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Mechanism of aromatic hydroxylation of lidocaine at a Pt electrode under acidic conditions

Turan Gül¹, Rainer Bischoff¹, Hjalmar P. Permentier^{1,2*}

¹Analytical Biochemistry, Groningen Research Institute of Pharmacy, University of Groningen, Groningen, The Netherlands

²Interfaculty Mass Spectrometry Center, University of Groningen, Groningen, The Netherlands

*Corresponding author: 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: h.p.permentier@rug.nl

Abstract

Aromatic hydroxylation reactions, which are mainly catalyzed by cytochrome P450 (CYP) enzymes *in vivo*, are some of the most important reactions of Phase I metabolism, because insertion of a hydroxyl group into a lipophilic drug compound increases its hydrophilicity and prepares it for subsequent Phase II metabolic conjugation reactions as a prerequisite to excretion. Aromatic hydroxylation metabolites of pharmaceuticals may be obtained through various synthetic and enzymatic methods. Electrochemical oxidation is an alternative with advantages in terms of mild reaction conditions and less hazardous chemicals. In the present study, we report that aromatic hydroxylation metabolites of lidocaine can be readily obtained electrochemically under aqueous acidic conditions at platinum electrodes. Our results show that the dominant N-dealkylation reaction can be suppressed by decreasing the solution pH below 0.5 resulting in selective 3-hydroxylidocaine, which is an *in vivo* metabolite of lidocaine. Experiments in ^{18}O labelled water indicated that water is the primary source of oxygen, while dissolved molecular oxygen contributes to a minor extent to the hydroxylation reaction.

Key words

- Drug metabolism
- Aromatic hydroxylation
- Pt-oxide layer
- Electrochemical synthesis
- ^{18}O labeled water

1. Introduction

Aromatic hydroxylation reactions of pharmaceuticals have been extensively studied due to their importance in the biotransformation of drugs and in wastewater treatment for the removal of drug residues [1–3]. *In vivo*, the hydroxylation reaction is mainly catalyzed by cytochrome P450 (CYP) enzymes in the liver, where it is an important reaction of Phase I metabolism, resulting in the bioactivation or detoxification of drug molecules [4–6]. Insertion of a hydroxyl group into a lipophilic drug compound increases solubility so that drugs can be more easily excreted, either directly or after conjugation with more polar moieties, such as sulfates or glucuronides [7].

In order to study hydroxylated drug metabolites and assess their toxicity during the drug development process, hydroxylation of drug substrates has been performed by a wide variety of enzymatic, organic and electrochemical synthesis methods. In the enzymatic conversions purified or enriched CYP enzymes are used to insert an oxygen atom into the C-H bond of an aromatic substrate [8–10]. In organic synthesis methods oxidizing reagents such as peroxides or peroxyacids and metal catalysts are employed to generate hydroxyl radicals (Fenton and Haber-Weiss reactions) which readily react with aromatic substrates to form hydroxylation products [2,11–14]. Compared to enzymatic reactions, these chemical methods often require harsh reaction conditions, have low regioselectivity and produce undesired byproducts [8,15].

Electrochemical (EC) oxidation methods have been used as alternatives to obtain aromatic hydroxylation metabolites of drug compounds by employing electrogenerated reactive oxygen species [16–18]. For example, the EC-assisted Fenton reaction, which produces hydroxyl radicals from hydrogen peroxide in solution, has been used for the hydroxylation of various drug substances including metoprolol, buspirone, promazine and 7-ethoxycoumarin [19,20]. We have extensively studied alternative electrochemical methods for the synthesis of specific oxidation products of the local anesthetic drug lidocaine. Lidocaine is oxidized *in vivo* to the aromatic hydroxylation products 3- and 4-hydroxylidocaine, and can also undergo benzylic hydroxylation, N-dealkylation and N-oxidation reactions [21,22]. By adjusting the electrochemical parameters, a certain degree of product selectivity can be achieved. Direct electrochemical oxidation of lidocaine in aqueous solution produces N-dealkylation and N-oxidation but no hydroxylation products. Aromatic hydroxylation was only observed at potentials higher than 3 V in the presence of 1% water resulting mainly in 4-hydroxylidocaine [23]. Oxidation in the presence of 1% hydrogen peroxide at 3 V led

to both 3- and 4-hydroxylidocaine, but no N-oxide was formed [24]. In the latter reaction, we proposed that hydrogen peroxide was electrocatalytically activated on the platinum electrode forming platinum-oxo species capable of direct insertion of an oxygen atom into the C-H bond of the aromatic ring [24].

