Received: 31 December 2010 Revised: 26 January 2011 Accepted: 26 January 2011

(www.drugtestinganalysis.com) DOI 10.1002/dta.276

Study of photodegradation kinetics of melatonin by multivariate curve resolution (MCR) with estimation of feasible band boundaries

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Multivariate curve resolution – alternating least squares (MCR-ALS) has been applied to data collected from UV spectrophotometric analysis of melatonin samples exposed to light with varying irradiance power. MCR-ALS was able to explain the degradation kinetics of this drug, deducing the pure spectra and concentration changes of the different species present throughout the entire process. Possible rotational ambiguities associated with MCR solutions were investigated and their extent was evaluated. The extent of the rotation ambiguity was estimated from the band boundaries of feasible solutions calculated using the MCR-BANDS procedure. The use of a non-linear fitting routine allowed improving kinetic information and provided a method of evaluation of the rate constants of the degradation process. The degradation pathway was found to follow a first-order reaction model, in which melatonin underwent photo-oxidation of the indole ring to give a formylamine group. Kinetics of the reaction was shown to be dependent on irradiation conditions, with an increase of the rate constants when light power also increased.

Keywords: melatonin; multivariate curve resolution; photodegradation; kinetics; band boundaries

Introduction

Spectrophotometric methods are widely used in the determination of several analytes and for the investigation of many analytical problems.[1] However, traditional spectrophotometric methods, using a few number of wavelengths, are often not sufficient to provide the information sought to solve a multicomponent system, especially when the spectra are highly overlapped. In recent years, different multivariate approaches have been proposed to extract useful information from UV data.[2] Multivariate methods have the advantage of exploiting all the information simultaneously, using a very large number of analytical signals.

Multivariate curve resolution – alternating least squares (MCR-ALS) is one of the recent chemometric techniques frequently used for the analysis of spectrophotometric data.[3–4] It is able to resolve the different sources of variance in a particular data set and it allows the study of complex evolving chemical processes, estimating the number of components, their pure spectra, and their concentration profiles. When the investigated process is a kinetic reaction, it also allows the estimation of rate constants (k).[5]

MCR-ALS, like principal component analysis (PCA),[6] performs a bilinear decomposition of the experimental data matrix in the product of two factor matrices of reduced size. This mathematical decomposition is performed under constraints with more physical meaning and easier interpretation than in PCA bilinear decomposition, in which the orthogonal type of constraints are used.[7] However, the solutions obtained by MCR methods are often not unique because of the intrinsic rotational and intensity ambiguities [8] and a set of feasible solutions that fits equally well the experimental data can be obtained instead. This extent of rotation ambiguity can be drastically reduced or eliminated depending on the applied constraints and on the intrinsic data structure. Non-negativity, closure, unimodality, selectivity, and local rank constraints are usually applied [9–10] to improve MCR solutions.

MCR-ALS methods have already been applied to UV spectrophotometric data for the interpretation of kinetics of drug photodegradation.[5,11] In these previous works, MCR-ALS methods have been used for the elucidation of photodegradation kinetic processes, allowing the estimation of the spectra of photoproducts and intermediates at the same time.

Photostability of drugs represents an important issue in pharmaceutical research and a very large number of compounds have been described to be photolabile.[12–15] A deep knowledge of the drug photoreactivity is effectively essential for a correct pharmaceutical formulation and its packaging. A standard protocol for photostability drug testing is described in the International Conference on Harmonization (ICH) Guidelines, which constitute the recommended set of procedures in pharmaceutical industry for key testing of new drugs.[16]

Melatonin (N-acetyl-5-methoxytryptamine) (ML) is a neurohormone produced mainly by the vertebrate pineal gland and synthesized from L-tryptophan.[17,18] It is an important compo-

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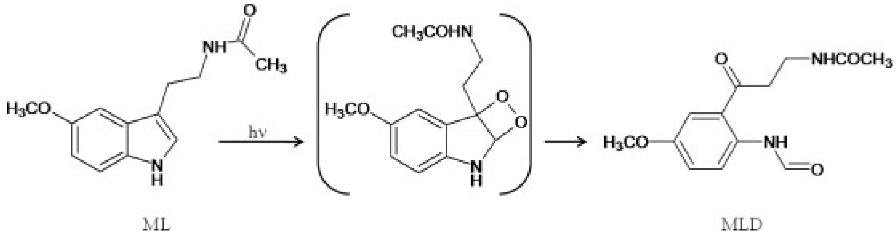

