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## UVC-induced degradation of cilastatin in natural water and treated wastewater

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# UVC-induced direct photolysis as an efficient process for the degradation of cilastatin in natural and waste water

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## Abstract

This work reports for the first time the UVC photodegradation of cilastatin, a renal dehydropeptidase inhibitor co-administered with the imipenem antibiotic. Initially, solutions of cilastatin at varying concentrations were prepared in ultra-pure water and the direct photolysis of cilastatin was monitored under 254-nm irradiation. Degradation was slower at higher initial cilastatin concentrations, due to absorption saturation. Of the different eluting photoproducts, only one was tentatively identified as oxidized cilastatin bearing a sulfoxide group. UV-254 photolysis occurred faster at lower pH values, because the protonated forms of the molecule ( $H_3A^+$ ,  $H_2A$ ) have both higher absorption coefficients and higher photolysis quantum yields than the non-protonated ones ( $HA^-$ ,  $A^{2-}$ ). The direct photolysis of cilastatin does not involve  $\bullet OH$ , as excluded by experiments in which t-butanol was added as  $\bullet OH$

26 scavenger, whereas the presence of humic acids inhibited photolysis due to competition for  
27 radiation absorption. The same explanation partially accounts for the observation that the  
28 photolysis kinetics of cilastatin was slower in tap water, river water and treated wastewater  
29 samples compared to ultra-pure water. Moreover, the direct photolysis quantum yield was  
30 also lower in water matrices compared to ultra-pure water. Similar findings reported for  
31 triclosan and the herbicide 2-methyl-4-chlorophenoxyacetic acid in previous studies might  
32 suggest that the water matrix components could carry out either physical quenching of  
33 cilastatin's excited states or back-reduction to cilastatin of the partially oxidized degradation  
34 intermediates. Overall, the present results demonstrate that UVC irradiation is a fast and  
35 efficient process for the degradation of cilastatin in natural water and wastewater.

36

37 **Keywords:** cilastatin; carbapenems; antibiotics; direct photolysis; waste water tertiary  
38 treatments; UVC photolysis.

39

## 40 **1. Introduction**

41 Antibiotics are frequently used in the treatment and prevention of infectious disease, as well  
42 as to treat humans and animals. Imipenem was the first member of the carbapenem class of  
43 antibiotics and an important broad-spectrum  $\beta$ -lactam antibiotic, often used as a “last-line  
44 antibiotic” when patients with infections became gravely ill or were suspected of harboring  
45 resistant bacteria (Proia et al., 2018; Reina et al., 2018; Briones et al., 2020). At early stages  
46 of development when imipenem was administered alone, low urinary recovery in animals and  
47 human volunteers were recorded, and further toxicological studies reported proximal tubular  
48 necrosis to rabbits (Drusano et al., 1984). The concept of co-administering imipenem together  
49 with a renal dehydropeptidase inhibitor to improve the urinary antibiotic profile resulted in  
50 the synthesis and use of cilastatin. The combination of imipenem and cilastatin in a ratio of  
51 1:1 yielded high urinary concentrations of imipenem (recoveries increased from 12-42% to  
52 70% of the dose when co-administered with cilastatin), and in addition, cilastatin prevented  
53 entry of imipenem into the proximal tubular epithelium (Hutt and O’Grady, 1996). At the  
54 same time, the urinary recoveries of cilastatin in the presence or absence of imipenem were  
55 reported to range between 70-80% after 6 h from administration (Norrby et al., 1984; Hsieh et  
56 al., 1985).

57 In recent years, the consumption of last-line antibiotics has been rapidly increasing across all  
58 income groups (Yılmaz and Özcengiz, 2017; Klein et al., 2018). In particular, the increased  
59 consumption of carbapenems such as imipenem, has resulted in their environmental release  
60 through various routes such as patients’ excretions, hospital wastewater and the  
61 pharmaceutical industry (Cheng et al., 2015; Szekeres et al., 2017) and in their widespread  
62 occurrence in aquatic systems (Tran et al., 2016; Szekeres et al., 2017; Proia et al., 2018). The  
63 latter findings were assumed to account for the developed resistance of some Gram-negative  
64 bacteria to carbapenems (Kumarasamy et al., 2010), which is now spreading throughout the

65 world and is threatening the efficiency of this essential class of life-saving antibiotics (Papp-  
66 Wallace et al., 2011; Szekeres et al., 2017; Reina et al., 2018).

67 The increasing consumption of imipenem inevitably entails environmental release of  
68 cilastatin. Although, the photochemical fate (Reina et al., 2018) and treatment (Cabrera-Reina  
69 et al., 2019) of imipenem have been the focus of past studies, very little is known on the  
70 photochemical fate of cilastatin in water, and its treatment was only recently proposed as part  
71 of an integrated advanced oxidation/reduction process for the degradation of  
72 imipenem/cilastatin antibiotic aqueous solutions (Godini et al., 2019). However, detailed data  
73 and discussion were not provided on the behavior/performance of cilastatin undergoing this  
74 process.

75 This contribution aims to explore for the first time the photolytic fate of cilastatin in water  
76 under 254-nm irradiation. Initially, ultra-pure water was used as matrix to study the effects of  
77 concentration, pH, presence of t-butanol and humic acids on the cilastatin  
78 (photo)transformation rate. Building on this knowledge, the photolysis of cilastatin spiked to  
79 tap water, river water and wastewater effluent was monitored as a function of irradiation time.  
80 At all times, modeling results are presented to confirm suggested hypotheses and discussion  
81 on experimental data.

