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Article

Photolytic Degradation of Chloramphenicol in Different Aqueous Matrices Using Artificial and Solar Radiation: Reaction Kinetics and Initial Transformation Products

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A fotodegradação de cloranfenicol (CAP) em água ultrapura (UW), água superficial não tratada (USW), e efluente tratado de estação de tratamento de esgoto (TESTP) em escala de laboratório e planta piloto, foi avaliada usando radiação artificial e solar. Os resultados mostram, em todos os casos, que a degradação de CAP ocorre segundo uma cinética de pseudo-primeira ordem, com as constantes de velocidade de degradação aparente (k_{app}) seguindo a ordem UW ≡ USW > TESTP. A k_{app} e o tempo de meia-vida foram influenciados pela fonte de radiação. Produtos de transformação mono e di-hidroxilados foram identificados em UW após 40 min de irradiação solar, enquanto toxicidade aguda para *Artemia salina* aumentou de 35% para 100%, respectivamente após 180 e 1440 min, sob irradiação artificial e solar (94 e 132 kJ L^{-1}), quando houve 99,2% e 97,7% de degradação de CAP. Os produtos de transformação não apresentaram atividade antimicrobiana.

The photodegradation of cloramphenicol (CAP) in ultrapure water (UW), untreated surface water (USW), and treated effluent from sewage treatment plant (TESTP) in laboratory scale and pilot scale, was evaluated using solar and artificial radiation. The results show, in all cases, that the CAP degradation occurs according to pseudo-first order kinetics, with the apparent degradation rate constants (k_{app}) following the order UW ≡ USW > TESTP. The k_{app} and half-life were strongly influenced by the radiation source. Mono- and di-hydroxyl transformation products were identified in UW after 40 min of solar irradiation, while the acute toxicity to *Artemia salina* increased from 35% to 100%, respectively after 180 and 1440 min of artificial and solar irradiation (94 and 132 kJ L^{-1}), when 99.2 and 97.7% of CAP degradation occurred. The transformation products did not present antimicrobial activity.

Keywords: antibiotics, photolysis, sunlight, antimicrobial activity, toxicity

Introduction

The presence of antibiotics in the environment has attracted attention within the scientific community due the high consumption, low biodegradability, toxic effects and contribution to the development of resistant bacteria in aqueous systems.[1-3] Their presence in the environment can be attributed to several sources, such as release during production, generation of domestic and hospital waste, human and animal excretion, among others.[1,3-5] However, municipal wastewater treatment plants have been identified as the main source of entry of antibiotics in aquatic environments due the low removal efficiency to this class of compounds promoted by conventional treatments. Thus, antibiotics and their metabolites are released constantly in aquatic environments.[6,7]

Into the environment, their elimination can occur by biotic (biodegradation) or non-biotic (sorption, hydrolysis, photolysis, oxidation and reduction) processes. It should be emphasized that antibiotics are difficult to be removed by biotic processes.[6,7] On the other hand, the non-biotic elimination by sorption depends on the physicochemical properties of the target-compound. In addition, the antibiotics are produced to be resistant to hydrolysis.

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In general, among these alternatives of elimination, the photolysis can be considered the main process of elimination of these compounds in surface waters. Several studies have showed that the photodegradation efficiency depends on conditions such as temperature, pH, composition of matrix, radiation source, latitude, etc.[8-10]

Photodegradation can occur by direct absorption of sunlight radiation (direct photolysis) or by means of reaction with transient reactive transformation products such as singlet oxygen, hydroxyl radicals or other reactive species formed in natural waters (indirect photolysis).[11-16] It has noted that for some compounds, the incomplete photodegradation can generate photoproducts more stable and toxic than the target compound.[9,17] Therefore the knowledge of the photodegradation pathways, kinetics, acute toxicity, transformation products and antimicrobial activity (AA) are essential to predict the behavior and the environmental impact of these pollutants in natural waters.

In this work, the photolytic degradation of chloramphenicol (CAP) (Figure 1), an antibiotic widely used due to its low cost and high efficiency in the treatment of various infectious diseases, which can cause serious toxic effects in humans, including bone marrow depression, particularly in the form of severe aplastic anemia,[18] was evaluated.

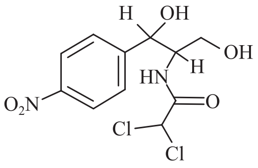


Figure 1. Chemical structure of CAP ($C_{11}H_{12}Cl_2N_2O_5$ = 323 g mol^{-1}).

CAP has been found at concentrations between 0.001 and 0.031 µg L^{-1} in surface waters in Singapore and Korea, respectively, while average concentrations of 2.08 and 26.6 µg L^{-1} were found in effluents of sewage treatment plants in China.[19-22] Considering it appears to resist biodegradation, the photolytic degradation was evaluated as a possible process for elimination of this antibiotic in aqueous media. Therefore, studies of CAP photolysis in different aqueous matrices at lab- and pilot plant scales, under artificial and solar radiation respectively, were done aiming to estimate the kinetics of these reactions, as well as to evaluate the influence of the matrix and radiation source. In addition, the initial transformation products formed in ultrapure water (UW) under natural sunlight, were identified by liquid chromatography - triple quadrupole mass spectrometry (LC-MS/MS). Furthermore, assays involving *Artemia salina* and *Escherichia coli* were applied to the samples obtained in the experiments using UW before and after photodegradation with artificial and solar radiation in order to assess the acute toxicity and AA of the photo-products.

