Minocycline detection in wastewaters

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Abstract

The tetracyclines constitute the third most consumed antibiotic group. Their low cost and broad spectrum of activity make them an attractive choice for people, especially in poor countries. However, the low metabolization rate - for instance, around 60% of the minocycline (MC) intake is excreted - of these compounds lead to of large quantities being discharged in urban and rural effluents. This represents a problem, given the fact that conventional WWTPs can't remove these micropollutants. Therefore, it is urgent to develop tertiary treatment methods that allow their elimination before discharge, with photocatalytic processes showing great promise in this regard. The purpose of this study is to evaluate the degradation capacity of MC via solar radiation under different experimental conditions in order to draw conclusions about the real applicability of the method. Therefore, the stability of MC was evaluated in water for 24 hours at two different temperatures. Furthermore, the ability of the solar simulator to degrade MC present in aqueous solutions was investigated, subjecting the system to variations in both the initial volume of solution and in the radiation source. The half-lives and kinetic constants were determined, which allowed the effectiveness of each of the variations in the system to be assessed. It was found that solar radiation could be a viable option for MC degradation.

Keywords: minocycline, photodegradation, tertiary treatment methods, solar simulator

1. Introduction

Over thousands of years, antibiotics have been applied in treatments - the Egyptians used to put a paste of mouldy bread on infected wounds - however, it was not until the end of the 19th century that scientists documented the effects of antibacterial chemicals. Paul Ehrlich, a German doctor, was the first to conclude that certain substances could kill certain bacterial cells without affecting others, revealing their selective character and in 1928, Alexander Fleming discovered penicillin, the first true antibiotic. The discovery of antibiotics is considered one of the greatest scientific achievements of the last century, having saved millions of lives [1] [2].

Minocycline (MC) is a second-generation semisynthetic tetracycline that has been used for therapeutic purposes for the past 40 years due to its antibiotic properties against gram-positive and gram-negative bacteria [3]. This antibiotic is predominantly applied in the treatment of acne and some sexually transmitted diseases. Recently, it has been reported that minocycline demonstrates a variety of biological characteristics independent of its antimicrobial

activity, including anti-inflammatory properties, capacity to inhibit tumor metastasis and effective neuroprotection [3].
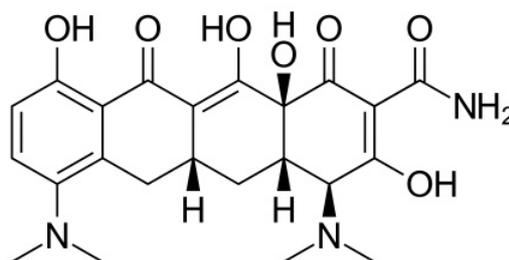

Fig. 1. Structure of minocycline (MC)

However, the increasing use of antibiotics (between 2000 and 2018 global antibiotic consumption increased by 46% [4]), often inappropriately, generates a significant amount of waste that is introduced directly and indirectly into aquatic and terrestrial environments and results in the proliferation of drug-resistant pathogens. This not only represents a biological threat to aquatic wildlife, but also to human health as contaminants progress

through the food chain [5]. Therefore, there is a need to develop mechanisms and technologies to purify wastewater, in particular to eliminate antibiotics such as MC, which is produced and marketed by pharmaceutical companies.

In the last decades, advances in analytical methods have allowed the detection of very low concentrations (in the order of ng/L) of various compounds in waters that have not been analysed so far. The tertiary treatment systems that are currently the most widely used in wastewater treatment plants, such as ionic exchange, membrane separation techniques, electrochemical processes and adsorption, are not effective enough to produce water with acceptable levels of these pollutants, so an additional treatment stage is often required to achieve this goal [6].

This is where advanced oxidation processes (AOPs) come into play. The AOPs are a group of chemical treatment methods with an interesting potential applicability. Due to their high capacity to remove organic compounds, including antibiotics, they have been suggested as a valid treatment solution to remove these pollutants from wastewater. The fundamental principle of advanced oxidation processes is the production of OH$^\bullet$, which results from H_2O_2, O_3, photocatalysis or oxidants using UV or sunlight. Hydroxyl radicals are known as strong, non-selective oxidizing agents. After their production, they start attacking organic compounds, which leads to the complete breakdown of these organic compounds [48]. Photocatalysis in particular has been focus of great attention for degrading antibiotics because of its economic, efficient and green feature. In this process, with the irradiation of UV or visible light, a semiconductor can generate hydroxyl and superoxide anion radicals; then, these radicals can mineralize a wide range of organic pollutants **[7]** (eq.1).