The role of platinum-oxide layers in electrochemical aromatic hydroxylation reactions has been extensively studied in the literature. It has been reported that oxide layers are formed on the Pt electrode surface under strong acidic conditions [25–29]. Conway *et al.* showed that a water molecule binds to the electrode surface and that a platinum oxide species is formed following the irreversible removal of one electron and one proton [27,28,30]. This Pt-oxide layer is claimed to increase electron transfer efficiency [31] thereby facilitating electrochemical oxidation of many organic substances. For example, oxidation of aromatic compounds, including benzene and phenol, has been achieved in aqueous acidic solutions (e.g. 0.5 M H₂SO₄) when oxide layers are present on the Pt electrode surface [32–34].

We showed recently that N-dealkylation of lidocaine is favored under basic conditions, while this reaction was completely suppressed at pH values below 0.5 in the presence of trifluoroacetic acid (TFA) [35]. In addition, we observed that aromatic hydroxylation products were readily produced under these conditions. In the present study we attempt to elucidate the mechanism of the aromatic hydroxylation reaction in the presence of TFA at platinum electrodes, the role of platinum-oxide layers and notably to define the source of oxygen.

2. Materials and Methods

2.1. Reagents

Lidocaine (L7757), trifluoroacetic acid (TFA, T6508) and H₂¹⁸O (329878) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Formic acid (HCOOH, 94318) and acetaminophen (00370) were purchased from Fluka and ultra-pure HPLC grade acetonitrile (ACN, 01203502) was purchased from Biosolve (Valkenswaard, The Netherlands). Sulfuric acid (H₂SO₄, 1007311) was purchased from Merck Millipore (Amsterdam, The Netherlands). Ultrapure water was obtained from a Milli-Q Advantage A10 Water Purification system (Millipore Corp., Billerica, MA, USA).

2.2. Electrochemical measurements

Constant potential measurements were performed with an Antec ROXY potentiostat (Antec Leyden, Zoeterwoude, The Netherlands) controlled by Antec Dialogue software. Cyclic voltammetry (CV) measurements were performed with an Autolab PGSTAT204 potentiostat (Metrohm, Schiedam, The Netherlands) controlled by Metrohm Nova software. Unless otherwise stated, electrochemical reactions were performed in a one-compartment three-electrode cell in which the working electrode was a Pt coil (P3640/88, Fischer Scientific, Landsmeer, The Netherlands) and the auxiliary electrode a platinum wire (MW-4130, BASi, West Lafayette, IN, USA). During CV measurements, a Ag/AgCl (in 3 M NaCl) reference electrode was used, while for constant potential experiments a silver wire pseudo-reference electrode (MF-2017, BASi) was used to avoid chloride contamination during electrochemical reactions. The potential shift of the Ag wire pseudo reference compared with the Ag/AgCl 3 M KCl reference electrode is -0.4 V, measured in an aqueous 0.1 M NaCl solution with 10 mM potassium hexacyanoferrate. All electrochemical experiments were performed at ambient temperature. For deaeration, nitrogen, or helium was bubbled via a sparge tube (MW-4145, BASi). Working electrodes were cleaned by sonicating in ethanol followed by drying with nitrogen.

For strong acidic conditions (pH 0.5, measured with a S20 SevenEasy pH meter (Mettler-Toledo, Schwerzenbach, Switzerland) using a pH electrode), 10 μ M lidocaine solutions were prepared either in ACN/H₂O/TFA (90/5/5) or in ACN/TFA (95/5). For CV measurements, a 1 M TFA solution in water was prepared. Unless otherwise stated, 3 mL solutions of lidocaine were oxidized for 30 min in a one-compartment three electrode cell. For oxygen-free conditions, a three-neck round-bottom flask was used and the working (Pt coil), auxiliary (Pt coil) and reference (Ag wire) electrodes were fixed each into a separate neck of the flask by using a rubber septum. For purging with nitrogen and sampling, two different sparge tubes were also inserted through the septa. The necks of the flask were sealed and air was removed by purging with nitrogen gas for 30 min while simultaneously applying vacuum (Supplementary Figure S1). In order to remove dissolved oxygen from the lidocaine solutions, the solvent bottle was sealed and purged with helium gas for 15 min before introduction into the three-neck flask. After electrochemical oxidation, samples were diluted with water containing 100 μ M acetaminophen as internal standard

(IS). The final samples for LC-MS analysis contained 1 μM lidocaine (based on the initial concentration) and 10 μM acetaminophen.

