Antec
Industrieweg 12
2382 NV Zoeterwoude
The Netherlands

µ-PrepCell™

User manual

204.0010, Edition 5, 2013

T +31 71 5813333 | F +31 71 5813334 | info@myantec.com | www.myantec.com

Intended use

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.

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 bin”.

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 (info@myantec.com). To assure hygienic & personal safety all instrument must 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

ROHS directive

Our instruments are currently exempt from the RoHS directive because they fall under WEEE Annex IA categories 8 and 9, which includes medical devices and monitoring and control instruments. Nevertheless, we have taken steps to eliminate all restricted substances from our products.

Antec Leyden is an ISO 9001:2008 certified company.

Symbols

The following pictograms are used in this user manual:

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

The attention sign signals relevant information. Read this information, as it might be helpful.

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

Safety practices

Perform periodic leak checks on LC tubing and cell connections. Do not allow flammable and/or toxic solvents to accumulate. Follow a regulated, approved waste disposal program. Never dispose of such products through the municipal sewage system.

LC equipments 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.

Use of this product outside the scope of this guide may present a hazard and can lead to personal injury.

Spare parts and service availability

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 instrument. 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.

Table of contents

CHAPTER 1

The µ-PrepCell™

Introduction

Congratulations on your purchase of the µ-PrepCell™.

With more than 20 years of experience in Electrochemistry (EC), Antec introduces a new, dedicated EC cell for on-line EC/MS and EC/LC/MS. The µ-PrepCell™ in combination with 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 other possible applications are:

- Rapid risk assessments of drug-protein binding
- Signal enhancement in MS
- Electrochemical cleavage of proteins/peptides
- Mimicking natures redox reactions, e.g. oxidative stress/damage of proteins, DNA, lipids, etc

The µ-PrepCell is a thin-layer electrochemical cell designed for high yield synthesis of metabolites. The active surface of the working electrode is about 10 times the size of a standard ReactorCell™, and guarantees a significantly higher efficiency in formation of reactive metabolites. The cell can be used in the ROXY EC system for off-line collection of metabolites. Flow rate and working potential can be optimized using the Dialogue software.

The µ-PrepCell™ including kit (p/n 204.4300) is delivered with a set of two different working electrodes (WE): Glassy Carbon (GC) or Magic Diamond™ (MD). The PrepCell is delivered with the Magic Diamond electrode preinstalled.The use of an *exchangeable* working electrode offers maximum flexibility for multiple applications requiring different working electrode materials.

As a Reference electrode the HyREF™ Pd/H_2 electrode is supplied. This reference electrode is maintenance free and can be used under harsh conditions. The µ-PrepCell is operating in a three-electrode configuration (more details about three-electrode configuration can be found in the next paragraph).

Table 1 summarizes the reactions that can be simulated in the ReactorCell™ or µ-PrepCell™. The electrochemical oxidation reactions are among others: S-oxidation, N-dealkylation, hydroxylation and dehydrogenation.

For successful and efficient conversion, the parameters as the potential, mobile phase composition (organic solvent concentration, pH), and flow rate need to be optimized depending on the type of the analyzed compound. In general, the samples can be oxidized in the solutions containing supporting electrolyte at concentrations of 10mM or higher. The higher concentration of supporting electrolyte, ca. 100mM, can improve conversion, but from the other hand it can also affect the ESI response (ionization suppression). An additional concern focuses on whether the mobile phase is compatible with ESI MS and to fulfill this requirement ammonium acetate, ammonium formate, formic acid or acetic acid can be used (It is not recommended to use acetic acid and its derivatives with Magic Diamond electrode). pH should be considered when optimizing the mobile phase composition. Although, in the most cases pH is adjusted to 7.4 (physiological value) with ammonium hydroxide, the oxidation reactions are pH dependent. E.g., N-oxidation can occur only under basic conditions but some desalkylation reactions happen only in the acidic medium. An optimization of the mobile phase composition requires the addition of an organic solvent. Acetonitrile or methanol can be added depending on the solubility of the sample. Furthermore, the higher % of acetonitrile can diminish the adsorption of the most hydrophobic compounds. For the samples that are difficult to soluble in the aqueous buffer the non-aqueous solution as 0.1M tetrabutylammonium perchlorate (TBAP) dissolved in ACN/H_2O 99/1 (v/v) can be used. The examples of the mobile phases in relation to the specific compound are presented in the Table 2.
The flow rate is another factor that can influence the conversion rate. In general lowering the flow rate will increase the conversion efficiency. The recommended flow rate for µ-PrepCell™ is 20-50µL/min, for ReactorCell™ the optimal flow rate is in the range of 5-20µL/min.
The driving force of the electrochemical reaction is potential applied between the working and counter electrodes. The ROXY™ Potentiostat allows using cell potentials in the range from -4.9V to +4.9V. To optimize the potential it is recommended to run the MS Voltammogram (ramp the potential within specified range) and estimate the optimum value for the desired metabolite formation. Furthermore, the MS Voltammogram is the fingerprint of the compound itself and provides the information about oxidative processes occurring in the cell. In the ROXY™ potentiostat the DC and Scan mode are available for efficient metabolite synthesis. The DC mode is based on applying a static (single) potential during the whole conversion process. Note that the synthesis of different metabolites of one compound may require operation at different potential settings. In the Scan mode stabile oxidation conditions are obtained by continuous scanning between two preset potentials values (E1 and E2) with a certain scan rate (unit: mV/s).

Table 1. Typical CYP reactions that can be simulated electrochemically. Compound indicated with asterisk is drawn as an example. Table is adapted from Lohmann et al. 2010.

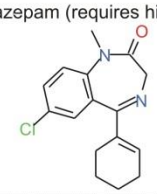
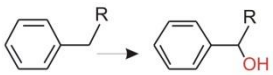
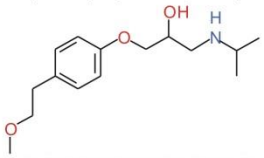
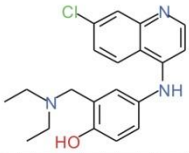
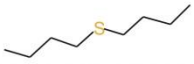
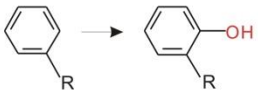
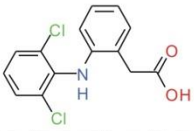
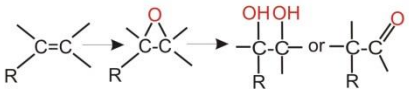
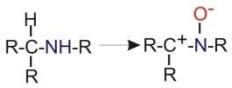
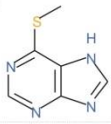
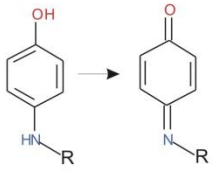
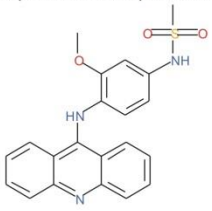
Enzyme-catalyzed	Electrochemically-simulated	Reference
Allylic and aliphatic hydroxylation 	Tetrazepam (requires high potential) 	1
Benzylic hydroxylation 	Metoprolol (only in low yields) 	2
Desalkylation of amines 	Amodiaquine*, lidocaine, clozapine 	3; 4
Desalkylation of ethers 	Metoprolol; dibutylsulphide* (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

Table 2. Examples of mobile phases used for electrochemical oxidation.