Experimental

Reagents

The CAP standard (Sigma-Aldrich) was used as received. HPLC grade methanol was supplied by Vetec. UW was used throughout this study, in the analyses and photodegradation experiments, except in the experiments carried out with untreated surface water and treated effluent from a Sewage Treatment Plants, named as USW and TESTP, respectively.

Sampling of surface water and sewage treatment plant effluent

In order to evaluate the matrix effects on CAP photodegradation, samples of USW and TESTP effluent were collected during autumn, winter and spring 2013 (March to December, in Brazil), respectively. The sample of USW was collected directly from a river whose water, after conventional treatment, is supplied to the city of Uberlândia. The TESTP where the collection was done serves about 95% of the population of Uberlândia (18°55'08"S; 48°16'37"W, at a mean altitude of 863 m), a city with more than 650,000 inhabitants. This plant is based on up flow anaerobic reactor. The sample of TESTP was collected after a complete treatment. Once collected, the samples were kept under refrigeration, for a week at most, so that the experiments were performed.

Photodegradation procedures

In lab-scale

The experiments were performed in lab-scale using a 400 W high pressure mercury vapor lamp as irradiation source. The photocatalytic reactor consists of an annular recipient of borosilicate glass, acting as radiation filter for photons with wavelength lower than 290 nm.[23] The annular reactor has an irradiated surface of 4.0 × 10^{-2} m^2 (outside diameter of 8.6 cm, 3.6 cm internal diameter and 23 cm height) and an irradiated volume of 0.850 L. The lamp was positioned at the center of the reactor, as described by Oliveira *et al.*.[24] The photonic flux provided by the lamp, considering a range of wavelengths between 295-390 and 295-710 nm, were respectively 6.0 × 10^{-7} and 3.3 × 10^{-6} einstein s^{-1},[23] with an average irradiance at UVA equal to 1100 W m^{-2}.[25]

A total volume of 5 L of 211 ± 19 mg L^{-1} solution of CAP (dissolved organic carbon, equal to 86 ± 7 mg C L^{-1}) without initial pH correction - at pH 6.6 ± 0.5, was recirculated by pumping at a flow rate of 2.14 L min^{-1} to the reactor with the lamp turned on. A thermostatic bath (Tecnal TE-184) was used to keep the temperature close 25 ± 5 °C, since due the high irradiance of the lamp, the temperature of the aqueous solutions reaches temperatures close to 40 ± 5 °C. Although CAP concentration is far from the expected levels in the environments, it can be assumed that the degradation pathway and the relative concentrations of the resulting transformation products are the same.

Three photodegradation experiments were performed at lab-scale, using different water matrices (UW, USW and TESTP), being monitored the CAP and dissolved organic carbon (DOC) removal. For the experiment in UW, it was also monitored the acute toxicity and AA evolution of the transformation products. Aliquots (30 mL) of the solutions containing the photodegraded material were collected at 20 min intervals up to 180 min, except to the experiment with UW, where aliquots of 100 mL were collected in 0, 40, 60, 120 and 180 min.

In a solar pilot plant

The experiments using solar radiation were carried out during autumn, winter and spring, under clear sky conditions, in the city of Uberlândia, Brazil, using a pilot plant based on a compound parabolic collector (CPC) reactor. The solar irradiance was measured using a radiometer (PMA 2100 Solar Light Co.) in the UVA region (320-400 nm), with the sensor placed at the same angle (19°, local latitude) as defined for the incidence of radiation in the reactor.

The CPC reactor is composed by 10 tubes of borosilicate glass of 15 cm length and 2.92 cm internal diameter, connected in series with irradiated surface and volume of, respectively, 1.62 m^2 and 12 L, and a reservoir with maximum capacity of 120 L.[23] In this case, the temperature of the aqueous solutions was not controlled.

Before beginning the irradiation, 10 g of CAP dissolved previously at 5 L of the respective water matrix (UW, USW or TESTP) was added into the CPC recirculation tank containing 45 L of the water matrix (resulting in 50 L of CAP solution), and homogenized by turbulent recirculation (33.3 L min^{-1}) during 30 min in darkness (a first control sample was taken to characterize the solution), generating 195 ± 10 mg L^{-1} of CAP solution (DOC = 80 ± 4 mg C L^{-1}). In the sequence, the CPC reactor was uncovered and samples (30 mL) of the CAP solutions without initial pH correction (pH = 7.1 ± 0.7) were collected at intervals of 20 min up to 140 and 240 min to USW and TESTP, respectively, and at 40 min intervals up to 300 min to UW, followed by sampling at 60 min intervals up to 2100 min. For UW, aliquots of 100 mL were collected in 0, 40, 240, 480, 720, 960, 1200, 1440, 1680, 1920 and 2100 min. For these experiments, an average solar UVA irradiance of 38.3 ± 11.4 W m^{-2} was obtained during 10 am and 14 pm.

