Application of multivariate curve resolution (MCR) to the photodegradation study of melatonin

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Abstract

A study of the degradation process of melatonin (ML) was carried out by means of a new chemometric approach known as multivariate curve resolution (MCR). MCR algorithm was applied on spectrophotometric data collected from UV analysis of ML solutions exposed to light under varying illuminance power (250, 350, 450 and 550 W/m²). Data elaboration by MCR was able to explain the kinetics of degradation, giving the pure spectra of the involved species and the respective concentration variation. The kinetic pathway was described to follow a first-order reaction, in which ML undergoes photo-oxidation of the indole ring to give a formylamine group. The degradation rate was demonstrated to depend from illuminance conditions, with an increase of the rate constants when the light power was increased. This modern multivariate approach demonstrated high ability to study in depth the kinetics of photolabile drugs by resolution of the components evolving along the degradation process.

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1. Introduction

The photostability of drugs represents an important field in the pharmaceutical research and a high number of compounds have been discovered to be photolabile [1-5]. A complete knowledge of the drug photo-reactivity is effectively essential for a correct pharmaceutical formulation and its packaging. A protocol for testing the photostability of new drugs is described in the ICH Guideline and recommended in the pharmaceutical industry as a key testing of the drugs [6].

Melatonin (N-acetyl-5-methoxytryptamine) (ML) is a neurohormone produced mainly by the vertebrate pineal gland and it’s synthesized from L-tryptophan [7-8]. ML is an important component of the body’s internal time keeping system and it is involved in important physiological events, as 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 disorder, breast and prostate cancer, hepatoma and melanoma [9-15]. In pharmaceutical therapy, ML is used to balance possible metabolic disorders or to regulate circadian rhythm, sleep disorders, insomnia in blind people, intercontinental flight disrhythmia (jet-lag syndrome) and insomnia in elderly patients [16].

ML is known as a photolabile drug and its exposure to light causes a deep transformation of the chemical structure with a probable loss of the therapeutic activity. The phodegradation process (Fig. 1), described in previous publications, 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 [17].
Fig.1 Hypothesis of photodegradation mechanism

Actually, the spectrophotometric methods are widely used in analytical chemistry because of the easy manipulation and interpretation of the spectral data. Nevertheless, the traditional spectrophotometric methods use few wavelengths that frequently are not enough to furnish the necessary information to resolve a system with components presenting spectra overlapping. In recent years, multivariate approaches for the extraction of a largest analytical information from UV spectra have been proposed [18]. Multivariate methods have the advantage of exploiting full spectral data points, by using simultaneously a very high number of analytical signals.

Multivariate curve resolution – alternative least squares (MCR-ALS) is one of the most recent approaches to elaborate the UV spectrophotometric data [19-20]. It allows to extract the pure spectra and concentration of the components in a mixture from a set of spectra with different composition. Its application is particularly useful to evaluate the kinetics of a chemical process, allowing also to calculate the concentration profiles of all the involved species.

This work aims at describing the kinetic pathway of ML photodegradation by MCR procedure, applied on spectral data from analysis of the drug when exposed under different illuminance power. The influence of the changing exposure conditions on the photodegradation rate was also investigated.
2. **Experimental**

2.1. **Chemicals and instruments**

Melatonin (ML), (N-acetyl-5-methoxytryptamine) was purchased from Sigma-Aldrich Co. (Italy). Spectrophotometric grade ethanol was from J.T. Baker (Holland).

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 recorded using an Agilent 8453 Diode Array spectrophotometer (Agilent Technologies. USA).

Chemometric elaboration was performed by MCR algorithm, available in the last version of the software “The Unscrambler®” (CAMO Software AS).

2.2. **Experimental procedures**

All the photodegradation experiments were performed following the ICH recommendations for the drug stability tests [6].

A stock solution of ML (1 mg/ml) in ethanol was properly diluted to obtain the samples (20 μg/ml) for degradation experiments. These solutions, 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 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 inner temperature was maintained constant at 25 °C in all the experiments.

UV spectra were recorded in the wavelength range between 200 and 450 nm, just after 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.
3. **Multivariate curve resolution (MCR)**

When a degradation process is analysed by UV spectroscopy, a series of spectra are recorded as a function of time. All the collected UV data contain the information about all species involved in the chemical process. The characterization of the various components represents a serious analytical problem and its resolution depends on the complexity of the transformation and on the number of the degradation products. These difficulties increase when some components are only intermediate compounds and are in their turn rapidly transformed.

The multivariate methods are very powerful tools to resolve multicomponent mixture systems. MCR-ALS modelling provides to decompose mathematically the analytical data into the contributions due to the pure components in the system [21-23], which can be written as bilinear model of pure component contributions.

\[ D_{(r \times c)} = C_{(r \times n)} S^{T}_{(n \times c)} + E_{(r \times c)} \]

The experimental data are disposed in the matrix D, rows \( r \) are the spectra at different times and columns \( c \) are the process signals at different wavelengths, \( C \) is the concentration matrix of \( n \) components, \( S \) is the spectra matrix and \( E \) represents the unexplained variance in the data set. The model appears equivalent at the general law of Lambert-Beer, therefore is well fit in elaborating an UV data matrix.

In MCR approach, the first step is a preliminary estimation of the number of involved components (\( n \)) in the studied system and an evaluation of \( S^{T} \) or \( C \). Afterward, these initial results are used to perform the alternative least square constrain (MCR-ALS) in such a way to optimize them through an iterative process. At each cycle, a new estimation of \( S^{T} \) and \( C \) is calculated by solving alternatively the two following least-squares matrix equations:

\[ 2) \quad S^{T} = (C)^{+}D \]

\[ 3) \quad C = D(S^{T})^{+} \]

where \((S^{T})^{+}\) and \((C)^{+}\) are the pseudoinverse of the \( S^{T} \) and \( C \) matrices, respectively [24-25].
During the optimization step, other constraints can be applied to drive the final solution towards a chemical meaning. *Non-negativity* constraint forces the concentrations and the spectra of the components so that must be positive; the concentration profiles in the degradation process are forced to give only one maximum per experiment by *unimodality* constraint; in the kinetic process the mass balance is assured by applying the *closure* constraint [25].

