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Biocompatible Supramolecular Systems Based on Chlorin e_6: Preparation, Photophysical Properties

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The synthesis methods as well as the data of spectral-fluorescent properties of novel supramolecular systems based on chlorin e_6 (Ce_6) are presented. The effect of various biocompatible excipients such as hydrolyzed polyvinyl alcohol (PVA), poly-N-vinylpyrrolidone (PVP), sodium salt of carboxymethyl cellulose (Na-KMC), dimethylsulfoxide (DMSO), Cremophor® PEG-40 (PEG) on the optical absorption and fluorescence of chlorin e_6 is demonstrated. A red shift of the chlorin e_6 absorption spectrum when using all presented here excipients is a good prerequisite for increasing tissue permeability for visible light. The fluorescence quantum yield φ_k of chlorin e_6 in systems with all excipients has been calculated. It has been proven, that in all obtained biocompatible systems, except DMSO – Ce_6 system, Ce_6 molecules disaggregate and charge transfer complexes "excipient – Ce_6" are formed. The high efficiency of such systems as PEG – Ce_6, PVP – Ce_6 and Na-KMC – Ce_6 for fluorescent diagnosis and photodynamic therapy is noted. The conclusions made in the work can be useful during the new photosensitizer controlled aggregation method development.

Keywords: Chlorin e_6, supramolecular systems, spectral characteristics, optical absorption, fluorescence, photodynamic therapy.

Биосовместимые супрамолекулярные системы на основе хлорина e_6: получение, фотофизические свойства

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В работе описаны методы синтеза, а также представлены данные изучения спектрально-флуоресцентных свойств новых супрамолекулярных систем на основе хлорина e_6 (Хл). Показано влияние различных биосовместимых вспомогательных веществ, таких как поливиниловый спирт гидролизованный (ПВС), поли-N-винилпирролидон (ПВП), натриевая соль карбоксиметилцеллюлозы (Na-КМЦ), диметилсульфоксид (ДМСО), кремофор® ПЭГ-40 (ПЭГ) на оптическое поглощение и флуоресценцию хлорина e_6. Сдвиг в красную область спектра оптического поглощения хлорина e_6 при использовании всех представленных здесь вспомогательных веществ является хорошей предпосылкой для повышения проницаемости ткани для видимого света. Рассчитан квантовый выход флюоресценции φ_k хлорина e_6 в системах со всеми вспомогательными веществами. Доказано, что во всех полученных биосовместимых системах, за исключением системы ДМСО – Хл, молекулы Хл дезагрегируют и образуются комплексы с переносом заряда «вспомогательное вещество – Хл». Отмечена высокая эффективность таких систем, как ПЭГ – Хл, ПВП – Хл и Na-КМЦ – Хл для флуоресцентной диагностики и фотодинамической терапии. Выводы, сделанные в работе, могут быть полезны при разработке нового метода управляемой агрегации фотосенсибилизатора в составе супрамолекулярного комплекса.

Ключевые слова: Хлорин e_6, супрамолекулярные системы, спектральные характеристики, оптическое поглощение, флуоресценция, фотодинамическая терапия.

Introduction

Photodynamic therapy (PDT) is now one of the most important treatments for cancer. This method is used in conservative treatment and based on the photosensitizers (PSs, natural or artificially synthesized substances capable of biological tissues photosensitizing) capability to accumulate selectively in tumour or other target tissues and generate singlet oxygen or oxygen-containing free radicals at local exposure of radiation of a certain wavelength corresponding to maximum absorption of PS.[1–6] These active oxygen forms are extremely cytotoxic and play a defining role in malignant cell death. Both in the Russian Federation and in other countries there are a number of drugs of the first to third generations with high antitumor activity for use in clinical practice, which can be conditionally combined into three groups: 1) based on porphyrin: a) derivatives of δ-aminolevulinic acid – precursor of endogenous photosensitizer protoporphyrin ("Alasens"); b) benzoporphyrin derivatives ("Visudine"); c) hematoporphyrin derivatives ("Photophrin", Photogem"); 2) chlorophyll-based PS (chlorins and purpurines ("Photolon", "Photoditazin", "Radachlorin", "Foscan"), as well as bacteriochlorins ("Tookad"); 3) dyes (phthalocyanine, naphthalocyanine ("Photosens", "Thiosens", "Octasens").

