PHOTOLYTIC BEHAVIOUR OF 1H-INDOLE-2,3-DIONE 3-[(2,4-DINITROPHENYL)HYDRAZONE AND 2-HYDROXY-4-METHOXYBENZOPHENONE

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The aim of this study was to investigate the photolytic behaviour of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone and compare it with the commercial photoprotective agent 2-hydroxy-4-methoxybenzophenone (Escalol 567, Eusolex 4360, Oxybenzone). The results show that the decrease of absorbance (5%) was proportional to continuous UV-irradiation during 5 minutes, while further irradiation during 60 min causes no significant changes of absorbance. On the basis of experimental data, the conclusion is that continuous UV-irradiation in three different ranges (UV-A, UV-B and UV-C) induced photolysis of these ultraviolet absorbers obeying the first-order kinetics. This study allows the insight into the photoprotective function of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone and its possible application as a UV absorber in the pharmaceutical and cosmetic industry.

Keywords: ultraviolet absorbers, photolysis, UV-irradiation, kinetic.

Introduction

The amount of ultraviolet (UV) radiation reaching the Earth's surface has markedly increased in recent years [1]. Overexposure to ultraviolet (UV) radiation can cause a number of skin disorders such as edema, hyperpigmentation, photo-aging and the most serious skin carcinogenesis, which involves direct and indirect damage to cellular DNA. The direct damage occurs because DNA absorbs ultraviolet light with the resultant gene damage while the indirect damage is caused by free radicals and singlet oxygen [2]. Sunlight is composed of various ultraviolet radiation which is divided into three categories: short wavelengths UV-C (100-290 nm), medium wavelengths UVB (290-320 nm), and long wavelengths UV-A (320-400 nm) [3].

UV-A radiation reportedly causes adverse effects such as the loss of collagen, a decrease in the quantity of blood vessels and an alteration of a connective tissue of the dermis. UVA radiation, known as aging rays, with effects that include roughening, blotchiness, sagging and wrinkles, represents more than ninety percent of UV radiation that reaches the Earth's surface. It can penetrate deeper into the epidermis and dermis of the skin (around 1 mm) and advance the generation of reactive oxygen species (ROS) [4]. UV-A also contributes to the development of the skin cancer. UVA absorbers are chemicals that absorb radiation in 320 – 360 nm, such as benzophenones, anthranylates and dibenzoylmethanes. Microfine zinc oxide is a physical blocker and is particularly effective in this region [5].

UV-B radiation is responsible for characteristic sunburn that humans acquire after a prolonged exposure to sunlight. This radiation inhibits DNA, RNA and protein synthesis and induces erythemal responses. Pigment darkening occurs above 310 nm so when the aim was to achieve a suntan without sunburn, sun filters showing maximum absorption (λmax) between 300 – 310 nm were used. UV-B is 1000 times more capable of causing sunburn than UV-A [6]. It induces direct and indirect adverse biological effects, including the formation of pyrimidine photoproducts [7], isomerization of trans- to cis-urocanic acid [8], induction of ornithine decarboxylase activity [9], stimulation of DNA synthesis [10], free radical production [11], photoaging [12] and photocarcinogenesis [13]. It is considered to be responsible for inducing skin cancer (squamous and basal cell carcinoma) and immunosuppression [5]. The principal UVB absorbers are para-aminobenzoic acid derivatives, salicylates, cinnamates and camphor derivatives, which were once widely used but which were reported to cause dermatitis [14].

UVC radiation is filtered out by the atmosphere and does not reach the earth's surface, so it is not an area of commercial interest to manufacturers of sun products [15]. Topical application of sunscreens is a strategy to protect the deleterious effect of UV radiation (UV-A and UV-B) on the skin. There are two main concerns responsible for limiting sunscreen chemicals for topical application:

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their potential for causing irritation and allergy and the possibility of photochemical instability. The majority of sunscreens are aromatic compounds conjugated with a carbonyl group with an electron-releasing amine or methoxyl group substituted in the ortho- or para-position of the aromatic ring [16,17]. Sunscreens work by absorbing high-energy short wave radiation, which excites them to a higher energy state. The material may dissipate this energy as long wave (>380nm) radiation and return to its original ground state or isomerization can occur, and the material may fragment into non-absorbing isomers. This not only destroys the effectiveness of the product, but isomers may cause irritation and allergic reactions [18]. Further studies by Herzog & Sommer showed that OMC with 4-methylbenzylidene camphor (MBC) were almost completely stable, even at high doses of radiation [19]. Recent studies have shown that the polarity of the solvent system can have a pronounced effect on photostability. It has been noted that stability is greater in polar solvents, and that adding small amounts of protic compounds such as butyloctylsalicylate or butyloctylbenzoate to the solvent retards instability [20]. Another way of improving photostability is by combining two or more sun filters with the aim of protecting the stability of the principal active ingredient. However, this has not always been successful and some filters can accelerate the photoinstability of others [21,22].

