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Degradation of indomethacin in river water under stress and non-stress laboratory conditions: degradation products, long-term evolution and adsorption to sediment

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ABSTRACT

The pharmaceutical compound indomethacin is not totally removed in wastewater treatment plants, whose effluents flow into aquatic environments; concentrations in the 0.1–100 ng/L range are commonly found in surface waters, and its fate is unknown. Here, biological, photochemical and thermal degradation assays were conducted under stress and non-stress conditions to estimate its degradation rate in river water and establish its degradation products over time. The results revealed that direct sunlight irradiation promoted the complete degradation of indomethacin (2 μg/L) in less than 6 hr, but indomethacin was detected over a period of 4 months when water was kept under the natural day–night cycle and the exposure to sunlight was partially limited, as occurs inside a body of water. The biological degradation in water was negligible, while the hydrolysis at pH 7.8 was slow. Residues were monitored by ultra-pressure liquid chromatography/quadrupole time-of-flight/mass spectrometry after solid-phase extraction, and six degradation products were found; their structures were proposed based on the molecular formulae and fragmentation observed in high-resolution tandem mass spectra. 4-Chlorobenzoic and 2-acetamido-5-methoxybenzoic acids were the long-term transformation products, persisting for at least 30 weeks in water kept under non-stress conditions. Furthermore, the degradation in the presence of sediment was also monitored over time, with some differences being noted. The adsorption coefficients of indomethacin and degradation products on river sediment were calculated; long-term degradation products did not have significant adsorption to sediment.

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Introduction

The presence of pharmaceutical compounds in the environment is a matter of increasing concern because they impact negatively on the environment. The effluents discharged from wastewater treatment plants (WWTPs) are the main introduction source of pharmaceuticals in surface waters. These compounds are found in the influents of the WWTPs mainly as a result of the inappropriate domestic disposal of unused medicinal products. Indomethacin (INDO) is a non-steroid

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anti-inflammatory drug detected in the primary influents that reach the WWTPs in concentrations, generally, of 20–100 ng/L, although concentrations of about 1 μg/L have also been reported (Sui et al., 2009; Radjenovic et al., 2009). Concentrations of about 10–20 ng/L have been found in depurated effluents (Zhou et al., 2009). In river water, INDO concentrations between 0.1 and 100 ng/L are frequent (Kim et al., 2009; Lewandowski et al., 2011; Yamamoto et al., 2009; Zhou et al., 2009). Concerning the efficiency of WWTPs at removing INDO, there are contradictory data; some authors have concluded that its removal in the global process is non-existent or slight (Radjenovic et al., 2009; Rosal et al., 2010; Sui et al., 2009; Tran et al., 2014), while other authors find that the removal rates are about 40%–100% (Huang et al., 2011; Matsuo et al., 2011; Zhou et al., 2009). Moreover, it has been stated that INDO infiltrates to subsurface waters (1 m depth) from surface waters, as has occurred for other pharmaceuticals (Lewandowski et al., 2011).

The frequent detection of INDO in surface waters and WWTP effluents discharged into rivers advises us not only to evaluate its persistence in the environment, but also to determine the possible transformation products in order to obtain an overall perspective and to assess possible risks, because the effects resulting from exposure to the parent pharmaceutical and the degradation products can be different (Celiz et al., 2009). So, INDO dissolved in different media has been subjected to degradation studies under stress conditions that indicate its photolability, and some degradation products generated in these conditions have been described (Temussi et al., 2011; Yamamoto et al., 2009), but there is no reliable information about its behavior in surface water and especially about its long-term fate in non-stress conditions.

In this context, river water spiked with INDO at trace levels was subjected to degradation studies in this work to ascertain the importance of the chemical, photochemical and biological processes in its degradation in surface water. In addition to assays under stress conditions, non-stress conditions were also applied to INDO in aqueous solution in order to simulate its behavior inside a body of water. Water aliquots were analyzed by ultra-pressure liquid chromatography/quadrupole time-of-flight/mass spectrometry, and the structures of the degradation products found were tentatively elucidated from the molecular formulae and fragmentation observed in high-resolution tandem mass spectra. The evolution of the degradation products was also monitored over time to estimate their occurrence, and a degradation pathway was outlined. In addition, the adsorption capacity of sediment for INDO and its degradation products was evaluated by calculating the corresponding adsorption coefficients.

