bridge.
8. Clean all parts with demi-water.
9. The frit in the salt bridge ensures electrical contact with the buffer. If the frit is discolored or dried out, it must be renewed—in which case, continue with step 1 in “Maintenance of the cotton wool frit”; otherwise, continue with step 7.

15.4.4.4 Maintenance of the cotton wool frit

Note: Use proper eye and skin protection when working with solvents.

Use the SB ref replacement tool (700013145) to push out the frit from the outside.

Figure 15–20: Pushing the cotton wool frit out with the SB ref replacement toot



Follow the procedure for the maintenance of the cotton wool frit:

1. Clean the salt bridge thoroughly with tap water then demi-water.
2. Saturate a small piece of cotton wool in KCl to prevent trapping of air within the wool.
3. Plug the salt bridge with the REF cap and fill the salt bridge to about 50%.
4. 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 firmly, because electrical conduction is essential.
5. Remove the cap.
6. Fill the salt bridge completely, add some KCl crystals out of a saturated solution to ensure prolonged saturation.
7. Place the O-ring into the groove of the salt bridge REF body and slowly insert the Ag/AgCl electrode into the chamber, at an angle of 45º. Make sure not to entrap an air bubble.
8. Tighten the fitting such that a small droplet appears at the end of the salt bridge, but do not over-tighten it.
9. Flush the complete, mounted REF with demi-water and dry it with a tissue, but keep the cotton wool frit soaked.
10. Visually inspect the REF for trapped air bubbles, and remove them if present (go back to step 7 or, if necessary, to step 1).

    Note: When not in use, please store the REF with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.

15.4.5 Working electrode

Cleaning of the working electrode block is necessary if the electrode surface has been electrochemically changed. This may happen due to fouling by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is evident from highly decreased sensitivity after prolonged use. As a rule of thumb, only polish if the surface of the working electrode lacks its mirror-like finish and cannot be restored by wiping the electrode surface with a tissue wetted with ethanol or acetone.

15.4.5.1 Decreased flow cell performance

Warning: Use proper eye and skin protection when working with solvents.

Several actions can be taken to address decreased flow cell performance. Avoid unnecessary polishing; take the next step only if the previous was not successful.

Steps to address decreased flow cell performance:

1. Electrochemical cleaning of glassy carbon WE: In the pulse mode, let the potential jump between +1 and -1 V for 10 minutes. Settings: t1 = 1000 ms, t2 = 1000 ms, t3 = 0 ms, E1 = +1V, and E2 = -1V.
2. Wiping the electrode surface with a tissue wetted with ethanol or acetone.
3. Polishing the electrode surface.

15.4.5.2 Polishing

Notice: Ensure that the SenCell is disassembled when setting it in position 0; otherwise, it may damage the WE electrode surface.

Disassemble the SenCell. Set the WE block in position 0 (polishing position) using the adjustment key (700013143) and turning it counterclockwise.

Figure 15–21: SenCell WE block with working volume set to position 0

To polish the SenCell:

1. Shake the diamond slurry (700001955) thoroughly before use.
2. Rinse the polishing disk with demi-water before applying the diamond slurry.
3. Apply a small amount (a few drops is sufficient) of slurry on the wetted polishing disk, and polish the electrode with a figure-eight motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with an ethanol-wetted tissue and check the surface visually. Repeat the procedure if necessary.
5. Reassemble the detector cell.
6. Clean the polishing disk with demi-water.
7. Store the polishing disk in its plastic bag.

15.5 Specifications

Table 15–5: Specifications for SenCell

Specifications	Description
Cell type	Three-electrode, wall-jet flow cell
Cell working volume (based on 2 mm Ø WE)	0 — 300 nL (steplessly adjustable)
Total cell volume	Approximately 0.5 mL
Working electrode diameter	2 mm
Working electrode (WE)	Glassy carbon
Reference electrodes	SB (Salt bridge Ag/AgCl), ISAAC (in situ Ag/AgCl), HyREF (Pd/H_2)
Auxiliary electrode	Stainless steel L316
Wetted materials	PCTFE, glassy carbon, stainless steel L316, PEEK, silicone, REF material (palladium or Ag and AgCl)
Max. pressure	5 bar (73 psi)
Fluidics connections	1/16-inch o.d. PEEK tubing with 10-32 PCTFE finger-tight connections
Electric connections	Cell cable for use with 3465 Detector

15.6 Parts list

Table 15–6: SenCell parts

Part number	Description
700013136	SenCell O-ring Silicone, 4 pcs
700013137	SenCell sb REF O-ring Silicone, 4 pcs
700013138	SenCell inlet block O-ring FKM, 1 pc
700013139	SenCell sb REF
700013141	SenCell sb REF body
700013142	SenCell sb REF cap
700013143	SenCell key for adjusting working volume
700013144	SenCell closing ring
700013052	SenCell 2 mm GC sb
700013070	SenCell 2 mm GC ISAAC
700013055	SenCell 2 mm GC HyREF
700013130	SenCell WE block 2 mm GC WE
700013133	SenCell inlet block sb REF (w/o sb REF)
700013134	SenCell inlet block ISAAC
700013135	SenCell inlet block HyREF
700001954	Polishing disc for WE
700002069	Polishing disc for REF
700013145	Sb REF replacement tool
700001955	10 mL diamond slurry 1 µm
700013253	30 mL KCl solution sat'd with AgCl
700001949	ISAAC solution 10 mL
700013151	Finger-tight fitting PCTFE 10-32, 4 pcs

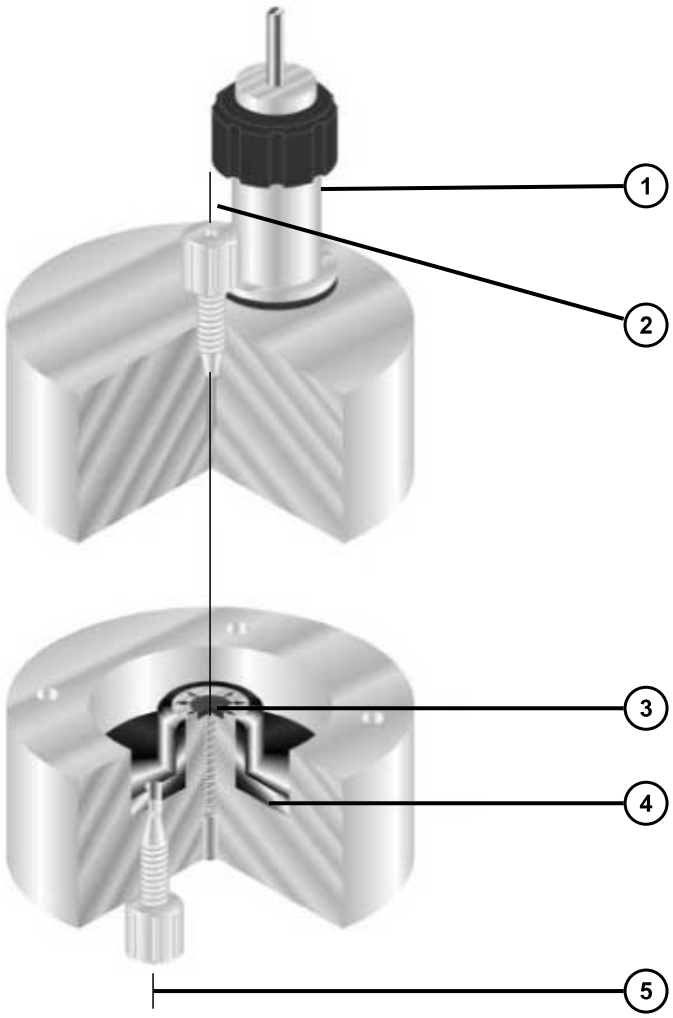
16 VT-03 flow cell

16.1 The FlexCell

16.1.1 Introduction

The VT-03 flow cell is available with a glassy carbon, platinum, gold, silver, or copper working electrode. In combination with the spacer set (25, 50 and 120 µm) a variety of detection volumes (down to 5 nL) can be attained. As a standard, the salt bridge Ag/AgCl reference electrode is recommended. For special applications the HyREF reference electrode is available. A third reference electrode is the in situ Ag/AgCl (ISAAC).

Figure 16–1: The VT-03 electrochemical flow cell



1 REF

2 Inlet

3 WE

4 AUX

5 Outlet

The upper part, the inlet block, is separated from the working electrode block by means of a gasket (spacer, not shown).

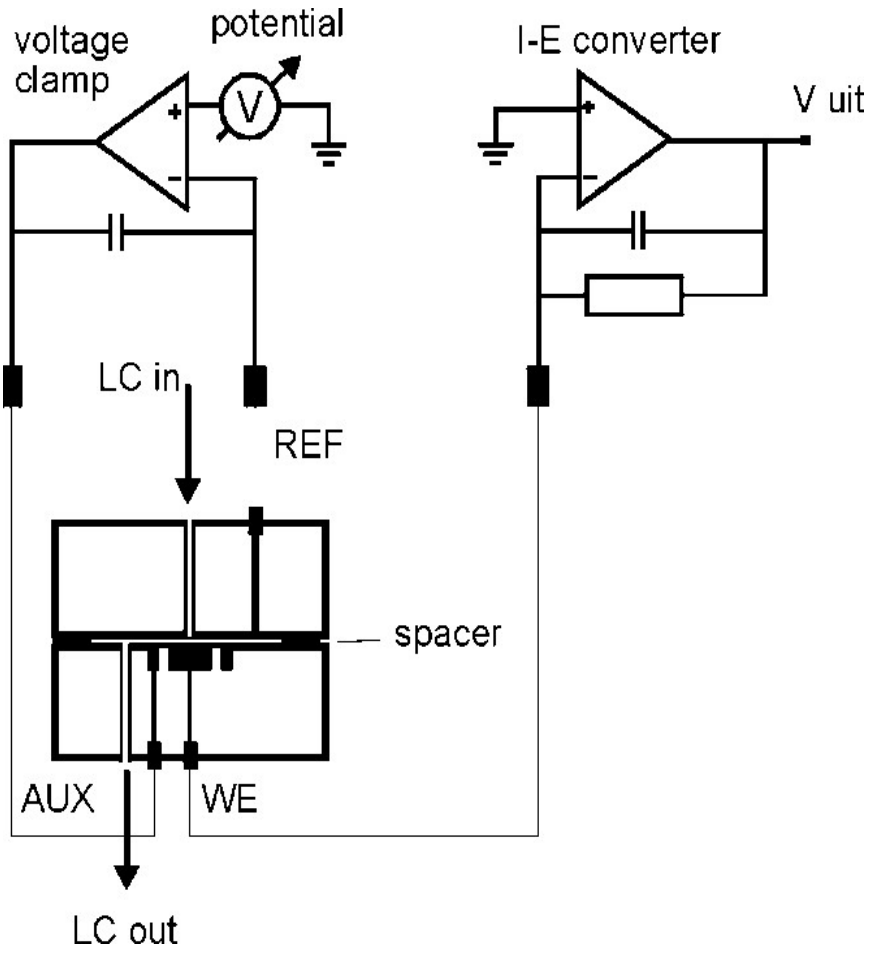
The VT-03 electrochemical flow cell was developed for ultra-trace analysis in standard, microbore, and capillary LC-EC. Extensive testing established that the confined wall-jet

configuration gives the best results. In addition, it was found that the quality and finishing of the electrode materials in the flow cell are decisive factors in the performance of an EC detector. While competitive designs usually deteriorate when in use, this flow cell, by design, improves in performance. The flow cell permits unusually short stabilization times. Trace analysis within half an hour after startup may be expected.