2.3. LC-MS analysis

LC-MS/MS analyses in the selected reaction monitoring (SRM) mode were carried out on an HPLC system with an Accela Autosampler and a Surveyor Pump coupled to a TSQ Quantum AM triple quadrupole mass spectrometer (Thermo Finnigan, San José, CA, USA) with an electrospray ionization interface in the positive mode. The MS parameters for the TSQ Quantum AM were as follows: spray voltage 3500 V, auxiliary gas pressure 20 (arbitrary unit), sheath gas pressure 40 (arbitrary unit), capillary temperature 350 °C, tube lens offset 90 V, skimmer offset -0 V, scan range m/z 100-300 (scan time 1 s, Q1 peak width 0.70 amu FWHM). The SRM transitions selected for lidocaine and its metabolites were: lidocaine: 235/86, N-dealkylation product: 207/58, aromatic hydroxylation products: 251/86. The transition 152/110 was selected for acetaminophen. SRM measurements were performed with a dwell time of 100 ms for each transition and Q3 peak widths of 0.70 amu FWHM.

The LC separation of lidocaine and its products was performed with a C_{18} reversed-phase column (GraceSmart RP 18, 5 μm particle size, 2.1 $\times$ 150 mm; Grace Davison, Lokeren, Belgium) at a flow rate of 250 $\mu\text{L}/\text{min}$. Solvent A was H_2O with 0.1% formic acid, while solvent B was ACN with 0.1% formic acid. A linear gradient was applied starting from 5% to 95% solvent B in solvent A over 11 min, which was held for 1 min. Solvent B was decreased rapidly to 5% in 20 s and the column was re-equilibrated at 5% solvent B for 4 min. Acetaminophen was used to normalize the peak areas of lidocaine and its oxidation products, resulting in normalized product yields.

3. Results and Discussion

3.1. Aromatic hydroxylation reactions of lidocaine under strong acidic conditions

As previously reported, the electrochemical N-dealkylation reaction of lidocaine is completely suppressed at pH values below 0.5 [35]. Unexpectedly, under these strongly acidic conditions aromatic hydroxylation metabolites of lidocaine are produced instead. Since aromatic hydroxylation of lidocaine has not been reported before at potentials as low as 1.5 V, we investigated this reaction in more detail. For this purpose, a solution of 10 μM lidocaine was prepared in ACN/TFA/ H_2O (90:5:5) and oxidized at 1.5 V for 30 min. Samples were analyzed by LC-MS/MS showing the generation of 3- and 4-hydroxylidocaine (Figure 1). The peak at 2.2 min

was assigned to 3-hydroxylidocaine using a synthetic standard, while the other peak at 1.9 min was assigned to 4-hydroxylidocaine based on literature data [36]. The ratio of the LC-MS peak areas of 3- and 4-hydroxylidocaine is 10:1. No other reaction products of lidocaine, including benzylic hydroxylation, N-oxide formation, and dihydroxylation were observed.

3.2. Investigation of the oxygen source

In order to gain a better understanding of the reaction mechanism, we first investigated whether water could serve as the source of oxygen and performed the reaction in ^{18}O -labelled water (97% ^{18}O atom). A solution of 10 μM lidocaine was prepared in ACN/TFA/ $^{18}\text{OH}_2$ (90:5:5) and oxidized at 1.5 V for 90 min in a three-neck round-bottom flask while purging with nitrogen gas. The LC-MS/MS results of collected samples showed that approximately 86% of the generated 3-hydroxylidocaine contained ^{18}O (Figure 2a). The remaining 14 % of 3-hydroxylidocaine contained ^{16}O which may have originated from residual unlabeled water in the reaction mixture. In support of this finding, we found that that same experiment using a 1:1 ratio of $^{18}\text{OH}_2$ / $^{16}\text{OH}_2$ yielded 58% of 3-hydroxylidocaine containing ^{16}O and 42% containing ^{18}O (Figure 2b).