Photolysis is the process that involves the use of light to degrade molecules toward their basic constituents. During photolysis, a direct photochemical transformed takes place when energy from light attacks bonds, thereby degrading the compound. The organic compounds generate a wide variety of photochemical reactions that can produce products more complex for degradation. A number of papers have been published on the photolysis in water using the Hg-lamp [23-25]. Photolysis conversion of bischromones has been investigated by Kumar and Yusf [26], the photolysis kinetic of the substituted N-methylcarbamates has been reported by Sanz-Asensio et al. [27] and the first photolysis of benzidine Schiff base has been published by Muhamed [28].

The aim of this study was to investigate the photolytic behaviour of one 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone as a potential photoprotective agent and its possibility for application in pharmaceutical and cosmetic industry. It was compared with photolytic stability of 2-hydroxy-4-methoxybenzophenone (Escalol 567, Eusolex 4360, Oxybenzone), a frequent constituent in cosmetic preparations.

Experimental

Materials

2-hydroxy-4-methoxybenzophenone was purchased from Merck KGaA, ethanol was purchased from Merck (Darmstadt, Germany).

Synthesis of 1H-indole-2,3-dione 3-[(2,4-dinitrophe nyl)hydrazone

1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone was prepared by a modified standard procedure: the solution mixture of 1H-indole-2,3-dione (0.01 mol, 1.47 g) and 2,4-dinitrophenylhydrazine (0.01 mol, 1.98 g) in ethanol (80 mL) was heated under reflux for 3h °C [29]. The formed precipitate was filtered, recrystallized from ethanol and dried to give the required light brown product in 61% yield, m.p. 167-168 °C. Microanalysis: $C_{14}H_9N_5O_5$, Calcd./Found: C51.33/51.62, H 2.75/2.78, N 21.41/21.23%. IR (cm^{-1}): 1605 υ(C=N); 1619^{-1}458 υ(C=Car); 3160 υ(NH $_{\text{isatin}}$); 3037 υ(C-Har), 1730 υ(C=O); 1336 υ(NO_{2sym}); 1458 υ(NO_{2asym}); 846-630 δ(HC=CHar) [29].

Sample preparations

35.71 mg of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone or 22.80 mg of 2-hydroxy-4-methoxybenzophenone was weighed, transferred to a 100 mL volumetric flask and diluted with ethanol to give bulk solutions. Exactly 1.00 mL of the solutions was transferred to the 10 mL volumetric flask to obtain solutions with a required concentration.

Methods

All UV spectra, before and after irradiation with UV light, were recorded on Varian Cary-100 Spectrophotometer from 200 to 400 nm using 1 cm quartz cell, and ethanol as a blank.

Continuous UV irradiations of the samples were performed in a cylindrical photochemical reactor “Rayonnet” with 8 symmetrically placed lamps, with the emission maxima at different ranges: 350 nm (UV-A), 300 nm (UV-B) and 254 nm (UV-C). The samples were irradiated in quartz cuvettes (1 x 1 x 4.5 cm) placed on a rotating circular holder. The total energy flux is 14.3 Wm^{-2} for 254 nm (UV-C), 12.0 Wm^{-2} for 300 nm (UV-B) and 10.3 Wm^{-2} for 350 nm (UV-A).

Statistical analysis

Data were expressed as mean ± standard errors (SE).

All data were analyzed by SigmaPlot 2002 for Windows Version 8.0.

Results

The absorption spectra of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone show strong π-π* at 239 and n-π* bands at 348 and 358 nm (Figure 1).

UV-A irradiation

Ethanolic solutions of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone (10^{-4}mol dm^{-3}) were irradiated with UV-A irradiation during increasing time periods. The analysis of the corresponding UV spectra showed that all maxima of absorbance are decreased proportionally to the irradiation time. UV-A induced photolysis of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone is a first-order reaction:

$InA_1 = InA_0 - K_t$ ....(1)

A_1 - absorbance at time t_1

A_0 - absorbance at the start of the reaction t_0

K – rate constant

$t = t_1 – t_0$, irradiation time.

