

Nanodiamonds + bacteriochlorin as an infrared photosensitizer for deep-lying tumor diagnostics and therapy

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Abstract. The spectroscopic properties of potentially perspective nanostructure: diamond nanoparticles with a surface layer of IR-photosensitizer, bacteriochlorin, were experimentally investigated in this study. Such specific structure of the object encourages enhancement of the drug tropism to the tumor, as well as increasing of photodynamic penetration depth. The size distribution spectra of diamond nanoparticles; diamond nanoparticles, artificially covered with bacteriochlorin molecules layer, in aqueous solution, were obtained during the study. Based on the absorption and fluorescence spectra analysis, the benefits of functional nanostructure as a drug for deep-lying tumor diagnostics and therapy were reviewed.

1. Introduction

Fluorescence diagnostics (FD) and photodynamic therapy (PDT) are the most effective methods of cancer identification and treatment. These methods are based on the laser radiation influence on a photosensitive agent such as photosensitizer. For successful PDT and FD three key components are necessary: photosensitizer, light and oxygen. [1-4]

1.1. FD and PDT mechanisms

FD is a preparatory stage of PDT. The intensity of the fluorescent signal shows an accumulation rate of the photosensitizer in damaged cells that gives information about tumor localization, its possible proliferation and malignization.

PDT involves two stages. At first, photosensitizer, which is capable to accumulate in tumors at higher concentrations, compared with healthy tissues, is injected into patient's body (intravenously or orally). At the second stage, the damaged areas are illuminated with a certain laser light wavelength, corresponding to the photosensitizer absorption peak. Photosensitizer molecules' absorption of photons in the presence of oxygen (triplet form) induces photochemical reaction with highly active oxygen (singlet form) generation leading to the damage of biomolecules' structures and destruction of sensitized cells.



The need of photodynamic therapy and fluorescent diagnostics methods' improvement makes the task of creating of the photosensitizer with improved properties actual.

2. Purposes

2.1. *Increasing of photodynamic penetration depth*

The important characteristics of a photosensitizer, other than the ability to accumulate in tissues, are its ability to fluoresce and its spectral range of optical absorption. The greatest penetration depth into the biological tissue can be achieved by infrared (IR) spectral range radiation, where the proper absorption of normal tissue is minimal. In this way, the deepest photodynamic treatment effect can be realized by using photosensitizers with the absorption maximum in infrared (IR) and near-IR (740-1050 nm) spectral ranges. The use of such near-IR photosensitizers will allow analyzing of deep bio tissue layers, what is significant for deep-lying tumor diagnostics and therapy [5, 6].

2.2. *Enhancement of drug accumulation selectivity in the tumor*

An important role in ensuring of PDT/ FD efficiency and selectivity plays the photosensitizer accumulation level control in cells. In this regard, one of the photodynamic therapy' actual problem is to increase the selectivity of drug accumulation in tumor cells relative to normal tissue. The mechanism of preferential photosensitizer accumulation in the tumor is associated with the angiogenesis specificity in the area of malignant neoplasms.

Organism cells receive oxygen and nutrients with the use of blood vessels, which normally have pores sizes ~ 2 nm. The principle of photosensitizer accumulation selectivity in the pathological tissues is explained by the fact, that drug molecules are not able to overcome the biological barrier and penetrate into the healthy tissues due to relatively small pores sizes existing in vessels. However, in the region of malignant cells biological barriers change. Tumor cells divide so actively that the blood vessels, "feeding" them, forced to stretch. The pores modify and increase in size ($\sim 100 \div 500$ nm). The drug penetrates them and accumulates in malignancy. Furthermore, it should be noted that the capillaries pores of such excretory organs as liver, spleen, lungs and kidneys have sizes of ~ 80 -150 nm. Thus, the photosensitizer is able to penetrate not only into the injured targeted organs, where it must show a therapeutic effect in combination with light impact, but also in other healthy tissues where its effect may be negative in certain conditions. This fact reduces the selectivity of drug accumulation in biological target.

