

Photosensitizing properties of supramolecular systems based on chlorin e₆

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Abstract. The novel supramolecular systems based on chlorine e₆ (Ce₆) are presented, and their optical absorption and fluorescence have been investigated. The influence of different excipients as poly-N-vinylpyrrolidone (PVP), polyethyleneglycol (PEG), bovine serum albumin (BSA), chitosan, Triton X-100 (TX-100) on spectral characteristics of these systems has been studied. The obtained spectral-fluorescence characteristics of Ce₆-PVP, Ce₆-PEG, Ce₆-BSA, Ce₆-TX-100 indicate disaggregation of chlorine e₆ molecules and their consecutive interaction with excipients in solutions and formation of molecular associates and molecular complexes. The system Ce₆-chitosan is characterized by aggregation of pigments in solution that reduces photochemical activity of a photosensitizer. The fluorescence quantum yield ϕ_k of mentioned above supramolecular systems has been calculated. The results can be useful during the new method of controlled aggregation of photosensitizers as a part of supramolecular complexes development as well as for the purposes of new medicines of predictable photodynamic activity creation. © 2016 Journal of Biomedical Photonics & Engineering.

Keywords: chlorin e₆, photodynamic therapy, supramolecular systems, spectral characteristics, optical absorption, fluorescence

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References

1. A. B. Uzdensky, Cellular and molecular mechanisms of photodynamic therapy, Science, SPb, Russia (2010).
2. A. L. Akopov, N. V. Kazakov, A. A. Rusanov, and A. Karlson, "The mechanisms of photodynamic action for treating of cancer patients," Photodynamic therapy and photodiagnosics 4(2), 9-16 (2015) [in Russian].
3. E. V. Pinyaskina, "Photodynamic processes in cells. Primary mechanisms," Bulletin of DSPU 1, 60-67 (2010) [in Russian].
4. E. Zenkevich, E. Sagun, V. Knyukshto, A. Shulga, A. Mironov, O. Efremova, R. Bonnett, S. P. Songca, and M. Kassem, "Photophysical and photochemical properties of potential porphyrin and chlorin photosensitizers for PDT," J. of Photochemistry and Photobiology B: Biology 33(2) 171-180 (1996).
5. M. V. Parkhats, V. A. Galievsky, A. S. Stashevsky, T. V. Trukhacheva, and B. M. Dzhagarov, "Dynamics and efficiency of the photosensitized singlet oxygen formation by chlorin e₆: the effects of the solution pH and polyvinylpyrrolidone," Optics and Spectroscopy 107(6), 974-980 (2009).
6. C. T. H. Yen, G. V. Ramenskaya, and N. A. Oborotova, "Chlorin derivatives in cancer photodynamic therapy," Rus. J. of Biotherapy 8(4), 99-104 (2009) [in Russian].
7. M. V. Parkhats, V. N. Knyukshto, H. A. Isakau, P. T. Petrov, and B. M. Dzhagarov, "Photophysical properties of photosensitizer chlorin e₆ incorporated into polyvinylpyrrolidone," Proc. SPIE 6727, 67272L (2007).
8. T. E. Zorina, I. V. Yankovsky, I. E. Kravchenko, T. V. Shman, M. V. Belevtsev, and V. P. Zorin, "Evaluation of Phototoxicity and cytotoxicity for chlorin e₆ ester derivatives and their liposomal forms," Biophysics 60(5), 759-766 (2015).
9. E. A. Genina, Biophotonics methods: Phototherapy, New Wind, Saratov, Russia (2012).