We investigated other possible sources of oxygen by performing the aromatic hydroxylation reaction in the absence of water (ACN/TFA (95:5)). The generation of 3-hydroxylidocaine under these conditions, albeit in lower amounts (Figure 3), confirmed that there must be another source, possibly dissolved molecular oxygen. To study this further, electrochemical hydroxylation was performed in the absence of water under oxygen-free conditions. Lidocaine solution in ACN/TFA (95:5) was deaerated and subsequently introduced into the reaction flask and the reaction was carried out under continuous purging with nitrogen gas. As a control, 10 μM lidocaine solution, prepared in ACN/TFA (95:5), was purged with zero air and both solutions were oxidized at 1.5 V for 90 min. The generation of 3-hydroxylidocaine and the consumption of lidocaine were followed by LC-MS/MS and compared to a reaction in the presence of water by plotting product yield against oxidation time. While very low amounts of 3-hydroxylidocaine were produced under oxygen-free conditions (Figure 3), it was readily observed when purging with zero air. The consumption of lidocaine under oxygen-free conditions was negligible which shows that no reaction takes place under these conditions. Approximately 0.8 μM of 3-hydroxylidocaine was detected when purging with zero air and 1.6 μM of 3-hydroxylidocaine was detected in the presence of water under nitrogen purging, corresponding to yields of 8% and 16%, respectively. Moreover, under both

conditions the ratio of 3- to 4-hydroxylidocaine was approximately 10 to 1 as was also observed in the experiments shown in Figure 2. Taken together these results indicate that dissolved molecular oxygen may also serve as oxygen source. We assume that molecular oxygen participates in the aromatic hydroxylation reaction after its reduction to water at the counter electrode (Eq. 1), a reaction that is catalyzed by metal oxide electrodes [37], although we cannot exclude that other reactive oxygen intermediates derived from oxygen play a role. Since the counter electrode and working electrode compartments are not separated, water may diffuse to the working electrode and thus ultimately serve as the source of oxygen.

3.3. Investigation of the aromatic hydroxylation mechanism

The aromatic hydroxylation reaction of lidocaine in the presence of water is thought to proceed through the formation of hydroxyl radicals upon electrochemical oxidation of water [17,38–40]. Comninellis *et al.* reported that oxidation of water at metal electrodes generates physically adsorbed hydroxyl radicals (Eq. (3)) [39–41]. Organic compounds are presumed to be oxidized by either the physically adsorbed hydroxyl radicals (Eq. (4) and (5)) or by chemically adsorbed oxygen (Eq. (6)) or by a combination of these pathways [40].

Since previous work has shown that oxide layers on the Pt surface are involved in hydroxylation reactions, we performed experiments in $^{18}\text{OH}_2$ to produce a Pt- ^{18}O layer, and checked whether ^{18}O from this layer was subsequently incorporated into lidocaine. Initially, cyclic voltammetry between -0.1 V and 1.5 V at a 100 mV/s scan rate was performed in the presence of a 1 M aqueous TFA solution using a Pt coil working electrode to follow consecutive oxide layer formation and removal. The cyclic voltammograms indicated that oxide layers were formed at

potentials above 1.0 V and removed again from the Pt electrode surface between 0.3 V and 0.6 V (Figure 4) [42]. In order to grow ^{18}O layers on the Pt surface 1 M $\text{H}_2\text{S}^{16}\text{O}_4$ was prepared in $^{18}\text{OH}_2$ and CV scans were performed by following the protocol reported in the literature [43]. The coverage of the oxygen atoms was estimated to be equivalent to 0.5-1 monolayers (ML), based on the charge density of $\sim 300 \mu\text{C}/\text{cm}^2$ [44]. The number of the Pt atoms on the 8 mm^2 working electrode was calculated to be 6.3×10^{13} considering a smooth surface. This corresponds to approximately 0.1 nmol Pt [45] translating into 0.05-0.1 nmol oxygen atoms. The ^{18}O covered electrode was used as a working electrode to oxidize a $10 \mu\text{M}$ lidocaine solution prepared in ACN/TFA (95:5). The LC-MS/MS results of this experiment showed that the aromatic hydroxylation products only contained ^{16}O (16% yield, corresponding to 1.6 nmol), whereas we would expect up to 6% ^{18}O contribution. Assuming that the Pt- ^{18}O layer was successfully generated, this leads to the conclusion that the oxygen atoms of the Pt-oxide layers are at most a minor source of oxygen in the aromatic hydroxylation reaction.

