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TOXICITY REDUCTION FOR PHARMACEUTICALS MIXTURE IN WATER BY ELECTRON BEAM IRRADIATION

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ABSTRACT

The incorrect disposal of products is committing the environment quality once the aquatic environment is the main vehicle for dispersion of pollutants. Among the highlighted contaminants there are the pharmaceuticals, which are also released to the aquatic environment through the domestic sewage, hospitals and effluents. The monitoring of these pharmaceuticals in the environment has grown, showing many of them as persistent pollutants. Pharmaceuticals from different therapeutic classes have been detected in domestic sewage, surface water and groundwater around the world. Several studies evidenced Fluoxetine Hydrochloride residues in waters. Another important product is the Propranolol, used for heart disease treatments as far as fluoxetine is applied for treating mental diseases. The objective of this study was to apply the radiation processing for the abatment of pollutant in waters. Electron beam accelerator was used during irradiation of the mixture (Propranolol + Fluoxetine Hydrochloride) in aqueous solution. Acute toxicity assays were carried out for *Vibrio fischeri* marine bacterium, 15 minutes exposure. The results showed that irradiation (2.5kGy and 5.0kGy) enhanced the average effective concentration of the mixture, which means reduction of toxicity (56.34%, 55.70% respectively). Inverse effect was obtained with 7.5 kGy and 10 kGy.

1. INTRODUCTION

The water contamination remains a current topic in national and international level, since the aquatic environment is the main vehicle for dispersion of chemical pollutant, receiving domestic sewage, industrial and agricultural sewer [1]. The release of wastewater into the aquatic environments has reduced the survival conditions for aquatic organisms. For meeting the needs of the modern man, the disposal of products and by-products is jeopardizing the environment health. Among the highlighted contaminants are pharmaceuticals drugs, since they are also released to the aquatic environment and due to large-scale production [2].

The most significant entry of pharmaceuticals into the aquatic environment is through the sewage treatment plants [3]. Low biodegradability was evidenced once many products are not completely removed in the sewage treatment plants [4]. Several are the pathways of water contamination due to the discharge of pharmaceuticals and their metabolites. The improper disposal of medicines into the environment include the expired validity products, [5] [6]. Pharmaceuticals from different therapeutic classes have been detected in domestic sewage, surface water and groundwater around the world [7].

The Fluoxetine Hydrochloride, known worldwide under the trade name Prozac®, is one of the earliest developed and marketed drugs for treatment of mental diseases [8] [9] [10]. It is

adopted as a psychotropic drug treatment in depressive disorders and anxiety [11]. Another class of pharmaceuticals drugs, widely found in surface water is Propranolol, extensively used for heart diseases treatments [12].

Several studies have reported the presence of Propranolol and Fluoxetine hydrochloride in different environments, especially in effluents from sewage treatment plants and surface water [12] [13]. These pharmaceuticals are always present into the environment due to its wide commercialization and also by the wastewater treatment system to be inadequate in its removal.

Today it is clear that combined techniques for the improvement of wastewaters are necessary. Advanced oxidation processes (AOP) may increase efficiency for pharmaceuticals decomposition [14]. Among AOPs we find the ionizing radiation, which has been shown as an alternative or supplement to biological wastewater treatment, since they contribute in the removal of organic contaminants and help in improving biodegradability of wastewater [15] [7].

Aquatic Ecotoxicology allows us to assess contamination and predict future impacts in different ecosystems due to introduction of wastes and effluents. The use of living organisms for environmental monitoring is regulated by legislation. It may help identifying the causes and harmful effects of pollutants on individuals and group systems and to stand the safety levels once chronic effects may be avoided [16]. *Vibrio fischeri* have been widely used for the evaluation of industrial effluents, assessment of toxic charges, to measure the toxic potency of new chemicals and others applications [17].

In this paper ecotoxicity assays were used for the evaluation of both the mixture of pharmaceuticals itself and the efficacy of ionizing radiation for treating this mixture (Propranolol + Fluoxetine Hydrochloride).

2. MATERIAL AND METHODS

Propranolol and fluoxetine hydrochloride were dissolved in destiled water and the mixture of both was obtained as the goal sample to be decomposed by radiation. Toxicity assessment is the main target parameter as a positive radiation effect.

2.1. SAMPLE SOLUBILIZATION

The pharmaceutical fluoxetine hydrochloride is presented in the form of capsules containing 20mg fluoxetine in capsules with 30 units; produced by Eli Lilly do Brasil LTDA laboratory. Its formulation comprises 22.40mg fluoxetine hydrochloride, equivalent to 20 mg of fluoxetine and the pharmaceutical excipients, starch powder and starch powder 50% of silicone.