$$Org.\,pollut. + O_2 \xrightarrow[h\nu]{semiconductor} CO_2 + H_2O + inorganic\ ions \quad (1)$$
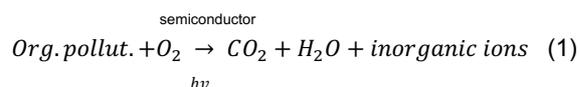
In this work, the aim was to evaluate the degree of photodegradation achieved without the presence of a catalyst, which would simulate a scenario where MC would be present in water courses exposed to sunlight. Accordingly, the mechanism of this reaction can be expressed by eq. 2:

$$R - R + h\nu \rightarrow 2R^\bullet \qquad (2)$$
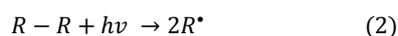
where molecules absorb radiant energy through photons, which is required to excite specific electrons and form free radicals, which undergo a succession of chain reactions to give reaction products. These free radicals can be generated by homolysis of weak bonds; by electron transfer from the excited state of the organic molecule to molecular oxygen, resulting in the superoxide radical ($O_2^{\bullet-}$) or in other chemical reagents, such as ozone or hydrogen peroxide, so that hydroxyl radicals are produced [6].

2. Materials and Methods

2.1. Materials

MC was provided by a pharmaceutical company from an intermediate step of the production process. Samples were collected in glass vials and refrigerated at 2 ºC. Reagents of analytical grade and distilled water were used for the preparation of the solutions. Acetonitrile was obtained from Fisher Chemical, orthophosphoric acid from Merck and triethylamine from Scharlau.

2.2 Material characterization

The MC powder was characterized by UV-Vis spectroscopy (Jasco V-750, Jasco Corporation, USA) before and after irradiation were evaluated in the wavelength range of 190-900 nm. TGA/DTG analysis was carried out on the Thermogravimetry/Differential Thermal Analyzer STA7200RV (Hitachi High-Tech Science Corporation) using an α-alumina sample holder. The experimental parameters for the TGA curves were the following: heating rate of 10 °C/min, under a flow rate of 200 mL/min of N2, from room temperature to 600 °C. The FTIR spectrum of MC powder was obtained by operating a Perkin Elmer spectrometer, model Spectrum Two™, in the wavenumber range 400-4000 cm^{-1} and resolution of 1 cm^{-1}.

2.3. Photocatalytic degradation experiments

The photodegradation of MC occurred under simulated solar radiation, whose source was a Newport solar simulator, model LCS-100 and ABB class with a 100 W Xenon lamp. The reference cell and meter used are of the Newport brand, model 91150V, with an error of 0.0001 Sun in the irradiance range used. A filter and a light collimator were used to evaluate the setup's influence. The procedure started with the measurement of how far the reactor should be from the lamp, so that the irradiance was equivalent to 1 Sun (1360 W/m2). The tests were performed in a 100 mL Erlenmayer flask wrapped in aluminium foil and filled with volumes of 10, 50 or 100 mL of minocycline solution of concentration 10 mg/L. The container with the solution had two tubes connected to it: one that carried the solution to the reactor and the other that brought it back. The solution circulation was done through a Hirschmann pump (Rotarus® Standard 50) operating at 24 rotations per minute. The beginning of the reaction was considered to be the moment at which the simulator was turned on and light hit the reactor. Periodically, aliquots of 3

mL were taken and the temperature was monitored, oscillating between 20 and 22 ºC during the experiments.

Table 1. System properties.

Property (units)	Value
Irradiance (W/m^2)	1360
$c_{solution}$ (mg/L)	10
$D_{i\ tubes}$ (mm)	3,2
$L_{i\ tubes}$ (m)	1,57
Q_{pump} (mL/s)	0,36
$V_{reactor}$ (mL)	0,16

It was determined that the residence time of 0.16 mL of solution in the reactor would be 0.43 seconds and that 275 seconds would be required for the 100 mL of solution to pass through the reactor. The following table illustrates a hypothetical case of an 8-hour experiment and the actual exposure time of an initial volume of 100 mL solution to sunlight.

Table 2. Number of cycles and effective exposure time in an 8-hour experiment.

Item (units)	Value
t_{exp} (h)	8
Number of cycles	105
t_{cell} (s)	0,43
t_r (s)	45

t_{exp} is the total time of the experiment, nº of cycles is the number of times that 100 mL pass in the cell during that period, t_{cell} is the time that a volume of solution equal to the reactor volume remains in the cell, and t_r is the effective exposure time of a solution volume equal to the reactor volume during the total time of the experiment.

2.4. Photocatalytic degradation measurements

Two methods were used to obtain the MC concentrations. First, the MC concentration in solution was measured by a UV-Vis spectrophotometer (Jasco V-750, Jasco Corporation, USA) in the wavelength range of 190-600 nm. The chosen wavelengths were 274,2 nm and 347,4 nm. Second, the MC concentration was determined by an Agilent (1200 series) high performance liquid chromatography (HPLC) system with diode-array detector at wavelengths 274 and 347 nm. The chromatographic conditions were defined based on (Matos et al., 2017) [8]. The column used was Purospher 120 Star RP-18 (125x4mm 5um, Lichrocart, Merck, Darmstadt, Germany) and the eluent was composed of a mixture of acetonitrile and water in a volumetric ratio of 15:85, respectively, and 0.6% (V/V) triethylamine (TEA), with subsequent adjustment to pH 3 with orthophosphoric acid (85%). The composition of the mobile phase was kept constant and the flow rate of fluid passing through the column was 1.2 mL/min throughout the process. The column temperature was 25 °C. The volume of sample removed was programmed to be 20 µL and the passage time in the column was 8 minutes, with a post-time of 2 minutes between sample suction. The selection of the wavelengths of maximum absorption was based on the UV-Vis absorption spectra and previous work by (Prasad, A.; Rao, V., 2010) [9] and (Matos et al., 2017) [8], thus the wavelengths 274 and 347 nm were considered for the quantification of minocycline.

