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Electrochemical and enzymatic synthesis of oxidative drug metabolites for metabolism studies
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Electrosynthesis methods and approaches for the preparative production of metabolites from parent drugs

Identification of potentially toxic metabolites is important for drug discovery and development. Synthesis of drug metabolites is typically performed by organic synthesis or enzymatic methods, but is not always straightforward. Electrochemical (EC) methods are increasingly used to study drug oxidation and to identify potential metabolites of new drug candidates, but the absolute yield of metabolites of these methods is low. This review discusses the challenges and recent developments of electrochemical synthesis in terms of instrumental aspects, EC reaction parameters and reaction monitoring approaches, in the effort to selectively produce drug metabolites from parent drugs on a preparative scale (1-10 mg).

2.1. Introduction

Drug discovery and development is a high-cost and long-term process. It is therefore important to identify possibly toxic drug metabolites at the early stages of drug development [1–3]. Prior to clinical trials, drug metabolism studies are often performed in experimental models in vivo and in vitro [4, 5]. Animal and human liver microsomes, whole animal models, and isolated enzymes are used to investigate cytochrome P450 (CYP450)-mediated metabolic oxidation reactions [4–7]. However, scaling up of in vivo or in vitro metabolite synthesis to mg levels, which is required for toxicity testing and structural characterization (e.g. by NMR), is not straightforward, and metabolites have to be extensively purified. In addition, these systems have limitations for the isolation of Phase I metabolites, which can have a short half-life, and eventually bind to cellular macromolecules or are further converted to Phase II metabolites [8].

Drug metabolites are generally synthesized via organic chemistry methods. Electrochemical (EC) synthesis methods, utilizing electron transfer processes in an electrochemical cell to oxidize drug compounds in a controlled manner, can offer an alternative for the production of drug metabolites [9, 10]. Compared to organic synthesis methods, electrochemical synthesis has several advantages including a limited number of reaction steps, mild reaction conditions, limited use of organic solvents and hazardous chemicals and the use of fairly simple equipment [11–13]. Electrochemistry can be readily combined with mass spectrometry (EC-MS) for product monitoring facilitating optimization of reaction conditions and is widely used as an analytical technique to study oxidative drug metabolism [14, 15]. However, production of sufficient amounts of drug metabolites (1-10 mg) in a fast and specific manner requires careful adaptation of the existing analytical EC and EC-MS methods.

Conventional electrosynthesis methods have been used for a wide range of synthetic reactions using building blocks with redox-active functional groups. However, there are challenges in the synthesis of drug metabolites by electrochemical modification of the parent drug compound, due to the complex structure of many drug molecules. Moreover, there are technical challenges to the scaling up of electrosynthesis methods, including the geometry and surface area of electrodes, which affect mass transfer and conversion rates. Finally, the chemical properties of electrodes and substrates may lead to adsorption on the electrode surface in particular at the high substrate concentrations required for preparative reactions [1, 3, 16]. Recent technological developments have led to the more intensive application of electrochemistry for the synthesis of drug metabolites or active compounds in the
pharmaceutical industry [17]. For instance, Nematollahi et al. [18] have synthesized phenylpiperazine derivatives, which are biologically active compounds used in various therapeutic areas, in mg amounts using electrochemistry on carbon rod electrodes (Figure 1).

\[
\begin{align*}
\text{H}_3\text{C} & \text{N} \text{H} \text{N} & \text{OH} \quad \text{H}_3\text{C} & \text{O} \\
\text{ } & \text{N} & \text{=O} & \quad -2e^- \quad -2H^+ \\
\text{ } & \text{pH} < 4 & & \\
\end{align*}
\]

Figure 1. Electrochemical synthesis of phenylpiperazine derivatives. Electrochemical oxidation of 4-acetyl-1-(4-hydroxyphenyl)piperazin-1-ium (1) forms a quinone-imine derivative (2) which allows a coupling reaction in the presence of arylsulfonic acid derivatives (3) to form phenypiperazine derivatives (4) [18].

