Available online at www.sciencedirect.com

ScienceDirect

Energy Procedia 153 (2018) 461–465

Energy Procedia

www.elsevier.com/locate/procedia

5th International Conference on Energy and Environment Research, ICEER 2018

# Pharmaceutical compounds electrotreatment by Pt anodes and effect on synaptic function

Morgana Bosio[a,d,] *, Bianca Souza[a,d], Enrico Saggioro[b], Marcia Dezotti[a], João Paulo Bassin[a], Emília Quinta-Ferreira[c] and Rosa Quinta-Ferreira[d].

[a]*Federal University of Rio de Janeiro, COPPE – Chemical Engineering Program, Centro de Tecnologia – Cidade Universitária – Bloco G – Sala 115, Rio de Janeiro – 21941-909, Brazil*

[b]*Sanitation and Environment Health Department, Sergio Arouca National School of Public Health, Oswaldo Cruz Foundation, Avenida Leopoldo Bulhões, 1480, Rio de Janeiro – 21041-210, Brazil*

[c]*Center for Neurosciences and Cell Biology and Physics Department, University of Coimbra, Rua Larga, Coimbra – 3004-516, Portugal*

[d]*CIEPQPF – Chemical Engineering Processes and Forest Products Research Center, Department of Chemical Engineering, University of Coimbra, Rua Sílvio Lima, Coimbra – 3030-790, Portugal*

**Abstract**

Thousands of compounds are used and disposed of every day and many of them are not degraded in conventional treatment plants. It is necessary an alternative to eliminate these compounds. This can be done through electrooxidation technology, which was applied in this work to a mixture of pharmaceutical compounds including alprazolam (ALP), clonazepam (CLP), diazepam (DZP), lorazepam (LZP) and carbamazepine (CBZ) at 100 $\mu g.L^{-1}$. The mixture was studied with different types of electrolytes and the neurotoxic effect of the treatment was evaluated. The best result was obtained with NaCl (0.5 $g.L^{-1}$), leading to complete degradation of CLP, LZP and CBZ.



*Keywords:* electrooxidation; benzodiazepines; hippocampal; neurotoxicity

* Corresponding author. Tel.: +351-239-798700; fax: +351-239-79803.
*E-mail address:* morganabosio@yahoo.com.br


10.1016/j.egypro.2018.10.012

## 1. Introduction

A few decades ago it was possible to find many compounds in rivers and oceans, mainly plastics, discarded by humans. These visible compounds directly affect aquatic animals, in many cases leading to their death. However, there are thousands of invisible compounds that can affect the entire food chain, being released into waterbodies every day. These contaminants, ranging from beauty and cleaning products to certain medicines, are giving rise to an emerging concern. Many of these compounds, found under concentrations of $ng.L^{-1}$ to $\mu g.L^{-1}$ [1], can have a cumulative action in the environment.

A major problem of some of these substances is that they are recalcitrant and bioaccumulative. Therefore, they are able to remain in the environment for a long time, affecting both animals and plants [2]. This way, the water quality of many rivers has been affected by the discharge of both untreated and treated industrial and domestic wastewaters? [3]

Among the different contaminants of emerging concern, benzodiazepines that are used for treatment of the central nervous system as sedative, muscle relaxant and anxiolytic, play an important role. Some studies report the existence of these compounds in rivers from many parts of the world [4-6]. Another compound that proves to be very influential is carbamazepine, an antiepileptic drug used in the treatment of seizure disorders. Its biodegradation cannot be performed in conventional sewage treatment plants. As a consequence many studies show that it is present in rivers and at the end of a biological treatment [7-9].

Since the mentioned compounds are not degraded by conventional methods it is necessary to employ processes that provide for the degradation of such recalcitrant compounds. As an alternative, advanced oxidative processes, which are characterized mainly by the degradation through highly reactive radicals with the capacity to degrade more complex molecules, are used [10]. Electrooxidation is one of the advanced oxidation processes that can be used to degrade compounds like benzodiazepines and carbamazepines. In this process, in which the anode may be Pt, BDD (boron-doped diamond), $IrO_2$ or $RuO_2$, the hydroxyl radicals, generated from water discharge at the surface of the anode, oxidize the organic compounds [11-13].

