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Photochemical transformation of flufenamic acid by artificial sunlight in aqueous solutions

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ABSTRACT

In the present article, we have studied the photochemical behavior of a common non-steroidal anti-inflammatory drug (NSAIDs) namely flufenamic acid (FLUA) in aqueous solution. The absorption spectrum of such compound shows a significant absorption beyond 290 nm and the photochemical irradiation within the range 300–450 nm leads to its complete transformation in roughly 6 h. The quantum yield of FLUA transformation measured at 290 and 310 nm was evaluated to about 1.1×10^{-4} without any significant effect of the excitation wavelength. The degradation process was inhibited in acidic solutions owing to the sharp increment in the absorption of FLUA in the wavelength region between 300 and 350 nm. The quantum yield was estimated to 1.2×10^{-4} at pH 7. The effect of oxygen on the photochemical behavior of FLUA has also been investigated. The obtained results clearly indicate that oxygen is not significantly involved in the photochemical degradation process. The phototransformation of the flufenamic acid appears to occur through one pathway that involves the photohydrolysis of trifluoromethyl group, and thus leads to the formation of one specific photoproduct, namely 2,3′-iminodibenzoic acid. This result was confirmed by the formation of the fluoride ions after irradiation during the irradiation of flufenamic acid. A mechanistic scheme for such transformation of flufenamic acid was proposed.

1. Introduction

Many pharmaceutical compounds used as human and veterinary drugs pass, at least in part, through sewage treatment plants (SWT) to end up in environmental natural waters. They are suspected to reach every environmental compartment. Indeed, it has been frequently reported that they have been detected in lakes, groundwater, and also in drinking water [1–5]. It has been largely reported that biodegradation and photodegradation are among major transformation processes influencing the fate of such pollutants in natural waters. However, Biological processes can induce a limited degree of transformation because of the biopersistence of many organic compounds as well as their generated byproducts that result from the incomplete biodegradation [6,7].

Since several pharmaceutical compounds are usually resistant to biodegradation, either direct or indirect photolysis may be considered as potential degradation routes of their transformation in surface waters [8]. Exposure to sunlight has already been confirmed as one of the most important way of their transformation in natural aquatic environments [9–11]. However, the photochemical transformation process efficiency in surface waters is dependent on various environmental factors such as the depth, turbidity, geographic latitude, season, weather and shadow [12].

Non-steroidal anti-inflammatory drugs (NSAIDs) are a group of drugs of diverse chemical composition and most often used in human and veterinary medicine, since they are available without prescription for treatment of fever and minor pains [13]. Many NSAIDs have been found or are expected to be present in the aquatic environment at significant concentrations [14–16]. In addition, this family compounds showed a remarkable reactivity toward the light. Indeed, they rapidly react under irradiation and can be largely eliminated from surface waters, owing to photolysis reactions. Direct photolysis by sunlight or artificial light constitutes the dominant degradation mechanism and the removal process for these products [17–20].

N-(α,α,α-trifluoro-*m*-tolyl) anthranilic acid known as flufenamic acid (FLUA) is a common non-steroidal anti-inflammatory

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drugs (NSAIDs) that belongs to the family of *N*-phenylanthranilic acid and resembles chemically to mefenamic and tolfenamic acids and other fenamates that are largely used in clinical issues [21]. flufenamic acid presents analgesic, anti-inflammatory and antipyretic properties and has been used in musculoskeletal as well as joint disorders [22].

FLUA is often found in the environment at significant concentrations [16]. The presence of mixtures of flufenamic and mefenamic acids in human urine samples in relatively important concentrations has also been demonstrated [23,24]. A very recent study also revealed the presence of flufenamic acid at considerable concentration, in rivers water [16]. Until now, however, there is very limited information concerning the photochemical behavior of flufenamic acid in aqueous solutions. In the present paper, we report results of its photochemical behavior from kinetic as well as analytical points of view. Our approach includes the selection of appropriate conditions in order to obtain the best results and to evaluate the effect of environmental parameters on the photolysis efficiency of flufenamic acid.

