



Kinetic studies of nitrofurazone photodegradation by multivariate curve resolution applied to UV-spectral data

Michele De Luca^{a,*}, Sílvia Mas^b, Giuseppina Ioele^a, Filomena Oliverio^a, Gaetano Ragno^a, Romà Tauler^b

^a Department of Pharmaceutical Sciences, University of Calabria, Via P. Bucci, 87036 Rende, Cosenza, Italy

^b Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Catalonia, Spain

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ABSTRACT

This work aims at describing the kinetic model of nitrofurazone photodegradation by a novel chemometric technique, hybrid hard-soft multivariate curve resolution (HS-MCR). The study was applied to UV-spectral data from the photolysis of nitrofurazone solutions at different concentrations and exposed under varying illuminance power. The HS-MCR method was able to elucidate the kinetics of the photodegradation process and to determine the rate constants, and estimating at the same time the pure spectra of the degradation products. Exposure to light of the drug gave a first rapid isomerization to the *syn*-form that in turn underwent degradation furnishing a mixture of yellow-red products. The photodegradation process could be explained with a kinetic model based on three consecutive first-order reactions ($A > B$, $B > C$ and $C > D$). These results were confirmed by application of the MCR procedure to the analysis of the data obtained from HPLC-DAD analysis of the nitrofurazone samples at different reaction times. The kinetic model was observed to be dependent on experimental conditions. The samples at higher concentrations showed rate constants lower than the diluted samples, whereas an increase of the rate of all degradation processes was observed when the light power also increased. This work shows the power of the hybrid hard- and soft-multivariate curve resolution as a method to deeply study degradation processes of photolabile drugs.

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1. Introduction

Nitrofurazone (2-((5-nitro-2-furanyl)methylene)hydrazinecarboxamide) (NFZ) is a well-known antibacterial agent effective against a wide variety of gram-negative and gram-positive organisms, applied to the treatment of many human and animal infections (Chowdhury, 1977; Lowenthal et al., 1977; McCalla, 1979). Nowadays, its use in medicine has become less frequent and it is above all used topically as a local anti-infective drug, prescribed in the prophylaxis and treatment of infections of the skin and mucous membranes (Erdur et al., 2008).

Since the drug, when exposed to light, is susceptible to photodegradation (Shahjahan and Enever, 1996a,b), its use in topical preparations represents a serious problem about the loss of the pharmacological activity. In previous publications the photodecomposition of this drug under controlled conditions has been investigated, hypothesizing a very complex degradation pathway of the drug (Shahjahan and Enever, 1996b; Tao et al., 2005). The photolability of NFZ was firstly reported in 1966 (Iwahara et al.,

1966; Uriach and Pozo, 1966) but the main photoproducts were separated by HPLC and identified only in 1987 (Quilliam et al., 1987) as the *syn*-isomer of NFZ (*syn*-NFZ) and the bis(5-nitrofurylidene)azine (AZI), respectively. The 5-nitro-2-furaldehyde (NFA) and the hydrazone of 5-nitro-2-furaldehyde (HYD), were also reported as further potential photodegradation products but their presence was not observed.

The reaction rate of photodegradation of NFZ in solution has been reported to increase with the decrease of the drug concentration and with the increase of the illuminance power (Lin and Lachman, 1969; Allowood and Plane, 1986; Tao et al., 2005). The influence of various factors including pH of the medium, irradiation wavelengths, presence of surfactants, metal ions or ultraviolet light absorbers has been also investigated (Shahjahan and Enever, 1996a,b).

Actually, the details of the photodegradation mechanism still remain unclear. The published studies are focused on the identification of the photodegradation products but do not investigate the sequential degradation steps that may take place. The prediction of the exact photochemical behaviour of a specific molecule is really difficult, as Greenhill has pointed out in his review (Greenhill, 1995). This is due to the fact that the photodegradation mechanism depends in a complex way on the structure and on experimental

* Corresponding author. Tel.: +39 0984493178; fax: +39 0984493201.
E-mail address: michele.deluca@unical.it (M. De Luca).

conditions and then the kinetics and photoproducts distribution may vary significantly even among closely related compounds (Albini and Fasani, 1997).

Nowadays, the spectrophotometric techniques remain largely used in the field of analytical chemistry because of the easy interpretation and handling of the spectral data. On the other hand, the conventional spectrophotometric methods use a discrete number of wavelengths that frequently are not enough to furnish the necessary information to resolve a system with severe spectra overlapping. In recent years, chemometrics approaches for the extraction of analytical information from multivariate data have been proposed (Massart et al., 1998; Breton, 2003). Multivariate methods have the advantage of exploiting full spectral information, by using simultaneously all measured analytical signals.

In this paper, we report the investigation on the photochemical degradation process of the NFZ drug and we give an interpretation of the complete kinetic pathway obtained by a recently proposed hybrid hard- and soft-modelling multivariate curve resolution (HS-MCR) method applied to UV spectrophotometric data. This chemometric method can be applied to the simultaneous treatment of several experiments conducted under different experimental conditions. It allows the extraction of the pure spectra of the involved components together with their concentration profiles and, at the same time, it evaluates the kinetic model and their various rate constants (de Juan et al., 2000a,b; Amigo et al., 2006; Mas et al., 2008).

The photodegradation experiments were performed following the ICH recommendations for the drug stability tests (ICH, 1996). An independent HPLC-DAD method was simultaneously developed to check the results obtained by the analysis of the UV kinetic photodegradation studies. The chromatographic data were then also analyzed by means of the same MCR approach. From the results obtained by these two approaches for synthesized and commercially obtained pure compounds, as well as for their degradation products, the chemical structure of the main photoproducts are proposed. Dependence of the kinetics pathway from sample concentrations and from different illuminance power is also verified.

