Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 5, pp. 441–453, 1987
Printed in Great Britain

0731-7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Review

Principles and practice of drug photodegradation studies

DOUGLAS E. MOORE

Department of Pharmacy, The University of Sydney, Sydney 2006, Australia

Abstract: A review is presented of the underlying principles and experimental techniques used for the study of the photochemical degradation of pharmaceuticals. Examples are given to illustrate their application.

Keywords: *Photodegradation; fluorescence; phosphorescence; actinometry; drugs.*

Introduction

The study of the degradation of drug substances under the action of UV or visible light is relevant to the drug development process since the photodegradation products may be toxic and might appear in the formulation, as a result of inappropriate exposure to light of the raw materials or the final preparation. Such photoproducts may also be formed by the action of sunlight on the epidermal layers of the skin of patients receiving the drug and may thereby cause adverse photosensitivity effects [1]. The untoward effects of light are generally due to the UVB (290–310 nm) and UVA (310–400 nm) ranges of sunlight acting directly on the compound; therefore, most studies are concerned with irradiation by light of these wavelengths. This should not exclude consideration of reactions sensitized by dyes and other compounds that absorb visible light.

Photochemical and Photophysical Processes

The possible photophysical and photochemical processes are summarised in Scheme 1 below:

$$S_0 + h\nu \rightarrow {}^1S, \quad (1)$$

$${}^1S \rightarrow S_0 + \text{heat}, \quad (2)$$

$${}^1S \rightarrow S_0 + h\nu', \quad (3)$$

$${}^1S \rightarrow {}^3S, \quad (4)$$

$${}^3S \rightarrow S_0 + h\nu''. \quad (5)$$

A molecule absorbs light only when an absorption band exists which overlaps to some extent the incident light energy, and a valence electron in the relevant chromophore is raised to the first excited state (^1S), which is a singlet state, spin-matched to the ground state (S_0) [equation (1)]. Return to the ground state may occur by internal conversion, a radiationless transition between isoenergetic states of the same spin multiplicity [equation (2)]. Alternatively, return to the ground state may occur with fluorescence emission of light of slightly lower energy (UVA or visible) [equation (3)].

Intersystem crossing to the triplet state [^3S, equation (4)] is a radiationless transition between isoenergetic states of different multiplicity. This is a low probability transition; the reverse movement is also of low probability so that the triplet state is relatively long-lived, usually 10–100 μs. Return to the ground state by emission of phosphorescence is expressed by equation (5).

Fluorescence and phosphorescence

Two mechanisms of energy dissipation, fluorescence and phosphorescence, can be used to indicate the population of the excited states. A comparison of the fluorescence and/or phosphorescence yielded for various drugs indicates those for which the excited states are long-lived and which have a relatively high population, thus giving greater chances for other events such as photochemical reactions to occur. Phosphorescence is the better guide since photochemical reactions take place predominantly from the relatively long-lived triplet state, which is said to have some bi-radical character by virtue of the unpaired spins.

The efficiency of these luminescence processes can be expressed in terms of the quantum yield, Φ, where

$$\Phi = \frac{\text{Number of photons emitted}}{\text{Number of photons absorbed}}.$$

The quantum yield, in principle, has a maximum value of 1.0, and the difference from unity provides information on the extent of occurrence of radiationless transitions. A number of factors can affect Φ, such as inner filter and self-absorption effects, sample–solvent impurities, chemical instability due to photodecomposition and other reactions, and polarisation–anisotropy effects [2].

Measurement of the fluorescence quantum yield requires either: (i) a high sensitivity quantum-counting photomultiplier, a sample which behaves as an ideal light-scattering source, and an absolute knowledge of the geometry in the fluorescence apparatus, for determination of the total light emission of the solution in relation to the amount of incident light absorbed; or, more readily, (ii) comparison with a compound of known fluorescence quantum yield, such as quinine in 0.1 M H_2SO_4 ($\Phi_F = 0.546$) [3]. The total fluorescence (area under the spectral curve of intensity against wavelength) is obtained for both compounds in a spectrofluorimeter operated in the corrected spectral mode; Φ_F is calculated from:

$$\Phi_F = \frac{\text{AUC}_{\text{drug}}}{\text{AUC}_{\text{quinine}}} \times \frac{A_{\text{quinine}}}{A_{\text{drug}}} \times 0.546,$$

where A_{drug} and A_{quinine} are the absorbances of the drug and the quinine solutions at their respective excitation wavelengths. For maximum precision of the results, these two

A values should be the same, and less than 0.02, so that the amount of light absorbed is negligible, and uncertainties (such as reflections at windows) are constant [4].

