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*Research Article*

# Impact of Hydrogen Peroxide on the UVC Photolysis of Diclofenac and Toxicity of the Phototransformation Products

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The aim of this study was to investigate the effect of hydrogen peroxide on the UVC photolysis of diclofenac (DCF) in aqueous solution. The experimental results confirmed very high effectivity of UVC direct photolysis of diclofenac. Moreover, it was found that $H_2O_2$/UV only slightly improved photodegradation; however, the addition of hydrogen peroxide into the reaction system affected the mechanism of DCF decomposition. Kinetics of the DCF reaction with $^{\cdot}OH$ radicals in the UV/$H_2O_2$ process was determined. For both processes, namely, photolysis and UV/$H_2O_2$, an in-depth analysis focused on the formation of phototransformation products of DCF (TPs) was performed. To the best of our knowledge, such comprehensive comparison of diclofenac photodegradation via UVC photolysis and UV/$H_2O_2$ has not been presented so far. Although there were no significant differences with regard to the rate of diclofenac degradation by photolysis and UV/$H_2O_2$, different oxidation products were found to be associated with the two considered processes. Furthermore, the $H_2O_2$/UV treatment increased toxicity towards *Vibrio fischeri*, while direct UVC photolysis had no significant effect on toxicity. The increase in toxicity can be attributed to the breakdown of DCF and formation of much more toxic TPs in the course of the $H_2O_2$/UVC process.

## 1. Introduction

The presence of pharmaceutical compounds in the aquatic environment has been the major subject of research in the recent years. Pharmaceuticals constitute a class of water pollutants that can be potentially dangerous even in trace amounts [1]. A number of marketed pharmaceutical and personal care products (PPCP) continues to increase because they are used to treat human and animal diseases and improve the quality of life. This leads to elevated levels of these compounds in aquatic systems [2]. In addition, many pharmaceuticals are not completely removed during the wastewater treatment process. High PPCP concentrations can be associated with negative impact on aquatic ecosystems and human health [3].

This study focused on diclofenac (DCF), one of the most popular painkillers, representing the group of nonsteroidal anti-inflammatory drugs (NSAIDs). Because of their availability as over-the-counter products in many countries, NSAIDs are widely used to treat inflammation and pain [4]. DCF is a drug commonly applied in the treatment of rheumatic and nonrheumatic diseases, which are typically associated with inflammation [1]. It is well tolerated by the human organism so it has found widespread use throughout the world [5]. Importantly, diclofenac is relatively persistent in water.

Over the past few years, the concentration of PPCPs has increased [6]. Many PPCPs susceptible to photodegradation can be found in water, sediments, fauna, and flora. Pharmaceuticals that are not completely metabolized in the human body are excreted with urine and reach the wastewater. Diclofenac can be found in surface waters, groundwater, and sewage from households, hospitals, or animal husbandry [7]. Importantly, the processes commonly used in wastewater

treatment plants are not sufficiently effective in the context of the removal of pharmaceuticals, which lead to serious environmental risks [5]. Many of these pollutants are not degraded by typical biological procedures used in wastewater treatment plants, which is why compounds such as diclofenac are found in surface waters all over Europe [8]. Previous studies demonstrated negative effects of DCF on trout [1, 2]. According to the literature, diclofenac was also detected in the bile of two wild fish near the Finnish wastewater treatment plant [9]. On the basis of the adsorption, biodegradation, photolysis, and hydrolysis experiments, it was found that diclofenac present in lake water is most effectively degraded by photolysis [8–10]. Diclofenac exposed to natural sunlight undergoes phototransformation. Currently, the sun is considered to be responsible for the transformation of many drugs in the environment [11]. UV radiation is a promising technology more and more often used for wastewater treatment. Notably, introducing photoproducts (as photosensitive components) into the aquatic environment has been suggested in the previous works [11].

Nowadays, it is extremely important to develop effective and nontoxic methods for the disposal of pharmaceuticals. This goal could be achieved by the application of advanced oxidation processes (AOPs) or direct photolysis. Advanced oxidation processes are well-accepted technologies for removing initial contaminants, but not necessarily postreaction by-products.

The main goal of the present work was to compare the outcomes of UVC photolysis and UV/$H_2O_2$ in the context of TPs analysis and toxicity assessment. As the previous efforts focused primarily on the photolysis process [12, 13], the studies on the application of UV/$H_2O_2$ towards diclofenac degradation are still rather scarce [1]. Therefore, there is a need for broad-scope experimental work aimed at providing a comparative perspective on the diclofenac-related outcomes of photolysis and UV/$H_2O_2$.

## 2. Materials and Methods

*2.1. Chemicals.* Diclofenac sodium salt (DCF) of analytical grade (CAS: 15307-79-6, Sigma (Germany), purity ≥98%), without any further purification, was dissolved in water to prepare experimental solutions. Hydrogen peroxide ($H_2O_2$) (30%) was provided by CHEMPUR (Poland). Catalase (2000–5000 units/mg protein) from bovine liver was purchased from Sigma-Aldrich (Germany).

*2.2. Experimental Procedures.* All solutions were prepared using distilled water. The used concentrations of DCF were prepared from the stock solution 1 g/dm$^{-3}$. The experiments were performed at room temperature (25° ± 1°C). 30% hydrogen peroxide was used, and an appropriative volume of it was added to the solution. The initial pH of the solutions was 4.5, and it was left to run freely during experiments. The experiments were performed in duplicate to assure accurate data acquisition, and error bars in figures represent the standard deviation.

The experiments were performed on a "merry-go-round" device with quartz tubes placed between two exposure panels (Luzchem). Each panel was composed of four low-pressure mercury lamps (USHIO, model G8T5 Hg (7.2 W each)) with a maximum emission of 254 nm. The photon flux rate equal to $7.33 \times 10^{-6}$ einstein dm$^{-3}$ s$^{-1}$ was previously determined using uranyl oxalate and confirmed by measuring by radiometer (UV–vis–NIR, Ocean Optics USB4000, USA). The volume of quartz tube was equal to 10 mL, with an average optical path length of 0.85 cm. At the beginning of each experiment, the samples were sealed with parafilm and put into the merry-go-round device. While the lamps were switching on, all samples were covered by a special barrier while lamps warmed up. To achieve the intensity of radiation, stable power stabilizer was used. For UV/$H_2O_2$ experiments, the hydrogen peroxide was spiked at an appropriative concentration to reaction solution before the lamps were turned on.