The metabolite electrosynthesis approaches described in this review typically use the parent drug as the EC substrate, although analogues or prodrugs can also be considered in cases where they provide easier starting points for EC synthesis. For instance, the analgesic drug phenacetin is initially metabolized by O-dealkylation to paracetamol (APAP, also known as acetaminophen) and consequently to N-acetyl-p-benzoquinone imine (NAPQI) by dehydrogenation (Figure 2). The electrochemical synthesis of NAPQI is considerably more straightforward starting with APAP rather than the parent drug [19]. In addition, the reactive NAPQI metabolite is rapidly conjugated in vivo with glutathione during Phase II metabolism. The absence of Phase II enzymes and reactive compounds such as glutathione makes EC methods more suitable for synthesis of Phase I metabolites, notably reactive intermediates [20].

Enzyme catalyzed reactions are often stereospecific, whereas many chemical and electrochemical reactions produce racemic mixtures. During the electron transfer process, addition or removal of an electron from the electrode surface to a substrate can result in an inversion of polarity of a functional group (known as umpoling),[11] which can provide stereoselectivity in electrochemical reactions for some substrates.

Electrochemical generation of oxidation products almost always involves multiple steps: the initial electrochemical oxidation produces a reactive (radical) intermediate which proceeds to react intramolecularly or, more commonly, intermolecularly with solvent molecules. This Electrochemistry – Chemistry (E-C) process may be followed by additional reactions, including electrochemical reactions, leading to E-C-E, and E-C-E-C processes, and so on. The solvent and electrolyte compositions and their relative concentrations therefore play a crucial role in selectivity, as illustrated
for NAPQI in Figure 2 [21,22]. Depending on solvent pH, NAPQI readily reacts with water or its parent drug paracetamol, while at basic pH an E-C-E-C process leads to the doubly hydroxylated product.

Figure 2. Metabolic pathway of phenacetin to NAPQI. The NAPQI metabolite of phenacetin reacts at neutral pH to form a dimer. Hydroxylation occurs at basic pH and the amide of NAPQI is hydrolysed to the p-quinone at acidic pH. *In vivo* NAPQI forms a Phase II metabolite in the presence of glutathione (GSH) [66,71].

Characterization and purification of metabolites of interest is a crucial step prior to toxicity testing. Since electrochemical reactions are rarely selective and often not complete, preparative liquid chromatography (LC) is required as the final step for metabolite purification. Chiral LC separation may be employed to purify the metabolite stereoisomers of interest.

This review will focus on the challenges and new developments in EC synthesis of drug metabolites from parent drugs on a preparative scale (1-10 mg) in terms of electrosynthesis methods, instrumental aspects and EC reaction parameters.

### 2.2. Electrochemical Techniques Used in Drug Metabolism

A range of oxidation reactions is mediated by CYP450 enzymes in living organisms, and the products of most of these reactions can be made with EC synthesis methods, albeit through different oxidation reaction pathways (Table 1) [2,15,23]. Applications have mainly focused on direct electrochemical methods on non-modified, mostly carbon electrodes, due to their simplicity [1,2,24].
The scope of electrochemical metabolite synthesis can be expanded using indirect EC methods involving electrochemically generated reactive oxygen species or the EC-assisted Fenton reaction. However, indirect EC methods are often less selective due to the generation of very reactive radical species, such as the hydroxyl radical [15,25,26].

Table 1. Drug molecules used in EC-MS studies, classified by reaction type and electrode material.

<table>
<thead>
<tr>
<th>Reaction type</th>
<th>Glassy carbon</th>
<th>Gold</th>
<th>Platinum</th>
<th>Boron-doped diamond</th>
</tr>
</thead>
</table>
Chemical modification of electrode surfaces with metalloporphyrins and immobilization of enzymes on electrode materials are other promising approaches to produce oxidative drug metabolites with higher selectivity and yield [9,27–29].

Cyclic voltammetry (CV) is often employed as an analytical EC technique to study the redox activity of drug compounds [9,30]. CV and linear sweep voltammetry allow to study the effect of potential on oxidation or reduction reactions and slow linear sweep voltammetry can be performed on-line with MS detection of products [30,31]. These analytical EC methods are therefore useful to determine the optimal potential for production of the metabolites of choice.