Phosphorescence is normally measured at low temperatures (e.g. 77 K) where the compound is held in a rigid matrix and its energy loss by collisional deactivation with solvent molecules is minimised. A phosphorescence accessory that can be fitted to the more advanced spectrofluorimeters consists of a low-temperature Dewar vessel and a chopper which is tuned to interrupt alternately the excitation and emission light paths such that the fluorescence ($t_{1/2} < 1\ \mu s$) is not collected. The substance under investigation must be dissolved in a solvent which will form a transparent glass at 77 K. Ethanol is usually satisfactory. A substance used as a standard for phosphorescence is indole in EPA (ether–isopentane–ethanol, 3:2:1, v/v/v), although quantum yield measurements for phosphorescence by means of total emission are subject to many variations due to the particular experimental arrangement [5]. For a rigid chromophore, e.g. a polycyclic aromatic hydrocarbon, in a relatively non-polar solvent, there may not be a great loss of the excitation energy to the solvent and

$$\Phi_F + \Phi_P = 1$$

may be used as a good approximation, although this does not apply to a significant number of drug molecules. The other main method for determining Φ_P is by competitive reaction kinetics with known triplet state quenching reagents [6].

Singlet oxygen

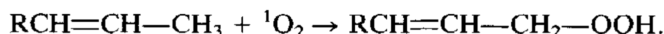
One very important reaction of the triplet state is electronic energy transfer to ground state molecular oxygen, which is spin-matched, i.e. a triplet state, thereby forming excited singlet molecular oxygen $^1O_2$:

$$^3S + {}^3O_2 \rightarrow S_0 + {}^1O_2. \qquad (6)$$

The highly reactive excited singlet oxygen is now spin-matched with ground state molecules susceptible to oxidation [equation (7)]:

$$^1O_2 + AH \rightarrow AOOH. \qquad (7)$$

Susceptible molecules contain olefinic groups with allylic H atoms, or possess the capability to produce an endoperoxide by trans-annular O_2 addition, e.g. anthracene, imidazole and furan derivatives [7, 8]:



$$RCH{=}CH{-}CH_3 + {}^1O_2 \rightarrow RCH{=}CH{-}CH_2{-}OOH.$$

Typical photosensitizers which generate $^1O_2$ include the dyes, Methylene Blue, Rose Bengal and Rhodamine B, and the process has been referred to as *photodynamic action* [9]. Many drug molecules are capable of generating $^1O_2$ including quinine, frusemide, hydrochlorothiazide, chloroquine, naproxen, nalidixic acid and tetracyclines [10–13]. The formation of $^1O_2$ by a sensitizer can be diagnosed by the appearance of a specific product pattern from certain acceptors, such as cholesterol, or by the measurement of rates of degradation following the addition of certain specific substrates, such as histidine

or 2,5-dimethylfuran, and quenchers, such as sodium azide or 2,2,2-diazabicyclo-octane [8, 9].

It is possible for a drug molecule to act as a sensitizer for its own oxidation by the singlet oxygen mechanism. Examples are 6-mercaptopurine [14] and chlorpromazine [15]. In many cases, no single mechanism applies, and free radical reactions may also complicate the scheme.

Free radical mechanisms

Photooxidation occurs by a free radical mechanism when the photoexcited molecule undergoes dissociation [equation (8)] and the free radical so formed is scavenged by oxygen [equation (9)]:

$$^3DH \rightarrow D\cdot + H\cdot, \tag{8}$$

$$D\cdot + O_2 \rightarrow DOO\cdot. \tag{9}$$

The peroxy free radical may then undergo a variety of reactions yielding a complex mixture of oxidized products, the very complexity of which renders the task of product identification difficult. In the absence of oxygen, the free radicals may recombine or generate a reaction with the solvent or other species present in the solution.

Superoxide. A further process that may occur from the excited singlet state is photoionization, in which photoelectrons are ejected to combine with oxygen to form the superoxide radical anion, O_2^-. Superoxide is also produced by some enzymic oxidation systems in nature, being removed by the enzyme, superoxide dismutase. The presence of superoxide can be diagnosed by reaction with the dye Nitro Blue Tetrazolium [16], or by electron spin resonance measurements [17], with and without superoxide dismutase. Since photoionization is found to occur particularly from molecules containing one or more hetero-atoms, there are a significant number of drugs which produce it although in general higher energy radiation is required (<300 nm). Photoionization and superoxide formation are supported to a greater extent by an aqueous medium. Examples of drugs which can generate superoxide are 6-mercaptopurine [18], chlorpromazine [15] and psoralens [19].

