Degradation of diclofenac by electron beam irradiaton: Toxicitiy removal, by-products identification and effect of another pharmaceutical compound

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ABSTRACT

Water contamination by the anti-inflammatory drug diclofenac (DCF) is a consequence of its incomplete removal in wastewater and sewage treatment plants, which is potentialized by interactions with other pharmaceutical contaminants. In this context, electron beam irradiation (EBI) has been considered a clean technology for degrading pharmaceutical compounds in water. Nevertheless, the identification of DCF by-products and their correlation with biological recalcitrance and acute toxicity are poorly understood. In this study, the *V. fischeri* test was used to characterize DCF toxicity in the absence and presence of fluoxetine (FLX), prior and after irradiation. The results showed complete DCF degradation at low dose (5 kGy). DCF concentration followed pseudo first-order decay with respect to the absorbed, with $k_0 = (1.33 \pm 0.10)$ kGy^{-1} (DCF) and $k_0 = (0.90 \pm 0.12)$ kGy^{-1} (DCF + FLX). In contrast, negligible TOC removal was observed even at 7.5 kGy, with the formation of recalcitrant, non-biodegradable by-products, as also suggested by the respirometry test. Despite that, the toxicity of the DCF solution diminished from (19.6 ± 1.6) TU to (6.2 ± 2.3) TU, and from (6.8 ± 0.9) TU to (3.1 ± 0.2) TU, in the absence and presence of FLX, respectively, after irradiation up to 5 kGy. Four of the eleven by-products identified by direct-injection MS were easily degraded by EBI, and one ($C_{13}H_{14}ClNO_5$) was considered the least recalcitrant but the most toxic. Based on these results, a possible DCF degradation pathway is proposed, involving hydroxylation and oxidation of aromatic rings, dehalogenation and $C - N$ bond cleavage.

1. Introduction

Different advanced oxidation processes (AOPs) (e.g., Fenton, photo-Fenton, TiO_2 photocatalysis and photoelectrocatalysis, ozonation, direct photolysis) have been applied as technological alternatives for degrading diclofenac (DCF) in aqueous systems, generally with incomplete mineralization [1–8]. These processes may generate unwanted and toxic by-products that must be considered as contaminants as well, which is a concern in any real application. Moreover, AOP do have practical constraints associated with operational variables such as solution pH, catalyst and auxiliary oxidant concentrations [9–11]. For example, pH and the iron/oxalic acid molar ratio were found to play a critical role in DCF degradation by the photo-Fenton reaction [6,8]. Likewise, ozonation of DCF is limited in acidic conditions due to the inhibition of ozone decomposition into oxidizing radical species [7,12]. Finally, although TiO_2 may promote DCF degradation under visible light, the catalyst is difficult to separate from aqueous suspensions for reuse [13,14].

In contrast to these processes, electron beam irradiation (EBI) has been considered a clean process technology that offers an attractive environmentally friendly alternative for degrading pollutants in water matrices [15–20], including pharmaceutical compounds [21–24], with the advantage of short degradation times, inline processing, insensitiveness to color and suspended solids [25] and no need of pH control and/or catalysts [15,23]. In addition, radiolysis efficiency can be increased by adding oxidizing additives, such as H_2O_2, $S_2O_8^{2-}$ or ozone [26–28].

EBI leads to water radiolysis, generating hydroxyl radicals (HO), hydrogen atoms (H), electronically excited species, ions, and solvated electrons (e_{aq}^-), as summarized by Eq. (1). The terms in brackets give the maximum amounts of each species formed (in μmol) per 1 J of electron energy absorbed in pure water, usually referred as the *G*-values [28]:
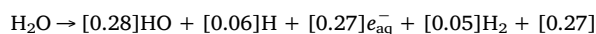
$$H_2O \rightarrow [0.28]HO + [0.06]H + [0.27]e_{aq}^- + [0.05]H_2 + [0.27]$$

$$H_3O_{aq}^+ + [0.07]H_2O_2 \tag{1}$$

All the three species H, e^-_{aq} and HO may react with organic contaminants. Solvated electrons and hydrogen atoms are reductive species. The former react through single electron transfer with substrates M having more positive reduction potentials by single-electron transfer processes (Eq. (2)), and also act as nucleophiles, readily removi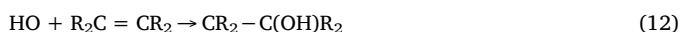ng halide ions from halogenated contaminants (Eq. (3)) [28,29]. Nevertheless, e^-_{aq} are easily scavenged by H_3O^+ ions in acidic medium (Eq. (4)), and by dissolved molecular oxygen in aerated solutions, with formation of superoxide radical anions, $O_2^{\cdot-}$ (Eq. (5)), which are protonated to yield hydroperoxyl radicals, HO_2 (Eq. (6)). The $O_2^{\cdot-}$ / HO_2 pair, however, has low reactivity with aromatic compounds [29]. Hydroperoxyl radicals can further disproportionate, yielding H_2O_2 and molecular oxygen (Eq. (7)) [29]. Solvated electrons also react with hydroxyl radicals (Eq. (8)) [25].