The main objective of this work was to analyze the effect of Ti/Pt anode material in the oxidation of a synthetic mixture of five pharmaceutical compounds, consisting of ALP, CZP, DZP, LZP and CBZ, that are usually present in water treatment plants (WTPs). Besides that, the neurotoxicity of a treated mixture was assessed in rat brain slices, using a fluorescent reactive oxygen species (ROS) indicator.

## 2. Material and methods

### *2.1. Chemicals and setup*

ALP, CZP, DZP, LZP and CBZ drugs were acquired from commercial sources and used as obtained. They were spiked at the concentration of 100 $\mu g.L^{-1}$. HPLC-grade acetonitrile was purchased from Sigma-Aldrich. For the brain slices experiments extracellular artificial cerebrospinal fluid (ACSF) was prepared with the following composition (in mM): 124 NaCl, 3.5 KCl, 24 $NaHCO_3$, 1.25 $NaH_2PO_4$, 2.0 $MgCl_2$, 2.0 $CaCl_2$ and 10 D-Glucose, pH 7.4. All chemicals were obtained from Sigma-Aldrich and used as received (analytical grade) without any further purification. The ROS indicator $H_2$DCFDA was purchased from Life Technologies. Ultrapure water was obtained by using an Interlab Direct-Pure purification system. The reactor, with a volume of 500 mL and magnetic stirring, contained electrodes composed of Ti/Pt (anode) and stainless steel (cathode).

The concentrations of benzodiazepines and of carbamazepine were determined by HPLC (High performance liquid chromatography) from Shimadzu, with a diode-array detector, flow rate of 1 $mL.min^{-1}$ and column C18 at 25 ºC. The initial gradient of the mobile phase was 70:30 of acetonitrile:water.

A fluorescence microscope (Zeiss Axioskop) equipped with a water immersion objective (40x, N.A. 0.75, 1.6 mm working distance) was used for the detection of the optical signals. Light from a tungsten/halogen lamp (12 V, 100 W) was selected using an excitation wavelength of 480 nm (10 nm bandwidth) being emission collected for wavelengths above 500 nm. The fluorescence signals were acquired through a photodiode system (Hammamatsu K2G 1336, 1.0 $mm^2$) using a 16 bit analog to digital converter (National Instruments) and the data were processed using the Signal Express® software (National Instruments). The optical signals represent the ratio of the fluorescence

intensity (F) over the baseline fluorescence ($F_0$, s the average of the first 10 points), after correction for the autofluorescence determined from non-incubated slices.

### *2.2. Toxicity tests procedure*

Toxicity assays were conducted in hippocampal slices (400 μm thick) from pregnant female Wistar rats (8-16 weeks old, 14-18 days of gestation), at the mossy fiber synaptic system. The slices were incubated in the oxygenated ACSF solution containing 20 μM of $H_2$DCFDA (ROS indicator), for 1h, at room temperature. After that period, the slices were transferred to the control oxygenated ACSF medium until they were used. For this purpose, the incubated slices were transferred to the experimental chamber inserted in the microscope setup, being continuously perfused with the desired medium, at 32º C and a flow rate of 1.5 mL.min$^{-1}$. Following the initial application of ACSF, the treated solution, containing the oxidized pharmaceutical compounds, was perfused for 30 min. Then, the ACSF medium was perfused again, also for 30 min, to investigate the ability of the synaptic activity to return to the initial condition. Each plotted point is an average of 100 points collected with a frequency of 1.6 Hz. All experiments were run in triplicate.