16.1.2 Three-electrode configuration

The VT-03 flow cell uses a three-electrode configuration (Figure 16–2: Schematic of an electrochemical cell with a three-electrode configuration (Page 152)). The working potential is set between the working electrode (WE) and the auxiliary electrode (AUX). The AUX is kept at a precisely defined reference electrode (REF) potential by means of a “voltage clamp”, an electronic feedback circuit that compensates for polarization effects at the electrodes. At the WE, which is kept at virtual ground, the electrochemical reaction takes place (that is, electrons are transferred at the WE). This results in an electrical current to the I/E converter, which is a special type of operational amplifier.

Figure 16–2: Schematic of an electrochemical cell with a three-electrode configuration



Essentially, for the oxidation or reduction reaction, it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration.

If the working potential were applied only over an AUX versus the WE (without REF), the working potential would continuously change due to polarization effects at the electrodes, resulting in highly unstable working conditions.

If the working potential were applied only over the REF versus the WE (without AUX), the working potential would be very well defined. However, the potential of a REF is only well defined if the current drawn is extremely low (pico-amperes), resulting in a very limited dynamic range.

A three-electrode configuration combines the best of both electrodes. The REF stabilizes the working potential and the AUX can supply high currents. This results in a tremendous dynamic range.

16.1.3 Working electrode

Electrochemical detection puts high demands on the WE material. The WE should be made of an electrochemically inert material. Furthermore, to avoid an irregular flow profile over the electrode, it should have a very well defined surface. Finally, it is important that the analyte of interest can be oxidized (or reduced) with favorable I/E characteristics. This in fact means that a high signal must be obtained at a low working potential. For most applications, glassy carbon is the WE material of choice. Under certain circumstances, other materials are favorable.

For example, in the analysis of iodide, a silver WE can be used. At a silver WE, the following oxidation reaction occurs for iodide: $Ag + I^- \rightarrow AgI + e^-$

This reaction already takes place at a very low working potential (1 mV), which results in extremely high selectivity. This allows the identification of iodide in urine samples almost without any sample pretreatment.

Table 16–1: Working potential limits and application areas for various WE materials

WE material	Potential limits (V)				Major application
	Alkaline		Acidic		
Glassy carbon	-1.50	+0.60	-0.80	+1.30	Catecholamines
Gold	-1.25	+0.75	-0.35	+1.10	Carbohydrates
Platinum	-0.90	+0.65	-0.20	+1.30	Alcohols, glycols
Silver	-1.20	+0.10	-0.55	+0.40	Halides, cyanide
Copper	-	+0.60	-	-	Amino acids, carbohydrates

Another consideration in choosing a WE is the oxidation or reduction of mobile phase constituents or WE material that occurs when the potential exceeds the limits as given in the above table. At high positive working potentials, water in the mobile phase electrolyzes and results in strong increases in background current and noise. Formation of metal oxides, resulting in an increase in 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 ECD. For negative potentials, the use of platinum electrodes is limited by the ease of reducing hydrogen ions to hydrogen gas.

16.1.4 Detection limit

One of the most important parameters used to characterize the performance of a detection system is the signal-to-noise ratio (S/N ratio) from which the concentration detection limit derives. It enables objective comparison not only between different electrochemical detectors but also between complete analytical methods, regardless of which detection system is used.

Table 16–2: LC-EC conditions for analysis of norepinephrine

Column	ODS-2, 3 µm, 100 x 4.6 mm
Flow rate	1.0 mL/min
Mobile phase	H_3PO_4 50 mM, citric acid 50 mM, 20 mg/L EDTA, 100 mg/L octane sulphonic acid (OSA), pH=3.1 with KOH, 5% methanol
Sample	1.0 µmol/L norepinephrine, 20-µL injection
Temperature	30 °C
Flow cell	VT-03 flow cell with 3-mm GC WE mounted with 50-µm spacer
E cell	800 mV (vs. Ag/AgCl, filled with saturated KCl)
I_{cell}	Approx. 3 nA

Industry literature describes several ways to determine the detection limit. In principle, it does not matter which definition of detection limit is used, as long as the definition is precisely described.

In this manual, the concentration detection limit (cLOD) for a compound is defined as the analyte concentration that results in a signal that is three times the standard deviation of the noise:

$$c_{LOD} = \frac{3 \cdot \sigma_{noise}}{signal} c_A$$

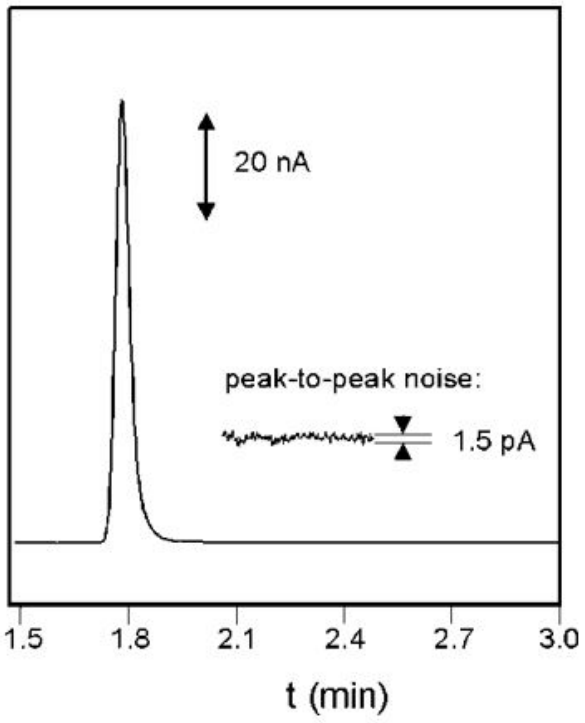
where sigma-noise is 0.2 x peak-to-peak noise and cA is the concentration of analyte injected.

Figure 16–3: Typical S/N ratio for norepinephrine measured with a VT-03 glassy carbon flow cell (peak height: 80 nA, peak-to-peak noise: 1.5 pA) (Page 155) shows a typical S/N ratio of a VT-03 glassy carbon flow cell with 2.74-mm WE. In this example the concentration detection limit for norepinephrine, based on three times the sigma-noise, is 11 pmol/L (refer to the "LC-EC conditions for analysis of norepinephrine" table).

Expressing the performance of a detection system by only the peak height makes no sense. A system can easily be changed so that a larger peak height is obtained. However, if the noise increases similarly, it has the same effect as switching a recorder to a higher sensitivity: peaks appear higher but the S/N ratio is the same.

Expressing the limit of detection in an absolute amount (that is, in picomoles) without mentioning the injection volume makes comparison between different systems difficult.

Figure 16–3: Typical S/N ratio for norepinephrine measured with a VT-03 glassy carbon flow cell (peak height: 80 nA, peak-to-peak noise: 1.5 pA)



The amount injected is 20 pmol (1.0 µmol/L). The concentration detection limit based on three times the sigma-noise is 11 pmol/L.

16.1.5 Working electrode diameter

The size of the WE is an important factor in LC-EC; it affects both the signal and the noise. For the VT-03 flow cell, several glassy carbon WE diameters are available (0.7, 2, and 3 mm). In a standard LC system, the signal and the noise increase linearly with the WE diameter. This means that the S/N ratio remains more or less the same. In micro-LC, an increase in WE diameter increases the noise more than the signal. In micro-LC, a decrease in WE diameter results in a better S/N ratio.

Table 16–3: Flow cell recommendations

Column diameter (mm)	Recommended flow cell
3 and higher	3 mm GC
3 - 1	2 mm GC
1 and below	0.7 mm µGC

The choice of flow cell is based primarily on the HPLC column diameter. This way the best possible detection limit for a standard, microbore, or capillary column is warranted.

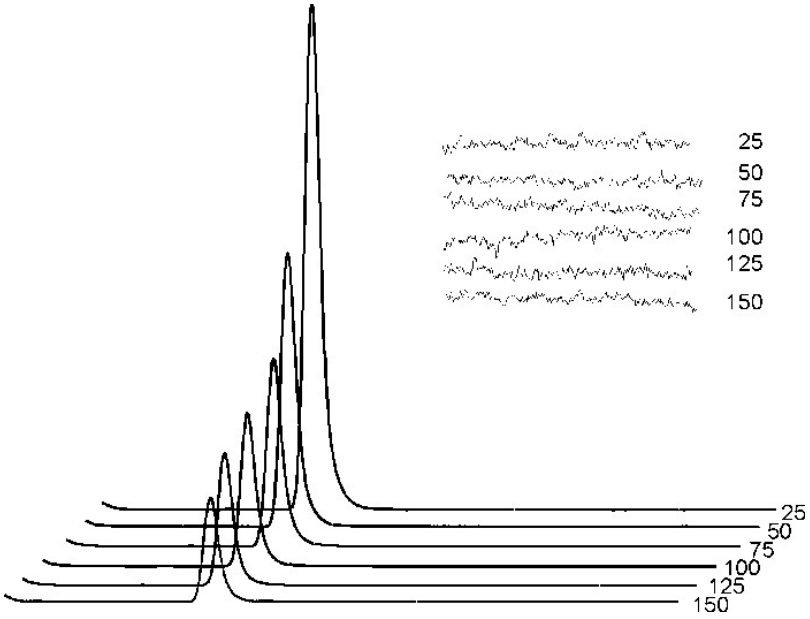
The recommended combinations give the best S/N ratios. Keep in mind that other combinations are possible that still result in acceptable sensitivities for many applications. All VT-03 flow cells are individually tested and meet our high standards of quality and detection sensitivity.

16.1.6 Spacer thickness

The thickness of the gasket affects the linear flow velocity in the cell. With a thinner spacer the cell volume decreases (Table 16–4: Flow cell volume (Page 158)), resulting in higher linear flow velocity. The signal increases with thinner spacers, while the noise remains more or less constant (Figure 16–4: Signal to Noise for 1.0 µmol/L norepinephrine (Page 157)).

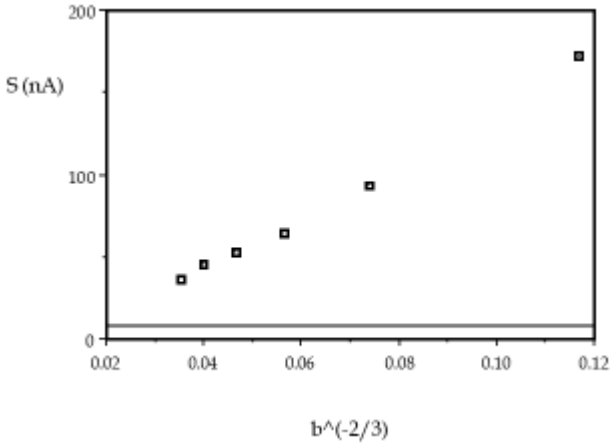
Several authors have described the relation between the layer thickness (spacer thickness) in a thin-layer flow cell and the measured current (S) as $S = k\ b^{-2/3}$, where b is the spacer thickness and k a constant. Also, for the VT-03 flow cell the relation between S and b-2/3 results in a straight line (Figure 16–5: Peak height versus spacer thickness to the power -2/3 (Page 158)).