Figure 1. Hypothesis of photodegradation mechanism of ML giving MLD.

nent of the body's internal time-keeping system and is involved in important physiological events, such as the circadian rhythms (sleep-wake cycle). Alterations in ML metabolism have been demonstrated in circadian rhythm sleep disorders, Alzheimer's and Parkinson's diseases, glaucoma, depressive disorders, breast and prostate cancer, hepatoma, and melanoma.[19,20] In pharmaceutical therapy, ML is used to balance possible metabolic disorders or regulate circadian rhythm, sleep disorders, insomnia in blind people, intercontinental flight dysrhythmia (jet-lag syndrome), and insomnia in elderly patients.[21]

ML is known as a photolabile drug and its exposure to light causes a deep transformation of its chemical structure with a probable loss of therapeutic activity. The phodegradation process (Figure 1), described in previous publications,[22] consists in the oxidation of the indole ring to give the N-{3-[2-(formylamino)-5-methoxyphenyl]-3-oxopropyl}acetamide (MLD) through an endoperoxide intermediate.

In our previous application note,[23] the kinetic pathway involved in the photodegradation process of ML was resolved by MCR-ALS. However, these results suffered a moderate uncertainty in estimating both the spectra of the degradation products and the rate constants of the reaction. In this work, the photodegradation mechanism of ML was investigated more thoroughly by applying MCR-ALS on spectrophotometric data. In particular, rotational and scale ambiguities affecting MCR results were studied in detail using a procedure proposed by Gemperline for the calculation of their extent under a set of constraints.[24] In particular, a non-linear curve-fitting routine (hard/soft-MCR-ALS) was used to constrain the MCR-ALS solutions to follow the proposed kinetic reaction model and reduce the extent of rotational ambiguities. Unique solutions and rate constants were thus obtained. The study allowed elucidation of the dependence of the kinetic rates of ML degradation on different values of light irradiation power.

Experimental

Chemicals and instruments

ML was purchased from Sigma-Aldrich Co. (Milan, Italy). Spectrophotometric grade ethanol was from J.T. Baker (Milan, Italy).

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 light irradiation and temperature measuring and controlling. The system was able to closely simulate sunlight and to select spectral regions by interposition of appropriate filters.

Spectrophotometric measurements were recorded using an Agilent 8453 Diode Array spectrophotometer (Agilent Technologies, USA).

All chemometric analyses were performed under MATLAB computer environment (The Mathworks Spa, Milan, Italy). MCR-ALS (both command line and GUI versions), HS-MCR-ALS (only command-line version) and MCR-BANDS (both command line and GUI versions) computer methods were implemented as MATLAB functions. They have been used as described in previous works.[3,25,26] Source files containing these algorithms are available under request to one of the authors of this paper or visiting the web site www.mcrals.info. MCR-BANDS additionally requires the use of *fminuncon, fminsearch,* and *fmincon* functions from the version 3 or higher of the MATLAB Optimization Toolbox.

Experimental procedures

All photodegradation experiments were performed following the ICH recommendations for drug stability tests.[16]

Stock solutions of ML (1 mg ml^{-1}) in ethanol were properly diluted to obtain the samples (20 µg ml^{-1}) for degradation experiments. These samples, in quartz cells perfectly stoppered, were directly light irradiated according to the ID65 standard of the ICH rules. The wavelength range was set between 300 and 800 nm, by means of a glass filter, and the irradiation power was changed at four different levels: 250, 350, 450, and 550 W m^{-2}, corresponding to energy values of 15, 21, 27, and 33 kJ (min $\text{m}^2)^{-1}$, respectively. The inner temperature was maintained constant at 25 °C in all these experiments.

UV spectra were recorded in the wavelength range of 200–450 nm, just after sample preparation ($t = 0$) and at the following times: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, 260, 280, 300, 320, 340, and 360 min.

