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## Electrochemical oxidation of paraben compounds and the effects of byproducts on neuronal activity

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### Abstract

Some organic recalcitrant compounds are not degraded by conventional water treatment systems, making necessary the use of advanced technologies to eliminate these substances. Advanced Oxidation Processes (AOPs) have been extensively proposed to remove emerging contaminants aiming potable water reuse, but literature barely addresses neurotoxic effects of AOPs residual byproducts. These processes involve high costs associated with the electricity, maintenance and oxidizing agent used. However, electrochemical AOPs are techniques based on electron transfer, thus being a clean form of energy and very efficient in the degradation of organic pollutants. Parabens are naturally found in plant sources but most are chemically synthesized, requiring careful treatment to not disturb the environment. In this study, a mixture of parabens (10 mg L<sup>-1</sup> each) was degraded by an electrochemical oxidation (EO) system with a Ti/Pt anode. Some parameters, such as the current density (25, 75 and 125 A m<sup>-2</sup>) and the electrolyte type and concentration (1.5, 3.0 and 5.0 g NaCl L<sup>-1</sup> and 3.0 g Na<sub>2</sub>SO<sub>4</sub> L<sup>-1</sup>) were changed. The best results were obtained with 125 A m<sup>-2</sup> and 3.0 g NaCl L<sup>-1</sup>, which led to the complete degradation of the parabens present in the mixture, after 10 min. In addition to these studies neurotoxicity tests were also performed using the solutions of interest, before and after the EO treatment. It was observed, using the reactive oxygen species (ROS) fluorescent indicator H<sub>2</sub>DCFDA, that the non-treated solution caused an increase in ROS formation with a signal amplitude of 0.84 ± 0.20 above the baseline. After the EO process the parabens mixture did not lead to a significant ROS change.

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The solution to bridge the problem of high electricity costs may be replacing it with solar energy, low cost catalysts and other treatment processes involving renewable and eco-friendly energy.

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**Keywords:** Bioenergy; Advanced oxidative process (AOPs); Contaminants of emerging concern (CECs); Hippocampal slices; Neurotoxicity; Reactive oxygen species (ROS)

## 1. Introduction

The use of renewable energy has been an alternative to the use of fossil fuels, which have a harmful effect on the environment [1]. Biomass is an abundant resource for bioenergy production, allowing the technological advancement the extraction of some products and the conversion of waste for bioenergy purposes [2]. Frequent use of natural paraben, especially in cosmetics, can generate a large amount of biomass that can be used to produce bioenergy [3].

The appearance of thousands of non-biodegradable compounds hinders the correct disposal of solid or liquid materials. An aggravating factor occurs when compounds are disposed directly in surface water without any or inadequate treatment. In these cases these substances can affect the local ecosystem and can bioaccumulate in plants and animals and even cause behavioral changes in small animals [4,5].

Contaminants of Emerging Concern (CECs) are any chemical discovered in water or environment that had not previously been detected, with potential significant impact on human health and aquatic life. CECs are present in several regions of the world such as India [6], Brazil [7] and Portugal [8]. As conventional systems do not allow efficient CECs removal, great attention has been raised towards Advanced Oxidative Processes (AOPs) that have the capacity to degrade recalcitrant compounds due to the presence of hydroxyl radicals (.OH), and can lead to complete mineralization in CO<sub>2</sub> and H<sub>2</sub>O [9,10].

The Electrochemical Oxidation (EO) process is an AOP that generally requires lower temperature, less space, and produce fewer by-products. In the EO system, various types of anode can be used and the degradation of the compounds can occur directly on the surface of the electrode, or indirectly in the reaction medium, promoted mainly by radicals from the electrolytes [11]. Many studies were carried out using this process and it was successful in removing several compounds such as pharmaceuticals [12], dyes [13] and phenol [14]. In anodic oxidation, pollutant destruction occurs by electron transfer on the electrode surface. This being a direct method, the effectiveness of pollutant removal is greater as it avoids two fundamental aspects: mass transfer limitations and electrode surface poisoning [12,13].