Therefore, studies of drug photodegradation should be carried out both in the absence and presence of oxygen. When oxygen is excluded, the reactions tend to give a less complex array of products which will assist in elucidating the pathways of photo-oxidation. It is often advantageous to compare the results with those of thermal degradation as well as with the metabolic pathways since some common mechanistic elements may occur. For instance, both metabolic and photochemical oxidation of protriptyline leads to a reactive epoxide intermediate, with oxygen introduction at the same position in the molecule [20].

Apparatus for Photodegradation Studies

The studies may be performed on an analytical or preparative scale. The important considerations are: control of the wavelength range of irradiation by means of filters; and minimization of secondary reactions by which the primary products are lost so that the mechanism is obscured. Thus, the irradiated solution should be very well stirred

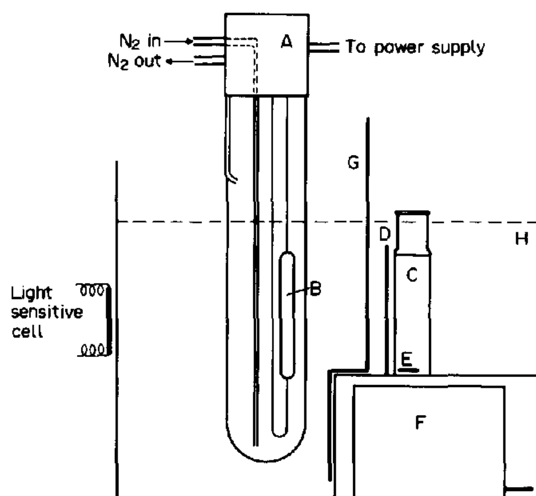


Figure 1
Irradiation apparatus for analytical scale photodegradation studies. Explanation of symbols: A, medium pressure mercury lamp; B, mercury arc; C, reaction vessel (volume about 30 ml); D, glass filter; E, magnetic stirring bar; F, magnetic stirrer motor; G, removable shutter; H, thermostatted water-bath.

throughout, and its composition monitored in order to determine the time of irradiation giving optimal yield of the primary product(s).

The analytical apparatus (Fig. 1) enables the reaction vessel to be moved to varying distances from the light source to control the speed of the reaction; in addition, it permits a shutter to be placed between the lamp and the vessel so that the mixture can be analysed without the lamp being switched off and restarted. This is useful since a warm-up time of about 5 min is required before a constant intensity is achieved. The small reaction vessel can be connected to a filling flask so that the solution can be rigorously degassed before irradiation, or it can be filled completely with an air-saturated aqueous solution of the drug and the consumption of oxygen determined by means of an oxygen electrode [21].

Use of the preparative scale apparatus (Fig. 2) results in a higher yield of photoproducts, although vigorous stirring is essential, because the major absorption of light takes place in the layer of solution next to the light source.

UV light sources

The choice of light source depends on the absorption spectrum of the drug under study. However, for the relevant region of 300–400 nm, the most widely used sources of ultraviolet and visible light for photochemical experiments with steady illumination are the xenon and mercury arc lamps.

The xenon arc lamp produces a continuous output most closely resembling sunlight when used with a Pyrex glass filter which transmits wavelengths above 300 nm. However, for useful intensity levels it is usually necessary to employ at least the 150 W version which generates a large amount of light in the visible and IR regions; such light is relatively ineffective for photochemical reactions. The major application of the xenon lamp is in association with a monochromator for irradiation with specific wavelengths or for obtaining an action spectrum of the degradation.

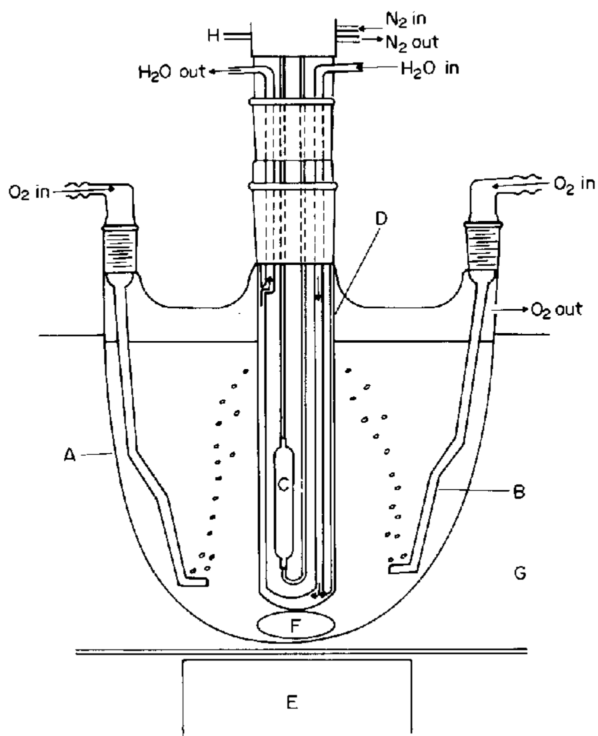


Figure 2
Preparative scale irradiation apparatus. A, 1 l reaction vessel; B, gas bubbler; C, mercury arc; D, waterjacket; E, magnetic stirrer; F, magnetic stirring bar; G, thermostatted water-bath; H, to power supply.

