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Spectral-luminescent properties of new photocontrolled multimodal diamond-containing nanocomplexes for theranostics of significant diseases

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Hybrid complexes of tetraaminophenylporphyrin with nanodiamonds and hyaluronic acid have been synthesized. Their basic photophysical properties have been studied by spectroscopic methods. It has been shown that porphyrins in the complexes are capable of acting as photosensitizers, since they retain their ability to generate singlet oxygen. The values of the quantum yields of singlet oxygen generation by the relative method are estimated. Such complexes may be promising for the development of optical theranostics methods.

Keywords: nanoscale diamonds, hybrid complexes, tetraaminophenylporphyrin, hyaluronic acid, spectral-luminescent properties, singlet oxygen generation, optical theranostics.

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Introduction

One of the main directions of biomedical optics is development of multimodal nanostructures for optical theranostics of various diseases, combining diagnostic and therapeutic functions united in a single sequential or simultaneous process within a common technological platform [1,2]. Nanocomposites for optical theranostics consist of a carrier nanoparticle, an optically active therapeutic agent and a selective vector. The search for such compositions to be used in biomedicine is a relevant problem.

Today the nanocomposites are well known for theranostics, which include different nanocarriers (micelles, polymer matrices, liposomes, quantum dots, nanocarbon particles etc.) and substances encapsulated in them for treatment and diagnostics of tumors that are rather promising for medicine [3,4]. New opportunities for such research open due to use of various carbon nanostructures as a carrier platform. Surface activation of carbon nanostructures with biologically active compounds, for instance, porphyrins, is one of the ways to create medical agents, in particular, new types of photosensitizers [5,6]

Porphyrins are invaluable synthetic building blocks for production of various complexes, since they have great photophysical properties that may be used both for optical visualization and for photodynamic therapy [7]. Thanks to their structure, the presence of tetrapyrrol macrocycles, they are well positioned both for chemical modification of the coordination sphere and the periphery of macrocycles to develop various chemical structures. Besides, the macrocyclic structure with high degree of π-coupling of porphyrins provides for high degree of light absorption in the ultraviolet and visible optical ranges.

The targeting agent chosen was hyaluronic acid (Hyal). Hyaluronic acid - is a natural biopolymer from the class of glycosaminoglycans with high affinity to biological tissues that contain significant quantities of it [8]. It may provide for the protection of the delivered medicine and improve the solubility of hydrophobic medicines. Hyaluronic acid plays a signal role in various cellular processes such as morphogenesis, inflammation, recovery of damaged tissues, processes happening in the tumor tissues [9]. It was found that it also had high affinity to RHAMM receptors (mediated mobility receptor, hyaluronan), which are re-expressed by the cells of certain cancers and are localized on their surface [10]. It is shown that hyaluronic acid — is a promising modifier of carbon preparations on their basis [11].

The surface of nanodiamonds (ND) contains oxygen-containing groups, for instance, carboxyl, carbonyl and hydroxyl ones, and also the primary amino groups, therefore various porphyrins and biomacromolecules may be immobilized on them with rather high efficiency. Nanoparticles based on hyaluronic acid may recognize RHAMM-expressing cells, bind with them and thus precisely deliver the medications bound to the particles to a tumor cell.

The purpose of this paper is development of a photocontrolled multimodal composition based on ND-particles with photodynamic activity for use in theranostics of diseases. The solution of the objective at hand is based on a combination of unique properties of porphyrins (ability to bind with ND-particles, effective photoluminescent properties, ability to generate a singlet oxygen) and exclusive properties of ND (nanoscale, high chemical stability, ability for modification, biocompatibility, absence of toxicity).

