Application Note
Electrochemical Synthesis

Electrochemical Reactions upfront
MS – EC/MS

Proteomics & Protein Chemistry
S-S bond reduction
HDX
Peptide bond cleavage
Na+, K+ removal
Drug-protein binding

Lipidomics & Fatty Acids
Cholesterol
Oxysterol
FAME Biodiesel

Drug Metabolism
Mimicking CYP 450
Phase I & II
Biotransformation

Synthesis (mg)
Metabolites & Degradants

Pharmaceutical Stability
Purposeful degradation
API testing
Antioxidants

Environmental
Degradation & persistence
Transformation products
Surface & drinking water

Food & Beverages
Oxidative stability
Antioxidants

Forensic Toxicology
Designer drugs
Illicit drugs

Healthcare & Cosmetics
Skin sensitizers

Genomics
DNA Damage
Adduct formation
Nucleic acid oxidation

SynthesisCell™ – Efficient Synthesis of Metabolites/ Degradants

- **Rapid and cost-efficient synthesis of mg quantities**
- **Superior than traditional wet chemistry/microsomal techniques**
- **Various large surface-area working electrodes**
- **Proven track record in Big Pharma**

Summary

A fast and efficient method for electrosynthesis of metabolites, degradants and reference materials is presented. Using the SynthesisCell oxidation and reduction products can be produced in milligram quantities in a short period of time [1-7]. The Oxidation of 3-methoxy 4-hydroxyphenylglycol (MOPEG) Lidocaine and two drug compounds from Big Pharma (Pfizer and Novartis) are used to demonstrate the electrochemical synthesis of their major metabolites. Almost complete conversion of 0.1 mmol/L MOPEG (1.4 mg) was achieved in 10 min. For Lidocaine 5 µmol/L (ca. 94 µg) was converted by almost 80% in 15 min into the relevant oxidation products. For the drug compound Cipargamin (Novartis) a key secondary metabolite M16 could be synthesized for the fist time and for Fesoterodine (Pfizer) two degradants (oxidation products) could be synthesized with almost 100% yield.

SynthesisCell™ – Efficient Synthesis of Metabolites and Reference Materials

Introduction

In most areas of drug discovery & development, including environmental degradation of drugs/xenobiotics, there is a severe need for reference materials. The same need exists for most bio-degradation and bio-transformation reactions, which lead to small amounts of REDOX products. In addition, scale-up to mg quantities of these REDOX products are required for comprehensive structural identification by MS, NMR and subsequent toxicology studies.

Conventional methods for synthesis include classical organic synthesis, microsomal incubation or porphyrin-catalyzed chemical oxidation. However, these methods are usually time consuming, cumbersome and not always successful. Electrochemical synthesis is a purely instrumental technique often capable to synthesize such REDOX products in absence of biological matrix in a very short period of time (less than 1 hour).

Figure 1: ROXY™ Potentiostat with SynthesisCell™. The cell contains a Reticulated Glassy Carbon (RGC) working electrode (WE), a Pd/H2 reference electrode (HyREF), and a Pt auxiliary electrode (AUX).

Method

A ROXY™ Potentiostat with extended current range (up to 20 mA) was used with Dialogue Elite software (version 2.0.0.81). The SynthesisCell was equipped with a Reticulated Glassy Carbon (RGC) working electrode, a HyREF™ reference electrode and an auxiliary electrode without frit.

Table 1

Synthesis Conditions

EC	ROXY™ EC System
Cell	SynthesisCell™ with RGC WE, Pt coil AUX and HyREF™
Volume	80mL
Solution A	50 mmol/L acetic acid, pH 4.4, with 5% methanol
Sample	10 or 100µmol/L MOPEG in solution A
Potential	1000 mV
Range	10mA

The SynthesisCell was filled with 80 mL of 10 or 100 µmol/L MOPEG dissolved in solution A (see Table 1). A constant potential of 1V was applied to oxidize MOPEG. The progress of the synthesis was checked each 5 min by taking an aliquot of 100 µL of the SynthesisCell solution. The sample was diluted a factor 20 (10 µmol/L) or 200 (100 µmol/L) prior to HPLC/ECD analysis (see Table 2).

A porous frit can be used to prevent mixing of products that are formed at the working and auxiliary electrodes and was also compared in this study. The conversion is calculated by the % decrease in MOPEG peak area when switching on the cell.

