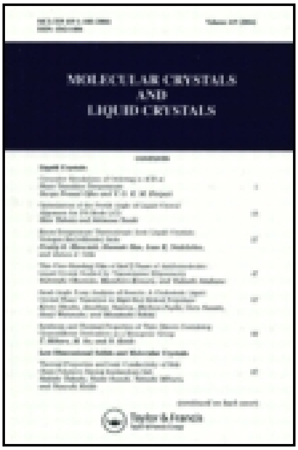

Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

Porphyrins and Phthalocyanines - Functional Molecular Materials for Optoelectronics and Medicine

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Version of record first published: 31 Jan 2007

To cite this article: Danuta Wróbel & Alina Dudkowiak (2006): Porphyrins and Phthalocyanines - Functional Molecular Materials for Optoelectronics and Medicine, Molecular Crystals and Liquid Crystals, 448:1, 15/[617]-38/[640]

To link to this article: http://dx.doi.org/10.1080/15421400500377354

Mol. Cryst. Liq. Cryst., Vol. 448, pp. 15/[617]–38/[640], 2006
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ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421400500377354

Porphyrins and Phthalocyanines – Functional Molecular Materials for Optoelectronics and Medicine

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This paper deals with photophysical characterization of some synthetic porphyrins and phthalocyanines as well as natural chlorophylls and bacteriochlorophylls as attractive multifunctional organic materials for novel molecular photovoltaics and for photodynamic therapy or diagnosis of cancerous tissues. The following spectral methods were used in our investigations: electronic absorption, fluorescence, steady-state photoacoustic spectroscopy and time-resolved optoacoustic spectroscopy to get insight into the mechanisms responsible for molecular processes occurring in dyes upon light illumination. Spectral study was supported by photoelectric examination. The spectral parameters of dyes which are essential for photovoltaics and/or photodynamic therapy/diagnosis were estimated: absorption molar coefficients, fluorescence quantum yields, natural life-times, thermal deactivation parameters, quantum yields of triplet-state population, triplet-state thermal relaxation decay. It was shown that some synthetic porphyrins, phthalocyanines and natural (bacterio)chlorophylls can be potentially used as functional materials for both optoelectronics and/or medicine.

Keywords: chlorophyll; photodynamic therapy and diagnosis; photoelectric examination; photovoltaics; phthalocyanine; porphyrin; singlet-state; spectral methods; triplet-state

1. INTRODUCTION

Organic dyes that contain π-electrons in their molecular structure provide interesting materials in fields for novel science and technology. In

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This work was supported by the Poznan University of Technology, BW 62-207/2005. Some synthetic dyes were kindly provided from Prof. R.M. Ion in the frame of the COST D20 Project. The authors thank to Dr Eng. A. Boguta and MSc Eng. Staśkowiak for their assistance in manuscript preparation.

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the last decades they are being wider and wider applied in laser technology, in photography, as radiative power indicators, environmental pollution probes [1–3]. Since they have the potential to manipulate light with high precision they can also serve as excellent materials for novel photonics and medicine. The most attractive dyes that can be used as photoconverters in photonics and photosensitizers or photomarkers in medicine are a family of both natural and synthetic porphyrin (P) and phthalocyanine (Pc) dyes [1–3]. Porphyrins and phthalocyanines as multifunctional materials are very attractive because they can be chemically easily modified by embedding a metal ion in the molecular core and by substituting the outer rings with various peripheral groups. Chlorophyll (Chl), bacteriochlorophyll (BChl) pigments and their metal-free derivatives are natural pigments, whose molecular structure is based on the porphyrin skeleton [4] and they are also promising in photovoltaics (PV) [5] and photodynamic therapy (PDT) or diagnosis (PDD) of cancerous tissues [6].

In this paper we focus our attention on some photophysical properties of chosen porphyrins, phthalocyanines and (bacterio)chlorophylls. Also spectral parameters which could be essential in their potential applications in molecular PV, PDT and PDD are described.

In our study we present spectral characterization of porphyrin/phthalocyanine dyes and (bacterio)chlorophylls, as well as mechanisms of photocurrent generation in a photoelectrochemical cell (PEC) based on synthetic dyes and the potential of porphyrins/phthalocyanines as photosensitizers or photomarkers. In the part concerning light energy to electric energy conversion we indicate the processes that are involved in photoconversion and basic spectroscopic investigations that can get insight into radiative and non-radiative processes of deactivation of the dyes excited states which are competitive to charge separation process. The excited singlet-states play an essential role in photocurrent generation but there are some evidences of a contribution of the excited triplet-states of dyes in this processes [7]. Therefore we study photophysical features of both singlet- and triplet-states to have full description of light-induced molecular processes responsible for photovoltaic effects. The structural differences in porphyrins and phthalocyanines provide to the differentiations in their spectroscopic behavior and therefore can affect the dyes effectiveness in photoelectric response. The knowledge about the correlation between the dye molecular structure and dye ability for photocurrent generation are essential in understanding of the mechanisms of processes occurring upon illumination and in controlling some molecular processes in molecular solar cells based on organic dyes.

In PDT the light, O_2, and a photosensitizing dye are combined to produce a selective therapeutic effect [8]. PDT is an innovative and attractive modality for the treatment based on the photooxidative damage sensitized by a dye calls a photosensitizer. As a treatment procedure, PDT requires (i) the dye absorbs light in the red part of the visible spectrum to minimize both scattering and absorption of light by the human tissue and (ii) a powerful light source which matches the absorption spectrum of the photosensitizer. The PDT could be applied in treatment of cancer, in antimicrobial chemiotherapy, in the sterilization of blood and blood products, in immunosuppression [8].

The intersystem crossing transition ($S_1 \rightarrow T_1$) is spin-forbidden but the photodynamic action is mostly mediated by the T_1-state therefore a good photosensitizer has to have a high triplet-state yield sufficiently long-lived to take part in chemical reactions. The light-activated sensitizers can transfer energy from their triplet state: (i) directly to molecular oxygen to yield singlet oxygen and (ii) by interaction with the solvent or the target tissue via an electron or a proton transfer leading to generation of radicals. Essential photophysical parameters, for the therapeutic application, that determine the photosensitizing capability of a compound are the singlet oxygen quantum-yield ($\Phi_{\triangle}$), the triplet-state yield (Φ_T), the triplet-state life-time (τ_T), and the triplet-state energy (E_T) [8]. The energy of the dye triplet-state needs to be $\geq 94\,\mathrm{kJmol}^{-1}$ for efficient energy transfer to the ground- state of dioxygen. To improve PDT, i.e., to increase photodynamic efficiency in the treatment of animal tumors and reduce phototoxic side effects second-generation photosensitizers, including the dyes belonging to the groups of porphyrins, phthalocyanines, texaphyrins, chlorins or bacteriochlorins, have been developed [8,9].