The medium pressure (MP) mercury lamp has been found to be most useful when the irradiation conditions are intended to resemble the UVA component of sunlight, even though it has a discontinuous output. The major lines transmitted through a Pyrex glass filter are at 303, 313, 334 and 365 nm with relative energies of 24, 50, 9 and 100, respectively [4]. Output at 405, 436 and 546 nm is also useful for studies that use dyes as photosensitizers. A 100 W mercury MP arc will overheat unless some arrangement is made to cool the arc. Thus, the apparatus is immersed in a thermostatted water-bath as shown in Figs 1 and 2.

The low pressure mercury arc lamp with 90% of its total output at 254 nm does not have a significant application in photodegradation studies since that wavelength region can be accessed with the MP arc and a quartz sleeve. The high pressure mercury arc lamp has essentially continuous output but mainly in the visible region.

Selection of a particular wavelength range for irradiation can be achieved by means of cut-off filters (such as those available from Corning Glass) or a high throughput monochromator (e.g. Schoeffels). For most purposes, the use of 2-mm Pyrex glass for the construction of the reaction vessel in the analytical scale apparatus, or a 2-mm Pyrex glass sleeve around the MP mercury arc for the preparative scale apparatus, will cut off all wavelengths below 300 nm.

Light intensity measurement

Because of the inherent variability of experimental arrangements, photochemical studies are often based on relative light intensity. That is, the amount of decomposition occurring following a given period of irradiation can be reproduced with a given set of experimental parameters, but may not be the same with a different apparatus. Some measure of comparison can be achieved by measuring the light intensity at a point by means of a radiometer, which consists of a photocell and a series of filters to transmit various wavelength regions. The reading is given as light energy incident at the point of measurement in J cm^{-2} s^{-1} or W m^{-2}.

For absolute determination of the extent of a photoreaction, which may be performed using different sets of apparatus, a calculation of the number of molecules reacted per photon absorbed must be made; this process is known as *actinometry*, and is discussed in detail below. Because different compounds have different absorption characteristics for the same light source and experimental arrangement, a comparison of the extent of photoreaction within a series of drugs must be made on the basis of quantum-corrected values, following actinometry. An alternative approach is to normalize by the "Relative Absorption Factor", which is obtained by multiplying the absorbance of the solution by the relative intensity of the incident radiation, and integrating that function over the range of wavelengths involved in the experiment [22].

Initial Investigations

(i) *Spectral changes*

The first study to determine that a substance undergoes photochemical degradation is most simply carried out by observing whether any change occurs in the UV absorption spectrum upon irradiation. This experiment is most readily performed on the analytical scale using a cuvette which can be directly inserted into the sample compartment of a spectrophotometer. For example, a cylindrical quartz cuvette of 20-mm pathlength can be used with a glass filter to absorb the incident light below 300 nm. The irradiation solution should have an initial absorbance of about 1 at the wavelength corresponding to the major output of the lamp, so that the relative absorption of light is 99% in the 20-mm cuvette.

Figure 3 shows the change in absorbance observed on irradiation of azathioprine in aqueous solution. The presence of isosbestic points (at 246 and 324 nm) is a reasonable indication of a single transformation in the chromophore, so that a relatively simple reaction might be postulated. As time progresses, if the spectral traces begin to distort the isosbestic points, that provides evidence of secondary photodecomposition. Isosbestic traces are seen for photolysis of the various barbituric acid derivatives, where the principal reaction is ring opening similar to hydrolysis [23].

A change in absorbance will only result if the chemical reaction occurs at a site involving the chromophore or an associated auxochrome. Thus, photolysis of frusemide involves loss of Cl from the parent chromophore, yielding a minor but clearly discernible change in the major absorption peak at 271 nm [24, 25]. On the other hand, photolysis of benoxaprofen results in decarboxylation of the β-COOH group which is sufficiently removed from the chromophore so that no change is evident in the spectrum; however, in an aqueous solution of the drug, decarboxy-benoxaprofen is detected readily as a precipitate [26, 27]. TLC analysis of the solution which has been subjected to irradiation is necessary when no obvious changes occur.