1. Experimental

1.1. Materials and reagents

Water samples were collected from the rivers Pisuerga (pH value 7.8, chemical oxygen demand value 4.6 mg/L), in the urban area of the city of Valladolid, and Tuerto (pH value 7.4, chemical oxygen demand value 3.9 mg/L), in the rural area of the La Bañeza, province of León; chemical oxygen demand was determined by the potassium dichromate method. A sediment sample (total organic carbon 1.2%; clay 11%, silt 44%, sand 45%) was collected from the river Pisuerga. Total organic carbon was measured by a combustion method with a LECO CS-225 elemental analyzer (St. Joseph, MI, USA). Sediment particle size analysis was based on the Bouyoucos hydrometer method; soil aggregates were dispersed by chemical means.

Cellulose nitrate disks from Sartorius (Barcelona, Spain) were used: river water was filtered through 0.2 μm pore-size disks for the estimation of adsorption coefficients, through 3 μm pore-size disks to carry out biodegradation experiments, and through 0.45 μm pore-size disks for other degradation experiments.

Indomethacin (99% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). LC–MS grade methanol, acetonitrile and formic acid were supplied by Panreac (Barcelona, Spain) and ultrapure water was obtained from a Milli-Q plus apparatus (Millipore, Milford, MA, USA). Analysis-grade sodium hydroxide, potassium dihydrogen phosphate and sodium azide were purchased from Panreac. EBH cartridges (60 mg) for solid-phase extraction (SPE) and PTFE disposable syringe filter units, 0.20 μm pore size, were obtained from Scharlab (Barcelona, Spain). Tryptone soya broth (TSB), a highly nutrient liquid culture medium for general purpose use, was purchased from Scharlab; its composition can be seen in the supplementary material (Appendix A Table S1). A vacuum centrifuge evaporator, Myvac model, was provided by Genevac (Ipswich, UK), a PK120 centrifuge by ALC (Winchester, VA, USA) and a Promax 2020 reciprocating platform shaker by Heidolph (Germany).

1.2. Biological degradation

1.2.1. Aerobic degradation

Biological degradation assays were carried out with water from the river Pisuerga (pH 7.8) which was spiked with INDO to achieve a concentration of 2 μg/L. A volume of 50 mL of river water was transferred into a 100 mL Erlenmeyer flask, which was then coated with aluminum foil to avoid exposure to sunlight but allowing the exchange of air with the atmosphere. An INDO control solution was similarly prepared in ultrapure water (pH 7.8 adjusted with NaOH) containing 0.02% (W/V) sodium azide as a biocide. Water blanks were prepared as well. Samples were run in parallel; flasks were shaken in a reciprocating shaker at a rotation speed of 130 r/min for 5 weeks, within a temperature range of 18–21°C. Aliquots of 5 mL were collected each week and subjected to analysis. Evaporation water losses were periodically restored by addition of water of the same type. All biological experiments were carried out in duplicate.

1.2.2. Anaerobic degradation

River water (pH 7.8) spiked at 2 μg/L was placed in 15 mL vials, completely filled to avoid the presence of air in the headspace. The vials were closed, protected from light by coating them with aluminum foil and kept in a temperature range of 18–21°C during experimentation. Control solutions with INDO in ultrapure water (pH 7.8 adjusted) containing 0.02% sodium azide, and the corresponding blanks, were also run in parallel. A batch of vials was assembled to withdraw weekly samples over a period of 5 weeks; a volume of 5 mL from each withdrawn vial was collected for analysis.

1.2.3. Degradation in culture medium

A 20/80 (V/V) mixture of TSB culture medium and river water was spiked with INDO at 2 μg/L, and 100 mL of the mixture was placed in a 250 mL glass container. A control solution in ultrapure water (pH 7.8 adjusted) containing 0.02% sodium azide and INDO, and a blank of the medium–water mixture, were also prepared and run in parallel. Closed vessels were heated at 35°C in darkness for 5 weeks. Aliquots of 10 mL were sampled weekly and filtered through 0.45 μm pore-size cellulose nitrate; 5 mL samples of filtrate were collected for analysis.

1.3. Photochemical and thermal degradation

River water (pH = 7.8) spiked with INDO (2 μg/L) was placed in a quartz cuvette, which was closed and placed on the outer edge of a window, south-facing, to allow its direct exposure to sunlight. The assay was performed in the city of Valladolid (latitude: 41°38′15″N, longitude: 4°44′17″W) in one day, in the month of January. Aliquots of 0.3 mL were withdrawn at regular time intervals and injected into the chromatographic system. Control samples of INDO in river water, protected from sunlight with aluminum foil, were prepared as well.

For thermal degradation, a volume of 100 mL of spiked river water (2 μg/L) was placed in a closed 250 mL glass flask. This was coated with aluminum foil and placed in an oven at 70 °C. Aliquots of 5 mL were collected hourly and subjected to analysis. All experiments were done in duplicate.