$$e^-_{aq} + M^n \rightarrow M^{n-1} \tag{2}$$

$$e^-_{aq} + R - X \rightarrow (R - X)^- \rightarrow R + X^- \tag{3}$$

$$e^-_{aq} + H_3O^+ \rightarrow H + H_2O \tag{4}$$

$$e^-_{aq} + O_{2aq} \rightarrow O_2^{\cdot-} \tag{5}$$

$$O_2^{\cdot-} + H_3O^+ \rightleftharpoons HO_2 + H_2O \; (pK_a = 4.8) \tag{6}$$

$$2HO_2 \rightarrow O_2 + H_2O_2 \tag{7}$$

$$e^-_{aq} + HO \rightarrow HO^- \tag{8}$$
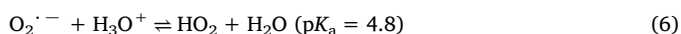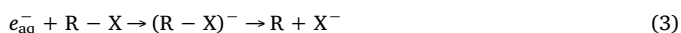
Hydrogen atoms, in turn, are the conjugated acid of solvated electrons and react with organic contaminants through hydrogen abstraction and addition, resulting in carbon-centered organic radicals; H are also readily trapped by O_2, with the formation of hydroperoxyl radicals (HO_2) (Eq. (9)) [29]:

$$H + O_{2aq} \rightarrow HO_2 \tag{9}$$

Hydroxyl radicals are extremely oxidizing, unselective species, which react with target substrates by electron transfer, hydrogen abstraction and electrophilic addition (Eqs. (10)–(12), respectively), leading to rapid degradation of organic pollutants [29]:

$$HO + M^n \rightarrow M^{n+1} + HO^- \tag{10}$$

$$HO + R - H \rightarrow R + H_2O \tag{11}$$

$$HO + R_2C = CR_2 \rightarrow CR_2 - C(OH)R_2 \tag{12}$$

Hydrogen abstraction involves saturated and many unsaturared molecules, while addition reactions occur with unsaturated aliphatic chains and aromatic rings, yielding hydroxylated carbon-centered radicals [28]. In the case o DCF, hydroxylated aromatic molecules are expected to dominate among initial DCF by-products [1,30–33]. The carbon centered radicals formed (Eqs. (3), (11) and (12)) react with dissolved molecular oxygen, giving peroxyl radicals (R–O–O·) that decompose through a complex series of radical reactions, yielding oxidation products [29].

Previous studies on diclofenac degradation using water radiolysis are found in the literature [22,25,34,35]. Homlok et al. [25] achieved complete DCF removal ($[DCF]_0 = 0.1$ mmol L^{-1}) using ^{60}Co γ-irradiation at 1 kGy absorbed dose. The authors concluded that 5–10 times higher doses were needed for complete mineralization and expressive decrease in toxicity in aerated medium. The removal of nineteen multi-class pharmaceutical products present in the influent of a WWTP was investigated by Reinholds et al. [36], who observed 99% DCF removal using EBI (5 kGy) and γ-irradiation (3 kGy) ($[DCF]_0 = 2.2$ ng L^{-1}). He et al. [22] reported almost 100% DCF removal using EBI at 0.5 kGy dose, for initial pollutant concentrations in the range 10–40 mg L^{-1}.

Nevertheless, there is a lack of studies that identify DCF by-products and evaluate the toxicity of aqueous solutions prior and after the radiolytic degradation of DCF through EBI, particularly in association with other pharmaceutical compounds. In fact, wastewater and sewage treatment plant effluents are complex systems containing several pharmaceutical compounds [37–39], whose interactions may be synergetic, additive or antagonistic. Ecotoxicological tests can therefore be used as valuable tools for evaluating the toxicity of aqueous solutions containing DCF, its by-products, and other pharmaceutical compounds. Among them, the inhibition of *Vibrio fischeri* luminescence is a sensitive, quick, reliable and standardized test [23,40]. For example, He et al. [22] treated a 10 mg L^{-1} DCF solution using EBI at a dose of 1 kGy, observing bioluminescence inhibition varying from 80% to 75%. For a 50 mg L^{-1} initial DCF concentration, Trojanowicz et al. [34] achieved DCF removals of 80–100% for ^{60}Co γ-radiation doses ranging from 0.5 to 1.0 kGy or higher. The authors reported an increase in toxicity for lower doses (0.5–0.8 kGy) and complete toxicity removal for doses up to 5 kGy. Nevertheless, although by-products may be more toxic than DCF [41], their correlation with toxicity measurements is still poorly understood.

In the present study, DCF removal by electron beam irradiation (EBI) in aqueous solution was investigated. DCF by-products were identified and a possible degradation pathway is proposed. The *V. fischeri* test was used to characterize DCF toxicity in the presence and absence of another pharmaceutical compound (fluoxetine, FLX), prior and after irradiation. The major research gap in the current literature concerning the correlation of DCF by-products with toxicity measurements is also addressed.

2. Experimental

2.1. Reagents

Diclofenac ($C_{14}H_{11}Cl_2NO_2$, 296.14 g mol^{-1}; 2-[2-[(2.6-dichlorophenyl)amino]-phenyl]acetic acid; CAS 315307-86-5; Pharmadium Ltd.; 98.8%) was used as a standard in the chromatographic analysis. Commercial DCF, marketed as Voltaren® (Novartis) was used in all the degradation experiments. Fluoxetine hydrochloride ($C_{17}H_{18}F_3NO\cdot HCl$, 309.33 g mol^{-1}; methyl[(3S)-3-phenyl-3-[4-(trifluoromethyl)phenoxy]propyl]amine]; CAS 54910-89-3; Divis Pharmaceuticals Pvt. Ltd.; 98.8%) was used as a standard in the chromatographic analysis. Commercial FLX, marketed as Prozac® (Lilly) was used in all the experiments. Methanol, acetonitrile, and acetic acid (HPLC grade) were purchased from Sigma-Aldrich. All the solutions used in EBI experiments were prepared using ultra-pure water (Millipore Milli-Q).

