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Metal chelates of porphyrin derivatives as sensitizers in photooxidation processes of sulfur compounds and in photodynamic therapy of cancer

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A number of porphyrin derivatives based on hematoporphyrin, 5,10,15,20-tetrasubstituted porphyrins, phthalocyanines, and naphthalocyanines were prepared either as low-molecular compounds or bonded with methoxypoly(ethylene glycol) or attached to silica of low surface area. The low-molecular weight and the polymer-bonded porphyrins exhibit comparable triplet lifetimes and activities in the photosensitized formation of singlet oxygen. For photon-induced processes, the monomeric state of sensitizers is fundamentally important. The porphyrins have been investigated as sensitizers for photooxidation of thiolates and sulfides, which occurs *via* singlet oxygen, and, therefore, is much more efficient than the corresponding catalytic dark oxidation. Polymer-bonded porphyrins and long-wavelength absorbing naphthalocyanines incorporated in liposomes exhibit *in vivo* high accumulation in tumor tissues. Under irradiation, singlet oxygen is produced, and efficient phototherapeutic effects are observed, which may be used for photodynamic cancer therapy.

Key words: metal chelates of porphyrin derivatives; photodynamic therapy of cancer.

Low-molecular-weight, polymeric, and polymer-embedded porphyrins, such as hematoporphyrin (**1**), 5,10,15,20-tetraphenylporphyrin (**2**), phthalocyanine (**3**), and naphthalocyanine (**4**)[1,2] are being intensively investigated because of their role in a number of visible light-driven processes, for example, in organic solar cells,[3,4] organic photoconducting devices,[5] photoelectrochemical cells,[6–10] and in photochemical reactions in solutions,[1,11–13] and also because of their use in the photodynamic therapy of cancer.[1,14–17] Basic investigations of reactions occurring in solutions under the action of visible light photons are of great importance for the analysis of various processes involving the conversion of matter and energy, for environmental protection, and for medicine.

This paper summarizes some results of our recent studies on employing porphyrin derivatives as sensitizers in photochemical reactions occurring in solutions under the action of photons of visible light in the presence of oxygen. Porphyrins **1**—**4** absorb visible light in the region of either the Soret band (320—450 nm) or the Q-band (600—800 nm) with high extinction coefficients ($\varepsilon \sim 10^5$ L mol^{-1} cm^{-1}) (Fig. 1).[1,2]

These compounds can be converted into excited states either by irradiation with sunlight or by using an

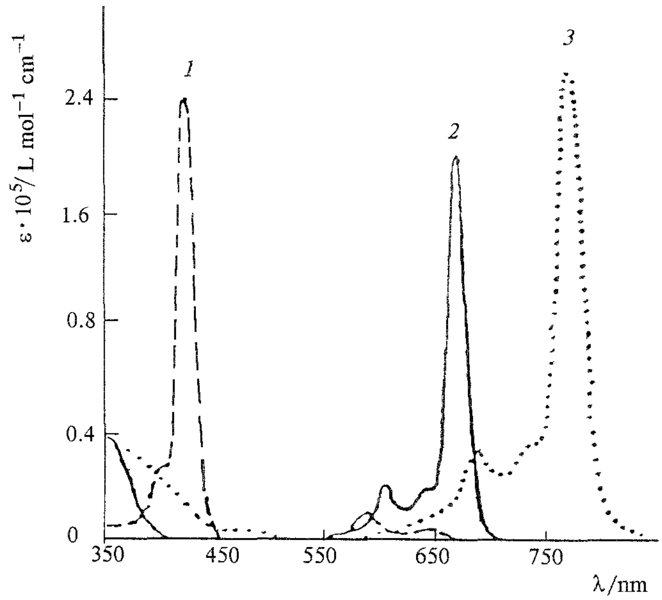

Fig. 1. The VIS spectra of Zn^{II} complexes of 5,10,15,20-tetraphenylporphyrin **2** (*1*), phthalocyanine **3** (*2*), and naphthalocyanine **4** (*3*) in DMF.

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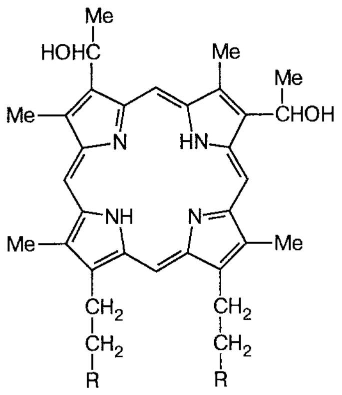
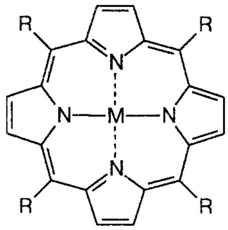


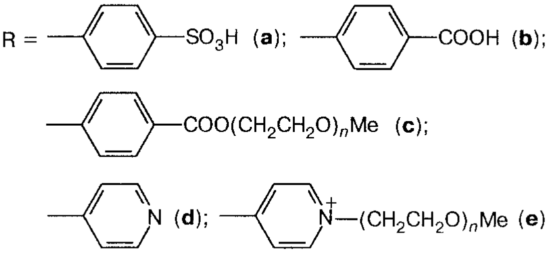
1a,b

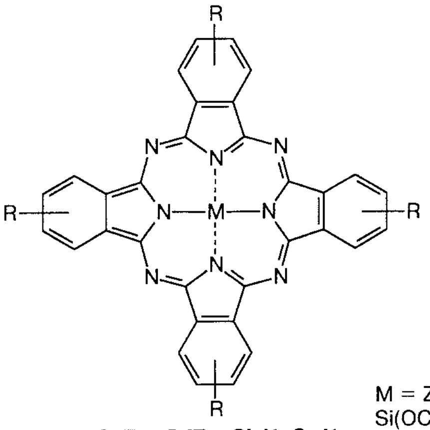
R = COOH (**a**); $COO(CH_2CH_2O)_nMe$ (**b**)



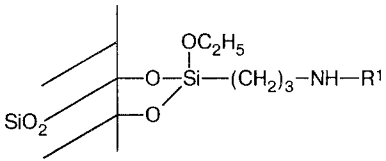
2a—e, **2a**Zn, **2d**Zn, **2e**Zn

M = 2H (**2a—e**); Zn (**2a**Zn, **2d**Zn, **2e**Zn)





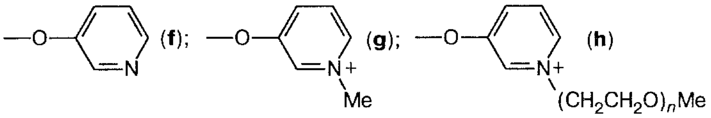
3aZn—3dZn, **3bAl**, **3cAl**, **3fZn—3hZn**, **3iSi**, **3jSi**



3eZn

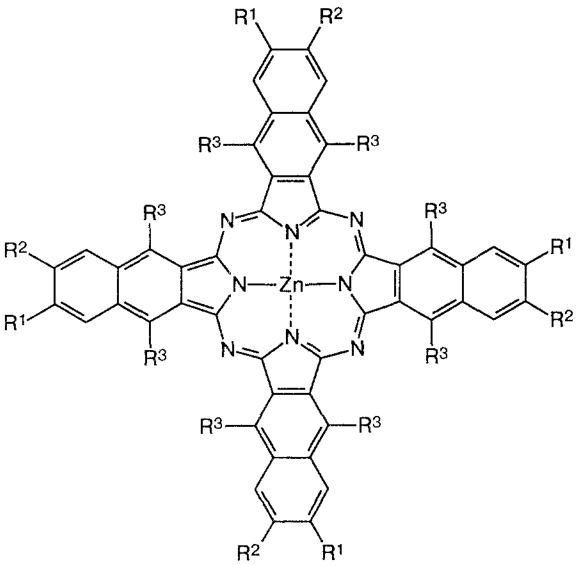
R^1 = —CO(ZnPc(COOH)$_3$)

