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Electrochemical Oxidation by Square-Wave Potential Pulses in the Imitation of Oxidative Drug Metabolism

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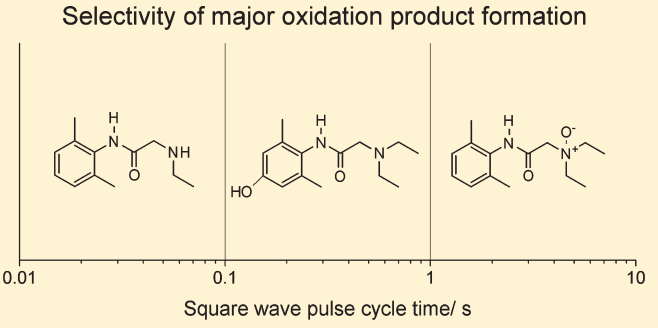
Supporting Information

ABSTRACT: Electrochemistry combined with mass spectrometry (EC−MS) is an emerging analytical technique in the imitation of oxidative drug metabolism at the early stages of new drug development. Here, we present the benefits of electrochemical oxidation by square-wave potential pulses for the oxidation of lidocaine, a test drug compound, on a platinum electrode. Lidocaine was oxidized at constant potential and by square-wave potential pulses with different cycle times, and the reaction products were analyzed by liquid chromatography−mass spectrometry [LC−MS(/MS)]. Application of constant potentials of up to +5.0 V resulted in relatively low yields of N-dealkylation and 4-hydroxylation products, while oxidation by square-wave potential pulses generated up to 50 times more of the 4-hydroxylation product at cycle times between 0.2 and 12 s (estimated yield of 10%). The highest yield of the N-dealkylation product was obtained at cycle times shorter than 0.2 s. Tuning of the cycle time is thus an important parameter to modulate the selectivity of electrochemical oxidation reactions. The N-oxidation product was only obtained by electrochemical oxidation under air atmosphere due to reaction with electrogenerated hydrogen peroxide. Square-wave potential pulses may also be applicable to modulate the selectivity of electrochemical reactions with other drug compounds in order to generate oxidation products with greater selectivity and higher yield based on the optimization of cycle times and potentials. This considerably widens the scope of direct electrochemistry-based oxidation reactions for the imitation of in vivo oxidative drug metabolism.



The imitation of oxidative drug metabolism by cytochrome P450s (P450) at the early stages of new drug development requires fast and accurate analytical techniques.[1,2] Electrochemistry combined with mass spectrometry (EC−MS) is emerging as a versatile analytical technique capable of the generation and identification of many known in vivo metabolites.[3−5] Although different oxidation products may be generated at different potentials, constant potential oxidations often lack in selectivity when it comes to generation of defined metabolites. In addition, electrode fouling and/or passivation associated with constant potential oxidation of organic compounds may attenuate surface reactivity and thereby reduce product yield.[6]

Platinum electrodes have been widely used for organic compound oxidation and detection due to their excellent conductivity and chemical stability even at high positive potentials.[7] Johnson and co-workers showed that electrochemical detection of various organic compounds on Pt electrodes can be significantly improved using square-wave potential pulses, as well as other potential waveforms, with cycle times between 0.2 and 2 s. Adsorbed molecules generated during oxidation are usually desorbed during the anodic formation of a surface oxide. Because the formation of surface oxide can attenuate surface reactivity, a subsequent negative potential step quickly allows the cathodic dissolution of the surface oxide to restore the reactivity of the fresh surface.[6,8]

A second function of square-wave potential pulses is claimed to involve the generation of intermediate reactive oxygen species during the oxidation of water on the Pt electrode. Production of OH radicals adsorbed on the Pt electrode may participate in O-transfer reactions to the preadsorbed organic molecules on the surface. Square-wave potential pulses have been applied successfully to achieve anodic detection of numerous polar aliphatic compounds.[9]

The aim of pulsed potential electrochemical detection is sensitive and reproducible current measurement, but the reaction products are not characterized by spectrometric methods.[10−14] Synthetic organic electrochemistry, on the other hand,[15] aims at the production and characterization of specific molecules. In this field, there have been no reports thus far of the use of square-wave potential pulses.

