



Triple state properties of tetrasubstituted zinc phthalocyanine derivatives

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Abstract

Photophysical properties of zinc phthalocyanine and its three novel tetrasubstituted derivatives were determined with laser flash photolysis. The substituents containing either glycol ether groups or benzene rings with different functions are attached to the aromatic nuclei of phthalocyanines (Pcs) through ether bonds. The triplet lifetimes in deoxygenated solutions in ethanol and toluene are around 300 μs while in air saturated solutions between 0.2 and 0.4 μs . Oxygen quenching rate constants are between 1 and $2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ and the quantum yields of singlet oxygen formation are between 0.4 and 0.6. Oxygen quenching of a Pc derivative embedded in phospholipid vesicles shows strong temperature dependence below the main phase transition temperature of the phospholipid, with activation energy of 61 kJ mol^{-1} . The triplet state parameters obtained in this study, together with the ground state absorption properties suggest that the new compounds may be tested as photosensitizers in photodynamic therapy.

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1. Introduction

Among the recent applications of phthalocyanine (Pc) derivatives (e.g. in display devices [1], data storage [2], chemical sensors [3], solar cells [4]), one of the most promising fields is the use of Pcs as sensitizers in photodynamic therapy (PDT), a rapidly developing modality in cancer therapy [5]. PDT is based on the interaction of a photosensitizer with visible light in the malignant tissues. The therapy starts with the administration of the drug, which then accumulates more or less selectively in the tumour. Illuminated with red light, the photosensitizer transforms into the first singlet excited state and then by intersystem crossing it reaches the triplet state. The triplet molecule in a Type I electron transfer process reacts with biological substrates to form radicals or radical ions or in

a Type II energy transfer process interacts with ground state (triplet) oxygen molecules resulting in highly reactive singlet oxygen, which is regarded as the key agent that kills tumours [6].

The first and still frequently used photosensitizer is hematoporphyrin derivative (HpD), which has, however, several drawbacks. Firstly, its longest wavelength absorption maximum is around 630 nm, where the penetration depth of light in soft tissues is quite small. Secondly, it is not a pure compound but a mixture of porphyrin dimers and oligomers. Finally, it causes prolonged skin photosensitivity after treatment. Therefore, there is an intensive research effort to find better photosensitizers. The most frequently studied classes of compounds are porphyrins, chlorins, bacteriochlorins, phthalocyanines and porphycenes [6].

The major advantage of Pcs over porphyrins is that their Q bands are at longer wavelengths and have much higher intensity than the Q bands of porphyrins. Some complexes of Pcs with non-transition elements show photobiological activity against tumours. The photophysical properties

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and photodynamic activities of Zn(II)Pc [7,8] and substituted Zn(II)Pcs [9,10] have been extensively studied. Most Pcs are practically insoluble in water, so their application requires lipid based delivery systems [11,12]. On the other hand, the water-soluble derivatives such as Zn(II)Pc-tetrasulfonates have a tendency for aggregation, reducing their photodynamic activity [13]. The nature of peripheral substituents influences the degree of aggregation, with bulky groups reducing, long alkyl chains increasing this phenomenon [14].

The aim of the present work was to study the photophysical parameters of some Pc derivatives synthesized in our laboratory [15]. Since the synthesis was based on the tetramerization of 4-*O*-substituted-phthalonitriles, therefore the products were obtained as unseparable mixtures of regioisomers. To increase the amphiphilic character of Pcs, glycol ether and tertiary amine groups were introduced in the four benzene rings of the Pc skeleton (see Fig. 1.) The tertiary amino groups can be quaternized to obtain water-soluble cationic Pcs, which are expected to exhibit decreased aggregation in solution, and the possibility of photodynamic inactivation of certain bacteria [16]. The introduction of benzene rings in the substituents is aimed to increase the lipophilic character of Pcs. Besides recording steady-state absorption and fluorescence spectra, triplet absorption spectra, triplet lifetimes and quantum yields of singlet oxygen formation were also determined by

laser flash photolysis. These latter parameters are of particular importance in PDT. It was our purpose to study the influence of the substituents on these photophysical properties.