The samples were drawn at time intervals of 20, 40, 60, 120, 300, and 600 seconds.

*2.3. Analytical Methods.* The reaction progress was monitored by determination of diclofenac concentration using Agilent 1220 Infinity LC HPLC apparatus equipped with Zorbax rapid resolution HD Eclipse plus C18 column 3.0 × 100 mm (1.8 $\mu$m) (Agilent Technologies Inc., Germany). The column temperature during analysis was 40°C. Flow rate was 0.4 mL/min, and the gradient elution was applied. Water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B) were used as eluents. The gradient started with 50% of B and increased to 100% of B for 15 minutes, then decreased from 100 to 50%. The detection wavelength was 273 nm. Formation yields of DCF were determined by comparing integrated peak areas with external calibration curves obtained for 0.99758.

The phototransformation products of diclofenac degradation were analyzed with the use of ultraperformance liquid chromatography system ACQUITY coupled with high-resolution mass spectrometer SYNAPT G2 (both from Waters, USA). The chromatographic runs were performed with the use of Waters ACQUITY UPLC BEH Shield RP18 column (2.1 mm × 100 mm × 1.7 $\mu$m). The linear $H_2O$/acetonitrile gradient (40% acetonitrile at 1 min to 60% at 10 min) was applied throughout the analysis. The eluents were acidified with formic acid (final concentration: 0.1% *v*/*v*).

The parameters of mass spectrometric analysis were as follows: ESI$^-$ (electrospray ionization in negative mode), capillary voltage: 2.5 kV, desolvation gas flow: 600 L/h, and source temperature: 80°C. TargetLynx software (Waters, USA) was employed for semiquantitative analysis involving the determination of peak area values corresponding to respective degradation products.

The toxicity tests on the marine luminescent bacteria *Vibrio fischeri* were performed according to ISO 11348-3 (ISO, 2007) and the methodology using a Microtox Model 500 Analyzer (Modern Water Inc., Newark, Delaware, USA). The tests were performed by running the 81.9% basic test protocol (MicrotoxOmni 4.2, Modern Water Inc.), which consisted of nine dilutions. According to the applied procedure, the freeze dried-bacteria was reconstituted with water, to provide a stock suspension of test organisms, which was

kept at 5°C and used to perform the test. Toxicity was expressed as EC50, which is the pollutant concentration reducing 50% of the initial luminescence. In this work, measurements were made at 15 min of exposures. Dose-response data were fitted using the Hill equation by setting the minimum inhibition to 0% and the maximum effect to 100%. The pH of the samples was corrected to $7.0 \pm 0.5$ before toxicity assessment tests using 0.1 M NaOH. Residual $H_2O_2$ was degraded by catalase from bovine liver (1 unit will decompose 1.0 $\mu$mole of $H_2O_2$/min at pH 7.0; 25°C) prior to toxicity measurements.

## 3. Results and Discussion

*3.1. Kinetics of Diclofenac Removal.* The DCF photolysis proceed very fast, and the initial reaction rates of direct photolysis and advanced oxidation in the $H_2O_2$/UV system are very similar. In order to estimate the extent of direct reaction of DCF with hydrogen peroxide ("dark reaction"), no changes of concentration was observed, thereby excluding the $H_2O_2$ role in the degradation process. According to the literature, the quantum yield of the DCF photolysis under 254 nm irradiation is $0.2727 \pm 0.046$ [14]. Whereas, the quantum yield of DCF photolysis determined for unbuffered solution (pH dropping from 7.0 to 4.8 during the treatment) is equal to $0.19 \pm 0.02$ [12]. It was detected that applying of diclofenac concentration higher that 0.156 and 0.231 mM contributed to a slight slowdown of advanced oxidation and photolysis reaction rate, respectively (Figure 1(a)). A similar phenomenon was observed for a series of tests using 6 lamps (studies not shown). This might be caused by the formation of TPs with strong absorption properties. The determined optimal dose of hydrogen peroxide for DCF degradation in $H_2O_2$/UV system was 8.82 mM (Figure 1(b)).

In the UVC irradiated reaction solution in the presence of hydrogen peroxide, the following reaction may occur:

$$H_2O_2 + hv \rightarrow 2^{\cdot}OH \quad r_1 = \phi_{H_2O_2} E_0 F_{H_2O_2} \left[1 - \exp \cdot \left(-2.303b \sum \varepsilon_i C_i\right)\right] \tag{1}$$

$$H_2O_2 \leftrightarrow H^+ + HO_2^- \quad Ka = 2.51 \cdot 10^{-12} \tag{2}$$

$$^{\cdot}OH + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \quad r_3 = k_3 C_{H_2O_2} C_{\cdot OH} \tag{3}$$

$$^{\cdot}OH + HO_2^- \rightarrow H_2O + O_2^{\cdot -} \quad r_4 = k_4 C_{HO_2} C_{\cdot OH} \tag{4}$$

$$DCF + {}^{\cdot}OH \rightarrow \text{products} \quad r_5 = k_{\cdot OH} C_{DCF} C_{\cdot OH} \tag{5}$$

$$DCF + hv \rightarrow \text{products} \quad r_6 = \phi_{DCF} E_0 F_{DCF} \left[1 - \exp \cdot \left(-2.303b \sum \varepsilon_i C_i\right)\right] \tag{6}$$

The occurrence of DCF direct photolysis (6) can be neglected, when the hydrogen peroxide concentration is high enough to absorb almost all radiation incident to the reaction solution. It was calculated from equation (7) that 98% of the UVC radiation is absorbed by $H_2O_2$ at its concentration equal to 1 M and at initial DCF concentration equal to 68.3 $\mu$M.

$$F_{H_2O_2} = \frac{\varepsilon_{H_2O_2} C_{H_2O_2}}{\varepsilon_{H_2O_2} C_{H_2O_2} + \varepsilon_{DCF} C_{DCF}} \tag{7}$$

where C and ε are the molar concentration (M) and molar absorption coefficients at 254 nm ($M^{-1}$ $cm^{-1}$) of DCF ($C_{DCF}$ and $\varepsilon_{DCF}$) and hydrogen peroxide ($C_{H2O2}$ and $\varepsilon_{H2O2}$).