Table 2

Detection Conditions

HPLC	LC 110; AS 110; DECADE II
Flow cell	VT03 flow cell with ISAAC and GC WE
Column	Antec HPLC Column for PQ
Detection potential	650 mV
Range	10mA

Table 3

Cleaning Conditions

Detection mode	scan
E1	- 200mV
E2	+1000mV
Scan rate	50 mV/s
Cycle	continuous
Time	30min

Results

Case Study 1 - MOPEG

Figure 2 depicts the progress of electrosynthesis for different experimental conditions using 100 and 10 µmol/L MOPEG and an AUX electrode with or without frit. No significant difference in conversion efficiency was observed for both AUX configurations. The complete oxidation of MOPEG was achieved in less than 30 minutes and near 100% conversion was reached in only 10 minutes. The current response was measured during the electrosynthesis using the Dialogue software (Figure 3). Evidently, only during first 15 minutes of oxidation the current response was significantly declining from 7.5 mA to approx. 0.5 mA. After 25 minutes of oxidation the current stabilized at about 130 µA. This observation corresponds to conversion efficiency (Fig. 2), which reached 100% after 15 min of electrosynthesis. Registering of the current response can give an insight in the electrosynthesis progress even without the control sample measurement.

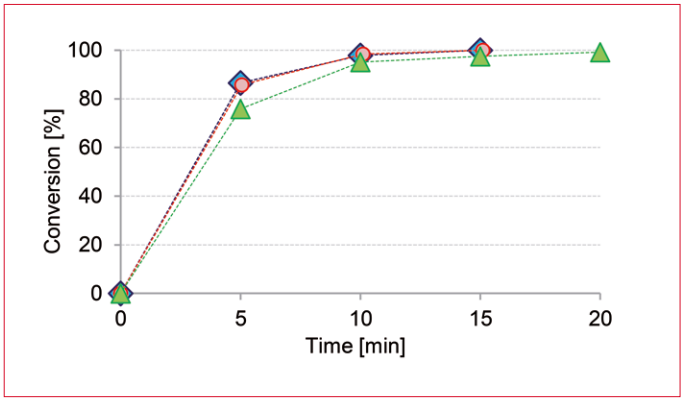

Figure 2: Oxidation of MOPEG. Green/Red: 10 µmol/L MOPEG. Blue: 100 µmol/L MOPEG. Green: using AUX with frit, the others are without frit.

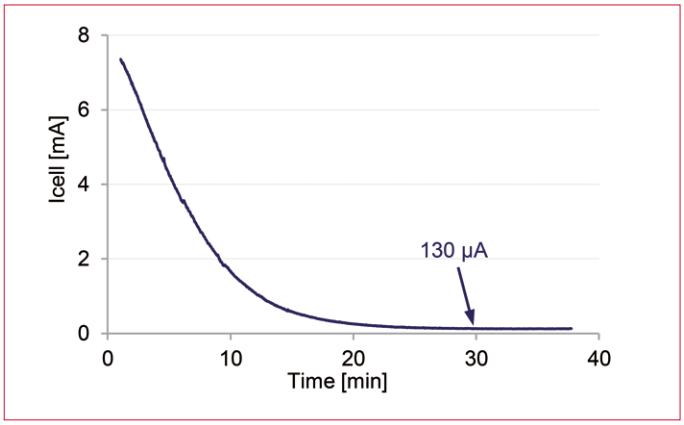

Figure 3: The current (I-cell) measured in the SynthesisCell during oxidation of 10 µmol/L MOPEG, using Dialogue.

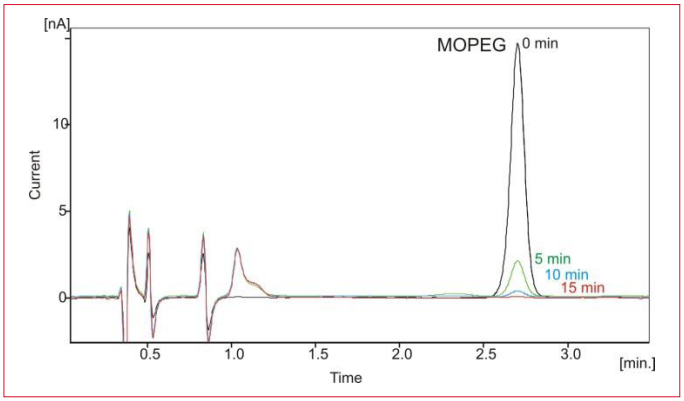

Figure 4: Oxidation of MOPEG. At time 0, 5 ,10 and 15 min. After 15 min almost 100% conversion.