But since most marketed PS and drugs do not exhibit the necessary combination of physical, chemical and biological properties for PDT,[7] the evolution of the PDT method is also towards the creation of the so-called "ideal PS" with high selectivity, low phototoxicity and rapid pharmacodynamics. Synthesis of new PS and search for new antitumor drugs are constantly in progress.[7–20] Among the promising ones are chlorin-based compounds, including chlorin e_6 (Ce_6),[21] as they meet the core requirements for the "ideal PS",[7,9,22] namely, the existence of the intense absorption band in the long-wave part of the spectrum (600–800 nm), in which biological tissues are more optically transparent (so-called "therapeutic window"[13]); the high capacity to accumulate in the target tissue; the existence of intense fluorescence, which actually provides photodynamic effect, as well as allows to carry out fluorescence diagnostics; the storage stability and virtually no toxicity.[9] At the same time due to the characteristics (λ_{max} = 660 ± 5 nm, ε = 41000 $mol^{-1}\cdot cm^{-1}$) chlorin-based compounds show higher depth of light penetration into tissues compared to porphyrins,[7,11,12] and the excitation coefficient of chlorins is 10 times larger than that of the porphyrins.[23]

The availability of raw materials plays a major role in the development of chlorin-based compounds, as chlorins precursor chlorophyll *a* is produced, for example, from *Spirulina platensis* microalgae or nettle leaves.[11,12] Chlorophyll, as an element of the usual nutrient media, is not a poison for the humans. Chlorin e_6 is synthesized by the anaerobic alkaline hydrolysis of pheophorbide *a*, formed in turn by treating chlorophyll *a* with concentrated acids.[11,24] The energy potential of Ce_6, as a chlorophyll derivative, is identical to the organism's civilized cells, and the selective accumulation of it in the tumor tissues is due to the capture of Ce_6 as a "nutrient medium" for them.

The absence of PS aggregation in solutions, which, in turn, leads to a drop in a quantum yield of singlet oxygen generation, is essential when developing medicines for PDT. It is known, however, Ce_6 molecules form aggregates in water solutions, that considerably reduces their photodynamic activity. The high application potential of Ce_6 in supramolecular systems with various excipients was confirmed in our previous works.[11,12] Studies of the spectral luminescent properties of Ce_6 based supramolecular systems with various excipients such as poly-*N*-vinylpyrrolidone, polyethyleneglycol PEG-200, bovine serum albumin, chitosan, Triton X-100, sodium hexametaphosphate, polydimethyldiallylammonium chloride were conducted. Some recommendations were made for the future use of poly-*N*-vinylpyrrolidone – Ce_6, polyethyleneglycol PEG-200 – Ce_6, bovine serum albumin – Ce_6 and Triton X-100 – Ce_6 systems for diagnostics and therapy. However, a search of more effective chlorin-based dosage forms is still under way. Therefore, we have synthesized new supramolecular compounds based on Ce_6 and investigated their photophysical properties.

Experimental

In the present work optical absorption and fluorescence of supramolecular systems based on Ce_6 with various excipients, such as *polyvinyl alcohol hydrolyzed* (PVA), *poly-N-vinylpyrrolidone* (PVP), *sodium salt of carboxymethylcellulose* (Na-KMC), *dimethyl sulfoxide* (DMSO), *Cremophor® PEG-40 (polyethyleneglycol*, PEG) have been investigated.

