Antec Scientific
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The Netherlands

ROXY Potentiostat

User manual

210.7010, Edition 10, 2018

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CE Declaration of conformity

We Antec Leyden B.V., Zoeterwoude, The Netherlands, declare that the product:

ROXY Potentiostat **type 210**

to which this declaration relates, is in conformity with the following CE directives:

Low Voltage Directive (2014/35/EU)

Safety requirements for electrical equipment for measurement, control, and laboratory use:

- Part 1: General Requirements	IEC61010-1 2ed :2001
- Part 2-010: Particular requirements for laboratory equipment for the heating of materials	IEC61010-2-010 2ed :2003

EMC Directive (2014/30/EU)

Electrical equipment for measurement, control and laboratory use - EMC requirements

- Part 1: General Requirements	EN61326-1:2006
Electromagnetic immunity	EN61000-4-2:2009 EN61000-4-3:2006 + A1:2008 + A2:2010 EN61000-4-4:2012, EN61000-4-5:2006 EN61000-4-6:2009, EN61000-4-8:2010 EN61000-4-11:2004
Electromagnetic emission	EN55011 (Class B): 2009 + A1:2010 EN61000-3-2:2006 + A1:2009 +A2:2009 EN61000-3-3:2008

RoHS directive (2011/65/EU) Restriction of Hazardous Substances
WEEE directive (2012/19/EC) Waste Electrical and Electronic Equipment

Only use manufacturer-supplied cable(s) 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 which do not meet relevant safety standards.

Zoeterwoude, The Netherlands, April 20th, 2016
Dr. N.J. Reinhoud (Managing Director)

Warning Symbols

The following symbols are used in this guide:

This sign warns about the risk of electric shock. It calls attention to a procedure or practice which, if not adhered to, could result in loss of life by electrocution. Do not proceed beyond a danger sign until the indicated conditions are fully understood and met.

The warning sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in severe injury, loss of life or damage or destruction of parts or all of the equipment. Do not proceed beyond a warning sign until the indicated conditions are fully understood and met.

The caution sign denotes a hazard. It calls attention to a procedure or practice which, if not adhered to, could result in damage or destruction of parts or all of the equipment and/or erratic results. Do not proceed beyond a cautions sign until the indicated conditions are fully understood and met.

The biohazard sign draws attention to the fact that use of biological materials, viral samples may carry a significant health risk.

The toxic hazard sign draws attention to the fact that use of toxic solvents or samples may carry a significant health risk.

The attention sign signals relevant information. Read this information.

The note sign signals additional information. It provides advice or a suggestion that may support you in using the equipment.

Intended use

The ROXY potentiostat in combination with flow-through reactor (µ-PrepCell or ReactorCell) is used for controlled REDOX reactions up-front Mass Spectrometric detection. It can be used in a wide range of application, for example:

- Fast synthesis of metabolites (µ-preparative)
- Rapid risk assessments of drug-protein binding
- Signal enhancement in MS
- Electrochemical cleavage of proteins/peptides
- Reduction of disulfide bonds in proteins/peptides
- Oxidative stress/damage of proteins, DNA, lipids, etc.

For research purposes only. **While clinical applications may be shown, this instrument is not tested by the manufacturer to comply with the In Vitro Diagnostics Directive.**

Operation of the ROXY potentiostat in combination with a flow cell can involve the use of hazardous materials including corrosive fluids and flammable liquids. The instrumentation should only be operated by users with the following expertise:

- Completed degree as chemical laboratory technician or comparable vocational training.
- Fundamental knowledge of liquid chromatography & mass spectrometry
- Participation in an installation of the system performed by the manufacturer or a company authorized by the manufacturer and suitable training on the system, flow cell and control 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 equipment operation manuals. Before using your equipment or accessories, you must thoroughly read these safety practices. This manual is written for laboratory technicians skilled in the art.

Unskilled, improper, or careless use of this equipment can create fire hazards, or other hazards which can cause death, serious injury to personnel, or severe damage to equipment and property. Observe all relevant safety practices at all times. Only use the device for applications that fall within the scope of the specified intended use. Else the protective and safety equipment of the device could fail

WEEE directive

Antec Leyden is a Business-to-Business producer of analytical analysis equipment which fall under WEEE Annex IA categories 8 and 9 (includes medical devices and monitoring and control instruments). All equipment of Antec Leyden which are subjected to the WEEE directive (shipped after August 13, 2005) are labelled with the “crossed out wheelie”.

The symbol on the product indicates that the product must not be disposed as unsorted municipality waste.

Collection & recycling information (business-to-business)
Antec Leyden offers the possibility for disposal and recycling of their instrument at an appropriate recycling facility if requested (there may be costs involved with this service). Please contact Antec Leyden for more information about this service and to register the return and disposal of end-of-life instruments. To assure hygienic & personal safety all instrument should be returned with a signed decontamination form which is available on the website.

Shipping address for end-of-life products:

Antec Leyden
Industrieweg 12
2382NV Zoeterwoude, The Netherlands

In case of questions, or if further information is required about the collection & recycling procedure, please contact Antec or your local distributor.

The ROXY potentiostat is ROHS compliant and in conformity with Directive 2011/65/EU Restricted use of Hazardous Substances in electrical and electronic Equipment (ROHS).

Antec Leyden B.V. is an ISO 9001 certified company.

Warranty, spare parts and service

The warranty period of this instrument is 1 year on workmanship, wear and tear parts are excluded. Manufacturer provides operational spare parts of the instrument and current accessories for a period of five years after shipment of the final production run of the flow cell. Spare parts will be available after this five years period on an 'as available' basis.

Manufacturer provides a variety of services to support her customers after warranty expiration. Repair service can be provided on a time and material basis. Contact your local supplier for servicing. Technical support and training can be provided by qualified chemists on both contractual and as-needed basis.

Safety Instructions

Adhere to the following guidelines when using the instrument. The safety practices are intended to ensure safe operation of the flow cell.

Working environment & safety

The intended use of the ROXY potentiostat is to perform controlled REDOX reactions of target compounds (in a suitable liquid electrolyte medium) up-front Mass Spectrometric detection. Operators using the system should have the appropriate education an extensive understanding of GLP rules and be skilled in the art. Use this system ONLY for the intended use. Use of the system for any other purpose might cause unsafe situations.

Operation

To assure optimal performance keep of the instrument we recommend that the flow cell is checked regularly and maintenance procedures are carried out. Preventive maintenance contracts are available for that Purpose. Please contact your local dealer or the nearest sales office for more information.

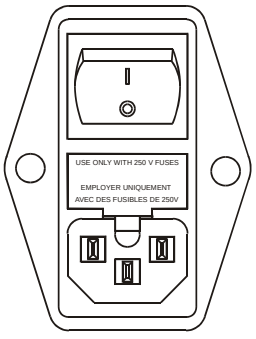
Electrical safety

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

WARNING - RISK OF ELECTRIC SHOCK
DISCONNECT POWER BEFORE SERVICING

AVERTISSEMENT - RISQUE DE CHOC ELECTRIQUE
COUPER L'ALIMENTATION AVANT LA MAINTENANCE

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. Check that the actual power voltage is the same as the voltage for which the instruments are wired. Make sure power cords are connected to correct voltage sources: grounded AC power source, line voltage 100 – 240 VAC. The instrument should be connected to a protective earth via a ground socket. The ROXY potentiostat must only be used 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.



WARNING - RISK OF FIRE
REPLACE FUSE AS MARKED

AVERTISSEMENT - RISQUE DE FEU
REMPLACEZ LE FUSIBLE COMME INDIQUÉ

V ~ 100 - 240V FUSE RATING
50 / 60 Hz
260VA 2.5 AT / 250 V

Replace blown fuses with fuses of proper type and rating as indicated on the rear panel and as listed in the list of accessories and spares (appendix D). 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. Only use manufacturer-supplied I/O cable(s) 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 which do not meet relevant safety standards. Place the potentiostat on a flat and smooth surface. Do not block the fan located at the bottom of the potentiostat. Blocking the fan will impair the cooling capability of the power supply

Solvents

The solvents used may be flammable, toxic or corrosive. The room in which the system is installed should be well ventilated to prevent that solvent vapors cause poisoning or ignite and cause a fire. Use of open fire in the vicinity of this system must be strictly prohibited. Do not install the system in the same room with any 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, it must be flushed away immediately. Provide equipment, such eye wash stations and safety showers, as close to system as possible. Use proper eye and skin protection when working with solvents. Additional safety requirements or protection may be necessary depending on the chemicals used in combination with this equipment. Make sure that you understand the hazards associated with the chemicals used and take appropriate measures with regards to safety and protection. Sample containers (vials) should be sealed to minimize any risks related to solvent vapor.

Biological Hazard

When you analyze biological fluids you need possible precautions and treat all specimens as potentially infectious. Always wear protective and gloves when handling toxic or biologically infectious samples to prevent bio hazards or hazards while working with the flow cell. If necessary the flow cell must be decontaminated before decommissioning or shipment of the flow cell for repair to Antec or its representatives. When shipped to Antec every flow cell has to be accompanied with a decontamination form which should be completely filled in and signed by the customer. Without this decontamination form the flow cell will not be processed by Antec (either repaired or disposed).

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 and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of flammable and/ toxic solvents through the municipal sewage system.

Using the ROXY potentiostat **in other ways than indicated in the manual might result in erratic or unsafe operation.**

Table of contents

C H A P T E R 1

ROXY Potentiostat

Congratulations on your purchase of the ROXY Potentiostat.

With more than 20 years of experience in Electrochemistry (EC), Antec introduces a new, dedicated Potentiostat for on-line EC/MS and EC/LC/MS. The ROXY Potentiostat generates metabolites of drugs or xenobiotics, similar to those generated during in vivo metabolic processes, in a significantly shorter time span (seconds vs. days or weeks) without any interfering components (no isolation steps required). The ROXY Potentiostat is based on state-of-the-art electronics with a large voltage range of ± 4.9 V, and a push button electrode regeneration program. Operational parameters and external equipment can be controlled through programmable timed events. Consequently, the generation of specific oxidation products, e.g., metabolites, cleavage products, etc., and supreme control of any conceivable Redox reaction is assured.

Notification: from the end of 2013 onwards the ROXY potentiostat is delivered with updated electronics which has an extended current range up to 20 mA full scale (was 200 uA). Instruments with updated electronics can be identified by their part number (210.00xxA) and firmware version (FW version 5.23 or higher). From this point onward the ROXY potentiostat with updated hardware will be abbreviated as high current version in this manual in the relevant sections.

This manual covers the installation, set-up and operation of the ROXY potentiostat only. Detailed operation instructions for other peripheral LC equipment and parts such as flow cells, pumps, auto samplers, valves, column heaters etc. are given in the manuals accompanying those accessories.

C H A P T E R 2

Installation guide

It is evident that (for as far it is not specified in this document) the installation site must comply with all applicable local laws and regulations with regard to electrical and mechanical installations, building safety, and use of potentially hazardous materials/chemical and disposal thereof, etc.

Storage requirements

The ROXY potentiostat is shipped in one box to your facility with the following dimensions:

Equipment	Dimensions storage box
ROXY potentiostat	59 (D) x 41 (W) x 56 (H) cm (23.2 x 16.1 x 22.0 in)

Make sure to have sufficient space to store the packed instrument under the following storage conditions:

Parameter	Requirement
Storage temperature	-10 – 50 °C (14 – 122 °F)
Storage humidity	20 – 80%, non-condensing

Site Preparation Requirements

For a successful onsite installation of the instrument, please arrange the following requirements at your location in advance:

Personal Computer

In case the instrument is used via remote control by PC software (Dialogue Elite, Clarity) or firmware (FW) update needs to be performed a desktop computer is required with the following requirements:

- Free RS232 port (onboard, PCI, PCI express or PCI-X)
- Free USB port (for software license key)
- Microsoft Windows 7 Operating System or higher. An US or European Microsoft® Windows™ version is recommended. The Windows operating system should be updated with the latest Service Packs.

Computer requirements can be downloaded from website.

Installing software requires a computer with administrator access. Make sure that the PC and its USB ports is authorized/able to install third-party software. Inform your IT department well in advance to arrange authorization to avoid unnecessary delays during the installation.

For uninterrupted operation of the ROXY EC system and control software Antec advises to turn off:

- Screensavers
- (USB, LAN) hibernate mode
- Auto hard disk shut down (energy saving)
- Automatic Windows updates
- Avoid exhaustive scanning by virus scanners*

*) In your antivirus SW, turn off the option "Check Files at Change" for the relevant Dialogue Elite data storage directory.

Environmental requirements

Your instrument is intended for indoor use only in an industrial or commercial environment. It is suitable for the following categories: Installation category II, Pollution degree 2, equipment class I. The ROXY potentiostat is specified for operation under the following conditions:

Parameter	Requirement
Operating temperature	10 – 35 °C (50 – 95 °F)
Maximum Altitude	2000 meter (7500 ft)
Operating humidity	20 – 80%, non-condensing

For optimum performance it is recommended that the ambient temperature of the laboratory be between 18-25 °C and be held constant to within ± 2 °C throughout the entire working day. Note: that for optimal performance of the oven the oven temperature should be set at least 7 degrees higher as ambient temperature.