Due to the differences inherent to the architecture of the reactors used in this study, like the average irradiance, irradiated surface area and total volume of solution, as well as the average solar or artificial UVA radiation, the accumulated UVA energy was calculated for each time of sampling for each reactor, to allow a good correlation between the results. Equation 1 allows to estimate the accumulated dose of UVA radiation *per* unit of volume ($Q_{UVA,n}$ kJ L^{-1}), received on any surface, for a solution inside a reactor, in a time interval Δt.[26]

$$Q_{UVA.n} = Q_{UVA.n-1} + \Delta t_n \overline{UVA}_{G.n}\left(\frac{A_r}{V_t}\right) \qquad (1)$$

where t_n is the sampling time, V_t is the total volume of CAP solution (5 and 50 L, respectively, for experiments using artificial and solar radiation), A_r the illuminated collector surface area and $UVA_{G,n}$ the average accumulated dose solar or artificial UVA radiation, measured during the period Δt_n (= $t_n - t_{n-1}$). Considering this equation, it was possible to correlate quantitatively the results at lab- and pilot scales.

Analytical determinations

Before all the analyses, the samples were filtered in membranes with 0.45 µm of porosity.

The CAP oxidation during the photodegradation experiments was followed by HPLC-UV (Shimadzu LC-10AD chromatograph and UV-Vis SPD-10A detector) equipped with a Phenomenex® reversed phase Luna C18 column (250 mm × 4.6 mm, 5 µm) set at 276 nm. In each essay, 20 µL of the sample was eluted using a water/methanol mixture of (50:50, % v/v), at a flow rate of 1 mL min^{-1}. The retention time for CAP was 6.8 ± 0.1 min. The CAP mineralization was followed by measuring the DOC using a TOC analyser (Shimadzu TOC-VCPH/CPN) equipped with an ASI-V autosampler.

The CAP transformation products were analysed by liquid chromatography/triple quadrupole mass spectrometry (LC-MS/MS), in negative ionisation mode (due the better response in relation the positive ionisation mode), using an high performance liquid chromatography (HPLC) (Agilent Infinity 1290) equipped with a ZORBAX RRHD SB-C18 (50 × 3 mm) reverse-phase C_{18} analytical column, 1.8 µm particle size (Agilent). The mobile phases A and B were respectively water with 0.1% acetic acid, and acetonitrile, at a 0.25 mL min^{-1} flow rate. The injection volume was 50 µL. During the first minute of run time, a mixture (90:10 - % v/v) of A and B, respectively, was used as mobile phase. After, a linear gradient for the B mobile phase progressed from 10 to 80% in 6 min, and maintained at 80% B for 0.6 min. Subsequently, a 1 min post-run time back to the initial mobile-phase composition was allowed after each analysis. Under these conditions, CAP retention time was of 4.9 ± 0.1 min. This HPLC system was connected to an Agilent 6460 mass spectrometer with an electrospray interface operating under the following conditions: capillary of −3.5 kV; flow rate of the gas of 1.5 L min^{-1} and temperature at 200 °C.

Acute toxicity and antimicrobial activity tests

The acute toxicity tests were done evaluating the immobilization of *Artemia salina*, as described by Trovó *et al.*,[27] and the AA using colonies of *Escherichia coli*, as described by Trovó *et al.*.[28]

Results and Discussion

Characterization of USW and TESTP

Relevant parameters of the USW and TESTP are shown in Table 1. Comparing these two aqueous matrices samples, mainly in relation to the parameters that can influence the CAP photodegradation (dissolved organic carbon, anions, turbity, total dissolved solids and solids in suspension), it can be observed that the TESTP presents values significantly higher that can probably contribute to reduce the CAP photodegradation efficiency.

Table 1. Main parameters determined in samples of USW and TESTP

Parameter	USW	TESTP
pH^a	6.8	6.4
Turbidity / (nephelometric units)^a	3.9	159.0
Total dissolved solids / (mg L^{-1})^a	20.0	600.0
Solids in suspension / (mg L^{-1})^a	n.d.	144.0
Dissolved oxygen / (mg L^{-1})^a	7.0	0.2
Chemical oxygen demand / (mg L^{-1})^a	3.3	306.0
Biochemical oxygen demand / (mg L^{-1})^a	2.0	127.0
Dissolved organic carbon / (mg L^{-1})^b	12.6	54.6
Nitrate / (mg L^{-1})^a	0.1	1.2
Total N-NH_4^+ / (mg L^{-1})^a	0.03	52.3
Dissolved iron / (mg L^{-1})^a	0.3	25.7
Sulphate / (mg L^{-1})^a	2.0	21.0
Total chloride / (mg L^{-1})^a	2.0	222.7
Total phosphorus / (mg L^{-1})^a	0.05	1.7
Fluoride / (mg L^{-1})^a	0.1	0.9

^{a}Data furnished by the Department of Water and Sewage in Uberlândia (DMAE); ^{b}measured in our laboratory. n.d. = not determined.

