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ECOTOXICOLOGICAL STUDY OF PHARMACEUTICAL MIXTURE IN WATER SOLUTION AND ITS TREATABILITY

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ABSTRACT

Residual pharmaceuticals are found in natural waters as well as in wastewater and in drinckable water treatment plants. In the environment such compounds may affect aquatic biota, biodiversity and cause severe risks to human health due to synergistic effects and represent environmental risks. The anti-inflammatory diclofenac and the antidepressant fluoxetine are some of the compounds found in surface water. They present persistent physicochemical properties and low biodegradability and can not be completely removed by conventional water treatments. Advanced Oxidation Processes are reported as efficient possibility for removing organic compounds and toxicity. The aim of this study was to evaluate the acute toxicity of the mixture of pharmaceutical diclofenac and fluoxetine on *Vibrio fischeri* marine bacteria. An industrial electron beam accelerator was used as the radiation source. The radiation induced degradation of the organic matter was determined by Total Organic Carbon analysis. Samples were exposed to different radiation doses: 2.5 kGy; 5.0 kGy; 7.5 kGy and 10 kGy. The toxicity values allow classifying the mixture as very toxic. After irradiation the toxicity decreased.

1. INTRODUCTION

Residual pharmaceutical substances have been monitored in various environmental matrices since the late 90's [1]. Several studies have shown the presence of pharmaceutical drugs and their metabolites in aquatic environments in different countries such as Germany, Brazil , Canada , Holland, England , Italy, Sweden, United States and United Kingdom [2].

Among this class of contaminants, various types of pharmaceutical drugs such as hormones, anesthetics, antilipemics, antidepressants, anti-inflammatories, are found in the range $\mu g.L^{-1}$ and $ng.L^{-1}$ [2]. A compound found in many different environments matrices in greater concentration is the anti - inflammatory diclofenac [3]. This substance has been detected in surface waters [4] and wastewater treatment plants [5]. Another substance detected in different studies is the antidepressant fluoxetine. This substance can also been detected in surface waters [6] [7] and wastewater treatment plants [8].

Pharmaceutical drugs are a class of emerging environmental contaminants, which can be defined generically for compounds used in both human medicine and veterinary. There is a great concern about the possible effects of chemical compounds in exposed aquatic organisms; the possible bioaccumulating effects, which can cause changes in the links in the food chain and also the possible toxic effects that these substances may affect in human health in a long term [9] [10].

These drugs are lipophilic, bioaccumulative and they have persistent physico-chemical properties, since they must maintain chemical properties to be used for therapeutic purposes. In addition, they have low vapor pressure, which facilitates their dispersion in the environment [11].

Previous studies have shown that pharmaceutical drugs have low biodegradability in wastewater treatment plants. It was analyzed the degradation of different drugs such as analgesics, hormones, antibiotic, ant depressive, anti-inflammatory and it was verified that the biodegradation varies depending on the compound [8].

The advanced oxidation processes (AOPs) have been described as an efficient alternative for the removal of pollutants and persistent effluents with high organic load when conventional treatments do not achieve the necessary efficiency. In this process, free radicals as hydroxyl ($OH^{\cdot}$) are generated. These radicals are able to lyse the molecules into less harmful products, and many cases, mineralize them [12].

Some studies have shown the efficiency of AOPs for removal of pharmaceutical drugs in aqueous solution [13] [14]. It was analyzed the removal of many drugs using different AOP such as Fenton systems, ozonation, hydrodynamic and acoustic cavitation, homogeneous ultraviolet irradiation, heterogeneous, photocatalysis using semiconductors, radiolysis and a number of electric and electrochemical methods and they presented a great removal.

Bioassays rely on measuring the response of organisms exposed to contaminants compared to a control. These assays are realized under specific and controlled experimental conditions, used to establish the toxicity levels of target contaminants and complex aqueous matrices (e.g., surface water, groundwater, wastewater) for aquatic organisms [15] [16].

Toxicity assays are desirable tools for evaluating the quality of water and the pollution load of effluents, since physic-chemical analysis are not enough to assess the possible interactions of these substances on the organism such as additive, synergistic or antagonistic effect and to forecast the environmental risk potential of contaminants [15] [17].

The degradation of complex organic contaminants submitted to advanced oxidation processes produces oxidation intermediate products form. These products may be more toxic than the

parent compounds. So toxicity test are very useful tool to evaluate the operative AOP conditions to make the treated aqueous matrix safer [17].

