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RADIATION EFFECTS ON THE FLUOXETINE HYDROCHLORIDE TOXICITY IN THE PRESENCE OF DOMESTIC SEWAGE

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ABSTRACT

The sanitation field is directly related to environmental quality and health. The improvements in wastewater treatment systems provide benefits to the environment and their population. It is clearly understood that the conventional wastewater treatment does not remove many micropollutants, including some medicinal products, and that these products can be toxic to living organisms. The goal of this research was the assessment of toxicity of fluoxetine hydrochloride (FH) as well as the irradiation application to remove FH from waters. The FH was irradiated in water solutions and also contained in domestic sewage. Both types of samples were irradiated at a Dynamitron® Electron Beam Accelerator (EBA). The *Vibrio fischeri* bacteria was applied as biological assay to the samples (water solution of FH; untreated sewage and the mixture of untreated sewage + FH). The efficiency was 1.44% to 26.21% less toxic after treatments. UV-Vis Spectrometry showed the degradation of FH by radiation. 2.5 kGy was a suitable dose that could be suggested for environmental application of Electron Beam Technology.

1. INTRODUCTION

To keep the quality of water resources is imperative for population health as well as for the maintenance of ecological balance. Nonetheless several factors have caused the degradation of these resources, bringing negative consequences for the biota, for the economy, and social as well.

According to the United Nations World Water Development Report more than 80% of the water used in the world is not collected or treated and goes through the water bodies [1].

Among several pollutants identified in the rivers there are the emerging pollutants, as new products or chemicals, that do not have regulated standard limits. Their effects to the environment and human health are unknown [2]. In this category we have the pharmaceuticals spread into waters due to the increasing use of these substances. The fluoxetine hydrochloride, FH, popularly known as Prozac®, is an example and it is a drug indicated for the treatment of depression, anxiety, etc [3].

During Metcalfe et al. studies, the fluoxetine was detected in three of four sampled systems: 0.038, 0.050 and 0.099 mg L^{-1}(WWTP) and 0.013 and 0.046 mg L^{-1} at surface water [4]. In the survey of Deblonde et al. [2] concentrations of fluoxetine were 5.85 mg L^{-1} in the influent and 0.112 mg L^{-1} in the effluent. In general environmental samples are contaminated by mixtures of compounds and their effects may be synergistic, antagonistic, adding and harmful to biota [5]. These evidences have contributed to the search of complementary wastewater treatments such as tertiary, Advanced Oxidation Processes (AOP) among others.

An advanced technology in development is the irradiation of effluents with electron beam accelerator. Through the direct and indirect action, known as radiolysis of water, the produced ions, excited species, ionized molecules and free electrons, will induce the oxidation, reduction, dissociation or degradation of given pollutants [6].

The Applied Ecotoxicology is an important tool for pollution prevention. For example, new chemicals registration requires this type of results. Several researches using AOP for treating effluents and chemicals, applied toxicity assays to measure the efficacy of EBA for removal of residual toxicity in different effluents and chemicals, at IPEN and other institutes [7, 8, 9]. Brazilian Projects demonstrated the potentially of radiation technology for pollution prevention and related toxicity [7, 8].

Homlak et al. demonstrated the elimination of diclofenac from water using irradiation and their work suggested 1kGy as possible radiation dose [9]. The Electron Beam Designs for environmental purposes have gained important knowledge nowadays specially by South Korean Group [10].

Regarding to ecotoxicity data and fluoxetine, it was noted that low concentrations (ng L^{-1}) were able to alter the metabolism of steroids and reproduction on *Dreissena polymorpha* mussel [11]; fluoxetine was evidenced also in muscle tissue, liver and brain of fish, collected in the stream Pecan Creek (Texas, USA) downstream of effluent discharges. Long persistence and bioaccumulation potential of fluoxetine were determined into *Oryzias latipes* fish [12, 13, 14].

The Hungarian studies for diclofenac decomposition by radiation were also evaluated for toxicity removal with *Vibrio fischeri* bacteria [9].

