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 and Reference Materials

- **Metabolite and Reference Material "Synthesizer"**
- **Fast Electrosynthesis of mg Quantities of Products for MS, NMR, etc.**
- **Various Large Surface-area Working Electrodes**
- **Proven Track Record in Big Pharma**

Introduction

In most areas of drug discovery & development, drug metabolism, and for degradation studies such as pharmaceuticals and environmental pollutants, there is a severe need for reference materials. The same need exist for most bio-degradation and -transformation reactions, which lead to small amounts of REDOX products that may requier full identification and /or quantification. For comprehensive structural identification of these REDOX products by MS and NMR, or for subsequent toxicology studies, mg quantities of these metabolites, degradants, (bio)-transformation products, are required.

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 method often capable to synthesize such REDOX products in absence of biological matrix and without the need of wet chemistry in a very short period of time (less than 1 hour).

Summary

A fast and efficient method for electrosynthesis of metabolites is presented. Using the SynthesisCell reactive intermediates and other oxidation and reduction products can be produced in milligram quantities in a short period of time. A proof of principle is demonstrated using 3-methoxy 4-hydroxyphenylglycol (MOPEG) and the oxidation of Lidocaine to synthesis its 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) were converted by almost 80% in 15 min into the relevant oxidation products.

Figure 1: SynthesisCell™ with 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 control software (version 2.02.194). 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% metha-nol
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 synthe-sis 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).

An AUX electrode with and without porous frit was compared. The porous frit can be used to prevent mixing of products that are formed at the working and auxiliary electrode. 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 electro-synthesis 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 pro-gress even without the control sample measurement.

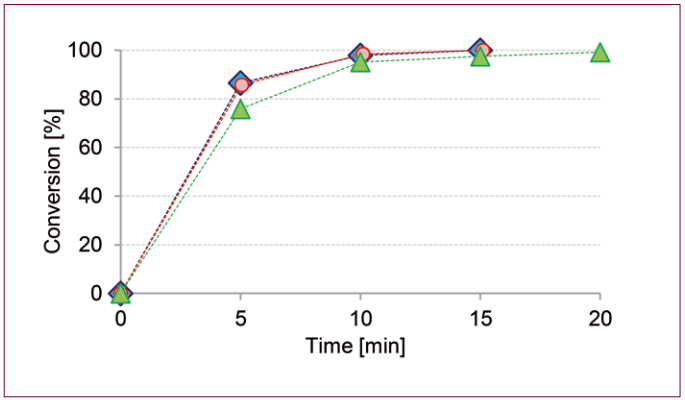

Figure 2: Oxidation of MOPEG. Green/Red: 10 µmol/L MOPEG. Blue: 100 µmol/L MOPEG. Green: using AUX with frit, the others are without frit.

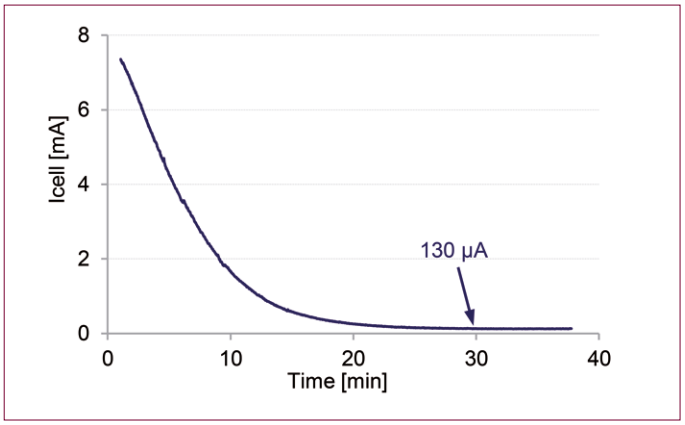

Figure 3: The current (I-cell) measured in the SynthesisCell during oxidation of 10 µmol/L MOPEG, using Dialogue.

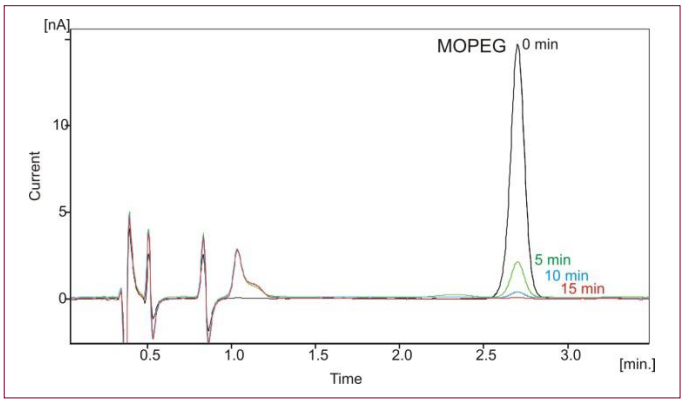

Figure 4: Oxidation of MOPEG. Green/Red: 10 µmol/L MOPEG. Blue: 100 µmol/L MOPEG. Green: using AUX with frit, the others are without frit.

Figure 4 shows the oxidation of MOPEG in the SynthesisCell during the first 15 min. MOPEG was not detectable after 15 minutes. Figure 5 presents the comparison between the sample collected from the AUX and WE compartments.