2. OBJECTIVES

The focus of the present study were to evaluate the degradation of the pharmaceutical drug mixture, diclofenac and fluoxetine hidrochloride, after the exposure to ionizing radiation from established radiation doses, and also, to assess the reduction of acute toxicity of the drug mixture.

3. MATERIAL AND METHODS

3.1. MIXTURE PHARMACEUTICAL DRUGS

The fluoxetine and diclofenac solutions were prepared at concentrations of 100 and 10 mgL^{-1}, respectively. The preparation of the mixture was carried out in a 50/50 ratio. Each drug capsule of diclofenac contains 50mg of diclofenac in its formulation besides some excipients (silicon dioxide, microcrystalline cellulose, lactose, magnesium stearate, povidone starch, macrogol, polysorbate 80, talc, yellow ferric oxide and red ferric oxide, sodium starch glycolate, polymer methacrylate, titanium dioxide, and hypromellose). The diclofenac solubility is 19 $mg.mL^{-1}$ in water [18]. The fluoxetine drug capsule contains 20 mg of fluoxetine hydrochloride in its formulation, besides some excipients (aerosil, sodium lauryl sulfate, micro-crystalline cellulose and maize starch).The FH solubility is 14 $mg.mL^{-1}$ in water [19].

3.2. IRRADIATION OF TEST SOLUTIONS

The irradiations were performed at the Radiation Technology Center (CTR), Instituto de Pesquisas Energéticas e Nucleares (IPEN), São Paulo, Brazil. The drug aqueous solution was irradiated at the Dynamitron electron beam accelerator and the energy machine was fixed at 1.4 MeV during the expriments. The samples were taken to irradiation field contained in a glass recipient (Pyrex®), covered with plastic wrap. In the Pyrex, the solutions were added until the volume reaches a 4mm of thickness. The applied radiation doses were: 2.5 kGy, 5.0kGy, 7.5 kGy and 10 kGy.

3.3. TOTAL ORGANIC CARBON ANALYSIS (TOC)

The TOC analyses were performed at the Chemical Institute (IQ), Universidade de São Paulo, Brazil, using the TOC Shimadzu analyser, 5000A model. The TOC is obtained from the difference from Total Carbon (CT) and Inorganic Carbon (IC).

3.4. *Vibrio fischeri* TOXICITY ASSAYS

The acute toxicity tests with *Vibrio fischeri* were performed following the recommendations of the normative ABNT NBR-15411-2 [20]. At first, reactivation of the bacteria was carried out (lyophilized form). The entire content of a vial containing the bacteria was passed to a glass cuvette and 1000 mL of buffer reactivation solution was added.

The serial sample dilution was performed using diluent solution (1:10 - Biolux solution) and the controls. The sample concentrations used for the acute toxicity tests were equivalent to 40.95%, 20.47%, 10.23% and 5,11%. Two sequences of cuvettes were prepared in the Microbics® analyzer: the first of them contained the samples and the suitable osmotic adjustment. The second line of cuvettes received the bacteria and the first signs of luminescence were measured in order to calibrate the analyser. The I0 counting were taken from this second line cuvettes. After that each cuvette received the correspondent sample in order to get the I15, after the exposition.

3.5. STATISTICAL ANALYSIS

The statistical analysis for the *Vibrio fischeri* assay was based on the gamma value (ratio between the light lost and the remanescent light from each pair of cuvets) and the sample concentrations (7.82 version of the program developed by 500 Microbics®).

4. RESULTS AND DISCUSSION

The results are presented at Figures 1 and 2 as well as Table 1. TOC analysis indicated that the mixture has low mineralization while exposed to irradiation (Figure 1). The mixture carried out a concentration of 50 $mg.L^{-1}$ diclofenac and 5 $mg.L^{-1}$ fluoxetine, from the TOC analysis it was found an average of 50 $mg.L^{-1}$ organic carbon.

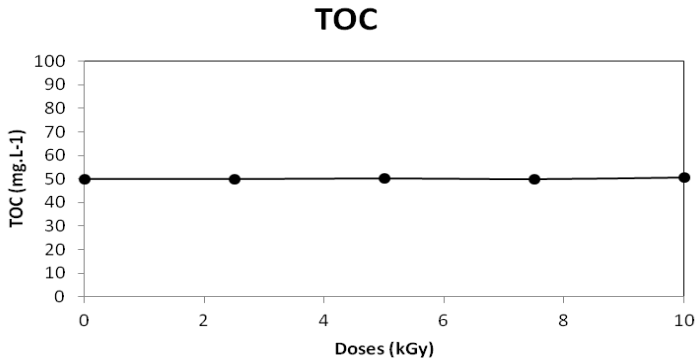

Figure 1 – Total organic carbon versus radiation dose

The treatment with ionizing radiation using the electron accelerator has shown a great efficiency in degradation of these pharmaceutical drugs [21] [22]. The advanced oxidation of complex organic contaminants typically does not result in fast mineralization, with formation of carbon dioxide and inorganic species, but oxidation intermediate products form [16]. In spite of, some studies have has shown that for mineralization, it is necessary high doses of radiation or the combination of different kinds of treatment [13] [22].

