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Short communication

Spectrophotometric monitoring of nimesulide photodegradation by a combined hard–soft multivariate curve resolution-alternative least square method

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Abstract

Nimesulide, a Cox-2 inhibitor anti-inflammatory drug, is a light sensitive compound and its biological activity is decreased upon photodegradation. The photodegradation kinetic of nimesulide was investigated spectrophotometrically using multivariate curve resolution analysis to overcome spectral overlapping of reactant and degradation products. The absorbance spectra of the nimesulide methanolic solution, exposed to daily sunlight, were recorded at different times. Three absorbing chemical species, coexisted in the reaction system, were detected by application of factor analysis to the absorbance data matrix. The soft-modeling multivariate curve resolution-alternative least square (MCR-ALS) analysis of the evolutionary absorbance data revealed that nimesulide undergoes photodegradation through a two-step consecutive manner, where both steps obey first-order kinetic. By application of the kinetic hard model constraint to the MCR-ALS analysis, an excellent agreement was obtained between the fitted concentration profiles and those obtained by soft method. The first-order rate constants of the first and second degradation products were calculated as 0.052 (±0.007) and 0.009 (±0.001) h^{-1}, respectively. Finally, the pure spectra of the resolved chemical species were calculated, where that of nimesulide was the same as that obtained experimentally.

Keywords: Nimesulide; Photodegradation; Chemometrics; Multivariate curve resolution

1. Introduction

The pharmacological effects of non-steroidal anti-inflammatory drugs (NSAIDs) are due to inhibition of a membrane enzyme called cyclooxygenase (COX), which is involved in the prostaglandin biosynthesis and existed as two isoforms, COX-1 and COX-2 [1–3]. Nimesulide, *N*-(4-nitro-2-phenoxiphenyl)methane sulfonamide (Scheme 1), is one of the first NSAIDs marketed, with a preferential COX-2 inhibition profile [4]. Nimesulide is widely used for the treatment of rheumatoid arthritis and other antipyretic properties [5–7]. It is a light yellow crystalline powder, which is practically odorless.

Drug stability research is critical in pharmaceutical studies as the increased degradation decreases the potency of the drug and can create compounds with undesirable pharmacological effects [7]. An increasing number of drugs belonging to different therapeutic classes (calcium channel blockers, non-steroidal anti-inflammatory drugs, chemotherapeutic agents, diuretics, benzodiazepines, beta-blockers, etc.) were found to be photolabile, consequently light protection is prescribed for their storage by the pharmacopoeias [8]. Information about the stability of the drug substance is an integral part of the systematic approach on the stability evaluation. Therefore, investigating the influence of light on the stability of drugs has gained more and more importance in recent years [9].

The analytical methods for analysis of nimesulide include different chromatographic methods [10–12] and differential pulse voltammetry using modified carbon electrode [13]. Although certain information about the photo stability of some NSAID's has been reported [14–17], reports about the photo stability of nimesulide as a modern agent with different structure are rare. Recently, Kovarikova et al. studied the photochemical stability of nimesulide and described a sensitive analytical method using

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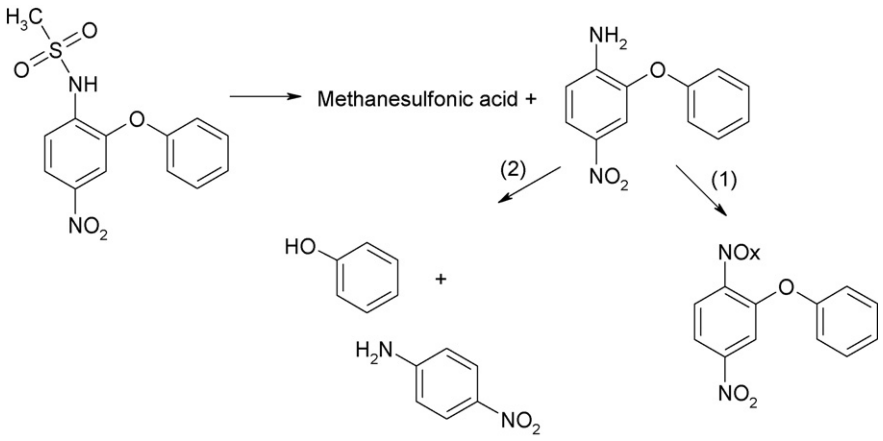

Scheme 1.