2. Experimental

2.1. Chemicals

Nitrofurazone (NFZ), 5-nitro-2-furaldehyde (NFA), 2-furaldehyde (FA), 5-nitro-2-furaldehyde diacetate and hydrazine hydrate were purchased from Sigma-Aldrich Co. (Italy). Spectrophotometric grade ethanol, HPLC grade water and acetonitrile were from J.T. Baker (Holland). The 5-nitro-2-furaldehyde azine (AZI), was prepared by condensation of 3.0 g (0.02 mol) of 5-nitro-2-furaldehyde with 0.5 g (0.01 mol) of hydrazine hydrate (Bird and Stevens, 1962). The hydrazone of 5-nitro-2-furaldehyde (HYD) was prepared from 2.5 g (0.01 mol) of 5-nitro-2-furaldehyde diacetate and 0.5 g (0.01 mol) of hydrazine hydrate (Quilliam et al., 1987).

2.2. Instruments

Light exposure was performed in a light cabinet Suntest CPS+ (Heraeus, Milan, Italy), equipped with a Xenon lamp. The apparatus was fitted up with an electronic device for irradiation and temperature measuring and controlling inside the box. The system was able to closely simulate sunlight and to appropriately select spectral regions by interposition of filters.

Spectrophotometric measurements were performed using an Agilent 8453 Diode Array spectrophotometer (Agilent Technologies, USA). HPLC analysis was performed by using a chromatograph Agilent 1100 series (Agilent Technologies, CA), equipped with a binary pump delivery system and a diode array UV-vis detector.

2.3. Experimental procedures

A stock solution of NFZ (1.0 mg/ml) in ethanol was properly diluted to obtain two samples for degradation experiments: sample solution A (NFZ at 100.0 µg/ml) and sample solution B (NFZ at 20.0 µg/ml). These two samples, in quartz cells perfectly stoppered, were light irradiated directly at the wavelength range between 300 and 800 nm, by means of a glass filter, according to the ID65 standard of the ICH rules. This light exposure was repeated by varying the irradiation power at four different levels: 250, 350, 450 and 550 W/m², corresponding to an energy value of 15, 21, 27 and 33 kJ/min/m², respectively. The solution temperature was maintained constant at 25 °C.

UV spectra were recorded in the range between 200 and 650 nm, just after preparation (*t*=0) and at the following times: 1, 3, 5, 7, 10, 15, 20, 25, 30, 40, 50, 60 and 70 min. The spectra of solution B were directly recorded, while solution A needed to be diluted before analysis with ethanol to obtain a concentration of 20 µg/ml.

HPLC separations were carried out on a LC column, 5 µm C18 Gemini column (250 × 4.60 mm) (Phenomenex, Torrance, CA), by gradient elution, using water and acetonitrile as mobile phase, filtered through 0.45 µm filter before use. Mobile phase was water/acetonitrile (95:5, v/v) for 5 min, gradually changed to 70:30 in 10 min and then maintained for 10 min. The injection volume was 5 µl and mobile phase flow rate was 1 ml/min.

HPLC analysis was performed after irradiating a 1 mg/ml ethanolic solution of NFZ placed in a quartz cell. The irradiance power was fixed at 250 W/m², at a temperature of 25 °C. Aliquots of 5 µl of the sample were directly analyzed by HPLC after preparation (*t*=0) and at the following times: 10, 20, 30, 40, 50, 60, 90, 120, 150, 180, 210, 240, 270 and 300 min.

3. Chemometric techniques

3.1. Multivariate curve resolution-alternative least squares (MCR-ALS)

When a degradation process is monitored by UV spectroscopy, a series of spectra are collected as a function of time. Changes in these spectra can be used to extract the analytical information necessary to resolve the system, including the pure spectra and concentration profiles of the single components and the kinetics parameters.

The recorded experimental data can be ordered in a data matrix **D**, whose rows (*r*) are the spectra at different times and whose columns (*c*) are the process signals at different wavelengths. Multivariate curve resolution (MCR) methods decompose mathematically these data into the contributions due to the pure components of the system (Lawton and Sylvestre, 1971; Sylvestre et al., 1974; Hamilton and Gemperline, 1990; de Juan et al., 2001; de Juan and Tauler, 2003; Jiang et al., 2004), following a bilinear model derived from the generalized Lambert-Beer's absorption law.

$$\mathbf{D}_{(r \times c)} = \mathbf{C}_{(r \times n)} \mathbf{S}_{(n \times c)}^T + \mathbf{E}_{(r \times c)} \quad (1)$$

where **C** is the concentration matrix of *n* components and **S**^T is the matrix of their spectra. **E** represents the unexplained variance in the data set. In MCR methods, the first step is the estimation of the number of involved components (*n*) in the kinetic system, which is initially obtained by application of singular value decomposition (SVD) (Golub and Van Loan, 1989). Moreover, iterative MCR methods, like MCR-ALS, need a preliminary estimation of **S**^T or **C**, calculated either by evolving factor analysis (EFA) (Gampp et al., 1987), by selection of the pure variables (Windig and Guilment, 1991; Sánchez et al., 1996), or by any previously estimation of them. These initial estimates are used to start the alternating least squares (ALS) constrained optimization through an iterative pro-

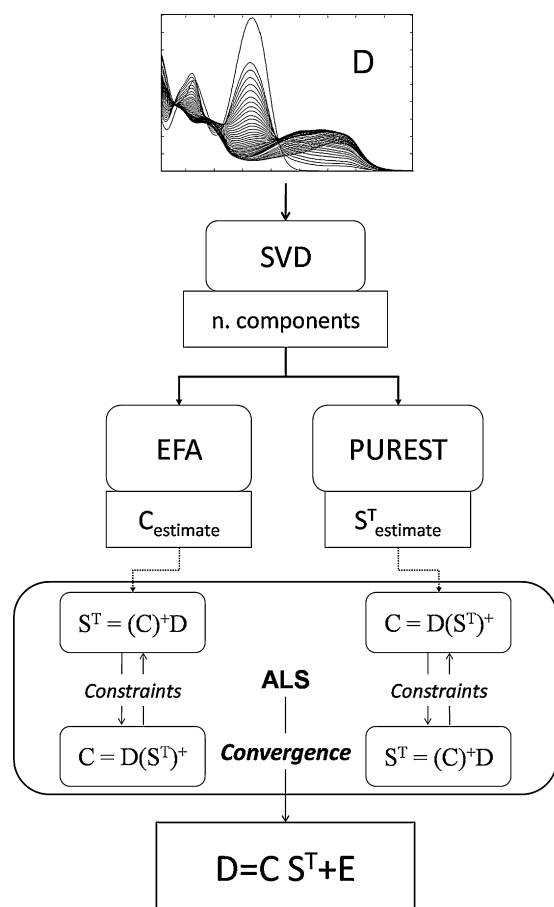


Fig. 1. Flow chart of the multivariate curve resolution-alternating least squares (MCR-ALS) procedure.

