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Short communication

Application of a self-modeling curve resolution method for studying the photodegradation kinetics of nitrendipine and felodipine

Katayoun Javidnia [a], Bahram Hemmateenejad [a,b,*], Ramin Miri [a], Mehdi Saeidi-Boroujeni [a]

[a] *Medicinal and Natural Products Chemistry Research Center, Shiraz University of Medical Sciences, Shiraz 71345, Iran*
[b] *Department of Chemistry, Shiraz University, Shiraz 71454, Iran*

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Abstract

Dihydropyridine (DHP) derivatives, as calcium channel blockers with cardiovascular activity, are highly photosensitive and converted in the presence of light to compounds that are inactive. In this work, a self-modeling curve resolution method was applied to study the photodegradation kinetics of nitrendipine and felodipine by spectrophotometric method. The methanolic solutions of drugs were separately exposed to UV and daylight, respectively. A fully soft-modeling multivariate curve resolution method based on the combination of iterative target transformation and Kubista methods were used to analyze the recorded absorbance data, extracting the concentration profiles and pure spectra of the drugs and their photodegradation products. By fitting the concentration profiles of the studied DHP drugs to different kinetic equations, it was found that at the beginning of lighting, the reaction is zero-order and in the case of nitrendipine it changes to a first-order kinetic when the concentration of products exceeded than that of the initial compounds.

Keywords: Photodegradation; Multivariate curve resolution; Nitrendipine; Felodipine

1. Introduction

Nitrendipine, 4-(3-nitrophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate and felodipine, 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylate (see Fig. 1), are calcium channel antagonists of dihydropyridine (DHP) class used in the treatment of hypertension [1–3]. DHP derivatives are highly photosensitive and converted in the presence of light to compounds that are inactive, and the most of the methods used for these studies were different chromatographic methods [4–12]. Of course chromatographic methods are difficult to operate and use relatively expensive instruments. In addition, further degradation of drugs may occur during the chromatographic analysis.

In the other hand, spectrophotometric methods are in general simple, sensitive and very suitable for studying chemical reactions in solutions. The spectral overlapping, as the major problem in almost all of the spectrochemical methods, can be overcome utilizing different chemometric methods [13]. For example, spectral curve deconvolution or multivariate curve resolution (MCR) methods are chemometrics techniques concerning with the extraction of the pure spectra and concentration profiles of the components in a chemical reaction preceded in an evolutionary process [14–16]. There are some literature reports on the use of different MCR methods for studying the forced degradation kinetics of drugs and other biologically important compounds [17,18].

In our research group, we have some interests on DHP derivatives and many papers have been published from this group regarding this type of molecules in different subjects [19–24]. We employed a constrained self-modeling MCR method for monitoring the photodegradation kinetics of nifedipine, the prototype of DHP drugs [25]. Since the photodegradation kinetic of DHP-based drugs is complex and it is preceded in zero- and first-order manners, application of hard-modeling methods is not straightforward. Thus, we extend our previously self-modeling method for studying the photodegradation monitoring

* Corresponding author at: Department of Chemistry, Shiraz University, Adabiat Four-way, Shiraz, Fars 71454, Iran. Tel.: +98 711 2284822; fax: +98 711 2286008.
E-mail address: hemmatb@sums.ac.ir (B. Hemmateenejad).

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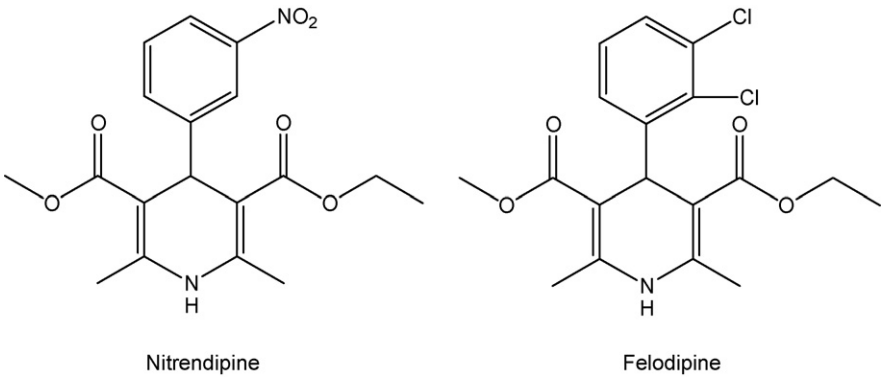

Fig. 1. Chemical structures of nitrendipine and felodipine.

of two other DHP derivatives, i.e., nitrendipine and felodipine.