2.5. Kinetic measurement

The kinetics of minocycline photodegradation using solar radiation tested were based on the assumptions that this would be a pseudo first order reaction and the volume change caused by the sample run would be negligible (S. Wu et al., 2020) **[10]**. It was also considered that the system would behave similarly to a batch reactor with recirculation. Thus, equation 3 translates the reaction kinetics:

$$\ln\left(\frac{c}{c_0}\right) = -kt \quad (3)$$

where c_0 is the initial concentration of minocycline in mg/L, c is the concentration of minocycline in mg/L after a given time, t, in minutes and k is the pseudo first order kinetic constant.

2.6. Calibration standard solutions

For the quantification of minocycline by UV-Vis spectrophotometry, solutions of concentration 0.02 - 40 mg/L were used (n=12). This procedure was repeated to improve the accuracy of calibration. As for the quantification of minocycline by HPLC-DAD, calibration solutions of concentration 0.08 - 10 mg/L (n=5) were used. The calibration curves were obtained by direct linear regression. In the case of UV-Vis spectrophotometry, the absorbance at the defined wavelengths was associated with the concentration of the standard solutions. For the chromatographic method, the relation was established between the area of the peaks and the concentration of the standard solutions.

3. Results and discussion

3.1. Sample Characterization

Minocycline was subjected to 3 different laboratory techniques: UV-Vis spectrophotometry, thermogravimetric analysis and Fourier transform infrared spectroscopy.

Fig.2 shows the absorption spectrum of minocycline before and after being subjected to simulated solar radiation for 24 hours. It can be seen that there are no significant changes between two

spectra, and both are characterised by a substantial increase in absorbance from 550 nm (0.4) to 400 nm (1.5).
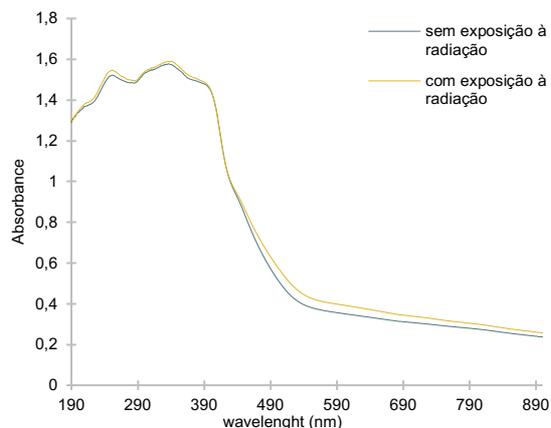

Fig 2. UV-Vis spectrum of minocycline powder in the wavelength range 190-900 nm

The DTG results allow the stability of the compound to be studied as a function of temperature (Fig. 3).
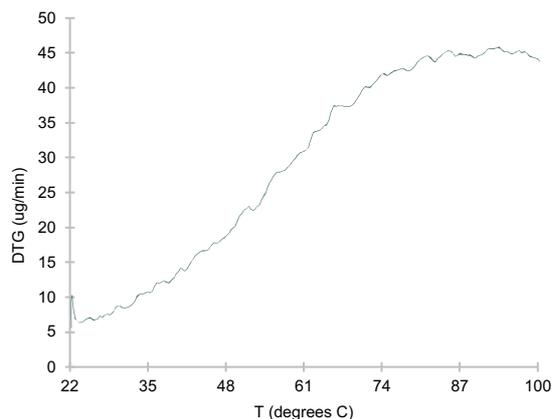

Fig 3. Derivative thermogravimetry in the temperature range 22-100 ºC

The compound shows an increase in mass loss in this temperature range, with a more pronounced incidence from 80 to 100 °C, which could be indicative of the loss of hydration of the sample **[11]**.

Figure 4 shows the FTIR spectrum of the minocycline sample. It was concluded that the stoichiometry of ordinary minocycline hydrochloride (α-minocycline) corresponds to approximately 2 molecules of water per molecule of minocycline.
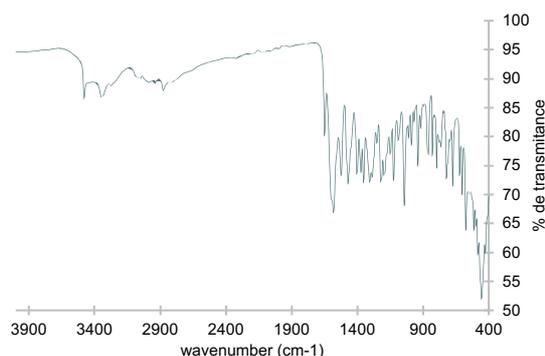

Fig 4. FTIR spectrum: α-minocycline

3.2. MC stability in an aqueous solution

The stability of minocycline was evaluated in water for 24 hours in a bench top environment (20 ºC) and in the freezer (-20 ºC). The solution had an initial concentration of 10 mg/L, prepared from a fresh stock solution. It was analyzed in the UV-Vis spectrophotometer and in HPLC-DAD and then compared with the solutions stored at 20 and -20 ºC, also analyzed in the same equipment.

