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

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ABSTRACT

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.

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

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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 clofibric acid, ibuprofen and diclofenac by means of GC/MS [31]. The removal efficiency for diclofenac was almost quantitative (~97% for ozone and 99.9% 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 (Barloworld 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, Böblingen, 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 dampener, an AS 100 autosampler, a Roxy potentiostat and a column oven. The TPs were detected with a time-of-flight (ToF) mass spectrometer (micrOTOF, 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 (Philaqua 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