82

## 83 **2. Material and Methods**

### 84 ***2.1. Chemicals and samples***

85 Cilastatin sodium salt (purity grade  $\geq 99.8\%$ ) was purchased from Sigma-Aldrich (Steinheim,  
86 Germany). All organic solvents used were LC/MS grade. t-Butanol ( $\geq 99\%$ ) was supplied by  
87 Fisher Chemicals (Pittsburgh, Pennsylvania). Analytical grade formic acid and humic acid  
88 were supplied by Fluka Chemie GmbH (Bucks, Switzerland). Aqueous solutions of sodium

89 hydroxide (Fluka Chemie GmbH) or buffer solutions were used to adjust the pH value of the  
90 irradiated solutions. The following chemicals were used to prepare the buffer solutions:  
91 glacial acetic acid and boric acid (99.8%) supplied by Merck, KGaA (Darmstadt, Germany),  
92 potassium phosphate dibasic trihydrate ( $\geq 99.0\%$ ) from Sigma-Aldrich (Steinheim, Germany),  
93 sodium acetate trihydrate ( $\geq 99.5\%$ ) and potassium dihydrogen phosphate ( $\geq 99.5\%$ ), both  
94 supplied by Fluka Chemie GmbH (Germany).

95 An EASYpure RF water purification system supplied by Barnstead/Thermolyne Corporation  
96 (Dubuque, IA, USA) was used to prepare ultra-pure water. The matrix effect on cilastatin  
97 photolysis was studied using: (i) freshwater sampled from the river Koiliaris at Kyani Akti  
98 (Kalyves, Crete, Greece); (ii) tap water from the University campus (Chania, Crete, Greece),  
99 and (iii) secondary treated wastewater effluent (WW) from the municipal wastewater  
100 treatment plant of Chania (Crete, Greece), serving approximately 70,000 inhabitants. See  
101 **Table A.1** in the Appendix for the chemical composition of the three water types used in this  
102 work. Samples were collected the day before conducting the photo-experiments, and were  
103 stored in the dark at 4 °C. All samples were initially analyzed and found free of cilastatin.

104

## 105 **2.2. Photolysis experiments**

106 For all photolysis experiments, a home-made laboratory photoreactor (28.0 cm height  $\times$  28.4  
107 cm length  $\times$  28.0 cm width) was used. The photoreactor was equipped with two 8 W low-  
108 pressure mercury lamps having a strong emission line at 254 nm (Osram 8W G8 T5), each  
109 mounted on an opposing sidewall of the photoreactor. The distance between each lamp and  
110 the quartz vial used in the experiments was 13 cm. The rate of the incident UV light intensity  
111 entering the solution ( $I_0$ , given in  $\text{E L}^{-1} \text{s}^{-1}$ , where E = Einstein) was determined using  $\text{H}_2\text{O}_2$   
112 as chemical actinometer, and was  $I_0 = (6.24 \pm 0.19) \times 10^{-6} \text{ E L}^{-1} \text{ s}^{-1}$ .

113 Aqueous solutions of cilastatin (CIL) were prepared daily by transferring the appropriate  
114 volume from a 7 g L<sup>-1</sup> methanolic stock solution to a tailor-made quartz vial (2.4 cm outer  
115 diameter × 5.2 cm height). A gentle nitrogen stream was then applied to the headspace and,  
116 after complete evaporation of methanol, 9 mL of water were added (ultra-pure H<sub>2</sub>O, H<sub>2</sub>O with  
117 additives, tap water, natural water or WW effluent, depending on the experiment). The  
118 mixture was then sonicated for a short time to facilitate CIL solubilization. The quartz vial  
119 containing this aqueous solution was then capped and submitted to UV irradiation for the  
120 preset time under mild magnetic agitation. The inner diameter of the quartz vial (2.0 cm) was  
121 used as the optical path length of radiation. Blank experiments were also conducted by  
122 placing the water samples inside the photoreactor with the lamps switched off. CIL removal  
123 in the dark was below the error of the analytical method (varied between 0.1 and 2.6%) after  
124 60 min, confirming that changes in the analytical signal after photolytic treatment were due to  
125 photochemical processes only. All experiments were run at least in duplicates.

126

### 127 ***2.3 Analytical methods***

128 The absorbance of aqueous samples at 254 nm was measured using a single-beam UV-visible  
129 spectrophotometer (UVmini-240, Shimadzu, Tokyo Japan), equipped with quartz cuvettes  
130 having a 1 cm optical path length. The total organic carbon (TOC) content of water solutions  
131 was measured using a TOC-5000 analyzer (Shimadzu, Kyoto, Japan; catalytic oxidation on Pt  
132 at 680°C). The calibration was performed using standards of potassium phthalate.

133 All liquid chromatography/mass spectrometry (LC/MS) analyses on CIL were carried out  
134 using an Agilent 1200 Series high-performance liquid chromatography (HPLC) system  
135 equipped with a binary pump, autosampler, degasser and thermostated column compartment,  
136 coupled to a diode array detector (DAD; the CIL analytical wavelength was 200 nm), and to  
137 an Agilent 6110 single quadrupole MS system equipped with a multimode ionization source.

138 A Thermo-Electron Betasil C18 column (Waltham, MA, USA) of dimensions 2.1 mm ID ×  
139 100 mm length with 5 μm particle size was used for separation. The mobile phase consisted  
140 of 85% of water containing 0.1% formic acid and of 15% of acetonitrile containing 0.1%  
141 formic acid. The flow rate of the mobile phase was 250 μL min<sup>-1</sup>, and the total analysis time  
142 was 10 min. For analysis, 60 μL of the sample were added to 100 μL polypropylene inserts  
143 and placed in 2 mL polypropylene autosampler vials equipped with caps, all purchased from  
144 Agilent (Palo Alto, USA). The injection volume was 20 μL. The MS conditions were: drying  
145 gas flow, 5 L min<sup>-1</sup>; drying gas temperature, 350 °C; nebulizer pressure, 40 psi; collector  
146 capillary voltage, 2.0 kV; fragmentor voltage, 70 V; scan range (m/z), 100-1000 amu. The  
147 mass spectrometry data were recorded using the positive electrospray ionization (ESI) mode.  
148 The linearity of the method was tested using five concentration levels ranging from 1 to 100  
149 mg L<sup>-1</sup> (correlation coefficient  $r^2=0.9982$ ). A 1.3 mg L<sup>-1</sup> limit of quantification (LOQ) was  
150 estimated. This concentration level was not sufficiently to monitor the photolysis of 2.5 mg  
151 L<sup>-1</sup> CIL (*i.e.*,  $7 \times 10^{-6}$  M). To increase sensitivity in these experiments, 100 μL of the  
152 irradiated sample were added to 250 μL vial inserts instead, the injection volume was set at 60  
153 μL, and the 350-365 amu mass spectrometry signal was used to monitor changes in CIL  
154 concentration. The linearity of this method was assessed using a five-point calibration curve  
155 in the concentration range 0.5-5 mg L<sup>-1</sup> and the estimated LOQ was 0.36 mg L<sup>-1</sup>, which was  
156 sufficient for monitoring close to 85% CIL conversion. The linearity of the method was tested  
157 in each water matrix studied here and it was concluded that the matrix did not affect the  
158 analytical performance of the method.