Chemometric techniques

Multivariate curve resolution – alternating least squares (MCR-ALS)

When a degradation process is monitored by UV spectroscopy, a series of spectra is collected and stored, furnishing the temporal spectral evolution of the system. These data contain the information about all species involved in the chemical process. The characterization of the various components in a chemical process represents a serious analytical problem because the resolution depends on the complexity of the transformation, the overlap of the spectra and the number of the degradation products. These difficulties increase in the presence of intermediate compounds that are rapidly transformed and their concentration profiles are merged with those of reagents and products. Chemometric methods have been proposed to resolve complex multicomponent systems such as the MCR-ALS method.[27]

MCR-ALS is based on a bilinear modelling which decomposes the experimental data into the concentration and spectral contributions of the pure components present in the analyzed mixtures or evolving during a particular chemical process.[28,29] This bilinear decomposition on pure component contributions can be described according to the following equation:

$$\mathbf{D} = \mathbf{C}\,\mathbf{S}^{\mathrm{T}} + \mathbf{E} \tag{1}$$

In the rows of the experimental data matrix **D** are the spectra collected at different reaction times and in the columns are the signals at the different wavelengths evolving with the reaction time. **C** is the concentration matrix of the *n* components involved in the process, $\mathbf{S}^{\mathrm{T}}$ is the spectral matrix of the pure components and **E** contains the unexplained data variance. This model is analogous to the multiwavelength and multisample extension of Lambert-Beer's Law.[1]

In the MCR applications, the first step is the estimation of the number of components, which may be simply performed through a chemical rank (mathematical rank in absence of noise) analysis of the data matrix **D**. Principal component analysis (PCA) and singular value decomposition (SVD)[30] can be used for this purpose. MCR-ALS also requires an initial estimate of either $\mathbf{S}^{\mathrm{T}}$ or **C** matrix, which can be obtained by selection of the purest variables (rows or columns) of the data matrix **D.**[31–33] These initial estimates are then optimized using an alternating least squares (ALS) algorithm under constraints. At each iterative cycle, a new estimation of $\mathbf{S}^{\mathbf{T}}$ or **C** is obtained solving alternatively Eqn 1 for these two unknown matrices. In absence of constraints, the linear least-squares solutions of Eqn 1 are given by the matrix equations:

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

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

where $(\mathbf{S}^{\mathrm{T}})^{+}$ and $(\mathbf{C})^{+}$ are the pseudoinverses[30] of $\mathbf{S}^{\mathrm{T}}$ and **C** matrices, respectively. In order to have MCR solutions with physical meaning, constraints are applied to **C** and $\mathbf{S}^{\mathrm{T}}$ matrices during the ALS procedure. The constraint more commonly used for absorption spectroscopic data is the non-negativity constraint, for both concentration and spectra profiles (matrices **C** and $\mathbf{S}^{\mathrm{T}}$ in Eqn 1). This can be achieved applying non-negative least squares procedures for solving Eqns 2 and 3. Other constraints like unimodality on peak-shaped profiles or closure for the fulfilment of mass-balance equations can be also applied. See references for more details about constraints implementation and examples of their application.[9,10] The ALS iteration procedure is stopped when convergence is achieved, by fixing a preselected number of cycles or by evaluating the value of lack of fit (*%lof*). Another parameter commonly used to indicate the quality of MCR-ALS modelling is the percentage of explained variance ($\%R^2$).[8,34] Their mathematical equations are listed below:

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

$$\%R^2 = 100 \times \frac{\sum_{ij} d_{ij}^{2*}}{\sum_{ij} d_{ij}^2} \tag{5}$$

MCR-ALS presents also the feature that allows the simultaneous analysis of multiple data sets, obtained from independent experiments at different experimental conditions and/or using different analytical and instrumental techniques. In this case, the whole set of photodegradation experiments performed on ML at different irradiation power conditions were arranged in a column-wise augmented data matrix and processed according to the extended bilinear model equation:[35,36]

$$\begin{aligned}\mathbf{D}_{\mathrm{aug}} &= [\mathbf{D}_1;\ldots;\mathbf{D}_{\mathrm{N}}] = [\mathbf{C}_1;\ldots;\mathbf{C}_{\mathrm{N}}]\mathbf{S}^{\mathrm{T}} + [\mathbf{E}_1;\ldots;\mathbf{E}_{\mathrm{N}}]\\ &= \mathbf{C}_{\mathrm{aug}}\mathbf{S}^{\mathrm{T}} + \mathbf{E}_{\mathrm{aug}}\end{aligned} \tag{6}$$

In Eqn 6, column-wise matrix augmentation is shown by using the MATLAB semicolon notation ';'. This notation indicates that every individual $\mathbf{D}_{\mathrm{i}}$ matrix (i=1,. . .,N), obtained in a different kinetic experiment, is arranged one on the top of the other, keeping the same number of columns (wavelengths) in common, and consequently increasing the number of spectra rows of the corresponding augmented matrix $\mathbf{D}_{\mathrm{aug}} = [\mathbf{D}_1;\ldots;\mathbf{D}_{\mathrm{N}}]$.

