Determination of Dacarbazine Φ–order photokinetics, quantum yields and potential for actinometry.

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Abstract

The characterization of drugs' Photodegradation kinetics is more accurately achieved by means of the recently developed Φ–order kinetics than by the 0^{th}–, 1^{st}– and/or 2^{nd}–order classical treatments. The photodegradation of anti–cancer Dacarbazine (*DBZ*) in ethanol has been investigated and found to obey Φ–order kinetics when subjected to continuous and monochromatic irradiation of various wavelengths. Its photochemical efficiency was proven to be wavelength–dependent in the 220-350 nm range, undergoing a 50–fold increase. Albeit this variation was well defined by a sigmoid pattern, the overall photoreactivity of *DBZ* was proven to depend also on the contributions of reactants' and experimental attributes. The usefulness of *DBZ* to serve as a drug–actinometer has been investigated using the mathematical framework of Φ–order kinetics. It has been shown that *DBZ* in ethanol can represent a good candidate for reliable actinometry in the range 270–350 nm. A detailed and easy–to–implement procedure has been proposed for *DBZ*–actinometry. This procedure could advantageously be implemented prior to the determination of the photodegradation quantum yields. This approach might be found useful for the development of many drug–actinometers as alternatives to quinine hydrochloride.

***Keywords**:* Dacarbazine, photodegradation, Φ–order kinetics, quantum yield, actinometry.

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1. Introduction

Dacarbazine (*DBZ*) is an anti–cancer drug used for the treatment of metastatic malignant melanoma and Hodgin's disease.[1] It is also sometimes used in combination with other drugs for soft tissue sarcoma.[2] *DBZ* is supplied as a sterile, lyophilized powder that can be reconstituted for intravenous injection.[3,4] *DBZ* can also be dispensed orally but this way of administration is limited due to its incomplete absorption in the body.[5]

The photodegradation of *DBZ* in aqueous solution, when subjected to both sunlight and fluorescent light, has been reported.[3,6-8] *DBZ* (0.1 mg/mL solutions) was found to rapidly photodegrade (in less than an hour) under the forced irradiation conditions standardly used for photostability testing.[9,10]

Despite a large number of studies reported, so far, on the photodegradation and photostabilisation of *DBZ*, only a few included kinetic studies. Nevertheless, the reaction order corresponding to *DBZ* photodegradation is still controversial as it was reported to obey both zero-order[11] and pseudo-first-order[9] kinetics in solution.

In fact, such an ambiguity on the reaction order, is ubiquitous in pharmaceutical studies in relation to the photodegradation of drugs.[12,13] In our team, we have recently proposed a new approach based on Φ–order kinetics that was found useful for the description and

quantification of unimolecular AB(1Φ) and reversible AB(2Φ) photodegradation reactions of drugs[14-18]. The Φ–order kinetics solves the uncertainty on drugs photodegradation order for these types of reaction systems.

In this study, *DBZ* kinetics was investigated in ethanolic solutions using Φ–order kinetics. This tool was employed to screen the change of *DBZ* photodegradation quantum yield in relation to the irradiation wavelength. It was also used to explore and demonstrate the potential of DBZ in actinometry.

2. Materials and methods

2.1. *Materials*

Dacarbazine, 5-(3,3-dimethyltriazin-1-yl)imidazole-4-carboamide, (*DBZ*), and spectrophotometric grade ethanol were purchased from Sigma-Aldrich.

2.2. Chromatographic Conditions

Dacarbazine and its photoproducts were separated on a Gemini C_{18} reverse phase column 5 µm, 2.1 x 50 mm (Phenomenex, UK) fitted to a Perkin Elmer Series 200 Pump and UV/Vis Detector, and a 600 Series Link interface; all of which were controlled remotely using TotalChrom software (PerkinElmer, USA). Separation was achieved at a flow rate of 0.5 ml/min with a mobile phase of 70% water (adjusted to pH 3 with glacial acetic acid) and 30% acetonitrile. An injection loop of 20 µl was used and the detector wavelength was set at 236 nm. The overall run time of the assay was 10 min. The retention times for DBZ and its photoproduct were 3.87 and 4.21 min, respectively. The linearity range of DBZ calibration graph ranged between 4.8×10^{-5} M and 5.5×10^{-4} M.

2.3. Monochromatic continuous irradiation

For irradiation experiments, a Ushio 1000 W xenon arc-lamp light source housed in a housing shell model A6000 and powered by a power supply model LPS–1200, was used. This set up was cooled by tap water circulation through a pipe system. The lamp housing was connected to a monochromator model 101 that allows the selection of specific irradiation wavelengths since it

consists of a special f/2.5 monochromator with a 1200 groove/mm at 300nm blaze grating. The excitation beam was guided through an optical fibre to impinge from the top of the sample cuvette i.e. the excitation and the analysis light beams were perpendicular to each other. The set up was manufactured by Photon Technology International Corporation.

2.4. The monitoring system

A diode array spectrophotometer (Agilent 8453) was used to measure the various absorption spectra and kinetic profiles for the irradiation and calibration experiments. This spectrophotometer was equipped with a 1–cm cuvette sample holder and a Peltier system model Agilent 8453 for temperature control. As such, the sample was kept at 22°C, stirred continuously during the experiment, and completely shielded from ambient light. The spectrophotometer was monitored by an Agilent 8453 Chemstation kinetics–software.

A Radiant Power/Energy meter model 70260 was used to measure the radiant power of the incident excitation beams.

The radiant power, given in $mW.cm^{-2}.s^{-1}$ by the radiant power meter, is converted to the appropriate unit ($einstein.dm^{-3}.s^{-1}$) for each wavelength, to be used in Eq.2. Accordingly, the reaction rate-constant ($k_{DBZ}^{\lambda_{irr}}$) is expressed in s^{-1}.

2.5. Kinetic data treatment

In order to carry out non−linear fittings and to determine best-fit curves, a Levenberg-Marquardt iterative program within the Origin 6.0 software was used.

2.6. DBZ solutions

DBZ stock and diluted solutions were prepared in ethanol, protected from light by aluminium foil wrapping and kept in the fridge while not in use. As such DBZ solutions remained stable for several weeks. The stock solution was diluted to prepare fresh analytical solutions (*ca.* 5×10^{-6} M) for analysis used to perform irradiation experiments at various wavelengths.

Experiments were conducted at least in triplicates.

3. Results and discussion

3.1. The Mathematical framework

The long lasting problem of achieving closed–form integration of photoreaction differential equations, has meant that no integrated rate–laws were available to describe photochemical reactions.[12,19] This situation represents a major issue for photokinetic studies and the reliable quantification of phototransformations.