2.2. Degradation experiments

Electron beam irradiation (EBI) was performed using a Dynamitron® Electron Beam Accelerator at 37.5 kW and 1.4 MeV, as previously described [23]. Due to operational constraints of the equipment, radiation doses of 2.5, 5.0, and 7.5 kGy were used; an additional run was performed at 1.0 kGy in order to follow DCF degradation. Doses were measured using a Perspex Harwell Red Batch KZ-4034 dosimeter with less than 5% variation. Samples were placed in rectangular glass recipients (Pyrex®) and irradiated in batch; a volume of 246 mL was used in order to ensure a maximum exposed liquid thickness of 4 mm for adequate electron beam penetration. The recipients passed twice under the electron beam on an automated conveyor at 6.72 m min^{-1}. Two replicates of each experiment were performed.

2.3. Ultra-fast liquid chromatography (UFLC) analyses

UFLC was employed to determine diclofenac (DCF) and fluoxetine (FLX) concentrations in aqueous solution using a Shimadzu equipment (LC 20 AD) with UV/Vis (SPD 20 A) and fluorescence (RF-10Axl) detectors. A C18 column (Kinetex Phenomenex 150 mm × 4.6 mm, 5 μm)
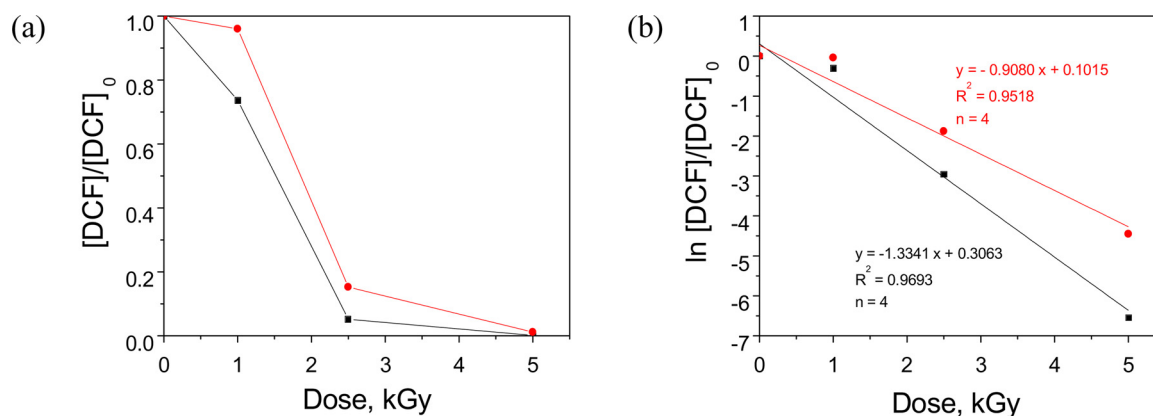
Fig. 1. (a) Diclofenac (DCF) degradation by electron beam irradiation (EBI) vs. radiation dose and (b) logarithm of relative DCF concentration vs. radiation dose, in the absence (-■-) and presence (-●-) of FLX. Experiments run in triplicate. The average relative errors of the original [DCF] vs. dose data were 5.8% and 2.8% for the runs in the absence and presence of FLX, respectively. Initial conditions: $[DCF]_0 = (51.8 \pm 1.1)$ mg L^{-1}; $[FLX]_0 = (4.1 \pm 0.8)$ mg L^{-1}; $pH_0 = 6.8 \pm 0.8$.

was used; the oven temperature was 40 °C. Gradient elution was performed with (A) acetic acid 1% and (B) acetonitrile (0–7 min, A:B = 71:29; 7–11 min, A:B = 48:52; 11–14 min, A:B = 71:29), at a flow rate 1.8 mL min^{-1}. The fluorescence detector was used for FLX identification at 230 nm (excitation) and 290 nm (emission). DCF was detected by UV/Vis absorption at 276 nm. The retention times were 9.9 min and 5.9 min for DCF and FLX, respectively. The injection volumes were 10 μL (1–40 mg L^{-1}, calibration curve 1) and 70 μL (< 1 mg L^{-1}, calibration curve 2), respectively. Calibration was performed using external standards prepared with known FLX and DCF concentrations. Two calibration curves were used depending on the concentration range. For FLX: curve 1 ($R^2 = 0.9997$; DL = 11 μg L^{-1}; QL = 33.3 μg L^{-1}) and curve 2 ($R^2 = 0.999$; DL = 1090 μg L^{-1}; QL = 3320 μg L^{-1}); for DCF: curve 1 ($R^2 = 0.9995$; DL = 8.44 μg L^{-1}; QL = 25.6 μg L^{-1}) and curve 2 ($R^2 = 0.9998$; DL = 918 μg L^{-1}; QL = 2780 μg L^{-1}), where DL and QL refer to the detection and quantification limits, respectively.

2.4. Total organic carbon measurement

The total organic carbon (TOC) of selected samples was measured using a Shimadzu TOC-5000 A equipment.

