Photophysical and photochemical properties of potential porphyrin and chlorin photosensitizers for PDT

Eduard Zenkevich a,*, Evgenii Sagun a, Valentin Knyukshto a, Alexander Shulga a, Andrei Mironov b, Olga Efremova b, Raymond Bonnett c, S. Phinda Songca c, Mohammed Kassem d

a Institute of Molecular and Atomic Physics, Academy of Sciences of Belarus, F. Skaryna Avenue 70, Minsk 220072, Belarus
b Moscow State Academy of Fine Chemical Technology, Vernadskogo Avenue 86, Moscow 117951, Russia
c University of London, Queen Mary and Westfield College, Mile End Road, London E1 4NS, UK
d Beirut Arab University, P.O. Box 115020, Beirut, Lebanon

Received 21 June 1995; accepted 11 September 1995

Abstract

Structural and optical properties as well as photophysical and photochemical parameters (excited S1 and T1 state lifetimes at 77 K and in the presence of O2 in solution at 293 K; efficiencies of singlet oxygen, 1Δg generation) are presented for porphyrins and chlorins with potential for the PDT of cancer: chlorin pC, and its trimethyl ester, chlorin e6 and its Na+, and K+ salts, purpurin-18 and its mono-methyl ester, 5,10,15,20-tetrakis(3-methoxyphenyl) porphyrin (TPP), 5,10,15,20-tetrakis(2,4-diflimo-3-methoxyphenyl)porphyrin (TPPMF) and Ga-TPP in different solvents (ethanol, toluene, pyridine and buffer pH 7.4) at 77-300 K. It has been shown that for monomeric chlorin e6, chlorin pC, and its derivatives the photophysical parameters are similar, as follows: fluorescence lifetimes τf in the presence of oxygen are 3.2-4.5 ns at 293 K; fluorescence quantum yields φf vary from 0.1 to 0.2 depending on the solvent; phosphorescence quantum yields φp are of the order 10-4, T1 state lifetimes τT1 = 1.5-2.0 ns at 77 K and 250-390 ns at 293 K in the presence of O2.

By use of the direct kinetic measurement of singlet oxygen emission at 1.27 μm on laser excitation the quantum yields of 1Δg generation by chlorins have been measured: φ1Δg = 0.35-0.68. In this case values of φ1Δg and φp depend strongly on the solvent, probably because of the formation of aggregates. For TPPM, TPPMF and Ga-TPP the φ1Δg values measured are higher (0.87-0.98) and are explained by the higher intersystem crossing S1 → T1 quantum yields.

Keywords: Porphyrins; Chlorins; Photosensitizers; PDT

1. Introduction

In the last decade there has been increasing interest in the use of porphyrins and chlorins as photosensitizers for selective and effective destruction of animal and human malignant tumours [1-14]. Photosensitization with different tetrapyrrole compounds has been shown to be an efficient process for the killing of cancerous and bacterial cells. It has been suggested that singlet oxygen, produced by energy transfer from the sensitizer triplet state to molecular oxygen, is the cytotoxic agent active in phototherapy in malignant tissues in vivo [9,15,16].

This developing medical application has stimulated a search for photosensitizers which are more effective than the previously used haematoporphyrin derivatives and related compounds [3-7]. The new, "second-generation" photosensitizers must fulfill a number of criteria if they are to act efficiently [7]. In addition to meeting the biological requirements, which appear to be best satisfied by a balance of hydrophobic and hydrophilic characteristics, the photosensitizer should have strong absorption in the red region of the visible spectrum. From a photophysical point of view, photosensitizers for this application also need to have a high intersystem crossing probability, leading to effective population of the triplet state, coupled with an efficient generation of singlet oxygen.

There is consequently considerable current interest in the synthesis and application of "new generation" photosensitizers based on various molecules containing a tetrapyrrole...
unit with increased red absorption, such as phthalocyanines [3,9], chlorins and purpurins [3-7,9-11], naphthaloporphyrins [17], and bacteriochlorins [11,18]. In this context, it should be noted that the choice and optimization of new tetrapyrrole-based photosensitizers is facilitated by the investigation of their photophysical and photochemical parameters in homogeneous solution as the first step. In addition, it is interesting to analyse the behaviour of their properties in solvents of different nature to control possible aggregation effects which can influence the photodynamic efficiency dramatically.

In the work reported here, optical, photophysical and photochemical parameters of purpurin-18 (P18) and chlorin \( \rho_n \) (Chl \( \rho_n \)) and their derivatives as well as of some tetraphenylporphyrins are presented. The main emphasis is directed to the comparative analysis of the values obtained for novel compounds in different solvents with the analogous parameters known for Chl \( \rho_n \).