159

160

### 161 3. Results and Discussion



### 162 3.1. Photodegradation kinetics

163 CIL undergoes several acid-base equilibria, which are described by the acid dissociation  
164 constants  $pK_{a1} = 2.0$ ,  $pK_{a2} = 4.4$  and  $pK_{a3} = 9.2$  (Drugbank, 2020). These data allow for the  
165 distribution of the different species to be derived as a function of pH (Nourmoradi et al.,  
166 2019). In particular, the species' fractions  $\alpha$  can be obtained as follows from the  
167 deprotonation equilibria:

168

$$169 \quad \alpha_{H_3A^+} = \frac{[H^+]^3}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (1)$$

$$170 \quad \alpha_{H_2A} = \frac{K_{a1}[H^+]^2}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (2)$$

$$171 \quad \alpha_{HA^-} = \frac{K_{a1}K_{a2}[H^+]}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (3)$$

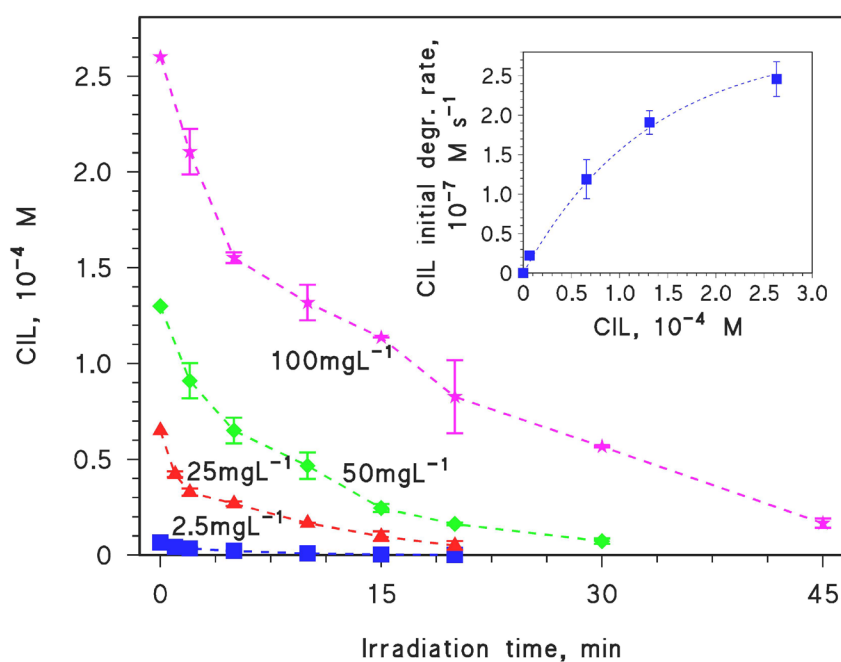
$$172 \quad \alpha_{A^{2-}} = \frac{K_{a1}K_{a2}K_{a3}}{[H^+]^3 + K_{a1}[H^+]^2 + K_{a1}K_{a2}[H^+] + K_{a1}K_{a2}K_{a3}} \quad (4)$$

173

174 Note that  $H_3A^+$  is the form of CIL with the amino and the two carboxylic groups all  
175 protonated, and the progressive deprotonation of these groups yields  $H_2A$ ,  $HA^-$  and  $A^{2-}$ . The  
176 distribution of the different CIL forms as a function of pH is reported in **Fig. A.1** in the  
177 Appendix. It can be observed that  $HA^-$  strongly prevails at pH 6-8, *i.e.*, in the conditions  
178 around neutrality that are most often found in water treatment.

179 A first series of degradation experiments was carried out at pH 7, for concentration values of  
180 CIL ranging from 2.5 to 100 mg L<sup>-1</sup> (note that 100 mg L<sup>-1</sup> correspond to  $2.6 \times 10^{-4}$  M). The  
181 2.5 mg L<sup>-1</sup> concentration represented the lowest concentration level where conversion of up  
182 to 90% of the irradiated samples could be successfully monitored and quantified by the  
183 analytical instrumentation. The time evolution of CIL (**Fig. 1**) shows that degradation was

184 more effective as the initial concentration was lower. Data fitting of the concentration profiles  
 185 from **Fig. 1** to  $C_t = C_o e^{-kt}$  gave the  $k$  values used to compute the initial degradation rates  $R_0$   
 186 (*vide infra*) shown in the inset graph, which increased with increasing  $C_o$ , from  $2.2 \times 10^{-8} \text{ M}$   
 187  $\text{s}^{-1}$  at  $2.5 \text{ mg L}^{-1}$  to  $2.5 \times 10^{-7} \text{ M s}^{-1}$  at  $100 \text{ mg L}^{-1}$ .



188  
 189 **Fig. 1.** Time trends of CIL concentration upon 254-nm irradiation at different initial  
 190 concentration values, under ~neutral conditions (pH ~ 7). Data points are linked with dashed  
 191 lines to visualize trends. **Inset:** initial degradation rates ( $R_0 = k C_o$ ) for the different  
 192 concentrations examined here ( $C_o$ ). The dashed connecting curve represents data fit with **Eq.**  
 193 **(5)**. Some error bars are too small to be visible.

194  
 195 In a pseudo-first order approximation the initial degradation rate  $R_0$  can be obtained as  $R_0 = k$   
 196  $C_o$ , and the  $R_0$  vs.  $C_o$  trend is reported in the inset of **Fig. 1**. The observed increase with  
 197 plateau may suggest that the substrate absorbs radiation and gets photolyzed as a  
 198 consequence, up to saturation of absorption (Kourouniotti et al., 2019; Alberti et al., 2021).