Ce_6 kindly provided by the colleagues from Moscow Technological University (Preobrazhensky Department of chemistry and technology of biologically active compounds), PVA ($[\text{-}CH_2CH(OH)\text{-}]_n$, Aldrich, 130 kDa), Na-KMC (food supplement E466, $[C_6H_7O_2(OH)_x(CH_2COONa)_y]_n$ Acros, 250 kDa), DMSO (M = 78.13 g/mol, ρ = 1.1004 g/cm^3), PVP (Sigma, 10 kDa), *Cremophor*® (hydrogenated castor oil solubilizer, polyethyleneglycol, PEG-40) were used (Figure 1). Purity and identity of Ce_6 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 excipients (Figure 1) is explained by the fact that they are the most widespread biologically-compatible substances which are the part of pharmacological drugs and cosmetic products. Stock Ce_6 solutions with the concentration of $5.0\cdot10^{-3}$ mol/L were prepared by dissolution of Ce_6 dry sample weight in 20 mL of dimethylformamide (DMF) double-distilled. The received concentration was specified on electron absorption spectrum. For spectral measurements 2 mL of the aqueous solution of one of the excipients and 10 ÷ 30 μL of aqua-diluted by 10 times stock Ce_6 solution were poured into a quartz 1 cm thick cuvette under stirring. The DMSO, PVP, PVA, PEG, Na-KMC aqueous solutions with the weight concentration of 0.1÷1 % were prepared separately. Solutions were stored in the dark at +4 ^{0}C.

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. The quantum yield of fluorescence of Ce_6 in complexes with excipients was calculated by the relative method using the formula $\varphi_k = (\varphi_0 A_0 I^s_k)/(A_k I^s_0)$,[25] where A and A_0 are the optical densities of solutions of Ce_6 complexed with excipients and of Ce_6 aqueous solution, respectively, at a wavelength of 410 nm; I and I_0 are the integrated fluorescence intensities (area under the fluorescence spectrum curve) of Ce_6 complexed

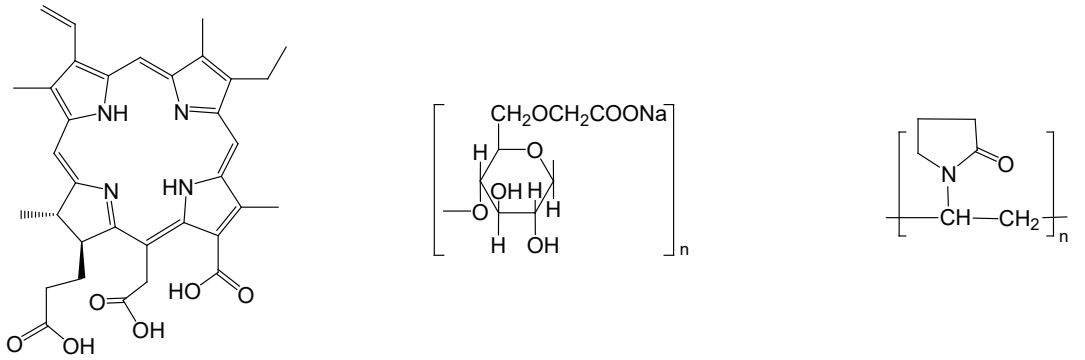

Figure 1. Structures of chlorin e_6, sodium salt of carboxymethylcellulose (Na-KMC), Cremophor® PEG-40, poly-*N*-vinylpyrrolidone (PVP), dimethyl sulfoxide (DMSO), polyvinyl alcohol hydrolyzed (PVA).

with excipients and in aqueous solution, respectively. The standard was the free form of Ce_6 in an aqueous solution, for which $I_0 = 0.15$[26] (Table 1). The relative error in φ_k determining did not exceed 7 %.

Results and Discussion

The absorption spectrum of chlorin e_6 (Figure 2) contains a specific Soret band (*B*-band) with the maximum at ~ 404 nm, three peaks of low intensity in the region of 500–600 nm, and, due to the partially hydrated porphyrin rings, a strongly marked peak at λ = 653 nm corresponding to the *Q*-band maximum. Analysis of the spectra in the region of Soret peak shows the same hydrophobicity of all presented systems.[11] We have already said that usually "a blue shift" is formed as a result of strengthening of Ce_6 interaction with polar molecules and is connected

