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Photophysical properties and photodynamic activity of 13,15-*N*-methoxycycloimide chlorin p_6 methyl ester in micellar surfactant solutions

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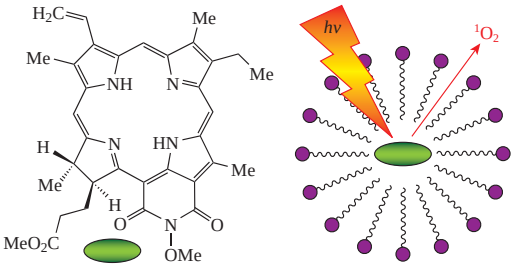
Photophysical parameters, photostability, aggregation behavior and photochemical activity of a new chlorin p_6-based photosensitizer in micellar surfactant solutions were estimated. Despite the low fluorescence quantum yields, this compound demonstrates high photostability, and its photodynamic activity in micellar systems is comparable to that of the commercial photosensitizer Fotoditazin®, which makes this chlorin p_6 derivative suitable for the interstitial photodynamic therapy of solid tumors.

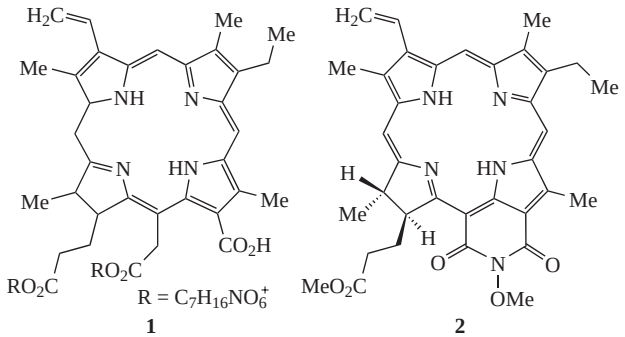


Photodynamic therapy (PDT) is a promising method for tumor treatment based on the photogeneration of reactive oxygen species by excited photosensitizer (PS) molecules. The main requirements imposed on an ideal PS include high molar extinction coefficients within a 600–800 nm spectral range, which is the most transparent for biological tissues, high singlet oxygen quantum yield, stability against aggregation in aqueous media, significant photostability without photobleaching, low dark cytotoxicity and selective accumulation in tumor cells.[1–5] The selective accumulation and photostability of PSs is crucial for the interstitial PDT of large-volume or thick solid tumors, which require significant irradiation time for neoplasmic tissue destruction.[6,7] Synthetic chlorophyll *a* derivatives with the peripheral modifications of the chlorin macrocycle increasing their water solubility are probably the most intensively studied second-generation photosensitizers.[8,9] A wide variety of modified chlorins with intense absorption bands in the near-IR region have been synthesized.[10–14] Many of them were characterized by high fluorescence and singlet oxygen quantum yields, significant stability against aggregation and photostability; however, not all of them demonstrated sufficient phototoxicity *in vivo*.[15] This is mostly due to the specific parameters of the local microenvironment of PS molecules in biological media, which are highly ordered (organized) microheterogeneous systems. In such systems, the local parameters of the microenvironment (polarity, acidity, viscosity and dielectric constant) significantly influence the photophysical properties of the PS molecules in both the ground and excited states and determine their photochemical activity in energy and electron transfer processes.[16,17] In this regard, it is important to study the photophysical properties and photochemical activity of potential photosensitizers in model microheterogeneous systems, such as micellar surfactant solutions,[18] polymer nanoparticles and micelles,[19,20] as well as in liposomal and vesicular membrane-mimetic systems.[21,22]

Among the above nanocarriers, micellar surfactant solutions are available synthetic systems considered as the simplest models of biological membranous structures and the prototypes of drug delivery systems. Hence, the studies of the preliminary solubilization of PSs allow one to predict their behavior in biological media and provide useful information for the further design of appropriate biocompatible systems for PS administration.

Here, we compared the spectral properties, aggregation behavior, photostability and photochemical activity of 13,15-*N*-methoxycycloimide chlorin p_6 methyl ester† **1** and bis-*N*-methyl-D-glucamine salt of chlorin e_6 **2** (Fotoditazin®) in the micellar solutions of different surfactants.