2. Experimental

2.1. Reagents and chemicals

Pure powders of drugs (nitrendipine and felodipine) were prepared from Sigma Chemical Co. The 100 μg/mL stock solution of each drug was prepared by dissolving appropriate amounts of drugs in methanol (Merck) under sodium lamp and used to prepare daily solutions.

2.2. Apparatus

The UV–vis absorbance spectra were recorded by a Shimadzu spectrophotometer (Tokyo, Japan) equipped with a 10.0 mm quartz cell and a water-thermostated cell holder. A home-made dark woody cabinet equipped with UV lamps of 254 nm wavelengths was used.

2.3. Procedure

The radiation tests were employed utilizing a 254-nm UV lamp for nitrendipine and natural sunlight for felodipine. In the case of nitrendipine, to protect samples from extraneous light, irradiation was conducted in a dark room with controlled temperature. The UV–vis absorbance spectra of the methanolic solution of nitrendipine, exposed to the UV lamp, were recorded between 220 and 400 nm in 24-h intervals for 15 days. The digitized absorbance data (in 1.0 nm intervals) were collected in a data matrix with a dimension of (182 × 15).

In the case of felodipine, the photodegradation was monitored in sequential sunny days in the time duration of 10 a.m. till 4 p.m. At the end of each day, the resulting solution was protected by aluminum foil and stored in the refrigerator to prevent further degradation and then in the new day, its temperature was raised to room temperature and then subjected to sunlight. The absorbance spectra of the irradiated solutions were recorded between 230 and 400 nm in 2 h intervals for 4 days. Since in each day the drug was exposed to sunlight for 6 h, the total lighting time was 24 h and 14 absorbance spectra were collected through-

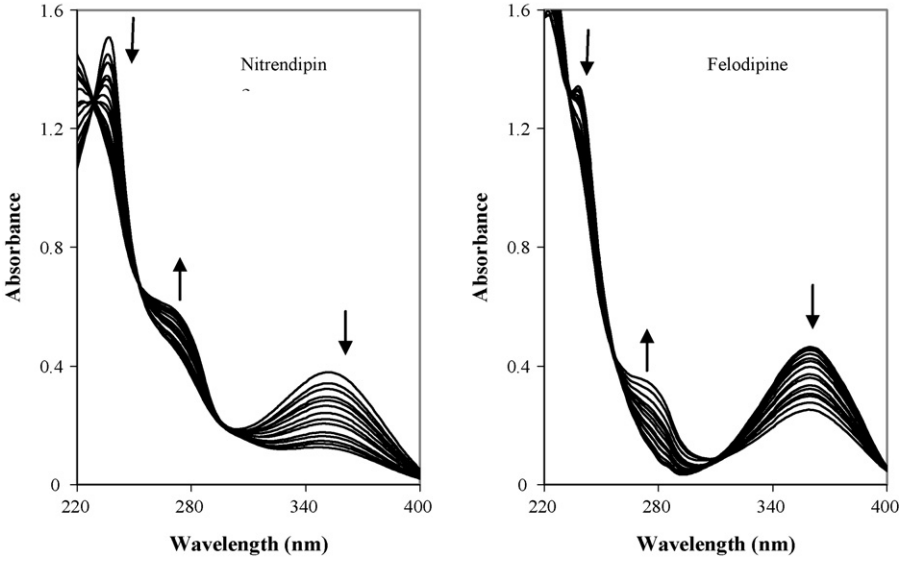

Fig. 2. Absorbance spectra of nitrendipine (6.90×10^{-5} M methanolic solutions, exposure to 254 nm UV lamp) and felodipine (6.50×10^{-5} M methanolic solution exposure to natural sunlight).