Compound	Mobile phase	Electrode
Acetaminophen	20mM ammonium acetate in 25% methanol	Glassy Carbon, Magic Diamond
Amodiaquine (1)	20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% acetonitrile	Glassy Carbon, Magic Diamond
Amodiaquine (2)	50% methanol and 50% 10 mM aqueous formic acid	Glassy Carbon
Irinotecan	20mM ammonium formate with 0.1% formic acid (pH 3.3) in 50% acetonitrile	Glassy Carbon, Magic Diamond (on both different oxidation profiles were obtained)
Angiotensin	0.1% formic acid in 50% acetonitrile	Magic Diamond
Adenosine	20mM ammonium formate (pH 7.3 adjusted with ammonium hydroxide) in 50% acetonitrile	Magic Diamond
Tetrazepam	10mM formic acid (pH 3.1)	Platinum
Lidocaine	0.1 M TBAP dissolved in ACN or ACN/H2O 99/1 (v/v)	Gold
Toremifene	20mM ammonium formate (pH 7.4 adjusted with ammonium hydroxide) in 50% methanol	Glassy Carbon
LYL, LWL (peptides)	90/10/1 (v/v/v) water/acetonitrile/formic acid	Glassy Carbon

The information how to acquire the MS Voltammogram and use the Scan or DC mode for metabolite synthesis are described in the ROXY Potentiostat User manual (210.7010) and Dialogue for ROXY user guide (210.7017).

References:

1. A. Baumann et al., *J. Chromatogr. A*, **121 6**, 3192–3198 (2009).
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).

An exploded view of the µ-PrepCell is shown in the Figure 1.

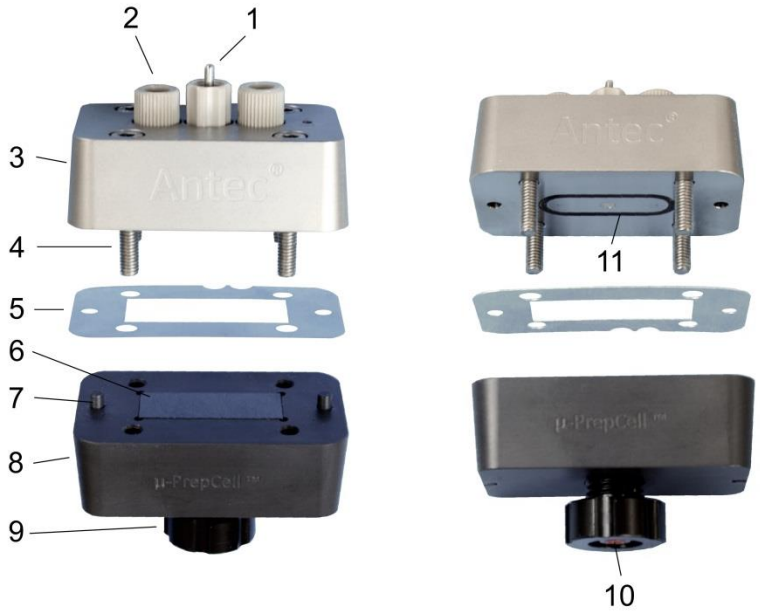


Fig. 1. Exploded view of the µ-PrepCell™.

1 HyREF™ Pd/H_2 electrode
2 Inlet / outlet
3 Inlet block (titanium)
4 Mounting screws (stainless steel)
5 Spacer 50 or 100 µm (stainless steel)
6 Working electrode (GC or MD)
7 Positioning pins for spacer
7 Positioning pins for spacer (PEEK)
8 Working electrode block (PEEK)
9 Nut (POM)
10 WE contact
11 O-ring (Viton® fluoroelastomer)

Warning: the spacers can be considered as SHARP METAL OBJECT. Take care handling this part during assembly of the cell, avoid contact with the sharp metal edges of the spacer.

Note that for use of a Magic Diamond™ electrode a ROXY potentiostat DCC or QCC (p/n 210.0050 or 210.0060, respectively) with an extended Ec range (±4.90 V) is required. Please contact your local supplier for additional information or advice.

The construction of the µ-PrepCell is symmetrically so the fluid connections can be used as either inlet or outlet.

Three-electrode configuration

In the µ-PrepCell™ a three-electrode configuration is used (Fig. 2). 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 polarisation 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 output voltage can be measured by an integrator or recorder.

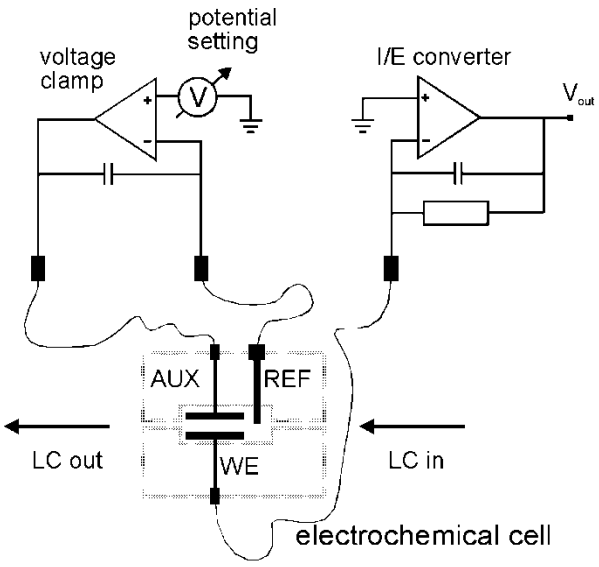


Fig. 2. Schematic representation of an electrochemical cell with a three-electrode configuration.

Essentially, for the oxidation or reduction reaction it would be sufficient to use only two electrodes. However, the three-electrode configuration has several advantages over a two-electrode configuration.
If the working potential would be applied only over an auxiliary electrode versus the working electrode (without reference electrode), the working potential would continuously change due to polarisation 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) resulting in a very limited dynamic range.

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

HyREF™ reference electrode

The µ-PrepCell™ is standard equipped with a HyREF (Pd/H_2) reference electrode. The HyREF can be used under harsh conditions and is free of maintenance under normal use. An important characteristic of the HyREF is the pH dependence of the reference potential.

It is important to realise that if the pH of the mobile phase is changed, also the optimum conversion potential changes. In such case it is advisable to re-determine the optimum conversion potential by constructing a voltammogram.

HyREF versus Ag/AgCl Reference electrode

The reference potential of an Ag/AgCl and HyREF are different (Fig. 3).

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

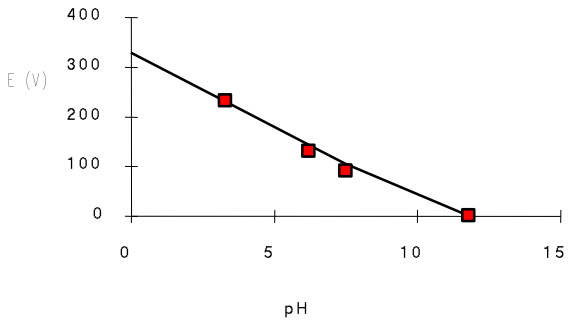


Fig. 3. Potential difference between HyREF and Ag/AgCl REF versus pH.