Do not place the system next to heating or cooling pipes or expose the instrument to direct sun light or expose it to air drafts (AC system / open windows).

ze Requirements

The size requirement for the ROXY potentiostat are as follow:

Description	Dimensions (D x W x H)	Weight
ROXY Potentiostat	44 x 22 x 44 cm (17 x 9 x 17 inch)	14 kg (30.9 lbs)

1. The potentiostat should be installed on a stable, flat and smooth table, which is able to hold the weight of the system and other peripheral which might stand next to it (like computer equipment etc.).

2. Sufficient space around the potentiostat must be kept clear for ventilation and safe working. If other equipment is placed next to the system:

    - keep at least 5 cm, if there is another device on one side.
    - Keep at least 10 cm, if there are devices on both sides

3. The electrochemical flow cell should be positioned as close as possible to the inlet of the ionization source of the MS. The ROXY potentiostat controlling the cell should also be positioned as close as possible to the MS, in any case within a distance of max 2.5 meter of the ESI inlet.

4. The PC for control and data-acquisition of the system should be placed in the vicinity of the ROXY potentiostat, within a distance of max 2.5 meter.

Tip: Installation of the ROXY potentiostat + PC on a suitable movable laboratory cart/table has multiple advantages:

- Easy access to all sides of the instrumentation
- System can be easily manoeuvred next to the (ESI inlet) MS
- Flexibility with respect to use of both ROXY potentiostat and MS.

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 be in compliance with all local regulations and safety standards.

2. The power source should exhibit minimal power transients and fluctuations. The AC mains supply voltage source should not fluctuate more than +/- 10% from the nominal voltage. If your mains voltage is unstable (>10% of nominal voltage use an Uninterruptable Power Supply (UPS) The mains supply must include a correctly installed protective earth conductor.

To protect against power transients (voltage spikes and power surges) it is advised to connect the equipment over an electrical surge protector.

3. The Roxy potentiostat is equipped with an universal AC/DC switched-mode power adapter rated for 100–240 V AC and 50/60 Hz. Every potentiostat is delivered with a set of 2 power cords for the following regions:

    - EUR (CEE7/7 plug to IEC60320 C13 plug)
    - US (NEMA 5-15 plug to IEC60320 C13 plug)

For regions with deviating mains plugs/sockets like (for example UK, Switzerland, Brazil etc.) make sure to have the appropriate power cords available on-site at the date of installation. Make sure these power cables are properly grounded and meet the relevant safety standards which apply in your country. In case of questions contact your local distributor.

4. The maximum power consumption of the ROXY potentiostat on full power) is < 200 Watt. The typical power consumption is < 50 Watt.

5. Connect the potentiostat to a grounded AC wall socket with a line voltage of 100 – 240 VAC (as specified in the sections above) using the supplied power cables. The instrument should be connected to a protective earth via the socket. Make sure the detector is placed in such a way that the mains power connection can be reached easily to disconnect it from the mains power by removing the power cable.

Only use manufacturer-supplied cable(s) 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 which do not meet relevant safety standards.

Chemicals

Mobile phase and flush/storage solutions must be clean as it is in direct contact with the working electrode in the electrochemical flow cell. High purity chemicals including water is a prerequisite. So all chemicals should be electrochemically clean, HPLC/ MS grade or better. For water used for the preparation of mobile phases a water purification apparatus is advised which is able to supply high purity deionized water with resistivity of >18 MOhm.cm and low TOC level (<10 ppb).

Unpacking

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

See check list below for reference:

(1)	Delivery is in accordance with order	O
(2)	Delivery is undamaged	O
(3)	All items on checklist(s) are included	O
(4)	Certificates of performance are included:	
	- ROXY potentiostat	O
	- flow cell(s)*	O
(5)	User manual(s) is (are) included on USB stick	O

*) Note that flow cells are not part of the ROXY potentiostat and have to be ordered separately.

To unpack the ROXY Potentiostat, lift it from its box by both hands (Fig. 1). **Never lift the ROXY Potentiostat at its front door**, but at its sides.

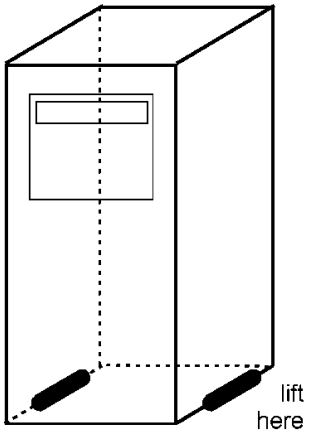


Fig. 1. Lift instructions ROXY Potentiostat.

Install the potentiostat in an area which meets the environmental conditions listed below, for further requirements read the :

Table I. Environmental conditions

Parameter	Requirement
Operating temperature	10 – 35 °C (50 – 95 °F)
Maximum Altitude	2000 meter (7500 ft)
Operating humidity	20 – 80%, non-condensing

Place the potentiostat on a flat and smooth surface.

Do not block the fan located at the bottom of the potentiostat (Fig. 2.). Blocking the fan will impair the cooling capability of the power supply.

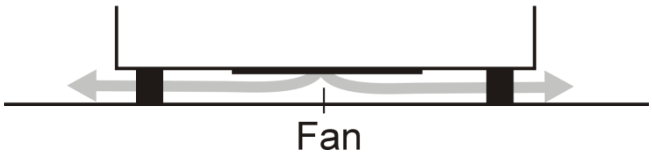


Fig. 2. Location of power supply fan ROXY Potentiostat.

Inspect the potentiostat for possible damage and make sure that all marked (and ordered) items on the checklist are included.

Switch ON the ROXY Potentiostat by the mains switch on the rear panel. Ensure that the power (on/off) switch and power cord are always accessible.

LC connections

Use proper eye and skin protection when working with solvents.

1. The manufacturer will not accept any liability for damage, direct or indirect, caused by connecting this instrument to devices that do not meet the relevant safety standards.
2. The ROXY™ EC system requires a syringe pump to deliver mobile phase or sample solution.
3. Consult your flow cell manual for installation details. Connect the cell to the corresponding cell connector in the oven compartment. All cell

connectors are marked with a label for identification. The cell connector inside the oven compartment is ESD sensitive. Make sure that the electrochemical cell is OFF when removing or connecting the cell cable.

Never switch ON the electrochemical cell when:
- the cell cable is not correctly connected
- the cell is only partly (or not at all) filled with mobile phase containing the supporting electrolyte (e.g., ammonium formate, formic acid)
- the outside of the electrochemical cell is wet, particularly the part between the auxiliary and working electrode connection
because substantial damage to the working electrode or electronics may occur.

4. Before switching ON the cell, make sure that the buffer contains sufficient electrolyte (buffer ions). A stable working conditions will never be obtained if the cell is switched ON with only water or another non-conducting mobile phase. Also be sure that no air bubbles are trapped in the electrochemical cell.
5. Connect the data system to the output (see page 38).
6. Set the cell potential (see page 49 for optimization of the potential), switch ON the flow cell (see page 27) to oxidize the sample. In case electrochemical detection will be used allow the system to stabilize for approximately 30 min. A ‘good’ stabilization curve shows a mono-exponential decline without jumps and/or spikes.

Your system is now ready for use.

C H A P T E R 3

Maintenance & Shutdown

Maintenance

In this paragraph all maintenance is described which can be performed by the end-user, all other maintenance & service procedures may only be performed by authorized service engineers only.

Periodic check for leakage

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

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 ROXY potentiostat.

In case the actual temperature exceeds 55°C switch off the detector and contact the manufacturer or its representatives for service.

Cleaning

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

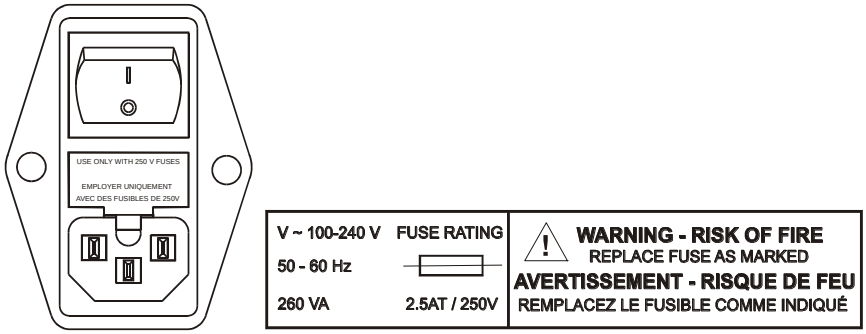
Do not use any organic solvents to clean the exterior of the potentiostat, because this may lead to damage of the paint layer.

In case of leakage in the cell cabinet (tubing, connectors, cell, column etc.) remove the spilled mobile phase or other solutions as soon as possible because this may damage the paint layer, or result in the deposition of salt

crusts (in case of 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.

Replacement of fuses

Replace blown fuses with fuses of proper type and rating as stipulated on the rear panel and specified in the installation section of this manual. 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.



In case the fuses blow out repetitively contact Antec or its representatives for instructions and/or service of the instrument.

Shutting down the system

There are a couple of steps to take to switch off an system with potentiostat for a longer period of time. Shutting down is not different from most other HPLC systems. Perform the following procedure:

- Switch off the flow cell using the keyboard (standalone) or via the software (Clarity or Dialogue Elite).
- If a column is installed: check the column(s) documentation for the appropriate storage liquid, apply this and make sure the column is properly flushed. A reversed phase C18 column is usually stored with 50% Acetonitrile/water.
- Take out the column, mount the corresponding end-caps and store the column in an appropriate place.

Avoid precipitation of high salt concentrations in organic solvent, first wash out salts with water if necessary.

- Flush and store the system with 50% water/acetonitrile (or methanol). Switch the injector valve between load and inject a few times. Make sure all tubing etc. are flushed so no traces of salt are left that could precipitate and clog the system.
- Remove the flow cell from the system by disconnecting the inlet and outlet capillary.
- Open the cell, flush with water, use some tissues to carefully dry the cell.
- Close the cell and store dry.
- Switch off the ROXY potentiostat (and other LC equipment) via the mains switch (switch to position ‘0’) on the rear panel.

C H A P T E R 4

ROXY Potentiostat

Introduction

The ROXY Potentiostat has been designed for maximum functionality and ease of use. The control of EC parameters is such that without reading this chapter, it should be possible to operate the potentiostat. This chapter is intended as a reference guide in case questions arise during operation.
The information shown in the numerous screens is presented in alphabetical order. For each item an explanation is given, together with the item's nature and the screen(s) of appearance. 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 - F5.
The 'Enter' button is only used to accept changes in cell potential. In the top right corner of each screen the name of the present screen is displayed. If available, the bottom left function button displays a previous screen, and the bottom right one the next screen.

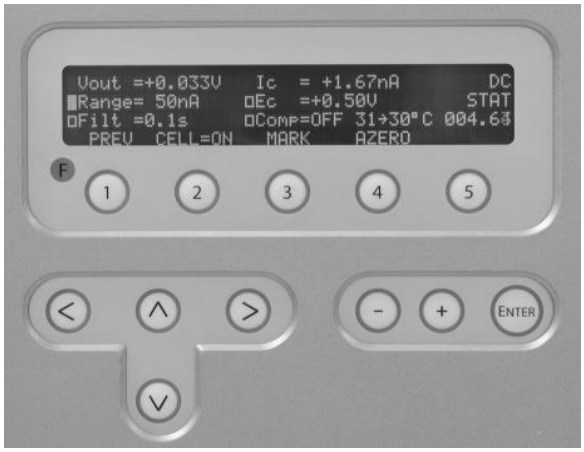


Fig. 3. ROXY Potentiostat keyboard. The cursor is on 'Range' which allows changes using the value buttons '+' and '-'. The 'Enter' button is only used to confirm changes in potential (Ec).