cess. At each cycle, a new estimation of \mathbf{S}^T and \mathbf{C} is calculated by solving alternatively the two following least-squares matrix equations, according to the scheme of Fig. 1:

$$\mathbf{S}^T = (\mathbf{C})^+ \mathbf{D} \quad (2)$$

$$\mathbf{C} = \mathbf{D} (\mathbf{S}^T)^+ \quad (3)$$

where $(\mathbf{S}^T)^+$ and $(\mathbf{C})^+$ are the pseudoinverses of the \mathbf{S}^T and \mathbf{C} matrices (s), respectively (Golub and Van Loan, 1989; Tauler, 1995; Tauler et al., 1995).

At every iteration, constraints like non-negativity, unimodality and concentration closure are applied to drive the optimization process towards a final solution with chemical meaning fulfilling the applied constraints (Tauler et al., 1995). This iteration procedure is stopped when convergence is achieved, by fixing a preselected number of cycles or when the value of lack of fit (LOF) does not change significantly between consecutive iterations:

$$\%LOF = 100 \times \sqrt{\frac{\sum_{ij} (d_{ij} - d_{ij}^*)^2}{\sum_{ij} d_{ij}^2}} \quad (4)$$

where d_{ij} and d_{ij}^* were, respectively, the experimental and calculated (by MCR-ALS) absorbance values. A different parameter used to indicate the quality of MCR-ALS modelling results is the percentage of explained variance (r^2):

$$r^2 = 100 \times \frac{\sum_{ij} d_{ij}^{2*}}{\sum_{ij} d_{ij}^2} \quad (5)$$

Another important feature of the MCR-ALS method is its possibility to perform the simultaneous analysis of multiple

data sets coming from more than one experiment, using different experimental conditions and/or conducted by different analytical techniques. The whole set of photodegradation experiments performed at different NFZ initial concentrations exposed under different illuminance power conditions were arranged in a column-wise augmented data matrix and analyzed using MCR-ALS according to the extended bilinear model equation (Tauler, 1995;

$$\begin{aligned} \mathbf{D}_{Aug.} &= \mathbf{C}_{Aug.} \mathbf{S}^T + \mathbf{E}_{Aug.} = [\mathbf{D}_1; \mathbf{D}_2; \dots; \mathbf{D}_N] \\ &= [\mathbf{C}_1; \mathbf{C}_2; \dots; \mathbf{C}_N] \mathbf{S}^T + [\mathbf{E}_1; \mathbf{E}_2; \dots; \mathbf{E}_N] \end{aligned} \quad (6)$$

In this matrix equation, column-wise matrix augmentation is indicated using MATLAB notation ‘;’, which indicates that every individual data block obtained in a single kinetic experiment is set on top of the other, with the same number of columns (wavelengths) in common, and consequently increasing the number of rows of the corresponding augmented matrix. As indicated in this equation, the same spectral matrix \mathbf{S}^T is resolved for all \mathbf{D}_i , $i = 1, \dots, N$ matrices, i.e. the same pure spectra are resolved for the common species in the different experiments. On the contrary, the concentration profiles of the different resolved components in \mathbf{C}_i matrices are allowed to be different for the different kinetic experiments. This is in agreement with the possibility of having degradation (concentration) profiles with different shapes in the different experimental data matrices obtained at different initial NFZ concentration and power irradiances. These degradation profiles will follow different patterns which cannot be described by the same set of concentration profiles. In contrast, however, the same chemical species will have the same characteristic UV spectrum in the different kinetic experiments. In conclusion, the model proposed in Eq. (6) will fit well with the expected behaviour of the measured system if the generalized bilinear Beer’s Lambert law of UV absorption holds for the system under investigation.

3.2. Hybrid hard- and soft-multivariate curve resolution-alternating least squares (HS-MCR) approach

In this work, a variation of the MCR-ALS method, recently proposed and named hard-soft multivariate curve resolution (HS-MCR-ALS) modelling, was used (de Juan et al., 2000a,b). This procedure introduces a new constraint able to force some or all concentration profiles in \mathbf{C} to fit a previously selected kinetic model. At each iteration, a non-linear kinetic fitting routine handles the concentration profiles in \mathbf{C} matrices to fulfil a preselected kinetic model (Maeder and Zuberbuhler, 1990; de Juan et al., 2000a,b, 2001; Maeder and Neuhold, 2007; Muñoz and de Juan, 2007; Mas et al., 2008). The resulting concentration profiles will fulfil the proposed kinetic model and the corresponding rate constants of the process will be obtained as additional information.

This HS-MCR-ALS algorithm is able to resolve some limitation of pure hard- and pure soft-modelling in the study of photodegradation processes. On one hand, HS-MCR-ALS minimizes the rotational ambiguity associated to the estimation of concentration profiles only using non-negativity and closure constraints (Tauler, 1995; Tauler et al., 1995) and on the other hand it allows for the estimation of the best kinetics constants associated to a postulated kinetic model (de Juan et al., 2000a,b). In comparison with hard-modelling approaches, in HS-MCR-ALS the kinetic model can be applied in a flexible way for some or all the species involved in the process, while in classical hard-modelling approaches, all contributions in the \mathbf{D} matrix are constrained according to the preselected model (Maeder and Zuberbuhler, 1990; Maeder and Neuhold, 2007). This possibility can be used, for instance, when the absorbing species in the samples are more than those following the postulated kinetic process (de Juan et al., 2000a,b).

Furthermore the proposed HS-MCR-ALS method allows the use of different kinetic models for the simultaneous analysis of different kinetic experiments in the augmented **D** matrix. In this work, different kinetic models were considered to investigate the effects of the concentration and illumination power on the degradation process.

3.3. Chemometric software

The computations were performed in MATLAB environment (Mathwork Inc., version 6.5) and MCR-ALS user-friendly interface tool (Jaumot et al., 2005). Hybrid hard-soft MCR-ALS (HS-MCR-ALS) has been described and used in de Juan et al. (2000a,b, 2001), Muñoz and de Juan (2007) and Mas et al. (2008).