The electrochemical production of drug metabolites by means of square-wave potential pulses might allow both higher yields

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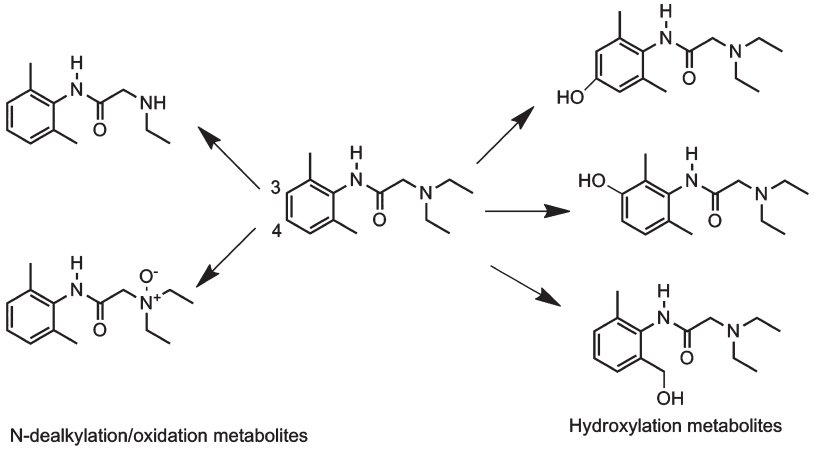
Scheme 1. In Vivo Metabolites of Lidocaine Due to Oxidative Metabolism by Cytochrome P450s[a]



[a] Refs 5, 16, and 17.

through surface renewal and access to additional products through O-transfer reactions. In the present study, lidocaine, a local anesthetic drug, was used as a test compound. The in vivo metabolites of lidocaine result from N-dealkylation, N-oxidation, and aromatic and benzylic hydroxylation (Scheme 1).[5,16,17] Direct electrochemical oxidation of lidocaine on a porous graphite electrode gave only the N-dealkylated product.[18] N-Oxidation of lidocaine was observed in the presence of electrochemically generated hydrogen peroxide.[19] Here, we show that square-wave potential pulses with different cycle times lead to a marked change in the distribution of lidocaine reaction products as analyzed and identified by liquid chromatography−mass spectrometry [LC−MS(/MS)].

EXPERIMENTAL PROCEDURES

Reagents. Tetrabutylammonium perchlorate (TBAP, 86893) and lidocaine (L7757) were purchased from Sigma-Aldrich. Water was purified by a Maxima Ultrapure water system (ELGA, High Wycombe, Bucks, U.K.). Ultrapure HPLC grade acetonitrile (ACN) was purchased from Merck. $H_2{}^{18}O$ with 97 atom % ^{18}O ($H_2{}^{18}O$, 329878) was purchased from Sigma-Aldrich. 3-Hydroxylidocaine (CAS no. 34604-55-2) was purchased from Toronto Research Chemicals Inc.

Electrode Preparation. The surface of the platinum disk working electrode was polished with a lapping sheet (Micromesh grade 3200) prior to each experiment. After mechanical polishing the surface was washed with ethanol and air-dried.

Electrochemical Measurements. Electrochemical experiments were performed with a homemade potentiostat controlled by a MacLab system (ADInstruments, Castle Hill, NSW, Australia) and EChem v.1.52 software (eDAQ, Denistone East, NSW, Australia). A two-compartment electrochemical cell was constructed by using a porous Vycor tip with Teflon heat shrink (MF-2064, Bioanalytical Systems (BASi), West Lafayette, IN, U.S.A.) to separate working and auxiliary half-cells. The working electrode was a 2 mm diameter platinum disk (MF-2013, BASi), and the auxiliary electrode was a platinum wire (MW-4130, BASi). Potentials were measured against a silver wire pseudo-reference electrode (MF-2017, BASi), to eliminate the possibility of chloride contamination of the working solution during prolonged electrolysis in the presence of conventional reference electrodes. The reference electrode was placed in the working compartment. All experiments were performed at ambient temperature. For deaeration and aeration, argon and synthetic air, respectively, were bubbled at 25 mL/min via a sparge tube (MW-4145, BASi) through the 1 mL solution during all experiments (4 mL of solution was used for the experiments presented in Supporting Information Figure S2). The auxiliary compartment was always under argon purging. In the case of pulsed potential experiments, the upper and lower potential steps within one cycle were of equal duration (e.g., 1 s steps for 2 s cycle time).

