BJRS

BRAZILIAN JOURNAL
OF
RADIATION SCIENCES
10-03B (2022) 01-08

Degradation and Toxicity of Amoxicillin After Electron Beam Irradiation

Borrely, S.I.; Redígolo, M.M.; Villard, B.D.; Lebre, D.T.; Tominaga, F.K.

Instituto de Pesquisas Energéticas e Nucleares (IPEN/CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP, Brazil
marcelo.redigolo@alumni.usp.br

ABSTRACT

A recent and growing concern in environmental studies is the presence of antibiotics in wastewater, which contributes to antimicrobial resistance building. Amoxicillin, according to the World Health Organization, is one of the most consumed antibiotics worldwide, for being a first line therapy for common infections. Among several drug degradation methodologies, electron beam irradiation (EBI) is presented as an efficient and green treatment. This work presents data on amoxicillin degradation via EBI. Degradation rate was evaluated by LC/MS-MS, carbon removal efficiency was evaluated by TOC and ecotoxicity assays were performed employing *Vibrio fischeri*. Chromatographic results indicate an efficiency removal of 97.65% at 0.75 kGy and concentration below the limit of detection with increasing absorbed dose. Low mineralization (up to 10%) was achieved at 3.0 kGy. Regarding toxicity, approximately 81% of toxicity removal was obtained at 0.75 kGy and a decrease in efficiency was achieved with higher doses. In conclusion, results indicate the low doses (0.75 kGy) as most effective for drug removal employing EBI.

Keywords: **Electron Beam Irradiation, Antibiotic, Toxicity.**

ISSN: 2319-0612
DOI: https://doi.org/10.15392/2319-0612.2022.1894
Submitted: 2022-01-28
Accepted: 2022-09-21

1. INTRODUCTION

Pharmaceutical and personal care products (PPCPs) have been a major concern in water pollution studies, over the past decade. These substances are used for medical care, cosmetics, hygiene and health care purposes and they possess a high global production and consumption rates, and a rapid discharge into the environment without control [1]. Among PPCPs, antibiotics are especially important due to their drug resistance building potential. It is estimated that 75% of all consumed antibiotic may be excreted unmetabolized in the domestic sewage wastewater treatment (WWT) facilities [2].

Amoxicillin is a β-lactam antibiotic (Fig. 1), which is frequently used in human and veterinary medicine around the world for the treatment of both gram-positive and negative bacteria. According to the WHO reports in 2015, amoxicillin is one of the most essential medicines used in basic health care [3]. Its impact on human health and the environment is uncertain and there is still a lack of information about ecotoxicity, however, few works show adverse effects on the biota. For instance, bacteria have developed resistance against this antibiotic due to the extensive consumption [4]. At a higher dose, amoxicillin is found to be a potential mutagen, carcinogen, and teratogen [3].

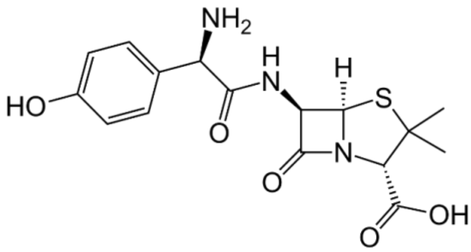

Figure 1: *Molecular structure of amoxicillin*

Several degradation methodologies for β-lactam antibiotics have been reported, such as sorption, biodegradation, photodegradation, oxidation and irradiation [3]. Electron beam irradiation (EBI) treatment is a green technology, being efficient and safe. The starting reaction occurring during EB irradiation in liquid wastewater samples is known as water radiolysis (Equation 1), and it gives a sequential list of interactions, which promote the degradation of organic compounds, as discussed by many authors [5]. For instance, when Zhu and coworkers (2021) discussed the kinetics, removal mechanisms, degradation products and toxicity during EB irradiation of sulfonamide antibiotics [6].

$$H_2O \overset{EB}{\rightarrow} e^-_{(aq.)} + H^\bullet + HO^\bullet + H_2O_2 + H_2 + H_3O^+ \quad (1)$$

From the literature, we would like to emphasize that for environmental use many advances are in constant development and that EBI and gamma irradiations were applied for the degradation of several pharmaceuticals. Takacs and coworkers (2021) evidenced that the oxacillin biodegradability is improved by γ-irradiation, producing non-toxic degradation products to microbes in a WWT plant. In addition, due to altering the bicyclic structure, elimination of antimicrobial activity was noted [7]. Tegze and coworkers (2021) showed > 99% removal of ciprofloxacin and norfloxacin at 2 kGy (8 kGy h^{-1} dose rate), demonstrating also that the degradation products had no antibacterial activity and that non-biodegradable antibiotics were transform to biodegradable ones [8].

The objective of this present paper is to show the possible use of ionizing radiation for the degradation and detoxification of amoxicillin antibiotic, one product frequently released through wastewater.

