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

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Abstract

Structural and optical properties as well as photophysical and photochemical parameters (excited S_1 and T_1 state lifetimes at 77 K and in the presence of O_2 in solution at 293 K; efficiencies of singlet oxygen, $^1\Delta_g$, generation) are presented for porphyrins and chlorins with potential for the PDT of cancer: chlorin p_6 and its trimethyl ester, chlorin e_6 and its Na_3 and K_3 salts, purpurin-18 and its monomethylester, 5,10,15,20-tetrakis(3-methoxyphenyl)porphyrin (TPPM), 5,10,15,20-tetrakis(2,4-difluoro-3-methoxyphenyl)porphyrin (TPPMF) and GaTPP in different solvents (ethanol, toluene, pyridine and buffer pH 7.4) at 77–300 K. It has been shown that for monomeric chlorin e_6, chlorin p_6 and its derivatives the photophysical parameters are similar, as follows: fluorescence lifetimes τ_f in the presence of oxygen are 3.2–4.5 ns at 293 K; fluorescence quantum yields ϕ_f vary from 0.1 to 0.2 depending on the solvent; phosphorescence quantum yields ϕ_f are of the order 10^{-5}; T_1 state lifetimes $\tau_T = 1.5$–2.0 ms at 77 K and 250–390 ns at 293 K in the presence of O_2.

By use of the direct kinetic measurement of singlet oxygen emission at 1.27 μm on laser excitation the quantum yields of $^1\Delta_g$ generation by chlorins have been measured: $\phi_\Delta = 0.35$–0.68. In this case values of ϕ_f and ϕ_Δ depend strongly on the solvent, probably because of the formation of aggregates. For TPPM, TPPMF and Ga-TPP the ϕ_Δ values measured are higher (0.87–0.98) and are explained by the higher intersystem crossing $S_1 \rightarrow T_1$ 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 fulfil 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. This criterion arises because, owing to lower scattering and to weak absorption by natural chromophores, red light penetrates tissue much better than do the other parts 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

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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 p_6 (Chl p_6) 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 new compounds in different solvents with the analogous parameters known for Chl e_6.

2. Materials and methods

2.1. Materials

Chlorophyll *a* has been isolated from plant materials by well-known methods [19] and has been converted chemically into P18 and Chl e_6 or Chl p_6 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 τ_s 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 $\phi_f \geq 0.1$, and the limit of emission quantum yield ϕ_f 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 ($\phi_f = 0.09$ 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

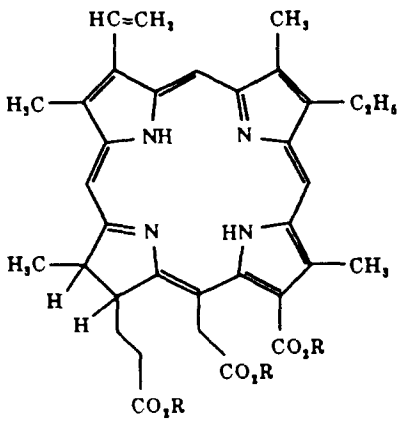
Spectra, kinetics and quantum yields of singlet oxygen emission were obtained using a laboratory experimental set-up, described in detail in Refs. [26–28]. The samples were excited by the pulses of the second harmonic of an AlG:Nd^{3+} laser ($\lambda = 532$ nm) or ruby laser ($\lambda = 347$ nm) with 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 filament 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 ϕ_Δ of the sensitized production of singlet oxygen is not trivial.

Usually ϕ_Δ 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 ϕ_Δ 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 ($\lambda = 1.27$ μm). 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 ϕ_Δ 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 ϕ_Δ^x 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_x) and by the standard (intensity I_0):

$$\phi_\Delta^x = \phi_\Delta^0 \frac{I_x}{I_0} \frac{\beta_0}{\beta_x} \quad (1)$$

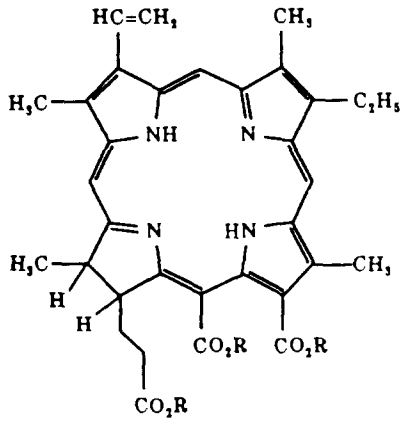
where ϕ_Δ^0 is the quantum yield of singlet oxygen formation for the standard, and $\beta_x = 1 - 10^{D_x}$ and $\beta_0 = 1 - 10^{-D_0}$ are the