Table 1. Properties of used ND-powders

Characteristics	Units of measurement	ND specimens
Average size of microcrystallites	nm	4–8
Average size of primary aggregates	nm	20–30
Specific area of surface (BET)	m^2/g	300–350
Electrokinetic potential (pH 7.36)	mV	−20 – 25

Experimental part

The objective of this paper was to design a structure of a potential photosensitizer (PC) in the form of a hybrid organic-inorganic complex to maintain the key characteristics of the porphyrin macrocycle, thanks to which it may be the maximum effective PC, and to minimize the side effects potentially causing unwanted additional effects in the subsequent experiments *in vitro*. In particular, fixation of porphyrin molecules on the ND particles will prevent their interaction with each other forming dimers and trimers etc. which helps to preserve their spectral and luminescent properties. Taking into account these conditions and analyzing the available approaches to synthesis of substituted porphyrins with nanoparticles, 5,10,15,20-(tetra-4-aminophenyl)porphyrin (TAPP) was chosen, as well as ND, the characteristics of which are presented in table 1 [12].

Preparation of specimens

Synthesis of the hybrid complex of tetraaminophenylporphyrin with ND and hyaluronic acid (ND-TAPP-Hyal) was carried out in two stages:

1) interaction of ND particles in the reaction of acylation of carboxyl groups in functionalized particles of ND and amino groups of peripheral porphyrin substitutes with formation of an amide bond,

2) activation of carboxyl groups on hyaluronic acid and reaction of free amino groups acylation on 5,10,15,20-(tetra-4-aminophenyl)porphyrin in ND-TAPP conjugate and carboxyl groups on hyaluronic acid.

Synthesis of ND-TAPP conjugate

For synthesis of porphyrin conjugate with ND we used tetraaminophenylporphyrin from PorphyChem (France), detonation synthesis ND by „Sinta“ NP CJSC (Minsk,The Republic of Belarus), 1,1′-carbonyl diimidazole (CDI), dimethylaminopyridinium (DMAP) and dimethylsulfoxide (DMSO) by „Sigma-Aldrich“ diethyl ether of (chemically pure) reagent grade .

Chemical synthesis: 20 ml of nonaqueous DMSO were added to ND powder (100 mg) in a round flask with capacity of 50 ml, mixed in a magnetic mixer for 1 h and dispersed for 10 min. The produced colloid was treated with ultrasound (22 kHz) for 40 min and mixed for 3 h at 40°C in the magnetic mixer in the argon atmosphere. Then 15 mg TAPP were added to DMSO and mixed in the magnetic mixer for 48 h at room temperature. The reaction mixture was centrifuged (20 min at 10000 rpm), and the dark residue with light green tint was produced.

Synthesis of ND-TAPP-Hyal conjugate

15 ml of anhydrous DMSO and 3 ml of anhydrous pyridine were added to a round flask with capacity of 50 ml and 50 mg hyaluronic acid and mixed in the magnetic mixer for 1 h, and then added 30 mg 1,1-carbonyl diimidazole for 3 h at 60°C in the magnetic mixer in the argon atmosphere. Then ND-TAPP (50 mg) produced in process of the previous synthesis were added to 10 ml of anhydrous DMSO and treated with ultrasound (22 kHz) for 40 min. Then 10 mg DMAP were added, and mixing in the magnetic mixer continued for 24 h at 60°C.

Upon completion of the reaction the mixture was centrifuged (20 min at 10000 rpm) and after separation of the liquid-filler the residue with greenish tint was produced. The produced ND-TAPP-Hyal conjugate was cleaned from free hyaluronic acid and side products of the reaction by triple washing in 10 ml DMSO and 10 ml diethyl ether with subsequent centrifuging (20 min at 10000 rpm). 70 mg of finely dispersed grey-green powder of ND-TAPP-Hyal conjugate were obtained. Fig. 1 showed the scheme of ND-TAPP-Hyal complex synthesis.

Spectral measurements

To record the absorption spectra, and fluorescence, fluorescence excitation and fluorescence kinetics spectra, the free base solutions of TAPP and hybrid complexes of ND-TAPP, ND-TAPP-Hyal in DMSO were prepared at $T = 293$ K. Absorption spectra of TAPP solutions and within complexes in DMSO were recorded with double-beam spectrophotometer Varian Cary 500 (USA). Measurements were conducted in quartz cells with a thickness of 1 cm.