M = Zn (**3aZn—3dZn**, **3fZn—3hZn**); AlX (**3bAl**, **3cAl**); $Si(OCH_2CH_2OMe)_2$ (**3iSi**); $Si(OCH_2CH_2O)_nMe)_2$ (**3jSi**)
X = OH^-, Cl^-
R = H (**a**,**i**,**j**); SO_3H (**b**); COOH (**c**); $COO(CH_2CH_2O)_nMe$ (**d**);



artificial light source. The proportion of visible light in the solar radiation falling on earth (AM1) is about 50 %, and it is three thousand times greater than the world energy consumption. Therefore, it is very reasonable to use excited states obtained by the action of solar radiation, as is done on an industrial scale in inorganic photochemical current sources (excitation: valence band → conduction band) and in pilot plants for detoxification of water (using TiO_2). In addition, increasing interest is attracted by the chemical conversion of energy (cleavage of water) and by the chemical synthesis of compounds through the action of solar radiation. On the other hand, artificial sources of visible light like halogen or Xenon arc lamps and monochromatic laser systems have been applied to study photochemical reactions on a laboratory scale and find increasing application for the photodynamic therapy of cancer. Chemists engaged in basic research can use both solar radiation and artificial light sources for the optimization of the structure of sensitizers and the efficiency of visible-light-driven reactions and conditions of their use for medical treatment.

Porphyrins such as phthalocyanines **3** incorporating a metal ion with a closed-shell electron configuration (*e.g.*, Zn^{II}, Al^{III}) exhibit great lifetimes and high quantum yields of the triplet excited state (*e.g.* **3**, M = Zn^{II}, $\tau_T^{300\,K} \sim 250$ μsec, $\Phi_T \sim 0.6$).[11—13,18,19] Therefore, after excitation in solution (reaction (1)), these compounds



4a—gZn

R^1 = H (**a,g**); NHCOMe (**b**); NH_2 (**c**); OMe (**d**); SC_8H_{17} (**e,f**)
R^2 = H (**a—e,g**); SC_8H_{17} (**f**)
R^3 = H (**a—f**); SC_8H_{17} (**g**)

can act as photocatalysts (sensitizers) of reactions involving electron transfer[11–13,20] or energy transfer.[18,21–26] In the presence of oxygen, the transfer of an electron from the sensitizer in the triplet excited state to the acceptor, *viz.* a molecule of O_2, may afford reactive intermediates, which oxidize the substrate (reactions (2), (3)), and energy transfer may convert triplet oxygen ($^3O_2$, $^3\Sigma_g$) into singlet oxygen ($^1O_2$, $^1\Delta_g$), which then oxidizes the substrate (reactions (4), (5)).

Excitation

$$\text{Sens} + h\nu \rightarrow {}^1\text{Sens}^* \rightarrow {}^3\text{Sens}^* \qquad (1)$$

Type I mechanism: electron transfer

$$^3\text{Sens}^* + O_2 \rightarrow \text{Sens}^{+\cdot} \rightarrow O_2^{-\cdot} \qquad (2)$$

$$\text{Sens}^{+\cdot}\text{ and/or } O_2^{-\cdot} + \text{substrate} \rightarrow \text{products of the substrate oxidation} \qquad (3)$$

Type II mechanism: energy transfer

$$^3\text{Sens}^* + {}^3O_2 \rightarrow \text{Sens} + {}^1O_2 \qquad (4)$$

$$^1O_2 + \text{substrate} \rightarrow \text{products of the substrate oxidation} \qquad (5)$$

It will be shown below that in the presence of oxygen, the type II energy transfer is more important than the type I electron transfer, because of the high quantum yield in the formation of $^1O_2$. Reactions of the photooxidation of amino acids (cysteine, tryptophan, and histidine),[27,28] unsaturated fatty acids,[29] and hydroxylamines[30,31] in the presence of porphyrins and other sensitizers have been investigated. Some papers[32,33] are devoted to the ability of thiols to quench $^1O_2$ in D_2O. The reactions involving $^1O_2$ are of interest because of its reactivity with respect to biological molecules in the photodynamic therapy of malignancies.

We have studied the photooxidation of thiols and sulfides in detail.[34–36] These reactions are important, on the one hand, as a method for purification of waste water by solar radiation and, on the other hand, since they simulate photooxidation of peptides containing mercapto groups. The photooxidation of thiols and sulfides in the presence of porphyrins such as phthalocyanines **3** readily occurs to give sulfonic acids and sulfates, according to reactions (6) and (7) (the Roman numerals correspond to the oxidation states of sulfur).

$$2\,(\text{RS}^{-\text{II}})^- + 3\,O_2 \xrightarrow{h\nu} 2\text{RS}^{+\text{IV}}O_3^-\ (\text{and } \text{S}^{+\text{VI}}O_4^{2-}) \qquad (6)$$

$$(\text{HS}^{-\text{II}})^- + 2\,O_2 + \text{OH}^- \xrightarrow{h\nu} \text{S}^{+\text{VI}}O_4^{2-} + H_2O \qquad (7)$$

Photodynamic therapy (PDT) is a method of cancer treatment based on the combined action of a sensitizer, light, and oxygen.[15–17] Relatively nontoxic compounds accumulate in a tumor after their intravenous injection (i.v.). Irradiation in the region of the absorption of the sensitizer results in the appearance of phototoxicity, which causes irreversible loss of vital functions of cells and, consequently, heavy necrosis. At present, a hematoporphyrin derivative, Photofrin, is used as the sensitizer in clinical practice. Several types of cancer have been treated.[17] However, the derivatives of hematoporphyrin used are not ideal drugs, because they are complex mixtures of porphyrins and have low selectivity toward tumor tissues, and because of the low efficiency of absorption of red light. Much wider application of PDT may be achieved (1) by the use of long-wavelength absorbing sensitizers ($\lambda \sim 680$—800 nm) for deeper penetration of light, (2) by combining a sensitizer with a carrier for its better accumulation in tumor tissues, and (3) by using cheaper semiconductor laser systems in combination with long-wavelength absorbing sensitizers in clinical practice. We investigated on the laboratory level porphyrins **1—4** either covalently bound to a polymeric carrier or incorporated into liposomes.