Figure 4 shows the oxidation of MOPEG in the SynthesisCell. After 15 min almost full conversion of the the MOPEG was obtained.

Case Study 2 - Lidocaine

Lidocaine is a common local anesthetic and class-1b antiarrhythmic drug. Lidocaine is used topically to relieve itching, burning, and pain from skin inflammations, injected as a dental anesthetic, or as a local anesthetic for minor surgery. It is listed as essential medicine by WHO and applied in numerous healthcare products.

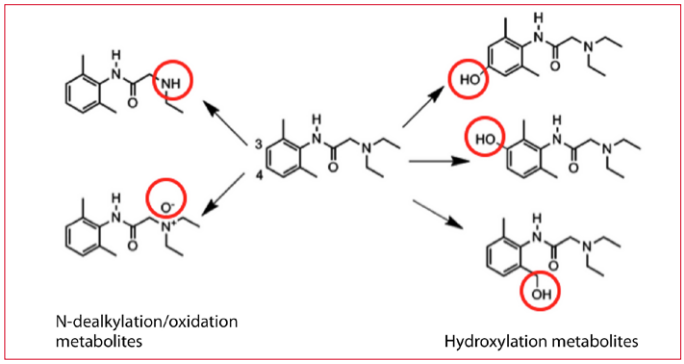

Figure 5: *In-vivo* metabolites of Lidocaine due to oxidative metabolism by Cytochrome P450. Metabolites result from N-dealkylation, N-oxidation, and aromatic and benzylic hydroxylation

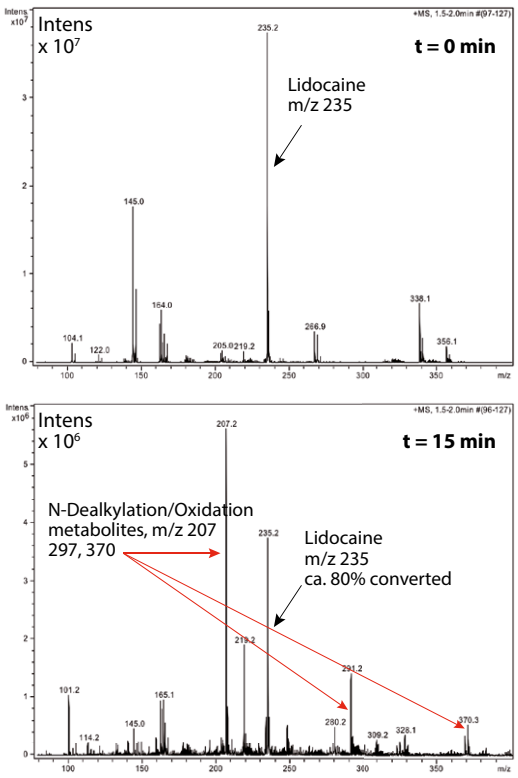

Figure 6: MS spectra of aliquots taken from the SynthesisCell at t=0 and t=15 minutes. Ca. 80 % of the Lidocaine was converted into 3 main reaction products (N-Dealkylation and N-Oxidation metabolites using the conditions listed in Table 4 and the Reticulated Glassy Carbon (RGC) electrode.

In Figure 6 the MS spectra are shown for aliquots taken at 0 and 15 minutes from the 80 mL SyntesisCell analyzed by direct infusion ESI/MS. At 0 minutes only Lidocaine is present. After 15 minutes of electrolysis ca. 80% of Lidocaine was converted into the oxidation products with m/z 207, 297 and 370, which correspond to the N-dealkylation and N-Oxide metabolites of Lidocaine. For the generation of larger amounts of hydroxylation metabolites, the use of Boron Doped Diamond (BBD) working electrode is required. Data not shown.