For use in the diagnostic mode (in PDD), the compound's ability for fluorescence needs to be appreciable which is quantified as its quantum yield of fluorescence (Φ_F). Fluorescence emitted by photosensitizer is useful in tumor detection, in the estimation of tumor size, and in the determination of photosensitizer concentration [8]. However, the compounds that would be developed as photosensitizer for PDT are different from those intended to act as photomarkers for tumor detection.

Recently, it has been shown [7,10–16] that a combination of the optical spectroscopy and photothermal methods can provide valuable information helpful for selection of dyes useful in PV and medicine. The information concerns depopulation of the dye excited states and the total energy dissipation through non- and radiative processes. In order to study the dye triplet-state properties and its population as well as the decay times of thermal deactivation process, the group of dyes were investigated by means of thermal spectroscopy named the laser induced optoacoustic spectroscopy (LIOAS) [17,18]. This method detects the fate of the energy absorbed by dye/pigment and its lost through non-radiative channels.

However, the high value of triplet-state generation is not only the one or the most important criterion for preliminary selection of potential sensitizers [10,11,19]. The photostability of the pigment is also important. Hence, the properties of the singlet-states of dyes and their photobleaching were determined using steady-state absorption and fluorescence techniques. The dye photostability can be assessed on the basis of the changes in the absorption and fluorescence spectra recorded for the samples illuminated with the light wavelength matching the pigment absorption in a region of weak absorption of the living cell material.

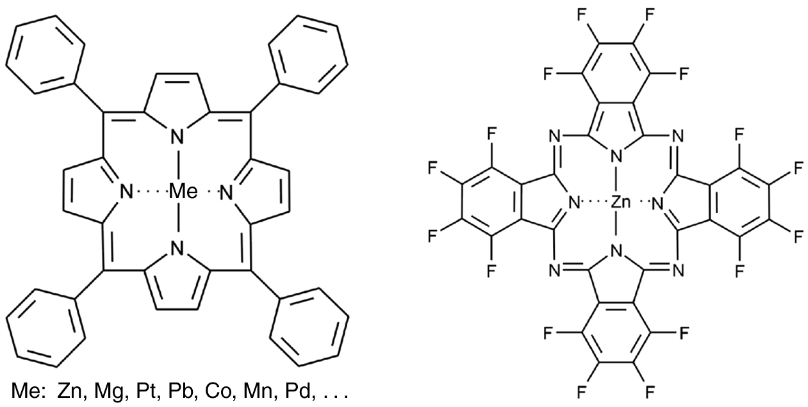
The main purpose of this paper is presentation of the spectral studies of some synthetic porphyrins/phthalocyanines and natural (bacterio)chlorophylls as well as evaluation of spectral parameters (absorption range, absorption coefficient, natural life-time, fluorescence quantum yield, triplet-state generation quantum yield, triplet-state decay-time, yield of singlet oxygen production) which are essential in PV, PDT and/or PDD.

2. MATERIALS AND EXPERIMENTAL METHODS

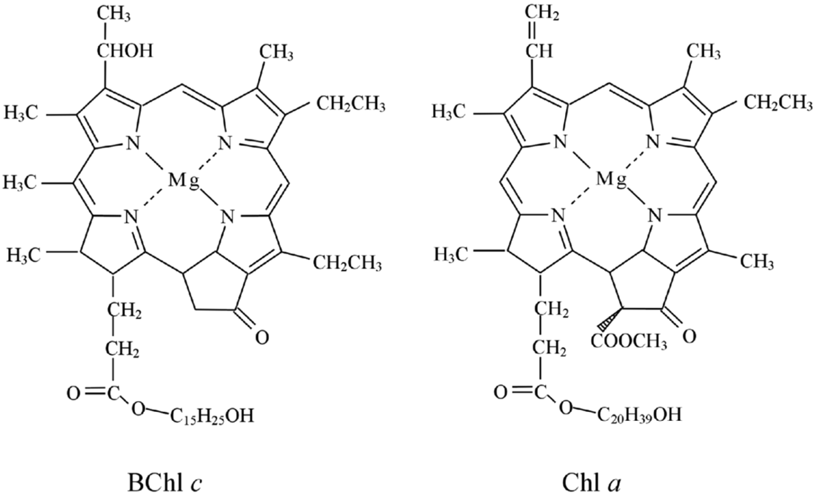
Chl *a* was purchased from Sigma-Aldrich. Pheophytin (Phe) *a* was prepared as described in [20], whereas BChl *c* and bacteriophoephytin (BPhe) *c* were extracted from the green bacterium *Prosthecochloris aestuarii* [21]. P and Pc were also purchased from Sigma-Aldrich or received from R.M. Ion. Figure 1 presents the molecular structures of some dyes and pigments under investigation.

In spectroscopic measurements and for photovoltaic purposes P and Pc were dissolved in dimethyl sulfoxide (DMSO) and dye concentrations were ranged from 10^{-5} to 10^{-3}M depending on experiments. For PDT and PDD examinations Chl *a*, BChl *c* and their metal-depleted derivative (Phe *a* and BPhe *c*, respectively) were dissolved in ethanol (POCh, Poland) [10,14,16] whereas phthalocyanines in DMSO with 0.5% H_2O [8] to a concentration of about 10^{-6} M for spectroscopic measurements and about 10^{-4}–10^{-2} M (depending on the dyes) for incubation with human cells. Organic solvents used in the measurements were of spectroscopic grade.

The leukemia T cells, described in details in [19], were obtained from two continuous culture lines (CCRF and MOLT). Lymphocytes were isolated from samples of heparinized venous blood from healthy donors [10,11]. The lymphocyte stimulation and the incubation of the



A - porphyrin, phthalocyanine



B - bacteriochlorophyll *c*, chlorophyll *a*

FIGURE 1 Molecular structure of selected synthetic dyes (A) and natural pigments (B) under investigation.

cells (resting (unstimulated), stimulated as well as the leukemia T cells) with pigments in organic solvent solution was described elsewhere [10,11,19].

The absorption spectra were measured with a Specord M40 (Carl Zeiss, Germany). The emission spectra were recorded by means of a fluorescence spectrophotometer F4500 (Hitachi, Japan). The photostability and photochemical activity of investigated pigments being in contact with air was measured under their illumination by a He-Ne laser source ($\lambda_{\mathrm{las}} = 632.8\,\mathrm{nm}$).