However, an expression has previously been established for the photodegradation of an initial species (*A*) into a photoproduct (*B*) via a unimolecular photoreaction involving a single photochemical step ($\Phi_{A\to B}^{\lambda_{irr}}$), the so–called AB(1Φ) photoreaction. The closed–form integration was achieved for the case where *A* is the only absorbing species in the reactive medium ($\varepsilon_B^{\lambda_{irr}} = 0$).[14] This integrated rate–law was then used as a template to develop a semi–empirical rate–law model for the situation where both (*A* and *B*) species absorb the excitation light, whose reaction differential equation cannot be solved. Optimization, testing and validation of the developed semi–empirical rate–law (Eq.1) were achieved by using data generated by Runge–Kutta numerical integration method.[16]

The general AB(1Φ) semi–empirical rate–law equation describing the time variation of the cumulative absorbance of the reactive medium involving the unimolecular phototransformation of an initial species *A*, into its photoproduct (*B*) is given by the logarithmic Eq.1.[16]

Eq.1 has been established for a homogeneously and continuously stirred solution, which is subjected to a monochromatic, non–isosbestic and continuous irradiation (λ_{irr}) at constant temperature. Also, it is assumed that the concentration of the excited–state species is negligible and at the irradiation wavelength (λ_{irr}) species *A* and *B* may absorb different amounts of light ($P_{\lambda_{irr}}$), i.e. the absorption coefficients of the species may be different ($\varepsilon_A^{\lambda_{irr}} \neq \varepsilon_B^{\lambda_{irr}} \neq 0$). For the present study, the species *A* and *B* correspond to dacarbazine, *DBZ*, and its photoproduct, *PP*. (The photo product's chemical structure has not been identified with certainty).

$$A_{tot}^{\lambda_{irr}/\lambda_{obs}}(t) = A_{PP}^{\lambda_{irr}/\lambda_{obs}}(\infty) + \frac{A_{DBZ}^{\lambda_{irr}/\lambda_{obs}}(0) - A_{PP}^{\lambda_{irr}/\lambda_{obs}}(\infty)}{A_{DBZ}^{\lambda_{irr}/\lambda_{irr}}(0) - A_{PP}^{\lambda_{irr}/\lambda_{irr}}(\infty)} \times \frac{l_{\lambda_{obs}}}{l_{\lambda_{irr}}} \times Log\left[1 + \left(10^{-\left[\left(A_{DBZ}^{\lambda_{irr}/\lambda_{irr}}(0) - A_{PP}^{\lambda_{irr}/\lambda_{irr}}(\infty)\right) \times \frac{l_{\lambda_{irr}}}{l_{\lambda_{obs}}}\right]} - 1\right) \times e^{k_{DBZ}^{\lambda_{irr}} \times t}\right] \quad (1)$$

where $l_{\lambda_{irr}}$represents the optical path length of the irradiation beam inside the sample and $l_{\lambda_{obs}}$is the optical path length of the spectrophotometer monitoring light. The absorbances ($A^{\lambda\ /\lambda}$) in Eq.1 correspond to the actual measurements by the instrument (i.e. along $l_{\lambda_{obs}}$) performed on the reactive medium, at a given reaction time ($A_{tot}^{\lambda_{irr}/\lambda_{obs}}(t)$), at the initial time (t=0, $A_{DBZ}^{\lambda_{irr}/\lambda_{obs}}(0)$), and at the end of the reaction (t = ∞, $A_{PP}^{\lambda_{irr}/\lambda_{obs}}(\infty)$ where only the photoproduct is present). The absorbances *vs*. time (the traces) can be read out from the spectra at the particular wavelengths of irradiation (λ_{irr}) and observation (λ_{obs}) which might or

not be the same. Therefore, each kinetic profile is dually labelled by the irradiation and the observation wavelengths ($\lambda_{irr}/\lambda_{irr}$ or $\lambda_{irr}/\lambda_{obs}$).

The exponential factor in Eq.1, $k_{DBZ}^{\lambda_{irr}}$, representing the overall photoreaction rate–constant, is analytically expressed by

$$k_{DBZ}^{\lambda_{irr}} = \Phi_{DBZ\rightarrow PP}^{\lambda_{irr}} \times \varepsilon_{DBZ}^{\lambda_{irr}} \times l_{\lambda_{irr}} \times F_{\infty}^{\lambda_{irr}} \times P_{\lambda_{irr}} = \beta_{\lambda_{irr}} \times P_{\lambda_{irr}} \quad (2)$$

where $\Phi_{DBZ\rightarrow PP}^{\lambda_{irr}}$ is the forward quantum yield of *DBZ* photodegradation, realised at the non–isosbestic irradiation wavelength (λ_{irr}), $\varepsilon_{DBZ}^{\lambda_{irr}}$ is the molar absorption coefficient of *DBZ*, $P_{\lambda_{irr}}$ is the radiant power, and $F_{\infty}^{\lambda_{irr}}$ the photokinetic factor expressed as

$$F_{\infty}^{\lambda_{irr}} = \frac{1 - 10^{-\left(A_{tot}^{\lambda_{irr}/\lambda_{irr}}(\infty) \times \frac{l_{\lambda_{irr}}}{l_{\lambda_{obs}}}\right)}}{A_{tot}^{\lambda_{irr}/\lambda_{irr}}(\infty) \times \frac{l_{\lambda_{irr}}}{l_{\lambda_{obs}}}} \quad (3)$$

The set of Eqs.1–3, are also applicable for the case where only species *A* absorbs as the corresponding equations can be retrieved from Eqs.1–3 by considering the quantities relative to the photoproduct equal to zero (i.e. $A_{tot}^{\lambda_{irr}/\lambda_{irr}}(\infty) = A_{PP}^{\lambda_{irr}/\lambda_{obs}}(\infty) = \varepsilon_{PP}^{\lambda_{irr}} = 0$).

Nevertheless, the application of Eqs.1–3 to the case where both species (*A* and *B*) absorb the excitation light was conditional to $F_{\infty}^{\lambda_{irr}}$ being higher than 1.2 ($A_{PP}^{\lambda_{irr}/\lambda_{irr}}(\infty) < 0.65$). This has proven to be a simple limitation as the former condition can easily be met by lowering the concentration of the initial species or possibly by reducing the path length of the irradiation ($l_{\lambda_{irr}}$).

The AB(1Φ) photodegradation reactions that are well described by Eq.1 are considered to obey Φ–order kinetics.

Since the differential equations of thermal and photochemical reactions are different, it is expected that the mathematical formulae of their respective integrated rate-law equations be different, and therefore a specific order for each reaction should, as a consequence, be established. This statement holds equally for the case where two different thermal reactions are considered (e.g., zeroth– and first–order reactions) as well as for reactions of different nature (photochemical and thermal reactions). As a matter of fact, Φ–order kinetics would be ill-described by for instance 1st–order kinetics (as it would be if either 0th or 2nd order kinetics were used). From this point of view, the photodegradation kinetics of drugs should be better described by Φ–order kinetics instead of the usually employed treatments that correspond to thermal reactions. A comprehensive discussion of the properties Φ–order kinetics and its flexibility compared to thermal reaction orders has been previously provided.[16]

Also, the Φ–order kinetics presents the advantage of providing the absolute values of the quantum yield (and/or the $\beta_{\lambda_{irr}}$ factors) irrespective of the radiant power used (as set out in

Eq.2). It therefore provides a plausible tool to perform high concentration/light radiant flux tests such as those recommended by ICH.