2. METHODOLOGY

The Electron Beam Accelerator was used for the decomposition of fluoxetine hydrochloride from water solutions. This paper reports the procedures carried out in order to evaluate chemical and ecotoxicological correlated aspects of the compound treated by radiation and untreated. The samples were irradiated and subjected to toxicity assays with *Vibrio fischeri* and qualitative analysis was performed by UV-Vis spectrophotometry.

An Dynamitron® Electron Beam Accelerator was used during the irradiations and fixed parameters were: 37.5 kW power and 1.4 MeV energy. The electric current varied according to the required dose up to 25 mA. Radiation doses applied were 2.5, 5.0, 7.5, 15.0 and 20.0 kGy. During the *Vibrio fischeri* luminescence assays, screening information was obtained to verify the effective radiation doses for the decomposition of 5 mg L^{-1} FH.

Biological assays were performed for measuring the acute toxicity to the *Vibrio fischeri* luminescent bacterium, according to ABNT NBR 15411-3/06 [15]. These assays were applied to fluoxetine hydrochloride solutions and to evaluate the efficacy of EB irradiation for reducing the whole toxicity.

The studied samples were FH standard solution (a), untreated sewage (b) and FH standard solution mixed with untreated sewage (c). The purpose of mixing the FH with untreated sewage was to evaluate the degradation pattern of FH in different irradiation conditions and mainly thinking about a further and real application of irradiation. The toxicity of those solutions was measured before and after radiation treatment.

The standard solutions were prepared with distilled water. Fluoxetine Hydrochloride (Divis Pharmaceuticals Pvt. Ltda, 98.78%) was used as a standard in chemical analysis and in degradation experiments.

Qualitative analysis was applied, by UV-Vis spectrophotometry, using a Varian equipment model Cary 50, obtaining the absorbances at wavelengths 200 to 800 nm (quartz cuvettes with 1 cm optical path).

3. RESULTS AND DISCUSSION

The toxicity values were expressed by the medium effective concentration (EC50), which refers to the concentration of the pharmaceutical compound that is lethal to 50% of a given organism within a specified exposure time. It is important to mention that a lower EC50, implies to a greater toxicity. An alternative way of expressing toxicity data is based on the toxic unit (TU) and is defined as 100/EC50.

The radiation processing efficiency was calculated based on TU, according to the formula: $((TU_{0kGy} - TU_{xkGy}) \times 100/TU_{0kGy})$. Where: TU_{0kGy} = Toxic Unit to untreated sample and TU_{xkGy} = Toxic Unit to sample irradiated with a given radiation dose. All the results related to the whole toxicity of studied solutions and irradiated solution are presented.

At Table 1 it is possible to note that the first experiment was carried out for doses from 0 to 7.5 kGy, while the second experiment included 5, 7.5, 15.0 and 20.0 kGy.

Table 1: Acute toxicity expressed by EC50, TU and efficacy of radiation for removal of toxicity on *Vibrio fischeri* bacteria

Samples	Doses (kGy)	EC50 (%) and confidence interval	TU	Efficiency (%)
F.H. (Exp. 1)	0	22.54 (9.25 – 54.92)	4.44	-
	2.5	27.13 (17.72 – 41.52)	3.69	16.92
	5.0	22.87 (9.07 – 57.65)	4.37	1.44
	7.5	29.96 (16.17 – 55.51)	3.34	24.77
F.H. (Exp. 2)	0	23.96 (6.53 – 87.88)	4.17	-
	5.0	28.60 (10.52 – 77.76)	3.50	16.22
	7.5	26.00 (9.79 – 69.06)	3.85	7.85
	15.0	26.35 (9.81 -70.79)	3.80	9.07
	20.0	26.57 (11.70 – 60.32)	3.76	9.82
Untreated sewage (Exp. 1)	0	16.36 (6.36 – 42.05)	6.11	-
	2.5	18.83 (5.38 – 65.90)	5.31	13.12
	5.0	20.58 (5.97 – 70.93)	4.86	20.51
	7.5	20.88 (6.39 – 68.22)	4.79	21.65
Untreated sewage (Exp. 2)	0	18.15 (8.79 – 37.47)	5.51	-
	5.0	21.43 (6.32 – 72.62)	4.67	15.31
	7.5	20.07 (6.05 – 66.53)	4.98	9.57
	15.0	22.35 (7.73 – 64.58)	4.47	18.79
	20.0	21.78 (7.19 – 65.93)	4.59	16.67
Mixture (Exp. 1)	0	16.05 (6.92 – 37.22)	6.23	-
	2.5	19.40 (5.61 – 67.00)	5.15	17.27
	5.0	21.75 (9.10 – 50.03)	4.60	26.21
	7.5	19.82 (5.58 – 70.36)	5.05	19.02
Mixture (Exp. 2)	0	18.53 (8.67 – 39.59)	5.40	-
	5.0	21.29 (7.30 – 62.09)	4.70	12.96
	7.5	23.90 (7.78 – 73.42)	4.18	22.47
	15.0	21.72 (6.76 – 69.74)	4.60	14.69
	20.0	21.32 (8.95 – 50.77)	4.69	13.09