The same spectral matrix $\mathbf{S}^{\mathrm{T}}$ is resolved for all the $\mathbf{D}_{\mathrm{i}}$ matrices. In contrast, every $\mathbf{C}_{\mathrm{i}}$ concentration profile matrix in $[\mathbf{C}_1;\ldots;\mathbf{C}_{\mathrm{N}}]$ (i=1,. . .,N), is coupled to a different kinetic experiment.

In conclusion, the model proposed in Eqn 6 will fit well with the expected behaviour of the measured system if the generalized bilinear Lambert-Beer's Law holds for this system. The concentration profiles in $\mathbf{C}_{\mathrm{aug}}$ may change due to difference irradiance power and kinetic rate law.

Evaluation of the extent of rotation ambiguities using the MCR-BANDS procedure

As already mentioned, MCR-ALS solutions, under a particular set of constraints, can still not be unique because of the presence of unsolved rotational ambiguities. In this case, instead of getting a unique solution, a set of feasible solutions that fit equally well the data matrix **D** can be obtained. In the literature, this problem has been reported as factor analysis ambiguity.[8,37–39] Rotational and intensity ambiguities can be represented through Eqn 7:

$$\mathbf{D} = \mathbf{C}_{\mathrm{old}}\mathbf{S}_{\mathrm{old}}{}^{\mathrm{T}} = (\mathbf{C}_{\mathrm{old}}\mathbf{T})(\mathbf{T}^{-1}\mathbf{S}_{\mathrm{old}}{}^{\mathrm{T}}) = (\mathbf{C}_{\mathrm{new}}\mathbf{S}_{\mathrm{new}}{}^{\mathrm{T}}) \tag{7}$$

For any non-singular matrix **T** (**T** should be invertible), a new set of solutions is obtained which describes equally well the data matrix **D**. The number of feasible solutions and possible **T** values (new **C** and $\mathbf{S}^{\mathrm{T}}$ matrices) can be drastically reduced by applying constraints derived from the physical nature and previous knowledge of the studied system. In our case, as was previously mentioned, non-negativity (concentrations and spectra of the components must be positive), unimodality (concentration profiles throughout the degradation process present only one maximum per experiment), and closure (mass balance in the kinetic process) constraints were adopted.[8,10] In some favourable cases, it is also possible to perform an improved resolution of the system using other constraints related to selectivity in concentration or spectral regions [8] or related to the knowledge of local rank conditions [40] if they are present.

For each species profile, the set of feasible solutions under constraints defines a range or band of feasible solutions, and this band may be delimited by maximum and minimum band boundaries estimated using a particular criterion. These boundaries will be related to specific rotation matrices **T** for each species *k*, which will be called $\mathbf{T}_{\max,k}$ and $\mathbf{T}_{\min,k}$. By considering a particular pair of solutions to Equation 1, $\mathbf{C}_{\mathrm{inic}}$ and $\mathbf{S}_{\mathrm{inic}}{}^{\mathrm{T}}$, the maximum band boundaries, $\mathbf{C}_{\max,k}$ and $\mathbf{S}_{\max,k}{}^{\mathrm{T}}$, and the minimum band boundaries, $\mathbf{C}_{\min,k}$ and $\mathbf{S}_{\min,k}{}^{\mathrm{T}}$, may be defined, respectively,