Moreover, in the above described situation the concentration of $H_2O_2$ in the initial stage of process can be treated as constant. Thus, the reaction rate can be described by equation (5). Assuming steady state for hydroxyl radicals concentration, the reaction rate can be expressed by pseudofirst order equation:

$$-\frac{dC_{DCF}}{dt} = k_z C_{DCF} \tag{8}$$

where $k_z$ is the pseudofirst order rate constant defined as follows:

$$k_z = k_{\cdot OH} C_{\cdot OH} \tag{9}$$

The slope of the integrated form of equation (8) corresponds to $k_z$. The combination of equations (1), (3), (4), and (5) under the assumption that the formation and consumption rates of hydroxyl radicals are equal leads to the expression of stationary concentration of hydroxyl radicals:

$$C_{\cdot OH} = \frac{2\phi_{H_2O_2} E_0 F_{H_2O_2} [1 - \exp(-2.303b \sum \varepsilon_i C_i)]}{k_2 C_{H_2O_2} + k_3 C_{HO_2} + k_{\cdot OH} C_{DCF}} \tag{10}$$

The concentration of hydroperoxide anion ($C_{HO2}$) can be calculated from the equilibrium constant (11):

$$C_{HO_2} = \frac{KaC_{H_2O_2}}{\exp(-2.303pH) + Ka} \tag{11}$$

Combining of equation (9) with (10) and after rearranging, we get

$$k_{\cdot OH} = \frac{k_z \left(k_2 C_{H_2O_2} + k_3 C_{HO_2}\right)}{2\phi_{H_2O_2} E_0 F_{H_2O_2} [1 - \exp(-2.303b \sum \varepsilon_i C_i) - k_z C_{DCF}]} \tag{12}$$

The above relationship was used for calculation of reaction rate constants ($k_{\cdot OH}$) of hydroxyl radicals with DCF. Table 1 presents the constants necessary for calculations and obtained results. The determined $k_{\cdot OH}$ value is higher than reported in the literature [3, 15, 16]. For example, the $k_{\cdot OH}$ determined by Huber et al. [15] during ozonation process equaled to $(7.5 \pm 1.5) \times 10^9$ $M^{-1}$ $s^{-1}$, the parameter determined by using competition kinetics with *p*-chlorobenzoic acid as a competitor equaled to $2.45 \times 10^9$ 1/(M s) [16], and the bimolecular reaction rate constant determined by

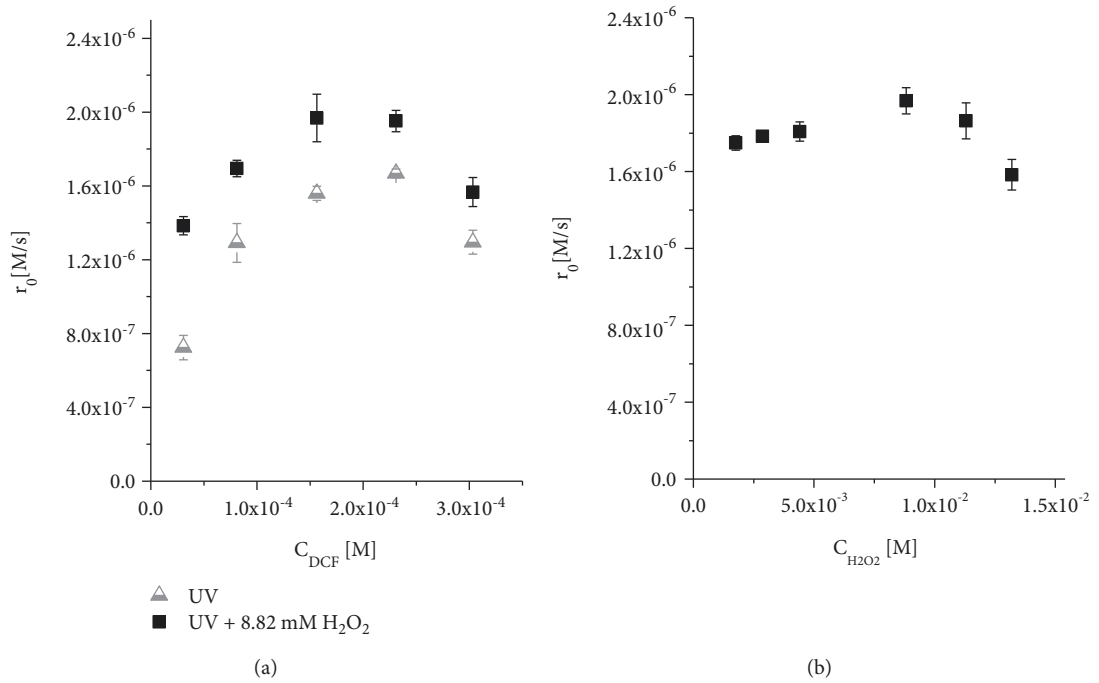


Figure 1: The influence of initial concentration of DCF (a) ($C_{H2O2} = 8.82 \times 10^{-3}$ M, pH = 4.5, $E_0 = 7.33 \times 10^{-6}$ einstein $dm^{-3}$ $s^{-1}$) and $H_2O_2$ (b) ($C_{DCF} = 1.56 \times 10^{-4}$ M, pH = 4.5, $E_0 = 7.33 \times 10^{-6}$ einstein $dm^{-3}$ $s^{-1}$) concentration on initial reaction rate.

Table 1: The literature data used for kinetic calculations and determined values of reaction rate constants.

| Constant | Value | References |
|---|---|---|
| $\varphi_{H2O2}$ | 0.5 | [17] |
| $\varepsilon_{H2O2}$ at 254 nm, $M^{-1}$ $cm^{-1}$ | 18.6 | [18] |
| $\varepsilon_{DCF}$ at 254 nm and pH 4.5, $M^{-1}$ $cm^{-1}$ | $5952 \pm 41$ | Determined in this work |
| $E_0$, einstein $dm^{-3}$ $s^{-1}$ | $7.33 \times 10^{-6}$ | [19] |
| $pK_a$ for $H_2O_2$ | 11.3 | [20] |
| $pK_a$ for DCF | 3.99 | [21] |
| $k_2$, $M^{-1}$ $s^{-1}$ | $2.7 \times 10^7$ | [22] |
| $k_3$, $M^{-1}$ $s^{-1}$ | $7.5 \times 10^9$ | [22] |
| $k_z$, $s^{-1}$ | $0.00348 \pm 0.000206$ | Determined in this work |
| $k_{\cdot OH}$, $M^{-1}$ $s^{-1}$ | $(1.36 \pm 0.080) \times 10^{10}$ | Determined in this work |

Yu et al. [3] was $(9.29 \pm 0.11) \times 10^9$ $M^{-1}$ $s^{-1}$. The differences between the reported $k_{\cdot OH}$ values and the values determined in the present study may be caused by employing different determination methods and reaction conditions.

