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

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3	UVC-induced direct photolysis as an efficient process
4	for the degradation of cilastatin in natural and waste
5	water
6	
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15 Abstract

16 This work reports for the first time the UVC photodegradation of cilastatin, a renal dehydropeptidase inhibitor co-adminstered with the imipenem antibiotic. Initially, solutions 17 of cilastatin at varying concentrations were prepared in ultra-pure water and the direct 18 19 photolysis of cilastatin was monitored under 254-nm irradiation. Degradation was slower at 20 higher initial cilastatin concentrations, due to absorption saturation. Of the different eluting photoproducts, only one was tentatively identified as oxidized cilastatin bearing a sulfoxide 21 22 group. UV-254 photolysis occurred faster at lower pH values, because the protonated forms of the molecule (H₃A⁺, H₂A) have both higher absorption coefficients and higher photolysis 23 quantum yields than the non-protonated ones (HA-, A2-). The direct photolysis of cilastatin 24 25 does not involve 'OH, as excluded by experiments in which t-butanol was added as '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 suggest that the water matrix components could carry out either physical quenching of 32 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.

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

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 β -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 pharmaceutical industry (Cheng et al., 2015; Szekeres et al., 2017) and in their widespread 61 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 world and is threatening the efficiency of this essential class of life-saving antibiotics (PappWallace 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 integrated advanced oxidation/reduction process for the degradation of of an 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.

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

82

83 2. Material and Methods

84 2.1. Chemicals and samples

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

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

For all photolysis experiments, a home-made laboratory photoreactor (28.0 cm height × 28.4 cm length × 28.0 cm width) was used. The photoreactor was equipped with two 8 W lowpressure mercury lamps having a strong emission line at 254 nm (Osram 8W G8 T5), each mounted on an opposing sidewall of the photoreactor. The distance between each lamp and the quartz vial used in the experiments was 13 cm. The rate of the incident UV light intensity entering the solution (I_0 , given in E L⁻¹ s⁻¹, where E = Einstein) was determined using H₂O₂ as chemical actinometer, and was $I_0 = (6.24 \pm 0.19) \times 10^{-6}$ E L⁻¹ s⁻¹.

113 Aqueous solutions of cilastatin (CIL) were prepared daily by transferring the appropriate volume from a 7 g L^{-1} methanolic stock solution to a tailor-made quartz vial (2.4 cm outer 114 115 diameter \times 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 H₂O, H₂O 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

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

All liquid chromatography/mass spectrometry (LC/MS) analyses on CIL were carried out using an Agilent 1200 Series high-performance liquid chromatography (HPLC) system equipped with a binary pump, autosampler, degasser and thermostated column compartment, coupled to a diode array detector (DAD; the CIL analytical wavelength was 200 nm), and to 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 \times 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% formic acid. The flow rate of the mobile phase was 250 μ L min⁻¹, and the total analysis time 141 was 10 min. For analysis, 60 uL of the sample were added to 100 uL polypropylene inserts 142 and placed in 2 mL polypropylene autosampler vials equipped with caps, all purchased from 143 144 Agilent (Palo Alto, USA). The injection volume was 20 µL. The MS conditions were: drying gas flow, 5 L min⁻¹; drying gas temperature, 350 °C; nebulizer pressure, 40 psi; collector 145 146 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 mg L⁻¹ (correlation coefficient r^2 =0.9982). A 1.3 mg L⁻¹ limit of quantification (LOQ) was 149 150 estimated. This concentration level was not sufficiently to monitor the photolysis of 2.5 mg L^{-1} CIL (*i.e.*, 7 × 10⁻⁶ M). To increase sensitivity in these experiments, 100 µL of the 151 irradiated sample were added to 250 µL vial inserts instead, the injection volume was set at 60 152 153 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 in the concentration range 0.5-5 mg L^{-1} and the estimated LOQ was 0.36 mg L^{-1} , which was 155 156 sufficient for monitoring close to 85% CIL conversion. The linearity of the method was tested in each water matrix studied here and it was concluded that the matrix did not affect the 157 158 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 α can be obtained as follows from the 167 deprotonation equilibria:

168

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

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

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

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

173

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

A first series of degradation experiments was carried out at pH 7, for concentration values of CIL ranging from 2.5 to 100 mg L⁻¹ (note that 100 mg L⁻¹ correspond to 2.6×10^{-4} M). The 2.5 mg L⁻¹ concentration represented the lowest concentration level where conversion of up to 90% of the irradiated samples could be successfully monitored and quantified by the analytical instrumentation. The time evolution of CIL (**Fig. 1**) shows that degradation was more effective as the initial concentration was lower. Data fitting of the concentration profiles from **Fig. 1** to $C_t = C_o e^{-kt}$ gave the *k* values used to compute the initial degradation rates R_0 (*vide infra*) shown in the inset graph, which increased with increasing C_o , from 2.2×10⁻⁸ M s⁻¹ at 2.5 mg L⁻¹ to 2.5×10⁻⁷ M s⁻¹ at 100 mg L⁻¹.