## 3. Results and discussion

### *3.1. Electrotreatment studies*

#### *3.1.1 Compounds degradation*

The degradation (C/Co) and the percentage of degradation of ALP, CLP, DZP, LZP and CBZ, both mixed and applied individually, are shown in Figure 1 (A and B), after 15 min. In the mixture (A) all compounds were degraded between about 70 and 85 %, being the behavior of the degradations similar in both types of experiments. The degradation was higher when the compounds were used individually (B) than when they were mixed. In the first case, LZP and CBZ were completely removed, while ALP, CLP and DZP were partially degraded (Figure 1, right side). The higher degradation observed for the individual solutions may be explained by the fact that there is less organic matter in the media. Thus, all radicals produced by the electrotreatment were used to degrade a single compound, while in the mixture several compounds had to be degraded by the same amount of radicals. These different levels of degradation are in agreement with previous results [14-15].

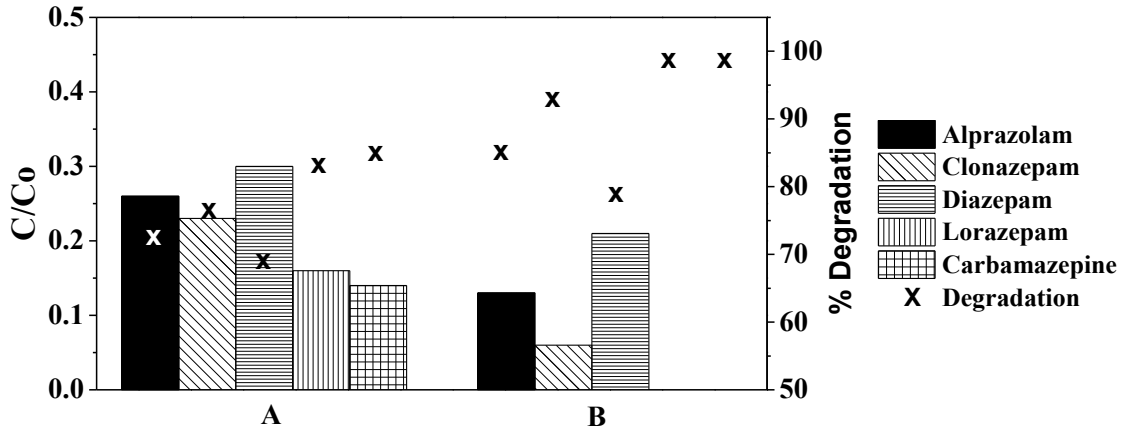


Fig. 1 Degradation and percentage of degradation of ALP, CZP, DZP, LZP and CBZ in ultrapure water. Bar graph for comparison of the degradation (C/Co) and of the percentage of degradation (x) of the mixed (A) and individually applied (B) compounds. T = 25º C, pH = 7, t = 15 min, I = 0.05 A, NaCl = 1.5 g.L$^{-1}$.

### *3.1.2. Effect of the electrolyte*

Figure 2 shows the degradation (C/Co) and the percentage of degradation of ALP, CZP, DZP, LZP and CBZ with different electrolyte types (NaCl and $Na_2SO_4$). The results indicate that NaCl was better at degrading the compounds. With NaCl, the CZP, LZP and CBZ pharmaceutical agents were completely degraded after 30 minutes, while with $Na_2SO_4$ the degradation of all compounds was between approximately 12 and 35 %. This difference in the amount of degradation may be explained by the formation of different oxidizing species [16].