Reaction kinetics

CAP solutions, prepared in the above mentioned aqueous media, were irradiated without control of pH during the photodegradation experiments, since no changes in maximum CAP absorption occurred for the different values of pH evaluated (2.5, 6.4 and 9.0) (Figure 2).

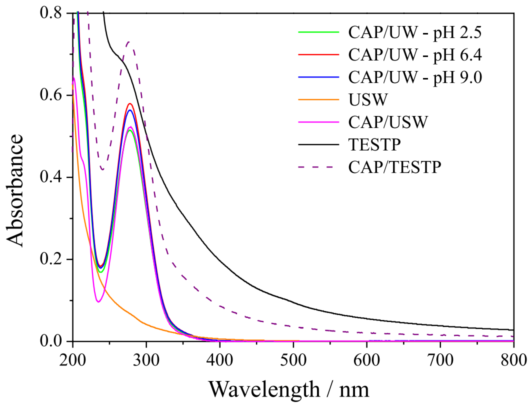


Figure 2. Absorption spectra of 20 mg L^{-1} CAP solutions in different aqueous media and of the isolated USW and TESTP matrices.

A fast decay of CAP concentration in UW and USW was obtained in relation the TESTP matrix for both radiation sources. In addition no difference on CAP degradation between the matrices of UW and USW was observed (Figure 3).

Observed kinetic data for artificial and solar photolysis of CAP in different aqueous matrices are shown in Figure 3, whereas the relative rates of CAP transformation in each of the three matrices and the respective radiation sources are shown in Table 2. For all the experiments using artificial or solar radiation, CAP photodegradation can described by a pseudo-first order rate law, since a plot of natural logarithm of CAP concentration *versus* irradiation time results in a straight line whose slope is the apparent rate constant (k_{app}), and the regression analysis usually results in correlation coefficients (R^2) higher than 0.98, which demonstrates the validity of the assumed rate law (Figure 3 and Table 2).

Measured k_{app} and $t_{1/2}$ follow the trend of UW ≡ USW > TESTP matrix for both radiation sources. These results are in accordance with previous study, which also reported that natural organic matter (NOM) from secondary effluent of sewage treatment plant inhibited the photochemical degradation of oxytetracycline and doxycycline.[10]

Comparing the results obtained using a same radiation font, but different aqueous matrices, it can be observed that

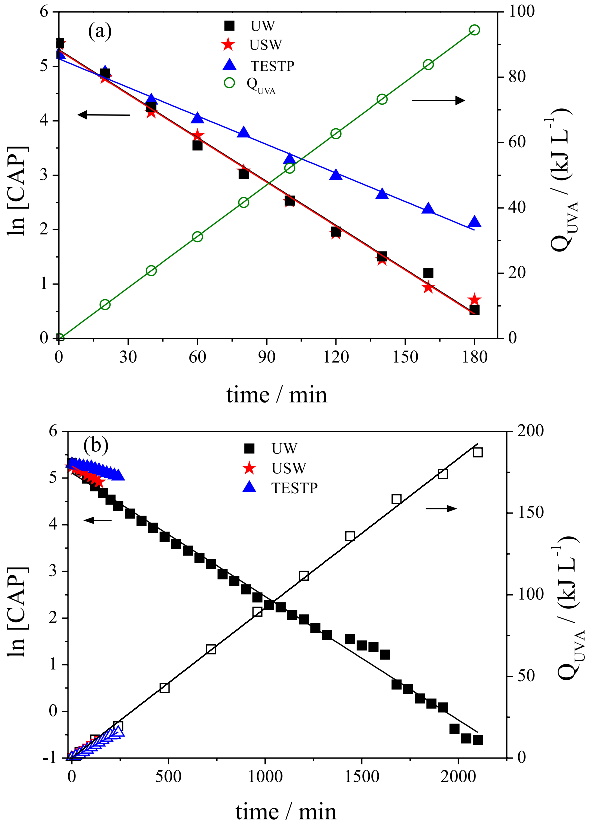


Figure 3. Kinetics for CAP photodegradation in different aqueous matrices using (a) artificial and (b) solar radiation. Initial conditions: [CAP] = 203 ± 17 mg L^{-1}; pH = 6.8 ± 0.6.

Table 2. Kinetic parameters (apparent kinetic constants (k_{app})), half-life ($t_{1/2}$) and correlation coefficients (R^2) estimated for CAP photodegradation in different aqueous matrices under artificial and solar radiation and the accumulated UVA dose *per* unit of volume (Q_{UVA}) needed to reach CAP half-life in each experiment

Exp.	k_{app} / (min^{-1})	$t_{1/2}$ / min	R^2	Q_{UVA} / (kJ L^{-1})
^{a}UW	0.0269	26	0.99	13
^{b}UW	0.0027	257	0.99	24
^{a}USW	0.0269	26	0.99	13
^{b}USW	0.0023	301	0.98	22
^{a}TESTP	0.0175	40	0.99	21
^{b}TESTP	0.0011	630	0.98	42

^{a} Artificial radiation; ^{b}solar radiation.

the same value of k_{app} and $t_{1/2}$ was obtained after obtaining the same Q_{UVA} for matrices of DW and USW, for both radiation fonts.

