RESEARCH ARTICLE

DICLOFENAC SODIUM UV DEGRADATION

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ARTICLE INFO	ABSTRACT

Diclofenac sodium (2- [2 - [(2,6-dicloro-fenil) amino] phenyl] sodium acetate) is an nonsteroidal anti-inflammatory drug widely used today as an analgesic, anti-arthritic and anti-rheumatic. It is susceptible to stress caused by UV radiation. In this work photodegradation of diclofenac was monitored in batch mode using a photolysis orthogonal chamber. Diclofenac aqueous solutions of pH 9 were subjected to the effect of UV radiation of 254nm from a mercury lamp UVP with wood glass, located at 15 cm from the liquid surface. UV Spectra of solutions exposed for periods of time in the range of 5 minutes to 48 h were scanned. Diclofenac molecule without UV treatment exhibits a maximum at 195 nm and another at 276 nm. At very short treatment times a peak appears around 239 nm, where in the original molecule the absorbance is minimal. Another peak begins to emerge at 289 nm, which becomes more apparent at times of treatment longer than 1 hour. Simultaneously in greater times peaks at 225 and 250 nm appear. The absorptiometric measurements were complemented by the application of quantum theoretical chemistry using the static approximation and density functional theory, B3LYP. The excitations energies B3LYP for diclofenac and for the photolysis products are closed with the experimental measurements. Based on this, a mechanism for the reaction of photolytic degradation of diclofenac in the tested conditions is proposed.

INTRODUCTION

Photolytic reactions can result in the decreased potency of the active ingredient of a drug and adverse reactions attributable to the occurrence of photodegradation products during storage or administration to patients (Jiménez Cerezo *et al*., 2006; Sánchez-Quiles, 2011). The photostability study of a pharmaceutical preparation allows to adapt the processing areas, storage and distribution of the drug, the characteristics required of the primary packaging and the precautions to be taken during their analytical control and conservation by patients (Ercolano *et al.*, 2010; ICHQ1B, 1997). Diclofenac sodium (2- [2 - [(2,6-diclo-rofenil) amino] phenyl] sodium acetate) is an NSAID, nonsteroidal anti-inflammatory drug (Goodman y Gilman, 2003) widely used today as an analgesic, anti-arthritic and anti-rheumatic. It is susceptible to stress caused by UV radiation. The Health Ministry of the Province of Corrientes (Argentina), through its Processing Medicinal Plant (PLAMECOR) provides this specialty, in tablets of 50 mg which are distributed in health centers across the province to be given to the most vulnerable population sectors. Photolysis of these tablets at short exposure times to UV radiation has been proposed as a quantitative analysis method (Monzón, 2014).

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Diclofenac photochemical degradation by sunlight in water bodies has been demonstrated (Poiger *et al*, 2001; Agüera, *et al.*, 2005; Agüera, *et al.*, 2006; Pérez Estrada, 2008). The detected concentrations of active ingredient not degraded at surface layers were much lower than those at deepest levels (Buser, *et al.*, 1998; Tixier *et al*., 2003). The influence of oxygen and free radicals as promoters of photolysis were analyzed (Rivas *et al.*, 2010) as well as the impact of TiO_2 or Fe (II) ion and H_2O_2 as catalysts of the process, resulting photochemical reaction speed dependent of the medium pH (Pérez Estrada, 2008). Diclofenac degradation by UV light of 365 nm was studied by Moore *et al.*, 1990.

The analysis of UV absorptiometry spectra of aqueous solutions of diclofenac allowed to monitor its evolution at different treatment times under the action of UV light of 254 nm. The absorptiometric measurements were complemented by the application of quantum theoretical chemistry using the static approximation and density functional theory, B3LYP (Parr *et al., 1*989; Gross *et al.*, 1996; Casida *et al.*, 1998). Based on this, mechanism for the reaction of photolytic degradation of diclofenac in the tested conditions are proposed.