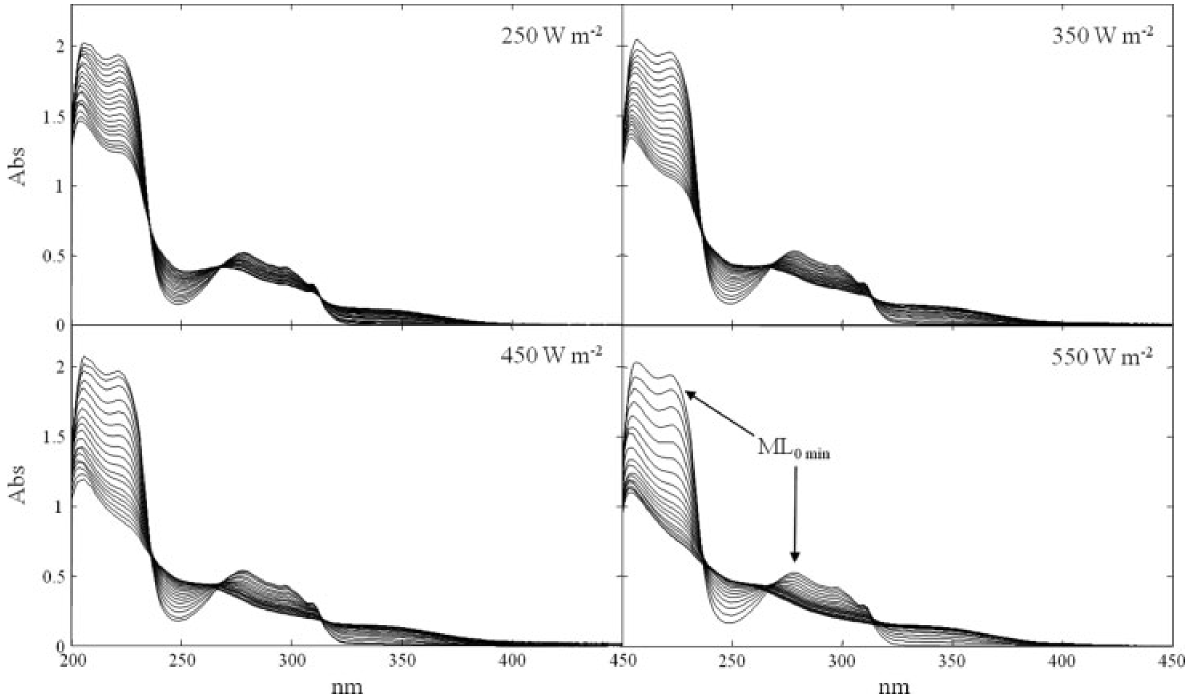

Figure 2. UV spectra of photodegradation experiments of ML (20.0 μg ml^{-1}), exposed under light at 250, 350, 450, and 550 W m^{-2}.

by the following equation:

$$\begin{aligned}\mathbf{D} &= \mathbf{C}_{\text{inic}}\mathbf{S}_{\text{inic}}^{\mathrm{T}} = \mathbf{C}_{\text{inic}}\mathbf{T}_{\text{min},k}\mathbf{T}_{\text{min},k}^{-1}\mathbf{S}_{\text{inic}}^{\mathrm{T}} = \mathbf{C}_{\text{min},k}\mathbf{S}_{\text{min},k}^{\mathrm{T}} \\ &= \mathbf{C}_{\text{inic}}\mathbf{T}_{\text{max},k}\mathbf{T}_{\text{max},k}^{-1}\mathbf{S}_{\text{inic}}^{\mathrm{T}} = \mathbf{C}_{\text{max},k}\mathbf{S}_{\text{max},k}^{\mathrm{T}} \end{aligned} \qquad (8)$$

Different procedures to define the band boundaries have been reported.[23,36,41–43] In this work, the optimization algorithm for the calculation of the band boundaries of feasible solutions was based on a non-linear constrained optimization of an objective function defined by the ratio between the signal contribution of a particular species and the whole measured signal (Signal Contribution Function, *SCF*).[24] It is calculated by Eqn 9:

$$SCF_k = \frac{\left\| \mathbf{c}_k \mathbf{s}_k^T \right\|}{\left\| \mathbf{CS}^T \right\|} \qquad (9)$$