So, if an Ag/AgCl REF is compared to a HyREF, the pH effect on HyREF, compared to Ag/AgCl must be taken into account. The pH-voltage relation is described by:

$E_{HyREF} = E_{Ag/AgCl} - 328 + 29.9\ pH$ (1)

Example

If an Ag/AgCl REF is exchanged for a HyREF a working potential of 800 mV (*vs*. Ag/AgCl) at pH 3, has to be changed to:

$E_{HyREF} = 800 - 328 + 29.9*3 = 560$ mV (*vs*. HyREF)

As a rule of thumb, the working potential should be chosen such that a similar background signal (I-cell) is measured with the HyREF as in using the Ag/AgCl REF.

Working electrodes

Electrochemical conversion puts high demands on the working electrode material. The working electrode should be made of a (electro-)chemically inert material. Furthermore, to avoid an irregular flow profile over the electrode, it should have a very well defined surface. In the table I the working potential limits are listed of the WE materials available for µ-PrepCell. The values presented in the table are only the estimation and will depend on mobile phase composition (pH, supporting electrolyte) and the analyzed compound itself. At high positive working potentials the water in the mobile phase electrolyses and results in decrease in metabolites formation. In case of electrolysis of the water/mobile phase the cell current (Icell) readout will display the message “overload” and auxiliary potential (Eaux) will have the extreme value (-9.9V) (With the default range value of 200 µA). Furthermore, the electrospray signal will become very unstable because of gas formed in the cell and loss of the signal can be observed. When such a phenomenon is observed it is recommended to adjust the potential to a lower value.

Table 3. Working potential limits for electrodes (WE) used with µ-PrepCell.

WE material	potential limits *vs*. HyREF (V)	
	Oxidation	reduction
Glassy carbon	+2.5V	-1.5V
Magic diamond	+3.5V	-2.5V

Magic Diamond™ is a new working electrode material available for the µ -PrepCell. The MD electrode consisting of an ultra-thin film of doped diamond material deposited on a Si wafer. The special properties of doped diamond electrodes, such as a wide potential window, their inertness and excellent response stability, makes them well suited for electrochemical conversion of a wide variety electro-analytical application. The MD electrode is perfect choice if the oxidation of the analyte requires high potential in aqueous electrolytes.

Recently a novel Titanium electrode became available for the u-PrepCell for fast & efficient online reduction of disulfide bonds in proteins and peptides.

Fig. 4. Working electrodes: MD and GC, respectively.

The working electrodes have the following part numbers:

Pn	Description	Active material
204.5007	WE GC for µ-PrepCell	Glassy Carbon
204.5010	WE S-S reduction for µ-PrepCell	Titanium
204.5050	WE MD for µ-PrepCell	Boron-doped Diamond

The part number can be found on the packaging bag of the electrode. The different type of Working Electrodes can be identified quite easily on their outer appearance. See photo on the next page.

The surface of the Titanium electrode has a grey matt appearance, which is identical on both sides. The Titanium electrode can therefore in principle be used on both sides.

The Magic Diamond electrode has a dark blue/grey metallic appearance on the top side (boron-doped diamond). The back side of the electrode is a film of gold (backside Au- metallized) to assure good electrical contact with the substrate. This electrode can therefore only be used on one side (top).

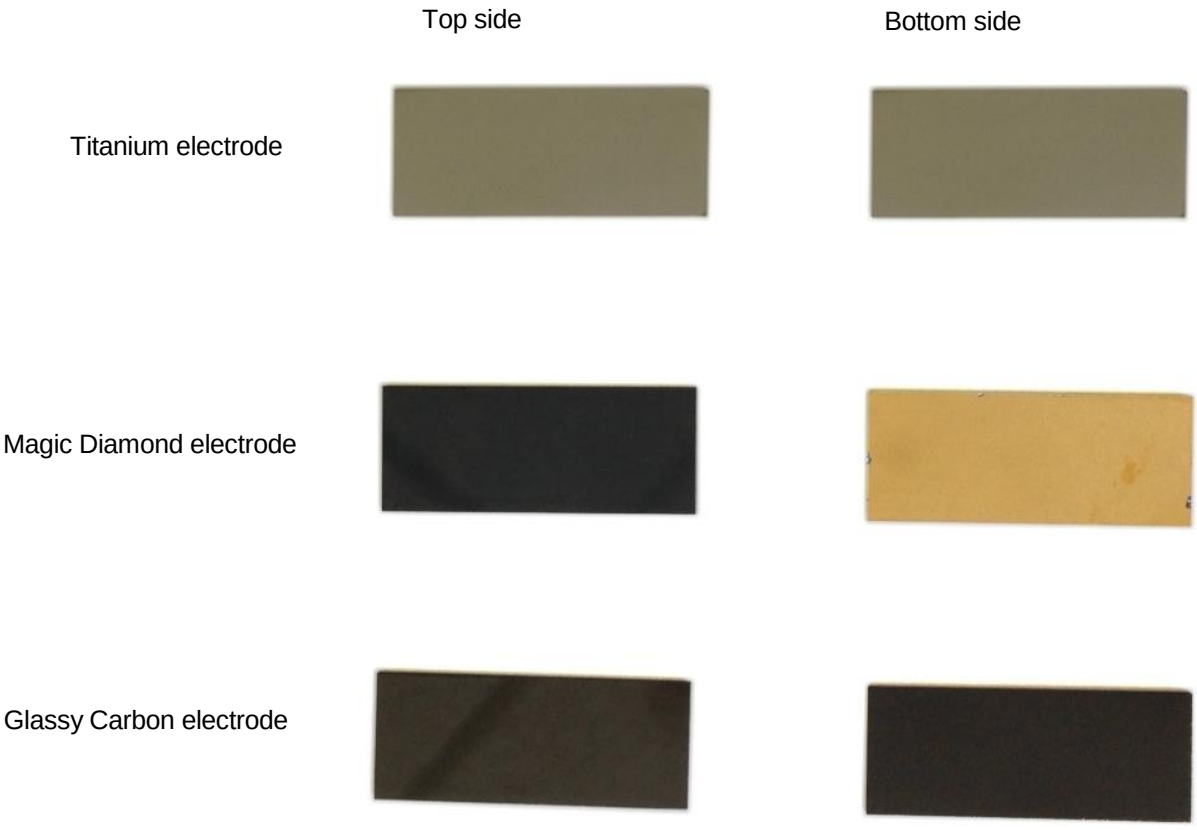


Fig. 5. µ-PrepCell working electrode identification. Photo of top and bottom side for all available electrodes.

The Glassy Carbon electrode has a black color, the polished top side has a shiny mirror like appearance, the unpolished back-side has a matt black color. This electrode should only be used on the polished top side.

Make sure that the working electrode is properly positioned in the prepcell with the correct active surface facing upwards in the working electrode block. If not it may lead to irreproducible erratic measurements.

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 µ-PrepCell has been thoroughly inspected and tested to meet the highest possible demands. The results of all tests are included.

Installation of the µ-PrepCell holder

The µ-PrepCell is delivered with a special holder to mount it inside the oven compartment of the ROXY potentiostat. The µ-PrepCell holder is fixed to the back-panel of the oven compartment using the M4 screw (red arrow) supplied with part 204.0102 the µ-PrepCell holder kit.

Fig. 6. µ-PrepCell holder mounted on the centre position in the ROXY potentiostat oven compartment.

The µ-PrepCell holder has a lip on both sides (blue-dotted circle) which clamp into the grooves on the sides of the µ-PrepCell. Insert the cell from the top into the clamp as indicated by the arrow.