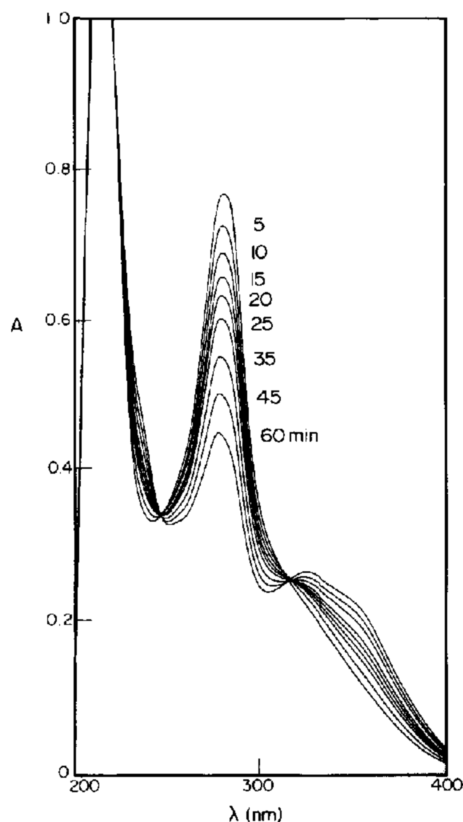


Figure 3
Absorption spectrum of azathioprine (2.1×10^{-5}M) in acetate buffer (pH 4). The spectra were recorded after irradiation with a medium pressure mercury arc through a Pyrex glass filter (incident intensity 25 W m^{-2}). The time of irradiation is indicated on each spectrum.

(ii) *Photodehalogenation*

As noted above for frusemide, photodehalogenation of aromatic compounds is a relatively common process, and in such cases can be detected and quantified by potentiometric titration with silver nitrate using a silver electrode.

Photodehalogenation was observed also for chlorpromazine, prochlorperazine, hydrochlorothiazide and chloroquine, but not for chlortetracycline, chlordiazepoxide or hexachlorophene under similar conditions of irradiation [11, 28].

Detailed Analysis of the Photolysis Mixture

HPLC is the most informative instrumental technique for describing the progress of the photolysis reaction, as it enables the analyst to determine not only the number of products being formed but also whether any of them are being degraded subsequently by either thermal or photochemical means. In the development of an HPLC method, a good starting point is a stability-indicating assay, if one is known for the drug under study. Often, the majority of products from photolysis in aqueous solutions are more polar than the parent substance, so that mostly the products will be eluted first in a reversed-phase system. Thus, it is advisable that a mobile phase is developed to expand the retention of the compounds that are eluted near the solvent front. The use of a high-speed spectrophotometric detector is a definite advantage in the determination of: (i) the

spectroscopic homogeneity of each eluted peak: and (ii) the possible relationship of the product to the parent substance, by the extent of the similarities in the UV absorption spectra. Figure 4 illustrates this use in the photolysis of naproxen where the HPLC trace of an irradiated sample shows two products (**1** and **2**) with the naproxen chromophore intact, while product **3** has a chromophore of distinctly different character, with stronger absorption at the long wavelength band.

Gas chromatography is of limited applicability because the photoproducts are frequently not sufficiently volatile or stable at the temperatures of operation. On-column derivatization with diazomethane or trimethylanilinium hydroxide was used for frusemide and hydrochlorothiazide, so that the GC–MS combination provided molecular weight data for the photoproducts [29]. The existence of several acidic protons in the molecules does require strong conditions to ensure complete derivatization.

The advent of a reliable and moderately priced interface between HPLC and a mass spectrometer will provide a much easier route to the determination of the molecular weight of the photoproduct. Otherwise, preparative scale chromatography is required to generate measurable fractions of the products for identification purposes.

