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Data fusion applied to the photodegradation study of ciprofloxacin using hyphenated detection systems (UV–Vis and fluorescence) and multivariate curve resolution

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ABSTRACT

In this article, a new method was developed based on a flow system with hyphenated UV and fluorescence detection to study the photodegradation of ciprofloxacin. The photodegradation was investigated at both pH 7.0 and 9.0 (Britton-Robinson buffer), i.e. the conditions normally found in hospital wastewaters (HWWs) and urban wastewaters (UWWs). The flow system included a reactor (12 m in length and 0.5 mm internal diameter) coiled on a germicide UV lamp (λ_{max} 254 nm, 15 W). Ciprofloxacin solution was pumped through the system at the optimal conditions (0.1 mL min^{-1} flow rate), with a maximal time of residence in the photoreactor of 18.8 min. Both UV and fluorescence spectra were taken every 4 s. The UV spectra were recorded from 210 to 400 nm ($\Delta\lambda = 2.0$ nm), while the fluorescence spectra were recorded from 372 to 620 nm ($\Delta\lambda = 1.7$ nm). A data fusion strategy was carried out to analyze the obtained spectroscopic signals and the Hard and Soft Multivariate Curve Resolution (HS-MCR) was applied to obtain the pure spectra and concentration profiles related to ciprofloxacin and its main degradation products, as well as the corresponding kinetic rate constants. Previously, photodegradation pathways were proposed, taking into account the concentration profiles recovered by pure soft modeling analysis. The rate constants for ciprofloxacin obtained after HS-MCR analysis were 0.0302 s^{-1} and 0.0322 s^{-1} for pH 7.0 and 9.0, respectively. The reaction pathway and the kinetic rate constants were in close agreement with the ones reported in the bibliography and with the HPLC analysis previously performed. The proposed method is simple, rapid, and represents a greener and cheaper alternative to HPLC for studying photodegradation processes.

1. Introduction

Multivariate curve resolution-alternating least squares (MCR-ALS) is a broadly used chemometric technique of inestimable value. It has a wide range of applications including investigation of chemical reactions (both kinetics and equilibria) using multivariate spectroscopic measurements (UV, fluorometric, near infrared, etc.) [1–4], macromolecular [5–6] and biochemical processes [7–8], mixtures analysis [9], environmental data analysis [10–11], improving of resolution for separation techniques (HPLC, gas chromatography, capillary electrophoresis) [12–14] and images resolution [15–16], among others.

In addition, this versatile technique offers the possibility to simultaneously analyze different information from the same system, a procedure known as *data fusion* [17]. The data fusion is able to integrate different kind of data to extend the knowledge about the system and also to overcome, if present, the rank deficiency problem [18].

Despite the versatility of the soft modeling approaches —including MCR-ALS— these methods do not provide estimates of process parameters, such as kinetic rate constants. This drawback can be solved by introducing a kinetic model as a hard constraint in the ALS algorithm. Thus, this hybrid of hard and soft modeling —the so-called HS-MCR— provides the rate constants as additional output information [19–20]. HS-MCR also helps to minimize the rotational ambiguity typically associated to the soft resolution methods [21]. As was previously mentioned, these techniques are useful to study reaction kinetics. In this sense, the photodegradation of pharmaceuticals is a topic of great importance at present, mainly from the remediation processes point of view [22]. Fluoroquinolones constitute a group of drugs susceptible of being photodegraded by the effects of natural and artificial radiation

Abbreviations: MCR-ALS, multivariate curve resolution-alternating least squares; UV, ultraviolet; F, fluorescence; HPLC, high performance liquid chromatography; HS-MCR, hard and soft multivariate curve resolution; CIP, ciprofloxacin hydrochloride; DAD, diode array detector; SVD, singular value decomposition; LOF, lack of fit; EV, explained variance; HWWs, hospital wastewaters; UWWs, urban wastewaters; LC–MS/MS, liquid chromatography coupled to tandem mass detection; UV-F/HS-MCR, HS-MCR applied to UV-F augmented matrices; UV–UV/HS-MCR, HS-MCR applied to UV–UV augmented matrices; *k*, kinetic rate constants; GAC, green analytical chemistry.

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[23–24]. They belong to a group of synthetic antibiotics, widely used in both human and veterinary medicine. It is estimated that about 100,000–200,000 t of these antibiotics are consumed worldwide [25].

Fluoroquinolones can reach the environment through the elimination of the unused drugs into the sewage system or via animal and human wastes. Once absorbed into the body, a high proportion of unmetabolized fluoroquinolones are eliminated through the kidneys (the fraction of dose excreted unchanged in urine for ciprofloxacin is about 65%) [26]. Therefore, if wastewater is not treated — mainly in hospitals — it could be assumed that large amounts of these antibiotics are released into the environment while they are still pharmacologically active [27]. Moreover, the presence of antibiotics in the environment leads to the phenomenon of bacterial resistance, problem of great relevance for the human health [28].

Biodegradation is still the predominant treatment of wastewater. However, this method is not useful when the compounds are not-biodegradable or are toxic for the organisms responsible of biodegradation. In these cases, photolysis is an interesting alternative [29]. Hence, the knowledge about the photodegradation kinetics of the parent compounds and its possible photoproducts is an important issue.

Degradation of fluoroquinolones is usually studied in batch by analysis of aliquots taken from the reacting mixture at predetermined times using UV [30], HPLC [31–32] or gas chromatography [33]. This procedure could be tedious and the analysis not always reliable. On the one hand, a considerable time could elapse between the sampling and the injection in the chromatograph, during which the degradation is still taking place. Moreover, additional errors may arise from the sample handling. One way to solve these disadvantages is performing the photodegradation in a continuous flow system [34–35].

The goal of this study is to develop a rapid, simple and economical method to study the photodegradation of ciprofloxacin (CIP) by using a continuous flow system with hyphenated detection techniques (i.e. UV and fluorescence). The data set recorded during the photodegradation was analyzed by HS-MCR using the data fusion approach. In this way, spectral and concentration profiles of the main components involved in the system and the corresponding kinetic rate constants were obtained. Reaction pathways were proposed on the basis of the concentration profiles obtained from pure soft modeling approach (MCR-ALS). The proposed kinetic models were compared with the information available in the literature.