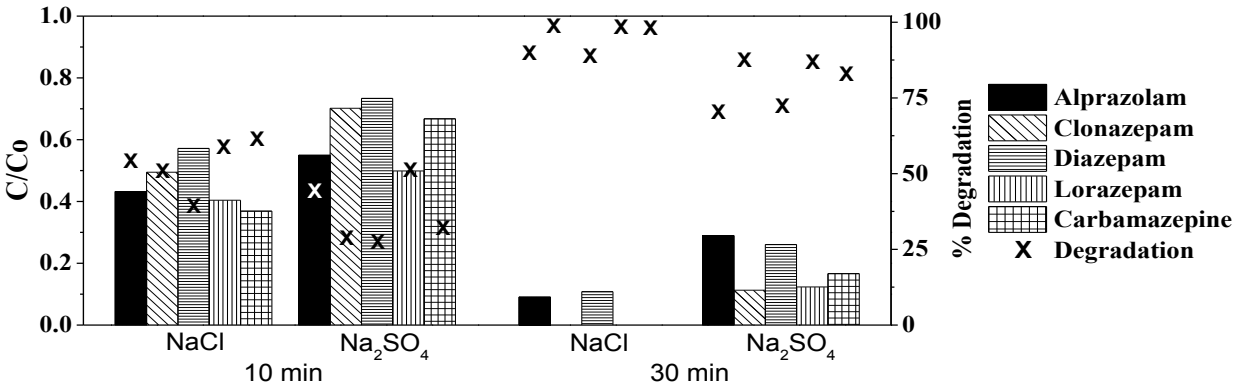


Fig. 2. Degradation and percentage of degradation of ALP, CZP, DZP, LZP and CBZ in ultrapure water. Bar graph representing the degradation (C/Co) and the percentage of degradation (x) with the electrolytes NaCl (0.5 $g.L^{-1}$) and $Na_2SO_4$ (0.5 $g.L^{-1}$), for different electrooxidation reaction times, 10 min and 30 min. T = 25º C, pH = 7, I = 0.05 A.

### *3.2. Neurotoxicity studies*

The effect of the mixture of the pharmaceutical compounds used (100 $\mu g.L^{-1}$, each) treated by the electrooxidation process, in neuronal metabolism, was evaluated in brain slices. For this purpose, fluorescence ROS signals were detected at the hippocampal mossy fiber synapses from slices incubated with the ROS indicator $H_2$DCFDA, (Figure 3). In the normal medium (ACSF) the fluorescence values of the ROS signal remained stable. When the treated solution was perfused, the ROS signal decreased significantly. Upon returning to the control (ACSF) medium, the fluorescence values increased towards the baseline, reaching afterwards a potentiated level. These results suggest that, in the presence of the treated water, there is less ROS production.

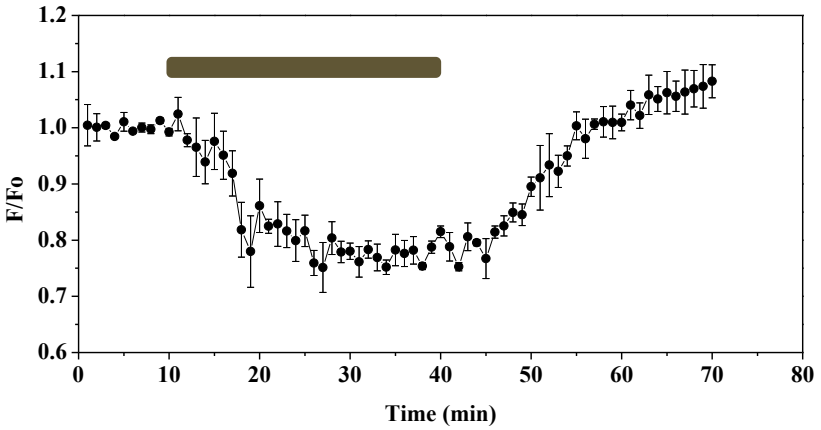


Fig. 3. Electrotreated water depresses the fluorescence ROS signals from brain slices. The data points represent the mean ± standard deviation of normalized signals (F, fluorescence intensity; Fo, baseline fluorescence). The bar indicates the period of the treated water perfusion.

## 4. Conclusions

The individual pharmaceutical compounds (alprazolam, clonazepam, diazepam, lorazepam and carbamazepine) were better degraded by the electrotreatment (Ti/Pt) than when mixed, probably due to the fact that, in the first case, there is less organic matter to be eliminated. The use of different electrolytes (NaCl, $Na_2SO_4$) lead to different amounts of drug degradation, possibly because the nature of the electrolytes generates different oxidative species. The treated water mixture caused a depression of the brain slices ROS signal in agreement with the idea that, in this medium, there was less physiological ROS formation.

## Acknowledgements

The authors acknowledge financial support of CNPq and CAPES. The neuronal studies were funded by the strategic project UID/NEU//04539/2013.