2.5. Toxicity assays

Acute toxicity assays were performed with the luminescent bacteria *Vibrio fischeri*, as previously described [23]. *V. fischeri* bioluminescence was detected using a Microbics 500® photometer and four sample dilutions were measured after 15-min exposure time. The stock solutions of DCF (100 mg L^{-1}), FLX (10 mg L^{-1}), and the mixture (containing 50 and 5 mg L^{-1} of DCF and FLX, respectively), as well as the irradiated samples, were used in the assays. The pH of the irradiated solutions was not corrected. The acute toxicity is expressed in toxicity units (TU = 100/EC50) and corresponds to the average effect concentrations that promoted 50% bioluminescence inhibition of exposed living-organisms (EC50%). The EC50 values were calculated using standard statistical procedures; standard deviations were calculated from three replicates of the measurements for each toxicity measurement. Tukey's multiple comparison significance test (at $p = 0.05$) was applied to assess the existence of significant differences between the results obtained.

2.6. Respirometry assays

Respirometry tests were performed using biological sludge samples collected from a sewage treatment plant. An open type semicontinuous

respirometer (Beluga model S32c, Biotec) was used. In order to minimize possible atmospheric oxygen transfer to the liquid, a small reactor lid with openings for the propeller stirrer and aerators was employed. The tests followed the methodology used for determining the oxygen uptake rate (OUR), considering the variations in the sludge respiration rate as a result of the type of substrate added and biomass degradation rate (Eq. (13)).

$$OUR = \frac{DO_{max} - DO_{min}}{t_2 - t_1} \tag{13}$$

Dissolved oxygen (DO) was continuously monitored and the results were automatically recorded using the Software S4.0C at 5-min intervals. The biological sludge activity prior and after the tests was evaluated by measuring the oxygen consumption using sodium acetate.

2.7. LC-MS Analysis

The by-products generated from the EBI-driven DCF degradation were identified by direct-injection mass spectrometry (UHR-QqTOF, Ultra-High Resolution Qq-Time-of-Flight, impact II, Bruker Daltonics). The time-of-flight mass spectra were obtained in positive electrospray (+ESI) mode in the range m/z 50–1300 at the following optimized operating conditions: nebulizer 3.0 bar; dry gas 8.0 L min^{-1}; dry heater 220 °C; and capillary 4500 V. The software ACD/ChemSketch (Advanced Chemistry Development, Inc., Toronto, Canada) was used for interpreting mass spectra and for establishing chemical structures.

3. Results and discussion

3.1. DCF removal in aqueous solution by EBI and degradation kinetics

Fig. 1 shows the results of diclofenac (DCF) degradation in aqueous solution by electron beam irradiation (EBI). As low DCF concentrations were undetectable by the HPLC method used, a high initial concentration (50 mg L^{-1}) was selected to investigate DCF degradation individually and in association with another pharmaceutical compound (fluoxetine, FLX).

According to Eq. (1), the EBI dose determines the amount of reactive species (HO, e_{aq}^-, H) generated by irradiation of aqueous solutions with electron beams. As a consequence, DCF concentrations decrease with increasing dose (Fig. 1a), falling below the detection limit (8.44 μg L^{-1}) at 5 kGy, both in the absence and presence of FLX; the $[DCF]/[DCF]_0$ behavior is in good agreement with previous investigations [25]. Regarding degradation kinetics, the linear $\ln([DCF]/[DCF]_0)$ vs. dose curve indicates that DCF degradation up to 5 kGy clearly followed pseudo first-order behavior with respect to the absorbed radiation dose, with $k_0 = (1.33 \pm 0.10)$ kGy^{-1} ($R^2 = 0.9693$) and $k_0 =$

(0.90 ± 0.12) kGy^{-1} (R^2 = 0.9518) for DCF removal in the absence and presence of FLX, respectively (Fig. 1b). It is worth observing that the reactions of organic molecules with the reactive species HO, e_{aq}^-, and H are bimolecular processes. The sum of the products of those species concentrations and the respective second-order rate constants corresponds to the pseudo-first-order rate constant (k_0) [28], which is here expressed with respect to the absorbed radiation dose rather than exposed time [23,28].

In addition, Fig. 1a reveals that at 1 kGy approximately 30% and 3% of the initial DCF was removed in the absence and presence of FLX, respectively. These results suggest that DCF degradation may be affected by other pharmaceutical compounds due to the potential competition for the reactive species generated from water radiolysis; in the present case, DCF and FLX competition can be inhibited and DCF degradation favored by selecting the appropriate electron beam radiation dose.

Negligible TOC removal was achieved in the presence of FLX despite of increasing radiation doses up to 7.5 kGy, in agreement with prior studies with other pharmaceutical compounds [23]. This clearly indicates that the EBI-driven DCF degradation did not reach complete mineralization, with the formation of recalcitrant by-products. Based on TOC measurements, He et al. [22] observed only 6.5% DCF mineralization for an EBI dose of 2 kGy. In contrast, Homlok et al. [25] obtained 35% TOC removal during DCF degradation using ^{60}Co γ-irradiation at 7.5 kGy, starting with an aqueous DCF solution of about 90 mgC L^{-1}; according to the authors, an absorbed dose of 40 kGy was needed for about 90% mineralization. Although the actual efficiency depends on the type and energy of ionizing radiation (electron beam or γ) [28], in the present study TOC removal might have been influenced by the presence of FLX, as observed for DCF degradation as well.