Direct extrapolation of the results obtained in homogeneous solution to the photodynamic behaviour of sensitizers in biological systems is very difficult [17]. Therefore, it is important to study the photophysical properties of sensitizers incorporated into microheterogeneous systems, such as phospholipid vesicles. Lipophilic sensitizers tend to accumulate in cell membranes [18]. Since vesicles can be regarded as simple models of cell membranes, we also determined some photophysical parameters of Pcs embedded in small unilamellar vesicles (SUVs).

2. Experimental

Compounds **1–4** (Fig. 1) were prepared in a two-step synthesis described in Refs. [19,20]. In the first step, 4-alkoxy/aryloxy-phthalonitriles were formed from 4-nitrophthalonitrile and the corresponding alcohols or phenols. The second step was the base catalyzed cyclotetramerization of the phthalonitriles. This way regioisomers of **1–4** were formed. The reaction times were significantly

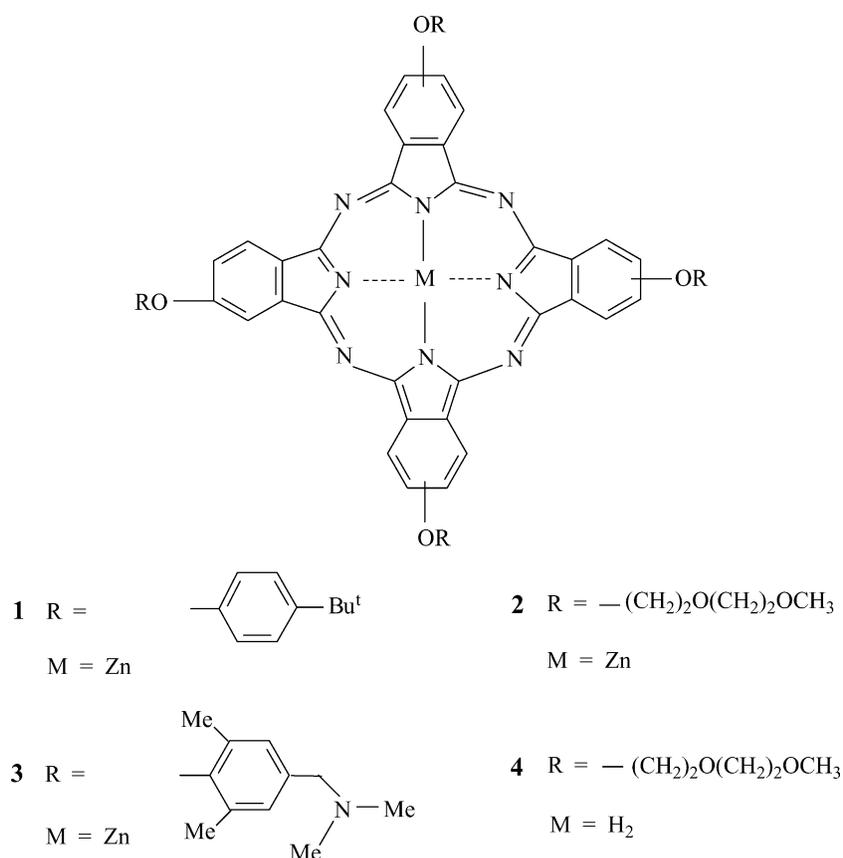


Fig. 1. Molecular structure of the phthalocyanine derivatives.

reduced by using microwave irradiation. Details of the synthesis are described in our former paper [15].

Spectroscopic and flash photolysis measurements were carried out with solutions in ethanol and toluene. Pcs **1** and **2** are soluble in both solvents, **3** is soluble in ethanol but scarcely soluble in toluene and **4** is soluble in toluene only. For comparison, the ethanolic solution of the unsubstituted Zn(II)Pc (**5**) was used.

SUVs were prepared by the ethanol injection method [21]. Thus, 5 mg dipalmitoylphosphatidylcholine (DPPC) was dissolved in 700 μl ethanol containing 0.112 μmol Pc and this solution was slowly injected into 10 cm^3 phosphate buffer (PBS) stirred magnetically at 55 $^\circ\text{C}$. After cooling down to room temperature vesicles of 50–100 nm were formed.