In addition to the application of constant potentials, pulsed potentials, switching alternately between positive and negative voltages, have been used to tune the selectivity of drug metabolite synthesis [30,32]. For example, it has been reported that the N-dealkylation product of lidocaine is obtained at short cycle times (below 0.2 s) whereas 4-hydroxylidocaine was the main product at cycle times of 1 s or more, presumably due to different reaction kinetics or the stability of reactive intermediates [33]. Another important benefit of pulsed potentials is that they may prevent adsorption to the electrode surface, reducing the need for regular cleaning of the electrode in order to obtain consistent yields [30,33].

In general, electrochemical reactions are performed either at controlled potential (potentiostatic) or at controlled current (galvanostatic). Most drug metabolism studies have been performed using a potentiostat where the potential of the working electrode is held constant with respect to a reference electrode. In addition, the current flow between working and counter electrode is allowed to vary in order to keep the potential difference between working and the reference electrode constant [9,12,24,26,34–38]. In a constant current experiment, in which a galvanostat is used, a controlled current is applied between the counter and working electrode and the potential of the working electrode is varied depending on the changes in the double layer and solvent resistance [12,35]. Selectivity of the reaction is rather low since the potential is not controlled and will increase over time when the initial substrate is consumed [12]. Nevertheless, Roth et. al [16] successfully used a continuous-flow electrosynthesis cell to synthesize oxidation products of various drug molecules, including diclofenac, tolbutamide, primidone, albendazole and chlorpromazine at the mg-scale at constant current. A broad range of oxidation reactions namely aliphatic and aromatic hydroxylation, S- and N-oxidation and dehydrogenation were achieved. The sulfoxide product of chlorpromazine was selectively obtained in high yield (83 % of isolated product yield), while for albendazole the sulfone
was also observed in addition to the sulfoxide. Therefore, this technique is of particular interest for drugs for which no competitive reactions are possible, i.e. drugs with a single oxidizable group.

2.3. Design of Electrochemical Cells for Drug Metabolite Synthesis

There are a number of general challenges of electrochemical cell design which have to be addressed to enable the synthesis of drug metabolites in amounts higher than the μg range [39]. The cell geometry has a significant effect on metabolite synthesis not only in terms of yield, but also of selectivity [39,40]. A commonly observed problem relates to the IR-drop, caused by uncompensated resistance between working and counter electrodes which is in turn caused by low solution conductivity and a large distance between the electrodes [9,40,41]. In order to reduce the IR-drop (ohmic drop), supporting electrolytes can be added to increase the conductivity of the solution, but the choice of appropriate electrolytes is often limited, since a change in the chemical composition of the solution may greatly affect the reaction pathways, in particular in E-C and E-C-E reactions [39]. Moreover, the working, counter and reference electrodes have to be in close proximity to minimize the IR-drop. However, this reduces the effective cell volume which in turn limits product yield [39].

2.3.1. Electrode Materials

Widely-used electrode materials in the synthesis of drug metabolites are metal electrodes, primarily gold, and platinum, and the carbon-based electrodes glassy carbon (GC), and boron-doped diamond (BDD) [7,42]. Each of these electrodes offers unique properties in terms of chemical and physical stability, potential window and adsorption properties, as well as availability in forms with a large surface area (e.g. porous or mesh electrodes) [40]. The advantages and disadvantages of various working electrodes for EC synthesis are listed in Table 2. Pt and carbon electrodes are generally preferred for oxidation of organic compounds due to their high stability even at elevated positive potentials. In particular, GC electrodes have been extensively used in reactions such as the dealkylation of amines and ethers, heteroatom (N-, S-, P-) oxidations, aromatic hydroxylations, alcohol oxidations and dehydrogenations [1]. Table 1 classifies oxidation reactions of drug compounds reported in the literature according to the electrode type. It should be noted that absolute product yields were not reported in any of these studies.