2. Materials and methods

2.1. Chemical and reagents

The "*N*-(α,α,α-trifluoro-*m*-tolyl) anthranilic acid" known as flufenamic acid (FLUA) was purchased from Fluka and used without any further purification. 2,3′-imino-dibenzoic acid was obtained from Alfa Chemistry as the purest grade available. Acetonitrile was purchased from Carlo Erba (HPLC grade). All solutions were prepared with deionized ultrapure water, which was purified with Milli-Q device (Millipore) and its purity was controlled by its resistivity (>18 MΩ cm). The measurements of pH were undertaken using a JENWAY 3310 pH-meter to ±0.01 pH unit and the ionic strength was not controlled during the irradiation experiments. The pH of the solutions was adjusted using dilute solutions of $HClO_4$ or NaOH.

Solutions were deoxygenated by nitrogen bubbling or oxygenated by oxygen bubbling for 30 min prior to irradiation at room temperature. For prolonged irradiations, the bubbling was maintained during the entire irradiation process.

2.2. Irradiation systems

For kinetic purposes, aqueous solutions were irradiated in a quartz cell (1 cm optical path length) using an arc Xenon lamp from OSRAM (XBO 1600 W/XL OFR). The emission of the lamp extends from 270 nm to 850 nm with a maximum at 650 nm. The entire system is equipped with a Schoeffel monochromator to select the appropriate wavelengths for monochromatic irradiations. Two different wavelengths were used: 290 nm and 310 nm. The bandwidth was set to 10 nm. The initial concentration of the solution was checked by HPLC analysis after oxygen or nitrogen bubbling. Potassium ferrioxalate was used as a chemical actinometer [25]. The light intensity was found equal to 1.5×10^{15} photons $cm^{-2}\,s^{-1}$ and 2.2×10^{15} photons $cm^{-2}\,s^{-1}$ at 290 nm and 310 nm respectively. By modifying the bandwidth modified the light intensity changes. For analyses purposes, excitations within the range 300–450 nm were performed. The irradiation device consists of a vertical Pyrex tube (20 mm internal diameter with a total volume of 100 mL) equipped with a water cooling jacket to limit thermal reactions. It is located along one of the focal axes of a cylindrical mirror with an elliptic base. A fluorescent lamp TLD15 W/05 emitting within the range 300–450 nm is located along the other focal axis. The distance between the lamp and the reactor was constant and equal to approximately 13 cm.

2.3. Analysis

The disappearance of FLUA and the formation of the byproducts were followed by HPLC technique that consists on a Waters 540HPLC chromatograph system equipped with a Waters 996 photodiode array detector. The chromatograms were extracted at 288 nm as detection wavelength. The separation of the solutions components was accomplished by using a reverse phase Nucleodur column (C18–5 μm; 250–4.6 mm). The flow rate was 1.0 mL/min and the injected volume was set to 50 μL. The elution was accomplished with water that was acidified with formic acid (0.1%) and acetonitrile using an isocratic program (60% water and 40% acetonitrile).

The quantum yield of FLUA disappearance was measured at 290 and 310 nm by using the following expression: Φ = number of decomposed molecules of pollutant/number of photons absorbed by pollutant.

LC/MS studies were carried out with Q-TOF-Micro/water 2699 from UBSTART center at the University Blaise Pascal. It is equipped with an electrospray ionization source (ESI) and a Waters photodiode array detector. Each single experiment permitted the simultaneous recording of both UV chromatogram at a preselected wavelength and an ESIMS full scan. Data acquisition and processing were performed by MassLynx NT 4.0 system.

The evolution of fluoride ions concentration as a function of irradiation time was obtained by ionic chromatography (IC) using a Dionex ICS-1500 equipped with an ionPac CG16 (analytical column 5 × 250 mm).

UV–vis spectra were recorded on a Cary 300 scan (Varian) spectrophotometer.

3. Results and discussion

3.1. Spectrophotometric study

The absorption spectrum of flufenamic acid at a concentration of 5.0×10^{-5} mol L^{-1} in aqueous solution and at pH of 5.6 exhibits a band with a maximum at 288 nm (ε_{288} = 19,400 $mol^{-1}\,L\,cm^{-1}$) and a shoulder at about 325 nm (ε_{325} = 8800 $mol^{-1}\,L\,cm^{-1}$). For acidic conditions, namely a pH 2.1, the absorption spectrum consisted of two well-defined bands at 283 nm (ε_{283} = 6500 $mol^{-1}\,L\,cm^{-1}$) and 345 nm (ε_{345} = 3650 $mol^{-1}\,L\,cm^{-1}$). It is worth noting that a significant absorption at λ > 300 nm is observed (Fig. 1), which indicates a significant overlaps with the emission spectrum of the solar radiation that reaches the biosphere.