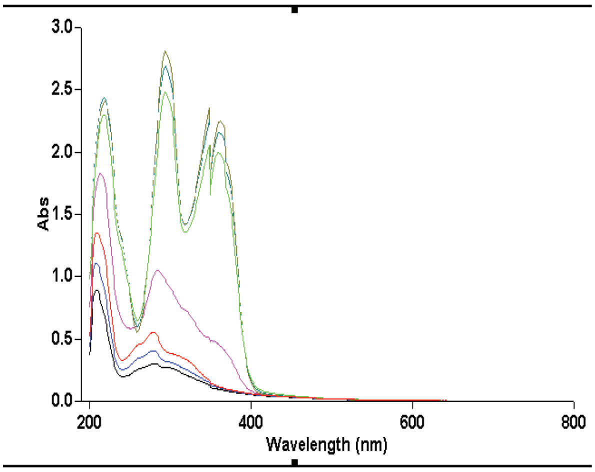
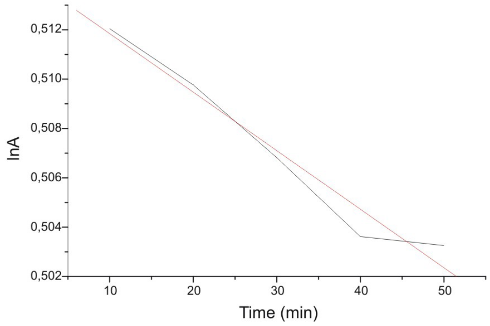
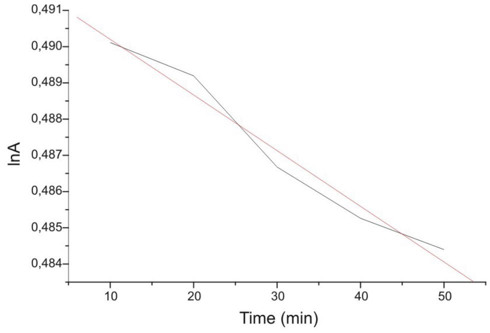


Figure 1. Spectral changes in ethanolic solutions of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone after UV-A irradiation: the decrease of absorbance is proportional to the irradiation time

The decrease of lnA values of 1H-indole-2,3-dione 3--[(2,4-dinitrophenyl)hydrazone during the increase of the irradiation times has been shown in Figure 2.a) absorption maximum at 358 nm, b) absorption maximum at 348 nm and c) absorption maximum at 293 nm.



(a)



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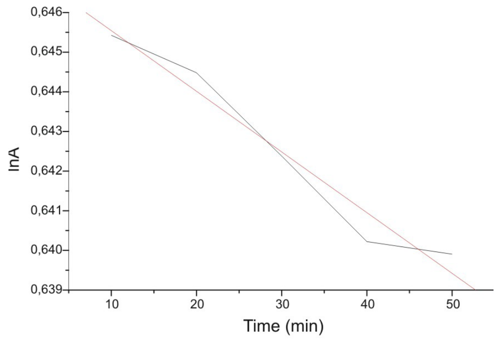


(c)

Figure 2. The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone photolysis in ethanol during increasing UV-A intervals at 358 nm (a), at 348 nm (b) and 293 nm (c)

The rate constants K of UV-A induced photolysis of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone at absorption maximum and R values are shown in Table 1.

Table 1. The rate constants K of UV-A,i UV-B and UV-C induced photolysis of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl) hydrazone at absorption maximum and R values

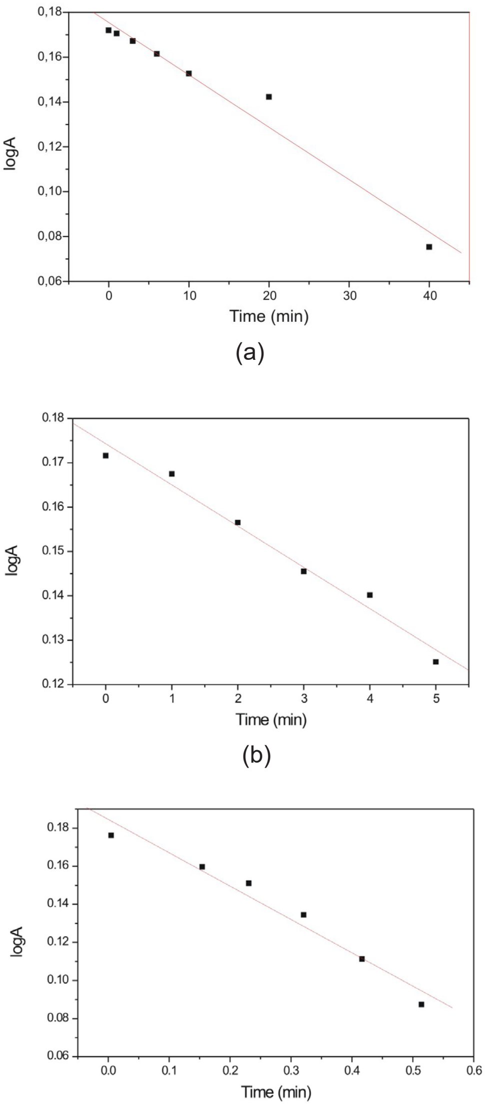
UV irradiation	λ_{max}(nm)	K (10^{-4}min^{-1})	R
UV-A	358	-2.37210	0.98062
	348	-1.53578	0.98510
	294	-1.53047	0.97907
UV-B	357	-2.47787	0.98891
	294	-1.94693	0.99918
UV-C	357	-2.58001	0.93199