On the basis of the electronic absorption and fluorescence spectra, the quantum yield of pigment fluorescence (Φ_{F}) and the natural lifetimes (τ_{N}) of the studied dyes were evaluated. The Φ_{F} values were estimated according to the method described in [22] using the following equation:

$$\Phi_F = \Phi_R \frac{I}{I_R} \frac{OD_R}{OD} \frac{n^2}{n_R^2}, \tag{1}$$

where Φ_{R} – the fluorescence quantum yield of the reference, I and $\mathrm{I_R}$ – the areas under the fluorescence curves of the sample and reference, respectively, OD and $\mathrm{OD_R}$ – the absorption of the sample and reference, respectively, n and $\mathrm{n_R}$ – refractive indices for the sample and reference, respectively. The estimated Φ_{F} values were further used for calculation the quantum yield of triplet-state generation (Φ_{T}) values based on Eq. (4) (see below). The τ_{N} values were calculated using the following equation:

$$\frac{1}{\tau_N} = 2.88 \cdot 10^{-9} n^2 \frac{\int \frac{F(\lambda)}{\lambda^2} d\lambda}{\int F(\lambda) \cdot \lambda d\lambda} \int \frac{\varepsilon(\lambda)}{\lambda} d\lambda, \quad [1/s] \tag{2}$$

where n – a refractive index, and integrals of $\mathrm{F}(\lambda)$ and $\varepsilon(\lambda)$ – the area under the fluorescence and absorption spectra, respectively.

The steady-state photoacoustic signal (PAS) measurements were done with a one-beam spectrometer described elsewhere [23]. Thermal deactivation parameter was determined as PAS/A. The time-resolved photothermal signal was recorded by means of the LIOAS setup [17,18]. Before the LIOAS experiments, some of the samples were deaerated (or oxygenated) by bubbling nitrogen (or oxygen) through the solution for 30 min. For the LIOAS measurements the samples were illuminated by a nitrogen-dye laser (flash duration 0.2 ns). At the laser flash wavelengths used the absorption of the investigated and the reference dyes were adjusted the same. The bromocresol purple (BCP), whose total excitation energy is promptly thermally deactivated (in time shorter than time resolution of setup) [17,18], was used as a reference in the LIOAS performed measurements. The quenching wave-form LIOAS signals were measured in the time range up to 5 μs (limited by the setup). Two methods of signal analysis proposed by Marti *et al.* [24] and Small *et al.* [25] were applied.

The method proposed by Marti *et al.* [24] was based on a comparison of the first maximum of the signal (H_{max}, Eq. (3)) measured for the sample and the reference. According to [24] the part of the energy exchanged promptly into heat, i.e., in a time shorter than τ_1 – the time resolution of the experimental setup used (estimated as about 0.5 μs [26]), is denoted as α. It can be obtained from equation:

$$H_{max} = k\alpha E_{las}(1 - 10^{-A}), \tag{3}$$

where H_{max} – the amplitude of the first maximum of the LIOAS signal, A – sample absorbance at the laser pulse wavelength, E_{las} – the average laser pulse energy, k – the coefficient related to the optical geometry, electronic impedance and thermoelastic properties of a sample. The coefficient k is eliminated by the measuring both LIOAS signals for the investigated and reference samples.

Assuming that the photochemical reactions and other non- or thermal paths of energy deactivation are not realised in the system investigated, the approximate value of the yield of triplet-state generation (Φ_T) can be obtained from equation:

$$\Phi_T E_T = (1 - \alpha)E_{h\nu} - \Phi_F E_F. \tag{4}$$

For calculation, the energy of dye triplet state (E_T) dyes was taken from literature [27,28], $E_{h\nu}$ – the molar energy of the incident photons, E_F – the energy of singlet state and Φ_F – the fluorescence yield of dye.

The method of LIOAS signal analysis proposed by Small *et al.* [25] consisted in the deconvolution of both sample and reference signals and the sample thermal signal is described by the sum of the exponential components ($H \sim \sum k_i \exp(-t/\tau_i)$), where τ_i – the decay time of the ith component, and $k_i{}^i$– a pre-exponential factor [25]. In our experiments, this method is applied only to the thermally deactivated energy in the time range available (from 0 to 5 μs). In this procedure we supposed that the k_1 value was related to the decay time τ_1 ($\tau_1 \leq 0.5$ μs) and described the non-radiative processes taking place in the time range below 0.5 μs (the setup time resolution). Hence, the values of k_1 and α obtained by deconvolution [25] and Marti *et al.* [24] methods, respectively, should be similar. The contributions from thermal deactivation processes occurring in the time range from 0.5 to 5 μs are represented by k_2 and τ_2 values.

Photovoltaic and photocurrent studies were done for dyes (P and Pc in DMSO) in a PEC constructed of the semiconducting (In_2O_3) and metallic (gold-Au) electrodes distanced of a 60 μm Teflon® spacer.

The solution of dyes was immersed between the two electrodes [7]. The PEC was illuminated on the semiconducting electrode side with the light of intensity 140 mW/cm^2. The photovoltaic signal was corrected for a number of incident photons. The arrangement details of photoelectric examinations are described in [26].

3. RESULTS AND DISCUSSION

3.1. Spectral Characterization of Porphyrin and Phthalocyanine Dyes

The ability of dyes for photoconversion of light energy in electric energy and for photodynamic reaction depends very strongly on the dye molecular structure [1]. For that reason we have investigated a large family of natural/synthetic porphyrins and phthalocyanines to make a good choice among dyes which could be promising photoconverters, photosensitizers or photomarkers. The natural porphyrins studied were chlorophylls and bacteriochlorophylls which are substituted derivatives of chlorins and bacteriochlorins, respectively.

In this chapter we describe briefly the basic properties of the singlet- and triplet-states of porphyrin and phthalocyanine dyes which are important in PV, PDT and PDD. In the light of the literature data, the photophysical properties of porphyrins and phthalocyanines are shown to be dependent markedly on central metal ion complexed with the dye skeleton and substituents attached to the main molecular corn [1].

