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Short communication

A study of the photo-degradation kinetics of nifedipine by multivariate curve resolution analysis

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Abstract

A multivariate curve resolution method based on the combination of Kubista approach and iterative target transformation method of Gemperline has been proposed. This method is a soft model and need no information about the spectrum of the product and mechanism of the reaction. The method was used to study the degradation kinetics of nifedipin, 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxilic acid dimethyl ester, upon exposure to the light of a 40 W tungsten lamp. The spectra of the nifedipine, collected at different lighting times, were subjected to the factor analysis and two chemical components were detected in the reaction system. Pure spectra of the components involved and their concentration profiles were obtained. The results revealed that the photodecomposition kinetics of nifedipine is zero-order at the beginning of the reaction. However, when the reaction preceded more than 50%, the kinetics of reaction changed to a first-order manner. The rate constants for the zero-order and first order regions were estimated as regions $(4.96 \pm 0.13) \times 10^{-9}$ M^{-1} s^{-1} and $(6.22 \pm 0.10) \times 10^{-5}$ s^{-1}, respectively.

Keywords: Nifedipine; 1,4-Dihydropyridine; Photo-degradation kinetics; Chemometrics; Multivariate curve resolution

1. Introduction

The influx of extracellular Ca^{2+} through the L-type potential dependent calcium channel is responsible for the regulation of many physiological functions, including smooth and cardiac muscle contraction [1,2]. It is discovered that the 1,4-dihydropyridine class of calcium channel antagonist inhibits this Ca^{2+} influx. Nifedipine (NIF), 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxilic acid dimethyl ester, as the prototype compound of the dihydropyridine class of calcium channel antagonist, is a selective arterial dilator and is frequently used for the treatment of hypertension, angina pectoris and other cardiovascular disorders [3].

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NIF is a sensitive substance which decomposes in UV light to give the 4-(2-nitrophenyl) pyridine homologue, and under daylight to give the 4-(2-nitrosophenyl)-pyridine homologue [4,5]. Several studies in the past have been conducted to determine the photostability of nifedipine in solution and or in solid state [4,6,12]. The methods used for these studies were all chromatography based [i.e. gas–liquid chromatography (GLC), GLC–mass spectrometry and high performance liquid chromatography (HPLC)] which, of course, are difficult to operate and use expensive instruments and solvents. In addition, further degradation of NIF may occur during the chromatographic analysis.

In the other hand, spectroscopic methods are in general simple, highly sensitive and very suitable for the study of chemical reactions in solutions. When the components involved in the chemical reaction have distinct spectral responses, their concentration can be monitored directly. However, in many cases, the spectral responses of two and, sometimes, even more components overlap considerably and the analysis is no longer straightforward. The common approach has been single-point measurements at a wavelength where one component dominates the spectral response and the contributions from the other components are neglected. However, by the use of chemometrics methods [13], one can analyze whole spectral, thereby utilizing all spectral information. This approach is superior to any single-point measurement, since several hundreds of data points per spectrum can be treated simultaneously.

Spectral curve deconvolution or multivariate curve resolution methods are chemometrics techniques which concern with the extracting of the pure spectra of components involved and their corresponding concentration profiles from evolutionary processes. These methods can be classified into two groups: (1) modeling methods and (2) self-modeling methods. Kankare is the originator of the modeling method [14]. While other workers such as Shrager [15] and Frans and Harris [16] tried to develop this method. Recently, Kubista et al. [17] proposed a modeling method based on the fact that in many cases, the spectrum of one of the components is known which, in turn, makes the calculations simpler.

Self-modeling methods extract the concentration profiles without having any information about the shape of the spectra. Several self-modeling approaches have been developed since the pioneering work by Lawton and Sylvestre in 1971 [18]. Among these are the factor analysis-based methods such as automated spectral isolation (ASI) [19], iterative target transformation factor analysis (ITTFA) [20], evolving factor analysis (EFA) [21], iterative key set factor analysis (IKS-FA) [22], windows factor analysis (WFA) [23] and alternative least squares (ALS) [24].