2. MATERIALS AND METHODS

2.1. Reagents

Amoxicillin trihydrate [$C_{16}H_{19}N_3O_5S.3H_2O$, MM = 419.5 g.$mol^{-1}$; (2S,5R,6R)-6-[[(2R)-2-amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate; CAS 61336-70-7] was purchased from Sigma-Aldrich. Formic acid and acetonitrile, chromatographic grade, was purchased from Supelco/Millipore.

Amoxicillin samples were prepared by diluting standard reagent in ultra-pure water (Millipore Milli-Q) prepared at 10 mg. L^{-1}.

2.2. Irradiation process

Irradiation of the aqueous samples was carried out at Dynamitron Electron Beam Accelerator. Samples were irradiated using a batch system in borosilicate containers (Pyrex®) with a volume of 246 mL to ensure a suitable beam penetration. The beam energy was fixed at a 1.4 MeV electron beam accelerator, through the variation of electric current. Applied doses were 0.75, 1.50 and 3.0 kGy. Absorbed doses were confirmed using a Perspex Harwell Red dosimeter, batch KZ-4034, with less than 5% variation.

2.3. Chemical analysis

Chemical characterization was carried out using Agilent HPLC model 1290 coupled to Sciex QTrap model 3200. Separation conditions were: Restek Ultra Aqueous (150 x 2.1 mm x 3.0 µm) column, mobile phase (A) H_2O + 0.1% formic acid, (B) acetonitrile + 0.1% formic acid, sample injection volume of 5.0 µL. MRM (multiple reaction monitoring) scan type was employed, with Q1 (366.11 Da) and Q3 (208.1 Da). Calibration curve (R^2 = 0.9968, LOD = 3 µg L^{-1}; LOQ = 11 µg L^{-1}) were determined for quantification, respectively, where LOD and LOQ refer to the limits of detection and quantification, respectively.

Total Organic Carbon (TOC) was analyzed on a Shimadzu equipment, TOC-L model, to determine organic carbon removal after irradiation.

2.4. Toxicity assays using *Vibrio fischeri*

Vibrio fischeri toxicity assays were applied for measuring acute effects for irradiated and non-irradiated samples. The acute toxicity assays were performed according to Brazilian standard methods (NBR 15411/2012) [9]. The exposure time was 15 minutes, and the negative effects were related to bacterial luminescence decreasing and associated to the gamma values (relation between lost and remaining light). 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.

3. RESULTS AND DISCUSSION

Chromatographic results (Fig. 2) indicated that peak intensity has dramatically decreased for the absorbed dose of 0.75 kGy, presenting an efficiency removal of 97.65 ± 0.33%. By increasing dose, the values for samples irradiated at 1.5 and 3.0 kGy are below the detection limit (3 µg. L^{-1}), showing efficient antibiotic removal at low doses. Regarding the total organic carbon removal, the irradiation of the antibiotic samples decreased TOC content with increase of with absorbed dose (Table 1). Nevertheless, negligible TOC removal was obtained at the proposed doses, reaching up approximately 10% at 3.0 kGy, indicating low decomposition of the pollutant into H_2O and CO_2.

Ionizing radiation has been demonstrated suitable for degradation of antibiotics at low doses [6, 7,8]. However, in terms of mineralization TOC removal is less effective than organic pollutants removal since extremely high absorbed doses are needed for complete mineralization [10]. Therefore, toxicity assays are necessary to evaluate the toxicity of the generated byproducts.