2. Materials and methods

2.1. Reagents and solutions

Analytical reagent-grade chemicals and ultra-pure deionized water (18.2 MΩcm, Barnstead, Dubuque, USA) was used. Britton-Robinson buffer solution pH 7.0 and 9.0 were prepared by mixing appropriate volumes of 0.04 mol L^{-1} of acid solutions: acetic acid (Mallinckrodt), phosphoric acid (Tejon) and boric acid (Sigma), and a 0.2 mol L^{-1} NaOH solution (Anedra). Ciprofloxacin hydrochloride (BioChemika Fluka) stock solution of 1000 mg L^{-1} was prepared in water. Ciprofloxacin working solution of 60 mg L^{-1} was prepared by appropriate dilution of the ciprofloxacin stock solution in buffer.

2.2. Apparatus

A germicide lamp with a maximum emission line at 254 nm was used to carry out the photodegradation reactions. The source of radiation was a low mercury UV lamp with a power of 15 W (Philips). The flow system components were made of PTFE (0.5 mm i.d). Photoreactor (12 m in length) was helically arranged around the lamp.

A Gilson Minipuls 3 peristaltic pump was used to propel the fluids into the system. A diode array Agilent 8453 spectrophotometer and an Ocean Optics fluorometer, both equipped with a Hellma 176752-QS quartz flow cell (25 μL inner volume, 1.5 mm light path), were used.

A modular Agilent 1100 Series instrument (Agilent Technologies, Waldbronn, Germany) with UV diode array detector (DAD) were used to carry out the chromatographic analysis. The separation was performed in a 5_m Zorbax Eclipse XDB-C18 column (4.6 mm × 150 mm). The experimental conditions of the chromatographic separation can be found elsewhere [24]. All measurements were done at room temperature.

2.3. Experimental procedure

The continuous flow system used in this study is shown in Fig. 1. The ciprofloxacin solution is pumped through the system at a constant flow rate of 0.1 mL min^{-1}. When the photoreactor (12 m) is completely filled, the UV-lamp is switched on. The UV and fluorescence spectra are recorded as the solution inside the reactor reaches the respective detectors.

In this way, it is assumed that the first spectra taken at each instrument correspond to $t_0 = 0$ s of photodegradation time (no degraded ciprofloxacin), whereas the last one corresponds to a reacting mixture with a maximum time of residence in the photoreactor. The maximum time of photodegradation achieved in the system was 18.8 min. During this period of time, the spectra were taken on both instruments every 4 s. The UV spectra were recorded from 210 to 400 nm ($\Delta\lambda = 2.0$ nm), while the fluorescence spectra from 372 to 620 nm ($\Delta\lambda = 1.7$ nm). All recorded spectra were exported and converted into MATLAB® binary files [36].

The experimental data were arranged in matrices. Their rows correspond to the spectra taken at each photodegradation time and their columns are related to the change in signal intensity at each wavelength as a function of time (Fig. 2). Two different individual data matrices were obtained for each experiment conducted at pH 7.0 and 9.0: the UV data matrix, named **A** (282 × 96 in size) and the fluorescence data matrix, called **B** (282 × 147 in size). The row-wise augmented data matrix **D** (282 × 243) was arranged with the individual **A** and **B** matrices. Accordingly, $\mathbf{D_1}$ and $\mathbf{D_2}$ augmented matrices correspond to the degradation at pH 7.0 and 9.0, respectively.

For the chromatographic analysis, the ciprofloxacin solution was photodegraded in the same system. Five aliquots were collected from the system at different times during the photodegradation experiment (0, 64, 152, 400 and 800 s) and immediately placed in amber vials until the chromatographic analysis.

2.4. Data analysis

The data analysis was carried out using soft-modeling multivariate curve resolution [37] and this subroutine was modified in our laboratory to perform HS-MCR. In this algorithm, a hard-modeling constraint is included in the iterative soft-modeling MCR-ALS to force the concentration profiles to satisfy a proposed kinetic model [20,34].

Previously to the application of HS-MCR, the determination of the number of components and the construction of the initial estimate were carried out. The number of components or factors in matrices $\mathbf{D_1}$ and $\mathbf{D_2}$ (chemical rank) was investigated by Singular Value Decomposition (SVD) [34,38]. It was expected that singular values associated with chemical components would be larger than singular values related to noise and experimental error. Thus, it was assumed that the rank of the data matrix was equal to the number of species that contribute to the signal and no other contributions, such as instrumental noise, were present. When rank deficiency is present, i.e. when the number of factors is lower than the expected sources of variability, augmented matrices strategy makes it possible to overcome this problem [39–40]. On the other hand, in this study, an initial estimate for the spectra was obtained by using the SIMPLISMA algorithm, a technique based on selecting of the *purest* variables [41]. Thus, SIMPLISMA was applied to both augmented matrices $\mathbf{D_1}$ and $\mathbf{D_2}$ to search for spectral estimates of the different components.