The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone photolysis in ethanol during increasing UV-A irradiation at 358 nm is described by equation $lnA= -2.3721x10^{-4}t + 0.51422$, with R value 0.98062; at 348 nm it is described by equation $lnA= -1.53578x10^{-4}$ t + +0.49174, with R value 0.9851 and at 293 nm it is described by equation $lnA= -1.53047x10^{-4}t + 0.64707$, with R value 0.97907.

The analysis of UV spectra of ethanol solvents of commercial UV absorber 2-hydroxy-4-methoxybenzophenone show that maximum of absorbance at 350 and 288 nm are decreased proportionally to the irradiation time [7].

The decrease of lnA during increasing irradiation times at 288 nm has been shown in Figure 3a. UV-A photolysis of 2-hydroxy-4-methoxybenzophenone is described by equation (1).

The kinetic ln-plot of 2-hydroxy-4-methoxybenzophenone photolysis during increasing UV-A intervals at 288 nm is described by equation $lnA= -5.3878x10^{-3}t +0.4039$, with R value 0.98207.



(a)

(b)

(c)

Figure 3. The kinetic log-plot of 2-hydroxy-4-methoxybenzophenone photolysis in ethanol during increasing a) UV-A , b) UV-B and c) UV-C intervals at 288 nm

UV-B irradiation

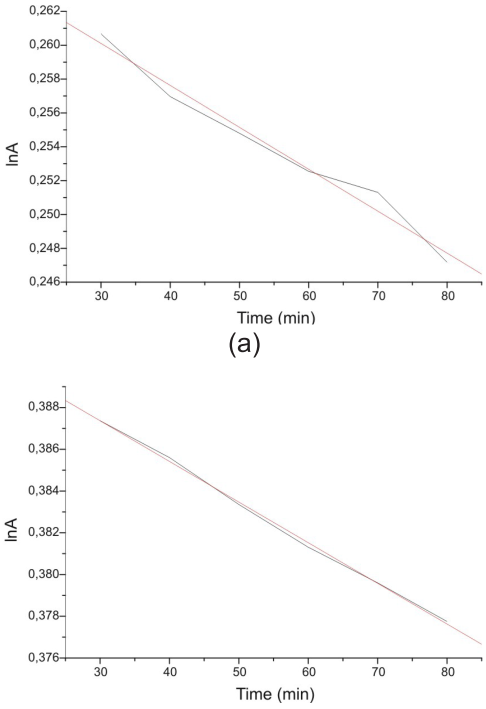
Ethanolic solutions of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone (10^{-4}mol dm^{-3}) were irradiated with UV-B light during increasing time periods. The absorption spectra showed very similar behaviour during a similar irradiation regime with UV-B light. The decrease of lnA during increasing irradiation times has been shown in Figure 4 a) at apsorption maximum at 357 nm and b) at apsorption maximum at 294 nm. UV-B photolysis of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone is described by equation (1).

The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone photolysis in ethanol during increasing UV-B intervals at 357 nm is described by equation $\ln A = -2.47787\times10^{-4}t + 0.26754$, with R value 0.98891 and at 294 nm it is described by equation $\ln A = -1.94693\times10^{-4}t + +0.39321$, with R value 0.99918 . The kinetic logarithmic plots obtained using UV-B light showed a linear behavior with average R values (Table 1).

The kinetic ln-plot of 2-hydroxy-4-methoxybenzophenone photolysis during increasing UV-B intervals at 288 nm is described by equation $\ln A = -2.139\times10^{-2}t + 0.4013$, with R value 0.98973 (Figure 3b.)