Steady-state spectra of fluorescence, spectra of fluorescence excitation were measured using Fluorolog-3 (Horiba Scientific, USA-France-Japan) multifunctional spectrofluorometer. In stationary measurements a 450 W continuous xenon lamp Ushio UXL-450SO was used as a source of fluorescence excitation, which radiation was passed through a double 180DF monochromator to extract the required wavelength of the excitation.

All spectral measurements were conducted in the standard 90-degree geometry at a room temperature. The fluorescence recording channel used iHR320 (Horiba Scientific) diffraction spectrometer and PPD-850 (Horiba Scientific)

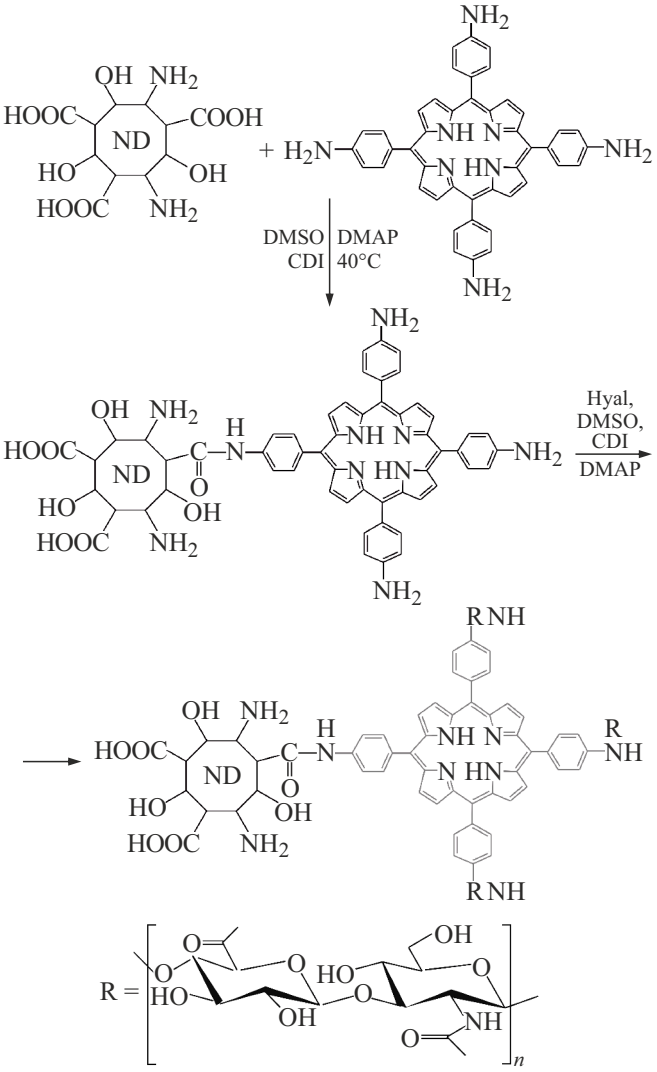

Figure 1. Scheme of ND-TAPP-Hyal complex synthesis.

thermoelectrically cooled PMT as a detector. Fluorescence spectra and fluorescence excitation spectra were corrected for the spectral sensitivity of the instrument.

Kinetics of fluorescence decay were recorded by the method of time-correlated single photon counting. The source of fluorescence excitation was a pulse light diode PLS-400 (PicoQuant, Germany) with wavelength 406 nm and pulse duration at half maximum 780 ps. Decay of the fluorescence signal was approximated in DAS6 software (Horiba Scientific) by two- or three- exponential dependence using the following relationship [13]:

$$I_f(t) = B + \Sigma_i A_i \exp\left(-\frac{t}{\tau_i}\right),$$

where $I_f(t)$ — fluorescence intensity as a functuion of time, τ_i — decay time constants, B, A_i — experimental constants.