Development and improvement of directed delivery system of drug to the tumor cells will reduce the concentration of the administered substance, decrease the drug accumulation time and provide drug accumulation only in malignant neoplasms. Due to the purpose of increasing of drug accumulation selectivity and its tropism to the tumor much attention, currently, is paid to the study of directed drugs delivery to damaged and modified cells using a variety of nanostructures. [7-17]

3. Materials and methods

Diamond nanoparticles are perspective materials for drug delivery due to their optical-spectroscopic, structural, electrochemical and surface properties, size variability within wide limits. [18-20]

An aqueous suspension of diamond nanoparticles was synthesized for this study (*Lomonosov Moscow State University (MSU), Chemical faculty). Prescribed by manufacturer* diameter of nanoparticles is $\sim 5 \div 10$ nm. However, the aggregation tendency (property) of such nanoparticles leads to the following experimental size distribution in an aqueous colloid: 12,8 nm; 215,4 nm. The initial concentration of the diamond nanoparticles in the solution - 8 mg / ml.

Meso-tetra (3-pyridyl) bacteriochlorin*(hereinafter "bacteriochlorin"), was used as the near-IR photosensitizer, chemical structure of which is shown in Figure 1a. The concentration of the aqueous bacteriochlorin solution- 10 mg / ml.

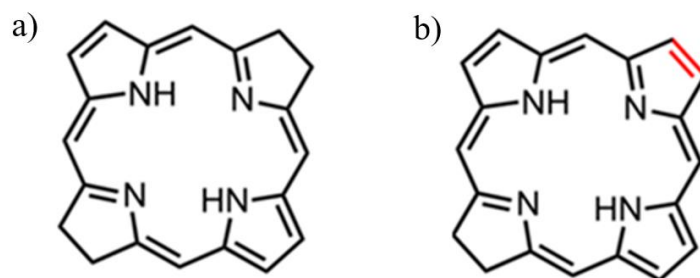


Figure 1. Chemical structure of a) meso-tetra(3-pyridyl)bacteriochlorin*
b) chlorin

In order to increase PDT efficiency, for investigation were selected diamond nanoparticles artificially covered with IR -photosensitizer (bacteriochlorin) molecules layer. Such specific structure of the investigated object encourage enhancement of the drug tropism to the tumor, as well as increasing of photodynamic impact depth (Figure 2).

Therefore, the diamond nanoparticles bounded with bacteriochlorin, in aqueous solution, were studied. The initial concentration of diamond nanoparticles in solution – 8 mg/ml, the concentration of bacteriochlorin - 2 mg/ml.

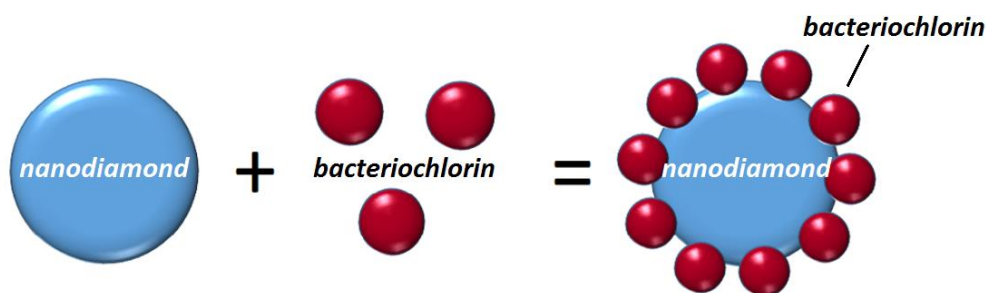


Figure 2. The nanostructure with improved properties (diamond nanoparticles bonded with bacteriochlorin molecules).

4. Results and discussion

4.1. Measuring of nanoparticles sizes

The sizes of original diamond nanoparticles; diamond nanoparticles coated with bacteriochlorin molecules were measured during the study. The measurements were carried out in aqueous solution, with the use of multi-angle dynamic scattering light spectrometer Photocor Complex, which allows getting the size distribution of nanoparticles by analyzing of correlation function of scattered light intensity fluctuations. Obtained slurry was dispersed in an ultrasonic homogenizer Bandelin SONOPLUS HD2070 with KE76 attachment (20 kHz, 165 microns amplitude) before the measurement. Thus, the following experimental size distributions were obtained for various samples:

- The diamond nanoparticles in aqueous solution-12,8 nm; 215,4 nm (Figure 3a).
- The diamond nanoparticles coated with bacteriochlorin, in aqueous solution-12 nm, 71,5 and 495 nm (Figure 3b).

