Electrochemical and enzymatic synthesis of oxidative drug metabolites for metabolism studies
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Future Perspectives

In this project lidocaine was used as a test drug molecule to study Phase I metabolism reactions by employing newly developed electrochemical methods. Although lidocaine covers a number of important CYP450 catalyzed reactions, it is a relatively simple drug molecule. Therefore, to extend the scope of our EC methods it is important to test a wider range of more complex drug compounds. We have two compounds available, darunavir and felodipine (kindly contributed by Janssen Pharmaceuticals and AstraZeneca, respectively) (Figure 1), which are attractive compounds since their known in vivo metabolites are available as standard reference materials. For most other complex drug compounds metabolites are not available except through custom synthesis. Availability of standards makes the characterization of their metabolites via LC-MS analysis much more straightforward, in particular for isomeric metabolites, such as the various hydroxylation products of lidocaine. Based on the results of Chapter 3 we expect that EC reaction parameters of these compounds can be optimized efficiently in a short time period by employing the DOE approach. We have performed preliminary experiments with darunavir and observed only one aromatic hydroxylation metabolite and the sulfonamide hydrolysis product (which is a postulated metabolite, but was not yet observed in vivo) under optimized EC conditions. The other in vivo metabolites of darunavir could not be obtained within the scope of the DOE study. Previously established EC-methods, including the pulsing method, EC-Fenton and Pt-electrocatalysis with H₂O₂ were not yet employed for these drug molecules. While these methods may provide selective formation of other darunavir metabolites, screening all methods with a range of parameters is time-consuming even with the DOE approach.
We also focused on the development of methods to improve the electrochemical synthesis of the oxidative metabolites of lidocaine in an effort to obtain them at the mg scale. In general, scaling-up of chemical processes has always been an important problem, and in EC synthesis this was particularly difficult to solve. Our initial approach was to use the EC parameters which were previously optimized for lidocaine N-dealkylation in a small batch cell by DOE (chapter 3), and simply translate these parameters to a larger synthesis batch cell. Instead of a small carbon disk electrode a large-surface-area reticulated carbon electrode was used as a working electrode. However, higher absolute yields were not achieved, and we ascribed this to technical problems in the form of a high IR-drop and low mass transport efficiency, which were related to the design of the electrochemical batch cell. In recent years, there has been an increasing interest in flow-chemistry, which is claimed to allow direct scalability of protocols using relatively small reaction cells in a fast manner. Compared to batch reactor cells, continuous flow cells offer some advantages. For example, in a flow cell conversion can be achieved in a single pass without formation of side products due to overoxidation or secondary reactions. Moreover, this technology offers the possibility of monitoring unstable or very reactive compounds on-line. Microfluidic systems offer the use of multiple parallel channels. Cell design and the use of various electrode materials can be efficiently optimized in a microfluidic electrochemical cell, and hence the rate of the electrochemical reactions can be easily controlled. Building on these advantages of flow-chemistry, a flow-through electrochemical cell may be combined with microfluidic technology to produce oxidative drug metabolites in preparative amounts. Continuous-flow electrochemical synthesis cells are commercially available (e.g. Asia Flow Chemistry System (Syrris), Ammonite family of Electrolysis Cell (Cambridge Reactor Design)) but they can also be custom-made in the lab. For example, as can be seen in Figure 2, a commercially available Antec μ-PrepCell can be modified for this purpose. In this cell, a spacer and a Viton O-ring are used to adjust the volume of the cell, and the spacer is modified by addition of microchannels, which may be prepared by laser
cutting. The surface area of the electrode materials in these cells can be further increased by making the material porous. Fabrication of novel nanoporous electrode materials (e.g. gold, platinum, glassy carbon) offers large surface area electrodes which can be used for scaling-up purposes. Combination of these nanoporous electrode materials in continuous-flow electrochemical cells with long channels may allow the production of metabolites with high conversion rates in short reaction times.

Figure 2. Proposed modification for the Antec μ-PrepCell. The spacer can be modified with meandering channels to increase the effective surface area.

In this thesis, enzymatic drug metabolite synthesis in the form of FMO enzyme-mediated conversion of sulfide and nitrogen containing drugs was studied in collaboration with the Department of Biotechnology at the University of Groningen (Prof. Marco Fraaije) as an alternative approach to (electro)chemical metabolite synthesis. Our results showed that each of the tested microbial FMO enzymes is capable of providing very good enantioselectivity for sulfide containing compounds. For preparative, stereospecific production of these drug metabolites, FMO enzymes may be covalently immobilized on electrode materials, such as gold, by linking the flavin cofactor to the electrode surface (Figure 3). In this system, a clean gold electrode surface was initially functionalized with a cysteamine linker. In the second step, the amine-terminated linker can be covalently linked to the FAD cofactor and finally the apo-flavoenzyme can be reconstituted. Modification of the gold surface with the FAD cofactor was monitored by cyclic voltammetry (CV). This FMO-immobilized gold electrode is expected to transfer electrons efficiently between the enzyme, electrode and the substrate to catalyze the oxidation of a variety of soft nucleophile containing drugs.
In conclusion, synthesis of specific metabolites in higher amounts is especially crucial for their further characterization such as toxicity testing and structural analyses by NMR. There is a still room for improvement of the design of electrochemical cells in order to upscale metabolite synthesis. Moreover, the toolbox needs to be extended by developing efficient electrochemical synthesis methods in order to imitate a variety of reactions occurring in biological systems (e.g. CYP450-catalyzed reactions). Therefore, the focus of the EC-MS work should be on gaining a better understanding of EC-driven reactions notably on the electrode surface.

Figure 3. Representation of the covalent immobilization of an Flavin monooxygenase enzyme on the surface of a gold electrode via the FAD cofactor.