Weight contribution of the f_i component with time constant τ_i was estimated by the following equation:

$$f_i = \frac{A_i \tau_i}{\Sigma_i A_i \tau_i}.$$

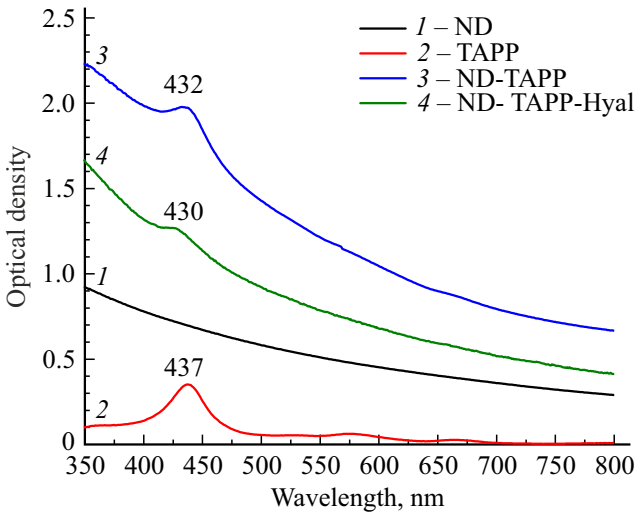

Figure 2. Absorbtion spectra of solutions: *1* — ND, *2* — TAPP, *3* — ND-TAPP, *4* — ND-TAPP-Hyal in DMSO.

Mean lifetime of excited state for the case of recording the fluorescence decay, mean excited state lifetimeτ_0 was determined as:

$$\tau_0 = \frac{\Sigma_i A_i \tau_i^2}{\Sigma_i A_i \tau_i}.$$

The approximation was made using the least square method, the approximation quality was estimated by the reduced parameterχ_2.

Luminescence of singlet oxygen ($^1O_2$) was recorded by direct method by measurement of luminescence $^1O_2$ in the area $\lambda = 1270$ nm using CCD detector cooled by liquid nitrogen in Symphony II spectrofluorometer Fluorolog-3 (Horiba Scientific, France). In the recording channel they additionally installed the optic filter LP02-1064RE-25 (Semrock, USA), which cuts the radiation with wavelength of less than 1064 nm. Singlet oxygen generation excitation was carried out in quartz cuvettes with optic path length $l = 10$ mm with a xenon lamp, the excitation wave length was $\lambda_{ex} = 430$ nm. Optical axes of excitation and registration made the angle of 90°.

Results and discussion

Fig. 2 provides the absorption spectra of ND, TAPP solutions and ND-TAPP, ND-TAPP-Hyal complexes in DMSO. In the absorption spectra of the complexes there is a scattering contribution due to the presence of diamond nanoparticles in the solutions with particle size much less than the wavelength of light, which leads to a visible difference in the optical density in the maximum of absorption.

For pure TAPP there is an intensive main band Cope is observed in the range of 410 – 440 nm (B-band) with the maximum at 437 nm and mild so called Q-bands in the field of 550 – 700 nm with maxima at 576 nm and 668 nm. You can see that during complex formation the Cope band

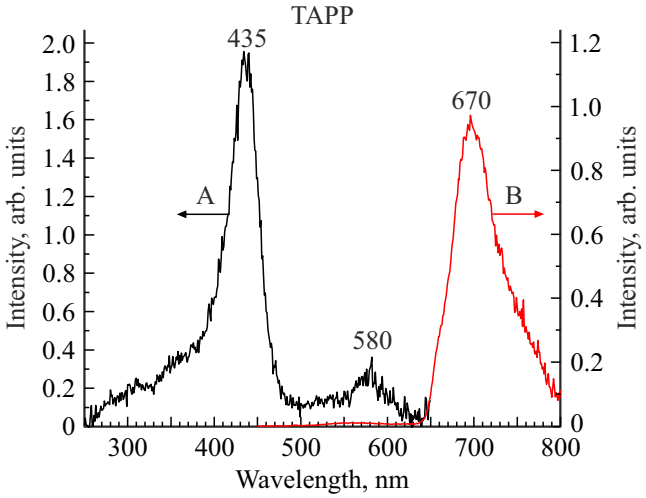

Figure 3. Fluorescence excitation (A) and fluorescence spectra (B) of TAPP in DMSO. Excitation wavelength is 430 nm, recording wavelength is 670 nm.