UV-C irradiation

Ethanolic solutions of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl) hydrazone (10^{-4} mol dm^{-3}) were irradiated with UV-C light during increasing time periods. The analysis of UV spectra show that maximum of absorbance at 357 is decreased proportionally to the irradiation time during 30 minutes (Figure 4).



(a)

(b)

Figure 4. The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone photolysis in ethanol during increasing UV-B intervals at 357 nm (a) and at 294 nm (b)

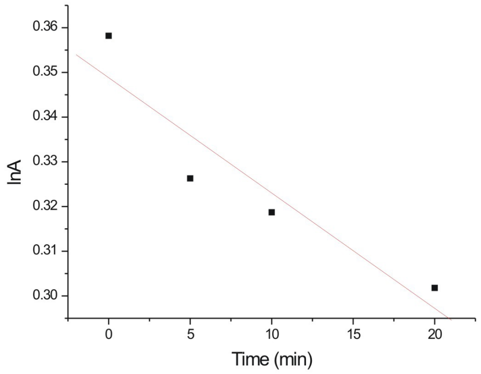


Figure 5. The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl) hydrazone photolysis in ethanol during increasing UV-C intervals at 357 nm

The kinetic ln-plot of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl) hydrazone photolysis in ethanol during increasing UV-C intervals at 357 nm is described by equation $\ln A = -2.58001 \times 10^{-3} t + 0.34882$, with R value 0.93199.

The kinetic ln-plot of 2-hydroxy-4-methoxybenzophenone photolysis during increasing UV-C intervals at 288 nm is described by equation $\ln A = -0.4031 \times t + 1.9479$, with R value 0.98146 (Figure 3c.). The kinetic logarithmic plots obtained with UV-C light showed a linear behavior and had very similar shapes to the presented ones.

Discussion

In the present study we examined the photolytic stability of 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone against UV-A, UV-B and UV-C irradiation. The absorption spectra showed very similar behavior during the time of UV irradiation. The investigated compound contains a photosensitive carbonyl group, so it is assumed that this group is responsible for photoreduction in 96% ethanol. On the basis of the spectroscopic analysis, it is clear that the decrease at 358, 348 and 294 is proportional to continuous UV-A, UV-B and UV-C irradiation during 5 minutes, while further irradiation causes no significant changes of absorbance. The observed kinetic plots fit the first-order law for the investigated molecules, 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone and 2-hydroxy-4-methoxybenzophenone as well.

Conclusions

In conclusion, this study demonstrated that ethanol solutions of investigated 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone and 2-hydroxy-4-methoxybenzophenone show only 5% degradation and similar photolytic behaviour against continuous UV-irradiation. This investigation confirms the efficacy of 2-hydroxy-4-methoxybenzophenone as a UV absorber. Furthermore, 1H-indole-2,3-dione 3-[(2,4-dinitrophenyl)hydrazone shows photostability against UV irradiation, so there is a possibility for its application in pharmaceutical and cosmetic industry as an effective UV photoprotective agent.

Acknowledgements

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Izvod

FOTOLITIČKO PONAŠANJE 1H-INDOL-2,3-DION 3-[(2,4-DINITROFENIL)HIDRAZONA I 2-HIDROKSI-4-METOKSIBENZOFENONA

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(ORIGINALNI NAUČNI RAD)
UDK 581.132.1:614.875

Cilj ove studije je bio ispitivanje fotolitičkog ponašanja 1H-indol-2,3-dion 3-[(2,4-dinitrofenil)hidrazona i 2-hidroksi-4-metoksibenzofenona (Escalol 567, Eusolex 4360, Oxybenzon). Rezultati pokazuju da je smanjenje apsorpcije (5%) proporcionalno kontinuiranom UV zračenju tokom 5 minuta, dok dalje ozračivanje tokom 60 min. izaziva značajne promene absorbance. Na osnovu eksperimentalnih podataka, može se zaključiti da kontinurano UV -zračenje u tri različita opsega (UV-A, UV-B i UV-C) indukuje fotolizu ispitivanih jedinjenja prateći kinetiku prvog reda. Ovo istraživanje omogućava uvid u fotozaštitnu funkciju 1H-indol-2,3-dion 3-[(2,4-dinitrofenil)hidrazona i potencijanu njegovu primenu kao UV apsorbera u farmaceutskoj i kozmetičkoj industriji.

Ključne reči: fotoliza, UV-ozračuvanje, kinetika