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Toxicity Removal of Pharmaceuticals Mixtures through Electron Beam Irradiation

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ABSTRACT

Contamination of the aquatic environment by pharmaceuticals is becoming a global phenomenon of growing concern. Pharmaceuticals are partially metabolized, resulting in the excretion and release of residual into sewage, unaltered or metabolites. The wastewater treatment plants are not designed to eliminate these compounds, leading the residues into the aquatic environment. Besides, pharmaceuticals are not detected individually but as a complex mixture. Advanced oxidative processes have been applied as an alternative or complement to conventional sewage treatment processes, aiming the degradation and removal of toxic pollutants. The objective of this study was to evaluate the toxicity removal of mixtures of pharmaceuticals subjected to electron beam treatment. The aqueous solutions of each pharmaceutical were diluted in ultra-pure water and prepared in three pharmaceuticals combinations: Propranolol + Fluoxetine + Sulfadiazine; Propranolol + Fluoxetine + Diclofenac; Acetylsalicylic acid + Fluoxetine + Metformin). Electron Beam Accelerator was applied for the irradiations and the absorbed doses were 2.5-5.0 kGy. Acute toxicity tests with *Daphnia similis* were performed to evaluate the toxicity, before and after irradiation.. The data analyzed showed toxicity removal efficiency around 80% for the mixture of Propranolol, Fluoxetine and Diclofenac; 75% for the mixture of Propranolol, Fluoxetine and Sulfadiazine; and 30% for the mixture of Acetylsalicylic acid, Fluoxetine and Metformin. According to the literature, this is a viable technology for the removal of toxicity from pharmaceuticals, and the results demonstrated the potential of electron beam irradiation in reducing the toxicity of pharmaceutical from different classes.

Keywords: **Irradiation, Pharmaceutical, Toxicity.**

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1. INTRODUCTION

Contamination of the aquatic environment by pharmaceuticals is becoming a global phenomenon of growing concern due to the significant risks it can bring to [1,2,3]. A wide range of inorganic and organic contaminants are being detected in surface waters around the world. These contaminants have their origin associated with the mechanisms of production and disposal, sanitation and excretion, with domestic sewage being the main route of entry of these contaminants into the environment, which reach sewage networks and subsequently surface water [4,5].

Pharmaceuticals can be only partially metabolized during therapeutic use, resulting in the excretion and release of residual fractions into sewage, unaltered or in the form of metabolites, and may remain active in sewage treatment facilities for a long time [6,7,8]. Many studies have shown that wastewater treatment plants are not designed to eliminate these compounds, as such the main source of drug residues in the aquatic environment [6,7,9].

Pharmaceuticals concentrations in water are generally low, in the range of $\mu g.L^{-1}$ to $ng.L^{-1}$. Despite these low concentrations, there are uncertainties about the risks arising from the exposure [4,10]. A factor to be taken into account, which has been showing that the presence of these chemicals in the environment becomes increasingly worrying, is that these substances do not appear individually, but as a complex mixture of contaminants, which can lead to unwanted effects [5].

Due to the frequency of its detection in the environment, its persistence and toxicity, the most studied pharmaceutical groups are antibiotics, psychiatric drugs, hormones, analgesics and anti-inflammatory, β-blockers, and antidiabetic drugs [9,11].

Conventional treatment techniques are insufficient to remove traces of different pharmaceuticals and organic and inorganic compounds. Biological processes are frequently used in the treatment of large volumes of effluents, since they allow the removal of high rates of organic matter, and it has lower cost. However, some compounds are recalcitrant, and can be toxic to microorganisms. Physical processes - decantation, flotation, filtration, adsorption - are characterized by the phase transfer of the contaminant, without it being degraded, but they can be useful as a pre- or post-treatment of the final process. Chemical processes are based on the oxidation of contaminants by reaction with strong oxidants, such as hydrogen peroxide (H_2O^2), chlorine (Cl_2), chlorine dioxide (ClO_2) and permanganate (MnO_4^-), however, in most cases the use

of this type of treatment does not promote the complete mineralization of contaminants, with the formation of degradation by-products [12,13]. The efficiency of pharmaceuticals removal in WWTP depends on the physicochemical properties of each compound. Several studies report that the elimination of these compounds is often incomplete, as the removal rate is variable.