Adsorption or fouling is a significant problem for most electrode materials [35,40]. The popular GC electrodes unfortunately strongly adsorb hydrophobic organic molecules [3,35]. Flat-surface working electrodes can be polished manually, but for metabolite synthesis applications, rapid fouling
at high substrate concentrations can severely limit the yield [39,40]. In addition, porous, mesh and reticulated electrodes, which are attractive for synthesis purposes due to their high surface area, are difficult to access and cannot be mechanically polished. Cell blockage or inactivation may be observed [43]. Various acidic solutions or organic solvents as well as potential cycling are used to clean these electrodes, but reproducibility problems are often observed even after thorough flushing [33,43]. Inexpensive, single-use electrodes are a possible solution to the irreversible fouling problem.

Table 2. Advantages and disadvantages of working electrode materials that have been used in the production of drug metabolites (adapted from Analytical Sciences Digital Library (ASDL), Analytical Electrochemistry: The Basic Concepts).

<table>
<thead>
<tr>
<th>Working electrode material</th>
<th>Advantages</th>
<th>Drawbacks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassy carbon</td>
<td>- Cheap</td>
<td>- Physicochemical form not well defined</td>
</tr>
<tr>
<td></td>
<td>- Large potential window</td>
<td>- Brittle, hard to shape</td>
</tr>
<tr>
<td></td>
<td>- Many types available (rod, flat plate, paste, reticulated)</td>
<td>- High adsorption</td>
</tr>
<tr>
<td>Gold</td>
<td>- Easy to shape into many types</td>
<td>- Expensive</td>
</tr>
<tr>
<td></td>
<td>- Large potential window</td>
<td>- Anodic window is limited by surface oxidation</td>
</tr>
<tr>
<td></td>
<td>- Easy to modify with enzymes through thiol groups</td>
<td>- Adsorption of compounds with thiol groups</td>
</tr>
<tr>
<td>Platinum</td>
<td>- Many types available (wire, mesh, flat plate)</td>
<td>- Expensive</td>
</tr>
<tr>
<td></td>
<td>- Low adsorption</td>
<td>- Limited potential window due to low hydrogen overpotential</td>
</tr>
<tr>
<td>Boron-doped diamond</td>
<td>- Large potential window</td>
<td>- Expensive</td>
</tr>
<tr>
<td></td>
<td>- Low adsorption</td>
<td>- Difficult to make (reproducibility of doping)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Cannot be polished physically</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Fragile, hard to shape</td>
</tr>
</tbody>
</table>

Physicochemical properties of electrodes can also be employed for indirect electrochemical oxidation reactions: Pt is known to activate H₂O₂ leading to electrooxidative formation of molecular oxygen [44]. Activated H₂O₂ on a Pt electrode in the presence of drug substrate has been shown to produce aromatic hydroxylation metabolites, presumably through platinum-oxo intermediates. This reaction does not occur on GC under the same conditions [45].
The surface area of the electrode material clearly has a major impact on conversion yield and the reaction rate. Commercially available electrode materials having large surface areas (in the cm\(^2\) range) include mesh platinum electrodes and reticulated GC electrodes that are used in batch synthesis cells, and porous GC electrodes used in flow-through cells (see next section) [9].

2.3.2. Electrochemical Cell Types

2.3.2.1. Thin-layer Flow Cells

Thin-layer flow cells are typically used for amperometric measurements but in general they have been also used to study drug metabolism [7,36,46,47]. Thin-layer cells have planar working and counter electrode surfaces, separated by a thin spacer (typically 10-100 μm) [42]. A schematic representation is shown in Figure 3a. Thin-layer cells allow a wide choice of working electrode materials, including Pt, Au, Ag, Cu, GC and BDD which are relevant for drug oxidation, and the working electrode is easily removed for cleaning or exchange [7,42,48].

![Schematic representation of a (a) thin-layer cell, (b) porous flow-through cell and (c) batch electrochemical cell.](image)

The major limitation of thin layer cells for synthetic purposes is their low surface area and cell volume [3]. The effective surface area of working electrodes commonly used in thin-layer cells is in the mm\(^2\) range. Due to the low surface area, low flow rates (<100 μL/min) have to be used in order to obtain conversion yields of 95% or higher [48,49].

