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Photoprotection of 1,4-dihydropyridine derivatives by dyes

Jadwiga Mielcarek[a,*], Włodzimierz Augustyniak[b], Paweł Grobelny[a], Grażyna Nowacka[b]

[a] *Department of Inorganic and Analytical Chemistry, Poznan University of Medical Sciences, Grunwaldzka 6, 60-780 Poznań, Poland*
[b] *Faculty of Chemistry, Department of Photochemistry, A. Mickiewicz University, Grunwaldzka 6, 60-780 Poznań, Poland*

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Abstract

The possibility of increasing the photochemical stability of nisoldipine by using indigotine and azorubine as photoprotectors has been studied. The course of the photodegradation was monitored by means of UV–vis spectrophotometry and HPLC. Quantitative assessments of the nisoldipine photodegradation included evaluation of the quantum yields and kinetic parameters. In order to establish the light intensity absorbed by a system, Reinecke salt was used as a chemical actinometer. The values of the quantum yields (φ) of photodegradation decreased with increasing dye concentration and were 0.24–0.15 and 0.27–0.25 for indigotine and azorubine, respectively.

Furthermore, our attention was focused on determination the role of the selected dyes during the photodegradation of nisoldipine and the calculations were made to eliminate an inner filter effect. The values obtained were used to construct a Stern–Volmer plot and calculate the Stern–Volmer constants (K_{SV}). On the basis of the K_{SV} and the values of the quenching constant (k_q) the exited state lifetime (τ_o) of nisoldipine in the presence of indigotine and azorubine were estimated. The calculated values of τ_o for NS were 6.66×10^{-6} s – in the presence of indigotine and 2.76×10^{-6} s – in the presence of azorubine, indicating that the photodegradation of nisoldipine occurs from the lowest triplet excited state.

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Keywords: Nisoldipine; Dihydropyridine derivatives; Photodegradation; Photostability; Photoprotection; Dyes

1. Introduction

Dihydropyridine derivatives (DHP) are considered extremely effective drugs used in the treatment of cardiovascular disorders, such as hypertension, cardiac arrhythmia and angina pectoris (Klocke, 1999; Vries et al., 2000; Cleophas and Marum, 2001). The next generations of DHP are characterised by a variety of beneficial properties and have been recently successfully applied in the treatment of many non-cardiological conditions, such as migraine, asthma, enterospasm, in preventing the immature labour

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[*] Corresponding author. Tel.: +48 61 8659566; fax: +48 61 8659566.
E-mail address: jmielcar@am.poznan.pl (J. Mielcarek).

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(Silberstein and Goadsby, 2002; Pepine et al., 2003; Eisenberg et al., 2004). Their effectiveness in the treatment of so diverse conditions reflects the fact that in the majority of cells the Ca^{2+} ions are important carriers of intracellular information. On the other hand, the most inconvenient feature of the drugs having a dihydropyridine ring is their excessive sensitivity to light leading to irreversible structural changes resulting in the loss of potency or the therapeutical inactivity (Alvarez-Lueje et al., 1998). Light induced transformation has been found to lead to oxidation of the dihydropyridine ring (Alvarez-Lueje et al., 2002; Ragno et al., 2003). Moreover, exposure of the compounds containing the nitro group to irradiation induces reduction of the nitro group to the nitroso group. The compounds containing the nitro group at *ortho* position of the phenyl ring, such as nisoldipine (NS), are particularly photosensitive (Ragno et al., 2002; Yàñez et al., 2004). Marinkovic et al. (2000, 2003) indicate that exposure of NS to UV light results in the oxidation of the DHP ring. On the other hand, daylight induces both the aromatization of the DHP ring and the reduction of the nitro group to the nitroso group (Marinkovic et al., 2000; Marinkovic et al., 2003). Other photodegradation products of NS have been identified by Michelitsch et al. (1995). According to these authors, the nitroso derivatives can form dimers capable of undergoing further transformations.