Table 3. Main data of the stability study of minocycline from UV-Vis spectra.

T (ºC)	time (h)	Ratio betweeen peaks	c/c_0 (347 nm)
20	0	1,03	-
20	24	1,02	1,01
-20	24	1,02	1,01

Tabela 4. Key data from the stability study of minocycline from HPLC chromatograms

T (ºC)	time (h)	Area (mAU.s)	c (mg/L)	c/c_0 (347 nm)
20	0	181,74	11,71	-
20	24	178,28	11,49	0,98
-20	24	184,69	11,91	1,02

Based on the tables, there are no significant differences between the two storage types after 24 hours. The ratio between the final and initial concentrations of 1.01 obtained from the spectra points to the stability of the solution in both forms of storage. The results of the chromatograms in Table 13 show a slight decrease in concentration in the solution that remained on the countertop, however, this method is not error-free since the base of the peaks is difficult to define manually and the program is used to trace it automatically. Thus, the areas may suffer discrepancies that do not correspond to a real change in concentrations. Despite this issue, the results suggest that minocycline in 10 mg/L solution is stable.

It has been reported that for 6 hours and at a temperature of 20 ºC a 12 mg/L minocycline solution would suffer a degradation in the order of 15% on the countertop and 5% in the freezer [8], so the results obtained in this experiment contradict this previous study.

3.3. Influence of initial solution volume

The tests consisted in preparing three solutions of minocycline of equal concentration (10 mg/L) and different volumes: 10, 50 and 100 mL. Each volume was circulated in the system for 24 hours, with and without exposure to solar radiation. Tables 5 and 6 illustrate the results without radiation.

Table 5. Main analysis from experiments without solar exposure and from spectra

V_0 (mL)	time (h)	Ratio between abs peaks	c/c_0 (347,4 nm)
100	0	0,99	1,00
	24,6	0,98	
50	0	1,00	1,00
	24,5	0,99	
10	0	1,02	1,02
	24,4	1,04	

Table 6. Key analysis data from experiments without sunlight exposure and chromatograms

V_0 (mL)	Time (h)	Area (mAU.s)	c (mg/L) 347 nm	c/c_0 (347 nm)
100	0	144,29	9,3	0,99
	24,6	143,11	9,2	
50	0	124,06	8,0	0,71
	24,5	87,72	5,6	
10	0	115,50	7,4	1,17
	24,4	134,75	8,7	

Table 5, referring to the spectra, points, once again, to a scenario of possible evaporation of the 10 mg/L solution during the experiment, since c/c0 shows a value greater than unity. Still, it is possible to infer that the concentration of minocycline in solution was maintained. However, in the case of Table 15, we see that the areas of the chromatogram peaks do not allow a clear interpretation. If, on the one hand, the

chromatogram of the solution with an initial volume of 100 mL shows identical areas at the beginning and end of the experiment, on the other hand, the 50 mL and 10 mL solutions differ in the conclusions that can be drawn. The 50 mL solution shows a considerable degree of degradation of minocycline in the period analyzed, contrary to the data provided by the spectra. The spectra of the initial and final samples of the 10 mL solution indicated that the concentration was maintained, which was not the case in the chromatograms, with a substantial increase in the area of the final sample compared with the initial one. Although the results of this experiment and the stability studies performed in IV.3 are not totally congruent, the evidence points to the maintenance of the concentration of CM in aqueous solution for 24 hours, highlighting the importance of studying mechanisms that allow its degradation in water lines. The procedure of the experiment described above was repeated, but this time exposing the solutions to solar radiation. Figures 5 and 6 show the variation of MC concentration as a function of actual irradiation time and initial solution volume.
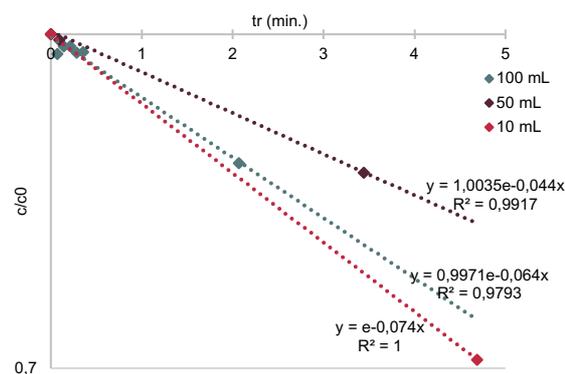