In this work, we developed a curve deconvolution method by combining the Kubista method with the ITTFA procedure of Gemperline. The method was used to study the decomposition kinetics of nifedipine in methanol solution exposed to a 40 W lamp. Since the kinetic behavior of the nifedipine decomposition is not well known [12], a hard model such as Kubista method cannot be applied successfully, and some ambiguity is shown up in the resolved spectral and concentration profiles. The transformation matrix obtained by the application of Kubista method was improved by the ITTFA procedure and using some constraints such as non-negativity and closure.

2. Theory

In this section, the following convention will be used. A capital letter in boldface demonstrates a matrix and a lowercase letter in boldface denotes a vector. Lowercase italic letters denote the scalars. The spectral data, recorded under the light of 40 W lamp in the 20 min intervals, were collected in a data matrix (**D**) with $m \times n$ dimension, m being the number of data points per spectrum and n being the number of spectra collected at various reaction times. If there are k absorbing components in the reaction system, the recorded absorbance at each wavelength is assumed to be the sum of contributions of all components:

$$d_j(\lambda) = \sum_{i=1}^{k} s_i(\lambda) c_{ij} \quad (j = 1,\ n) \tag{1}$$

where d_j (λ) is the spectrum of sample j, c_{ij} is the concentration of component i in sample j and n is the number of samples. The above equation can be written in a matrix notation as $\mathbf{D} = \mathbf{X}\ \mathbf{Y}$, where $\mathbf{X}$ is an $m \times k$ matrix of the molar absorbencies and $\mathbf{Y}$ is $k \times n$ matrix containing concentration profiles. The following steps were employed to carry out the curve resolution procedure on the data matrix $\mathbf{D}$.

(1) The number of components or chemical species (k) present in the system is estimated by factor analysis [25]. For this purpose, the data matrix was decomposed to row and column matrices by singular value decomposition (SVD)

$$\mathbf{D} = \mathbf{R}\ \mathbf{C} \tag{2}$$

where $\mathbf{R}$ contains the ortho-normal eigenvectors spanning the row space of original data matrix and $\mathbf{C}$ spans the column space. Some methods such as real error (RE) imbedded error (IE) chi value (χ) and indicator function are available which can determine the number of factors [25]. In our case, we have two absorbing species, the nifedipine and its decomposition product (i.e. nitroso-pyridine homologue). Thus, we have limited the analysis to the two first columns of $\mathbf{R}$ and two first rows of $\mathbf{C}$:

$$\mathbf{D} = [\mathbf{r}_1 \quad \mathbf{r}_2] \begin{bmatrix} \mathbf{c}_1 \\ \mathbf{c}_2 \end{bmatrix} \tag{3}$$

where $\mathbf{r}_1$ and $\mathbf{r}_2$ concern with the spectral characteristics of the components and $\mathbf{c}_1$ and $\mathbf{c}_2$ are related to their concentration profiles. These are mathematical factors and do not have any physical or chemical meaning.

(2) A 2×2 transformation matrix, $\mathbf{T}$, and its inverse, $\mathbf{T}^{-1}$, are introduced to transform the abstract matrices $\mathbf{R}$ and $\mathbf{C}$ into the real matrices, $\mathbf{X}$ and $\mathbf{Y}$ (i.e. real spectral and concentration profiles, respectively):

$$\mathbf{D} = (\mathbf{R}\ \ \mathbf{T})(\mathbf{T}^{-1}\ \ \mathbf{C}) = \mathbf{X}\mathbf{Y} \tag{4}$$

$$\mathbf{T} = \begin{bmatrix} t_1 & t_3 \\ t_2 & t_4 \end{bmatrix} \quad \text{and} \quad \mathbf{T}^{-1} = \frac{1}{t_1 t_4 - t_2 t_3} \begin{bmatrix} t_4 & -t_3 \\ -t_2 & t_1 \end{bmatrix} = \begin{bmatrix} tt_1 & tt_3 \\ tt_2 & tt_4 \end{bmatrix} \tag{5}$$

At this point, $\mathbf{T}$ is unknown while $\mathbf{R}$ and $\mathbf{C}$ are known from the SVD analysis. Once the transformation matrix, $\mathbf{T}$ obtained, the pure spectrum and concentration profile of the species could be determined.

