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Optimization of reaction parameters for the electrochemical oxidation of lidocaine with a Design of Experiments approach

Turan Gul [a], Rainer Bischoff [a], Hjalmar P. Permentier [a,b,*]

[a] Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands
[b] Interfaculty Mass Spectrometry Center, University of Groningen, Groningen, The Netherlands

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ABSTRACT

Identification of potentially toxic oxidative drug metabolites is a crucial step in the development of new drugs. Electrochemical methods are useful to study oxidative drug metabolism, but are not widely used to synthesize metabolites for follow-up studies. Careful optimization of reaction parameters is important for scaling up the electrochemical synthesis of metabolites. In the present study, lidocaine was used as a drug compound in order to optimize electrochemical reaction parameters employing a design of experiments approach to improve the yield of N-dealkylated lidocaine, a major *in vivo* metabolite. pH and electrode material were found to have a major effect on the final yield.

1. Introduction

The development of new drugs is a costly and time-consuming process. The detection and evaluation of possibly toxic oxidative drug metabolites at the early stages of drug discovery and development is therefore quite important [1–3]. Drug metabolism is initially studied in *in vivo* and *in vitro* experimental models, for example in human or animal liver microsomes [4,5]. However, these methods are not adequate to produce metabolites in sufficiently large quantities for follow-up studies [6]. In order to characterize the structure and study the toxicology of metabolites, it is necessary to have quantities in excess of 1 mg [7,8]. While organic synthesis is the standard approach, an emerging technique to produce oxidative metabolites is electrochemistry (EC), which can be combined with mass spectrometry (EC-MS) for product monitoring to optimize the conditions of metabolite synthesis [9,10].

The local anesthetic lidocaine (Fig. 1) has been used as a model compound for the study of oxidative metabolism by electrochemistry. The *in vivo* reactions, catalyzed by enzymes of the cytochrome P450 family, are N-dealkylation and N-oxidation of the tertiary amine, aromatic hydroxylation at the 3 and 4 positions and benzylic hydroxylation (Fig. 1) [11–14]. Previously, our group reproduced most of these reactions by various electrochemical approaches. Jurva et al. [1] showed that direct electrochemical oxidation results in N-dealkylation of lidocaine. Nouri-Nigjeh et al. [15] showed further that indirect oxidation of lidocaine by electrochemically generated reactive oxygen species leads to N-oxide formation. Moreover, aromatic hydroxylations were observed at high potentials (3 V or more) in acetonitrile/water (99:1) containing 0.1 M tetrabutylammonium perchlorate as background electrolyte [16]. In addition, Nouri-Nigjeh et al. [16] showed that using square-wave potential pulses with long cycle times of around 1 s led to formation of the 4-hydroxylation product, but that short cycle times of 10 ms or less resulted in N-dealkylation. Although various electrochemical methods have been shown to produce different lidocaine metabolites, they were not optimized with respect to yield and reproducibility. Optimizing electrochemical synthetic methods is a multi-parameter problem with several interconnected factors affecting selectivity and yield. Electrochemical reaction parameters include substrate concentration, solvent, supporting electrolyte, pH, temperature, oxidation potential, potential cycle times, electrode material, flow rate (for flow-through cells) or reaction time (for batch cells) and cell dimensions. A better understanding of the effects of the reaction conditions is thus needed prior to scaling the synthesis up.

* Corresponding author at: Hjalmar Permentier, Interfaculty Mass Spectrometry Center, University of Groningen, Antonius Deusinglaan 1, 9713 AV Groningen, The Netherlands. Tel.: +31 50 363 3262; fax: +31 50 363 7582
E-mail address: h.p.permentier@rug.nl (H.P. Permentier).

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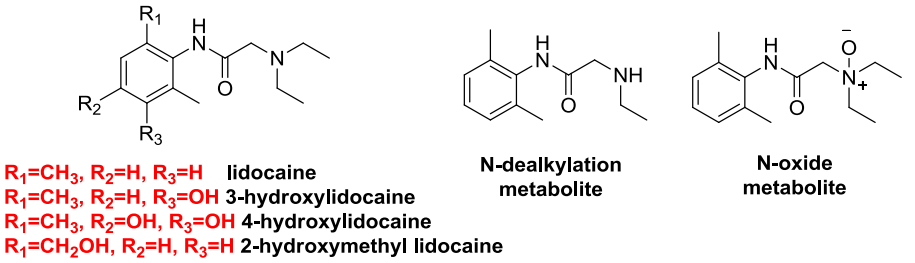

Fig. 1. Lidocaine and its major metabolites.

Optimization of EC parameters has thus far mostly been done using on-line EC-MS with commercially available flow-through cells [5,10,17–26]. In these systems, drug molecules are prepared in solvents of different compositions at different pH values, and these samples are pumped through a flow-through cell at various flow rates. A potential ramp may be applied over a desired range, and the electroactive species monitored by mass spectrometry and identified from the recorded mass voltammograms. While convenient with respect to selecting the optimal potential, this approach is time-consuming when it comes to parameters such as solvent, pH and electrode material and notably for assessing interdependent parameters. In addition, on-line EC-MS limits the range of parameters, such as solvent, pH and electrolytes, since a compromise between MS and EC conditions must be found. The above-mentioned problems can be avoided by working off-line so that EC parameters can be optimized independent of the MS conditions [15,27–32].