Overview of ROXY Potentiostat screens

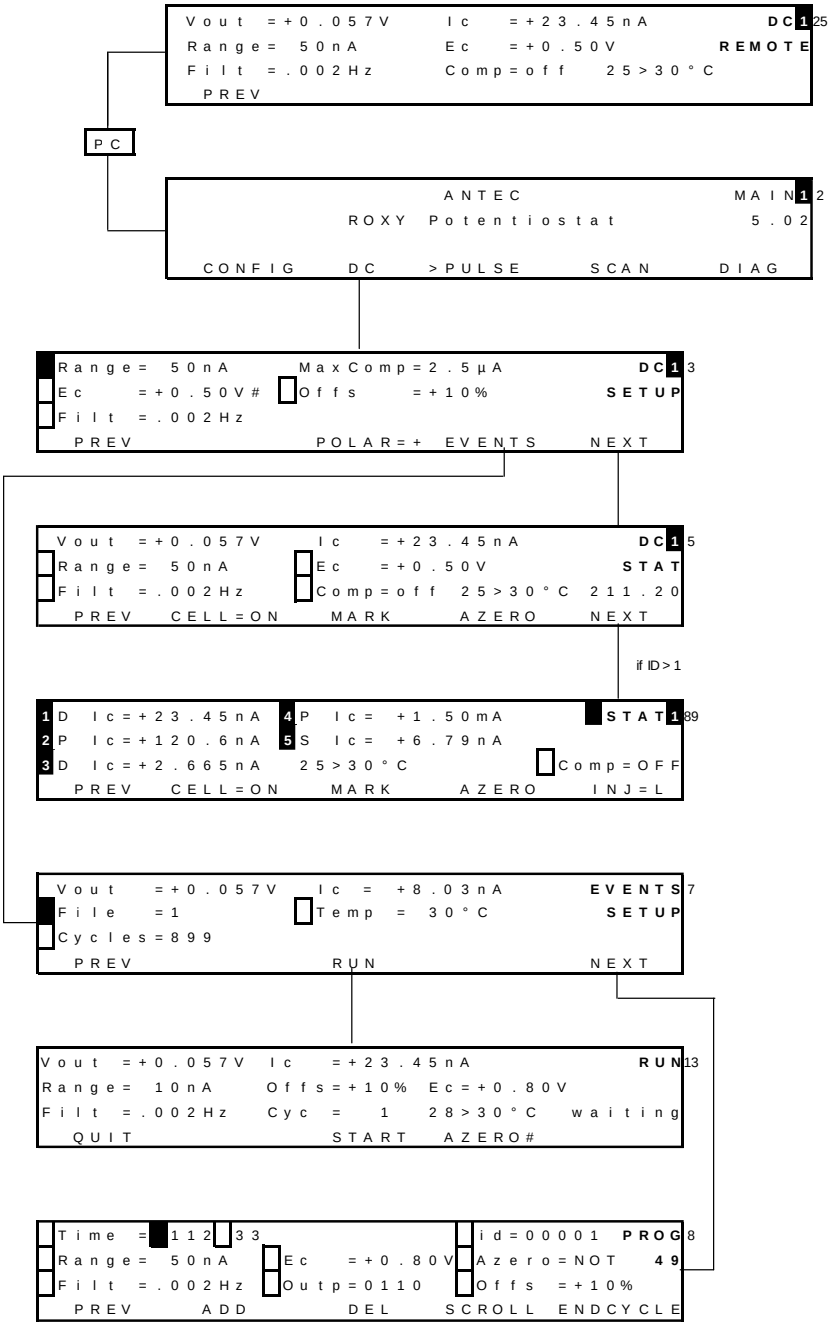


Fig. 4. DC mode.

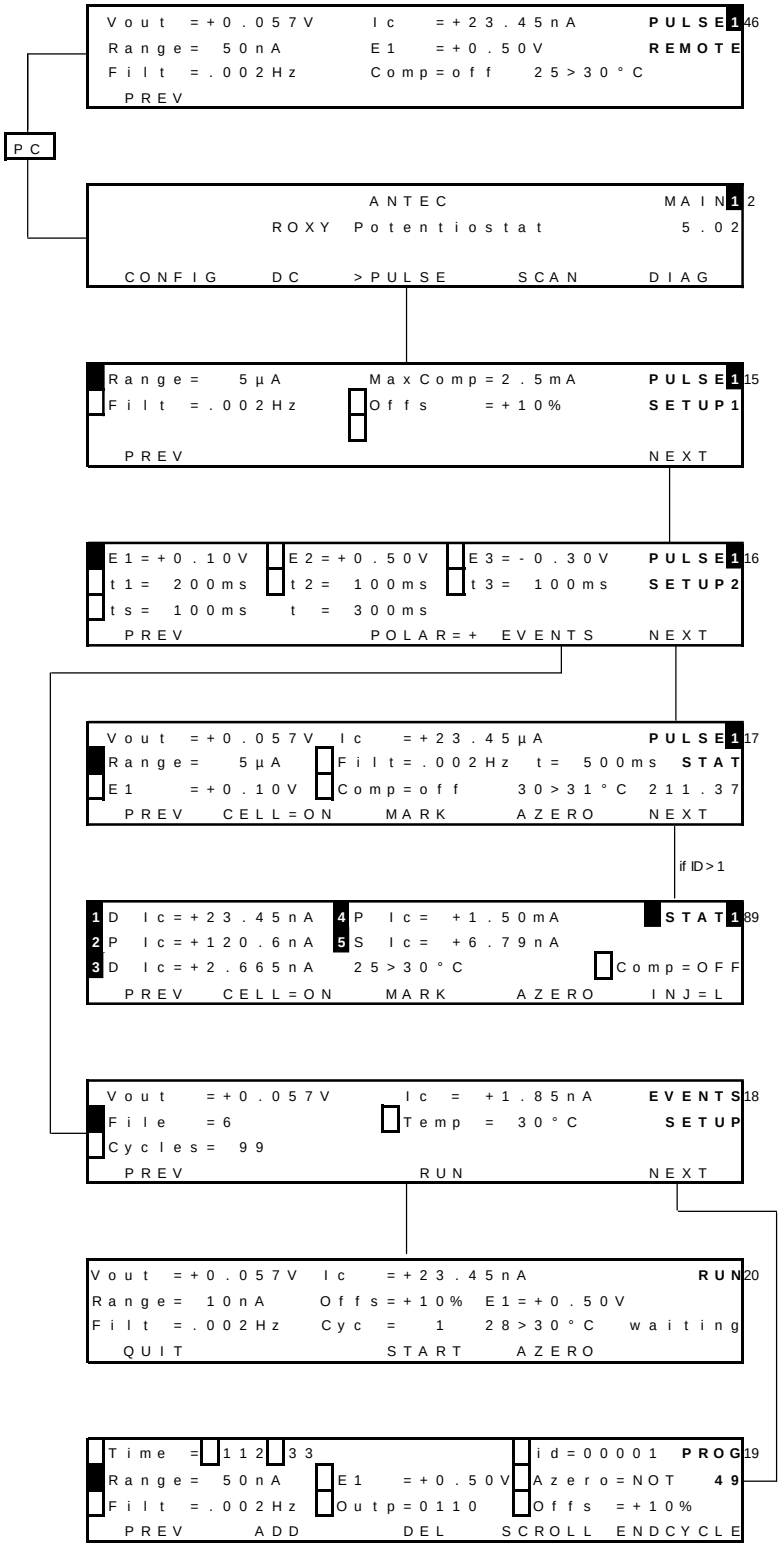


Fig. 5. PULSE mode.

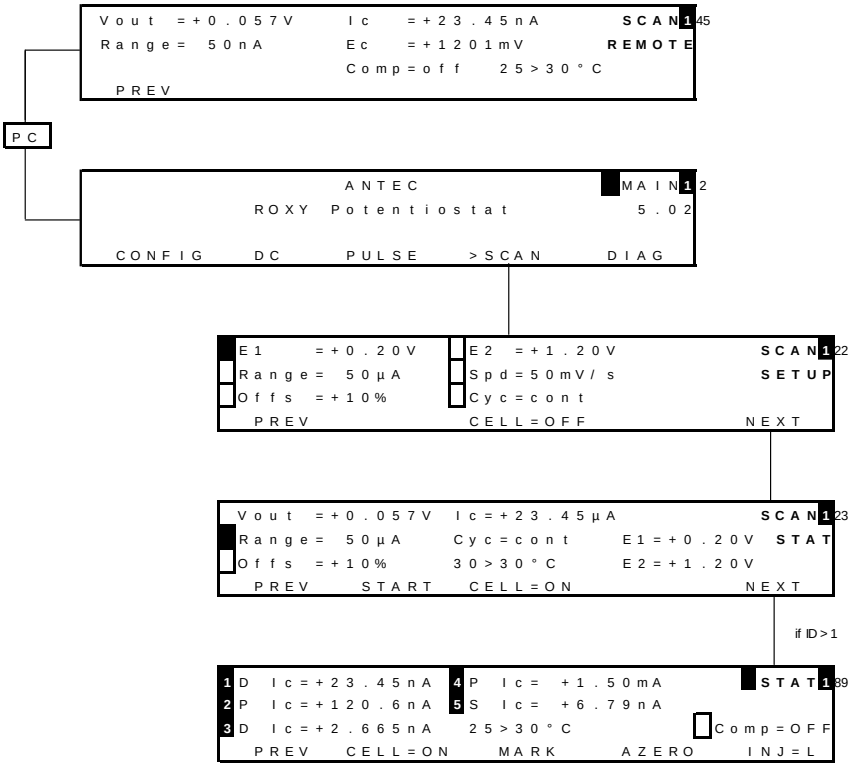


Fig. 6. SCAN mode

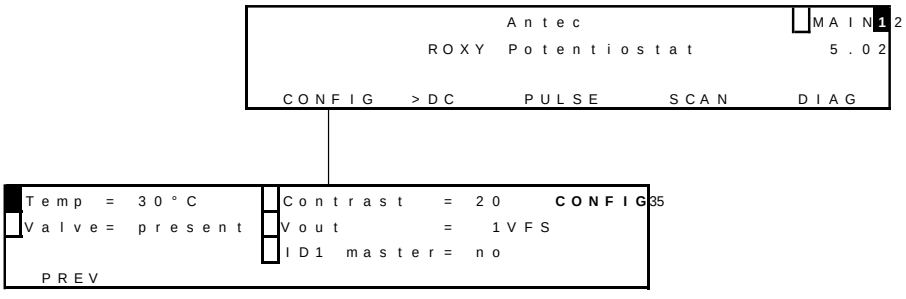


Fig. 7. CONFIG screens.

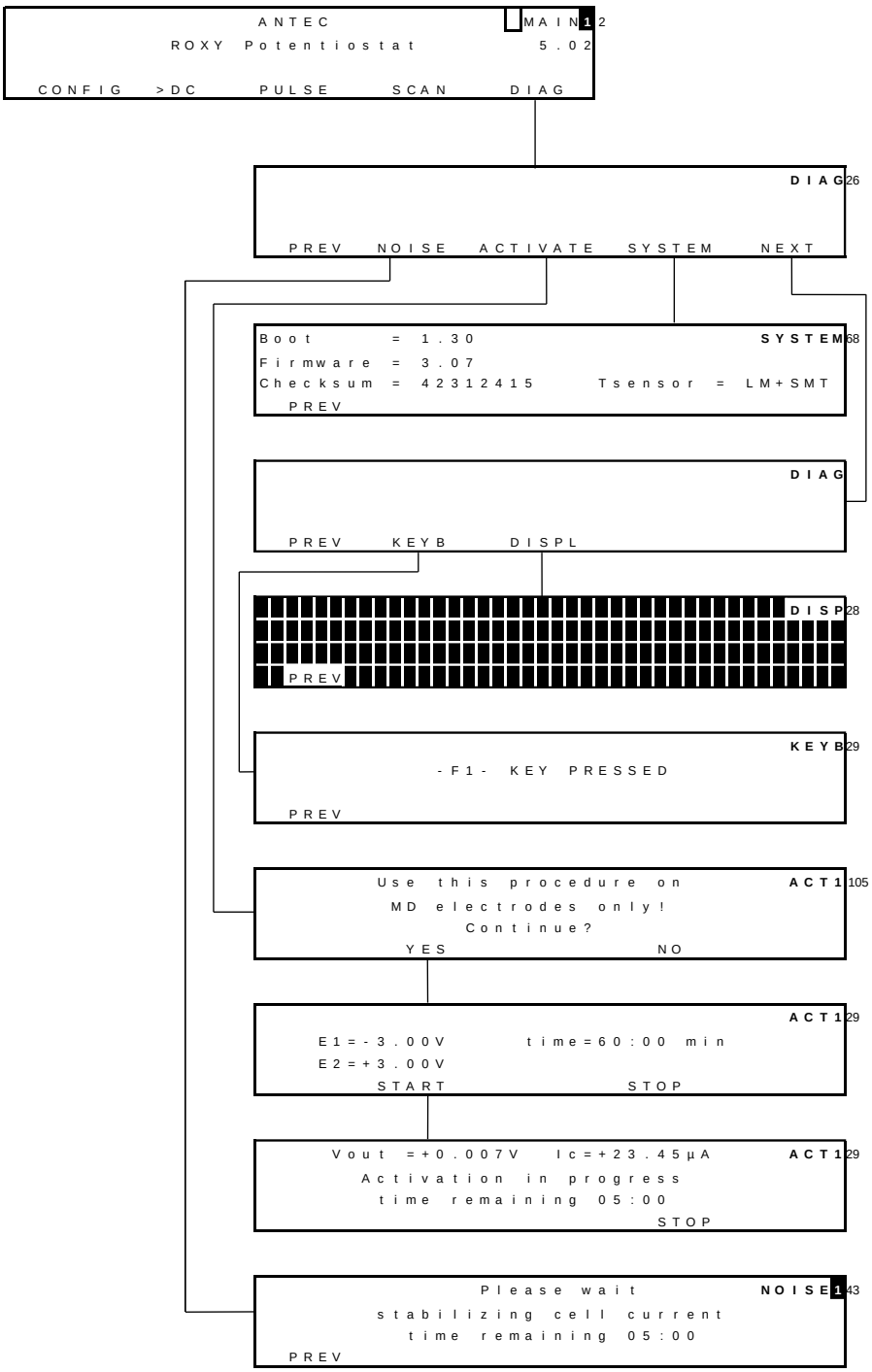


Fig. 8. DIAG screens

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 value) and the pre-set oven temperature (right value).	S
ADD	prog	Adds the active data line to the time file . Confirmation is asked for if an existing time is overwritten. As time 0.00 always exists, changing this time results in an overwrite warning (see page 43).	F
EVENTS	dc setup, pulse setup2	Enters EVENTS (‘EVENTS SETUP’ screen) for editing and running a time file.	F
AZERO	dc stat, run, pulse stat, scan stat	Sets the output voltage to 0 V, or to the offset voltage (see page 38). Control Comp = off changes to Comp = on. If cell current exceeds the max. compensation a message “cell current exceeds max. compensation” appears. In that case max. compensation will be applied, which may not be the 0 Volt level but higher.	F
Azero	prog	Controls auto zero, which can be programmed in a time file (see page 43). Toggles between ‘set’ and ‘not’.	
Boot	system	Displays boot firmware version	S
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 which screen is 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