where SCF_k is a scalar value which gives the relative signal contribution of a particular component to the whole signal for the mixture of N components ($k = 1, \ldots N$). This relative signal contribution function, SCF_k, is measured as the quotient between two norms (Frobenious norm), the first one from the signal of the considered component k, $||\mathbf{c}_k\mathbf{s}_k^{\mathrm{T}}||$, and the other from the whole signal considering all the components, $||\mathbf{CS}^{\mathrm{T}}||$. The product $\mathbf{CS}^{\mathrm{T}}$ is constant, as fixed in Eqn 7, and it remains constant for any invertible matrix **T**. On the contrary, every **T** matrix gives a different set of $\mathbf{c}_k$ and $\mathbf{s}_k^{\mathrm{T}}$, so their product $c_k s_k^{\mathrm{T}}$ will be also different, as well as its norm $||\mathbf{c}_k\mathbf{s}_k^{\mathrm{T}}||$ and SCF_k defined in Eqn 9. Therefore, the SCF_k is a scalar value, which will depend on the considered **T** matrix, which will depend on a particular set of constraints. The goal of the procedure is to find the **T** matrices ($\mathbf{T}_{\text{max},k}$ and $\mathbf{T}_{\text{min},k}$) that give the maximum and minimum SCF_k values ($SCF_{\text{max},k}$ and $SCF_{\text{min},k}$) for every k component involved in a system **C** and $\mathbf{S}^{\mathrm{T}}$.[24,41]

The profiles giving these maximum and minimum band boundaries were calculated using the MCR-ALS solutions obtained in the analysis of the augmented matrix obtained from the photodegradation experiments of ML samples at every irradiance power condition.

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

A new constraint can be introduced during the ALS optimization to force the concentration profiles in matrix **C** to fit a previously selected kinetic model. This procedure, which has been named as hard–soft multivariate curve resolution – alternating least squares (HS-MCR-ALS) modelling, applies a hard modelling constraint based on an additional non-linear kinetic curve fitting routine during each ALS iteration, which forces concentration profiles in matrix **C** to fulfil a preselected kinetic model with their corresponding rate constants[3,44] adjusted during the ALS procedure. The resulting concentration profiles will fit the proposed kinetic model and the corresponding rate constants of the process will be obtained as additional valuable information.

Furthermore, HS-MCR-ALS permits the simultaneous analysis of multiple data matrices obtained under different conditions and having different kinetic reaction models and rate constant values, in such a way to optimally resolve the experimental augmented data matrix $\mathbf{D}_{\text{aug}}$ (Eqn 6) from different photodegradation experiments.[11,27]

In this work, the ML photodegradation mechanism was adequately described by a first order kinetic model, with a rate constant dependent on the irradiance power of the light source used during the degradation experiments.

Results and discussion

MCR analysis of the photodegradation experiments

Figure 2 shows the spectral sequences of the photodegradation experiments. Spectra from four ML sample solutions (20 μg ml^{-1})