3.2. DBZ Photodegradation in ethanol

In ethanol, the absorption spectrum of *DBZ* in the dark is characterised by two main and broad absorption 200–250 and 280–400 nm regions, spanning the UVB, UVA and a small section of the visible spectral ranges (Fig. 1). The maxima for *DBZ* are located at 235 and 325 nm, with the latter much broader and higher in intensity than the former. The long–wavelength absorption band is probably due to the $\pi \rightarrow \pi^*$ transitions occurring in the extended double bond system formed between the ring and its adjacent unsaturated azo–group (Scheme 1). It could also be suggested that this band might correspond to a charge–transfer (CT) transition. In the case of such an interpretation, the intramolecular CT transitions might be attributed to a resonance interaction involving $n \rightarrow \pi$ conjugation. Since the π^* orbital is situated on the double bond system then the effect of the transition is to transfer a charge from the electron-donating substituent (e.g. $–N(CH_3)_2$) to the electron accepting group (e.g. pyrazole ring).[20,21]

A number of spectral changes occur when subjecting the reactive medium to monochromatic irradiation, including the gradual decrease of both peaks, with a complete disappearance of the long–wavelength band at the end of the reaction. During the reaction progress a new peak emerges at *ca*. 206 nm. This behaviour is observed irrespective of the wavelength of the irradiation beam. Such features indicate a significant disturbance of the π system, and overall,

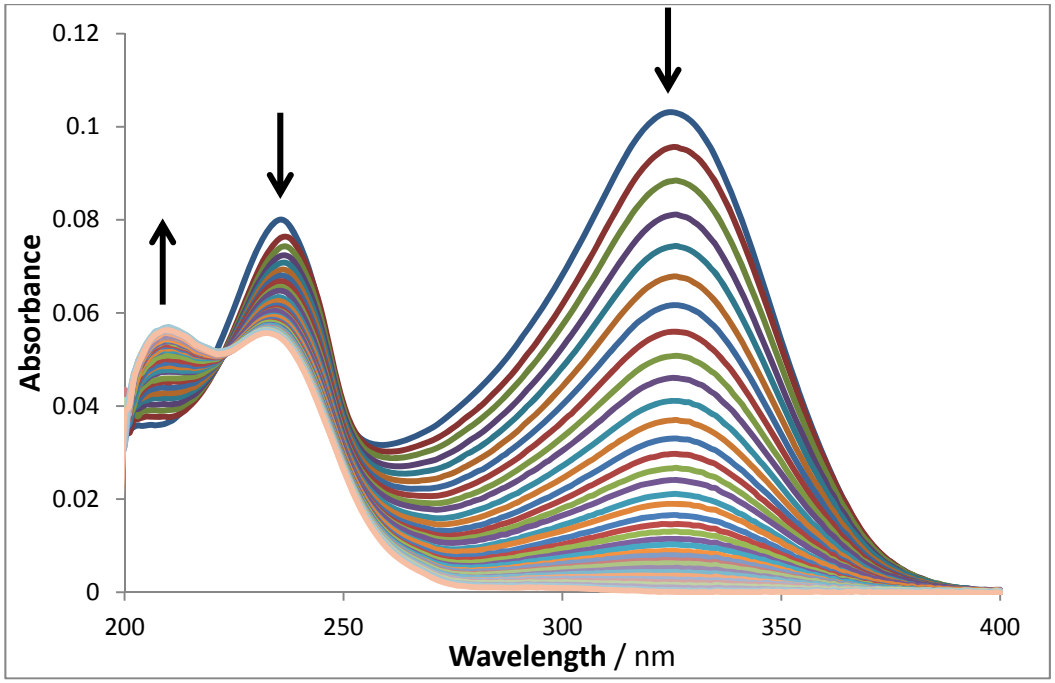


Figure 1: Evolution of the electronic absorption spectra of 5.47 x 10^{-6} M Dacarbazine (*DBZ*) in ethanol, when irradiated continuously with a monochromatic beam at 325-nm (total irradiation time 1500 s at a radiant power of P_{325} = 1.84 x 10^{-6} einstein.s^{-1}.dm^{-3}. The arrows indicate the direction of the evolution of the absorption bands during the photoreaction.

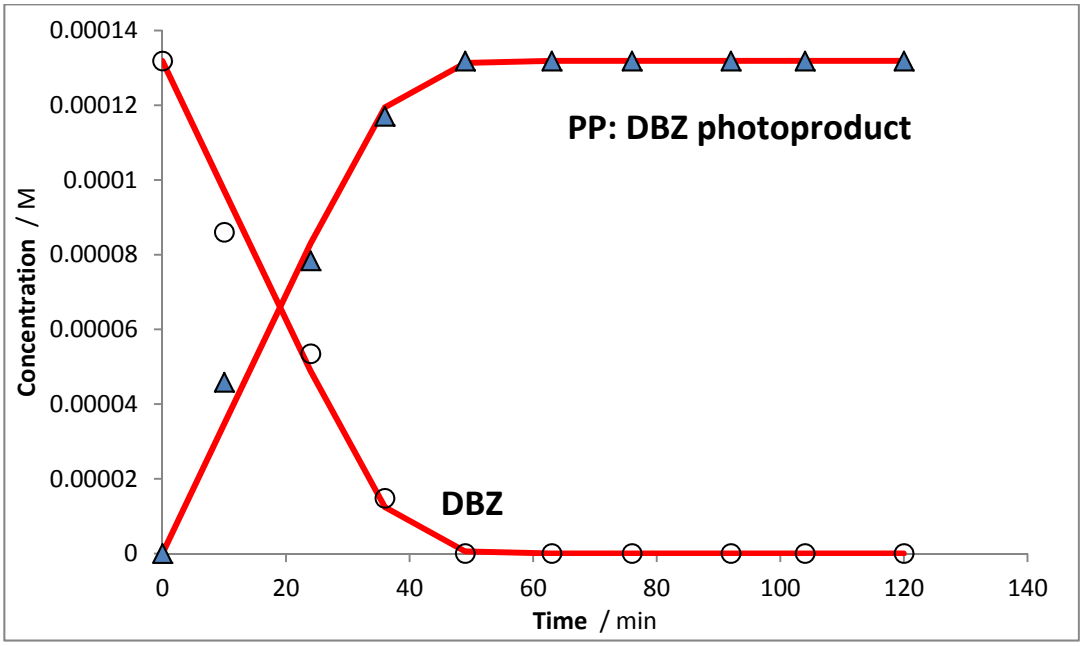
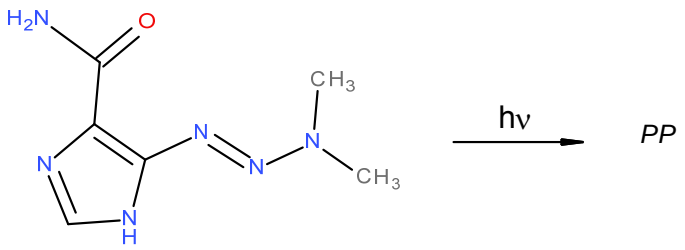