Chl e_6: R=H

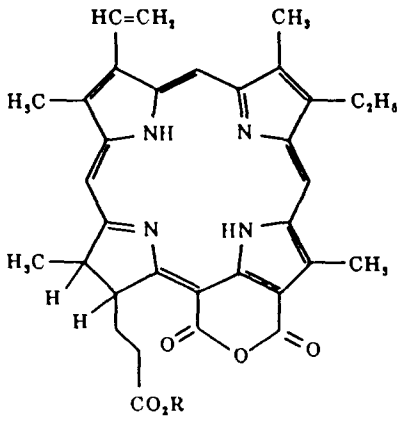
Chl e_6 K_3: R=K

Chl e_6 Na_3: R=Na



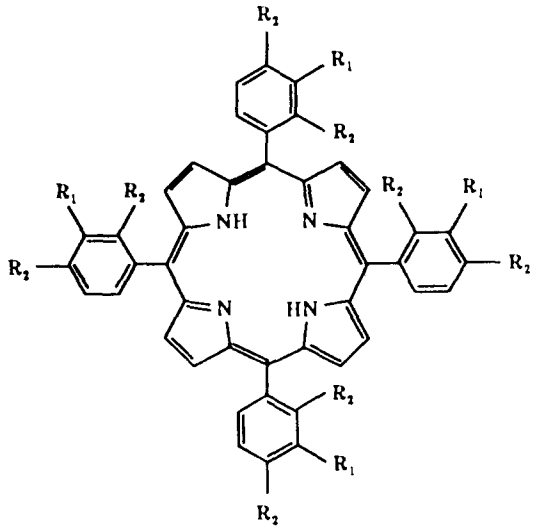
Chl p_6: R=H

Chl p_6-$(OMe)_3$: R=CH_3



P18: R=H

P18-OMe: R=CH_3



TPPM: R_1=OCH_3, R_2=H

TPPMF: R_1=OCH_3, R_2=F

Fig. 1. The structural formulae and acronyms of the purpurins, chlorins and tetraphenylporphyrins studied here.

absorbed exciting light portions at excitation wavelength for the investigated and standard compounds respectively. I_x 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 ϵ values were taken from Refs. [26,31]. Various compounds were used as standards: (i) chlorophyll a ($\phi_\Delta^0 = 0.6$ [26]) and octaethylporphyrin ($\phi_\Delta^0 = 0.75$ [32]) in toluene solutions; (ii) methylene blue ($\phi_\Delta^0 = 0.52$ [33]) in ethanol; (iii) Chl e_6 ($\phi_\Delta^0 = 0.74$ [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 ϕ_Δ^x determination did not exceed 10%–15%.

The experimental set-up described permitted us to measure τ_T 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,

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

where τ_T^0 and τ_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,

$$k_s = \frac{(\tau_s)^{-1} - (\tau_s^0)^{-1}}{[O_2]} \quad (3)$$

where τ_s^0 and τ_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 τ_s, $\pm 5\%$ for τ_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 $Q_x(0,0)$ electronic transition [35]. The maximum of the Soret band is observed at 408 nm (see Fig. 2(a)). The ratio $A(\text{Soret})/A(Q_x) = 2.7$ reflects a situation which is typical for tetrapyrrole 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 $Q_x(0,0)$ 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 $Q_x(0,0)$ 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 ϕ_f of P18 is strongly dependent on the solvent, changing from 0.08 in ethanol to