Conclusions

We report that aromatic hydroxylation of lidocaine can be readily achieved electrochemically under strongly acidic conditions (ACN/TFA) at Pt electrodes. Both water and dissolved molecular oxygen may function as sources of oxygen. An important prerequisite is that the dominant N-dealkylation reaction is suppressed at pH values below 0.5. Experiments in the presence of ^{18}O -labelled water indicate that water is the primary source of oxygen and we assume that dissolved molecular oxygen is reduced to water at the counter electrode thus contributing to the reaction, notably in the absence of added water. The aromatic hydroxylation mechanism in the presence of water is assumed to proceed through activation of water to generate reactive oxygen species particularly hydroxyl radicals.

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References

- [1] D.A. Alonso, C. Nájera, I.M. Pastor, M. Yus, Transition-Metal-Catalyzed Synthesis of Hydroxylated Arenes, *Chem. Eur. J.* 16 (2010) 5274–5284.
- [2] A. Raba, M. Cokoja, W.A. Herrmann, F.E. Kühn, Catalytic hydroxylation of benzene and toluene by an iron complex bearing a chelating di-pyridyl-di-NHC ligand, *Chem. Commun.* 50 (2014) 11454–11457.
- [3] L.F. D’Elia, R.L. Ortiz, Electrochemical Oxidation of Toluene on Glassy Carbon in Organic-Aqueous Acid Solution, *J. Electrochem. Soc.* 153 (2006) D187–D192.
- [4] F.P. Guengerich, Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity, *Chem. Res. Toxicol.* 14 (2001) 611–650.
- [5] F.P. Guengerich, J.S. MacDonald, Applying mechanisms of chemical toxicity to predict drug safety, *Chem. Res. Toxicol.* 20 (2007) 344–369.
- [6] C.M. Bathelt, L. Ridder, A.J. Mulholland, J.N. Harvey, Mechanism and structure-reactivity relationships for aromatic hydroxylation by cytochrome P450, *Org. Biomol. Chem.* 2 (2004) 2998–3005.
- [7] C.M. Brown, B. Reisfeld, A.N. Mayeno, Cytochromes P450: A Structure-Based Summary of Biotransformations Using Representative Substrates, *Drug Metab. Rev.* 40 (2008) 1–100.
- [8] R. Ullrich, M. Hofrichter, Enzymatic hydroxylation of aromatic compounds, *Cell. Mol. Life Sci.* 64 (2007) 271–293.
- [9] D. Korolev, K. V Balakin, Y. Nikolsky, E. Kirillov, Y.A. Ivanenkov, N.P. Savchuk, Modeling of Human Cytochrome P450-Mediated Drug Metabolism Using Unsupervised Machine Learning Approach, *J. Med. Chem.* 46 (2003) 3631–3643.
- [10] F.P. Guengerich, Cytochrome p450 and chemical toxicology, *Chem. Res. Toxicol.* 21 (2008) 70–83.
- [11] J.P. Kehrer, The Haber-Weiss reaction and mechanisms of toxicity, *Toxicology.* 149 (2000) 43–50.
- [12] H.J.H. Fenton, Oxidation of tartaric acid in presence of iron, *J Chem Soc.* 65 (1894) 899–910.
- [13] S. Goldstein, D. Meyerstein, G. Czapski, The Fenton reagents, *Free Radic. Biol. Med.* 15 (1993) 435–445.