In addition, the remarkable decrease of solution pH from 6.8 to 4.1 (Fig. 2) can be associated with HCl elimination following HO radical addition to chlorine bearing carbon atoms in the DCF molecules [25], and to the formation of recalcitrant carboxylic acids through hydroxylated aromatic rings opening, as a consequence of hydroxyl radical attack [25].

Fig. 3 presents the results of the biological respirometry experiment. The time variation of the oxygen uptake rate (OUR) was assessed by monitoring dissolved oxygen concentration, for different types of substrate (non-irradiated and irradiated DCF samples, sodium acetate) added to a sample of STP biological sludge. Such experiments have been used for evaluating the effect of toxic substances and the biodegradability of treated water matrices [42,43].

Biological activity was observed following every addition of sodium acetate (Fig. 3a), with OUR peak values of about 75 and 65 mg L^{-1} h^{-1}
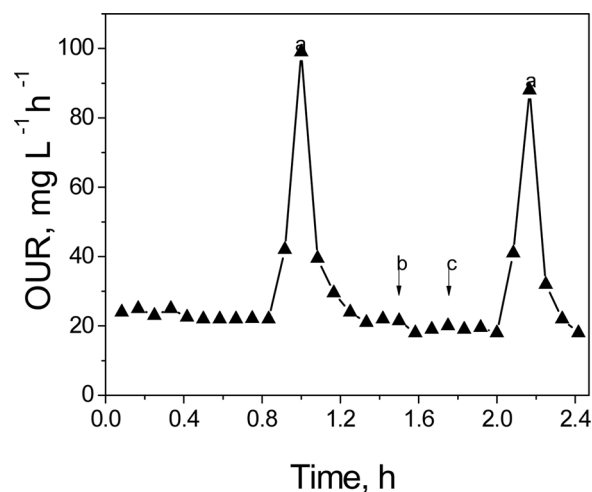
Fig. 3. Evaluation of aerobic biodegradation through the determination of oxygen uptake rate (OUR) for (a) sodium acetate (reference substance), (b) non-irradiated DCF solutions, and (c) DCF solution irradiated at 5 kGy in the absence of FLX. Addition times: 0.75 h (sodium acetate), 1.5 h (non-irradiated solution sample), 1.75 h (irradiated solution sample), and 2 h (sodium acetate).

above the base line. In contrast, no oxygen consumption occurred during the respirometry assay for the irradiated and non-irradiated DCF solutions, indicating negligible biodegradation (Figs. 3b and c). This suggests the presence of recalcitrant compounds formed during EBI degradation [42]. Previous investigations also reported no DCF biodegradation [22].

3.2. Toxicity measurements

Fig. 4a shows that the toxicity of the mixture containing both pharmaceutical compounds was lower than the sum of toxicity responses exhibited by each individual compound, indicating antagonistic interactions between DCF and FLX. The acute toxicities of the non-irradiated DCF and FLX solutions were (19.6 ± 1.6) TU and (2.2 ± 0.1) TU, respectively, while (6.8 ± 0.9) TU was obtained for the aqueous solution containing both pharmaceutical compounds. A previous study mentioned the antagonistic interaction of DCF with estrone, an estrogenic hormone [44].

The acute toxicities of non-irradiated samples are compared to those of irradiated samples in Figs. 4b and 4c, for different radiation doses. DCF was observed to be more toxic to *V. fischeri* than its by-products. The solution with DCF concentration below the detection limit after EBI irradiation exhibited a significant decrease in acute toxicity, despite the presence of remaining recalcitrant by-products, as indicated by the TOC analysis. In fact, the toxicity of the DCF solution diminished from (19.6 ± 1.6) TU to (6.2 ± 2.3) TU (Fig. 4b), and from (6.8 ± 0.9) TU to (3.1 ± 0.2) TU (Fig. 4c), in the absence and presence of FLX, respectively, up to 5 kGy. The Tukey's test indicates no significant difference in the acute toxicity of DCF solutions at 5 kGy and 7.5 kGy, neither in the absence of FLX or in its presence (*p*-values of 0.725 and 0.493, respectively). Previous studies reported an increase in DCF toxicity following sunlight-driven degradation processes [41].

Since distinct degradation processes have been proposed for degrading DCF in aqueous solution and different transformation products and degradation pathways reported [1,30–32], the identification of DCF by-products following EBI, and their correlation with toxicity measurements is discussed in the following section.

3.3. Identification of DCF by-products and proposed degradation pathway

Figure S1 (Supplementary Material) shows the mass spectra obtained by direct-injection mass spectrometry (UHR-QqTOF) for the
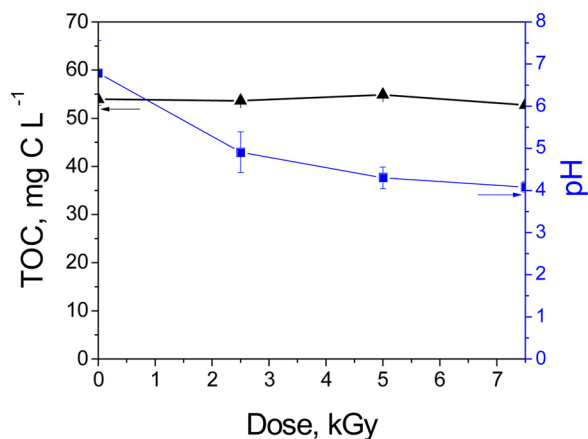