HPLC and TLC for determination of nimesulide in the presence of its degradation products [17]. The major degradation pathway of nimesulide is hydrolysis of methyl sulfonamide group and producing 2-phenoxy-4-nitroaniline and methanesulfonic acid. They also found an unknown photodecomposition product.

The aim of this study was to develop a simple chemometrics-based spectrophotometric method for monitoring the photodegradation of nimesulide. Spectroscopic 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 chemometrics methods [18]. 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 [19–24].

In MCR methods, data analysis can be achieved by hard-modeling method (when a chemical model is available) or soft-modeling method (when there is no robust idea about the model of the chemical reaction) [19–24]. Recently, attentions have been directed toward the combination of hard- and soft-modeling methods to have the advantages of both methods and therefore to obtain more reliable results [25–27]. Among the different available MCR methods, the multivariate curve resolution-alternative least square (MCR-ALS) has been found the major popularity, especially in the case of combining hard and soft modeling methods [28,29].

Since, there are no prior evidences about the photodegradation mechanism of nimesulide, we employed first a soft-modeling method and then a combined hard–soft modeling method to give a more deep insight about the degradation mechanism of nimesulide.

2. Experimental

2.1. Chemical and reagents

The 100.0 μg/mL stock solution of nimesulide (Sigma) was prepared by dissolving appropriate amounts of the pure powder of drug in spectroscopic grade methanol (Merck) and was used to prepare daily solutions.

2.2. Apparatus and software

The UV–vis absorbance spectra were recorded by a Shimadzu spectrophotometer equipped with a 10.0 mm quartz cell and a water-thermostated cell holder. The computations were performed in MATLAB environment (Mathwork Inc., version 7) utilizing a personal computer with Windows XP operating system.

2.3. Procedure

Natural sunlight was used for photodegradation monitoring of nimesulide. The photodegradation was monitored by exposing a 50.0 mL 8.0×10^{-5} M methanolic solution of nimesulide to sunlight in the sequential sunny days in the time duration of 10:00 a.m. till 4:00 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. 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 in the wavelength interval of 230–400 nm at 4:00 p.m. in each day. Therefore, each absorbance measurement was performed after 6 h lighting. The experiment was continued for 30 days. The resulted absorbance spectra were digitized in 1.0 nm intervals and collected in a data matrix of (30×172) dimension.

2.4. Combined-hard model MCR-ALS analysis

The theory of MCR-ALS and its combination with hard modeling has been developed by de Juan and coworkers [28,29]. Here, a brief description of the methodology we used is given. If there are k absorbing species in the reaction system, the recorded absorbance at each wavelength ($d_j(\lambda)$), according to the Beer-Lambert law, is assumed to be the sum of contributions of all components:

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

where $d_j(\lambda)$ is the spectrum of sample j, $s_i(\lambda)$ the molar absorptivity of component i, c_{ij} the concentration of component i in sample j and m is the number of samples. Eq. (1) can be written in a matrix notation as Eq. (2), which is the basis of multivariate curve resolution analyses.

$$\mathbf{D} = \mathbf{C}\,\mathbf{S} + \mathbf{E} \quad (2)$$

where $\mathbf{D}$ ($m \times n$) is the original raw data matrix with the mixed experimental information of all absorbing chemical species coexisted in the reaction system, the columns in $\mathbf{C}$ ($m \times k$) and the rows in $\mathbf{S}$ ($k \times n$) contain the pure response profiles of the n mixture components associated with the row direction and the column direction of $\mathbf{D}$, respectively, and $\mathbf{E}$ ($m \times n$) is the error-related matrix. MCR-ALS is an iterative curve resolution method applicable to data sets formed by one or more data matrices (two- or three-way data sets), respectively [25–29]. The initial estimate of the concentration profiles can be obtained by evolving factor analysis (EFA) process [31].