2. Materials and methods

2.1. Materials

Chlorophyll \( \alpha \) has been isolated from plant materials by well-known methods [19] and has been converted chemically into P18 and Chl \( \epsilon_n \) or Chl \( \rho_n \), and their derivatives with different substituents [20]. The synthesis of the tetraphenylporphyrin derivatives 5,10,15,20-tetrakis(3-methoxyphenyl)porphyrin (TPPM) and 5,10,15,20-tetrakis(2,4-difluoro-3-methoxyphenyl)porphyrin (TPPMF) has been carried out using methods based on those described elsewhere [21]. The compounds have been characterized by the use of electronic and \( ^1 \)H nuclear magnetic resonance spectroscopy and by mass spectrometry. The structural formulae of the compounds, and the abbreviations used here, are given in Fig. 1.

All solvents (ethanol, toluene, pyridine, isopropanol, diethyl ester (spectroscopic grade)) have been used without further purification. The experimental results have been obtained at ambient temperature or at 77 K in some cases.

2.2. Spectral and kinetic measurements

Electronic absorption spectra were recorded on Beckman 5270 and Specord M40 spectrophotometers. The photosensitizer fluorescence lifetime \( \tau \), was measured using a PRA-3000 pulse fluorometer operating in the single-photon counting mode.

Corrected fluorescence and phosphorescence spectra as well as fluorescence and phosphorescence excitation spectra were recorded on a laboratory spectral luminescent set-up, equipped with a personal computer. The operating spectral region was from 200 nm to 1100 nm, and the exciting light sources were xenon and tungsten lamps or an argon laser. For better signal-to-noise ratio the experimental set-up was equipped with a thermocooling system for the photomultiplier (FEU-83 or FEU-100). The reproducability of the system is 5%, the accuracy of emission quantum yield measurements is 5%-7% for \( \phi_\lambda \approx 0.1 \), and the limit of emission quantum yield \( \phi_\lambda \) measured is \( 10^{-5} \). The apparatus has been described in detail [22]. The emission quantum yields of the compounds under investigation were determined by the relative method [23]. Tetraphenylporphyrin in toluene (\( \phi_\lambda = 0.09 \) at 293 K) was used as a standard [43]. Quantum yields of phosphorescence were estimated by comparison with the fluorescence quantum yields of the same compound.

The kinetics of triplet-triplet absorption was studied on a standard laboratory flash photolysis set-up with a time resolution of \( 10^{-7} \) s [24-26].

2.3. Singlet oxygen laser fluorometry

Spectra, kinetics and quantum yields of singlet oxygen emission were obtained using a laboratory experimental set-up, described in detail in Refs. [26-28]. The samples were excited by the pulses of the second harmonic of an AIG:Nd\(^{3+}\) laser (\( \lambda = 532 \) nm) or ruby laser (\( \lambda = 347 \) nm) with a duration of 20-30 ns and an energy density of 0.3-3.0 mJ cm\(^{-2}\). The probing of induced absorption was performed by a fllament lamp. The recording was carried out by a germanium photodiode or a photomultiplier tube and by a digital oscilloscope connected with a personal computer. The separate determination of quantum yield \( \phi_\Delta \) of the sensitized production of singlet oxygen is not trivial.

Usually \( \phi_\Delta \) values are determined relative to each other or to sensitizers with a known yield. The determination of the quantum yield of photosensitized formation of singlet oxygen \( (O_2(1\Delta _g)) \) based on using standard compounds with well-documented \( \phi_\Delta \) values in different solvents has been described in detail [29]. In contrast with methods based on the photochemical oxidation of the substrate [17,30] we used the direct spectral kinetic method for the measurement of singlet oxygen IR emission (\( \lambda = 1.27 \) \( \mu \)m). In this case uncertainties in the quantitative estimation of the role of singlet oxygen in the photochemical oxidation of the substrate are excluded. In this respect we consider that our method provides more accurate values of the quantum yields of singlet oxygen formation.

The main procedure for the determination of the quantum yield \( \phi_\Delta \) of singlet oxygen formation may be presented as follows. When the compound under consideration and the standard were in the same solvent the value of \( \phi_\Delta \) for the compound was determined by the relative method, i.e. by the comparison of the emission intensity of singlet oxygen, photosensitized by the compound under investigation (intensity \( I_\lambda \)) and by the standard (intensity \( I_\nu \)):

\[
\phi_\Delta \ = \ \phi_\Delta \ \frac{I_\lambda}{I_\nu} \ \frac{\beta_\lambda}{\beta_\nu}
\]

where \( \phi_\Delta \) is the quantum yield of singlet oxygen formation for the standard, and \( \beta_\lambda = 1 - 10^{0.02} \) and \( \beta_\nu = 1 - 10^{-5} \) are the
absorbed exciting light portions at excitation wavelength for the investigated and standard compounds respectively. \( I_s \) and \( I_0 \) values were obtained for no less than 30 laser pulses and then were averaged and extrapolated to the maximal pulse intensity.

