Chapter 6
Summary and Future Perspective

6.1 Summary
The thesis commences in Chapter 1 with reviewing known electrochemical techniques and their advantages and limitations in the context of oxidative drug metabolism. The thesis continues with development of new electrochemical techniques to cover certain in vivo oxidative drug metabolism reactions which were not possible by constant potential oxidation. Electrochemically generated reactive oxygen species (ROS) in Chapter 2, electrochemical oxidation by square-wave potential pulses in Chapters 3 and 4, as well as electrocatalytic activation of hydrogen peroxide on a platinum electrode in Chapter 5 are among the electrochemical techniques developed throughout my research.

Chapter 1 presents Cytochrome P450s (CYP), their discovery, structure, and role in in vivo oxidative drug metabolism by catalytic activation of molecular oxygen and the generation of ROS, mainly oxo-ferryl radical cations. Chapter 1 further reviews different electrochemical techniques that have been developed so far, including direct electrochemical oxidation (in combination with mass spectrometry), oxidation by electrochemically generated ROS, and oxidation with modified electrodes containing metalloporphyrines and enzymes.

Direct electrochemical oxidation in combination with mass spectrometry, introduced in Chapter 1, has been used widely to imitate in vivo oxidative drug metabolism. The oxidation mechanisms were discussed in relation to in vivo oxidative drug metabolism. Direct electrochemical oxidation, accordingly, is capable of imitating oxidative reactions that are initiated by electron transfer, such as N-dealkylation and hydroxylation of substituted aromatic rings, whereas oxidation reactions which are initiated by hydrogen atom transfer (HAT) or oxygen atom insertion are difficult to imitate. Another major obstacle in imitation emerges when the oxidation products are oxidized more easily than the drug substrates, which prevents isolation of the latter. Finally the regioselectivity of direct electrochemical oxidation leading to charged intermediates, which presumably are
not present during in vivo oxidation by CYP, can limit the extent of imitation by favoring specific reaction pathways.

Electrochemically generated ROS may be able to imitate the oxidation reactions initiated by HAT. Electrochemically assisted Fenton, and Gif reactions activating hydrogen peroxide through homolytic and heterolytic bond cleavage to generate hydroxyl radicals and high valency iron species, respectively, are reviewed in Chapter 1. A wide range of oxidation reactions, in particular hydroxylations of non-activated aromatic rings, were achieved by these methods.

Metalloporphyrines, as surrogates for the active site of CYP, can be immobilized on the electrode surface to imitate oxidative drug metabolism by generation of reactive intermediates resembling those generated by CYP. Selection of metalloporphyrines with different substituents and metallic centers could determine the chemo- and stereoselectivity of the oxidation reactions.

Chapter 1 also addresses my work on the immobilization of metalloporphyrines via self-assembled monolayers (SAM) of alkanethiols, and their corresponding surface analysis using Surface Enhanced Resonance Raman Spectroscopy (SERRS). These might open a new road in the application of modified surfaces on-line with mass spectrometry that can be controlled by electrochemistry. Finally, chemoselective and stereoselective oxidation reactions can be mimicked by using electrodes that have been modified with immobilized enzymes, especially CYP itself.

Chapter 2 illustrates the use of electrochemically generated ROS, generated by electrochemical reduction of molecular oxygen and further radical reactions through the Haber-Weiss reaction, for oxidation of the test compound lidocaine. A two-compartment electrochemical cell was successfully constructed to isolate the reaction products from working and auxiliary compartments. N-oxidation occurred in the working compartment. I suggest that electrochemical reduction of molecular oxygen to hydrogen peroxide followed by reaction with the tertiary amine part of lidocaine to a peroxide intermediate and its subsequent decomposition resulted in the N-oxidation product. N-dealkylation was observed in the auxiliary compartment and occurs through direct electrochemical oxidation of lidocaine to an iminium intermediate followed by hydrolysis and intramolecular rearrangement. The use of electrochemically generated ROS can therefore extend the application of electrochemistry in the selective imitation of oxidative drug metabolism.

The N-oxidation product was identified using Atmospheric Pressure Chemical Ionization (APCI) through thermally-induced degradation reactions. This emphasizes the importance of using various ionization techniques in the identification of drug metabolites.