In this study were used parabens which are esters of 4-hydroxybenzoic acid. They are found naturally in some vegetables, plants and can be produced by bacteria. When synthesized they can be applied in the chemical and electrical industries as a material for polymer and cosmetic production. Although a high energy demand is required to degrade the compounds by EO, this process is necessary since the compounds must not reach the environment as they may bioaccumulate or cause adverse effects on animals and plants [3].

The goal of this study was to investigate the ROS production in the CA3 region of Wistar rat hippocampus with a mixture of parabens before and after the EO treatment. The electrochemical oxidation was performed with Ti/Pt anode and different parameters such as current density (25, 75 or 125 A m<sup>-2</sup>) and the concentration of different electrolytes (1.5 g NaCl L<sup>-1</sup>; 3.0 g NaCl L<sup>-1</sup>, 5.0 g NaCl L<sup>-1</sup>, or 3.0 g Na<sub>2</sub>SO<sub>4</sub> L<sup>-1</sup>) were assessed to select the best condition. A mixture of five parabens (10 mg L<sup>-1</sup> each) namely methylparaben (MeP), ethylparaben (EtP), propylparaben (PrP), butylparaben (BuP) and benzylparaben (BeP) was used in the experiments

In electrochemical AOPs the advantage is that the energy spent can be reduced by placing electrochemical reagents *in situ*. In this way, the application of this technique is optimized through new low cost catalytic materials, reducing energy production and developing more efficient and economically viable techniques [9,11]. Several studies have addressed the replacement of energy from thermoelectric and hydroelectric plants by the generation of electricity through solar energy. Thus, solar energy is capable of meeting the needs of processes such as AOPs, especially in countries with high insolation rates [2,10].

## 2. Material and methods

### 2.1. Chemicals and setup

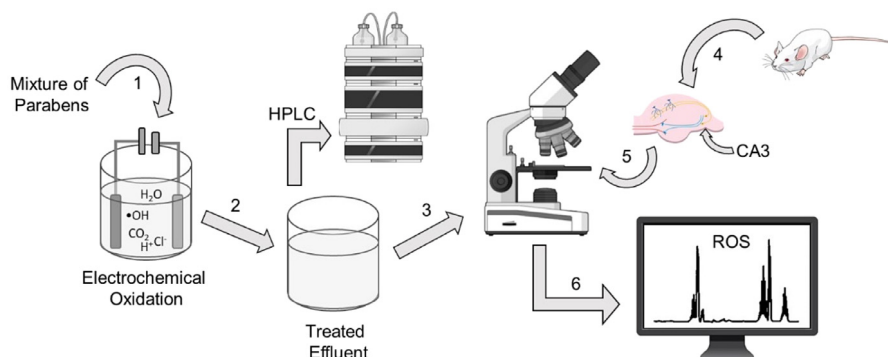
The parabens MeP, EtP, PrP, BuP, BeP and the reagents  $\text{CH}_3\text{CN}$ ,  $\text{NaCl}$ ,  $\text{Na}_2\text{SO}_4$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{KCl}$ ,  $\text{NaHCO}_3$ ,  $\text{C}_6\text{H}_{12}\text{O}_6$ ,  $\text{NaH}_2\text{PO}_4$  were obtained from Sigma-Aldrich and  $\text{H}_2\text{DCFDA}$  (2', 7'-dichlorodihydrofluorescein diacetate) was obtained from Life Technologies. The solution of these parabens ( $10 \text{ mg L}^{-1}$  each) in ultrapure water (Interlab Direct-Pure) was used in electrochemical oxidation with different current density ( $25$ ,  $75$  or  $125 \text{ A m}^{-2}$ ) and concentration and type of electrolyte ( $1.5 \text{ g NaCl L}^{-1}$ ;  $3.0 \text{ g NaCl L}^{-1}$ ,  $5.0 \text{ g NaCl L}^{-1}$ , or  $3.0 \text{ g Na}_2\text{SO}_4 \text{ L}^{-1}$ ). The reactor had a volume of  $500 \text{ mL}$  magnetic stirring and the electrodes used were titanium-platinum (Ti/Pt) as anode and a stainless steel as cathode, with the area of  $20 \text{ cm}^2$  ( $8.0 \text{ cm} \times 2.5 \text{ cm}$ ).