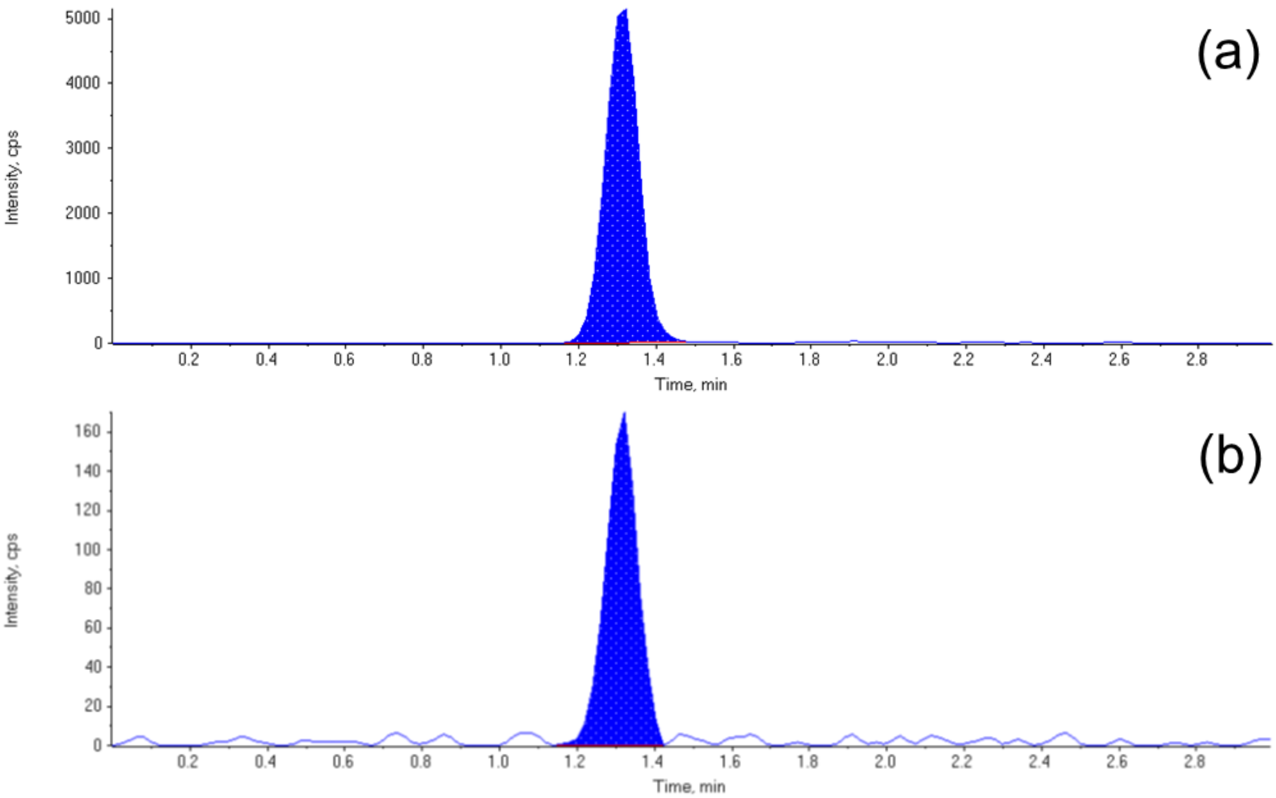

Figure 2: *Comparative chromatogram of non-irradiated (a) and irradiated (b) amoxicillin samples at 0.75 kGy*

Table 1: Comparative effect of irradiation on mineralization.

Doses (kGy)	Total Organic Carbon (TOC) (mg. L^{-1})	TOC Removal (%)
0.00	14.44 ± 0.07	-
0.75	13.85 ± 0.12	4.13 ± 0.80
1.50	13.28 ± 0.03	8.03 ± 0.17
3.00	13.04 ± 0.05	9.74 ± 0.33

The results on toxicity removal were reported at Table 2. The average concentration of amoxicillin to inhibit 50% luminescence was 10.50%, while the 0.75 kGy irradiated solution corresponded to 54.14% of the sample. These values indicated 80% of toxicity removal at studied conditions. Note that lower dose (0.75 kGy) was more effective than 1.50 and 3.0 kGy for toxicity removal (acute effects).

Toxicity assays are important tools for evaluating the impact of the effluents since after the treatment, there might occur the formation of more toxic byproducts. Toxicity reduction of antibiotics, such as tetracycline, sulfanilamide has been reported employing *V. fischeri* after irradiation treatment at low doses (<1 kGy) [11, 12]. In contrast, Sági and coworkers (2018) found out negligible changes in toxicity to *Vibrio fischeri* in irradiated samples of sulfaguanidine, while more toxic byproducts were generated after the treatment for sulfathiazole and sulfamethoxazole [12]. Therefore, further studies should be conducted to correlate the generated products with toxicity.

Table 2: Comparative effect of irradiation on toxicity assessed by bioluminescent bacteria *V. fischeri* (15 min exposure)

Doses (kGy)	CE50% (15min)	Toxicity Removal (%)
0.00	10.50 (2.30 – 47.82)	-
0.75	54.14 (31.36 – 93.46)	80.60
1.50	33.81 (13.37 – 92.43)	68.94
3.00	29.39 (12.08 – 71.50)	64.27

The variety of pollutants dissolved into the aquatic environment is increasing for many reasons such as wastewater introduced by WWT facilities, industrial discharges of liquid effluents, solid residues and excessive water usage for several applications. Healthcare care products, including pharmaceuticals, have been detected in many parts of globe. Regarding negative effects of antibiotics to aquatic biota, they are associated to many types of pollutants affecting reproduction and behavior of aquatic organisms and bacterial resistance to antibiotics [6]. Generally, the effects of antibiotics on bacteria and micro algae are 2 to 3 orders of magnitude below the toxic values for higher trophic levels. But still, if antibiotics exert adverse effects on crustaceans in nature, these effects could be an indirect result of an influence on their food organisms.

4. CONCLUSION

An important reduction of amoxicillin was achieved at 0.75 kGy, at neutral pH solutions, while there was an effective reduction on toxicity. Further studies will include the decomposition of antibiotics contained into wastewater samples (spiked).

ACKNOWLEDGMENT

The authors thank CAPES and CNPq Brazilian councils for supporting the PhD students and International Atomic Energy Agency (IAEA) for fundings.

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