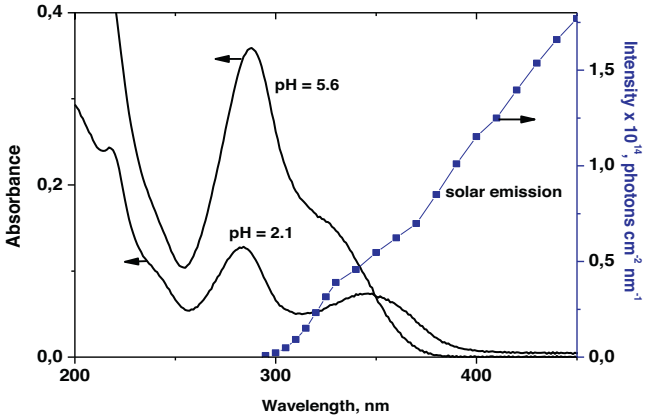


Fig. 1. Characteristic UV spectra of FLUA (2.0×10^{-5} mol L^{-1}) at pH 5.6 and pH 2.1 in aqueous solution compared to the solar emission spectrum.

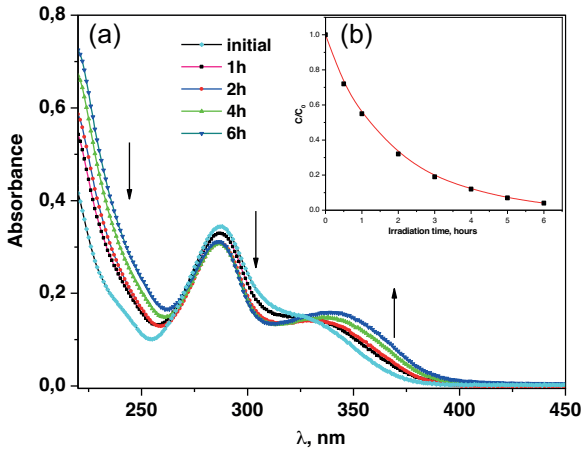


Fig. 2. (a) Evolution of the UV absorption spectrum of FLUA in aqueous solution upon irradiation within the range 300–450 nm. (b) Evolution of the concentration as a function of irradiation time. ([FLUA] = 5.0×10^{-5} mol L^{-1}, pH 4.8).

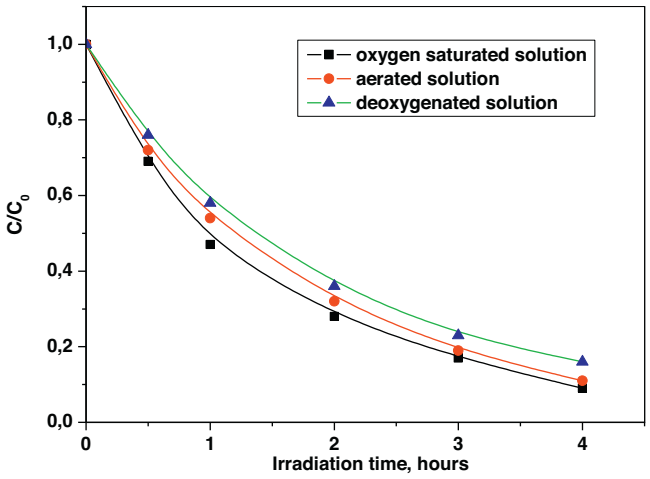


Fig. 4. Effect of the oxygen concentration on the photodegradation of FLUA upon irradiation at the range 300–450 nm.

These spectroscopic data indicate that FLUA is able to absorb solar light and thus to undergo direct phototransformation, which is very interesting from the environmental point of view. Indeed, the direct photolysis of FLUA under artificial light within the range 300–450 nm in aerated aqueous solutions was investigated. Dark control samples were analyzed in order to ensure a negligible effect of thermal reactions in the same conditions.