Solutions containing 10 mM lidocaine and 0.1 M TBAP dissolved in ACN/H_2O 99/1 (v/v) (0.1 M TBAP used as electrolyte to provide sufficient conductivity for electrochemical experiments) were subjected to constant potential oxidation and to oxidation by square-wave potential pulses with continuous gas flow, for 30 min prior to LC−MS analysis. Samples were collected from the working compartment and diluted 100 times in water containing 10 μM acetaminophen, as an internal standard for LC−MS signal normalization, immediately after the batch oxidations and stored at room temperature until LC−MS analysis.

LC−MS Analysis. LC−MS experiments were carried out on an LC Packings Ultimate HPLC system (LC Packings, Amsterdam, The Netherlands) coupled to an API 365 triple quadrupole mass spectrometer (MDS Sciex, Concord, ON, Canada) upgraded to EP10+ (Ionics, Bolton, ON, Canada) with electrospray ionization in the positive mode in the TurboIonSpray source. The MS spectra were acquired between m/z 100 and 600 (step size 1.0 amu, dwell time 1 ms).

A C_{18} reversed-phase column (GraceSmart RP 18 5 μm, 2.1 mm × 150 mm; Grace Davison, Lokeren, Belgium) was used at a flow rate of 200 μL/min: solvent A, H_2O/ACN 95/5 (v/v) with 0.1% formic acid; solvent B, ACN/H_2O 95/5 (v/v) with 0.1% formic acid. An amount of 5 μL of a diluted oxidation product mixture was injected, and a linear gradient of 5−50% B in 20 min was used for elution. Peak heights were normalized with respect to the peak height of the acetaminophen internal reference compound. In case the ion counting detector of the MH^+ ion was saturated, the $^{13}C_1$ isotope intensity was taken, and the corresponding monoisotopic ^{12}C ion intensity was calculated using the theoretical isotope distribution.