Absorption spectra are always monitored to provide information on the energetic range of the light energy being absorbed by dyes and probability of absorption transitions between the ground and excited states. All investigated dyes are characterized by absorption in the visible range (from about 300 to about 700 nm depending on dye–Fig. 2). The absorption band in the “blue” part (415–430 nm – Soret band) and the much less pronounced bands in the “red” region (660–680 nm – Q bands) are characteristic for porphyrins. Phthalocyanines absorb very effectively in the “red” region of the solar irradiation (640–750 nm) and also in the near UV (about 330 nm) (Fig. 2A). The absorption bands in the Soret region are assigned to the $S_0 \rightarrow S_2$ transition and $S_0 \rightarrow S_1$ transition is responsible for the appearance of the bands in the “red” part of the spectra [29]. In metal-free porphyrins (e.g., H_2TPP) four bands in the Q region are observed which turn to two bands in metallic dyes due to the changes in the dye symmetry from D_{4h} to D_{2h} [29]. The difference in the shapes and energetic positions of the absorption bands are due to the difference between the porphin ring (porphyrins) and porphyrazine (phthalocyanines) macrocycle; porphyrins resemble

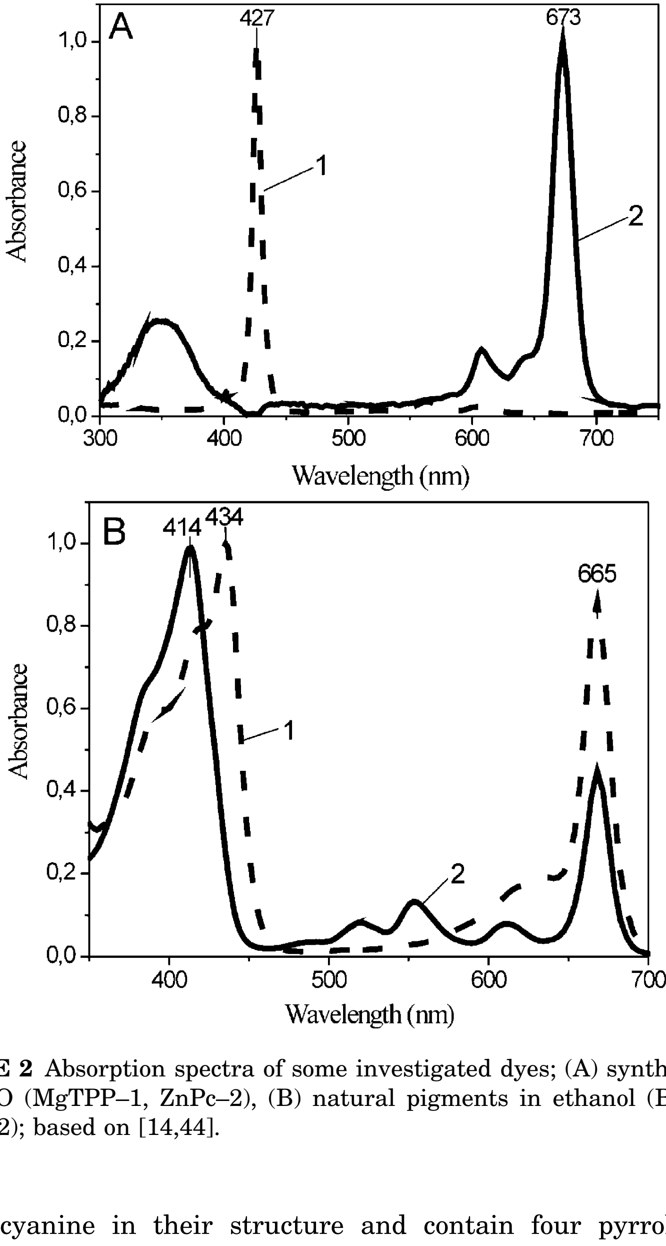


FIGURE 2 Absorption spectra of some investigated dyes; (A) synthetic dyes in DMSO (MgTPP–1, ZnPc–2), (B) natural pigments in ethanol (BChl *c*–1, BPhe *c*–2); based on [14,44].

phthalocyanine in their structure and contain four pyrrole rings linked by methane carbon bridges, whereas phthalocyanines are build up from four indole groups linked by the nitrogen atoms. As shown in some papers [3,29,30] the electronic HOMO-LUMO transition in porphyrin/phthalocyanine dyes can be strongly affected by central metal ion; the higher metal ion reactivity is the more absorption bathochromic shift can be registered [1,29]. It can be assigned to the π-electrons in the molecular conjugated system and/or formation of molecular aggregates or oligomers [2,3]. In our experiments aggregation creation could be neglected as due to alcohols and DMSO that protect against aggregation and keep dye in a monomeric form.

Chlorophyll and bacteriochlorophyll properties are rather very well known owing to their wide occurrence in nature [8,20], therefore, in Table 1 are gathered only the main spectral features important for our purpose and describe the synthetic dyes and natural pigments under investigation. There are some of the most important photophysical parameters: the absorption range, position of main absorption bands and the absorption molar coefficients. As seen from this table the absorption regions for porphyrins and phthalocyanines are different. Moreover metal ion, the benzene and aromatic groups or fluorine atoms can cause some changes in the molar absorption coefficients and energetic band positions.

The fluorescence behaviour of the studied dyes is typical for porphyrins and phthalocyanines. Porphyrins usually have two fluorescence bands (in the range from 625 to 720 nm dependent on dye and substituent) and phthalocyanines are characterized also with two bands at about 690 and 750 nm (some fluorescence data are also collected in Table 1). As seen the attachment of the peripheral groups can lead to the lowering of the singlet-states of the dye molecules observed as a

TABLE 1 Absorption, Fluorescence and Thermal Deactivation Parameters of Dyes/Pigments. Synthetic Dyes in DMSO, Natural Pigments in Ethanol; Based on [14,16,35,54]

Dye/pigment	λ_{max} (nm) absorption wavelength	ε ($10^4\,M^{-1}\,cm^{-1}$)	λ_{max} (nm) fluorescence wavelength	τ_N (ns)	Φ_F	TD (a.u.)
MgTPP	427	8.2	625	31.8	0.08	1.00
ZnTPP	427	5.7	605	25.1	0.07	1.20
ZnPcF	638	6.1	688	16.5	0.21	3.52
ZnPcAromatic	680	13.1	694	14.0	0.14	3.51
ZnPcAlkyl	690	10.2	706	14.0	0.10	7.12
Chl *a*	665	9.9	670	21.3	0.36	–
Phe *a*	666	4.4	670	23.3	0.17	–
BChl *c*	665	6.4	668	20.9	0.22	–
BPhe *c*	668	7.7	670	19.3	0.15	–

ε – molar absorption coefficient, τ_N – natural life-time, Φ_F – fluorescence quantum yield, TD – thermal deactivation parameter.

longwavelength shift with respect to that of the non-substituted dyes. On the basis of the fluorescence and absorption data we can estimate the natural life-time (τ_N) and fluorescence quantum yield (Φ_F). The estimated life-time is in order of nanoseconds as expected. For example MgTPP is characterized by the longest τ_N and Chl *a* by the largest value of Φ_F. On the other hand there are some dyes which do not show fluorescence or fluoresce very weakly (e.g., PbTPP, CuPc).

Energy absorbed by molecules can be also released more or less in non-radiative processes as heat. PAS gives input in a global thermal energy of dyes. A part of absorbed energy converted into heat is estimated as thermal deactivated parameters (TD) which are also collected in Table 1. For example the dyes with the long alkyl- or alkyloxy chains loose much energy in thermal relaxation when compared with the parent dye (ZnPc).

