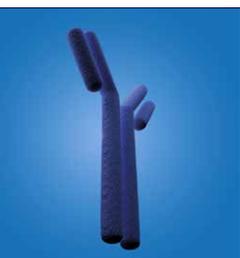
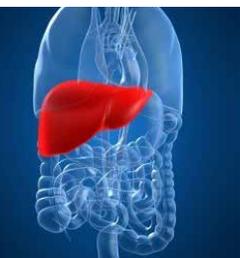


Roxy Exceed

Electrochemistry System for EC-MS



Proteomics



Metabolism



Drug Stability



Environmental



Synthesis



Antec
Scientific

Electrochemistry – Mass Spectrometry (EC–MS)

Why?

Over 90% of the world's existing compounds possess REDOX properties making them either oxidizable or reducible. By injecting your analyte into an electrochemical cell and identifying the reaction products by MS, many of nature's REDOX reactions can be mimicked (simulated) within seconds without any biological interactions, making on-line EC-MS the technique of choice.

Electrochemistry-MS the technique of choice for fast simulation of any REDOX reactions!

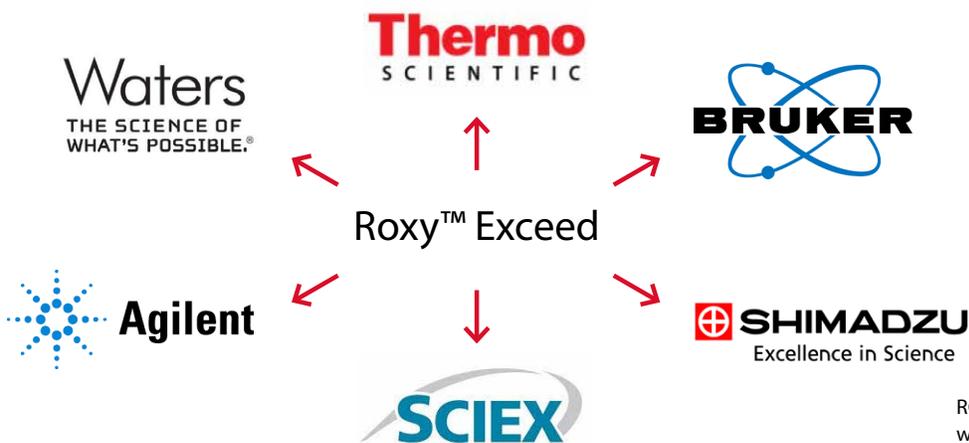
"In-electro" a rapid and much cleaner alternative to costly and time consuming in-vivo, in- vitro, microbial, enzymatic or chemical reactions.

When?

EC-MS is the perfect analytical technique for:

- **Fast generation of (drug)metabolites, intermediates, and degradants**
- **No interferences with matrix (e.g., cell membranes, microsomes)**
- **No need of costly enzymes**
- **Direct identification of reaction products including short lived components**
- **Substantial time and cost savings compared to chemical or enzymatic reactions**
- **Superior MS data: better Id and sequence coverage**
- **Easy scale-up for synthesis of mg quantities**

Typical applications are in the fast prediction and mimicking (simulation) of drug metabolism (phase I and II), drug stability testing and degradation, rapid environmental degradation and persistence of pollutants (POPs), superior reduction of disulfide bonds in proteins/biopharmaceuticals beside many other enzymatic, aquatic or microbial initiated reactions. The rapid electrochemical synthesis of reference materials (e.g., metabolites, degradants) is another emerging application.



ROXY Exceed compatible
with all major LC-MS suppliers

ROXY™ Exceed for seamless EC-MS

- Dedicated for on- and off-line EC-MS
- Fully programmable: DC, Scan and Pulse mode
- Full control of ReactorCell, μ -PrepCell, and SynthesisCell
- Compatible with all LC-MS systems

The ROXY™ Exceed is a new generation Potentiostat dedicated for on- and off-line coupling of Electrochemistry with Mass Spectrometry (EC-MS). With over 130 peer reviewed scientific publications of its predecessor, the Exceed is the best choice for seamless EC-MS.

On-line Mode

In on-line mode the ROXY Exceed can be used in (a) direct infusion mode, (b) for flow injection analysis, or (c) with any type of LC-MS system, e.g., HDX-MS, top-down or bottom-up LC-MS proteomics.