As clearly shown in Fig. 2, upon irradiation of FLUA at a concentration of 2.0×10^{-5} mol L^{-1} and pH 4.8, we observe a decrease of the absorbance at $\lambda < 320$ nm and a significant increase for higher wavelengths without a significant change of the pH. These are owing to the disappearance of FLUA and the formation of byproducts respectively indicating that flufenamic acid is efficiently photodegraded by direct irradiation. The HPLC analysis of the irradiated solution showed that about 50% conversion was obtained in 1 hour and a complete transformation was achieved by an irradiation period of roughly 6 h.

The quantum yield of FLUA phototransformation was measured in air-saturated solutions and pH of 4.8, by using monochromatic lights at 290 and 310 nm. It was evaluated (in both irradiation conditions) to about 1.1×10^{-4} indicating that the photochemical behavior in not excitation wavelength dependent. Moreover, no effect of the light intensity was observed under our experimental conditions.

3.2. The effect of pH

The kinetics of FLUA photolysis in aqueous solution has been studied at different pH values, 3.0, 4.8 and 7.0. As clearly shown in Fig. 3a, the pH of the FLUA solution has an obvious influence on the direct photolysis rate. The degradation rate increased when the pH increased. It is evident that different pH values of the solution make difference in the absorption spectrum of the solution. Thus, an increase of the absorbance is clearly observed within the wavelength rage 300–350 nm when the pH decreases (Fig. 3b). This significant change is attributed to the ionization process of FLUA in aqueous solution owing to the protolytic equilibrium with a pK_a of 4.17 [26]. In order to take into account for this change in absorption, we calculated the disappearance quantum yield. This was evaluated to 1.2×10^{-5}, 1.1×10^{-4} and 1.2×10^{-4}, at pH 3.0, 4.8 and 7.0 respectively. No difference in the disappearance quantum yield was observed within the pH range 5.0–11.0 indicating that the higher photoreactivity of the anionic form.

3.3. Effect of oxygen concentration

It is well known that in aqueous medium, molecular oxygen may have dual roles in the photochemical behavior of organic pollutants. For example, It could quench the molecules excited triplet states resulting in the decrease of the conversion rate by the

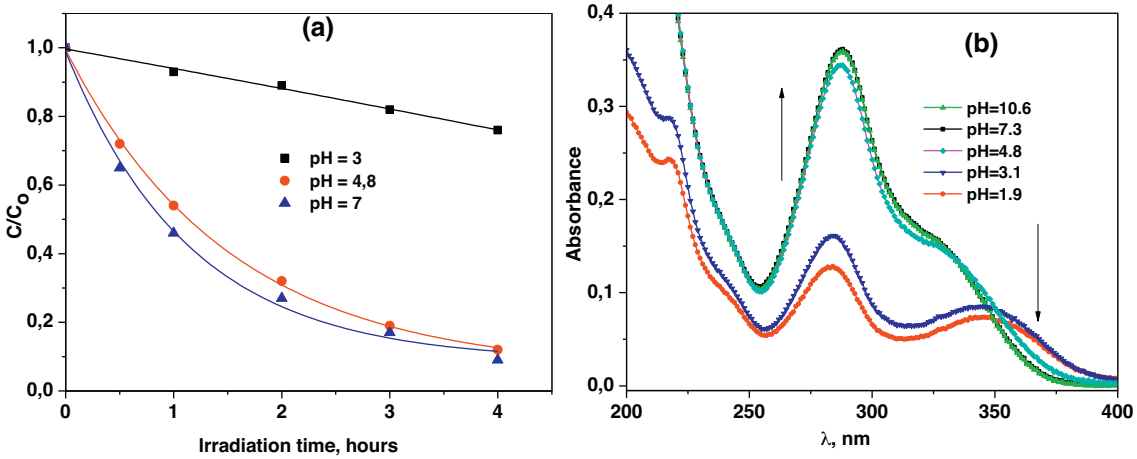


Fig. 3. (a) Effect of pH on the photodegradation rate of FLUA in aqueous solution irradiated within the range 300–450 nm. (b) Effect of pH on the absorption spectrum of FLUA.