Fig 5. Variation of the concentration of minocycline under different volumes of initial solution, calculated from the absorbances of the spectra.
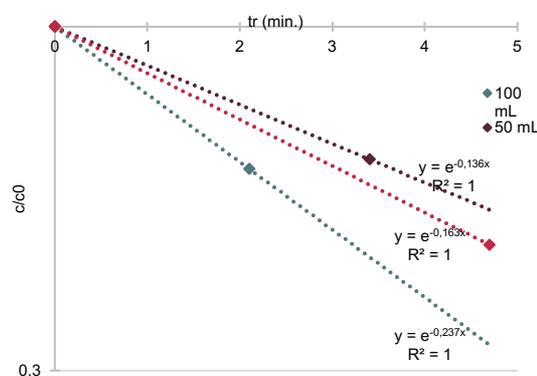

Fig 6. Variation of minocycline concentration under different volumes of initial solution, calculated from the peak areas of the chromatogram

R^2 value was higher than 0.97 in all cases, which validates the correlation. Since the initial solution volume will determine the number of cycles that this volume fulfils in the system, and consequently the radiation exposure time, it would be expected that the 10 mL solution would be the one that would present the steepest slope line in both graphs, indicating a higher kinetic constant. This assumption was verified in the case of the data obtained from the spectra, however, in the case of the chromatograms, the 100 mL solution exhibited the highest degradation rate. In both cases, the 50 mL solution exhibited the lowest degradation rate.

Table 7. Main data from initial volume change experiments and spectra

V_0 (mL)	time (h)	Real exposure time, t_r (min)	Ratio between Abs peaks	c/c0 (347,4 nm)	k (min^{-1})	$t_{1/2}$ (min.)
100	0,0	0,0	1,06	0,87	0,064	10,8
	22,1	2,1	1,19			
50	0,0	0,0	1,03	0,86	0,044	15,8
	18,4	3,4	1,13			
10	0	0	1,02	0,71	0,074	9,4
	5	4,7	1,26			

Table 8. Main data from initial volume variation experiments and chromatograms

V_0 (mL)	Time (h)	Real exposure time, t_r (min.)	Area (mAU.s)	c (mg/L)	c/c$_0$ (347 mn)	k (min^{-1})	$t_{1/2}$ (min.)
100	0	0	137,47	8,85	0,61	0,237	2,9
	22,1	2,1	83,85	5,38			
50	0	0	110,56	7,11	0,63	0,136	5,1
	18,4	3,4	69,79	4,47			
10	0	0	115,37	7,42	0,47	0,163	4,3
	5	4,7	54,11	3,46			

In Table 7, the initial ratio between the first wavelength and the second at the concentration of 10 mg/L is approximately 1. However, in all the tests a more marked decrease in absorbance occurred at 347.4 nm than at 274.2 nm, which may suggest degradation in a specific area of the molecule. This phenomenon was documented by Wiebe and Moore [12], who attributed this variation in the ratio between

absorbance peaks to the fact that the chromophore cluster on the BCD ring contains an ionizable system. For the 10, 50 and 100 mL solutions, the half-lives determined via spectra are 9.4, 15.8 and 10.8 minutes, which correspond, in the system in question, to an experiment time of 100, 169 and 115 hours, respectively. For the same 10, 50 and 100 mL solutions, the half-lives calculated from peak areas are 4.3, 5.1 and 2.9 minutes, which, in the same order, correspond to 46, 54 and 31 hours in the system.

3.4. Influence of the setup

In this subchapter the impact that changes in the sunlight source could have on the MC degradation in the system was appreciated. For that, two experiments were performed: in the first, a filter was placed at the light output and, in the second, a collimator was installed in the optical path between the lamp and the light output. The filter reduced the irradiation intensity by about 30%, to 0.7 Sun. Regarding the collimator, it will act on the divergent light beam emitted by the source, turning it into a collimated beam, i.e. a beam in which the light propagates essentially in one direction. In theoretical terms, a perfectly collimated beam does not scatter with distance. Figures 7 to 12 illustrate the spectra and chromatograms of the samples taken from the experimental activities, including those of the original system (without the collimator and the filter):
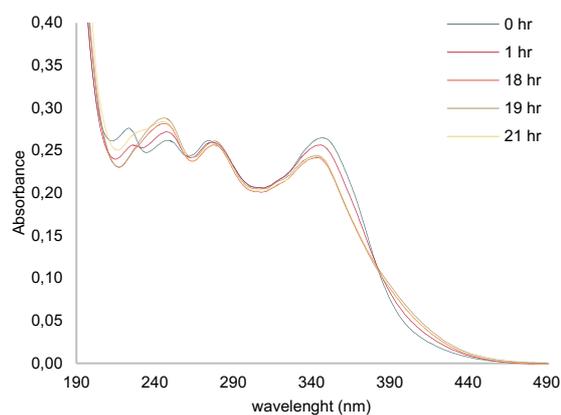

Fig 7. UV-Vis spectrum in the wavelength range 190-490 nm. V0 = 100 mL c0 = 10 mg/L with filter