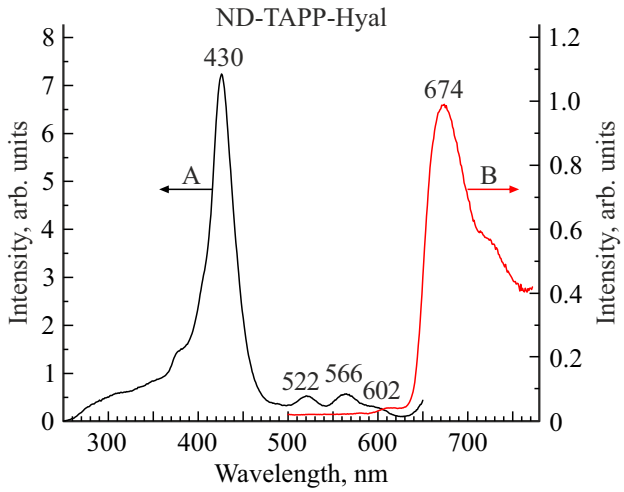

Figure 5. Fluorescence excitation (A) and fluorescence spectra (B) of ND-TAPP-Hyal in DMSO. Excitation wavelength is 425 nm, recording wavelength is 665 nm.

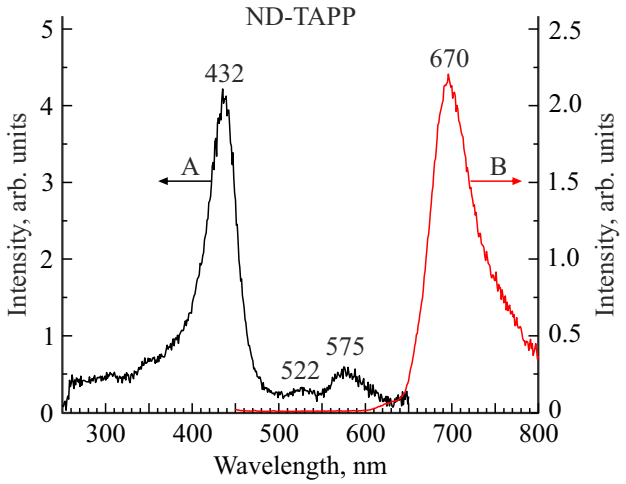

Figure 4. Fluorescence excitation (A) and fluorescence spectra (B) of ND-TAPP in DMSO. Excitation wavelength is 430 nm, recording wavelength is 670 nm.

undergoes a small hypsochromic shift (5 nm) (curve *3*) and 7 nm (curve *4*), which indicates there is a bond between porphyrin molecules, diamond nanoparticles (curves *3*, *4*) and hyaluronic acid.

Fig. 3, 4, 5 provides fluorescence spectra (excitation wavelength 430 nm) and fluorescence excitation spectra (registration wavelength 670 nm) of TAPP and ND-TAPP, ND-TAPP-Hyal complexes solution in DMSO. Spectral slit-width at the monochromator output was 1.5 nm.

From the given spectra you can see that all fluorescence excitation spectra show an intense Cope band specific for porphyrins, in the area of 430 nm and Q absorption band, usually used for photodynamic therapy, in the optic range 450–650 nm. Changes are observed both in fluorescence spectra and in fluorescence excitation spectra. The spectrum of ND-TAPP complex shows clear Q-bands at 522 and 575 nm, and for pure TAPP one Q-band is observed at 580 nm, and for the complex with Hyal — three Q-bands. Fluorescence excitation intensity in the Cope band for porphyrin in the complex with ND-particles of ND-TAPP is 2.2 times higher than for pure porphyrin. All observed changes of the spectrum indicate the formation of a covalent bond between diamond nanoparticles and porphyrin molecules. These changes are confirmed by data on fluorescence kinetics (table 2). The analysis of the fluorescence spectra also shows that the fluorescence spectrum of porphyrin within the ND-TAPP-Hyal nanocomplex has changes in the main fluorescence bands. Two radiation bands are observed in the area of 650–750 nm. From the spectra you can see that conjugation of porphyrin with nanoparticles and hyaluronic acid causes change in the state of the porphyrin emitting centers.