(3) The elements of the $\mathbf{T}$ matrix can be determined by target factor analysis (TFA) procedure [25] as suggested by Kubista [17].

$$\mathbf{X} = [\mathbf{x}_1 \quad \mathbf{x}_2] = \mathbf{R} \begin{bmatrix} t_1 & t_3 \\ t_2 & t_4 \end{bmatrix} = \mathbf{R} \begin{bmatrix} t_1 \\ t_2 \end{bmatrix} + \mathbf{R} \begin{bmatrix} t_3 \\ t_4 \end{bmatrix} \tag{6}$$

$$\mathbf{x}_1 = \mathbf{R} \begin{bmatrix} t_1 \\ t_2 \end{bmatrix} \quad \text{and} \quad \mathbf{x}_2 = \mathbf{R} \begin{bmatrix} t_3 \\ t_4 \end{bmatrix} \tag{7}$$

$\mathbf{x}_1$ and $\mathbf{x}_2$ are the pure spectral of nifedipine and its decomposition product, respectively. By knowing the spectrum of the nifedipine, t_1 and t_2 are calculated by Eq. (8):

$$\begin{bmatrix} t_1 \\ t_2 \end{bmatrix} = \mathbf{R}^{+} \mathbf{x}_1 \tag{8}$$

where $\mathbf{R}^{+}$ is the pseudo inverse of the matrix $\mathbf{R}$. Since the spectrum of the nifedipine decomposition product is unknown, t_3 and t_4 cannot be determined by this procedure. However, since the sum of concentrations of the two components during the reaction is a constant value (i.e. $c_t = c_1 + c_2$), t_3 and t_4 can be estimated by the following procedure:

$$\mathbf{Y} = [\mathbf{y}_1 \quad \mathbf{y}_2] = \mathbf{T}^{-1}\mathbf{C} = \begin{bmatrix} tt_1 & tt_3 \\ tt_2 & tt_4 \end{bmatrix} \mathbf{C} = \begin{bmatrix} tt_1 \\ tt_2 \end{bmatrix} \mathbf{C} + \begin{bmatrix} tt_3 \\ tt_4 \end{bmatrix} \mathbf{C} \tag{9}$$

$$\mathbf{y}_1 = \begin{bmatrix} tt_1 \\ tt_2 \end{bmatrix} \mathbf{C} \quad \text{and} \quad \mathbf{y}_2 = \begin{bmatrix} tt_3 \\ tt_4 \end{bmatrix} \mathbf{C} \tag{10}$$

where, $\mathbf{y}_1$ and $\mathbf{y}_2$ are row vectors containing the concentration of the components during the photodecomposition reaction. The sum of $\mathbf{y}_1$ and $\mathbf{y}_2$ is equal to the initial concentration of the nifedipin (c_t). Thus:

$$\mathbf{c}_t = \mathbf{y}_1 + \mathbf{y}_2 = \begin{bmatrix} tt_1 \\ tt_2 \end{bmatrix}\mathbf{C} + \begin{bmatrix} tt_3 \\ tt_4 \end{bmatrix}\mathbf{C} = \begin{bmatrix} tt_1 + tt_3 \\ tt_2 + tt_4 \end{bmatrix}\mathbf{C} \quad (11)$$

$\mathbf{c}_t$ is a vector whose all elements are the same (initial concentration of the nifedipine). By solving the above equation, (tt_1+tt_3) and (tt_2+tt_4) can be determined. Then, by the use of the relationship existed between the elements of **T** and its inverse (Eq. (5)) and knowing the values of t_1, t_2, (tt_1+tt_3) and (tt_2+tt_4), all of the elements of the **T** and $\mathbf{T}^{-1}$ matrices can be calculated. Thus, it is possible to determine the pure spectrum of the nifedipine decomposition's product and their concentration profiles (Eqs. (7)–(10)). However, there is some ambiguity in the derived results; the negative values of the $\mathbf{x}_2$ vector are meaningless.