Figure 16–4: Signal to Noise for 1.0 µmol/L norepinephrine



The signal and noise for 1.0 µmol/L norepinephrine measured at variable spacer thickness (given in µm). See Table 16–2: LC-EC conditions for analysis of norepinephrine (Page 154) for other conditions.

Figure 16–5: Peak height versus spacer thickness to the power -2/3



Decreasing the spacer thickness is limited by an increased pressure drop over the flow cell that eventually leads to an obstruction of the flow. The minimum spacer thickness available is 25 µm. Applying these small spacers should be done with care. Over-tightening of the bolts may cause an excessive pressure buildup over the flow cell and increase the noise considerably.

Table 16–4: Flow cell volume

	WE diameter (mm)							
	3.00	2.74	2.54	2.00	1.90	1.00	0.75	0.50
Spacer (µm)	Cell volume (µL)							
25	0.18	0.15	0.13	0.08	0.07	0.020	0.011	0.005
50	0.35	0.29	0.25	0.16	0.14	0.039	0.022	0.010
120	0.85	0.71	0.61	0.38	0.34	0.094	0.053	0.024

16.2 Reference electrodes

The VT-03 flow cell is available with an ISAAC (in situ Ag/AgCl) reference electrode, a salt bridge Ag/AgCl reference electrode, or a HyREF reference electrode.

16.2.1 ISAAC reference electrode

The ISAAC reference electrode is in direct contact with the mobile phase, which contains chloride ions. The chloride concentration determines the potential, so each time a fresh mobile phase is prepared it should contain exactly the same concentration of chloride ions.

The standard electrode potential of the Ag/AgCl electrode (in 1.0 mol/L Cl solution) for the following half-reaction is defined as E^0:

$AgCl(s) + e^- <=> Ag(s) + Cl^-$ $E^0 = 0.222\ V$

The potential of the reference electrode (REF) depends on the chloride concentration as described by the following equation:

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

where R is the gas constant (8.314 $Jmol\text{-}1K^{-1}$), T is the absolute temperature (293 K), and F is the Faraday constant (96485 $Cmol^{-1}$). The potential of the ISAAC at 2 mmol/L KCl is 379 mV. The potential difference (dE) between the saturated KCl Ag/AgCl reference electrode and the ISAAC is 189 mV. If an application is running at 800 mV (vs. Ag/AgCl with saturated KCl), the potential setting using the ISAAC should be 611 mV (vs. Ag/AgCl in 2 mmol/L KCl).

Figure 16–6: Dependence of the Ag/AgCl REF potential on the chloride concentration

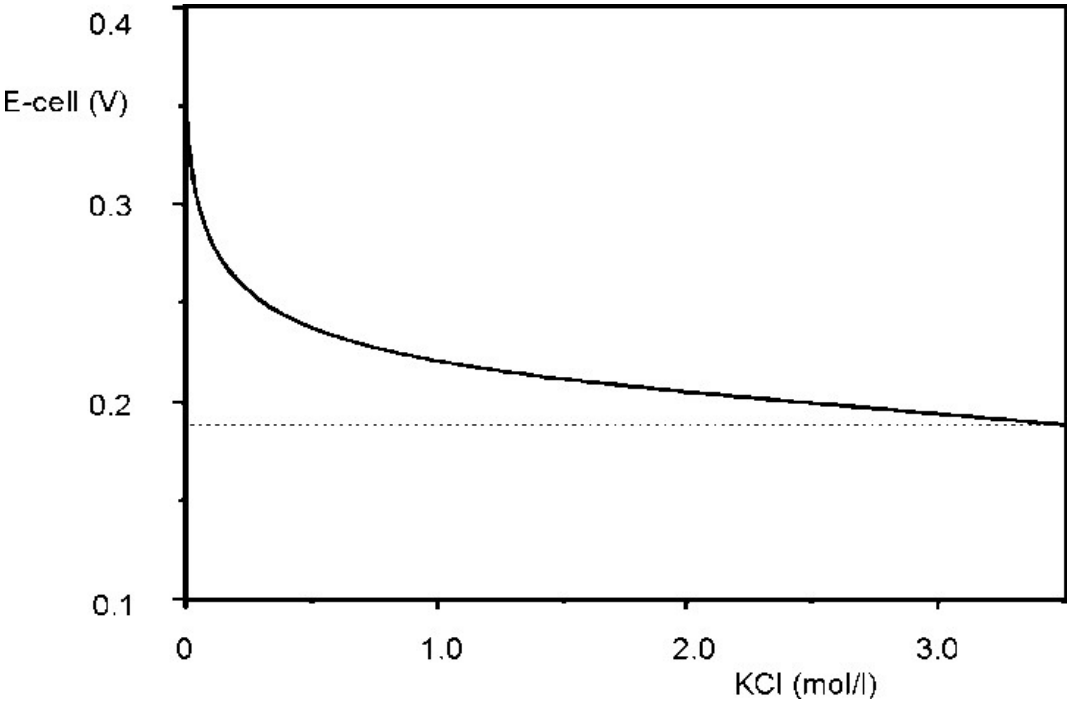


Table 16–5: Potential of the Ag/AgCl reference electrode; dE is the potential difference with EAg/AgCl in saturated KCl

Cl^-(mmol/L)	$E_{Ag/AgCl}$ (mV)[a]	dE (mV) [a]
3500	190	0
2500	199	8
1500	212	21
500	240	49
100	280	90
20	321	130

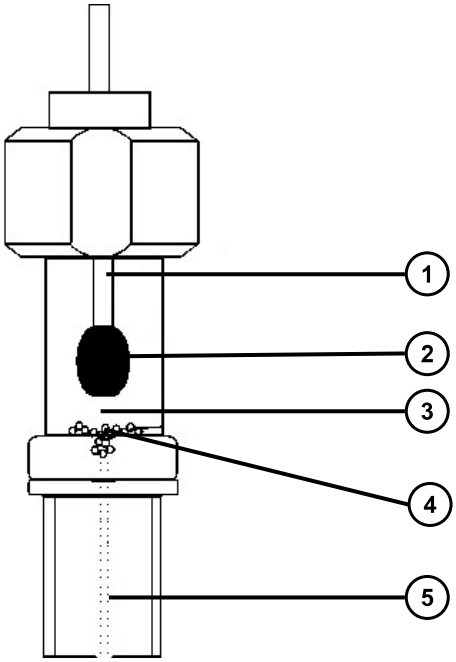
Table 16–5: Potential of the Ag/AgCl reference electrode; dE is the potential difference with EAg/AgCl in saturated KCl (continued)

Cl^-(mmol/L)	$E_{Ag/AgCl}$ (mV)[a]	dE (mV) [a]
10	338	148
8.0	344	154
6.0	351	161
4.0	361	171
2.0	379	189
1.0	396	206
0.5	414	224

a. Rounded to the nearest whole number

The addition of chloride to the mobile phase has a few restrictions. For example, the ISAAC is not recommended at a high working potential (> 1.2 V vs. Ag/AgCl in 2 mmol/L KCl) because Cl- is oxidized and contributes to the background current. In ion chromatography, the addition of Cl- may lead to undesired chromatographic changes. With a silver working electrode, the addition of Cl- to the mobile phase causes formation of an AgCl coating on the working electrode, leading to inactivation. At high pH or high modifier concentrations, the ISAAC is less suitable and a HyREF is recommended.

Figure 16–7: Schematic representation of the Ag/AgCl reference electrode



1. Ag
2. AgCl coating
3. KCl saturated
4. KCl crystals
5. Cotton wool

16.2.2 Salt bridge Ag/AgCl reference electrode

The Ag/AgCl reference electrode with salt bridge consists of a silver rod, coated with solid AgCl, immersed in a solution of saturated KCl containing KCl crystals. 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. This REF for the VT-03 flow cell is filled with KCl at the factory. For certain applications, another chloride salt may be preferable. With perchlorate-containing mobile phases, sodium chloride is mandatory, because potassium perchlorate precipitates and will clog the cotton wool frit. At high modifier percentages, the REF must be filled with lithium chloride for similar reasons.

16.2.3 HyREF reference electrode

The HyREF is a hydrogen reference electrode. Its potential depends on the pH of the mobile phase. The HyREF is comparable to the standard Ag/AgCl REF in baseline stability and S/N ratio. The HyREF is more user-friendly and, in principle, requires no maintenance. Trapping of air bubbles, like in the salt bridge Ag/AgCl type, is impossible because of the absence of a salt bridge. Consequently, refilling the REF with saturated KCl is not required. Due to the absence of a salt bridge and its inertness, the HyREF is an excellent alternative to the Ag/AgCl REF, especially in high modifier concentrations (as in analysis of fat-soluble vitamins) or high pH (as in analysis of carbohydrates, PAD).

Depending on the pH of the mobile phase, the potential setting of the working electrode vs. the HyREF may differ significantly compared with Ag/AgCl. I/E curves show a shift of more than 200 mV at pH 3.1 (for example, catecholamines), but no shift appears at pH 12 (for example, PAD of carbohydrates). It is advisable to first construct a hydrodynamic (or scanning) voltammogram when using the HyREF. In the following "Measured cell potential (HyREF - Ag/AgCl) versus pH" table, the potential of the HyREF is measured against the Ag/AgCl (in saturated KCl) electrode at different pH values.

Table 16–6: Measured cell potential (HyREF - Ag/AgCl) versus pH

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

So, if an Ag/AgCl REF is replaced by a HyREF, the pH effect must be taken into account (refer to the "Measured cell potential (HyREF - Ag/AgCl) versus pH" table). The pH vs. voltage relation is described by: $E_{HyREF} = E_{Ag/AgCl} - 328 + 29.9\ pH$

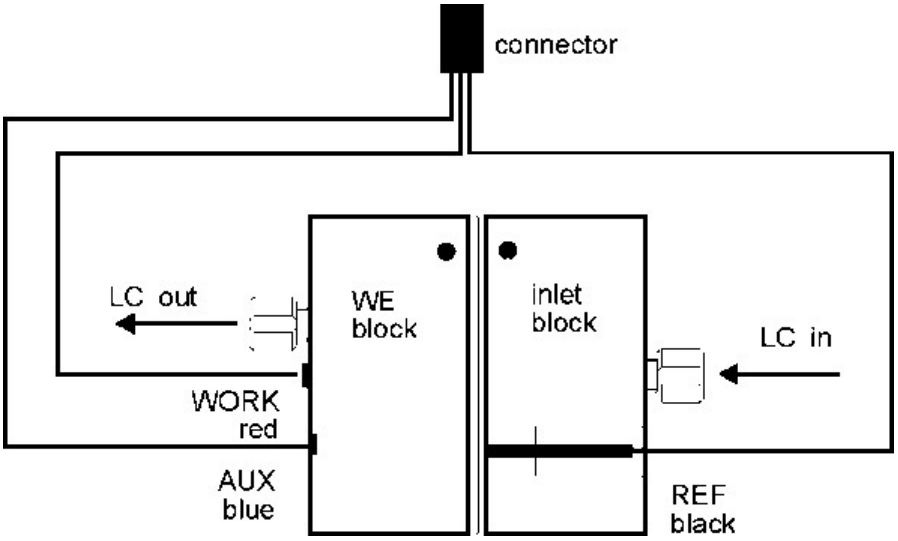
Example: A working potential of 800 mV (vs. Ag/AgCl with saturated KCl) at pH 3 must be changed to $E_{HyREF} = 800 - 328 + 29.9*3 = 561.7$ mV (vs. HyREF).