199 The monochromatic irradiation set-up allows for a simple Lambert-Beer approach to be  
200 applied, where the initial degradation rate can be expressed as follows (Braslavsky, 2007):

201

$$202 \quad R_o = \gamma \left[ 1 - 10^{-\varepsilon_{CIL} b C_o} \right] \quad (5)$$

203

204 where  $\gamma$  is a constant proportionality factor,  $\varepsilon_{CIL}$  is the molar absorption coefficient of CIL at  
205 254 nm,  $b = 2$  cm the optical path length in solution, and  $C_o$  the initial CIL concentration. The  
206 experimental values of  $k$  and  $R_o$  in the different experiments conducted in this work are listed  
207 in **Table A.2** in the Appendix.

208

209 Independent spectrophotometric measurements gave  $\varepsilon_{CIL}(254 \text{ nm}) = 1660 \text{ L mol}^{-1} \text{ cm}^{-1}$  at pH  
210 7 (molar absorption of the  $\text{HA}^-$  species, see **Fig. A.1** in the Appendix). Therefore, by  
211 introducing in **Eq. (5)** all the known parameters it is possible to fit the experimental data of  
212 **Fig. 1** (insert) with  $\gamma$  as the only free-floating variable. The good fit results show that the  
213 phenomenon of absorption saturation indeed accounts for the plateau trend of  $R_o$  vs.  $C_o$ .

214 In an attempt to identify the transformation products formed, the photolysis of  $100 \text{ mg L}^{-1}$   
215 CIL solutions was monitored for 60 min, that is 15 min longer than the time required to  
216 remove CIL itself. During LC-MS analysis, the elution of several photoproducts was  
217 recorded. However, considering the parent compound, the use of a single quadrupole mass  
218 spectrometer could not provide a large amount of structural information, and specificity was  
219 limited compared to other more sophisticated types of mass spectrometers such as tandem  
220 quadrupole mass spectrometers (Kourounioti et al., 2019; Petrovic et al., 2007). Moreover,  
221 co-elution of products formed further obstructed their identification. The only photoproduct  
222 that could be tentatively identified was P375 at  $[\text{M-H}]^+ = 375 \text{ m/z}$ , where the sulfide moiety of  
223 CIL was oxidized to a sulfoxide group. In the Appendix, **Fig. A.2** depicts the tentative

224 mechanism and **Table A.3** gives the summary of analytical characteristics. P375 appeared after  
225 exposing CIL to 254-nm radiation for 10 min and reached its maximum at 45 min. The  
226 amount of organics remaining in the system after complete conversion of CIL was also  
227 evaluated, by measuring the TOC of 15 mg L<sup>-1</sup> CIL water solutions before irradiation and for  
228 up to 240 min of UV<sub>254</sub> exposure, after which time only ~10% TOC removal was recorded.  
229 This observation pointed towards the formation and accumulation of degradation products  
230 during irradiation, which were more recalcitrant than the parent compound (Yazdanbakhsh et  
231 al., 2018).

232 Another series of experiments studied the effect of pH on CIL degradation. The relevant time  
233 trends (see **Fig. 2**) suggest that degradation was faster at low pH and slowed down as the pH  
234 increased. A change in pH modifies the prevailing species that occur in solution, and the pH  
235 effect is most likely explained by different photochemical reactivity. The experimental  
236 degradation rate can be expressed as the sum of the contributions of the different species, each  
237 weighted for the relevant fraction, as follows:

238

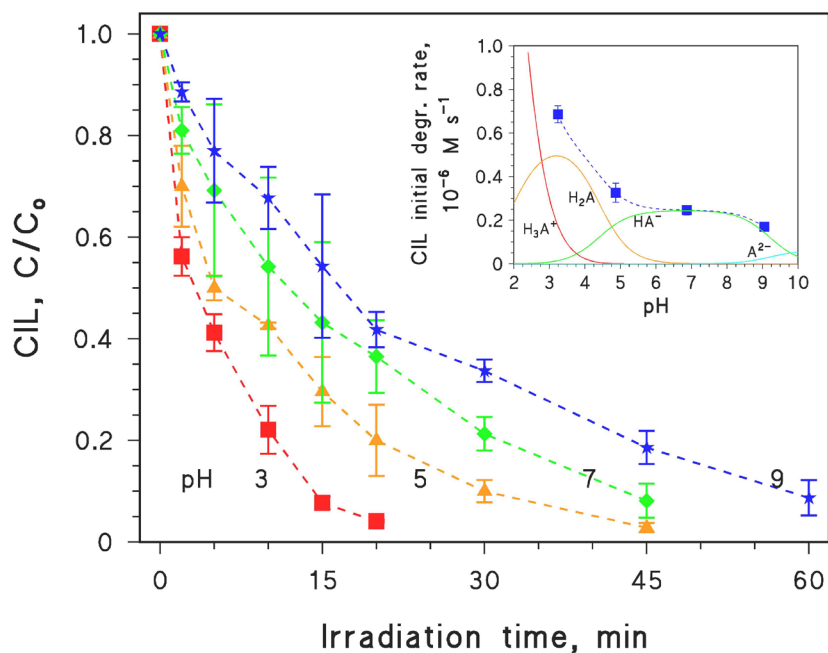
$$239 \quad R_o = \alpha_{H_3A^+} R_{H_3A^+} + \alpha_{H_2A} R_{H_2A} + \alpha_{HA^-} R_{HA^-} + \alpha_{A^{2-}} R_{A^{2-}} \quad (6)$$

240

241 where  $\alpha_{H_3A^+}$ ,  $\alpha_{H_2A}$ ,  $\alpha_{HA^-}$  and  $\alpha_{A^{2-}}$  are expressed as per **Eqs. (1-4)** and are reported in **Fig.**

242 **A.1** in the Appendix.