(4) The ITTFA procedure combined with non-negativity and closure constrains, was used to reduce this rational ambiguity. First, the negative values of $\mathbf{x}_2$ were forced to zero, and a new transformation matrix was calculated as

$$\mathbf{T} = \mathbf{R}^{+}\mathbf{X} \quad (12)$$

Then, by using the newly calculated **T** matrix, the **Y** matrix was calculated by Eq. (9) and, subsequently, the **X** matrix recalculated as:

$$\mathbf{X} = \mathbf{D}\mathbf{Y}^{+} \quad (13)$$

After application of non-negativity constrain to this calculated **X** and **Y** matrices, the calculation of **T**, **Y** and **X** matrices was repeated iteratively until reaching to convergence. The degree of convergence was determined by two criteria: (1) comparison of calculated **T** matrix at each iteration step with that calculated at the previous iteration step and (2) the difference between the experimental **D** matrix and the calculated $\hat{\mathbf{D}}$ matrix using the **X** and **Y** matrices.

3. Experimental

3.1. Apparatus and reagents

Nifedipine was purchased from the Sigma Chemical Co. A 1.20×10^{-4} mol l^{-1} methanolic solution of nifedipine was prepared by dissolving an appropriate amount of nifedipine in pure methanol, (Merck).

All spectra were recorded on an Ultrospec 3000 pro (Pharmacia Biotech) UV–Vis spectrophotometer equipped with 10-mm quartz cells. The Swift (II) software was used to collect the absorbance data of the solution into a spreadsheet.

A multivariate curve resolution program was written in MATLAB (Ver. 5.1, the MathWork Inc.). This program contains all the necessary calculations mentioned in the previous section.

3.2. Procedure

The irradiation test employed utilized a 40 W lamp placed 50 cm from the nifedipine solution. Irradiation was conducted inside a dark room with controlled temperature to protect samples from extraneous light. The UV–Vis spectra of solutions (220–450 nm) were recorded in 20 min intervals, up to 300 min. At a given temperature of 25 ± 1 °C, 16 digitized absorbance spectra were recorded in 0.5 nm intervals and the data were collected in a (460×16) data matrix, **D**. This data matrix was subjected to the multivariate curve resolution analysis. From the resulted concentration profile of the components, the reaction rate constants were calculated.

4. Results and discussion

The photodecomposition of nifedipine has been the subject of many studies [4,6–12]. However, the employed chromatographic methods were not sufficient for studying the kinetics of this reaction. In this work, we used a multivariate curve resolution method for this purpose. It is well known that, upon exposure of nifedipine to daylight, it is converted to its nitrosopyridine homologue. In order to overcome the potential problems inherent when using natural sunlight, such as daytime, regional, weather and seasonal variability, a 40 W lamp which emit visible light and some portion of UV light is used.

Fig. 1 shows the spectra of a nifedipine solution, collected at 20 min intervals, upon exposure to the light at 25 °C. As is obvious, the gradual decrease

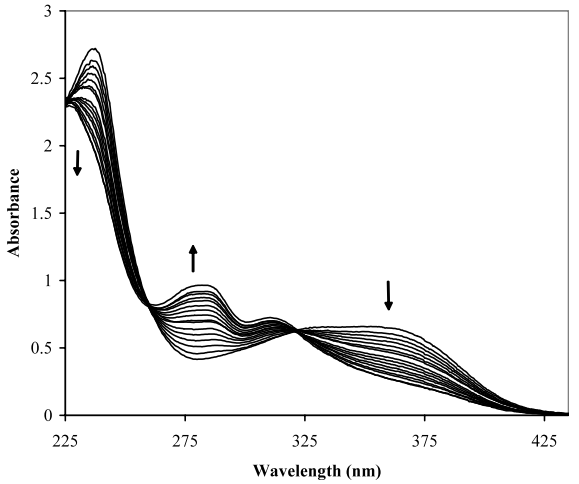

Fig. 1. Absorption spectra of nifedipine solution at different lighting times. The spectra are collected at 20 min intervals.

in nifedipine absorbance at 238 and 361 nm is accompanied by the appearance of two new peaks at 284 and 312 nm, possessing two narrow isosbestic points at 322 and 261 nm. In order to resolve the spectra of nifedipine and its degradation product, the MCR procedure was conducted. Thus, the resulting absorbance data matrix was subjected to factor analysis, in order to find the number of chemical components co-existing in the system. The results are plotted in Figs. 2 and 3. In Fig. 2, the evolution of the eigenvalues is plotted as a function of the reaction time. The large change

