

Photobleaching of 1,3-diphenylisobenzofuran by novel phthalocyanine dye derivatives

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Abstract

As part of a wider programme to identify novel photosensitizers for photodynamic therapy, the ability of a number of phthalocyanine dyes, including some novel copper phthalocyanine derivatives with a range of water solubilities, to produce potentially cytotoxic species in solution was examined. The experiments were performed in dimethylformamide using 1,3-diphenylisobenzofuran (DPIBF) as the scavenger. The study revealed that all the dyes tested produced DPIBF photobleaching on illumination *in vitro*, but with widely different (greater than 12x) rates. The possible correlation of DPIBF photobleaching rates with a number of the dyes' properties is discussed.

Keywords: Phthalocyanine dyes, hydrophobicity, photodynamic therapy.

1. Introduction

A photoactive preparation used for the photodynamic therapy (PDT) of various types of cancer, haematoporphyrin derivative (HpD), has been the object of intense research interest over the past few years [1]. However, since HpD is a drug of uncertain composition, has limited tumour-localizing and photokilling properties and has associated with its use unwanted side effects, much effort is at present being expended in the search for alternative photosensitizers. One class of dyes that has some potential as a source of HpD replacements is the porphyrin-like phthalocyanine class.

The photoactivity of the phthalocyanines has been tested against various cell lines in culture including V79 Chinese hamster cells [2–7], non-lymphoblastic leukaemia cells [8], human NHIK 3025 cells [9] and the NIH/3T3 fibroblast and UV-2237 fibrosarcoma cells [10, 11]. Also worth mentioning in this context is the recent study of the photoactivity of sulphonated phthalocyanines against erythrocytes [12]. Phthalocyanines have also been tested in animal models including chloroaluminium phthalocyanine tetrasulphonate against the transplantable rat *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]-formamide(FANFT)-induced bladder tumour [13], various phthalocyanines against the EMT-6 mouse mammary tumour [4], chloroaluminium phthalocyanine

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sulphonate against the Colo 26 colorectal mouse carcinoma [11] and zinc phthalocyanine against the MS-2 mouse fibrosarcoma [14]. Finally, zinc phthalocyanine has been shown to be photoactive against *Herpes simplex virus in vitro* [15].

The phthalocyanines have been of interest as possible tumour localizers for some time, although published studies have been rather scarce [16]. Phthalocyanines have been shown to be preferentially taken up in mouse intracranial neoplasms [17, 18], rat mammary carcinomas [4, 9, 19–22], rat fibrosarcoma [23], mouse pancreatic tumours [24], rat S180 tumours [25] and normal rat liver [26]. A study by Selman *et al.* [13] suggests that chloroaluminium phthalocyanine is selectively taken up in rat FANFT-induced bladder tumours after administration by intravenous injection in a saline solution. Similarly, phthalocyanine localization probably occurs in mouse colorectal carcinomas [11]; in the latter study, most of the water-soluble phthalocyanines were administered by intravenous injection of the dye made up in saline solution, whilst the water-insoluble dyes were injected in the solvent dimethylsulphoxide (see for example ref. 9). In a 1984 paper, El Far and Pimstone [27] studied the localization of 28 different porphyrin-type dyes in mouse mammary carcinoma; one of the substances that did not localize very well *in vivo*, as assessed by fluorescence measurements, was phthalocyanine. In a study on a number of different dyes, Moan and coworkers [9, 28] studied dye uptake into mouse mammary carcinomas. Aluminium phthalocyanine tetrasulphonate was found to be taken up in tumour to a greater extent than aluminium phthalocyanine was, whilst the former dye gave a lower tumour-to-skin ratio than the latter. In animal work by Straight and Spikes [29], zinc phthalocyanine sulphonate was shown to be retained as effectively in murine S180 tumours as in “model tumours” made from pieces of polyvinyl alcohol sponge implanted subcutaneously in mice, which become heavily vascularized and infiltrated with connective tissue elements after a few days. In cell culture, however, a study by Chan *et al.* [10] showed that a fibroblast cell line and a fibrosarcoma cell line exhibited essentially identical behaviour with respect to both kinetics and photokilling by a water-soluble chloroaluminium phthalocyanine.