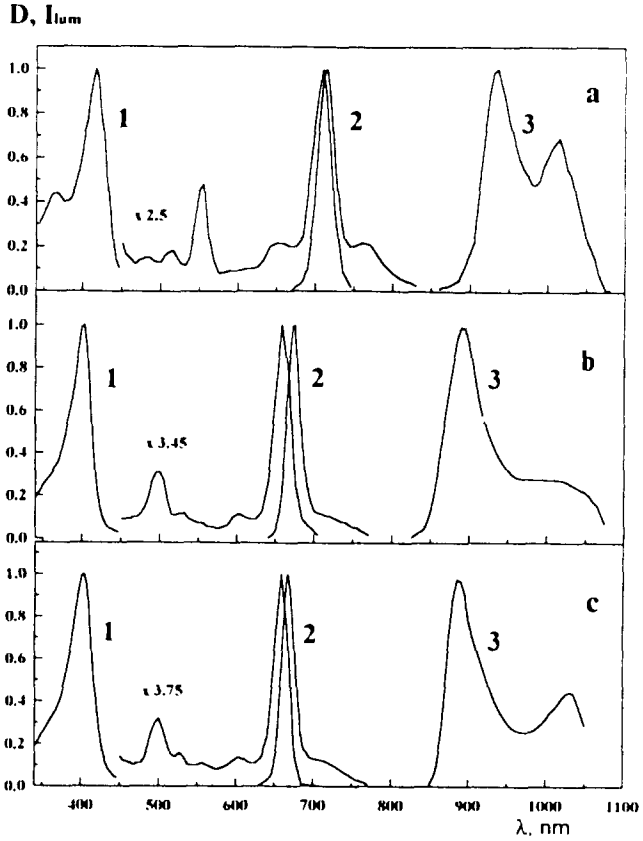


Fig. 2. Absorption (spectra 1; 293 K) and corrected fluorescence (spectra 2; 293 K) and phosphorescence (spectra 3; 77 K) spectra of (a) P18 in pyridine and (b) Chl p_6 and (c) Chl e_6 in ethanol.

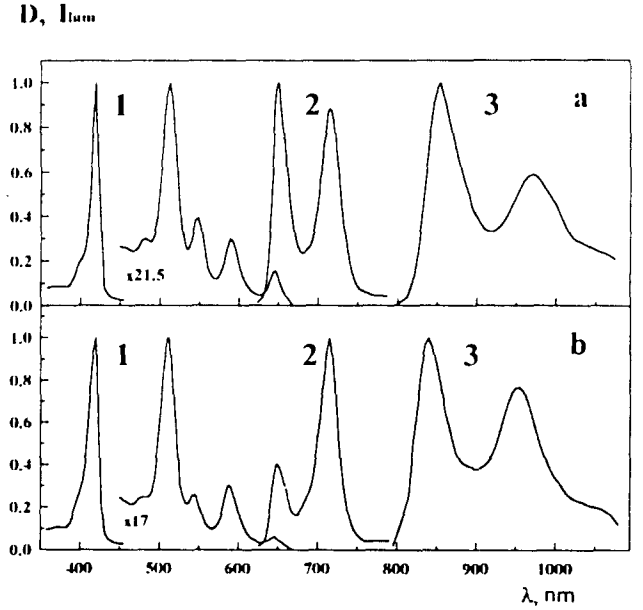


Fig. 3. Absorption (spectra 1; 293 K) and corrected fluorescence (spectra 2; 293 K) and phosphorescence (spectra 3; 77 K) spectra of (a) TPPM and (b) TPPMF in toluene.

0.13 in pyridine. Nevertheless the fluorescence lifetime τ_s 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

Table 1
Spectral luminescent parameters of chlorins, purpurins and tetraphenylporphyrin derivatives

Compound	Solvent	λ_{max}				$\Delta E_{S_1-T_1}$ (cm^{-1})	ϕ_f at 293 K	τ_s at 293 K (ns)	ϕ_p at 77 K ($\times 10^{-4}$)	τ_p at 77 K (ms)
		Absorbance at 293 K	Fluorescence at 293 K	Fluorescence at 77 K	Phosphorescence at 77 K					
Chl p_6–Me_3	Toluene	674	683	680	910	3720	0.19	3.8	0.75	1.1
Chl p_6	Ethanol	664	672	670	898	3790	0.10	3.2	1.0	1.2
Chl e_6	Ethanol	663	668	664	886	3770	0.13	4.4	0.9	1.6
(Chl e_6)Na_3	Ethanol	663	668	666	888	3750	0.15	4.3	1.2	1.4
(Chl e_6)K_3	Ethanol	664	670	665	900	3920	0.12	4.5	1.0	1.2
Chl p_6	Buffer, pH 7.4	656	664				0.17	3.3		
Chl e_6	Buffer, pH 7.4	654	662				0.18	3.7		
(Chl e_6)Na_3	Buffer, pH 7.4	654	662				0.22	3.7		
(Chl e_6)K_3	Buffer, pH 7.4	656	662				0.16	3.6		
P18	Ethanol	700	712				0.095	2.4		
	Toluene	704	711				0.08	2.6		
	Pyridine	707	714	712	930	3290	0.13	2.6	2.9	1.7
P18–Me	Toluene	702	710	709	917	3200	0.11	2.6	2.8	1.8
TPP	Toluene	648	653	645	859	3860	0.09	10.7	6.7	5.8
TPPM	Toluene	648	652	645	858	3850	0.08	9.7	5.3	5.3
TPPMF	Toluene	645	648	640	840	3720	0.045	10.0	5.5	7.2

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 or P18–Me has been established by the coincidence of the phosphorescence excitation spectra with the absorption spectra of the corresponding compounds. Phosphorescent data permitted us to estimate directly the energy of the triplet states.

