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SUCCESSFUL REDUCTION OF PHARMACEUTICALS AND HERBICIDES FROM WATER: FIRST LABORATORY-SCALE RESULTS OF LIFE PharmDegrade PROJECT

Andrej Meglič, Gorazd Lakovič, Tinkara Rozina, Anita Klančar, Jurij Trontelj, Albin Kristl, Robert Roškar, Neža Finžgar, Maja Zupančič Justin, Marko Gerl

SUMMARY: Numerous studies have been published about the presence of pharmaceuticals and phytopharmaceuticals in wastewater, ground and even in drinking waters. They present a significant burden for the environment and may cause health risks for the general population. Biological wastewater treatment plants are usually insufficiently equipped to treat pharmaceuticals. To introduce an efficient and financially viable technology for the removal of pharmaceuticals the demonstration project "LIFE PharmDegrade" has been supported. The technology is based on the advanced oxidation processes. The goal of the preliminary tests was to define degradability rate of 11 different pharmaceutical and phytopharmaceutical compounds and in different concentration ranges and presence of other organic material in drinking water. The selected compounds were subjected to LC-MS/MS analysis. Our results show that BDD exhibited high removal of pollutants, reaching up to 100 % degradation in 60 minutes of treatment. At higher concentration mixtures (100 µg/L per pharmaceutical), the removal capacity was lower. The present study shows that the advanced oxidation processes have a promising potential to be developed into an efficient wastewater treatment device for removal of potentially harmful pharmaceutical and phytopharmaceutical pollutants.

KEY WORDS: Oxidative treatment, Pharmaceuticals, Herbicides, Carbamazepine, Boron doped diamond electrode, LC-MS/MS

USPJEŠNA REDUKCIJA FARMACEUTSKIH PROIZVODA I HERBICIDA IZ VODE: PRVI LABORATORIJSKI REZULTATI PROJEKTA LIFE PharmDegrade

SAŽETAK: Brojne studije izvješćuju o ostacima farmaceutskih i fitofarmaceutskih proizvoda u otpadnim vodama, podzemnim vodama, pa čak i vodi za piće. Oni predstavljaju značajno opterećenje za okoliš i mogu prouzročiti zdravstvene rizike za stanovništvo. Biološki uređaji za pročišćavanje otpadnih voda obično su nedovoljno opremljeni za

pročišćavanje farmaceutskih proizvoda. Pilot projekt "LIFE PharmDegrade" podržan je kako se uvela učinkovita i financijski isplativa tehnologija uklanjanja farmaceutskih proizvoda. Ova se tehnologija zasniva na naprednim procesima oksidacije. Svrha preliminarnih testova bila je definirati stupanj razgradivosti 11 različitih farmaceutskih i fitofarmaceutskih spojeva u različitim rasponima koncentracija i prisutnosti drugog organskog materijala u vodi za piće. Odabrani spojevi podvrgnuti su LC-MS/MS analizi. Naši rezultati pokazuju da dijamantna elektroda dopirana borom (BDD) u visokom stupnju uklanja onečišćujuće tvari, čak do 100% za 60 minuta pročišćavanja. Pri višim koncentracijama mješavina (100 µg/L po farmaceutskom proizvodu), redukcijski kapacitet je bio niži. Ova studija pokazuje da napredni oksidacijski procesi imaju veliki potencijal za razvijanje u učinkovit uređaj za uklanjanje potencijalno štetnih onečišćujući tvari farmaceutskog ili fitofarmaceutskog porijekla iz otpadnih voda.

KLJUČNE RIJEČI: Oksidacijsko pročišćavanje, Farmaceutski proizvodi, Herbicidi, Dijamantna elektroda dopirana borom, LC-MS/MS

1. INTRODUCTION

Modern lifestyle, longer life expectancy, intensive agriculture, livestock production and industrialisation result in use and consequently release of huge amounts of so-called new emerging pollutants, like pharmaceuticals, phytopharmaceuticals, personal care products, pigments and other anthropogenic molecules into the environment (Richardson et al., 2014). Numerous reports on their presence in freshwater and groundwater can be found (Fick et al., 2010). Their harmful effects on human and animal fertility as well as other effects have been confirmed (Cleuvers, 2003; Bolong et al., 2009; Donner et al., 2013). Besides increased use of these complex organic compounds, the reason of their presence in the environment can be found also in insufficient wastewater treatment (Gomez et al., 2007, Deblonde et al., 2011). Existing, predominately biological municipal wastewater treatment (WWT) plants are namely not designed for the removal of complex and biologically persistent organic compounds. High stability of these organic compounds is a consequence of their complex structure. This means that their biological degradation (mineralization) takes place very slowly.