Off-line Mode

In off-line mode the ROXY Exceed is mainly used for electrochemical synthesis and for the collection of electrochemically generated products.

Specifications

The Potentiostat is based on state-of-the-art electronics, has a large voltage and current range of ± 4.9 V and

1 nA - 100 mA, respectively. It supports different modes of operation, such as DC, Scan, and Pulse, assuring the highest yields, reproducibility and robustness.

DC mode: In Direct Current (DC) mode a constant potential is applied to the EC flow cell to oxidize or reduce a target compound.

Scan mode: In Scan mode a scanning or MS voltammogram is obtained by applying a voltage ramp between two potentials using a certain scan speed (in mV/s) during the sweep.

Pulse and Pulse 2: In Pulse mode the working electrode is dynamically and continuously regenerated by a series of potential steps in a cyclic manner. Up to 5 potential steps can be programmed. Pulse 2 mode is an extended pulse mode. It allows for freely programming a multi-step waveform, with up to 30 time potential (t,E) coordinates.



ROXY Exceed Potentiostat

Software

The Exceed can be controlled by using Dialogue (Antec Scientific) or Chromeleon CDS (Thermo Scientific) software.

Flow Cells

Different electrochemical flow cells are available, e.g., ReactorCell, μ -PrepCell (2.0 and SS), and SynthesisCell, to master the most demanding applications.

Electrochemical Flow Cells

ReactorCell™ - universal starter cell

- Flowrate 1-20ul
- Oxidation / reduction / activation
- Virtually zero sample adsorption
- Easy electrode exchange, supplied with various electrodes



The ReactorCell is a basic starter cell, ideally suited for oxidation, reduction and/or activation of compounds that pass through the cell. For single component analysis, the cell can be used in infusion mode by simply connecting it to the syringe pump for direct EC-MS experiments.

For multi-component analyses, the electrochemical cell can be positioned after the LC column to perform LC/EC/MS experiments on the separated components. The ReactorCell is based on a thin-layer flow cell concept, comprising of a very smooth working electrode surface over which the sample is flowing. This results in virtually zero sample adsorption unlike porous flow through electrodes which are prone to adsorption and carry over. The ReactorCell is supplied with 4 working electrodes, Pt, Au, Glassy Carbon (GC), and Magic Diamond (Boron Doped Diamond, BDD). BDD is the workhorse and electrode of choice for oxidation reactions up to a maximum applicable voltage of 4.0 V. Pt and Au are typically employed for specific reactions such as hydroxylation or N-oxidation.

μ-PrepCell™ 2.0 - workhorse for efficient oxidations

- Robust and reproducible oxidations (20 -100 μL/min)
- Fast generation of metabolites, transformation products, intermediates
- Large working range (potential)
- Excellent correlation with metabolic & degradation pathway



The μ-PrepCell™ 2.0 is specifically designed for high yields and conversion rates. Compounds passing through the flow cell are instantaneously oxidized, making the cell ideally suited for (mimicking and/or predicting) on-line EC/MS or LC/EC/MS. The cell is routinely applied in drug metabolism, drug stability testing (degradation studies), environmental degradation/persistence of organic pollutants, POPs, stability of nutrients and healthcare products, to name a few. The surface area of the working electrode is approximately 10 times larger than that of the ReactorCell; therefore, a significantly higher yield is obtained using the μ-PrepCell. Like the ReactorCell, the μ-PrepCell 2.0 is based on a thin-layer flow cell concept, resulting in almost zero carry-over. An additional advantage of using this cell in amperometric mode is the formation of intermediates including reactive metabolites, useful in the understanding and prediction of the metabolic-, (bio)degradation- or (bio)transformation pathway. For highest conversion rate and wide applicability, the cell is supplied with two electrode materials, Glassy Carbon (GC) and Magic Diamond™ (Boron Doped Diamond)

μ -PrepCell™ SS - reduction of disulfide bonds in proteins

- Fast and efficient reduction of S-S bonds in proteins/peptides
- Long term stability and reproducibility
- Reagent free, no interfering DTT, TCEP
- Superior peptide sequencing and S-S bond assignment