1.4. Non-stress degradation assays

A simple, although slow, approach was adopted in this work to simulate the concurrent natural process in a body of water. River water was placed in a transparent sodium calcium silicate glass container with air-tight seal, which was opened weekly to collect a sample aliquot and replace the air inside in contact with the water surface. The container was kept at laboratory temperature (18–21°C) under the natural day–night cycle and directly exposed to sunlight for 30 weeks, at which time the degradation assay was ended. In these conditions, the solar radiation must pass through the laboratory window glass and the glass of the container to reach the body of water; the glass absorbs UV radiation and the behavior of INDO in the glass container simulates, in a greater or lesser extent, that in a mass of water where the penetration of solar UV radiation is diminished with depth. The attenuation of the radiation was estimated by measurements of transmittance through the two types of glass (container and window), the UV–visible absorption spectra of the glasses were recorded; so, it was quantified that the percentages of radiation transmitted to the body of water were 40%, 1.3%, 0.02%, 8×10^{-4}% and 8×10^{-5}% at wavelengths of 350, 320, 310, 305 and 290 nm, respectively.

A volume of 2500 mL of river water placed in glass container was spiked with INDO to achieve an initial concentration of 2 μg/L in each degradation assay. Aliquots of 25 mL were collected periodically and subjected to SPE; extracts were injected in the chromatographic system to follow the degradation of INDO, identify degradation products and monitor them. Degradation experiments were carried out between the months of November and June with waters from the rivers Pisuerga (W1 sample) and Tuerto (W2 sample). Simultaneously, the degradation of the W1 sample was also studied in the presence of sediment by adding river sediment to the container in a sediment-to-solution ratio of 0.3 g/mL (SED sample), in the absence of sunlight by coating the container with aluminum foil (DARK sample), and in the presence of nutrients by adding culture medium at the percentage of 1% (BIO sample).

It was verified that the W1 and W2 river water samples were free of INDO residues. First, water blanks were subjected to analysis to test for the absence of the parent compound. Once the degradation products yielded in the stress assays were known, tests were also performed to verify that they were not present in the water extracts. Similarly, the absence of residues in the sediment sample was confirmed; to this aim, sediment was extracted with methanol by mechanical shaking and the extract was concentrated for analysis.

1.5. Study of adsorption to sediment

Experiments were conducted to investigate the adsorption capacity of the sediment for INDO and its degradation products. The adsorption coefficient (K_d) of INDO to sediment (sieved through a 0.5 mm mesh) was determined by using a batch approach. River water (100 mL) spiked with INDO was added to sediment (20 g) to achieve a sediment-to-solution ratio of 0.20 g/mL; INDO concentrations were 200, 400, 600, 800 and 1000 ng/L. River water contained 0.02% sodium azide as a biocide to minimize any possible microbial activity and pH (7.8) was controlled with phosphate buffer (0.02 mol/L). Control solutions without sediment were prepared as well. The flasks, protected from sunlight with aluminum foil, were manually shaken for 1 min and left standing at 20 ± 1°C for a period of 24 hr. Afterwards, an aliquot of 10 mL, previously centrifuged to remove solids, was collected to determine the INDO concentration at equilibrium. An adsorption isotherm was drawn in duplicate. The concentration adsorbed on the sediment was not directly measured.

Analytical standards are unavailable for most of the degradation products, however their K_d were estimated based on the assumption that there is a linear relationship between peak area and concentration for each compound. Experiments similar to the above-described were devised by spiking water with a solution containing the degradation products and an appropriate sediment-to-solution ratio. The decrease percentage of peak area in water was assumed to be the percentage of compound adsorbed onto sediment (A%), and K_d was calculated by Eq. (1), which is valid if the adsorption equation is linear (OECD, 2000). Peak areas of the adsorption experiments were compared with those of control solutions by a *t*-test ($n = 5$) to confirm the existence of significant differences before applying the equation.

$$\log K_d = \log\left(\frac{A\%/100}{1-A\%/100}\right) - \log R \quad (1)$$

1.6. Sample preparation

Except for the photochemical degradation study, river water aliquots were eluted through EBH cartridges previously conditioned by successive elution of methanol (6 mL) and

water (6 mL). The cartridges were washed with 3 mL of a water–methanol (80:20, V/V) mixture after sample elution. The stationary phase was dried with air for 3 min and the target compounds were eluted with methanol (4 mL) by gravity. Then, the extract was evaporated in 30 min by a vacuum centrifuge evaporator heated at 40 °C, and the dry residue was re-dissolved in 0.5 mL of methanol, which was filtered through a 0.20 μm pore-size PTFE filter for chromatographic analysis.