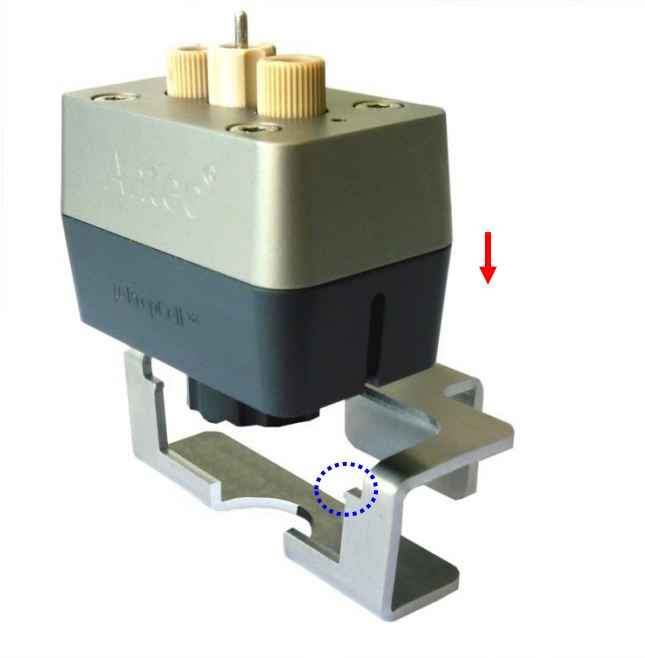


Fig. 7. Inserting the µ-PrepCell into the cell holder.

General precautions

Before starting to use the µ-PrepCell please take into account the following precautions when handling the cell.

1. Always make sure that the surfaces of the spacer and working electrode are dry and free from particulate matter before assembling the cell.
2. Assure that the spring-loaded WE electrode contact holder (see fig 13) is never exposed to (corrosive) liquids because it may lead to corrosion of the spring inside of the contact and malfunction of the cell.
3. If the auxiliary electrode needs to be cleaned, wipe the surface careful with a soft tissue soaked in ethanol or methanol. In case of strong oxidation/coloration of the active area within the O-ring of the Titanium AUX block use the polishing kit supplied with the cell. For instructions see the maintenance section.
4. In case of a Glassy Carbon working electrode, make sure that the surface work has a mirror-like appearance before re-assembling the flow cell. The electrode can be wiped with a soft tissue soaked in acetone or methanol. In case of a heavily contaminated surface which cannot be cleaned with solvent or in case

the electrode lost its activity it can be polished using the 1 µm diamond slurry (p/n 250.1030). For instructions see the maintenance section.

The Magic Diamond electrode is an exception. This particular WE electrode has a crystalline blue/gray surface structure. This electrode must <u>not</u> be polished mechanically. Polishing the MD electrode surface will damage the electrode and lead to loss of performance. Follow the specific maintenance instructions in chapter 3.

5. If the cell is not in use and removed out of the EC system, we recommend that you disassemble the cell and clean all surfaces (turn off the cell first) before storage.

Assembly of the µ-PrepCell

Never switch on the flow cell if:

- **the (black, red and blue) cell cable is not correctly connected,**
- **the cell is only partly (or not at all) filled with mobile phase containing the supporting electrolyte (e.g., ammonium formate, ammonium acetate, formic acid), because damage to the working electrode or the electronics may occur.**

Use proper eye and skin protection when working with solvents.

<u>Warning:</u> the spacers can be considered as <u>SHARP METAL OBJECT</u>. Take care handling this part during assembly of the cell, avoid contact with the sharp metal edges of the spacer.

Execute the following steps to assemble the µ-PrepCell:

1. Take the HyREF reference electrode, make sure that the Viton O-ring is mounted on the tip of the PEEK body. See figure 8. The Viton O-ring assures that no leakage can occur during operation.

Fig. 8. Reference electrode with Viton O-ring (black arrow).

2. Screw the reference electrode in the Titanium inlet block in the centre hole, check if the reference electrode is not protruding from the titanium block, otherwise it can lead to short circuits or damage to the working electrode.

Fig. 9. The Reference electrode (centre) properly mounted in the inlet block.

3. Place the Magic Diamond (MD) or Glassy Carbon (GC) working electrode in the recess of the PEEK WE block.

   Note: the WE contact holder should not be installed in the WE block yet. The MD electrode should be positioned with the gold side of the electrode facing downwards, the GC electrode should be positioned with the mirror-like surface upwards. Make sure that the electrode is laying levelled in the recess and that it is free of any (hard) particulate matter below and on top of the electrode. It may lead to damage to the electrode when tightening the cell.

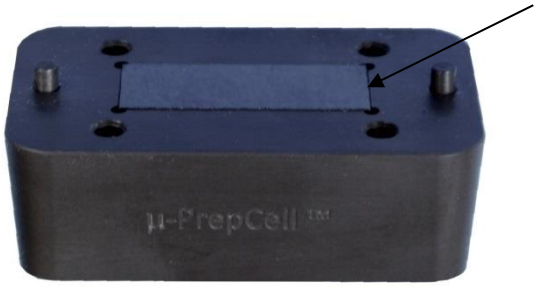


Fig. 10. The PEEK WE block with MD working electrode inserted (arrow).

4. Place the spacers onto the PEEK WE block. There are two positioning pins on the WE block to fix the metal spacers on the right position. The volume of the µ-PrepCell™ chamber (area within the Viton O-ring) can be adjusted by varying the amount of spacers. There are 2 types of spacers available for the µ-PrepCell: an 50 µm stainless steel spacers and 100 µm stainless steel spacers.

The µ-PrepCell is standard assembled at the factory with 1x100µm and 1x 50µm spacer, so in total a spacer thickness of 150µm . A minimum spacer thickness of 150µm and maximum of 300µm should be applied. A spacer thickness larger then 300µm can result in leakage.

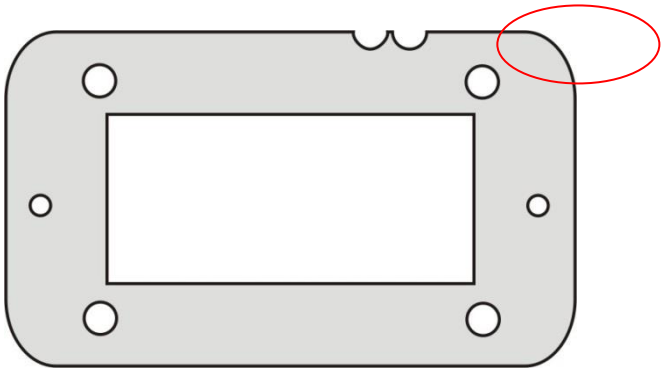


Fig. 11. Schematic drawing of 100 µm µ-PrepCell spacer. Red circle: marking of the spacer thickness. One notch: 50 µm, two notches: 100 µm.

Effective spacer thickness

The effective spacer thickness and thus the volume of the cell is determined by the total thickness of the metal spacers minus the height which the WE electrode is protruding above the top plane of the PEEK WE block.

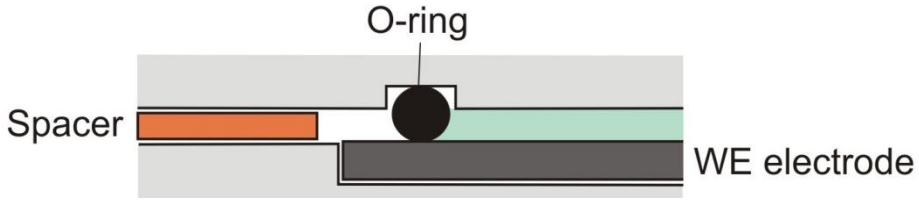


Fig. 12. Schematic drawing of the sealing construction in an µ-PrepCell.