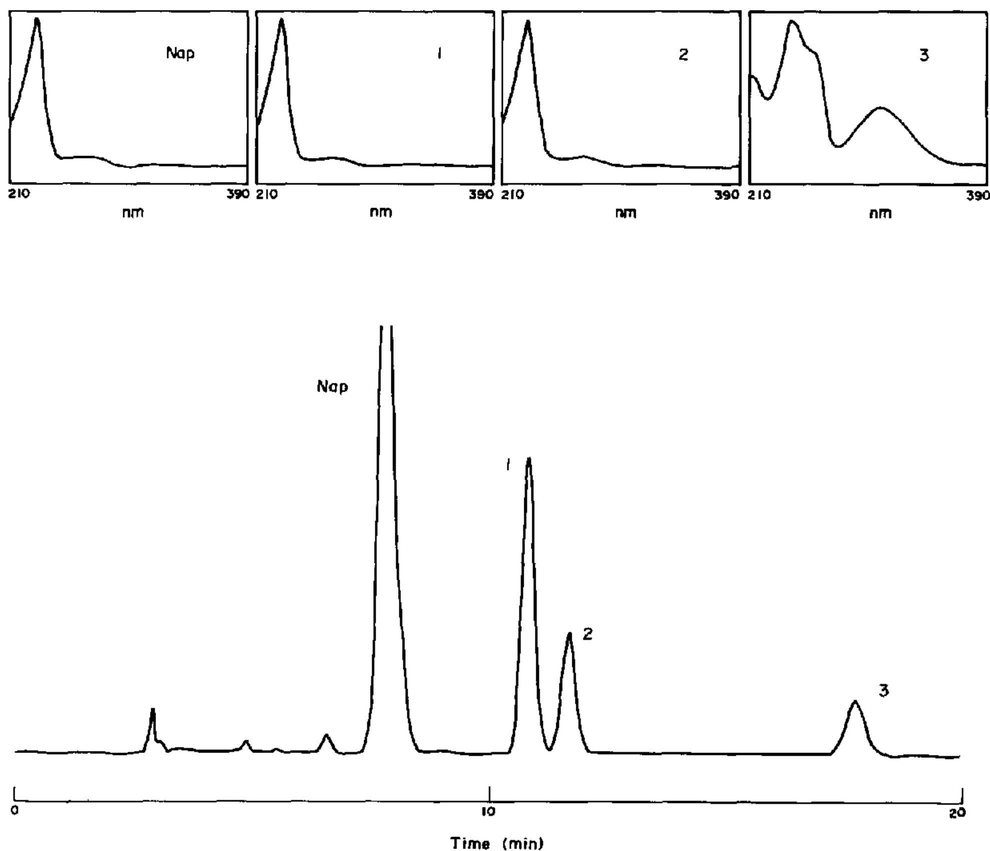


Figure 4
HPLC signal recorded at 230 nm for a solution of naproxen (2×10^{-4}M) in phosphate buffer (pH 7) after irradiation for 30 min. The upper panel shows the absorption spectrum of each of the major peaks taken as eluted.

The identity of the photoproducts must be deduced on the basis of MS and NMR data, together with a logical assessment of possible pathways. Where this is not enough to provide an unequivocal identification, it may be necessary to collect sufficient of the products to enable the structure to be assigned by X-ray crystallography.

The common primary photochemical reactions are [5]: addition to unsaturated systems; substitution; *cis–trans* isomerization; structural rearrangements; fragmentation; photooxidation and photoreduction.

Addition and substitution reactions will depend on the presence of particular substrates and solvents, and therefore can be controlled by appropriate selection of the medium. Similarly, photooxidation occurs in the presence of oxygen or oxidizing agents, and photoreduction requires the absence of oxygen.

Some product patterns have emerged as a result of a number of studies on structurally related compounds. For example, from studies on photodehalogenation of aromatic compounds, two types of product can be predicted: reduction (Cl replaced by H); and substitution (Cl replaced by RO from the solvent ROH). Thus, chlorpromazine irradiated in 2-propanol is converted to promazine and iso-propoxypromazine [30]. The relative yields of the two types of product are related to the formation of a pair of radical ions from the triplet state. The precursor of the reduction product (Ar–H) is suggested to be a radical anion ($Ar{-}Cl^{-\cdot}$) while a radical cation ($Ar{-}Cl^{+\cdot}$) is postulated as the precursor of the substitution product (Ar–OR) [31].

The benzodiazepine group shows a variability of photochemical behaviour dictated by the particular substituents. On extended irradiation with UV light at 254 nm, diazepam yields a mixture of products — 8% benzophenones, 15% 4-phenylquinazolinones and 70% 4-phenylquinazolines [32]. From chlordiazepoxide, an oxaziridine is produced which is more phototoxic than the parent compound [33]. The nitro-derivatives, nitrazepam and flunitrazepam, yield the amino reduction product on irradiation [34, 35]. On the other hand, the nitroimidazole antibacterial substance, metronidazole, undergoes only partial reduction before rearrangement to an oxadiazole [36].

The barbiturates are susceptible to hydrolysis (ring opening) and this is greatly facilitated by UV irradiation [23, 37–39]. Indapamide undergoes hydrolytic cleavage at three places following UV irradiation [40]. Photohydrolysis is also evident for frusemide [25] and hydrochlorothiazide [29], as well as the dechlorination reaction in both cases.

Adrenaline, isoprenaline and noradrenaline are oxidized to the corresponding adrenochromes upon UV irradiation [41].

Quantum Yield Determination — Actinometry

Actinometry is performed with either a physical device such as a photocell or a chemical system for determining the amount of light absorbed by a sample. The most commonly employed technique uses the ferrioxalate chemical actinometer [4, 42].