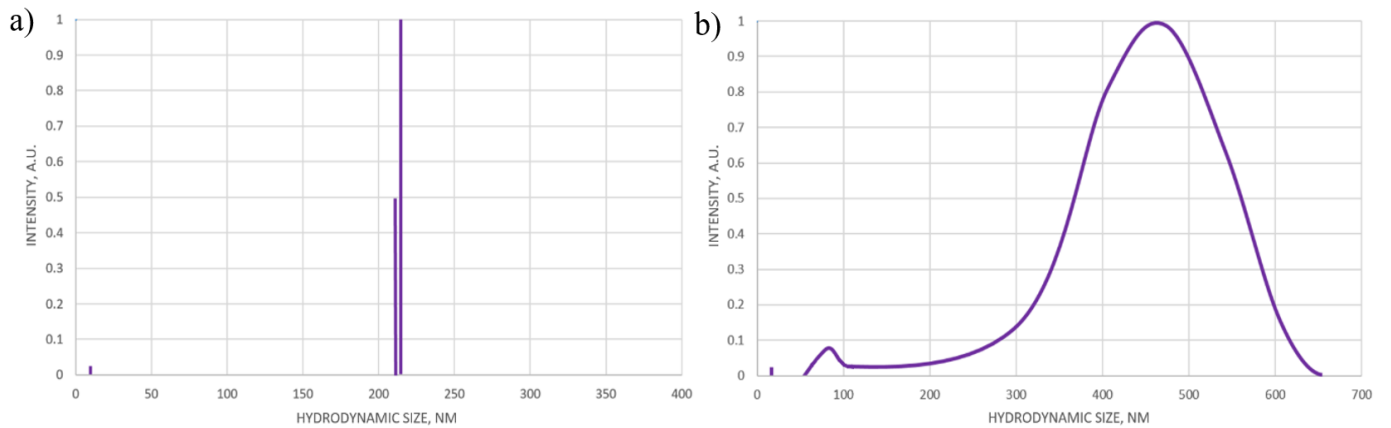


Figure 3. The range of size distribution of a) diamond nanoparticles b) diamond nanoparticles, covered with bacteriochlorin, in aqueous solution. The Spectrum is normalized to the nanoparticles light scattering intensity.

4.2. Determination of absorption and fluorescence spectra

The absorption spectra of the samples were obtained with the use of computerized spectrophotometer HitachiU-3400.

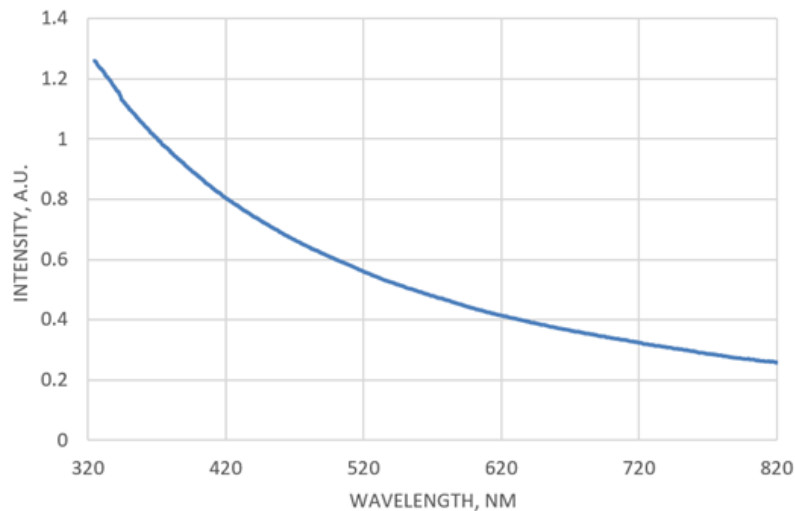


Figure 4. The absorption spectrum of uncovered diamond nanoparticles.