Experimental

Hematoporphyrin **1a** from Fluka (No. 257566) was used. Compound **1b** was prepared by the reaction of **1a** with methoxypoly(ethylene glycol) (mol. weight 2000, 5000),[37] 5,10,15,20-tetrakis(4-sulfophenyl)porphyrin **2a** was prepared by sulfonation of 5,10,15,20-tetraphenylporphyrin.[38] The synthesis of **2e** (mol. weight 2000, 5000)[37] was carried out by the covalent bonding of 5,10,15,20-tetrakis(4-carboxyphenyl)-porphyrin **2b** (see Refs. 39, 40) with methoxypoly(ethylene glycol) (mol. weight 2000, 5000). The uncharged porphyrin **2c** (2000, 5000) and positively charged **2e** (2000, 5000), both metal-free and as zinc(II) complexes, were obtained by the reaction of the metal-free or Zn-containing 5,10,15,20-tetrakis(4-pyridylporphyrin) (**2d** or **2d**Zn, respectively) with activated methoxypoly(ethylene glycol).[37] The complexes of 2,9,16,23-tetrasulfophthalocyanine and -tetracarboxyphthalocyanine with zinc(II) (**3b**Zn and **3c**Zn) and aluminum(III) (**3b**Al and **3c**Al) were obtained from the corresponding substituted phthalic anhydride derivatives.[35] Compound **3c**Zn was covalently bonded to methoxypoly(ethylene glycol) (compounds **3d**Zn (2000, 5000)) or various 3-aminopropyl substituted silica carriers, such as monosphere silica of grain size 500 nm with a surface area of 6.9 $m^2 g^{-1}$ (sample **3e**Zn (MPA-500)) or Lichrosphere of grain size 500 nm with a surface area of 40 $m^2 g^{-1}$ (sample **3e**Zn (LPA-500)).[35] The zinc(II) complex of 2,9,16,23-tetrakis(3-methylpyridiniumoxy)phthalocyanine **3g** and positively charged phthalocyanines (metal-free compound **3h** (2000, 5000) and Zn complex **3h**Zn (2000, 5000)) were prepared by the reaction of metal-free or zinc(II)-containing 2,9,16,23-tetrakis(3-pyridyl-oxy)phthalocyanine **3f** or **3f**Zn with methyl iodide or activated methoxypoly(ethylene glycol).[37,41] Compounds **3i**Si and **3j**Si (5000) are Si^{IV}-containing phthalocyanines with 2-methoxyethanol or methoxypoly(ethylene glycol) residues at the silicon atom.[42]

The zinc(II) complexes of 2,3-naphthalocyanine, **4a**Zn,[43] 2,11,20,29-tetraacetamino-2,3-naphthalocyanine, **4b**Zn, 2,11,20,29-tetramethoxy-2,3-naphthalocyanine **4d**Zn,[44—46] 2,11,20,29-tetra(octylthio)-2,3-naphthalocyanine **4e**Zn, 2,3,11,12,20,21,29,30-octa(octylthio)-2,3-naphthalocyanine **4f**Zn, and 5,9,14,18,23,27,32,36-octa(octylthio)-2,3-naphthalocyanine **4g**Zn[47—49] were prepared according to known procedures. The purity and stability of all of the compounds were checked by UV/VIS electronic spectroscopy (reflectance spectroscopy for compound **3e**), IR spectroscopy, elemental analysis, and, where possible, by mass spectroscopy (*e.g.*, using the MALDI technique) and thin layer chromatography. Methoxypoly(ethylene glycol) of molecular weight equal to 2000 and 5000 was used. The degree of substitution by the polymer was[37] 1.7—1.9 for **1b** and 3.2—3.5 for **2c**, **2e**, **3d**, and **3h**. Samples of **3e** contained ~1 μmol of the chelate per 1 g of the carrier.[35]

Determination of $^1O_2$. The activity of the porphyrins with respect to the formation of singlet oxygen was studied using 1,3-diphenylisobenzofuran (DPBF) as an $^1O_2$ quencher (see Refs. 35, 46).

The activity with respect to the electron transfer was investigated in a photochemical redox system consisting of the methylviologen dication as the acceptor and 2-mercaptoethanol as a donor.[35,46]

Photooxidation of 2-mercaptoethanol and hydrogen sulfide. The activities in photooxidation were determined by the volumetric method, from the consumption of oxygen under irradiation with 180 mW cm^{-2} of visible light (see Refs. 35, 50). The turnover frequencies (TOF) were calculated from the initial consumption of O_2 in moles per mole of photocatalyst per min.

Pharmacokinetic and phototherapeutic studies. Sensitizers incorporated into DL-α-dipalmitoylphosphatidylcholine (DPPC) liposomal vesicles were studied with experimental tumor models.[46,51]

Results and Discussion

Solubility and absorption spectra

A crucial factor for the photochemical activity of porphyrins is that they should exist in the monomeric state either in appropriate solvents or immobilized on carrier systems. The samples of porphyrins prepared possess dissimilar solubilities:

(a) water soluble (pH > 7 is preferred) low-molecular-weight negatively charged porphyrins, **1a**, **2a**, **2b**, **3b**, and **3c**;

(b) water soluble low-molecular-weight positively charged porphyrin **3g**;

(c) water soluble neutral porphyrins bound to methoxypoly(ethylene glycol): **1b**, **2c**, **3d**;

(d) water soluble positively charged porphyrins bound to methoxypoly(ethylene glycol): **2e**, **3h**;

(e) all of the porphyrins mentioned in (a)—(d) are also soluble in DMF;

(f) porphyrins **2d**, **3a**, **3e**, **3f**, **3i**, and **4a—g** are insoluble in water but soluble in some organic solvents.

The water-insoluble compounds listed in (f) can be converted to a water-soluble state by incorporation into self-organizing microheterogeneous systems such as micelles and liposomes.[35,46,51] Solubility in water has also been achieved by covalent bonding with a hydrophilic polymer of either the ligand (compounds **1b**, **2c**, **3d**) or the central metal ion (compound **3j**). The covalent bonding of porphyrins with the side chains of positively or negatively charged or uncharged hydrophilic polymers also affords water-soluble compounds.[52] By covalent binding to silica with a low surface area, porphyrins are immobilized on chemically, mechanically, and thermally stable carrier systems.[35,53] Another example of immobilization is the monomeric encapsulation of porphyrins, such as phthalocyanines, in the lattice of molecular sieves, such as zeolites and $AlPO_4$-5 (see Ref. 54).

The porphyrins absorb the visible light in a broad region: the Soret band ~390—430 (**1**, **2**), ~330—370 (**3**), and ~340—360 (**4**) nm; the Q band ~590—650 (**1**, **2**); ~670—700 (**3**), and ~750—820 (**4**) nm. The association of the molecules of porphyrins is very important.[11,12] The monomeric state of molecules is fundamentally important for photoinduced processes, since in the case of associates, the decay of the triplet state under irradiation occurs as a bimolecular process, *viz.* the triplet—triplet annihilation. In the general case, association of molecules in an aqueous solution is more pronounced for more extended aromatic systems (**1**, **2** < **3** < **4**). All of these compounds are soluble in DMF and exist in solutions in the monomeric state. For example, Fig. 2 shows that **3b**Zn is strongly associated in aqueous solutions. The absorption band at 630 nm is characteristic of associates, and the band of a lower intensity at

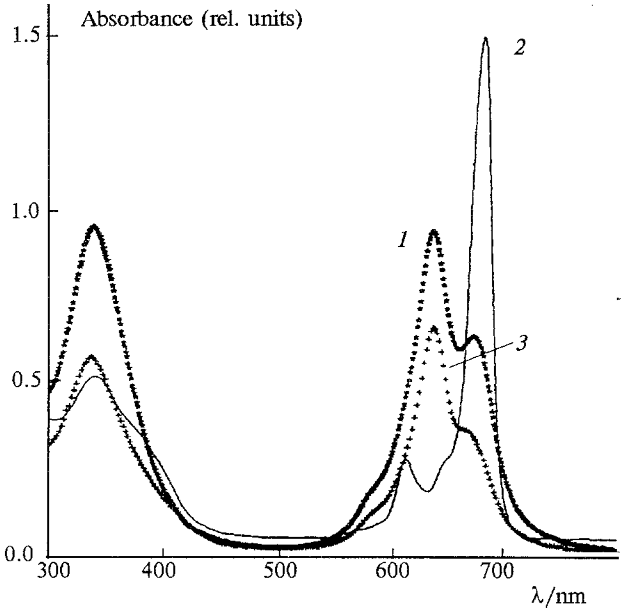


Fig. 2. The VIS spectra of **3b**Zn (~10^{-3} *M*) in an aqueous solution: at pH 7 (*1*); in the presence of 0.1 *M* CTAC (*2*); in the presence of 0.1 *M* of SDS (*3*).