Experimental

MATERIALS AND METHODS

- Raw Material: supplied by the drug plant of Corrientes, Argentina (Plamecor)
- Active ingredient: Diclofenac sodium Lot 20010321 origin: China. 99.6% purity
- Sodium diclofenac 103 ppm solution: prepared by dissolving an accurately weighed mass of diclofenac active ingredient in distilled water, pH was adjusted with 0.1 N NaOH.
- *Equipment*
- Mercury lamp UVP, UVGL-58 model with Wood glass, 6 Watts of power, short wavelength: 254 nm.
- Photolysis Orthogonal Chamber of 30 x 25 x 20 cm
- Jasco V-630 Spectrophotometer Bio.
- *Software*
- Gaussian 09 quantum chemistry computational package.

Procedure

Diclofenac photodegradation was done in batch mode. Two Petri dishes of 9 cm diameter containing 15 mL of diclofenac aqueous solution were introduced into the photolysis chamber. One dish covered with aluminum foil served as control. At time zero the UV lamp, located at a fixed distance of 15 cm from the liquid surface, was turned on. After the set period, the samples were extracted from the chamber, and wrapped in aluminum foil until analysis. Afterwards, samples were centrifuged and diluted in order to perform spectrophotometric readings. Spectra of aqueous solutions of diclofenac exposed to UV light for periods of time in the range of 5 minutes to 48 h were scanned. The study was performed in triplicate for each time.

RESULTS AND DISCUSSION

Spectrophotometric Analysis

Selection of the working pH

Diclofenac is a weekly acidic molecule (pKa = 4.15), therefore spectra of aqueous solution at pH 7; 9 and 11, without UV treatment, called control, were scanned (Figure 1). Figure 1 shows very little difference between the curves, corresponding the greatest absorbance spectrum to the solution of pH = 9. Similarly, the spectra of solutions UV irradiated during 1h at different pH were monitored (Figure 2). As seen in Figure 2, the highest absorbance corresponds to pH 9 diclofenac solution, which was the value chosen for diclofenac spectra readings at different times of treatment.

Spectrophotometric monitoring of diclofenac solutions irradiated at different times

Diclofenac solutions of pH 9 were subjected to the effect of UV radiation of 254nm during times between 5 min and 48 h. The analysis of the changes observed in the obtained spectra, led us to divide them into three groups: a) Photolysis at short times: 10-60 min of treatment (Figure 3); b) Photolysis at intermediate times: 120-180 min of treatment (Figure 4) and c) Photolysis at long times:

24 to 48 hours of treatment (Figure 5). Figures 1 and Figures 3 to 5 show that at zero (control) diclofenac molecule exhibits a maximum at 195 nm and another maximum at 276 nm. At very short treatment times a maximum appears at 239 nm, where in the original molecule the absorbance is minimal and begins to sketch a maximum around 289 nm (Figure 3), which becomes more apparent at times of treatment longer than 1 hour (Figure 4). The latter peak becomes most evident at 24 h of treatment. Simultaneously in greater times peaks at 225 and 250 nm (Figure 5) appear.

Quantum Chemistry Methods

All DFT (Parr *et al.*, 1989) and TD-DFT (Gross *et al.*, 1996; Casida *et al.*, 1998) calculations were performed with the Gaussian 09 quantum chemistry computational package (Frisch *et al.*, 2004). For the specie shown in Figure 6 in this study (diclofenac, N-2,6-dichlorophenylamine and N-2-chlorophenylamine) (Pérez Estrada, 2008) the conformational stability as well as the conformational analysis, were carried out using the hybrid version of the B3LYP (Becke, 1993) and the 6-311þG(d, p) basis set (Cossi *et al.*, 2003). Minimum energy conformation of specie was reoptimized using the same functional and basis set in conjunction with the polarizable continuum model (PCM) (Cossi *et al.*, 2003) to obtain the minimum energy structure in solution for the specie. We confirmed that the structure corresponded to a minimum on the potential energy surface by an analysis of vibrational frequencies at the same level of theory. Vertical electronic excitation energies (λ_{theor}^{excit}, nm) of the studied molecule was calculated in the minimum energy conformation. For this task, the upmost three excitations were used in all the calculations. The level of theory for calculating excitation energies was B3LYP/6-311G (d,p). Solution environments, where the experimental absorption measurements are performed, was treated with PCM under the same solvent. To compare with experimentally detected λ_{exp}^{max}, we only considered the theoretical excitation energies λ_{theor}^{excit} with the highest oscillator strengths