16.3 Installation

16.3.1 VT-03 flow cell with HyREF or ISAAC

The flow cell is assembled when it arrives. The force on the bolts is preset to 13 Ncm (a little bit beyond finger-tight). Familiarize yourself with this force, because over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. For instructions on assembling the cell, see Assembling the micro flow cell (Page 167).

Figure 16–8: Installation of flow cell



WORK, AUX, and REF are connected using the red, blue, and black cell cables.

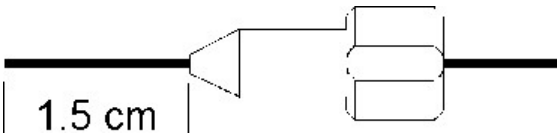
Note: LC out should be on top, to prevent entrapment of bubbles.

Warning: The ISAAC reference electrode requires 2 mmol/L chloride ions (KCl or NaCL) in the mobile phase. Add and equilibrate before installation of the ISAAC.

To install the flow cell with HyREF or ISAAC:

1. Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (0.3-mm ID) and one of the finger-tights supplied. Let the tubing protrude approximately 1.5 cm from the finger-tight fitting, and tighten it so that the tubing is not indented or only slightly indented by the fitting.

   Warning: Use only the factory-supplied finger-tights in the flow cell; others may cause serious damage.



2. Do not over-tighten the finger-tight. Over-tightening affects the flow pattern through the tubing (turbulence) and may strongly decrease flow cell performance.
3. Connect 0.5 mm ID PEEK tubing to the outlet of the flow cell. Use only the factory-supplied finger-tights in the flow cell. Others may cause serious damage. Again (see above), do not over-tighten the finger-tight.
4. Turn on the HPLC pump. Keep some tissues at hand because you will probably spill some mobile phase during this mounting procedure.
5. Fill the flow cell, keeping it at an angle of about 45° with the outlet (LC out) on top to force the air through the outlet.
6. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This prevents bubble entrapment.
7. Connect the cell cable as illustrated in Figure 16–8: Installation of flow cell (Page 163).

Warning: Never switch ON the flow cell when:

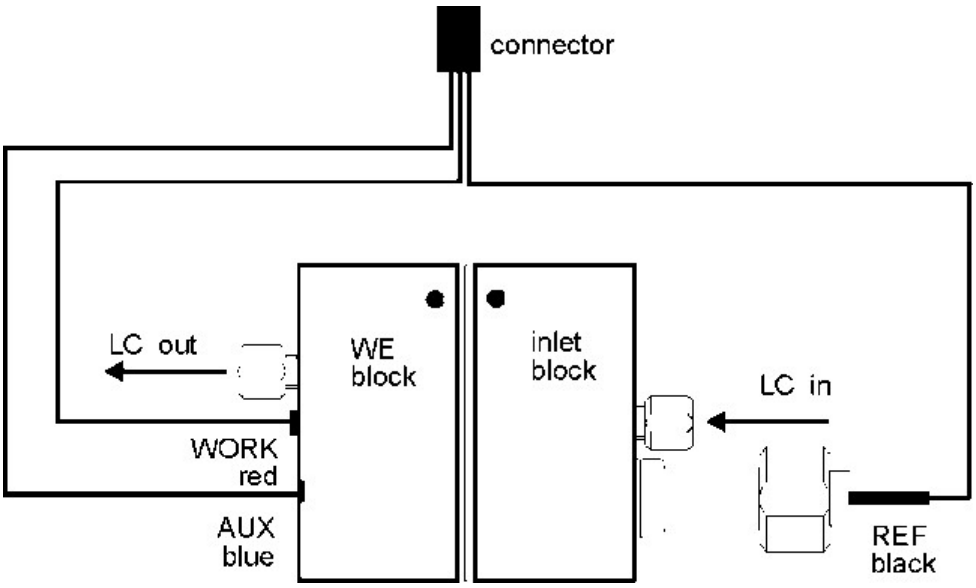
- The cell cable is not correctly connected.
- The cell is only partly (or not at all) filled with buffer/electrolyte.
- The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection, because substantial damage to the working electrode or electronics may occur.

Maximum detection stability is attained when not only the flow cell but also the HPLC column is incorporated in the controller. The controller has an integrated Faraday cage and an accurately thermostatted oven compartment that ensures stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

16.3.2 VT-03 flow cell with salt bridge REF

The flow cell is assembled when it arrives. The force on the bolts is preset to 13 Ncm (a little bit beyond finger-tight). Familiarize yourself with this force, because over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line. For instructions on assembling the cell, see Assembling the micro flow cell (Page 167).

Figure 16–9: Installation of flow cell



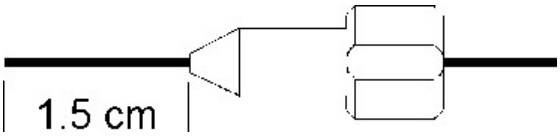
WORK, AUX, and REF are connected using the red, blue, and black cell cables.

Note: The LC outlet is placed on top, to prevent entrapment of bubbles.

Warning: Use proper eye and skin protection when working with solvents.

To install the flow cell with salt bridge REF:

1. Inspect the REF for air bubbles and saturation with KCl. Some KCl crystals should be visible. When no crystals are visible, or when air bubbles are trapped, the REF requires maintenance. To prevent the REF from drying out, it is sealed with a cap on arrival. Remove the cap.
2. Tighten the black swivel of the REF. A small droplet should appear at the cotton-wool frit. Do not remove this droplet because it ensures proper contact of the REF with the mobile phase.
3. Turn on the HPLC pump. Place some tissues because mobile phase may spill during this mounting procedure. Connect the column outlet to the flow cell inlet, using small-bore PEEK tubing (0.3-mm ID) and one of the finger-tights supplied. Use only the factory-supplied finger-tights in the flow cell; others may cause serious damage. Let the tubing protrude approximately 1.5 cm from the finger-tight and tighten it carefully.



Note: Over-tightening affects the flow through the tubing (turbulence) and decreases flow cell performance.

4. Connect 0.5-mm ID PEEK tubing to the outlet of the flow cell. Use only the factory-supplied finger-tights in the flow cell; others may cause serious damage. Again, do not over-tighten the finger-tight.
5. Fill the flow cell, keeping it in an angle of 45° with the REF fitting on top. Filling is best done by blocking the outlet with a finger and letting the air escape via the REF fitting. Carefully check the thread of the fitting for trapped air bubbles.
6. When the REF fitting is completely filled with mobile phase, mount the REF, while slowly releasing the outlet. Ensure that you do not include an air bubble.
7. Position the flow cell in its clamp in the controller with the REF at the lower side and the outlet at the upper side. This prevents bubble entrapment.
8. Connect the cell cable as illustrated in Figure 16–9: Installation of flow cell (Page 165) .

   Maximum detection stability is attained when not only the flow cell but also the HPLC column is incorporated in the controller. The controller has an integrated Faraday cage and an accurately thermostatted oven compartment that ensures stable working conditions. Installing the flow cell and column within such a controlled environment is the minimum requirement for high-quality LC-EC trace analyses.

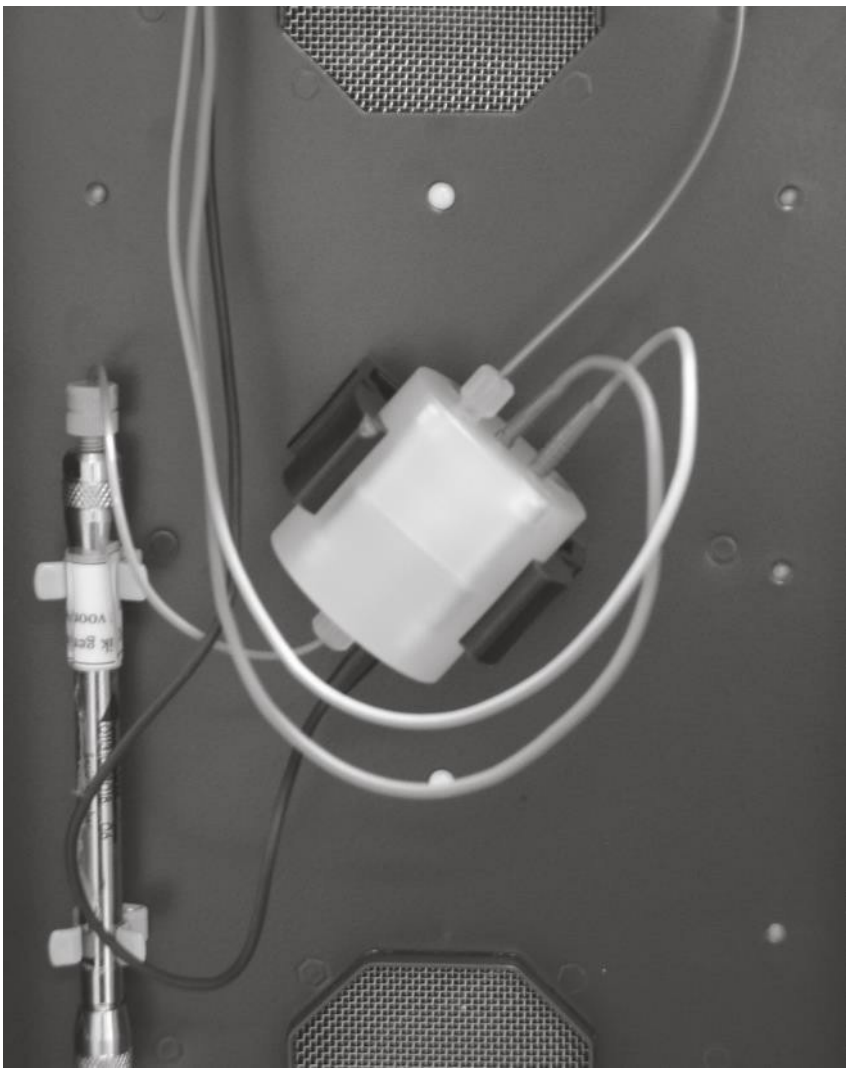
   

   Warning: Never switch ON the flow cell when:

   - The cell cable is not correctly connected.
   - The cell is only partly (or not at all) filled with buffer/electrolyte.
   - The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection, because substantial damage to the working electrode or electronics may occur.

   When not in use, store the REF with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.

Figure 16–10: Always install flow cell with outlet on top



16.3.3 VT-03 micro flow cell

The micro flow cell is assembled when it arrives. The force on the bolts is preset to 13 Ncm (“a little bit beyond finger-tight”). Familiarize yourself with this force, because over-tightening of the bolts strongly deteriorates the S/N ratio and eventually the cell itself. Also, be aware that the black marks on both blocks should be in line.

16.3.3.1 Assembling the micro flow cell

The VT-03 micro flow cell has a 0.7-mm WE and is equipped with a 25-µm spacer. Older models of the VT-03 micro are equipped with a metal centering ring, in which case both blocks (WE and inlet block) have an edge/groove to accommodate the metal ring and are fixed in the metal ring to align the inlet in the center of the WE.