Therefore, advanced techniques are sought, such as ozonation, advanced oxidative processes or filtration with the use of membranes to increase efficiency in reducing waste and by-products of these compounds in water [14]. These Advanced Oxidative Processes (AOPs) have been shown as an alternative or complement to conventional wastewater treatment processes. Characterized by chemical oxidation reactions, mediated by the hydroxyl radical (HO•), this species is poorly selective and highly reactive, acting in the chemical oxidation of many substances. Thus, POAs have been used in wastewater treatment, contributing to the reduction of organic contaminants and favoring the biodegradability of industrial effluents [15].

Pharmaceuticals do not occur individually but as a complex mixture. Most works focus on the degradation and toxicity removal of a single compound or binary mixtures [16,17,18,19]. In this study, we focused on the toxicity removal of tertiary mixtures containing fluoxetine, propranolol, diclofenac, acetylsalicylic acid, metformin, and sulfadiazine (PRP+FLX+SDZ; PRP+FLX+DIC; ASA+FLX+MET).

2. MATERIALS AND METHODS

2.1.Reagents

Fluoxetine hydrochloride [$C_{17}H_{18}F_3NO$. HCl; MM = 309.33 g/mol; methyl[(3S)-3-phenyl-3-[4-(trifluoromethyl) phenoxy] propyl] amine]; CAS 54910-89-3]; Propranolol [$C_{16}H_{21}NO_2$; MM = 259.34 g/mol; (*RS*)-1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-ol; CAS 525-66-6] and Diclofenac [$C_{14}H_{11}Cl_2NO_2$; MM = 296.148 g/mol; 2-[2-(2,6-dichloroanilino)phenyl]acetic acid; CAS: 15307-86-5] were purchased from Divis Pharmaceuticals Pvt. Ltd. (98.8% of purity). Sulfadiazine [$C_{10}H_{10}N_4O_2S$; MM = 250.270 g mol-1; 4-amino-N-pirimidina-2-il-benzenosulfonamida; CAS 68-35-9, 99.9% of purity] and Metformin [$C_4H_{11}N_5$; MM = 129.164 g/mol; 1,1-dimethylbiguanide; CAS 657-24-9, 97% of purity] were obtained from Sigma-

Aldrich. Acetylsalicylic acid [$C_9H_8O_4$; MM = 180.16 g mol^{-1}; 2-acetoxybenzoic acid; CAS 50-78-2] was purchased from Labsynth (99.5% of purity).

All aqueous solution prepared for irradiations experiments were diluted using ultra-pure water (Millipore Milli-Q) and prepared at concentrations of 10-20mg/L (FLX), 80mg/L (PRP), 50 mg/L (DIC), 50mg/L (SDZ), 10 mg/L (ASA) and 10 mg/L(MET). These compounds were combined into three different tertiary mixtures, following the proportions 1:1:1.