Oxygen concentrations at ambient conditions in the solvents being used, and dielectric constant \( \varepsilon \) values were taken from Refs. [26,31]. Various compounds were used as standards: (i) chlorophyll \( a (\phi_x^0=0.6 \ [26]) \) and octaethylporphyrin \( (\phi_x^0=0.75 \ [32]) \) in toluene solutions; (ii) methylene blue \( (\phi_x^0=0.52 \ [33]) \) in ethanol; (iii) Chl \( e \), \( (\phi_x^0=0.74 \ [34]) \) in pyridine. In all cases solution absorbance at the excitation wavelength was not higher than 0.2 at a path length of 10 mm. The relative error of the \( \phi_x \) determination did not exceed 10%-15%.

The experimental set-up described permitted us to measure \( \tau_1 \) values for the compounds of interest in liquid solutions at 293 K in the presence of molecular oxygen as well. The corresponding values of singlet and triplet state quenching rate constants by molecular oxygen were calculated by using oxygen solubility data and physicochemical properties of solvents from Ref. [31].

The following formulae were used.

(i) For the rate constant \( k_T \) of triplet state quenching by molecular oxygen,

\[
k_T = \frac{(\tau_T)^{-1} - (\tau_T^0)^{-1}}{[O_2]} \tag{2}
\]

where \( \tau_T^0 \) and \( \tau_T \) are triplet lifetimes of the compound under consideration in liquid solution in the absence and the pres-
ence respectively of oxygen and \([O_2]\) is the dissolved molecular oxygen concentration. As a rule in calculations of \(k_T\) the value of \((\tau_T^0)^{-1}\) is neglected because of \(\tau_T^0 \gg \tau_T\) (over 2–3 orders of magnitude).

(ii) For the rate constant \(k_s\) of singlet state quenching by molecular oxygen,

\[
k_s = \frac{(\tau_s)^{-1} - (\tau_s^0)^{-1}}{[O_2]}
\]

where \(\tau_s^0\) and \(\tau_s\) are singlet (fluorescent) lifetimes of the compound of interest in liquid solution in the absence and the presence respectively of oxygen. The relative error in the measurements is estimated to be \(\pm 3\%\) for \(\tau_s\), \(\pm 5\%\) for \(\tau_T\) and \(\pm 10\%–12\%\) for \(k_s\) and \(k_T\).

3. Results and discussion

3.1. Singlet and triplet parameters

It is convenient to separate and discuss the results of our investigation by presenting them in three different parts: (i) purpurins, (ii) chlorins and (iii) tetraphenylporphyrins.

Figs. 2 and 3 show the absorption and corrected luminescence (fluorescence and phosphorescence) spectra of some representatives of these three groups of the compounds. The results of the study of their photophysical parameters are summarized in Table 1.

3.1.1. Purpurins

P18 in ethanol is characterized by a long-wavelength absorption band at 700 nm which is attributed to a \(Q_s(0,0)\) electronic transition [35]. The maximum of the Soret band is observed at 408 nm (see Fig. 2(a)). The ratio \(A(\text{Soret})/A(Q_s) = 2.7\) reflects a situation which is typical for tetrapyrrrole compounds with hydrogenated pyrrole rings [36]. Table 1 shows that when the solvent is changed to toluene and pyridine the absorption spectra of P18 are red shifted by 4–7 nm. In all solvents the weak vibronic structure of the first electronic \(Q_s(0,0)\) transition is observed. In addition, a significant band at 547 nm manifests itself in contrast to the low intensity band for various “normal” chlorins in this region. According to our polarized fluorescence measurements for chlorins this band belongs to the second \(Q_s(0,0)\) electronic transition [36]. The energy of this transition is almost independent of the substituents and the solvent and does not change with temperature decrease down to 77 K.

The fluorescence spectrum of P18 (Fig. 2(a)) is roughly the mirror image of the long-wavelength region of the absorption spectrum and is characterized by weak vibrational structure. This means that, on excitation, the structural changes of P18 are quite small and the Frank–Condon principle, which determines the form of the absorption band, is applicable to the emission processes for this molecule.