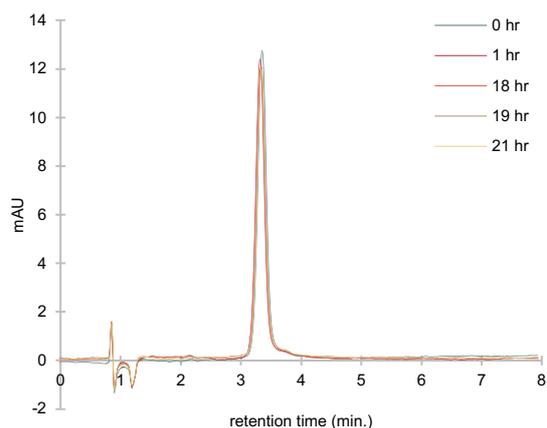Fig 8. Chromatogram (347 nm) of the samples taken from the solution with V0 = 100 mL c0 = 10 mg/L with filter

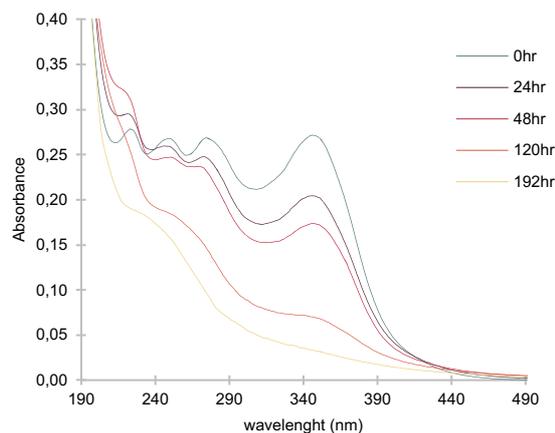

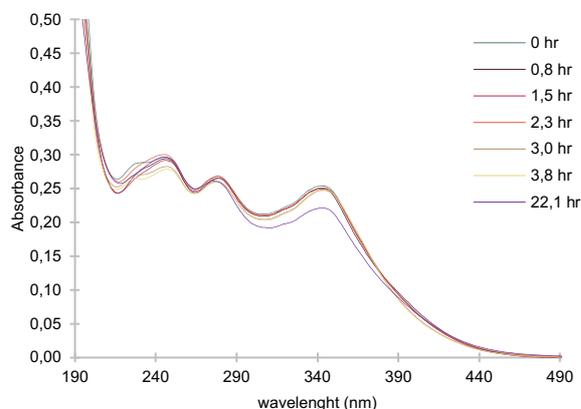Fig 11. UV-Vis spectrum in the wavelength range 190-490 nm. V0 = 100 mL c0 = 10 mg/L with collimator

Fig 9. UV-Vis spectrum in the wavelength range 190-490nm. V0 = 100 mL c0 = 10 mg/L
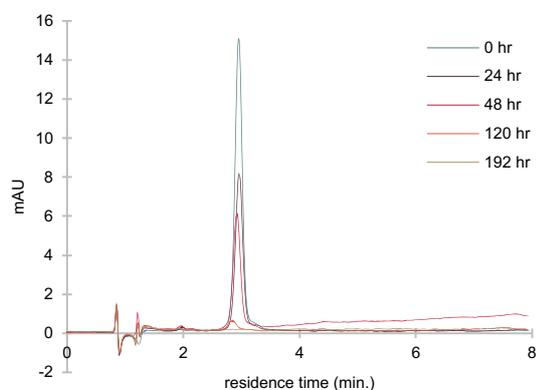

Figura 12. Chromatogram (347 nm) of the samples taken from the solution with V0 = 100 mL c0 = 10 mg/L with collimator

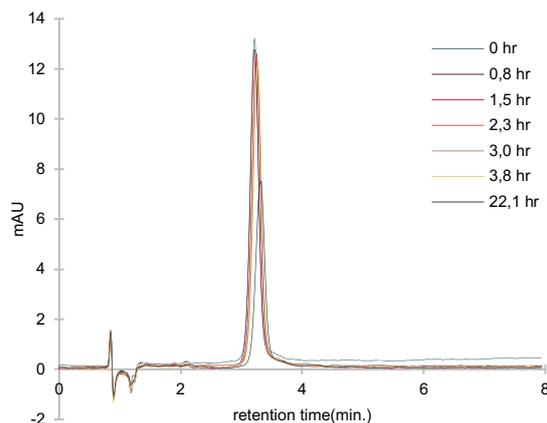The spectrum of the samples irradiated by simulated filtered sunlight indicates a reduction in absorbance at wavelength 347.4 nm and maintenance at 274.2 nm of the absorbance values - a behaviour characteristic of tetracyclines when irradiated with sunlight, as discussed earlier. The chromatogram reflects a slight decrease in peak area over time. As for the collimated light experiment, we tried to study the evolution of the samples' spectrum up to the point where MC was no longer detected in solution. In the spectra of the samples taken after 0, 24 and 48 hours it is possible to identify the characteristics of the typical spectrum of the CM, however, the samples obtained after 120 and 192 hours reveal an atypical spectrum, which suggests that, although it is possible to assign an absorbance value in the wavelengths 247.2 and 347.4 nm, the compound may no longer be in solution. The HPLC analysis confirms this possibility. Figure 12, concerning the chromatogram of the samples taken

Fig 10. Chromatogram (347 nm) of the samples taken from the solution with V0 = 100 mL c0 = 10 mg/L

from the solution irradiated by collimated light, shows that the peaks progressively decrease as the samples are taken from the solution; after 120 hours, the peak is barely perceptible and at the end of the experiment, after 192 hours, the peak is not detected.