2.3.2.2. Porous Flow-through Cells

Electrochemical synthesis of oxidative drug metabolites has thus far mostly been performed using commercially available coulometric flow-through cells with a porous GC working electrode
(Figure 3b) [15,20,50–52]. A significant advantage over thin-layer cells is the high conversion rate, which for some compounds can reach 100% even at flow rates of 0.5 mL/min due to the large surface area (estimated to be in the cm² range) of a ~5 mm³ porous GC electrode [3,38,50].

Flow-through cells provide a continuous flow of fresh substrate which limits over-oxidation at the electrode surface, although this cannot be prevented completely. It has, for example, been shown that during the oxidation of rotigotine the phenol functional group is easily oxidized to the corresponding catechol or β-hydroquinone derivative, but that these derivatives are immediately oxidized further to the quinone [6]. Disadvantages of flow-through cells are their limited scalability and the limited control over dissolved gas parameters, which can only be adjusted prior to infusion into the cell. These drawbacks limit the suitability of flow-through cells for large-scale metabolite synthesis (see also Table 3).

Table 3. Comparison of parameters applicable to various cell types required for production of about 1 mg of metabolite product from 100 μM substrate concentration. Calculations were done for a metabolite of diclofenac (5-hydroxydiclofenac) using the most favorable EC conditions based on the literature [36,75].

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimated cell volume</th>
<th>Estimated surface area</th>
<th>Flow rate</th>
<th>Oxidation time</th>
<th>Conversion yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Porous flow-through (GC)</td>
<td>0.2 mL</td>
<td>5 cm²</td>
<td>500 μL/min</td>
<td>80 min</td>
<td>~90%</td>
</tr>
<tr>
<td>Thin-layer (BDD)</td>
<td>0.5 μL</td>
<td>10 mm²</td>
<td>10 μL/min</td>
<td>66.6 h</td>
<td>~90%</td>
</tr>
<tr>
<td>Batch (GC)</td>
<td>75 mL</td>
<td>10 cm²</td>
<td>Not applicable</td>
<td>60 min</td>
<td>~50%</td>
</tr>
</tbody>
</table>

2.3.2.3. Batch Cell Reactors

A batch cell reactor is typically constructed as a conventional three-electrode electrochemical cell (Figure 3c). Since the cell volume, the electrode size and the surface area can be readily increased, batch cells are better suited for scaling metabolite synthesis up [4,53]. Another important advantage of batch cells over flow-through cells is that solvent and gas parameters can be controlled more easily. Batch cells share the advantage with thin-layer cells of a wide choice of working electrode materials in the form of flat-surfaced electrodes that can be easily polished [7,9]. Large, spongelike electrodes like Pt mesh or reticulated carbon electrodes (RGC) with surface areas of 10 cm² or more are also available, but cannot be polished and cleaned due to their fragile and/or inaccessible surface structures. Alternatively, nanostructured electrode materials employing carbon nanotubes, metal nanoparticles
and nanowires can be used for synthesis purposes due to their significantly larger surface area. The surface area of a planar electrode can be increased up to 1000 times by rendering it nanoporous [54].

In a batch cell configuration, working and counter electrodes can be separated from each other, most commonly by a porous Vycor glass frit [9,26]. This porous frit enables the transfer of ions and small organic molecules, but blocks the extensive mixing of solutions between the working and counter electrode compartments. Two-compartment cells therefore provide additional selectivity, by preventing (1) mixing of oxidation and reduction products of the parent drug, (2) back-reduction of drug oxidation products at the counter electrode, and (3) unwanted reaction with reactive (oxygen) species generated by reduction of oxygen at the counter electrode [9,55]. For example, for lidocaine it has been reported that under conditions where the N-dealkylation product is formed at the working electrode, the N-oxide is formed at the counter electrode [26]. Although batch cells provide relatively high absolute yields compared to flow-through cells, undesirable over-oxidation products due to multiple oxidation steps may occur (e.g. due to E-C-E processes). For example, sulfide-containing drug molecules (e.g. albendazole) are primarily oxidized to the sulfoxide but this can be further oxidized to the sulfone at longer reaction times, if the potential is sufficiently high [23]. In order to prevent over-oxidation of sulfoxides in a batch cell, EC parameters such as the potential can be adjusted and the reaction can be stopped in time by monitoring the products via MS by sampling either on-line or off-line as shown in Figure 4.