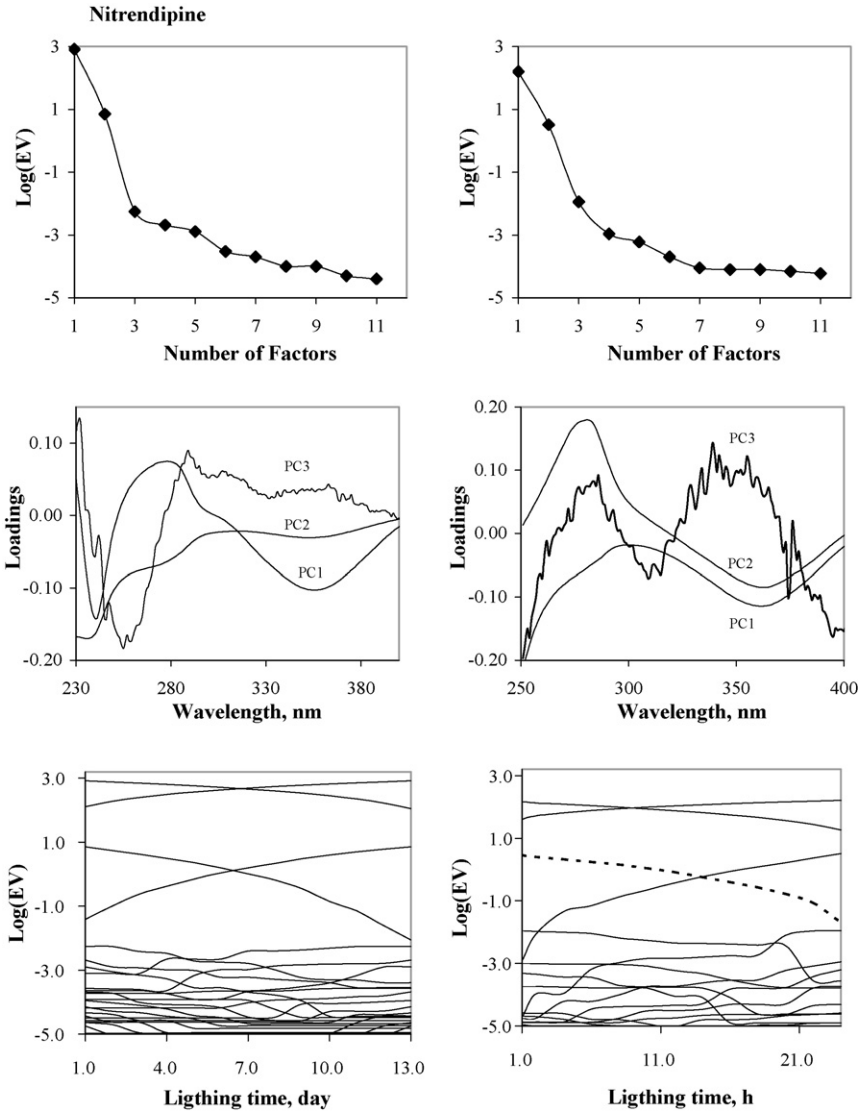

Fig. 3. Plots of data for the determination of the number of chemical components in the reaction system for nitrendipine (left) and felodipine (right); (upper) variation of the logarithm of Eigen-value as a function of number of factors; (middle) plots of normalized loadings for PCs 1–3 and (lower) EFA plots; solid lines for forward analysis and dashed lines for backward analysis.

out. The resulted absorbance spectra were digitized in 1.0-nm intervals and collected in a data matrix of (172 × 14) dimension.

2.4. Data analysis

Previously, we developed a curve deconvolution method by combining the Kubista method with ITTFA procedure using non-negativity and closure constraints [25], and in this article we used this method. This method is briefly described here and interested readers can refer to the original paper for more details. If there are k absorbing species 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 $d_j(\lambda)$ is the spectrum of sample j, $s_i(\lambda)$ is the molar absorptivity of component i, c_{ij} is the concentration of component i in sample j and n is the number of samples. Eq. (1) can be written in a matrix notation as $\mathbf{D} = \mathbf{SC}$, where $\mathbf{S}$ is a $(n \times k)$ matrix of the molar absorbencies and $\mathbf{C}$ is a $(k \times n)$ containing concentration profiles of the chemical species in the reaction system. The aim of MCR analysis is to determine $\mathbf{C}$ and $\mathbf{S}$. First, the number of chemical species coexisted in the reaction system was obtained by principal component or factor analysis (PCA or FA, respectively) employing different methods such as Eigen-value ratio, indicator function (IND) and loading plots [26]. By application of FA to the resultant absorbance data matrix of each drug, it decomposed into two matrices named score ($\mathbf{T}$) and loading ($\mathbf{P}$) spanning the row and column spaces of $\mathbf{D}$, respectively.