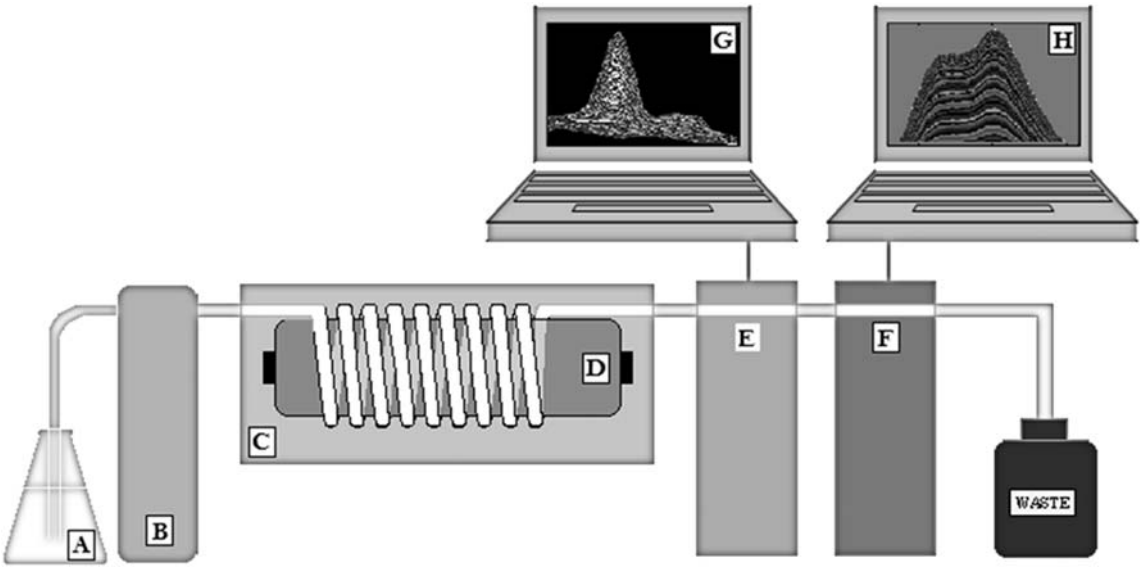

Fig. 1. Schematic representation of the flow system for ciprofloxacin photodegradation. Ciprofloxacin solution (A), peristaltic pump (B), protector box (C), UV lamp (D), spectrophotometer (E), fluorometer (F) and personal computers (G and H).

During the optimization by alternating least squares (ALS) the following constrains were applied:

a) The values of the spectra of each component must be non-negative.
b) The values of the concentration profiles as a function of time must be non-negative.

When HS-MCR was applied, the additional hard-modeling constraint was fulfilled and therefore the concentration profiles must satisfy the imposed kinetic model.

The parameters used to indicate the fit quality of the model were the percentage of lack of fit (% LOF) and the percentage of explained variance (% EV) [42].

3. Results and discussion

3.1. Proposed kinetic model

The spectra obtained from the UV and fluorescence monitoring of the ciprofloxacin solutions confirmed the degradation of this compound under UV-light. As an example, Fig. 2 shows a three dimensional view of the spectra contained in the $\mathbf{D_1}$ matrix, corresponding to the photodegradation of ciprofloxacin at pH 7.0. Both UV and fluorescence spectra change as a function of the degradation time but they vary in a different manner. While the absorption bands in the UV spectra decrease throughout the degradation reaction, the fluorescence spectra show increases and decreases in the signal over time. This is an important point because this fact could indicate that the information provided by each technique is complementary to each other and then contributes to enhance the knowledge about the system.

The current study is focused on the ciprofloxacin degradation at pH 7.0 and 9.0. Since the photodegradation is a procedure normally used for the remediation of polluted wastewaters, we investigated the ciprofloxacin degradation at the pH values usually found in hospital wastewaters (HWWs) and urban wastewaters (UWWs). Some authors report pH values in the range of 7.7–8.1 and 7.5–8.5 for HWWs and UWWs, respectively [43]. Likewise, Akin et al. performed a study of wastewater from a Turkish hospital, and they found that pH values varied between 7.9 and 9.4 [44]. Furthermore, Prayitno et al. found pH values around 7.0 in wastewaters of Malang City [45]. Thus, the ciprofloxacin degradation was conducted at both pH 7.0 and 9.0.

The kinetic model for the ciprofloxacin photodegradation was mainly based on the shape of the concentration profiles obtained by

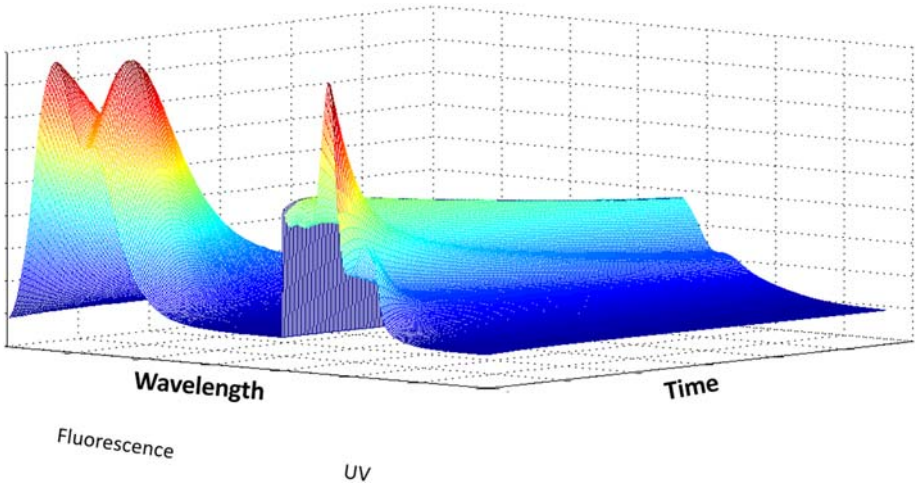

Fig. 2. Three-dimensional view of the $\mathbf{D_1}$ matrix, corresponding to the photodegradation of ciprofloxacin at pH 7.0.

soft-modeling analysis (MCR-ALS) of the UV and fluorescence data (Fig. S1). As mentioned in Section 2.3, each augmented matrix was constructed with two individual matrices (matrices **A** and **B**, i.e. UV and fluorescence data matrices, respectively). The simultaneous analysis of different data from the same system (in a unique augmented matrix) make possible to extract more information about it [46]. In addition, the selection of the number of components for the system is a crucial step that should be made prior to the application of MCR-ALS. The number of components investigated by Singular Value Decomposition (SVD) for the augmented data matrices corresponding to the degradation of ciprofloxacin at both pH 7.0 and 9.0 is shown in Table S1. Six sources of variability, different of noise were observed in both augmented matrices. Thus, six compounds were considered for the MCR-ALS analysis. The recovered concentration profiles are shown as a supplementary material (Fig. S1).

Fig. 3 shows the proposed photodegradation kinetic model postulated on the basis of the concentration profiles recovered by soft-modeling MCR-ALS analysis of the augmented matrices (Fig. S1). Also, information found in scientific articles about the possible photoproducts of ciprofloxacin was used [47–52]. This model is the same that the proposed one in our previous article, because the degradation was performed under the same conditions (air-equilibrated solutions, type of buffer, pH, etc.) [34].

