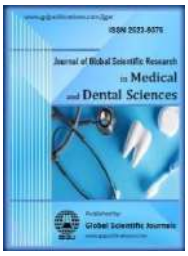
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Structural and Kinetic Characteristics of Metatetrahydroxy-phenylchlorine Photosensitizer Complexes with Stimuli Dependent Nanocarriers

Mohammed S. Altaee, M.A. Al-Akash, Dhamiaa H. Muhsen

Department of Radiology, Technical Institute of Mosul, Northern Technical University, Mosul, Iraq.

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ABSTRACT

Photodynamic therapy (PDT) is a form of therapy that uses light along with special chemicals known as photosensitizers (PS) to treat cancer and many other diseases [1]. The effectiveness of PDT depends on the photophysical and spectral properties of the photosensitizer, the presence of a high quantum yield of reactive oxygen species (ROS) generation [2]. also, the outcome of PDT is largely determined by the pharmacodynamic properties of PS. The main intermediary in PDT is singlet oxygen. As its lifetime is short, its ability to diffuse into cells is limited to 10-55 nm Thus, photodynamic damage to biological structures is observed in a small area around the PS [3]. Therefore, photosensitizer delivery to cellular and tissue targets and its localization in them is important for PDT. Currently, one way of developing photodynamic therapy is through the use of special photosensitizer delivery systems, which increase the selectivity of PS delivery. Examples of such an approach are the use of nanostructured and supramolecular complexes of PS with nanoparticles, polymers, cyclodextrins, etc. [4].

1. Introduction

PDT is a photochemical-based technique that involves a light-activatable drug PS and a specific wavelength of light to harm tumor tissue by creating reactive molecular species. Clinically, depending on the particular PS and the target illness, the photosensitizer is often injected intravenously, and the tumor is treated with an appropriate light source after a particular time delay referred to as the drug-light interval. Currently, the creation of unique nanocarriers to carry photosensitizers to tumor targets has emerged as a prominent trend in PDT research to adjust treatment procedures while avoiding adverse effects and improving PDT efficacy. meta-tetra(hydroxyphenyl)chlorin (mTHPC) is a highly approved the 2nd generation photosensitizer for the palliative treatment of head and neck cancer. Due to the high hydrophobicity of this photosensitizer, the therapeutic application of mTHPC faces multiple challenges. mTHPC significantly aggregates in aqueous media, such as blood plasma, and as a result is inefficient in generating singlet oxygen, resulting in a decrease in photosensitizing efficacy. The hydrophobic property of mTHPC further complicates medication administration. To boost its

Corresponding author:
E-mail address: mohammed95altaee@gmail.com
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bioavailability and efficacy, mTHPC is loaded into different nanocarrier particles, which have several appealing features. for anticancer drug delivery. Numerous investigations on mTHPC formulations based on lipid vesicles, cyclodextrins, protein nanoparticles, polymers, and others have been conducted The incorporation of a photosensitizer into such vesicles can modify its pharmacokinetic and photophysical characteristics dramatically. A thorough characterization of a nano delivery drug system is required for extensive knowledge of the system-target interactions that produce the in vivo result. It is unknown from anticancer drug research what the most important parameter to consider when optimizing nanocarrier PDT. Along with blood circulation and spatio-temporal absorption in the tumor, drug release is regarded as a critical characteristic of liposomal drug formulation [1-5]. This project aims to evaluate the applicability of temperature-dependent copolymer of dextran and PNIPAM as nanocarriers for mTHPC, mTHPP, and other arylporphyrins. For this purpose, we designed special techniques for assessing the release of PSs from carrier polymers in vitro and in vivo which is based on the study of the spectroscopic properties of the photosensitizer.

2. Fundamentals of Photodynamic Therapy

The method of photodynamic therapy (PDT) is based on the generation of highly active intermediates by photosensitizer (PS) molecules when they are activated by light of a certain wavelength, resulting in phototoxic effects that lead to the death of selectively accumulated PS cells and/or damage to target tissues [2]. To date, the main photophysical and photochemical processes involved in PDT have been studied in detail. The organic PS molecule, after absorbing a photon, passes from the ground singlet state S_0 to the short-lived (0.1-100 ns) excited singlet state S_1 without changing the electron spin. Decontamination of S_1 can occur by several mechanisms [3]. From the excited state, the PS can quickly relax to the ground state S_0 with the emission of a fluorescence photon or by thermal energy dissipation, as well as by inter-combination conversion to the first excited triplet state Sn. Most S has a high quantum efficiency of such a transition.