To contribute towards the solving of this issue, the demonstration project "Degradation of pharmaceuticals in wastewaters from nursing homes and hospitals LIFE PharmDegrade" has been supported. The aim of the project is to demonstrate an efficient tertiary WWT system for the removal of persistent organic compounds, placed close to their origin of emergence. Treatment of sewage from hospitals and nursing homes was considered as an appropriate approach, since drugs are applied there in increased amounts. The main approach to the tertiary WWT in the project is an application of electrolytic cell to achieve electrochemical oxidation of complex organic compounds. Advanced electrode material is used, such as boron doped diamond (BDD) and mixed metal oxide electrodes. They enable a formation of hydroxyl ($HO^{\bullet}$) radicals directly from water with the help of electric power (Chen, 2004; Feng et al., 2013). Hydroxyl radicals are very strong oxidants. They rapidly and unselectively react with nearly all organic compounds. Complex organic compounds in water, like residuals of drugs, can be degraded in their presence to carbon dioxide and water, or to smaller molecules, which are less toxic and further easier biologically degradable.

The preliminary tests, presented in the paper, have been conducted with an aim to define degradability rate of pharmaceuticals in different concentration ranges and presence of other organic material. A removal efficiency in terms of concentration reduction of a mixture of 11 pharmaceuticals in two different inflow concentration ranges is presented in the paper and a comparison of efficiency of carbamazepine removal in six different experimental sets with different organic load and concentrations of pharmaceuticals. The carbamazepine has been chosen due to its known difficult biodegradability and frequent occurrence in freshwater surveys (Donner et al., 2013). Further steps of the LIFE PharmDegrade project`s preparatory actions will be oriented in testing of different settings of electrolytic cell (flow rate, current density, power supply mode, electrode material and spacing) presenting the starting point for the design of the pilot plant.

2. MATERIALS AND METHODS

The experiments were performed with bottled drinking water Dana® to which different concentrations of medical drugs, herbicides (referred here as Active Pharmaceutical Substances, APS) and other organic substances (urea, cysteine, microcrystalline cellulose) were added. The standards of APS and organic substances were obtained from standard material (atrazine, carbamazepine, clofibric acid, diclofenac, fluoxetine, metoprolol and simazine from Sigma-Aldrich (Germany), imatinib and bisoprolol fumarate from Sequoia Researcher products (UK), ciprofloxacin and amoxicillin from AppliChem GmbH (Germany). Urea, cysteine and microcrystalline cellulose used to simulate wastewater organic load, were obtained from Sigma. Standard stock solutions of each APS were prepared by dissolving 5 mg of accurately weighed standard in 5 mL methanol (Merck, Germany) to concentration of 1 mg/mL. With further dilution of APS in commercial bottled drinking water Dana® a water mixture solutions of all the APSs was prepared with preferred tested concentrations (10 – 100 μg/L).

The electrochemical water treatment was carried out in a single-compartment electrolytic cell made of Plexiglas in continuous flow mode (Figure 1). The electrode set consisted of one anode and one cathode placed in parallel 2 mm apart. The boron doped diamond (BDD) plate (Diachem, Condias, Germany) with a surface area of 8 cm^2 served as the anode and the same size stainless steel plate as the cathode. The working volume of the electrolytic cell was 1.6 mL. The electrical current was applied using a laboratory DC power supply GPS-4303 (Gwinstek, China) in constant current mode. The current density was 37.5 mA/cm^2. The experiments were performed at room temperature. Peristaltic pump (Masterflex EW-07553-75, Cole-Parmer, US) provided water circulation (50 mL/min) within the experimental set. The processed volume was 500 mL in each run. The samples were collected every 10 minutes during the 60 minutes treatment and stored in glass containers or 96-well microtiter plates in darkness at 8 °C until further analysis. Each treatment was repeated twice.