Relatively low reduction on whole toxicity was obtained by irradiation and the doses from 2.5, 5.0 and 7.5kGy were the more effective for detoxification of irradiated samples. On the other hand, the decreasing concentration of FH was presented at Figures 1 and 2.

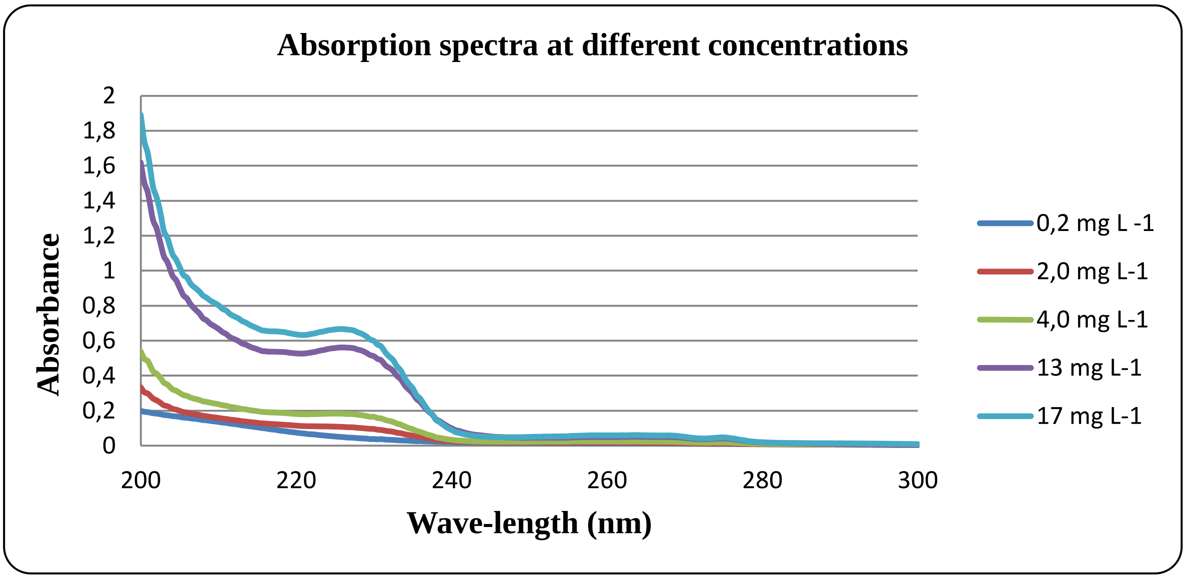

Figure 1: Absorption spectra of FH according to solution concentration

By UV-Vis spectrophotometric analysis the absorption peak of fluoxetine was identified at 226 nm. It is observed that there is no compound capable of absorbing wavelengths between 300-800 nm. The same behavior was observed in the irradiated samples.

The initial concentration of studied solution was 20 mg L^{-1} (figure 2). Through absorption spectra it was possible to verify the degradation of FH drug by radiation.