The lifetime of PS molecules in the triplet state is much longer (>500 ns), which significantly increases the probability of their participation in reactions with other molecules, which is a condition for the effectiveness of photosensitized reactions. From the Sn state, the PS molecule can return to the ground state by emitting a phosphorescence photon or transfer energy to another molecule by nonradiative transfer in a collision [2]. An activated PS molecule in the Sn state, when interacting with another molecule, can cause chemical changes in it. By the mechanisms of such reactions, it is customary to divide them, according to the Ft classification [4], into photochemical reactions of type I and II. Photochemical reactions of type I include reactions with the transfer of an electron or proton between the PS in the Sn state and neighboring molecules with the formation of anionic or cationic radicals. These radicals can then react with molecular oxygen to generate reactive oxygen species (ROS). Photochemical reactions of type I often result in the formation of superoxide anions by electron transfer from PS to molecular oxygen. In biological systems, superoxide anions are not very active, but they can react to produce hydrogen peroxide (H_2O_2), which, in turn, in reaction with the hypochlorite anion, can generate singlet oxygen. At high concentrations, H_2O_2 can react with superoxide anions to form a very active hydroxyl radical, whose redox potential (E_0=1.35 V) exceeds the activation energy of the oxidation reactions of most biomolecules.

In the presence of metal ions (for example, iron or copper), hydroxyl radicals can also be produced in the Fenton reaction [5]. Hydroxyl radicals and H_2O_2, which have high penetrating power, can cause damage to various cell compartments and may play an important role in the photocytotoxic effect of PDT. The transfer of energy (but not electrons) by a PS molecule in the Sn state to molecular oxygen is called a type II photochemical reaction. Molecular oxygen differs from most molecules in that it contains unpaired electrons in valence orbitals, that is, for O_2, the main state is a triplet $^3O_2$. In the process of energy transfer, a type II photochemical reaction produces very active singlet oxygen $^1O_2$, which can be deactivated either by returning to the ground state or by reactions with electron-donor sites of

biomolecules, forming oxidation products. Since the PS returns to the ground state during the reactions, it can generate many times higher amounts of reactive intermediates. Photochemical reactions of type I and II usually proceed in parallel, and for the same PS, the relative yield of these reactions may vary depending on the conditions, the most important of which are the properties of the PS, the microenvironment conditions, and the oxygen concentration [7,8]. For example, when the O_2 concentration decreases due to tissue hypoxia-induced during PDT, type I reactions may become the predominant type of photochemical reactions instead of type II reactions [5]. These are represented in the Jablonski diagram (Figure 1.1). The same effect is observed at high concentrations of the substrate [6]. The polar microenvironment contributes to the development of type I reactions due to the stabilization of the formed radial pairs in them and the prohibition of the reverse electron transfer reaction [7]. In hydrophobic media, where the lifetime and solubility of oxygen are higher, type II reactions are more likely [8].

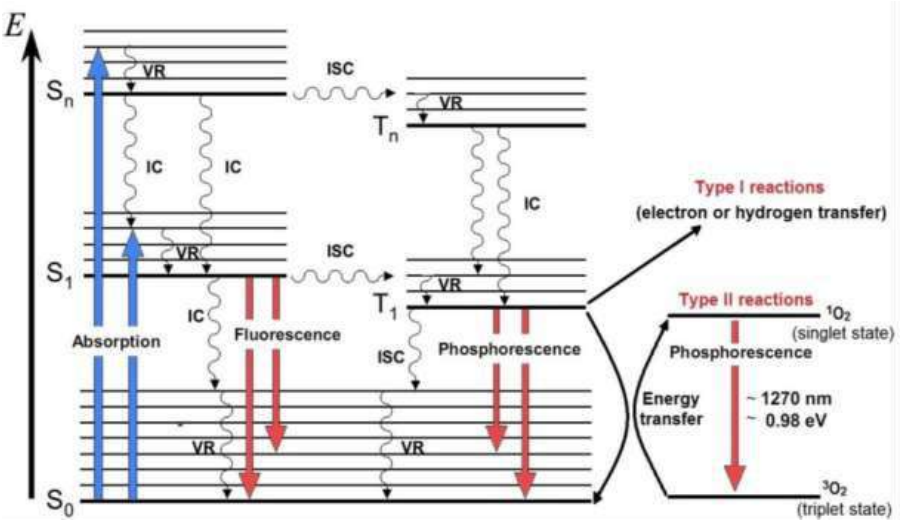


Figure 1.1 - Jablonski energy diagram for PS. VR – vibrational relaxation, IC internal conversion, ISC – intersystem crossing (from [1]).