Parameter	screen	Description	Type
Cyc	run	Displays the cycle counter. If a time file has to be executed more than once (‘Cycles’>1), this is the number of times a time file has been started (see page 43). RESET (external) or QUIT sets Cy to 1 and returns to EVENTS SETUP screen.	S
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, and 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
Cycles	events setup	Controls the number of times a time file has to be repeated. This number can be 1 - 999 or continuous.	C
DEL	prog	Deletes the current data line from the time file . Deleting time 00.00, results in deleting the complete time file . Confirmation is required.	F
DIAG	main	Enters Diag screen	F
DISPL	test	Enters DISP screen for display test.	F
E1, E2, E3	pulse setup2, act	Controls the cell potential settings of the pulse or activation	C
Ec	prog (dc), events setup (dc), dc setup	Controls the cell potential is 10 mV steps between +4.90 and -4.90V for the ROXY Potentiostat. Can only be set or changed after confirmation with the ‘enter’ button. Controls the cell potential in a time file (without confirmation).	c
Ec	run (dc), scan stat	Reflects the set cell potential. Displays the actual cell potential in the scan mode.	S

Parameter	screen	Description	Type
EndCycle	prog	Enters a screen to set EndCycleTime. Controls duration of a time file (max. 999.99 min). When this time is reached the execution of the time file stops. If programmed, the next run is started. Cannot be smaller than smallest time in time file +0.01 min. Is therefore never smaller than 0.01 min.	F
Events	dc setup, pulse setupup2	Enter events menu	F
File	events setup	Selected time file number. In the DC mode file numbers 1 - 5 are available, in the pulse mode file numbers 6 - 9 can be selected. The time files remain stored in RAM, also after switching off the ROXY Potentiostat. Time files can be uploaded via RS232.	C
Filt	dc setup, dc stat, prog	Filter settings: 0.5 to 0.001 Hz cut off frequency, in 1, 2, 5 steps.	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 time file or scan.	F
HOLD=0,1	run, scan stat	Holds or continues execution of time file or scan. Toggles between 1 and 0. Pressing hold again continues time file or scan were it has been hold.	F
Ic	stat, events setup, run, noise, act	Displays the true, non-compensated cell current, unaffected by auto zero or offset.	S
Id	prog	Board identifier for multi cell purpose. Indicates for which boards time file settings apply. Binary coded.	C
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.	F/S

Parameter	screen	Description	Type
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
Offs	setup, stat	Percentage offset, can be set between -50 and +50%.	C
Offs	run	Displays percentage offset during execution of a time file .	S
Outp	prog	Control of four output functions in EVENTS. Is open/high if '0', is closed/low if '1'. AUX1: 0001, AUX2: 0010, relay 1: 0100, relay 2: 1000. Combinations are possible.	C
POLAR	dc setup, pulse setup2	Inverts output polarity, toggle between + and -. Requires confirmation.	F
PREV	several screens	Return to previous screen	F
QUIT	run	Aborts the time file and returns to the 'EVENTS SETUP' screen. The cycle counter ('Cy') is reset to 1. Outputs Aux 1 and 2, and Relays 1 and 2 are reset (status: 0000).	F
Range	setup, stat, prog	Range setting, varying from 10 pA to 20 mA* full scale, in 1, 2 and 5 steps. In the pulse and scan mode 10 nA to 20 mA* full scale can be used.	C
RUN	events setup	Enters RUN screen. System waits ("waiting") for the 'START' input trigger (external or keyboard) to start a run.	F
S	scan setup	Scan speed, can be set from 1 - 50 mV/s in 1, 2, 5 steps.	C
SCROLL	prog	Scrolls through a time file .	F
SPD	scan stat	Scan speed, can be set from 1 - 50 mV/s in 1, 2, 5 steps.	C

Parameter	screen	Description	Type
START	run, scan stat	In DC and pulse mode: toggle between STOP and START execution of a time file . Starts a scan in scan mode.	F
STOP	run, scan stat	Scan mode: STOP aborts scan and resets cell potential to E1. DC and pulse mode: toggle between STOP and START to control execution of a time file . Pressing 'STOP' aborts this run, cycle counter (Cy) is reset to 1. STOP also deactivates the outputs Aux 1 and 2, and Relays 1 and 2 (status: 0000) and sets the electric valve to load (if present).	F
t	pulse setup2, pulse stat	Displays the total duration of one pulse (t1 + t2 + t3).	S
t1, t2, t3	pulse setup2	Duration of potential step E1, E2, or E3. Time can be set between 0 (t2, t3) or 100 (t1) and 2000 ms in 10 ms increments.	C
Temp	config	Controls the temperature of the oven. Range: off, 15 - 45˚C, selectable in 1˚C steps. The oven is stable from 5 ˚C above ambient oven temperature.	C
Temp	events setup	Controls the temperature of the oven, is stored with time file. Temperature of active time file temperature overrules other temperature setting after selecting START.	C
Time	prog	Controls the time to execute a data line in a time file , can be set with 0.01 min resolution. Maximum time is 999.99 min. The time to stop the execution of a time file must be programmed by EndCycleTime.	C
Toven	dc setup, pulse setup1	Controls the temperature of the oven. Range: off, 15 - 45˚C, selectable in 1˚C steps. The oven is stable from 5 ˚C above ambient.	C

Parameter	screen	Description	Type
ts	pulse setup2	Controls the duration of the sampling time in the pulse mode. The time can be set between 20 and 100 ms in 20 ms increments.	C
Tsensor	system	Displays active temperature sensor	S
Valve	prog	Controls the electrically actuated injector, during execution of a time file. Forces this valve to load (‘LD’) or inject (‘INJ’).	C
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, events setup, run, noise, act	Displays output signal.	S
Vout	config	Controls max output signal (1 or 10 V full scale) for all sensor boards.	C

*) Specifications marked with * are only valid for the ROXY potentiostat high current version. For the previous model the max current range is 200µA for all measurement modes.

ROXY Potentiostat data systems

The ROXY Potentiostat in the ROXY EC system can be fully controlled by Dialogue Elite™ software (Fig. 9). Additionally, Dialogue Elite can control syringe pump, which is delivering sample solution. The software package contains a set of event table files (*.evt) for the automated recording of mass voltammograms and sample oxidation or reduction. The exemplary methods can be easily adapted to any demands. (Fig. 10). Detailed information about Dialogue Elite and event programming can be found in document 175.0015 Dialogue Elite user manual.

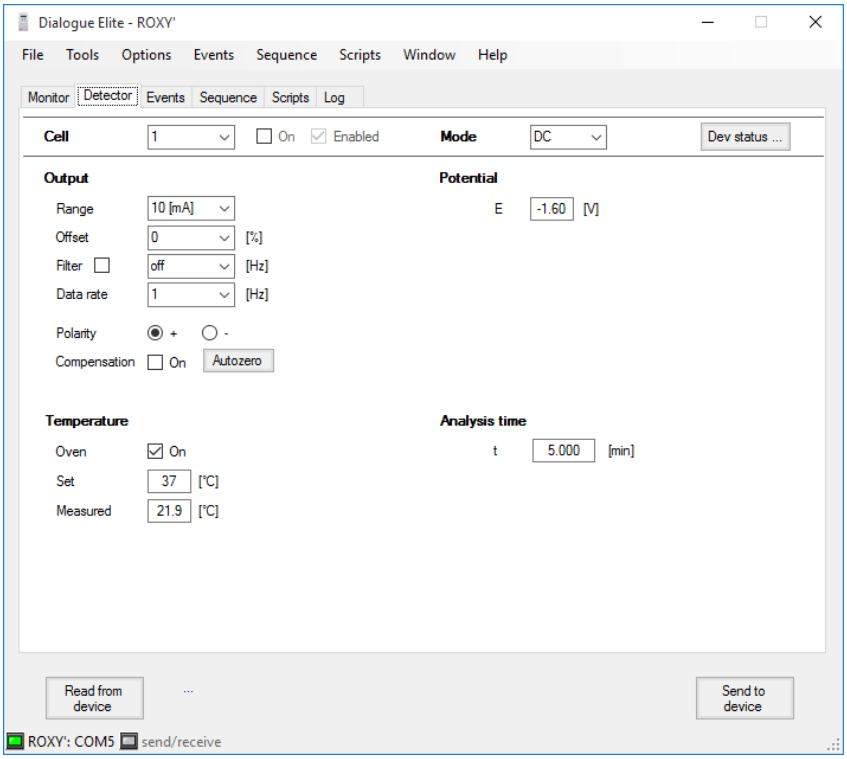


Fig. 9. Dialogue Elite for ROXY. Cell control window (Detector tab).

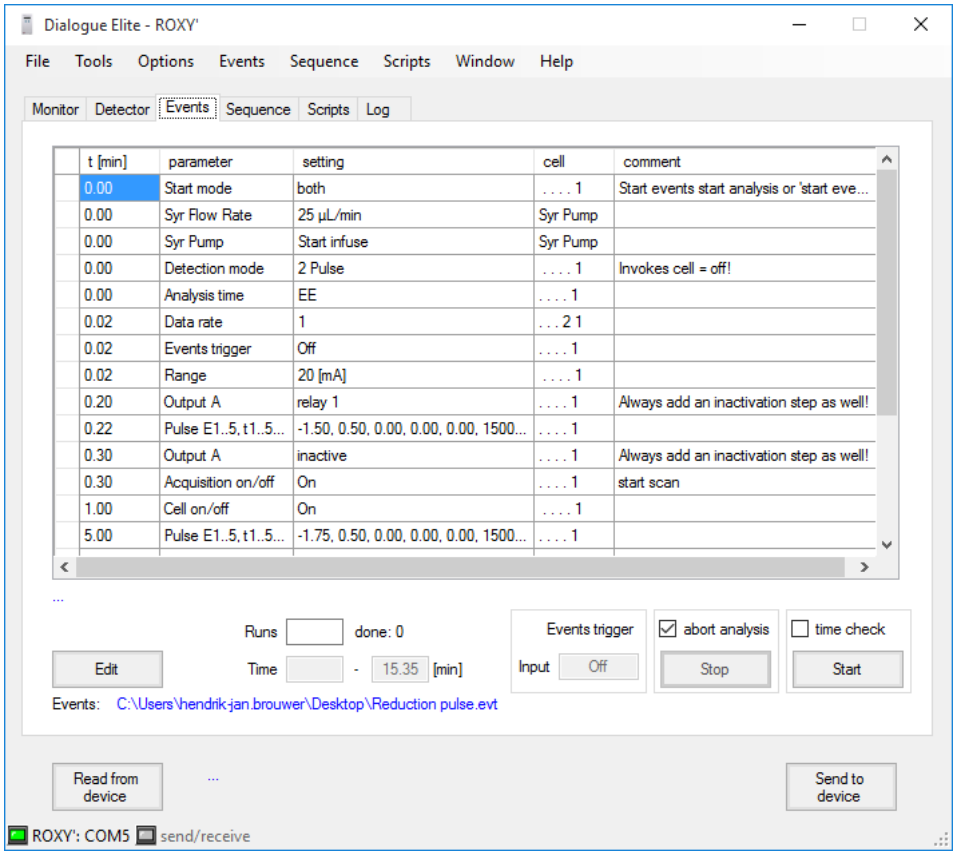


Fig. 10. Dialogue for ROXY. Events programming window.

The ROXY Potentiostat (delivered before 2013, with FW5.11) in ROXY EC/LC system is controlled by Clarity software (Fig.11). Programming of all functions of the potentiostat is performed in the Clarity: Main, Output and Time Table tabs of the Potentiostat control window. The user defined programs are delivered with the Dialogue software for the ROXY EC/LC system. The phase I (oxidation of the substrate) and phase II (conjugation with any reactant) experiments are automated. A detailed explanation of the User Defined Programs is given in the appendix of application note 210.002A ROXY™ EC/LC system – user defined programs for AS110.

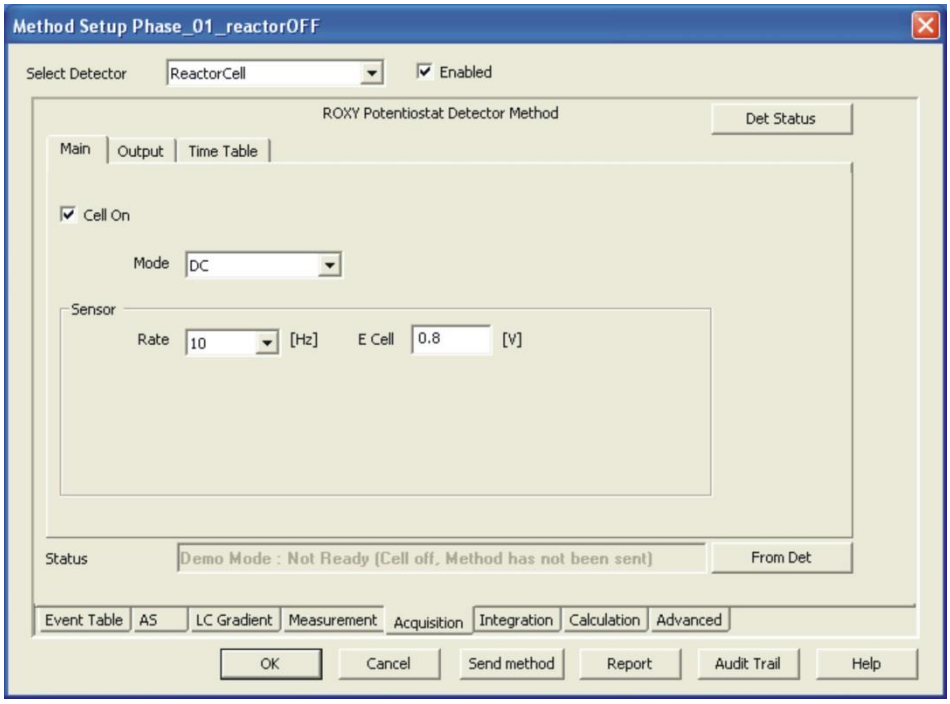


Fig. 11. Clarity software. The potentiostat control window.