Theoretical Predictions Assessment

It is important to evaluate the error incurred by selected functional in calculating the λ_{theor}^{excit} at the used level of theory, and how λ_{theor}^{excit} compares with the experimental determinations. The results of the assessment for B3LYP gives 195 and 278 nm better predictions, the excitations energies B3LYP for diclofenac are closed with the experimental measurements respectively, B3LYP is recommended for calculating excitation energy. (Table 1) The same goes for the first photolysis product, N-2,6-dichlorophenyl amine its values of wavelengths values are 242 and 293 nm, having a difference of 3 and 4 nm with the experimental respectively.
For the second photolysis product, N-2-chlorophenyl amine its values of wavelengths values are 225 and 249 nm are very close to the obtained experimental values.

Table 1. Theoretical UV spectrum

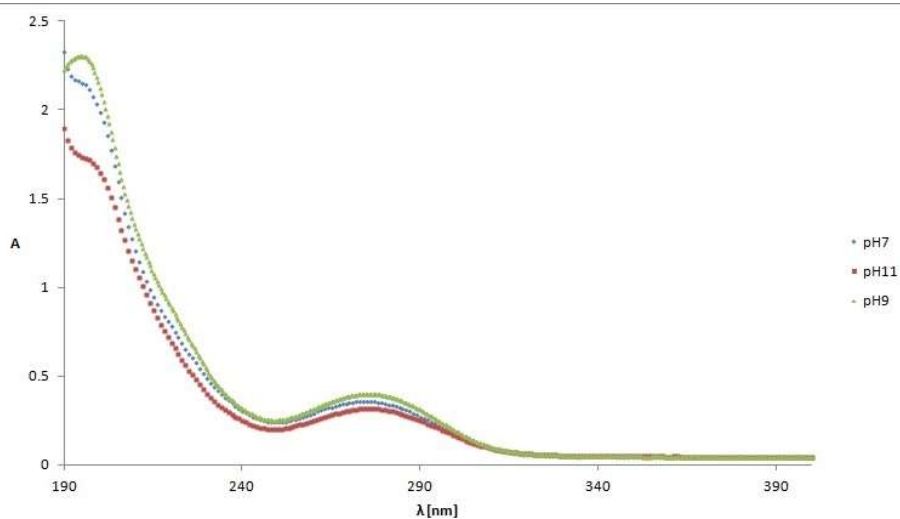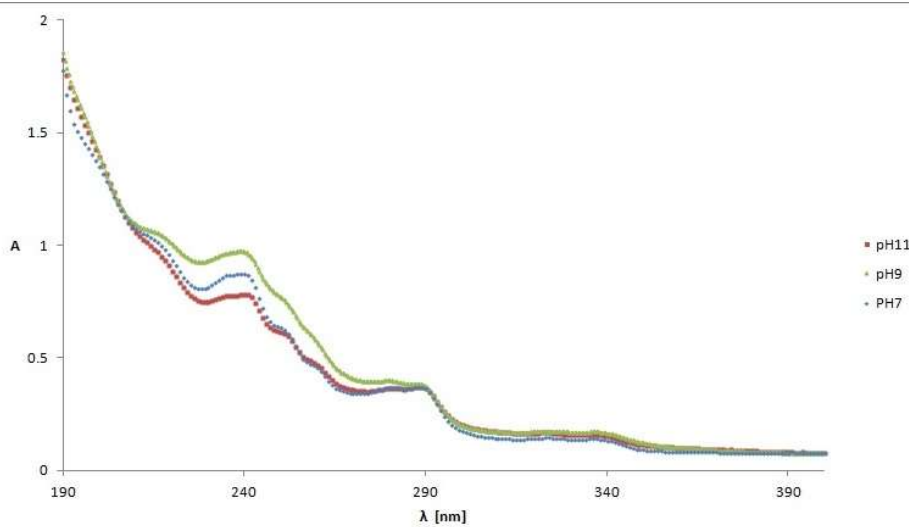| Species or molecules	$\lambda_{max.\ exp}$ [nm]	$\lambda_{max.\ calc}$ [nm]	MOs	Assignment
Diclofenac	195	195	49→50	$\pi\to\pi^*$
	276	278	49→53	$\pi\to\pi^*$
N-2,6-dichlorophenyl amine	239	242	38→40	$\pi\to\pi^*$
	289	293	37→39	$\pi\to\pi^*$
N-2-chlorophenyl amine	225	225	35→38	$\pi\to\pi^*$
	250	249	35→40	$\pi\to\pi^*$