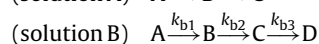
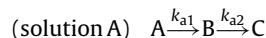
4. Results and discussion

4.1. Photodegradation experiments

Fig. 2 shows the spectral sequence for the degradation of NFZ sample solution A (100.0 µg/ml) and sample solution B (20.0 µg/ml), both exposed to an irradiation power of 250 W/m². The different spectra profiles observed in these two cases confirmed the dependence of the degradation on the concentration (Tao et al., 2005). Sample B presented a degradation process more complex than sample A with the probable formation of a higher number of photoproducts. Anyhow, a very rapid shift of the main absorbance peak from 368 to 356 nm was observed in both samples. Afterwards, this peak decreased constantly with the contemporary increase of absorbance bands above 450 nm, responsible of the rising of orange colour.

Experimental data were processed by the MCR-ALS procedure using non-negativity (concentrations and spectra of the components must be positive), unimodality (concentration profiles in the degradation process present only one maximum per experiment) and closure (mass balance in the kinetic process) constraints. MCR-ALS resolved three and four species for solution A and B, respectively. Fig. 3 shows the calculated concentration profiles (**C**) and the relative pure spectra (**S^T**) for both systems. Percentage of lack of fit (%LOF) values resulted to be in both cases below 1%. The spectral shapes of the common components did not show any significant difference between the two experiments. Evolution of the concentration profile plots were in agreement with kinetic models with two and three consecutive first-order reactions,

respectively:



where k_{ai} are the different rate constants.

In these kinetic models, however, the possible existence of mixtures of subproducts identified as a single component (C or D) cannot be excluded. In reaction networks such as the one proposed in this work, the formation of multiple species with the same kinetic profile (for instance, in a reaction like $A \rightarrow B + C$, profiles B and C would have been exactly equal) produce the so-called rank deficiency of spectral matrices measured on them (Izquierdo-Ridorsa et al., 1997; Saurina et al., 1998). Therefore, in this work, subproducts C and D could be considered to be a mixture of chemical compounds with different molecular structures instead of a pure chemical compound with a single molecular structure.

4.2. Hypothesis of photodegradation pathway

MCR-ALS analysis clearly suggested the very rapid isomerization of NFZ into its *syn*-form, as reported in previous literature (Iwahara et al., 1966; Tao et al., 2005), with a shift of the main peak from 368 to 356 nm and of the secondary peak from 265 to 259 nm. A significant change in the absorptivity of these peaks was also recorded, and the ratio of the first to the second peak varied from 0.587 for NFZ to 1.485 for the isomer. Isomerization resulted to be occurring in all the investigated cases but, in contrast, the subsequent degradation steps seemed to be strongly influenced by the NFZ initial concentration.

In sample A, the isomer degraded into a new compound characterized by an intense absorbance above 450 nm and the simultaneous disappearance of the peak at 359 nm, which is characteristic of the parent compound. This bathochromic shift of the absorption band is confirmed by the progressive colouring of the solution. Sample B at lower NFZ concentration, showed an analogous degradation pathway, but in this case MCR-ALS resolved a third photoproduct, with chromophoric features slightly different to the previously formed compound. Isolation and characterization of these two coloured photoproducts resulted very difficult, due to their simultaneous presence at high concentrations and to their further easy degradation.

In order to identify the main photoproducts of NFZ, the 5-nitro-2-furaldehyde azine (AZI) and the hydrazone of 5-nitro-2-furaldehyde (HYD) compounds were synthesized. Quilliam et al.

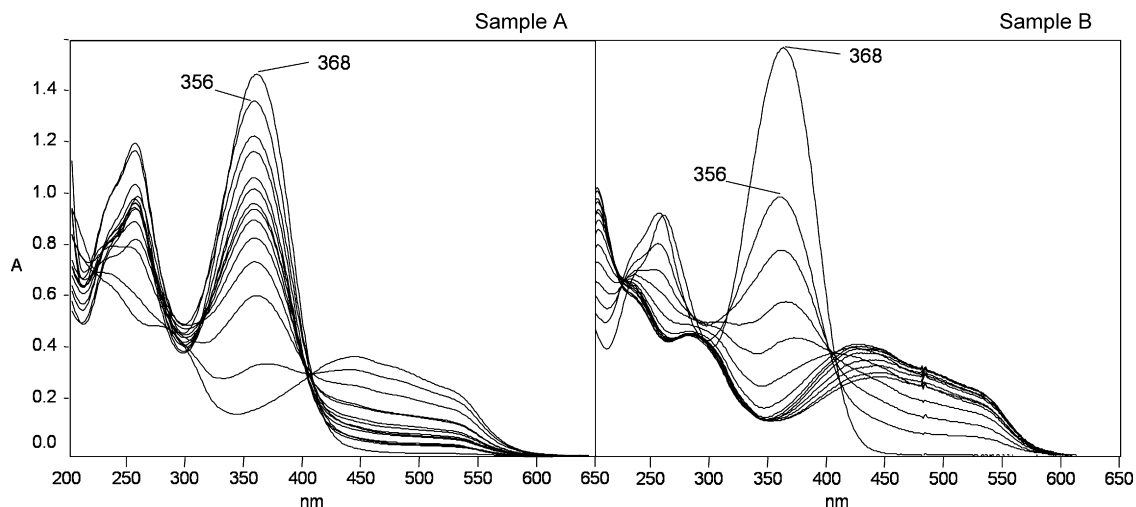


Fig. 2. UV spectra of photodegradation experiments of NFZ sample A (100.0 µg/ml) and NFZ sample B (20.0 µg/ml), exposed under light at 250 W/m².