The pharmaceutical drug stock solutions were prepared by dilution of a 20mg fluoxetine capsule in 2 liters of water. The solution was stirred to ensure complete solubilization of the active substance.

Propranolol pharmaceutical drug is presented in tablet form containing 80mg of propranolol in packs of 30 tablets, produced by EMS S / A laboratory. Its formulation comprises 80mg of propranolol in the form of hydrochloride and the pharmaceutical excipients, stearic acid, lactose, silicon dioxide, microcrystalline cellulose and magnesium stearate.

The pharmaceutical drug stock solution was prepared by diluting one tablet of 80mg of propranolol in 1 liter of water. The solution was stirred to ensure complete solubilization of the active substance.

For the mixture of pharmaceuticals it was prepared one liter using volumetric flask, by mixing 500 ml of each drug a stock solution.

2.2. RADIATION OF TEST SOLUTIONS

The irradiations were performed at the Radiation Technology Center (CTR), Instituto de Pesquisas Energéticas e Nucleares (IPEN), São Paulo, Brazil. A Dynamitron Electron Beam Accelerator was applied for irradiations. Machine Job188, model DC 1500/25/4, manufactured by RDI-Radiation Dynamics INC. The energy was fixed at 1.4 MeV during the experiments.

The aqueous solution of the mixture of fluoxetine hydrochloride (10mg/L-1) and propranolol (80mg/L-1) was considered as an effluent sample. Liquid samples were irradiated using batch system in borosilicate containers (Pyrex type). Samples were covered with plastic wrap during irradiation for protection. The applied radiation doses were: 2.5 kGy , 5.0 kGy, 7.5 kGy e 10 kGy.

2.3. TOXICITY ASSAYS - *Vibrio fischeri*

The *Vibrio fischeri* bacterium was the biological indicator of toxicity and it was purchased in lyophilized form, as Biolux®. The *Vibrio fischeri* bacterium was hydrated using 1000µl reactivation buffer. All the samples were tested at Microbics® (M500 Toxicity Analyzer) for the measurement of luminescence. Samples were stored in a chamber at 15 °C. and the stock solution of bacteria was maintained at 4°C ± 0.3. Serial dilution of samples was carried out as far as the samples measurements according to ABNT NBR 15411-3/12 standard method. After obtaining I_0 and I_{15} bioluminescence readings the EC_{50} was calculated.

2.4. STATISTICAL ANALYSIS

The statistical analysis for *Vibrio fischeri* acute toxicity tests were based on the gamma value

(ratio between the light lost and the remanescent light from each pair) and the concentration of the sample. The data I_0 and I_{15} were analysed by linear regression. Besides the EC_{50} values the 95% confidence interval was determined.

3. RESULTS AND DISCUSSION

Acute toxicity tests were carried out with *Vibrio fischeri* in the mixture of pharmaceuticals Propranolol and Hydrochloride Fluoxetine. The values for untreated samples, not irradiated, were shown in Table 1. The data for irradiated samples were organized at Table 2. The decrease in bioluminescence emitted by *V. fischeri* was the basis for this study.

Table 1. Acute toxicity of the original mixture to *Vibrio fischeri* (EC_{50})

Assay	EC 50 (mg/L^{-1})	Confidence Interval (mg/L^{-1})
1	40.46	35.67 – 45.90
2	47.28	32.14 – 69.54
3	45.62	36.69 – 56.72
X ± S	**44.45 ± 3.55**	

Table 2. Acute toxicity of the irradiated mixture to *Vibrio fischeri* (EC_{50})

Assay	Dose (kGy)	EC 50 (mg/L^{-1})	Confidence Interval (mg/L^{-1})
1		60.02	39.27 – 91.73
2	2.5	55.21	38.44 – 79.31
3		53.80	30.99 – 93.38
X ± S		**56.34 ± 0.99**	
1		55.22	40.33 – 75.61
2	5.0	54.60	33.78 – 88.27
3		57.29	45.56 – 72.05
X ± S		**55.70 ± 1.40**	
1		25.46	14.44 – 44.90
2	7.5	27.74	16.16 – 47.62
3		27.58	12.51 – 60.79