The concentration of the parabens was quantified on high performance liquid chromatography (HPLC) from Shimadzu with a diode-array detector. A C18 chromatography column from SiliaChrom was used at  $40 \text{ }^\circ\text{C}$  and the injection volume of the samples was  $20 \text{ } \mu\text{L}$  with a mobile phase consisted by a mixture of methanol 50% and acid water (0.1% orthophosphoric acid) 50% and a flow rate of  $0.5 \text{ mL min}^{-1}$ .

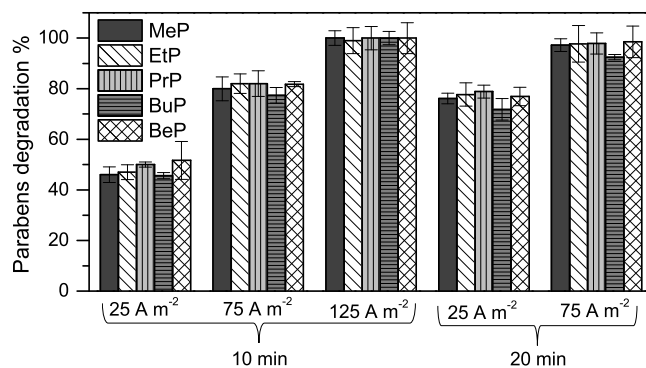
### 2.2. Neurotoxicity procedure

The neurotoxicity studies were performed in hippocampal slices ( $400 \text{ } \mu\text{m}$  thick) from pregnant Wistar rats (8–16 weeks old and 14–18 days of gestation), at CA3 region. After extracting the hippocampal slices, they were incubated in oxygenated (5%  $\text{O}_2$ , 95%  $\text{CO}_2$ ) extracellular artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 10 D-Glucose, 24  $\text{NaHCO}_3$ , 1.25  $\text{NaH}_2\text{PO}_4$ , 2.0  $\text{MgCl}_2$ , 2.0  $\text{CaCl}_2$ , 124  $\text{NaCl}$  and 3.5  $\text{KCl}$ , at pH 7.4. Subsequently the slices were incubated in ACSF solution containing the ROS indicator  $\text{H}_2\text{DCFDA}$  ( $20 \text{ } \mu\text{M}$ ), for one hour, at room temperature. After that period the slices were transferred to a chamber in the microscope setup where they were perfused, for 30 min periods, with ACSF or a parabens medium, at  $32 \text{ }^\circ\text{C}$ , with a flow rate of  $1.5 \text{ mL min}^{-1}$ . The final ACSF perfusion aimed to ascertain if the parabens evoked signals returned to the initial level. All the neurotoxicity experiments were run in triplicate.

A fluorescence microscope, Zeiss Axioskop, equipped with a water immersion objective (40x, N.A. 0.75, 1.6 mm working distance), a tungsten/halogen lamp (12 V, 100 W) and excitation and emission filters of 480 nm and  $> 500 \text{ nm}$ , respectively, were used for the detection of the optical signals. These were recorded through a photodiode from Hamamatsu (K2G 1336,  $1.0 \text{ mm}^2$ ), using a 16 bit analog to digital converter from National Instruments, that processed the data using the Signal Express<sup>®</sup> software from National Instruments. The correction of the optical signals was performed by constant autofluorescence, represent the ratio of the total fluorescence ( $F_T$ ) over baseline fluorescence ( $F_0$ ) obtained as the average of the first 10 points. Data represent the mean  $\pm$  S.E.M. Statistical significance was assessed by means of the Mann–Whitney U test,  $p < 0.05$  was considered significant. Processes and operating systems of electrochemical oxidation and neurotoxicity studies can be visualized in Fig. 1.