10. H. A. Isakau, M. V. Parkhats, V. N. Knyukshto, B. M. Dzhagarov, E. P. Petrov, and P. T. Petrov, "Toward understanding the high PDT efficacy of chlorin e₆-polyvinylpyrrolidone formulations: Photophysical and molecular aspects of photosensitizer-polymer interaction in vitro," *J. of Photochem. and Photobiol. B: Biology* 92(3), 165-174 (2008).
11. A. K. Visheratina, A. O. Orlova, V. G. Maslov, A. V. Fedorov, and A. V. Baranov, "Optical properties of conjugates of CdSe/ZnS quantum dots and chlorin e₆ in aqueous solution," *J. of Opt. Tech.* 82(11), 738-742 (2015).
12. S. L. Fiedaruk, T. V. Trukhacheva, S. N. Sokolov, K. A. Frolenko, and V. P. Kheidorov, "The study of spectral properties of dimethylester of chlorin e₆ in the presence of polyvinylpyrrolidone," *Vestnik farmacii* 3(65), 82-88 (2014) [in Russian].
13. V. Yu. Plavskii, V. A. Mostovnikov, G. R. Mostovnikova, A. I. Tret'yakova, and A. V. Mikulich, "Spectral-luminescent properties of chlorin e₆ and malate dehydrogenase complexes," *J. of Appl. Spectroscopy* 71(6), 818-828 (2004).
14. G. P. Gurinovich, A. I. Sevchenko, and K. N. Solov'ev, *Spectroscopy of a chlorophyll and related compounds*, Science and technology, Minsk, USSR (1978).
15. H. Podbielska, A. Ulatowska-Jarza, G. Muller, I. Holowacz, J. Bauer, and U. Bindig, "Silica sol-gel matrix doped with Photolon molecules for sensing and medical therapy purposes," *Biomolecular Engineering* 24(5), 425-433 (2007).
16. A. A. Krasnovsky, "Photodynamic action and singlet oxygen," *Biophysics* 49(2), 289-306 (2004) [in Russian].
17. G. V. Golovina, V. A. Ol'shevskaya, V. V. Vargin, V. A. Kuzmin, and A. B. Shevelev, "Solubility of porphyrine derivatives in vivo affected by aggregate formation and complexes formation with serum albumin," *Health of the population and habitat* 12, 41-43 (2013) [in Russian].
18. N. H. Karapetyan, and V. N. Madakyan, "The Interaction of new pyridylporphyrins with bovine serum albumin," *Russ. J. of Bioorganic Chemistry* 30(2), 172-177 (2004) [in Russian].

1 Introduction

Photodynamic therapy (PDT) is clinically used as a nonsurgical treatment in oncology, ophthalmology, dermatovenerology, and others, for various diseases, for example, cancers and bacterial cells. It is based on the use of a special chemical agent – photosensitizer (PS), which is accumulated in pathological target cells and initiated by laser light of an appropriate wavelength, corresponding photosensitizer absorption maxima. As a result, photochemical reactions between PS and oxygen, leading to the formation of cytotoxic agents in tumor tissue, damaging a tumor, take place. At this, photosensitizer transits into excited state, and PS molecule undergoes or oxidation-reduction reactions with electrons or protons transfer and formation of intermediate products - the radicals, further interacting with oxygen, or transfers excess of energy to the molecule of oxygen transiting at the same time into the excited singlet state [1].

In real biological systems both mechanisms can take place. Their relative contribution to bioprocesses depends on the PS identity, a concentration and a nature of the oxidized reagents, a concentration of oxygen and some other factors.

Nowadays most of the clinically used and experimentally tested PSs belong to the class of porphyrines or their reduced analogues, chlorins [2]. In biosystems in the presence of oxygen they usually enter photochemical reactions of the II type as the first triplet state energy of porphyrines (200 kJ/mol) is sufficient for ¹O₂ (95 kJ/mol) development [3].

Chlorin e₆ (Ce₆) belongs to the class of the plant porphyrines purchasing from chlorophyll a. It is

anticancer immune response modifier, activating the system of cell-mediated immunity and it possesses the following spectral and energy characteristics: the maximum of absorption is in the region of 660±5 nm that corresponds to the region of the greatest optical transparency for biological tissue, and a quantum yield of singlet oxygen reaches 0.7 [4, 5]. Three COOH groups provide the Ce₆ molecule hydrophilic properties, effective transport and accumulation in tumour tissue. High photodynamic activity when using small doses and low toxicity are also characteristics of Ce₆. Moreover, the availability of raw materials (microalgae *Spirulina platensis* or leaves of a nettle) plays a large role in the development of compounds based on chlorin.