Fig 2: Evolution of *DBZ* concentration ($C_{DBZ}(0)$ = 1.32 x 10^{-4} M) over the photodegradation time and the simultaneous formation of the photoproduct. The reaction medium was monitored by HPLC while irradiated continuously with a monochromatic beam at 325 nm (P_{325} = 1.02 x 10^{-6} einstein s^{-1} dm^{-3}). Circles and triangles: experimental data; Lines: fitting of the data with Eq.1.

clearly point out to a chemical change due to irradiation. It is worth noting that *DBZ* is stable in the dark with no recorded variation of its spectrum over a long time.

The chromatograms obtained by monitoring the reaction medium by HPLC showed the presence of only two peaks attributable to *DBZ* and its photoproduct, *PP* (as laid out in Scheme 1). The concentration of *DBZ* decreased continuously with irradiation time until reaching a zero value while that of *PP* increased (Fig.2). The good fit of the experimental data with Eq.1 (with the same rate-constant for both species) corroborates the occurrence of a unimolecular mechanism of the AB(1Φ) type (Scheme 1).



Scheme 1. Proposed photodegradation reaction of *DBZ* in ethanol.

3.3. Photodegradation kinetics of DBZ

The investigation of *DBZ* kinetics was performed on freshly prepared and equally concentrated solutions subjected to a series of irradiations whose beams' wavelengths (λ_{irr} = 350, 325, 300, 275, 250, 236 and 221 nm) covered almost entirely the absorption spectrum of the drug.

The kinetic traces were collected by observing the timely variation of the absorbance of the reactive medium at a unique observation wavelength (λ_{obs} = 325 nm) for each experiment performed at a given irradiation wavelength (Fig. 3). These traces are labelled by their irradiation/observation wavelengths ($\lambda_{irr}/\lambda_{obs}$).

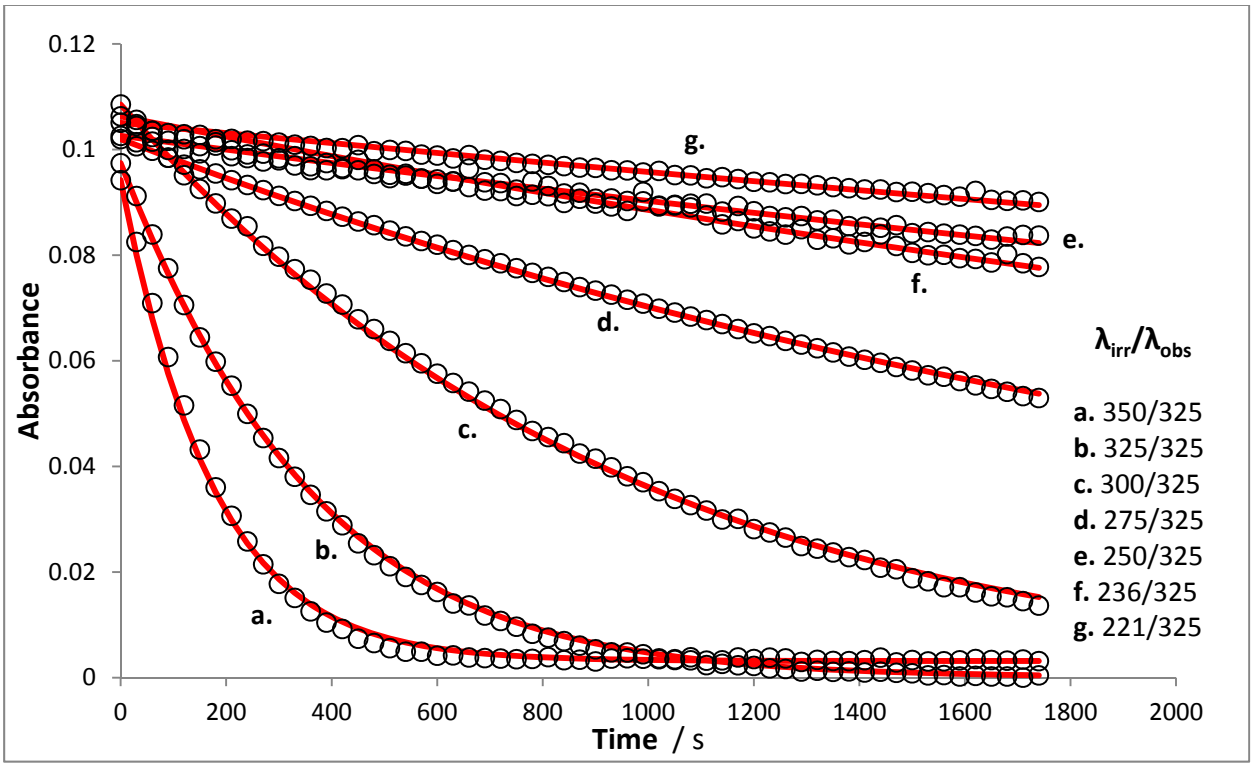


Fig. 3: Photokinetic profiles of *DBZ* in ethanol (5.47 x 10^{-6} M) obtained at different irradiation wavelengths (λ_{irr} = 350, 325, 300, 275, 250, 236 and 221 nm) and observed at λ_{obs} = 325 nm. The circles represent experimental data whereas the solid lines represent the fitting of the traces with Eq.1.

The subsequent good fitting of these traces with Eq.1 further confirmed that *DBZ* obeyed a unimolecular phototransformation which was well described by Φ–order kinetics. This also underlines the usefulness of the model equation (Eq.1) in depicting AB(1Φ) photoreactions kinetics at any non–isosbestic and monochromatic irradiation.