3.2. Porphyrins and Phthalocyanines as Photoconverters

Molecular Mechanism of Photovoltaic Signal Generation

Examination of the dyes ability as photoconverters of solar energy to electric energy can usually be realized with a simple device – a photoelectrochemical cell (PEC). In the light of the experimental results obtained in our and others laboratories the mechanism responsible for photovoltage signal is an electron injection from the excited dye molecule (acts as a preliminary electron donor) to the semiconducting electrode (a preliminary electron acceptor) [30–32]. After dye excitation by a photon, charge separation can occur followed by electron transfer at the semiconductor – thin dye layer interface. In the short circuit the gold electrode plays a role of a secondary donor to provide an electron back in order to make a dye molecule to its ground state. The enhancement of the charge density at the electrode-dye layer interface was shown as due to the change in the electric permitivity and conductivity at the semiconductor and dye layer boundary [33].

Molecular Structure and Dye Ability for Photocurrent Generation

In this chapter we will show how the presence of (i) the metal ion, (ii) aromatic rings, (iii) peripheral groups like alkyl-, alkyloxy chains, or (iv) atoms (fluorine) attached to the main molecular structure can affect the photocurrent signal generated in the PEC based on porphyrins or phthalocyanines upon illumination.

(i) We will start with presentation of the results concerning the influence of metal ion on photosignal. In Figure 3 the photocurrent

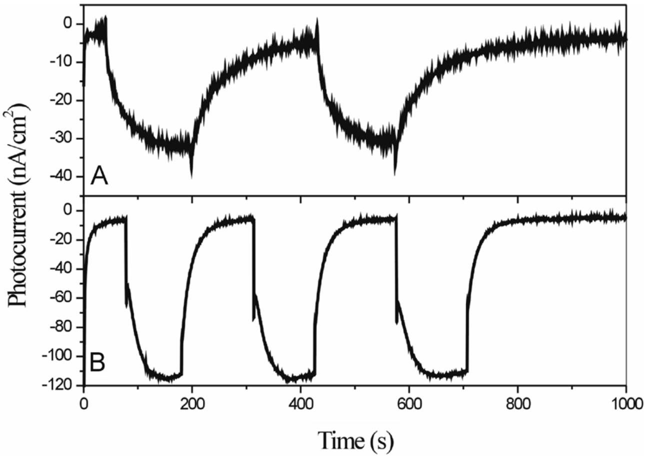


FIGURE 3 Photocurrent kinetics (in second time scale) in PEC with dye; based on [35].

kinetics (in the second time scale) are shown for some investigated dyes – ZnPc and ZnPcF. Free-metal porphyrin (H_2TPP) is taken as a standard sample since its photocurrent intensity in the PEC is near zero. A rise of the photosignal observed for MgTPP and ZnPc results in the presence of the metal ion Mg or Zn in the dye molecular structure. The metal ion like Pt, Cu, Mn, Pd and some others also affect the intensity of photocurrent but only slightly (PbTPP does not give signal). In all our experiments Mg and Zn when complexed both with porphyrins and phthalocyanines give the highest photoresponse [7]. These observations we could connect with metal activity. Mg and Zn are chemically very active, while remaining metals show rather low activity [3]. In the light of the ESR examination, the (g-factor value (2.0043÷2.0046) and spectral band width, ΔB (0.28÷0.35) [34]) it was showed that the π-electron in the macrocyclic rings rather than in metal is involved in charge separation and contribute to photoresponse. In the light of our photocurrent and ESR examinations it is evident that metal ion is pre-requested for high photocurrent. Its presence in the dye structure can change the distribution and delocalization of the π-electrons which lead to more intensive photoelectric effects.

(ii) Metal-free TPP (H_2TPP) gives photocurrent near zero whereas metal-free TNP (in which naphtyl groups are attached to the molecular skeleton instead of the benzene rings), even not complexed with metal ion gives a better photoresponse [7]. The quite intensive photocurrent was also observed for ZnTPP and also for ZnPc substituted with benzene rings [35]. These results evidently confirm that not only metal ion is able to give rise in photocurrent but also the aromatic peripheral groups can enhance photoresponse. In the light of this observation done for porphyrins and phthalocyanines without aromatic substituent and those with attached aromatic groups we can suppose that both higher number of π-electrons and their delocalization lead to enhancement of photosignal in the PEC.

(iii) Substituents like alkyl- or alkyloxy chains attached as peripheral groups change markedly ability of dyes for photocurrent. This result is coherent with our photoacoustic study. Alkyl- and alkyloxy chained phthalocyanines change absorbed energy in non-radiative processes and a part of energy is lost for heat (Table 1) and as a result these dyes show diminishing photosignal due to the mobile long chains. Thus we can suppose that the long alkyl/alkyloxy chains lead to thermal relaxation rather than charge separation [35].

(iv) When the indole units in ZnPc are substituted with the fluorine atoms (ZnPcF) the drastic rise in photocurrent is observed. The Φ_F value of ZnPcF is high, its τ_N value of the first singlet-state is long (Table 1) but its thermal deactivation is rather low. The large value of τ_N and weak thermal deactivation lead to high contribution of other deactivation processes like for example charge separation. In the light of the literature data the fluorine atom has very unique photophysical properties due to its high electronegativity (4 eV in Pauling's scale) and therefore is expected to has electron withdrawing character. However when it is attached to the phthalocyanine macrocyclic system it can turn into the electro-donating system due to the mesomeric resonance coupling [36]. As a result ZnPcF has an electro-donor feature and it can markedly enhance photocurrent.

The results presented in this chapter evidently proof the close correlation between the molecular structure of porphyrins and phthalocyanines and dyes ability for photocurrent generated in the PEC.

Singlet and Triplet States in Photocurrent Generation

In the light of occurrence of photosignal in the PEC for fluorescent and non-fluorescent dyes [7,37] and the expected affection of triplet-state behavior in the presence of metal ion we have to take into consideration not only the influence of the dye molecular structure but also the possible participation of both singlet- and triplet-states in generation of photosignal.

In the chapter above we have shown that the first excited singlet-state of a dye contribute to the charge separation process. However in opinion of some authors [38,39] for intensive photoactivity the dye triplet-state should be involved by effective intersystem crossing [3,26,38] and in a good photoconverter the triplet-state is expected to be involved in photoresponse [38,39]. As shown in our study some investigated porphyrins and some metal free substituted analogues do not show fluorescence or their fluorescence is rather modest [7,40]. In spite of that these dyes are able to generate photovoltage in the PEC. It could mean that in charge separation process (examined by photovoltage generation in the PEC) the triplet-state of organic dyes can also be involved.