The ground state absorption spectra were recorded on a Cintra 10e UV/vis spectrophotometer. Fluorescence spectra were acquired on a Perkin Elmer LS 50B luminescence spectrometer. For the measurement of triplet absorption spectra, triplet lifetimes and singlet oxygen formation, a laser flash photolysis system was employed. The excitation source was the third harmonic (355 nm) of a Nd:YAG laser (Continuum, Surelite I-10). Pulse energy was measured by an energy meter (Rj 7100, Laser Precision Corporation). The analysing light from a 150 W xenon lamp was sent into the cell (perpendicularly to the exciting laser beam), and focussed to the entrance slit of a monochromator. The signal was detected by a photomultiplier tube. For detecting the infrared emission of singlet oxygen, a liquid nitrogen cooled germanium photodiode (North Coast EO817P) was applied. Data acquisition was performed by a digital oscilloscope (Tektronix TDS 210) and a personal computer.

The measurements were done at room temperature (298 K) except for the temperature-dependent lifetime determinations in phospholipid vesicles. For oxygen free measurements, deoxygenation of the sample was carried out by bubbling high purity nitrogen gas through the solution. Zinc phthalocyanine and phenalenone used in the determination of quantum yield of singlet oxygen formation were purchased from Aldrich and were used without further purification. DPPC was purchased from Sigma. The solvents (ethanol and toluene) were of HPLC grade.

3. Results and discussion

The data of the bands observed in the steady-state absorption and fluorescence spectra are given in Table 1. As an example, the spectra of compound **2** in ethanol solution are shown in Fig. 2. All the studied compounds have strong absorption around 680 nm, which is favourable for PDT. The visible spectra were not influenced significantly by the side-chain substituents, whereas the lack of the central Zn ion resulted in the splitting of the most intense absorption

Table 1
Ground state absorption maxima and emission maxima of compounds **1–4**

Compound (solvent)	Absorption maxima (nm) [ϵ ($10^5 \text{ M}^{-1} \text{ cm}^{-1}$)]			Emission maxima (nm) ($\lambda_{\text{ex}} = 610\text{nm}$)	
1 (ethanol)	348	614	675 [1.05]	693	754
1 (toluene)	355	613	681 [1.54]	697	761
2 (ethanol)	343	612	676 [0.93]	695	757
2 (toluene)	355	616	685 [1.21]	698	762
3 (ethanol)	347	612	677 [0.85]	694	756
3 (toluene)	356	615	685 [1.33]	704	763
4 (toluene)	324	603	666	711	789
5 (ethanol)	385	638	702 [0.28]	743	
5 (ethanol)	341	601	666 [3.02]	671	740

The molar absorption coefficients (ϵ) for the longest wavelength absorption bands are given in brackets.

band near 680 nm. A similar but less pronounced effect was observed in the fluorescence spectra. No significant dimerization occurred, except for compound **2** in concentrated ethanol solution (indicated by a new band emerging at 630 nm). The fluorescence bands near 700 nm may be used for diagnostic purposes in cancer therapy.

The triplet properties are summarised in Table 2. Because of solubility problems only compounds **1** and **2** could be measured in both solvents. Triplet absorption spectra (unstructured broad bands with maxima around 500 nm) were recorded in deoxygenated solutions. Triplet lifetimes were around 300 μs in the absence of air and well below 1 μs (see third column of Table 2) in air saturated solutions. This great difference shows that in the presence of oxygen the only decay pathway of the triplets is quenching by oxygen, which results in the formation of singlet oxygen. Under our experimental conditions, the kinetics of the triplet Pcs show pseudo first-order characteristics due to the great excess of molecular oxygen. The second-order quenching rate constants were also evaluated, taking the solubilities of oxygen in these solvents from Ref. [22]. The values determined are typical of quenching of porphyrin/Pc triplets

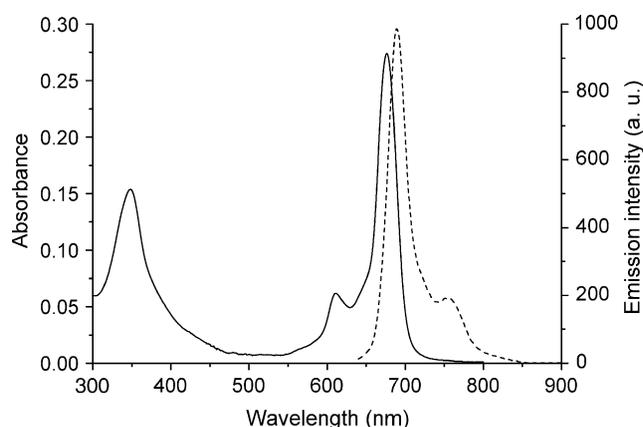


Fig. 2. Absorption (solid line) and fluorescence (dashed line) spectrum of **2** in ethanol ($c = 3 \times 10^{-6} \text{ mol dm}^{-3}$). The excitation wavelength for the fluorescence spectrum is 610 nm.