The kinetic studies on *DBZ* are very scarce. As far as we are aware, no data is available on *DBZ* photodegradation in organic media. In water solution, *DBZ* degradation under polychromatic fluorescent light was reported to obey apparent first–order kinetics even though the data presented showed a good linear correlation between the remaining *DBZ* concentration (in percentage) with reaction time.[9] Similar treatment of the kinetic data are predominant in photodegradation studies.[12] However, owing to the fact that the 0^{th}–, 1^{st}–, and 2^{nd}–order kinetic classical models were basically developed for thermal reaction, they should not in principal be applicable to photodegradation data due to the differences found in the differential equations of thermal and photochemical reactions. It is also worth noting that there are many examples where the same experimental data of a given drug photoreaction could be fitted with equations corresponding to two different classical reaction-orders,[12] hence, raising some serious issues about the applicability of the aforementioned approach.[16] This situation does not seem to be specific to AB(1Φ) reactions.[12,13] In this respect, the Φ–order kinetics seems to be a viable and more precise alternative to the classical treatments.

The analysis of Fig.3 suggests that *DBZ* photodegradation increases with irradiation wavelength, with the fastest reactions observed for λ_{irr} > 300 nm. However, a definite conclusion on the basis of this observation might be dubious as the formula of the photodegradation rate–constant, involves a contribution of reaction attributes, reactants'

parameters and some experimental conditions whose values might considerably differ between wavelengths (e.g. the absorption coefficients, the radiant power...etc). Therefore, it is not reliable to directly compare the overall rate–constant values. In a more consistent strategy, the evaluation of all the reaction parameters is needed. For instance, the determination of the photochemical quantum yield values and their possible variation with irradiation would shed light on the efficiency of the photodegradation as well as on their contribution to the rate–constant value.

3.4. DBZ photochemical quantum yields

The fitting of the experimental kinetic profiles of *DBZ* photodegradation to Eq.1 (Fig.3) allows the extraction of the overall rate–constant values for each individual experiment. These values together with the analytical expression of $k_{DBZ}^{\lambda_{irr}}$ (Eq. 2) are used to work out the values of *DBZ* quantum yield values under any monochromatic irradiation condition (Table 1).

The efficiency of *DBZ* photodegradation is wavelength–dependent with a significant 50–fold increase between 235 and 350 nm. Its highest values ($\Phi_{DBZ\rightarrow PP}^{\lambda_{irr}}$ > 0.015) were recorded for wavelengths higher than 300 nm, which indicates that the lowest excited state produced from the long-wavelength transition plays a major role in the photodegradation of the anti–cancer drug.

The phenomenological trend of the experimental data relative to our *DBZ* photodegradation quantum yields in ethanol is well reproduced by a sigmoidal pattern with respect to the

variation of the irradiation wavelength (Fig. 4). Even though the plateau region of the sigmoid was not reached for DBZ, the general pattern is consistent with the data observed for other drugs.[16,18,22-24] The sigmoid function, given by Eq.4, yields values for $\Phi_{DBZ\rightarrow PP}^{\lambda_{irr}}$ which agree well with their experimental counterparts listed in Table 1. The two sets of values are linearly correlated as indicated by the inset of Fig.4. In this respect, Eq.4 can be used to predict the quantum yield absolute values at any irradiation wavelength between 220 and 350 nm.

$$\Phi_{\mathrm{DBZ\rightarrow PP}}^{\lambda_{\mathrm{irr}}} = \frac{0.263}{1+399\times e^{-0.0265\times(\lambda_{irr}-180)}} \qquad (4)$$

The acute evolution of the quantum yield values over the 150–nm spectral range, clearly indicate that monochromatic light is the best tool to characterise the photoreactivity of drugs. Incidentally, such a result on quantum yields cannot be achieved using the classical thermal kinetic models. This also points out the challenge of obtaining reliable measurements of reaction quantum yields when using polychromatic and pseudo–monochromatic lights (the latter are most often obtained as filtered light from lamps whose beams' bandwidths may vary from a few tens to more than a hundred nanometres).[25] The results obtained from such measurements for the evaluation of quantum yield values can only be considered as approximations as they ought to provide only average values.

Even though no quantum yield values are available in the literature for *DBZ*, the results of this study can be compared to those obtained for other drugs since, conversely to the reaction rate–constants, the photochemical photodegradation quantum yields are absolute parameters.

Table 1

Quantum yields, overall rate–constants, absorption coefficient and radiant power values for *DBZ* photodegradation reactions under various monochromatic irradiation wavelengths.

λ_{irr}	$\varepsilon_{DBZ}^{\lambda irr}$ / $M^{-1}.cm^{-1}$	$P_{\lambda irr}$ / einstein $s^{-1}dm^{-3}$	$k_{DBZ}^{\lambda_{irr}}$ x 10^3 /s^{-1}	$\Phi_{DBZ\rightarrow PP}^{\lambda_{irr}}$
350	9896	2.99 x 10^{-6}	6.63	(49 ± 1.11) x 10^{-3}
345	12580	2.17 x 10^{-6}	5.55	(42 ± 1.65) x 10^{-3}
325	18864	2.16 x 10^{-6}	4.17	(23 ± 5.84) x 10^{-3}
300	12469	1.45 x 10^{-6}	1.37	(16 ± 3.31) x 10^{-3}
275	7068	1.44 x 10^{-6}	0.45	(9 ± 1.80) x 10^{-3}
250	7157	1.26 x 10^{-6}	0.23	(4 ± 2.81) x 10^{-3}
236	14639	1.49 x 10^{-6}	0.20	(1 ± 1.00) x 10^{-3}
221	9476	1.53 x 10^{-6}	0.13	(1 ± 1.00) x 10^{-3}