As can be seen in Fig. 3, ciprofloxacin (at pH 7.0 and 9.0 in buffer Britton-Robinson) could photodegrade following two different pathways. One of them involves one compound (pathway 1), while the other gives rise to a reaction chain with four compounds (pathway 2). The compound 'a' (Pathway 1, Fig. 3) is produced by a photosubstitution of the fluorine atom of ciprofloxacin by a hydroxyl group [50]. This product is generated mainly under alkaline conditions and with an air-saturated solution [51]. Among others, Batchu et al. [52] and Salma et al. [47] have reported the compound 'a' as a photoproduct of ciprofloxacin, as well. These authors have found that this compound is also photodegraded, giving rise to several photoproducts. However, in our work we could not found photoproducts of compound 'a', probably due to the low sensibility of the optical techniques in comparison with the HPLC-MS/MS determination reported in the bibliography [47,52]. Indeed, the presence of phosphate ion could produce a decrease in the fluorescence quantum yield [48].

Pathway 2 is a reaction chain consisting of four compounds. The first one is 'b', which arise from reductive dehalogenation of ciprofloxacin [53]. Several authors have reported this compound in the ciprofloxacin photodegradation process [48,52,53]. On the other side, Salma et al. [47] have not found it, but only reported this species as an intermediate product, which subsequently gives rise to compounds 'c' and 'd'. Following Pathway 2, the compound 'b' originates the compound 'c' that is obtained by oxidation of the piperazine ring, which in turn generates compound 'd', after reduction. Several authors have reported this reaction sequence [47–49]. Additionally, the compound 'd' gives rise to the compound 'e' by a decarboxylation reaction. This reaction could be favored by the presence of free electrons in the nitrogen atom of the amino group [54].

The whole reaction pathway proposed in the current article was partially confirmed by Salma et al. [47]. In their article, ciprofloxacin photodegradation process was performed at different pH values (including pH 7.0 and 9.0) using the same source of UV radiation (254 nm and 15 W). The photodegradation reaction was investigated using deuterated ciprofloxacin and liquid chromatography coupled to tandem mass detection (LC–MS/MS). The number of compounds and the photodegradation pathways for both pH 7.0 and 9.0 reported by Salma et al. [47] are very similar to the ones depicted in Fig. 3. Besides the differences already discussed, they proposed a third pathway (of one compound) [47], which involves fluorinated photoproducts. However, photodegradation of ciprofloxacin in the presence of electron

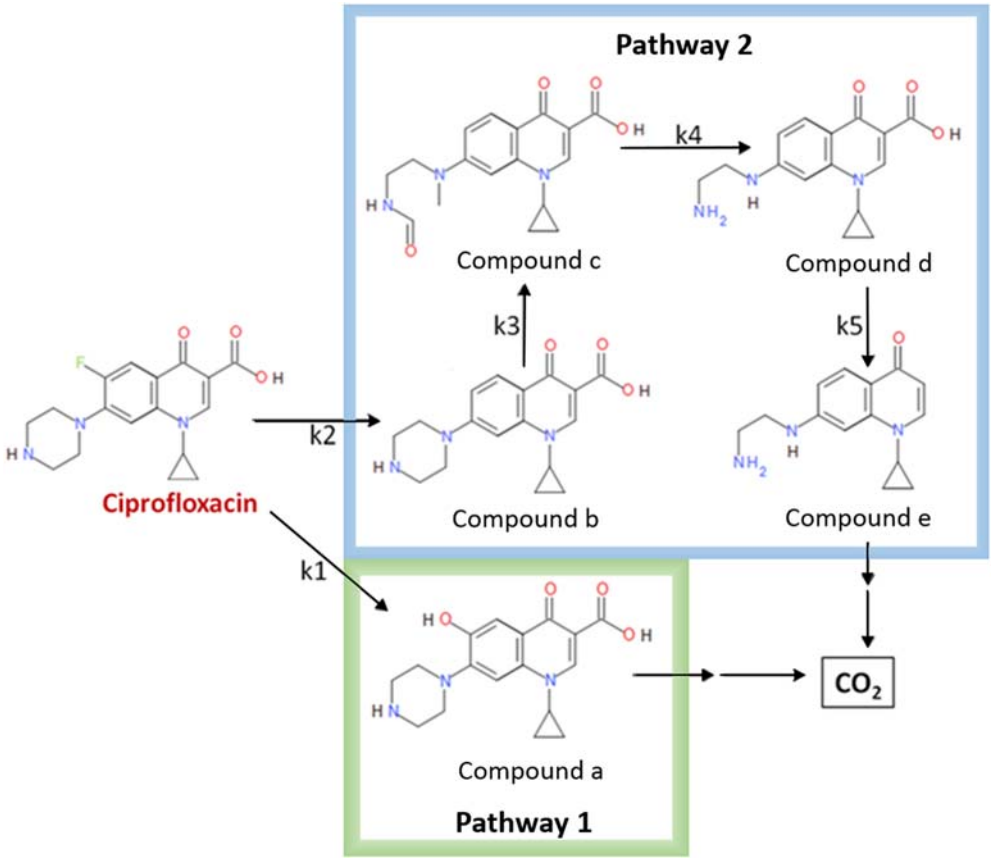

Fig. 3. Reaction pathways proposed for the ciprofloxacin photodegradation reaction.

donors such as phosphate ions mainly leads to defluorination, so fluorinated photoproducts are less favored in our experiments [49].

In accordance with the reaction pathways show in Fig. 3, the following kinetic equations were proposed:

$$d[\text{Cipro}]/dt = -k_1[\text{Cipro}] - k_2[\text{Cipro}]$$

$$d[\text{a}]/dt = k_1[\text{Cipro}]$$

$$d[\text{b}]/dt = k_2[\text{Cipro}] - k_3[\text{b}]$$

$$d[\text{c}]/dt = k_3[\text{b}] - k_4[\text{c}]$$

$$d[\text{d}]/dt = k_4[\text{c}] - k_5[\text{d}]$$

$$d[\text{e}]/dt = k_5[\text{d}]$$

3.2. HS-MCR resolution

The HS-MCR algorithm was applied to the data set as mentioned in Section 2.4 and the concentration profiles were fitted to the proposed kinetic model at each iteration step in the ALS optimization. Table 1 shows the quality parameters obtained from the HS-MCR analysis of both augmented matrices (UV-F/HS-MCR) and their rate constants. The obtained quality parameters are satisfactory enough to suppose that no overfitting is present and the proposed pathways seem to be correct.