The μ -PrepCell SS consists of a dual electrode set-up, i.e., working electrode and counter electrode. This dedicated electrode cell configuration results in distinguished advantages such as: superior stability and longevity, no undesired peptide/protein oxidation during the reduction and overall much easier in use. Another advantage of this cell is the higher pressure stability of up to 350 bar, allowing for its use in HDX-MS and precolumn LC settings (EC-LC-MS). The inlet block of the cell is made out of titanium and serves as working electrode, meanwhile the lower part contains the rectangular counter electrode made of platinum. For highest stability and reduction efficiency of the S-S bonds the μ -PrepCell SS should be operated in pulse mode. For most peptides/proteins including mAbs a pulse of 1 to 1.5 V is sufficient for the reduction of inter- and intra-molecular disulfide bonds.

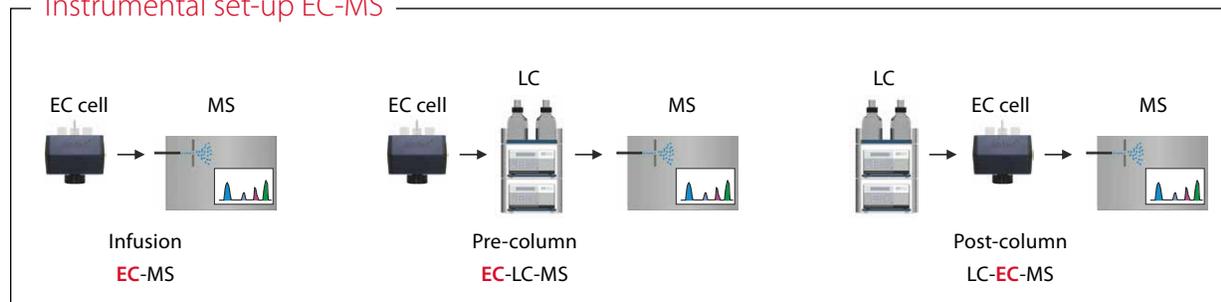
SynthesisCell™ - batch reactor for mg quantities

- Rapid electrochemical synthesis of reference materials, metabolites, degradants
- Complete electrolysis of electroactive species in solution
- Various large surface working electrodes
- Ideal for small-scale electrosynthesis studies (up to 60 mL)



The SynthesisCell is designed for small-scale electrosynthesis of mg quantities of compounds that are difficult to synthesize by other methods, e.g., wet chemistry. Typical compounds that can be synthesized are (drug) metabolites, degradants, and almost any type of REDOX products. The large surface-area of the working electrode and active stirring of the bulk solution assures for complete electrolysis of any electroactive compound and the generation of REDOX products in approximately 1 hour. Various large surface-area working electrodes such as carbon based, Magic Diamond™ (Boron Doped Diamond, BDD), Pt are available for optimal selectivity and maximum yield.

Instrumental set-up EC-MS



ROXY™ Exceed System

The ROXY Exceed system consists of the ROXY Potentiostat, the electrochemical flow cell (reactor) an additional dual syringe infusion pump that allows for direct infusion of the analyte through the electrochemical cell into the MS.



Applications



Proteomics

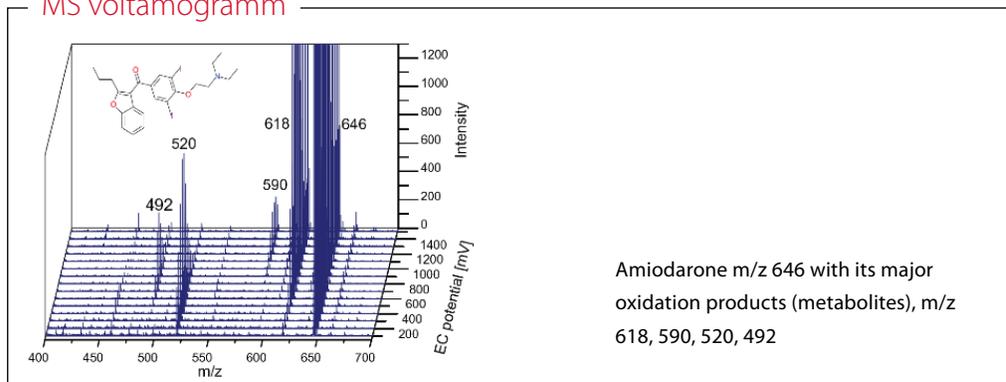
In proteomics and protein chemistry, Electrochemistry (EC) is mainly used for the **reduction of disulfide bonds**, thereby replacing the use of the often harsh and insufficient chemical reduction (DTT, TCEP). The reduction is instantaneous to allow its on-line integration into the HDX-MS, top-down and recently also in bottom-up LC-MS workflows. Superior characterization of proteins/peptides including immunotherapeutics (mAbs) has been published.