It has been shown that the products formed as a result of DHP irradiation are pharmacologically inactive (Alvarez-Lueje et al., 1998; Michelitsch et al., 1995). Thus, to avoid the photodegradation, efforts are made to find new methods to enhance the photostability of DHP and so far the technique most often used to improve the photochemical stability within the DHP group has been complexation with cyclodextrins (CD). It has been shown (Mielcarek, 1995, 1997; Pomponio et al., 2004) that the use of modified CD ensured much more effective photoprotection when compared with native CD. Some other reports (Teraoka et al., 1989) have shown that titanium dioxide can play a photoprotective role.

Therefore, the aim of our study was to check the effect of dyes on the photostability of NS in solutions as well as to determine their photoprotective mechanism. From among the dyes used in pharmaceutical industry to colour drugs and therapeutical substances azorubine and indigotine have been selected for our studies.

2. Experimental methods

2.1. Materials and instrumentation

Nisoldipine [(±)3-isobutyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-pyridine-3,5-dicarboxylate ($C_{20}H_{24}N_2O_6$; $M_r = 388.4$ g/mol) was kindly supplied by Lusochimica Milano, Italy. Azorubine and indigotine were purchased from Hoffmann and LCW, respectively. Methanol and acetonitrile (HPLC grade) were purchased from J. T. Backer.

Water was twice distilled and purified by Milipore Water Purification System Milipore Corp., USA. The potassium Reinecke salt was synthesised from ammonium salt made by Fluka.

Spectrophotometer UV-160A, Shimadzu and PC 160 Plus software. High-performance liquid chromatograph, equipped with a pump Waters 616, a Photodiode Array Detector Waters 996 and Millennium 2000 software.

2.2. Irradiation conditions

Photodegradation was performed following the recommendations of the International Conference on Harmonisation (ICH)—option I, currently in force in the study of photostability of drugs and therapeutic substances, using a high-pressure lamp, type HBO-50, equipped with a mercury burner emitting the radiation in the range of 300–400 nm. The maximum absorption at $\lambda = 365$ nm was obtained using the interference filter and Wood's filter.

Methanol solution of nisoldipine with the concentration 7.77×10^{-6} M was transferred to a cylindrical quartz cell ($V = 2.8$ ml, $l = 1$ cm) and exposed to irradiation for 30 min. At different times of irradiation the absorption spectra were recorded in the range of 200–500 nm against methanol (Fig. 1).

2.3. High-performance liquid chromatography conditions

The HPLC method employed a LiChrospher RP-18 analytical column (250 mm × 4 mm) (Waters). The column operated at temperature 20 °C. All analyses were performed under isocratic conditions with mobile phase consisting of methanol–water–acetonitrile (5:4:1, v/v/v), at the flow rate 0.6 ml/min. Sample

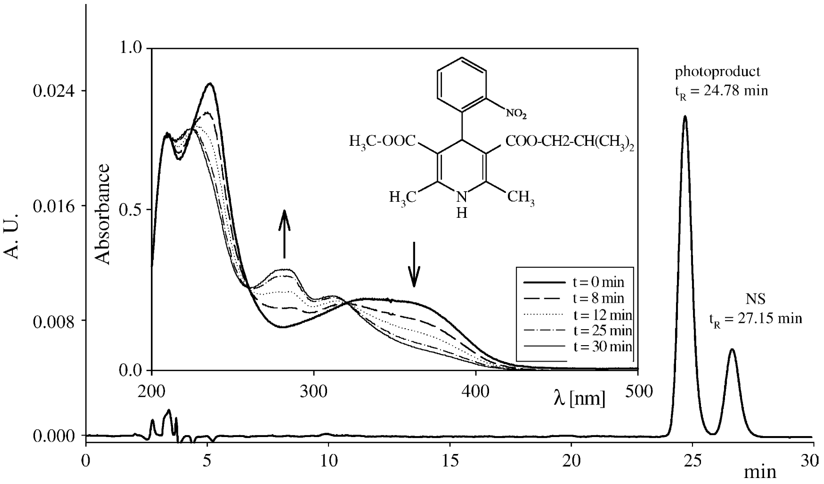

Fig. 1. The chemical structure, spectral changes of nisoldipine after different time of irradiation and chromatogram corresponding to 12 min exposure to light source.

injection volume was 20 μL, and the run time was 35 min. The chromatogram of NS after exposure to irradiation for 12 min is presented in Fig. 1.