Table 4

Synthesis Conditions	
EC	ROXY™ EC System
Cell	SynthesisCell™ with RGC WE, perforated glass tube as AUX and HyREF™
Volume	80mL
Solution A	20 mM NH4Ac + 0.1M Acetic Acid in ACN:H2O (90:10)
Sample	5 µM Lidocaine*)
Potential	1500 mV, DC mode
Range	20mA

*) Up to 100 x higher concentrations are typically used. This low concentration was chosen for direct infusion MS of aliquots from the SynthesisCell without any sample preparation, i.e., filtration or dilution.

Examples from Pharma

Cipargamin (KAE609), Novartis

In case of Novartis, a key secondary metabolite M16 of the antimalarial drug Cipargamin (KAE609) was identified in all biological matrices at very low levels.

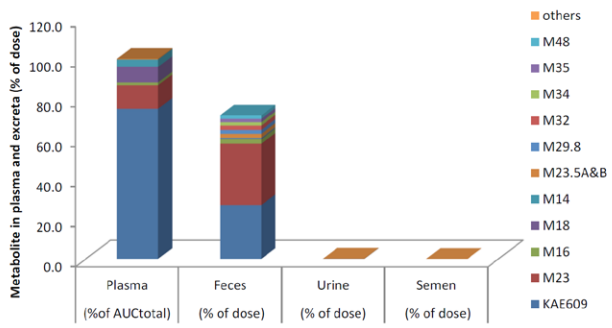

Figure 7: Overall metabolism of Cipargamin (KAE609)

All 19 recombinant human CYP enzymes were capable of catalyzing the hydroxylation of M23 to form M16 but with insufficient turn-over for structural characterization by NMR.

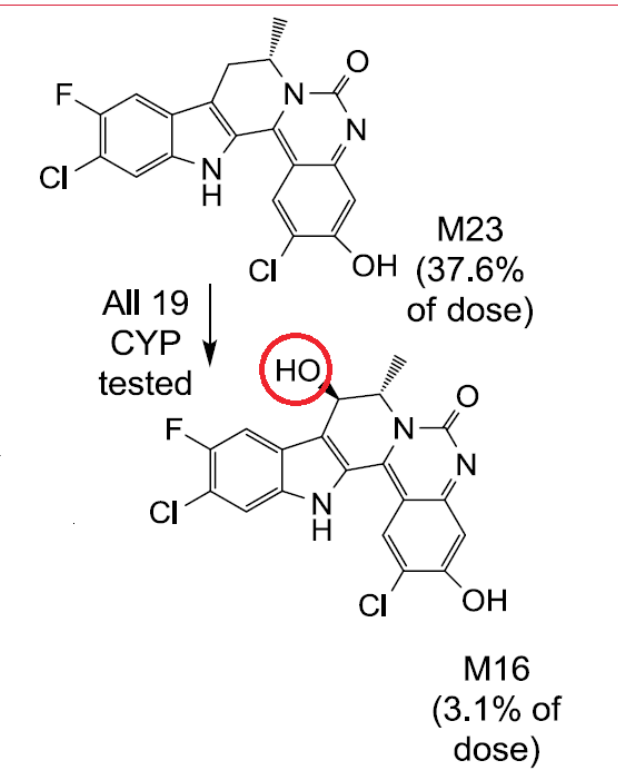

Figure 8: Excerpts of the metabolic pathway of Cipargamin. Hydroxylation of metabolite M23 to M16

As the proposed structure of M16 suggest benzylic oxidation, electrochemical synthesis was applied using the ROXY EC system equipped with SynthesisCell. A boron doped diamond electrode under acidic conditions gave the desired stereoselective product in 10% yield. For the first time ever, sufficient quantities of M16 could be synthesized, to allow full structural characterization by NMR, previously unable using traditional enzymatic techniques [5].

Fesoterodine, Pfizer

At Pfizer, electrochemical synthesis was used for the fast and convenient synthesis of pharmaceutical oxidation products (degradation products) of N-dealkylation reactions of Fesoterodine.

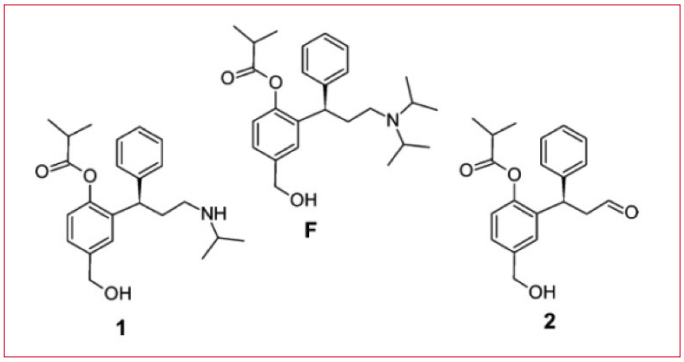

Figure 9: Chemical structure of Fesoterodine (F) and its two oxidative N-dealkylation products (degradants) 1 and 2.