Monomolecular form of compound **1** in DMF is characterized by an intense absorption band at 705 nm ($\lg\varepsilon$ = 4.56) and a fluorescence maximum near 725 nm.‡ Since this compound is



1 **2**

† Compound **1** was synthesized by the alkylation of chlorin p_6 *N*-hydroxycycloimide with iodomethane in the presence of DBU in a yield higher than 80%.[23]

‡ Electronic absorption spectra were recorded using a Hach DR-4000V spectrophotometer within 320–1100 nm spectral range in 10 mm quartz cells at room temperature. Emission spectra were measured on a Perkin Elmer LS-50B luminescence spectrometer in 10 mm quartz cells with the excitation wavelength corresponding to the Soret band absorption maximum of the photosensitizers. The final molar concentration of PSs **1** and **2** was about 5 μM.

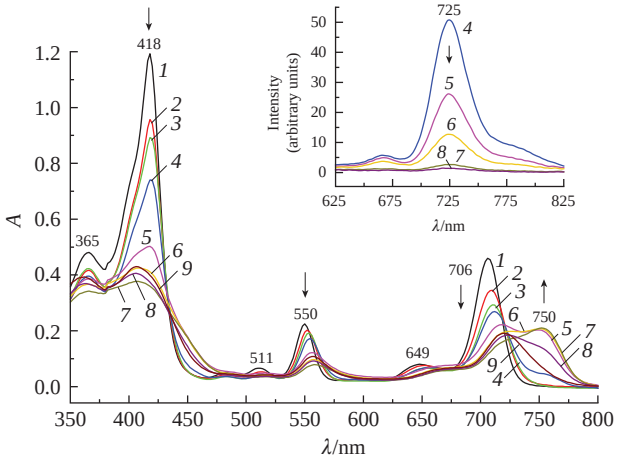


Figure 1 Absorption and (insert) fluorescence spectra of PS **1** in H_2O/DMF mixtures, where DMF content is (*1*) 100, (*2*) 65, (*3*) 50, (*4*) 40, (*5*) 30, (*6*) 20, (*7*) 10, (*8*) 5 and (*9*) 2 vol%.

hydrophobic, it was necessary to study its aggregation behavior in aqueous-organic media in order to reveal the aggregation threshold. A series of electronic absorption spectra of **1** in DMF–H_2O mixtures with different volume ratios is shown in Figure 1.

The absorbance decreased with increasing water content accompanied by a significant broadening of absorption bands indicating the self-associated state of the macrocycles. Within a range of 10–30 vol% DMF, the formation of *J*-type aggregates was observed for **1**, resulting in a 40–50 nm bathochromic shift of the *Q*-band and complete fluorescence quenching with decreased photodynamic activity. Unlike **1**, PS **2** demonstrated no significant tendency to aggregation in aqueous-organic solutions due to its more polar anionic molecule resulting from the dissociation of methylglucamine cations bound to carboxyl residues. The above results confirmed the need to select suitable supramolecular systems for the stabilization of a monomolecular fluorescent form of **1** in polar aqueous media. In 0.1% aqueous solutions of biocompatible polymers (poly-*N*-vinylpyrrolidone, polyethylene glycol, poly-L-lysine and human serum albumin), the spectral parameters of **1** corresponded to the associated form of PS (data not shown). Figure 2 depicts the absorption spectra of **1** in the micellar solutions of surfactants: anionic SDS (50 mM), cationic CTAB (2.5 mM) and nonionic TX-100 (1 mM).

The above data suggests that chlorin binding to the surfactant micelles leads to a 10 nm bathochromic shift of the absorption Q-band and the corresponding emission band. Wherein, micellar solubilization prevents **1** from aggregation and provides effective stabilization of the monomeric fluorescent form of the PS. Despite the presence of hydrophilic substituents, PS **2** also reveals an inherent tendency to aggregation in aqueous media with the corresponding decrease of the fluorescence intensity and photodynamic activity. A similar effect observed for **2** in the micellar solution of cationic CTAB may be due to the intermolecular interactions of the neighboring macrocycles with anionic carboxyl residues bound to the cationic micellar surface. In this case, spectral signs of *J*-type aggregate formation can be observed for **2** in micellar CTAB solutions upon storage for several hours with the absorption maximum at 675 nm, which confirms the possibility of ionic self-assembly of the supramolecular aggregates on the micellar surface.[24]

In addition to aggregation behavior, another essential parameter of PS for PDT is photostability, so the photobleaching rates for **1** and **2** in micellar surfactant solutions under visible light irradiation ($\lambda \geqslant 600$ nm)[§] have been compared (Figure 3).

