Liquid chromatography/mass spectrometry to study oxidative degradation of environmentally relevant pharmaceuticals by electrochemistry and ozonation

Helene Faber a, Holger Lutze b, Pablo Lores Lareo b, Lisa Frensemeier a, Martin Vogel a, Torsten C. Schmidt b, Uwe Karst a,∗

a Westfälische Wilhelms-Universität Münster, Institut für Anorganische und Analytische Chemie, Corrensstraße 30, 48149 Münster, Germany
b Universität Duisburg-Essen, Instrumentelle Analytische Chemie, Universitätstraße 4, 45141 Essen, Germany

A R T I C L E   I N F O

Article history:
Received 18 November 2013
Received in revised form 27 March 2014
Accepted 30 March 2014
Available online 4 April 2014

Keywords:
Electrochemistry
LC/MS
Ozonation
Diclofenac
Metoprolol
Transformation products

A B S T R A C T

In this work, the potential of electrochemical oxidation as a tool for the rapid prediction of transformation products in water appearing after ozonation is investigated. These two approaches were compared by choosing the two environmentally relevant model compounds diclofenac and metoprolol and comparison of their transformation products after electrochemical oxidation and treatment with ozone. Within these two approaches, certain similarities were observed in the resulting chromatograms: Six transformation products of the electrochemical oxidation of metoprolol were also detected in the ozone samples. For diclofenac two transformation products matched. Additionally, five of the electrochemically generated oxidation products were reported in literature to occur after water treatment processes. The application of a boron-doped diamond working electrode for electrochemical oxidation allowed the generation of hydroxyl radicals, which was shown by spin trapping experiments with p-chlorobenzoic acid. This allowed the generation of certain transformation products previously not obtained by electrochemical oxidation. Concluding, the hyphenation of electrochemistry with liquid chromatography and mass spectrometry offers a useful tool in transformation studies.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Today, a wide range of pharmaceutical residues originating from different sources, e.g., chemotherapy, contrast agents, hormone therapy, etc., can be detected both in surface or ground waters [1–3]. Already 40 years ago, the first pharmaceutical metabolite from clofibric acid was detected by means of GC/MS in treated wastewater [4]. Going along with the development of more sensitive analytical techniques, the number of publications dealing with pharmaceuticals in waters increased in the last decades [5]. Pharmaceuticals are released into the environment via different ways [3]. For example, the high load of pharmaceuticals in waste water treatment plants (WWTP) is a result of an increase in household pharmaceutical consumption and hospital wastewaters. For a more detailed overview on possible ways of entry, compare ref. [3] and [6]. Many compounds cannot be completely degraded with the commonly applied biological treatment processes during waste water treatment [7,8] and are thus present in the WWTP’s effluents. In Germany, 32 pharmaceutically active compounds can nowadays be detected in WWTP effluents or river waters [9]. Usually the determined residual concentrations are within the ng/L to µg/L range, thus, acute toxic effects are rather unlikely. Nevertheless, data on ecotoxicity upon longtime exposure are hardly available. Therefore, chronic effects on the environment cannot be excluded [10].

Hence, pharmaceuticals have to be removed from waste waters with other processes than biological or mechanical treatment. One example is the oxidative treatment of water. Among these oxidative methods, ozonation has proven to be an efficient method for the removal of pharmaceuticals [7,11,12]. Rosal et al. have shown that the application of 90 µM ozone resulted in a complete disappearance of many pollutants in municipal and industrial wastewaters including frequently prescribed drugs like non-steroidal anti-inflammatory drugs (NSAID) or β-blockers, whereas the efficiency of the biological treatment was below 20% for most compounds investigated [7]. In another study by Huber et al. regarding spiked
wastewater effluents, an applied dose of 2 mg/L ozone led to a decrease of 90–99% for some pharmaceutically active compounds including for example diclofenac and naproxen [11].

The degradation of micro pollutants with oxidative water treatment normally does not result in quantitative mineralization, thus, transformation products (TPs) are formed [13,14]. Since it is possible that TPs are as toxic or even more toxic than the parent compounds [15,16], the formation of TPs is increasingly investigated. However, the analysis and identification of TPs is a complex, time consuming and expensive process.