ROXY potentiostats delivered after 2013 with updated electronics (which can be recognized by its FW version: FW 5.2x or higher) cannot be controlled in Clarity. For this instrument Dialogue Elite should be used for remote control.

C H A P T E R 5

Events and time files

Introduction

Running an Events table (time file) enables a time-based, automated and full parametric control of the analysis. This is particularly useful when during a run or between runs settings have to be changed such as the sensitivity, auto zero or control of external equipment (i.e. trigger to start integration software etc.). A time file contains a series of data lines (maximum of 50) in which the settings of the ROXY Potentiostat can be changed with 0.01 min (0.6 s) time resolution.

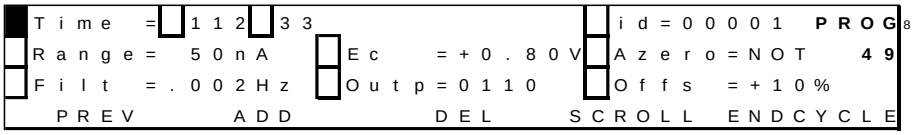


Fig. 12. Programming a time file using the 'PROG' screen.

The time file is made using the ‘PROG’ screen. Programmable parameters comprise cell potential, range, auto zero, offset, filter, board id, electrically actuated injector (if present) and the ROXY Potentiostat output contacts to control the status of external equipment.

Table II. A time file used for offset programming at 5.00 min.

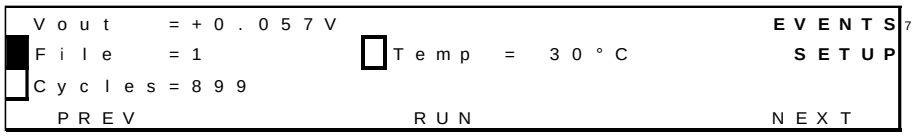
time	range	filter	Valve	auto zero	output	offset	E cell	id
00.00	1 nA	0.5 Hz	Load	not	0000	00%	0.80 V	00001
00.02	1 nA	0.5 Hz	Load	set	0000	00%	0.80 V	00001
05.00	1 nA	0.5 Hz	Load	not	0000	-30%	0.80 V	00001
05.02	1 nA	0.5 Hz	Load	set	0000	-30%	0.80 V	00001
14.96	1 nA	0.5 Hz	Load	not	0000	00%	0.80 V	00001
14.98	1 nA	0.5 Hz	Load	set	0000	00%	0.80 V	00001
15.00	EndCycleTime (end of run)							

Step by step example

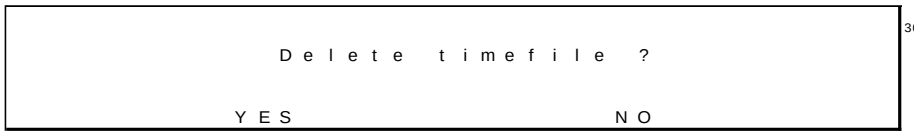
In this step-by-step guide the example from Table II will be programmed. The data acquisition software controls the potentiostat, and the autosampler. There is no external valve connected to the ‘C’ or ‘B’ on the rear panel of the

potentiostat. For some reason, a user wants to give an –30% offset after t=5.00 min.

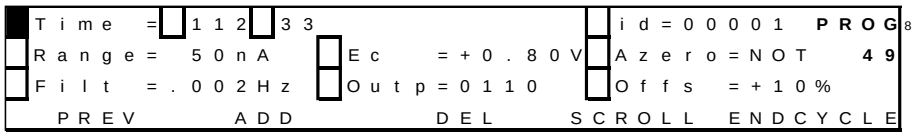
Go from MAIN, DC SETUP to DC STAT screen to see if the cell is ON or not. Set the cell to the desired status and return to DC SETUP. From the DC SETUP screen select 'EVENTS'.
In the EVENTS SETUP screen, select file number 'File = 1', actual cell potential 'Ec = 0.80 V', and the number of cycles 'Cycles = 1'. Vout and Ic show the actual cell current and output signal. In DC mode file nr. 1..5 is available, in PULSE mode file 6..9.



Press PROG to enter the PROG screen. Before programming, first the contents of file 1 is checked to make sure that the file is not already in use. Press SCROLL to see the contents of the file. If the file is still relevant and contains timed events, another file can be selected in the EVENTS SETUP screen. If the file contains data that are no longer used, the contents of the file can be erased. Scroll to Time = 0.00 min and press DEL. Answer 'Yes' to the question:



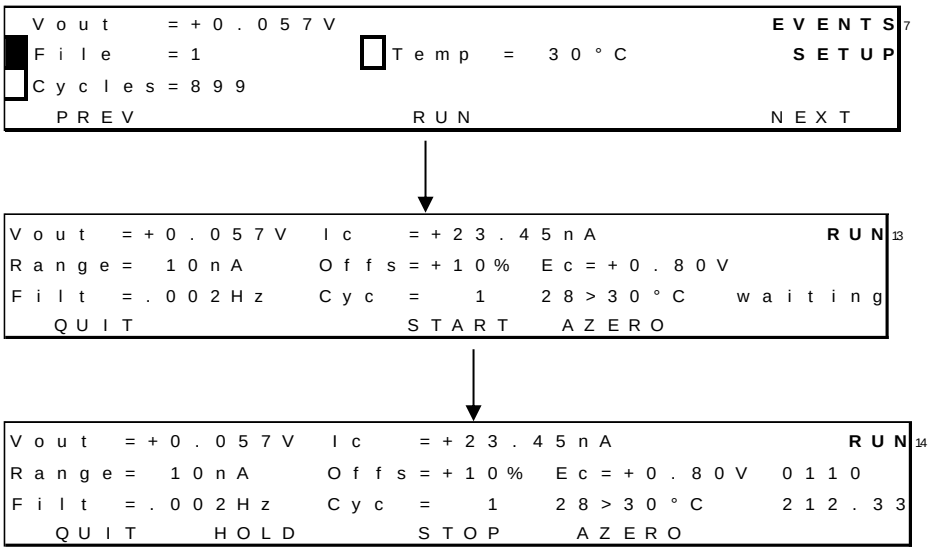
Programming the time file is done by entering all parameters for Time = 0.00 and pressing ADD. This is repeated for each time line in Table II.



If a time already exists, a message appears "Overwrite time x.xx ?". Confirm this and continue programming by entering the new time with its corresponding settings. Note that in the example at Time = 14.96 min the % offset is set to

00% to prepare for the next run. An auto zero event is programmed 0.02 min later at Time = 14.98 min.
After entering all events, press PREV (or ENDCYCLE) to enter the EndCycle screen. Program the EndCycleTime. This time is always 0.01 min higher than the last programmed events.

To start the time file , select RUN from the EVENTS SETUP screen. The RUN screen appears and the system is waiting for a start command. This can be a keyboard command, or an external trigger (line 13 from connector A on the rear panel).



Output events

Connector A and B on the rear panel enable control of (or by) external equipment. Together with time files this supplies a powerful tool for development of automated methods.

Inject marker

A manual valve with position sensor can be connected to ‘C’ on the rear panel of the ROXY Potentiostat which enables the inject marker on connector B. The

contact is high when the valve is in ‘load’ position, and low in the ‘inject’ position. It can be used to start the integration software when injection is done.

Overload

Activated when a overload occurs, see also page 63 for details.

Auto zero

Enables external activation of the auto zero command. This function is active only when the ‘I-cell’ is displayed.

To pos I, L

Forces the electrically actuated injector to position L (load) or I (inject).

Cell on, off

Switches on (off) the cell. This input command can be used for example to switch on and stabilize the cell early in the morning by means of a timer.

Table III. I/O contacts connector A.

No.	Name	I/O	Function
1,2,3	Relay 1	Out	Contact between 1 (common) and 2 (default) or 3. Activated by time file Outp 0100
4,5,6	Relay 2	Out	Contact between 4 (common) and 5 (default) or 6. Activated by time file Outp 1000
7	Cell on	In	Trigger to switch on cell
8	Reset	In	Resets a running time file
9	Overload	Out	Active when overload occurs (‘out of range’)
10	AUX1	Out	Free programmable TTL output Activated by time file Outp 0001
11	AUX2	Out	Free programmable TTL output Activated by time file Outp 0010
12	Cell off	In	Trigger to switch off cell
13	Start	In	Starts a time file
14	Auto zero	In	Auto zero command, always accessible when ‘I-cell’ is in display
15	Common		Ground

Outputs 7, 8,12,13 and 14 are level triggered. When active, output status 9, 10 and 11 is low (default is high).

Table IV. I/O contacts connector B.

No.	Name	I/O	Function
1 - 3	Common		Ground
4	Free TTL input	In	
5	Mark	In	Baseline spike of 10% FS, duration: 0.1 s
6	Status I	In	Status read of electric valve, pos B (inject)
7	Status L	In	Status read of electric valve, pos A (load)
8 - 11	Common		Ground
12	Free TTL output	Out	
13	Inject marker	Out	In combination with manual valve connected to connector C, high: 'load', low: 'inject'.
14	To I	Out	Forces electric injector to "inject"
15	To L	Out	Forces electric injector to "load"

Outputs 4 – 7, 12, 14 and 15 are level triggered.

Level triggered TTL input: 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 will occur.
TTL output: default = high (5 Volt)

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

C H A P T E R 6

Optimization of working potential

Introduction

A current - voltage (I/E) relationship (Electrochemical Detection), or voltammogram (ROXY applications), characterizes an analyte. It gives information on the optimum working potential, which can be used to improve detection sensitivity and selectivity or REDOX products formation.
There are several ways to obtain a voltammogram. A *hydrodynamic* voltammogram is obtained in the 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 can be acquired with ROXY EC/LC system with automated sample oxidation, separation of the products and MS detection. A *scanning* voltammogram is obtained in the so-called scan mode of the ROXY Potentiostat: the voltage runs between two pre-set values and the current is measured. Hydrodynamic and scanning voltammetry are common methods to obtain the optimized potential for a target compound in EC/LC/MS or EC/MS when a mass spectrometer is used as a detector. A MS Voltammogram can be obtained also in DC mode by ramping the WE electrode potential within required range. All operational modes of the ROXY Potentiostat are programmable in the Dialogue (events table). A MS voltammogram can be visualized in a 3-D or 2-D plots.
Information about MS voltammogram acquisition can be found in the Dialogue for ROXY™ EC system User guide (p/n 210.7017).
Optimization of the working potential and the construction of a hydrodynamic and scanning voltammogram using ROXY Potentiostat keyboard are described below.

Electrochemical reactions

In an electrochemical reactor a reaction of the analyte at an electrode surface occurs. 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.

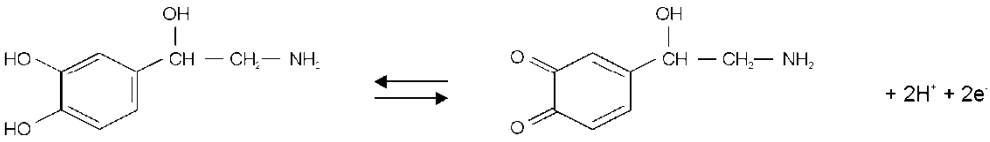


Fig. 13. Oxidation/reduction reaction of norepinephrine.

The mechanism of the REDOX reactions is the same for the ROXY applications. The potential is the reactions driving force, but the mass spectrometry (MS) is applied for the oxidation or reduction products detection. An example of an electrochemical reaction is shown in Fig. 13, 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. The norepinephrine and its quinone product itself will be detected in mass spectrometer in ROXY applications.

Because of the same nature of electrochemical reactions in electrochemical detection and ROXY applications with MS detection some details that are strictly related to the electrochemical detection are presented in the following paragraphs. The purpose of these fragments is to explain the processes occurring in the electrochemical cell.

Hydrodynamic and scanning voltammogram

A *hydrodynamic* voltammogram is constructed when the pure analyte is not available and separation over an analytical column is required. Simply, the analyte is separated over the column and detected in the electrochemical cell with different potentials applied. To construct hydrodynamic voltammogram the peak heights are plotted vs. the potential (Fig 14). Furthermore, under real chromatographic conditions reliable information about the S/N ratio is obtained. Additionally, the hydrodynamic voltammogram can be used to optimize potential when ROXY EC/LC system is used. The drug compound /xenobiotic isoxidized in the electrochemical cell to the appropriate metabolites/oxidation products, prior to the injection into the HPLC and the metabolites are detected in MS. When mass spectrometer is used as detector, the extracted

ion chromatogram (EIC) representing m/z ratios (mass to charge) of specific metabolites will be plotted and the optimal potential can be estimated.