Drug Metabolism

EC-MS has been applied successfully for **fast prediction** and mimicking of oxidative **drug metabolism** ("in-electro" vs. *in-vivo* or *in-vitro*). The metabolites are formed instantaneously in the electrochemical cell (**biomimetic oxidation**), mimicking the enzymatic biotransformation of the Cytochrome P450 reactions of the liver (Phase I reaction) including adduct formation (Phase II reactions). Compared to conventional *in-vivo* methods (e.g., rodents, humans) or *in-vitro* methods (e.g., microsomes), considerable time and cost savings have been published.

MS voltammogramm



Environmental Degradation

EC-MS is used for simulating most major reactions that take place in the environmental degradation process, i.e., **aquatic** (aqueous), **photolytic** (photochemical), **microbial** conversions including adduct formation (phase II). Relevant reactive intermediates useful in the understanding of the transformation (degradation) pathway have been generated by EC – impossible to extract from environmental samples. Again EC-MS is an easy inexpensive and extremely fast approach to get a first insight into the degradation process of **persistent organic pollutants** (POP's)



Drug Stability

Forced degradation studies are important to obtain information about potential degradation products and their pathways. Oxidative reactions are the most commonly observed degradation pathways for pharmaceuticals. EC as “green” and **rapid oxidative stress test** to study the degradation of APIs or the influence of excipients has been used successfully, making the use of harsh and toxic oxidizing reagents (peroxide, radical initiators) obsolete. Furthermore the EC approach is tunable, selective and can be scaled up for fast synthesis of mg quantities of degradants.



Electrochemical Synthesis

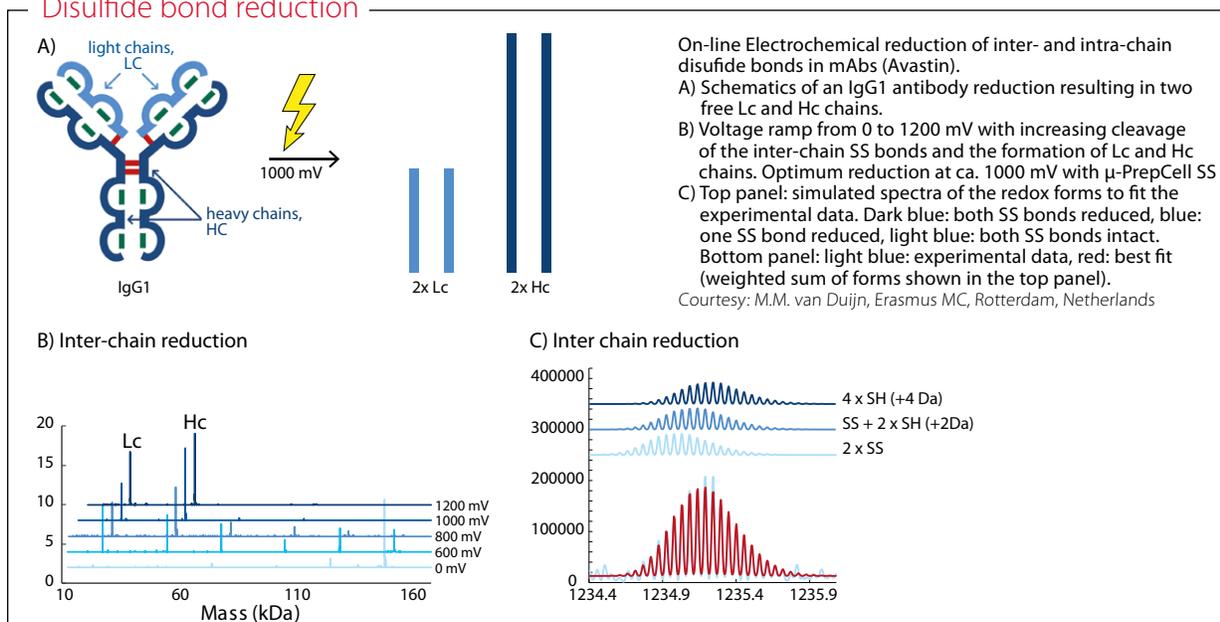
In most areas of drug discovery & development and for degradation studies of pharmaceuticals/xenobiotics in environment, there is a need for **reference materials** (i.e., metabolites, degradants, etc.). Scale-up to mg quantities is required for structural identification of these products by MS and NMR, and for subsequent toxicology studies. Conventional strategies such as wet chemistry or enzymatic approaches are time consuming, cumbersome, expensive and often unsuccessful. Electrochemical synthesis is a purely instrumental technique capable of **rapid synthesis of mg quantities in less than 1 hour**.