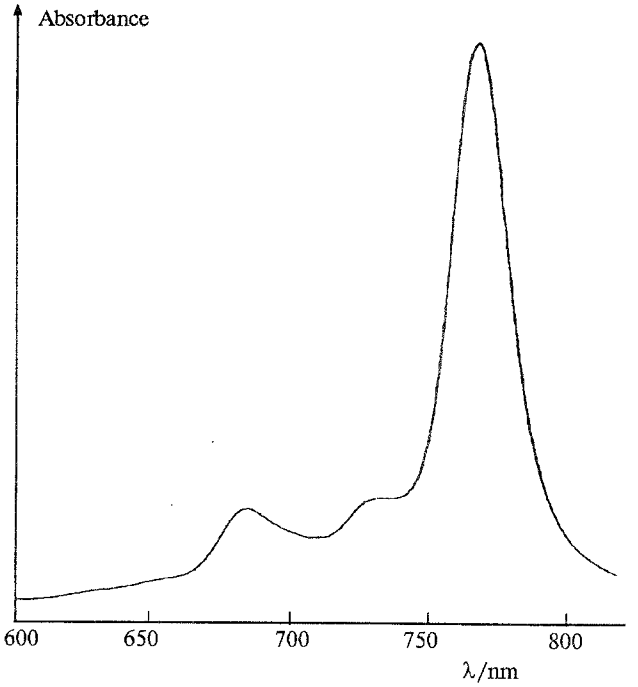


Fig. 3. The VIS spectrum of **4a**Zn (1.6 · 10^{-5} *M*) in an aqueous dispersion of small unilamellar DPPC liposomes.

672 nm corresponds to the remaining monomers.[11,12,55] Addition of the oppositely charged cetyltrimethylammonium chloride (CTAC) results in decomposition of the associates, whereas a combination of similar charges, *e.g.*, **3b** and sodium dodecyl sulfate (SDS), yields associated complexes (see Fig. 2). In the case of the positively charged **3g**, on the contrary, monomerization occurs as SDS is added, and the addition of CTAC favors association. For $Al^{III}X$ complexes the situation is different. **3b**Al remains mostly monomeric in aqueous solutions in the absence of CTAC[56] because the X substituent (X = Cl or OH) may partly separate the macrocycles. The complete monomerization and solubility in water is also realized when hydrophobic porphyrins are incorporated in liposomes.[57] Even the strongly associating naphthalocyanines are monomeric[46,51] in an aqueous liposomal DPPC solution, as shown in Fig. 3 for **4a**Zn. The complete monomerization in water is achieved due to an axial addition of polymer chains, ensuring the solubility in water, to the tetravalent silicon atom (compound **3j**) (Fig. 4).[42] At the same time, the peripheral addition of four hydrophilic methoxypoly(ethylene glycol) chains to the ligand only results in a low decrease in the degree of association (see Fig. 4, curve *1*). This is probably caused by the interaction of polymer coils in the solution, which is additionally favored by the π—π interaction in the aromatic systems. Immobilization of porphyrins on silica with a low surface area is mostly monomeric (Fig. 5).

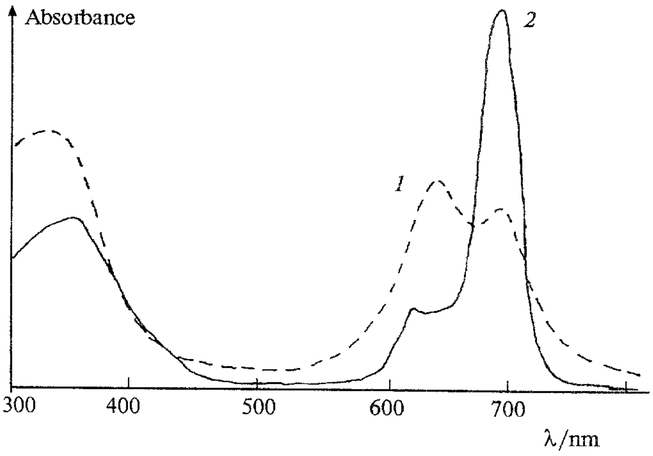


Fig. 4. The VIS spectrum of **3d**Zn (*1*) and **3j**Si (*2*) (~10^{-6} *M* of porphyrin units) in an aqueous solution at pH 7.

Thus, it can be concluded that all of the samples studied dissolve in DMF as monomers. The incorporation of these compounds into micelles/liposomes and axial substitution are the most efficient methods used to inhibit their association in aqueous solutions. In Fig. 6, the energy levels of porphyrins are compared with the energy of $^3O_2/^1O_2$.

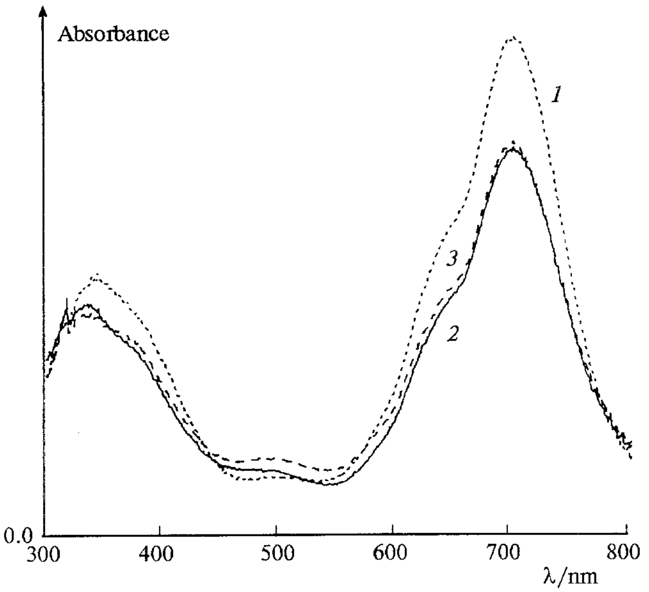


Fig. 5. The VIS reflectance spectrum of **3e**Zn (MPA-500) after preparation (*1*); after photocatalytic oxidation of 2-mercaptoethanol at pH 10 (*2*); the same at pH 12 (*3*).

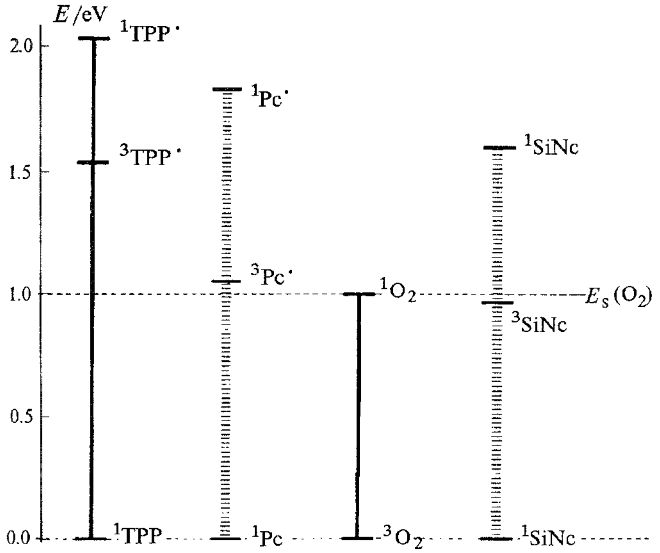


Fig. 6. Comparison of triplet energy levels of zinc(II)tetraphenylporphyrin, zinc(II)phthalocyanine, and silicon(IV)-naphthalocyanine with the singlet energy of O_2 (see Refs. 11, 58).