Figure 16–11: Disassembled VT-03 micro flow cell with spacer

Note: VT- 03 micro cells purchased after 2009 are delivered without the metal centering ring and have no groove in the WE and inlet block.

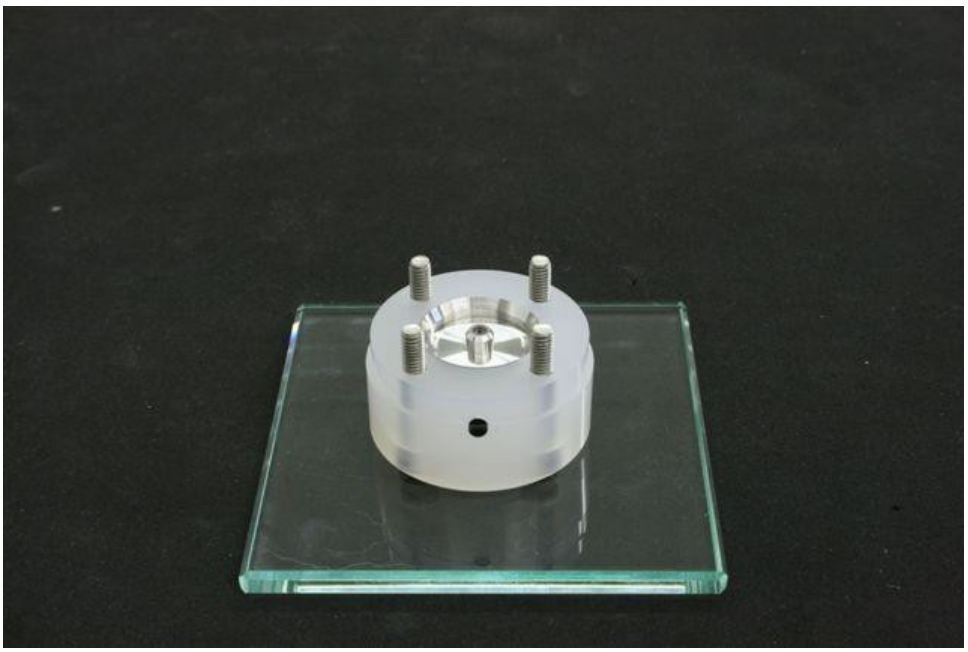
To ensure proper installation and optimal performance, follow the instructions below for assembling a VT-03 micro flow cell. It is also strongly recommended that you assemble normal VT-03 flow cells (2-mm and 3-mm GC) using a similar procedure.

Note: Mounting the cell in another manner may lead to damage of the spacer and flow cell blocks. For cells without a metal centering ring, skip the sections in the procedure related to placement of the centering ring.

Instructions for assembling a VT-03 micro flow cell:

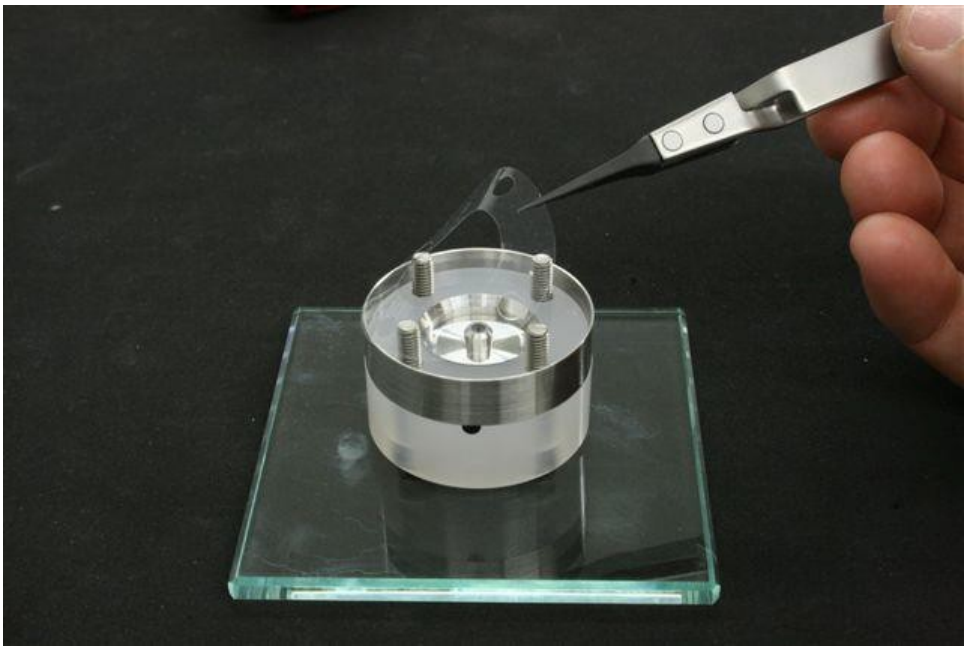
1. Hold the WE block in a horizontal position with the WE facing downward, and insert the four bolts in the four mounting holes of the WE block. Place the glass mounting plate on top, turn the glass plate with WE block upside down, and place it on a flat surface.

Figure 16–12: WE block with inserted bolds on the glass mounting plate



2. Place the metal ring on the WE block. Make sure that you hold the metal ring horizontally when pushing it onto the block. Subsequently, gently place the 25-µm spacer on top of the WE block using a pair of tweezers. The spacer should lay flat on the WE surface, with the bolts centered in the spacer bolt holes.

Figure 16–13: Placement of 25-µm spacer on the WE block



3. Place the inlet block on top of the WE block as depicted in the next figure. When placing the block, keep it tilted and gently try to position the two bolt holes in the inlet block on top of the bolts without touching the surface of the inlet block with the bolt ends (this minimizes

the chance of scratching the inlet block surface). Then, turn the inlet block downward in horizontal position. The inlet block now rests on top of the WE block with the bolts positioned on the mounting holes. The black positioning markers on the sides of the blocks should now be aligned in vertical position.

Figure 16–14: Positioning the inlet block on top of the WE block

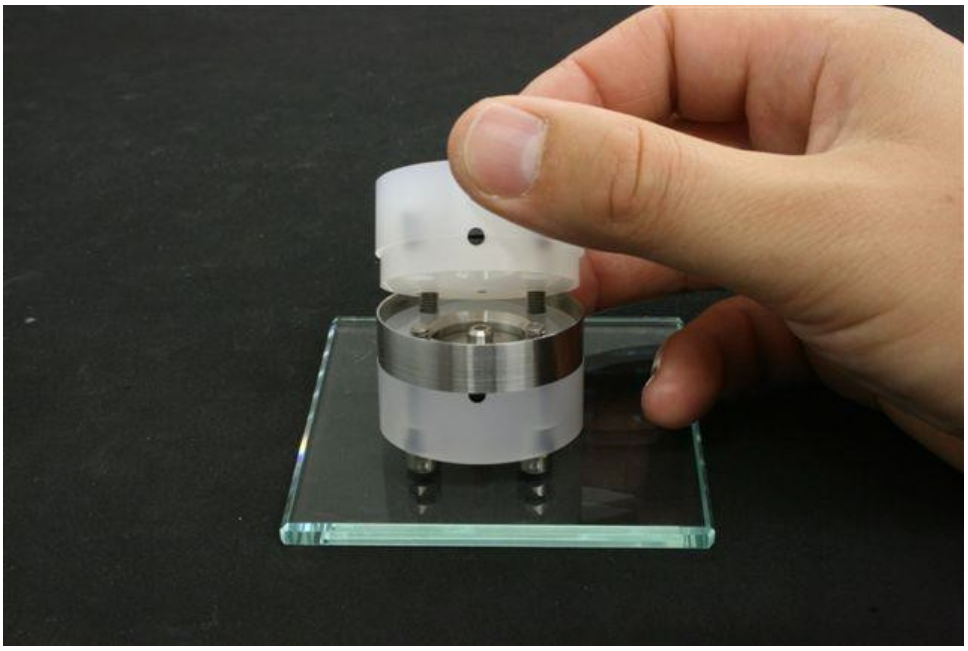
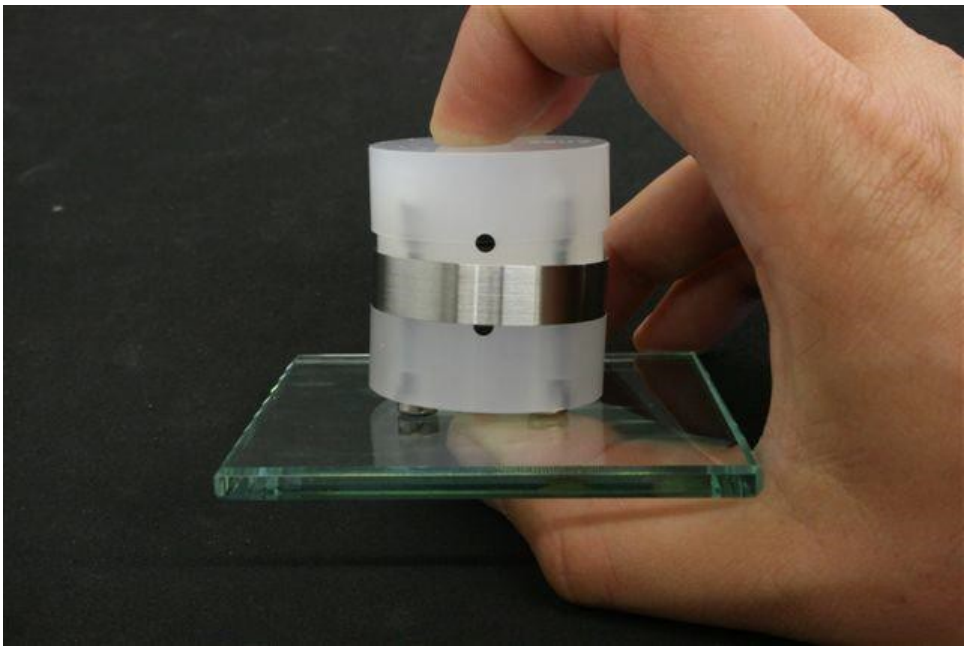


Figure 16–15: Inlet block positioned on top of WE electrode

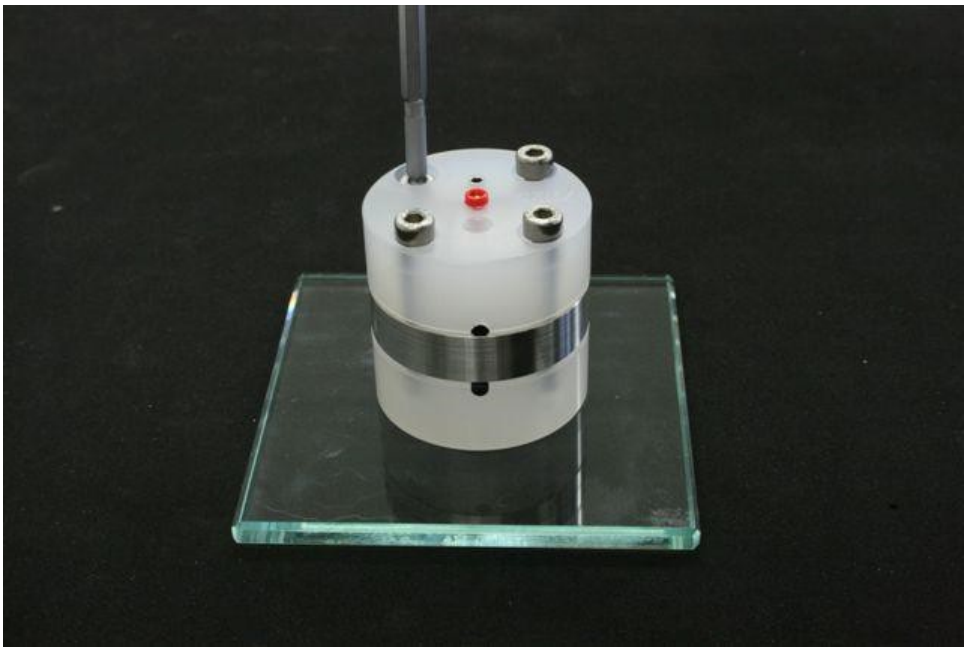


Note that the black positioning marks are aligned in vertical position.