The presence of type I and II reactions in living cells and tissues have been proven, so an additional assessment of the relative yield of such reactions is often required to analyze the physicochemical mechanisms of damage to specific cellular and tissue systems. It is well known that for most of the PS used in modern PDT, the photochemical reaction of type II is dominant [9]. Most ROS formed in photosensitized reactions have short lifetimes in the biological environment, so they are not able to diffuse over significant distances. For example, for hydroxyl radical, the path length is only(2.1-14) nm in isolated nuclei of Ehrlich ascitic carcinoma cells and (6.1) nm in (HL-60) cells, the path length of $^1O_2$ is (11±2) nm in thin aqueous films [1] and 10-20 nm in cells, and in Escherichia coli its value is not more than (50) nm from the above data, it follows that in biological objects, the main destructive effect during PDT will be observed near the sites of localization of PS molecules. Thus, an important condition for achieving the high efficiency of PDT is the predominant accumulation of PS in critical targets of the cell or tissue. A significant role in the photosensitization process can be played by chemical changes in the PS itself during PDT, which also occur as a result of both types I and type II reactions usually when analyzing the phototransformation of PS molecules [11]. The processes of photodegradation and photo modification are distinguished. (Photodegradation – Photobleaching) is the chemical destruction of PS molecules, leading to their splitting into small fragments that do not absorb in the visible spectral region. As a consequence, photodegraded PS molecules perform their functions in PDT Photo modification is a less profound chemical

change that leads to the modification of the side groups and/or the molecular skeleton of the PS. For example, photo modification of protoporphyrin IX (PPIX) is characterized by the appearance of chlorins, accompanied by a red shift in fluorescence, and the photoproducts are more effective PS than the original molecule. In addition, photo modification can change the physical and chemical properties of PS molecules, which often leads to changes in their intracellular localization It is important that the phototransformation products of PS are not toxic and do not cause mutational effects, for example, when they are relocalized into the cell nucleus. Thus, the processes of PS photodegradation can play a significant role in determining the effectiveness of PDT. At the same time, a decrease in the photodegradation of the PS guarantees the stability of photosensitization but may prolong the period of the patient's general photosensitivity. On the other hand, photodegradation of PS can be used to validate the doses of the administered drug and to develop treatment regimens that require a rapid decrease in the content of PS in the tissues [13]. PS participate in photochemical reactions in the Sn state, so the quantum yield and lifetime of the triplet state are important photophysical characteristics of PS. With a high quantum yield of the longlived triplet state of PS, the possibility of generating cytotoxic intermediates, for example, ROS, increases. The ability of PS to fluoresce is used for visual diagnosis of a tumor determination of the irradiation area or to assess the success of treatment by changing the fluorescence of tissues before and after PDT [1]. Among the factors that affect the efficiency of PDT, we can also distinguish the wavelength of light used to activate the PS. This is due to the depth of light penetration into the tissues, which is maximal in the red region of the spectrum and is limited by the strong absorption of light by the main endogenous acceptors (melanin, hemoglobin, H_2O). Therefore, the wavelength of the maximum absorption of the PS must correspond to the so-called "photodynamic window" 650-850 nm, in which, on the one hand, the relatively high light transmission of tissues, and on the other hand, the energy of the triplet state of the PS is sufficient to generate $^1O_2$.

