The New Panacea in Metabolomics, Proteomics and Genomics — Electrochemistry / MS

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Recently, the use of electrochemistry (EC) in conjunction with LC/MS has been extended from mimicking drug metabolism to new applications such as: protein/peptide cleavage, disulfide bonds reduction in proteins/peptides, DNA damage, covalent DNA adduct formation, covalent drug-protein binding, etc. In this poster we will show the application of online EC/MS as a powerful tool to simulate various oxidation and reduction processes in life sciences.

The coupling of electrochemistry for protein and peptide cleavage before MS analysis is a very promising new approach to enzymatic digestion. Electrochemical cleavage of proteins and peptides typically occurs specifically at C-terminal of the Tyrosine and Tryptophan peptide bonds. Examples of oxidative cleavage will be presented.

Disulfide bonds are one of the most important post-translational modifications for proteins. In this poster we present the structural analysis of biologically active peptides and proteins containing disulfide bonds (e.g., GSSG, insulin, etc.) using electrochemistry (EC) combined with mass spectrometry. In this approach, the sample undergoes electrolytic disulfide cleavage in the electrochemical flow cell followed by online MS analysis.

Furthermore, quick electrochemical activation of electrode and new scanning method for efficient metabolite synthesis will be presented. A new scanning method was applied for oxidation of the highly concentrated samples (mM range) to achieve high yield in the metabolites formation. Stable oxidation conditions were obtained without the need of any cell maintenance for a prolonged period of time.

EC was used to initiate adduct formation with DNA. The obtained reaction products were separated by LC and detected by MS. Tandem MS experiments were used for structural confirmation. In a proof of principle study acetaminophen was selected as model compound. Covalent adduct formation was observed for electrochemical activated mixtures of acetaminophen and guanosine.

These applications illustrate the tremendous power and broad applicability of electrochemistry as a tool to mimic nature’s Redox reactions within a few seconds or minutes and can aid in the understanding of biochemical processes.
On-line Electrochemistry/MS — A Powerful Technique for Rapid Prediction of Phase I and II Drug Metabolism

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The use of electrochemistry is a complementary approach to traditional methods such as in vivo (human, rodent) or in vitro (liver microsomes) metabolism studies and can deliver the oxidative metabolic fingerprint of a (drug) molecule in a very short time. In this poster a dedicated electrochemical system will be described that can provide for the rapid screening of drugs to obtain their metabolic fingerprint. Furthermore, a novel micro preparative electrochemical cell for highly efficient metabolite synthesis will be described. The cell can be used in conjunction with MS or LC/MS to perform the separation and identification of the newly created metabolites. Alternatively the cell can be used off-line and the generated metabolites can be collected for identification by NMR or used as reference substance in MS.

Amodiaquine, an anti-malaria drug was chosen as one of the model compounds to investigate oxidative metabolism using the on-line EC/MS system with automated MS voltammogram acquisition. A MS voltammogram visualizes the ion abundance versus m/z as a function of applied potential to the electrochemical cell. With a MS voltammogram the optimal potential can be determined for electrochemical generation of the desired metabolite for further research such as a phase II metabolism study (i.e. adduct formation).

The easy and fast electrochemical conversion of Amodiaquine into its major phase I metabolites will be presented in both analytical and preparative cells. In a second step, Glutathione (GSH) is added to the electrochemically generated metabolites to form the appropriate GSH-metabolite adducts, mimicking phase II reactions. All known adducts were successfully formed and identified with MS. Additionally, the applicability of on-line EC/MS to oxidize other drugs and xenobiotics (e.g., acetaminophen, irinotecan, and others) will be presented.

The data demonstrate that hyphenation of electrochemistry with electrospray mass spectrometry provides a versatile and user-friendly technique for rapid and cost efficient screening of target compounds (drugs, xenobiotics, etc.) in phase I and phase II metabolomics studies.