*3.2. Detected Products of Diclofenac Degradation.* The process of diclofenac degradation was associated with the formation of several molecules (Figures 2(a) and 2(b)) that could be tentatively identified by comparing the experimental $m/z$ values with the previously published datasets. The recorded $ESI^- m/z$ values turned out to be in agreement with the previous studies and provided a basis for proposing the structures of detected compounds (Figure 2(c)). Whenever the mass spectral data alone proved to be insufficient to distinguish between the isomers that had been suggested by other authors, multiple structures consistent with the observed spectra were depicted as possible degradation products (Figure 2(c)).

The first two eluted molecules (marked as "A" and "B" in Figure 2) were both associated with the $ESI^- m/z$ values of 254.0459, which could be attributed to two isomeric compounds "A/B" presented in Figure 2(c). The following three eluted species, referred to as "C", "D", and "E", exhibited the experimental $m/z$ values of 290.0226, 240.0666, and 224.0717, respectively. The molecules "F" and "G" both displayed the $m/z$ values equal to 310.0043, which could be assigned to isomeric structures differing with regard to the position of hydroxyl moiety (structures "F/G" in Figure 2(c)), whereas the "H" compound, represented by $m/z = 547.0469$, was probably a result of dimerization (discussed in further detail by [12]). Finally, the presence of the degradation product "I" was reflected by the mass peak at $m/z = 258.0327$ (Figure 2(b)). All the above-mentioned observations regarding the degradation products of diclofenac were in good agreement with the previous reports and structure-related suggestions [1, 8, 12, 23–27].

The peaks "A" and "B" can be associated with two "A/B" isomers of possible structures presented in Figure 2(c). Similarly, the "F" and "G" peaks can be attributed to three "F/G" isomers (c) that differ solely with respect to the position of hydroxyl group.

As can be seen in Figure 3, different TPs are formed during these both processes. It was found that four of the seven products identified were probably the products of photolysis.

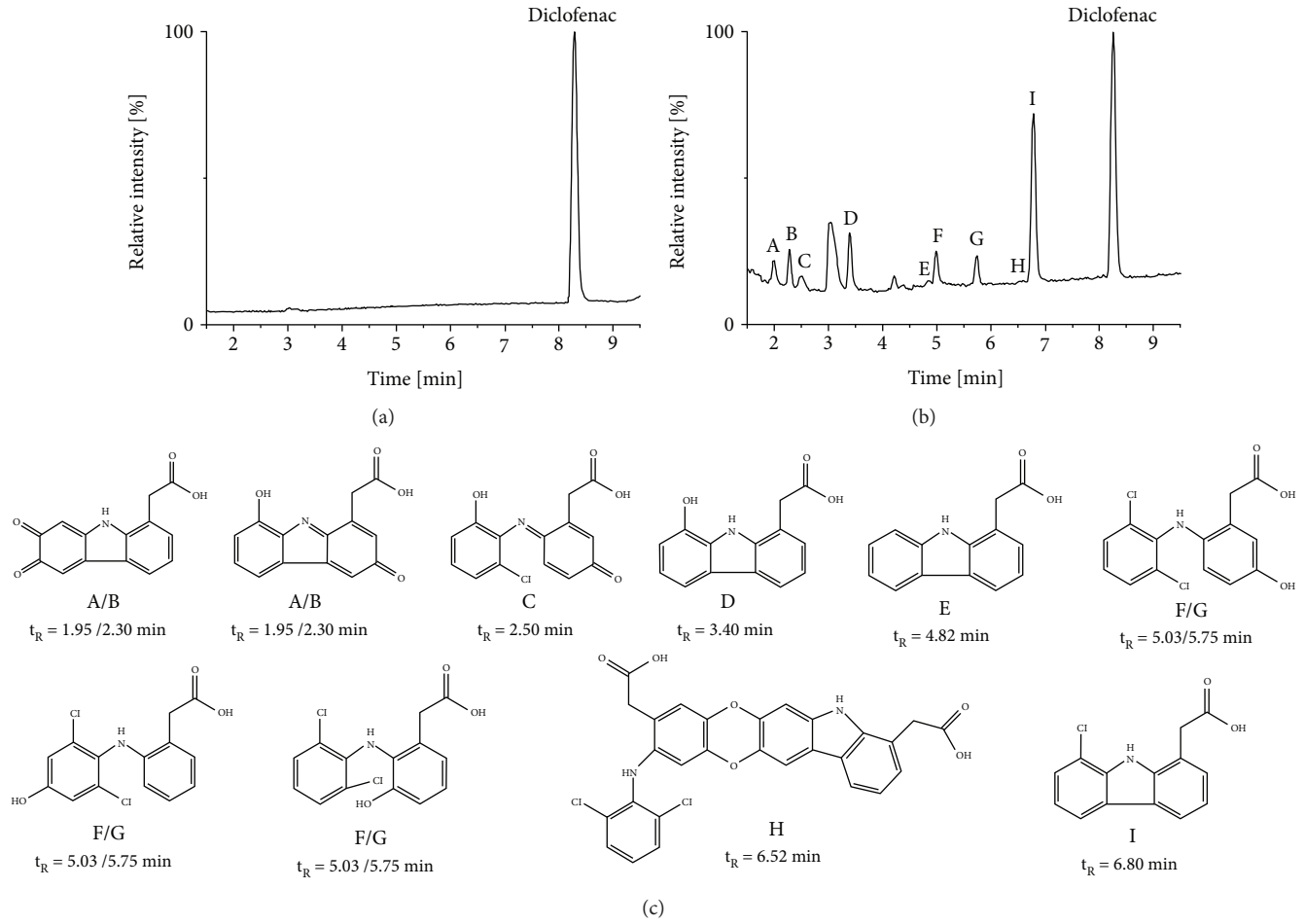


Figure 2: Total ion chromatograms (TICs) recorded for diclofenac samples before (a) and after (b) the degradation process involving 4 UV lamps, with corresponding structural formulae (c).