Fig. 2. TOC concentration and solution pH vs. electron beam radiation dose: (-▲-) TOC; -■- pH. Experiments run in triplicate. Initial conditions: [DCF]$_0$ = (51.8 ± 1.1) mg L^{-1}; [FLX]$_0$ = (4.1 ± 0.8) mg L^{-1}; pH$_0$ = 6.8 ± 0.8; TOC$_0$ = (53.9 ± 0.3) mg C L^{-1}.
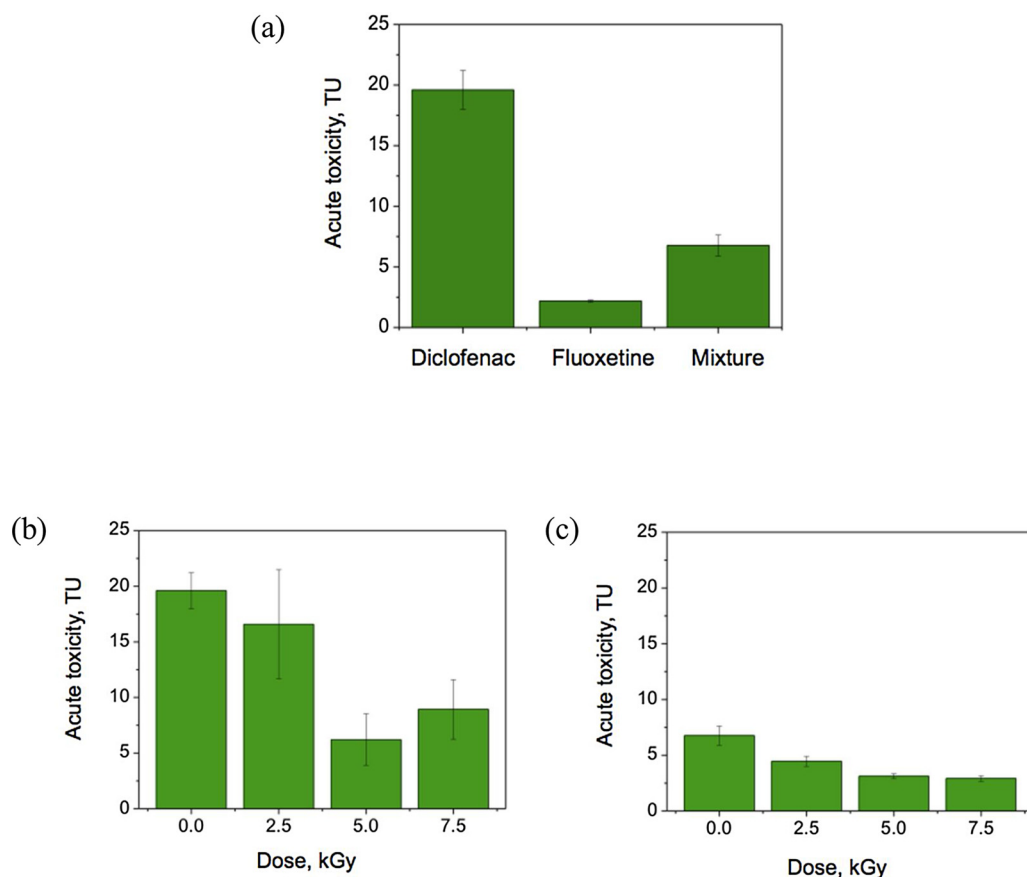
Fig. 4. Results of acute toxicity (TU = 100/EC50%) measurements with V. *fischeri*. (a) Non-irradiated samples of diclofenac (DCF), fluoxetine (FLX) and mixture (DCF + FLX). (b) Irradiated samples of DCF in the absence of FLX. (c) Irradiated samples of DCF in the presence of FLX. Data in triplicate. Initial conditions: [DCF] = (51.8 ± 1.1) mg L^{-1}; [FLX] = (4.1 ± 0.8) mg L^{-1}; pH = 6.8 ± 0.8.

Table 1
DCF and related by-products for irradiated DCF solutions in the absence of FLX.

Compound	Molecular formula	Measured mass [M+H]$^+$	Radiation dose (kGy)
DCF	$C_{14}H_{11}Cl_2NO_2$	296.03	–
P1	$C_{14}H_{20}ClNO_5$	318.01	2.5; 5.0 and 7.5
P2	$C_{14}H_{20}ClNO_6$	334.00	2.5 and 5
P3	$C_8H_{10}O_6$	203.06	2.5; 5.0 and 7.5
P4	$C_{14}H_{15}Cl_2NO_5$	349.98	2.5; 5.0 and 7.5
P5	$C_{14}H_{15}Cl_2NO_6$	365.11	2.5 and 5
P6	$C_{14}H_{15}Cl_2NO_7$	381.11	2.5 and 5
P7	$C_9H_{11}N$	134.06	2.5; 5.0 and 7.5
P8	C_7H_7N	106.07	2.5; 5.0 and 7.5
P9	$C_9H_9Cl_2NO$	219.05	2.5; 5.0 and 7.5
P10	$C_8H_9NO_2$	152.07	2.5; 5.0 and 7.5
P11	$C_{13}H_{14}ClNO_5$	300.05	2.5

Table 2
By-products and related acute toxicity (in Toxic Units, TU = 100/EC50%) for irradiated DCF solutions in the absence of FLX.