3. The Main Molecular, Cellular, and Tissue Targets of PDT and the Mechanisms of their Photosensitized Damage

When considering photosensitized processes in real biological systems, an important role is played by the determination of the main targets of PDT. Depending on the level of structural organization of the objects under consideration, molecular, cellular, and tissue targets can be identified. At the same time, the specificity of damage to molecular targets is determined by the differences in the interaction constants of (ROS) generated in photosensitized reactions with these biological targets. For target cells and tissues, their sensitivity to PDT is due to the processes of distribution and accumulation of PS in such biological objects.To identify the main molecular targets of PDT in biological systems, numerous studies have been conducted to compare the sensitivity of different biological molecules to PDT. It has been shown that such targets include proteins whose sensitivity to PDT is mainly due to photosensitized damage of 5 amino acids (cysteine, methionine, histidine, tyrosine, and tryptophan) out of 20 that are part of natural proteins [13]. Lipid molecules, whose damage occurs due to the destruction of unsaturated double bonds during peroxidation, are also important targets of PDT. In some cases, damage to nucleic acids has been reported, with purine bases more prone to photo-oxidation than pyrimidine bases, and photosensitized damage to guanine under physiological conditions is the main cause of single - and double-stranded DNA breaks. However, for most of the porphyrin PS used in modern PDT, the damage efficiency of nucleic acids is low. It is noted that such biologically important molecules as biotin, folic acid, and glutathione are effectively oxidized in the presence of PS. Although in some cases it was possible to register the contribution of a certain type of molecular damage to the effectiveness of PDT, for example, in photosensitized cell damage, there was no strict determination of the effectiveness of this process from the type of molecular targets. Studies at the cellular level have made it possible to more accurately determine the main intracellular targets of PDT. It is shown that depending on the intracellular localization of PS, certain cellular organelles play the main role in the processes of photosensitized

cell damage. According to fluorescence microscopy, most porphyrin PS are localized in cytosolic targets, such as the Golgi apparatus, endoplasmic reticulum, mitochondria, lysosomes, and in the cytoplasmic membrane Thus, almost all cellular organelles can be used as targets for PDT. Mitochondria are a very sensitive target for PDT since they produce adenosine triphosphoric acid (ATP), which is necessary for the energy supply of the cell's functioning. Electron microscopy records the presence of mitochondrial damage after PDT lasting about several minutes [1,2]. PDT with the production of hematoporphyrin(HpD) in vivo has been shown to inhibit individual mitochondrial proteins necessary to create an electrochemical gradient across the inner mitochondrial membrane. For example, the inhibition of malate dehydrogenase, succinate dehydrogenase, and cytochrome- C oxidase reached 15%, 20-25%, and 50-60%, respectively Under in vitro conditions, PDT with Hp reduces the activity of ATPase [16]. and photoinduced cell death occurs after a decrease in the mitochondrial membrane potential and the cessation of oxidative phosphorylation (for example, due to photooxidation of cytochrome C oxidase), preceding mitochondrial swelling. Tumor cells with smaller mitochondria with a higher crust density (RIF–1 sarcoma) accumulate less Photofrin on the inner mitochondrial membrane compared to normal cells and demonstrate resistance to PDT [3]. Lysosomes are shown to be very sensitive to PDT because many PS accumulate in them. For example, PDT using sulfo-derivatives of meso-tetraphenyl porphine (mTHPP) does destroy lysosomes, but this does not cause a significant decrease in cell viability Moreover, it has been shown that the destruction of lysosomes is not cytotoxic in itself, and hydrolases and acids released from the destroyed lysosomes can be neutralized by cell components [14]. It was found that lysosomes serve as sites of primary localization of some aluminum phthalocyanine sulfonates (AlFcS2 and AlFcS4), but are not PDT targets. After treatment with small doses of light, a strong increase in the fluorescence intensity of these PS inside the cells was observed, due to the disaggregation of PS molecules in the process of leaving damaged lysosomes and moving to new, more sensitive intracellular targets damage to the plasma membrane is also often seen in PDT. For example, the disappearance of microfilaments and the swelling of the membrane surface, which led to the destruction of the cell membrane and a decrease in cell volume, were observed after PDT with Photofrin (melanoma cells A-Mel-3) and with AlFcS4 (human bladder cancer cells) [18]. The plasma membrane of neutrophils is the main target of PDT using merocyanine-540 since in experiments the membrane effects were much more pronounced than the effects in the cytosol. Another cellular target is microtubules, the effect of PDT on which was manifested in the destruction of the structure of microtubules a decrease in their number [14], and a delay in metaphase When analyzing the mechanism of cell damage in PDT, it is important not only to identify the role of various cellular organelles in this process but also to establish the cellular mechanism of PS cytotoxicity. It is well known that various cytotoxic factors can induce cell death by necrosis and apoptosis in this case, the response of cells to PDT depends on both external (for example, the concentration of PS and the light dose used) and internal (for example, the state of cellular metabolism of factors and the phase of cell growth) factors that affect the sensitivity of cells [12]. Cell necrosis, including cell swelling, destruction of the membrane, pathological changes in the cytoplasm, and karyolysis is observed when using high concentrations of PS and large light exposures Peroxidation of membrane cholesterol (CH) and other unsaturated phospholipids during PDT leads to changes in membrane permeability, loss of fluidity, and inactivation of membrane-bound enzyme complexes and receptors [7]. These processes cause loss of membrane integrity and disruption of ion homeostasis. The key event in the process of PDT-induced cell death leading to necrosis is considered by many to be the inhibition of mitochondrial enzymes and the resulting termination of the cell's energy supply [8].