243



244

245 **Fig. 2.** Time trends of CIL ( $C_0 = 100 \text{ mg L}^{-1}$  or  $2.6 \times 10^{-4} \text{ M}$ ) upon 254-nm irradiation, as a

246 function of pH. Data points are linked with dashed lines to visualize trends. **Inset:** trend of  $R_0$

247 (initial degradation rate of CIL) vs. pH. The solid blue squares are the experimental data, the

248 dashed curve is the data fit with **Eq. (6)**, using  $R_{H_3A^+}$ ,  $R_{H_2A}$ ,  $R_{HA^-}$ ,  $R_{A^{2-}}$  as free-floating

249 parameters. The solid curves represent the contributions to photodegradation of the different

250 CIL species. Some error bars are too small to be visible.

251

252 The trend of  $R_0$  vs. pH is shown in the inset of **Fig. 2**, and the experimental data (solid

253 squares) were fitted with **Eq. (6)** using  $R_{H_3A^+}$ ,  $R_{H_2A}$ ,  $R_{HA^-}$  and  $R_{A^{2-}}$  as floating parameters.

254 The fit (see dashed curve in **Fig. 2**, inset) yielded  $R_{H_3A^+} > R_{H_2A} > R_{HA^-} > R_{A^{2-}}$ , which

255 accounts for the observed pH trend. The contributions of the different CIL species to

256 photodegradation (respectively,  $\alpha_{H_3A^+} R_{H_3A^+}$ ,  $\alpha_{H_2A} R_{H_2A}$ ,  $\alpha_{HA^-} R_{HA^-}$  and  $\alpha_{A^{2-}} R_{A^{2-}}$ ) are also

257 reported in the inset of **Fig. 2**, as solid curves as a function of pH. Notably, degradation at pH

258 6-8 would mainly be accounted for by  $HA^-$ , and its kinetics is not expected to vary much in

259 that pH interval.

260 The rate data derived for the different species ( $R_{H_3A^+}$ ,  $R_{H_2A}$ ,  $R_{HA^-}$  and  $R_{A^{2-}}$ ) allow for the  
 261 calculation of the relevant direct photolysis quantum yields. For a generic species  $x$   
 262 undergoing monochromatic 254-nm irradiation, the following relationship holds between the  
 263 degradation rate  $R_x$  and the molar absorption coefficient at 254 nm,  $\varepsilon_x(254\text{nm})$ :

264

$$265 \quad R_x = \Phi_x I_o (1 - 10^{-\varepsilon_x(254\text{nm})b C_o}) \quad (7)$$

266

267 where  $x = H_3A^+$ ,  $H_2A$ ,  $HA^-$  or  $A^{2-}$ ,  $\Phi_x$  is the direct photolysis quantum yield for the  $x$  species,  
 268  $I_o = (6.24 \pm 0.19) \times 10^{-6} \text{ E L}^{-1} \text{ s}^{-1}$  the incident photon flux,  $b = 2 \text{ cm}$  the optical path length,  
 269 and the concentration  $C_o = 2.6 \times 10^{-4} \text{ M}$  (note that  $R_x$  is the contribution of the species  $x$  to the  
 270 photodegradation of CIL when its molar fraction  $\alpha_x = 1$ , thus we could use  $C_x = C_o$ ). The  
 271 absorbance of CIL was measured spectrophotometrically at 254 nm and at different pH  
 272 values, and the experimental data of the molar absorption coefficients  $\varepsilon_{CIL}(254\text{nm})$  are  
 273 reported in **Fig. 3** as a function of pH (solid squares). The pH trend of  $\varepsilon_{CIL}(254\text{nm})$  is  
 274 accounted for by fact that the different CIL species have different absorption coefficients  
 275  $\varepsilon_x(254\text{nm})$ . Therefore, the experimental data can be fitted with the following equation:

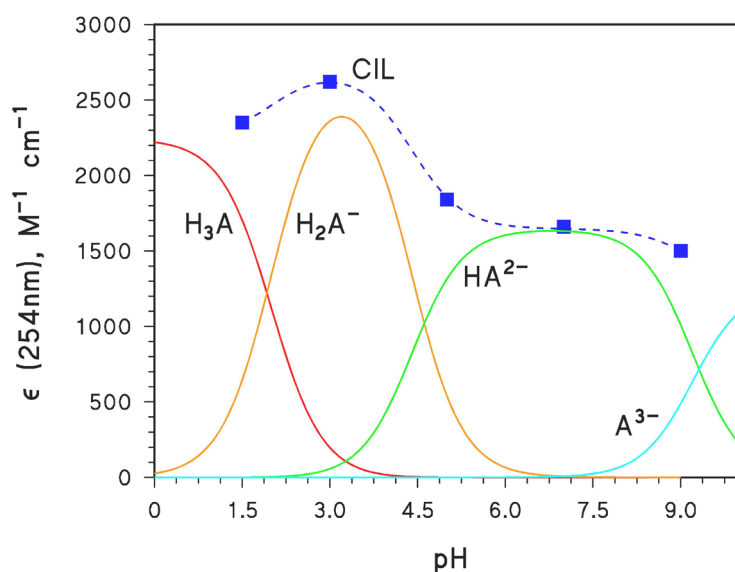
276

$$277 \quad \varepsilon_{CIL} = \alpha_{H_3A^+} \varepsilon_{H_3A^+} + \alpha_{H_2A} \varepsilon_{H_2A} + \alpha_{HA^-} \varepsilon_{HA^-} + \alpha_{A^{2-}} \varepsilon_{A^{2-}} \quad (8)$$

278

279 where all the molar absorption coefficients are referred to 254 nm. The fit of the  $\varepsilon_{CIL}$   
 280 experimental data with **Eq. (8)**, using  $\varepsilon_{H_3A^+}$ ,  $\varepsilon_{H_2A}$ ,  $\varepsilon_{HA^-}$  and  $\varepsilon_{A^{2-}}$  as floating parameters,  
 281 yielded the values of the 254-nm molar absorption coefficients of  $H_3A^+$ ,  $H_2A$ ,  $HA^-$  and  $A^{2-}$   
 282 that are reported in **Table A.4** in the Appendix. With these values and those of  $R_x$  it is  
 283 possible to obtain the direct photolysis quantum yields of the various species at 254 nm, using