However, an amount Q_{UVA} twice higher was necessary to obtain the same values when using TESTP matrix for both radiation sources (Table 2). The lower k_{app} of CAP in TESTP matrix is probably due the NOM present in the TESTP matrix, that show strong absorbance in the same wavelength that CAP, acting as a filter (Figure 2), thus reducing the photodegradation efficiency (Figure 3). This was not observed with the NOM present in the USW, being obtained the same k_{app} to that verified in UW (Table 2). The small difference when using UW or USW and sunlight is within of the experimental error (± 10%). Therefore, the higher difference of the Q_{UVA} to reach the same $t_{1/2}$, obtained in the experiments carried out with the TESTP matrix, may be associated with their composition, the spectral distribution (between 295 and 800 nm) of the radiation emitted by a 400 W high pressure vapor mercury lamp, and the solar spectral distribution in the same spectral range.[23] Probably, in the experiments using artificial radiation, there is a lower competition by radiation between CAP and the NOM present in the TESTP matrix, being possible to reach the same $t_{1/2}$ using a lower Q_{UVA}. On the other hand, a higher competition should occurr using sunlight, and consequently a higher Q_{UVA} is necessary to reach the same $t_{1/2}$ obtained with artificial irradiation (Table 2).

In addition to the matrix effects, the k_{app} and $t_{1/2}$ of CAP were strongly influenced by the radiation sources (Table 2). Comparing the results obtained for the same matrices, but using artificial or solar radiation, it can be observed that k_{app} increases while $t_{1/2}$ decreases in magnitude of 11.8 ± 2.9 times when artificial radiation was used, in comparison with the use of solar radiation (Table 2).

The higher k_{app} and lower $t_{1/2}$ of CAP using artificial radiation in comparison with the solar experiments is due the high irradiance furnished by the higher pressure mercury lamp (1100 W m^{-2}) against 38.3 ± 11.4 W m^{-2} to the solar experiments. Besides, comparing the results obtained for the same matrices but with different radiation sources, it can be observed that a higher Q_{UVA} to the solar experiments (almost twice), was necessary to reach the same $t_{1/2}$ than the experiments carried out with artificial radiation (Table 2). This can be due the higher absorption of CAP inside of the spectral distribution of the radiation emitted by a 400 W high pressure vapor mercury lamp in the 295 to 800 nm spectral range, in relation the solar spectral distribution to these same spectral range.[23]

As shown in Figure 3, 99.2 and 99.7% of CAP transformation was obtained respectively in UW after 180 (Q_{UVA} = 94 kJ L^{-1}) and 2100 min (Q_{UVA} = 193 kJ L^{-1}), using artificial and solar radiation. On the other hand, only 24% of COD removal (Figure 3) were obtained in both experiments, demonstrating the formation of transformation products more persistent to photodegradation than CAP and with considerable acid character, since the average initial pH decreases from 6.9 ± 0.1 to 3.6 ± 0.2 (Figure 4). Control experiments after 49 days showed that no hydrolysis occurred at pH values of 2.5 and 6.4 (data not shown).

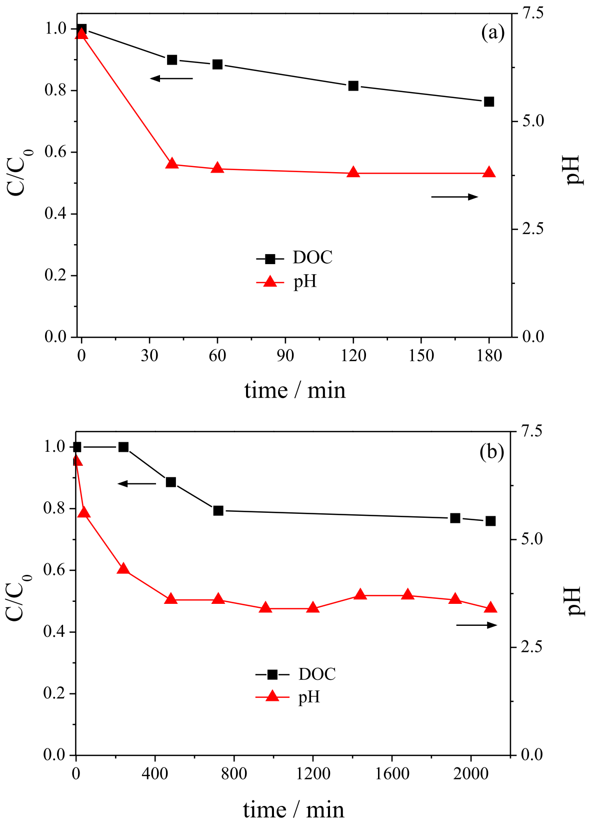


Figure 4. Evolution of DOC decay and pH during the photodegradation of CAP in UW using (a) artificial and (b) solar radiation. Initial conditions: [CAP] = 203 ± 17 mg L^{-1} (DOC = 83 ± 7 mg C L^{-1}); pH = 6.4 ± 0.4.