In PDT-induced apoptosis, there is no leakage of intracellular material into the environment during controlled cell destruction, which prevents damage to neighboring cells. There is cell compression, nuclear fragmentation, and cell division into apoptotic bodies. which are recognized and absorbed by phagocytes [9]. It should be noted that apoptosis is one of the main

mechanisms of cell damage during PDT. It is shown that for several PS, the apoptotic type of cell death in PDT reaches 80-100% which causes a reduced inflammatory response of the treated tumor tissues in comparison with other methods of treatment. The implementation of a particular mechanism of cell death as a result of PDT depends on many factors, for example, on the severity of cell damage at high (lethal) doses of PDT, i.e., when the concentrations of PS and acid and the value of the light dose are high, the signaling pathways of cell death start immediately, leading to their rapid death. Schematically, the principle of PDT-induced destruction of a tumor is depicted in (Figure 1.2) The administration of the PS is followed by the irradiation of the tumor site after a certain drug-light interval DLI to allow for PS accumulation.

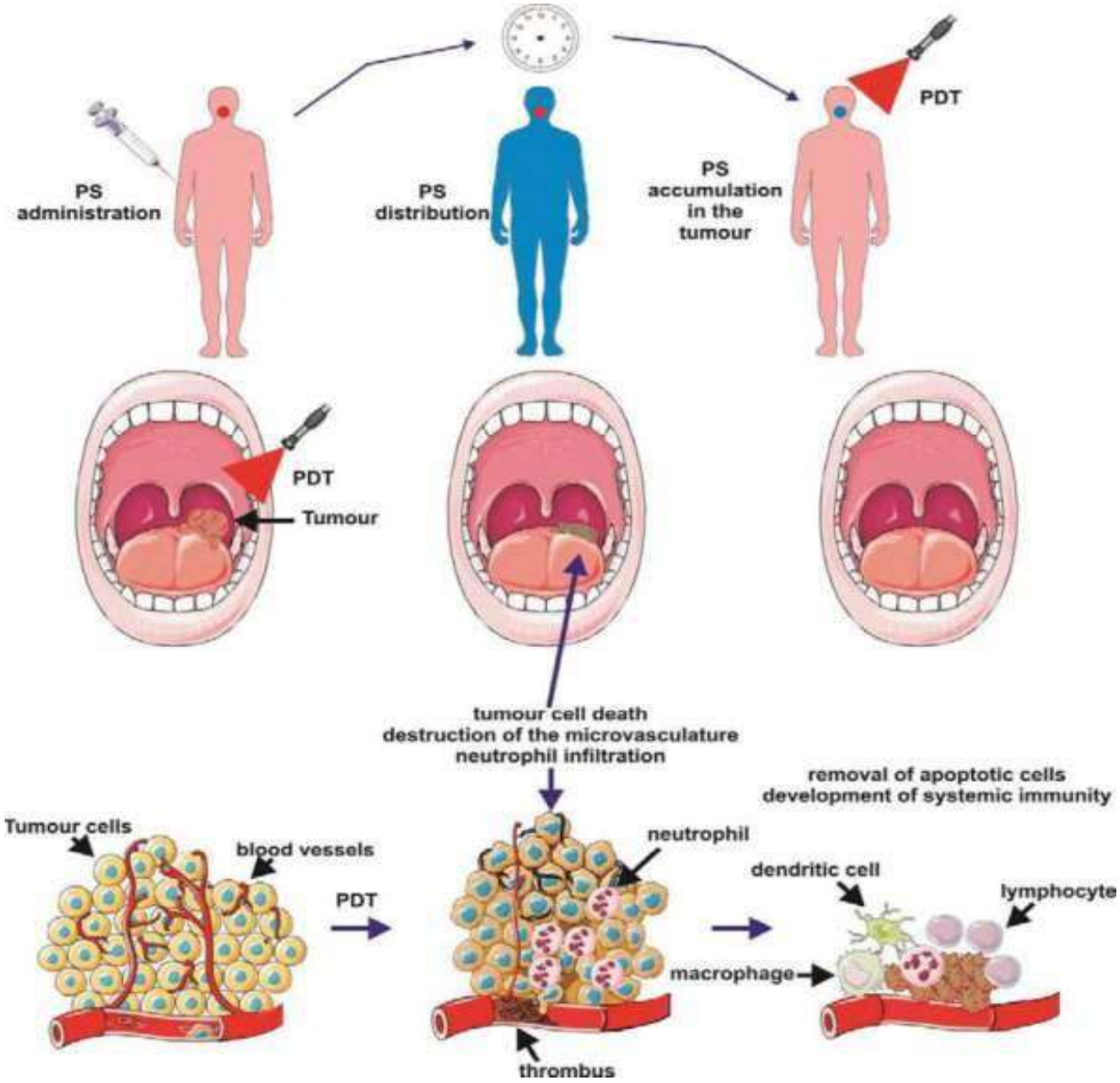


Figure 1.2 - Principle of PDT and tumor photoirradiation (from [1]).

However, at low (sublethal) doses of PDT (usually characteristic of PDT), the death of tumor cells will be delayed and will depend on the balance between cell death signaling and PDT-induced cell survival signaling. The cell survival signal can be determined by innate protective cellular responses against oxidative damage and against the signaling of apoptotic death (for example, the

expression of heat shock protein, anti-oxidant enzymes, and anti-apoptotic proteins), Inhibition of such survival signals increases the effects of PDT both in vitro and in vivo [22]. Higher concentrations of PS in cells lead to more severe post-irradiation injuries and the duration of the light exposure and the power density of the light flux affect the time dynamics of the (ROS) generation [18]. An increase in the intensity of PDT seems to shift the mechanism of cell death towards necrosis [19]. In solid tumors, in addition to the characteristics of PS accumulation, the depth of light penetration also plays an important role. For example, when photoprocessing bulk tumors, a light gradient can form inside the tissue, which causes different cytotoxicity and/or different types of cell death in different areas of the tumor.

4. Applications, Advantages, and Limitations of Photodynamic Therapy

There are several major clinical applications of PDT which have evolved in time with the development of new drugs, light sources, and the understanding of the fundamental processes involved in PDT [8].