Photophysical and photochemical properties

Closed-shell metal porphyrins exhibit great lifetimes and good quantum yields of the triplet state, whereas the triplet lifetimes of the compounds containing paramagnetic transition metal ions are normally low:[11,12] for example, for **3b**Zn, $\tau_T^{300\ K} \approx 245$ μsec, $\Phi_T \approx 0.6$; for **3b**Co, $\tau_T^{300\ K} \approx 0.06$ μsec, $\Phi_T = 0.92$. Of the porphyrins containing closed-shell metal ions, those with Zn^{II}, Al^{III}, and Si^{IV} exhibit the best combination of lifetime and quantum yield of the triplet state and, therefore, they are the best photosensitizers.[18,19,21] The sensitizers which are most often employed in the clinical practice are mixtures of metal-free hematoporphyrins, for example, of a hematoporphyrin derivative (HPD) with Photofrin (PII). In this case, the Q-transition at ~620 nm is used for excitation *in vivo*.[14,15,17] The second generation of sensitizers will be based on compounds absorbing in the longer-wavelength region, such as phthalocyanines and naphthalocyanines containing Al^{III}, Zn^{II} and perhaps also Si^{IV} as the central ion surrounded by ligands. Several papers, which are not considered here in detail, describe the photophysical properties of these compounds (see selected Refs. 11, 12, 18, 19, 21).

It can be seen from Table 1 that under an inert gas, all of the porphyrins synthesized exhibit triplet lifetimes of ~1—0.1 μsec depending on the solvent. The covalent bonding with methoxypoly(ethylene glycol) does not lead to significant changes in the lifetimes of the triplet states. In the presence of oxygen ($^3O_2$), all of the porphyrins are efficient in the energy transfer giving singlet oxygen ($^1O_2$). The quantum yield of the formation of $^1O_2$ was measured on the basis of luminescence at 1270 nm or was determined in the presence of physical quenchers, such as sodium azide, or chemical quenchers, for example, DBPF.[35,58] In the case of associated phthalocyanines, the yield of $^1O_2$ is negligibly low.[22,25] For monomeric phthalocyanines, the quantum yields of $^1O_2$, according to the literature data, are 0.1—0.7 (see selected Refs. 22, 25, 26, 55, 58). As can be seen from Table 1, all of the porphyrins studied can sensitize the formation of $^1O_2$ in quantum yields of 0.06—0.67. For comparison, a typical sensitizer of the formation of $^1O_2$, Rose Bengale, provides $\Phi \approx 0.2$. The covalent binding of a porphyrin to methoxypoly(ethylene glycol) slightly decreases or increases the amount of $^1O_2$ formed. Even naphthalocyanines absorbing in the long-wavelength region (λ = 760 to 810 nm) are efficient in the photoinduced energy transfer. Naphthalocyanines in the triplet state reversibly interact with $^3O_2$, according to reaction (8).[59]

$$^3Nc^* + O_2(^3\Sigma_g^-) \rightleftharpoons Nc(S_0) + O_2(^1\Delta_g) \qquad (8)$$

The interaction of **3b**Zn or **3c**Zn with detergents results in a decrease in the quantum yield of $^1O_2$: it is ~ 0.35 in DMF and ~ 0.06 in an aqueous solution containing CTAC. As discussed above, the porphyrins are monomerized in micellar (also liposomal) solutions. The hydrophobic quencher DBPF may be located in the hydrophobic center of the micellar phase. In the presence of the cationic CTAC, the locus of the negatively charged **3b** and **3c** may be at the interface between the micellar and aqueous phases, due to electrostatic interactions.[55,58] Despite the fact that during their lifetimes (~1 μsec), the $^1O_2$ molecules pass several

Table 1. Absorption wavelengths, triplet lifetimes, and quantum yields in the photooxidation of DPBF sensitized by compounds **1—4** [35,46,47,51]

Compound	Main absorption bands in DMF, λ/nm	Triplet lifetime /μsec	Φ of the $^1O_2$ formation[a]
1a	398, 567, 620	103 (DMSO)	0.30 (DMF)
1b(2000, 5000)	398, 567, 620	1000, 3100 (Triton/H_2O)	0.43, 0.56 (DMF)
2a	419, 590, 647	300 (DMSO)	0.28 (DMF)
2aZn	425, 559, 599	980 (DMSO)	0.49 (DMF)
2b	419, 590, 647	100 (DMSO)	0.60 (DMF)
2c(2000, 5000)	419, 592, 648	1800, 1900 (Triton/H_2O), 670, 710 (H_2O)	0.37, 0.34 (DMF)
2d			0.47 (DMF)
2e(2000, 5000)	415, 508, 581, 637		0.28, 0.24 (DMF)
2dZn	423, 556, 595		0.67 (DMF)
2eZn(2000, 5000)	450, 574, 621		0.23, 0.15 (DMF)
3aZn	345, 678	~250 (DMF)[1]	0.25 (DMSO)
3bZn	328, 672	245 (DMF)[11]	0.36 (DMF)
3bZn		100 (H_2O/CTAC)	0.064 (H_2O/CTAC)
3cZn	342, 686	140 (DMSO)	0.32 (DMF)
3cZn			0.061 (H_2O/CTAC)
3dZn(2000, 5000)	344, 687	122, 140 (DMSO)	0.27, 0.25 (DMF)
3f	342, 606, 670, 694		0.2 (DMF)
3h(2000, 5000)	342, 607, 668, 691		0.1, 0.15 (DMF)
3fZn	352, 607, 674		0.58 (DMF)
3hZn(2000, 3000)	345, 607, 674		0.29, 0.42 (DMF)
3iSi	351, 601, 669		
3jSi	354, 604, 671		
4a—d Zn	764—777	100—300	0.14—0.16 (DMSO)
4eZn	350, 767		0.24 (DMF)
4fZn	346, 779		0.20 (DMF
4gZn	338, 814		0.15 (DMF)

Note. The solvents employed are given in parentheses.

[a] Values measured for comparison: Φ = 0.16 to 0.24 (Rose Bengale); 0.26 (HPD); 0.39 (Photofrin in DMF); 0.31 (PII).

micelles (~3 nm in diameter), the rate of the reaction depends on the partition and localization of the reacting $^1O_2$ and DBPF molecules, as has been shown for other substrates.[58]

Under an inert gas in the presence of a donor (2-mercaptoethanol, cysteine, EDTA), and an acceptor (methylviologen dication, MV^{2+}), a photoinduced electron transfer can occur according to the equations which are given below for zinc phthalocyanines (reactions (9) and (10)).

$$^3ZnPc + RS^- \rightarrow ZnPc^{-\cdot} + RS^{\cdot} (2\ RS^{\cdot} \rightarrow RSSR) \qquad (9)$$

$$ZnPc^{-\cdot} + MV^{2+} \rightarrow ZnPc + MV^{+\cdot} \qquad (10)$$

Taking into account the redox potentials, these reactions are thermodynamically possible:[11,35,44,52] $E^0(^3ZnPc^*/ZnPc^{-\cdot}) = +0.48$ V, $E^0(RSSR/RS^-) = -0.30$ V (at pH 11), $E^0(ZnPc/ZnPc^{-\cdot}) = -0.65$ V, $E^0(MV^{2+}/MV^{+\cdot}) = -0.45$ V (with respect to NHE). The process of the formation of the blue-colored $MV^{+\cdot}$ has been studied in a large number of papers,[11] and it was shown that the photoinduced electron transfer can occur even for the covalently bonded porphyrins having great triplet lifetimes.[44,52] In the presence of oxygen, the photochemical reduction product, $MV^{+\cdot}$, can reduce oxygen (E^0 $(O_2/O_2^{-\cdot}) = -0.33$ V) (reaction (11)).