3.1.2. Chlorins

Comparison of Figs. 2(a) and 2(b) shows that on moving from P18 (or P18–Me) to Chl p_6 (or Chl p_6–$(Me)_3$) the $Q_x(0,0)$ band in the absorption spectrum shifts to the short-wavelength region by 36 nm. The vibrational structure of the first $Q_x(0,0)$ electronic transition practically disappears. The intensity ratio $A(\text{Soret})/A[Q_x(0,0)]$ is approximately the same as for P18. However, the relative intensity of the $Q_x(0,0)$ transition is drastically decreased and its position must be at 530 nm, according to polarized fluorescence measurements on Chl e_6 [36].

The fluorescence spectrum of Chl p_6 is essentially a single band (see Fig. 2(b)). As one can see from Table 1 the fluorescence quantum yield of Chl p_6 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 ϕ_f in ethanol is connected with the partial aggregation of the pigment which is due to the low solubility of this compound. For Chl p_6–Me_3 which has a better solubility in ethanol and toluene in comparison with Chl p_6, the fluorescence quantum yield is near 0.2 (see Table 1).

A noteworthy feature of Chl p_6–Me_3 is that its absorption $Q_x(0,0)$ band maximum in non-polar solvents ($\lambda_{max} = 674$ nm) coincides with a generation line of the Kr laser. This feature makes Chl p_6–Me_3 an attractive possibility for PDT when using laser excitation.

The phosphorescence parameters of Chl p_6–Me_3 (ϕ_p, τ_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 pheophytin *b* [41].

A spectral polarized study of Chl e_6 and its energetics 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_6 (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_6 and Chl p_6–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_6, its salts ((Chl e_6)Na_3 and (Chl e_6)K_3) and Chl p_6 and Chl p_6–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_6 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 (ϕ_f, τ_f, and τ_p) 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 π electronic system of the main chromophore. The changes in electronic spectra when moving from TPP to TPPM are in a 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 $Q_x(0,0)$ and $Q_y(0,0)$ bands in absorption ("phyllo-type 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 –C–C– bond and, on the other hand, to an increase in the energy of the highest occupied molecular orbital a_{1u} (for symmetry group D_{4h}).

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 π 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_6 in ethanol are practically the same as the corresponding values for Chl e_6. It should be mentioned that the rate constants k_s for these compounds are practically the same as the diffusionally controlled values of k_{dif} 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 $g_t = 1/9$, 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_{T_x}) which are not connected with singlet oxygen generation. For instance, the following process may occur:

$$^3M_1 + {}^3O_2 \underset{k_{dis}}{\overset{g_t k_{dif}}{\rightleftharpoons}} {}^3[{}^1M_1 \cdots {}^3O_2] \xrightarrow{k_{T_x}} {}^3[{}^1M_0 \cdots {}^3O_2] \xrightarrow{k_{dis}} {}^1M_0 + {}^3O_2 \quad (4)$$

where $g_t = 1/3$ is the spin statistical factor, k_{dif} is the diffusion rate constant, and k_{dis} 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 ϕ_Δ and the rate constant k_T of triplet state quenching on the medium polarity for Chl e_6 (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_x of the spin-forbidden transition between triplet and singlet states of the collision complex:

$$^3[{}^3M_1 \cdots {}^3O_2] \xrightarrow{k_x} {}^1[{}^1M_0 \cdots {}^1O_2({}^1\Delta_g)] \quad (5)$$

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 $^1\Delta_g$ state is not so large for chlorophyll-type molecules. Hence, this transition is characterized by a higher value of the Franck–Condon 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_x, is not excluded. On salt formation (from Chl e_6 to (Chl e_6)K_3 or (Chl e_6)Na_3) we do not observe

Table 2
Photophysical properties and parameters of interaction of the compounds with molecular oxygen

