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SynthesisCell™

User manual

206.0010, Edition 5, 2026

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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 personal injury or even 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 SynthesisCell is used in combination with the ROXY Exceed potentiostat for controlled redox reactions.

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 SynthesisCell in combination with the ROXY Exceed EC(/LC) system or ROXY Exceed potentiostat can involve the use of hazardous materials including corrosive fluids and flammable liquids. The cell 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, 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. Always observe all relevant safety practices. 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

ISO 9001 certified

Antec Scientific is an ISO 9001:2015 certified company.

Warranty, spare parts and service

The warranty period of this cell is 1 year on workmanship, wear and tear parts are excluded. The 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 cell. Spare parts will be available after these 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 SynthesisCell. The safety practices are intended to ensure safe operation of the cell.

Working environment & safety

The intended use of the SynthesisCell is to perform controlled REDOX reactions of target compounds (in a suitable liquid electrolyte medium). 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 cell we recommend that the 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.

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 gear and gloves when handling toxic or biologically infectious samples to prevent biohazards or hazards while working with the cell. If necessary, the cell must be decontaminated before decommissioning or shipment of the cell for repair to Antec or its representatives. When shipped to Antec every cell has to be accompanied by a decontamination form which should be completely filled in and signed by the customer. Without this decontamination form the 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 cell in other ways than indicated in the manual might result in erratic or unsafe operation.

Table of contents

CHAPTER 1

The SynthesisCell

Introduction

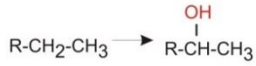
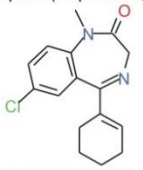
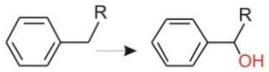
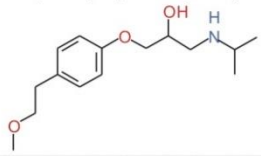
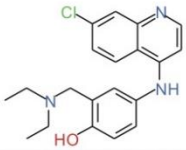
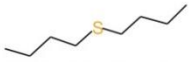
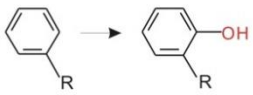
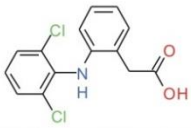
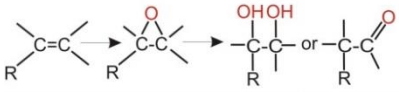
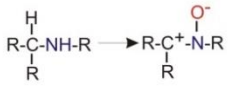
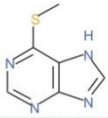
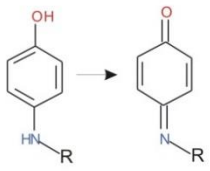
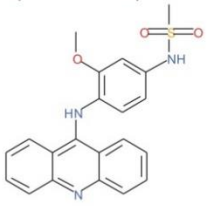
Congratulations on your purchase of the SynthesisCell.

The SynthesisCell is a bulk electrochemical cell, designed for small-scale electrosynthesis of metabolites, reactive intermediates and other oxidation and reduction products in milligram quantities. The SynthesisCell comes with the following electrodes: a tubular Reticulated Glassy Carbon (RGC) working electrode (WE), a salt bridge Ag/AgCl reference electrode (SB REF) and a coiled platinum wire as the auxiliary electrode (AUX).

For successful and efficient conversion, the parameters as the potential, composition of the solution (supporting electrolyte, organic solvent concentration, pH), need to be optimized depending on the type of compounds or desired reaction. In general, the samples can be oxidized in the solutions containing supporting electrolyte at concentrations of 10 mM or higher. The higher concentration of supporting electrolyte, ca. 100 mM, can improve conversion. In case MS is used for analysis, the solvent also affects the ESI response (ionization suppression). In that case the solvent must be compatible with ESI MS and to fulfill this requirement ammonium acetate, ammonium formate, formic acid or acetic acid can be used.

The information about setting the potentiostat parameters for metabolite synthesis are described in the ROXY Exceed User manual (211.0010) and Dialogue for ROXY user guide (210.7017).

Table 1. Typical enzymatic CYP reactions that can be done electrochemically. Compound indicated with asterisk is drawn as an example. Table is adapted from Lohmann et al. 2010. The electrochemical oxidation reactions are among others: S-oxidation, N-dealkylation, hydroxylation and dehydrogenation.