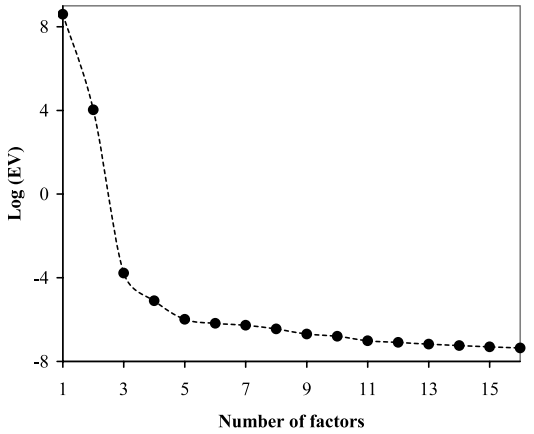

Fig. 2. Plot of the logarithm of eigenvalue as a function of the number of factors.

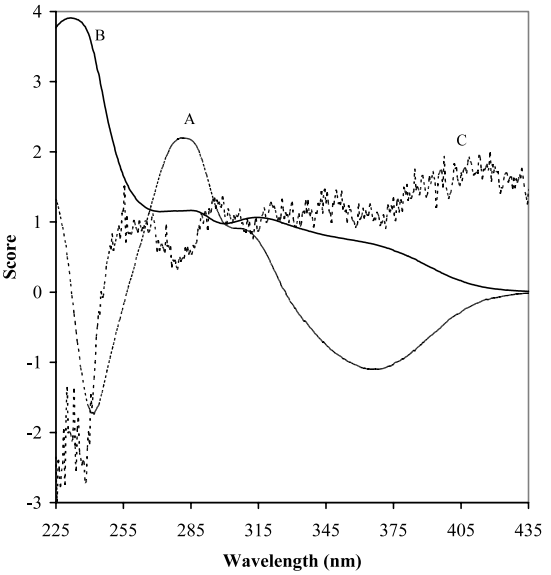

Fig. 3. Plot of the scores obtained from the absorbance data matrix of the nifedipine: (A) first score, (B) second score, (C) third score.

observed between the eigenvalues 2 and 3 emphasizes that only two components are involved in the process. Furthermore, score (or loading) plot would also provide an estimation of the number of significant components or factors present in the system. Fig. 3 shows the plot of the first three scores as a function of wavelength. These plots obviously confirm that only the first two factors reveal systematic variations of the data and the third factor models the noise. Thus, we limited our further calculations on a two-component system.

The pure spectra of the components and their corresponding concentration profiles were determined by the Kubista method (target transformation factor analysis). The resolved spectrum for nifedipine was similar to that obtained experimentally. However, the resulting product spectrum revealed negative absorbance in some spectral regions. This is most probably due to the inaccurate calculation of t_3 and t_4, since calculation of these elements was only done by using closure constrain which is in fact insufficient. Thus the ITTFA combined with non-negativity and closure constrains were used for the adjustment of the transformation matrix. When the calculation con-

verged, a fitting error of 0.75% was observed. The resulting fitting error shows how much the raw data matrix reproduced by the calculated **X** and **Y** matrices. The optimal pure spectra and corresponding concentration–time profiles are shown in Figs. 4 and 5, respectively. The experimentally determined spectra of nifedipine and its reaction mixture after 300 min are also included in Fig. 4, for comparison. As is quite obvious, there is a fair agreement between the resolved nifedipine spectrum and its experimentally determined spectrum.