2.4. Photodegradation studies

Solutions of indigotine and azorubine with the concentrations 6.95×10^{-6} and 2.98×10^{-6} M, respectively, were subjected to photodegradation. After the appropriate time of exposure, absorption spectra of the compounds were recorded in the range of 200–700 nm. The exposure of the methanol solutions of the dyes to irradiation had no effect on their absorption spectra (Fig. 2).

Subsequently, two series of eight combinations were prepared including NS with the same concentration

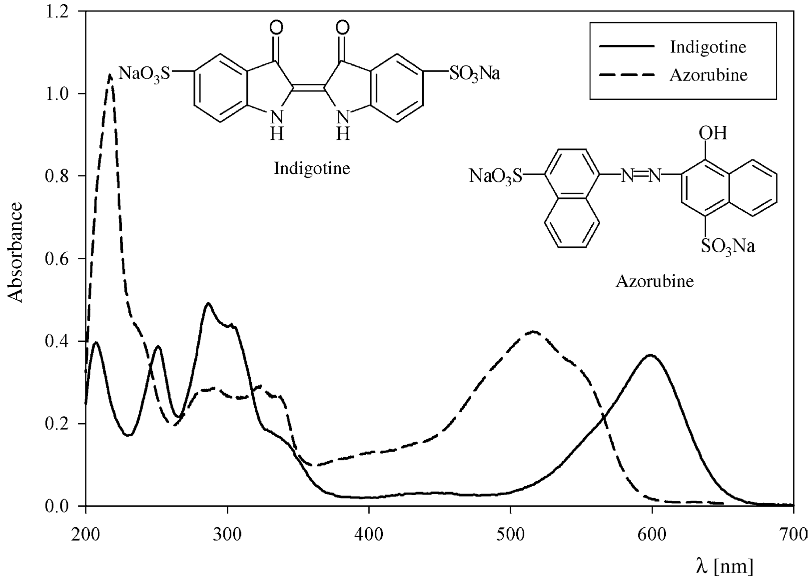

Fig. 2. The chemical structures and absorption spectra of indigotine and azorubine before and after irradiation for 40 min.

Table 1
Real quantum yields for nisoldipine irradiated in the presence of indigotine and azorubine

Indigotine concentration (10^6 M)	φ_R	Azorubine concentration (10^6 M)	φ_R
–	0.24	–	0.35
1.39	0.21	0.89	0.25
2.08	0.20	1.78	0.23
2.78	0.19	2.98	0.28
4.17	0.17	4.17	0.28
5.56	0.20	5.36	0.26
6.25	0.16	5.95	0.27
6.95	0.15	7.14	0.25

φ_R, Real quantum yield.

each and increasing concentrations of dyes (Table 1). The solutions were subjected to photodegradation for 30 min in the conditions described in Section 2.2. Changes were monitored at regular time intervals by means of the spectrophotometric method and HPLC (Figs. 3 and 4).

2.5. Kinetics of photodegradation

The results obtained by means of the spectrophotometric method and HPLC were used for quantitative assessment of photochemical degradation of NS. The photodegradation of NS both in the absence and presence of the dyes was determined. Changes in the concentration of NS during irradiation can be described by the equation:

$$\ln A = \ln A_0 - kt$$

Table 2
Kinetic parameters of photodegradation of nisoldipine obtained by spectroscopic method (SP) and high-performance liquid chromatography (HPLC)

Method	Correlation coefficient (r)	Rate constant, $k \times 10^2$ (min^{-1})	$t_{0,5}$ (min)	$t_{0,1}$ (min)
SP	0.999	2.99	23.10	3.51
HPLC	0.998	2.96	23.38	3.56

On the basis of the above equation the following kinetic parameters were estimated: the rate constant of photodegradation (k), the half-life time ($t_{0,5}$) and the time of degradation of 10% of the compound ($t_{0,1}$), see Table 2.

In order to determine the kinetic changes of nisoldipine photochemical degradation the quantum yields were calculated. To establish the number of quanta absorbed by a system, Reinecke salt was used as a chemical actinometer.