Other Applications

The ROXY EC system is used successfully in many other fields such as: **Forensic Toxicology**, for the fast mimicking and prediction of designer/illegal drug metabolites and their toxicity; **Health-care Products** to study the skin-sensitizing effect of oxidised ingredients and their metabolic pathway, including phase II reactions (hapten-protein complex); **Lipidomics**, oxidation of cholesterol to oxysterols to investigate the enzymatic (CYP450) and radical-induced peroxidation reactions including fatty acid methyl esters (FAME) and phospholipids oxidation. Stability of **Antioxidants** in F&B, pharmaceuticals, etc. For a complete listing visit <https://antecscientific.com/>

Disulfide bond reduction



Specifications

General Specifications ROXY Exceed

Power	110-240 VAC, 50/60 Hz, 260 VA, autosensing
Operating modes	DC, Pulse, Pulse2 and SCAN
Single channel	Full control of all Antec flow cells. Optional dual channel control (DCC)
PC control	Parametric control and data-acquisition via LAN port (USB service port)
Potential range	between ± 4.90 V in 10 mV increments
Analog output (DAC)	-1 to +1 V full scale (via 16-bit D/A converter)
LC or MS trigger	Digital I/O (HW) 2x Relay, 5x TTL outputs (CMOS 3.3V logic), 13 TTL inputs (programmable), 1x GND
Oven	height 37 cm; from 7°C above ambient to 60°C, accuracy 0.5°C, stability 0.1°C
Regulatory	CE, UL/CSA, RoHS compliant

DC mode

Ranges	1 nA - 100 mA in 1, 2, 5 increments
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PULSE mode

Range	200 nA - 100 mA in 1, 2, 5 increments
Waveform	Max 5 potential steps
Pulse times	t1: 100 ms - 2000 ms; t2, t3, t4, t5: 0 - 2000
Sampling times	20 ms - [t1 - 60] ms

PULSE2 mode

Range	200 nA - 100 mA in 1, 2, 5 increments
Waveform	Free programmable multi-step waveform with up to 30 time-potential (t, E) coordinates and max. pulse duration of 4 s. Time points in 10 ms increments.
Sampling times	Sampling interval is free programmable

SCAN mode

Range	200 nA - 100 mA in 1, 2, 5 increments
Scan rate	1 - 100 mV/s in 1, 2, 5 increments
Cycle	half, full, continuous

Rear panel

I/O connections	Mains, LAN, USB, Analog data, Valve and Digital IO connector
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Physical specifications

Dimensions	43 (D) x 22 (W) x 44 (H) cm = 16.9" (D) x 8.7" (W) x 17.3" (H)
Weight	14.4 kg (32 lbs) without flow cell

Part no	Description
211.0070	ROXY Exceed system with ReactorCell and syringe pump
211.0074	ROXY Exceed system with μ -PrepCell 2.0 and syringe pump
211.0072	ROXY Exceed system for S-S reduction and syringe pump
211.0073	ROXY Exceed for HDX w/o syringe pump

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ROXY, ROXY Exceed, ReactorCell, μ -PrepCell, SynthesisCell are official trade marks of Antec Scientific. Granted patents on ROXY Exceed: EP 2572188 B1, EP 2706066 A1