For this experiment an AUX with porous frit was used. Clearly, no mixing of oxidation products occurred. After 30 min the MOPEG completely disappeared from the SynthesisCell. However, the MOPEG concentration measured in AUX compartment corresponds to the level before the electrosynthesis has started.

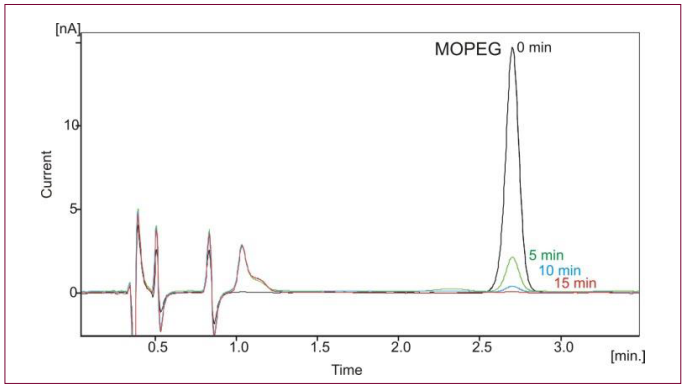

Figure 5: Oxidation 10 µmol/L MOPEG using the SynthesisCell and an AUX with porous frit to prevent mixing of products formed at WE and AUX. After 30 min a sample was taken from the AUX compartment (black) and the WE compartment (red).

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 in less than 30 minutes.

Moreover, other type of working electrodes such as Magic Diamond (BDD) and Platinum (Pt) are available for increased selectivity e.g., aromatic and benzylic hydroxylation reactions on BDD.

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.

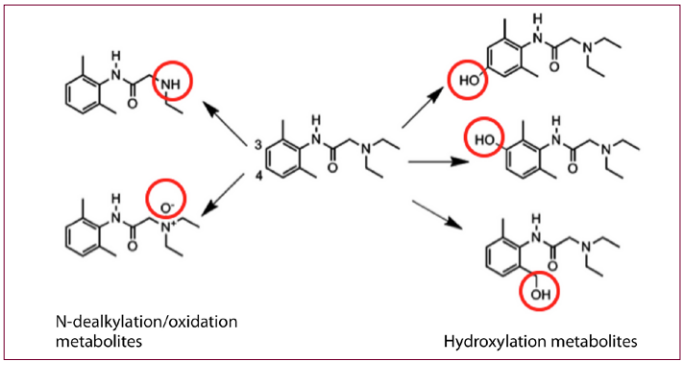

Figure 6: *In-vivo* metabolites of Lidocaine due to oxidative metabolism by Cytochrome P450. Metabolites result from N-dealkylation, N-oxidation, and aromatic and benzylic hydroxylation

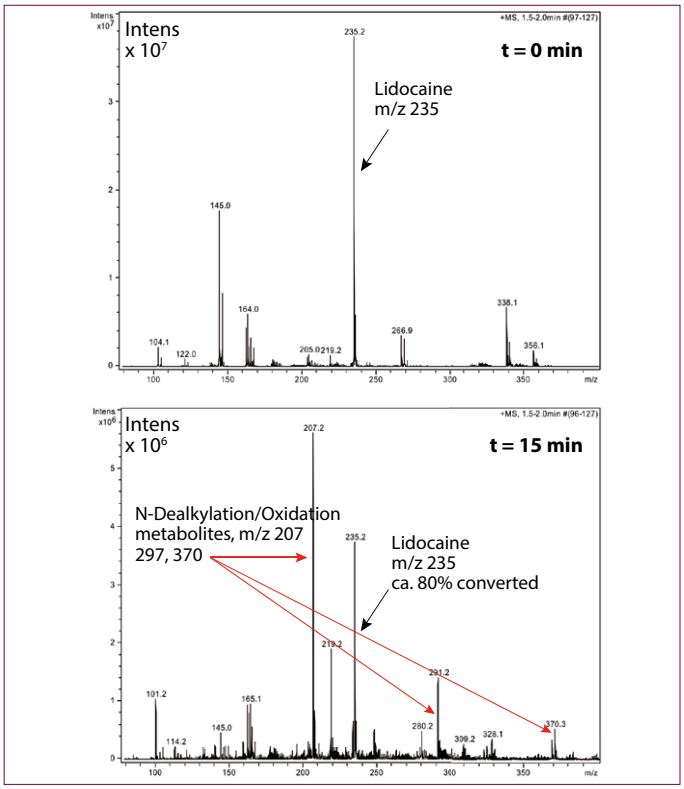

Figure 7: MS spectra of aliquots taken from the SynthesisCell at t=0 and t=15 minutes. Ca. 80 % of the Lidocaine was converted into 3 main reaction products (N-Dealkylation and N-Oxidation metabolites using the conditions listed in Table 4 and the Reticulated Glassy Carbon (RGC) electrode.

In Figure 7 the MS spectra are shown for aliquots taken at 0 and 15 minutes from the 80 mL bulk 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.

PART NUMBERS AND CONFIGURATIONS

SynthesisCell	
206.0037	Complete 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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