The calculations of the variation of MC concentration as a function of the real irradiation time and of the volume of the initial solution according to the spectra and chromatograms are given by Figures 13 and 14, respectively:
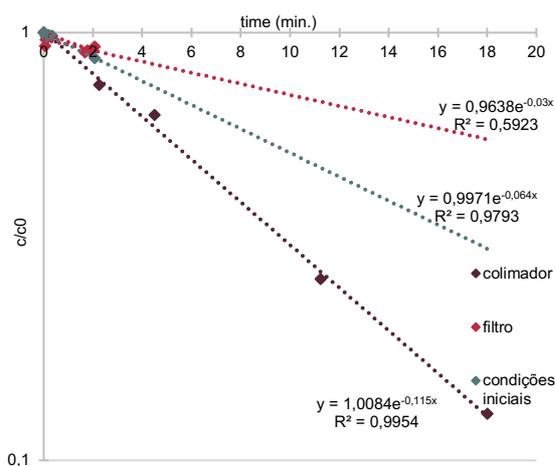

Fig 13. Variation of minocycline concentration under different setup variations, calculated from the absorbances of the spectra
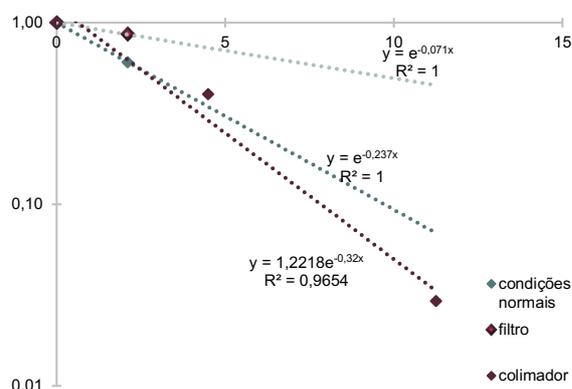

Figura 14. Variation of the concentration of minocycline under different volumes of initial solution, calculated from the absorbances of the spectra

In both graphs, where also the results of the "standard" irradiation were included, that is, without filter or collimator, the system with collimated light presents a higher degradation rate than the other two. On the contrary, the filtered system has the lowest degradation rate, even though the R^2 of the trend line has a low value in the case of the spectra-based method, calling into question the validity of this result. Once again, the graph of the variation of concentration as a function of irradiation time referring to the chromatogram reveals higher kinetic constants than that obtained through the spectra.

Table 9. Main data from setup variation experiments and spectra

V_0 (mL)	Irradiation (Sun)	time (h)	Real exposure time, t_r (min.)	peak ratio	c/c_0 (347,4 nm)	k (min^{-1})	$t_{1/2}$ (min.)
100	1,00	0,0	0,0	1,06	0,87	0,064	10,8
		22,1	2,1	1,19			
100 (colimator)	1,00	0,0	0,0	0,99	0,13	0,115	6,1
		192,0	18,0	2,99			
100 (filter)	0,70	0,0	0,0	0,99	0,93	0,030	21,9
		22,2	2,1	1,05			

Table 10. Main analysis data from setup variation experiments and chromatograms

V_0 (mL)	Experiment time (h)	Real exposure time, t_r (min.)	Area (mAU.s)	c (mg/L)	c/c_0	k (min^{-1})	$t_{1/2}$ (min.)
100 (filter)	0	0	138,29	8,90	0,86	0,071	9,8
	22,2	2,1	119,34	7,68			
100 (collimator)	0	0	142,16	9,15	0,03	0,32	2,8
	120	11,3	4,86	0,27			
100	0	0	137,47	8,85	0,61	0,237	2,9
	22,1	2,1	83,85	5,38			

It should be noted that, in formal terms, the kinetic constants obtained in the experiments performed are relative to the system and not to the reaction, since the constants vary with the conditions of the system, i.e. with the initial volume of solution and the differences in the Setup. Based on the values of the kinetic constants shown in the tables, it is concluded that the rate of photodegradation is proportional to the intensity of sunlight and that collimated light is more efficient in degrading the compound than scattered light, so the introduction of the collimator constitutes an optimization of the process. This improvement is more evident in the results obtained by the calculation method via spectra. The placement of a filter, which represents a scenario of irradiation with lower intensity, affected the performance of the system in a negative way in the degradation of minocycline. In this

case, the introduction of a catalyst (e.g. TiO2 [10]) could be an alternative to improve the efficiency of the system.