$$MV^{+\cdot} + O_2 \rightarrow MV^{2+} + O_2^{-\cdot} \qquad (11)$$

Analysis of the redox potentials allows one to infer that in a donor (for example, thiol)/sensitizer (porphyrin)/acceptor (O_2) system, along with the photoinduced energy transfer yielding $^1O_2$, a photoinduced electron transfer giving $O_2^{-\cdot}$ is also thermodynamically possible. However, the quantum yield of $O_2^{-\cdot}$ in the electron transfer occurring in the presence of sulfonated phthalocyanines is one or two orders of magnitude lower than the quantum yield of $^1O_2$ in the energy transfer.[22] The triplet lifetime of **3b**Zn, which is equal to 100 μsec in an aqueous solution in the presence of CTAC under an inert gas, decreases to 3.4 μsec when oxygen is introduced and decreases only to 94 μsec as a result of the addition of 2-mercaptoethanol.[35] Therefore, quenching with O_2 to give $^1O_2$, as has been noted previously, is the predominant process. $^1O_2$ efficiently oxidizes such substrates as thiolates or sulfides, and, as will be shown below, cell constituents *in vivo.*

Photooxidation of 2-mercaptoethanol and sulfide

In the RS^- (or SH^-)/sensitizer (porphyrin)/O_2 system, photooxidation of the sulfur compound occurs efficiently, according to the type II mechanism (reactions (4), (5)), rather than according to the type I mechanism (reactions (2), (3)).[35,36] The photooxidation is described by reactions (6) and (7).

Figures 7 and 8 present some of the results obtained in the study of the oxidation/photooxidation of 2-mercaptoethanol or sulfide.[35,36] In the presence of a porphyrin incorporating a paramagnetic metal ion, for example, cobalt(II), the amount of O_2 consumed in the dark and under irradiation corresponds to the formation of the disulfide (from sulfide) and the thiosulfate (from thiol) (see Fig. 7, curves *1, 2*; Fig. 8, curves *1, 2*; reactions (12), (13)). These catalytic processes are widely applied in the desulfuration of gasoline fractions (the MEROX process).[53]

$$4\ RS^- + O_2 + 2\ H_2O \xrightarrow{\text{Cat., dark reaction}} \longrightarrow 2\ RSSR + 4\ OH^- \qquad (12)$$

$$2\ HS^- + 2\ O_2 \xrightarrow{\text{Cat., dark reaction}} S_2O_3^{2-} + H_2O \qquad (13)$$

Under irradiation at pH 13, a deeper oxidation can occur (compare the oxidation states of sulfur in the compounds participating in reactions (6), (7), (12), and (13).) When the negatively charged Zn^{II} and Al^{III} complexes of **3b** and **3c** are used for the dark reaction, no real catalytic effect is seen (see, for example, Fig. 7, curve *3*). However, under irradiation, the photocatalytic effect is manifested and the reaction yields the sulfonic acid (reaction (6)) or sulfate (reaction (7)) (see Fig. 7, curve *4*; Fig. 8, curves *4* and *5*). When Zn^{II}-containing chelates of **3b,c** are used, addition of the oppositely charged detergent, CTAC, is necessary, whereas addition of SDS results only in a slight increase in the O_2 consumption (see Ref. 35). Al^{III}-containing chelates exhibit high photocatalytic activity even without a detergent (see Fig. 8, curve *3*); and the reaction rate slightly increases after the addition of CTAC (see Fig. 8, curve *7*). The positively charged complex **3gZn** shows a comparable activity in the photooxidation in the presence of SDS, but not with CTAC. Figure 9 presents the activity at pH 10 of a Zn^{II}-containing chelate covalently bonded to silica with low surface area (compound **3eZn**). In the dark, the catalytic activity is low (curve *2*); it also increases under the action of irradiation without addition of CTAC (curve *3*). The low-molecular-weight **3bZn** provides a higher TOF but a lower consumption of O_2 in the presence of CTAC.

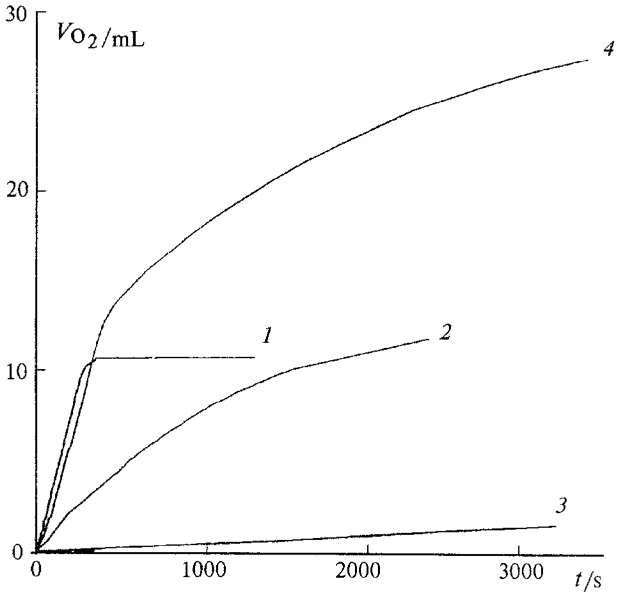


Fig. 7. Catalytic and photocatalytic oxidation of 2-mercaptoethanol in an aqueous solution at pH 13: with 1.4 mmol of thiol and 0.5 μmol of **2bCo** (the molar ratio is 2800 : 1) under irradiation in the dark (*1*); with 1.4 mmol of thiol and 0.5 μmol of **2bCo** (the molar ratio is 2800 : 1) and 0.1 *M* of CTAC under irradiation in the dark (*2*); with 0.7 mmol of thiol and 0.5 μmol of **2bZn** (the molar ratio is 1400 : 1) and 0.1 *M* of CTAC in the dark (*3*); with 0.7 mmol of thiol and 0.5 μmol of **2bZn** (the molar ratio is 1400 : 1) and 0.1 *M* of CTAC under irradiation (*4*).

It is very important to consider the stability of the photocatalysts, since $^1O_2$ can also decompose phthalocyanines.[60] Chelates **3bZn** and **3cZn** are decomposed by 50 to 70 % in one run, while **3bAl** and **3cAl** are decomposed in one run by only 4 %, therefore the latter can be employed several times.[35] The stability of the chelates can also be increased by their deposition onto a silica carrier (samples **3eZn**; see Fig. 5).

The photocatalytic activities of phthalocyanines **3** (similarly to porphyrins **2**) change in the following sequences:

(a) metal-free chelates < Zn^{II} or Al^{III} chelates;

(b) in the absence of a detergent < in the presence of detergents or on a silica carrier without a detergent;

(c) Zn^{II}-containing chelates in the absence of detergents << Al^{III} chelates in the absence of detergents.

The mechanism of the photocatalytic oxidation has been discussed in detail previously:[35,36]

(1). The photoactivation spectrum corresponds to the absorption spectrum.