X ± S		**26.92 ± 1.27**	
1		19.13	4.75 – 77.09
2	10.0	20.55	8.00 – 52.79
3		22.39	8.7 – 57.31
X ± S		**20.69 ± 1.63**	

It was noted that 44.45 ± 3.55 was the EC50 for *V.fischeri*, which means the average concentration that reduced 50% of initial light produced by bacteria. These numbers allows us to conclude that is a toxic sample. After electron beam irradiation it was possible to observe two interesting responses. The first was an important reduction in toxicity, obtained at 2.5kGy and 5 kGy. When higher doses were applied, 7.5 kGy and 10.0 kGy, it was noted increasing toxicity in relation to the unirradiated samples (the initias 44.45 ± 3.55 was reduced to 20.69 ± 1.63 for 10kGy). It is important to note that the lower the values of EC50, higher is the effect and the toxicity, once we have inverse numbers. The decreasing in pH values by irradiation may be related to organic acids formation. At Table 3 the pH, dissolved oxygen and conductivity of samples irradiated and non irradiated were presented.

Table 3. pH, Dissolved Oxygen and Conductivity of samples of the mixture (Fluoxetine + Propranolol) used in the trials with *Vibrio fischeri.*

Dose (kGy)	Assay	pH	Dissolved Oxygen (mg/L^{-1})	Conductivity ($\mu S/cm^{-1}$)
0.0	1	5.50	8.07	46.1
	2	5.76	7.58	48.5
	3	6.20	8.95	50.2
	X ± S	**5.82 ± 0.35**	**8.20 ± 0.69**	**48.26 ± 2.05**
2.5	1	4.68	7.47	60.9
	2	5.65	7.86	68.7
	3	6.10	8.74	67.5
	X ± S	**5.47 ± 0.72**	**8.02 ± 0.65**	**65.7 ± 4.20**
5.0	1	4.68	7.85	80.0
	2	4.53	8.76	85.7
	X ± S	**4.60 ± 0.10**	**8.30 ± 0.64**	**82.85 ± 4.03**
7.5	1	4.08	7.96	97.0
	2	3.92	8.81	100.4

	X ± S	**4.00 ± 0.11**	**8.38 ± 0.60**	**98.7 ± 2.40**
	1	3.76	7.95	114.4
10.0	2	3.74	8.84	113.6
	X ± S	**3.75 ± 0.01**	**8.39 ± 0.62**	**114 ± 0.56**

The decrease in pH due to degradation of organic compounds from the irradiation treatment has been demonstrated by several authors [15] [18] [13]. It has also been observed the increase in conductivity due to the increase of the applied dose. The higher radiation dose means that we may have higher number of ionized molecules [18] [13]. There were no significant variations in dissolved oxygen value.

Doses of 2.5 kGy and 5.0 kGy were the best in reducing the toxicity of the mixture of pharmaceuticals drugs. The dose more reduced toxicity to the *V. fischeri* bacterium was 2.5 kGy with 26.74% efficiency, then the dose of 5.0 kGy with 25.30%, significant in reduction of toxicity.

In ecotoxicological studies with Fluoxetine Hydrochloride, better results for the reduction of acute toxicity were obtained as doses of 2.5 kGy - 2 times less toxic [18], and 5.0 kGy, with efficiency removal of 17.26% [13].

Propranolol drug degradation studies through advanced oxidation processes (POAs) proved promising results. The processes H_2O_2/UV and photo-Fenton present high rate of degradation with few minutes of treatment, reaching 90% of the removal efficiency [19]. These results can be explained by a possible transformation of the molecule of pharmaceuticals, due to hydroxyl radicals formed by radiolysis of water and the direct irradiation of the molecule [18].

Acute toxicity tests with the *Vibrio fischeri* bacterium, and *Daphnia similis* crustacean, are used to evaluate the toxicity of industrial and domestic effluents exposed to ionizing radiation, showing significant reduction of pharmaceuticals toxicity.

4. CONCLUSIONS

In this paper the effects of ionizing radiation on acute toxicity were determined using *Vibrio fischeri* luminescence test. Aqueous solution of the mixture of Fluoxetine Hydrochloride and Propranolol when irradiated by accelerated electrons resulted that 2.5 kGy was the more effective dose (26.74% less toxic) and 5.0 kGy (25.30% less toxic) than the unirradiated sample. Nonetheless at 7.5 and 10.0 kGy there was an opposite effect, increased toxicity, which may be attributed to decrease in pH and possibility of by products formation that may be more toxic than the target compounds in the not irradiated mixture. On the other hand, the efficacy of 2.5 kGy is important once as lower is the dose for treatment, lower are the prices for the technology application.

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