Chapter 3 explores square-wave potential pulses for the selective generation of metabolites of lidocaine with increased yield. This study shows that using square-wave potential pulses instead of constant potential oxidation increases the
yield of 4-hydroxylation up to fifty times depending on the cycle time. Whereas 4-
hydroxylation of lidocaine was observed at cycle times of around one second, N-
dealkylation was favored at shorter pulse times. The oxidation mechanism under
square-wave potential pulses was studied by stripping linear voltammograms,
which suggested the regeneration of the electrode surface under square-wave
potential pulse conditions as the main reason behind the high yield oxidation.
Isotope studies revealed the source of the oxygen atom in the N-oxidation and 4-
hydroxylation: the oxygen atom in the N-oxide was derived from dissolved
molecular oxygen and in the 4-hydroxylation product it originated from water. The
exact mechanism leading to selectivity of oxidation during long and short pulses
remains, however, unclear.

The use of square-wave potential pulses is not limited to high yield and
selective oxidation reactions. Square-wave potential pulses may also be used to
promote reactions that are not possible by constant potential oxidation. Chapter 4
shows how to promote O-dealkylation of phenacetin to acetaminophen, a reaction
that is not possible by direct electrochemical oxidation. Oxidation intermediates
were successfully stabilized by scavenging them with nucleophiles and analyzed
using LC-MS/MS. Stable isotope labeling allowed studying the mechanism of
hydrolysis and bond cleavage under square-wave potential pulses. While constant
potential oxidation resulted in the p-quinone as the end product, fast-pulsed
conditions transformed phenacetin to acetaminophen. Taken together Chapters 3
and 4 show that square-wave potential pulses with different cycle times are a novel
way to the high-yield and selective oxidation of drug compounds, as well as in
promoting oxidation reactions that are not possible by constant potential oxidation.

Oxygen insertion by oxo-ferryl radical cations, in the in vivo oxidative drug
metabolism by CYP, is the main mechanism behind aromatic hydroxylation. In
Chapter 5, we present a novel approach based on the electrocatalytic activation of
hydrogen peroxide on a platinum electrode that is thought to generate platinum-
oxo species capable of promoting oxygen insertion reactions. The putative
platinum-oxo species allows the generation of 3-hydroxylidocaine, which cannot be
obtained through direct electrochemical oxidation. We suggest that oxygen
insertion by the putative platinum-oxo species and formation of an arene
intermediate is the reason behind this hydroxylation reaction. The absence of
benzylic hydroxylation excludes the generation of freely diffusing radicals. The
nature of the putative platinum-oxo species on the surface was studied further
using an excess of pyridine as a competitive substrate. Pyridine quenched the
reaction towards 3-hydroxylidocaine rather leading to the oxidation of pyridine.
Catalytic activation of hydrogen peroxide on a platinum electrode surface, hence,
further extends the application of electrochemistry in the mimicry of oxidative drug
metabolism.

In conclusion, whereas direct electrochemical oxidation is merely capable of
imitation of oxidative metabolisms which are initiated by electron tranfer, I extended
the application of electrochemistry for the oxidation of drug compounds by adding
new methods to the electrochemical toolbox. Scheme 1 illustrates all the electrochemical techniques that have been developed throughout this research in the oxidation of lidocaine as a test drug compound.

6.2 Future Perspective

The most promising research areas for continued development of electrochemistry in the mimicry of oxidative drug metabolism are the development of modified electrode surfaces, extending the application of square-wave potential pulses, and further studying the electrocatalytic activation of hydrogen peroxide.

Our study of the modification of electrode surfaces should be continued as it intrinsically represents a better mimicry model for CYP than bare electrodes. Electrochemical cells with modified surfaces that can be coupled to mass spectrometry are of great interest, but several technical problems were encountered which need to be overcome. First, axial coordination was used to anchor metalloporphyrines on the surface through SAM, which may lead to direct reduction of oxidative intermediates by the electrode, rather than oxidation of drug substrates. As a possible solution, square-wave potential pulses can be used to generate the reactive species in one step, and to allow the oxidation reaction to happen in the next step. Naturally, direct reduction of molecular oxygen to hydrogen peroxide on the electrode followed by chemical activation by adjacent metalloporphyrines would be a simpler solution. Chemoselectivity may be obtained by using different metalloporphyrines, with various metallic centers and substituents, based on preliminary results screening several drug substrates.