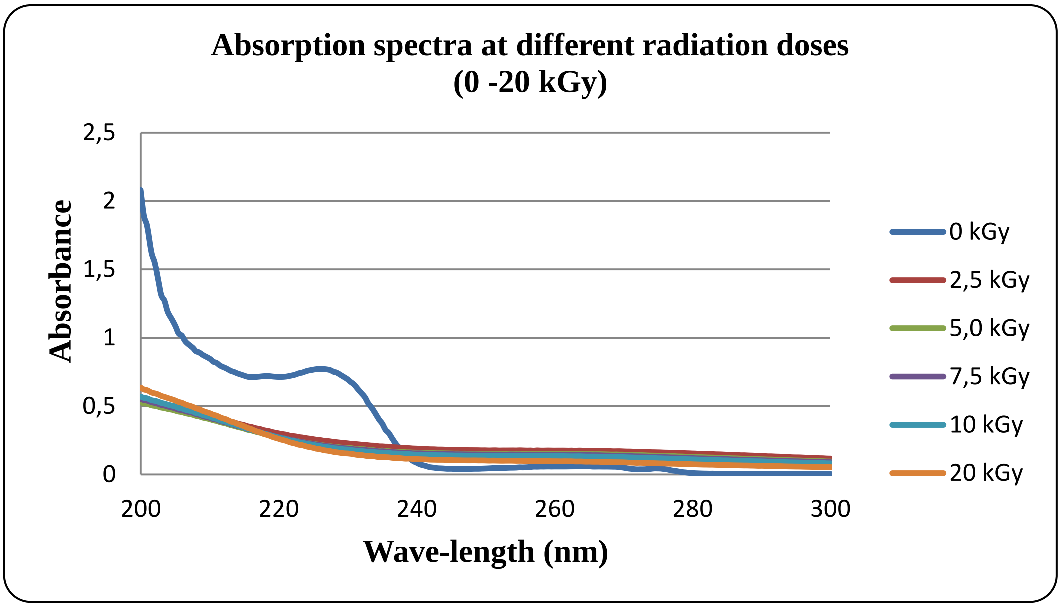

Figure 2: Absorption spectra of FH versus radiation dose

It have also been reported that organic compounds degradation induced by ionizing radiation generate organic acids and this may be confirmed by decreasing pH values at irradiated solutions. In relation the mixture it was not observed any additional effect for acute toxicity, once values of Toxic Units were very similar. It is a positive result since there were no increasing values for toxicity even in the mixture, and decreasing concentration of FH at relatively low dose. The technology discussed here will be effective for real situations according to economic vantages and for this the lower radiation doses means low costs and suitable irradiations systems as well.

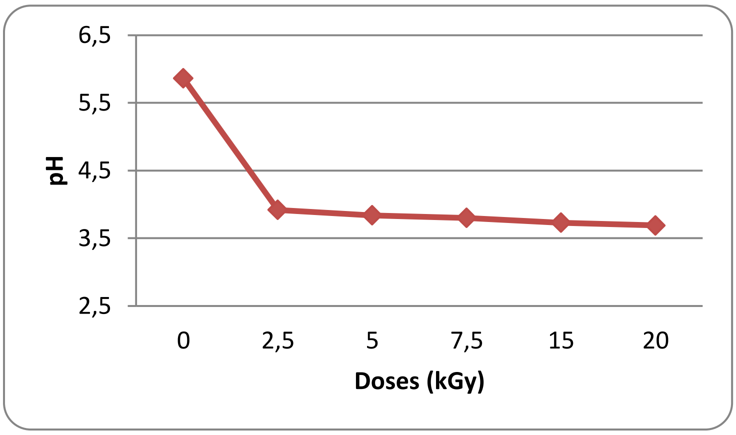

Figure 3: Standard FH solution pH versus radiation dose

4. CONCLUSIONS

The degradation of Fluoxetine Hydrochloride by radiation was obtained. The average EC50 for FH was 1.16 mg L^{-1} on *Vibrio fischeri* and after irradiation it was obtained toxicity removal from 1.44% to 26.21%. Despite the variable efficiency of radiation to reduce toxicity of *Vibrio fischeri*, preliminary studies with microcrustacean *Daphnia similis* showed better results at irradiated samples.

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