In this work, the use of electrochemistry (EC) for the fast prediction of TPs is investigated. Currently, this technique has mostly been implemented in studies dealing with the oxidative liver metabolism of pharmaceuticals [17–20]. The application of EC in metabolism studies allows the detection of reactive species in contrast to the conventionally used in vitro incubations such as liver cell microsomes [21,22]. This is mainly due to the absence of interfering biological matrices. For many compounds, the comparison between the instrumental and the biological approach has shown a good correlation [23,24]. In order to evaluate if the complementary use of EC is also applicable to study the generation of TPs in oxidative water treatment, two model compounds were chosen and compared regarding their resulting TPs after electrochemical oxidation or ozone treatment: Diclofenac is a frequently used NSAID and can be freely obtained at the pharmacy. It can be found in surface and ground waters in concentrations up to 2–3 µg/L [25,26]. The prescription drug metoprolol is detected in lower concentrations in surface waters and only rarely in ground waters [27]. However, the prescriptions for this β-blocker have increased by 90% during the past ten years in Germany [28]. Thus, it might become even more environmentally relevant in the future. Following the guidelines for risk assessment of the EMEA (European Medicines Agency, 2005), the State of North Rhine-Westphalia’s environmental agency (LUA NRW) classifies a compound as environmentally relevant if it is detected in surface waters in concentrations higher than 0.01 µg/L. This is the case for both selected model compounds [2].

Ozonation TPs of diclofenac and metoprolol have been previously investigated, so that not only a comparison of TPs with own experiments is possible, but also with literature data. Benner et al. studied the degradation of metoprolol at two different pH values and detected a total number of 23 TPs. For the majority of these TPs, they could propose structures based on detailed MS fragmentation experiments [29]. TPs of diclofenac have been investigated by Sein et al. [30]. In their work, six TPs could be detected, from which three, however, remained unidentified. Zwiener et al. compared the degradation efficiency for the application of ozone and the peroxone process for the pharmaceuticals clobifacic acid, ibuprofen and diclofenac by means of GC/MS [31]. The removal efficiency for diclofenac was almost quantitative (~97% for ozone and 99.8% for the peroxone process) but no degradation products could be detected with the GC/MS approach. In contrast, Coelho et al. were able to detect 18 different TPs after ozonation using LC/ToF-MS [13].

The oxidation of compounds during ozonation can occur via ozone or OH• radicals or a combination thereof. Ozone shows a high selectivity during oxidation reactions. It predominantly reacts with activated aromatic rings, double bonds and amine groups. The reaction of ozone with natural organic matter present in wastewater yields OH• radicals, which can then react with a wider variety of functional groups than ozone. In many cases the rate constant of such radical reactions is much higher than that of the first mentioned selective reaction with ozone. However, the degradation of pollutants via OH• radicals is very inefficient due to the scavenging effect of the water matrix [32].

If the comparison of TPs generated electrochemically with TPs formed after treatment with ozone yields a good correlation, then EC might be implemented in future studies for investigation of the oxidative behavior of pollutants during ozonation. For electrochemical oxidations, the generation of OH• radicals at the surface of boron doped diamond (BDD) electrodes has been reported in the literature [33]. Therefore, ozonation and electrochemical oxidation may yield similar transformation products. Until now, a synoptic comparison of both approaches has not been carried out and should therefore be investigated.

2. Experimental

2.1. Chemicals

Diclofenac sodium salt, metoprolol tartrate salt, N,N-dimethyl-p-nitrosoaniline (RNO) and p-chlorobenzoic acid (pCBA) were ordered from Sigma Aldrich (Steinheim, Germany). 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO, >96%) and potassium dihydrogen phosphate were purchased from Fluka (Buchs, Switzerland). Potassium phosphate was from Riedel-de Haën (Seetze, Germany). Formic acid (FA) was ordered from Th. Geyer GmbH & Co. KG (Renningen, Germany), and acetonitrile (ACN, gradient grade) was purchased from VWR (Darmstadt, Germany). Oxygen (>99.9%) was ordered from Liquid Air. Water was purified with an Aquatron 4000D system (Barlowlow Scientific, Nemours, France) prior to use.