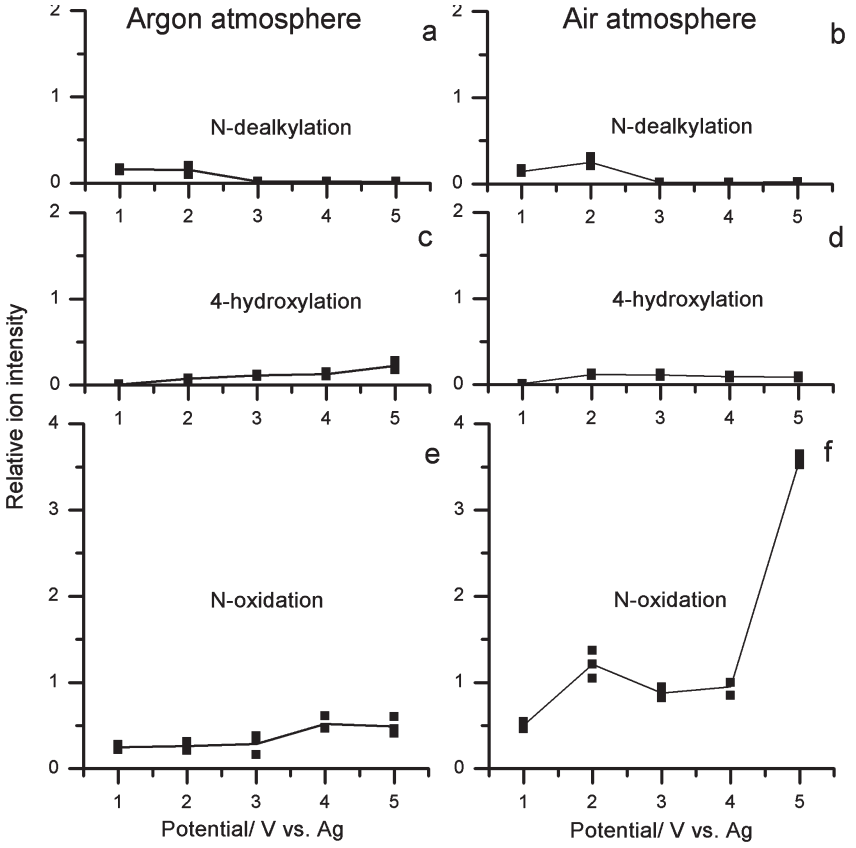


Figure 1. Relative MH^+ ion intensities of the N-dealkylation (a and b), the 4-hydroxylation (c and d), and the N-oxidation (e and f) products of lidocaine from a solution of 10 mM lidocaine in 0.1 M tetrabutylammonium perchlorate in acetonitrile/water 99/1 (v/v) at different oxidation potentials under argon and air atmosphere, respectively. Ion intensities were normalized relative to the intensity of the signal for acetaminophen, which was added as internal standard to all LC−MS analyses. Experiments were performed in triplicate.

N-Dealkylation, N-oxidation, and aromatic and benzylic hydroxylation products were identified by LC−MS/MS analysis, and in the case of the aromatic hydroxylation at the 4-position further evidence was obtained by coinjection with the 3-hydroxylidocaine as reference compound, as described before.[19]

RESULTS AND DISCUSSION

Constant Potential Oxidation. In a previous study, N-dealkylation of lidocaine was achieved by online EC−MS in a porous graphite flow-through cell with a potential ramp from 0 to +1.8 V in a solution of ACN/H_2O 50/50 (v/v).[18,20] The amount of N-dealkylation product was shown to decrease above +0.8 V in a potential ramp experiment, although no other oxidation products were observed at higher potentials.[18] The oxidation conditions in our current batch cell with a Pt working electrode at potentials below +2.0 V resulted in N-dealkylation and N-oxidation. At potentials of +2.0 V and higher, 4-hydroxylation, as well as small amounts of doubly oxidized products (mainly 3,4-dihydroxylation), was observed. Figure 1 shows the distribution of oxidation products as a function of the applied potential on the Pt working electrode when purged with argon and air, respectively.

The N-dealkylation product was barely detected at potentials above +2.0 V, both under argon and under air atmosphere (Figure 1, parts a and b). The N-dealkylation yield was not affected by the presence of air, which is consistent with the proposed reaction mechanism, which does not involve dissolved molecular oxygen and proceeds via direct electron transfer from the tertiary amine to the electrode followed by deprotonation to give an iminium intermediate that, after hydrolysis and intramolecular rearrangement, leads to the N-dealkylation product.[21]

Hydroxylation at the 4-position of the aromatic ring in lidocaine was observed at potentials of +2.0 V or more (Figure 1, parts c and d), although overall yield was low. The 4-hydroxylation product is most likely generated through an anodic substitution reaction by water molecules, which is initiated by a two-electron transfer to the electrode simultaneously with the formation of a Wheland-type intermediate, resulting in the aromatic hydroxylation product after deprotonation (Scheme 2).[22,23] Although unsubstituted aromatic rings can be oxidized at higher positive potentials,[20] the electron-donating amide and the two methyl substituents presumably decrease the oxidation potential of the aromatic ring in lidocaine and direct the hydroxylation reaction toward the 4-position through resonance stabilization of the charge of the radical cation intermediate. Whereas direct electrochemical oxidation is apparently unable to generate the 3-hydroxylation product, in vivo oxidative metabolism by P450 produces both the 3- and the 4-hydroxylation products (Scheme 1) indicating that in vivo aromatic hydroxylation does not involve a radical cation intermediate, but that it proceeds via an oxygen insertion mechanism as proposed for reaction of the oxo-ferryl radical cation.[24] The range of aromatic hydroxylation reactions by direct electrochemistry is thus restricted in