Enzyme-catalyzed	Electrochemically-simulated	Reference
Allylic and aliphatic hydroxylation 	Tetrazepam (requires high potential) 	1
Benzylic hydroxylation 	Metoprolol (only in low yields) 	2
Desalkylation of amines 	Amodiaquine*, lidocaine, clozapine 	3; 4
Desalkylation of ethers 	Metoprolol; dibutylsulphide* (low yield, only under acidic conditions) 	2; 5
Hydroxylation of aromatics 	Metoprolol; mephenytoin; diclofenac* (especially for aromatics with electron-donating groups) 	2; 6
Epoxide formation 	Benzo[a]pyrene; Not mimicked electrochemically	7
Oxidation of heteroatom (N, S) 	S-methyltiopurine*, lidocaine; phenothiazines under basic conditions 	2; 7
Dehydrogenation 	Acetaminophen; amodiaquine; amsacrine*; mitoxantrone 	3; 8

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2. T. Johansson, L. Weidolf and U. Jurva, *Rapid Commun. Mass Spectrom.*, **21**, 2323–2331 (2007).
3. S.M. van Leeuwen et al., *Anal. Bioanal. Chem.*, **382**, 742–750 (2005).
4. W. Lohmann and U. Karst, *Anal. Chem.*, **79**, 6831–6839 (2007).
5. K.G. Madsen et al., *Chem. Res. Toxicol.*, **21**, 1107–1119 (2008).
6. S.M. van Leeuwen, H. Hayen and U. Karst, *Anal. Bioanal. Chem.*, **378**, 917–925 (2004).
7. B. Blankert et al., *Electroanalysis*, **17**, 1501–1510 (2005).
8. W. Lohmann and U. Karst, *Anal. Bioanal. Chem.*, **386**, 1701–1708 (2006).

Table 2. Examples of solvents used for electrochemical oxidation on glassy carbon WE.

Compound	Solvent
Acetaminophen	20mM ammonium acetate in 25% methanol
Amodiaquine (1)	20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% acetonitrile
Amodiaquine (2)	50% methanol and 50% 10 mM aqueous formic acid
Irinotecan	20mM ammonium formate with 0.1% formic acid (pH 3.3) in 50% acetonitrile
Toremifene	20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% methanol
LYL, LWL (peptides)	90/10/1 (v/v/v) water/acetonitrile/formic acid

Three-electrode configuration

In the SynthesisCell a three-electrode configuration is used. The working potential is set between the working electrode and the auxiliary electrode (AUX). The auxiliary electrode is kept at a precisely defined reference electrode potential by means of the so-called voltage clamp. This is 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, i.e. 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 current can be recorded digitally.

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 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 would be 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) which would make this configuration useless for our purpose.

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, with sufficient yield in electrosynthesis.

Salt bridge (Ag/AgCl) reference electrode

The SynthesisCell is standard equipped with a salt bridge (Ag/AgCl) reference electrode. The salt bridge Ag/AgCl reference electrode consists of a small glass container, with a solid AgCl coated silver rod immersed in a solution of saturated (3 M) KCl (Figure 1). This type of reference electrode needs regular maintenance. Electrical contact with the other two electrodes in the SynthesisCell is made through a ceramic frit (the salt bridge), which is electrically conducting and slows down the leakage of KCl.

Use of the salt bridge reference electrode in high levels of organic modifier

The Ag/AgCl salt bridge is suited for use in aqueous solutions. In case the solution contains high levels of organic modifier, this could lead to precipitation at the ceramic frit. For such cases it is advised to replace the KCl solution by lithium chloride.

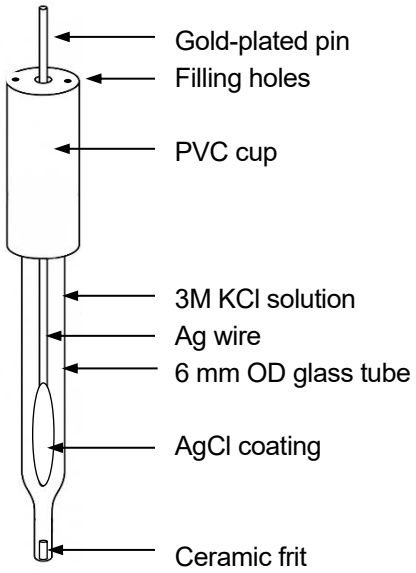


Figure 1. Schematic drawing of the Ag/AgCl salt bridge reference electrode.