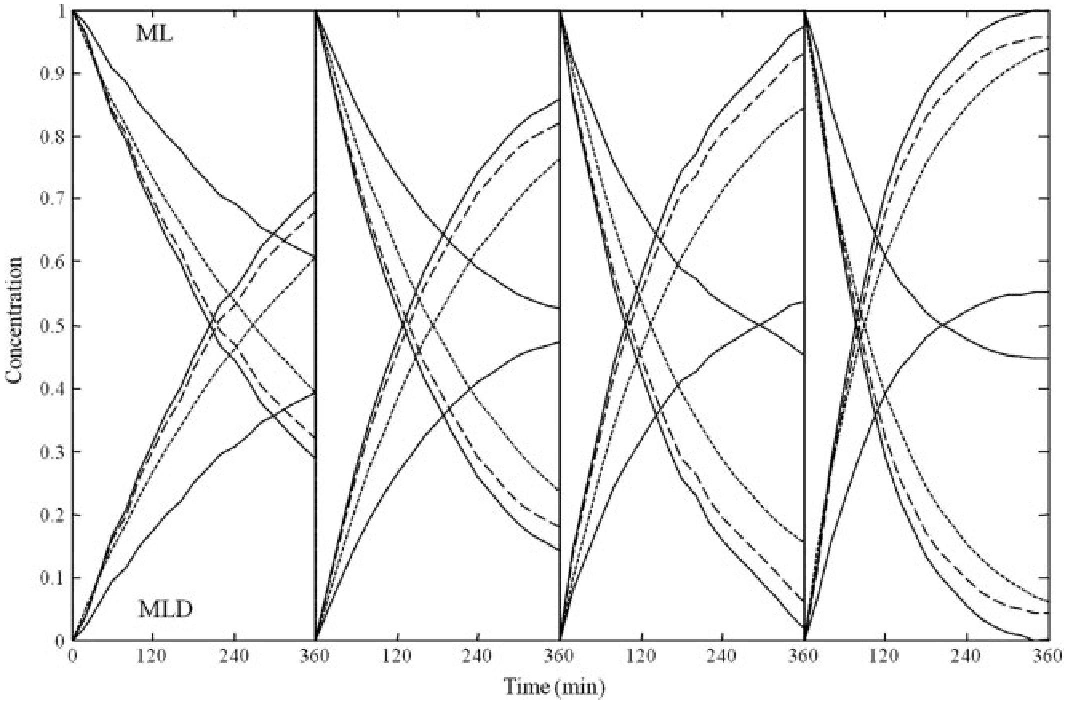

Figure 3. Concentration profiles obtained from the simultaneous analysis of the four photo-degradation experiments of ML solution (20.0 μg ml^{-1}): full lines are the band boundaries obtained by MCR-BANDS, broken lines are the profiles obtained using MCR-ALS, dotted lines are the profiles calculated by HS-MCR-ALS.

under different conditions of light irradiance were recorded during 360 min. Light power was set at 250, 350, 450, and 550 W m^{-2}, respectively. The differences observed in Figure 2 confirmed a significant increase in the photodegradation rate when the irradiation power increased.

Rank analysis (SVD) of the augmented column-wise data set, $\mathbf{D}_{aug}$ in Eqn 6, consisting of the data from the four degradation processes, showed the presence of two different species. Initial estimations of the pure spectra of these two species were already obtained using the purest variable detection method. These spectra were identified to correspond very closely to the known pure spectra of ML and MLD. It was not possible to detect the peroxide intermediate by ordinary UV absorption spectroscopy, probably due to its extremely short life. The spectral data set of Figure 2 was analyzed using the MCR-ALS column-wise matrix augmentation procedure, applying non-negativity (both concentrations and spectra), unimodality (only concentrations), and closure (only concentrations) constraints. Values of *%lof* and *%R*^{2} resulted to be 1.53% and 99.96%, respectively.

The shapes of the pure species concentration profiles were in agreement with a first order reaction kinetic model (ML → MLD).

Calculation of the extent of rotation ambiguities and of the boundaries of feasible solutions

Figures 3 and 4 show the extent of rotation ambiguities associated with concentration and spectra profiles obtained using MCR-ALS (broken lines) as initial estimates (C_{inic} and S_{inic}^T in Eqn 8). The band boundaries (continuous lines) were obtained using the non-linear optimization MCR-BANDS procedure, previously described in the Method section. The dotted lines are the profiles obtained by the HS-MCR-ALS procedure. All these plots were obtained considering the simultaneous analysis of the four kinetic experiments and they show clearly that although the system was relatively simple (only two components), the extent of rotation ambiguities can still be relatively large.

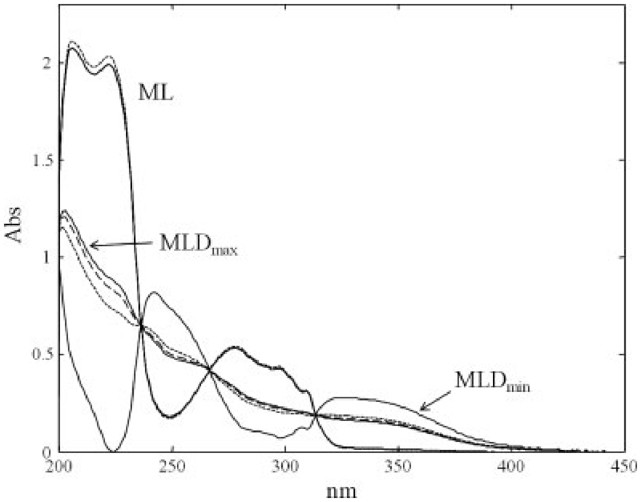

Figure 4. Spectra profiles obtained from simultaneous analysis of the four photodegradation experiments of ML solution (20.0 μg ml^{-1}): full lines are the band boundaries obtained by the MCR-BANDS procedure, broken lines are the profiles obtained using MCR-ALS, dotted lines are the true profiles calculated by HS-MCR-ALS, which are coincident with the known (normalized) pure spectra profiles of ML and MLD.