Potassium ferrioxalate is readily prepared by reaction of ferric chloride with potassium oxalate. When acidic solutions (6 mM) of $K_3Fe(C_2O_4)_3$ are irradiated by light in the range 250–570 nm, 99% of the light is absorbed; Fe(III) is reduced while oxalate is oxidized. The quantum yield of Fe(II) formation (Φ_{Fe}) varies with the wavelength of irradiation, but for the 300–400 nm region the value can be taken as 1.2; the stoichiometry of the reaction indicates that two atoms of Fe(III) are reduced per photon absorbed, i.e. a theoretical maximum quantum yield of 2. The product Fe(II) and its oxalate complex do not absorb the incident radiation measurably, so there is no back

reaction. After the required time of irradiation, the Fe(II) is assayed by colorimetry with 1,10-phenanthroline.

The purpose of determining the amount of light absorbed by a substance when undergoing a photochemical reaction, is to be able to calculate the photochemical efficiency or quantum yield:

$$\Phi = \frac{\text{Number of molecules reacted per unit volume per unit time}}{\text{Number of photons absorbed per unit volume per unit time}}. \quad (10)$$

Experimentally, a two-cell arrangement is required, as shown in Fig. 5. Cell B is filled with the ferrioxalate solution, and two sets of irradiations are performed. In Set 1, cell A contains the solution of drug being tested at a concentration such that its absorbance is between 0.5 and 0.8, while in Set 2 cell A is filled with the solvent. Typical results are shown in Fig. 6, with the Fe(II) yield, expressed by the absorbance of the phenanthroline complex at 510 nm, plotted as a function of time of irradiation. The difference between the slopes for Sets 1 and 2 represents the amount of light absorbed by the drug sample. Thus, the number of photons absorbed per unit time is calculated by:

$$N_{\text{drug}} = [\text{slope(Set 2)} - \text{slope(Set 1)}]\ N_A\ \Phi_{\text{Fe}}\ R/\varepsilon_{510},$$

where N_A is Avogadro's Number, ε_{510} is the molar absorptivity of the Fe(II)–phenanthroline complex at 510 nm (1110 m^2 mol^{-1}), and R is a factor that takes into account the dilutions involved in the colorimetric assay.

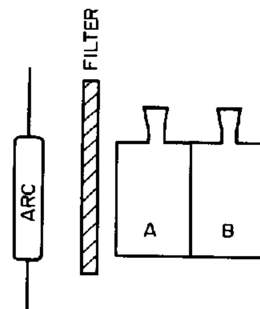


Figure 5
Schematic representation of arrangement of cells A and B used for actinometry studies.

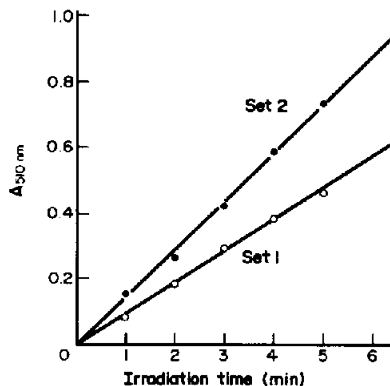


Figure 6
Typical results obtained from an actinometry experiment. For explanation see text.

In parallel, the extent of reaction of the drug sample in cell A is determined by the appropriate method of analysis, so that the number of molecules reacted per unit time can be calculated for insertion in equation (10). For the majority of drug photodegradation studies, the time of irradiation needed to give adequately measurable changes in the drug concentration is much longer than that giving significant changes in the actinometer solution. However, the change in parent drug concentration should not be greater than 15% in order that the degradation remains linear with the time of irradiation.

The quantum yield of a photochemical reaction can be expressed in terms of the loss of starting material or the appearance of a particular product, and provides an absolute measure of the extent of the process independent of the apparatus used.