3. Results and discussion

The possibility of increasing photochemical stability of NS by taking advantage of the dyes, frequently

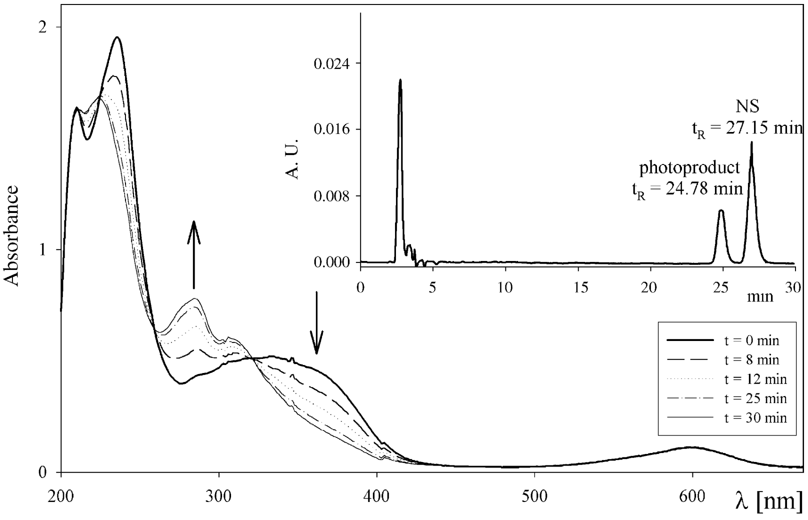

Fig. 3. Spectral changes of nisoldipine in the presence of indigotine and chromatogram corresponding to 12 min exposure to irradiation.

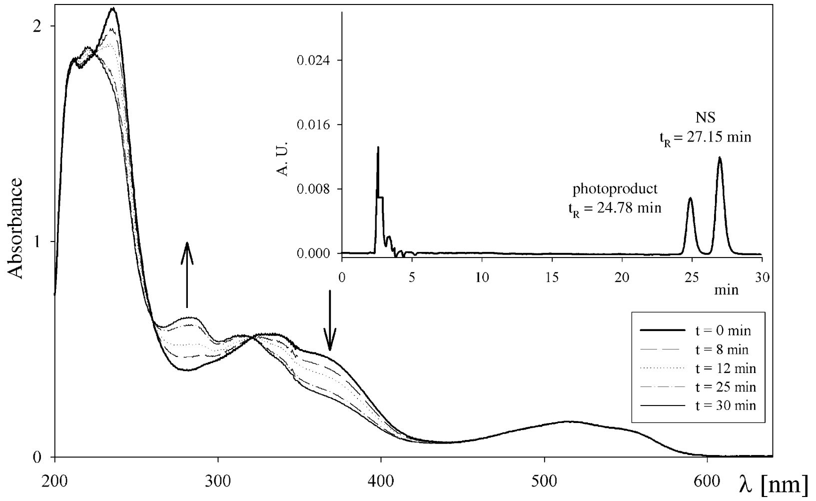

Fig. 4. Spectral changes of nisoldipine in the presence of azorubine and chromatogram corresponding to 12 min exposure to irradiation.

used by pharmaceutical industry to colour drug presentations has been studied. From among numerous derivatives within the 1,4-dihydropyridines group, NS has been selected for our research, because of its extreme photochemical sensitivity resulting from the presence of the nitro group at the *ortho* position of the phenyl ring.

Indigotine and azorubine were used as photoprotectors. The photodegradation of nisoldipine in the presence of dyes was carried out following the recommendations of the International Conference on Harmonisation—option I. The photodegradation was assayed by means of UV–vis spectrophotometry and HPLC.

As shown in Fig. 1, the irradiation caused changes in the UV spectrum of the compound. The hipsochromic effect of the absorption band in the range of 320–420 nm, appearing as a result of the irradiation, is assigned to the $\pi \rightarrow \pi^*$ electronic transition in the heterocyclic dihydropyridine ring. The hyperchromic effect in the range of 260–320 nm, is related to the $\pi \rightarrow \pi^*$ electronic transitions in the aromatic ring.