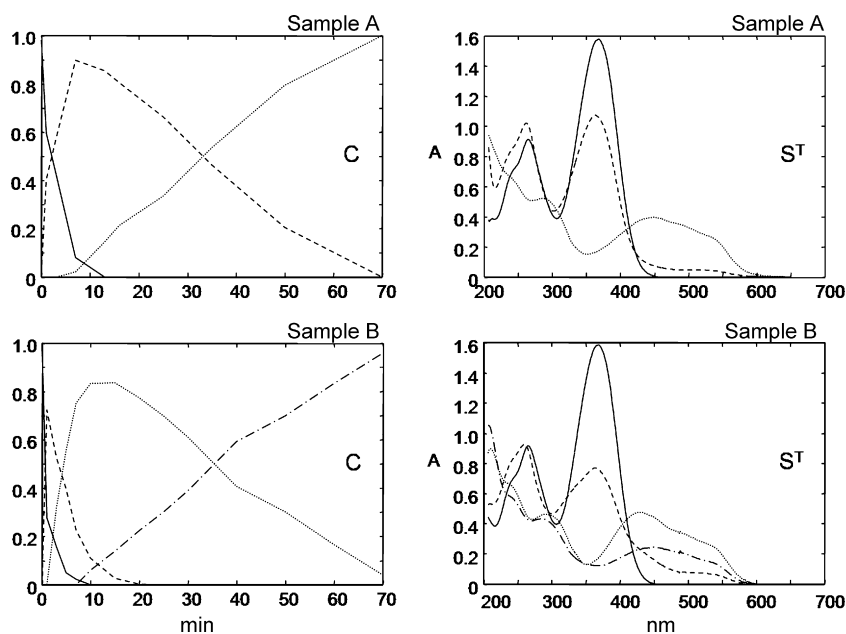


Fig. 3. MCR-ALS resolved kinetic profiles and related pure spectra obtained in the analysis of individual photodegradation experiments: samples A (100.0 µg/ml) and B (20.0 µg/ml) exposed under light at 250 W/m².

(1987) reported AZI as one of the main photodegradation products whereas HYD as a potential photoproduct. However, the recorded spectra of these two compounds demonstrated that these two compounds were not formed during the degradation process of NFZ. This was also confirmed afterwards by HPLC analysis of the NFZ photodegraded samples, which in no case did confirm the chromatographic signals of these two products. AZI (20.1 µg/ml) and HYD (20.0 µg/ml) were then exposed to light irradiation under the same conditions adopted for NFZ, with the aim to highlight what chemical groups could be involved in the formation of the coloured photoproducts. The UV spectra of the AZI and HYD samples recorded during degradation are shown in Fig. 4. Exposure to light of AZI and HYD gave an analogous degradation behaviour than NFZ, showing an intense colouring of their solutions and the appearance of broad bands above 450 nm. The formation of similar photodegradation compounds from NFZ, AZI and HYD demonstrated the importance of the methylene-hydrazine group in the

furanic cycle. The absorbance rising in the visible region could be associated to the transformation of the nitro group into the hydroxymimic group, in agreement with the previous results described by other authors (Borodulin et al., 1999; Edlund et al., 2006). Photodegradation mechanism of NFZ could be so described (Fig. 5) as a rapid isomerization to its *syn*-form that in turn undergoes degradation to a series of coloured photoproducts, highly unstable. These products could directly arise from the NFZ isomer as well as from the azine (AZI) or hydrazone (HYD) derivatives of the drug, which could be formed during degradation but they were rapidly transformed (not detected in our kinetic experiments) in the same final photoproducts as NFZ.

Since 5-nitro-2-furaldehyde (NFA) and 2-furaldehyde (FA), were also suggested (Quilliam et al., 1987; Tao et al., 2005) as possible secondary photodegradation products of NFZ, they were also subdued to analogous irradiance tests. The UV-spectral sequences recorded over the degradation on solutions of NFA (20.4 µg/ml)

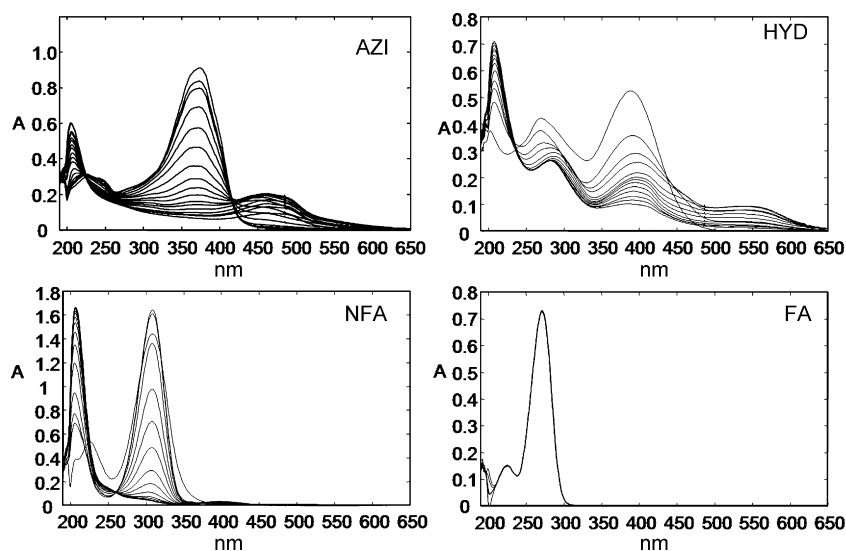


Fig. 4. UV spectra of photodegradation experiments of solutions of AZI (20.1 µg/ml), HYD (20.0 µg/ml), NFA (20.4 µg/ml) and FA (20.0 µg/ml).