Such "second-generation" photosensitizers as Radachlorine, Photolon, Potoditazin and others of chlorin alignment are in medical application in Russia. Various physical, chemical and pharmacological properties, and also various commercial costs are the characteristic of all these medicines [6]. However, these PSs have the high degree of phototoxicity connected with a high quantum yield of the singlet oxygen which is formed under the light influence when carrying out PDT. Besides, Ce₆, as a substance, has a number of shortcomings limiting its broad consumption, for example, a small period of storage and a loss of optical properties, elimination within 48 hours or more. It should be noted that chlorin molecules also tend to form aggregates in water solutions that leads to considerable decrease in photodynamic activity [5, 7]. Besides, the aggregation processes of the porphyrine pigments take place in polar solvents because of hydrophobic and π-π interactions of tetrapyrrol structures [8]. To avoid these

it's reasonable to develop and study the new effective systems with the optimum photophysical and pharmacological properties.

In this work the novel supramolecular systems based on chlorin e_6 have been obtained and the influence of various excipients on spectral characteristic of these systems as well as the influence of intermolecular interactions on aggregation behavior of chlorin e_6 in solutions have been studied.

2 Materials and methods

Ce₆ (Fig. 1) was kindly provided by the colleagues from Moscow Technological University (Preobrazhensky Department of chemistry and technology of biologically active compounds). Basic Ce₆ solutions with the concentration of $2.0 \cdot 10^{-3}$ mol/l were prepared by dissolution of Ce₆ dry sample weight in 20 ml of dimethylformamide (DMF) double-distilled. The received concentration was specified on electron absorption spectrum. Solutions stored in the dark at +4 °C. Purity and identity of Ce₆ were confirmed with the MALDI - mass spectrometry method on the Thermo Scientific DSQ II single quadrupole mass spectrometer (Thermo Scientific, USA)

The choice of water-soluble polymers (Fig. 1) is explained by the fact that they are the most widespread biologically-compatible substances which are the part of physiological solutions applied as a part of pharmacological drug. Supramolecular systems based on Ce₆ ($2 \cdot 10^{-5}$ M) in various excipients were studied: poly-N-vinylpyrrolidone (PVP) - Ce₆, polyethyleneglycol (PEG) - Ce₆, Triton X-100 (TX-100) - Ce₆, bovine serum albumin (BSA) - Ce₆, chitosan - Ce₆. Water solutions of Triton X-100 micelles (TX-100, C₃₄H₆₂O₁₁, LAB-SCAN analytical sciences, Poland) of concentration $2.5 \cdot 10^{-4}$ mol/l, and 2% (weight) solutions of poly-N-vinylpyrrolidone (PVP, (M=26500), Sigma-Aldrich, Germany) and polyethyleneglycol (PEG, (M=1000) MERCK, Germany) were prepared separately.

Absorption spectra (200-900 nm) were recorded with TU-1901 UV-Vis spectrophotometer from Beijing Purkinje General Instruments Co Ltd. Poorly resolved spectra were analyzed by decomposing the spectra into their Gaussian constituents.

The fluorescence spectra in the range of 550-800 nm were recorded with Fluorat-02-Panorama spectrofluorimeter (Lumex, Russia). The excitation wavelength was 410 nm. All measurements were conducted at 20 °C in standard K10 quartz cuvettes with optical path lengths of 1 cm. Registration of singlet oxygen was carried out by the method of "chemical traps"; 1,3-diphenylisobenzofuran was used as a "trap".

3 Results and discussion

Obtained in this experiment optical absorption spectrum of Ce₆ aqueous solution practically corresponds to the data of Ce₆ optical absorption spectrum presented in [9-13]. It is characterized by a strong Soret band with a

peak at $\lambda=403$ nm, typical for all porphyrines [14] and weaker Q-band in the region of 450-750 nm, which include a plateau at $\lambda=450-615$ nm with small peaks at 504, 533 and 599 nm, and also high-intensity peak at $\lambda=653$ nm. It should be noted that the penetration depth of light is limited by absorption and scattering in tissue [15]. Besides, the Ce₆ line shift to the long-wavelength region increases tissue permeability for visible light and at the same time reduces light absorption by blood pigment at 500-600 nm. All of these play an essential role for increase of PDT effectiveness [9].