Taking into account relative values of surface areas, product "I" ($t_R = 6.8$ min) ((2-(8-chloro-9H-carbazol-1-yl) acetic acid) was probably formed most quickly as one of the first products (Figure 3). It is known that 2-(8-chloro-9H-carbazol-1-yl) acetic acid is the main by-product of direct photolysis of DCF [11, 13, 23, 24, 28–30]. Irradiation of DCF with UV light leads to the elimination of chlorine substituents in the DCF structure, followed by ring closure to form carbazole-1-acetic acid, which is considered as the basic product of photolytic degradation [13, 28]. The product is unstable. Its maximum concentration was recorded at 120 seconds, after which the product began to fade. A similar result was presented in [13].

The second product of direct photolysis is probably product "D" ($t_R = 3.4$ min), which is formed by the substitution of a chlorine atom with the OH group in the molecule of "I" product. A similar conclusion was presented by other researchers who studied the mechanism of diclofenac photolysis [11, 23, 24, 28]. The product can be regarded as stable. Maximum concentration obtained after 5 minutes practically slightly decreased after 10 minutes of the reaction. Lekkerkerker-Teunissen et al. [28] in their transformation scheme assumed that product "D" is transformed into a product that in our work is defined as an "A/B" isomer ($t_R = 1.95/2.30$ min). It follows from Figure 3 that isomers of the "A/B" products are likely to be formed during direct photolysis. These are relatively stable products. The maximum concentration of these products was maintained for 5 to 10 minutes of the reaction (Figure 4).

Based on the current analysis of surface areas corresponding to diclofenac oxidation products (Figure 3), three products resulting from radical transformation in the presence of hydrogen peroxide were identified, i.e., product "C" ($t_R = 2.5$ min), product "E" ($t_R = 4.82$ min), and isomers "F/G" ($t_R = 5.03/5.75$ min). Product "E" ($t_R = 4.82$ min) is probably a transformation product of product "D", in which the -OH group was removed from the molecule. Yu et al. [3] proposed the mechanism of decomposition of diclofenac by acting with hydrated electrons $e^-_{aq}$, in which product "I" and then product "E" were produced.

As can be seen in Figure 3, the "F/G" isomers ($t_R = 5.75$ min) are mainly formed in the presence of hydrogen peroxide, which may indicate the involvement of $HO^{\cdot}$ radicals. This is confirmed by the literature data [3]. "F/G" isomers are found in the literature as intermediates of various oxidation processes in which hydroxyl radicals are the dominant oxidizing agent. Michael et al. [31] identified the abovementioned diclofenac degradation products in the sono-

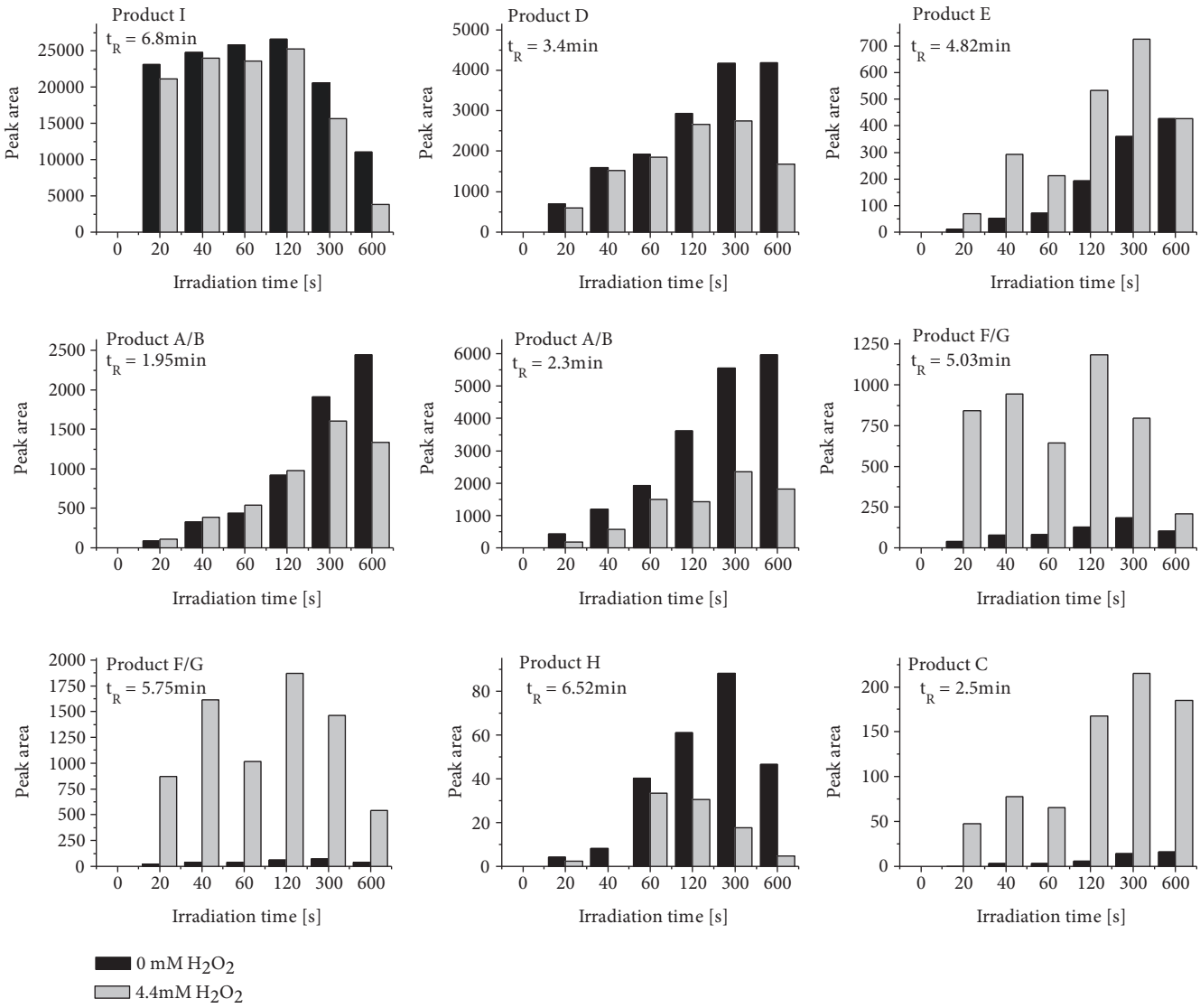


Figure 3: Evolution of main transformation photoproducts formed during degradation of DCF by direct UVC photolysis and $H_2O_2$/UVC process as determined by LC–MS.

photocatalysis reaction. Vogna et al. [1] proposed such products while conducting studies on the use of UV/$H_2O_2$ process for diclofenac degradation. A similar mechanism was proposed by Yu et al. [3] who conducted research using pulse-radiolysis. The study was carried out in the $N_2O$ environment, which enabled the in situ generation of hydroxyl radicals.