2.2. Electrochemical oxidation and chromatographic separation of transformation products

For the comparison of ozone treated and electrochemically oxidized solutions, a liquid chromatographic separation was carried out. With respect to the electrochemically oxidized samples, an online setup was used: The analyte (5 × 10–5 M in 10 mM phosphate buffer, pH 8) was passed through a preparative electrochemical cell (flow: 20 µL/min) and oxidized at a BDD working electrode (PrepCell, Antec Leyden, Zoeterwoude, The Netherlands). The effluent from the oxidation in the cell was collected in an injection loop (5 µL), which was mounted on a six port switching valve. By switching the valve, the solution was injected onto the column. For diclofenac a C18 column (Hypersil Gold, 150 × 2.1 mm, 3 µm, Thermo Fisher Scientific, Bremen, Germany) was used, whereas metoprolol and its TPs were separated on a C8 column (Zorbax Eclipse XDB-C8, 150 × 4.6 mm, 5 µm, Agilent, Büblingen, Germany). The LC system was from Antec Leyden and comprised two LC 100 pumps, an OR 110 organizer rack with a degasser and a pulse damper, an AS 100 autosampler, a Roxy potentiostat and a column oven. The TPs were detected with a time-of-flight (ToF) mass spectrometer (micrTOF, Bruker Daltonics, Bremen, Germany), equipped with an ESI source in the positive (for metoprolol) and in the negative (for diclofenac) ionization mode. For detailed mass spectrometric and chromatographic parameters see supplementary (Table 1 and Table 2).

2.3. Preparation of ozone samples

An aqueous ozone stock solution was prepared by purging an ozone enriched gas through ice cooled pure water. At stationary conditions a steady state concentration of ≈1 mM ozone could be achieved. This solution was used to dose specific amounts of ozone to the samples. Therefore glass syringes with stainless steel cannulas were used. In order to maintain a constant ozone concentration, continuous purging with the ozone enriched gas was necessary. Ozone was generated by an ozone generator purchased from BMT Messtechnik Berlin (Phiulaqua 802x) with oxygen as feed gas.

In order to investigate the effect of OH• radicals on the degradation of the parent compounds, experiments were performed in presence and absence of a radical scavenger (tert-butanol). The
buffer and concentration of the respective parent compound was the same as for the electrochemical approach.

2.4. LC-MS/MS measurements

For a deeper insight into the formation of TPs, fragmentation was carried out for several TPs. Therefore the following LC/MS instrument was used: The LC part was from Shimadzu (Duisburg, Germany) and included two LC pumps (LC-20AD) with a degasser (DGU-20A3), an autosampler (20AC HT), a column oven (CTO-20AC) and a controller unit (CBM-20A). After the chromatographic separation, selected TPs were fragmented using an ion trap MS (esquire6000, Bruker Daltonics, Bremen, Germany). For metoprolol, the fragment spectra were also obtained by higher energy collisional dissociation (HCD, 25 eV) on an Orbitrap MS (Exactive, Thermo Fisher Scientific, Bremen, Germany) in order to gain exact masses for some fragments. The LC system used in this approach was also from Thermo Fisher Scientific and comprised an autosampler and an Accela 600 HPLC pump. For the detailed mass spectrometric parameters please refer to the supplementary (Table 3 and Table 4).

2.5. Spin trapping with p-chlorobenzoic acid

In order to evaluate whether OH• radicals are generated during the electrochemical oxidation under the applied conditions, a spin trapping agent was used to enable an indirect detection of OH• radicals. Such trapping compounds have to fulfill the prerequisite of being inert towards anodic oxidation, but readily react with OH• radicals. The electrochemical stability of three different compounds was tested: N,N-dimethyl-p-nitrosoaniline (RNO) [34,35], 5,5-dimethyl-1-pyrrole-N-oxide (DMPO) [33,36] and p-chlorobenzoic acid (pCBA) [37–39], which all react fast with OH• radicals (k > 10³ M⁻¹ s⁻¹). Current voltage diagrams were recorded for the oxidation of these compounds and compared to the oxidation of the pure buffer solution. This has been done in order to assess if the trapping agents themselves are transformed through a direct electrochemical oxidation. Accordingly, it was found that RNO and DMPO were unsuitable for OH• radical trapping (see supplementary Figs. 1–3). Finally, pCBA was chosen, oxidized at different oxidation potentials, and analyzed by the online-LC/MS setup described in section 2.2. The reaction was followed by means of ESI(−) MS detection (m/z 155), pCBA and its oxidation products were separated on a C8 column (Zorbax Eclipse XDB-C8, 150 × 4.6 mm, 5 µm, Agilent, Böblingen, Germany) using isocratic conditions (0.1% formic acid/ACN, 50/50) at a flow rate of 0.5 mL/min.