4.1. *Vibrio fischeri* TOXICITY ASSAYS

One important parameter is the EC50 which is the effective concentration for 50% luminescence reduction for *Vibrio fischeri* assays [15]. The *Vibrio fischeri EC50* values published for diclofenac and fluoxetine were 4.17 $mg.L^{-1}$ [22] and 1.15 mg.L-1 [21]. At the present paper the EC50 values 5.03 $mg.L^{-1}$ ± 0.52 (diclofenac) ; 4.25 $mg.L^{-1}$ ± 0,50 (fluoxetine) and 14.69 $mg.L^{-1}$ ± 1,77 for the mixture of both. The numerical value of acute and chronic toxicity, expressed in EC50, shows an inverse relationship, so, the lower the value the higher is the toxicity. A directly proportional value may be expressed by toxic units (TU):

$$TU = 100/EC50 \quad (1)$$

The mixture of different substances can result in interactions such as antagonism, synergetic or addictive effects. This interaction can be measured by using the TU values [15]. The TU values for diclofenac, fluoxetine and the mixture are, respectively, 19,87%; 23.55% and 6.89%. The results demonstrate an antagonist effect of the mixture, since the addition of TU of the pure substances are lower than the UT of the mixture [UT(A+B) < UT(A) + UT(B)].

At Figure 2 it was presented the reduction of TU after irradiation. Bioassays, today known as ecotoxicity, have a great importance in evaluating the reduction of toxicity of AOP's [16].

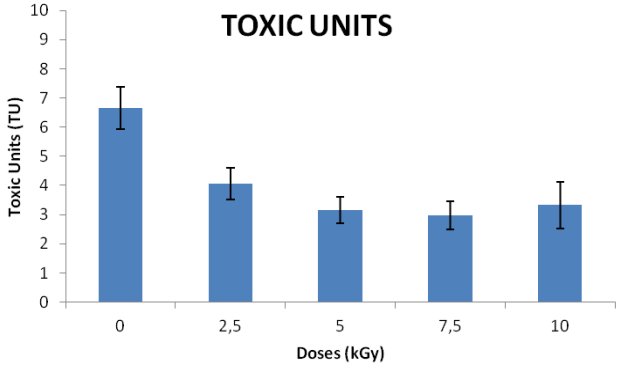

Figure 2 – Toxic Units versus radiation dose (*Vibrio fischeri* – 15 min)

After irradiation the toxicity expressed as Toxic Unitis varied from 2 and 4 (Figure 2). HOMLOK (2011) noted that the toxicity remains unchanged after 1 kGy for the pharmaceutical diclofenac. Another advanced oxidative process, the pulse radiolysis and γ-radiolisis, verified that in low doses, the toxicity remains constant. However, in higher doses, there's a toxicity increase. [23], depending on the contaminant. The toxicity removal efficiency of the treatment can be calculated from toxicity unit, as demonstrated at **Erro! Fonte de referência não encontrada.**.

Table 1: Toxicity Removal (%) of irradiated samples containg a mixture of diclofenac and fluoxetine

Doses (kGy)	Removal Efficiency (%)
2.5	43.76
5	54.13
7.5	54.58
10	49.03

The results indicated that the 5.0 and 7.5 kGy are more efficient for removal of toxicity for the mixture. SILVA (2014) found out 5,0 kGy as the best dose for removing fluoxetine, achieving 17,26% removal.

5. CONCLUSIONS

This present study showed the acute toxicity effects caused by the mixture of fluoxetine and diclofenac to *Vibrio fischeri* and it was observed an antagonist effect of the mixture. From the doses applied for the decomposition of the mixture as effluent the 7.5 kGy was the more effective dose. TOC analysis indicated a low mineralization by irradiation treatment. Different authors reported radiation degradation of organic substances, suggesting the generation of organic acids as intermediate compounds.

It's important to encourage further studies that aim the minimization of the impact caused by pharmaceutical residues to human and environmental health.

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