Glassy carbon working electrode and working potential

Electrochemical conversion puts high demands on the working electrode material. Therefore, the working electrode should be made of a (electro-)chemical inert material. The estimation of the limits to the working potential that can be applied depends on the solvent composition (pH, supporting electrolyte) and the analyzed compound itself. The maximum working potential that can be applied is limited by the potential that results in electrolysis of the water (about +2.7 V), which also results in decrease in metabolites formation. At high positive working potentials, in case of electrolysis of the water/solvent, the cell current (Icell) readout will display the message “overload” and auxiliary potential (Eaux) will show an extreme value (-9.9V). The lowest working potential is also limited by the water/solvent features, and it is about -1.3 V.

CHAPTER 2

Installation

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. Contact your supplier in case of damage or if not all marked items on the checklist are included.
Prior to shipment, your SynthesisCell has been thoroughly inspected and tested to meet the highest possible demands. The results of all tests are included.

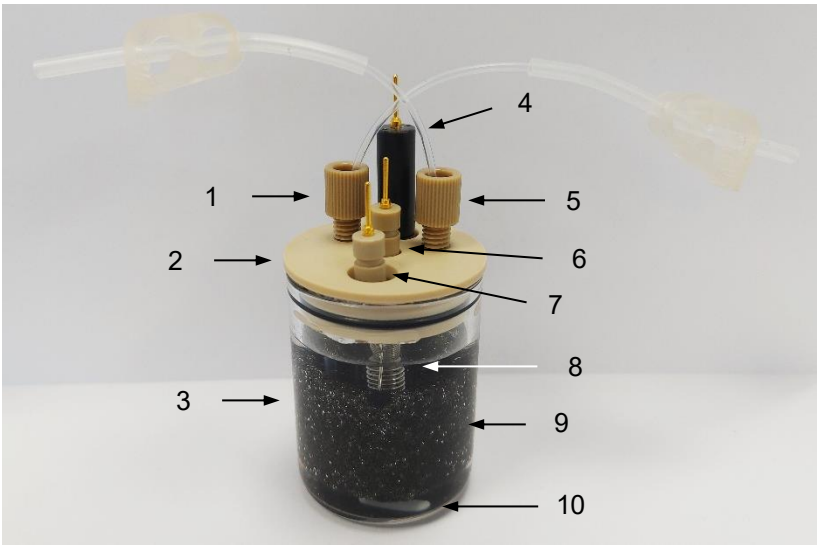


Figure 2. SynthesisCell with RGC WE and salt bridge reference electrode. 1: Sampling outlet, 2: SynthesisCell cap, 3: Glass reaction vessel, 4: Salt bridge contact, 5: Sampling inlet, 6: AUX contact, 7: WE contact, 8: AUX electrode in glass chamber, 9: RGC WE, 10: Stir bar.

This hardware should be used by trained laboratory personnel only. 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.

The SynthesisCell is delivered pre-assembled however some protectors for safe transportation must be removed before use.

Never switch ON the flow cell when:
- the cell cable is not correctly connected
- the cell is not filled with buffer/electrolyte
as substantial damage to the working electrode or electronics may occur.

Preparations

1. Take the SynthesisCell from the shipping box and remove the protecting material.
2. Prepare a solution containing the substance(s) and a supporting electrolyte (ammonium formate, formic acid, or acetic acid, 0.1 - 1% in water).
3. Download and install the latest version of Dialogue software from our website.

Assembling the SynthesisCell

A. Remove the vessel cap and fill the glass reaction vessel with a solution containing electrolyte(s) and substance(s). The maximum allowed volume is 30 mL. Put the stirrer in the vessel. See pictures in Figure 3
B. Install the auxiliary electrode in the vessel cap by pressing it in from the bottom side of the cap. The auxiliary electrode will not yet be fixed. Place the glass tube of the auxiliary electrode over the auxiliary electrode. By pressing the glass tube in the vessel cap, this will fixate the electrode.
C. Carefully press the contact of the working electrode from the bottom side upwards into the vessel cap. Make sure that the O-ring is positioned on the lower groove to assure that the electrode is placed high enough.
D. Gently place the vessel cap on the reaction vessel and turn while closing. If not being turned, the glass could break. Since the closure is tight, it is recommended to leave a small groove between the cap and vessel by not fully pressing it downwards. The ceramic frit of the small glass tube is permeable, so the tube should be filled within a few minutes after being submerged.
E. Install the reference electrode by inserting it into the vessel cap. Make sure that it is submerged into the solution and avoid direct contact with the working electrode.
F. Make sure the potential is OFF when connecting the cell cable. Carefully push each connector on the correct electrode contact while holding it to avoid it from coming loose and falling into the vessel.