The nature of the central metal atom in a phthalocyanine derivative affects ring configuration and aggregation behaviour. As a consequence this affects, for example, *in vivo* tumour localization of the dye. This has been shown in a study by Rousseau *et al.* [20] in which a technetium phthalocyanine derivative showed a much lower tumour-to-muscle localization ratio than a gallium derivative did. Van Lier *et al.* [21] noted that the tissue distribution of the phthalocyanines was strongly influenced by their charge and water solubility. Alcian Blue (a phthalocyanine dye) binds *in vivo* to damaged (*i.e.* not tumorous) urothelium after intravesical instillation, but not to healthy urothelium [30, 31]. Also, it has been suggested that one of the major sites of photodamage by sulphonated chloroaluminium phthalocyanine is the subendothelial zone in the walls of tumour blood vessels, a region of complex composition containing microfibrils, elastin, glycosaminoglycans, collagen fibres etc. [32, 33].

Apart from the tumour-localizing properties of phthalocyanines discussed above, another interesting property of the phthalocyanine dyes is their ability to generate singlet oxygen *in vitro* on photoexcitation. *In vitro* singlet oxygen generation by a number of phthalocyanines has been reported (see, for example, recent work by Kimel *et al.* [34]) and direct observations of singlet oxygen luminescence at 1270 nm produced by photoexcitation of phthalocyanines have been made [35]. It should also be mentioned, however, that Firey *et al.* [36] were unable to detect singlet oxygen luminescence during the photoexcitation of zinc phthalocyanine in cultured mouse myeloma cells; only phthalocyanine triplet states were observed.

As part of a programme to assess novel dyes as potential photosensitizers for photodynamic therapy [37–39], we have assessed the relative photosensitizing abilities of some phthalocyanine dyes, including some novel copper phthalocyanine derivatives, using as a test the bleaching of the scavenger 1,3-diphenylisobenzofuran (DPIBF) in solution, in the presence of phthalocyanine dye and light. The photo-oxidizing abilities of a number of structurally dissimilar dyes, as assessed with DPIBF, have been shown to have a remarkable correlation with the dyes' ability to photosensitize living cells [9]; so the results obtained using this scavenger in the present work may be relevant to photobiological studies, and hence to studies concerning the potential use of phthalocyanines in PDT.

2. Materials and method

2.1. Chemicals

The phthalocyanine dyes used (*e.g.* disulphonated, trisulphonated and tetrasulphonated copper phthalocyanines, chloroaluminium phthalocyanine and bis-aluminium phthalocyanine), as well as some novel phthalocyanine derivatives, were obtained as a gift from Dr. W. Rigby at I.C.I. Organics Division. The phthalocyanine dyes used in this study are listed in Table 1. The solvent used was dimethylformamide (DMF) (May and Baker) and the chemical used as a scavenger was DPIBF (Aldrich). It is well known that commercially available dye preparations can be very impure [40]. The sulphonated phthalocyanines are named so as to describe their major constituent (see below), although in practice a number of isomers or dyes with different degrees of sulphonation may have been present; the procedures for separating these dye mixtures are difficult [16, 41].

TABLE 1
1,3-Diphenylisobenzofuran photobleaching rates of various phthalocyanines

Phthalocyanine dye	k^a (s^{-1})
Zinc phthalocyanine	0.49 (0.03) ^b
Magnesium phthalocyanine	0.32 (0.03) ^c
Chloroaluminium phthalocyanine	0.89 (0.03)
bis-Aluminium phthalocyanine	1.18 (0.02)
Pentachloro-copper phthalocyanine	0.096 (0.002)
Copper phthalocyanine tetrasulphonate	0.93 (0.02)
Copper phthalocyanine trisulphonate	0.75 (0.04)
Copper phthalocyanine disulphonate	0.61 (0.05)
Copper phthalocyanine disulphonate-R ₂ ^d	0.40 (0.01)
Copper phthalocyanine-R ₃ ^d	0.46 (0.02)
Copper phthalocyanine-R ₄ ^d	0.43 (0.02)

^a k represents the mean first-order rate of decay of DPIBF in DMF, corrected to account for the power absorbed by each dye solution (results from five experiments). The values in parentheses are the standard deviations.

^bTaking DPIBF decay from first 40 s only.

^cPhthalocyanine barely soluble in chloroform.

^dR ≡ SOONHCH₂CH₂NHCH₂CH₂OH.