**Fig. 1.** Operational system: 1 - Mixture of parabens added to the reactor for electrochemical oxidation; 2 - Solution of parabens after electrochemical oxidation; 3 - Treated effluent used in neurotoxicity studies; 4 - Extraction of Wistar rat hippocampus for the neurotoxicity assays; 5 - Hippocampal slice to be perfused by a treated or untreated solution; 6 - Fluorescence ROS signals.



**Fig. 2.** Degradation of the parabens MeP, EtP, PrP, BuP and BeP in ultrapure water by electrochemical oxidation with different times and current densities (10 min: 25, 75 and 125 A m<sup>-2</sup> and 20 min: 25 and 75 A m<sup>-2</sup>). 3.0 g NaCl L<sup>-1</sup>, pH = 7 and T = 25 °C.

### 3. Results and discussion

#### 3.1. Effects of the current density

The increase in current density generates a greater production of radicals, which will degrade the compounds faster and with greater efficiency [15]. According to Fig. 2, the increase in current density led to an increase in the degradation of all the parabens. Applying a current density of 125 A m<sup>-2</sup> the complete parabens degradation occurred in 10 min. However when 25 A m<sup>-2</sup> and 75 A m<sup>-2</sup> were applied, the degradation of the compounds varied between 48 ± 3% and 82 ± 5%, respectively. After 20 min of EO applying 75 A m<sup>-2</sup> the complete degradation of the parabens occurred, whereas for 25 A m<sup>-2</sup> the degradation ranged between 71 and 78%. For the complete degradation of the parabens with 25 A m<sup>-2</sup>, 30 min was necessary to eliminate BuP and BeP and 45 min to degrade MeP, EtP and PrP (Data not shown). Similar results are observed in the literature [16–18].

#### 3.2. Effect of the electrolytes

The addition of electrolytes increases conductivity and promotes the production of radicals that will eliminate the organic compounds [19]. However, the concentration or type of electrolyte can directly influence the reactions, since each compound has properties and generates different radicals [20]. Here the degradation of the parabens was evaluated using NaCl (1.5, 3.0 and 5.0 g L<sup>-1</sup>) and Na<sub>2</sub>SO<sub>4</sub> (3.0 g L<sup>-1</sup>).

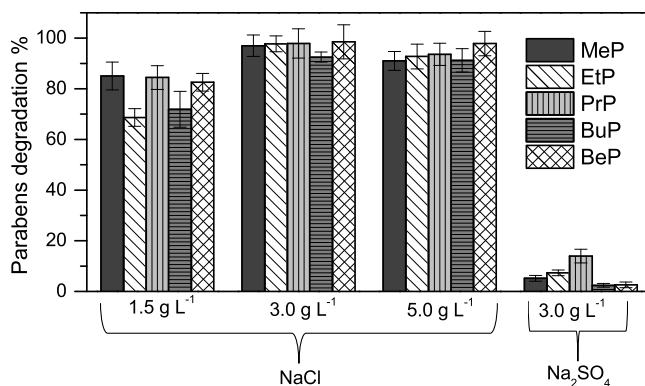
According to Fig. 3, the increase of NaCl concentration from 1.5 g L<sup>-1</sup> to 3.0 g L<sup>-1</sup> promoted an increase in the elimination of parabens that varied from 12 to 29%. When increasing NaCl concentration to 5.0 g L<sup>-1</sup>, similar degradation profiles were achieved compared to 3.0 g L<sup>-1</sup>, and the excess of electrolyte did not contribute to the parabens removal. Similar results were previously described [21]. There is a high energy demand for the complete elimination of compounds, however this contributes to the effluent being disposed cleaner into the environment.

Fig. 3. also present the low efficiency in the degradation of the parabens when using 3.0 g Na<sub>2</sub>SO<sub>4</sub> L<sup>-1</sup>. The removal of MeP, EtP, PrP, BuP and BeP was 5.1, 7.3, 13.9, 2.4 and 2.5%, respectively. The decrease in the efficiency when applying Na<sub>2</sub>SO<sub>4</sub> compared to NaCl was also observed by [22] and is probably associated to the production of radicals of species S<sub>2</sub>O<sub>8</sub><sup>2-</sup>, which are less reactive with organic compounds than the radicals formed by NaCl [23].