The fluorescence quantum yield \(\phi\) of P18 is strongly dependent on the solvent, changing from 0.08 in ethanol to 0.13 in pyridine. Nevertheless the fluorescence lifetime \(\tau_f\) remains practically constant in all solvents (see Table 1). If one takes into account that the solubility of P18 in ethanol and toluene is significantly lower than that in pyridine, this...
observation may be due to the partial aggregation of P18 in ethanol and toluene at 293 K. The same situation cannot be excluded in ethanol–ether mixtures used in Ref. [37] for the same compounds. It should be noted that for chlorins and chlorophyll-type molecules aggregation does not significantly change the spectral properties of solutions in some cases but does lead to fluorescence quenching [38,39]. In our case, at 77 K the fluorescence of P18 in ethanol and toluene decreases by about 100 times. So, we stress that for P18 aggregation effects must be taken into consideration when using different solvents.

In this regard, P18-Me, having a higher solubility than P18, is characterized by the same parameters of fluorescence in toluene as for P18 in pyridine (see Table 1). So, P18-Me may be considered as a potential sensitizer for PDT purposes. Below we discuss its efficiency of singlet oxygen generation.

Finally, weak phosphorescence of P18 in pyridine and of P18-Me in toluene has been observed at 77 K (see Table 1 and Fig. 2(a)). The assignment of this emission to P18-Me has been established by the coincidence of the phosphorescence spectrum with the absorption spectrum of the corresponding compound. Phosphorescent data permitted us to estimate directly the energy of the triplet states.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$\lambda_{max}$ (nm)</th>
<th>Absorbance at 293 K</th>
<th>Fluorescence at 293 K</th>
<th>Fluorescence at 77 K</th>
<th>Phosphorescence at 77 K</th>
<th>$\Delta E_{00}$ (cm$^{-1}$)</th>
<th>$\phi_p$ at 293 K</th>
<th>$\tau_p$ at 293 K</th>
<th>$\phi_p$ at 77 K</th>
<th>$\tau_p$ at 77 K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $p_n$-Me$_3$</td>
<td>Toluene</td>
<td>674</td>
<td>683</td>
<td>680</td>
<td>910</td>
<td>3720</td>
<td>0.19</td>
<td>3.8</td>
<td>0.75</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>Chl $p_n$</td>
<td>Ethanol</td>
<td>664</td>
<td>672</td>
<td>670</td>
<td>898</td>
<td>3790</td>
<td>0.10</td>
<td>3.2</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td>Chl $e_o$</td>
<td>Ethanol</td>
<td>663</td>
<td>668</td>
<td>664</td>
<td>886</td>
<td>3770</td>
<td>0.13</td>
<td>4.4</td>
<td>0.9</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>(Chl $e_o$)Na$_2$</td>
<td>Ethanol</td>
<td>663</td>
<td>668</td>
<td>666</td>
<td>888</td>
<td>3750</td>
<td>0.15</td>
<td>4.3</td>
<td>1.2</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>(Chl $e_o$)K$_2$</td>
<td>Ethanol</td>
<td>664</td>
<td>670</td>
<td>665</td>
<td>900</td>
<td>3920</td>
<td>0.12</td>
<td>4.5</td>
<td>1.0</td>
<td>1.2</td>
<td></td>
</tr>
</tbody>
</table>

The fluorescence spectrum of Chl $p_n$ is essentially a single band (see Fig. 2(b)). As one can see from Table 1 the fluorescence quantum yield of Chl $p_n$ in ethanol is lower by 1.5–2.0 times in comparison with the analogous values measured for chlorins in different systems [34,36,40]. As with P18 (see Section 3.1.1) we believe that the decrease in $\phi_p$ in ethanol is connected with the partial aggregation of the pigment which is due to the low solubility of this compound. For Chl $p_n$–Me$_3$ which has a better solubility in ethanol and toluene in comparison with Chl $p_n$, the fluorescence quantum yield is near 0.2 (see Table 1).

A noteworthy feature of Chl $p_n$–Me$_3$ is that its absorption $Q(0,0)$ band maximum in non-polar solvents ($\lambda_{max}$ = 674 nm) coincides with a generation line of the Kr laser. This feature makes Chl $p_n$–Me$_3$ an attractive possibility for PDT when using laser excitation.

The phosphorescence parameters of Chl $p_n$–Me$_3$ ( $\phi_p$, $\tau_p$) see Table 1) as well as the $S_1$–$T_1$ energy gap seem to be typical for chlorins [36]. Their energetic characteristics resemble the corresponding values of phycophytin $b$ [41].