Photoproducts analysis

Before identification of the transformation products, a previous study was done using aliquots of the CAP standard solution (10 mg L^{-1}) aiming to evaluate the ionization mode (negative or positive), obtaining the negative mode as better ionization mode (results not shown). Using the negative ionization mode, different fragmentation intensities were evaluated: 5, 10, 15 and 20 V (Figure 5). Using 15 and 20 V, it was not possible to observe the peak of mass/charge (*m/z*) equal to 321, for the deprotonated CAP. For these voltages were obtained only the ions with *m/z* 78, 121, 152, 176 and 257 (Figures 5c and 5d).

Reducing the fragmentation intensity for 10 and 5 V (respectively, Figures 5a and 5b), the ions with *m/z* 121 and 78 disappeared, appearing the ion with *m/z* 194 and ions formed from deprotonated CAP. Since the transformation products are proposed considering the *m/z* ratio of the deprotonated molecule, as well as the respective fragments ions, it is important to use a fragmentation voltage that provides a mass spectrum containing a large amount of ions and, if possible, with high abundance. Within this context, and based on the spectra obtained (Figure 5), the fragmentation voltage chosen and used in the LC/MS/MS analysed was equal to 5 V.

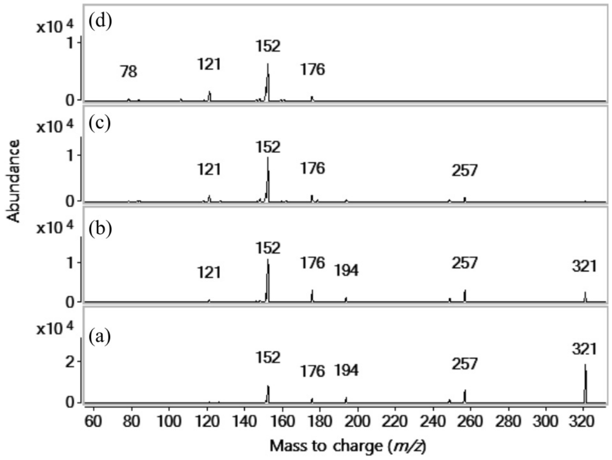


Figure 5. Mass spectrum of CAP standard solutions (10 mg L^{-1}) using different intensity of fragmentation: (a) 5, (b) 10, (c) 15 and (d) 20 V in negative mode.

The total ion chromatogram obtained after 40 min of CAP photolysis in UW show four new peaks (C1, C2, C3 and C4) (Figure 6b), not present in the initial sample (Figure 6a). The mass spectra of the transformation products were then obtained, and the structures proposed based on the deprotonated molecules [M–H]$^-$ and the main fragments observed in the mass spectra of each intermediate (Table 3).

The mass spectrum of CAP, presents ions with *m/z* 321/323 [M–H]$^-$, characteristic of the isotopic distribution of CAP, besides more six fragments with *m/z* 121, 152, 166, 176, 194 and 257/259 (Table 3). These results agree with CAP fragmentation elucidation reported by Berendsen *et al.*.[29]

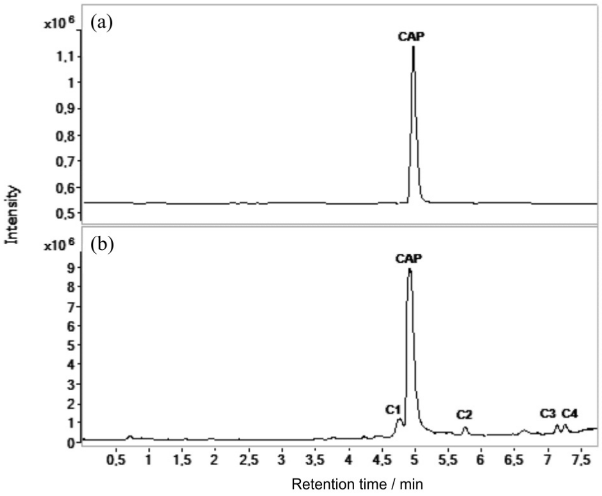
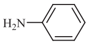
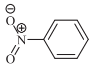
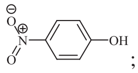
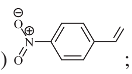
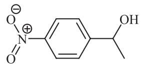
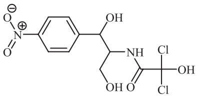
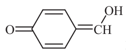
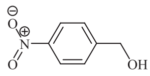
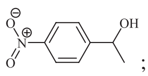
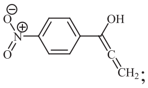
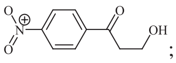
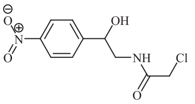
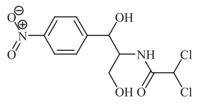
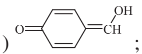
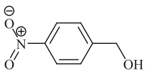
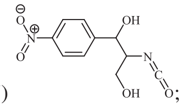
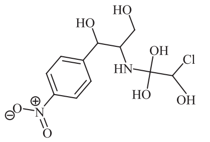
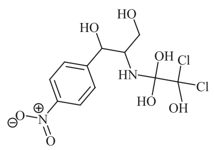
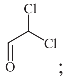
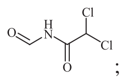
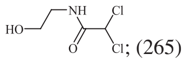
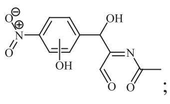
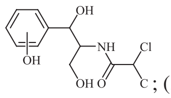
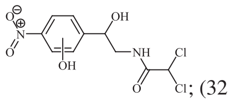
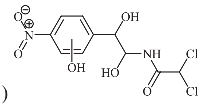
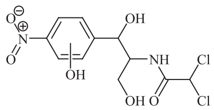
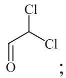
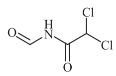
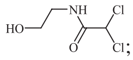
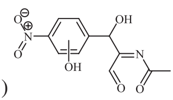
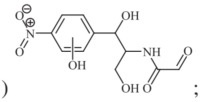
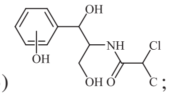
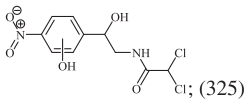
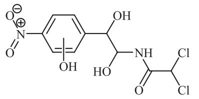
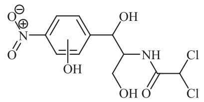