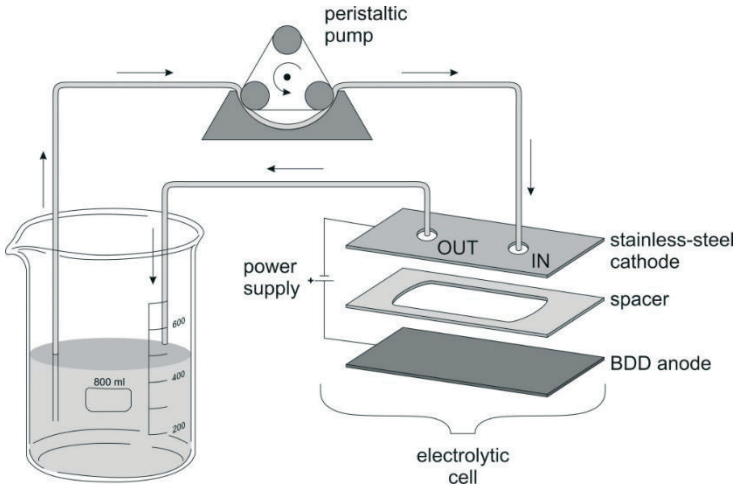

Figure 1. Experimental setup. Electrolytic cell with stainless-steel cathode, spacer and BDD anode was enclosed in Plexiglas housing

Six experimental treatments with different water mixture solutions of APSs and additional common organic substances are presented in the paper. The concentrations of added substances in each experimental treatment are presented in Table 1. The treatments T_{100} and T_{10} are referred to the water solutions with 100 and 10 µg/L of each APS added. The treatments T_{10u}, T_{10cy} and T_{10mc} are referred to water solutions with 10 µg/L of each APS to which urea, cysteine and microcrystalline cellulose were added, respectively. The treatment T_{mix} refers to water solution with APS added in concentrations most often found in the literature reporting on the presence of APS in wastewater (Gomez et al., 2007; Fick e al., 2010; Deblonde et al., 2011).

The concentration of APS in treated (TS) and untreated samples (US) were determined by Liquid chromatography-tandem mass spectrometry (LC-MS/MS) in Agilent 1290 Infinity Ultra high performance liquid Chromatograph coupled to an Agilent 6460 triple quadrupole mass spectrometer (Agilent Technologies, USA). The injection volume was 1µL and 5µL for 100 µg/L and 10 µg/L concentration samples, respectively. The mobile phase A was 0.1% HCOOH in MilliQ water and mobile phase B was acetonitrile. The chromatographic separation was performed on a Kinetex 50 2.1 mm C18 column with 2.6 µm particles by the following gradient: time points: 0-0.5-1.1- 1.2-1.3-2.0-2.5 min, % mobile phase B:5-5-9-40-50-60-60 and flow rates:0.35-0.35-0.35-0.65-0.65-0.65.-0.65 mL/min. Total run time was 3.2 min. Instrument control, data acquisition and quantification were performed by MassHunter Workstation software. The removal efficiency (RE) was calculated as (1).

$$RE\ (\%) = (US - TS)/US \times 100 \quad (1)$$

Chemical oxygen demand (COD) of the prepared synthetic water mixtures was measured by Nanocolor tests 0-29 and 0-26 (Macherey-Nagel, Germany) and spectrophotometrically determined (ISO 15705). Electrical conductivity of the water samples was measured with conductivity sensor Multi 3430 (WTW, Germany).

Table 1. Prepared water solutions with different concentrations of added active pharmaceutical substances (APS) and common organic substances treated in a single compartment electrolytic cell with boron doped diamond anode (T_{100} and T_{10} refer to the water solutions with 100 and 10 μg/L of each APS added; T_{10u}, T_{10cy}, T_{10mc} refer to treatments with added 10 μg/L of each APS, urea, cysteine and microcrystalline cellulose, respectively; T_{mix} refers to water solution with different APS concentrations).