1.7. Determination by liquid chromatography – mass spectrometry

An Acquity ultra-pressure liquid chromatograph from Waters (Milford, MA, USA) coupled to a Maxis Impact quadrupole time-of-flight tandem mass spectrometer from Bruker Daltonics (Bremen, Germany) was used. Analyses were performed with electrospray ionization in negative mode. The chromatograph was fitted with a Waters BEH ODS column (50 mm × 2.1 mm, 1.7 μm particle size). The mobile phase flow rate was 0.5 mL/min and consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B); assays were performed under gradient conditions: from 20% B to 55% B in 4.5 min, and then 60% B in 1 min. Re-equilibration time was 1 min. Injection volume was 7 μL.

The operating parameters of the electrospray ionization source for the MS and MS/MS experiments were as follows: nebulizing gas pressure, 2 bar; end plate offset voltage, 1000 V; capillary voltage, 4500 V; drying gas temperature, 200°C; dry gas flow, 6 L/min. Nitrogen was used as drying and nebulizing gas. Mass calibration adjustments were performed by using a 10 mmol/L sodium formate solution in 2-propanol/water. MS/MS experiments based on collision-induced dissociation with nitrogen gas were performed. The quantitation of INDO was accomplished using linear calibration graphs based on the measurement of peak areas in the chromatograms extracted for the fragment-ion $[M–H–CO_2]^-$ generated in the electrospray source by MS experiments, with a mass range of ±0.01 Da. Similarly, peak areas of degradation products were integrated in the chromatograms extracted for the corresponding $[M–H]^-$ ions.

As regards the performance of the analytical method, the mean recovery of INDO (2 μg/L) was about 95% with a repeatability of 1.8% for W1 sample (n = 5). The repeatability in the determination of the degradation products varied from 1.6% to 5.5% (n = 5, Appendix A Table S2).

2. Results and discussion

2.1. Degradation of indomethacin

Fig. 1 shows the variation of the INDO concentration over time in biological and stress assays; data are the average of two independent experiments, and individual data are shown in Appendix A Tables S3 and S4. After 5 weeks, the degradation of INDO in river water was negligible under aerobic and anaerobic conditions at room temperature, but when a nutrient was added to river water and the mixture was incubated at 35°C, the concentration of INDO decreased by

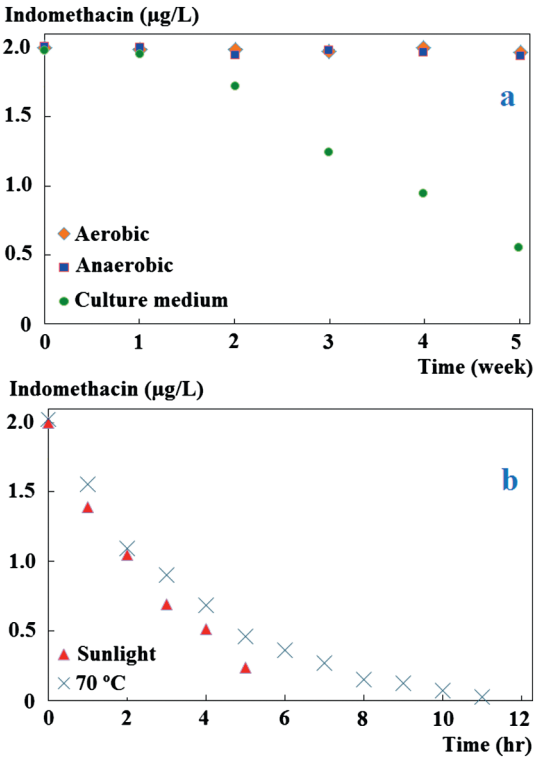

Fig. 1 – Degradation of indomethacin in river water according to biological (a) or stress (b) assays. Data are the mean of two experiments.

about 68%. A 1 week lag period was observed in this last experiment; if the initial concentration (time 0 weeks) was excluded, the variation of the remaining experimental concentrations with time could be fitted to a linear equation (R^2 = 0.98), from which a half-life of 3.70 weeks was calculated. On the other hand, heating at 70°C and UV radiation from the sunlight promoted faster degradation of INDO in river water; it could be detected for only 11 and 5 hr, respectively. Degradation data were fitted to a first-order kinetics equation with half-lives of 1.97 h (R^2 = 0.97) and 1.60 h (R^2 = 0.97) for the heating and direct irradiation assays, respectively. The UV radiation is the main factor that boosts the degradation of INDO.