Fig. 4 shows the concentration profiles obtained for the experiments conducted at both pH 7.0 and 9.0. The photodegradation profiles of ciprofloxacin are almost equal in shape for both experiments, but degradation is faster at pH 9.0. This fact is also reflected in the kinetic rate constants shown in Table 1; k_{cip} is slightly higher at pH 9.0 (k_{cip} 0.0322 s^{-1}) than at pH 7.0 (k_{cip} 0.0302 s^{-1}), which is in agreement with the information found in the bibliography [49,52,53,55]. The photodegradation kinetic of the photoproducts is also faster at pH 9.0. At pH 7.0 the concentration profiles of all photoproducts show a shift to higher degradation times. In fact, at the end of the experiment at pH 9.0 only the compound 'e' is present, whereas at pH of 7.0 both the compounds 'e' and 'd' are detected since the degradation of the compound 'd' is not complete. Therefore, despite the fact that ciprofloxacin is no longer present after 100 s in both experiments; from the remediation point of view the best medium to carry out the photodegradation of this antibiotic in wastewater would involve a pH value close to 9.0. Moreover, compound 'a' (pathway 1) achieves higher concentration at pH 9.0 [34]. This fact is in accordance with Salma et al., which have reported that pathway 1 is the dominant transformation when the photolysis is carried out above pH 7 [47]. This is reflected in the k_1 values shown in Table 1.

Table 1
Results obtained performing UV-F/HS-MCR and UV–UV/HS-MCR analysis at pH 7.0 and pH 9.0.

	UV-F/HS-MCR		UV–UV/HS-MCR	
	pH 7.0	pH 9.0	pH 7.0	pH 9.0
Lack of fit (%)	0.3	0.5	3.5	6.5
Variance explained (%)	99.99	99.99	99.88	99.58
Number of components	6	6	6	6
Kinetic rate constants (s^{-1})				
k_1	0.0211	0.0218	0.0153	0.0156
k_2	0.0091	0.0104	0.0165	0.0093
k_3	0.0045	0.0033	0.0128	0.0050
k_4	0.0023	0.0016	0.0059	0.0033
k_5	0.0005	0.0027	0.0019	0.0013
k_{cip}	0.0302	0.0322	0.0318	0.0248

The recovered UV and fluorescence spectra are shown in Fig. 5. The UV spectra at the different pH are very similar. The maximum absorption bands appear at the same wavelengths either for pH 7.0 or 9.0, for all compounds (including ciprofloxacin). Only slight variations were observed in the intensity of the signal. The fluorescence spectra show a different behavior. The highest signals correspond to ciprofloxacin and compounds 'a' and 'b', which is in agreement with the spectral features reported by Hidalgo et al. [54]. They inform that the quinolones containing a piperazine ring exhibit the highest fluorescence; such is the case of the mentioned compounds. On the other side, the fluorescence spectra have significant differences at the different pH values. The major changes involve variations in the spectral shape and in the wavelengths of maximum fluorescence emission. For instance, at pH 9.0 the compound 'a' has a maximum around 430 nm, similar to ciprofloxacin. However, this maximum disappears at pH 7.0, possibly due to the protonation of the hydroxyl group at C6. On the other hand, the absence of fluoride in C6 of compound 'b' respect to the ciprofloxacin could favor the florescence around 495 nm, but the band at 430 nm become less important at pH 9.0. Probably, the electronic changes in such alkaline medium (pKa of piperazinyl ring for ciprofloxacin around 8.7 [56]) disadvantage the fluorescence at 430 nm when no substituent is present at C6.

A comparative analysis was also performed to evaluate the improvements of the proposed UV-fluorescence hyphenated arrangement (UV-F/HS-MCR) respect to our previous article in which HS-MCR was applied to the column-wise augmented matrices from the UV monitoring of the photodegradation conducted under different CIP concentrations (UV–UV/HS-MCR) [34]. Table 1 shows the quality parameters obtained for the HS-MCR application. As can be seen, both LOF and EV were improved when HS-MCR was applied to the row-wise augmented matrices from the UV-fluorescence combined monitoring of the reaction.

Moreover, Fig. 6 shows the degradation profiles of ciprofloxacin obtained using HPLC-DAD, UV-F/HS-MCR and UV–UV/HS-MCR. As can be seen, at both pH values the concentration profiles obtained using UV-F/HS-MCR (solid line) are more similar to the ones obtained by HPLC analysis (dashed line) than the concentration profiles found using UV–UV/HS-MCR (dotted line). The new information supplied by fluorescence allows the current method to predict the ciprofloxacin behavior better than our last method, where only the UV spectra were used.

The UV spectra recorded during HPLC-DAD separation for the main degradation products were also compared with the ones recovered by UV-F/HS-MCR and UV–UV/HS-MCR, the corresponding similarity coefficients were calculated for the degradation at both pH 7.0 and 9.0. The coefficients obtained for the comparison among HPLC and UV–UV/HS-MCR were around 0.85–0.96 and 0.83–0.92 for pH 7.0 and 9.0, respectively. On the other hand, the correlation coefficients calculated when HPLC and UV-F/HS-MCR were compared, ranged between 0.87–0.99 and 0.85–0.97 for pH 7.0 and pH 9.0, respectively. Thus, using UV-F/HS-MCR makes it possible to improve the resolution of the system in comparison with UV–UV augmented matrices.