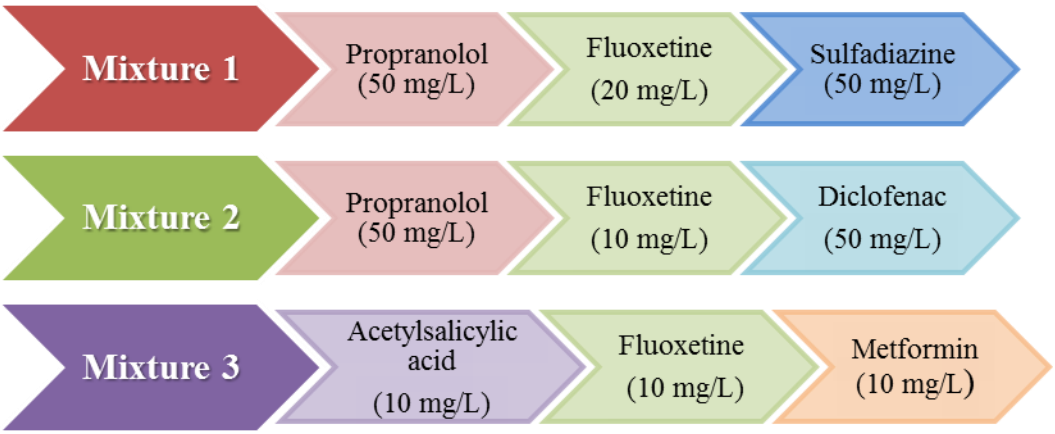

Figure 1: *Different tertiary mixtures prepared for the tests.*

2.2.Irradiation process

A Dynamitron Electron Beam Accelerator was applied for the irradiations. The beam energy was fixed at 1.4 MeV during all the experiments. Liquid samples were irradiated using a batch system in borosilicate containers (Pyrex) a volume of 246 mL was used in order to ensure a suitable beam penetration, 4mm thickness for aqueous samples. The recipient's speed was 6.72 m min^{-1} for samples passing under the electron beam. Absorbed doses were confirmed using a Perspex Harwell Red dosimeter, batch KZ-4034, with less than 5% variation.

2.3.Toxicity assays using *Daphnia similis*

The acute toxicity tests with D. similis were performed according to Brazilian standard methods [20]. The effect observed was the immobility to organisms after 48 hours of exposure to the samples. The results of the toxicity tests were obtained based on the mean value of solutions concentration, which affects the exposed organism (EC50%), as well as the 95% confidence intervals, calculated from the estimated endpoint by the Trimmed Spearman-Karber method [21]. The tests were performed in duplicate, three different tertiary mixtures of pharmaceuticals were analysed.

2.4. Total Organic Carbon (TOC)

Total Organic Carbon was analysed on a Shimadzu TOC equipment in order to determine organic carbon removal after irradiation.

3. RESULTS AND DISCUSSION

The obtained results herein demonstrate that EBI was effective in removing toxicity of both compounds mixtures at low doses. Figure 2 presents the tertiary mixtures detoxification results for 2.5 and 5.0 kGy. The mixture toxicities of PRP+FLX+SDZ decreased from 10.6 to 2.5 and 2.3 TU at 2.5 and 5 kGy, respectively, corresponding to toxicity removal efficiencies of 76.4% and 78.1%. For the tertiary mixture of PRP + FLX + DIC, the toxicities decreased from 19.2 to 3.9 and 5.6 TU at 2.5 and 5 kGy, respectively, corresponding to toxicity removal efficiencies of 80% and 70%. The ASA + FLX + MET mixture detoxification results for 2.5 and 5.0 kGy, were a toxicity decrease from 3.3 to 2.2 TU in both doses of radiation applied, resulting to toxicity removal efficiencies around 30%.