A spectral polarized study of Chl $e_o$ and its energetic has been performed [36] in which a glassy matrix of diethyl ether–petroleum ether (1:1) at 77 K has been used. In addition, the photodynamic action of this compound in several systems has been investigated [3,34]. Here we compare these data with present results (Table 1). The experimental data known for Chl $e_o$ (see Fig. 2(c)) and observed for its derivatives in this investigation are comprehensively compared with spectral energetic characteristics and photosensitization efficiency for Chl $p_n$ and Chl $p_n$–Me$_3$.

The experimental results given in Table 1 and in Fig. 2 lead to a number of conclusions. The spectral, energetic and kinetic characteristics of luminescence of Chl $e_o$, its salts ((Chl $e_o$)Na$_2$ and (Chl $e_o$)K$_2$) and Chl $p_n$ and Chl $p_n$–Me$_3$
are rather similar. Structural changes (here, the introduction of ionic or ester peripheral substituents) have only a small influence on the photophysics of the compounds being investigated. This conclusion agrees with the conclusions presented in Ref. [35]. However, the nature of the solvent may influence the state of the pigment molecules. In some cases (especially, for ethanol) it leads to aggregation effects. In other cases (for buffer solutions; see Table 1) spectral shifts of absorption and luminescence bands are observed without noticeable aggregation phenomena. The last observation for Chl $e_c$ is mentioned in Refs. [34,40,42] and is attributed to changes in dielectric constant in the medium surrounding the chromophore.

### 3.1.3. Tetraphenylporphyrin derivatives

As will be shown below, TPPM (with methoxy substitution) and TPPMF (with additional 2,4-difluoro substitution, Fig. 1) are of interest in PDT owing to their high efficiency of singlet oxygen formation. This led us to compare their spectral and luminescence parameters, given in Fig. 3 and Table 1, with data for various halogen-substituted TPP compounds [43-46]. It must be noted that the spectral parameters of TPPM practically coincide with those for TPP. At equal intensities of the fluorescence vibronic Q(0,1) bands, the electronic Q(0,0) band intensity for TPPM is 1.4 times lower than the corresponding band intensity for TPP. Furthermore, photophysical properties of TPPM ($\phi_h$, $\tau_i$, and $\tau_0$) do not differ considerably from the same values for TPP. So, the introduction of OMe groups at the meta position of the phenyl rings does not markedly influence the $\pi$ electronic system of the main chromophore. The changes in electronic spectra when moving from TPP to TPPM are in good agreement with our previous results [45], which have shown that para-methoxy substitution, and fluoro substitution at the meta and ortho positions of the phenyl ring, resulted in a noticeable weakening of the intensity of the long-wavelength transition Q(0,0) band in the absorption spectrum. It should be noted that the weakening of the intensities of the Q(0,0) and Q(1,0) bands in absorption ("phyllotype spectrum") has been observed previously [43,45]. The observed spectral features in this case have been attributed to the steric interaction of the halogen atom with the pyrrole ring. This leads, on the one hand, to increased difficulty in the rotation of the benzene rings around the $\pi$-C-C- bond and, on the other hand, to an increase in the energy of the highest occupied molecular orbital $\epsilon_m$ (for symmetry group $D_{5h}$).

The same situation is observed in fluoro-substituted TPPMF (see Fig. 3(b)) where the intensity of the long-wavelength Q(0,0) band decreases almost fourfold relative to TPP. As a result, the fluorescence quantum yield of TPPMF is half that for TPP at the same fluorescence probability. We suppose that the decrease in fluorescence efficiency is due to the absolute decrease in the Q(0,0) transition intensity.

The experimental results obtained for TPPMF show that the incorporation of fluorine atoms at para and ortho positions of the phenyl ring does not cause a change in the intersystem crossing probabilities. However, it has been shown that para-Cl substitution in TPP reduces the phosphorescence lifetime and increases the phosphorescence quantum yield [45]. Thus in the case of TPPMF one should assume that the phenyl rings are rotated by a large angle (about 60°) relative to the $\pi$ electronic macrocycle and do not take an effective part in conjugation.

### 3.2. Interaction with molecular oxygen

#### 3.2.1. Chlorins and their derivatives

Table 2 summarizes the main results obtained for the compounds under consideration when interacting with molecular oxygen. The rate constants $k_s$ and $k_t$ of singlet and triplet state quenching by molecular oxygen for Chl $p_{n}$ in ethanol are practically the same as the corresponding values for Chl $e_c$. It should be mentioned that the rate constants $k_s$ for these compounds are practically the same as the diffusionally controlled values of $k_{diff}$ in this solvent. At the same time the experimental ratio $\beta = k_t/k_s = 0.22$ for these two compounds in ethanol is noticeably higher than the spin statistical factor $g_s = 1/9$, taking into account the spin states of sensitizer molecule and molecular oxygen. The last fact may be explained by the inclusion, in the quenching of T states by molecular oxygen, of the other spin states of the collision complex ($k_{1,1}$) which are not connected with singlet oxygen generation. For example, the following process may occur:

$$\begin{align*}
3^1M_1 + O_2 & \xrightarrow{\kappa_{1,1}} 3^1M_1 \cdots O_2 \xrightarrow{\kappa_{int}} 3^1M_n + O_2
\end{align*}$$

where $g_s = 1/3$ is the spin statistical factor, $k_{diff}$ is the diffusion rate constant, and $k_{1,1}$ is the rate constant of complex dissociation.