Experimental treatment	**T_{100}**	**T_{10}**	**T_{10u}**	**T_{10cy}**	**T_{10mc}**	**T_{mix}**
Active pharmaceutical substance (μg/L)						
Amoxicillin	100.00	10.00	10.00	10.00	10.00	1.00
Ciprofloxacin						100.00
Metoprolol						1.20
Bisoprolol						0.42
Diclofenac						5.45
Clofibric acid						0.20
Fluoxetine						0.29
Carbamazepine						5.00
Imatinib						1.00
Simazine						0.69
Atrazine						0.037
Common organic substance (mg/L)						
Urea	/	/	90	/	/	/
Cysteine	/	/	/	179	/	/
Microcrystalline Cellulose	/	/	/	/	300	/

3. RESULTS

Based on reports from scientific literature (Gomez et al., 2007; Fick e al., 2010; Deblonde et al., 2011) on commonly occurring APS in wastewater and freshwater resources, 11 different APS have been selected to test their degradation potential in electrolytic cell. Pharmacological groups considered in the test were antibiotics amoxicillin and ciprofloxacin, beta blockers metoprolol and bisoprolol, nonsteroidal anti-inflammatory drug diclofenac, anticonvulsant carbamazepine, antidepressant fluoxetine, anti-cancer drug imatinib and metabolite of antilipidemic agent clofibric acid. The phytopharmaceuticals tested were atrazine and simazine.

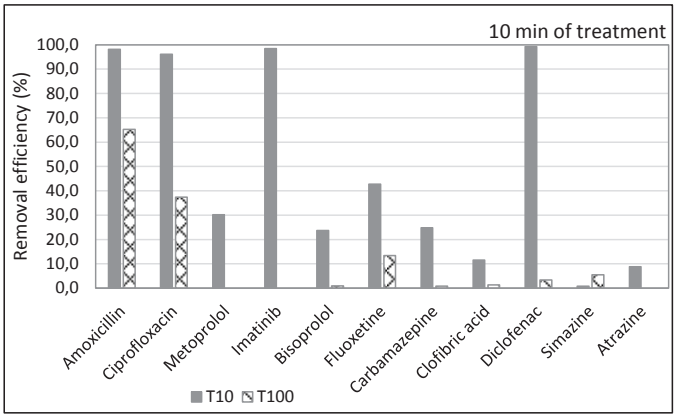

Figure 2. Removal efficiency of active pharmaceutical substances prepared in two concentration mixtures (T_{10} = 10 µg/L and T_{100} = 100 µg/L of each APS diluted in drinking water) after 10 minutes of electrochemical treatment in electrolytic cell with boron doped diamond electrode.

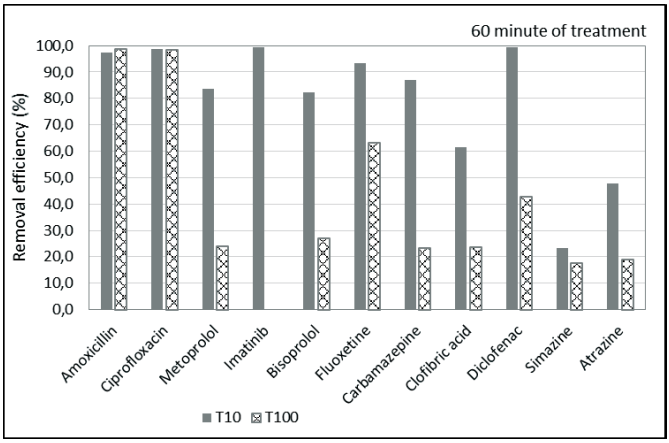

Figure 3. Removal efficiency of active pharmaceutical substances prepared in two concentration mixtures (T_{10} = 10 µg/L and T_{100} = 100 µg/L of each APS diluted in drinking water) after 60 minutes of electrochemical treatment in electrolytic cell with boron doped diamond anode.