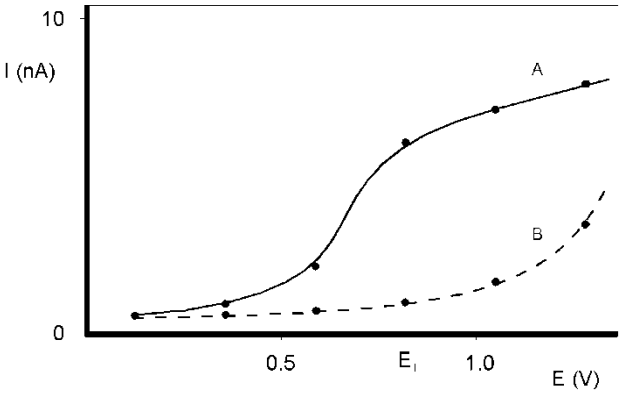


Fig. 14. Hydrodynamic voltammogram of norepinephrine (A) at a glassy carbon working electrode, and the current of the baseline (B). At E_1 the electrochemical signal becomes diffusion limited (Example with ECD).

Example of hydrodynamic voltammogram is presented in the figure 14. As peak heights are used, the signal in Fig. 14, line A is only due to the analyte.
An alternative for the chromatographic construction of an I/E relationship is the application of scanning voltammetry. The working potential runs between two pre-set values and the current is measured while the analyte is continuously flushed through the flow cell.
The signal in Fig. 15 (scanning voltammogram), line A is the sum of the analyte signal and the background signal. Subtracting both lines in Fig. 15 results in a similar I/E relationship as in Fig. 14, line A. It takes only a few minutes to construct a *scanning* voltammogram. This is an advantage, especially when a number of analytes have to be characterized. However, as the scan is obtained in flow injection analysis (FIA, without analytical column), it is a prerequisite to have the *pure* analyte dissolved in buffer. **Any contamination may lead to artifacts**. A blank scan of the buffer should be used to distinguish between solvent peaks and analyte peaks.

As can be seen in both Fig. 14 and Fig. 15, when the working potential is increased the electrochemical reaction is enhanced hence 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*.

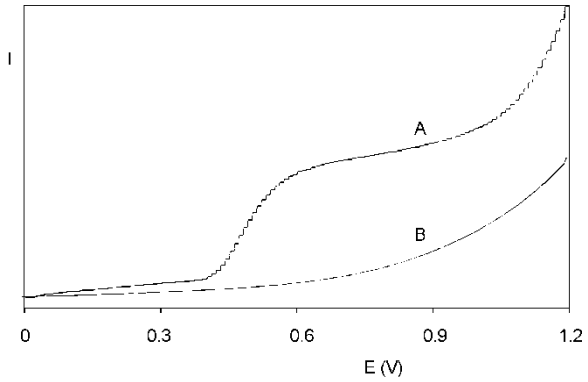


Fig. 15. Scanning voltammetry of 1.0 μmol/l norepinephrine (A) at a glassy carbon working electrode, at a scan speed of 10 mV/s. Scan (B) is the blank solvent.(Example with ECD).

In practice the choice of the working potential is a compromise between sensitivity, selectivity and reproducibility or the yield in desired metabolite formation (in ROXY Ec applications). In the example of Fig. 14 a working potential (E_1) of 0.8 V is chosen.

Scanning voltammetry can be also used in ROXY EC applications. With MS detection the I/E curves can be used only as supplementary data. Mass spectrometry allows the sample identification (determining the elemental composition, structure elucidation) and all ions having specific m/z ratios are plotted in the mass spectrum. Mass spectrometry data can be presented in form of mass chromatogram, e.g., the extracted ion chromatogram (EIC) in which a specific metabolites/oxidation products are monitored throughout the entire run, and a particular analyte's mass-to-charge ratios are plotted at every point during the analysis. The optimal potential can be estimated from EIC plots (Fig. 16).

With help of the figure 16, where EICs are presented , it is easy to estimate the potential range in which the desired metabolite will have the highest abundance. E.g., for metabolite at m/z 354 the best will be potential 300-400 mV, however to form the metabolites 326 and 299 the higher value of potential

(1200 mV) should be applied. In figure 17 the different mass spectra represents the different conditions: no potential, 300 mV or 1200 mV, respectively.
The mass spectra corresponds to the scanning voltammogram presented in the figure 17.

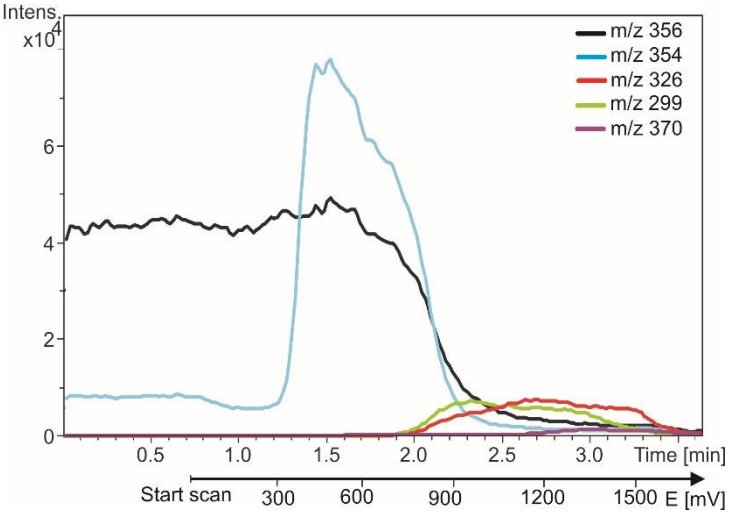


Fig. 16. Scanning voltammetry of 10 µmol/L amodiaquine at a glassy carbon working electrode, at a scan speed of 10 mV/s. The m/z ratios of different metabolites of Amodiaquine are plotted (see legend).

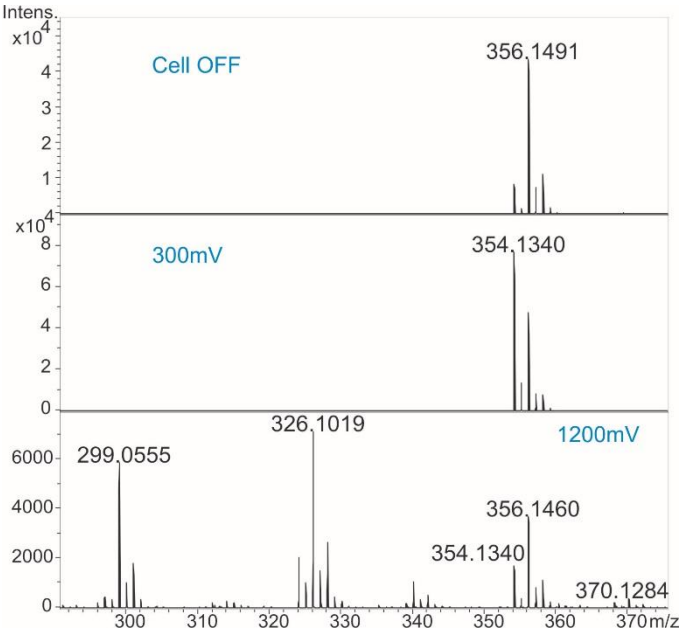


Fig. 17. Example of the mass spectra of 10 µmol/L amodiaquine oxidized at a glassy carbon working electrode, at a scan speed of 10 mV/s.

Optimisation using a voltammogram

Electrochemical detection

Sometimes, when interfering peaks appear in the chromatogram, it is 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, i.e. the largest difference in peak height. In the example of Fig. 18 the selectivity for compound X is improved considerably by decreasing the potential to E_2 or E_1. Obviously, if compound Y is the compound of interest, optimization of selectivity in this way is not possible and the chromatography has to 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 not only holds 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 of paramount importance for a stable baseline and reproducible detection conditions.

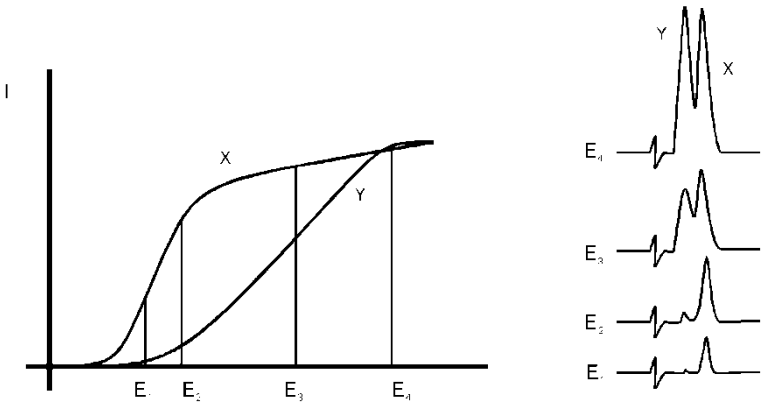


Fig. 18. Selectivity in LC-EC of compound X and Y is optimised by choosing the working potential with the largest difference in peak height.

Electrochemical reactions are pH sensitive (Fig. 19). For norepinephrine the I/E curve is shifted 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 will result 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 will strongly increase at higher pH. In both cases the background current increases at a higher pH.

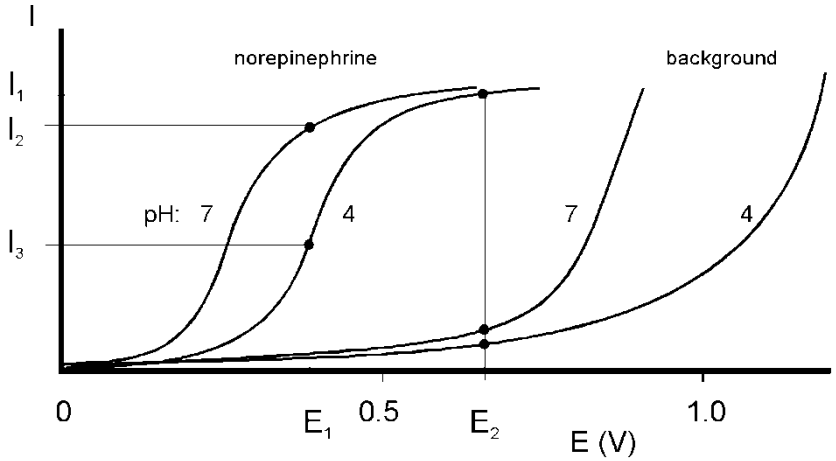


Fig. 19. At a higher pH the I/E curve of norepinephrine is shifted to the left.

Reaction kinetics predict that electrochemical detection is mass flow dependent. When the LC flow is stopped in LC-EC, the analyte will be oxidized completely and the signal decreases rapidly. This means that the flow rate not only affects temporal peak width and analysis time but also peak height. Also the

background signal is sensitive towards fluctuations in the flow rate. Therefore, it is important to use a pulse-free solvent delivery system.

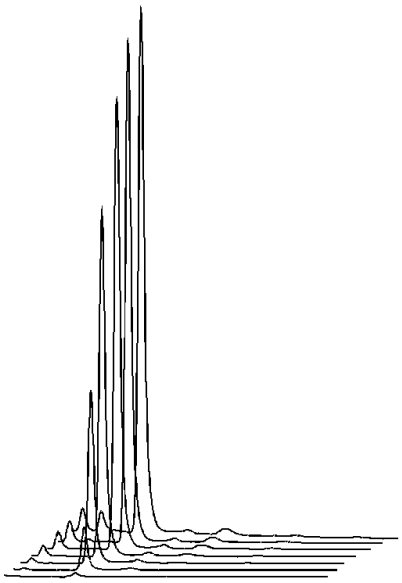


Fig. 20. Construction of a hydrodynamic voltammogram for norepinephrine. Chromatograms are obtained at cell potentials ranging from 1.0 V (back) to 0.4 V (front), with 100 mV steps.

On-line electrochemistry mass spectrometry

Information about potential optimization for ROXY EC system and the detailed background information about the supplied events files and relevant Dialogue settings are provided in the application note 210.001A ROXY™ EC system – events programming & settings

ROXY EC system delivers the oxidative metabolic fingerprint of the molecule in a very short time. The acquired mass spectra can be presented in three-dimensional plots, so-called MS voltammograms (Fig. 21). A MS voltammogram visualizes the ion abundance versus m/z as a function of applied potential to the electrochemical cell. With a mass voltammogram the optimal potential can be determined for electrochemical generation of the desired metabolite for further research, e.g., NMR.

In the figure 21, the 3-D MS voltammogram of amodiaquine is shown. To oxidized Amodiaquine to get dehydrogenated metabolite it is required to use lower (400mV) potential than to form to other metabolites (m/z 299 and 326)

and in this case the potential should be ca. 1200mV. For each cell potential mass spectra are recorded and saved in separate data files.