$$\mathbf{D} = \mathbf{TP}' \tag{2}$$

The prime over matrix denotes matrix transpose. **T** and **P**′ have dimensions of $(n \times k)$ and $(k \times m)$, respectively. By using a transformation matrix (**R**), the score and loading matrices were transformed to the pure spectra and concentration profiles, respectively.

$$\mathbf{D} = (\mathbf{TR})(\mathbf{R}^{-1}\mathbf{P}') = \mathbf{SC} \quad (3)$$

The main object of different spectral curve deconvolution methods is how to determine **R** so that accurate **S** and **C** are obtained. Kubista used a closure constraint-based method for calculating **R** [27] and we found that in a kinetic study this method does not give an accurate estimate of **R**. Thus, the calculated **R** matrix by Kubista method was used as starting point in ITTFA method [28]. Through ITTFA, non-negativity and closure constraints were applied to the concentration profiles.

3. Results and discussion

The photodegradation of drugs is highly dependent on the irradiation source. For MCR study, a stable irradiation source, which induced a detectable degradation, should be employed. Usually, the photodegradation of a chemical compound is caused by oxidation or by the breakdown of certain weak chemical bond, where both phenomena are energy related and consequently they should be preceded by photons with specified wavelengths. On the other hand, the rate of photodegradation reaction is dependent on light intensity so that the higher light intensity, the faster reaction rate. We first examined a 200 W tungsten lamp and then a 254 nm UV lamp. No significant degradation was observed in the presence of tungsten even for some days. Remarkable photodegradation was observed for nitrendipine, when it was exposed to UV lamp, whereas it could not degrade felodipine significantly. Therefore, natural sunlight, which has higher light intensity, was used for monitoring the photodegradation of felodipine.

The changes in the absorbance spectra of the methanol solution of nitrendipine and felodipine upon exposure to UV and sunlight, respectively, are represented in Fig. 2. As it is observed, when the methanolic solution of nitrendipine was exposed to a 254-nm UV irradiation, its absorbance spectrum showed gradual decreases at 238 and 352 nm accompanying with the appearance of a peak at about 275 nm. The absorbance changes represented three narrow isosbestic points at 229, 254 and 298 nm. Similar changes were observed for the absorbance spectrum of nitrendipine, when its methanolic solution was exposed to daily sunlight. The disappearance of the peak at 238 and 361 nm was accompanying with the appearance of a new peak at about 280 nm. The isosbestic points were observed at wavelength 232, 259 and 308 nm. It should be noted that the isosbestic points of felodipine were not as narrow as nitrendipine, which can be attributed to the sunlight instability at different days.

The first step in the MCR studies is to find the number of chemical species coexisted in a reaction system, which can be attained by application of factor analysis to the absorbance data matrices. The results are represented in Fig. 3. For both drugs there is a large separation between Eigen-values number 2 and 3. In addition, the loading plots indicate that the first and the second principal components (PC) contained systematic variances and the third PC represents noise variances. These indicate the presence of two coexisting chemical species in the photodegradation reaction of each of nitrendipine and felodipine. This result was confirmed by evolving factor analysis (EFA). In EFA method, which proposed by Gampp et al. [29], FA is performed on a series of matrices constructed by successively adding spectra to the previous matrix during the evolutionary process. This procedure is called forward-EFA. Backward-EFA is initiated by starting the FA on the last two spectra and systematically adding spectra in the reverse order of collection. As it is shown in Fig. 3, EFA plots represent a large separation between the first two Eigen-values and the rest of ones. This is another confirmation of the presence of two chemical species in photodegradation reaction of the studied drug.

The detected chemical species by FA results can be attributed to the drug and its photodegradation products. This means that the photodegradation of nitrendipine and felodipine, in the experimental conditions employed in this work, is preceded in a single step. It should be noted that the employed FA methods to access the number of chemical species are failed if photodegradation is followed by a two parallel reaction producing two degradation products. This is because of rank deficiency problem in the concentration profile of the reaction products. However, according to the literature reports the studied drugs do not produce photodegradation products in a parallel reactions. Given one reactant (i.e., pure drug) and one photodegradation product, the proposed MCR method was used to obtain the concentration profile and pure spectra of the components involved in the reaction. The results are represented in Figs. 4 and 5 for nitrendipine and felodipine, respectively. It should be noted that the MCR method used was fully soft model and no prior assumption about the mechanism of photodegradation was given.