Oxidative drug metabolism experiments can be performed with either on-line or off-line EC-(LC)-MS set-ups [4,34,35,39,56,57]. Figure 4 shows both configurations with the possibility of collecting metabolite products. In an on-line EC-MS system, a compromise between EC and MS conditions is required, in particular with respect to electrolytes. Electrospray ionization is very sensitive to the presence of non-volatile salts, high-proton affinity modifiers such as amines, and solvent composition, all of which may greatly suppress the signal of the metabolites of interest. In contrast, in an off-line EC-(LC)-MS system, where a batch cell is used, [4] sample conditions can be readily adjusted prior to (LC)-MS [37]. On-line sampling is also possible in a batch cell which is depicted in Figure 4a, but solution compatibility with ESI-MS becomes an issue again [58].
2.4. Electrochemical Reaction Parameters

The important electrochemical reaction parameters for metabolite synthesis are solvent type, dissolved gases, pH, electrolyte type and concentration, substrate concentration, and oxidation potential \([40,49,59,60]\). Since these parameters are often dependent on each other directly or indirectly, careful selection and tuning is crucial to synthesize oxidative metabolites at high selectivity and amounts.

2.4.1. Solvents

The solvent composition has several important effects: first of all, both the parent drug and its metabolites should remain in solution. Moreover, the nature of the solvent (e.g. protic or aprotic) can
affect the stability of reactive intermediates and oxidation products with a profound effect on selectivity and yield [61]. For example, molecular oxygen can be reduced in aprotic solvents to form superoxide anions which are stable in the absence of proton donors and subsequently lead to oxidation reactions of drug substrates [26]. Solvents used in electrochemical reactions should have a higher oxidation potential than the substrate, because reactions must proceed below the oxidation potential of the solvent [60,61]. Acetonitrile, methanol or ethanol and their aqueous mixtures are often used in EC-MS studies [1,15]. Among these solvents, acetonitrile is one of the most widely used solvents in electrochemical oxidation reactions [62]. It is a polar aprotic solvent with a high dielectric constant (ε=37), which provides a reasonable conductivity for salt solutions [62]. Moreover, acetonitrile may act as an inert solvent for many electrode materials which makes it suitable for electrochemical reactions.

2.4.2. Dissolved Gases

Dissolved gases, in particular oxygen, can affect selectivity and the yield of electrochemical reactions. In general, dissolved molecular oxygen is unreactive due to its triplet electronic structure. However, as discussed earlier, electrochemical reduction of molecular oxygen at the counter electrode generates hydrogen peroxide and reactive oxygen species such as superoxide anions and hydroxyl radicals which can oxidize various organic compounds [63]. It has been reported that electrochemical reduction of molecular oxygen was directly involved in the formation of the N-oxide metabolite of lidocaine [33]. In order to prevent generation of reactive oxygen species from molecular oxygen, EC cells or cell compartments are purged with inert gases such as nitrogen or argon (see also Figure 4).

2.4.3. Solution pH

Most electrochemical oxidation reactions are pH-dependent [8,15,23,37]. There are many literature examples of specific drug metabolites generated under either acidic, basic or neutral conditions. For example, aromatic hydroxylation, S-oxidation, dehydrogenation and O-dealkylation occur at acidic and neutral pH (pH 3-7), whereas benzylic hydroxylation, N-dealkylation and N-oxidation take place at pH 7-10 [1,2,7,8,20,64]. Selective production of a metabolite of interest from a parent drug with multiple reactive groups can therefore be induced by selecting the appropriate solution pH. When selecting solvent pH, it should be noted that the commonly used Pd/H₂ reference electrode shows a pH dependent shift of its oxidation potential [65].
The solution pH can also have an effect on the stability of intermediates. Madsen et al. [19,66] reported that the stability of reactive intermediates such as NAPQI (Figure 2) shows a strong pH dependence: EC oxidation of paracetamol in aqueous solutions does not generate NAPQI, but leads to a dimerization reaction between pH 5 to 7, a hydroxylation reaction at alkaline pH, and oxidation to the \( p \)-quinone under strongly acidic conditions. Moreover, Mali’n and co-workers [67] indicated that pH plays an important role in the further reaction of reactive intermediates with trapping agents (e.g. KCN) to form the corresponding cyanated products.