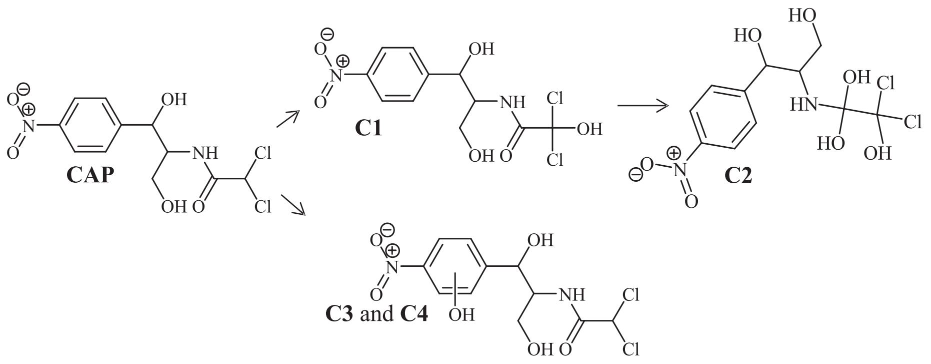
Figure 6. LC-MS total ion chromatogram to (a) CAP standard (10 mg L^{-1}) and (b) sample of CAP (200 mg L^{-1}) photodegradation after 40 min of solar irradiation at pH 6.8.

Table 3. Structures and mass spectral data for CAP and postulated transformation products, as determined from LC-MS/MS, obtained after 40 min of solar irradiation

Comp.	Retention time / min	Molecular mass	Main fragments (*m/z*) and possible structures of the respective compounds	Suggestion of possible transformation products structures
1	4.75	338/340	(92) ; (122) ; (138) ; (150) ; (166) 	
CAP	4.91	322/324	(121) ; (152) ; (166) ; (176) ; (194) ; (257/259) 	
2	5.74	356/358	(121) ; (152) ; (237) ; (321/323) 	
3	7.12	338/340	(113) ; (156) ; (172) ; (265) ; (293) ; (311) ; (325) 	
4	7.24	338/340	(113) ; (156) ; (172) ; (265) ; (283) ; (293) ; (311) ; (325) 	

The transformation products obtained were result of the formation of mono- and di-hydroxyl derivates of CAP (Table 3), typical reactions (hydroxylation) of the photolytic processes.[9]

Starting with CAP, there are two possible pathways for the initial photolytic CAP degradation (Scheme 1). The first one is the hydroxylation of the terminal carbon that contains two chloride atoms, yielding the compound 1, ion with *m/z* 337/339 $[M-H]^-$, which corresponds to the addition of 16 units of mass in relation to CAP. The presence of the fragment with *m/z* 166, the same fragment present in CAP fragmentation, indicates that the attack of the hydroxyl radical occurs on the terminal carbon containing two chloride atoms. The hydroxyl radicals are produced through the direct oxidation of water or the activation of dissolved oxygen, as presented in previous studies in which the photodegradation of the antibiotic ciprofloxacin was evaluated.[30,31] Besides, the other fragments reinforce the proposed structure (Table 3). The compound 2 presents a difference of 18 and 34 units of mass with respect to



Scheme 1. Possible initial pathway for CAP photodegradation in UW after 40 min of solar irradiation. Initial conditions: [CAP] = 205 mg L^{-1}; pH = 6.8.

compound 1 and CAP, respectively, as well as the same fragments of CAP, suggesting that the attack of the hydroxyl radical occurs on the carbonyl group, since the cleavage of the double bond of the carbonyl group also contributes to the addition of two chemical bonds (Table 3).