The concentration profile of nitrendipine (Fig. 4a) describes that the photodegradation kinetics of this molecule is composed of two distinct regions; one is before half-life and the other after it. In the first region, the concentration profiles of both reactant and products are obeyed to a zero-order kinetic law, whereas the concentration profiles are fitted to a first-order kinetic in the second region. Previously, we obtained similar results for photodegradation of nifedipine. According to the Einstein photochemical equivalence law, the kinetics of photochemical reactions is zero-order [30]. Alteration of the reaction kinetic from zero-order to first-order can be attributed to the inhibitory effect of the reaction product on the photodegradation kinetic. As it is observed from Fig. 4a, the second region is tightly fitted to the exponential concentration–time relationship. The error bars shown in Fig. 4a are the standard deviation of the calculated concentration profiles for three replications. As it is observed, the standard deviations are increased by increasing lighting time, which can be attributed the warming and flickering of UV lamp when it has been on for a long time. The resulted pure spectra of nitrendipine and its photodegradation product are shown in Fig. 4b. For comparison, the experimental pure spectrum of nitrendipine (the first spectrum of data matrix **D**) and number one and a spectrum after 15 days lighting (the first

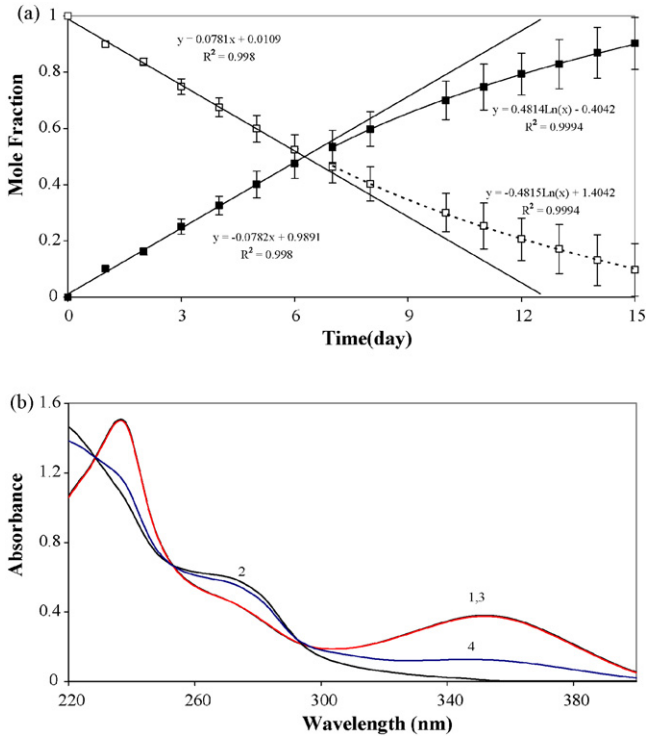

Fig. 4. (a) Concentration profiles of the reactant (open markers) and degradation product (filled markers) in photodegradation monitoring of nitrendipine; solid and broken lines are fitting to linear and exponential equations, respectively. Error bars are the standard deviations for three replicates. (b) Calculated pure spectra of nitrendipine (1) and its degradation product (2), experimental pure spectrum of nitrendipine (3) and the last recorded spectrum of nitrendipine through photodegradation (4).