284 the relationship reported in **Eq. (7)**. The quantum yield data thus calculated are reported in  
 285 **Table A.4** in the Appendix as well, and they follow the order  $\Phi_{H_3A^+} > \Phi_{H_2A} > \Phi_{HA^-} > \Phi_{A^{2-}}$ .  
 286 The quantum yield value of  $H_3A^+$  is remarkably high, which explains why  $H_3A^+$  undergoes  
 287 faster photodegradation compared to  $H_2A$  (**Fig. 2**), although it absorbs radiation at a  
 288 somewhat lesser extent (**Fig. 3**).  
 289



290  
 291 **Fig. 3.** Molar absorption coefficients (254 nm) of CIL and its different species, as a function  
 292 of pH. The solid squares are the experimental data; the dashed curve is the data fit with **Eq.**  
 293 **(8)**, using  $\epsilon_{H_3A^+}$ ,  $\epsilon_{H_2A}$ ,  $\epsilon_{HA^-}$ , and  $\epsilon_{A^{2-}}$  as floating parameters; the solid curves represent the  
 294 contributions to radiation absorption of the different CIL species (given by the respective  
 295 products  $\alpha_{H_3A^+}\epsilon_{H_3A^+}$ ,  $\alpha_{H_2A}\epsilon_{H_2A}$ ,  $\alpha_{HA^-}\epsilon_{HA^-}$ , and  $\alpha_{A^{2-}}\epsilon_{A^{2-}}$ ).  
 296

### 297 3.3. Effect of dissolved species

298 In some cases, the direct photolysis of organic molecules follows a self-sensitized pathway  
 299 where the excited state of a compound is involved in the degradation of the same compound  
 300 in the ground state. The occurrence of a bimolecular reaction between excited and ground

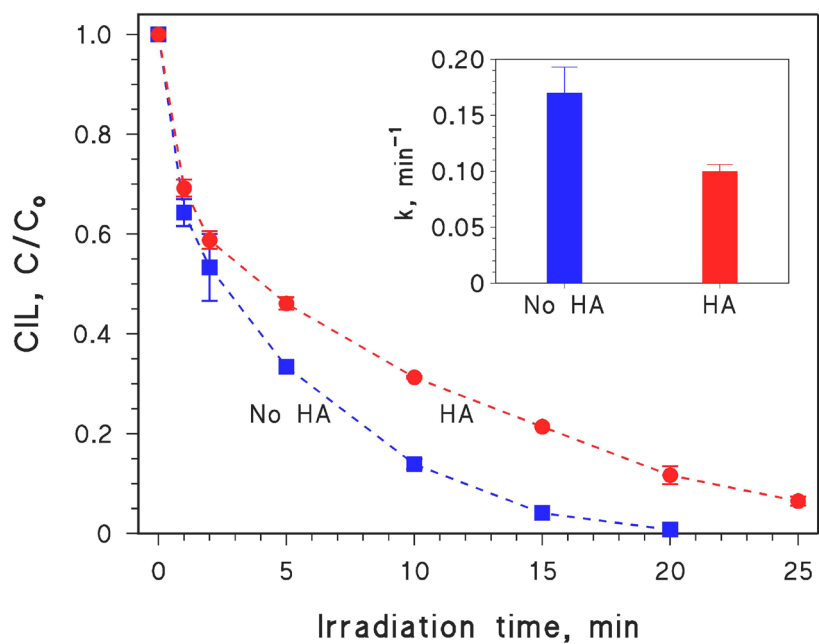
301 states ensures that the process kinetics becomes faster as the concentration  $C_0$  increases  
302 (Bedini et al., 2012). In such cases the trend of  $R_0$  vs.  $C_0$  would not follow absorption  
303 saturation, and the latter should underestimate the degradation kinetics at high  $C_0$ . The data  
304 reported in the inset of **Fig. 1** show that degradation kinetics in our study strictly adhered to  
305 the absorption saturation model. Therefore, a self-sensitized process can be excluded. The  
306 same data rule out as well the opposite phenomenon, that ground-state CIL quenches the  
307 excited states of the same molecule. Indeed, in such cases one expects  $R_0$  to grow more slowly  
308 with  $C_0$  compared to the case of absorption saturation, sometimes yielding a trend with a  
309 maximum (Minto et al., 1989). The experimental data of **Fig. 1** rather suggest that CIL  
310 follows direct photolysis triggered by evolution of the excited state(s), excluding important  
311 interactions between these and the ground state.

312 In some relatively rare circumstances, the irradiation of a molecule in water can trigger  $\bullet\text{OH}$   
313 production. This happens for instance if the excited state is able to oxidize water (Sur et al.,  
314 2011), and in such cases photogenerated  $\bullet\text{OH}$  could contribute to the degradation process.  
315 Such a hypothesis can be checked by addition of  $\bullet\text{OH}$  scavengers to the irradiated system. t-  
316 butanol is a common and rather selective  $\bullet\text{OH}$  scavenger that can be useful to this purpose  
317 (Buxton et al., 1988; Neta et al., 1988; Tsiampalis et al., 2019; Stathoulopoulos et al., 2020).  
318 As shown in **Fig. A.3** in the Appendix, the addition of 100 mg L<sup>-1</sup> t-butanol had very little  
319 effect on CIL degradation, thereby suggesting that the transformation process does not  
320 involve  $\bullet\text{OH}$  to a significant degree. It is noted that the experimental data obtained with  
321 alcohols should be treated with caution, because sometimes alcohols scavenge reactive states  
322 rather than  $\bullet\text{OH}$  (Vione et al., 2010). However, in the present case the alcohol had a minor  
323 effect that suggests a limited importance of both  $\bullet\text{OH}$  production and the possible  
324 confounding process (in contrast, the finding that t-butanol inhibits photolysis would not  
325 necessarily prove that the process involves  $\bullet\text{OH}$ ).