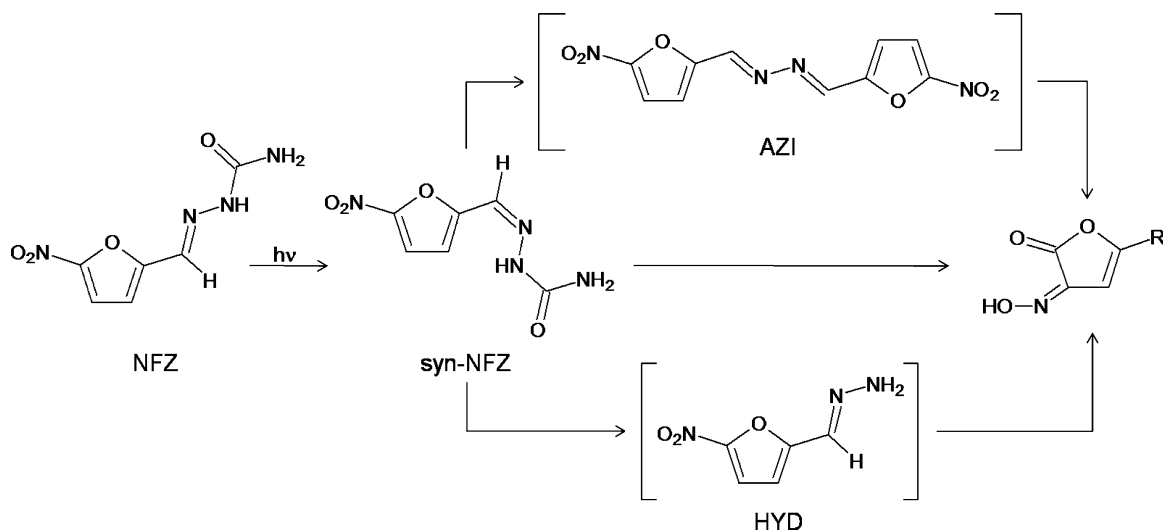


Fig. 5. Scheme of the proposed degradation pathway.

and FA (20.0 $\mu\text{g/ml}$) are also reported in Fig. 4. 2-furaldehyde, FA, resulted to be very stable under light exposure and no spectral changes were observed for this compound. On the other hand, UV irradiance of 5-nitro-2-furaldehyde (NFA), caused a clear decrease of its absorption maximum peak at 309 nm. However, in both experiments, no significant absorbance above 450 nm was observed, demonstrating that they could not be the responsible for the formation of the photoproducts C and D in the photodegradation of NFZ. In fact, FA and NFA do not have this chemical group and this is the reason why they did not show the sought degradation products.

4.3. Hybrid hard- and soft-multivariate curve resolution (HS-MCR)

In order to constrain the shape of the concentration profiles obtained by MCR-ALS analysis to fit exactly a particular kinetic model, the algorithm HS-MCR (Maeder and Zuberbuhler, 1990; de

Juan et al., 2000a,b; Maeder and Neuhold, 2007) was applied, to obtain a new set of kinetic profiles (C) and pure spectra (S^T) of the resolved components, as it is shown in Fig. 6. HS-MCR analysis confirmed the pure spectra for all the components previously estimated by MCR, but a new set of concentration profiles showing now a better agreement with a particular kinetic pathway are obtained. The kinetic rate constants of the different photolysis steps in both samples A and B are listed in Table 1. The values of LOF and r^2 are also reported in this table.

HS-MCR-ALS results confirmed the dependence of the photodegradation rate on the initial concentration of the drug. The more concentrated sample A gave a slower degradation than sample B, in agreement with previous literature results (Tao et al., 2005), furnishing a limited number of photoproducts. Indeed, the degradation process of the more diluted solution B resulted to be faster and more complex, with the formation of more photoproducts after the first common degradation steps. Nevertheless, the rate constants k_1 and k_2 , of both samples A and B, belong to the

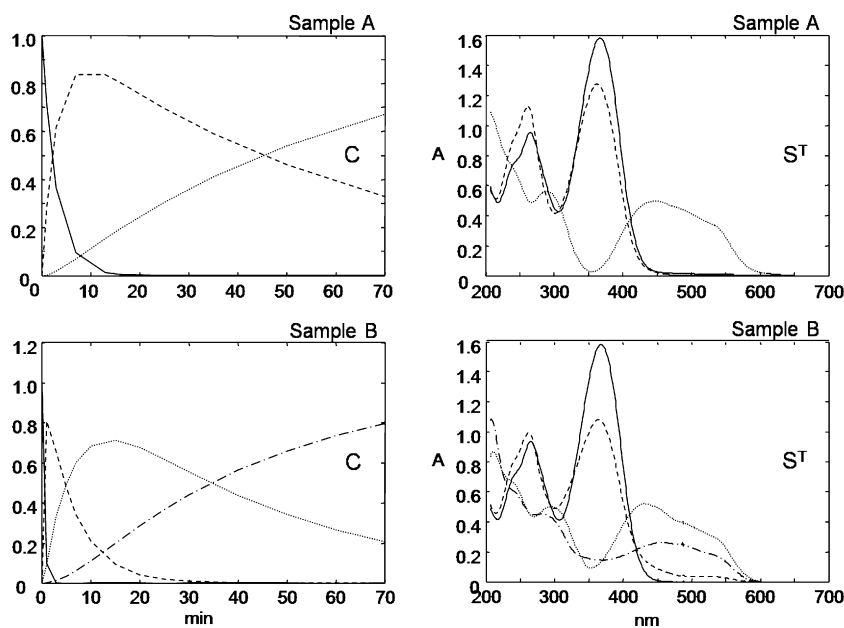


Fig. 6. HS-MCR resolved kinetic profiles and related pure spectra obtained in the analysis of individual photodegradation experiments: samples A (100.0 $\mu\text{g/ml}$) and B (20.0 $\mu\text{g/ml}$) exposed under light at 250 W/m².

Table 1
Kinetic rates for NFZ photodegradation resulted from HS-MCR.

| | k_1 | k_2 | k_3 | %LOF | r^2 |
|------------------------------------|-----------|-----------|-----------|-------|--------|
| Sample A (100.0 $\mu\text{g/ml}$) | 8.638e-02 | 2.076e-02 | – | 1.512 | 99.922 |
| Sample B (20.0 $\mu\text{g/ml}$) | 4.851e-01 | 3.070e-01 | 1.143e-02 | 1.381 | 99.957 |

%LOF = 3.31; r^2 = 99.95%.

photodegradation of NFZ and syn-NFZ, while k_3 (sample B) could be considered as a composite rate constant, because the specie D could be a mixture of compounds.

4.4. Simultaneous MCR analysis of photodegradation experiments under various conditions

A very powerful tool in MCR analysis is the possibility of processing simultaneously multiple data sets obtained in different experiments. In the present work the spectral data from sample solutions A and B when irradiated under various illuminance power (250, 350, 450 and 550 W/m^2) were simultaneously processed by MCR-ALS and HS-MCR-ALS. The analysis of these data sets comprised eight photolysis experiments arranged in a single column-wise augmented data matrix and modelled according to Eq. (6). MCR analysis was initialized, using the set of pure spectra (S^T) previously estimated in Sections 4.2 and 4.3 (analysis of individual data matrices). The concentration profiles and pure spectra finally obtained from HS-MCR-ALS analysis of the augmented data matrix are shown in Fig. 7.