The data obtained clearly indicates at least a threefold increase in photostability of **1** compared to the commercially available

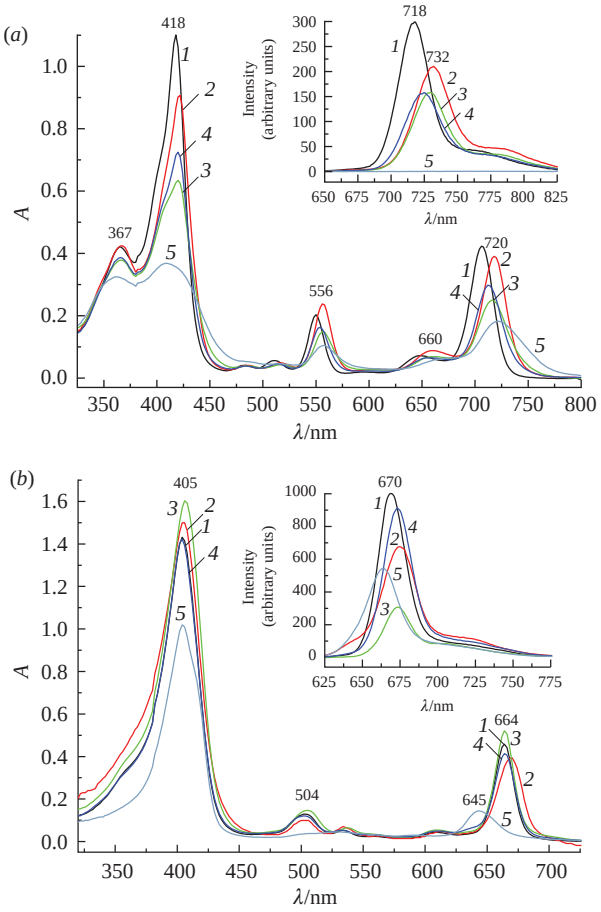


Figure 2 Absorption and (insert) fluorescence spectra of (*a*) PS **1** (λ_{ex} = = 420 nm**)** and (*b*) **2** (λ_{ex} = 405 nm**)** in micellar systems: (*1*) DMF, (*2*) SDS, (*3*) CTAB, (*4*) TX-100, (*5*) H_2O.

PS **2**. The rate of photobleaching upon irradiation for **1** in micellar systems was about 4–6% per hour, while for **2** it was about 13–16%, and in cationic CTAB micellar solution even 33%. The latter result can be related both to the more intense photosensitized self-oxidation of **2** bound to the cationic micellar surface, as compared to the same chromophore solubilized within the non-polar micellar core of either anionic SDS or neutral TX-100 micelles, and the simultaneously occurring aggregate formation, leading to the decrease of the monomer absorption.

Finally, photochemical activities of both photosensitizers in singlet oxygen generation upon visible light irradiation have been

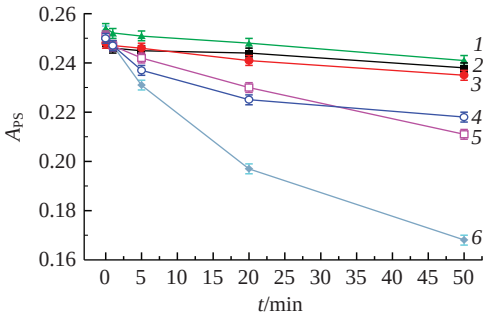


Figure 3 Photobleaching kinetics in micellar surfactant solutions for **1**: (*1*) TX-100, (*2*) SDS, (*3*) CTAB and **2**: (*4*) SDS, (*5*) TX-100, (*6*) CTAB.

[§] The sample irradiation was performed in quartz cells under ambient conditions in the air-saturated solutions. The light source included a halogen lamp (150 W), a three-lens spherical condenser with a reflector, heat and UV filters and an orange light filter (OS-13) with $\lambda \geqslant 600$ nm. The incident light intensity was 10 mW cm^{-2}.