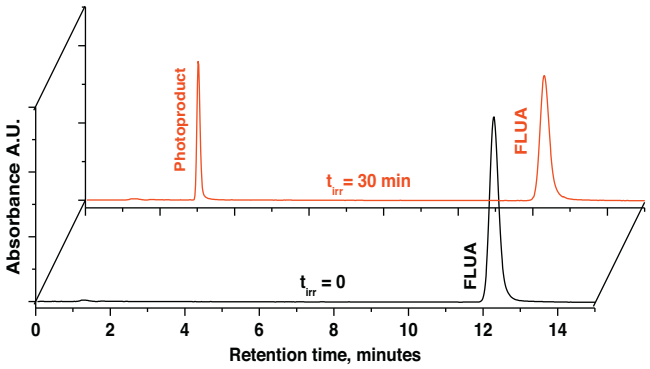


Fig. 5. HPLC chromatogram of the initial and irradiated solutions of FLUA at the initial concentration of 50 μmol L^{-1}. The conversion percentage was roughly 28%.

generation of singlet oxygen, $^1O_2$, or react with radical intermediates leading to oxidation processes [26–28].

To examine the effect of oxygen on the photodegradation rates of FLUA, photochemical irradiations were carried out in aqueous solutions at different oxygen concentrations with a stream of nitrogen gas ($[O_2] < 1.0 \times 10^{-5}$ mol L^{-1}) or of oxygen gas ($[O_2] = 1.29 \times 10^{-3}$ mol L^{-1}) an aerated solutions ($[O_2] = 2.6 \times 10^{-4}$ mol L^{-1}). As far as the initial degradation rate is concerned, Fig. 4 clearly shows that similar initial degradation rates were obtained for the three used oxygen concentration. This indicates, within the experimental errors, that oxygen is not significantly involved in the photochemical degradation process of FLUA.

These results were confirmed by evaluating the quantum yield in different conditions and by monochromatic excitation at 310 nm. This was found constant and equal to 1.1×10^{-4}.

3.4. Identification of photolysis products of FLUA

The irradiated solution of FLUA, at natural pH, was analyzed by using chromatographic technique such as HPLC (Fig. 5). Under these conditions, a single photoproduct resulting from the irradiation of FLUA was clearly seen at a retention time of 4.0 min.

HPLC coupled to mass spectrometry (LC–ESI–MS) screening method using a MS/MS spectral library for the identification of xenobiotic substances has been developed and validated. This method is a selective and accurate method that allowed the determination of selected non-steroidal anti-inflammatory drugs (NSAIDs), either individually or in mixtures [29].

First, flufenamic acid was analyzed in ESI(+) mode, giving a mass value at $m/z = 282$ together with a daughter fragment ion at $m/z = 264$. The latter corresponds to the loss of a water molecule. This was then followed by the elimination of hydrogen fluoride (HF) leading to a fragment ion at $m/z = 244$. The latter ion undergoes the elimination of carbonyl group and then a second elimination of hydrogen fluoride giving rise to two principal fragments at m/z 216 and 196 respectively (Fig. 6a). The determination of the chemical structure of the degradation product was conducted using the same conditions. The obtained MS–MS spectra are compiled in Fig. 6b.

FLUA parent ion and byproduct ion differed by 24 units of mass. These results clearly show that the direct photodegradation of FLUA involves an interesting reaction where the CF_3 group is converted to COOH more likely by hydrolysis reaction.