The evolution of the INDO concentration over weeks in the non-stress assays can be seen in Fig. 2 and Appendix A Table S5. INDO was detected for 16 and 15 weeks in water from the two rivers (W1 and W2 samples); the concentration decreased gradually until it was less than the detection limit achieved by the method, which was estimated to be about 10 ng/L. Experimental data were also well fitted to first-order kinetics; half-lives were 2.57 (R^2 = 0.97) and 2.47 weeks (R^2 = 0.94) for W1 and W2 samples, respectively. The degradation of INDO in river water at room temperature and in the absence of sunlight (DARK sample) was slower (half-life: 5.12 weeks, R^2 = 0.98), being detected throughout the 30 weeks of experimentation. These assays indicate that solar radiation is a notable factor in the behavior of INDO inside a body of water even though the transmission of UV radiation is attenuated. However, the photochemical reactions should not be the only

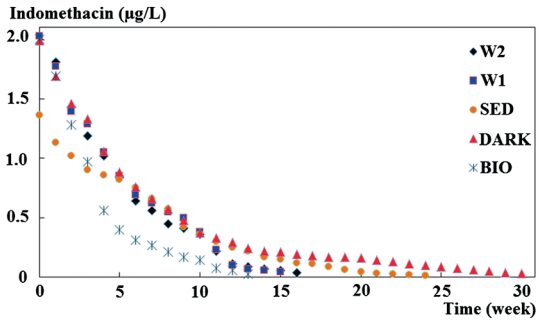

Fig. 2 – Degradation of indomethacin in river water under non-stress conditions. Data are the mean of two experiments.

factor considered because the degradation of INDO in darkness is not negligible.

INDO seems to be a non-biodegradable compound in unmodified river water. However, the degradation study over time in water whose nutrient content was enhanced (BIO sample) revealed that the degradation rate was slightly higher in comparison with that of the W1 sample. The half-life was now estimated to be 2.04 weeks (R^2 = 0.98, first-order kinetics). Otherwise, in the presence of aquatic sediment (SED sample) the half-life was higher, 3.39 weeks (R^2 = 0.96, first-order kinetics); INDO was found in the aqueous phase for 24 weeks. The measured initial concentrations in the SED sample were lower with regard to the previous assays, which suggested possible adsorption of INDO to sediment. This adsorption phenomenon could help protect INDO from sunlight exposure and increase its stability in water samples.

2.2. Detection and identification of degradation products

The presence of compounds related to degradation of INDO was established by the observation of new chromatographic peaks in the MS chromatograms, which were present neither in the previous chromatograms nor in blanks, and the gradual variation of their peak areas over time. In this way, six degradation products of INDO were found in the extracts. The $[M–H]^-$ ion of each degradation product, besides INDO, was isolated and fragmented by collision-induced dissociation to obtain structural information. MS/MS spectra (Appendix A Figs. S1–S7), discussion of the fragmentation patterns, interpretation of the fragment-ions and a brief explanation on the assignment of tentative structures to the degradation products can be found in the supplementary material. Table 1 shows the molecular formulae established and the identifications proposed for the compounds.

The compounds 5-methoxy-2-methyl-1H-indole-3-acetic acid (MMIA) and 4-chlorobenzoic acid (CBA) were unequivocally identified. They were the two basic constituent moieties of INDO. An unknown degradation product was identified as acid (DHINDO), a derivative of INDO hydroxylated at the C6 and C7 sites of the indole moiety, while the other unknown compound was thought to be 2-(4-chlorobenzamido)-5-methoxybenzoic acid (CMBA), which arose from the cleavage of the indole structure. The other two degradation products were identified as 5-methoxy-2-methyl-1H-indole-3-carbaldehyde (MMIC) and 2-acetamido-5-methoxybenzoic acid (AMBA) in agreement with two described photodegradation compounds (Temussi et al., 2011). Thus, a structure was ascribed to each compound considering spectral and bibliographic data.

2.3. Occurrence of degradation products in river water

The peak areas of the degradation products were monitored during the degradation studies; a value of 100 was assigned to the initial peak area of INDO (week 0) in each experiment, and all other peak areas were referred to this value. Fig. 3 illustrates the evolution of compounds in non-stress conditions. Data for all compounds and experiments are provided as Supplementary material. Four compounds were found in W1 and W2 samples (Appendix A Figs. S8 and S9), whose degradation conditions were intended to simulate the behavior of INDO inside a body of water; CBA, AMBA and MMIA were the major degradation products in the first 15 weeks, while CBA together with AMBA were the only compounds detected after 30 weeks. CBA was the most abundant degradation product in terms of peak area; its amount increased gradually until about the 10th week, and then decreased slowly. AMBA was a minor degradation product that seemed to persist over time. DHINDO was also a minor compound that reached its maximum occurrence at about the 9th–10th week; like MMIA, DHINDO was not detected after the 19th week. The evolution profile of the compounds was basically similar in waters from the two rivers (W1 and W2). The joint presence of CBA and

Table 1 – Retention times (RT), masses measured in the mass spectra for the $[M-H]^-$ ion, errors in the determination of exact masses, molecular formulae and number of rings and double bonds (rdb) of the corresponding structures for the detected degradation products.