The photodegradation of NS was also analysed by the HPLC method and optimisation of the analysis conditions permitted a satisfactory separation of the product of decomposition. The photoproduct appeared already after 3 min of irradiation and the retention times (t_R) for NS and the product of its photodegradation were 27.15 and 24.78 min, respectively.

It should be emphasised that the solutions of the dyes alone subjected to the irradiation in the same conditions as NS have been characterised by significant stability. As illustrated in the Fig. 2, no changes in the absorption spectra of indigotine and azarubine subjected to irradiation for 40 min have been detected.

Quantitative assessment of the photochemical degradation involved determination of the kinetic parameters: the rate constant of photodegradation (k), the half-time time ($t_{0,5}$) and the time of degradation of 10% of the compound ($t_{0,1}$) (Table 2).

Estimation of the photochemical degradation required measurements of the irradiation energy absorbed by the system studied. In our experiment, it was measured by the method of chemical actinometry. In order to determine the number of quanta emitted Reinecke salt was used. A Reinecke salt solution was irradiated with the wavelength $\lambda = 365$ nm for 85 s. The number of quanta absorbed by the actinometer was 3.626×10^{14} and was equal to the number of quanta falling on the nisoldipine solution studied in the time of 85 s.

The energy of a quantum of radiation of $\lambda = 365$ nm (E_q) was calculated from the formula:

$$E_q = h\frac{c}{\lambda} = 6.624 \times 10^{-34}\,\mathrm{Js}\,\frac{2.998 \times 10^8\,\mathrm{m\,s^{-1}}}{365 \times 10^{-9}\,\mathrm{m}}$$
$$= 5.442 \times 10^{-19}\,\mathrm{J}$$

The intensity of irradiation (P) absorbed by the actinometer was:

$$P = \frac{5.442 \times 10^{-19} \times 3.626 \times 10^{14}}{85} = 2.32 \times 10^{-6}\,\mathrm{W}$$

Given the area of the cell surface equal to $2.26\,\mathrm{cm}^2$, the energy of irradiation falling onto the area of $1\,\mathrm{m}^2$ in 1 h (E_s) was calculated:

$$E_s = 2.32 \times 10^{-2}\,\mathrm{W\,s\,m^{-2}} = 83.52\,\mathrm{Wh\,m^{-2}}$$

The experiments permitted determination of the apparent quantum yields and their extrapolation to the zero irradiation time gave the real quantum yields (φ_R) (Table 1). The quantum yields of nisoldipine photodegradation decreased with increasing concentration of the dye and changed from 0.21 to 0.15 with indigotine concentration increasing from 1.39×10^{-6} to 6.95×10^{-6} M and from 0.36 to 0.25 with azorubine concentration increasing from 0.90×10^{-6} to 7.14×10^{-6} M.

In photochemical processes, the substances added to the irradiated system can act as sensitizers, inner filter or quencher. In order to identify the role of the dyes studied in the process of NS photodegradation, the calculations of the absorption were performed in which the apparent quenching effect, so-called inner filter effect of the dye molecules was eliminated.

On the basis of the well-known relations for the intensity of irradiation absorbed by a system with one and with two absorbing components, the intensity of irradiation absorbed by NS was calculated as a function of the dye concentration.

In the following equations, the relations taking into account the inner filter effect due to the dye presence have been regarded:

$$I_{\mathrm{abs}}^{\mathrm{NS}} = I_0(1 - 10^{-\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} l})$$

$$I_{\mathrm{abs}}^{\mathrm{DYE}} = I_0(1 - 10^{-\varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}} l})$$

$$I^{\mathrm{NS(DYE)}} = I_0 \frac{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}}}{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}} \times (1 - 10^{-(\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}) l})$$

$$I_{\mathrm{abs}}^{\mathrm{NS+DYE}} = I_{\mathrm{abs}}^{\mathrm{NS(DYE)}} + I_{\mathrm{abs}}^{\mathrm{DYE(NS)}}$$