2.2. Measurement of relative 1,3-diphenylisobenzofuran bleaching rates

Measurements were performed in oxygen-saturated solutions of DMF. Use of the scavenger DPIBF for the assessment of phenothiazine dyes as photosensitizers has also been described in the literature [42]. The concentration of DPIBF in DMF was adjusted so that its absorbance at 412 nm was between 1.6 and 2.2; scavenger absorbance was measured with a Perkin-Elmer Lambda 3 UV-visible spectrophotometer. The concentration of each potentially photosensitizing dye was adjusted so that the absorbance at its major visible absorption peak lay between 1.0 and 2.0. The irradiation chamber received filtered light from a 70 W tungsten-halogen lamp. The filter used (Corning Glass 3-70, 3384) restricted light absorption to the phthalocyanine dye component of the DPIBF-dye solutions. The different relative photoexcitation rates of each of the dyes were derived from a knowledge of the respective dye absorption spectra and the spectral distributions of the exciting light; the light absorbed by a particular dye solution was calculated from the relevant absorption spectrum using a computer program. The total incident light flux at the irradiation chamber was measured using a U.D.T. model 40X Opto-Meter, whilst its spectral distribution was measured using an ISCO model SR spectroradiometer. A thermostatted water bath was used to control the temperature of both the irradiation chamber and the spectrophotometer cell holder; all measurements were carried out at 20 °C.

The raw kinetic data were in the form of absorbance values at a suitable DPIBF absorption peak chosen for monitoring (412 nm) as a function of time in minutes. The data from an individual experiment comprised two parts: a pre-illumination phase and a post-illumination phase. The pre-illumination data served to check for the absence of a dark reaction leading to scavenger bleaching. A computer program was used to fit post-illumination data to a first-order kinetic expression; fitting our data to a first-order kinetic expression gave higher correlation coefficients than fits of the data to either zero- or second-order kinetic expressions. In practice, this meant that data in the form of the natural logarithm of the absorbance *vs.* time were fitted using the least-mean-squares method, giving values for the first-order rate coefficient from the gradient and for the fitting correlation coefficient. Data from only the first 5 min of illumination were used in the analysis. The DPIBF photobleaching rates given in Table 1 are derived from first-order kinetic plots of DPIBF absorbance loss as a function of time and are corrected for differences in light absorption by the dyes (see above). The mean and standard deviation of slopes from corresponding sets of experiments were calculated, and it is these values that are quoted in the result table (Table 1) as being representative of the rates of DPIBF bleaching.

3. Results and discussion

The DPIBF bleaching rates obtained for the phthalocyanines in the solvent DMF are given in Table 1. Phthalocyanines containing light metals, such as zinc, aluminium and magnesium, give high fluorescence yields with nanosecond fluorescence lifetimes and moderate triplet yields with long triplet lifetimes [43]. In paramagnetic phthalocyanines, such as the copper derivatives, the metal unpaired electrons are available to couple with the promoted electrons of the excited singlet and triplet states, leading to their rapid deactivation.

In the present study, the ability to produce species that bleach DPIBF decreases for the unsulphonated phthalocyanines in the order chloroaluminium phthalocyanine > zinc phthalocyanine > magnesium phthalocyanine. This is the same order as

reported in the literature by, for example, Langlois *et al.* [43], who used L-tryptophan as a scavenger. These workers placed the activity of unsulphonated copper phthalocyanine between that of the zinc and magnesium phthalocyanines, whilst Wu *et al.* [44] give the following descending order of zinc phthalocyanine > magnesium phthalocyanine > copper phthalocyanine for the photo-oxidation of dimethylfuran in organic solvent mixtures. bis-Aluminium phthalocyanine has the highest rate of DPIBF bleaching of any of the phthalocyanines measured (about twice that of copper phthalocyanine disulphonate). Chloroaluminium phthalocyanine has a triplet lifetime approximately 8000 times that of copper phthalocyanine monosulphonate, with about half the triplet quantum yield [45], which helps to explain these observations. It has also been observed that aluminium phthalocyanine does not dimerize in aqueous solution [46]; if this is also true for chloroaluminium phthalocyanine, so that the dye is solely in a monomeric form in DMF, this would explain why it gives a higher DPIBF bleaching rate (*vide infra*).