RT (min)	Molecular formula	Exact mass (Da)	Measured mass (Da)	Error (ppm)	rdb	Compound	Abbreviation
0.89	$C_{10}H_{10}NO_4$	208.0615	208.0616	−0.5	6.5	2-acetamido-5-methoxybenzoic acid	AMBA
1.15	$C_{11}H_{10}NO_2$	188.0717	188.0724	−2.3	7.5	5-methoxy-2-methyl-1H-indole-3-carbaldehyde	MMIC
1.26	$C_{12}H_{12}NO_3$	218.0823	218.0824	−0.5	7.5	5-methoxy-2-methyl-1H-indole-3-acetic acid	MMIA
2.04	$C_7H_4ClO_2$	154.9905	154.9905	0.0	5.5	4-chlorobenzoic acid	CBA
2.82	$C_{19}H_{17}ClNO_6$	390.0750	390.0746	1.0	11.5	1-(4-chlorobenzoyl)-6,7-dihydro-6,7-dihydroxy-5-methoxy-2-methyl-1H-indole-3-acetic acid	DHINDO
3.80	$C_{15}H_{11}ClNO_4$	304.0382	304.0378	−1.3	10.5	2-(4-chlorobenzamido)-5-methoxybenzoic acid	CMBA
4.25	$C_{19}H_{15}ClNO_4$	356.0695	356.0696	−0.3	12.5	1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid	INDO

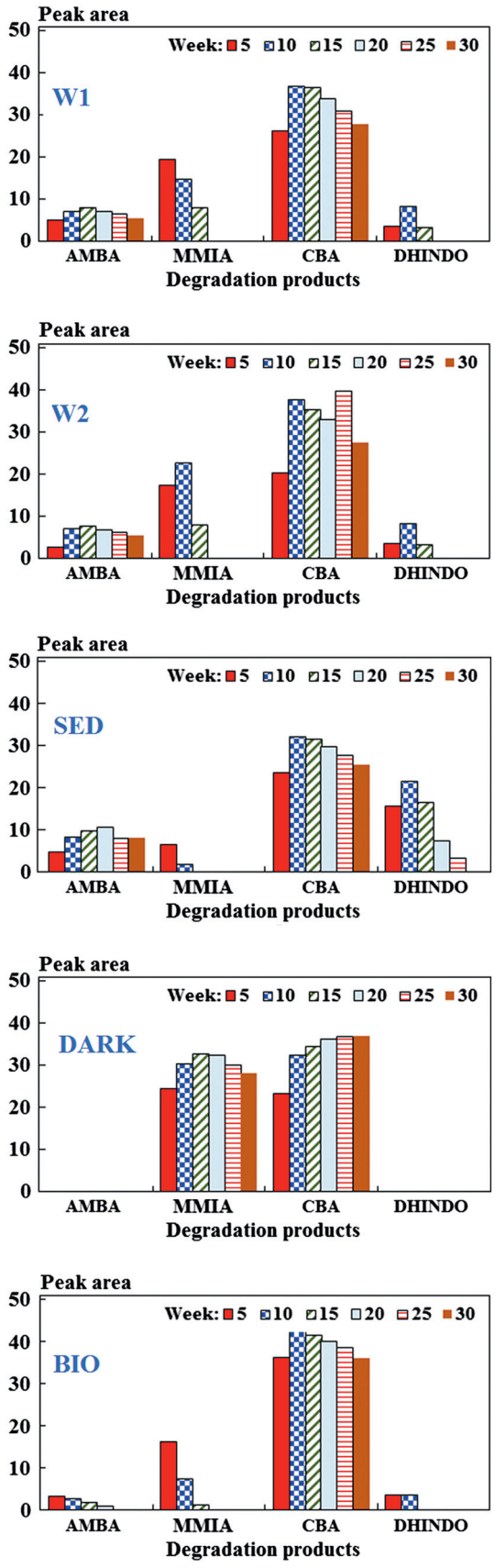

Fig. 3 – Variation of peak areas of the degradation products in river water under non-stress conditions. Mean of two experiments.

AMBA in surface water can constitute an indicator of the earlier presence of INDO in that body of water.

The evolution of the degradation products when the amount of nutrients in river water was increased to favor the growth of microorganisms (BIO sample) followed the pattern described for W1 and W2 samples, with the difference that the transformation rate of the compounds increased somewhat, as was previously stated for the degradation of INDO (Appendix A Fig. S10). Excepting CBA, all degradation products were detected during a smaller number of weeks; even AMBA was now fully degraded in 23 weeks.