Table 2

Triplet absorption maxima (λ_{\max}), triplet lifetimes in air saturated solutions (τ),^a oxygen quenching rate constants (k)^a and quantum yields of singlet oxygen formation (Φ)^a

Compound (solvent)	λ_{\max} (nm)	τ (ns)	k ($10^9 \text{ M}^{-1} \text{ s}^{-1}$)	Φ
1 (ethanol)	495	306	1.55	0.47
1 (toluene)	510	287	1.67	0.58
2 (ethanol)	490	381	1.25	0.42
2 (toluene)	505	263	1.81	0.44
3 (ethanol)	483	231	2.09	0.59
4 (toluene)	498	504	0.95	0.19
5 (ethanol)	478	220	2.17	0.43

^a Estimated accuracy $\pm 10\%$.

[23]. No significant effect of the substituents was observed but the lack of the central Zn ion lowered the quenching rate constant. In cases when the measurements could be done in both solvents (compounds **1** and **2**), lower triplet lifetimes and higher rate constants were obtained in toluene than in ethanol. This is probably due to the lower viscosity of toluene, resulting in a higher diffusion coefficient of oxygen in toluene than in ethanol.

For the determination of quantum yield of singlet oxygen formation the near IR emission of singlet oxygen ($\sim 1275 \text{ nm}$) was detected. This signal showed an exponential decay. The initial intensities (I_0) were determined by extrapolating to the time of the laser shot (time zero). Similar measurements were carried out with a reference compound having known quantum yield of singlet oxygen formation (Φ_{ref}). The emission signals were plotted against the laser intensities between 0.1 and 0.8 mJ. Fig. 3 shows the data points in toluene. The concentrations were adjusted to obtain the same absorbances for the Pcs and for the reference compound at the wavelength of excitation. The quantum yields of singlet oxygen formation (Φ) were calculated from the slopes of the linear parts of the plots

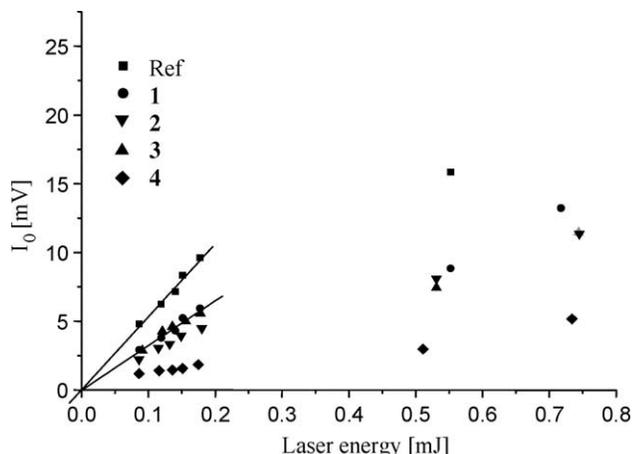


Fig. 3. Singlet oxygen emission intensities against pulse energy in toluene solutions: ■, phenalenon standard; ●, **1**; ▼, **2**; ▲, **3**; ◆, **4**. In the linear region, the slope for the standard and the slope for **1** are shown.

obtained for the Pcs (m), compared with the slope (m_{ref}) of the reference compound

$$\Phi = \frac{m}{m_{\text{ref}}} \Phi_{\text{ref}}$$

The reference compound in these experiments was phenalenone, an aromatic ketone with $\Phi_{\text{ref}} = 0.95 \pm 0.05$, the value which is practically independent of the solvent [23,24]. The results are shown in Table 2. (The literature value Φ for unsubstituted ZnPc (**5**) is 0.4 ± 0.1 in ethanol [25] and 0.58 in toluene [26].) It can be seen that the substituents on the benzene rings do not significantly influence the quantum yields of singlet oxygen formation, whereas the lack of the central Zn (compound **4**) reduces it considerably.