### 2.4.4. Supporting Electrolyte

Electrolytes are used to increase conductivity and minimize the IR-drop, and are preferably inert compounds which are not reduced or oxidized under the experimental conditions [9]. In aqueous solutions, a variety of acids, bases or salts (e.g. acetic acid, ammonia, and ammonium acetate) are used as supporting electrolytes in EC-MS applications, partly due to their compatibility with MS analysis, whereas in organic solvents salts such as tetraalkylammonium perchlorate and lithium triflate are commonly used [35,60,62]. Although high organic-soluble salt concentrations (ranging from 1 mM to 1 M) are preferred, these electrolytes are not suitable for on-line EC-ESI-MS [23,62]. Moreover, it has been reported that quaternary ammonium salts can be oxidized under EC conditions to form by-products [68].

### 2.4.5. Substrate Concentration

Elevated substrate concentrations are desired in electrochemical synthesis in order to obtain high amounts of products in a short time period. However, increased substrate concentrations will likely decrease conversion rates in electrochemical cells with limited electrode surface areas. For example, in syntheses using direct electrochemical methods, the maximum rate of chemical change per unit area of electrode surface is directly proportional to the substrate concentration in solution [69].

To illustrate this, the amount of metabolite product that is formed per time unit in different types of electrochemical cells is shown in Table 3. Using the most favorable EC-parameters found in the literature, we calculated the time required to synthesize approximately 1 mg of the hydroxyl metabolite of diclofenac in a typical thin-layer flow-through cell, a porous flow-through cell and a batch cell, with different surface areas.

Electrochemical reactions are heterogeneous electron transfer processes which take place at the electrode surface. Mass transport, the migration of oxidized or reduced species from the electrode
surface into solution, determines the rate of the reaction [35,40,69]. There are various modes of mass transport namely migration induced by a potential difference, diffusion due to a concentration gradient and convection (hydrodynamic transport, e.g. due to stirring or pumping) [13]. High substrate concentrations can more easily result in adsorption on the electrode surface. Dimerization of metabolites or other intermolecular reactions between parent drugs and oxidized intermediates are of significant concern at high substrate concentrations; for instance, drugs with phenol groups readily form oligomers, which tend to adsorb at the electrode surface [70]. Therefore, in most of the electrochemical cell setups used for drug oxidation reported to date, relatively low starting material concentrations (ranging from 5 to 100 μM) are used, which severely limits the absolute amount of metabolites synthesized in practice.

2.5. Conclusions and Future Trends

Existing analytical electrochemical techniques are adequate to study drug metabolites on a small scale, but in order to employ these EC techniques for the large scale synthesis of selected drug metabolites, many aspects of the electrosynthesis methods and cells need to be considered. The most obvious approach is to increase the size of the electrochemical cell and the electrodes, but in practice many other related parameters do not scale well, often leading to disappointing metabolite product yields, both in relative and absolute terms.

Commercially available electrochemical cells share some technical drawbacks when scaled up, such as a high IR-drop, low mass transport and low conversion yields due to cell geometry, which must be addressed in future cell designs for better scalability. In addition, the fabrication of physically and chemically stable electrodes with low adsorption properties and an effective surface area in the order of 100 cm² that can be used in a reasonable cell volume of about 100 mL is required for metabolite synthesis. Alternative options are large-surface area porous or nanoporous electrode materials which are cheap enough to be used as disposables. Finally, optimization of EC conditions is a multi-parameter problem which can benefit from using dedicated statistical optimization approaches (e.g. through a design-of-experiment approach) in order to maximize selectivity and yield. We conclude that electrosynthesis is a promising alternative tool to generate drug metabolites, even though for most metabolites it needs to be improved by at least an order of magnitude in absolute yield to reach mg amounts for follow-up studies.
2.6. References