However, the high values of the singlet oxygen generation quantum yield as well as the observed non-dependence of $\phi_A$ and the rate constant $k_t$ of triplet state quenching on the medium polarity for Chl $e_c$ (ethanol, toluene; see Table 2) do not support such a possibility. The same situation has been observed for chlorophyll-type molecules [26] and has been explained as a result of the increasing probability $k_s$ of the spin-forbidden transition between triplet and singlet states of the collision complex:

$$\begin{align*}
3^1M_1 + O_2 & \xrightarrow{\kappa_{1,1}} 3^1M_1 \cdots O_2 \xrightarrow{\kappa_{int}} 3^1M_n + O_2 + \Delta_\kappa
\end{align*}$$

where $\Delta_\kappa = 1/3$ is the spin statistical factor, $k_{diff}$ is the diffusion rate constant, and $k_{1,1}$ is the rate constant of complex dissociation. It should be stressed that such a transition is spin forbidden and is not generally observed for aromatic molecules. The energy gap between the sensitizer $T_1$ state and the oxygen $1\Delta \kappa$ state is not so large for chlorophyll-type molecules. Hence, this transition is characterized by a higher value of the Franck-Condor factor which leads to an increase in the non-radiative transition probability. Moreover, in this case the existence of a low-lying charge transfer state, which may also influence $k_s$, is not excluded. On salt formation (from Chl $e_c$ to (Chl $e_c$)K$^+$ or (Chl $e_c$)Na$^+$) we do not observe...
Table 2
Photophysical properties and parameters of interaction of the compounds with molecular oxygen

<table>
<thead>
<tr>
<th>Compound</th>
<th>Solvent</th>
<th>$\tau_\text{T}$ (ns)</th>
<th>$k_\text{r} \times 10^{-9}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$k_\text{a} \times 10^{-9}$ (M$^{-1}$ s$^{-1}$)</th>
<th>$\beta = \frac{k_\text{r}}{k_\text{a}}$</th>
<th>$\phi_\text{A}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chl $e_6$</td>
<td>Ethanol</td>
<td>290</td>
<td>2.1</td>
<td>9.4</td>
<td>0.22</td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>255</td>
<td>2.2</td>
<td>0.68 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Chl $e_6$)Na$_3$</td>
<td>Ethanol</td>
<td>305</td>
<td>2.0</td>
<td>0.68 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Chl $e_6$)K$_3$</td>
<td>Ethanol</td>
<td>260</td>
<td>2.3</td>
<td>9.3</td>
<td>0.24</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>Chl $p_6$</td>
<td>Ethanol</td>
<td>255</td>
<td>2.4</td>
<td>9.9</td>
<td>0.24</td>
<td>0.60 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.35 ± 0.05</td>
</tr>
<tr>
<td>Chl $p_6$-Me$_3$</td>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.65 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.79 ± 0.07</td>
</tr>
<tr>
<td>P18</td>
<td>Ethanol</td>
<td>195</td>
<td>3.1</td>
<td>0.45 ± 0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>250</td>
<td>2.2</td>
<td>0.70 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.75 ± 0.07</td>
</tr>
<tr>
<td>P18-Me</td>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.55 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.73 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.80 ± 0.08</td>
</tr>
<tr>
<td>TPP</td>
<td>Toluene</td>
<td>400</td>
<td>1.4</td>
<td>11.0</td>
<td>0.127</td>
<td>0.68 ± 0.06</td>
</tr>
<tr>
<td>GTPP</td>
<td>Ethanol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.95 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.85 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>Toluene</td>
<td>545</td>
<td>1.05</td>
<td>0.85 ± 0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acetonitrile</td>
<td>685</td>
<td>0.9</td>
<td>0.98 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPPM</td>
<td>Toluene</td>
<td>300</td>
<td>1.85</td>
<td>15.8</td>
<td>0.117</td>
<td>0.87 ± 0.08</td>
</tr>
<tr>
<td>TPPMF</td>
<td>Toluene</td>
<td>300</td>
<td>1.4</td>
<td>12.7</td>
<td>0.112</td>
<td>0.87 ± 0.08</td>
</tr>
</tbody>
</table>

* Taken from Refs. [25,32].
* These values have been determined by E.I. Sagan and B.M. Drzagurov.

noticeable changes in rate constants $k_\text{r}$ and $k_\text{a}$, but only a slight increase (about 5%) in the singlet oxygen generation quantum yield. A small increase in the ratio $\beta = 0.24$ for (Chl $e_6$)K$_3$ in this case may be connected with the lowering of the energy of its $T_1$ level. The position of the phosphorescence maximum of this compound and the shortening of its $\tau_p$ (see Table 1) support such an explanation.