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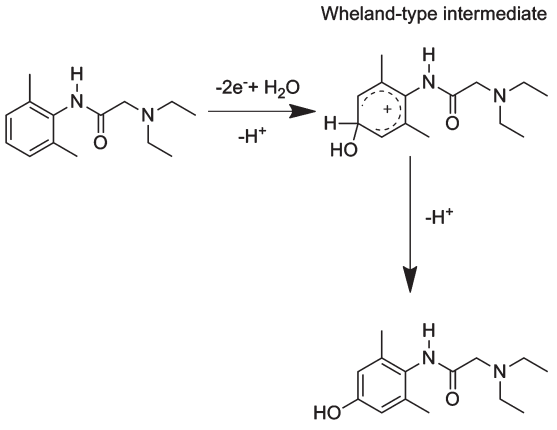
Scheme 2. Proposed Anodic Substitution Mechanism Initiated by the Abstraction of Two Electrons from the Aromatic Ring Followed by Substitution with Water Proceeding through a Wheland-Type Intermediate[a]



[a] Ref 23.

comparison to P450 due to the different nature of the reaction mechanism.

In contrast to N-dealkylation and 4-hydroxylation, formation of the N-oxidation product is strongly dependent on the presence of dissolved molecular oxygen in the solution with a sharp increase in yield between +4.0 and +5.0 V (Figure 1, parts e and f). N-Oxidation has been reported to result from the reaction between lidocaine and electrochemically generated hydrogen peroxide,[19] through peroxide intermediate formation and decomposition.[25] Hydrogen peroxide is generated in the cathodic compartment of the counter electrode and reaches the anodic compartment by diffusion through the porous frit membrane that separates both compartments, as previously reported.[19]

Stable isotope labeling was used to reveal the source of the oxygen atom in the N-oxidation product. According to the proposed N-oxidation mechanism, hydrogen peroxide is generated by reduction of molecular oxygen and the oxygen atom should originate from dissolved molecular oxygen. Since the only oxygen sources in our experiment are molecular oxygen and water, we replaced $H_2{}^{16}O$ by $H_2{}^{18}O$, to test this hypothesis. LC−MS analysis showed that the N-oxidation product incorporated only ^{16}O confirming that the oxygen atom in the N-oxidation product originated from dissolved molecular oxygen. The low amount of N-oxidation product observed under argon atmosphere (Figure 1e) may therefore be explained by incomplete removal of residual oxygen during argon purging.

Oxidation by Square-Wave Potential Pulses. The absolute yield of N-dealkylation and 4-hydroxylation products under constant potential conditions was below 1%. Yields were estimated by comparison of the MH^+ ion intensities of the products with that of lidocaine under the assumption that the electrospray ionization efficiencies for lidocaine and its oxidation products are similar. In order to affect the kinetics of the oxidation reaction, we modified constant potential oxidation by adding a lower potential step to generate a square-wave potential pulse with variable cycle time.

In view of the results presented in Figure 1, parts c and d, an upper oxidation potential of +3.0 V was selected for generation of the 4-hydroxylation product, while the lower potential step was

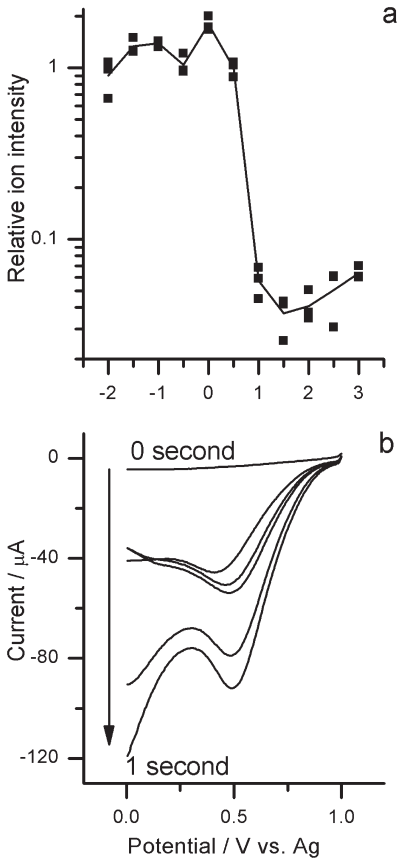


Figure 2. (a) Relative MH^+ ion intensities of the 4-hydroxylation product of lidocaine measured by LC−MS after electrochemical oxidation with square-wave potential pulses alternating between an upper potential of +3.0 V and lower potentials ranging from −2.0 to +3.0 V with a fixed cycle time of 2 s. Ion intensities were normalized relative to the intensity of the signal for acetaminophen, which was added as internal standard to all LC−MS analyses. Experiments were performed in triplicate. Ion intensities are plotted on a logarithmic scale. (b) Linear stripping voltammograms recorded at a scan rate of 1 V/s from +1.0 to 0.0 V after constant potential oxidation at +3.0 V (Pt electrode) for 0, 0.01, 0.05, 0.10, 0.5, and 1.0 s in the absence of lidocaine (the arrow shows the respective traces from top to bottom).