In addition, in the UV–UV/HS-MCR method, two experiments conducted under different conditions were required to overcome the rank deficiency problem, inherent to data from this kind of reactions [34]. However, in the UV-F/HS-MCR method only one run is required to obtain both matrices (UV and F) and correctly resolve the system. Thus, the method is faster, simpler and allows the data to

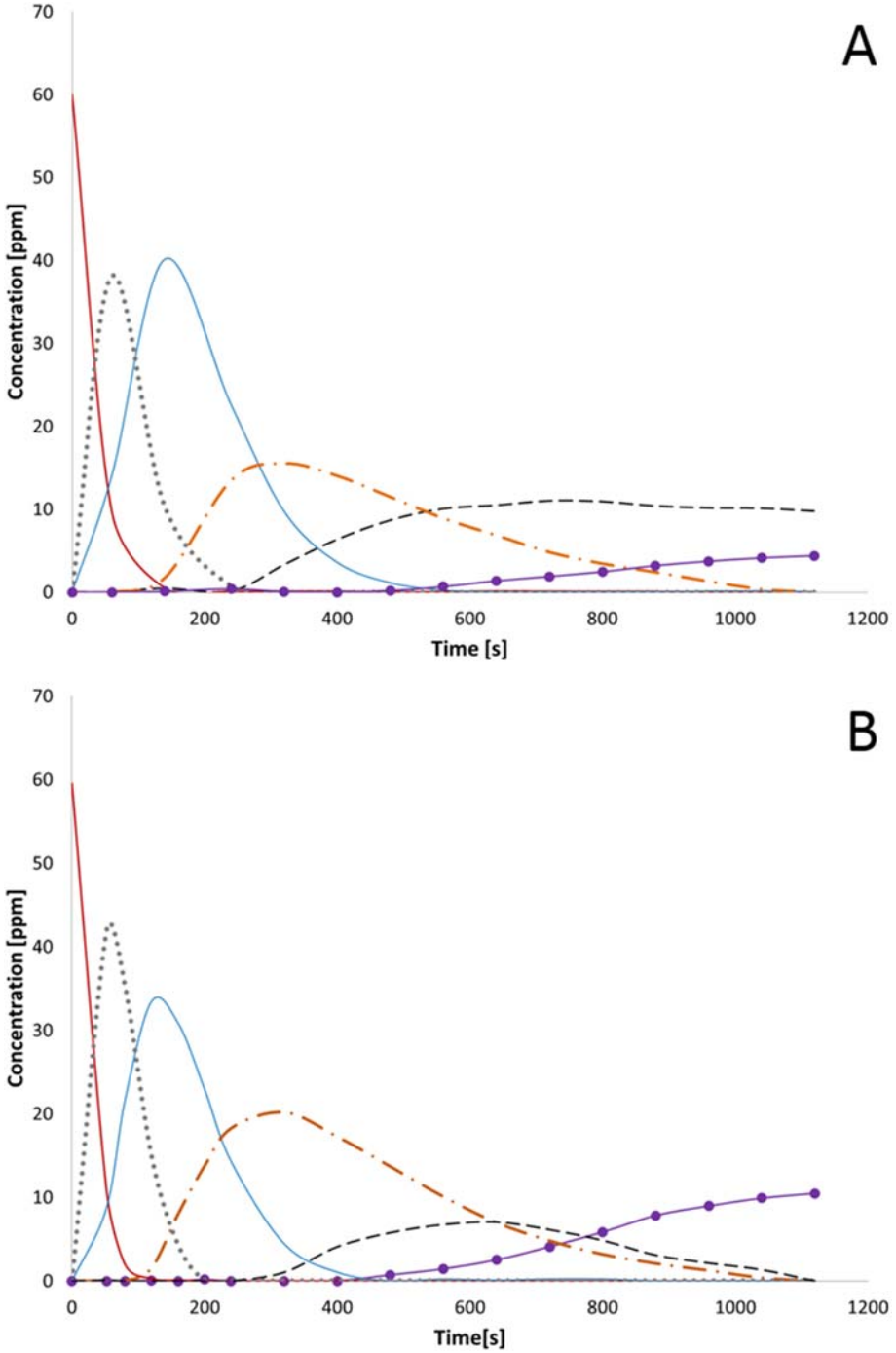

Fig. 4. Concentration profiles obtained by HS-MCR for ciprofloxacin and its main degradation products throughout the photodegradation time: (A) pH 7.0 and (B) pH 9.0. Ciprofloxacin (red line), compound 'a' (dotted line), compound 'b' (blue line), compound 'c' (dash-dotted line), compound 'd' (dashed line) and compound 'e' (●). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

be obtained in a single run, which reduce the possible handling errors.

Table 2 shows a comparison among the current investigation and several articles available in literature regarding to ciprofloxacin photodegradation. It can be noted that the photodegradation reactions, in almost all cases, were studied at pH values between 7.0 and 9.0 (either in natural waters or in aqueous solutions adjusted with buffers, acids or alkalis) [23,34,47–49,53–58]. The maximal photodegradation time studied in the literature ranged between 4 min and 130 hs [49,58]. This variability in the photodegradation time arises from the different radiation sources, the design of the system and the volumes of sample used for studying the reaction. In our case, the small volume of sample (2 mL) in the flow system and the closeness with the UV lamp made it possible to study the ciprofloxacin degradation in 18.8 min, which is a very short time. In addition, no separation techniques was used, so the time of analysis was reduced and organic solvents were not required, which is relevant from an environmental point of view. Another difference is the number of reaction times analyzed. As can be seen from Table 2, the maximal number of reaction times analyzed was 12 [58], whereas in the proposed flow system, we could analyze 282 photodegradation times in a single run. On the other hand, the shortest HPLC analysis reported took around 8 min [47] for each aliquot withdrawn from the reaction mixture and this time could be as higher as 53 min in other studies [58]. On the contrary, our analysis took