The WE electrode is protruding approximately100 µm above the WE block resulting in an *effective spacer thickness of 50 µm* in the case 150 µm of metal spacers are used.

5. Close the Cell by placing the auxiliary (inlet) block on the top of WE block. Place the four allen screws in the screw holes and tighten them gently in a cross wise manner using the hex key delivered in the accessory kit (max 0.13 Nm). Don't over-tighten the screws.

Before closing make sure that both blocks and the holes in the AUX block for the positioning pins are dry. In case the holes contain liquid it could result in wet spacers or liquid accumulating in the area between the Viton O-ring and the spacer. This could negatively affect the performance of the cell.

6. The spring-loaded contact pin from the WE holder will assure proper electrical contact with the working electrode (Fig. 12).

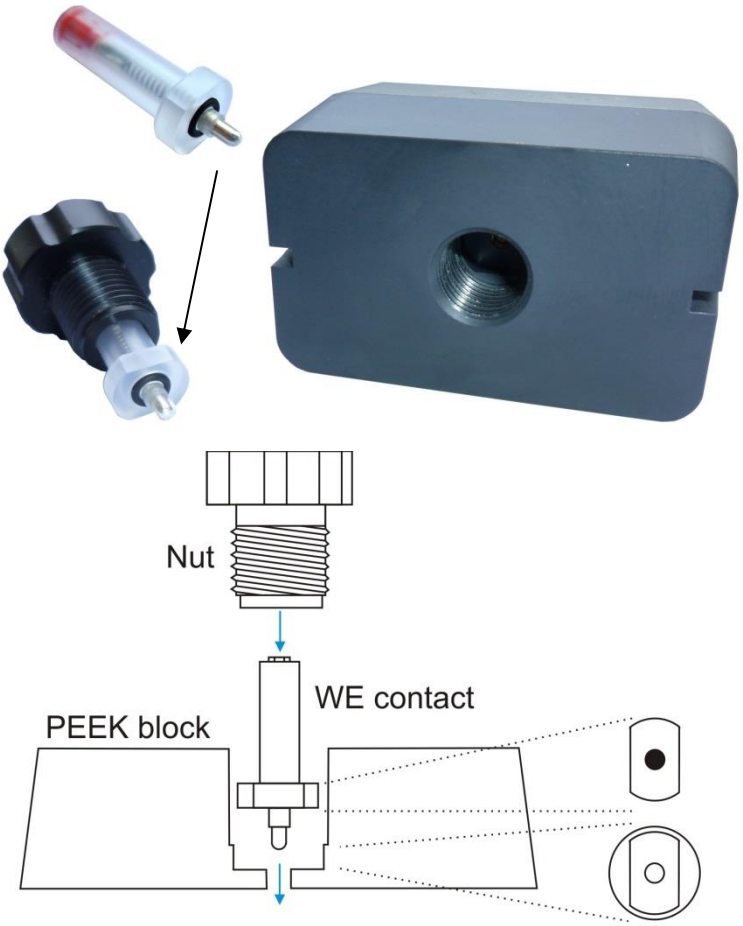


Fig. 13. µ-PrepCell – WE electrical contact assembly and WE holder. Below: schematic intersection.

7. First insert the WE contact holder in the centre hole of the PEEK WE block. Fix the holder by means of the black nut. Make sure that the holder is inserted deep enough and 'locked' within the hole, note that the round holder has two straight sides which uniquely fit within the bottom part of the centre hole. This prevents rotation of the WE contact holder when fixing the black nut.

Assure that the spring-loaded WE electrode contact holder is never exposed to (corrosive) liquids because it may lead to corrosion of the spring inside of the contact and malfunction of the cell.

8. Before using the µ-PrepCell, it is recommended to check if none of the electrodes are short-circuited. This can be done by measuring the ohmic resistance with a voltmeter between the WE–AUX; WE–REF and AUX–REF contacts. Note that the cell should be completely dry for a valid measurement.

For installation and priming of the µ-PrepCell see the installation section on the next page.

Installation of the µ-PrepCell

To install the µ-PrepCell in your ROXY EC system follow the steps below:

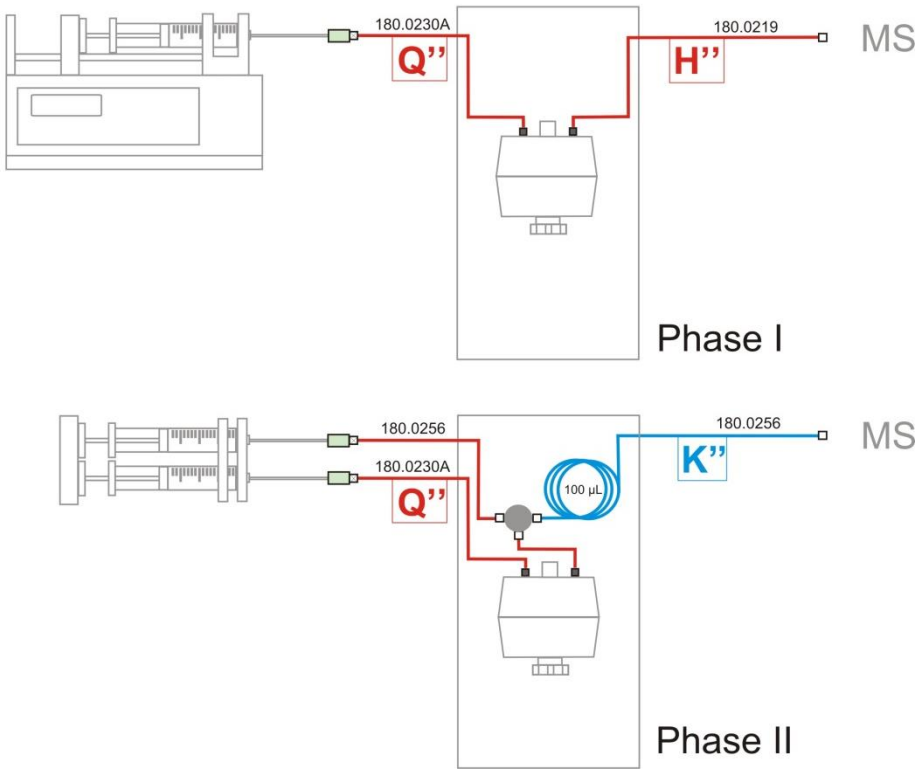


Fig. 14. Schematic drawing of the µ-PrepCell in a ROXY EC system. Top part: Phase I configuration, bottom part: Phase II configuration.

1. Have some tissues ready and wear protective gloves as you probably will spill some mobile phase during the mounting procedure.
2. Connect the syringe filled with mobile phase to the inlet of the µPrepCell using tubing assembly Q'' (p/n 180.0230A) and tighten it carefully. Make sure that the syringe and tubing is air-bubble free when connecting it to the cell.

The mobile phase should contain the supporting electrolyte (e.g., ammonium formate, ammonium acetate, formic acid) to provide proper working conditions and to prevent the damage of the working electrode or the electronics.