3.5. Case study

Relating the studies described above to a real situation, in which the presence of CM in Portuguese waters is estimated and, based on the data available about the maximum limits allowed by law, a prediction is made about the applicability of the proposed method. The scenario that is interpreted involves the assessment of the ability of sunlight to degrade the minocycline present in watercourses. Thus, of the experiments carried out, the one that seems to be closest to real conditions is the one performed with a solution with an initial volume of 100 mL, which, because it has a larger volume than the other cases studied, is less susceptible to variations in volume caused by evaporation that could affect the initial results. It will also be considered that the light is scattered and unfiltered, with an irradiance of 1 Sun, an intensity approximately equal to that experienced on the earth's surface. The kinetic constant considered will be the one obtained through the method that is based on the absorbance of the spectra, since this method presents lower values for the kinetic constants and, as such, the risk of simulating excessively optimistic scenarios is reduced. According to Pena et al. (2010) [13], MC levels of 0.318-0.532 mg/L were reported in four hospital effluents and 0.350-0.915 mg/L in tributaries of municipal wastewater treatment plants, in Coimbra. As there are no data concerning the Lisbon area, for calculation purposes the highest concentration of LC, 0.915 mg/L, in the waters to be treated will be considered, since Lisbon is a more populated area than Coimbra and, as such, a higher consumption of the compound is expected. MC concentration limits are lacking in most of the countries' legislations. The European Medicines Agency has defined the environmental risk by the minimum predictable no effect concentration [14]. As it was not possible to obtain information regarding this concentration for MC, the minimum predictable no-effect concentration of tetracycline in children, 4.3×10^5 ng/L [15], was considered as the concentration target to be achieved with the photodegradation method. As for the availability of sunlight, it is estimated that Lisbon has 3045 hours of sunlight annually, which gives a daily average of about 8 hours. The relevant data for the estimation of the time it takes to degrade the MC

present in the wastewater to safe values are shown in Table 11.

Tabela 11. System relevant data

Characteristic	Value
Concentration of MC in waters (mg/L), c_0	0,915
Safe concentration of MC (mg/L), c	0,430
c/c_0	0,47
k (min^{-1})	0,064
Necessary time of solar exposure in order to occur degradation (hours)	126
Daily sunlight hours	8
Necessary time for the desired degradation to happen (days)	15

The required concentration reduction was 53% and, according to the calculations, would take 15 days to occur. This prediction model has limitations. The kinetic constant used in the calculations was obtained in an experimental environment and with monitored temperature, contrary to the simulated scenario in which there would be sharp temperature oscillations. As previously mentioned, in the experimental activities, of the total duration time of the experiment, only a small fraction corresponded to effective radiation time. This principle was also applied in this model, considering that, of the solar exposure time required for the degradation to occur up to the desired concentration, only a part would correspond to effective solar exposure. It should be taken into account that the turbidity of the water and the gradient of sunlight intensity with depth were not quantified. Depending on the seasonality of CM consumption, the degradation time may vary [73].

4. Conclusion and future work

In the present work, the photodegradation of the antibiotic minocycline in aqueous solution was studied using solar radiation. The stability studies revealed that the compound is stable in aqueous solutions of 10 mg/L at -20 ºC and 20 ºC. It was found that the decrease in the initial solution volume translates into an increase in the degradation of MC, according to the quantification based on spectra, however, the quantification based on HPLC-DAD did not confirm this hypothesis. The variations in the setup, consisted in the introduction of a filter and the addition of a collimator. The half-lives, according to UV-Vis and HPLC-DAD based quantification, were 21.9 and 9.8 minutes for the system with filter, respectively, and 6.1

and 2.8 minutes for the system with the collimator. The half-life times calculated for the system without filter and without collimator were 10.8 and 2.9 minutes. In the 192-hour assay, where collimated light was used, it was possible to degrade all of the minocycline. These data support that the introduction of the collimator improves the performance of the system, representing an opportunity to optimise water treatment systems involving simulated sunlight. A case study was carried out in which the concentration of MC in wastewater in the Lisbon region was estimated and the results of the studied method were evaluated when applied to a real situation. The method proved to be effective, reducing the concentration of the compound under study to the desired limit after 15 days. It is important, however, to determine the environmental impact that the permanence of the CM would have on animal life and public health during that period. In order to refine this estimate, determining the influence of water turbidity and the gradient of sunlight intensity with depth on the degradation of the compound could be a future study. Investigating the photodegradation products and their toxicity is also of utmost relevance. This method is promising, not only from the perspective of practical design and application of the system, but also from the potential of optimising the degradation parameters in order to ensure a sustainable activity. In a region such as Lisbon and Tagus Valley, which has over 3000 hours of annual sunshine, the use of photolysis, alone or coupled with other mechanisms, for the degradation of antibiotics appears to be a potentially viable hypothesis, through the evaluation of the impact of its photodegradation products and their permanence in the system for a determined time.

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