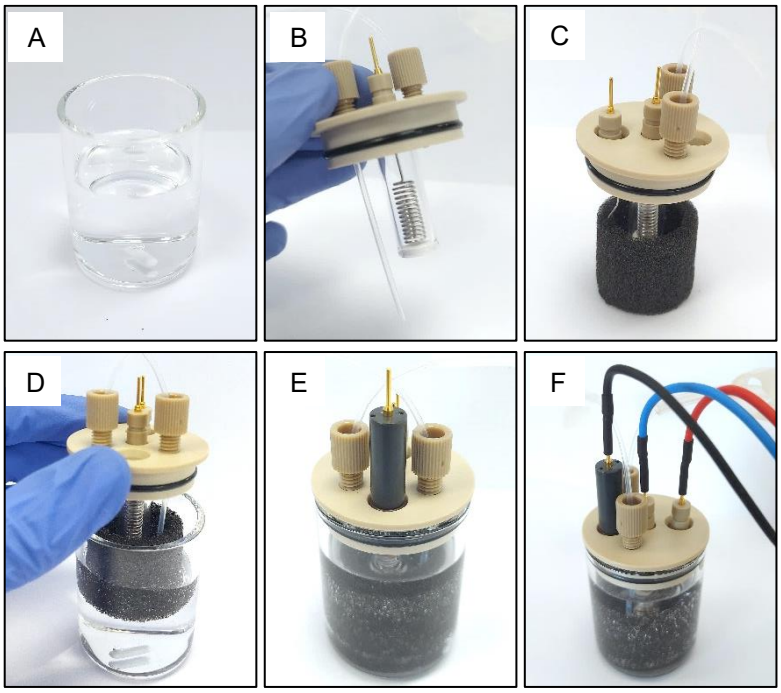


Figure 3. A: Glass reaction vessel filled with 30 mL solution and stir bar. B: AUX assembled in vessel cap. C: RGC WE mounted on vessel cap. D: Mounting of vessel cap on glass reaction vessel. E: Assembled cell with salt bridge REF. F: Cell cables installed on SynthesisCell, red: WE, blue: AUX and black: REF.

Operation

1. Place the SynthesisCell on the magnetic stirrer and adjust the rotation.
2. Make sure the cell cable is connected as shown in Figure 3F.
3. Set the measurement conditions in the Dialogue Elite software. The user manual (210.7017 – Dialogue for ROXY) is available on our website and on the provided USB stick with manuals.
4. The reaction starts as soon as the potential is set to ON at the SynthesisCell. Depending on the substance(s) and the applied potential, the cell current (I-Cell) can go up into the range of 2 – 20 mA.
5. Monitor the formation of product by taking aliquots from the vessel through the sampling port.
6. As an example: a 0.1 mmol/L (0.5 mg in 30 mL) MOPEG solution is for 90% converted in about 40 min under optimized conditions.
7. When finished, switch off the cell and take the vessel cap off. When mixing of the solutions from around the electrodes is not allowed, collect the solution from the AUX glass tube first.

C H A P T E R 3

Maintenance

Disassembly of the SynthesisCell

If the working electrodes needs maintenance, the SynthesisCell has to be disassembled.

Before disassembling the flow cell read General precautions.

1. Switch OFF the potential and disconnect the cell cable from the cell.
2. Remove the reference electrode by lifting it out of the vessel cap.
3. Carefully remove the vessel cap by slowly turning it. Avoid excess tension on the glass.
4. Remove the WE by gently pushing the beige WE contact downwards.
5. Remove the AUX: first remove the glass AUX compartment (which will release the grip on the AUX), then take out AUX electrode.

Use proper eye and skin protection when working with solvents.