In the hard model-combined MCR-ALS, the initial estimate of the concentration profile of reactants and products obtained by EFA was used to calculate the pure spectra of these chemicals as $\mathbf{S} = \mathbf{C}^{+}\mathbf{D}$, where the superscript '+' denotes matrix pseudo inverse. Then, another estimate of concentration profile was obtained using the calculated pure spectra as $\mathbf{C} = \mathbf{D}\mathbf{S}^{+}$. The iterative calculation of $\mathbf{C}$ and $\mathbf{S}$ was continued till the lack of fit error between the experimental $\mathbf{D}$ and that calculated by Eq. (2) was minimized or reached to a predefined value. The applied constraints through MCR-ALS analysis were non-negativity to pure spectra and non-negativity, unimodality and closure to concentration profiles. After convergence, the resulted concentration profile was fitted to a predefined kinetic model. Here, since factor analysis detected the presence of three coexisting chemical components, two-step consecutive kinetic models of the form of $A \rightarrow B \rightarrow C$ with the respective rate constants of k_1 and k_2 for the first and second steps of photodegradation reaction were considered. The differences in the considered kinetic models were based on the reaction orders of the first and second steps. Examples of the considered reaction orders are zero–zero, zero–first, first–first, first–zero and so on. It should be noted that in the case of each kinetic model a separate MCR-ALS analysis was run.

Once the resulted concentration profile by MCR-ALS was fitted to a kinetic model and rate constants were calculated, a new estimated of concentration profile was calculated based on these rate constants, and then it was used as initial estimate of a new MCR-ALS analysis. This operation was iteratively performed till no significant improvement in the lack of fit error was obtained. Among the examined kinetics model, one of them that reproduced the most reasonable results (with the aspects of the shape of the concentration and spectral profiles) and also resulted in the least lack of fit error was selected as the kinetic model for the photodegradation of nimesulide.

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 firstly examined a 200 W tungsten lamp and then a 254 nm UV lamp. No significant degradation was observed in the presence of both irradiation sources even for some days. However, remarkable changes in the absorbance spectra of nimesulide were observed when it was exposed to natural sunlight. Thus, the latter was used as the irradiation source for monitoring nimesulide photodegradation.

The changes in the absorbance spectra of the methanolic solution of nimesulide (8×10^{-5} M) upon exposure to sunlight at different lighting time are represented in Fig. 1. As it is observed, the nimesulide spectrum possess two absorbance maxima at 300 and 330 nm, whereas upon lighting the absorbance intensity is decreased at both peak maxima, accompanied with a shift of the former to lower wavelengths. In addition, an increase in the absorbance is observed at wavelengths lower than 280 nm. These changes in the absorbance spectra of nimesulide can be attributed to conversion of nimesulide to its degradation product(s). The absence of a clear and narrow isosbestic point suggests that the reaction may proceed in more than one step. As it was reported previously by Kovarikova et al. [17], nimesulide is

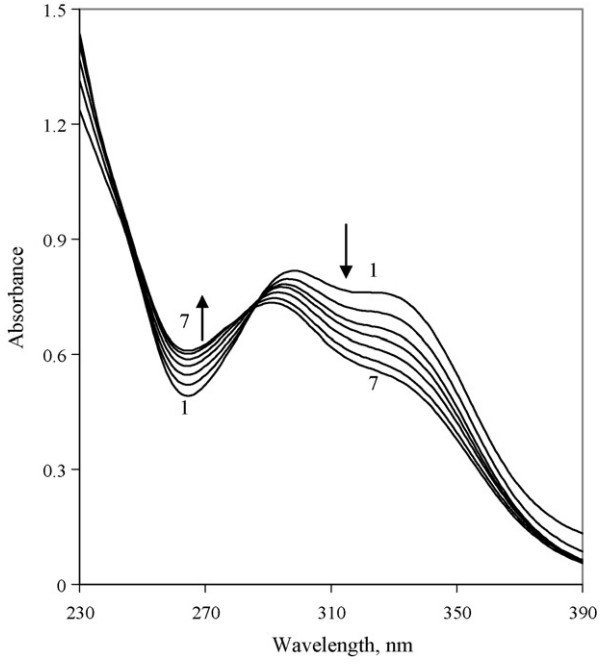

Fig. 1. Absorbance spectra of the 8×10^{-5} M methanolic solution of nimesulide exposure to sunlight in 30 h lighting time intervals: (1) before lighting and (7) after 180 h lighting.

degraded to 2-phenoxy4-nitroaniline and methanesolfunic acid, where the latter dose not have significant absorbance at the wavelength intervals depicted in Fig. 1. The authors also detected an unknown degradation product.