The product "H" ($t_R$ = 6.52 min) is likely to be formed by coupling the molecule of diclofenac with its oxidation products. From Figure 5, it can be concluded that the product is formed under high diclofenac concentration, as evidenced by a sharp peak at the maximum concentration of DCF (75 mg/L). Formation of this product may be the cause of a slight slowdown of the reaction rate at higher DCF concentrations. The product is unstable, as evidenced by a decrease in the surface areas after 10 minutes of the reaction. Keen et al. [12] investigated the mechanism of dimer formation during diclofenac photolysis. In particular, the effect of dissolved oxygen on dimer formation was studied. The role of diclofenac products as singlet sensitizers and singlet oxygen in the dimerization process was also evaluated. The authors found that singlet oxygen can participate in the reactions with unsaturated bonds and probably enhance the formation of radicals that lead to dimerization. Product "H" may be degraded again to diclofenac and the oxidation product.

*3.3. Acute Toxicity Analysis.* As has been shown and discussed above, DCF is transformed into a variety of TPs present in the solution at the same time. This mixture was tested for its cumulative toxicity activity toward *Vibrio fischeri*. Figure 6(a) shows the DCF removal efficiency during direct photolysis and in the presence of 4.4 mM $H_2O_2$, while Figures 6(b) and 6(c) and C show bioluminescence inhibition curves obtained for the DCF reaction solution after 40 s, 120 s, and 600 s of treatment.

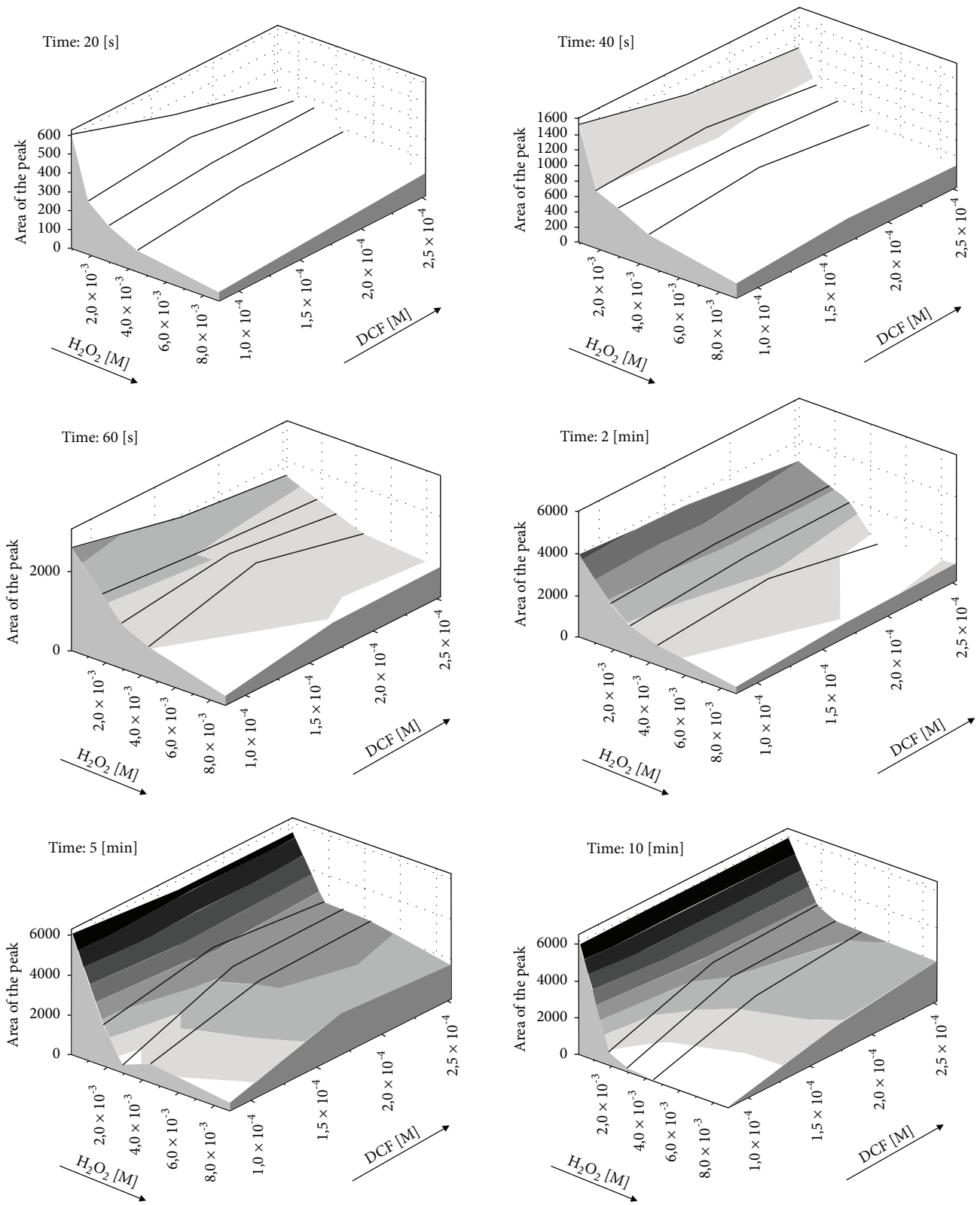


FIGURE 4: The influence of $H_2O_2$ and DCF concentration on product A/B ($t_R = 2.3$ min) formation during different irradiation time.