To get insight more deeply in this problem we have done fluorescence quenching and ESR experiments for a system containing porphyrin dyes (H_2TPP, MgTPP, ZnTPP, PbTPP, PtTPP – as electron donors) and antraquinone (an electron acceptor) in DMSO. The electro-donating porphyrin and an electron withdrawing antraquinone could be a model of the process of electron transfer from a dye molecule to a semiconducting electrode in the PEC. Interaction between porphyrins and antraquinone results in fluorescence quenching of the singlet excited state of porphyrin in the presence of antraquinone. Electron-donating character of the singlet-state was also reported for some phthalocyanines [38,41]. The evaluated photoinduced electron transfer rates ($0.30 \cdot 10^9 \div 4.17 \cdot 10^9\,\mathrm{s}^{-1}$, depending on dye) evidently confirm participation of the dye singlet-state in electron transfer process [34,38,41]. On the basis of Stern-Volmer plots the quenching rates were estimated and ranged from about $10^{14}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ (fast quenching in poor photoconverters) to a little less than $10^{13}\,\mathrm{M}^{-1}\,\mathrm{s}^{-1}$ (slower quenching in good photoconverters). The fluorescence quenching examination was coherent with the ESR study. The ESR experiment revealed the appearance of an “unpaired” electron, predominantly in MgTPP and ZnTPP and thus showed their good electro-donating character. Otherwise, H_2TPP and PtTPP which presented rather poor photosignal in the PEC were characterized by the higher bimolecular quenching rates and lower ESR signals with respect to those for MgTPP and ZnTPP. This result indicated that charge recombination processes in MgTPP and ZnTPP was slower than in e.g., H_2TPP and PtTPP. The slower recombination process in ZnTPP and MgTPP could explain their high ability for photocurrent response. The poor photoconversion observed in H_2TPP and PtTPP could be explained by their high fluorescence quenching rate, low ESR signal and fast charge recombination. The charge recombination process with triplet-state participation was proofed with the delayed luminescence experiments for some porphyrins [42].

Fluorescence decay time of some porphyrins and phthalocyanines is sufficient long and the dyes can interact being excited in the first singlet state with the electron acceptor for a long time. The long life-time of the singlet-state could indicate an intensive intersystem crossing transition which could lead to high population of the dye triplet-states. Therefore we did steady-state PAS (it gives information on the global thermal deactivation with singlet- and triplet-states participation) and time-resolved LIOAS experiment (it gives insight into triplet-state population) to answer a question as to the possibility of triplet-state contribution in photocurrent. The correlation factor estimated on the basis of the experimental dependence of the PAS value on light modulation frequency and predicted on the basis of Rosencwaig-Gersho theory [43] does not reach unity. It indicates that not only prompt internal conversion transitions but also slow relaxation processes with triplet-state participation are involved. The values of the quantum yield of triplet-state generation, Φ_T and the thermal relaxation time, τ_2 from the triplet-state to the ground-state are gathered in Table 2. The high Φ_T were found for MgTPP, ZnTPP, their phthalocyanines analogues (MgPc, ZnPc) and fluorinated phthalocyanine (ZnPcF) ranging from 0.72 to almost 1 [44]. The PbTPP dye is characterized by the lower probability of the triplet-state population (0.65) although its Φ_T is expected to rise owing to the spin-orbit coupling [44]. However due to the fast charge recombination process the Φ_T is diminished. The dissimilarity between MgTPP, ZnTPP and PbTPP can be also described as due to the charge transfer process in PbTPP

TABLE 2 LIOAS Data Evaluated on the Basis of Marti *et al.* [24] and Small *et al.* [25] Procedures of the Synthetic Dyes (Temperature 20°C); based on [44]

Dye	α	k_1	k_2	Σk_i	τ_2(μs)	Φ_T
MgTPP	0.52	0.55	0.12	0.67	1.74	0.95
ZnTPP	0.34	0.34	0.21	0.55	2.30	0.98
PbTPP	0.50	0.55	0.34	0.89	3.59	0.65
MgPc	0.54	0.55	0.12	0.67	1.74	0.72
ZnPc	0.52	0.52	0.18	0.70	2.00	0.81

α – a part of excitation exchange into heat promptly (in shorter time than time resolution of apparatus), Φ_T – quantum yield of triplet-state population, k_1 – pre-exponential factor for $\tau_1 \leq 0.5\,\mu s$, k_2 and τ_2 – pre-exponential factor and triplet-state thermal decay time, respectively; accuracy of calculated values α, $k_{1,2}$, τ_2 and Φ_T – about 10%.

and distortion in the planarity of the molecular frame owing to the presence of heavy Pb [45]. The extremely high value for ZnPcF is closely connected with the mesomeric effects occurring when the dye molecular scaffold is conjugated with the electronegative fluorine atoms [35,36]. Moreover the relaxation decay time τ_2 is enough long (about 2 μs) for a dye to interact with a proper electron-acceptor (in the PEC with a semiconducting electrode). The high population of the triplet-state and its long-live time allow the process of charge separation to occur.

The contribution of the singlet- and triplet-states in photocurrent generation in the PEC based on porphyrins or phthalocyanines could be also explained in the terms of the position of the excited dye level in the energy scale with respect to the conductive band of the semiconductor (the energy gap for In_2O_3 is 3.6 eV) and Fermi level (located at 0.6 eV below the conductive band and equalized to the dye S_0) [46]. The energy of $S_1 \rightarrow S_0$ transitions (calculated on the basis of fluorescence spectra) are nearly 1.8 eV and 2.0 eV for phthalocyanines and porphyrines, respectively. The energetic distance of the $T_1 \rightarrow S_0$ transitions for phthalocyanines and porphyrins are about 1.13 eV and 1.59 eV, respectively [19,20]. Thus we can suppose that both singlet- and triplet-states of Pc and P dyes under our investigation lie above the energy gap of the semiconductor (In_2O_3) and the triplet-state together with the singlet-state could be involved in charge separation and they both are active in photocurrent generation in the PEC due to their advantageous location in the energetic scale with respect to the conductive band of In_2O_3 [44,47].

3.3. Porphyrins and Phthalocyanines as Photosensitizers and Photomarkers

Procedure of Dye Selection

The optimal sensitizers have to be chosen in a step by step selection procedure based on the investigation of a group of dyes introduced into various model systems. To establish which dye is best suited for a particular application, the set of investigations concerning several model systems (started from very simple model till the model close to medical issue) should be performed.