To investigate the number of independent chemical absorbing species created through photodegradation of nimesulide, the data matrix of the recorded absorbance at different lighting times were subjected to factor analysis (FA) [30]. The variation in the eigen-values (EV) of the covariance data matrix as a function of number of factor is represented in Fig. 2A for the first 10 factors. As it is observed, there is a relatively large separation between the third and fourth eigen-values. The three first factors, which could explain about 99% of total variances in the absorbance data, contained the systematic variances and the rest of factors can be considered as noisy factors. The number of significant factors was also investigated by loadings plot as it is represented in Fig. 2B. Obviously, the first three ladings represent the systematic variations and the fourth one represents a noisy distribution. Therefore, both eigen-value and loading plots indicate the presence of three absorbing coexisting chemical species in the reaction system. This result was confirmed by evolving factor analysis (EFA) [31]. As it is shown in Fig. 3A, the forward and backward EFA plots represent a large separation between the first three eigen-values and the rest of ones. This is another confirmation of the presence of three chemical species in photodegradation reaction of the studied drug.

The detected chemical species by FA results can be attributed to the pure drug and its photodegradation products. This means that the photodegradation of nimesulide in the experimental conditions employed in this work may be preceded in a two-step manner. The calculated initial estimate of the concentration profile of the reactant and products by EFA is shown in Fig. 3B. The plotted concentration profiles are more likely to a consecutive two-step reaction of the form of A → B → C, where A is pure nimesulide and B and C are the intermediate and final products, respectively. According to the previous study [17], the first step of photodegradation can be attributed to the N–S bond cleavage, and formation of methanesolfunic acid and 2-phenoxy-4-nitroaniline (see Scheme 1). The second step of photodegradation reaction can be related to further degradation of 2-phenoxy-4-nitroaniline, which can be proceeded by two different reactions: (1) oxidation of $–NH_2$ to nitroso or nitro and (2) C–O bond cleavage and formation of 4-nitroaniline and phenol. It should be noted that in both reactions, the number of identified chemical species by FA is 3. In the second case, where two products are formed in the second step, the system is rank deficient and both products should be considered as a single absorbing species. Therefore, irrespective to the reaction course considered, the overall reaction mechanism can be considered as A → B → C.

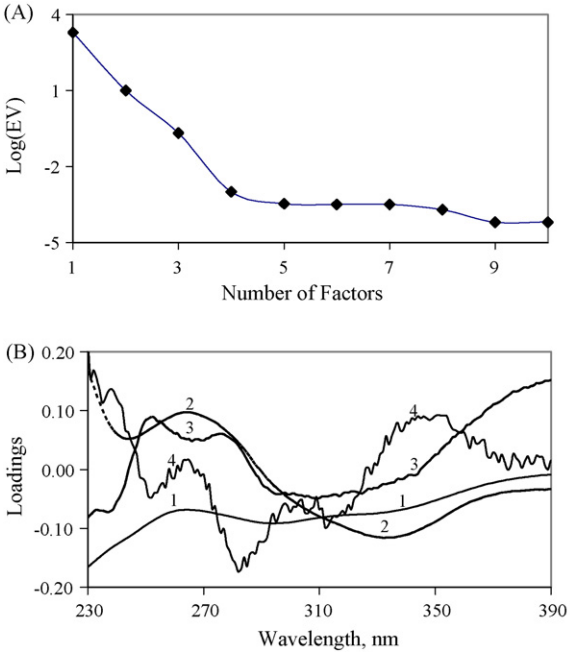

Fig. 2. FA results for determining the number of absorbing chemical species. (A) Variation of the logarithm of eigen-values as a function of the number of factors. (B) The normalized loadings plot for the first four factors. The numbers in the plot indicate factor's number.