The removal efficiency of 11 APS diluted in two synthetic water mixture after 10 and 60 minutes of treatment in the electrolytic cell is presented in the Figures 2 and 3, respectively. The synthetic mixtures were prepared in two concentration ranges of APS, 10 µg/L of each APS (T_{10}) and 100 µg/L of each APS (T_{100}), with consequently different chemical oxygen demand (COD), 200 and 1890 mgO_2/L, respectively. After 10 minutes of electrochemical treatment, four APS (amoxicillin, ciprofloxacin, imatinib, diclofenac) exhibited very high concentration reduction efficiency (> 96%) in T_{10} treatment (Figure 2). After 60 minutes of treatment, also metoprolol, bisoprolol, fluoxetine and carbamazepine

exhibited more that 80% concentration reduction efficiency in T_{10} treatment (Figure 3). The reduction efficiency in T_{100} treatment was much lower; only the concentration of both antibiotics, amoxicillin and ciprofloxacin dropped in a very high percentage (> 98%) after 60 minutes of treatment (Figure 3).

The results proved that the capacity of the electrolytic cell in the given arrangement (BDD/stainless steel electrode, 1.6 mL treatment working volume, current density 37.5 mA/cm^2) was sufficient for the complete degradation of the APS in their inflow concentration of 10 μg/L and COD 200 mgO_2/L after 60 minutes of treatment (Figure 2). More than 60% reduction efficiency for the above listed APS was achieved already after 30 minutes of treatment (data not shown). For the higher mass loads of complex organic compounds, as it was in T_{100} treatment, with COD 1890 mgO_2/L, longer exposure in the electrolytic cell would be needed or higher current densities used. It should be noted that the electric conductivity of the electrolyte (treated water in our case) was 564 μS/cm in average, which presents a low electric conductivity of the electrolyte and hinders the production of hydroxyl radicals. In the real sewage wastewater samples, a higher electric conductivity, around 1000 μS/cm can be expected (Davis, 2010). Lower treatment efficiency of high COD inflow indicates also a need of a sufficient pre-treatment of wastewater in primary and secondary processes.

Three tested APS exhibited low concentration reduction efficiency even at low inflow concentration range (T_{10}) and 60 minutes of treatment, 62% clofibric acid, 48% atrazine and 23% simazine (Figure 3). The degradation potential of clofibric acid has been studied by Sires et al. (2006), who confirmed that electrochemical oxidation with BDD can lead to 100% mineralization in aqueous solution of clofibric acid close to saturation (557 mg/L). In their experiments, this has been reached at higher applied current densities and longer treatment time (360 minutes, 100 mA/cm^2, 89 - 557 mg/L initial clofibric acid concentration) compared to our experiment. Polcaro et al. (2005) discussed the difficulties of atrazine (2-Cl-4-ethylamino-6-isopropylamino-1,3,5-triazine) and simazine (2-Cl-4,6-diamminoo-1,3,5-triazine) herbicides degradation, which degradation intermediate is chemically highly inert cyanuric acid. They improved the electrochemical degradation of herbicides on BDD anode by addition of H_2SO_4 as a supporting electrolyte. In this case, persulphate ions ($S_2O_8^{2-}$) emerge at the anode jointly with hydroxyl radicals. Although the persulphate ions are weaker oxidisers, they are longer-lived, diffuse to the bulk solution and provoke oxidation. Final mineralization of cyanuric acid was successful only under specific current densities and pH conditions.

The degradation potential of carbamazepine, for which relatively high removal efficiency was achieved (87%) at lower inflow concentration ranges (T_{10}) and much lower (23%) at high inflow concentration ranges of APS (T_{100}), was further tested in four other water mixture combinations. The variant of T_{10} was tested with a separate addition of three common organic substances (urea T_{10U}, cysteine T_{10cy} and cellulose T_{10mc}). The sixth treatment (T_{mix}) was performed with a water mixture of APS in concentrations expected in real wastewater with five μg/L of carbamazepine added (Table 1). The degradation of the carbamazepine increased with exposure time to electrochemical oxidation in electrolytic cell in all tested variants (Figure 4). Lowering the overall inflow mass load of APS resulted in increased carbamazepine reduction efficiency, evident from T_{100}, T_{10} and T_{mix} variants on the same Figure. The addition of common organic substances reduced the