This thesis has added oxidation by square-wave potential pulses to the toolbox of techniques and of course the oxidation conditions can be further optimized, especially by using different potential wave forms. In the case of electrocatalytic activation of hydrogen peroxide on a platinum electrode, hydrogen peroxide can be generated by electrochemical reduction of molecular oxygen. This can be done through square-wave potential pulses in which a reduction step to reduce molecular oxygen to hydrogen peroxide is followed by the electrocatalytic activation of hydrogen peroxide in the subsequent oxidation step.

In our studies we have focused mainly on lidocaine as a test drug compound to compare different approaches, as well as to overcome issues with the identification of metabolites. A major hindrance in these studies is the requirement for careful identification of oxidation products which are often isomeric. A combination of detailed MS analyses, using ionization, fragmentation (MS^n) and isotope labeling studies, and NMR should be employed for their definitive identification in the common case where no reference compounds are available. Misidentification of the location of a hydroxyl group in a metabolite can otherwise lead to errors in the interpretation of the reaction mechanism.
**Summary and Future Perspective**

**Scheme 1.** Various electrochemical techniques in the oxidation of lidocaine as a test drug compound in the light of oxidative drug metabolism. Electrochemical oxidation of lidocaine at the tertiary amine moiety under direct electrochemical oxidation leading to N-dealkylation through formation of an iminium intermediate (path \( A \)), direct electrochemical oxidation of lidocaine at the aromatic moiety to generate a Wheland-type intermediate which after deprotonation results in 4-hydroxylation (path \( B \)), electrochemical reduction of molecular oxygen to hydrogen peroxide followed by reaction with the tertiary amine moiety of lidocaine to generate a peroxide intermediate, which after decomposition results in N-oxidation (path \( C \)), and electrochemical activation of hydrogen peroxide on a platinum electrode to generate putative platinum-oxo species that react in an oxygen atom insertion manner to generate an arene intermediate that after tautomerization leads to 3-hydroxylation (path \( D \)).

**B: 4-Hydroxylation**

**Peroxide intermediate**

\[
\text{Wheland-type intermediate} \rightarrow \text{Iminium intermediate} \quad \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{Peroxide intermediate} \rightarrow \text{Arene intermediate}
\]

**C: N-Oxidation**

\[
\text{Iminium intermediate} \quad \text{PtO} \rightarrow \text{N-oxidation intermediate}
\]

**D: 3-Hydroxylation**

\[
\text{Arene intermediate} \rightarrow \text{3-hydroxylation intermediate}
\]

**A: N-Dealkylation**

\[
\text{N-dealkylation intermediate}
\]
Electrochemistry in the Mimicry of Oxidative Drug Metabolism

We have already checked the oxidation of various drug compounds under square-wave potential pulses, yet the other methods including electrocatalytically activated hydrogen peroxide can be applied to different substrates. A range of drug compounds with various functional groups and known metabolism by CYP should be tested both to verify the reaction mechanisms and to assess the specificity of the new oxidation techniques. It will remain to be investigated whether extensive optimization of reaction conditions is required for each new compound and desired metabolite, or whether more general guidelines for method development can be outlined.

Microfluidics where parallel channels can be operated separately by electrochemistry is an attractive format for optimization of conditions or simultaneous generation of different metabolites. Electrodes operated under various pulsed conditions or differently modified electrodes with a range of metalloporphyrines can be employed in such a microchannel array.

Finally, next to down-scaling, upscaling of specific reactions to increase the absolute yield should be a major focus for future work. Toxicity testing of potential metabolites of new drug compounds depends on the availability of a sufficient amount of material. The studies described in this thesis have not focused on maximizing product yield, although in several cases, much higher yields were obtained than with previous (direct) electrochemical oxidation techniques. Yields of up to 10% were observed in some cases in a batch cell. Improvements in cell and electrode dimensions and the switch to flow-through instead of batch cells is expected to increase both yield and product purity. The conversion yield in flow-through cells can reach almost 100% but selectivity of product formation will depend on the careful control of reaction conditions. Upscaling of absolute amounts without loss in relative yield is thus a challenge.