Dose (kGy)	By-products	Toxic Units (TU)
2.5	P1, P2, P3, P4, P5, P6, P7, P8, P9, P10, and P11	16.58 ± 4.90
5.0	P1, P2, P3, P4, P5, P6, P7, P8, P9, and P10	6.22 ± 2.31
7.5	P1, P3, P4, P7, P8, P9, and P10	8.93 ± 2.68

solutions irradiated at different EBI doses. The mass assigned to [M+H]$^+$ ions of the analytes (Table 1) was treated as an independent measurement. Only the [M+H]$^+$ ions with high intensity were considered significant for elucidating DCF by-products. The identification of DCF by-products was performed in the absence of FLX owing to the higher toxicity response shown by DCF alone.

For the solution irradiated at 2.5 kGy, eleven products from DCF degradation were identified, resulting in the highest acute toxicity (16.58 ± 4.90 TU) (Table 2). Seven DCF by-products (P1, P3, P4, P7, P8, P9, and P10) remained in the solution irradiated at the highest dose (7.5 kGy), suggesting they are recalcitrant compounds, corresponding to an acute toxicity of (8.93 ± 2.68) TU. In contrast, the by-products P2, P5, P6 were degraded for doses higher than 5.0 kGy, at which P11 had been already consumed, suggesting that these four products are easily degraded by EBI. Since the Tukey's test indicates no significant difference in toxicity for DCF solutions irradiated at 5 kGy and 7.5 kGy,

the results in Table 2 and Fig. 4b indicate compound P11 as the least recalcitrant by-product but exhibiting the highest toxicity.

A possible DCF degradation pathway is proposed (Fig. 5). According to previous studies [1,25,30,45,46], DCF molecules ($C_{14}H_{11}Cl_2NO_2$, [M+H]$^+$ at *m/z* 296.03) undergo electrophilic addition of hydroxyl radicals generated from water radiolysis (Eq. (1)) to the aromatic rings [45,46]. The DCF by-products identified are consistent with prior studies reporting hydroxylation followed by oxidation of hydroxide groups, dechlorination, and decarboxylation [1,30–33].

In this study we propose that DCF degradation occurs through hydroxylation and dechlorination steps to generate compounds P1 ($C_{14}H_{20}ClNO_5$, [M+H]$^+$ at *m/z* 318.01) and P2 ($C_{14}H_{20}ClNO_6$, [M+H]$^+$ at *m/z* 334.00), followed by C − N bond cleavage and further hydroxylation, giving compound P3 ($C_8H_{10}O_6$, [M+H]$^+$ at *m/z* 203.06) [47]. Hydroxyl radicals may attack different positions activated by substituent groups on the two rings in the DCF molecule [25]. On the other hand, the dissociative electron attachment to the aromatic ring bearing the two chlorine atoms (Eq. (3)) may contribute to DCF dehalogenation and release of Cl$^-$ anions [25], even though e_{aq}^- are less available for reaction, owing to scavenging by H$_3$O$^+$, dissolved oxygen and HO radicals (Eqs. (4), (5) and (8), respectively). Bojanowska-Czajka et al. [48] determined the second order reaction rate constants of DCF with HO radicals (1.24×10^{10} L mol s^{-1}) and e_{aq}^- (3.1×10^9 L mol
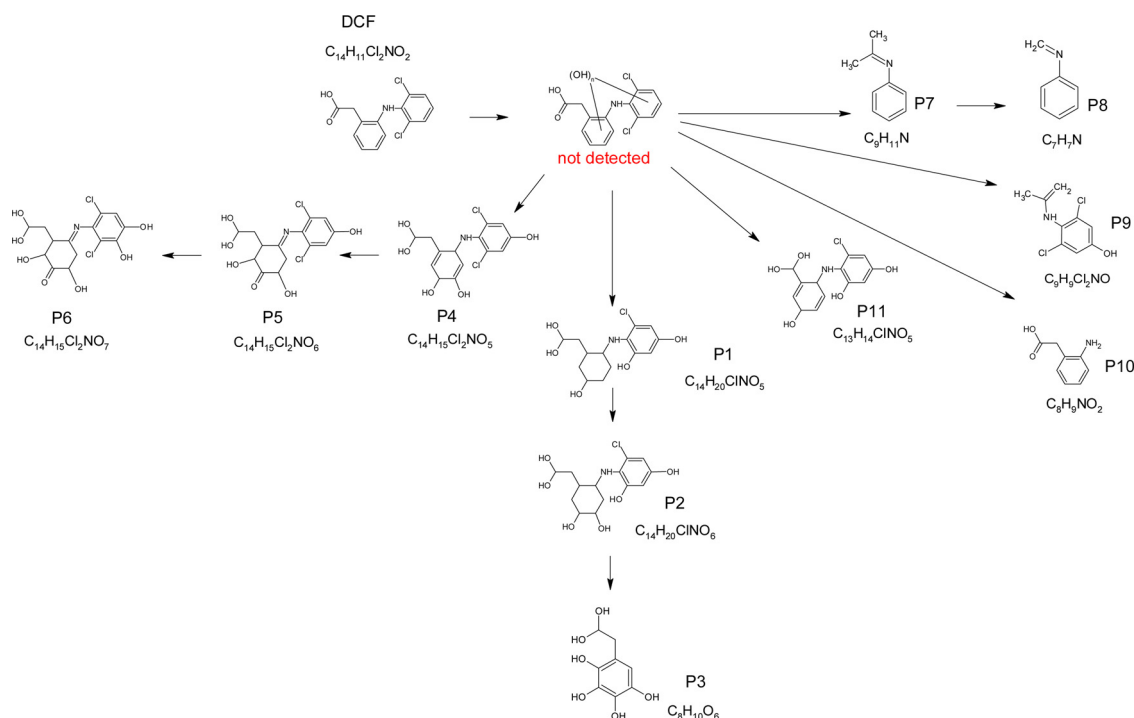
Fig. 5. Proposed DCF degradation pathway by EBI in the absence of FLX.