Cancer Treatment

Historically the destruction of solid tumors was the initial indication for the palliative use of modern PDT [9,10]. Today, as a major number of clinical trials in which different methods of cancer treatment have shown little difference in the treatment results the treatment of superficial and early disease may give a different therapeutic approach Refractory superficial bladder cancer was the first tumor approved for treatment [11,12]. Additional approvals include obstructive and earlystage bronchial treating cancer oesophageal dysplasia and in situ carcinoma Cholangiocarcinoma is unresectable. Excellent cosmetic outcomes make PDT suitable for patients with skin cancers PDT for basal cell carcinoma, including extensive or recurrent tumors, [13,14] has been shown to have great effectiveness. Several clinical trials of PDT for primary and recurrent tumors have been conducted with malignant brain tumors, which illuminate the institution cavity immediately after a radical resection to reduce the burden of the residual tumor and to increase the probability of long-term control over the disease. Prostate cancer PDT trials are ongoing, with the treatment of the whole prostate in patients who have recurred locally following radiation therapy or as primary therapy for focus point tumors effective clinical treatment of head and neck cancers is led via the generation of PSs of secondgeneration: widespread, unresectable and reoccurring tumors oral cancers of the early stage and nasopharyngeal cancers [15-19]. However, because of the paucity of welldesigned clinical trials, the effectiveness of treating many types of cancer with PDT remains to be demonstrated. PDT has several potential advantages over surgery and radiotherapy [20,1]:

1. minimally invasive.
2. Flexible fiber optic devices with exact treatment sites can be utilized to find PSs with cancerous lesions and administer precise light to them.
3. Repeated doses can be administered without causing considerable radiation resistance or exceeding the total dosage limit.
4. Few results from the healing process.
5. Neither of the clinically recognized PSs accumulate in the nucleus, limiting cell damage that might be carcinogenic or contribute to the generation of resistant clones.
6. There are no adverse effects from chemotherapy or radiation, and systemic toxicity is negligible.
7. PDT can be used before or after chemotherapy, irradiation, or surgery without interfering with the therapies.
8. PDT may be performed in ambulatory environments, which saves money and makes it easier for the patient. PDT works through several biological mechanisms, however, therapy is complicated and difficult to optimize because of the numerous interconnected components.