3. Connect tubing assembly H'' (p/n 180.0219) to the outlet of the cell but not yet to the MS. Flush µ-PrepCell with the mobile phase and ensure that all air bubbles are removed. Keep it under an angle of 45 degree to clear out the air in the cell. During clearing tick against the cell to promote removal of air bubbles.
4. Place the cell levelled in the cell holder and unscrew the HyREF electrode from the AUX inlet block at low flow rate of mobile phase. Visually inspect if there are no air bubbles present anymore in the hole of the reference electrode in the AUX block (one can clearly see the working electrode when looking inside). In case of air bubbles tick against the cell to promote removal. When all air bubbles are removed mount the HyREF electrode back in the AUX block and remove the excess mobile phase which is pushed out the chamber during fixing of the HyREF electrode.
5. Replace the mobile phase in the syringe with the desired sample solution and place the syringe in the syringe pump. Make sure that the syringe is air bubble free. If necessary the sample can be degassed or sparged with argon (deaerated conditions) before introducing the sample to the cell.

In case of high sample concentration, more frequent cleaning of the electrode may be required (See the cleaning and activation procedures in Maintenance section of this User manual) to recover the full performance of the cell.

6. Carefully connect the syringe to the inlet tubing assembly Q'' (180.0230A), avoid the introduction of air bubbles in the system.
7. Start the syringe pump with a sufficiently low flow rate for efficient electrochemical conversion (e.g. 10µL/min - 50 µL/min).
8. Connect the outlet tubing with the MS source (make sure that the MS inlet is proper grounded). If not use the ROXY grounding cable 250.0035 provided with the ROXY potentiostat (available for units purchased after 1 September 2010).
9. Connect the cell cable to the µ-PrepCell. The red connector should be connected to the Working electrode, the black one to the HyREF reference electrode and the blue connector to the Auxiliary electrode.

µ-PrepCell is now ready for use.

The µ-PrepCell should be flushed with demi water after use, ensure that the cell is switched off during flushing with water!

It is recommended to optimize the conditions for metabolite synthesis with on-line MS detection. At first instance recording of MS Voltammogram helps with estimation of the potential. Once the conditions are established the synthesis of metabolites can be performed with off-line sample collection. How to record MS Voltammogram and the detailed background information about the supplied events files and relevant Dialogue settings are provided in the Dialogue for ROXY EC system User guide (210.7017).

C H A P T E R 3

Maintenance

Working electrode maintenance

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

Decreased cell performance

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

1. Wiping the electrode surface with a tissue wetted with methanol or acetone.
2. Polishing the electrode surface. Only apply polishing on Metal and GC electrode only. **The MD electrode must <u>not</u> be polished mechanically.**
3. Electrochemical activation of the GC and MD electrodes using pulse mode (see separate section of this manual).
4. The (re)activation procedure for Diamond electrodes using scan mode (see separate section of this manual).

Disassembly of the µ-PrepCell

If the working electrode needs maintenance, the cell has to be disassembled.

Before disassembling the flow cell read General precautions.

1. Switch off the cell and syringe pump and disconnect both fingertights.
2. Unscrew the four screws from the titanium inlet block and open the cell.
3. Remove the Working electrode
4. Remove the HyREF reference electrode
5. Clean and dry the spacers.
6. Clean/polish the WE as described in the next section.
7. Note that the GC WE has only 1 side with a mirror-like surface. This side is facing the sample and should be polished.

Use proper eye and skin protection when working with solvents.

Polishing of the GC electrode

The polishing procedure is for GC working electrode only. Do <u>not</u> polish the thin-film Magic Diamond electrode, it will lead to damage of the electrode surface and a loss of performance. For Magic Diamond electrodes follow the activation procedure described in a separate section of this manual.

1. Shake diamond slurry thoroughly before use!
2. Rinse the polishing disc with demi water before applying the diamond slurry!
3. Apply a small amount of slurry on the wetted polishing disc, usually **one drop** is sufficient.
4. Put the working electrode with the face down on the disc and polish the electrode with a 'figure 8' motion for about one minute. Apply only gentle pressure with your fingers. The GC electrode is rather brittle and can break when applying too much force.
5. Clean the electrode with an ethanol-wetted tissue and check the surface visually; repeat the procedure if necessary.
6. Reassemble the cell.
7. Clean the polishing disc with demi water.
8. Store the polishing disc dust free in its plastic bag.

Polishing of the Inlet block

The Titanium inlet block can slightly oxidize during use and results in a yellowish/brownish coloration. There is no large effect on the conversion efficiency of the cell with a slightly oxidised block, however, if a significant impact on conversion efficiency is observed we recommend polishing the inlet block.

The two types of flattening plates are supplied for that purpose (yellow = 12 um, green = 30 um). The procedure includes two steps:

[1] Flattening step, coarse (30 um flattening plate)
[2] Flattening step, fine (12 um flattening plate)

Fig. 15. The Titanium inlet block with Viton-O-ring.

Please perform the procedure below to polishing the inlet block. Please read the procedure completely before executing the polishing procedure.

1. Remove the HyREF reference electrode from the Titanium inlet block.
2. Gently remove the O-ring using the supplied stainless steel needle supplied in the µ-PrepCell accessory kit. Carefully lift the Viton O-ring out of its chamber as depicted in the figure below. Make sure not to cut the O-ring with the needle point.

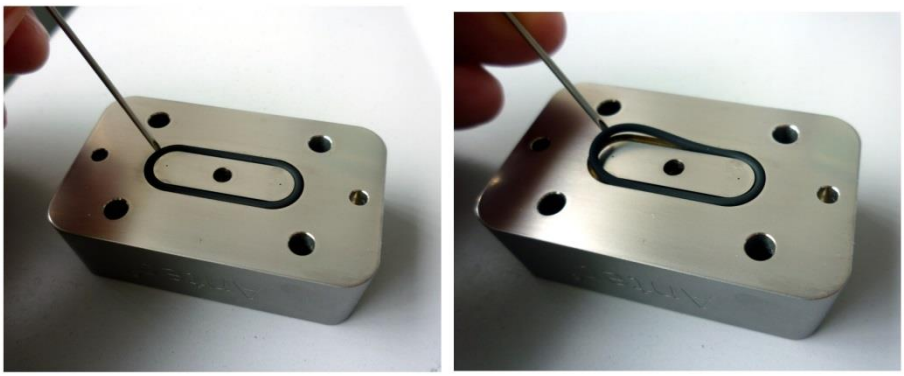


Fig. 16. Removal of Viton O-ring. Gently lift the Viton O-ring out of its chamber from the outside using the needle provided in the µ-PrepCell accessory kit.

<u>Warning:</u> the needle has a <u>SHARP TIP</u>. Take care not to cut/pierce yourself!

3. Take the green flattening plate 30 µm (p/n 250.1042), put it on a flat surface and wet it with a few droplets of demi water.
4. Put the inlet block with the bottom side on the flattening plate and polish it by moving it on the flattening plate in the direction indicated in the figure below. Apply gentle force with your fingers.