Figure 1. Diclofenac spectra at different pH

Figure 2. Diclofenac spectra of solutions of different pH irradiated for 1h
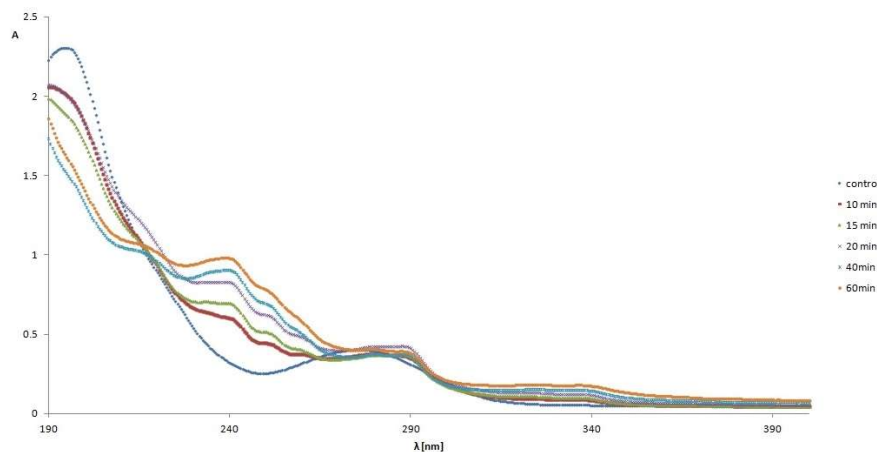

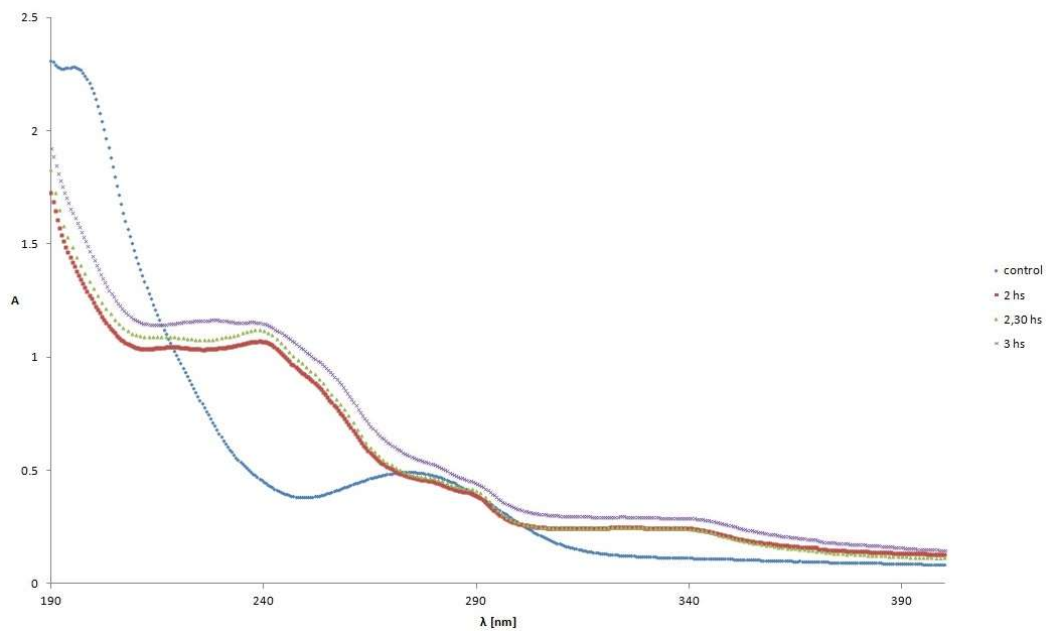**Figure 3. Absorbance spectra of diclofenac Photolysis at short times**