The data presented in Figures 6(b) and 6(c) were fitted using the Hill1 equation, while 100% mixture represents the undiluted sample drawn at the corresponding sampling time (after 0, 40, 120, and 600 s irradiation of DCF solution). The bioluminescence inhibition curves shifted to lower concentrations over the irradiation time, i.e., an increasing

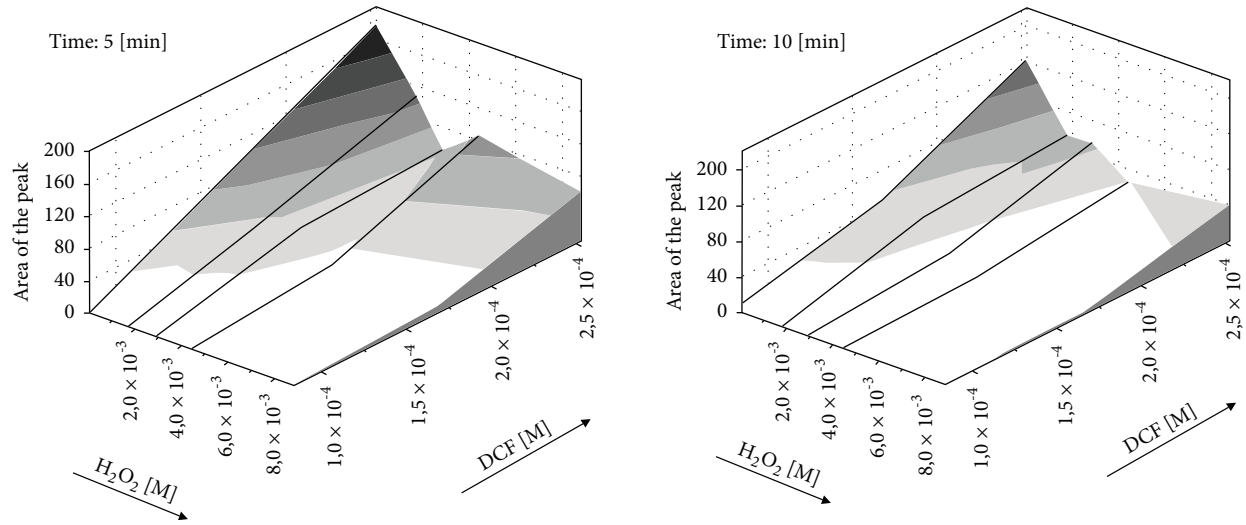


Figure 5: The influence of $H_2O_2$ and DCF concentration on product H formation.

bioluminescence inhibitory effect was observed in the case of $H_2O_2$/UVC. Average $EC_{50}$ obtained for the DCF solution was equal to $(31.92 \pm 1.14)\%$, which leads to $EC_{50} = (15.78 \pm 0.56)$ mg dm$^{-3}$. The determined value of $EC_{50}$ DCF toxicity towards *V. fischeri* is similar to the literature data 11.45 mg/L [32], 13.5 mg dm$^{-3}$ [33], 14.04 mg dm$^{-3}$ [34]. Based on toxic unit classification (TU, [35], DCF solution exhibited acute toxicity (class III). Moreover, according to the EU Directive 93/67/EEC, DCF is classified as "harmful to aquatic organisms" [36, 37]. This finding is in agreement with the literature based on the classifying chemicals as toxic with log $K$ ow < 6.0 (DCF has 4.51) as well as with the EU toxicity classification according to the Globally Harmonized System (GHS) of Classification and Labelling of Chemical [30].

As can be seen in Figure 6(b), the $H_2O_2$/UV treatment causes an increase of toxicity of DCF oxidation by-products. $EC_{50}$ after 40 s of the process decreased to $(23.58 \pm 1.24)\%$ and remained almost constant till 120 s of treatment $(23.16 \pm 2.73)\%$. Then, after 600 s of $H_2O_2$/UV treatment the toxicity increased even more ($EC_{50} = (13.55 \pm 0.5)\%$). As can be seen in Figure 6(c), direct UVC photolysis has not much influenced the toxicity. However, according to classification system proposed by Persoone et al. (2003) [35], the samples irradiated with and without $H_2O_2$ after 600 s still can be classified in class III, but TU is twice higher in the case of $H_2O_2$/UVC (TU = 7.3) then UVC process (TU = 3.3). Based on the photodegradation efficiency (Figure 6(a)), the removal of DCF during those both processes are very similar (pseudofirst order rate constants equal to $k^{UVC} = (9.09 \mp 0.48) \times 10^{-3}$ 1/s while $k^{H2O2/UVC} = (10.33 \mp 0.56) \times 10^{-3}$ 1/s). The increase of the toxicity can be attributed to breakdown of DCF and the formation of much more toxic TPs during $H_2O_2$/UVC process. It is known that during $^{\cdot}OH$ radical-driven photocatalytic removal of DCF toxicity of the degradation products increased during the oxidation [37–39]. As confirmed in the course of pulse radiolysis experiments, toxic breakdown products are formed at significant levels when DCF is oxidized by $^{\cdot}OH$ [3]. However, according to [13], the direct photolysis of DCF also leads to the increase of toxicity.

As has been shown (Figure 3), TP "I" with retention time of 6.8 min (2-(8-chloro-9H-carbazol-1-yl) acetic acid) is the main by-product of direct photolysis of DCF also confirmed by [12, 13, 23, 29, 30]. Moreover, this TP is also harmful to *Daphnia* [30] and according to [13, 29], this TP is mainly responsible for the toxicity increase. However, as can be seen in Figure 3 not many differences can be observed in relation with this by-product during photolysis and $H_2O_2$/UV degradation. Furthermore, after 600 s of $H_2O_2$/UV process the amount of this particular TP was lower than in the solution after photolysis, whereas the solution itself turned out to be more toxic compared to the one resulting from photolysis. It can be thus assumed that this TP has a rather limited influence on the toxicity. However, TPs "E", "C", and "F/G" with $t_R$ equal to 4.82 min, 2.5 min, 5.03 min, and 5.75 min, respectively, are responsible for the toxicity after the $H_2O_2$/UV treatment. While TPs "D", "A/B", and "H" with $t_R$ 3.4 min, 2.3 min, 1.95 min, and 6.52, respectively, have the impact on toxicity during UVC photolysis.