A second degradation route should be initiated by hydroxylation on the benzene ring, yielding isomers (C3 and C4), which differ by the hydroxyl radical position on the aromatic ring, since similar mass spectra were obtained (Table 3). Similar behavior was observed during the photolytic degradation of the antibiotic sulfamethoxazole.[9]

Acute toxicity and antimicrobial activity of CAP photoproducts

As shown in Figure 7, the CAP solutions (216 ± 11 mg L^{-1}) showed an average acute toxicity to *A. salina* (35 ± 5%). However, during the photolytic degradation of CAP using artificial radiation, the acute toxicity of the solution during the photodegradation grew quickly and consistently, reaching 100% after 180 min (Q_{UVA} = 94 kJ L^{-1}) (Figure 7a), when 99.2% of CAP transformation occurred (Figure 3a), while a similar result was obtained in the experiments using solar radiation after 1440 min (Q_{UVA} = 132 kJ L^{-1}) (Figure 7b), when 97.7% of CAP degradation was reached (Figure 3b). The same profile of acute toxicity with the time reaction and Q_{UVA} reinforces the results of HPLC-UV and DOC. The increase of acute toxicity with the irradiation time is associated with formation of photo-products more toxic than the target-compound. On the other hand, no AA was observed after 180 and 1440 min, (Q_{UVA} = 94 and 132 kJ L^{-1}), respectively (Figure 7). These results, therefore, suggest that artificial and solar direct photolyis promote CAP degradation (Figure 3), but did not lead to its complete mineralization (Figure 4), generating transformation products without AA, but with higher acute toxicity than CAP (Figure 7), which can affect the aquatic systems. Similar behavior was observed to *Daphnia magna*, during the photodegradation of the antibiotic sulfamethoxazole.[9]

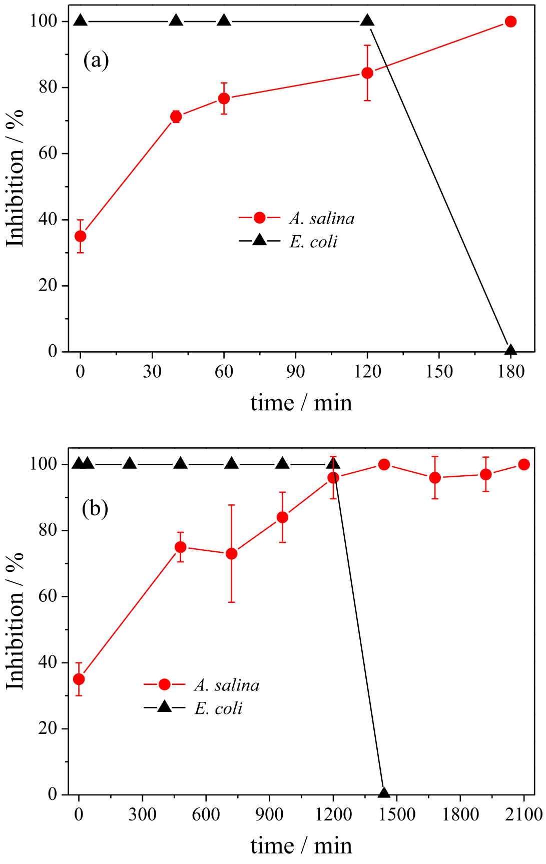


Figure 7. Evolution of the acute toxicity and AA during CAP photodegradation in UW using (a) artificial and (b) solar radiation. Initial conditions: [CAP] = 216 ± 11 mg L^{-1}; pH = 6.4 ± 0.4.

Conclusions

The results obtained in the present study showed no difference on CAP photodegradation in UW and USW for both radiation sources. On the other hand, the composition of TESTP strongly influenced the efficiency of CAP photodegradation, when compared with the UW and USW matrices, probably due the natural organic matter present in the TESTP that presents strong absorbance in the same wavelength of CAP, acting as a filter. In addition to the effect of matrices, CAP transformation was strongly influenced by the radiation source. A higher dose of UVA *per* unit of volume in the solar experiments was necessary to reach the same results obtained using artificial radiation due the higher absorption of photons by CAP inside the spectral distribution of the radiation furnished by a 400 W high pressure mercury lamp, between 295 and 800 nm, when compared to the solar spectral distribution in this same spectral range. For both radiation sources and matrices studied, the rates of CAP degradation were better fitted considering a pseudo-first order rate law, as well as, the measured rate constants follow the trend UW ≡ USW > TESTP.

Mono- and di-hydroxylated derivatives of CAP were obtained as initial transformation products after 40 min of solar irradiation. Although no antimicrobial activity was obtained with CAP photodegradation, an increase to 100% in the acute toxicity to *A. salina* occurred, showing that the application of a combined treatment is necessary to guarantee the total mineralization of CAP and transformation products, preventing deleterious effects in aquatic systems.

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