The overall results were similar, as regards to both involved species and kinetic models, to the results obtained by MCR-ALS on the analysis of single degradation experiments. The final resolved

pure spectra presented similar shapes. The influence of the total analyte concentration on the kinetic profiles and photodegradation rate was again confirmed, showing an increasing complexity of the degradation process in diluted solutions, at all illuminance conditions. For sample A, in fact, isomerization of NFZ was rather constant under the different irradiance power values, whereas the formation of a secondary main photoproduct seemed to increase slightly when the irradiance also increased. In sample B (NFZ diluted), on the contrary, the disappearance of both NFZ and its isomer form resulted to be very fast. The formation of the same coloured photoproduct as in sample A was shown, but in this case it was in turn degraded to give a third photoproduct with a rate constant highly influenced by the illuminance power. Table 2 lists the rate constants for all the experiments. The percentage values of LOF and r^2 resulted to be 3.31% and 99.95%, respectively, which are considered very good when the amount of experiments and constraints used during the analysis are considered. The residuals in the time direction showed larger values at the beginning of the experiments, than afterwards they decreased along time; moreover, the residuals in the wavelength direction showed that they were larger when absorptions were also larger. This indicates that probably errors were proportional to absorbance intensities. In all cases, however, the residuals were small and symmetric, equally

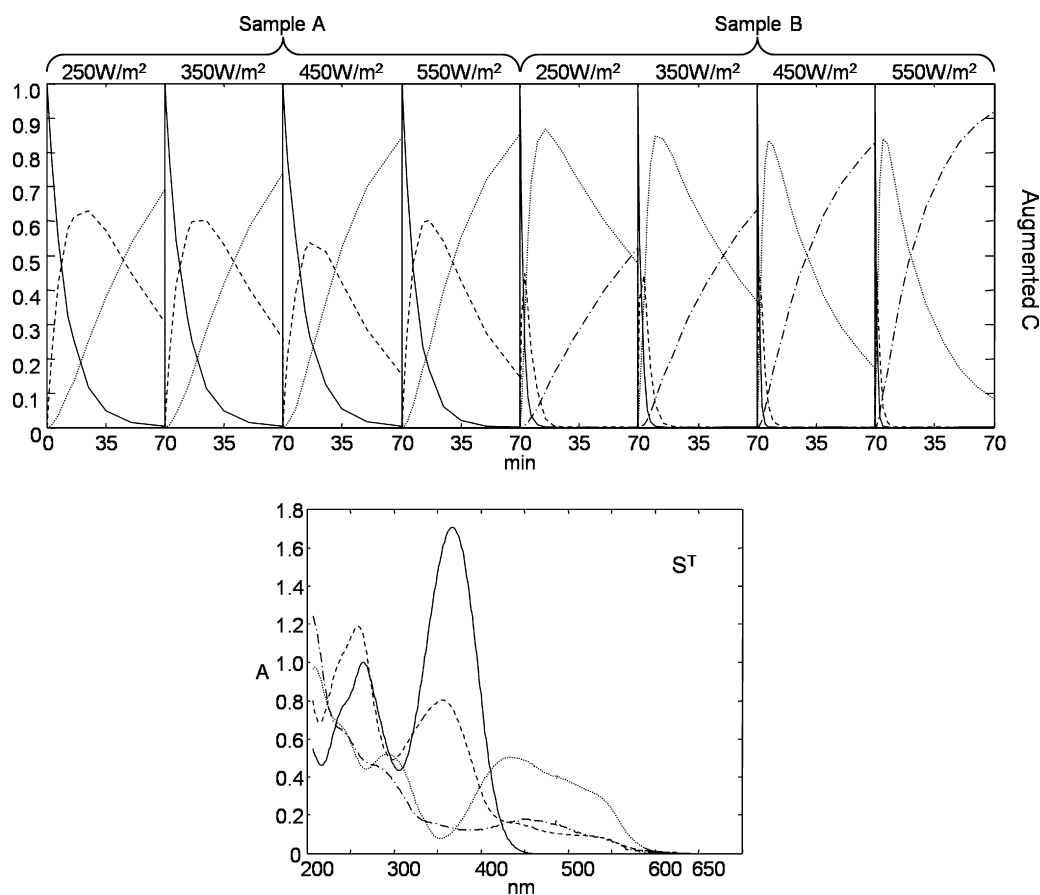


Fig. 7. Simultaneous HS-MCR resolution of eight photodegradation experiments: samples A (100.0 $\mu\text{g/ml}$) and B (20.0 $\mu\text{g/ml}$) exposed under light at 250, 350, 450 and 550 W/m^2 .

Table 2
Kinetic constants for NFZ photodegradation under different conditions of irradiance power and concentration resulted from simultaneous HS-MCR analysis.

| | Power (W/m ²) | k_1 | k_2 | k_3 |
|------------------------|---------------------------|-----------|-----------|-----------|
| Sample A (100.0 µg/ml) | 250 | 8.638e-02 | 2.076e-02 | – |
| | 350 | 8.988e-02 | 2.372e-02 | – |
| | 450 | 9.353e-02 | 3.390e-02 | – |
| | 550 | 1.121e-01 | 4.230e-02 | – |
| Sample B (20.0 µg/ml) | 250 | 4.851e-01 | 3.070e-01 | 1.143e-02 |
| | 350 | 5.982e-01 | 3.484e-01 | 1.529e-02 |
| | 450 | 9.252e-01 | 4.931e-01 | 2.637e-02 |
| | 550 | 1.176e+00 | 7.434e-01 | 3.689e-02 |

distributed in positive and negative sides, but they were not homocedastic.