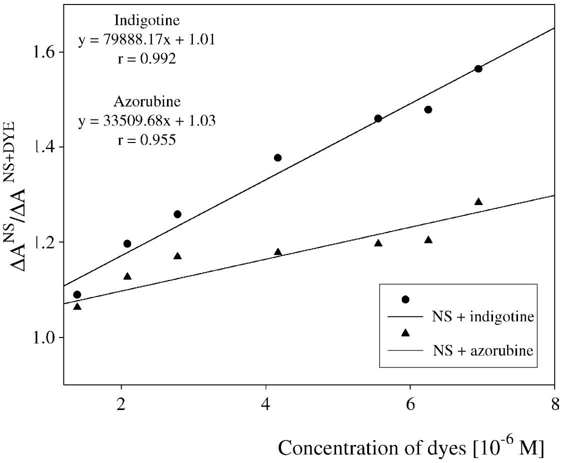

Fig. 5. Stern–Volmer plot for nisoldipine in the presence of dyes.

$$I_{\mathrm{abs}}^{\mathrm{NS+DYE}} = I_0(1 - 10^{-(\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}) l})$$

$$\frac{I_{\mathrm{abs}}^{\mathrm{NS(DYE)}}}{I_{\mathrm{abs}}^{\mathrm{DYE(NS)}}} = \frac{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}}}{\varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}}$$

$$\frac{I_{\mathrm{abs}}^{\mathrm{NS(DYE)}}}{I_{\mathrm{abs}}^{\mathrm{NS+DYE}}} = \frac{\varepsilon_{\mathrm{NS}} c_{\mathrm{DYE}}}{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}}$$

$$I_{\mathrm{abs}}^{\mathrm{NS}} = I_{\mathrm{abs}}^{\mathrm{NS(DYE)}} \frac{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}}}{\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}}} \times \left(\frac{1 - 10^{-\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} l}}{1 - 10^{-(\varepsilon_{\mathrm{NS}} c_{\mathrm{NS}} + \varepsilon_{\mathrm{DYE}} c_{\mathrm{DYE}})}} \right)$$

$I_{\mathrm{abs}}^{\mathrm{NS}}$ is the intensity of irradiation absorbed by NS; $I_{\mathrm{abs}}^{\mathrm{NS(DYE)}}$ the intensity of irradiation absorbed by NS in the presence of the dye, $\varepsilon_{\mathrm{NS}}$ the molar absorption coefficient of NS, $\varepsilon_{\mathrm{NS}} = 5590\,\mathrm{mol^{-1}\,l\,cm^{-1}}$ at $\lambda = 365$ nm; $\varepsilon_{\mathrm{DYE}}$ the molar absorption coefficient of the dye at $\lambda = 365$ nm; indigotine, $2528\,\mathrm{mol^{-1}\,l\,cm^{-1}}$; azorubine, $4365\,\mathrm{mol^{-1}\,l\,cm^{-1}}$; c_{NS} the concentration of NS [M]; with indigotine, 7.59×10^{-5} M; with azorubine, 7.18×10^{-5} M; c_{DYE} the dye concentration [M]; l the optical path length.

The obtained corrected values of the absorption were used to plot a Stern–Volmer type function of the ratio of the NS quenching rate in the absence and in the presence of the dye, versus the dye concentration (Fig. 5). Assuming that the quenching is diffusion-controlled, the quenching rate constant (k_q) can be compared to that of the diffusion-controlled processes (k_{dif}). The value of k_{dif} for methanol of the viscosity $\eta = 0.5513 \times 10^3$ Pa s at 25 °C is $1.2 \times 10^{10}\ l^3\ mol^{-1}\ s^{-1}$. Considering the above assumption, it was possible to estimate the lifetime of the excited state quenched (τ_o) involved in the NS photodegradation. The values of τ_o of nisoldipine with indigotine and azorubine applied as quenchers are 6.66×10^{-6} s and 2.76×10^{-6} s, respectively. The values of τ_o obtained suggest that the excited state of nisoldipine from which its photodegradation takes place is the lowest triplet state.

4. Conclusion

The dyes studied indigotine and azorubine can be potential photoprotectors of drugs from the group of 1,4-dihydropyridine as they quench the excited states of these compounds.

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