(2). The quantum yields in the thiolate/porphyrin/O_2 (detergent) system are ~ 0.25. For the DPBF/porphyrin/O_2 (detergent) system, the quantum yields are only ~ 0.06. This results in the molecules of compound **3bZn**, along with RS^- or HS^- anions, being accumulated at the outer hydrophilic part of the positively

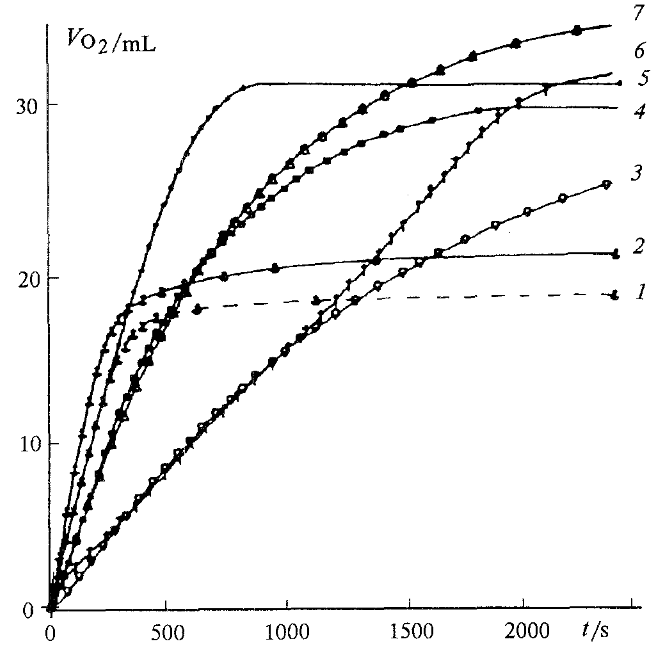


Fig. 8. Catalytic and photocatalytic oxidation of hydrogen sulfide (0.7 mmol) in an aqueous solution in the presence of a catalyst/photocatalyst (0.5 μmol) and CTAC (0.1 μmol): with **3bCo** at pH 9 without irradiation (*1*); with **3bCo** at pH 9 under irradiation (*2*); with **3bAl** at pH 9 without CTAC under irradiation (*3*); with **3cZn** at pH 9 under irradiation (*4*); with **3bZn** at pH 9 under irradiation (*5*); with Rose Bengale at pH 9 under irradiation for comparison (*6*); with **3bAl** at pH 13 under irradiation (*7*).

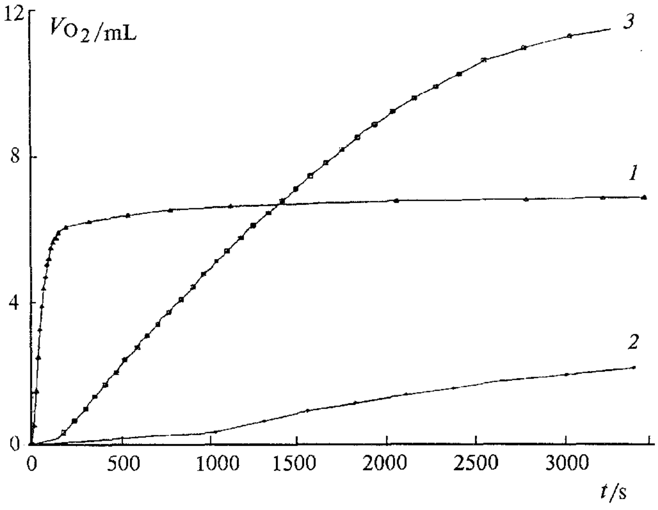


Fig. 9. Catalytic and photocatalytic oxidation of 0.7 mmol of 2-mercaptoethanol in an aqueous solution at pH 10 in the presence of 0.5 μmol of a phthalocyanine: **3bZn** in the presence of 0.1 *M* of CTAC without irradiation (*1*); **3eZn** (MPA-500) or (LPA-500) in the dark (*2*); **3eZn** (MDA-500) or LPAC (500) under irradiation (*3*).

charged CTAC and efficiently reacting with $^1O_2$ (Fig. 10).

(3). The experiments on quenching with DPBF or sodium azide, measurements in D_2O, and detection of such intermediates as H_2O_2 suggest the following mechanism of the photooxidation (the type II mechanism):

$$\mathrm{ZnPcR_4} \xrightarrow{h\nu} {}^{S}\mathrm{ZnPcR_4}^{*} \rightarrow {}^{T}\mathrm{ZnPcR_4}^{*} \tag{14}$$

$$ {}^{T}\mathrm{ZnPcR_4}^{*} + \mathrm{O_2} \rightarrow \mathrm{ZnPcR_4} + {}^{1}\mathrm{O_2} \tag{15}$$

$$\mathrm{RS^-} + {}^{1}\mathrm{O_2} \rightarrow \mathrm{RS^{\cdot}} + \mathrm{O_2^{-\cdot}} \tag{16}$$

Basic studies on the photodynamic therapy of cancer

Some of the compounds under consideration have been studied from the viewpoint of their potential application in PDT.

The *in vitro* experiments with various malignant cell lines have shown that positively charged phthalocyanines like **3gZn** exhibit a higher photodynamic activity than Photofrin.[41] The photodynamic activity increases as the length (and, correspondingly, the hydrophobic character) of the alkyl chain, the substituent in the pyridine ring, increases.

The distribution and photodynamic activity *in vivo* of porphyrins **1**, **2** and phthalocyanines **3** covalently bonded to methoxypoly(ethylene glycol) (for example, structures **1b**, **2c**, **3d**) have been determined scintigraphically (the tissues were labeled with ^{131}I) at the German Cancer Institute (Heidelberg).[61,62] As can be

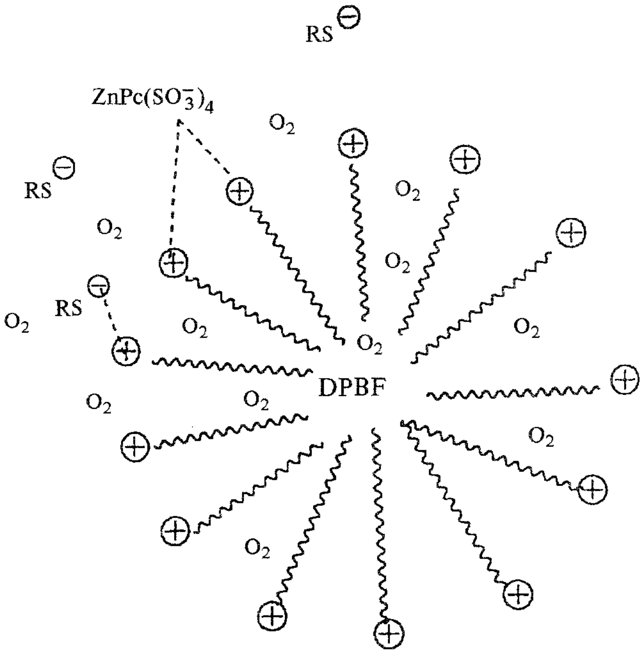


Fig. 10. The scheme of the localization of various compounds in an aqueous solution containing CTAC.