Compound	Solvent	τ_T (ns)	$k_T \times 10^{-9}$ ($M^{-1} s^{-1}$)	$k_s \times 10^{-9}$ ($M^{-1} s^{-1}$)	$\beta = \frac{k_T}{k_s}$	ϕ_Δ
Chl e_6	Ethanol	290	2.1	9.4	0.22	0.65 ± 0.06
	Toluene	255	2.2			0.61 ± 0.06
(Chl e_6)Na_3	Ethanol	305	2.0			0.68 ± 0.06
(Chl e_6)K_3	Ethanol	260	2.3	9.3	0.24	0.68 ± 0.06
Chl p_6	Ethanol	255	2.4	9.9	0.24	0.60 ± 0.06
	Pyridine					0.35 ± 0.05
Chl p_6–Me_3	Ethanol					0.69 ± 0.06
	Toluene					0.65 ± 0.06
	Pyridine					0.79 ± 0.07
P18	Ethanol	195	3.1			0.45 ± 0.10
	Toluene	250	2.2			0.70 ± 0.07
	Pyridine					0.75 ± 0.07
P18–Me	Ethanol					0.55 ± 0.10
	Toluene					0.73 ± 0.07
	Pyridine					0.80 ± 0.08
TPP	Toluene [a]	400	1.4	11.0	0.127	0.68 ± 0.06
GaTPP	Ethanol					0.95 ± 0.05
	Pyridine					0.85 ± 0.08
	Toluene [b]	545	1.05			0.85 ± 0.08
	Acetonitrile [b]	685	0.9			0.98 ± 0.02
TPPM	Toluene	300	1.85	15.8	0.117	0.87 ± 0.08
TPPMF	Toluene	390	1.4	12.7	0.112	0.87 ± 0.08

[a] Taken from Refs. [25,32].
[b] These values have been determined by E.I. Sagun and B.M. Dzhagarov.

noticeable changes in rate constants k_s and k_T, 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 τ_p (see Table 1) support such an explanation.

It must be noted that Chl p_6 is characterized by a 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_s, k_T, ϕ_Δ) 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 ($\phi_\Delta = 0.35 \pm 0.05$) 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 ϕ_Δ 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 $T_1 \rightsquigarrow S_0$ transitions resulting in a diminishing of quantum efficiency of singlet oxygen generation by these molecules.

Nevertheless Table 1 shows that the triplet lifetimes τ_p of P18 and P18–Me at 77 K ($\tau_p = 1.8 \times 10^{-3}$ 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 $T_1 \rightsquigarrow S_0$ probability k_q. 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 τ_T and the high rate constant k_T (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 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 ϕ_Δ, 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 ($\phi_\Delta = 0.45$–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_4, $-Cl_4$, $-Br_4$ and $-I_4$) [32] increasing the atomic number (Z) of the halogen atom the ϕ_Δ value becomes larger, being 0.65 ± 0.06 for TPP(*para*-F_4) up to 0.97 ± 0.03 for TPP(*para*-I_4). 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 ϕ_Δ depends not only on the Z value but is sensitive to the position of the halogen in the phenyl ring relative to π generated system of porphyrin molecule, being 0.71 for TPP(*para*-Cl)$_4$, 0.82 for TPP(*meta*-Cl)$_4$ and 0.84 for TPP(*ortho*-Cl)$_4$. 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 ϕ_Δ 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_q and k_r as well as increasing the ϕ_Δ value. Incorporation of the electron acceptor F atoms in TPPM (giving TPPMF) is accompanied by a small decrease in the k_q and k_r whereas the ϕ_Δ value scarcely changes. The same influence of F atoms on ϕ_Δ 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 τ_p at 77 K, whereas the incorporation of electron-attracting F atoms (to give to TPPMF) leads to longer τ_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 ($\phi_\Delta = 0.85$–0.98) in all solvents being used. This compound is an effective sensitizer of singlet oxygen.

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

$$^3M_t + {}^3O_2 \underset{k_{dis}}{\overset{g_t k_{diff}}{\rightleftharpoons}} {}^1[{}^3M_t \cdots {}^3O_2] \xrightarrow{k_\Delta}$$

$$^1[{}^1M_0 \cdots {}^1O_2(^1\Delta_g)] \xrightarrow{k_{dis}} {}^1M_0 + {}^1O_2(^1\Delta_g)$$

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_6 in ethanol coincides with the same parameter for Chl e_6.

(2) The singlet oxygen formation quantum yield ϕ_Δ for chlorins and purpurins does not appear to depend markedly on medium polarity. The decrease in ϕ_t and ϕ_Δ 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_6 and P18 (Chl p_6–Me_3 and P18–Me respectively), having higher solubility in comparison with the parent acids, are characterized by slightly higher ϕ_Δ 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, TPPMF and GaTPP photophysical properties depend strongly on the nature of substituents, and these compounds are highly effective photosensitizers of molecular oxygen in solution.

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