4. Hold the complete cell together with the glass mounting plate and turn it upside down again. Take the glass plate and position it underneath the cell again. Tighten the bolts in

a crosswise pattern with the hex key (tightening force approximately 13 Ncm). The cell is again ready for use.

Figure 16–16: Fixing the two cell blocks with the hex key (tighten the bolts in a crosswise pattern)



16.3.3.2 Capillary connections

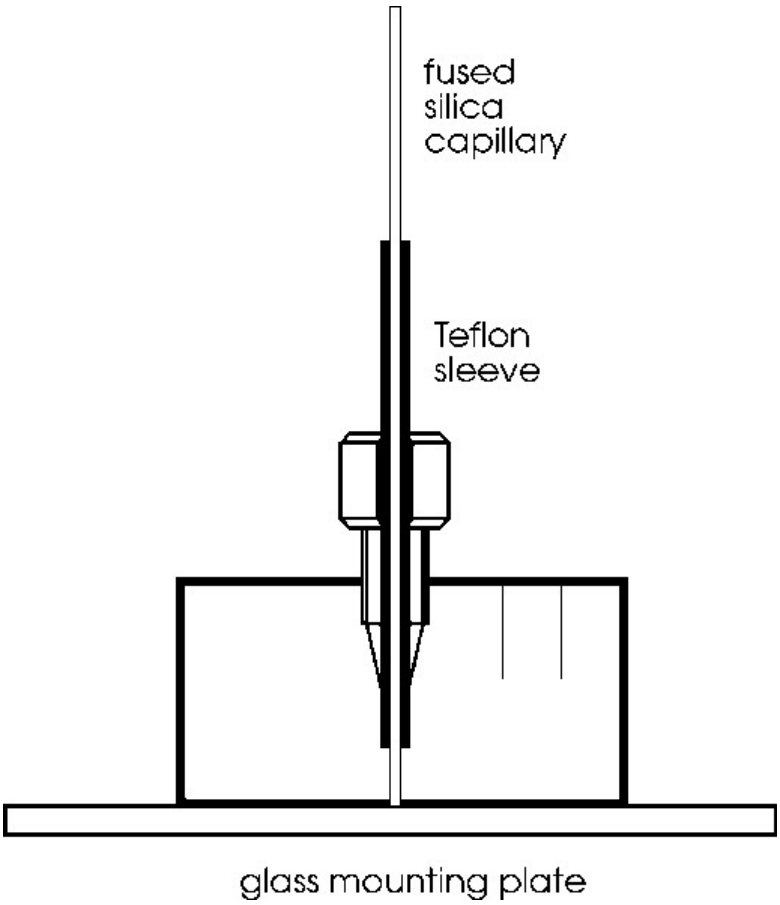
The micro flow cell is supplied with a low dead-volume fused silica capillary connection for coupling with capillary columns (< 1 mm ID fused silica columns) as a standard.

Important: Use proper eye and skin protection when working with solvents.

Note: If the capillary connection is already installed properly, continue with step 11. If not, start with step 1.

Warning: When disconnecting the column, always release pressure slowly, or damage to the column will occur. Tighten the low dead-volume coupling firmly to prevent it from snapping out of the capillary. A sudden pressure drop will destroy column performance and the pulse dampener.

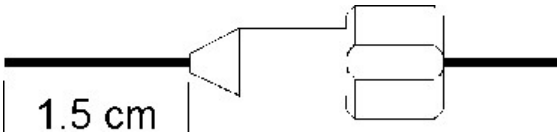
Figure 16–17: Mounting of the fused silica connector in the VT-03 micro flow cell



The capillary connector is not necessary with a 1-mm (or larger ID) column. With larger columns, use narrow-bore PEEK tubing (100-µm ID), and install it as described in VT-03 flow cell with HyREF or ISAAC (Page 163) (ISAAC REF) or in VT-03 flow cell with salt bridge REF (Page 164) (salt bridge REF).

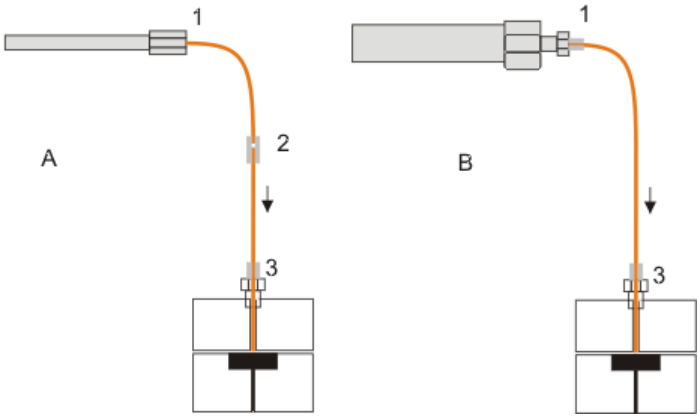
To prevent damage to the flow cell, carry out the following steps:

1. Connect the FS capillary to the pump (bypassing the column) and switch on the pump to confirm that the capillary is open. Liquid droplets must come out. Otherwise, install a new piece of 100-µm FS tubing. Note that both ends of the supplied FS capillary are cut and polished.
2. If the capillary is open, switch off the pump and wait until the pressure is zero. Disconnect the capillary from the pump.
3. Insert the fused silica capillary into the tightly fitting Teflon sleeve supplied.
4. Protrude both through the factory-supplied finger-tight fitting. Use only the factory-supplied finger-tights in the flow cell; others may cause serious damage.



5. Mount the combination carefully in the injection block (Figure 16–17: Mounting of the fused silica connector in the VT-03 micro flow cell (Page 172)).
6. Let the fused silica protrude slightly (< 0.5 mm) through the injection hole.
7. Clean the factory-supplied glass mounting plate from particles.
8. Carefully push the block on the glass plate until the silica capillary is flush with the surface.
9. Fix the fused silica capillary firmly with the finger-tight while keeping a slight pressure on the block against the glass plate.
10. Mount the two flow cell blocks by crosswise tightening the bolts (max. 13 Ncm).

Figure 16–18: Different column connectors with micro flow cell



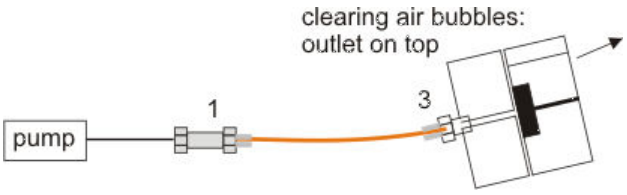
Column type A has no ferrule but ends with fused silica (1); column type B has a ferrule connector (1).

On the left (A), the standard installation with capillary connector (2) is shown. This is suitable for fused silica columns. On the right (B), installation with a ferrule and sleeve connected to the column is shown.

11. Connect the other end of the capillary connector as shown in "Different column connectors with micro flow cell" figure. Use a ferrule with sleeve, or couple to the capillary column using the connector sleeve. If leakage occurs, a low-dead coupler can be used with finger-tights and sleeve.
12. Continue installation as described at point 3 in step 3 (Page 172) (ISAAC REF) or at point 1 (skip point 3) in step 1 (Page 172) (salt bridge REF), depending on the type of reference electrode used.

Note: To speed up the filling of the micro flow cell, the cell can be connected directly to the HPLC, thus bypassing the column.

Figure 16–19: Filling a VT-03 micro flow cell at 200–400 µL/min using a low dead-volume connector (1); pressure must not rise > 200 bar



Read the column instructions first. The flow cell is then filled at a higher flow rate. After filling, reconnect the column. Be careful not to include air bubbles.

Warning: Never switch ON the flow cell when:

- The cell cable is not correctly connected.
- The cell is only partly (or not at all) filled with buffer/electrolyte.
- The outside of the flow cell is wet, particularly the part between the auxiliary and working electrode connection, because substantial damage to the working electrode or electronics may occur.

16.4 Maintenance

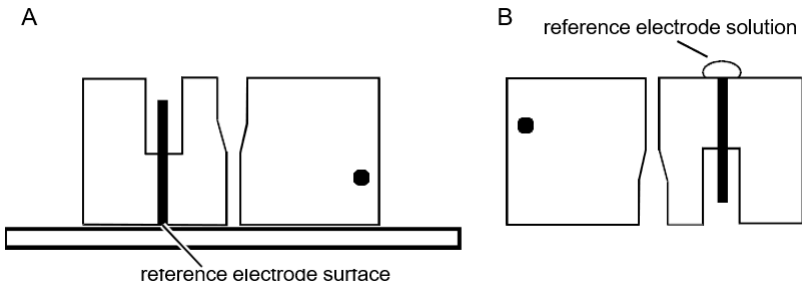
16.4.1 HyREF

The HyREF reference electrode is, in principle, maintenance-free. If not in use, store it dry after disassembling the flow cell.

16.4.2 ISAAC

The ISAAC reference electrode requires maintenance, usually not more than once every three months. In practice this means that when the flow cell is opened to service the working electrode, the reference electrode should be serviced as well.

Figure 16–20: Servicing the ISAAC reference electrode, polishing (A) and coating (B)



Servicing the reference electrode is done by polishing its surface until shining metal appears (Servicing the ISAAC reference electrode, polishing (A)). Immediately after polishing, the electrode is coated by applying a few drops of the reference electrode solution on the electrode surface (Servicing the ISAAC reference electrode, polishing (A)). After 20 minutes, flush away the reference solution with distilled water.

If not in use for a longer period of time, disassemble the flow cell. The flow cell, including the reference electrode, should be cleaned with distilled water, dried with a tissue, and stored dry.

16.4.2.1 Polishing

Polishing the reference electrode is done using the factory-supplied polishing kit, which contains diamond slurry and a polishing disc.

To initiate polishing:

1. Shake the diamond slurry thoroughly before use.
2. Rinse the polishing disc with demi-water before applying the diamond slurry.
3. Apply a few drops of slurry to the wetted polishing disc, and polish the electrode with a figure-8 motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with an ethanol-wetted tissue and inspect the surface visually. Repeat the procedure if necessary, until the shining metal REF surface appears.
5. Clean the polishing disc with demi-water.
6. Store the polishing disc in its plastic bag.