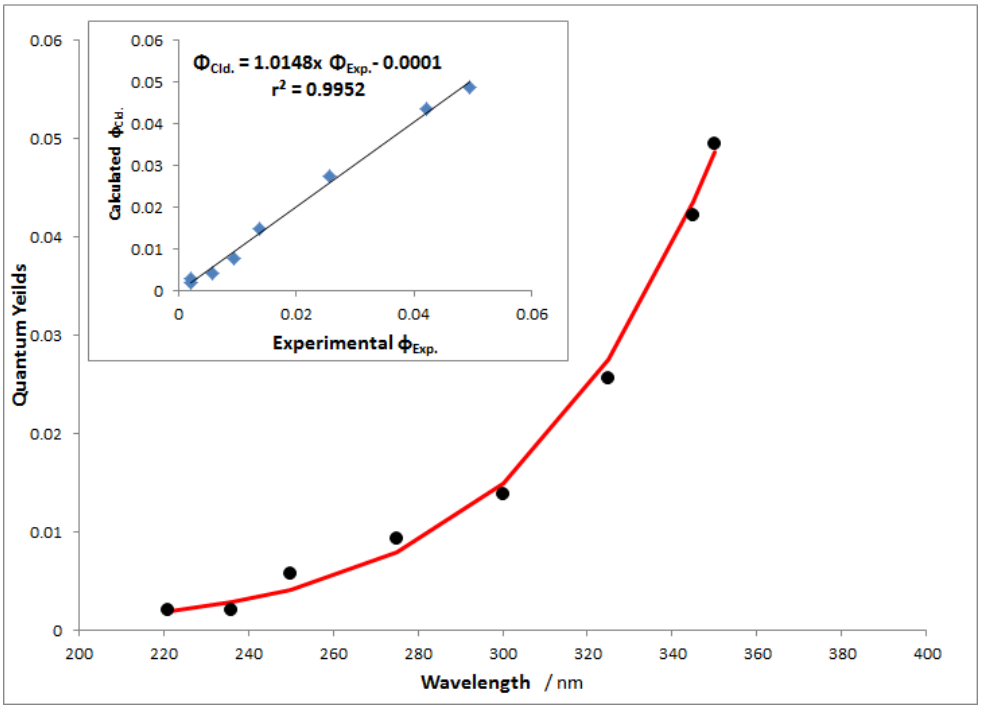


Fig. 4: Average forward $\Phi_{DBZ\rightarrow PP}^{\lambda_{irr}}$ (filled circles) quantum yields calculated for irradiation wavelengths λ_{irr} = 350, 345, 325, 300, 275, 250, 236 and 221 nm. The line represents the fitting of the data with Eq.7. Inset: Calculated (Eq.4), $\Phi_{Cld.}$, *vs.* experimental, $\Phi_{Exp.}$, values of photochemical quantum yields (Table1).

Our results for *DBZ* ($\Phi_{\mathrm{DBZ}\rightarrow\mathrm{PP}}^{\lambda_{\mathrm{irr}}}$) are about 10-fold lower than those obtained for nifedipine, a very light sensitive cardiovascular drug, which obeys AB(1Φ) Φ–order kinetics,[16] and 2-5 times lower than those obtained for *trans*–Montelukast, a photoreversible AB(2Φ) system.[18] However, for all these drugs, the quantum yield values increased with the irradiation wavelength and their photochemical efficiencies were the smallest for the shortest wavelength transitions (mostly below 270 nm). Obviously, our findings do not corroborate the affirmation, concerning the quantum yield dependency with irradiation wavelength, stating that "*the quantum yield is a wavelength independent quantity and thus can be determined at any wavelength that the substance absorbs, although a greater precision can be gained near the absorption maximum*".[13] Therefore, our results recommend that the photodegradation reaction quantum yields of drugs should be determined experimentally to confirm whether or not they are wavelength-dependent.

3.5. DBZ-actinometer

Drugs are not usually used as actinometers and no kinetic–based methods for actinometry have so far been reported.[12,13,25,26] In previous studies it has been shown that drugs and chemical systems which are well described by Φ–order kinetics may be exploited as actinometers.[15,16,18] Indeed, the Φ–order equations are flexible enough to bring forward the potential and facilitate the development of new drug–actinometers based on the kinetic data of their photodegradations. In the context of pharmaceutics, proposing new actinometers is important since the one recommended by ICH,[27] namely the quinine hydrochloride actinometer, presents various drawbacks that seriously hamper its application to photostability testing purposes.[28-31]

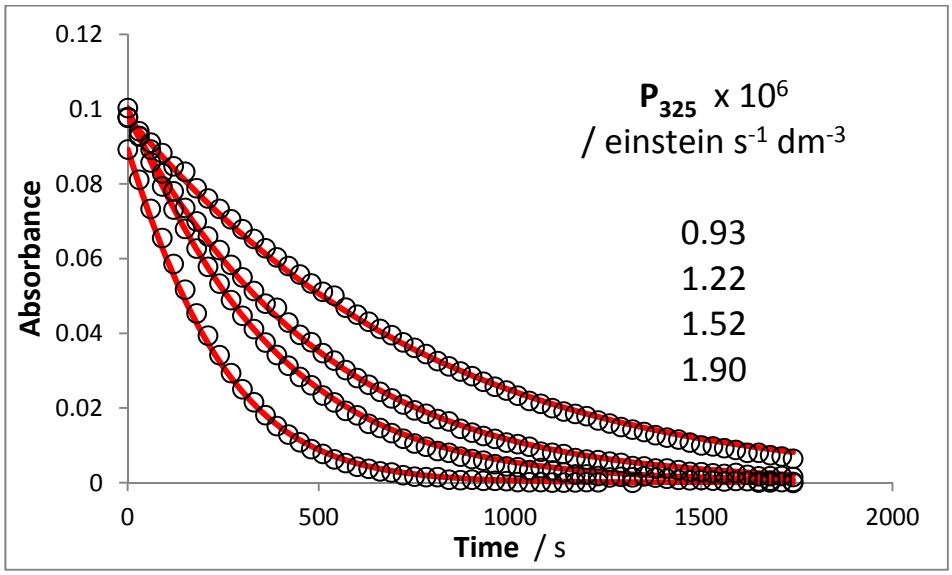


Fig. 5: Effect of increasing the radiant power, P_{325}, of a 325–nm monochromatic irradiation beam on the kinetic traces of *DBZ* (5.47 x 10^{-6} M). Circles represent experimental data (λ_{obs} = 325 nm) and the solid lines correspond to the best fit traces to Eq.1.

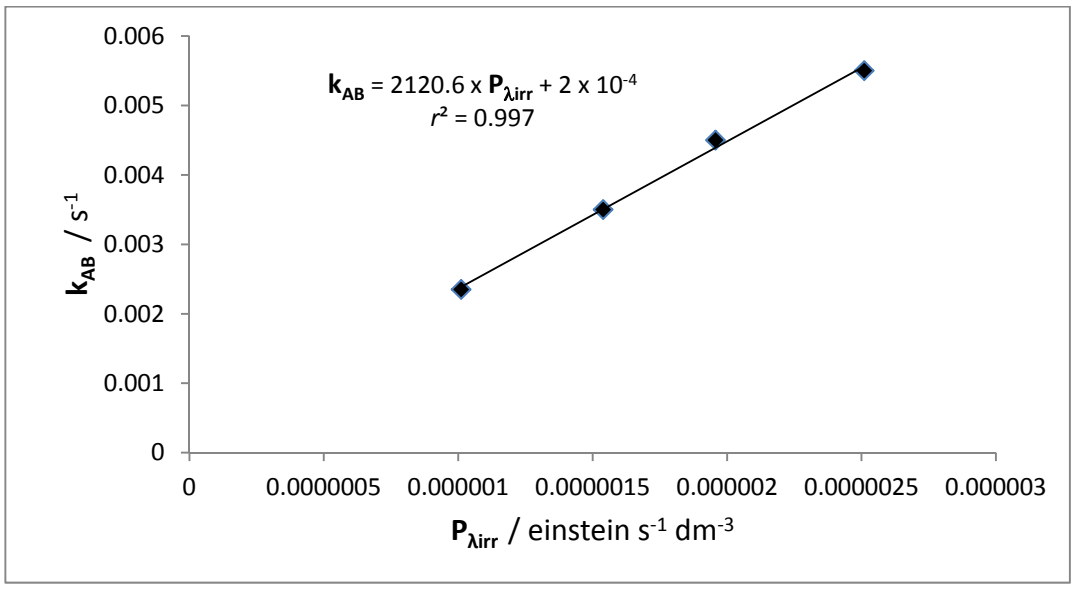


Fig. 6: An example of a linear correlation between of the k_{DBZ}^{350} parameter of the traces against the radiant power, P_{350} for *DBZ* in ethanol.