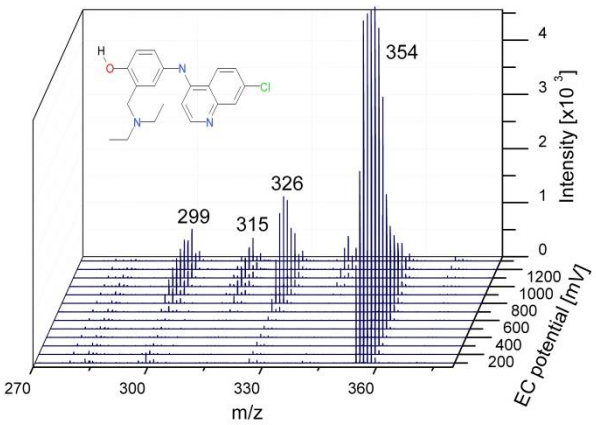


Fig. 21. 3-D MS Voltammogram of Amodiaquine. The plot is reconstructed from the separate mass spectra saved for each potential value.

Furthermore, the 2-D version of Voltammogram can be recorded and the data can be saved in one MS file, as presented in the figure 22. This plot can be quickly generated with any of MS software.
Both, 3-D and 2-D MS Voltammograms were acquired in the DC mode. The Dialogue controls the syringe pump, the potentiostat and triggers the acquisition of mass spectra at the designated cell potentials.

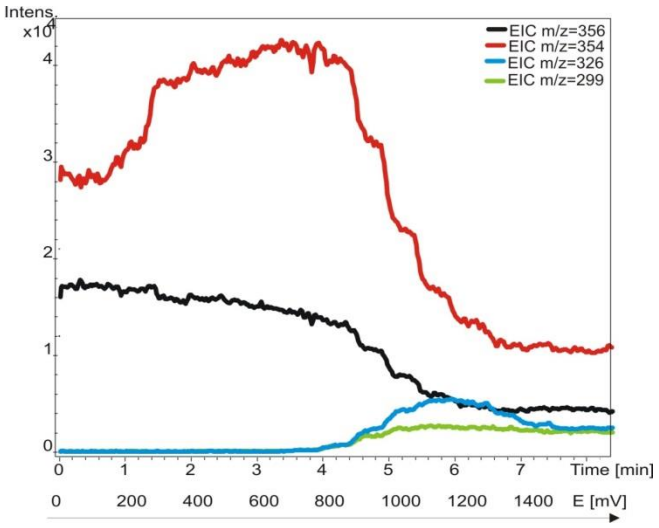


Fig. 22. 2-D MS Voltammogram of Amodiaquine. The mass spectra are saved in one file for whole analysis. EIC are representing the changes in oxidation of the Amodiaquine with respect to the potential applied.

Construction of a hydrodynamic voltammogram

Before a hydrodynamic voltammogram can be obtained, the chromatographic conditions should be optimized. Then the following steps are taken:
A solution of the analyte at a concentration between 1 - 100 μmol/l, is prepared in mobile phase.
The electrochemical potentiostat is stabilized in the DC mode at a high potential. After stabilization the background current is read from the display of the potentiostat (I-cell) and the noise is measured.
The run is started by injecting the compound. When at the high working potential no signal is obtained, it may be concluded that the compound is not electrochemically active. In such a case derivatization of the compound may be an option.
If a peak is measured, the working potential is decreased by 50 or 100 mV and step 2 to 4 is repeated until the lowest potential setting (Fig. 20).
The peak heights and the background currents are plotted against the working potential (Fig. 14).
The working potential which gives the best sensitivity is obtained by plotting the signal-to-noise ratio against the working potential.

Construction of a scanning voltammogram

The scan mode is programmed in the ‘SCAN SETUP’ screen of the ROXY Potentiostat. Depending on the data acquisition software that is used and the experimental set-up, a full, half or continuous scan cycle can be chosen.

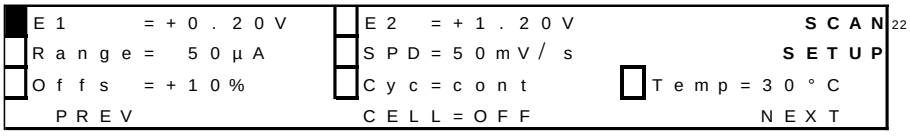


Fig. 23. Programming the scan mode in the 'SCAN SETUP' screen.

In the example of Fig. 15 and Fig. 24 a ‘half’ scan is used, sweeping the potential from for example 0.2 V to 1.2 V. A full scan would include the reverse

scan, i.e. from 0.2 V to 1.2 V and back to 0.2 V. In the continuous mode the voltage is swept up and down between both potentials.

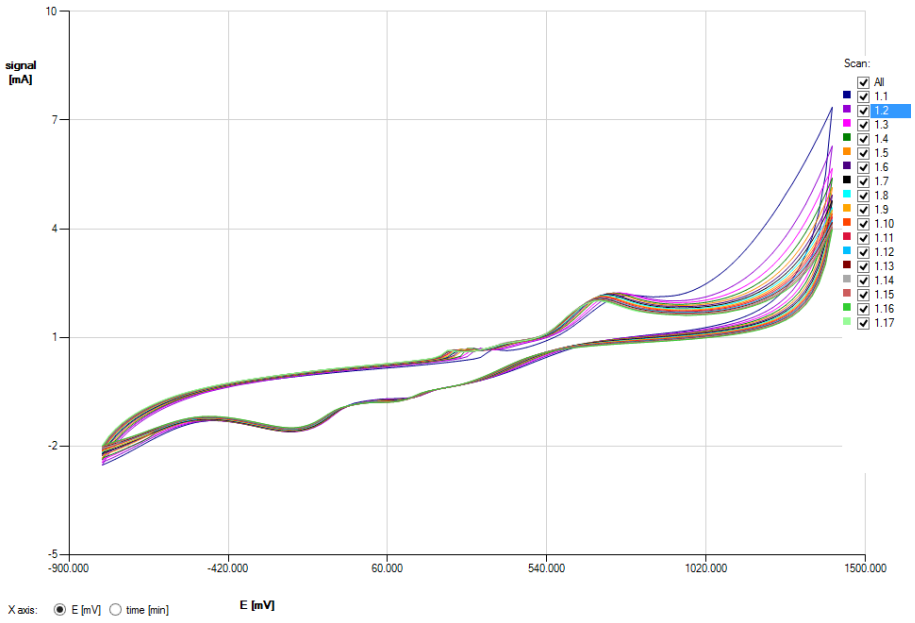


Fig. 24. A continuous scanning voltammogram in Dialogue Elite software. Example of multiple scans ('continuous') of Amodiaquine.

The voltammogram is recorded in the flow injection analysis (FIA) mode using a syringe pump to supply the substrate dissolved in solvent. The pure compound is dissolved at a concentration of ca. 10-100 μmol/l.
The sampling frequency of the integrator is set at 1 Hz. This is the same frequency as the voltage steps during the scan. If a higher sampling frequency is chosen a typical stepwise pattern may appear.
In Dialogue, the scan parameters are set and 'applied', and the actual scan is started by starting a run. In the Data menu the graph can be displayed as current vs time (I/t), or current vs potential (I/E).
Control of the syringe pump is under the 'S pump' tab (see Dialogue Elite manual).

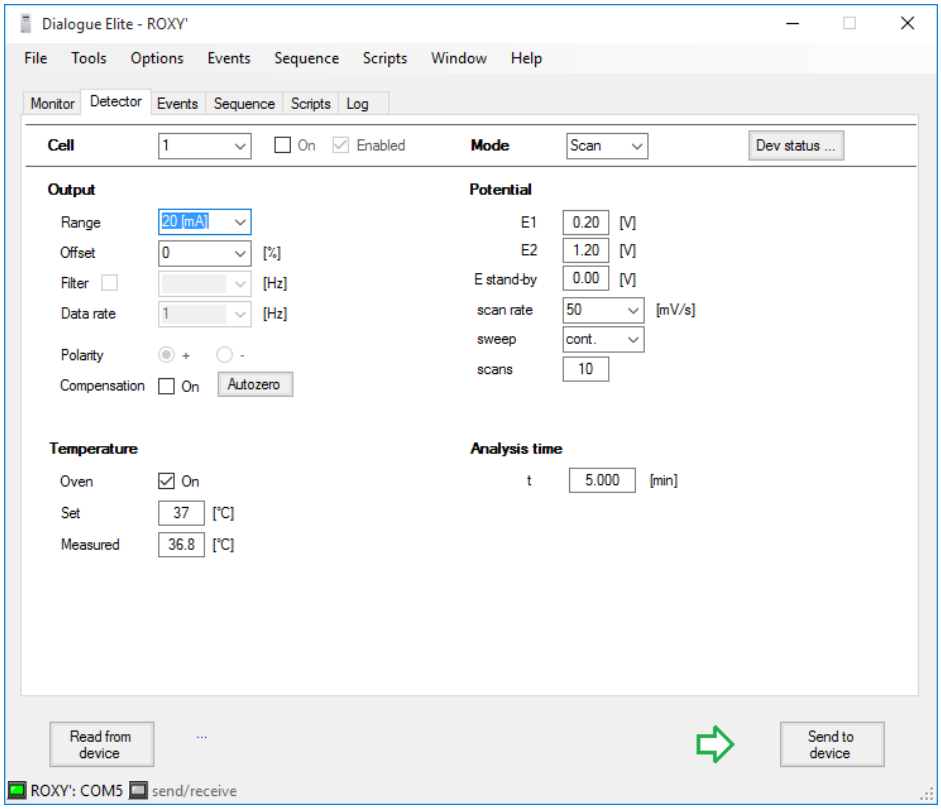


Fig. 25. Scan settings window (under detector tab) in Dialogue Elite software.

C H A P T E R 7

Specifications ROXY Potentiostat

General specifications

*) Specifications marked with * are only valid for the ROXY potentiostat high current version. For the previous model the max current range is 200µA for all measurement modes. The noise specification of the previous model is < 2 pA under the specified measurement conditions.

Power	110-240 VAC, 50/60 Hz, 260 VA, autosensing
Operating modes	DC, PAD and Scan
Potential range	between +4.90 and - 4.90 V in 10 mV increments
Output	between +1 and - 1 V or between +10 and -10 V (20 bit D/A converter)
Offset	between +50% and - 50% of max. output voltage, 5% steps
Event marker	pulse of 10% of max. output
Auto zero	triggered by keyboard, rear panel TTL input , or RS232C control
RS232C	Full parametric instrument control, data acquisition at 1, 2, 5 and 10 Hz
Injector sensor	Starts system clock at injection
Oven	height 37 cm, from 7°C above ambient to 45°C, accuracy 0.5°C, stability 0.1°C; accommodates column and flow cell(s)
Diagnostics	LCD screen, keyboard and noise (internal dummy cell)
Service mode	system settings & calibration parameters
Config mode	menu for system customisation and optimisation
Activation mode	Potential steps E1 - E2, duration programmable
Firmware	upgradeable via PC (RS232)
Environmental	operating temperature: 4 – 40 deg C, rel. humidity: 20 to 80% non-condensing
Second flow cell	Acquisition and control of second flow cell (option)

DC mode

Ranges	10 pA – 20 mA* in 1, 2, 5 steps
Filter (cut off)	0.5 – 0.01 Hz in 1, 2, 5 steps
Noise	better than 4 pA* with a dummy cell (load of 300 MOhm and 0.5 uF) with filter off, Ec +800mV and temperature of 35 ^{o}C.

PULSE mode

Range	10 nA – 20 mA* in 1, 2, 5 steps
Filter (cut off)	0.5 – 0.01 Hz in 1, 2, 5 steps
Pulse times	t1: 100 - 2000 ms; t2: 0 - 2000 ms; t3: 0 - 2000 ms in 10 ms steps
Sample times	20 ms - (t1 - 60 ms), with 20 ms increments

SCAN mode

Range	10 nA – 20 mA* in 1, 2, 5 steps
Scan rate	1 - 50 mV/s in 1, 2, 5 steps
Cycle	half, full or continuous

Events

DC mode (5 files) and pulse mode (4 files), end cycle time, number of cycles and oven temperature. Time-based control of 50 time points as to range, filter, output contacts (2 TTL, 2 relays), auto zero, offset, valve position (if present), and E-cell.

Rear panel I/O connections

Mains, Output, 2 Connectors 15 pins (A, B), manual valve (C), RS232C connector

Physical specifications

Dimensions	44 (D) x 22 (W) x 44 (H) cm = 17.3” (D) x 8.7” (W) x 17.3” (H)
Weight	14 kg without flow cell and column

Electrochemical cells

Working volume determined by spacer thickness and WE diameter

ReactorCell

Spacers	50 or 100 µm, stackable
WE diameters	8 mm
Cell volume	0.75 µl
WE electrodes	Glassy carbon, Magic Diamond™, gold, platinum, silver and copper
Reference electrodes	salt-bridge Ag/AgCl; in-situ Ag/AgCl (ISAAC); HyREF™
Auxiliary electrode	Graphite
Wetted materials	PCTFE, FEP, 316-SS, Viton, Ag, AgCl and WE
Max. pressure	40 psi / 2.8 bar

µ-PrepCell

Type	Thin-layer electrochemical cell (micro-preparative work)
Spacers	50 or 100 µm, stainless steel, stackable (max. stack thickness 250 µm)
WE dimensions	12 x 30 mm, thickness 1 mm
WE area (wetted)	1.9 cm^2
Cell volume	10 µl (effective spacer thickness 50 µm)
Working electrode	Glassy Carbon (GC), Boron-Doped Diamond (BDD), Titanium (Ti), Titanium oxide (TiBlue), Platinum (Pt) and Gold (Au)
Reference electrode	HyREF™ (Pd/H_2 electrode)
Auxiliary electrode	Conductive PEEK (PEEK, 30% carbon fiber-reinforced) or Titanium (previous version of µPC)
Wetted materials	PEEK, Carbon, Methyl-vinyl silicone rubber (Silicone VMQ-70), PCTFE and WE material (see working electrode section)
Fluidic connections	1/16” OD PEEK or PEEKsil tubing, ID 250 µm or less, with 10-32 PCTFE fingertight fitting
Electrical connections	ROXY cell cable incl 0.5uF, 3m (part 250.0139L)
Flow rate	Typically 20 – 100 µL/min
Working temp range	10 - 50 °C
Max. pressure	25 bar (GC electrode) , 50 bar (with MD electrode)

C H A P T E R 8

Error messages

Table V. Error messages.