16.4.3 Ag/AgCl salt bridge

Three aspects determine the proper function of an Ag/AgCl 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 conduction.
- Air bubbles inside or close to the salt bridge will lead to instability of the three-electrode configuration. Because of their extreme compressibility, changes in conductivity and the ionic equilibrium of the REF occur. This increases the noise considerably.

The REF is factory-filled with KCl unless specified otherwise. Other chloride salts should be used when the mobile phase contains perchlorate (use NaCl) or a high percentage of organic modifier (use LiCl).

16.4.3.1 Saturation and air bubbles

After prolonged use, the salt bridge in the REF will no longer be saturated, which usually leads to poor reproducibility in electrochemical detection. The potential of the REF is determined by the chloride concentration. If the salt bridge is not saturated and the KCl concentration changes:

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

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. Inspect your REF regularly. If you do not see chloride salt crystals, or if you see air bubbles, your REF needs maintenance.

16.4.3.2 Material

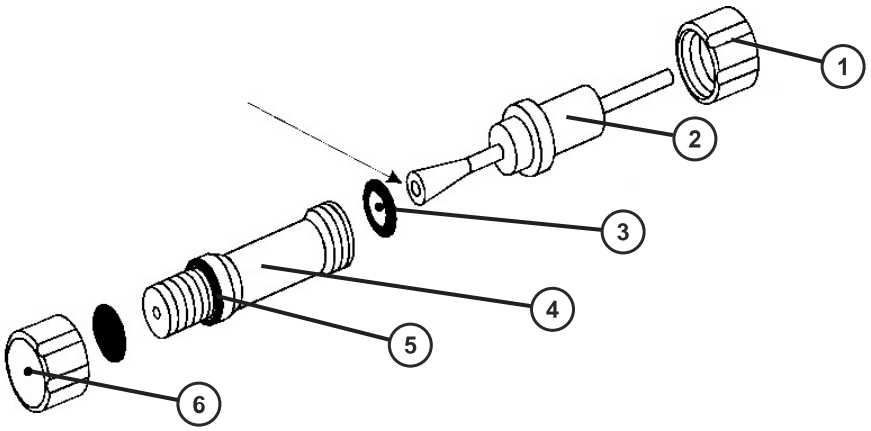
- An over-saturated and thoroughly degassed KCl solution
- Salt bridge REF tool (700013145), or a stainless steel rod of about 5 cm in length and 1 mm in diameter
- Ordinary cotton wool

16.4.3.3 Procedure

Note: Use proper eye and skin protection when working with solvents.

1. Turn the cell OFF on the controller.
2. Stop the HPLC pump.
3. Disconnect the cell from the controller.
4. Remove the REF from the inlet block.
5. Disassemble the REF by unscrewing the black fitting.
6. Inspect the O-ring for wear, and especially the cotton wool frit. Replace if necessary.

Figure 16–21: Exploded view of the reference electrode



1. Swivel for REF
2. Ag/AgCl electrode + fitting
3. Vyton ring (small)
4. Salt bridge
5. Vyton ring (large)
6. REF cap for storage and shipment

Note: The arrow indicates the tip of the AgCl-coated silver rod.

7. Remove the remaining KCl from the salt bridge.

8. Clean all parts with demi-water.
9. The Ag/AgCl electrode must be cleaned if the silver on the tip (Figure 16–21: Exploded view of the reference electrode (Page 176) arrow) has a non-metallic appearance by gently grinding it with sanding paper; also, the AgCl can be gently resurfaced in this way.
10. The frit in the salt bridge ensures electrical contact with the buffer. If the frit is discolored or dried out, it must be renewed. If that is the case, continue with “Maintenance of the cotton wool frit”, step 1. Otherwise, continue with step 7.

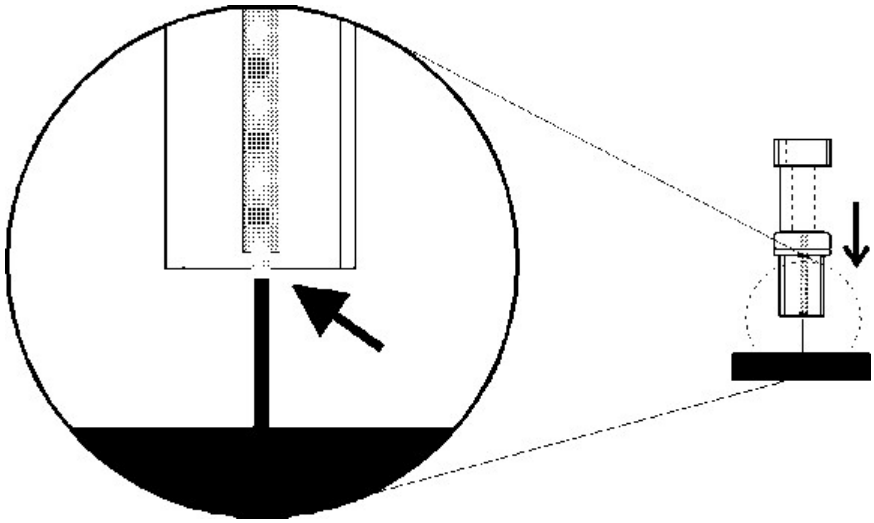
16.4.3.4 Maintenance of the cotton wool frit

Note: Use proper eye and skin protection when working with solvents.

To maintain the cotton wool frit:

1. Use a drill bit of approximately 1 mm (0.039 inch) to push out the frit from the outside. Be careful not to damage the frit constriction (first arrow).

Figure 16–22: Pushing out the cotton wool frit



2. Clean the salt bridge thoroughly with tap water and demi-water.
3. Saturate a small piece of cotton wool in KCl to prevent trapping of air within the wool.
4. Plug the salt bridge with the REF cap and fill the salt bridge to about 50%.
5. Use the drill bit to pack the wool, from above, through the KCl solution into the channel of the salt bridge. Compress it firmly, but not too much, because electrical conduction is essential.
6. Remove the cap.
7. Fill the salt bridge completely, and add some KCl crystals out of a saturated solution to ensure prolonged saturation.
8. Place the small Vyton 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.

9. Tighten the black swivel so that a small droplet appears at the end of the salt bridge, but do not over-tighten the swivel.
10. Flush the complete, mounted REF with demi-water and dry it with a tissue, but keep the cotton wool frit soaked.
11. Carefully inspect the REF for trapped air bubbles, and remove them if present (go back to step 7 or, if necessary, to step 1).

    Note: When not in use, store the REF with the cotton wool frit immersed in a saturated KCl solution to prevent drying out.

16.4.4 Working electrode

Cleaning of the working electrode block is necessary if the electrode surface has been electrochemically changed. This may happen due to fouling by oxidation (reduction) reaction products. Excessively high currents also may change the electrode surface. This is evident from highly decreased sensitivity after prolonged use.

As a rule of thumb, only polish if the surface of the working electrode lacks its mirror-like finish and cannot be restored by wiping the electrode surface with a tissue wetted with ethanol or acetone.

16.4.4.1 Decreased flow cell performance

Warning: Use proper eye and skin protection when working with solvents.

Several actions can be taken to address decreased flow cell performance. Avoid unnecessary polishing; take the next step only if the previous was not successful.

Steps to address decreased flow cell performance:

1. Electrochemical cleaning of glassy carbon WE: In the pulse mode, let the potential jump between +1 and -1 V for 10 minutes. Settings: t1 = 1000 ms, t2 = 1000 ms, t3 = 0 ms, E1 = +1V, and E2 = -1V.
2. Wiping the electrode surface with a tissue wetted with ethanol or acetone.
3. Polishing the electrode surface.

16.4.4.2 Polishing

Polishing the reference electrode is done using the factory-supplied polishing kit, which contains diamond slurry and a polishing disc.

To initiate polishing:

1. Shake the diamond slurry thoroughly before use.
2. Rinse the polishing disc with demi-water before applying the diamond slurry.

3. Apply a few drops of slurry to the wetted polishing disc, and polish the electrode with a figure-8 motion for about one minute. Apply only gentle pressure.
4. Clean the electrode with an ethanol-wetted tissue and inspect the surface visually. Repeat the procedure if necessary, until the shining metal REF surface appears.
5. Reassemble the detector cell.
6. Clean the polishing disc with demi-water.
7. Store the polishing disc in its plastic bag.

A Safety advisories

Waters products display safety symbols that identify hazards associated with the product's operation and maintenance. The symbols also appear in product manuals with statements that describe the hazards and advise how to avoid them. This appendix presents all safety symbols and statements that apply to Waters' product offerings. The symbols and statements can apply to a specific product, or apply to other products within the same system.

A.1 Warning symbols

Warning symbols alert you to the risk of death, injury, or seriously adverse physiological reactions associated with the misuse of an instrument or device. Heed all warnings when you install, repair, or operate any Waters instrument or device. Waters accepts no liability in cases of injury or property damage resulting from the failure of individuals to comply with any safety precaution when installing, repairing, or operating any of its instruments or devices.

The following symbols warn of risks that can arise when you operate or maintain a Waters instrument or device or component of an instrument or device. When one of these symbols appears in a manual's narrative sections or procedures, an accompanying statement identifies the applicable risk and explains how to avoid it.

Warning: (General risk of danger. When this symbol appears on an instrument, consult the instrument's user documentation for important safety-related information before you use the instrument.)

Warning: (Risk of burn injury from contacting hot surfaces.)

Warning: (Risk of electric shock.)

Warning: (Risk of fire.)

Warning: (Risk of sharp-point puncture injury.)

Warning: (Risk of hand crush injury.)

Warning: (Risk of injury caused by moving machinery.)

Warning: (Risk of exposure to ultraviolet radiation.)

Warning: (Risk of contacting corrosive substances.)

Warning: (Risk of exposure to a toxic substance.)

Warning: (Risk of personal exposure to laser radiation.)

Warning: (Risk of exposure to biological agents that can pose a serious health threat.)

Warning: (Risk of tipping.)

Warning: (Risk of explosion.)

Warning: (Risk of high-pressure gas release.)

A.1.1 Specific warnings

A.1.1.1 Burst warning

This warning applies to Waters instruments and devices fitted with nonmetallic tubing.

Warning: To avoid injury from bursting, nonmetallic tubing, heed these precautions when working in the vicinity of such tubing when it is pressurized:

- Wear eye protection.
- Extinguish all nearby flames.
- Do not use tubing that is, or has been, stressed or kinked.
- Do not expose nonmetallic tubing to compounds with which it is chemically incompatible: tetrahydrofuran, nitric acid, and sulfuric acid, for example.
- Be aware that some compounds, like methylene chloride and dimethyl sulfoxide, can cause nonmetallic tubing to swell, significantly reducing the pressure at which the tubing can rupture.

A.1.1.2 Biohazard warning

The following warning applies to Waters instruments and devices that can process biologically hazardous materials. Biologically hazardous materials are substances that contain biological agents capable of producing harmful effects in humans.

Warning: To avoid infection from blood-borne pathogens, inactivated microorganisms, and other biological materials, assume that all biological fluids that you handle are infectious.

Specific precautions appear in the latest edition of the US National Institutes of Health (NIH) publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL).

Warning: To avoid injury when working with hazardous materials, consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials.

A.1.1.3 Biohazard and chemical hazard warning

This warning applies to Waters instruments and devices that can process biohazards, corrosive materials, or toxic materials.