Testing the potential of DBZ for actinometry was performed within the 270-350 nm spectral range where *DBZ* is most photoactive (Table 1). Five irradiation wavelengths (λ_{irr} = 350, 345, 325, 300, and 275 nm) were selected for the study. A series of freshly prepared *DBZ* solutions were each irradiated at one of the selected wavelengths with a series of different radiant powers of the excitation beam ($P_{\lambda_{irr}}$, spanning the capacity of our instrumentation). The traces for *DBZ* photodegradations were collected while monitoring the medium at the maximum intensity of *DBZ* absorption spectrum (λ_{obs} = 325 nm). These traces have then been fitted to Eq.1 (Fig.5), and the respective values of the rate–constants ($k_{DBZ}^{\lambda_{irr}}$) determined.

According to Eq.2, the higher the $P_{\lambda_{irr}}$ values the faster the photodegradation (i.e. the higher the $k_{DBZ}^{\lambda_{irr}}$ values). Also, as predicted by the latter equation, $k_{DBZ}^{\lambda_{irr}}$ and $P_{\lambda_{irr}}$ values were found to linearly correlate with very small intercepts (close to zero values) and almost unitary correlation coefficients (Fig.6 and Table 2).

For the selected irradiation wavelengths, the gradients of these equations, expressed here as the factors $\beta_{\lambda_{irr}}$(Eq.2), produce a triangular correlation with the irradiation wavelength. This correlation can readily be represented by two linear relationships as given in Fig.7 (with correlation coefficients exceeding 0.99). Using the linear relationships, allows predicting the values of $\beta_{\lambda_{irr}}$ for any irradiation wavelength situated between 275 and 350 nm. But more importantly, such $\beta_{\lambda_{irr}}$ values effectively serve the determination of the radiant power of an unknown, though monochromatic, light source within the aforementioned range through a rearranged Eq.3, as

Table 4: Correlation equations for the variation of *DBZ* photodegradation overall rate–constants ($k_{NIS}^{\lambda_{irr}}$) with radiant power ($P_{\lambda_{irr}}$), the corresponding $\beta_{\lambda_{irr}}$ factors, and the span of radiant power employed for various monochromatic irradiations.

Irradiation wavelength λ_{irr} / nm	Equation of the line [a] $k_{DBZ}^{\lambda_{irr}} = \beta_{\lambda_{irr}} \times P_{\lambda_{irr}} + intercept$	Correlation coefficient, r^2	$P_{\lambda_{irr}} \times 10^7$ / einst.s^{-1}.dm^{-3}
350	2120.6 x P_{350} + 0.0002	0.9969	1.01 – 2.51
345	2589.8 x P_{345} - 0.0003	0.9830	0.95 – 2.20
325	4013.2 x P_{300} - 0.0024	0.9499	0.93 – 1.90
300	2499.3 x P_{300} - 0.0017	0.9987	1.05 – 1.31
275	326.26 x P_{275} - 0.0001	0.9991	0.78 – 1.64

[a] $k_{NIS}^{\lambda_{irr}}$ and intercepts expressed in s^{-1} and $\beta_{\lambda_{irr}}$ in einstein^{-1}.dm^3.

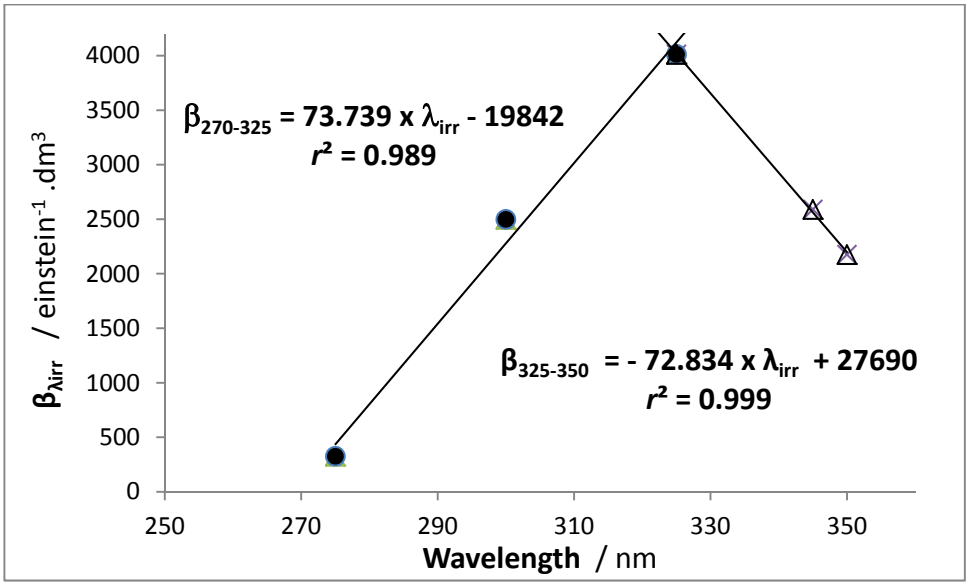


Fig.7: Correlations of $\beta_{\lambda_{irr}}$ with irradiation wavelength. $\beta_{\lambda_{irr}}$ is expressed in einstein^{-1} dm^{-3}

$$P_{\lambda_{irr}} = \frac{k_{DBZ}^{\lambda_{irr}}}{\beta_{\lambda_{irr}}} \quad (5)$$

Therefore, *DBZ* can act as an actinometer for that range of wavelengths by simply applying the following actinometric procedure: (i)– subject a freshly prepared (*ca*. 5 x 10^{-6} M) solution of the drug *DBZ* in ethanol to the monochromatic light (between 275 and 350 nm) of the unknown source, (ii)– collect its full kinetic trace by monitoring the variation of the absorbance at 325 nm (λ_{irr}/325), (iii)– fit it with Eq.1 and determine its $k_{DBZ}^{\lambda_{irr}}$, (iv)– determine the value of the $\beta_{\lambda_{irr}}$ factor from the linear relationships (Fig. 7) and finally (iv)– work out the value of the unknown light source radiant power ($P_{\lambda_{irr}}$) from Eq.5.