- [14] C. Walling, R. a Johnson, Fenton's reagent. V. Hydroxylation and side-chain cleavage of aromatics, *J. Am. Chem. Soc.* 97 (1975) 363–367.
- [15] R.P. Kreh, M.E. Tadros, H.M. Hand, M.P. Cockerham, E.K. Smith, Indirect hydroxylation of aromatic rings using electrochemical methods, *J. Appl. Electrochem.* 16 (1986) 440–446.
- [16] U. Jurva, H. V Wikström, A.P. Bruins, Electrochemically assisted Fenton reaction: reaction of hydroxyl radicals with xenobiotics followed by on-line analysis with high-performance liquid chromatography/tandem mass spectrometry, *Rapid Commun. Mass Spectrom.* 16 (2002) 1934–1940.
- [17] R. Oliveira, F. Bento, D. Geraldo, Aromatic hydroxylation reactions by electrogenerated HO radicals: A kinetic study, *J. Electroanal. Chem.* 682 (2012) 7–13.
- [18] T.A. Enache, A.-M. Chiorcea-Paquim, O. Fatibello-Filho, A.M. Oliveira-Brett, Hydroxyl radicals electrochemically generated in situ on a boron-doped diamond electrode, *Electrochem. Commun.* 11 (2009) 1342–1345.
- [19] T. Johansson, L. Weidolf, U. Jurva, Mimicry of phase I drug metabolism – novel methods for metabolite characterization and synthesis, *Rapid Commun. Mass Spectrom.* 21 (2007) 2323–2331.
- [20] M. Ruokolainen, T. Gul, H. Permentier, T. Sikanen, R. Kostainen, T. Kotiaho, Comparison of TiO₂ photocatalysis, electrochemically assisted Fenton reaction and direct electrochemistry for simulation of phase I metabolism reactions of drugs, *Eur. J. Pharm. Sci.* 83 (2016) 36–44.
- [21] L.E. Mather, J. Thomas, Metabolism of lidocaine in man, *Life Sci.* 11 (1972) 915–919.
- [22] Y. Oda, S. Imaoka, Y. Nakahira, A. Asada, M. Fujimori, S. Fujita, Metabolism of lidocaine by purified rat liver microsomal cytochrome P-450 isozymes, *Biochem. Pharmacol.* 38 (1989) 4439–4444.
- [23] E. Nouri-Nigjeh, H.P. Permentier, R. Bischoff, A.P. Bruins, Electrochemical oxidation by square-wave potential pulses in the imitation of oxidative drug metabolism, *Anal. Chem.* 83 (2011) 5519–5525.
- [24] E. Nouri-Nigjeh, A.P. Bruins, R. Bischoff, H.P. Permentier, Electrocatalytic oxidation of hydrogen peroxide on a platinum electrode in the imitation of oxidative drug metabolism of lidocaine, *Analyst* 137 (2012) 4698–4702.
- [25] F. Anson, J. Lingane, Chemical evidence for oxide films on platinum electrometric

- electrodes, *J. Am. Chem. Soc.* 1315 (1957) 4901–4904.
- [26] A. Damjanovic, Formation of Oxide Films at Platinum Anodes in Alkaline Solutions, *J. Electrochem. Soc.* 127 (1980) 1945–1950.
- [27] B.E. Conway, S. Gottesfeld, Real condition of oxidized platinum electrodes. Part 2.— Resolution of reversible and irreversible processes by optical and impedance studies, *J. Chem. Soc. Faraday Trans. 1 Phys. Chem. Condens. Phases.* 69 (1973) 1090–1107.
- [28] B.E. Conway, T.C. Liu, Characterization of electrocatalysis in the oxygen evolution reaction at platinum by evaluation of behavior of surface intermediate states at the oxide film, *Langmuir* 6 (1990) 268–276.
- [29] D. Gilroy, Oxide formation in the oxygen evolution region at Pt electrodes in M H₂SO₄, *J. Electroanal. Chem. Interfacial Electrochem.* 83 (1977) 329–339.
- [30] B.E. Conway, Electrochemical oxide film formation at noble metals as a surface-chemical process, *Prog. Surf. Sci.* 49 (1995) 331–452.
- [31] A. Damjanovic, Electron Transfer Through Thin Anodic Oxide Films during the Oxygen Evolution Reactions at Pt Electrodes, *J. Electrochem. Soc.* 138 (1991) 2549–2555.
- [32] M. Gattrell, D.W. Kirk, A Study of the Oxidation of Phenol at Platinum and Preoxidized Platinum Surfaces, *J. Electrochem. Soc.* 140 (1993) 1534–1540.
- [33] F. Montilla, F. Huerta, E. Morallon, J.L. Vazquez, Electrochemical behaviour of benzene on platinum electrodes, *Electrochim. Acta.* 45 (2000) 4271–4277.
- [34] P.I. Iotov, S.V. Kalcheva, Mechanistic approach to the oxidation of phenol at a platinum/gold electrode in an acid medium, *J. Electroanal. Chem.* 442 (1998) 19–26.
- [35] T. Gul, R. Bischoff, H.P. Permentier, Optimization of reaction parameters for the electrochemical oxidation of lidocaine with a Design of Experiments approach, *Electrochim. Acta.* 171 (2015) 23–28.
- [36] E. Nouri-Nigjeh, H.P. Permentier, R. Bischoff, A.P. Bruins, Lidocaine oxidation by electrogenerated reactive oxygen species in the light of oxidative drug metabolism, *Anal. Chem.* 82 (2010) 7625–7633.
- [37] J. Wilshire, D.T. Sawyer, Redox chemistry of dioxygen species, *Acc. Chem. Res.* 12 (1979) 105–110.
- [38] H.A. Laitinen, C.G. Enke, The Electrolytic Formation and Dissolution of Oxide Films on Platinum, *J. Electrochem. Soc.* 107 (1960) 773–781.