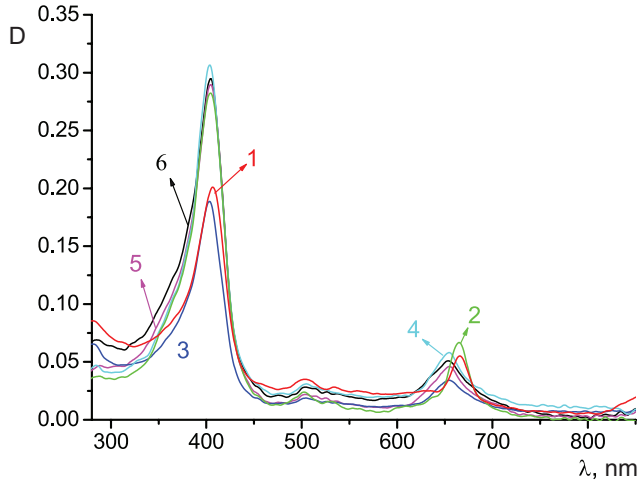

Figure 2. Absorption spectra of supramolecular systems based on Ce_6: 1. PEG – Ce_6, 2. PVP – Ce_6, 3. Na-KMC – Ce_6, 4. PVA – Ce_6, 5. DMSO – Ce_6, 6. Ce_6 in aqueous solution.

with chromophore transition to more polar environment, and, in our case, to aqua as a polar solvent.[11] Therefore, the absence of "a blue shift" suggests the identical in size interaction of chlorin e_6 molecules with polar aqua molecules, both in the presence of all the excipients used, and simply in the aqueous solution. The spectra of Ce_6 solutions with such excipients as PVP, PEG and Na-KMC in the region of Soret band have exhibited the decrease in absorption intensity, and in the case of PEG and Na-KMC a drop of intensity is significant (Table 1). There is also a broadening of the half-width of the absorption maximum by ~ 1–2 nm for the systems with PVP, PEG and Na-KMC, and for the system with PEG there is also a bathochromic shift of the absorption maximum by ~ 3 nm. These spectral hypochromic changes in the region of Cope band, most likely, can be explained by the occurring PVP – Ce_6, PEG – Ce_6 and Na-KMC – Ce_6 complexes in solution. Possibly, a chromophore in them is in more ordered form in comparison with its free state in the aqueous solution of Ce_6. A shift of the chlorin e_6 absorption spectrum to a long-wave region when using all presented here excipients is a good prerequisite for increasing tissue permeability for visible light and reducing the absorption of light by blood hemoglobin in the 500–600 nm region, which plays a significant role in increasing of the PDT efficiency.[3,11,27] The bathochromic shift of a *Q*-band maximum is observed for all presented here supramolecular biocompatible systems. Herewith, the highest shift to the region of long waves is observed for PVP – Ce_6 and PEG – Ce_6 systems by 12 and 13 nm, respectively. These changes in the absorption spectrum in the presence of excipients are likely related to the solvatochromic effect caused by the presence of a less polar, as compared to an aqueous solution only, environment of a chlorin e_6. That is not to deny a monomerization of the Ce_6 aggregated molecules in the presence of excipients. Similar changes in the absorption spectra of the PVP – Ce_6 system were also observed in.[28–31] The authors of the work[28] speak about the appearance of the second

Table 1. Spectral-fluorecsent properties of supramolecular Ce_6 based systems.

System	λ_B, nm	λ_Q, nm	$\Delta\lambda_B^{1/2}$, nm	$\Delta\lambda_Q^{1/2}$, nm	A_{410}	λ_{fl}, nm	$\Delta\lambda^{1/2}_{fl}$, nm	I^s	φ_k
PVP – Ce_6	404	665	24.4	21.36	0.26	668	21.6	33.2	0.19
PEG – Ce_6	407	666	23.8	16.99	0.20	670	19.6	60.2	0.47
Na-KMC – Ce_6	404	655	23.2	31.74	0.16	659	25.0	32.6	0.32
PVA – Ce_6	404	654	22.7	21.36	0.27	658	26.2	28.2	0.16
DMSO – Ce_6	404	655	22.7	35.39	0.27	655	30.4	26.5	0.15
Ce_6 – water	404	653	22.7	36.14	0.27	657	31.5	26.0	0.15*

peak of low intensity in the region of a *Q*-band (~ 672 nm) and assign its appearing to the influence of PVP on a monomerization of Ce_6 aggregates. Moreover, with the increasing of PVP concentration in solution the intensity of this peak increases. In our case, there is also a slight multiplicity of the spectrum line of supramolecular systems in the long wave region in the form of low intensity peaks. At the same time the authors of the works[29–30] attribute the hypochromic changes of the PVP – Ce_6 systems spectra in the region of a *Q*-band to the appearance of a complex due to the binding effect from PVP, explaining their point of view by small concentration of chlorin in a system. We believe that in our supramolecular systems both the disaggregation of chlorin e_6 molecules and its interaction with excipients with the formation of the charge transfer complexes take place. The appearance of the lines of a porphyrin molecular complex with excipients in the region of the spectra at ~ 600–640 nm is the confirmation of this assumption.