## 4. Conclusions

It was found that the addition of hydrogen peroxide into the photolysis system slightly accelerates the diclofenac decomposition (higher pseudofirst order rate constant in the case of $H_2O_2$/UV treatment); however, it significantly affects the reaction mechanism. Seven products of DCF photodegradation have been identified, with four of them formed in the process of direct photolysis, while the remaining three in the radical reaction process in the presence of $H_2O_2$. (2-(8-chloro-9H-carbazol-1-yl) acetic acid) was probably formed most quickly as one of the first by-products. DCF toxicity toward *V. fischeri* was similar to the literature data. Direct UVC photolysis does not have much influence on toxicity, while the $H_2O_2$/UV treatment causes increase of toxicity. The increase in toxicity can be attributed to the breakdown of DCF and formation of much more toxic TPs in the course of the $H_2O_2$/UVC process.

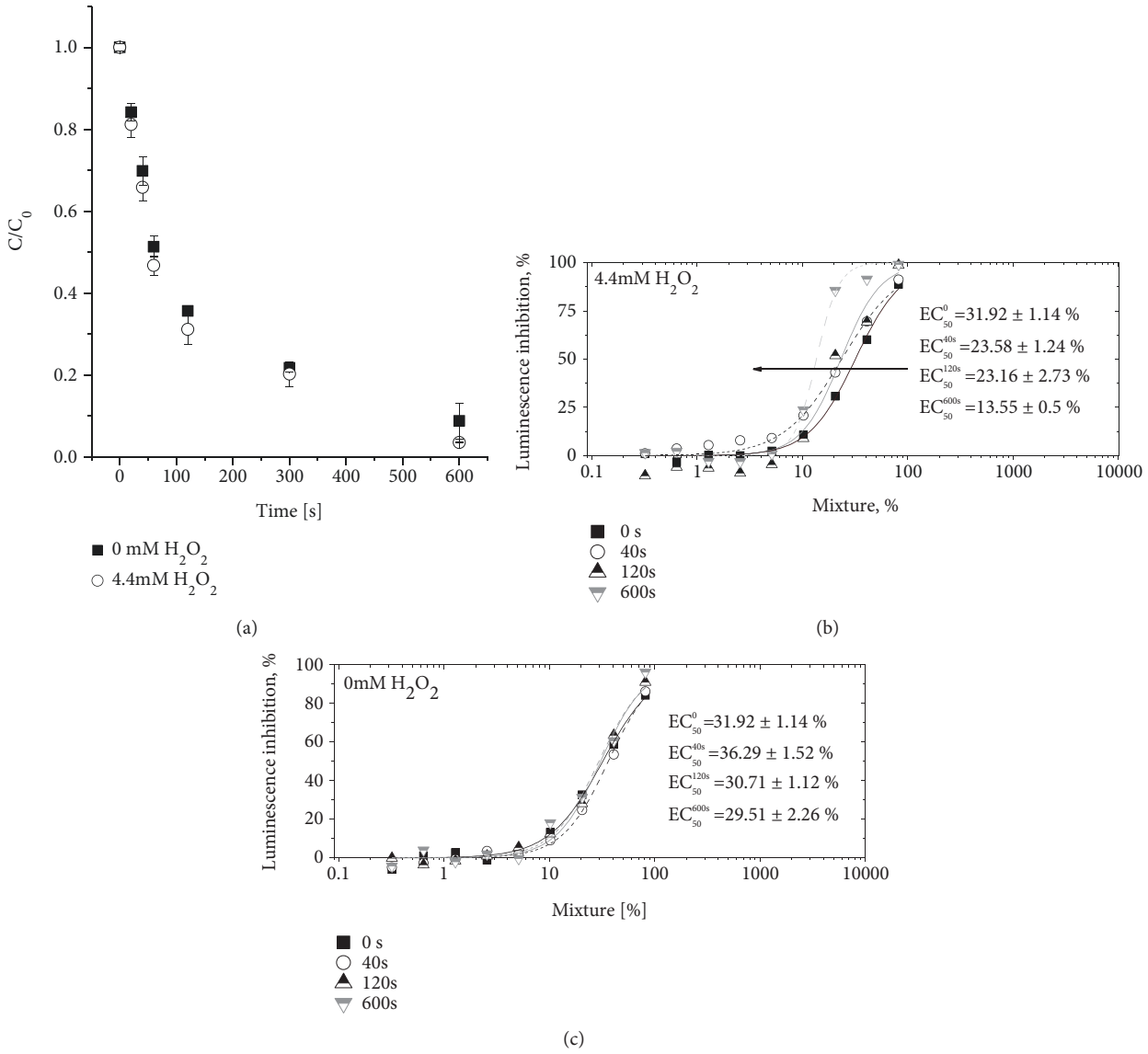


FIGURE 6: (a) Photodegradation efficiency during UVC direct photolysis (0 mM $H_2O_2$) and $H_2O_2$/UVC process (4.4 mM $H_2O_2$); dose-response curves for *V. fischeri* bioluminescence inhibition (15 min exposure) after $H_2O_2$/UVC process (b) and UVC direct photolysis (c).

## Nomenclature

DCF: Diclofenac
TPs: Phototransformation products of DCF
PPCP: Pharmaceutical and personal care products
NSAIDs: Nonsteroidal anti-inflammatory drugs
$H_2O_2$: Hydrogen peroxide
$^{\cdot}OH$: Hydroxyl radical
$E_0$: Volumic photon fluence rate (einstein $dm^{-1}$ $s^{-1}$)
$F_{H2O2}$: The fraction of UVC radiation absorbed by hydrogen peroxide
$F_{DCF}$: The fraction of UVC radiation absorbed by diclofenac
C: Molar concentration (M)
b: Optical path length (cm)
r: Initial reaction rate ($M\,s^{-1}$)
k: Reaction rate constant
t: Time (s)
$t_r$: Retention time during chromatographic analysis (s)
$k_z$: Pseudofirst order rate constant ($s^{-1}$).

*Greek Letters*

$\varepsilon$: Molar absorption coefficient ($M^{-1}$ $cm^{-1}$)
$\varphi$: Quantum yield.

*Subscripts*

0: Initial conditions.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## Conflicts of Interest

On behalf of all the authors, the corresponding author states that there is no conflict of interest.

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