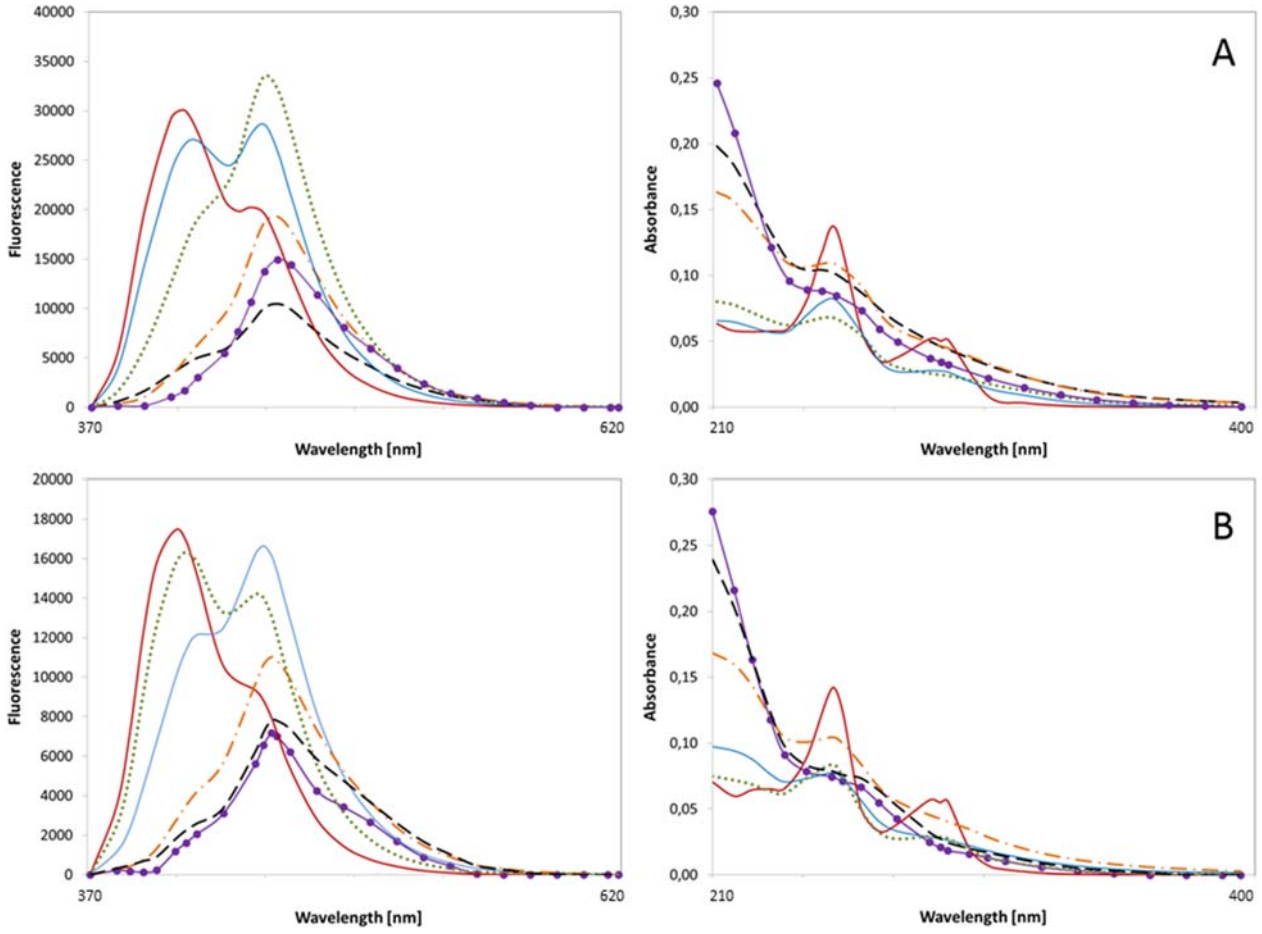

Fig. 5. UV–Vis and Fluorescence spectra recovered by HS-MCR: (A) pH 7.0 and (B) pH 9.0. Ciprofloxacin (red line), compound 'a' (dotted line), compound 'b' (blue line), compound 'c' (dash-dotted line), compound 'd' (dashed line) and compound 'e' (●). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

18.8 min to obtain the whole set of spectra needed for the HS-MCR analysis. Table 2 also shows the ciprofloxacin rate constants reported by several authors. However, only the current study and our previous work report the rate constants for the photoproducts. Thus, the proposed method is able to predict the rate constants for ciprofloxacin and their major photoproducts in a very short time, using a small sample volume, without using organic solvents and generating small volume of wastes in comparison with the studies reported in the literature.

3.3. Green approach

In the last years the Green Analytical Chemistry (GAC) [59] have took and important place in the development of new analytical methods. In these sense, not only the analytical parameters are enough to judge the goodness of a new analytical method, but also the effects that it could cause in the environment. Several reviews and articles were recently published in this field [60–63].

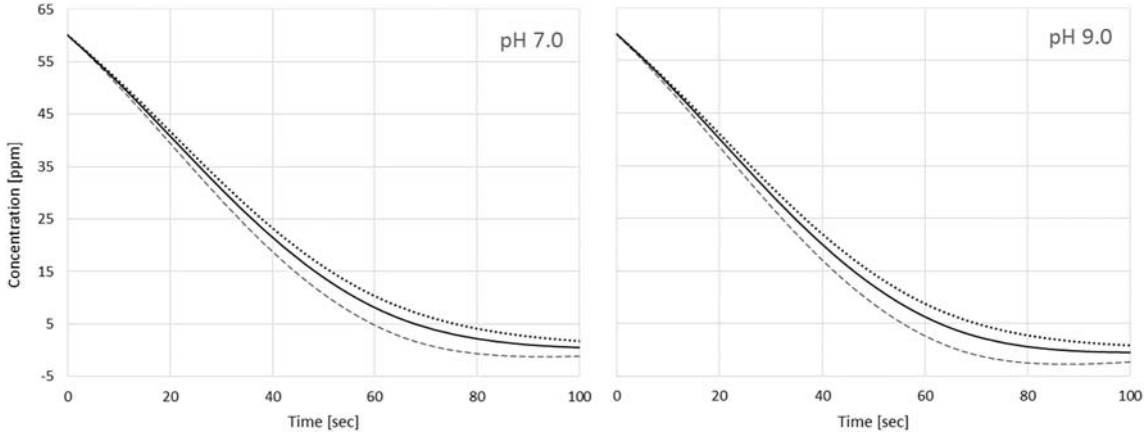

Fig. 6. Ciprofloxacin concentration profiles obtained at pH 7.0 and 9.0 by UV-F/HS-MCR (solid line), UV–UV/HS-MCR (dotted line) and HPLC-DAD (dashed line).