At first, the properties of dyes in solution were established [14–16] (i.e., absorption range, the fluorescence quantum yield, photostability, the quantum yield of triplet-state generation, thermal deactivation, the decay time of the dye triplet-state) on the basis of the experiments made in air, oxygen or nitrogen atmosphere at various temperatures.

The next step of the study was the examinations of the properties of dyes introduced into resting and stimulated lymphocytes. Stimulated cells can be used as a model for leukemic cells [10,11]. There are several differences between lymphocytes obtained from healthy donors, and artificially stimulated *in vitro* and leukemic cells, but they have at least one common feature, i.e., vigorous proliferation.

And finally, the human cells obtained from leukemia cell-lines are stained by the selected dyes [19]. In the last two steps of examinations, the efficiency and selectivity of the dyes incorporation into cells as well as photochemical properties of stained cells are investigated.

Synthetic Dyes

Lately, Pc's properties as a potential photosensitizers were established in several simple models: in fluid solvents [26], in anisotropic polymer matrix [48,49], and in the resting and stimulated human peripheral blood cells [49]. Comparison of sensitizer properties in various matrixes enable to predict its interactions with tissue and/or explained obtained spectral and photochemical properties of dye in complex anisotropic biological surroundings. Pc complexation with various metals and attachment of various side groups influence their interactions with macromolecules [48–50]. The spectral and photochemical properties of these dyes in a model system and in cells can be different. From fluorescence emission of dyes it follows that the Pc's were introduced selectively, i.e., with higher efficiency into stimulated than into resting (normal) cells [49,50]. The intrinsic fluorescence emission of normal and stimulated cells was also found different. It was suggested [49] that it makes the opportunity of the application of this emission in PDD to distinguish malignant and healthy cells. It was also shown [26] that the investigated Pc dyes exhibit efficient formation of triplet-state, therefore, they will probably be good candidates suitable for PDT.

Three metal substituted Pc dyes exhibited a high population of triplet-state [26] were chosen to incorporate into two different culture lines of human T leukemia cells [19]. The behavior of Pc dyes into leukemia cells were investigated using fluorescence spectroscopy because of perturbation of absorption spectra by light scattering. Fluorescence spectra of the samples were measured over two regions of excitation wavelengths: (i) predominant absorption of the cell material and (ii) strong absorption of photosensitizers as well as the emission was observed as a function of the time of sample illumination [19]. Dye incorporation and sample illumination affect membrane permeability of leukemia cells. This effect differs for a cell from various lines [51] and depends on types and amounts of lipid and proteins in the cells.

The performed experiments show (Fig. 4) that the kinetics of photoreactions in stained cells from the two lines are different and that these differences are dependent on the dye used. In solution, all Pc dyes investigated exhibit a similar high yields of triplet-state generation and degree of quenching by oxygen [26], but after incorporation into leukemia cells they show different photochemical properties (Fig. 4). It was found [48,49] that the Pc dyes with the same metal ion and different side groups exhibit different activity in PDT and can be ordered differently in an anisotropic matrix. It could suggest that Pc dyes interact with

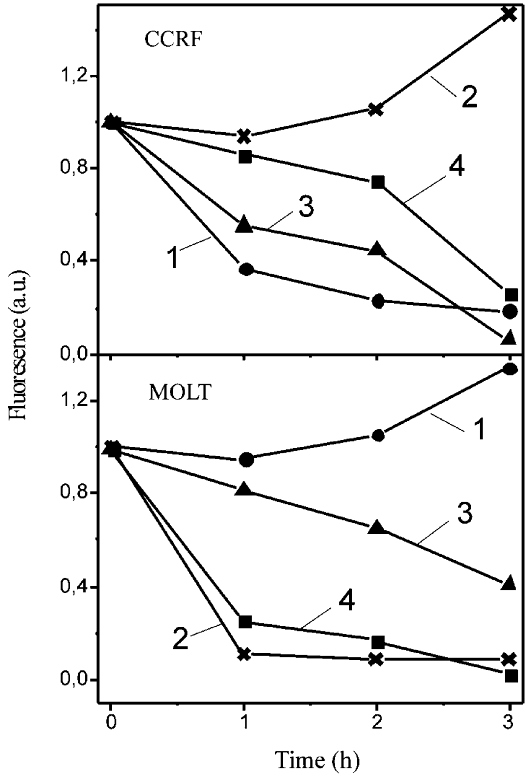


FIGURE 4 Changes in intensities of the main fluorescence bands *versus* illumination time recorded for Pc's (ZnPc - curves 1,3; MgPc - curves 2,4) introduced into two cell lines; wavelengths of fluorescence excitation (λ_{exc}) and observation (λ_{obs}): $\lambda_{exc} = 295\,nm$, $\lambda_{obs} = 335\,nm$ for curves 1,2 and $\lambda_{exc} = 610\,nm$, $\lambda_{obs} = 674\,nm$ and $675\,nm$ for curves 3 and 4, respectively; based on [19]. CCRF and MOLT are leukemia cells from the cell-lines.

non-similar surroundings, i.e., their localization and/or orientation within cell structures is probably different.

In the light of the results obtained [19] and of the current knowledge, the mechanism of interaction between Pc dyes and cell material is not yet established. The described results show that the same Pc dye exhibits different properties in each of the two cell lines [19]. Small differences in the leukemia T cell properties can have a strong influence on the efficiency of the Pc dye incorporation and on the course of photodynamic reaction (Fig. 4). It seems [19] that an optimal dye-sensitizer for a given patient should be established before photodynamic treatment by checking *in vitro* interaction of a photosensitizer with the patient's cells.

Natural Pigments

A similar series of experiments, as for synthetic dyes, were carried out for the natural tetrapyrrole macrocycle. (B)Chls aggregates in aqueous solvents and in nonpolar organic solvents, which hinders significantly their ability to generate $^1O_2$, therefore ethanol was chosen for the investigation, because in alcohol pigments exist in monomeric form (Fig. 2B) and ethanol is also non-toxic for human cells.

The attention is focused on natural pigments (chlorins and bacteriochlorins) to apply them for medical purposes because they have high optical extinction coefficients in the region of low cell material absorption (Table 1). BChl *c* which belongs to chlorosome Chls is an exception as to a differently substituted derivatives of Chl *a* (Fig. 1B). Chl *a* and BChl *c* as well as their closest Mg-free derivatives (Phe *a* and BPhe *c*) are hydrophobic photosensitizers. The most hydrophilic parts of (B)Chls are the central Mg ion, the 13^1 – oxy group of isocyclic ring, and the ester carbonyls. It is known [8] that hydrophobicity of a photosensitizer enhances its inclusion into the transformed cells but slows down its clearance from normal cells.