The plots in Fig. 5 show the respective consumption and evolution of the reactant and product as a function of reaction time. It is seen that, after 300 min, 65% of the reaction has been completed. According to the Einstein photochemical equivalence law, the kinetics of such reactions is zero order (i.e. $-dc/dt = k$) [26]. This is confirmed by the obtained concentration–time profiles up to about 175 min corresponding to 50% completion the reaction (Fig. 5). After this time, the reaction rate decreases and the reaction kinetics obeys a first-order behavior. A similar kinetic behavior has already been reported from the spectroscopic [4] and chromatographic methods [6–12]. However, some other researchers have used first order or more complex models to describe the degradation of nifedipine [27,28]. As it was reported previously, altering the reaction

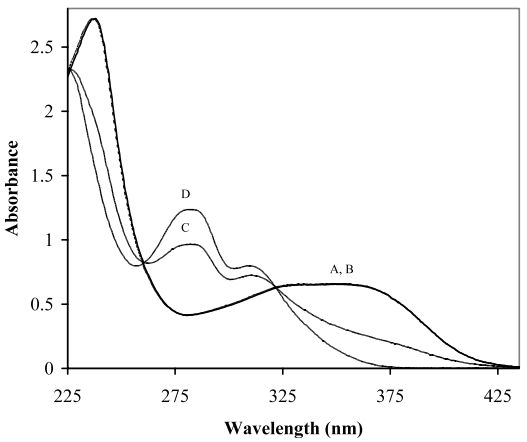

Fig. 4. Optimum pure spectra of the nifedipine and its degradation product resulted after convergence of the ITTFA method: (A) resolved nifedipine, (B) experimental nifedipine, (C) product, (D) nifedipine-product mixture after 300 min.

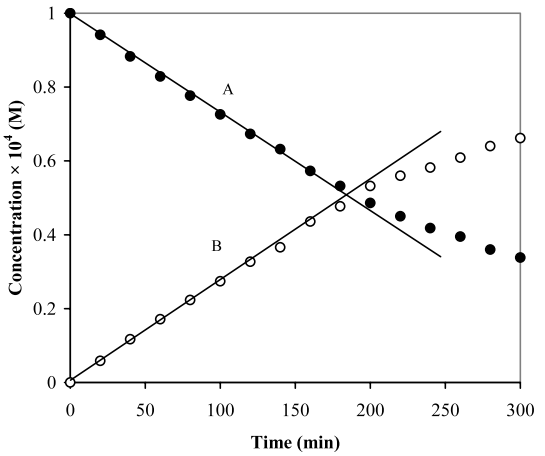

Fig. 5. Optimum concentration profiles of the nifedipine and its degradation product resulted after convergence of the ITTFA method.

rate from zero-order to first order one my attributed to the inhibition of the nifedipine degradation by its nitrosopyridine homologue, as the reaction product [4,12]. The resulted concentration–time profiles (Fig. 5) show that deviation from zero-order kinetics is occurred when the concentration of the product in the reaction mixture is exceeded that of nifedipine.

The reaction rate constants can be evaluated directly from the resulting concentration–time profiles by fitting the first segment to a linear model and second segment to an exponential model. The equation obtained for the linear and exponential segments are represented below:

$$C_{NIF} = 1.181(\pm 0.001) \times 10^{-4} - 4.96(\pm 0.13) \times 10^{-9} t$$
$$r^2 = 0.995$$

$$C_{NIF} = 1.197(\pm 0.003) \times 10^{-4} \exp(-6.22(\pm 0.10) \times 10^{-5} t)$$
$$r^2 = 0.998$$

Therefore, at 25 °C, the zero-order rate constant for decomposition of nifedipin is $(4.96 \pm 0.13) \times 10^{-9}$ M^{-1} s^{-1} and the first-order one is $(6.22 \pm 0.10) \times 10^{-5}$ s^{-1}.

5. Conclusion

A multivariate curve resolution method based on the combination of the Kubista approach and iterative target transformation method of Gemperline was applied to study the kinetics of nifedipine decomposition upon exposure to a 40 W lamp. Factor analysis showed that there are two chemical components in the reaction system; one of them is nifedipine and another one is the photodecomposition product of nifedipine, nitrosopyridine homologue of nifedipine. The resulting concentration–time profile of the components showed that the reaction is 65% completed after 300 min. In addition, it was found that kinetic profile of the degradation of nifedipine possesses two regions. The first region was linear with time which confirms a zero order kinetic and the second region showed a first-order kinetic pathway. The reaction rate constants were calculated for the zero and first order regions as $(4.96 \pm 0.13) \times 10^{-9}$ M^{-1} s^{-1} and $(6.22 \pm 0.10) \times 10^{-5}$ s^{-1}, respectively.

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