Error	Message
01	Incompatible boot version
02	Control board error
03	Sensor board x error (x = board number)
04	Firmware program error
05	Record error
06	Incompatible FW version
07	Incompatible FW
08	Control board FW erase failed
09	Sensor board x
10	Upload checksum error.
11	Checksum error.
12	Temperature sensor 1 error.
13	Disconnect flow cell x
14	Control board SRAM error.
15	Sensor board x SRAM error .

Please contact your local supplier if one of the above errors occur. Furthermore the following messages can be displayed on the LCD screen during a measurement:

Table VI. Messages.

Message	Advice
01 Out of range	Output is either above +1.0V or below –1.0V. Pressing AZERO may give an adequate read-out again. If the message remains after pressing AZERO, the autozero function is unable to compensate the background cell current. Advice: use a less sensitive range in the SETUP menu.
02 PAD overload	Charging current in pulse mode out of range. Pressing AZERO may give an adequate read-out again. If not, it is advisable to change the pulse settings (increase t1) or use a less sensitive range.

*) An ‘Out of range’ error appears when the cell current Icell exceeds the limit of the current range at which the measurement is performed.

C H A P T E R 9

Rear panel

Connectors A, B and C

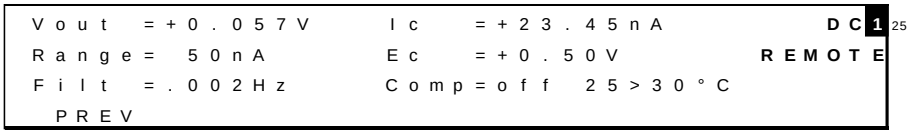
For detailed information on the I/O contacts see page 46.

Fig. 26. ROXY Potentiostat rear panel.

RS232C

The RS232 interface provides full parametric control from a PC. Programmable parameters comprise cell potential, range, auto zero, offset, filter, electrical injector and control of ROXY Potentiostat output contacts for control of external equipment. During operation a remote screen is shown and the keyboard is

locked. Keeping the PREV button (F1) pressed for 4 seconds disconnects from RS232 control and returns to MAIN.



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

C H A P T E R 1 0

Troubleshooting guide

No response, no product in MS

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
Cell disconnected, or switched off	Check connection
WE contact problem	uPrepcell: check the spring construction in the WE contact, should be flexible. If not: replace.
Air or gas bubbles in cell	Read filling instructions, check for bubbles at the outlet
Conditioning of WE	Follow conditioning instructions
Fouled WE	Clean WE

Make sure the ROXY potentiostat is connected to a grounded power source with a line voltage which is within the specified ratings. If the ROXY potentiostat does not respond, a fuse in the mains inlet may be blown.

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)
Normal behaviour	With high substrate concentrations it can be normal behaviour. Check with solvent (without substrate) if current is at expected value and in scale.

C H A P T E R 1 1

Dummy cell

External dummy cell

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.

The dummy cell has a resistor (R) of 300 MOhm and a capacitor (C) of 0.47 uF in parallel. The current is measured at a working potential of 800 mV and will be about 2.67 nA (I = V/R). Slight differences as to this (ideal) value are due to the tolerance of the resistor. The noise generated via the dummy should be less than 4 pA provided that the door is closed and the unit is stabilized.

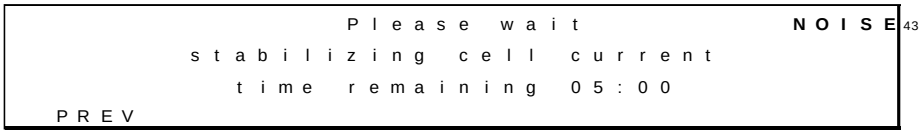
Table VII. Dummy cell test settings.

Parameter	Setting
Cell potential	800 mV
Cell current	2.67± 0.05 nA (read-out)
Oven	35 °C, stable
Filter	off (or as specified)
Range	100 pA/V (or 1 nA/V)

The results (cell current and noise) of the dummy cell test should be within the above mentioned 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. Please consult your local representative.

Internal dummy cell

From the MAIN screen DIAG can be selected to enter the DIAG screen, followed by selecting NOISE. This activates a timer in the NOISE screen, and after 5 min stabilisation auto zero 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 4 pA. Potentiostat settings in the NOISE screen are the same as in Table VII, with exception of the oven temperature. Temperature is switched off.



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

C H A P T E R 1 2

Potentiostat accessories

The electrochemical potentiostat is shipped together with a number of parts. The listing in Table below may not be complete, see check list of delivery for complete listing.

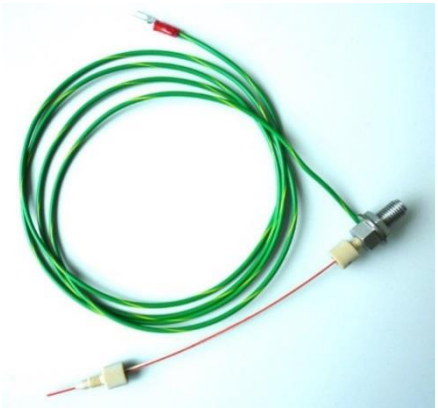
Table VIII. Accessories ROXY potentiostat.

Part number	Component
250.0040	External dummy flow cell
250.0107K	Column clamp kit, 12 mm
250.0113	Fuse 2.5 AT 250 V
250.0122	RS232 cable
250.0032E	ROXY potentiostat trigger cable
250.0128	Output cable
250.0116	Mains cable (Europe)
250.0118	Mains cable (USA)
250.0126	D/R cell cable

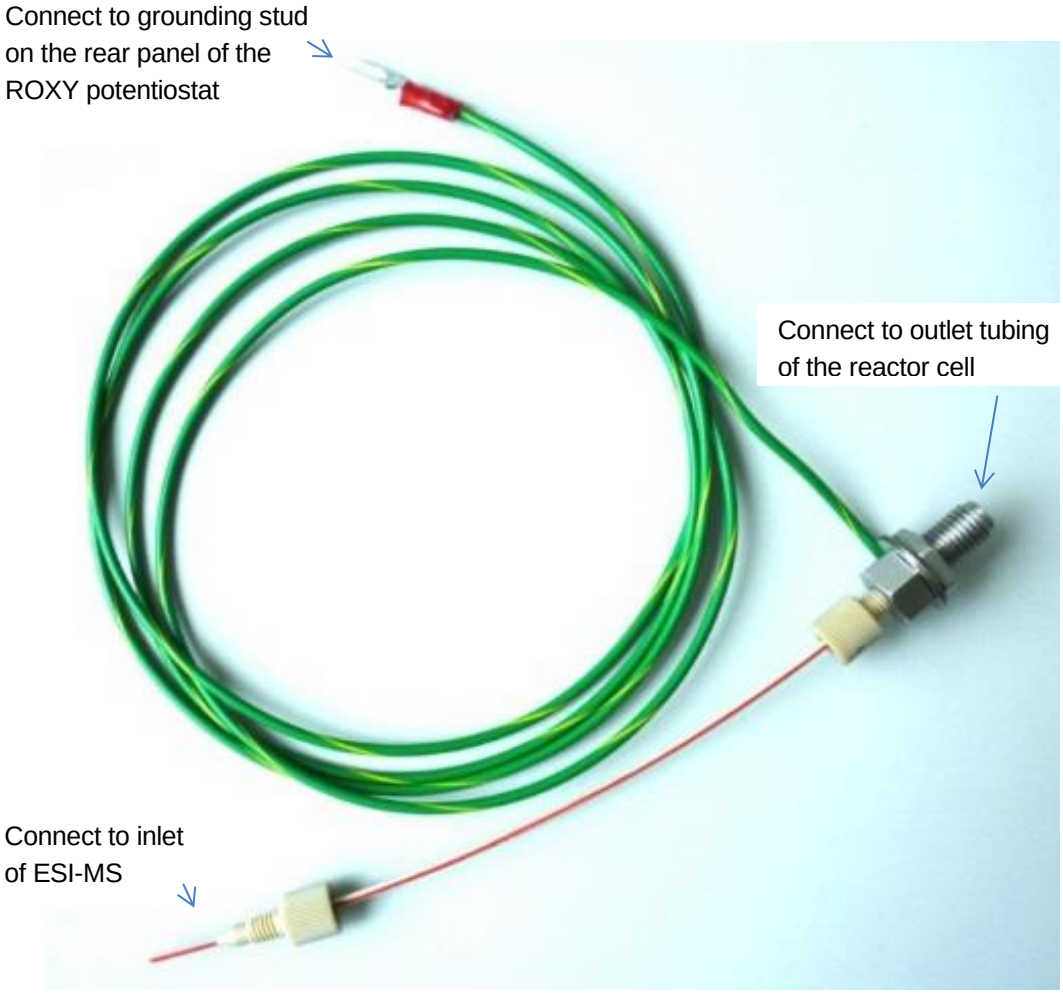
For these and other ROXY Potentiostat parts or flow cells contact your local supplier.

Grounding kit

For the ROXY potentiostat (pn) an optional grounding kit pn 250.0035 is available.



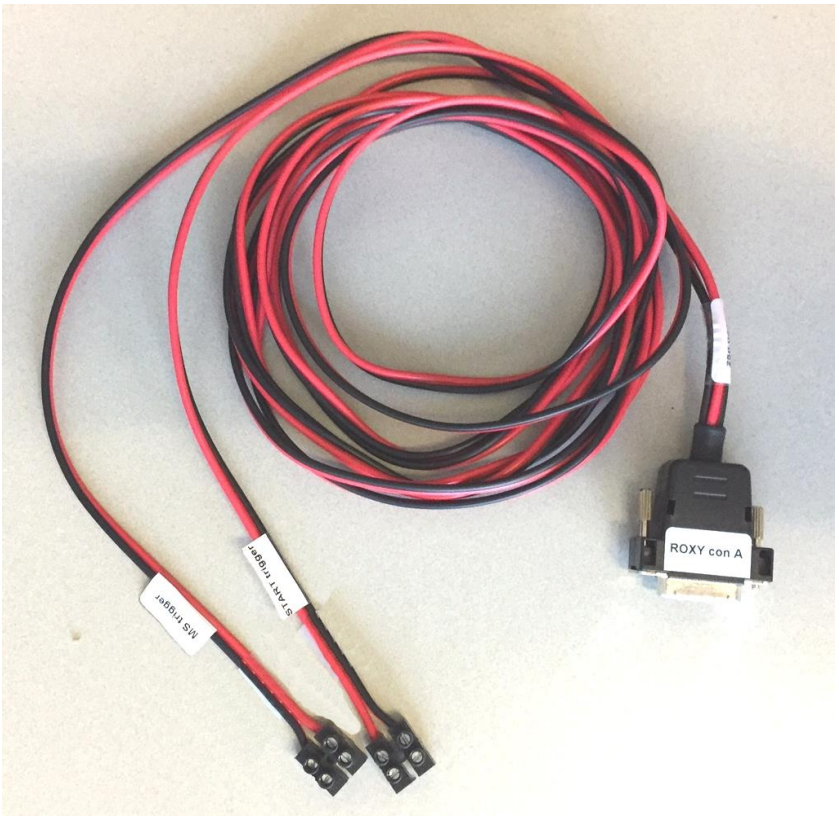
With a ROXY EC system (pn 210.0070) or ROXY EC/LC system (pn 210.0080C) this part is provided with the systems
An ESI interface of an MS is usually operating at high voltages of typically 3 – 5 kV. In cases where the inlet of the ESI-MS is not grounded, the grounding kit (pn 250.0035) must be used. If not used it may lead to damage of the ROXY potentiostat.



For detailed installation information please consult the relevant installation documentation of the ROXY EC or EC/LC system or contact Antec support.

Trigger cable

The ROXY potentiostat is delivered with 250.0032E ROXY potentiostat trigger cable.



The trigger cable 15-pins d-sub connector marked ‘ROXY con A’ should be connected to connector A of sensor board 1 (the sensor board located above the control board) on the rear panel of the ROXY potentiostat.

The output lead with screw terminal block marked ‘MS trigger’ can be used to automatically start acquisition of an MS via the Dialogue Elite software , by connecting it to an available start input of an MS. Check the technical documentation of your specific MS for details how to make the connections on the MS. The black wire on the cable should be connected to ground.

The input lead with screw terminal block marked ‘start trigger’ can be used to automatically start an event table of sequence in Dialogue Elite or a sample sequence in Clarity, by connecting it to an available external start output (relay/contact closure) of for example an LC system, autosampler (inject signal) or other external device. Check the technical documentation of your specific equipment for details how to make the connections on the external device. The black wire on the cable should be connected to ground.

Index