Band boundaries of Figures 3 and 4 describe the extent of rotation ambiguities and the set of possible solutions that fit the data equally well and fulfil the imposed constraints: non-negative spectra and concentration profiles, unimodal concentration profiles, and closed concentration profiles. For both species in the four experiments, one of the band boundaries is very close or

Table 1. Kinetic constants for ML photodegradation under different conditions of irradiance power

Power (W m^{-2})	Kinetic constants
250	3.185E-03 ± 2.121E-05
350	4.351E-03 ± 2.859E-05
450	6.460E-03 ± 6.828E-05
550	1.008E-02 ± 6.737E-05

coincides with the MCR-ALS solution; ML concentration profile is close to the minimum band boundary profile; while MLD concentration profile is close to the maximum band boundary profile. In particular, the concentration profiles of both species exactly coincide with the band at the beginning of the experiments, where the rotational ambiguity of the MCR-ALS solution is absent. This is due to the favourable local rank conditions at the start of degradation, when only ML is present. Moreover, the effect of the favourable local rank conditions is also clear in the band boundaries of the spectra profiles, where the ML maximum and minimum band boundaries are practically identical to the MCR-ALS solution and the rotational ambiguity is only present for MLD, which has very different maximum and minimum band boundary profiles.

In order to reduce the extent of rotation ambiguities and assure the recovery of the true spectra and concentration profiles, the HS-MCR-ALS procedure was then applied to fit the particular reaction kinetic model. Kinetics (**C**) and pure spectra ($\mathbf{S}^T$) profiles obtained by HS-MCR-ALS analysis (broken lines) are also plotted in Figures 3 and 4. These graphs confirmed that the HS-MCR-ALS solution is the one that recovers more perfectly the known spectra profiles (normalized) of ML and MLD. Therefore, by using the HS-MCR-ALS procedure, the ambiguity of the resolved solutions was practically totally removed. This confirms that HS-MCR-ALS eliminates rotation ambiguities associated to the application of MCR-ALS under soft constraints (like non-negativity, unimodality, and closure) in the study of kinetic chemical reactions.

Table 1 lists the values of the rate constants of the ML photodegradation for every experiment. The percentage values of *lof* and R^2 resulted to be 2.13% and 99.94%, respectively. The results from augmented MCR analysis pointed out a significant dependence of the photodegradation kinetics by the irradiation power. The rate constants of the postulated reaction were increasing with the increase of the light power, following an exponential tendency in according with Eqn 10:

$$\mathbf{y} = 0.0012e^{0.0039\mathbf{x}} \quad (R^2 = 0.994) \tag{10}$$

where *y* is the k value and *x* the illuminance power (Wm^{-2}).

The reaction rate of photodegradation of drugs in solution has been reported to increase with the decrease of the drug concentration and with the increase of the illuminance power.[45,46] These results could be interpreted with a dependence of the photodegradation rate from the number of incident quanta of energy. This number is scarce when the illuminance power is low and therefore the photodegradation rate is limited. On the contrary, the number of incident quanta is abundant when the illuminance power is high, so the rate tends to increase.

Conclusions

Application of MCR-ALS methods have been demonstrated to be suitable for investigation of photodegradation drug studies using spectrophotometric UV-DAD (ultraviolet diode array detector). MCR-ALS resolution of the experimental data obtained in the photodegradation of melatonin drug showed the presence of two species. Although the chemical complexity of this system was relatively low, the use of only natural constraints like non-negativity, unimodality, and closure, still caused a considerable amount of rotational ambiguity in the MCR-ALS solutions. The extent of this rotational ambiguity was evaluated from the band boundaries of the set of feasible solutions, calculated using the MCR-BANDS procedure. The extent of rotational ambiguities and the effect of applied constraints could be easily evaluated using this procedure. In particular, the application of the hard-modelling constrained HS-MCR-ALS procedure which takes into account the reaction mechanism and kinetic rate laws permitted the practical elimination of any ambiguity, furnishing very reliable estimations of the rate constants of the kinetic process. These rate constant values were demonstrated to be strongly dependent on irradiance power. A significant increase of the rate constant of the degradation processes of ML was clearly observed with the increasing of the light power. Summarizing, the proposed MCR techniques (MCR-ALS, MCR-BANDS, and HS-MCR-ALS) have shown to be very appropriate methods for the in-depth study of degradation processes of photolabile drugs and could be extended to more complex photodegradation processes.

Acknowledgement

The authors thank the Ministry of University and Research (MIUR) of Italy for financial support of this work.

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