For the copper phthalocyanine derivatives, the hydrophobicity of the compounds is decreased by sulphonate groups ($-\text{SO}_3\text{H}$) and increased by sulphonate groups containing aliphatic alcohol chain substituents R (where $\text{R} \equiv -\text{SO}_3\text{N}(\text{H})-\text{CH}_2\text{N}(\text{H})\text{CH}_2\text{CH}_2\text{OH}$) (see Table 1). In other words, the hydrophobicity of the phthalocyanines increases in the order copper phthalocyanine tetrasulphonate < copper phthalocyanine trisulphonate < copper phthalocyanine disulphonate < copper phthalocyanine disulphonate- R_2 < copper phthalocyanine- R_3 < copper phthalocyanine- R_4 ; for most of the dyes, increasing hydrophobicity seems to correlate with decreasing DPIBF bleaching in DMF. It should be remembered, however, that the sulphonated phthalocyanines used in this study may contain impurities with higher or lower degrees of sulphonation, as well as various isomers, as previously mentioned. Also, the DPIBF bleaching yields for the last three dyes mentioned above, copper phthalocyanine disulphonate- R_2 , copper phthalocyanine- R_3 and copper phthalocyanine- R_4 , do not seem to be significantly different from each other (see Table 1). It has been noted that, for the phthalocyanines, their ability to produce singlet oxygen decreases when they become aggregated [16, 47]; the less hydrophilic a dye is, the more likely it is to aggregate in polar solvents, and the less singlet oxygen it should produce. DMF is regarded as a polar solvent; the dielectric constant (relative permittivity) of DMF at 20 °C is 37.65 [48]. The degree of aggregation of copper phthalocyanine is not as high in DMF as in carbon tetrachloride or chloroform [49].

Figure 1, spectra a and b, shows the absorption spectra between 500 and 750 nm for copper phthalocyanine tetrasulphonate and copper phthalocyanine- R_4 respectively. The major peak in each spectrum, at a longer wavelength, represents the monomer form of the dye; this is borne out by the literature [50, 51]. From the shape of spectrum a, it would seem that copper phthalocyanine tetrasulphonate is mainly in the monomeric form, as there is no characteristic dimer peak at about 640 nm. In spectrum b, however, there is a characteristic dimer peak superimposed on the monomer spectrum of copper phthalocyanine- R_4 , indicating that this dye, more hydrophobic than copper phthalocyanine tetrasulphonate, has dimerized to some extent; this in turn would explain why copper phthalocyanine- R_4 gives a lower DPIBF bleaching rate than copper phthalocyanine tetrasulphonate.

It should also be mentioned that, according to McCubbin [45], unsulphonated copper phthalocyanine gives a lower triplet yield and has half the triplet lifetime of the monosulphonated derivative; this would also help to explain why phthalocyanines with a higher number of sulphonate groups, and therefore presumably longer-lived triplet states, gave higher DPIBF bleaching rates in the present study.

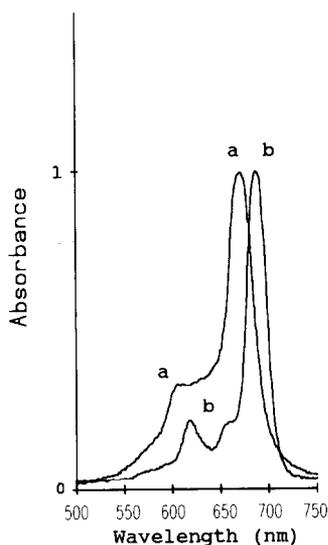


Fig. 1. Absorption spectrum of copper phthalocyanine tetrasulphonate (spectrum a) and copper phthalocyanine with four $-\text{SO}_3\text{NHCH}_2\text{NHCH}_2\text{CH}_2\text{OH}$ groups (spectrum b).

Wagner *et al.* [47] noted that, for chlorogallium phthalocyanines, the photosensitizing ability against L-tryptophan was unaffected by the degree of sulphonation of the dye; the yields of singlet oxygen were dictated by the tendency of the dyes to aggregate in solution. Against V-79 Chinese hamster cells *in vivo*, Brasseur *et al.* [3] noted that for zinc phthalocyanines a lower degree of sulphonation usually meant that the dye was more photoactive; this is opposite to the trend noted earlier and is probably more to do with dye localization than with singlet oxygen production.

Finally, the pentachloro copper phthalocyanine derivative seems to have the smallest rate of DPIBF bleaching of all and is also probably the most hydrophobic.

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