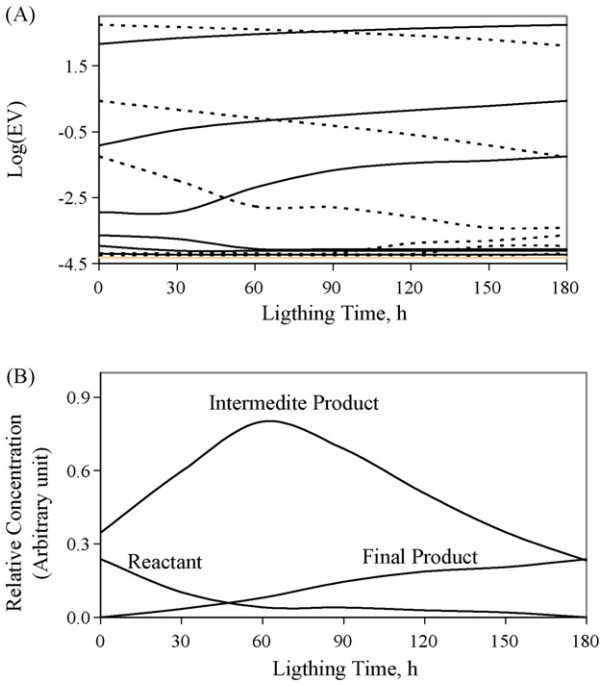

Fig. 3. Results of EFA: (A) plots of the logarithm of eigen-values as function of lighting time for forward (solid lines) and backward (dashed lines) analyses and (B) initial estimate of concentration profile.

To resolve the optimum concentration profile and pure spectra of A, B and C species, and to find the reaction orders of the first and second steps, at the first, soft-modeling MCR-ALS without hard-model constraint was employed. The resulted optimum concentration profiles, shown in Fig. 4A, are more likely to exponential decay for both reaction steps. This indicates that the reaction orders are first order with respect to nimesulide in the first step and 2-phenoxy-4-nitroaniline in the second step. To be insure about this hypothesis, the concentration profiles were fitted to different kinetic models considering the following reaction orders: zero–zero, first–zero, zero–first, first–first, zero–second, second–zero, first–second, second–first

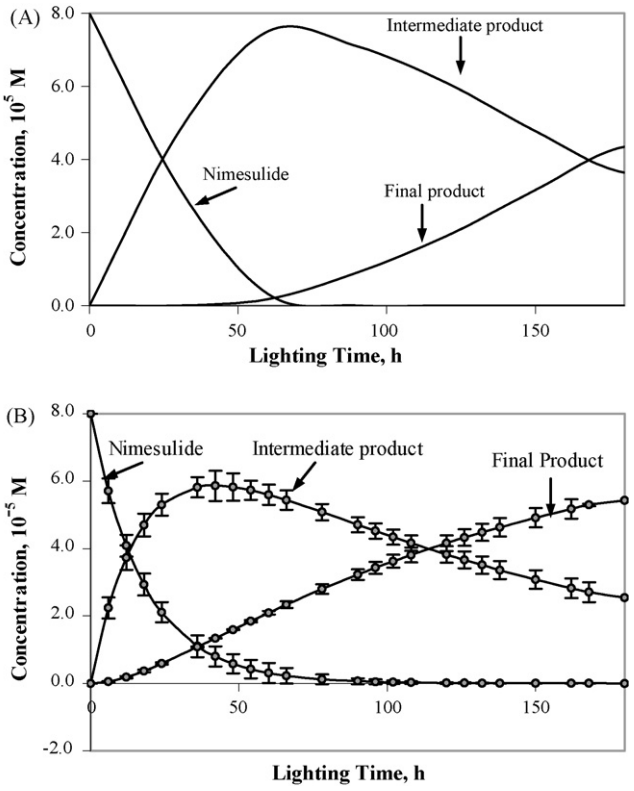

Fig. 4. Concentration profiles of the nimesulide and its degradation products as a function of lighting time calculated by (A) soft-modeling MCR-ALS and (B) combined hard–soft modeling MCR-ALS. The error bars shown in part B are the 95% confidence intervals of the concentrations calculated from three replicate measurements.

and second–second, where the words before and after '–' denotes the reaction order for the first and second reaction step, respectively. Among the different considered kinetic models, when both reaction steps were considered as first-order, the least fitting error was obtained.