4.5. Analysis of NFZ photodegradation by HPLC-DAD

With the aim to further interpret and confirm MCR-ALS results obtained in the analysis of time dependent UV data, the photodegradation of NFZ was also investigated by HPLC-DAD. Aliquot samples (0.05 ml) were taken during degradation experiments (at different times) of 1 mg/ml NFZ sample solution and analyzed by HPLC-DAD. The data set obtained in these experiments were then used to build a new set of data matrices (D_{time}) and they were analyzed by MCR-ALS. Fig. 8 shows the results obtained by MCR-ALS when it was applied to this series of chromatograms runs. The simultaneous formation of different components in the same sequence as in previous kinetic UV spectrophotometric studies was

confirmed. Although the chromatographic peak of the NFZ isomer was highly overlapped with the NFZ drug peak, it was clearly resolved by MCR, and its formation resulted to be complete after about 1 h of light exposure. An additional series of new chromatographic peaks were formed after about 40 min, all of them eluting in the first minutes of the chromatographic run and they were not further separated by chromatography. MCR-ALS achieved the resolution of at least four of these by-products, from which at least one peak presented the same spectral profile than the second photoproduct resolved in the previous spectrophotometric study.

The HPLC study confirmed therefore the intrinsic difficulties encountered in the isolation and characterization of the secondary photoproducts derived from the NFZ isomer. It is therefore concluded that the components resolved by MCR-ALS from UV kinetic spectrophotometric experiments were most probably an admix-

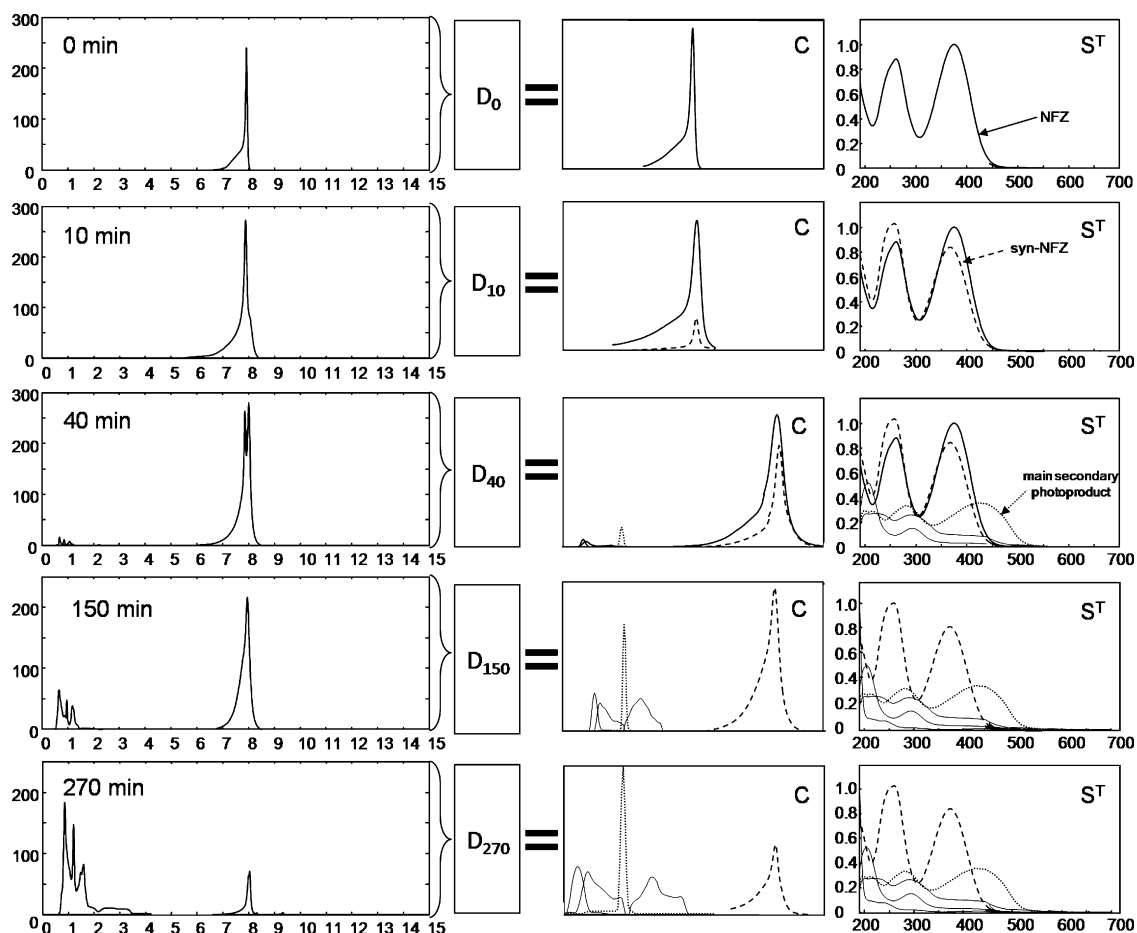


Fig. 8. MCR-ALS resolution of the DAD data from HPLC analysis of a NFZ solution (1 mg/ml), collected at different photodegradation times.

ture of several related compounds rather than single distinct components with different kinetic and spectra profiles. Further investigation is needed to identify and confirm the nature of all the formed subproducts.

5. Conclusions

Results obtained in this work have shown the power of using multivariate curve resolution derived methods to study the degradation processes of photolabile drugs. In particular, in this work, the application of MCR-ALS and of HS-MCR-ALS methods has given an exhaustive description of the kinetic pathway involved in the photodegradation process of the NFZ drug. By using the resolution power of these two methods when they are applied to the simultaneous analysis of multiple samples at different experimental conditions, it was possible to estimate the pure spectra of the degradation products and to determine their concentration profiles and rate constants. It is concluded that NFZ is rapidly transformed into its *syn*-isomer compound which in turn undergoes further degradation to give a series of coloured compounds, unstable and difficult to characterize. Calculated rate constants were shown to depend on both drug concentration and irradiance power. An increase of the rate constants of all degradation processes was observed with the increasing of the light power and with the sample dilution (with decreasing NFZ concentration). Isolation and characterization of all the components involved in the photodegradation process of NFZ were very challenging because of the easy photodegradation of the main photoproducts. Nevertheless, the ability of MCR-ALS method to propose a pathway for the UV photodegradation of the NFZ drug from UV spectrophotometric and LC-DAD data analysis has been proved.

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