varied between +3.0 and −2.0 V with a cycle time of 2 s. The yield of the 4-hydroxylation product at different lower potentials shows that alternating between +3.0 V and a lower potential of +0.5 V or less leads to a more than 10-fold increase in 4-hydroxylation product, indicating that the surface properties of the electrode are significantly affected by potential steps below +0.5 V (Figure 2a). It was suggested previously that, although surface reactivity could be attenuated by formation of an inert platinum oxide layer during oxidation, a subsequent reduction step can restore the reactivity of the fresh surface.[6,8] To test the oxide layer formation under our condition and its subsequent reduction, the surface was oxidized at +3.0 V with times ranging from 0, 0.01, 0.05, 0.1, 0.5, to 1.0 s in the absence of lidocaine followed by linear stripping voltammograms recorded from +1.0 to 0.0 V at a scan rate of 1 V/s (Figure 2b). A fresh surface (0 s) did not show any reduction peak across the recorded potential region that implies the absence of oxide layer on the fresh surface. However, application of the oxidation potential even for 0.01 s led to a negative current peak at approximately +0.5 V indicating

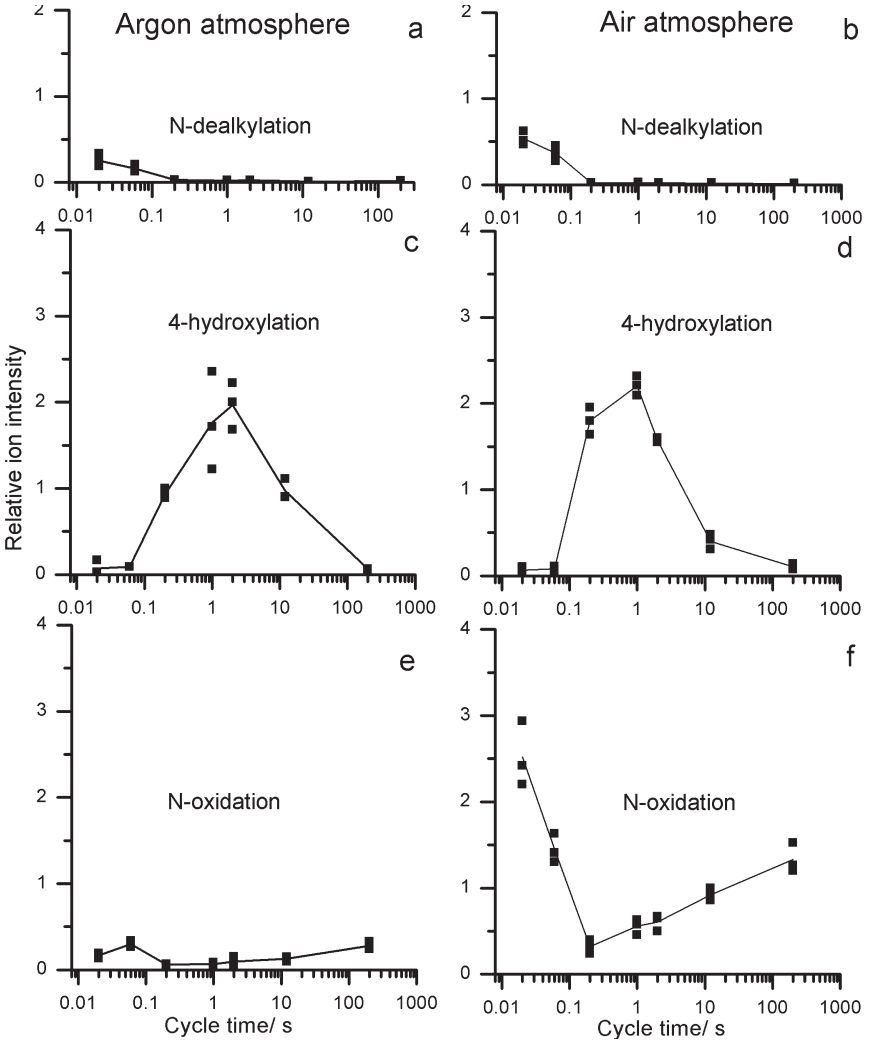


Figure 3. Relative MH^+ ion intensities of the N-dealkylation (a and b), 4-hydroxylation (c and d), and N-oxidation (e and f) products of lidocaine from a solution of 10 mM lidocaine in 0.1 M tetrabutylammonium perchlorate in acetonitrile/water 99/1 (v/v) at varying cycle times (plotted logarithmically) of square-wave potential pulses alternating between +3.0 and −1.0 V under argon and air atmosphere, respectively. Ion intensities were normalized relative to the intensity of the signal for acetaminophen, which was added as internal standard to all LC−MS analyses. Experiments were performed in triplicate.

rapid formation of an oxide layer on the Pt surface at +3.0 V, and its subsequent reduction at potentials lower than +0.5 V. In addition, reduction of the presumed oxide layer at +0.5 V coincides with the potential at which we observe a dramatic increase in the yield of 4-hydroxylation product of lidocaine (Figure 2a). This indicates strongly that reduction of the oxide layer on the Pt electrode surface at or below +0.5 V reactivates the electrode surface for the next pulse cycle leading to increased yields.