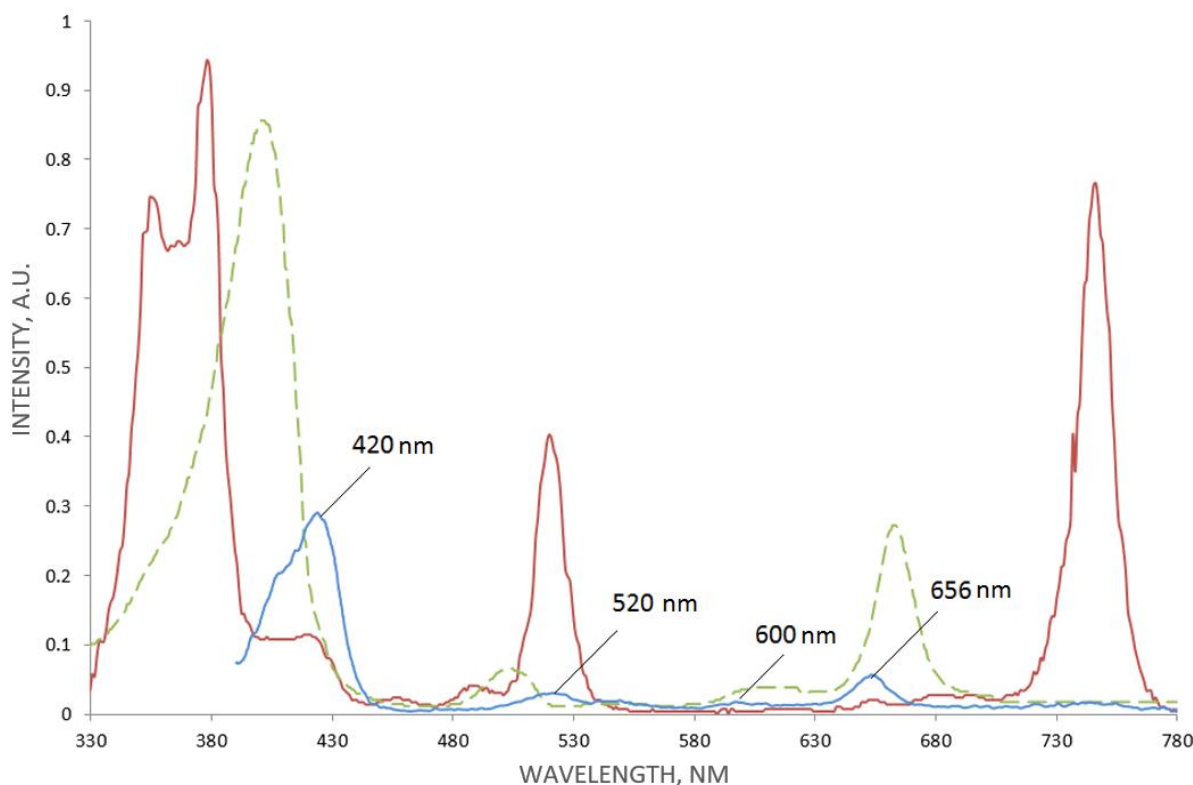


Figure 5. The absorption spectra of bacteriochlorin solution (●); chlorin (---); diamond nanoparticles, covered with bacteriochlorin (●).

The absorption spectrum of diamond nanoparticles changes intensely by coating them with bacteriochlorin. Maximums of absorption spectrum of diamond nanoparticles, covered with bacteriochlorin, occur at 420 nm, 520 nm, 600 nm and 656 nm wavelengths. The most evident peaks correspond to 420 nm, 656 nm wavelengths, where an active absorption of molecular chlorin form is registered (Figure 5). This circumstance can be explained by the fact that bacteriochlorin molecules on the surface of nanodiamond form a kind of antenna that effectively scatters the radiation in the spectral range corresponding to free bacteriochlorin molecules absorption. Evidently, surface bacteriochlorin molecules bond with nanodiamond surface and depending on the surroundings they may “lie” taking para- position according to nanodiamond surface, or “stand up” taking ortho- position, refraining on the nanodiamond top and showing bacteriochlorin and chlorin spectroscopic properties.

By the absorption spectra (Figure 5) analyzing it can be concluded that the most efficient fluorescence excitation of diamond nanoparticles covered with bacteriochlorin, should be realized at $\lambda = 405$ nm; 510 nm and 632.8 nm wavelengths.

Using NIR region (740-1400 nm) radiation considers to be the most perspective for fluorescence diagnosis and photodynamic therapy. However, during the study, it was found that the test samples of diamond nanoparticles coated with bacteriochlorin molecules, do not have pronounced IR -absorption peak. Therefore, choosing from three available sources with 400 nm, 510 nm and 632.8 nm wavelengths, in accordance with the absorption spectrum of the colloid test, the most efficient, from the standpoint of fluorescence excitation, would be a laser radiation with 405 nm wavelength. Experimental results confirmed this assumption. Thus, the fluorescence spectrum of colloidal solution of diamond nanoparticles with the surface layer of bacteriochlorin molecules was obtained with the use of fiber-optic spectrum analyzer LESA-01-“Biospec” (Figure 6).