188

Fig. 1. Time trends of CIL concentration upon 254-nm irradiation at different initial concentration values, under ~neutral conditions (pH ~ 7). Data points are linked with dashed lines to visualize trends. Inset: initial degradation rates ($R_0 = k C_0$) for the different concentrations examined here (C_0). The dashed connecting curve represents data fit with Eq. (5). Some error bars are too small to be visible.

194

In a pseudo-first order approximation the initial degradation rate R_0 can be obtained as $R_0 = k$ 196 C_0 , and the R_0 vs. C_0 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 (Kourounioti et al., 2019; Alberti et al., 2021). The monochromatic irradiation set-up allows for a simple Lambert-Beer approach to be
applied, where the initial degradation rate can be expressed as follows (Braslavsky, 2007):

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

203

where γ is a constant proportionality factor, ε_{CIL} is the molar absorption coefficient of CIL at 205 254 nm, b = 2 cm the optical path length in solution, and C_0 the initial CIL concentration. The 206 experimental values of k and R_0 in the different experiments conducted in this work are listed 207 in **Table A.2** in the Appendix.

208

Independent spectrophotometric measurements gave $\varepsilon_{CIL}(254 \text{ nm}) = 1660 \text{ L mol}^{-1} \text{ cm}^{-1}$ at pH 7 (molar absorption of the HA⁻ species, see **Fig. A.1** in the Appendix). Therefore, by introducing in **Eq. (5)** all the known parameters it is possible to fit the experimental data of **Fig. 1** (insert) with γ as the only free-floating variable. The good fit results show that the phenomenon of absorption saturation indeed accounts for the plateau trend of R_0 vs. C_0 .

214 In an attempt to identify the transformation products formed, the photolysis of 100 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 $[M-H]^+=375$ 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 sumary 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 evaluated, by measuring the TOC of 15 mg L^{-1} CIL water solutions before irradiation and for 227 228 up to 240 min of UV₂₅₄ exposure, after which time only $\sim 10\%$ TOC removal was recorded. This observation pointed towards the formation and accumulation of degradation products 229 230 during irradiation, which were more recalcitrant than the parent compound (Yazdanbakhsh et 231 al., 2018).

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

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

240

241 where $\alpha_{H_3A^+}$, α_{H_2A} , α_{HA^-} and $\alpha_{A^{2-}}$ are expressed as per Eqs. (1-4) and are reported in Fig. 242 A.1 in the Appendix.



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 function of pH. Data points are linked with dashed lines to visualize trends. Inset: trend of R_0 (initial degradation rate of CIL) *vs.* pH. The solid blue squares are the experimental data, the dashed curve is the data fit with Eq. (6), using $R_{H_3A^+}$, R_{H_2A} , R_{HA^-} , $R_{A^{2-}}$ as free-floating parameters. The solid curves represent the contributions to photodegradation of the different CIL species. Some error bars are too small to be visible.

251

244

252 The trend of R_0 vs. pH is shown in the inset of Fig. 2, and the experimental data (solid squares) were fitted with Eq. (6) using $R_{H_3A^+}$, R_{H_2A} , R_{HA^-} and $R_{A^{2-}}$ as floating parameters. 253 The fit (see dashed curve in Fig. 2, inset) yielded $R_{H_3A^+} > R_{H_2A} > R_{HA^-} > R_{A^{2-}}$, which 254 255 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, $\varepsilon_x(254$ nm):

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- 265

5
$$R_x = \Phi_x I_o \left(1 - 10^{-\varepsilon_x (254nm)b C_o} \right)$$
 (7)

266

where $x = H_3A^+$, H_2A , HA^- or A^{2-} , Φ_x is the direct photolysis quantum yield for the x species, 267 $I_{\rm o} = (6.24 \pm 0.19) \times 10^{-6} \text{ E L}^{-1} \text{ s}^{-1}$ the incident photon flux, b = 2 cm the optical path length, 268 and the concentration $C_0 = 2.6 \times 10^{-4}$ M (note that R_x is the contribution of the species x to the 269 photodegradation of CIL when its molar fraction $\alpha_x = 1$, thus we could use $C_x = C_0$). The 270 271 absorbance of CIL was measured spectrophotometrically at 254 nm and at different pH 272 values, and the experimental data of the molar absorption coefficients $\varepsilon_{CIL}(254nm)$ are 273 reported in Fig. 3 as a function of pH (solid squares). The pH trend of ε_{CII} (254nm) is 274 accounted for by fact that the different CIL species have different absorption coefficients 275 $\varepsilon_x(254$ nm). Therefore, the experimental data can be fitted with the following equation:

276

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

278

where all the molar absorption coefficients are referred to 254 nm. The fit of the ε_{CIL} experimental data with **Eq. (8)**, using $\varepsilon_{H_3A^+}$, ε_{H_2A} , ε_{HA^-} and $\varepsilon_{A^{2-}}$ as floating parameters, yielded the values of the 254-nm molar absorption coefficients of H₃A⁺, H₂A, HA⁻ and A²⁻ that are reported in **Table A.4** in the Appendix. With these values and those of R_x it is possible to obtain the direct photolysis quantum yields of the various species at 254 nm, using the relationship reported in Eq. (7). The quantum yield data thus calculated are reported in **Table A.4** in the Appendix as well, and they follow the order $\Phi_{H_3A^+} > \Phi_{H_2A} > \Phi_{HA^-} > \Phi_{A^{2-}}$. The quantum yield value of H₃A⁺ is remarkably high, which explains why H₃A⁺ undergoes faster photodegradation compared to H₂A (Fig. 2), although it absorbs radiation at a somewhat lesser extent (Fig. 3).

289



290

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

296

297 3.3. Effect of dissolved species

In some cases, the direct photolysis of organic molecules follows a self-sensitized pathway where the excited state of a compound is involved in the degradation of the same compound 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 •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 •OH could contribute to the degradation process. 315 Such a hypothesis can be checked by addition of 'OH scavengers to the irradiated system. t-316 butanol is a common and rather selective 'OH scavenger that can be useful to this purpose (Buxton et al., 1988; Neta et al., 1988; Tsiampalis et al., 2019; Stathoulopoulos et al., 2020). 317 As shown in Fig. A.3 in the Appendix, the addition of 100 mg L^{-1} t-butanol had very little 318 319 effect on CIL degradation, thereby suggesting that the transformation process does not 320 involve '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 'OH (Vione et al., 2010). However, in the present case the alcohol had a minor 323 effect that suggests a limited importance of both '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 •OH).

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

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



Fig. 4. Time trends of 2.5 mg L^{-1} CIL upon 254-nm irradiation at near-neutral pH, alone and upon addition of 2.5 mg L^{-1} humic acids (HA). Data points are linked with dashed lines to visualize trends. **Inset:** pseudo-first order photodegradation rate constants, together with their 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.



357

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

363

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

368
$$P_{a,CIL} = I_o [1 - 10^{-\varepsilon_{CIL}(254nm) \times b \times C_o}]$$
(9)

369

where $I_0 = 6.24 \times 10^{-6} \text{ E L}^{-1} \text{ s}^{-1}$ is the incident photon flux from the lamp, $\varepsilon_{\text{CIL}}(254\text{ nm}) =$ 1660 L mol⁻¹ cm⁻¹ the molar absorption coefficient of CIL (HA⁻) at the specified wavelength and pH 7, b = 2 cm the optical path length, and $C_0 = 7 \times 10^{-6}$ 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
$$P'_{a,CIL} = I_o \frac{\mathcal{E}_{CIL}(254nm) \times C_o}{A_W(254nm)} [1 - 10^{-A_W(254nm) \times b}]$$
(10)

378

where $A_w(254nm)$ [cm⁻¹] is the 254-nm absorbance of the water matrix spiked with CIL (in the present case, HA solution, tap water, river water or wastewater). In particular, it was $A_w(254nm) = 0.073$ cm⁻¹ for 2.5 mg L⁻¹ HA, 0.034 cm⁻¹ for tap water, 0.061 cm⁻¹ for river water, and 0.240 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 located above the dashed line) but rather inhibit the direct photolysis of CIL. Such a 394 395 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 al., 2020), and might involve either the physical quenching of CIL excited states (which
would be quenched back to the ground state, inhibiting photolysis), or the back-reduction to
CIL of partially oxidized degradation intermediates such as radicals or radical cations. The
back-reduction effect could be triggered by the anti-oxidant (mostly phenolic) moieties that
are ubiquitous in both HA and the natural organic matter (Wenk and Canonica, 2012).

402



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 faster at low pH values, thereby suggesting that altering the pH can synergistically accelerate 421 422 degradation kinetics. Moreover, we showed that the transformation process does not involve 423 •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 photolysis quantum yield. Nonetheless, cilastatin depletion in complex matrices can be 427 completed within reasonable exposure times. 428

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