Working electrode maintenance

Cleaning and reactivation of the working electrode is necessary if the electrode surface has been electrochemically changed. This is noticed by a strongly decreased conversion efficiency after prolonged use.
It may be due to fouling by oxidation (or reduction) reaction products. Excessively high currents also may change the electrode surface.

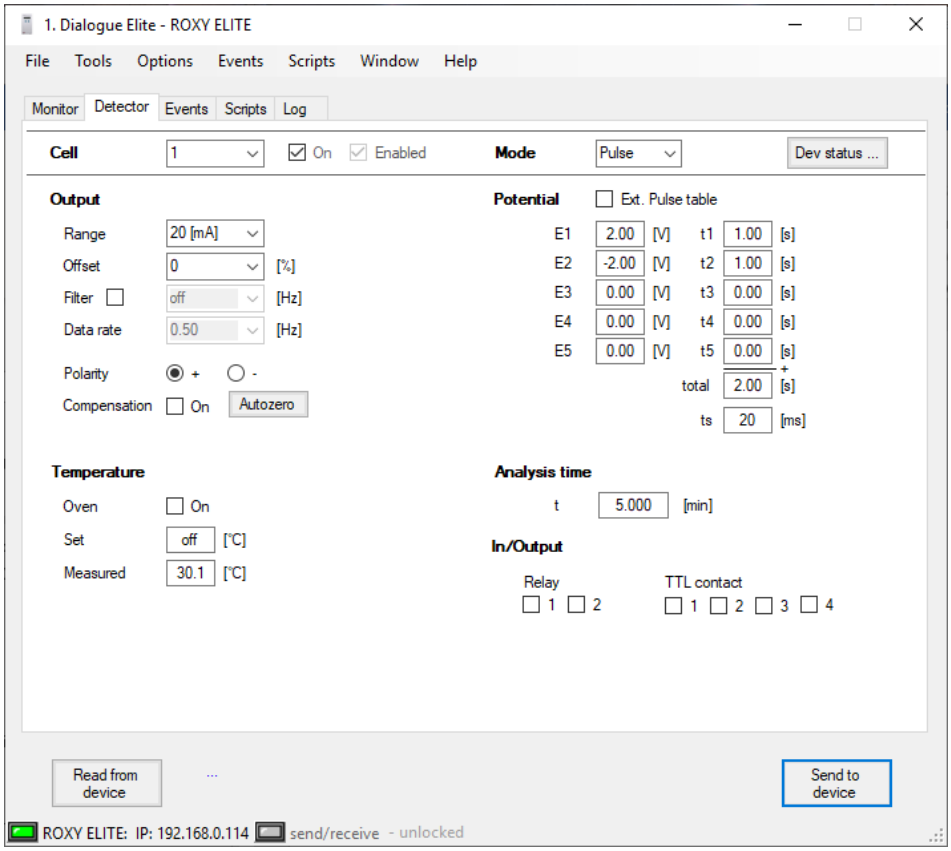
These actions can be taken to recuperate the electrodes after noticing a decreased conversion efficiency.

1. Rinse the WE and AUX electrodes with a clean 50/50 mixture of water and organic solvent.
2. Electrochemical activation of the GC electrode using pulse mode (see next paragraph of this manual).

Electrochemical pulse-cleaning of the electrodes

This activation procedure is suitable for GC electrodes.

1. Fill the vessel with clean solvent that contains the supporting electrolyte (but no sample)
2. Activate the tab “Detector” in Dialogue Elite software



3. Set the mode to Pulse and set the below pulse parameters.

Pulse parameter	*Value*
E1	+ 2 V
E2	- 2 V
t1	1 s
t2	1 s
ts	20 ms

4. Set t3, t4 and t5 to 0s. It is recommended to set the Range to 20 mA.
5. Set the Analysis time to 5 min.
6. Go to Options and click Start Analysis to run the procedure
7. After the 5 min activation procedure, turn OFF the cell.

The activation procedure is also available as a pre-programmed event file in Dialogue Elite software (Activation_pulse_rev01.evt) and can be found through Events\Open Events. The detailed background information about the supplied events files and relevant Dialogue settings are provided in the Dialogue for ROXY user guide (210.7017).

If the pulse-cleaning procedure hasn't resulted in significant improvement of the conversion efficiency, then replace the RGC electrode for a new one.