3. Results and discussion

3.1. Comparison of transformation products after ozone treatment and electrochemical oxidation

The scope of the present study is to evaluate, if the coupling of electrochemistry, liquid chromatography, and mass spectrometry is a valuable tool for the rapid and easy elucidation of transformation products in ozone transformation studies. During ozone treatment of water samples, OH• radicals play an important role in the formation of TPs. In order to compare electrochemical oxidation with the ozonation process suitable conditions for the electrochemical oxidation had to be determined: A BDD electrode was used as working electrode, since as a non-active electrode, they are more effective towards the generation of OH• radicals than active electrodes such as those of platinum [33]. Furthermore, the adsorption of non-polar compounds is low on BDD electrodes. This is important because the sample solution contained no organic modifier like ACN, which would prevent compounds from adsorption on the surface. ACN or acetate buffer cannot be used in this study, because they readily react with OH• radicals [37,40].

3.2. Metoprolol

To study the generation of TPs, metoprolol was treated with ozone concentrations ranging from 10 to 70 µM and oxidized electrochemically at varying potentials. Regarding ozone samples, most TPs were found in those samples treated with 70 µM O₃. For the electrochemical approach, 1.25 V vs. Pd/H₂ turned out to be the best suited potential to generate a maximum amount of TPs. Although the increase of the potential up to 2.0 V resulted in a complete disappearance of metoprolol, no detectable TPs were generated at all. Fig. 1 shows the extracted ion chromatograms of those TPs which are detected during ozonation with 70 µM O₃ (with and without radical scavenger t-BuOH) or during electrochemical oxidation and the respective liquid chromatographic separation. In order to display all TPs in a representative manner, the intensity of metoprolol was divided. The extent of degradation of metoprolol was estimated to be about 45–54% in presence of ozone and 61% for EC, based on the initial peak area. Five of the TPs (M1–M5) were detected at least in two approaches, whereas M6 (N-dealkylation) was generated in every approach. These TPs are highlighted in black in the chromatogram and labeled M1–M6. Their respective structures are depicted in Fig. 2. The corresponding sum formulae and the mass deviation are listed in Table 5 in the supplementary. The exact masses and retention times for the additionally generated TPs, which are displayed in grey, can be derived also from the supplementary (Fig. 4 and Table 6). The product spectrum of the electrochemical approach is quite similar to that of the ozonation without the addition of a radical scavenger. This might indicate that in both approaches OH• radicals play an important role in the oxidation process. However, for the electrochemical oxidation no water oxidation and OH• radical generation should be expected at the applied potential of 1.25 V [41]. Thus, it is likely that the identified TPs M1–M6 were formed through direct electrochemical oxidation. The comparison with literature data shows.

Fig. 1. Comparison of liquid chromatographic separations (LC/ESI-ToF-MS) of TPs of metoprolol generated during ozonation (70 µM O₃, chromatogram above), ozonation in presence of a radical scavenger (70 µM O₃ × 50 mM t-BuOH, chromatogram in the middle) or electrochemical oxidation (EC, chromatogram below). All extracted ion chromatograms of TPs are shown, while matching TPs are depicted in black. TPs which could only be generated in one single approach are colored grey.
good agreement: The TPs found in this work were also detected by Benner et al. [29]. However, they detected even more TPs by carrying out experiments in an ozone solution at pH = 3. At acidic pH the amine group of metoprolol is protonated, so that ozone will rather react with the aromatic ring than with the amine function, yielding additional products originating from a ring cleavage reaction [29]. In this study, only a buffer with a pH of 8 was used, so that the number of detectable TPs is smaller. The proposed structures of the detected TPs are shown in Fig. 2. The structures are based on the determination of exact masses and MS fragmentation experiments.

Metoprolol can be modified either at the secondary amine (N-dealkylation, M6), or at the side chain of the aromatic ring (M1–M5). It should be noted that the TP M5 was generated by both electrochemical oxidation and ozonation, but its generation has not been reported in literature for the latter method. The fragment spectrum of M5 is depicted in Fig. 3 (fragment spectrum above).