Some results obtained for the natural chlorins from LIOAS signal analysis are shown in Table 3. The (B)Phe pigments exchange less excitation energy into heat in a short time than (B)Chl (Table 3). From a comparison of the Φ_T values (Table 3) [14,16] with that described in literature [27,52,53] it follows that they are in good agreement with those obtained for (B)Chl-type pigments. The obtained results indicate that in a simple model system, using Marti *et al.* [24] method of the LIOAS signal analysis, we are able to establish the appropriate yield of triplet-state generation. In the light of the experiments performed [14,16], a more photochemically stable and generating more effectively triplet-states, (bacterio)pheophytines seem to be suitable for PDT. The results concern the pigments in alcoholic solvents imply also that

TABLE 3 LIOAS Data Obtained for the Pigments in Ethanol Under Air and N_2 Atmosphere (Temperature 20°C) and Evaluated on the Basis of Marti *et al.* [24] and Small *et al.* [25] procedures; based on [14,16]. Notation as in Table 2

Pigment	gas	α	k_1	k_2	Σk_i	τ_2 (μs)	Φ_T
Chl *a*	air	0.62	0.64	0.05	0.69	1.30	0.34
	N_2	0.54	0.55	0.03	0.58	1.65	0.52
Phe *a*	air	0.53	0.53	0.08	0.61	1.07	0.84
	N_2	0.50	0.51	0.03	0.54	2.42	0.91
BChl *c*	air	0.63	0.61	0.02	0.63	2.01	0.55
	N_2	0.58	0.59	0.03	0.62	1.54	0.58
BPhe *c*	air	0.59	0.59	0.06	0.65	2.13	0.74
	N_2	0.52	0.52	0.05	0.57	1.68	0.76

(B)Chls, which are photochemically less stable but exhibit higher Φ_F values (Table 1), can be promising candidate as photomarkers for diagnosis. It was found out that even at an excess amount of oxygen both pigments in the triplet-states are still able to participate directly in photodynamic reaction. The roughly estimated yield of singlet oxygen formation is about 0.22÷0.38 and 0.14÷0.18 for BChl *c* and BPhe *c*, respectively [14].

It is known that the yield of singlet oxygen formation ($\Phi_\triangle$) as a result of pigment triplet interaction with oxygen can be given by the relation: $\Phi_\triangle = \Phi_T \cdot S_\triangle$. The values of $S_\triangle$ – coefficient are known to be close to 1 for porphyrins and Chl [27]. Our data [16] agree with this suggestion because the Φ_T values estimated in our experiments, presented in Table 3, are similar to the $\Phi_\triangle$ values reported by others [27].

In order to establish the possibility of natural pigments applications and their interaction with the human lymphocytes the optical spectroscopy was applied. As it is clearly seen (Fig. 5) from the Q_y region of the absorption spectra that BChl molecules are incorporated mainly in the aggregated form and with higher degree into resting than into stimulated cells. After illumination, this pigment disaggregates and is not photochemicaly stable. BPhe molecules are predominantly in the monomeric form and show selectivity of incorporation into the cells (Fig. 5). It was shown also [10] that the stimulated cells stained by Mg-free chlorins undergo destruction (caused by photodynamic reaction) to a higher degree than the resting stained cells (not shown) whereas BChl molecules which are photobleached on the cell illumination cannot cause the target cell damage and cannot be effective in PDT.

It suggests again that the metal-depleted (B)Chl-like pigments seem to be good candidates for therapeutic purpose. They show high yields of excited triplet-state formation with life-times similar to those

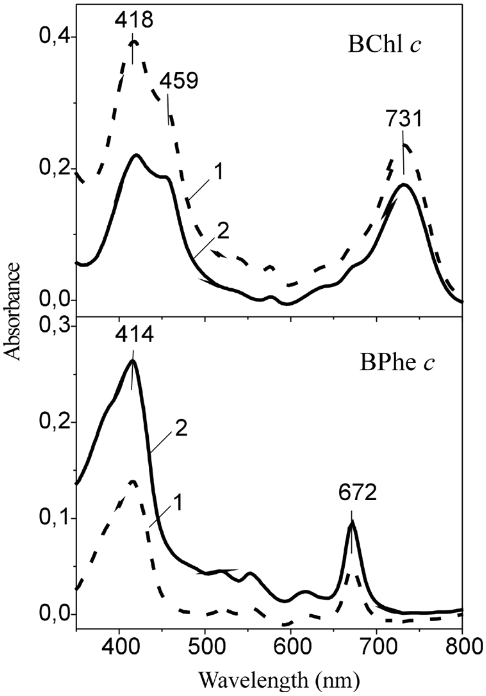


FIGURE 5 Absorption spectra of BChl *c* and BPhe *c* incorporated into resting (1) and stimulated (2) lymphocytes; based on [10].

observed for hematoporphyrin derivative [8]. Despite their promising photodynamic properties and low cytotoxicity, the poor solubility in physiological liquids has limited the use of them as a photosensitizer *in vitro* and *in vivo*.

CONCLUSIONS

In this article we have evidently shown that natural pigments as chlorophylls and bacteriochlorophylls and their synthetic derivatives porphyrins and phthalocyanines are perfect molecular materials which could be applied in molecular photovoltaics and photodynamic therapy/diagnosis of cancerous tissues. From the presented results some conclusion concerning possibility of dye applications in PV, PDT or PDD can be drawn:

(i) some porphyrins and phthalocyanines could be very good agents in molecular photovoltaics; the best dyes are those which are complexed with zinc or magnesium ion and substituted with the fluorine atoms,

(ii) the excited singlet-states of dyes are involved in photocurrent generation but participation of the dye triplet-states cannot be excluded,

(iii) the dyes exhibiting, not only high photostability but also efficient triplet-states generation and monomeric form after incorporation into biological environment are promising sensitizers for the application in PDT,

(iv) dyes selected on the basis of the optical spectroscopy and LIOAS analysis, suitable for diagnosis, should be at least highly fluorescent, whereas to prevent the photomarker phototoxicity, their triplet formation is rather unwanted,

(v) the same dye, in various human cancer cells exhibit different spectral properties and ability to trigger the photodynamic reaction and this fact should be taken into consideration in further clinical examinations.

ABBREVIATIONS

A	absorbance
(B)Chl	(bacterio)chlorophyll
(B)Phe	(bacterio)pheophytin
BCP	bromocresol purple
DMSO	dimethyl sulfoxide
ESR	electron spin resonance
LIOAS	laser induced optoacoustic spectroscopy
PAS	photoacoustic spectroscopy/signal
PDD	photodynamic diagnosis
PDT	photodynamic therapy
PEC	photoelectrochemical cell
TD	photothermal deactivation
PV	photovoltaics
Pc	phthalocyanine
P	porphyrin
TPP	tetraphenylporphyrin

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