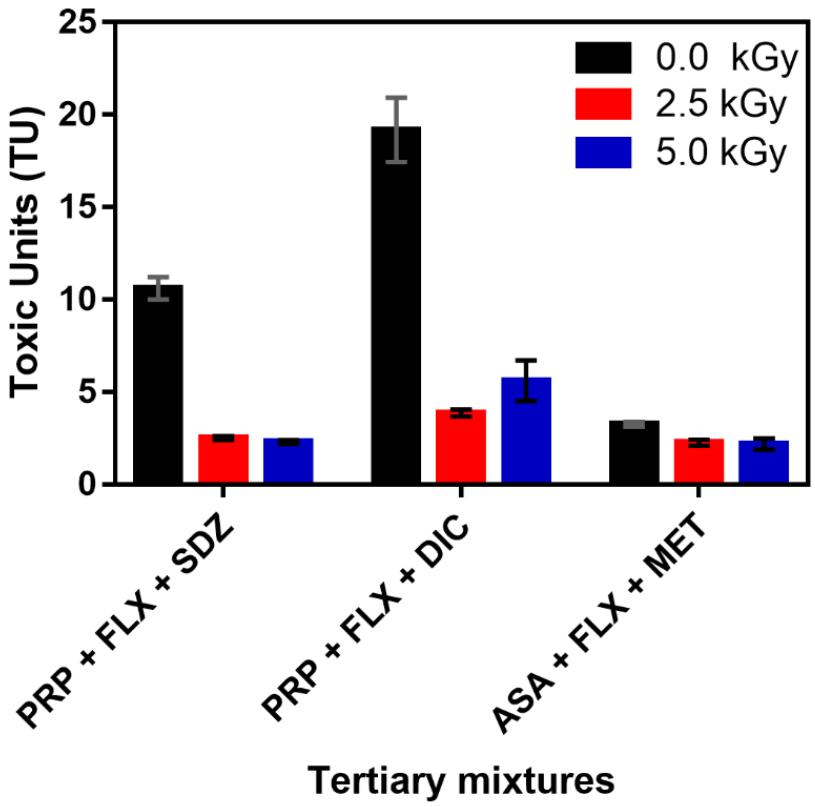

Figure 2: *Acute toxicity (in toxic units, TU = 100/EC50%) of tertiary mixture of PRP + FLX + SDZ; PRP + FLX + DIC and ASA + FLX + MET treated by electron beam irradiation assessed using D. similis.*

In real effluents, pharmaceuticals are present in combination with dozens of compounds, in a complex mixture of contaminants, which can lead to unwanted effects. The ubiquity of a number of potentially toxic emerging contaminants in the environment leads to the need to better understand their occurrence, fate and ecological impact [2].

In a binary mixture of acetylsalicylic acid with fluoxetine, toxicity reduction values of 60% were obtained for D. similis at doses of 1.0 and 2.5 kGy [17]. One study reported approximately 80% toxicity removal efficiency for D. similis exposed to binary mixture of fluoxetine and propranolol irradiated at 5.0 kGy [18]. The radiation effects of pharmaceuticals mixture were also evaluated, where a 50% reduction in toxicity was evidenced for a binary mixture containing

fluoxetine and diclofenac, irradiated at 5.0 kGy [16]. An 80% toxicity reduction for D. similis was demonstrated in samples of fluoxetine diluted in raw domestic sewage irradiated at 5.0 kGy [19].

Table 1 shows the behaviour of TOC concentration and removal obtained after electron beam treatment. The irradiation of the pharmaceutical mixtures decreased the TOC content of the solutions reaching up 40% at 5.0 kGy. Also, the increase of dose promoted a decrease in TOC content for PRP + FLX + SDZ and ASA + FLX + MET mixtures, indicating the decomposition of the pollutants into CO2 and H2O.

EBI leads to water radiolysis, which generates important reactive species for degrading contaminants such as •OH and eaq- radicals [22]. This technology has been demonstrating effective for removing pollutants at low doses [16,17,18,19]. Nevertheless, EBI is less effective for TOC removal, requiring high absorbed for complete mineralization into carbon dioxide, thus generating intermediates such as carboxylic acids and aldehydes, among others [23]. It worth noting that complete mineralization might not be necessary in water and wastewater treatment. Therefore, EBI is an interesting alternative process that can be combined with different techniques or can be applied as pretreatment for biological processes [18,23].

Table 1: TOC concentration and TOC removal vs. electron beam absorbed dose of tertiary mixture of PRP + FLX + SDZ; PRP + FLX + DIC and ASA + FLX + MET treated by electron beam irradiation.

Doses	**Total Organic Carbon (TOC)**			**TOC Removal**		
(kGy)	PRP + FLX + SDZ	PRP + FLX + DIC	ASA + FLX + MET	PRP + FLX + SDZ	PRP + FLX + DIC	ASA + FLX + MET
0.0	36.50 ± 0.26	27.94 ± 0.24	6.33 ± 0.14	-	-	-
2.5	32.88 ± 0.22	25.81 ± 0.05	4.46 ± 0.03	9.91 ± 0.60	7.62 ± 0.18	29.55 ± 0.53
5.0	30.69 ± 0.33	25.79 ± 0.22	3.64 ± 0.07	15.91 ± 0.91	7.72 ± 0.80	42.40 ± 1.07

4. CONCLUSION

The obtained results reinforce that low doses may be adequate for the detoxification of samples of pharmaceutical products, highlighting the importance of evaluating mixtures. According to the literature, EBI is a viable technology for the removal of toxicity from mixtures of pharmaceutical products, and the results demonstrated the potential of electron beam irradiation as an effective alternative in reducing the toxicity of pharmaceutical products of different classes. This technology proved to be efficient in removing the toxicity of the three different tertiary mixtures of pharmaceutical. These data as well as literature indicate the need for further studies on mixtures, and the EBI can be an interesting alternative process applied as a pre-treatment technology capable of detoxifying pharmaceutical products found in environmental matrices.

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