In the present study, we used a multi-parametric optimization approach to increase yield and selectivity of electrochemical drug metabolite synthesis based on a Design of Experiments (DOE) strategy. A set of experiments was planned to obtain information about inter-parameter correlations and interactions from a limited number of experiments [33]. Lidocaine was used as model compound focusing on optimizing the yield of the N-dealkylation product. Our data show that reaction parameters such as pH and electrode material have a major effect on the final yield.

Table 1
Experimental plan for the first round of optimizations of the lidocaine N-dealkylation reaction conditions. The plan includes three replicates (Exp 21–23). The D-optimal experimental design with a minimal number of experiments implies that only the extreme values of each parameter range were investigated.

Exp Name	Run order	pH	c (μmol dm^{-3})	Electrodes	Potential (E)
Exp 1	23	3	10	Carbon	0.75
Exp 2	10	3	1000	Carbon	0.75
Exp 3	15	12	1000	Carbon	0.75
Exp 4	12	3	10	Pt	0.75
Exp 5	5	12	10	Pt	0.75
Exp 6	11	3	1000	Pt	0.75
Exp 7	9	12	1000	Pt	0.75
Exp 8	14	3	10	Au	0.75
Exp 9	1	12	10	Au	0.75
Exp 10	16	3	1000	Au	0.75
Exp 11	19	3	10	Carbon	1.50
Exp 12	13	12	10	Carbon	1.50
Exp 13	6	3	1000	Carbon	1.50
Exp 14	7	3	10	Pt	1.50
Exp 15	21	12	10	Pt	1.50
Exp 16	3	3	1000	Pt	1.50
Exp 17	17	12	1000	Pt	1.50
Exp 18	8	3	10	Au	1.50
Exp 19	18	3	1000	Au	1.50
Exp 20	2	12	1000	Au	1.50
Exp 21	4	12	1000	Au	1.50
Exp 22	20	12	1000	Au	1.50
Exp 23	22	12	1000	Au	1.50

2. Experimental Procedures

2.1. Reagents

Lidocaine (L7757), monoethylglycinexylidide (MEGX, SML0087, the N-dealkylated form of lidocaine) and ammonium hydroxide (NH_4OH, 221228) were purchased from Sigma-Aldrich. Formic acid (HCOOH, 94318) and acetaminophen (00370) were purchased from Fluka and ultra-pure HPLC grade acetonitrile (ACN, 01203502) was purchased from Biosolve. Trifluoroacetic acid (TFA, 289084) was purchased from Thermo Fisher, and sulfuric acid (H_2SO_4, 100731100) from Merck Millipore. Ultrapure water was obtained from a Milli-Q Advantage A10 Water Purification system (Millipore Corp., Billerica, MA, USA).

2.2. Electrochemical measurements

All electrochemical measurements were performed with an Antec ROXY potentiostat (Antec Leyden, Zoeterwoude, The Netherlands) controlled by the Antec Dialogue software. Electrochemical reactions were performed in a one-compartment three-electrode cell in which the working electrodes were gold (MF-2014, 1.6 mm diameter, Bioanalytical System (BASi), West Lafayette, IN, USA), platinum (MF-2013, 1.6 mm diameter, BASi) or glassy carbon (GC) (MF-2012, 3.0 mm diameter, BASi) disk electrodes and the auxiliary electrode a platinum wire (MW-4130, BASi). Potentials were measured against a silver wire pseudo-reference electrode (MF-2017, BASi) to avoid possible chloride contamination from the traditional Ag/AgCl reference electrode during electrochemical oxidations. All electrochemical experiments were performed at ambient temperature, and for deaeration argon gas was bubbled at 20 mL/min via a sparge tube (MW-4145, BASi). Working electrodes

Table 2
Experimental plan of the second round of optimizations. The experiments were performed in duplicate and cover the full set of parameter combinations (pH 8, 10, 12, and potential 0.75, 1.00, 1.25 and 1.50 V) (including 3 replicates, Exp 13–15). 10 μM lidocaine concentration and glassy carbon electrode were used for all experiments.

Exp Name	Run Order	pH	Potential (E)
Exp 1	13	8	0.75
Exp 2	12	10	0.75
Exp 3	15	12	0.75
Exp 4	9	8	1.00
Exp 5	6	10	1.00
Exp 6	10	12	1.00
Exp 7	2	8	1.25
Exp 8	5	10	1.25
Exp 9	14	12	1.25
Exp 10	1	8	1.50
Exp 11	3	10	1.50
Exp 12	4	12	1.50
Exp 13	8	10	1.00
Exp 14	7	10	1.00
Exp 15	11	10	1.00