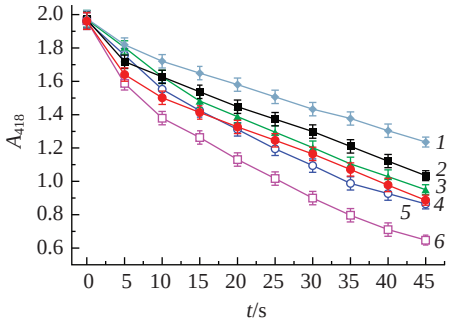


Figure 4 Photooxidation kinetics of DPBF in micellar surfactant solutions for **1**: (*1*) TX-100, (*2*) SDS, (*3*) CTAB and **2**: (*4*) SDS, (*5*) CTAB, (*6*) TX-100.

Table 1 Photophysical parameters of PSs **1** and **2** in different media.

Media	**2**				**1**				Φ_{F1}/Φ_{F2}	$\Phi_{\Delta1}/\Phi_{\Delta2}$
	λ_{max}/nm	lg ε	λ_{em}/nm	$\Delta\lambda_{st}$/nm	λ_{max}/nm	lgε	λ_{em}/nm	$\Delta\lambda_{st}$/nm		
DMF	406 666	5.46 4.92	669	3	418 708	5.17 4.83	718	10	0.42	0.71
H_2O	404 644	5.30 4.45	664	20	410 722	4.50 4.20	–	–	–	–
SDS	402 670	5.35 4.78	674	4	422 718	5.08 4.71	732	14	0.23	0.62
CTAB	406 670	5.35 4.78	673	3	422 716	4.99 4.66	730	14	0.65	0.54
TX-100	406 670	5.38 4.84	673	3	420 714	5.01 4.68	726	12	0.28	0.48

compared.[¶] Kinetic curves of DPBF photosensitized oxidation in micellar surfactant solutions are shown in Figure 4.

In micellar systems for both photosensitizers singlet oxygen generation efficiency was found to depend significantly on the surfactant nature. Thus, for **1** photodynamic activity increases in the series TX-100 < SDS < CTAB < DMF, which is in good agreement with the fluorescence data (Figure 2), while PS **2** demonstrates maximal photochemical activity in neutral TX-100 micelles. This indicates the importance of the chromophore localization within the micellar system in regulation of its photochemical activity. From the spectral data the main photophysical parameters for both photosensitizers were calculated (Table 1).[††] The results obtained indicate a number of advantages of PS **1** compared to the commercially available drug **2**. Particularly, its absorption Q-band is 40–50 nm-shifted to the low-frequency spectral region due to the presence of the additional imide cycle, while the Stokes shift ($\Delta\lambda_{st}$) value is 7–10 nm higher than that for **2**, providing a more efficient use of the light energy during PDT. However, PS **1** possesses 2–3 times lower Φ_F values in micellar systems compared to PS **2**. The calculations suggest that the absolute value of Φ_Δ for PS **1** in DMF is about 0.5 ± 0.05, which is consistent with the literature data,[15] while in micellar systems its Φ_Δ has been determined for the first time and appeared to be 1.5–2 times lower than that for PS **2**. However, despite the low fluorescence quantum yields of **1** in micellar systems, its photodynamic activity in ionic surfactant micelles is comparable to activity of **2**. Thus, considering its high photostability, it is possible to conclude that 13,15-*N*-methoxycycloimide chlorin p_6 methyl ester is a promising candidate for interstitial PDT photosensitizers. Since the above tested ionic surfactants are just the model compounds for the drug solubilization studies, it is impossible to use them directly in further biological tests. However, the results obtained clearly demonstrate the advantages of PS **1** solubilization within the micellar media and suggest the application of biocompatible polymeric micelles instead of the individual polymers and polyelectrolyte complexes as the most appropriate drug formulations.

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[¶] The efficiency of photosensitized singlet oxygen generation for both photosensitizers was estimated by a chemical trapping method using a selective $^1O_2$ acceptor – 1,3-diphenylisobenzofuran (DPBF).[25] In all the photochemical experiments concentration of the PSs was maintained at 2–3 μmol cm^{-3} resulting in the Q-band absorbance about 0.1.

[††] Calculations of the absolute values of fluorescence (Φ_F) and singlet oxygen (Φ_Δ) quantum yields were performed according to the known procedure[25] using zinc(II) phthalocyaninate solution as a standard (Φ_Δ = = 0.65 in DMF).

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