## References

[1] James, C. Andrew et al. “Evaluating Contaminants of Emerging Concern as Tracers of Wastewater from Septic Systems.” *Water Research* 101 (2016): 241–251.
[2] Wang, Shizong, and Jianlong Wang. “Carbamazepine Degradation by Gamma Irradiation Coupled to Biological Treatment.” *Journal of Hazardous Materials* 321 (2017): 639–646.
[3] Mohapatra, D. P. et al. “Analysis and Advanced Oxidation Treatment of a Persistent Pharmaceutical Compound in Wastewater and Wastewater Sludge-Carbamazepine.” *Science of the Total Environment* 470–471 (2014): 58–75.
[4] Mutiyar, Pravin K, Sanjay Kumar, and Atul Kumar. “Ecotoxicology and Environmental Safety Fate of Pharmaceutical Active Compounds (PhACs ) from River Yamuna , India : An Ecotoxicological Risk Assessment Approach.” *Ecotoxicology and Environmental Safety* 150.January (2018): 297–304.
[5] Fick, Jerker et al. “Chemosphere Screening of Benzodiazepines in Thirty European Rivers.” *Chemosphere* 176 (2017): 324–332.
[6] Wu, Minghong et al. “Occurrence and Fate of Psychiatric Pharmaceuticals in the Urban Water System of Shanghai, China.” *Chemosphere* 138 (2015): 486–493.
[7] Luo, Yunlong et al. “Science of the Total Environment A Review on the Occurrence of Micropollutants in the Aquatic Environment and Their Fate and Removal during Wastewater Treatment.” *Science of the Total Environment, The* 473–474 (2014): 619–641.
[8] Ramaswamy, Babu Rajendran et al. “GC-MS Analysis and Ecotoxicological Risk Assessment of Triclosan, Carbamazepine and Parabens in Indian Rivers.” *Journal of Hazardous Materials* 186.2–3 (2011): 1586–1593.
[9] González Alonso, Silvia et al. “Pollution by Psychoactive Pharmaceuticals in the Rivers of Madrid Metropolitan Area (Spain).” *Environment International* 36.2 (2010): 195–201.
[10] Garza-campos, Benjamin et al. “Salicylic Acid Degradation by Advanced Oxidation Processes . Coupling of Solar Photoelectro-Fenton and Solar Heterogeneous Photocatalysis.” *Journal of Hazardous Materials* 319 (2016): 34–42. Print.
[11] Klidi, Nizar et al. “Applicability of Electrochemical Methods to Paper Mill Wastewater for Reuse . Anodic Oxidation with BDD and TiRuSnO2 Anodes.” *Journal of Electroanalytical Chemistry* 815.February (2018): 16–23.
[12] Fajardo, Ana S, Rui C Martins, et al. “Electrochimica Acta Treatment of Amaranth Dye in Aqueous Solution by Using One Cell or Two Cells in Series with Active and Non-Active Anodes.” *Electrochimica Acta* 210 (2016): 96–104.
[13] Garza-campos, Benjamín R et al. “Chemosphere Coupling of Solar Photoelectro-Fenton with a BDD Anode and Solar Heterogeneous Photocatalysis for the Mineralization of the Herbicide Atrazine.” *Chemosphere* 97 (2014): 26–33.
[14] Bautitz, Ivonete Rossi, and Raquel F Pupo Nogueira. “Photodegradation of Lincomycin and Diazepam in Sewage Treatment Plant Effluent by Photo-Fenton Process.” *Catalysis today* 151.3 (2010): 94–99.
[15] He, Yujie et al. “Degradation of Pharmaceuticals in Wastewater Using Immobilized TiO2 Photocatalysis under Simulated Solar Irradiation.” *Applied Catalysis B: Environmental* 182 (2016): 132–141.
[16] Fajardo, Ana S, Helga F Seca, et al. “Electrochemical Oxidation of Phenolic Wastewaters Using a Batch-Stirred Reactor with NaCl Electrolyte and Ti / RuO2 Anodes.” *Journal of Electroanalytical Chemistry* 785 (2017): 180–189.