Finally, the transformation products found in the SED sample (Appendix A Fig. S11) were the same as those in the W1 and W2 samples, although the evolution profile was clearly different. Regardless of the lower degradation rate of INDO, MMIA was detected only in extracts for 10 weeks, and its peak areas were lower in relation to assays without sediment. Moreover, DHINDO was present in higher relative amounts, which was attributed not only to the lower initial peak area of INDO, but also to the higher occurrence of DHINDO, whose formation was enhanced in the presence of sediment. Furthermore, the persistence of DHINDO also increased, being detected in water for 28 weeks. However, CBA and AMBA remained as the only compounds detected after 30 weeks.

As regards the origin of the degradation products, CBA and MMIA were the only degradation products detected in the stress assay at 70°C (Appendix A Fig. S12), the DARK sample (Appendix A Fig. S13), and the water sample incubated at 35°C with added culture medium (Appendix A Fig. S14). Their occurrence in the two first samples, in addition to W1 and W2, could be easily explained by chemical hydrolysis, while biochemical hydrolysis in the water containing nutrients could not be discarded. On the other hand, the peak areas of CBA and MMIA in the DARK sample after 30 weeks were not very different between them, in contrast to W1 and W2 samples, where the amount of MMIA was clearly lower than that of CBA at the end of the monitoring; this suggested that MMIA is a relatively stable compound in the absence of sunlight.

Five degradation products were yielded by photochemical reactions. They were found when river water was directly exposed to sunlight, without passing through the glass, which absorbs UV radiation (Appendix A Fig. S15). DHINDO was the predominant product in terms of peak area, which was two-fold higher than that of INDO after 6 hr of exposure (see Supplementary material). CBA and AMBA were also detected, but not MMIA. Two other photo-induced degradation products that resulted were MMIC and CMBA, which were not noted in the samples under non-stress conditions, likely as a consequence of the attenuation of the UV radiation. It is plausible that the amount of DHINDO could be higher in a body of water if the UV radiation was less attenuated with depth.

The described photodegradation compounds can be yielded by direct or indirect photolysis; data to differentiate and discuss the two photolysis mechanisms in the degradation of INDO are not available. In direct photolysis, the molecule of INDO in the excited state (after absorbing a photon) would be unstable and would break down. In indirect photolysis, the radiation is

absorbed for one or more chemical species in the water solution (for instance, the dissolved organic matter), which could then decompose INDO by direct reaction with it or through new chemical species derived from those excited compounds.

2.4. Distribution between water and sediment. Adsorption coefficients

Fig. 4 shows the adsorption isotherm obtained for INDO in the batch experiments; the resulting K_d and K_{oc} (organic carbon normalized adsorption coefficient) values were 1.79 and 149 kg/L (RSD 5.0%, n = 10), respectively. The K_d value was comparable to that already described in the literature (Yamamoto et al., 2009). Adsorption coefficients were also calculated for the degradation products from the experimental adsorption percentages; the K_d and K_{oc} values and the sediment-to-water ratios used are given in Table 2. The adsorption coefficient of INDO was also calculated by this method and was found to be similar to the previous value. CBA, and particularly AMBA and DHINDO, had a lower capacity for adsorption than INDO. In fact, the low adsorption percentages (<10%) of the latter two degradation compounds resulted in a worse precision (28%) in the determination performed for DHINDO, and only a rough estimate of K_d for AMBA. On the other hand, the adsorption coefficients of MMIA and MMIC were relatively high, about 18-fold higher than that of INDO, which was consistent with the low peak areas observed for MMIA in the SED sample. After checking the structures of the compounds, the notable capacity for adsorption of MMIA and MMIC to sediment would be related to the presence of a N–H bond in the indole moiety, because higher adsorption coefficients have been reported in amine compounds (Yamamoto et al., 2009). It should be noted that the water–sediment interaction is not simple and that the adsorption coefficients were estimated for only a sample of water and sediment; consequently, the data provided in this work should be considered preliminary.

2.5. Degradation pathway

A main degradation pathway of INDO in river water was outlined (Fig. 5). First, the amide group of INDO is hydrolyzed to give its two constituent moieties: MMIA and CBA, whose presence would be expected (Krzek and Starek, 2001). Simultaneously, INDO undergoes a secondary degradation reaction in which the diol derivative DHINDO is formed through a photochemical reaction; it is thought that DHINDO could undergo a similar hydrolysis reaction, but its corresponding indole moiety was not found in this work.