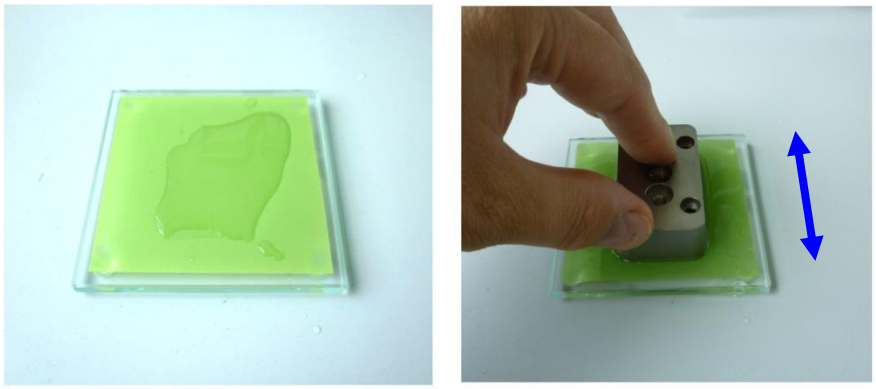


Fig. 17. Flattening plate 30 µm: put the plate on a flat surface. Wet the plate with some droplets of demi-water and polish the µ-PrepCell in the direction indicated by the blue arrow.

5. When all irregularities are polished away. Rinse the inlet block with a sufficient amount of demi water to remove all polishing debris and particles.
6. Repeat step 3 till 5 using the yellow flattening plate 12 µm (p/n 250.1040).
7. After polishing, flush the inlet block with sufficient amounts of water and let it dry.
8. Rinse the flattening plates with water to remove particulate matter and let it dry. When the flattening plates are getting blunt order new ones.

Maintenance Magic Diamond™ electrode

The Magic Diamond working electrode consists of an ultra-thin crystalline Diamond layer deposited on top of a Si substrate. Therefore, such electrode cannot be polished to restore the electrode surface in case of loss of sensitivity due to fouling. The backside of the MD electrode is backside metalized with Gold for better contact. Note that the electrode edges on the gold side may show some blackish marking. This is caused by the activation procedure executed at the manufacturer.

An effective method to restore the detection performance is by electrochemically reactivation of the electrode surface under acidic conditions.

Activation of the electrodes using pulse mode

This activation procedure is suitable for both MD and GC electrodes.

1. Refill the syringe with the mobile phase containing the supporting electrolyte, e.g., the same mobile phase used for oxidation of the sample and start the flow rate (20-50µL/min).
2. Go to the Options pull down menu and choose “Detection mode” (Fig. 17).

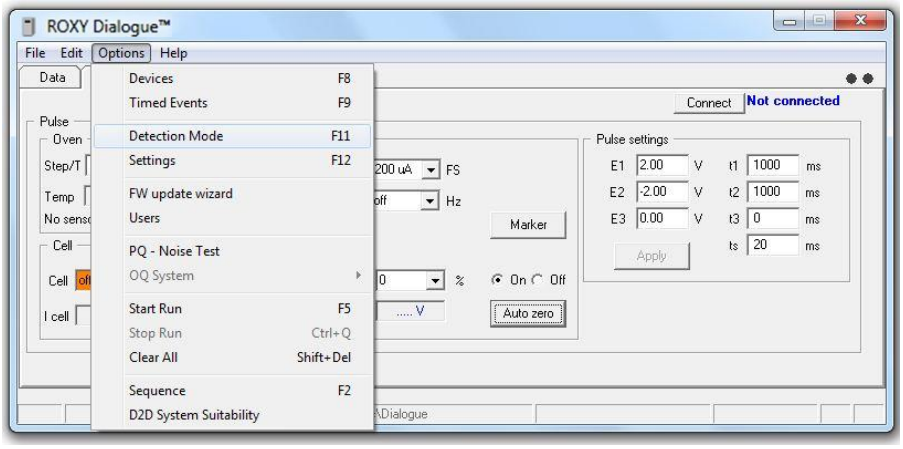


Fig. 18. Pull down menu: Options → Detection Mode.

3. Select Pulse mode (Fig.18) and click OK.

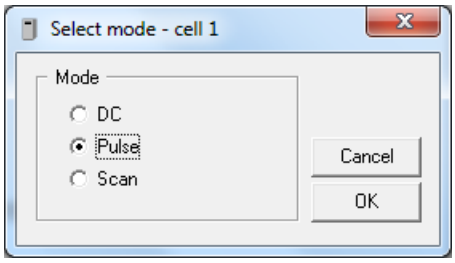


Fig. 19. Select mode window.

4. Set the parameters in the cell control window (Fig. 19).

Parameter	*Value*
E1	+ 2 V
E2	- 2 V
E3	0 V
t1	1000 ms
t2	1000 ms
t3	0 ms

ts	20 ms

(It is recommended to set the Output Range to 200 µA.)

5. Set Run time value to 5 min and turn on the cell.
6. Go to Options pull down menu and click Start Run (Fig. 20).

Program will ask to save the data (The excel file will be created). And start the acquisition.

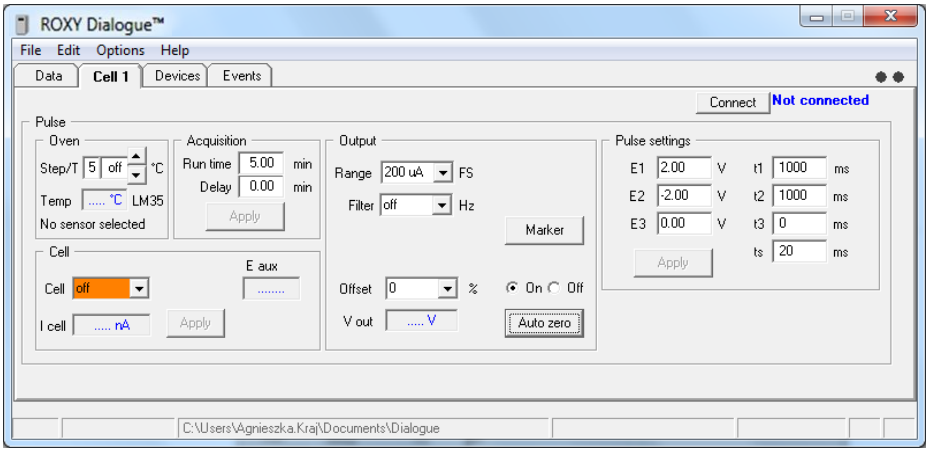


Fig. 20. The cell settings in the pulse mode for MD electrode activation.

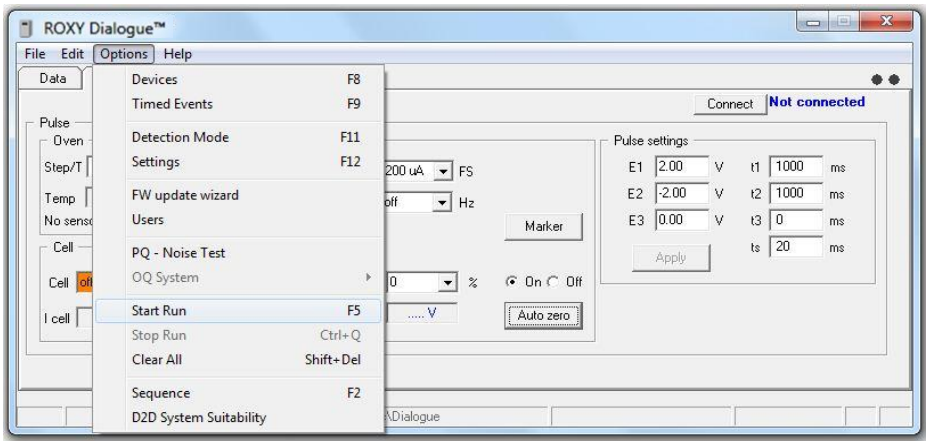


Fig. 21. Start the activation procedure.