The MS/MS spectrum of metoprolol is also shown in Fig. 3 (fragment spectrum below). The loss of 42 amu from M5 yielding a fragment with m/z 196 reveals the presence of an intact secondary amine. The same fragmentation can be observed for metoprolol which gives a fragment with m/z 226. The fragment m/z 116 indicates that either the aromatic ring or its side chain has been modified, since this fragment is observed for metoprolol as well. In comparison to the MS/MS spectrum of metoprolol, no loss of 48 amu (loss of methanol and water) is detected, which would lead to a fragment with m/z 218. Thus, one may conclude that the side chain must have been modified. The same structure for M5 which is shown in Fig. 3 has been postulated by Slegers et al., who detected M5 after electron-beam and gamma irradiation of a metoprolol solution [42]. The MS/MS spectrum obtained in their work is very similar to the spectrum shown in Fig. 3. MS/MS spectra of the other TPs found are given in the supplementary (Figs. 5–8). For TP M1 no MS/MS spectra could be obtained, since its intensity was insufficient for fragmentation.

3.3. Diclofenac

As for metoprolol, most TPs were detected in those samples treated with ozone at the maximum concentration of 70 μM. The electrochemical oxidation generated the highest amount of TPs at potentials of 1.5 V and 2.0 V vs. Pt/H2. For diclofenac, the extent of degradation has also been estimated based on the initial peak area of the parent compound. It was found to be 50% for the ozone samples or nearly quantitative for the electrochemical oxidation at a potential of 1.5 V. Fig. 4 shows the LC chromatograms, which were obtained after treatment of a diclofenac solution with ozone or electrochemical oxidation, respectively. TPs formed in ozonation and after electrochemical oxidation are presented in black. Their respective structures are presented in Fig. 5. The other TPs, which were detected only in one of the approaches, are depicted in grey.

The corresponding exact masses and their mass deviations are listed in Table 7 in the supplementary information. It should be noted that in Fig. 4 the extracted ion chromatogram of the parent compound is adjusted to the scale of the other compounds in order to show all generated TPs in a representative manner. In the electrochemical approach, diclofenac was almost quantitatively oxidized, so that its remaining intensity had to be multiplied by a factor of 10.
Diclofenac is hydroxylated at the aromatic ring (D1) and subsequently dehydrogenated yielding the corresponding quinone imine (D2). In the ESI-interface, however, the quinone imine is decarboxylated so that it is detected as m/z 264 (D2−CO2) (see Fig. 5). This observation had also been made in previous work of the authors [17]. D1 and D2 were also described in a transformation study published by Sein et al. [30]. In contrast to this study, the variety of TPs in this present work is smaller for the ozone samples. Sein et al. could additionally detect 2,6-dichloroaniline and three TPs with a m/z ratio of 160, which, however, remained unidentified. In order to exclude that the ionization may be responsible for the lack of the TPs, the samples were also analyzed in the positive ionization mode, but no additional peaks could be detected (data not shown).

D1 and D2 are described in other publications as well: Perez-Estrada et al. detected both TPs after photo-Fenton reaction [43]. Forrez et al. observed these TPs after treatment with biologically produced manganese oxide [44] and Coelho et al. observed both TPs after ozonation without the addition of a radical scavenger [13]. The chromatogram in Fig. 4, which was obtained after electrochemical oxidation, shows other TPs than D1 and D2. Increasing the potential up to 2.0 V resulted in a complete disappearance of D1 and D2, but simultaneously an even wider product range of additional eleven TPs occurred. The chromatograms at both oxidation potentials are displayed in Fig. 6.

Corresponding sum formulae and exact masses are summarized in the supplementary information in Table 8. None of these TPs were found by Sein et al. [30]. In comparison with other publications, four of the found TPs had already been mentioned in literature: D3 (m/z 177) [14,45,46], D5 (m/z 280) [13,17,43], D12 (m/z 282) [17] and D10/D13 (m/z 296) [13]. D5 (m/z 280) and D12 (m/z 282) were already detected in previous work [17]. They are most likely obtained by electrochemical decarboxylation of diclofenac, the subsequent nucleophilic addition of water (D12) and a further dehydrogenation (D5) related to a so called non-Kolbe reaction [47]. Coelho et al. also found D5 by means of LC/ESI(+)-ToF-MS after ozonation [13]. The same accounts for Perez-Estrada et al. who found D5 upon degradation via a photo-Fenton reaction [43]. D3 (2,6-dichlorhydroquinone, m/z 177) has also been detected by several authors after oxidative treatment of a diclofenac solution (UV/H2O2, sonolysis, anodic oxidation) [14,45,46]. The samples in this work were analyzed with LC/ESI-MS (negative ionization). A peak for D3 is clearly visible in the chromatogram depicted in Fig. 6, but fragmentation with the ion trap MS led to no detections of fragments at all. The LC/MS/MS analysis was repeated in the positive ionization mode, but D3 cannot be detected in this ionization mode. Therefore, the postulated structure is based on the sum formula.