Figure 4. Absorbance spectra of the Photolysis of diclofenac at intermediate times
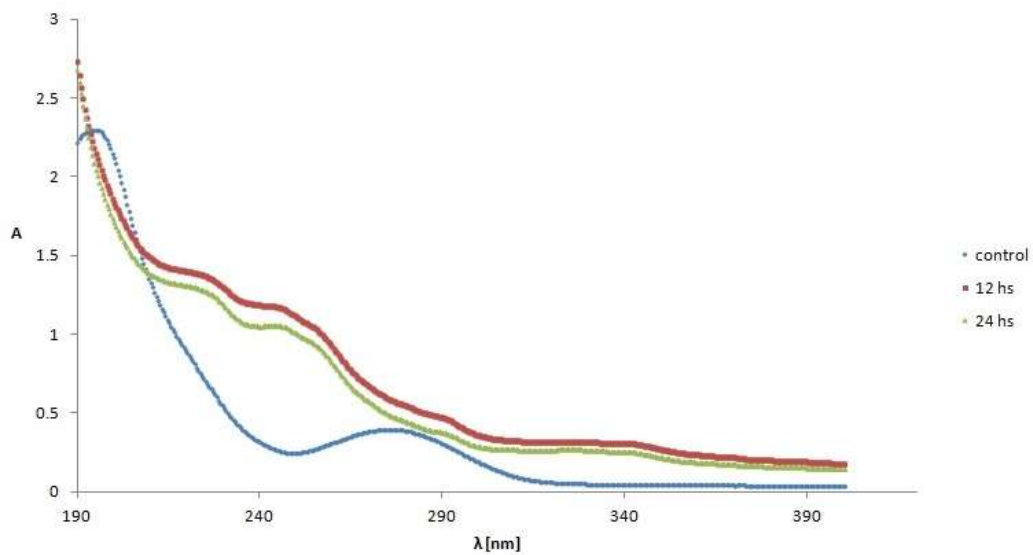

Figure 5. Absorbance spectra of diclofenac Photolysis at long times

Diclofenac   N-2,6-Dichlophenyl amine   N-2-Dichlophenyl amine

Figure 6. Diclofenac and photolysis reaction products

Absorption behavior in water

Behavior of diclofenac in aqueous solution has been previously characterized by UV-Vis spectroscopy. As long as the diclofenac amount in solution is lower than its saturation limit, the observed spectrum will be independent of the concentration but strongly dependent on the pH of the solution. Its theoretical excitation energy in water is 195 nm, very close to the experimental 195 nm (pH = 9). Thus, the neutral species is present under these conditions in water. The products obtained in the photolysis reaction coincide theoretically designed, as shown in the spectra obtained by calculation DF-TDFT.

Conclusions

Degradation of diclofenac molecule is enhanced as the pH increases until the value of pH = 9. At this pH diclofenac sodium, dissolved in aqueous medium, is degraded by 254 nm UV light action. The assignation of the detected signals assisted by TD-DFT calculation allows us to associate each experimental band to a specific minimum energy structure. In first stage acetate loss occurs at position 2 and characteristics bands of the molecule of N-2, 6-dichlorophenyl amine appear. At longer times the output of the chlorine at position 6 takes place and peaks corresponding to N-2-chlorophenyl amine appear.

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