It must be noted that Chl $p_6$ is characterized by its lower solubility in comparison with Chl $e_6$ in the solvents being used. For instance, Chl $p_6$ has a small solubility in pyridine and it is scarcely soluble in toluene. However, in ethanol, where its solubility is relatively high, the photophysical parameters of this compound ($k_\text{r}$, $k_\text{a}$, $\phi_\text{A}$) are practically the same as the analogous parameters of Chl $e_6$ (see Table 2). In this connection, the low value of the singlet oxygen generation quantum yield for Chl $p_6$ in pyridine ($\phi_\text{A} = 0.35 ± 0.05$) may be attributed to its low solubility and to aggregation effects.

In contrast to Chl $p_6$, the ester Chl $p_6$-Me$_3$ has a rather good solubility in all the solvents used and is characterized by higher $\phi_\text{A}$ values in corresponding media compared with Chl $e_6$ and Chl $p_6$, (by approximately 5%-10%). For the purposes of singlet oxygen generation, Chl $p_6$-Me$_3$ exhibits the best activity of this group of compounds.

3.2.2. Purpurins

As mentioned above the use of a long-wavelength band of the sensitizer is desirable because of the better tissue penetration of exciting light in vivo. Purpurin and its analogues may be considered as promising candidates in this respect. The bathochromic shift of the long-wavelength absorption band of P18 and P18-Me (see Table 1) in comparison with Chl $e_6$ reveals a noticeable lowering of the energies of their $S_1$ levels. In accordance with well-known photophysical correlations one would expect the simultaneous lowering of the $T_1$ levels and an increase in the probabilities of non-radiative $T_1 → S_0$ transitions resulting in a diminishing of quantum efficiency of singlet oxygen generation by these molecules.

Nevertheless Table 1 shows that the triplet lifetimes $\tau_\text{T}$ of P18 and P18-Me at 77 K ($\tau_\text{T} = 1.8 \times 10^{-3}$ s) are similar to the corresponding values for chlorins. This means that, on the one hand, the purpurins being investigated are not characterized by higher values of the non-radiative intersystem crossing $T_1 → S_0$ probability $k_\text{u}$. On the other hand, the long triplet lifetimes of these compounds facilitate the high probability of diffusional collision of P18 and P18-Me triplet molecules with oxygen saturated solutions. It must be noted that in liquid solution the small value for P18 $\tau_\text{T}$ and the high rate constant $k_\text{r}$ (see Table 2) points to the high quenching efficiency of the compound by molecular oxygen. In addition, the high values of quantum yields of singlet oxygen yields of singlet oxygen generation by P18 in pyridine and toluene resemble the same sensitizers for Chl $p_6$-Me$_3$. Table 2 shows also that, under the same conditions, the reduced derivative of P18, P18-Me, exhibits a slightly higher (by 5%-6%) value of $\phi_\text{A}$, and from this point of view P18-Me might be a better potential photosensitizer for cancer therapy than P18. However, in ethanol we obtained lower values of the singlet oxygen generation...
quantum yield ($\phi_s = 0.45-0.55$) for P18 and P18-Me. As discussed earlier, this effect may be due to the low solubility of these compounds in solution and consequent aggregation phenomena.