![Fig. 4. Comparison of the liquid chromatographic separations (LC/ESI-ToF-MS) of the TPs of diclofenac generated during ozonation (70 µM O3, chromatogram above), ozonation in presence of a radical scavenger (70 µM O3 + 50 mM t-BuOH, chromatogram in the middle) or electrochemical oxidation (EC, chromatogram below). All extracted ion chromatograms of TPs are shown. Matching TPs are depicted in black while TPs which could only be generated in one single approach are colored grey.](image-url)

![Fig. 5. Proposed structures for TPs. Above: TPs which were generated during the O3 and the EC experiment (peaks that are depicted in black in Fig. 4, D1, D2−CO2); below: TPs, which are only generated in the electrochemical approach and which were already reported in literature (D3, D5, D12, D10/D13).](image-url)
derived from the exact mass (C$_{14}$H$_{14}$O$_2$, 176.9511, mass deviation 3.1 ppm), the determined double bond equivalents and the isotopic pattern, which indicated the presence of two chlorine atoms. For TPs, which were detected with the ion trap MS the MS/MS spectra and corresponding structures are listed in the supplementary (Figures 9–14). The TPs D10/D13 were reported by Coelho et al. after ozonation in absence of a radical scavenger [13]. Thus, it can be assumed that OH$^*$ radicals are involved in the formation of these TPs. After electrochemical oxidation two peaks were obtained for the extracted mass trace of m/z 296 (LC/ESI(−)ToF-MS). The MS/MS spectrum for the first peak (D10) was achieved by fragmentation on an ion trap mass spectrometer and is displayed in Fig. 7. Some losses of CO or HCl were observed. However, these did not give much structural information. Since no –COOH amu fragment could be observed, which is characteristic for a carboxylic acid moiety, this functional group must have been modified through the anodic oxidation. Normally, the electrochemical oxidation of diclofenac leads to hydroxylation in the upper aromatic ring at 5 position [17,48]. The hydroxylation at 4’ position in the lower aromatic ring is hindered due to the two electron withdrawing chlorine atoms. However, the MS/MS spectrum of D10/D13 and more precisely the fragments m/z 121 and m/z 161 indicate that one oxygen must be located in each ring. Under the applied conditions (phosphate buffer, 2.0 V) it might be possible that the observed hydroxylation is related to reactive OH$^*$ radicals, which might be able to attack even unpreferred moieties such as the deactivated aromatic ring of diclofenac. OH$^*$ radicals are generated by the anodic oxidation of water. This leads to the formation of adsorbed OH$^*$ radicals at the electrode surface [33]. The potential necessary for the oxidation of water is highly dependent on the pH of the solution. At pH 8 the process can be initiated applying a potential of 1.6 V vs. Pd/H$_2$ [41]. The oxidation of diclofenac was conducted at 1.5 V and 2.0 V vs. Pd/H$_2$. Thus, it is conceivable that OH$^*$ radicals are involved in the degradation of diclofenac. In a previous work, no hydroxylation at 4’ was observed, but ammonium acetate was used as electrolyte which is reported to be an effective OH$^*$ radical scavenger [37,40]. Since it is difficult to detect OH$^*$ radicals directly, they were detected in an indirect way by the use of a spin-trapping agent (compare 3.2).