removal efficiency of carbamazepine. The carbamazepine degradation was hindered the most by the addition of amino acid cysteine, where only 26% removal efficiency after 60 minutes of treatment was achieved. A lower slowdown of the carbamazepine degradation was caused by the addition of urea and microcrystalline cellulose, achieving 75% and 85% degradation efficiency after 60 minutes of treatment, respectively (Figure 4). The influence of the added common organic substances on the carbamazepine degradation can be explained by the ease of their degradability and therefore the amount of the formed oxidative groups (e.g. hydroxyl radicals) that need to be spent for their degradation (Chen, 2004). The degradation efficiency of pure water solution of the same amount of cysteine as it was in T_{10cy}, was only 14%, while the efficiency of cellulose degradation reached 70% after 60 minutes of treatment in the electrolytic cell, supporting the explanation of the above results. These results indicate again on the need of a prior overall degradability testing of the treated wastewater and achievement of a sufficient reduction of easily degradable organic matter prior to the entrance into electrolytic cell to achieve energy-efficient electrochemical degradation of remained persistent organic compounds.

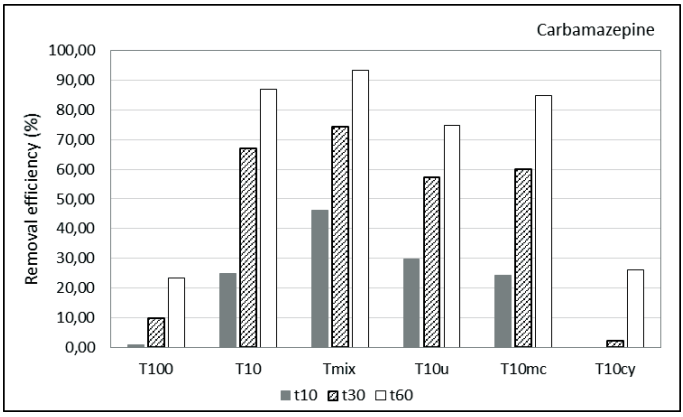

Figure 4. Removal efficiency of carbamazepine in six different water mixtures with added active pharmaceutical substances and common organic substances (described in Table 1) after 10 (t10), 30 (t30) and 60 (t60) minutes of electrochemical treatment in electrolytic cell equipped with boron doped diamond electrode.

CONCLUSION

The laboratory tests in the single compartment electrolytic cell equipped with BDD anode and stainless steel cathode gave optimistic answers on the potential of degradability of pharmaceuticals, which can be expected in sewage and outflows from WWT plants. Additionally, the results on degradability of two herbicides with BDD have been tested. The reduction efficiency of amoxicillin, ciprofloxacin, metoprolol, imatinib, bisoprolol, fluoxetine, carbamazepine and diclofenac was more than 80% from the water mixture with 10 μg/L of their initial concentrations, after 60 minutes of treatment and 37.5 mA/cm^2 current density applied. For the further determination of the pilot plant configuration, the experiments will be dedicated to the testing of other electrode material and their

placement, flow rate, power supply mode and optimisation of the current density. The level of completeness of mineralisation of APS is being followed by COD analysis and degradation metabolites are being followed by liquid chromatography with tandem mass spectrometry. Their presentation exceeds the frames of this paper.

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AUTHORS

Andrej Meglič [a]
Gorazd Lakovič [a]
Tinkara Rozina [b]
Anita Klančar [c]
Jurij Trontelj [c]
Albin Kristl [c]
Robert Roškar [c]
Neža Finžgar [b]
Maja Zupančič Justin [a]
Marko Gerl [a]

[a] Arhel d.o.o., Pustovrhova ulica 15, 1210 Ljubljana-Šentvid, Slovenia, andrej.meglic@arhel.si; gorazd.lakovic@arhel.si; maja.justin@arhel.si; marko.gerl@arhel.si;

[b] Envit d.o.o., Vojkova cesta 63, 1000 Ljubljana, Slovenia, tinkara.rozina@gmail.com; neza.finzgar@envit.si

[c] University of Ljubljana, Faculty of Pharmacy, Aškerčeva 7, 1000 Ljubljana, Slovenia, jurij.trontelj@ffa.uni-lj.si; robert.roskar@ffa.uni-lj.si; anita.klancar@ffa.uni-lj.si; albin.kristl@ffa.uni-lj.si