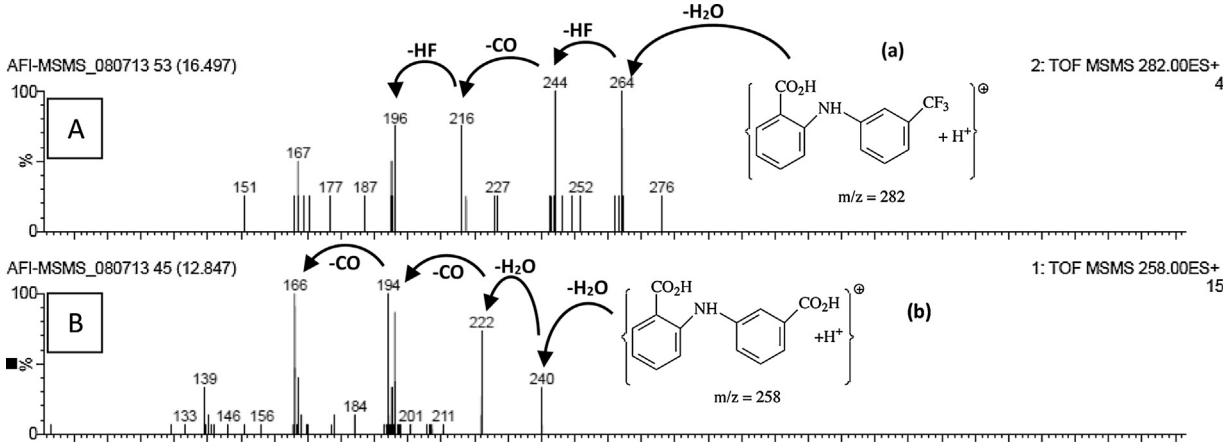
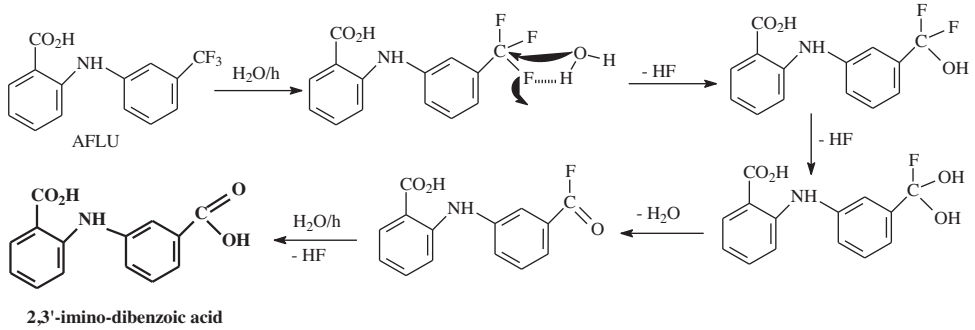


Fig. 6. LC/ESI/MS/MS chromatogram in positive mode for (A) FLUA and its byproduct (B).



Scheme 1. Proposed mechanism for the phototransformation of flufenamic acid in aqueous solution.

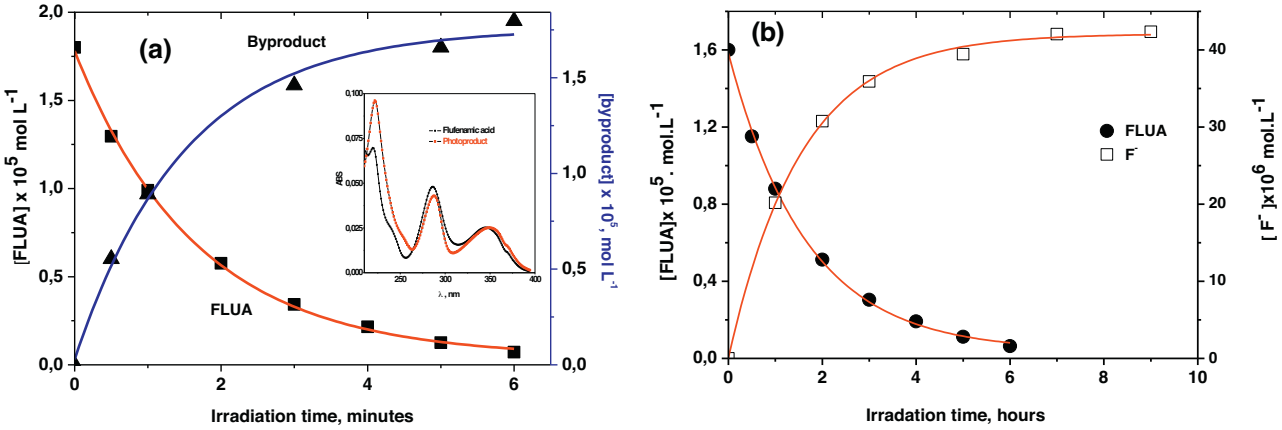


Fig. 7. Kinetic of FLUA phototransformation and formation of byproduct (2,3′-imino-dibenzoic acid (a) and fluoride ions (b) a function of irradiation time.