### 3.4. Spin trapping with p-chlorobenzoic acid

The OH$^*$ radical is one of the strongest oxidizing agents in water. During the anodic oxidation of aqueous solutions, it is generated at high potentials at the surface of modified BDD electrodes [33]. Due to its high reactivity resulting in low steady state concentrations, a direct detection is hardly possible. Therefore, it can be detected only indirectly by spin trapping with different reagents such as 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) [33,36], N,N-dimethyl-p-nitrosoaniline (RNO) [34,35] or p-chlorobenzoic acid (pCBA) [37–39]. The applicability of these three reagents for OH$^*$ radical trapping was tested using the electrochemical conditions described above. First, a potential ramp was applied to the cell and current-voltage diagrams were recorded. The diagrams showed that both DMPO and RNO are oxidized at lower potentials than that of the anodic oxidation of the aqueous solution (see supplementary Figs. 1–3). Thus, they are electrochemically transformed prior to the generation of OH$^*$ radicals and are not suited for an OH$^*$ radical spin trapping. pCBA was electrochemically stable, so that it was chosen as a suitable trapping agent. pCBA reacts with OH$^*$ radicals by hydroxylation of the aromatic ring. The peak height of pCBA depending on different oxidation potentials ranging from 1.0 to 2.3 V was monitored using LC/ESI-MS in the negative ionization mode (left part of Fig. 8). pCBA (m/z 155) eluted at t$_R$ = 6.7 min. When the oxidation potential was increased to 2.0 V vs. Pd/H$_2$, the peak height of pCBA decreased to about 50% of its initial height (light green chromatogram in Fig. 8). A further increase up to 2.3 V vs. Pd/H$_2$ resulted in a nearly quantitative disappearance of pCBA (chromatogram in pink). Simultaneously, an additional peak was detected at a retention time of t$_R$ = 4.5 min. This peak with m/z 187 corresponded to the doubly hydroxylated pCBA species (+2O pCBA). These data indicate that OH$^*$ radicals are generated at the here applied electrochemical conditions (2.0 V vs. Pd/H$_2$) and can be detected indirectly via degradation of pCBA. This explains some of the TPs for diclofenac, the generation of which is assumed to be related to the presence of OH$^*$ radicals in solution.

In the following, the radical scavenger t-BuOH was added in excess to the electrolyte solution, in order to prove whether the OH$^*$ radical-dependent reaction can be inhibited (right part of Fig. 8). The black line shows the chromatogram for the unmodified pCBA. The dark green chromatogram was recorded after applying an oxidation potential of 2.0 V vs. Pd/H$_2$. The rear chromatogram in light green was obtained after the same potential had been applied in presence of 50 mM t-BuOH. Obviously, the addition of t-BuOH can decrease the extent of hydroxylation of pCBA to at least 50%. However, it remains still unclear, why the scavenging reaction could not be inhibited quantitatively.

Unlike the samples treated with ozone only, the electrochemical oxidation of metoprolol and diclofenac yielded the same TPs independent of the addition of t-BuOH as a radical scavenger even though t-BuOH has been added in large excess over the probe compound (factor 10$^3$). This indicates the formation of other radical species, which might not be scavenged by t-BuOH. However, a
detailed discussion is not possible on basis of the present data and should be directed to future work.

4. Conclusion

In this work, a comparison of transformation products generated by electrochemical oxidation or by ozonation was carried out for two model compounds in order to evaluate if the complementary use of EC is useful in ozone transformation studies. It was shown for the two model compounds metoprolol and diclofenac that both the electrochemical oxidation and the treatment with ozone yielded several identical TPs. For metoprolol, a new TP (MS) could be detected after ozonation. The higher turnover after electrochemical oxidation compared to the ozone samples enabled an easy MS fragmentation of MS for structure elucidation.

Under selected conditions, the electrochemical oxidation allowed to generate OH• radicals, which gave rise to some TPs for diclofenac, which have not been generated by electrochemistry so far.

Initially, it was expected that especially this electrochemical generation of OH• radicals would lead to similar TPs which are formed during ozonation. However, for metoprolol, the application of a potential at which OH• radicals were generated, led to a quantitative degradation, but no detectable TPs were found. In contrast to this, the OH• radical mediated degradation of diclofenac generated four TPs, which were already reported in literature in other ozone transformation studies. Since the formation of TPs in the ozone samples analyzed in this work was low, it cannot be excluded that some TPs were not detected due to an insufficient intensity. The initial question, whether the use of EC allows the prediction of TPs in ozone water treatment may now be answered in the way that the complementary use might be attractive, e.g., for the structure elucidation of individual TPs. The detected pattern of TPs which was generated electrochemically is in good qualitative accordance to the TPs found after ozone treatment. If an online EC/LC/MS setup is established, the chromatograms will be recorded in a moderate time and do not acquire more time than in conventional transformation studies with ozone.

Acknowledgement

The German Federal Environmental Foundation (Deutsche Bundesstiftung Umwelt, DBU, Osnabrück, Germany) is gratefully acknowledged for financial support in form of a Ph.D. scholarship for Helene Faber.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.03.081.

References