It has been reported that AMBA arises from INDO through a photodegradation process involving the non-prominent intermediate MMIC (Temussi et al., 2011), however it seems quite possible that MMIA is transformed into AMBA by photo-induced reactions as well. In fact, the amount of MMIA remained high and relatively constant in the DARK sample, as already noted above. In order to verify the conversion of MMIA in AMBA, an INDO aliquot was subjected to hydrolysis by heating at 70°C to yield CBA and MMIA, and then was placed in a quartz cuvette for direct exposure to sunlight. It was observed that MMIA was completely removed in about 4 hr, that MMIC was very abundant after 2 hr, decreasing quickly afterwards, and that AMBA was effectively a photoproduct arising from MMIA (Appendix A Fig. S16); the CBA amount remained virtually constant in this assay. Finally, there were no data to ascertain the fate of CMBA, however it is reasonable to hypothesize its transformation to CBA by hydrolysis.

Table 2 – K_d and K_{oc} mean values (RSDs in parenthesis) calculated for the degradation products. Mean adsorption percentages (A%) onto sediment and experimental sediment to water ratios (R), n = 5.

Compound	K_d (kg/L)	K_{oc} (kg/L)	A%	R (kg/L)	Experimental t-value*
AMBA	<0.10	<8.7(–)	<5	0.507	0.6
MMIC	35	2938 (6.0%)	54.0	0.0333	10.8
MMIA	33	2756 (6.7%)	52.2	0.0331	18.2
CBA	1.1	89 (8.6%)	30.5	0.409	10.1
DHINDO	0.15	12.3 (28%)	7.0	0.509	2.57
CMBA	1.8	150 (8.1)	27.4	0.210	15.2
INDO	1.8	150 (7.8%)	27.0	0.206	16.9

* Critical t-value: 2.3; –: without data.

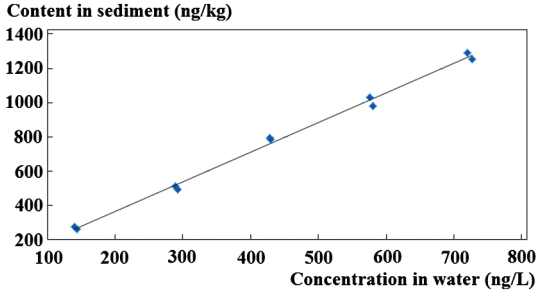

Fig. 4 – Adsorption isotherm of indomethacin (n = 2).

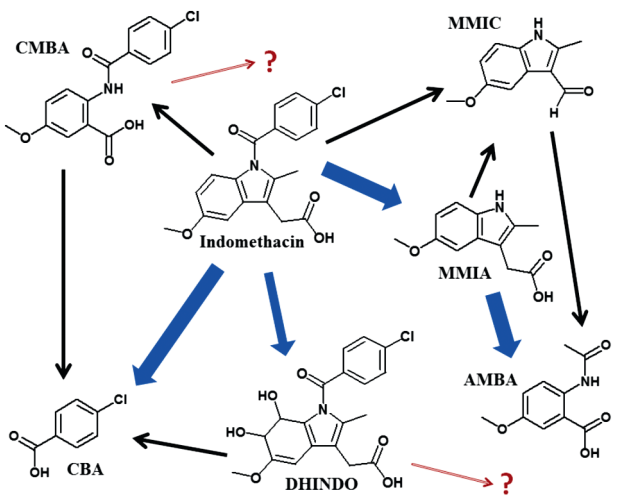

Fig. 5 – Degradation pathway of indomethacin in river water. Bold lines denote the possible predominance of the transformation process.

3. Conclusions

The hydrolysis of indomethacin into its two constituent moieties and the photochemical reactions undergone by the indole moiety were the main degradation pathways in river water. A relatively slow biological degradation took place only in the presence of high contents of nutrients. The degradation rate decreased notably when photochemical stress was avoided. The capability of the chemical and photochemical stress assays to properly predict the degradation products of indomethacin, or their relative amounts, inside a body of water was limited.

Four degradation products were found in river water under non-stress conditions and two of them, 4-chlorobenzoic acid and 2-acetamido-5-methoxy benzoic acid, were the only and persistent degradation products detected after 30 weeks. The half-life of indomethacin in river water under these conditions was estimated to be about 2.5 weeks. Two new degradation compounds of indomethacin were reported: acid and 2-(4-chlorobenzamido)-5-methoxybenzoic acid.

Indomethacin was partially adsorbed onto sediment and a degradation product, 5-methoxy-2-methyl-1H-indole-3-acetic acid, showed higher adsorption capacity. The degradation rate in contact with sediment was slower, likely as consequence of the protection supplied by the sediment against sunlight irradiation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jes.2016.08.021.

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