In fact the mass spectra of the photoproduct (*m*/*z* 258) and its generated daughter fragments corresponding to the loss of two consecutive water molecules (*m*/*z* 240 and 222) and two consecutive carbon monoxide molecules (*m*/*z* 194 and 166), were clearly observed. They perfectly confirm the proposed structure of the generated byproduct as 2,3′-imino-dibenzoic acid.

The proposed mechanism for the photodegradation of flufenamic acid is shown in (Scheme 1). The degradation pathway under UV-irradiation is the photohydrolysis of a C—F bond. This reaction has been previously observed for several compounds containing a trifluoromethyl group (Table 1).

The formation of the photoproduct 2,3′-imino-dibenzoic acid may follow the same mechanistic pathway. This photohydrolysis is owing to the electron-withdrawing effect of the carboxylic function, which inhibits the release of a halogen ion when it is not assisted by the heterolytic scission of a molecule of water. It is very important to note that the mefemanic acid, which presents a methylene group (CH_3) instead of CF_3 as in the flufenamic acid, did not undergo any degradation upon light irradiation under the same conditions.

Table 1
Photohydrolysis of the C—F bond of the compounds containing a trifluoromethyl group presented in litterature.

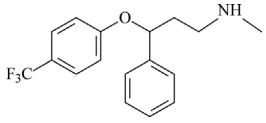
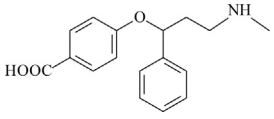
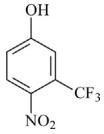
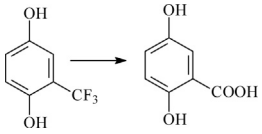
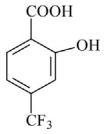
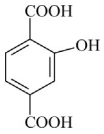
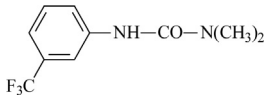
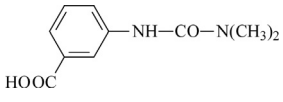
Products	Photoproducts	Ref
		[30,31]
		[32]
		[33]
		[34]

Fig. 7a shows a typical time course of FLUA photodegradation in aqueous solution as discussed previously. Since the generated product is commercial a quantitative analysis was undertaken by performing a calibration curve. Under our experimental conditions, similar values of the degradation rate of FLUA and the formation rate of the byproduct 2,3′-imino-dibenzoic acid were obtained indicating the selective photoreactivity of flufenamic acid.

These results show that this photodegradation product is obtained with nearly stoichiometric proportion to the parent since they have the same evolution kinetic curves. It is worth noting that flufenamic acid and its generated product present roughly the same absorption spectrum (Fig. 7a).

As shown in Fig. 7b, the phototransformation of FLUA generates fluoride species that were easily detected and analyzed by ionic chromatography. Such Fig. compares the released Fluoride ions concentration and the disappearance amount of FLUA. As clearly observed, under our experimental conditions, the initial rates for FLUA degradation and Fluoride ions are perfectly similar indicating the involvement of a unique photochemical. This was evaluated to about 1.2×10^{-7} mol L^{-1} min^{-1}. Moreover, when flufenamic acid at the initial concentration of 1.6×10^{-6} mol L^{-1}, is photochemically and entirely converted to 2,3′-imino-dibenzoic acid, the obtained concentration of fluoride ions was evaluated, as 4.2×10^{-5} mol L^{-1}. This result is compatible with that calculated while multiplying by a factor of three 3 the initial concentration of FLUA (analyzed by HPLC) in good agreement with the molecular structure (Fig. 7b).

4. Conclusion

The total transformation of the flufenamic acid under direct photochemical irradiation in solution was obtained in 6 h under our experimental conditions. The photochemical transformation was studied according to several parameters such as the effect of oxygen, pH and excitation wavelength. A single photoproduct was obtained namely 2,3′-imino-dibenzoic acid. Its structure as well as its selective formation was confirmed by LC-mass technique and by HPLC calibration using the standard compound. This is in agreement of the defluoration of the flufenamic acid in aqueous solution. Based on these results, a photochemical degradation mechanism of flufenamic acid was proposed.

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