Kinetics of fluorescence decay

Fluorescence lifetimes of TAPP and ND-TAPP, ND-TAPP-Hyal complexes in DMSO were measured for excitation wavelength $\lambda_{ex} = 405$ nm and registration wavelengths $\lambda_{reg} = 660$ and 685 nm. Fluorescence decay times with their relative contributions and mean lifetimes of excited state of porphyrin in three systems are presented in table 2.

From table 2 you can see that there are three decay components for pure TAPP, the main of which has lifetime 5.8 ns with significant contribution of 53.2%, the shorter component — 2.5 ns with contribution of 40.3% and the third component with the least decay time — 0.3 ns (6.5%). For ND-TAPP complex the redistribution of the emitting centers state is observed when they are excited. The largest changes are observed for the complex with hyaluronic acid. For ND-TAPP-Hyal complex a noticeable increase

Table 2. Lifetimes of fluorescence of TAPP and ND-TAPP and ND-TAPP-Hyal complexes DMSO solution for various registration wavelengths at excitation of 405 nm

Sample	λ_{ex}, nm	$\lambda_{reg} = 660$ nm			$\lambda_{reg} = 685$ nm		
		τ, ns; (f_i, %)	$< \tau >$, ns	χ^2	τ, ns; (f_i, %)	$< \tau >$, ns	χ^2
TAPP	405	$\tau_1 = 5.8$ (53.2) $\tau_2 = 2.5$(40.3) $\tau_3 = 0.3$ (6.5)	4.1	1.07	$\tau_1 = 4.6$ (62.6) $\tau_2 = 1.9$ (32.6) $\tau_3 = 0.2$ (4.8)	3.5	1.04
ND-TAPP	405	$\tau_1 = 6.8$ (56.8) $\tau_2 = 2.4$ (25.4) $\tau_3 = 0.6$ (17.8)	4.5	0.92	$\tau_1 = 4.8$ (54.6) $\tau_2 = 1.9$ (33.1) $\tau_3 = 0.1$ (12.4)	3.3	1.13
ND-TAPP-Hyal	405	$\tau_1 = 8.8$ (36.5) $\tau_2 = 2.6$ (36.5) $\tau_3 = 0.8$ (32.3)	3.9	1.14	$\tau_1 = 7.5$ (58.4) $\tau_2 = 2.6$ (24.8) $\tau_3 = 0.9$ (16.8)	5.2	0.96

was observed in the value of the shortest lifetime to 0.8 ns and growth of its contribution to 32.3%, which may be related to increase in the effect of the nonradiative deactivation mechanism for the excited electron state of porphyrin. Besides, changes are also observed for τ_1 and τ_2. At the same time the average lifetimes of the excited state for free TAPP ($< \tau >= 4.1$ ns) and for ND-TAPP-Hyal ($< \tau >= 3.9$ ns) complex differ insignificantly.

Singlet oxygen

Fig. 6 shows the luminescence spectra of the singlet oxygen upon excitation of TAPP and its ND-TAPP and ND-TAPP-Hyal complexes by radiation of xenon lamp with excitation wavelength $\lambda_{ex} = 430$ ns in DMSO.

You can see that all studied compounds when excited in the absorption band by optical radiation with wavelength