Maintenance of the salt bridge reference electrode

The SynthesisCell salt bridge reference electrode is a refillable electrode, meaning that the KCl solution can easily be refreshed or filled up. The reference electrode should be refilled in case of a decreased conversion efficiency, a discoloration of the KCl solution, or if the electrode is filled for less than 25%:

1. Start with rinsing the outside of the salt bridge reference electrode with water.
2. Wipe and dry the electrode using a tissue.
3. Carefully place a hollow needle in one of the holes on top of the electrode (Figure 4A), and make sure it pierces through the internal septum. This needle will serve as an outlet for the solution and/or gas (Figure 4B)
4. Fill a syringe with 3 M (saturated) KCl solution and make sure that no air is taken up.
5. Place the second needle on the syringe and carefully pierce through the septum. (Figure 4C)
6. Slowly infuse the solution into the reference electrode. Keep the electrode positioned upwards and release all the gas from the tube out through the vent hole. (Figure 4D)
7. When filled, remove both needles. The salt bridge reference electrode is ready for use again.

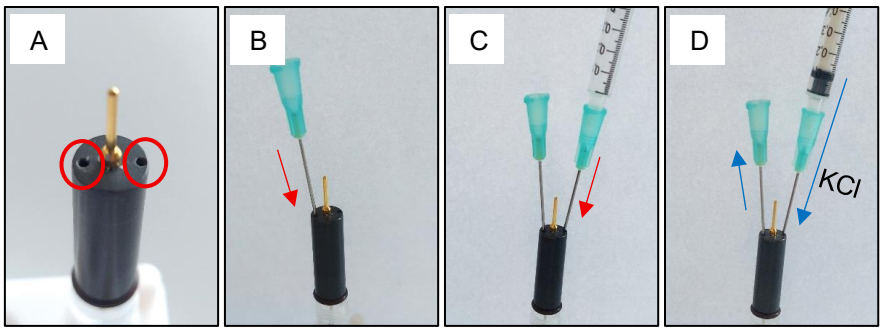


Figure 4. A: Salt bridge reference filling holes. B: Piercing the first (vent) hole with a hollow needle. C: Inserting a syringe with hollow needle in the second hole. D: Filling the reference electrode with saturated KCl.

Never fill the reference electrode without making a vent hole: over-pressurizing the glass chamber could lead to shattering of the glass.

Storage

If the SynthesisCell is not in use, switch OFF the cell, and rinse the electrodes with water. Store WE and AUX clean and dry. In addition, the salt bridge reference electrode should be capped by using the included rubber stopper to avoid it from running dry. To improve the lifetime of the salt bridge reference electrode, it is advised to store this electrode in the refrigerator. Before being taken into operation again, the salt bridge reference electrode should be brought to room temperature.

Before disconnecting the cell from the potentiostat, turn OFF the cell first!

CHAPTER 4

Specifications SynthesisCell

Cell type	Three electrode synthesis cell, consisting of working electrode (WE), reference electrode (REF) and auxiliary electrode (AUX)
Cell volume	Up to 30 mL of sample solution in glass reaction vessel
Working electrode (WE)	Tubular Reticulated Glassy Carbon (RGC)
Reference electrode (REF)	Ag/AgCl reference electrode
Auxiliary electrode (AUX)	Coiled platinum wire in glass isolation tube
Port plug	Access port for sample collection, dispensing of reagents, or venting of cell
Electric connections	SynthesisCell cables for use with ROXY Exceed

CHAPTER 5

Accessories SynthesisCell

The Antec SynthesisCell (p/n 206.0037B) is shipped together with a number of accessories. Use the information below to re-order those parts.

Part no	Description
206.0037B	Complete SynthesisCell, consisting of 30 mL glass reaction vessel with cap, WE (Tubular Reticulated Glassy Carbon), REF (salt bridge) and AUX electrode (Pt), stir bar, electrode cables, etc., all parts included for direct use with a ROXY Exceed potentiostat.
	Replacement parts
206.0304A	Tubular Reticulated Glassy Carbon (RGC) working electrode
206.0310A	Auxiliary Pt electrode in glass tube
206.0314A	Ag/AgCl reference electrode
206.0900A	Glass reaction vessel, 50 mL
206.0902A	Reaction vessel cap
206.0342	PTFE coated magnet stir bar, 13x3mm
250.0139S	ROXY cell cable for SynthesisCell incl 0.5uF, 3m