Due to the rotational ambiguity, we found a considerable difference between the fitted concentration profiles and those resolved by soft-modeling MCR-ALS analysis. Therefore, to obtain more accurate results, the hard model constraint was applied. The fitted concentration profile was used as the initial estimate of MCR-ALS and it was optimized again. This procedure was run many times till the least root mean square error was obtained between the optimized concentration profile resulted by MCR-ALS and the fitted one. The results are represented in Fig. 4B. Obviously, there is a close agreement between the fitted concentration profiles and those resolved by MCR-ALS. The following rate equations were used for fitting the resulted concentration profiles by MCR-ALS:

$$[A]_t = [A]_0 e^{-k_1 t} \quad (3)$$

$$[B]_t = \frac{[A]_0 k_1}{k_2 - k_1}[e^{-k_1 t} - e^{-k_2 t}] \quad (4)$$

$$[C]_t = [A]_0 + \frac{[A]_0}{k_1 - k_2}[k_2 e^{-k_1 t} - k_1 e^{-k_2 t}] \quad (5)$$

In the above equations, $[A]_0$ is the analytical concentration of drug before exposing to sunlight and $[A]_t$, $[B]_t$ and $[C]_t$ are time-dependent equilibrium concentration of nimesulide, and its intermediate (2-phenoxy-4-nitroaniline) and final photodegradation products, respectively. The respective first-order rate constants are denoted by k_1 and k_2, and t denotes lighting time. The calculated first-order rate constants of the first and second reaction steps were 0.052 (±0.007) and 0.009 (±0.001) h^{-1}, respectively. The kinetic profiles shown in Fig. 4B and the calculated rate constants suggest that the first photodegradation step of nimesulide is faster than the second one. The error bars shown in Fig. 4B are the confidence intervals in the concentrations calculated from three replicate samples. The low values of the error bars explain the reproducibility of the data collection as well as the MCR-ALS calculations.

An important application of MCR-based methods is calculating the pure spectra of the reaction products, especially unstable intermediate products. Once the concentration profile of the absorbing species coexisted in the reaction system was obtained, the pure spectra of these components can be easily calculated by pre-multiplication of experimental absorbance data matrix with the pseudo-inverse of concentration profile data matrix. The results are represented in Fig. 5. The resolved spectrum of nimesulide is the same as that obtained experimentally (see spectrum #1 of Fig. 1). The pure spectrum of the intermediate degradation product (i.e., 2-phenoxy-4-nitroaniline) is similar to that of nimesulide. The slight differences are the decreased and increased absorbencies at wavelengths higher and lower than 294 nm, respectively. This implies that N–S bond cleavage in nimesulide has not significant effect on its absorbance spectrum. On the other hand, the resolved pure spectrum of the final degradation products exhibited significant differences from that

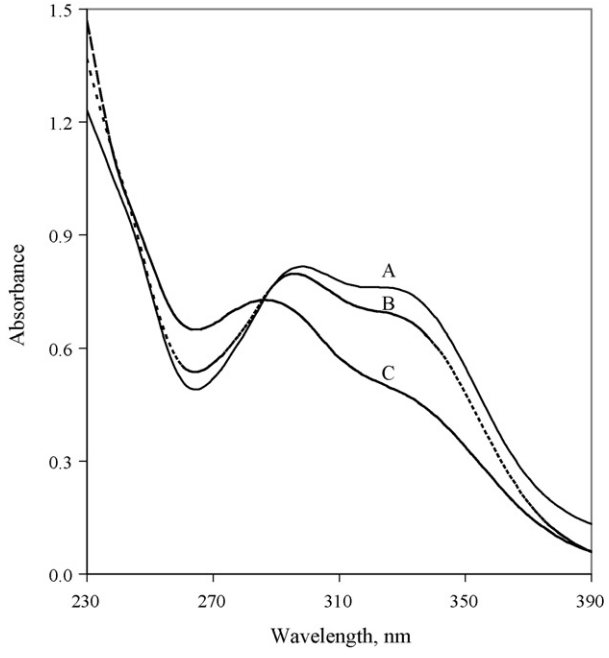

Fig. 5. The calculated pure spectra of nimesulide (A) and its degradation products: (B) intermediate product and (C) final product.

of both nimesulide and the intermediate product so that the peak maxima of nimesulide at 300 nm was shifted to 288 nm in the final degradation product.

As it was noted previously, the chromatographic-based photodegradation study of nimesulide has been previously reported by Kovarikova et al. [17], where the authors could separate the photodegradation products of nimesulide after a long time exposure (i.e., 80 h) of drug to 254 nm UV radiation. However, no kinetic study was conducted in that work. The proposed method in this article is the first kinetic report on the photodegradation of nimesulide. Besides, we employed UV–vis spectrophotometer, as an alternative to chromatographic method, for monitoring of nimesulide photodegradation.

Acknowledgement

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