- [39] C. Comninellis, Electrocatalysis in the electrochemical conversion/combustion of organic pollutants for waste water treatment, *Electrochim. Acta.* 39 (1994) 1857–1862.
- [40] O. Scialdone, Electrochemical oxidation of organic pollutants in water at metal oxide electrodes: A simple theoretical model including direct and indirect oxidation processes at the anodic surface, *Electrochim. Acta.* 54 (2009) 6140–6147.
- [41] N.D. Popović, D.C. Johnson, A Ring–Disk Study of the Competition between Anodic Oxygen-Transfer and Dioxygen-Evolution Reactions, *Anal. Chem.* 70 (1998) 468–472.
- [42] E.M. Hudak, J.T. Mortimer, H.B. Martin, Platinum for neural stimulation: voltammetry considerations, *J. Neural Eng.* 7 (2010) 26005 1-7.
- [43] J. Willsau, O. Wolter, J. Heitbaum, Does the oxide layer take part in the oxygen evolution reaction on platinum?, *J. Electroanal. Chem.* 195 (1985) 299–306.
- [44] A.A. McMath, J. Van Drunen, J. Kim, G. Jerkiewicz, Identification and Analysis of Electrochemical Instrumentation Limitations through the Study of Platinum Surface Oxide Formation and Reduction, *Anal. Chem.* 88 (2016) 3136–3143.
- [45] R. Holze, *Experimental Electrochemistry*, Wiley, Weinheim, 2009.

Figure Legends

Figure 1. Electrochemical conversion of lidocaine upon oxidation under strong basic or acidic conditions. LC-MS/MS chromatograms in the SRM mode were recorded as follows: N-dealkylation m/z 207/86, N-oxidation and aromatic hydroxylation m/z 251/86. Oxidation was performed for 30 min at 1.5 V. Solvent conditions: a) pH 12 (ACN/NH₄OH), and b) pH 0.5 (ACN/H₂O/TFA). N-dealkylation and N-oxidation products were observed at pH 12 while only aromatic hydroxylation products of lidocaine were observed at pH 0.5. Lidocaine elutes at 3.7 min and the peak in trace a (251/86 SRM chromatogram) is due to the partial in-source oxidation of lidocaine during the electrospray ionization process. Different intensity scales were used for chromatograms.

Figure 2. Aromatic hydroxylation product formation upon electrochemical oxidation of lidocaine a) in the presence of ACN/TFA/¹⁸OH₂ (90:5:5), and b) ACN/TFA/¹⁸OH₂/¹⁶OH₂ (90:5:2.5:2.5). Experiments were repeated two times, and the error bars indicate the standard error.

Figure 3. Effect of the presence of molecular oxygen and water on the formation of 3-hydroxylidocaine. Experiments were repeated 5 times for the oxygen-free experiment and the error bars show the standard error.

Figure 4. The reversible formation of Pt-oxide layers. Cyclic voltammetry experiments were performed using a Pt electrode in 1 M TFA in water. Potentials were cycled from -0.1 V to 1.5 V at 100 mV/s scan rate vs an Ag/AgCl (in 3 M NaCl) reference electrode.

Figure 1.

Figure 2.

Figure 3.

Figure 4.