On the basis of the above results, we selected −1.0 V as the lower potential level and monitored product distribution as a function of cycle time ranging from 0.02 to 200 s by LC−MS (Figure 3). N-Dealkylation was only detected at cycle times shorter than 0.2 s (Figure 3, parts a and b). Since N-dealkylation was nearly absent upon constant potential oxidation at +3.0 V (Figure 1, parts a and b), repeated and fast switching of the surface potential apparently facilitated electron transfer from the tertiary amine moiety of lidocaine to the electrode. The yield of the 4-hydroxylation product also showed a strong dependence on cycle time, with an optimum at cycle times around 1 s independent of an argon or air atmosphere (Figure 3, parts c and d). Whereas the yield of the 4-hydroxylation product was low at a constant potential of +3.0 V or at short cycle times, it increased about 50-fold (estimated total yield of 10%) at cycle times of 1 s (see Figures 3c and 1c). The selectivity of pulsed electrochemical oxidation of lidocaine can thus be directed at the N-dealkylation or at the 4-hydroxylation product by selection of the cycle time.

The cause of this change in selectivity is currently unclear. Orientation of lidocaine on the working electrode, the lifetime of different reactive intermediates of oxidized lidocaine, and recovery of a fresh Pt surface may all be controlled by the potential levels of the square-wave potential pulses and may have their effect on the competition between N-dealkylation and 4-hydroxylation.

A change of atmosphere from argon to air did not affect the yield of the 4-hydroxylation product (Figure 3, parts c and d), whereas a higher yield of the N-dealkylation product was observed under air atmosphere, possibly because oxygen reduction generates

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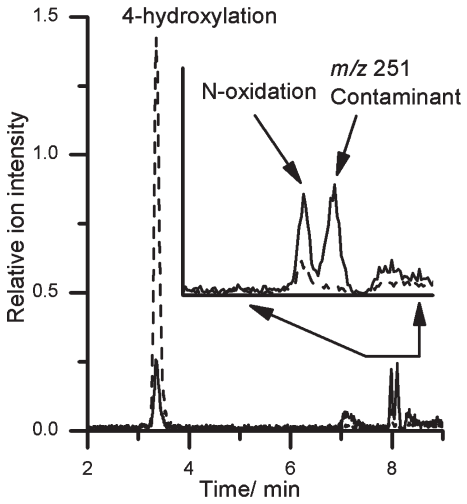