For the study of photophysical parameters of Pc molecules embedded in SUVs compound **3** was selected because of its good solubility in ethanol. Using a thermostatted cell, triplet lifetimes in the presence of oxygen were determined between 20 and 55 °C. Slow bubbling of air ensured oxygen saturation of the solution.

As can be seen in Table 3, the lifetimes in vesicles have strong temperature dependence, and are one order of magnitude longer than those in organic solvents (see Table 2). The main reason for the latter is the lower solubility of oxygen in water than in ethanol or toluene. (Direct comparison of the lifetimes in vesicles and in water was not possible because of the poor solubility of **3** in water.) Taking into account the temperature dependent solubility of oxygen in water [27], the oxygen quenching rate constants were also calculated.

The Arrhenius plot of these rate constants (Fig. 4) shows two distinct regions. Below the phase transition temperature ($\sim 41 \text{ °C}$) of the phospholipid bilayer, a linear function can be fitted to the experimental values with activation energy of 61 kJ mol^{-1} . Above this temperature, $\ln k$ is constant within the experimental error of the measurement. This behaviour suggests that below the phase transition temperature, the rate-determining step of oxygen quenching is the transfer of

Table 3

Temperature dependence of the triplet lifetime and oxygen quenching rate constant of compound **3** embedded in small unilamellar vesicles

t (°C)	τ (μs)	k ($10^9 \text{ M}^{-1} \text{ s}^{-1}$)
20.2	5.53	0.62
24.0	3.82	0.95
27.9	3.50	1.11
30.3	2.52	1.60
33.4	2.40	1.77
35.2	2.02	2.17
37.3	2.23	2.05
39.1	2.04	2.32
41.0	2.25	2.20
42.9	2.00	2.59
46.6	2.06	2.82
50.4	2.07	2.47
54.1	2.23	2.36

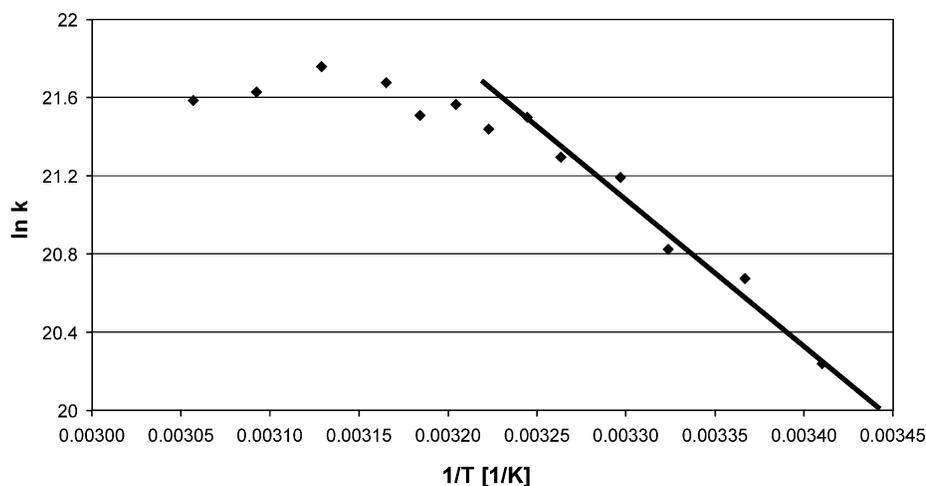


Fig. 4. Arrhenius plot of the oxygen quenching rate constants of **3** in DPPC vesicles. Activation energy calculated from the linear region is 61 kJ mol⁻¹.

oxygen from the aqueous solution to the Pc embedded in the lipid bilayer, and the calculated activation energy belongs to this process.

4. Conclusion

The triplet state parameters and the ground state absorption properties of compounds **1–3** fulfil the photo-physical requirements for a photosensitizer in PDT. There are, however, many other properties (selectivity, lack of dark toxicity, etc.) a good photosensitizer must possess. Further, both in vitro and in vivo studies are needed to characterise the photodynamic activity of these substances. The results presented here confirm the previous observations on porphyrin families of compounds having various side-chains, i.e. the difference in the substitution pattern in the side-chain does not change the photophysical properties, which are essential in PDT [28,29]. Thus, choice of the most suitable substituent can be made based on solubility and membrane compatibility characteristics.

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