After the activation procedure turn OFF the cell and leave the flow for additional 1-2min.

The activation procedure is available also as a program (Activation_pulse_rev01.evt) and can be executed automatically via events

window (Fig. 22). The method can be found in My Documents and in the subfolders: Dialogue\Templates.

The detailed background information about the supplied events files and relevant Dialogue settings are provided in the Dialogue for ROXY User guide (210.7017).

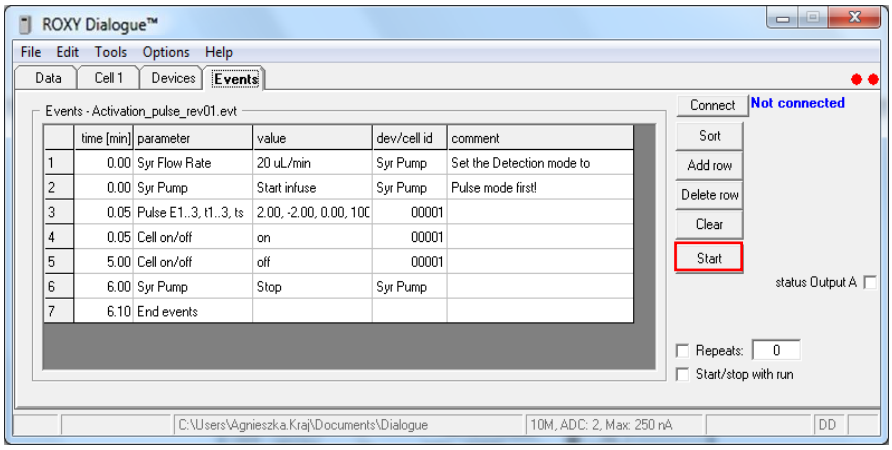


Fig. 22. Activation procedure event table.

In case that no significant improvement of the cell performance is seen:

A. For MD electrode use the additional procedure described in the next chapter (Maintenance Magic Diamond electrode).
B. For GC electrode you can polish the electrode manually as described in the previous chapter (Polishing of the GC electrode).

Activation of the electrodes using scan mode in acidic conditions

1. Disassemble the µ-PrepCell as described on page 12. Keep the MD electrode disc fixed inside the working electrode assembly.
2. Wipe the electrode surface with a tissue wetted with methanol or acetone and subsequently with demi water to clean the electrode surface. **Under all circumstances try to avoid direct contact of the electrode surface with fingers.** The skin contains fatty substances which will foul the electrode.
3. Assemble the µ-PrepCell with MD electrode as described in the previous section.
4. Prepare a mobile phase of 0.5 M Nitric acid (HNO_3), install the µ-PrepCell and prime it with mobile phase to remove any air bubbles.
5. Set the flow rate of 50 µL/min (You will need at least 5mL syringe).

Make sure that all parts that are not acid-resistant such as: nylon inlet filters, column are not connected in the system during this step.

6. Set the ROXY POTENTIOSTAT 5V detector in SCAN mode with the following settings: E1 = -3.00 Volt, E2 = +3 Volt, Scan rate: 50 mV/s, scan cycle: continuous and range 200 µA/V.
7. Start scanning under acidic conditions for 1 hours.
8. After scanning switch off the cell and the flow rate, replace the mobile phase by HPLC grade water and flush the cell to remove the acidic solution.
9. You can start up measurements again.

In the case there is no significant sensitivity improvement observed, repeat step 1 to 10 and increase the total scan time (step 7).
One can also try to perform the activation procedure under the mobile phase conditions of the application. Furthermore, in literature anodic treatments are reported (for example the application of a static potential of + 3 Volt vs. Ag/AgCl for 5 – 10 minutes) to restore the electrode response. Such procedure could be executed as an alternative, if the above-mentioned procedure does not lead to satisfactory results.

Electrochemical reactivation procedure for MD electrode is part of the ROXY potentiostat firmware and available via display panel (Diag→Activate). The scan settings and the duration are as described in points 6 and 7, and only “push button” action is needed to execute it (See ROXY Potentiostat user manual (210.7010)).

Replace the Magic Diamond electrode disc when no improvement is seen after repeated reactivation/conditioning attempts.

Chemical compatibility: The MD electrode exhibits an excellent inertness and can be used with a large variety of mobile phase and chemicals. However it has been observed that the MD electrode operational lifetime is strongly reduced when exposed to fluorinated acids, such as tri-fluoroacetic acid. Even at relatively low concentrations (2% in aqueous solution) significant damage of the diamond electrode was seen within days of operation (delamination/’blister’ formation of the MD layer).

Storage

If the flow cell is not in use, switch off the cell and flush with water. Disconnect from the LC system, we recommend that you disassemble the cell and clean and dry all surfaces.

Before removing the cell from the detector, turn off the cell first !

C H A P T E R 4

Specifications µ-PrepCell

Type	Thin-layer electrochemical cell
Spacers	50 or 100 µm, stainless steel, stackable
WE dimensions	12 x 30 mm, thickness 1 mm
WE area (wetted)	1.9 cm^2
Cell volume	11 µl (effective spacer thickness 50 µm)
Working electrode	Glassy Carbon or Magic Diamond™
Reference electrode	HyREF™ (Pd/H_2 electrode)
Auxiliary electrode	Titanium
Wetted materials	PEEK, Titanium, Viton® fluoroelastomer, Palladium and Glassy Carbon, Titanium or conductive Diamond (WE)
Max. pressure	25 bar (GC electrode) , 50 bar (with MD electrode)

C H A P T E R 5

Accessories µ-PrepCell

The Antec µ-PrepCell (p/n 204.4300) is shipped together with a number of parts listed below (in shipkit):

Table 4. Accessories µ-PrepCell.

Part number	Component
204.0500	µ-PrepCell O-ring inlet block
204.0502	µ-PrepCell O-ring REF electrode
204.2217	µ-PrepCell spacer 50 µm
204.2218	µ-PrepCell spacer 100 µm
204.5007	WE GC for µ-PrepCell
204.5050	WE MD for µ-PrepCell
250.1570	Fingertight fitting PEEK 10-32
250.0062	Hex key for flow cell assembly
250.1030	10 mL diamond slurry 1 µm
250.1025	Polishing disc for WE
250.1040	Flattening plate 12 µm
250.1042	Flattening plate 30 µm
204.0102	µ-PrepCell holder kit

Furthermore, the following (service) parts are available for the µ-PrepCell:

Table 5. (Service) parts µ-PrepCell.

Part number	Component
204.0102	holder kit for µ-PrepCell
204.0501	µ-PrepCell O-ring inl block,Silicone,5pcs
204.0503	µ-PrepCell O-ring REF electr,Silicone,5pcs
204.0510	µ-PrepCell O-ring tool (inlet block)
204.0512	WE contact for µ-PrepCell
204.0913	HyREF for µ-PrepCell
204.2207	inlet block µ-PrepCell
204.2209	WE block µ-PrepCell
204.2210	WE mounting nut for µ-PrepCell
204.2217	spacer 50 µm for µ-PrepCell
204.2218	spacer 100 µm for µ-PrepCell
204.5007	WE GC for µ-PrepCell
204.5010	WE S-S reduction for µ-PrepCell
204.5050	WE MD for µ-PrepCell

For these and other Antec µ-PrepCell parts contact your local supplier.