326 Natural water components can affect photodegradation either by competing with the substrate  
327 for the incoming photons, or by sensitizing its transformation (Canonica and Freiburghaus,  
328 2001). Differently from the case of self-sensitization, in this indirect photochemical path the  
329 excited state of another compound (i.e. the photosensitizer) triggers degradation by reacting  
330 with the ground-state substrate (McNeill and Canonica, 2016). Actually, while competition  
331 for lamp irradiance by other compounds inhibits degradation, the sensitization process favors  
332 it.

333 Humic substances are major light absorbers and photosensitizers in surface waters (Rosario-  
334 Ortiz and Canonica, 2016), thus they are very suitable compounds with which to test the  
335 inhibition/sensitization potential. The presence of humic compounds at environmental  
336 concentrations ( $2.5 \text{ mg L}^{-1}$ ) inhibited the photodegradation of CIL as shown in **Fig. 4**. This  
337 result suggests that humic acids can act as radiation absorbers, thereby inhibiting the direct  
338 photolysis of the substrate, and that this inhibition process prevails over photosensitization  
339 (*vide infra* for additional calculations over this issue).



340

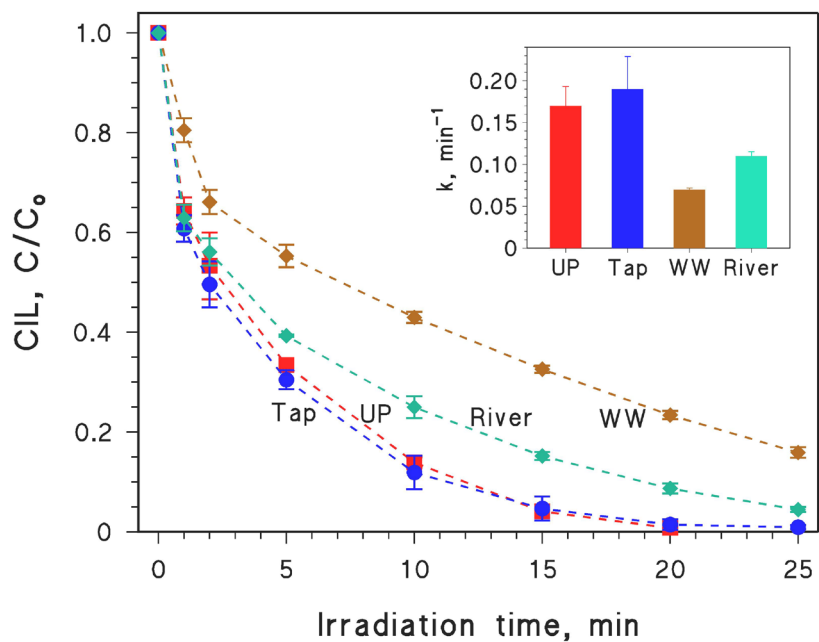
341 **Fig. 4.** Time trends of 2.5 mg L<sup>-1</sup> CIL upon 254-nm irradiation at near-neutral pH, alone and  
342 upon addition of 2.5 mg L<sup>-1</sup> humic acids (HA). Data points are linked with dashed lines to  
343 visualize trends. **Inset:** pseudo-first order photodegradation rate constants, together with their  
344 sigma-level error bounds. Some error bars are too small to be visible.

345

### 346 *3.4. CIL photodegradation in real water matrices*

347 In addition to humic substances, other components (organic and inorganic) that occur in  
348 natural waters might affect (either inhibit or enhance) the photodegradation kinetics of CIL.  
349 Therefore, photodegradation experiments were also carried out in the presence of natural  
350 water and treated wastewater samples spiked with CIL. The results of these experiments are  
351 shown in **Fig. 5**. Similar transformation rates were obtained in ultra-pure (Milli-Q quality)  
352 and tap water, while inhibition of CIL photodegradation was observed in river water and WW  
353 effluent. The recorded decrease in degradation kinetics with increasing matrix complexity,  
354 signified the importance of the organic and inorganic constituents typically found in natural  
355 waters and wastewaters.

356



357  
 358 **Fig. 5.** Time trends of  $2.5 \text{ mg L}^{-1}$  CIL upon 254-nm irradiation in different water matrices:  
 359 ultra-pure water (UP), tap water (Tap), treated wastewater (WW) and river water (River).  
 360 Data points are linked with dashed lines to visualize trends. **Inset:** respective pseudo-first  
 361 order photodegradation rate constants, together with their sigma-level error bounds. Some  
 362 error bars are too small to be visible.

363  
 364 The absorption of radiation by CIL at concentration  $C_0$  (in our case,  $C_0 = 2.5 \text{ mg L}^{-1}$  that  
 365 corresponds to  $7 \times 10^{-6} \text{ M}$ ) in solution can be expressed as the absorbed photon flux  $P_{a,CIL}$  [ $\text{E}$   
 366  $\text{L}^{-1} \text{ s}^{-1}$ ], which reads as follows according to the Lambert-Beer law (Braslavsky, 2007):

$$P_{a,CIL} = I_o [1 - 10^{-\epsilon_{CIL}(254nm) \times b \times C_o}] \quad (9)$$

369  
 370 where  $I_0 = 6.24 \times 10^{-6} \text{ E L}^{-1} \text{ s}^{-1}$  is the incident photon flux from the lamp,  $\epsilon_{CIL}(254nm) =$   
 371  $1660 \text{ L mol}^{-1} \text{ cm}^{-1}$  the molar absorption coefficient of CIL ( $\text{HA}^-$ ) at the specified wavelength  
 372 and pH 7,  $b = 2 \text{ cm}$  the optical path length, and  $C_0 = 7 \times 10^{-6} \text{ M}$  the initial CIL concentration.