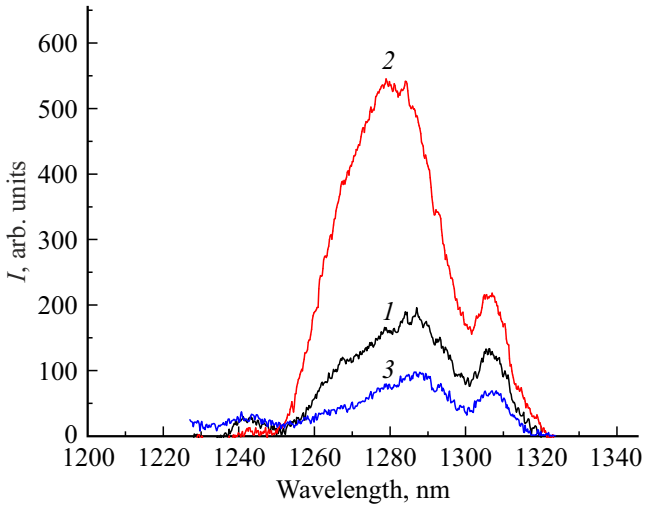

Figure 6. Luminescence spectra of singlet oxygen of porphyrin in TAPP (*1*), ND-TAPP (*2*) complex and ND-TAPP-Hyal (*3*) complex in DMSO solution, $\lambda_{ex} = 430$ nm.

Table 3. Values of quantum yields of the singlet oxygen generation (F_Δ) for TAPP and ND-TAPP and ND-TAPP-Hyal complexes at 293 K

Specimen	F_Δ
Phenalenone (standard)	0.99
TAPP	0.15
ND-TAPP	0.39
ND-TAPP-Hyal	0.16

$\lambda = 430$ nm may generate a singlet oxygen. This is confirmed by the presence of the peak in the luminescence spectra with maximum in the area of $\lambda = 1270$ nm.

Assessment of the values of singlet oxygen generation quantum yields F_Δ was carried out by the relative method [14]; by comparison of the surface area under the curves of the singlet oxygen luminescence spectra for the studied porphyrin TAPP and its ND-TAPP and ND-TAPP-Hyal complexes in DMSO solution with the standard-phenalenone in heavy water at 293 K with account of the difference in the solvent refractive indices for the studied specimen and the standard and the optical density of solutions at $\lambda = 430$ nm. Table 3 presents the results of measurement of the singlet oxygen generation quantum yield values F_Δ for porphyrin TAPP and its ND-TAPP and ND-TAPP-Hyal complexes in DMSO solution.

It should be noted that the efficiency of singlet oxygen generation by porphyrin in combination with ND is 2.6 times higher than in pure porphyrin. It may be related to the fact that in process of chemical synthesis several porphyrin molecules are bound, since TAPP molecules have 4 free amino groups that form a bond with carboxyl groups on an ND-particle, which helps to increase F_Δ for the double complex. It may also be related to the fact that conjugation of porphyrin with ND prevents formation of

dimers, trimers etc., which causes suppression of the triplet state of porphyrin.

In the triple complex with Hyal there is decrease of F_Δ compared to the double complex. Evidently it is related to the fact that whenever the triple complex is dissolved in DMSO, strong intermolecular hydrogen bonds are formed between hydrogen atoms within the complex (due to the presence of free $-$OH and COOH-groups on ND, $-$OH groups within Hyal, availability of acceptor amino groups within porphyrin macrocycles) with DMSO due to the non-divided electron pair on oxygen within DMSO, which results in the change of the emitting centers' microenvironment. The main contribution to these changes is made by Hyal through increase of solution viscosity [15], and, as a result, the oxygen availability reduces due to the reduction in its speed of diffusion to the complex.

Conclusion

Therefore, using methods of organic-inorganic synthesis, the hybrid nanostructured complexes of ND-porphyrin and ND-porphyrin-hyaluronic acid were produced. Their spectral-luminescent properties were studied. As you can see from the presented results, the structural changes in the state of the emitting centers of porphyrin (i.e. transition from free porphyrin to the one bound in the complex structure) impact its photophysical and photochemical properties. Changes are observed in the lifetimes of emitting centers, spectral-luminescent characteristics, efficiency of singlet oxygen generations. All of them may be explained by conformation changes to the complexes along with the change (complication) of their structure, and also by the processes of dissolution, change to the microenvironment, which impact the state of the emitting porphyrin centers when they are radiated.