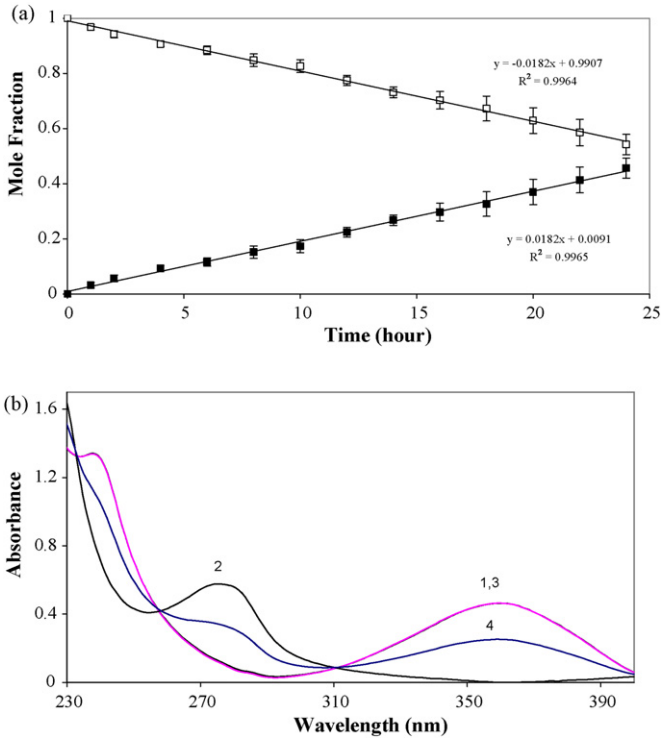

Fig. 5. (a) Concentration profiles of the reactant (open markers) and degradation product (filled markers) in photodegradation monitoring of felodipine; solid lines are fitting to zero-order kinetics. Error bars are the standard deviations for three replicates. (c) Calculated pure spectra of nitrendipine (1) and its degradation product (2), experimental pure spectrum of nitrendipine (3) and the last recorded spectrum of nitrendipine through photodegradation (4).

spectrum of data matrix **D**) are also shown. Obviously, there is very close agreement between the pure and experimental spectrum of nitrendipine, which confirms the accuracy the resulted data obtained by the proposed MCR analysis method.

The resulted concentration profile of felodipine is represented in Fig. 5a. Obviously, the concentration profiles of both reactant and product are fitted to a zero-order kinetic and up to 25-h UV lighting (254 nm), the concentration of reactant is still higher than that of product. Therefore, the absence of first-order region is not unexpected. To observe first-order region, more lighting time is necessary, which is difficult to operate. As it was mentioned in Section 2, the spectral data of felodipine were collected in 4 days, and it was difficult to find more days with similar sunlight stability. The higher values of error bars at higher lighting times can be attributed to this phenomenon. The error bars are the standard deviation of calculated concentration profiles for three replications. The resolved pure spectra of felodipine and its degradation product are shown in Fig. 5b. Obviously, the calculated pure spectrum of felodipine by the MCR analysis is the same of the experimental spectrum, which indicates the high accuracy of the results obtained by the proposed MCR analysis. Interestingly, the pure spectrum of photodegradation product exhibits a peak maximum at 275 nm. The experimental spectrum of felodipine exposed to UV light for 24 h, which a mixture of felodipine and its degradation product is also shown in Fig. 5b for comparison.

According to the concentration profiles depicted in Figs. 4a and 5a, the zero- and first-order rate constants of the photodegradation reactions were calculated by fitting the concentration–time data to the related kinetic equations. The results are represented in Table 1. For comparison, the related data of nifedipine, which were calculated previously [25], are also included in Table 1. Since, the drugs share the same structural backbone, a description of structural effect on rate constant will be beneficial. However, since different lighting conditions were used for each drug it is not feasible to derive a structure–kinetic relationship. Nevertheless, based on our experimental experiences with the photodegradation of the drugs, we found that nifedipine undergoes photodegradation reaction easier than nitrendipine and felodipine so that, in the presence of sunlight, it completely converted to its photodegradation product in a few minutes. On the other hand, nitrendipine represented

Table 1
The first- and zero-order rate constants for the photodegradation kinetics of the DHP drugs

Drug	Zero-order ($M^{-1}\ s^{-1}$)	First-order (s^{-1})
Felodipine	$1.18\ (\pm 0.01) \times 10^{-6}$	–
Nitrendipine	$5.48\ (\pm 0.01) \times 10^{-6}$	$1.90\ (\pm 0.02) \times 10^{-1}$
Nifedipine[a]	$4.96\ (\pm 0.13) \times 10^{-9}$	$6.22\ (\pm 0.10) \times 10^{-5}$

[a] The rate constant data of nifedipine was provided from our previous paper [25].

higher stability toward photodegradation so that when it was exposed to direct sunlight for a day only 10% photodegradation was detected.

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