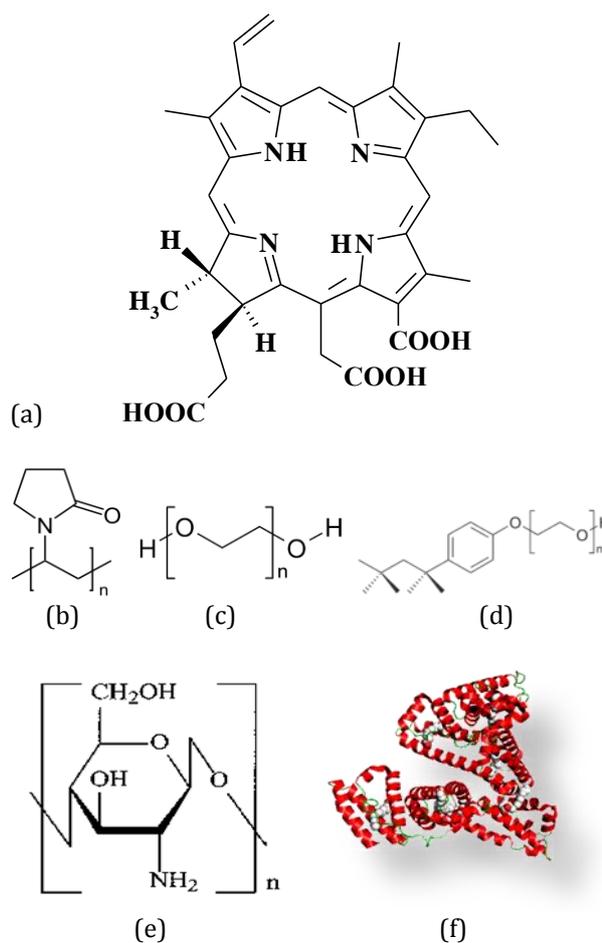


Fig. 1 Structures of chlorin e_6 (a), poly-N-vinylpyrrolidone (b), polyethyleneglycol (c), Triton X-100 (d), chitosan (e), bovine serum albumin (f).

When studying spectral properties of all presented supramolecular systems based on Ce₆, the hypsochromic shift in the field of Soret peak wasn't revealed (Fig. 2). According to the ideas of spectral shifts in biopolymers [12], the hypsochromic shift is connected with the transition of chromophore to more polar environment, and in our case, polar aqueous solvent. Absence of hypsochromic shift in the field of Soret peak testifies the identical hydrophobic behavior of all supramolecular systems, similar to hydrophobic behavior of Ce₆.

As illustrated in the Fig. 2, a small shift of Soret band to the long-wavelength region of optical

absorption spectra of Ce_6 -PVP and Ce_6 -BSA systems by 2 and 5 nanometers, respectively, in comparison to one of Ce_6 , is observed. Besides, some broadening of spectral band can be seen for the Ce_6 -PVP system. The system of Ce_6 -PEG shows a sharp splitting of Soret band with a form of Gaussian distribution and broadening by $\Delta\lambda^{1/2}_{Soret} = 13\%$. On the basis of absorbency fall in the region of Soret band for all systems and according to [16] one can say of single oxygen formation in all presented supramolecular systems. Besides, hypsochromic effect can be connected with the strengthening of intermolecular interactions (including interactions between the chromophores which are in more ordered form in comparison with the free state), and, as a result, the formation of molecular complexes takes place. This statement is consistent with the conclusions made by the authors of [5, 12], showed that, upon interactions, Ce_6 forms complexes with PVP. Moreover, the results of [17, 18] devoted to the investigation of porphyrines-serum albumin systems also confirm our hypotheses.

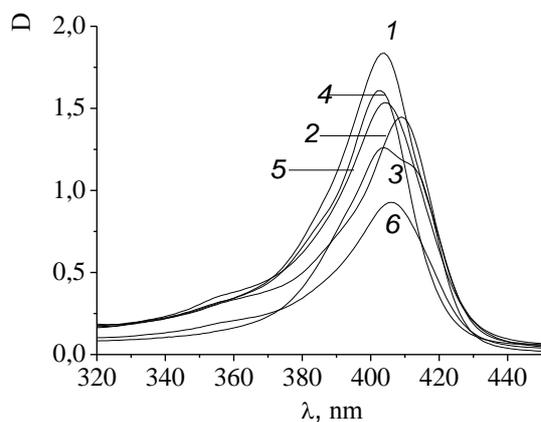


Fig. 2 Absorption spectra of supramolecular systems based on Ce_6 ($2 \cdot 10^{-5}$ M) in the region of the Soret peak: 1 - Ce_6 in aqueous solution, 2 - Ce_6 -BSA, 3 - Ce_6 -PEG, 4 - Ce_6 -chitosan, 5 - Ce_6 -TX-100, 6 - Ce_6 -PVP.