When interpreting the experimental data, the fact should be taken into account that hyaluronic acid may form viscous gel solutions, the properties and behavior of which to a large extent depend on its concentration. In this paper we used low molecular Hyal. Unique physical-chemical and biological properties of Hyal, including biocompatibility and high hydrophilic property, make it possible to use in various fields of medicine as cords, gels, films of various composition [16]. Taking into account the detected ability of porphyrin within the triple complex to generate the singlet oxygen, and also Hyal properties, it is necessary to do further research in the area of development of medications and dosages based on glycosaminoglycans, since they may provide for effective use of such complexes both due to the ability to generate singlet oxygen and at the expense of their target localization and extended effect.

The studied complexes may be used as visualizers of the sites of binding with pathologically changed cells for diagnostics, on the one hand, and may show photodynamic activity, influencing the processes in a live cell with the purpose to correct the pathological state, on the other hand. This will make it possible to diagnose pathogenesis induced by various damaging factors and to develop the means and methods to eliminate the damage to the cellular structures.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

[1] L.B. Josefsen, R.W. Boyle. Theranostics, **2** (9), 916 (2012). DOI: 10.7150/thno.4571
[2] M.J. Garland, C.M. Cassidy, D. Woolfson, R.F. Donnelly. Future Med. Chem., **1** (9), 667 (2009). DOI: 10.4155/fmc.09.55
[3] S.H. Cheng, S.H. Lee, M.-C. Chen, J.S. Souris, F.-G. Tseng, C.-S. Yang. J. Mater. Chem., **20** (29), 6149 (2010). DOI: org/10.1039/C0JM00645A
[4] R. Bardhan, W. Chen, M. Bartels, et al. Nano Letters, **10** (12), 4928 (2010). DOI: org/10.1021/nl102889y
[5] V.A. Lapina, S.B. Bushuk, T.A. Pavich, A.V. Vorobey. J. Appl. Spectrosc., **83** (3), 344 (2016). DOI: 10.1007/s10812-016-0292-3
[6] V. Lapina, T. Pavich, S. Bushuk, J. Kalvinkovskaya, A. Vorobey, B. Bushuk. J. Nanomed. Nanosci., **1**, 1 (2019). DOI: 10.29011/2577-1477.100057
[7] M. Imran, M. Ramzan, et al. Biosensors, **8** (4), 95 (2018). DOI: 10.3390/bios8040095
[8] L. Schaefer, R.M. Schaefer. Cell Tissue Res., **339** (1), 237 (2010). DOI: 10.1007/s00441-009-0821-y
[9] M.G. Neuman, R.M. Nanau, L. Oruña-Sanchez, G. Coto. J. Pharm. Pharm. Sci., **18** (1), 53 (2015). DOI: 10.18433/j3k89d
[10] R. Edelman, Y.G. Assaraf, I. Levitzky, T. Shahar, Y.D. Livney. Oncotarget, **8** (15) 24337 (2017). DOI: 10.18632/oncotarget.15363
[11] L. Hou, Q. Feng, Y. Wang, H. Zhang, et al. J. Nanoparticle Res., **17** (2) 285 (2015). DOI: 10.1088/0957-4484/27/1/015701
[12] V.A. Lapina, T.M. Gubarevich, G.S. Akhremkova, Yu. Schreiber. Rus. J. Phys. Chem. A., **84** (2), 267 (2010). DOI: 10.1134/S0036024410020184
[13] J.C. Lakowicz. *Principles of Fluorescence Spectroscopy,* 3rd ed (Springer Science+Business Media, NY., 2006).
[14] P. Sen, C. Hirel, A.G. Gurek, C. Andraud, Y. Bretonniere, M. Lindgren. J. Porphyrins Phthalocyanines, **17** (1), 965 (2013). DOI: 10.1142/S1088424613500739
[15] T. Kluge, A. Masuda, K. Yamashita, K. Ushida. Photochem. Photobiol., **68** (5), 771 (1998). DOI: 10.1111/j.1751-1097.1998.tb02543.x
[16] N.N. Sigaeva, S.V. Kolesov, P.V. Nazarov, R.R. Vildanova. Vestnik Bashkirskogo universiteta, **17** (3), 1220 (2012) (in Russian).

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