The iteration procedure is stopped when convergence is achieved. The values of the residuals, decreasing with optimization, are adopted to evaluate the quality of the fitting. In MCR three different residuals can be considered: variable residuals concerning the $D_{(c)}$, sample residuals for $D_{(r)}$ and total residuals (MCR fitting):

$$
MCR \text{ fitting} = \frac{\sum_{ij} (d_{ij} - d_{ij}^*)^2}{n-1}
$$

where $d_{ij}$ is the experimental absorbance at reaction time $i$ and wavelength $j$, $d_{ij}^*$ is the absorbance obtained by the MCR-ALS model and $n$ is the sample number.

In this work, different kinetic models have been considered to investigate the effects of the illuminance power on the degradation process.

4. **Result and discussion**

4.1. **Photodegradation experiments**

The UV spectra of four ML solutions (20 $\mu$g/ml) under different conditions of light irradiance were recorded along an exposure time of 360 min. In particular, light power was set at 250, 350, 450 and 550 W/m$^2$, respectively. Figure 2 shows the spectral sequence from all photodegradation experiments. The different graph profiles confirmed the dependence of the degradation from experimental conditions, with a significant increase of the photodegradation rate when the irradiation power rose. Exposure under light was prolonged to 360 min because at that time the change in the UV spectrum of the sample under 550 W/m$^2$ was considered not significant.
Fig. 2 UV-spectra of photodegradation experiments of ML (20.0 µg/ml), exposed under light at 250, 350, 450 and 550 W/m²

The spectral data were processed by MCR procedure, by applying non-negativity (both concentrations and spectra) and unimodality as constraints. Multivariate elaboration carried out a system of two species for all the experiments, whereas the peroxide intermediate was never found by MCR elaboration. In Figure 3 the $S^T$ matrix calculated by the resolution procedure of MCR is shown. The graph corresponded to the spectra of two species and their curves were comparable with the spectra in D matrix: the first coincided with the pure drug spectrum and the other curve resulted superimposing the ultimate spectrum recorded in the photodegradation experiment under 550 W/m², corresponding so to the photoproduct MLD.
In a second step of the MCR elaboration, the estimate spectra resolved in the $S^T$ matrix were used to perform a new MCR-ALS resolution. With the purpose to respect the kinetics of the reaction, the mass balance constraint (Closure) was used in addition to non-negativity and unimodality constraints. The $C$ and $S^T$ matrices from the four experiments are shown in Figure 4. MCR fitting values, listed in Table 1, resulted in every cases very small.
Fig. 4 Kinetic profiles and related pure spectra obtained from the analysis of photodegradation experiments of ML solution (20.0 µg/ml) exposed under light at 250, 350, 450 and 550 W/m².
Table 1
Values of total residues by MCR-ALS elaboration

<table>
<thead>
<tr>
<th>Power (W/m²)</th>
<th>MCR fitting</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>2.601E-05</td>
</tr>
<tr>
<td>350</td>
<td>1.918E-05</td>
</tr>
<tr>
<td>450</td>
<td>4.216E-05</td>
</tr>
<tr>
<td>550</td>
<td>4.681E-05</td>
</tr>
</tbody>
</table>

The kinetics study of ML degradation was carried out using the information given by the matrices C, showing the profile concentrations of the involved species (ML and MLD) in the photodegradation process. The curve profiles seemed obeying to a first order kinetics, in agreement with the following equation, calculated for each photodegradation experiment:

\[ \ln[ML] = \ln[ML]_0 - kt \]

where \([ML]\) is the concentration of the drug during degradation, \([ML]_0\) is the starting concentration, \(t\) is the time (min) and \(k\) is the rate constant. The kinetic rates of the photolysis processes are listed in Table 2. This table also reports the half times of the drug, when exposed to light, measured under the different conditions.

Table 2.
Kinetic constants for ML photodegradation under different conditions of irradiance power

<table>
<thead>
<tr>
<th>Power (W/m²)</th>
<th>(k) (min)</th>
<th>(t_{1/2}) (min)</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>3.36E-03</td>
<td>222.52</td>
<td>0.996</td>
</tr>
<tr>
<td>350</td>
<td>5.43E-03</td>
<td>143.28</td>
<td>0.998</td>
</tr>
<tr>
<td>450</td>
<td>7.62E-03</td>
<td>111.29</td>
<td>0.993</td>
</tr>
<tr>
<td>550</td>
<td>1.22E-02</td>
<td>71.15</td>
<td>0.991</td>
</tr>
</tbody>
</table>

The results from MCR-ALS analysis pointed out a significant dependence of the photodegradation kinetics by the illuminance power, as it is evident from the graph of Figure 5, with all the rate constants increasing with the increase of the light power.
5. **Conclusions**

Application of MCR methods has demonstrated to be able to extract the useful information from a set of UV spectral data in order to give an exhaustive description of the kinetic pathway involved in the photodegradation process of ML. By using the resolution power of MCR modelling at different experimental conditions, it was possible to estimate the spectra of the degradation products and to determine the rate constants of the degradation transformations.

The kinetic model was demonstrated to depend on irradiance power and an increase of the rate of all the degradation processes was observed with the increasing of the light power. This approach demonstrates the substantial power of the multivariate curve resolution techniques as a method to study in depth the degradation process of photolabile drugs.
**References**