### 3.2.3. Tetraphenylporphyrin derivatives

Let us consider the third group of the compounds being investigated (TPPM, and GaTPP). Various derivatives of TPP have been investigated and used widely as photosensitizers of singlet oxygen [47–49]. It has been shown that the photophysical properties and the efficiency of singlet oxygen depletion depend strongly on the nature of the central metal ion and the character of the peripheral substituents. For instance, for halogen-substituted TPP derivatives (para-F, –Cl, –Br and –I) [32] increasing the atomic number ($Z$) of the halogen atom the $\phi_s$ value becomes larger, being $0.65 \pm 0.06$ for TPP(para-F$_2$) up to $0.97 \pm 0.03$ for TPP(para-I$_2$). This effect is explained by the strengthening of spin-orbital interaction according to the heavy atom perturbation in the sensitizer molecule. In addition, it has been established that $\phi_s$ depends not only on the $Z$ value but is sensitive to the position of the halogen in the phenyl ring relative to $\pi$ generated system of porphyrin molecule, being 0.71 for TPP(para-Cl)$_2$, 0.82 for TPP(meta-Cl)$_2$, and 0.84 for TPP(ortho-Cl)$_2$. Moreover, in the same group of compounds the $k_r$ values decrease. This effect has been related to the increase in porphyrin oxidation potential and the decrease in the contribution of donor-acceptor interactions in the quenching process. Finally, it has been found that incorporating the electron donor amino group (NH$_2$) in the meso position of the porphyrin molecule increases $k_r$ and $\phi_s$ values whereas the electron acceptor meso-nitro group (NO$_2$) reduces singlet oxygen generation quantum yields [32].

Taking into account the results outlined above we can analyse the experimental data summarized in Table 2. It is seen that the incorporation of the electron donor methoxy group OCH$_3$ in the meta positions of the phenyl rings of the TPP molecule (in the case of TPPM) results in the increase in rate constants $k_r$ and $k_t$ as well as increasing the $\phi_s$ value. Incorporation of the electron acceptor F atoms in TPPM (giving TPFPMF) is accompanied by a small decrease in the $k_r$ and $k_t$ whereas the $\phi_s$ value scarcely changes. The same influence of F atoms on $\phi_s$ values has been noted earlier [32]. This effect may be related to a relatively small heavy atom perturbation ($Z = 9$ for F atom). For the compounds of interest the observed dependence of quenching rate constants on the sensitizer oxidation potential may be considered as supporting a quenching mechanism due to a donor–acceptor interaction [50].

Table 1 shows that the incorporation of the electron-donating –OCH$_3$ group into TPP (to give TPPM) results in the shortening of $\tau_p$ at 77 K, whereas the incorporation of electron-attracting F atoms (to give to TPFPMF) leads to longer $\tau_p$ values again. The analogous situation has been observed earlier [29] and has been connected with the change in local electron density on the central nitrogen atoms and the corresponding change in the N–H vibrations.

GaTPP is characterized by high solubility and appreciable photochemical stability. The experimental results show that in accordance with "the heavy atom effect", GaTPP has a relatively high quantum yield of $^1\Delta_g$ generation ($\phi_s = 0.85-0.97$) in all solvents being used. This compound is an effective sensitizer of singlet oxygen.

The direct estimation shows that for TPPM and TPFPMF the ratio $\beta = k_r/k_t$ in toluene (see Table 2) is close to the spin statistical factor $g_s = 1/9$. This fact, together with the high values of $\phi_s$, indicates that the quenching of the triplet states of these compounds occurs by the mechanism of singlet oxygen generation exclusively:

$$\text{M}_1 + ^1\text{O}_2 \xrightarrow{k_{\text{abs}}^+} [\text{M}_1 \cdots ^1\text{O}_2] \xrightarrow{k_{\text{esc}}} ^1\text{M}_0 + ^1\text{O}_2 (^1\Delta_g)$$

### 4. Conclusions

The results of the comprehensive study of optical and photophysical parameters of the compounds under consideration as well as the detailed mechanisms of their interaction with molecular oxygen lead to the following conclusions.

1) The quantum yield of the photosensitized generation of singlet oxygen by Chl $p_h$ in ethanol coincides with the same parameter for Chl $n$.

2) The singlet oxygen formation quantum yield $\phi_s$ for chlorins and purpurins does not appear to depend markedly on medium polarity. The decrease in $\phi_s$ and $\phi_s$ values in some cases is presumed to be due to the low solubility of the compounds and corresponding aggregation effects.

3) Esters of Chl $p_h$ and P18 (Chl $p_h$–Me$_1$ and P18–Me respectively), having higher solubility in comparison with the parent acids, are characterized by slightly higher $\phi_s$ values (by about 5%–10%). This property, combined with strong absorption bands in the red region (660–700 nm), makes these molecules potential candidates for biological photosensitization.

4) For TPPM, TPFPMF and GaTPP photophysical properties depend strongly on the nature of substituents, and these compounds are highly effective photosensitizers of molecular oxygen in solution.

### Acknowledgements

This research was supported by the INTAS Project (Grant 93-2810), the National Foundation for Basic Research of Belarus (Grant Ph5-111), the International Science Foundation (Grants RW 1000 and MD 2300), and the UN Education and Training Programme for Southern Africa.
The authors would like to thank S.M. Bachilo and N.N. Kruk for the measurements described in the first paragraph of Section 2.3.

References