Figure 4. LC−MS analysis of lidocaine oxidation products in the presence $H_2{}^{18}O$ with square-wave potential pulses alternating between +3.0 and −1.0 V and a cycle time of 2 s under air atmosphere. The extracted ion chromatograms correspond to the MH^+ ions of the 4-hydroxylation and the N-oxidation products at *m/z* 251 (solid line, ^{16}O) and 253 (dashed line, ^{18}O).

superoxide anions that may be sufficiently basic for proton abstraction to form the iminium intermediate, facilitating generation of the N-dealkylation product.[21] Generation of the N-oxidation product requires molecular oxygen (see Figure 1, parts e and f), but it is also dependent on cycle time (Figure 3, parts e and f). A lower yield of the N-oxidation product was observed at cycle times between 0.2 and 12 s. It is intriguing that a minimum in N-oxidation yield coincides with the optimum for the 4-hydroxylation reaction suggesting a competition between the electrochemical generation of hydrogen peroxide and oxidation of the aromatic ring for subsequent reaction with water.

The anodic substitution mechanism (see Scheme 2) implies that the oxygen atom in 4-hydroxylated lidocaine originates from water. To verify this we replaced $H_2{}^{16}O$ by $H_2{}^{18}O$ during oxidation of lidocaine using square-wave potential pulses. The extracted ion chromatograms (Figure 4) show that the major part of the 4-hydroxylation product contained ^{18}O, although about 15−20% of ^{16}O incorporation was observed, which is likely due to a remaining 3% of $H_2{}^{16}O$ in the commercial $H_2{}^{18}O$ vial and to residual water from the atmosphere. This result is in agreement with the proposed mechanism. Producing the N-oxidation product using square-wave potential pulses in the presence of $H_2{}^{18}O$ resulted in approximately 30% incorporation of ^{18}O, whereas the remainder contained ^{16}O in accordance with the proposed mechanism through which molecular oxygen is reduced to hydrogen peroxide. The 30% of ^{18}O incorporation in the N-oxidation product under pulsed potential conditions might be due to oxidation of $H_2{}^{18}O$ to $^{18}O_2$ followed by reduction to $H_2{}^{18}O_2$.

The only observed hydroxylation product of lidocaine upon pulsed potential oxidation was 4-hydroxylidocaine, whereas the Fenton reaction, which leads to generation of hydroxyl radicals, results additionally in 3-hydroxylation and benzylic hydroxylation.[19] The absence of the latter hydroxylation products indicates strongly that no adsorbed OH radicals are generated on the Pt electrode during water oxidation. Pulsed potential oxidation of lidocaine on a gold electrode showed the same response as on the Pt electrode, whereas the yield of the 4-hydroxylation product was much lower using a glassy carbon electrode. This supports further that this reaction requires a noble metal surface that can be reactivated through redox cycling (Supporting Information Figure S1). In addition, a linear increase in the generation of 4-hydroxylation product was observed under pulsed potential condition over a 30 min period, suggesting that successful surface reactivation was achieved under pulse condition (Supporting Information Figure S2).

CONCLUSIONS

Constant potential oxidation of lidocaine generates low yields of N-dealkylation and 4-hydroxylation products. By varying the cycle time of square-wave potential pulses and the voltage of the lower potential step, we increased the yield of 4-hydroxylidocaine by about 50-fold relative to constant potential conditions resulting in an overall yield of approximately 10% at a cycle time of 1 s, whereas N-dealkylation was favored at short cycle times below 0.2 s. Pulsed potentials are thus effective in modulating electrochemical oxidation reactions that are initiated by direct electron transfer. We show that the Pt electrode surface is rapidly passivated under oxidative conditions and that it is regenerated during the low-potential step of the square-wave pulse. How cycle time affects the selectivity of the reaction (N-dealkylation at short pulse times versus 4-hydroxylation at longer pulse times) remains to be elucidated. It is conceivable that the lidocaine molecule reorients itself on the electrode during pulsing and that different parts of the molecule are in contact with the electron-transferring surface. Further experiments are needed to study this in greater detail. Square-wave potential pulses may be applicable to other drug compounds in order to generate oxidation products with greater selectively and higher yield based on optimization of cycle times and potentials. This could widen the scope of direct electrochemistry-based oxidation reactions for the imitation of in vivo drug metabolism.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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