It is interesting to notice that in general, the variation of $\beta_{\lambda_{irr}}$ with λ_{irr} will depend on the various parameters making up the general expression of the $\beta_{\lambda_{irr}}$ factors (Eq.2). However, the obtained $\beta_{\lambda_{irr}}$ values for a given actinometer can be used for the determination of $P_{\lambda_{irr}}$ irrespective of how $\beta_{\lambda_{irr}}$ particularly correlate with λ_{irr}. A triangular pattern has been found here for *DBZ*, but linear relationships were obtained for Nifedipine,[16] Montelukast[18] and a diarylethene derivative, *DAE.*[15]

Furthermore, it is reasonable to envisage that a combination–actinometer, involving several individual drugs and chemical compounds can be proposed with the aim to cover a larger dynamic range (e.g. *DBZ* for 275–350 nm and *DAE* for 400–580 nm ranges).

433 The present actinometric overall approach has the double advantage of using kinetic data that
434 are easily collected and avoiding the usual problems encountered for other actinometers. In
435 addition of being simple–to–implement, it can be run without depending on the knowledge of
436 the reaction attributes (as the quantum yields) as long as the system employed obeys a
437 unimolecular AB(1Φ) kinetics. This approach might prove to be useful for the development of
438 other drug–actinometers.

439

440 If our actinometric method can certainly be applied to situations where high radiant power is
441 used for drugs absorbing below 350 nm, it can however only be used for monochromatic
442 irradiation beams. This might be seen as a limitation for applications using polychromatic light.
443 It is though important to stress the fact that integrated rate–laws of such a polychromatic
444 absorption have not yet been made available. The actual actinometers used for that purpose
445 present important limitations that shed doubt on their reliability. It is in our opinion a serious
446 issue that ought to be acknowledged and addressed in order to contribute to the improvement
447 of photostability and/or photosafety testing.

448

449 It is also important to notice that the $\beta_{\lambda_{irr}}$ factors (Eq.2), labelled lately the *pseudo-rate-*
450 *constants*,[23] offer an undeniable advantage of rendering possible the direct comparison of
451 reactivity for different photodegradation reactions performed in the same solvent but
452 irrespective of the molecule and experimental conditions used. Indeed, the pseudo-rate-
453 constants of the group of active pharmaceutical ingredients reported in Table 5, indicate that
454 Montelukast has the highest $\beta_{\lambda_{irr}}$ value and hence, in the optimal conditions, is the most
455 reactive drug of the series (if the absorption spectra of the molecules, i.e. the irradiation

wavelengths involved, are overlooked). From this point of view, Montelukast photodegrades faster than Nisoldipine, a cardiovascular drug similar to Nifedipine, that is considered one of the fastest known photoreactive drugs. This is a significant result as for these two molecules the maximal values of $\beta_{\lambda_{irr}}$ are recorded at relatively close irradiation wavelengths of 360 and 370 nm for Montelukast and Nisoldipine, repectively. In this context, DBZ has moderate photodegradation reactivity amongst the series studied (recording *ca*. half the $\beta_{\lambda_{irr}}$ factor of Nisoldipine).

As a matter of fact, since the pseudo-rate-constants of drugs depend on many factors (Eq.2), it is not possible to find a simple correlation of their values with those of the corresponding quantum yields (Table 5). For instance, the maximum experimental value of the quantum yield of Fluvoxamine is 2.4-folds higher than that of Montelukast but the maximum value of the $\beta_{\lambda_{irr}}$ factor of the latter is 13.5 times that of the former. This is therefore a compelling example demonstrating that a comparison of quantum yields would not be informative on photoractivity of transforming molecules in solution (such a comparison might eventually be considered for AB(1Φ) systems where only the initial species absorbs the irradiation light if the absorption coefficients of the compared systems do not show important differences at λ_{irr}, such as for DBZ in the range 300-400 nm). These findings might help with the difficulty of comparing photodegradation studies, as has been reported in the pharmaceutical literature,[12,13] and emphasises further a strong advice against using the thermal 0^{th}-, 1^{st}- and/or 2^{nd} order kinetic treatments that are usually employed in dealing with data of photodegradation reactions of drugs.

Table 5: Comparative ranges of photodegradation wavelengths (λ_{irr}), quantum yields (Φ) and pseudo-rate-constants ($\beta_{\lambda_{irr}}$) for a series of pharmaceutical drugs.

Drugs[a]	**Wavelength**[b] /nm	**Quantum yield**	**Pseudo-rate-constant**[c]	Ref.
E-Montelukast	258 – 360	0.065 – 0.180	2251 – 28069	18
Nisoldipine	320 – 390	0.304 – 0.351	7558 – 8851	24
Dacarbazine	300 – 350	0.016 – 0.049	2121 – 4013	-
E-Sunitinib	340 – 480	0.020 – 0.026	1560 – 5847	22
E-Fluvoxamine	260 – 290	0.013 – 0.435	818 – 2077	23

[a] : measurements performed in ethanol for drugs' concentration varying between 1.8 x 10^{-6} and 8.9 x 10^{-6} M.

[b] : wavelength domain of the photodegradation causative range (which includes the long wavelength peak of maximum absorption).

[c] : $\beta_{\lambda_{irr}}$ factors expressed in einstein^{-1} dm^3.

4. Conclusion

The photodegradation of *DBZ* in ethanol proceeds through a unimolecular reaction leading to the formation of a single photoproduct. The reaction progress with time has been proven to obey Φ–order kinetics. The *DBZ* photodegradation quantum yields, determined in the region 221–350 nm, are wavelength dependent and their variation has been modelled by a sigmoid function. Their absolute values are relatively low ($\Phi_{\mathrm{DBZ}\rightarrow\mathrm{PP}}^{\lambda_{\mathrm{irr}}}$ < 5 x 10^{-2}) but a rather large, 50-fold, increase of the quantum yield has been found between the UVB and UVA excitations. The

UVA region of the *DBZ* spectrum (with $\Phi_{\mathrm{DBZ}\rightarrow\mathrm{PP}}^{\lambda_{\mathrm{irr}}} > 0.01$) represents the main photodegradation causative range.

The Φ–order kinetic equations have also been used to develop a *DBZ*–actinometric procedure that makes this anti–cancer drug a functional actinometer in the 275–350 nm range. The procedure proposed here should work well for monochromatic irradiation. However, both the sigmoid variation of $\Phi_{\mathrm{DBZ}\rightarrow\mathrm{PP}}^{\lambda_{\mathrm{irr}}}$ and the triangular shape of the $\beta_{\lambda_{irr}}$ factor indicate that *DBZ*–actinometry is most likely not suitable for polychromatic light beams. In fact, this might be the case for many actinometers since their individual quantum yield values should *a priori* be considered wavelength dependent as should their $\beta_{\lambda_{irr}}$ factors. In this respect, the radiant power values that are generally reported for polychromatic light sources must be considered with caution as they might correspond to averages rather than absolute values.

The Φ–order kinetic equations prove to be much useful tools for the characterization and quantification of photodegradation of AB(1Φ) drug systems which in addition offer flexibility to develop new and reliable drug–actinometers.

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