A working potential of 950 mV was applied using the ROXY Potentiostat equipped with the SynthesisCell for the generation of the two oxidative N-dealkylation products (degradants). A glassy carbon working electrode (Reticulated Glassy Carbon – RGC) was used as the supporting electrolyte. The reaction was monitored over a 2 h period of time. Aliquots of the reacting solution were taken at given time points and analyzed using high-performance liquid chromatography with UV and mass detection. After turning on the cell voltage, a decrease in fesoterodine peak area was observed with concomitant formation of the two N-dealkylated oxidation products. These experimental conditions generated an almost complete conversion of fesoterodine into the two N-dealkylation products after 2 hrs of operation.

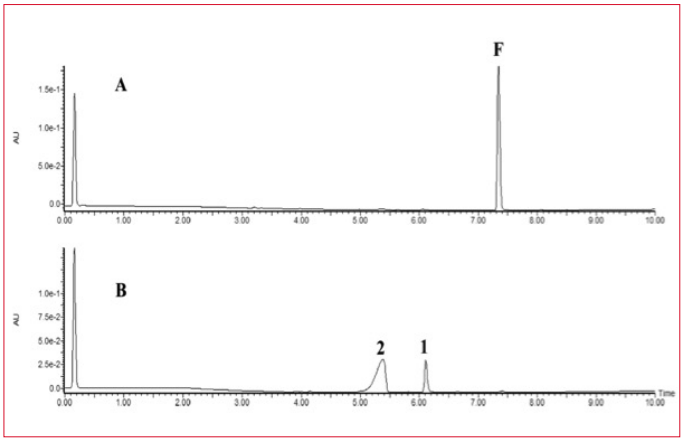

Figure 10: UV chromatograms at 224 nm. (A) 0.25 mg/mL fesoterodine fumarate solution in 50 mM aqueous ammonium acetate (no potential). The observed peak "F" corresponds to fesoterodine. (B) Reaction mixture after 2 hrs (a constant potential of 950 mV was applied to the cell) with the two oxidative N-dealkylation products (degradants) 1 and 2.

The two oxidation products were purified by reverse-phase preparative high-performance liquid chromatography and subsequent characterization by NMR.

Pfizer reported that the electrochemical procedure proved to be rapid, clean, and efficient compared to traditional synthetic methods and that it is particularly useful for generating milligram quantities of oxidative degradants [6].

Conclusion

The electrosynthesis using the SynthesisCell is fast, efficient and cost-effective. Full conversion in less than 30 min has been demonstrated for MOPEG, using the large surface area Reticulated Glassy Carbon working electrode. With same type of electrode all major N-dealkylation and N-oxide metabolites of Lidocaine can be produced. Moreover, other types of working electrodes such as Magic Diamond (BDD) and Platinum (Pt) are available for increased selectivity such as aromatic and benzylic hydroxylation reactions on BDD. In the examples of Novartis and Pfizer the obtained oxidation products could be synthesized for the first time for characterization by NMR.

References

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[3] L. Zhu et al., Science of the Total Environment (2018) 622–623: 1193-1201
[4] L. Zhu et al., Water Research (2016) 102: 52-62
[5] S.E.W. Huskey et al., Drug Metabolism and Disposition (2016) DOI: https://doi.org/10.1124/dmd.115.069187
[6] S. Torres et al., Org. Process Res. Dev. (2015) 19: 1596-1603
[7] L. Zhu et al., Chemosphere (2015) 131: 34-40

Ordering information

ROXY EC System	
210.0010A	ROXY Potentiostat, High Current
SynthesisCell	
206.0037	SynthesisCell, consisting of 80 mL reaction vessel with Teflon cap, WE (Reticulated Glassy Carbon), RE (HyREF) and AUX electrode, stir bar, all parts included for immediate use with high current ROXY Potentiostat
Optional	
206.0306	Magic Diamond (BDD) working electrode
206.0322	Platinum (Pt) working electrode

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