s^{-1}), concluding that both oxidative and reductive processes can occur in the radiolytic degradation of DCF. In turn, the role of reactive species towards DCF degradation was found to follow the order $HO > e_{aq}^- > H$ [49]. HO radical attack may also occur on the carbon atoms at the chlorine substituent, yielding conjugated cyclohexadienyl radicals as a result of fast HCl elimination, which may result in quinoid type compounds [25].

DCF molecules may also undergo HO radicals addition to the CH_2COOH bearing ring, giving compound P4 ($C_{14}H_{15}Cl_2NO_5$, $[M+H]^+$ at m/z 349.98), followed by oxidation of hydroxide groups to yield compounds P5 ($C_{14}H_{15}Cl_2NO_6$, $[M+H]^+$ at m/z 365.11), and P6 ($C_{14}H_{15}Cl_2NO_7$, $[M+H]^+$ at m/z 381.11). Compounds P7 ($C_9H_{11}N$, $[M+H]^+$ at m/z 134.06), P8 (C_7H_7N, $[M+H]^+$ at m/z 106.07), P9 ($C_9H_9Cl_2NO$, $[M+H]^+$ at m/z 219.05), and P10 ($C_8H_9NO_2$, $[M+H]^+$ at m/z 152.07) were detected as well, and are related to C—N bond cleavage [33], preserving the nitrogen-containing group on one aromatic moiety [30]. Hydroxylation, dechlorination and decarboxylation are also suggested to explain the formation of compound P11 ($C_{13}H_{14}ClNO_5$, $[M+H]^+$ at m/z 300.05).

4. Conclusion

In this study, complete degradation of the anti-inflammatory drug diclofenac (DCF) in aqueous solutions was achieved by electron beam irradiation (EBI) at a low radiation dose (5 kGy), with DCF concentrations falling below the detection limit of the chromatographic method (8.44 μg L^{-1}). DCF removals of about 30% and 3% were obtained at 1 kGy in the absence and presence of another pharmaceutical compound (fluoxetine, FLX); for 2.5 kGy, the removals were 95% and 85%, respectively. In addition, DCF concentration followed pseudo first-order decay with respect to the absorbed radiation dose, with $k_0 = (1.33 \pm 0.10)$ kGy^{-1} (DCF) and $k_0 = (0.90 \pm 0.12)$ kGy^{-1} (DCF + FLX). These results clearly demonstrate that DCF removal may be affected by other pharmaceutical compounds due to the potential competition for the reactive species (HO, e_{aq}^-, H) generated from water radiolysis.

In contrast, negligible TOC removal was observed even at 7.5 kGy,

showing that DCF mineralization was not achieved, with the formation of recalcitrant, non-biodegradable by-products, as also suggested by the respirometry assay. On the other hand, the toxicity of the DCF solution diminished from (19.6 ± 1.6) TU to (6.2 ± 2.3) TU, and from (6.8 ± 0.9) TU to (3.1 ± 0.2) TU, in the absence and presence of FLX, respectively, following irradiation up to 5 kGy.

Direct-injection mass spectrometry revealed eleven DCF by-products after irradiation at 2.5 kGy, resulting in the treated solution with the highest acute toxicity (16.58 ± 4.90 TU). Among them, seven recalcitrant compounds remained in solution even after irradiation at 7.5 kGy, corresponding to an acute toxicity of (8.93 ± 2.68) TU. Four of the eleven by-products identified were easily degraded by EBI, and one ($C_{13}H_{14}ClNO_5$) was identified as the least recalcitrant but the most toxic. The proposed degradation pathway includes: (i) electrophilic addition of hydroxyl radicals generated from water radiolysis to the two aromatic rings in the DCF molecule, followed by oxidation of hydroxide groups attached to the CH_2COOH bearing ring; (ii) dehalogenation through dissociative solvated electron attachment to the aromatic ring bearing the chlorine atoms and/or fast HCl elimination following HO radical attack on the carbon atoms at the chlorine substituent; (iii) C—N bond cleavage, preserving the nitrogen-containing group on one aromatic moiety.

In conclusion, the results of this work indicate that EBI using low doses (up to 5–7.5 kGy) can be a viable alternative for the efficient removal of DCF and residual toxicity of wastewater containing pharmaceutical pollutants. Nevertheless, studies using other advanced oxidation processes should not be disregarded for the treatment of complex wastewater from pharmaceutical formulation facilities.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jece.2018.06.065.

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