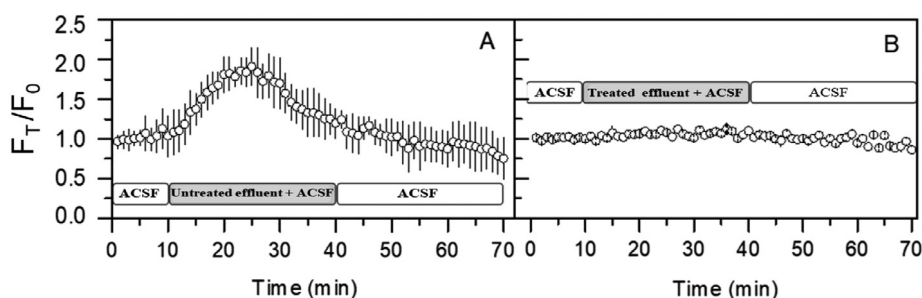
#### 3.3. Neurotoxicity studies

In Fig. 4 A it is possible to observe changes in ROS production, which occur when the mixture of parabens gets in contact with the hippocampal slices, during the period represented by the gray bar. The signals increase initially reaching a maximum amplitude (0.84 ± 0.20, at 20–30 min, n = 3) above the baseline.

This suggests that the compounds present in the parabens mixture acted within the cells, altering their normal functioning and consequently the synaptic activity that affects the energy production in mitochondria [24]. This



**Fig. 3.** Degradation of the parabens MeP, EtP, PrP, BuP and BeP in ultrapure water by electrochemical oxidation with different concentrations and types of electrolyte (NaCl: 1.5, 3.0 and 5.0 g L<sup>-1</sup> and Na<sub>2</sub>SO<sub>4</sub>: 3.0 g L<sup>-1</sup>). 75 A m<sup>-2</sup>, pH = 7 and T = 25 °C.



**Fig. 4.** ROS signals from brain slices evoked by the synthetic effluent (applied during the period indicated by the gray bar) of the paraben mixture: before EO treatment (A) and after EO treatment (pH 7.4, 3 g NaCl L<sup>-1</sup>, 125 A m<sup>-2</sup>) (B).

process causes oxidative stress, a behavior in which the cell produces ROS in high amounts that can lead to cell death [25]. However, upon returning to the ACSF solution, the signals decreased to the baseline, indicating that the effect is reversible.

In Fig. 4 B the slices were in contact with the EO treated (125 A m<sup>-2</sup>, 3.0 g NaCl L<sup>-1</sup>, 10 min) parabens solution, during the period indicated by the gray bar. In this case there were no significant changes in the ROS signals, supporting the idea that the applied treatment was efficient in the elimination of the neurotoxic effects. Another study [26] reported a similar result with a mixture of parabens (methylparaben, ethylparaben, propylparaben, benzylparaben and butylparaben, 10 mg L<sup>-1</sup> each), treated by ozonation.

#### 4. Conclusion

The use of biomass from paraben extraction plants as a source for renewable energy production is very promising. The degradation of the parabens was successfully achieved using an electrochemical oxidation process. The increase of current density led to an increase in the production of highly oxidative radicals that promoted the elimination of the paraben compounds. The electrolyte concentration may influence the degradation rate up to the limit of saturation, and the type of electrolyte has a high influence on the degradation of the compounds. Neurotoxicity assays showed a high effect of the non-treated solution, but the electrochemical oxidation was efficient in the degradation of the compounds that caused the ROS changes, thus eliminating, under the mentioned conditions, the neurotoxic actions. AOPs are growing processes for wastewater treatment, by optimizing some efficiency factors. One of the main ones is the cost of energy sources, in this case the chemical reagent, and another is the development of new catalytic materials that facilitate the use of sunlight.

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