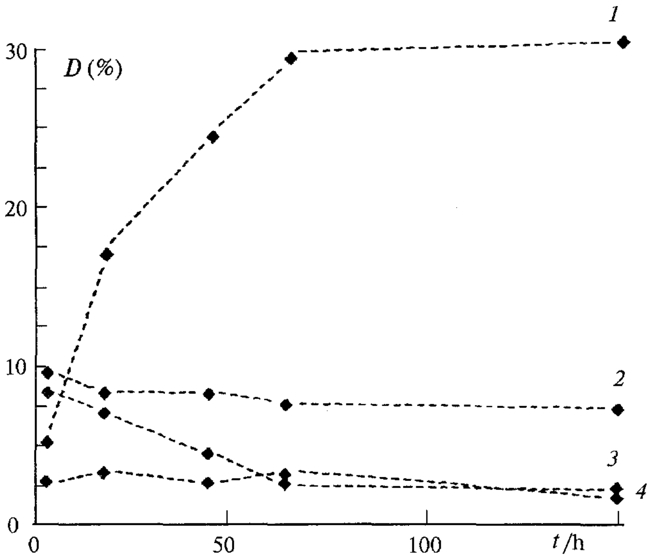


Fig. 11. The distribution of a hematoporphyrin covalently bonded with methoxypoly(ethylene glycol) in BD.IX rat with ovarian carcinoma (an i.v. injection of 0.5 mg kg^{-1} porphyrine in the left hind leg; 100 % corresponds to the total amount of the sensitizer in the entire rat): the tumor (*1*); the liver (*2*); the heart (*3*); muscles (*4*).

seen from Fig. 11, accumulation in tumor tissues of polymer-bonded porphyrins is 12 times higher than that of Photofrin. Irradiation of a tumor on the left hind leg of a rat results in a heavy necrosis and the complete tumor regression two weeks later.[61,62] In general, tumor vessels have wide interendothelial junctions, and a large number of fenestrae and transendothelial channels; in addition, they are characterized by discontinuous basement membranes or even by their absence. This may explain the high accumulation of polymer-bonded sensitizers.

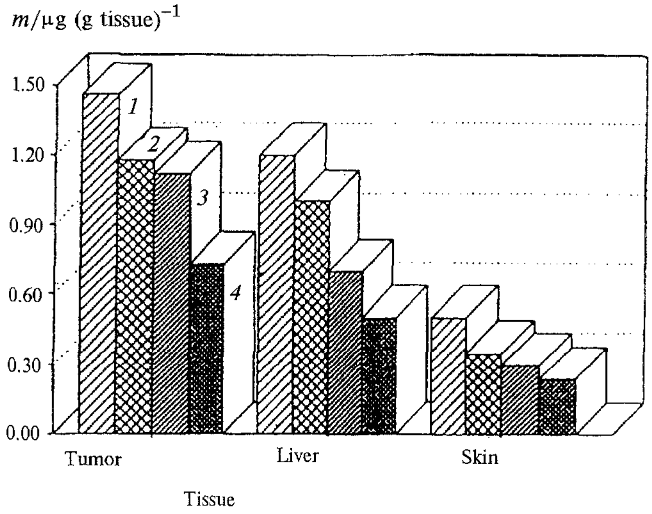


Fig. 12. The distribution of **4aZn** (column *1*), **4dZn** (*2*), **4bZn** (*3*), **4cZn** (*4*) among the tissues of male C57 black mice bearing Lewis lung carcinoma, within 20 h after administration.

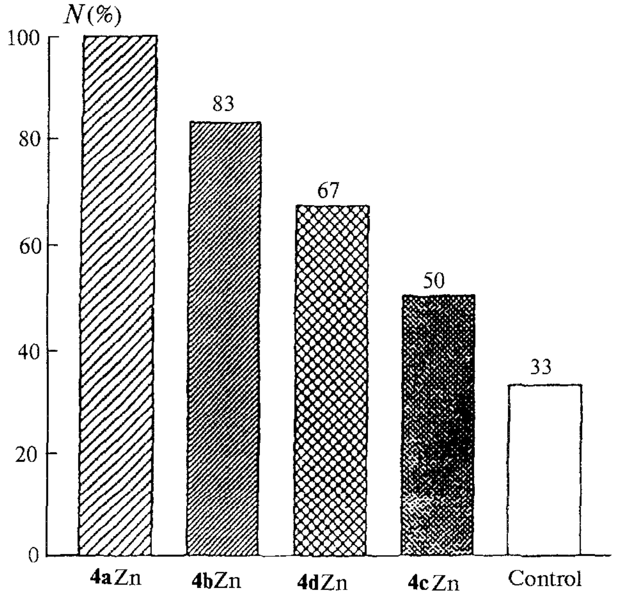


Fig. 13. Percentage of surviving male C57 black mice with Lewis lung carcinoma on the 17th day after PDT with **4a—dZn** (control means animals not treated).

Naphthalocyanines **4** absorb light in the long-wavelength region and cause the formation of $^1O_2$ under irradiation (see Fig. 6; Table 1). Their disadvantages compared with the other compounds are substantial association, the low stability during preparation and purification, and a more difficult synthesis. The monomerization of these compounds in aqueous solutions has been achieved by their incorporation in liposomes.[46,51] The incorporation of Zn^{II} naphthalocyanines in liposomes makes it possible to use derivatives that are more easily prepared, such as **4a—d**. The pharmacokinetic and phototherapeutic effects of the compounds **4a—dZn** in DPPC liposomes has been studied in hamsters and black mice bearing different tumor models (the preparation was injected intra-peritoneally (i.p.), 0.15—0.3 mg kg^{-1}).[46,51] The quantum yields of $^1O_2$ for all of compounds **4a—dZn** are nearly identical, irrespective of the substituent in the molecule. However, their *in vivo* activities are different. This may be due to the fact that they are differently accumulated, distributed, or retained in tissues. Figure 12 illustrates the contents of **4a—dZn** in various tissues within 20 h after their i.p. administration, *i.e.*, at the instant when the accumulation of the preparations in tissues is the highest. A nearly total cleaning of skin is achieved within 72 h after the i.p. administration. Phototherapeutic studies have shown that the highest survival percent within 17 days after PDT is achieved with **4aZn** and **4bZn** (Fig. 13). These compounds can act as efficient phototherapeutic agents even when only 0.2 mg per 1 kg of body weight is used (whereas HPD are used in amounts of 5 mg kg^{-1}).

It is important to study the localization and the mechanism of the action of the sensitizers in tumor tissues.[12,14,15,51] A morphological analysis has shown that compounds **4a**—**d**Zn delivered in liposomes cause direct photocytotoxic changes in neoplastic cells subjected to irradiation (*i.e.*, membranes, mitochondria, rough endoplasmatic reticulum). A similar effect has been observed for phthalocyanines in liposomes.[57] It was pointed out that lipophilic anionic sensitizers generally accumulate in the membrane structure (including plasma, mitochondria, endoplasmatic reticulum, nuclear membranes), hydrophilic anionic sensitizers accumulate in lysosomes, and certain cationic sensitizers accumulate in mitochondria. Photooxidation of the membrane cholesterol and other unsaturated phospholipids, aminolipids, and polypeptides as well as DNA damages resulting in disturbance of various cell functions occur.[14,15]

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A series of low-molecular-weight porphyrins **1**—**4** having various charges and also porphyrins covalently bonded to organic polymers or to the surface of inorganic polymers were prepared. It was found that the lifetimes of the triplet states and the photosensitized formation of singlet oxygen depend slightly on whether the porphyrins are low-molecular-weight or polymer-bonded. Even the sensitizers absorbing at ~810 nm can convert $^3O_2$ into $^1O_2$ under irradiation.

The monomeric state of porphyrins is an essential factor as regards their photophysical and photochemical activity. The sensitized photooxidation of thiolates and sulfides occurs more efficiently than the corresponding dark catalytic transformations. The photooxidation reactions involve singlet oxygen. The photooxidation reactions occurring in tumor tissues during PTD of cancer resulting in their heavy necrosis also mostly involve $^1O_2$. In addition, it was shown that the covalently bonded porphyrins and long-wavelength absorbing naphthalocyanines incorporated in liposomes exhibit high tumor accumulation and phototherapeutic effect.

In the future, it will be expedient to pay attention to the reactions occurring under the action of sunlight and to use long-wavelength absorbing sensitizers with a specific tumor accumulation in clinical practice.

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