Warning: To avoid personal contamination with biologically hazardous, toxic, or corrosive materials, you must understand the hazards associated with their handling.
Guidelines prescribing the proper use and handling of such materials appear in the latest edition of the National Research Council's publication, *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards*.
To avoid injury when working with hazardous materials, consult the Safety Data Sheets regarding the solvents you use. Additionally, consult the safety representative for your organization regarding its protocols for handling such materials and follow good laboratory practices.

A.2 Notices

Notice advisories appear where an instrument, device, or component can be subject to use or misuse that can damage it or compromise a sample’s integrity. The exclamation point symbol and its associated statement alert you to such risk.

Notice: To avoid damaging the case of the instrument or device, do not clean it with abrasives or solvents.

A.3 Bottles Prohibited symbol

The Bottles Prohibited symbol alerts you to the risk of equipment damage caused by solvent spills.

Prohibited: To avoid equipment damage caused by spilled solvent, do not place reservoir bottles directly atop an instrument or device or on its front ledge. Instead, place the bottles in the bottle tray, which serves as secondary containment in the event of spills.

A.4 Required protection

The Use Eye Protection and Wear Protective Gloves symbols alert you to the requirement for personal protective equipment. Select appropriate protective equipment according to your organization's standard operating procedures.

Requirement: Use eye protection when performing this procedure.

Requirement: Wear clean, chemical-resistant, powder-free gloves when performing this procedure.

A.5 Warnings that apply to all Waters instruments and devices

When operating this device, follow standard quality-control procedures and the equipment guidelines in this section.

Warning: 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.

Avertissement : 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.

Warnung: 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: Qualsiasi modifica o alterazione apportata a questa unità e non espressamente autorizzata dai responsabili per la conformità fa decadere il diritto all'utilizzo dell'apparecchiatura da parte dell'utente.

Advertencia: 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.

警告： 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。

警告： 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。

경고: 규정 준수를 책임지는 당사자의 명백한 승인 없이 이 장치를 개조 또는 변경할 경우, 이 장치를 운용할 수 있는 사용자 권한의 효력을 상실할 수 있습니다.

警告: 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、本装置のユーザーとしての承認が無効になる可能性があります。

Warning: 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 tubing that has been severely stressed or kinked.
- Do not use nonmetallic tubing with tetrahydrofuran (THF) or concentrated nitric or sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause nonmetallic tubing to swell, which greatly reduces the rupture pressure of the tubing.

Avertissement : Manipulez les tubes en polymère sous pression avec précaution:

- Portez systématiquement des lunettes de protection à proximité de tubes en polymère sous pression.
- Éteignez toute flamme se trouvant à proximité de l’instrument.
- Évitez d'utiliser des tubes sévèrement déformés ou endommagés.
- N’exposez pas les tuyaux non métalliques au tétrahydrofurane, ou THF, ou à de l’acide nitrique ou sulfurique concentré.
- Sachez que le chlorure de méthylène et le diméthylesulfoxyde entraînent le gonflement des tuyaux non métalliques, ce qui réduit considérablement leur pression de rupture.

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

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

Avvertenza: Fare attenzione quando si utilizzano tubi in materiale polimerico sotto pressione:

- Indossare sempre occhiali da lavoro protettivi nei pressi di tubi di polimero pressurizzati.
- Spegnere tutte le fiamme vive nell'ambiente circostante.
- Non utilizzare tubi eccessivamente logorati o piegati.
- Non utilizzare tubi non metallici con tetraidrofurano (THF) o acido solforico o nitrico concentrati.
- Tenere presente che il cloruro di metilene e il dimetilsolfossido provocano rigonfiamento nei tubi non metallici, riducendo notevolmente la resistenza alla rottura dei tubi stessi.

Advertencia: Se recomienda precaución cuando se trabaje con tubos de polímero sometidos a presión:

- El usuario deberá protegerse siempre los ojos cuando trabaje cerca de tubos de polímero sometidos a presión.
- Apagar cualquier llama que pueda estar encendida en las proximidades.
- No se debe trabajar con tubos que se hayan doblado o sometido a altas presiones.
- Es necesario utilizar tubos de metal cuando se trabaje con tetrahidrofurano (THF) o ácidos nítrico o sulfúrico concentrados.
- Hay que tener en cuenta que el diclorometano y el dimetilsulfóxido dilatan los tubos no metálicos, lo que reduce la presión de ruptura de los tubos.

警告： 当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管线。
- 不要在非金属管线中使用四氢呋喃或浓硝酸或浓硫酸。
- 要了解使用二氯甲烷及二甲基亚砜会导致非金属管线膨胀，大大降低管线的耐压能力。

警告： 當在有壓力的情況下使用聚合物管線時，小心注意以下幾點。

- 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管線。
- 不要在非金屬管線中使用四氫呋喃或濃硝酸或濃硫酸。
- 要了解使用二氯甲烷及二甲基亞碸會導致非金屬管線膨脹，大大降低管線的耐壓能力。

경고: 가압 폴리머 튜브로 작업할 경우에는 주의하십시오.

- 가압 폴리머 튜브 근처에서는 항상 보호 안경을 착용하십시오.
- 근처의 화기를 모두 끄십시오.
- 심하게 변형되거나 꼬인 튜브는 사용하지 마십시오.
- 비금속(Nonmetallic) 튜브를 테트라히드로푸란(Tetrahydrofuran: THF) 또는 농축 질산 또는 황산과 함께 사용하지 마십시오.
- 염화 메틸렌(Methylene chloride) 및 디메틸술폭시드(Dimethyl sulfoxide)는 비금속 튜브를 부풀려 튜브의 파열 압력을 크게 감소시킬 수 있으므로 유의하십시오.

警告: 圧力のかかったポリマーチューブを扱うときは、注意してください。

- 加圧されたポリマーチューブの付近では、必ず保護メガネを着用してください。
- 近くにある火を消してください。
- 著しく変形した、または折れ曲がったチューブは使用しないでください。
- 非金属チューブには、テトラヒドロフラン (THF) や高濃度の硝酸または硫酸などを流さないでください。
- 塩化メチレンやジメチルスルホキシドは、非金属チューブの膨張を引き起こす場合があり、その場合、チューブは極めて低い圧力で破裂します。

This warning applies to Waters instruments fitted with nonmetallic tubing or operated with flammable solvents.

Warning: 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.

Avertissement : 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 défectueuse.

Warnung: Der Benutzer wird darauf aufmerksam gemacht, dass bei unsachgemäßer Verwendung des Gerätes die eingebauten Sicherheitseinrichtungen unter Umständen nicht ordnungsgemäß funktionieren.

Avvertenza: Si rende noto all'utente che l'eventuale utilizzo dell'apparecchiatura secondo modalità non previste dal produttore può compromettere la protezione offerta dall'apparecchiatura.

Advertencia: El usuario debe 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削弱。

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

경고: 제조업체가 명시하지 않은 방식으로 장비를 사용할 경우 장비가 제공하는 보호 수단이 제대로 작동하지 않을 수 있다는 점을 사용자에게 반드시 인식시켜야 합니다.

警告: ユーザーは、製造元により指定されていない方法で機器を使用すると、機器が提供している保証が無効になる可能性があることに注意して下さい。

A.6 Warnings that address the replacement of fuses

The following warnings pertain to instruments and devices equipped with user-replaceable fuses. Information describing fuse types and ratings sometimes, but not always, appears on the instrument or device.

Finding fuse types and ratings when that information appears on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating printed on panels adjacent to instrument fuse covers.

Avertissement : Pour éviter tout risque d’incendie, remplacez toujours les fusibles par d’autres du type et de la puissance indiqués sur le panneau à proximité du couvercle de la boîte à fusible de l’instrument.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert auf den Tafeln neben den Sicherungsabdeckungen des Geräts gedruckt sind.

Avvertenza: Per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate sui pannelli adiacenti alla copertura fusibili dello strumento.

Advertencia: Para evitar incendios, sustituir los fusibles por otros del tipo y características impresos en los paneles adyacentes a las cubiertas de los fusibles del instrumento.

警告： 为了避免火灾，应更换与仪器保险丝盖旁边面板上印刷的类型和规格相同的保险丝。

警告： 為了避免火災，更換保險絲時，請使用與儀器保險絲蓋旁面板上所印刷之相同類型與規格的保險絲。

경고: 화재의 위험을 막으려면 기기 퓨즈 커버에 가까운 패널에 인쇄된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズを交換する場合は、装置ヒューズカバーの隣のパネルに記載されている種類および定格のヒューズをご使用ください。

Finding fuse types and ratings when that information does not appear on the instrument or device:

Warning: To protect against fire, replace fuses with those of the type and rating indicated in the “Replacing fuses” section of the Maintenance Procedures chapter.

Avertissement : Pour éviter tout risque d’incendie, remplacez toujours les fusibles par d’autres du type et de la puissance indiqués dans la rubrique « Remplacement des fusibles » du chapitre traitant des procédures de maintenance.

Warnung: Zum Schutz gegen Feuer die Sicherungen nur mit Sicherungen ersetzen, deren Typ und Nennwert im Abschnitt "Sicherungen ersetzen" des Kapitels "Wartungsverfahren" angegeben sind.

Avvertenza: Per garantire protezione contro gli incendi, sostituire i fusibili con altri dello stesso tipo aventi le caratteristiche indicate nel paragrafo "Sostituzione dei fusibili" del capitolo "Procedure di manutenzione".

Advertencia: Para evitar incendios, sustituir los fusibles por otros del tipo y características indicados en la sección "Sustituir fusibles" del capítulo Procedimientos de mantenimiento.

警告： 为了避免火灾，应更换“维护步骤”一章的“更换保险丝”一节中介绍的相同类型和规格的保险丝。

警告： 為了避免火災，更換保險絲時，應使用「維護步驟」章節中「更換保險絲」所指定之相同類型與規格的保險絲。

경고: 화재의 위험을 막으려면 유지관리 절차 단원의 “퓨즈 교체” 절에 설명된 것과 동일한 타입 및 정격의 제품으로 퓨즈를 교체하십시오.

警告: 火災予防のために、ヒューズ交換ではメンテナンス項目の「ヒューズの交換」に記載されているタイプおよび定格のヒューズをご使用ください。

A.7 Electrical symbols

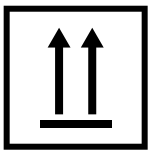
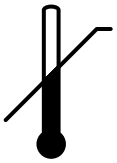
The following electrical symbols and their associated statements can appear in instrument manuals and on an instrument’s front or rear panels.

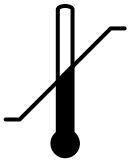
Symbol	Description
	Electrical power on
	Electrical power off
	Standby
	Direct current
	Alternating current
	Alternating current (three phase)
	Safety ground
	Frame or chassis terminal connection
	Fuse
	Functional ground
	Input
	Output

Symbol	Description
	Indicates that the device or assembly is susceptible to damage from electrostatic discharge (ESD)

A.8 Handling symbols

The following handling symbols and their associated statements can appear on labels affixed to the packaging in which instruments, devices, and component parts are shipped.

Symbol	Description
	Keep upright!
	Keep dry!
	Fragile!
	Use no hooks!
	Upper limit of temperature
	Lower limit of temperature

Symbol	Description
	Temperature limitation