References

[1] I. E. Kochevar, in *The Biological Effects of UVA Radiation* (F. Urbach and R. W. Gange, Eds), pp. 87–97. Praegar, New York (1986).
[2] J. N. Miller, in *Standards in Fluorescence Spectrometry*, pp. 68–77. Chapman and Hall, London (1981).
[3] J. N. Demas and G. A. Crosby, *J. Phys. Chem.* **75**, 991 (1971).
[4] J. G. Calvert and J. N. Pitts, in *Photochemistry*, pp. 696, 783–786, 798–804. John Wiley, New York (1966).
[5] N. J. Turro, in *Modern Molecular Photochemistry*, pp. 121–130. Benjamin-Cummings, Menlo Park, Calif. (1978).
[6] R. Hurley and A. C. Testa, *J. Am. Chem. Soc.* **90**, 1949–1952 (1968).
[7] C. S. Foote, *Acc. Chem. Res.* **1**, 104–110 (1968).
[8] A. A. Gorman and M. A. J. Rodgers, *Chem. Soc. Rev.* **10**, 205–231 (1981).
[9] J. D. Spikes, in *The Science of Photobiology* (K. C. Smith, Ed.), pp. 87–112. Plenum Press, New York (1977).
[10] J. A. Wiebe and D. E. Moore, *J. Pharm. Sci.* **66**, 186–189 (1977).
[11] D. E. Moore, *J. Pharm. Sci.* **66**, 1282–1284 (1977).
[12] D. E. Moore and V. J. Hemmens, *Photochem. Photobiol.* **36**, 71–77 (1982).
[13] D. E. Moore, V. J. Hemmens and H. Yip, *Photochem. Photobiol.* **39**, 57–61 (1984).
[14] V. J. Hemmens and D. E. Moore, *J. Chem. Soc. Perkin Trans.* **2**, 209–211 (1984).
[15] A. G. Motten, G. R. Beuttner and C. F. Chignell, *Photochem. Photobiol.* **42**, 9–15 (1985).
[16] C. Beauchamp and I. Fridovich, *Anal. Biochem.* **44**, 276–287 (1971).
[17] B. Kalyanaraman, C. Mottley and R. P. Mason, *J. Biol. Chem.* **258**, 3855–3858 (1983).
[18] V. J. Hemmens and D. E. Moore, *Photochem. Photobiol.* **47**, 247–255 (1986).
[19] P. C. Joshi and M. A. Pathak, *Biochem. Biophys. Res. Commun.* **112**, 638–646 (1983).
[20] G. E. Jones and D. Sharples, *J. Pharm. Pharmacol.* **36**, 46–48 (1984).
[21] D. E. Moore, *J. Pharm. Sci.* **65**, 1447–1451 (1976).
[22] I. E. Kochevar, R. B. Armstrong, J. Einbinder, R. R. Walther and L. C. Harber, *Photochem. Photobiol.* **36**, 65–69 (1979).
[23] J. Mokrosz, M. Klimczak, H. Barton and J. Bojarski, *Pharmazie* **35**, 205–208 (1982).
[24] P. C. Rowbotham, J. B. Stanford and J. K. Sugden, *Pharm. Acta Helv.* **51**, 304–307 (1976).
[25] D. E. Moore and V. Sithipitaks, *J. Pharm. Pharmacol.* **35**, 489–493 (1983).
[26] I. E. Kochevar, K. W. Hoover and M. Gawienowski, *J. Invest. Dermatol.* **82**, 214–218 (1984).
[27] K. Reszka and C. F. Chignell, *Photochem. Photobiol.* **38**, 281–291 (1984).
[28] D. E. Moore and S. R. Tamat, *J. Pharm. Pharmacol.* **32**, 172–177 (1980).
[29] S. R. Tamat and D. E. Moore, *J. Pharm. Sci.* **72**, 180–183 (1983).
[30] F. W. Grant, in *The Phenothiazines and Structurally Related Drugs* (I. S. Forrest, C. J. Carr and E. Usdin, Eds), p. 539. Raven Press, New York (1974).
[31] N. J. Bunce and L. Ravanal, *J. Am. Chem. Soc.* **99**, 4150–4152 (1977).
[32] P. J. G. Cornelissen, G. M. J. Beijersbergen van Henegouwen and K. W. Gerritsma, *Int. J. Pharm.* **1**, 173–181 (1978).
[33] P. J. G. Cornelissen, G. M. J. Beijersbergen van Henegouwen and K. W. Gerritsma, *Int. J. Pharm.* **3**, 205–220 (1979).
[34] P. J. G. Cornelissen and G. M. J. Beijersbergen van Henegouwen, *Photochem. Photobiol.* **30**, 337–341 (1979).
[35] R. S. Givens, J. Gingrich and S. Mecklenberg, *Int. J. Pharm.* **29**, 67–72 (1986).
[36] B. J. Wilkins, G. J. Gainsford and D. E. Moore, *J. Chem. Soc. Perkin Trans* **1**, in press (1987).

[37] H. Barton, J. Mokrosz, J. Bojarski and M. Klimczak, *Pharmazie* **35**, 155–158 (1980).
[38] J. Mokrosz and J. Bojarski, *Pharmazie* **37**, 768–773 (1982).
[39] J. Mokrosz, A. Zurowska and J. Bojarski, *Pharmazie* **37**, 832–835 (1982).
[40] R. Davis, C. H. J. Wells and A. R. Taylor, *J. Pharm. Sci.* **68**, 1063–1064 (1979).
[41] N. J. de Mol, G. M. J. Beijersbergen van Henegouwen and K. W. Gerritsma, *Photochem. Photobiol.* **29**, 479–482 (1979).
[42] C. G. Hatchard and C. A. Parker, *Proc. R. Soc. London* **A235**, 518–530 (1956).

[*Received for review 6 January 1987*]