Optical absorption spectra (Q-band region) of supramolecular systems based on Ce_6 ($2 \cdot 10^{-5}$ M) in various excipients are shown in Fig. 3. When comparing the received data with the data for Ce_6 aqueous solution, we have revealed that in a case of chitosan, PVP, TX-100 and BSA addition to Ce_6 the maximum of long-wavelength Q-band absorption ($\lambda = 653$ nm) undergoes bathochromic shift (1 nm, 10 nm, 12 nm and 13 nm, correspondingly), whereas in the Ce_6 -PEG system the hypsochromic shift of the Q-band maximum (14 nm) is observed. As a result, the maximum of Q-band absorption changes in row: PEG (639 nanometers) < chitosan (654 nm) < PVP (663 nm) < TX-100 (665 nm) < BSA (666 nm). Thus, absorption maximum shift to the red region of spectrum demonstrates that when performing photodynamic therapy with the systems having PVP, TX-100 and BSA as excipients there is a high probability of penetrating light absorption and big

prospects for invasion these systems through tissues in comparison with Ce_6 .

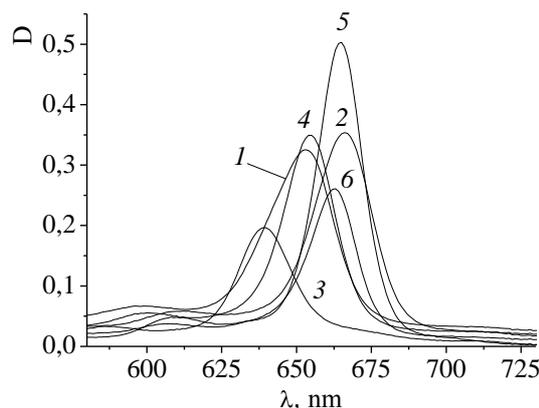


Fig. 3 Absorption spectra of supramolecular systems based on Ce_6 ($2 \cdot 10^{-5}$ M) in the region of the Q-band: 1 - Ce_6 in aqueous solution, 2 - Ce_6 -BSA, 3 - Ce_6 -PEG, 4 - Ce_6 -chitosan, 5 - Ce_6 -TX-100, 6 - Ce_6 -PVP.

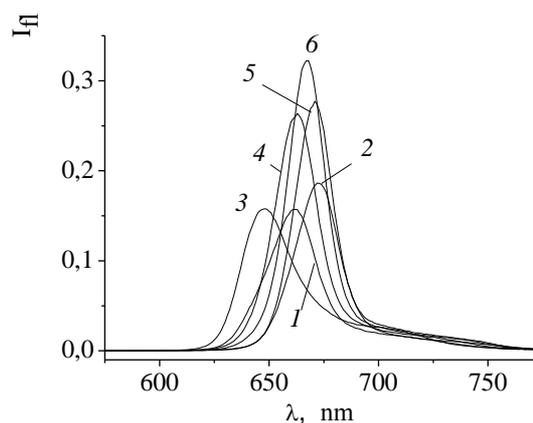


Fig. 4 Fluorescence spectra of supramolecular systems based on Ce_6 ($2 \cdot 10^{-5}$ M): 1 - Ce_6 in aqueous solution, 2 - Ce_6 -BSA, 3 - Ce_6 -PEG, 4 - Ce_6 -chitosan, 5 - Ce_6 -TX-100, 6 - Ce_6 -PVP.

The spectral changes in the Q-band region of absorption spectrum connected with the bathochromic shift of a long - wavelength absorption band of a sensitizer and essential reduction of half-width of a line also demonstrate disaggregation of Ce_6 molecules, their interaction with excipients in solutions and formation of a molecular complex. The appearance of a porphyrine molecular complex with TX-100, BSA and PVP absorption band at 615 nm (Fig. 3) is the confirmation of this assumption. Moreover, the disappearance of absorption in the region of 680-700 nm for Ce_6 -PEG system, specific for porphyrine aggregates, is also the confirmation of the porphyrine aggregates destruction and transition of a PS to molecular associates, and at a later stage, possibly, molecular complexes [17].