The analysis of the luminescent properties of the presented here systems (Figure 3) has shown that the addition of all excipients leads to the increase in intensity of Ce_6 luminescence. The maximum increase in luminescent intensity is specifically attributed to PEG – Ce_6, PVP – Ce_6, Na-KMC – Ce_6 and PVA – Ce_6 systems.

It should be noted that the luminescence intensity of the systems is markedly higher when excited with the light of the wavelength at λ = 410 nm than when excited with the light of the wavelength at λ = 620 nm. It is an indirect indicator of intracomplex energy transfer from excipients to Ce_6. The observed strong bathochromic shift of the luminescence maximum, especially in the presence of biocompatible excipients such as Cremophor® PEG-40 and poly-*N*-vinylpyrrolidone, also indicates the appearance of the excipient – Ce_6 complex. It is worth pointing out that chlorins are tetrapyrrole macrocyclic compounds with large areas of π-conjugation, which are also responsible for the hydrophobicity of the compound. On the other hand, strong intermolecular π-π interactions are responsible for the aggregation of chlorin e_6 in aqueous solutions. The excipients presented here, due to the presence of hydrogen bonds, are highly soluble in water. At their addition into a system based on Ce_6 there is an increase in its solubility both due to own hydrophobicity, and due to intermolecular interactions between heterocyclic aromatic rings of Ce_6 and hydrogen bonds of excipients.

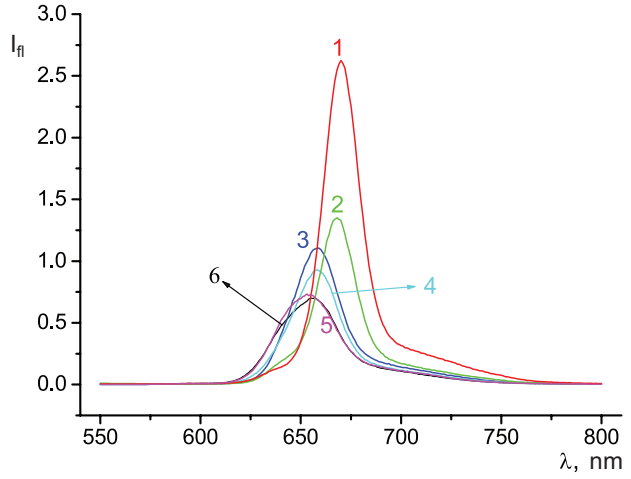

Figure 3. Fluorescence spectra of supramolecular systems based on Ce_6: 1. PEG – Ce_6, 2.PVP – Ce_6, 3. Na-KMC – Ce_6, 4. PVA – Ce_6, 5. DMSO – Ce_6, 6. Ce_6 in aqueous solution.

The analysis of the spectral-fluorescent data (Table 1) has shown the increase in the quantum yield of fluorescence value φ_k for PVP – Ce_6 , PEG – Ce_6, Na-KMC – Ce_6, and PVA – Ce_6 systems, which is one more confirmation of consecutive destruction of chlorin e_6 aggregates and excipient – Ce_6 complex formation. Moreover, the rate of Ce_6 aggregates destruction in the aqueous medium is different in the presence of different excipients. It should be noted that spectral-fluorescent parameters of DMSO – Ce_6 system remains almost invariable in comparison with similar characteristics of a Ce_6 – water system.

Conclusions

Based on all submittes data it is safe to say that application of such biocompatible supramolecular systems as Cremophor® PEG-40 – Ce_6, Na-KMC – Ce_6 and PVP – Ce_6 for PDT and diagnostics is obvious. At the same time solubilization in all solutions of presented excipients except for DMSO prevents aggregation of chlorin e_6, ensures its effective stabilization either in monomer fluorescent-active form or in the form of complex with charge transfer.

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