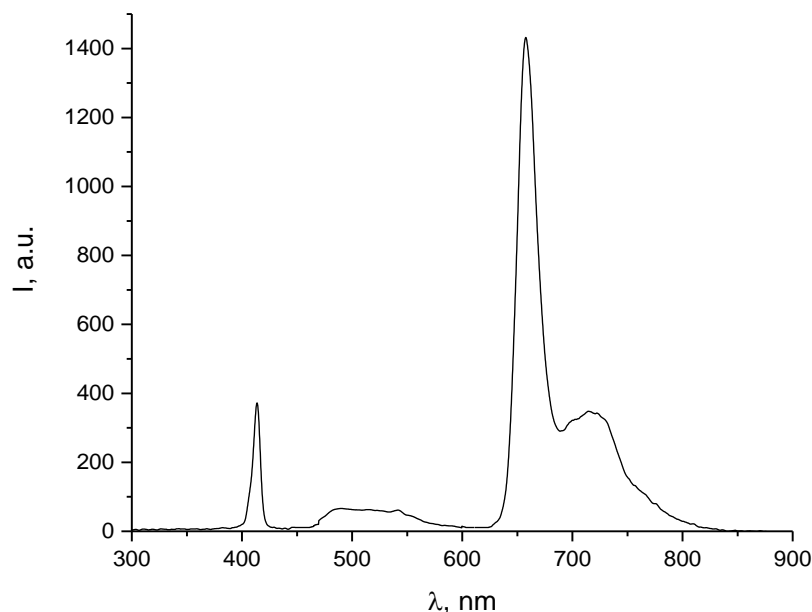


Figure 6. The fluorescent spectrum of diamond nanoparticles, covered with bacteriochlorin, in aqueous solution, with laser excitation at $\lambda_{\text{ex}} = 405$ nm wavelength.

The fluorescent spectrum of nanostructure created on the basis of diamond nanoparticles with a surface layer of bacteriochlorin molecules, excited with $\lambda_{\text{ex}} = 405$ nm laser radiation, has two fluorescence bands at $\lambda_{\text{ex}} = 749$ nm and $\lambda_{\text{ex}} = 662$ nm, corresponding to the molecular bacteriochlorin and chlorin forms. Apparently, the surface bacteriochlorin molecules can change their position relative to each other and to the surface of nanodiamond structure, showing the spectroscopic properties of bacteriochlorin ($\lambda_{\text{em}} = 758$ nm) and chlorin ($\lambda_{\text{em}} = 654$ nm) solutions. This phenomenon can probably be explained by the surface bacteriochlorin molecules interaction with a complex and heterogeneous surface structure of nanodiamond.

Depending on the localization of molecular bacteriochlorin crystals on the surface of the diamond nanoparticles, surface photosensitizer molecules can "lie", taking the para-position relative to the nanodiamond surface, or "stand up", taking the ortho-position, keeping on the surface and showing the spectroscopic properties of photosensitizer solutions that are demonstrated in Figure 6.

It is seen from Figure 1 that chlorin differs from bacteriochlorin with one more double bond in its structural formula. In this case, probably under the exciting laser radiation influence two close bacteriochlorin molecules on the nanodiamond surface may form joint temporary double bond between them. In this case, one of the molecules may show spectroscopic properties of chlorin. The fluorescence spectrum of the diamond nanoparticles (450-600 nm) has no pronounced peak (Figure 6).

5. Conclusion

During the study, it was found that bacteriochlorin molecules interact with each other and with a complex surface structure of nanodiamond. Depending on the interaction forces, location and surroundings of bacteriochlorin, they may take a different position relative to each other and to the surface of nanodiamond structures, showing spectroscopic properties of chlorin, which does not have an absorption band in IR spectral range. However, it was shown, that the investigated drug has an intense fluorescence signal in the NIR spectral range. In this way, using the drug in the form of specialized nanoparticles covered with IR-photosensitizer (bacteriochlorin) molecules may perspectively enhance the drug accumulation selectivity in biotissues and increase the photodynamic penetration depth.

Acknowledgments

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