The specific characteristic of fluorescence spectra of all supramolecular systems is their overlap with Q-band of absorption spectra (Fig. 4), that allows considering the formed molecular associates and complexes as donors of photoexcitation energy for Ce_6 molecules. As can be seen from the Fig. 4, the addition of all studied excipients into Ce_6 aqueous solution leads to the increase of fluorescence intensity of Ce_6 . The greatest increase in fluorescence intensity is observed in the presence of PVP. Moreover, the determined by a relative method [13] (the free form of the dye in the absence of the enzyme was as a standart with $\phi_0 = 0.15$) quantum yield ϕ_k of the fluorescence is depends on the excipients, changing from 0.12 for TX-100 and 0.13 for chitosan to 0.16, in PEG and PVP. Such supramolecular compound as Ce_6 -BSA shows the value of the fluorescence quantum yield as 0.15 equal to the fluorescence quantum yield for Ce_6 .

Some increase in ϕ_k value for Ce_6 – PEG and Ce_6 – PVP in comparison with ϕ_k value for Ce_6 aqueous solution confirms the data of optical absorption spectra and demonstrates consecutive destruction of Ce_6 aggregates and a complex formation. It should be noted that aggregates degradation rate in the aqueous environments is various in the presence of different excipients. Probably, the calculated for Ce_6 -BSA system value of a quantum yield, equal to the same value for Ce_6 , can be explained by this. Low ϕ_k value for Ce_6 – TX-100, possibly, is connected with the partial aggregation of a pigment which is due to low solubility and the formation of another type complex characterized by a low fluorescence quantum yield. As far as fluorescent characteristics of the Ce_6 – chitosan system, it is seen that they remain almost invariable in comparison with similar characteristics of Ce_6 aqueous solution, and some decrease in ϕ_k is explained, perhaps, by aggregation of dye in solution that reduces photochemical activity of a PS.

With the help of the method of "chemical traps" the registration of singlet oxygen was carried out. From this, one can assume that as a part of the supramolecular complexes, Ce_6 is an effective photosensitizer of singlet oxygen generation with a quantum yield 0.53 (Ce_6 -PVP), 0.50 (Ce_6 -PEG), 0.62 (Ce_6 -TX-100) and 0.56 (Ce_6 -BSA).

4 Conclusion

To sum up, the conducted study of spectral and fluorescent characteristics of various supramolecular systems on the basis of chlorin e_6 allows us to conclude promising method of controlled aggregation of photosensitizers in the composition of supramolecular complexes to create medicines of predictable photodynamic activity. The obtained Q-band absorption maxima are in a row: PEG (639 nanometers) < chitosan (654 nm) < PVP (663 nm) < TX-100 (665 nm) < BSA (666 nm), which clearly demonstrates the disaggregation of Ce_6 molecules in systems with TX-100, BSA and PVP. The appearance of a porphyrine

molecular complex absorption band at 615 nm for Ce_6 -TX-100, Ce_6 -BSA and Ce_6 -PVP systems also shows the Ce_6 molecules disaggregation. As far as Ce_6 -PEG system, the absence of absorption in the region of 680-700 nm testifies the porphyrine aggregates destruction. In addition, the obtained from fluorescence spectra value of $\phi_k=0.16$ for Ce_6 – PEG is consistent with the absorption data and confirms the appearance of disaggregated Ce_6 molecules fraction. A quantum yield of singlet oxygen generation falls in the range from 0.5 for PEG up to 0,62 for TX-100. According to [5], values received are close or exceed the corresponding value for chlorin e_6 in the aqueous solution (0.5 ± 0.05 at the medium of pH 6.3), which confirms the assumption on the disaggregating action of excipients.

Though, the presented systems call for the further investigations, spectral and fluorescence data clearly demonstrate the absence of aggregation in such systems as Ce_6 -PVP, Ce_6 -PEG, Ce_6 -BSA and Ce_6 -TX-100 and the prospects of use of these systems for diagnostics (and, as possible, therapy) are evident.