Table 2
Comparison among different ciprofloxacin photodegradation studies.

CIP conc. (mg L^{-1})	Photolysis medium	UV lamp	Photodegradation time (min)	Points measured over time	Type of system	Analytical technique	Solvents	Sample volume (mL)	Time analysis (min)	Kinetic rate constants (s^{-1})	Refs.
1	Pure (pH 5.5) and natural water (canal pH 8.1 and salt water pH 7.9)	16-mercury vapor lamps (UV 254 nm) Black light phosphor bulbs (UV 350 nm) Xenon lamp (750 W/cm^2)	60–360	6	Batch	LC–MS/MS	Methanol and water	30	25 (for each time analyzed)	1.10^{-4}–0.0125 (only for CIP)	[52]
0.050	Water and water with fine particulate organic matter (FPOM). pH 7.5 (HCl)	UVB compact lamp, 20 W	960	4	Batch	HPLC–fluorescence	Acetonitrile, methanol and water	60	13 (for each time analyzed)	–	[23]
≡115	Water (0.1 N HCl) Phosphate buffer (pH 7.4). Sulfite buffer (pH 7.2)	Pyrex-filtered medium-pressure Hg arc. (310 nm, 500 W)	NS	NS	Batch	HPLC	Acetonitrile, methanol and water	10	NS	–	[48]
≡23	Water pH 3.0, 5.0, 7.0 and 9.0 (HCl or NaOH solutions)	UV lamp, (254 nm, 15 W)	120	10	Flow system (only for photodegradation)	LC–MS/MS	Acetonitrile, methanol and water	600	8 (for each time analyzed)	≡ 0.0003 (only for CIP)	[47]
.0.050	Raw surface water (pH 7.7)	Natural sunlight	50	7	Batch	HPLC–UV. HPLC–MS/MS	Acetonitrile and water	500 mL	25 (for each time analyzed)	0.0036 (only for CIP)	[53]
0.050	Urban wastewater treatment plants effluents (pH 6.9)	Solar simulator (250 Wm^{-2})	70	8	Batch	HPLC–fluorescence HPLC–UV HPLC–MS/MS.	Acetonitrile and water	500	30 (for each time analyzed)	0.0018 (only for CIP)	[57]
1	0.001, 0.01 and 0.1 M HCl and phosphate buffer pH 3.1	High-pressure mercury lamps, TQ 150 and TQ 718	240	5	Batch	HPLC–UV	Acetonitrile and water	3	30 (for each time analyzed)	–	[24]
0.1	Water pH 9 (NH_4Cl 0.050 M and KOH 3 M)	Medium-pressure Hg-lamp, 150 W	4	4	Batch	LC–MS/MS	Acetonitrile and water	2500	30 (for each time analyzed)	0.0153 (only for CIP)	[49]
≡4	Buffer solutions (pH 3–10.6)	High-pressure mercury lamp (313 nm)	150	8	Flow system (only for photodegradation)	HPLC–UV	Acetonitrile and water	3 mL	30 (for each time analyzed)	–	[56]
0.25	Surface waters (pH 3.0, 7.3 and 7.8)	Econo-watt 34-W fluorescent shop lights; 33% Vita-light UVA-UVB	7800	6–12	Batch	LC–DAD/MS	Acetonitrile and water	3000	53 (for each time analyzed)	–	[58]
≡40	Phosphate buffer (pH 7.4)	UVA (350 nm)	NS	NS	Batch	HPLC	NS	4 mL	NS	0.0041 (only for CIP)	[55]
10 and 60	Britton Robinson buffer (pH 5.0, 7.0 and 9.0)	Low mercury UV lamp (254 nm, 15 W)	18.8	282	Flow system	DAD	water	4 mL	37.6 (for all times analyzed)	0.0045–0.0156 (for CIP) 0.0006–0.0165 (for photoproducts)	[34]
60	Britton Robinson buffer (pH 7.0 and 9.0)	Low mercury UV lamp (254 nm, 15 W)	18.8	282	Flow system	Fluorescence – DAD	water	2 mL	18.8 (for all times analyzed)	0.0302–0.0322 (for CIP) 0.0005–0.0218 (for photoproducts)	This study

NS: Not specified

The UV-F/HS-MCR method is in accordance with several principles of the GAC [60], and it could be considered as a cheaper and greener alternative to the HPLC analysis. In this sense, the water was the only solvent used, the volume of the sample required was very low (1.88 mL per sample, i.e. 0.1 mL min^{-1} during 18.8 min) and the waste generated during the analysis was also very low (not higher than 3.5 mL per sample analyzed, including washing operations). The use of energy could be considered lower in comparison with chromatographic analysis, taking into account that UV-F/HS-MCR is an automated, fast and simple method. In addition, the equipment employed, even though it involves two detection systems and a peristaltic pump, could be considered cheaper than HPLC, either with DAD or MS detection. Moreover, no high purity solvent was required.

4. Conclusion

The proposed method represents a simple and fast alternative to the separation techniques for the ciprofloxacin photodegradation study. The soft modeling approach made it possible to propose a kinetic model, even though no separation was performed and the detection techniques used were not able to identify the full chemical structure of the compounds involved in the reaction. The HS-MCR allows us to obtain the concentration and spectra profile of ciprofloxacin and its main photodegradation products, as well as the involved kinetic rate constants. According to the obtained results, the photodegradation was faster at pH 9.0 than at 7.0 and this fact would be taken into account when UV light is used for the decontamination of wastewaters containing ciprofloxacin.

The new method (UV-F/HS-MCR) has proven to improve the resolution of the system in comparison with our prior method, UV–UV/HS-MCR. In this sense, better fitting parameters were obtained and higher similarity with HPLC-DAD was observed. The improvements could be attributed to the use of two different detection techniques (UV and fluorescence), which increase the information about the system. In addition, this method is faster because only one run is required to resolve correctly the system.

A series of features of the proposed method make it also environmental friendly, since it fulfill several principles of the so-called Green Analytical Chemistry, because the time of analysis is short, organic solvents are not required, low amounts of sample are needed and small quantities of wastes are generated.

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