373 **Eq. (9)** holds if CIL is the only light-absorbing species in solution. In the presence of other  
374 light-absorbing compounds (Sheikhmohammadi et al., 2019), the photon flux absorbed by  
375 CIL gets modified as follows (Braslavsky, 2007):

376

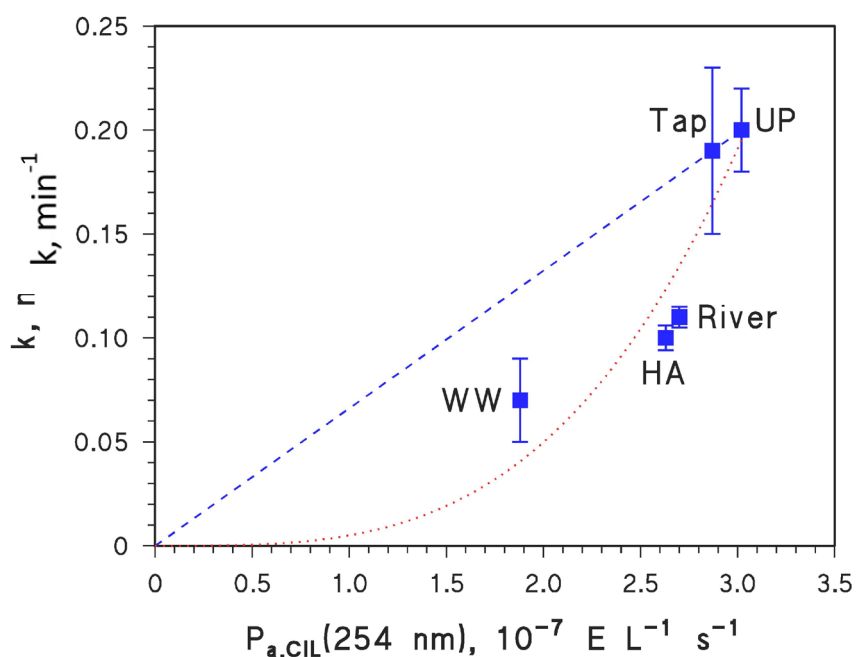
$$377 \quad P'_{a,CIL} = I_o \frac{\epsilon_{CIL}(254nm) \times C_o}{A_w(254nm)} [1 - 10^{-A_w(254nm) \times b}] \quad (10)$$

378

379 where  $A_w(254nm)$  [ $\text{cm}^{-1}$ ] is the 254-nm absorbance of the water matrix spiked with CIL (in  
380 the present case, HA solution, tap water, river water or wastewater). In particular, it was  
381  $A_w(254nm) = 0.073 \text{ cm}^{-1}$  for  $2.5 \text{ mg L}^{-1}$  HA,  $0.034 \text{ cm}^{-1}$  for tap water,  $0.061 \text{ cm}^{-1}$  for river  
382 water, and  $0.240 \text{ cm}^{-1}$  for wastewater.

383 If the water matrix only affected radiation absorption by CIL, leaving its direct photolysis  
384 quantum yield unchanged, one would expect the CIL photolysis kinetics to be directly  
385 proportional to  $P_{a,CIL}$ . The trend of  $k$  (pseudo first-order photodegradation rate constant) as a  
386 function of  $P_{a,CIL}$  is reported in **Fig. 6** for the different samples, where the dashed line shows  
387 the trend that would be expected at constant photolysis quantum yield. It is clearly shown that  
388 only tap water follows a comparable trend as ultra-pure water, while the other water matrices  
389 and the sample containing HA feature lower quantum yields for the direct photolysis of CIL  
390 (the relevant data points are unequivocally located below the dashed line, even when  
391 accounting for the experimental uncertainty). This finding suggests that the natural water  
392 components may on the one hand compete with CIL for the lamp irradiance; on the other  
393 hand, they do not behave as photosensitizers (in which case the relevant data points should be  
394 located above the dashed line) but rather inhibit the direct photolysis of CIL. Such a  
395 phenomenon of direct photolysis inhibition has already been observed in the presence of a  
396 range of natural organic compounds including humic substances (Vione et al., 2010; Liu et

397 al., 2020), and might involve either the physical quenching of CIL excited states (which  
 398 would be quenched back to the ground state, inhibiting photolysis), or the back-reduction to  
 399 CIL of partially oxidized degradation intermediates such as radicals or radical cations. The  
 400 back-reduction effect could be triggered by the anti-oxidant (mostly phenolic) moieties that  
 401 are ubiquitous in both HA and the natural organic matter (Wenk and Canonica, 2012).  
 402



403  
 404 **Fig. 6.** Correlation between the pseudo-first order rate constant of CIL degradation ( $k$ ) and the  
 405 photon flux absorbed by CIL in solution ( $P_{a,CIL}$ ). The dashed line shows the trend that would  
 406 be expected if the water matrix (UP = ultra-pure water; Tap = tap water; HA = humic acids;  
 407 River = river water; WW = wastewater) only acted as radiation absorber. The error bounds  
 408 represent the sigma-level uncertainty deriving from the exponential fit of the time trend data,  
 409 while the dotted curve is the result of data fit with a power function ( $k = \alpha (P_{a,CIL})^\beta$ ).  
 410

#### 411 **4. Conclusions**

412

413 The rising global consumption of antibiotics resulted in the widespread occurrence of  
414 antibiotic formulations in the aquatic environment. Although past investigations report the  
415 photolytic behavior of antibiotics, they overlook studying the entailed problem of co-  
416 administered agents released in water bodies. This is the first contribution studying in depth  
417 the direct photolysis of cilastatin, a co-administered agent for the antibiotic imipenem, under  
418 254-nm irradiation. The experimental data showed fast depletion of cilastatin in water under  
419 UVC, and suggested that the direct photolysis of cilastatin is an efficient degradation  
420 technique. Our experimental and modelling data demonstrated that degradation proceeded  
421 faster at low pH values, thereby suggesting that altering the pH can synergistically accelerate  
422 degradation kinetics. Moreover, we showed that the transformation process does not involve  
423  $\bullet\text{OH}$  to a significant degree, and that radiation absorbers may slow down degradation kinetics.  
424 Finally, degradation rates decreased with increasing matrix complexity (tap water ~ ultra-pure  
425 water > river water > wastewater effluent), suggesting that the natural water components may  
426 both compete with cilastatin for the 254 nm photon absorption and decrease its direct  
427 photolysis quantum yield. Nonetheless, cilastatin depletion in complex matrices can be  
428 completed within reasonable exposure times.

429

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