Localized Infections

Increased resistance to antibiotics among many pathogens may bring about the end of the antibiotic age of 60 years Increased study on alternative therapies has resulted in the growth of multi-drug resistant bacteria [21,7]. In the treatment of localized bacterial infections, PDT is

considered as an option. Even multi-drugresistant strains were demonstrated to be effective. PDT's advantages include equal killing efficacy regardless of antibiotic resistance and the absence of PDT induction. [22,23]. Disadvantages are the cessation of the antimicrobial effect when the light is turned off and rather poor selectivity for microbial cells over the host tissue [7].

Macular Degeneration

The clinical ophthalmological success in the treatment of macular degeneration linked to age and eye diseases linked to neovascularisation was one of the main achievements of PDT [24]. The use of thermal laser coagulation was the only treatment before PDT, but it was little effective as a first-line treatment, PDT clearance for Visudyne® in 2000 resulted in more than 2 million treatments to date [8,25].

Dermatology

Approved methods of treatment for actinic keratosis or sun-damaged skinare used in the treatment of aminolevulinic acid PDT, which is linked with skin cancer development. It is widely used in the treatment of acne and hair removal (treating hair follicle damage) and skin re-modeling in cosmetic dermatology, for instance. PDT with this prodrug is also widely used. [26,27]. The adverse effects of PDT relate to pain during treatment protocols and a persistent skin photosensitization that has been somewhat circumvented by the latest PSs. But this is a small price to pay for a potential cure of cancer compared to other treatments. The PDT treatment is localized and is not effective against one of the most frequent causes of death in metastatic lesions in cancer patients [1,8]. And not systemic disorders. and does not apply. In the present state of the art, PDT is primarily constrained by the availability of accurate dosimetry.

5. Results and Discussions

Experimental part

characterization, and pecupiarrities of copolymer molecular structure molecular

Polymer system like copolymer with Dextran core (Mw=70x105 g/mol) and 15 grafted poly-(N-isopropylacrylamide) chains was used as a polymer nanoplatform for photosensitizer encapsulation. Further, this polymer will be designed as D70-PIPAM 15. Synthesis,

parameters of D70-g-PNIPAM15 are given in (Table 4.1) Mw- weight average molecular weight; Mn - the number average molecular weight; Mw/Mn polymer polydispersity, n (%) – the content of dextran component in the polymer.

Table 4.1 - Molecular parameters of D70-g-PNIPAM15 copolymer, determined by SEC

Sample	Mw×10-6 g/mol	Mn×10-6 g/mol	M_w/M_n	n, % dextran component
D70-g-PNIPAM15	1,03	0,674	1,52	6,8

Photosensitizers

mTHPC was obtained from the Biolitec (Germany). Other PSs were obtained from Porphyrin Products (USA). The properties of these compounds were described earlier [128]. Stock solutions of pigments (10-3 M) were prepared in ethanol or other organic solvents and kept in dark at 4°C.

Spectral Measurements

Corrected absorption and fluorescence spectra were recorded on the SFL-1211A and CM 2303 spectrofluorimeter (Solar, Minsk). All spectral measurements were carried out with the thermostatic cell unit equipped with a magnetic stirrer.

Fluorescence polarization values were recorded by the standard methods. Measurements of PSs

fluorescence polarization degree (P) were performed on Solar CM 2303 equipped with polarizers. In this case, samples were excited at 430-435 nm and fluorescence was registered at 650 nm as published before [125].

The polarization degree was calculated using a mean value of fluorescence intensities with the polarizers in parallel and perpendicular positions. The absolute error value for all measurements was less than 0.02.

Quasy Elastic Light Scattering (QELS)

QELS measurements were carried out using Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK).

The apparatus contains a 4 mW He-Ne laser with a wavelength of 632.8 nm and the scattered light is detected at an angle 173° (backscattering). For an accurate transition study, correlogram of 0.1 mg/mL aqueous D70-PNIPAM15 were collected in the temperature of physiological temperatures, namely at 25, 37, and 40°C.

Each temperature point was held for 5 min before measurements to equilibrate the sample. At least 10 correlation curves for each temperature point were treated by CONTIN algorithm [125] that is known to be reliable for complicated systems [126] to get hydrodynamic diameter (DH) distributions.

Spectroscopic Properties

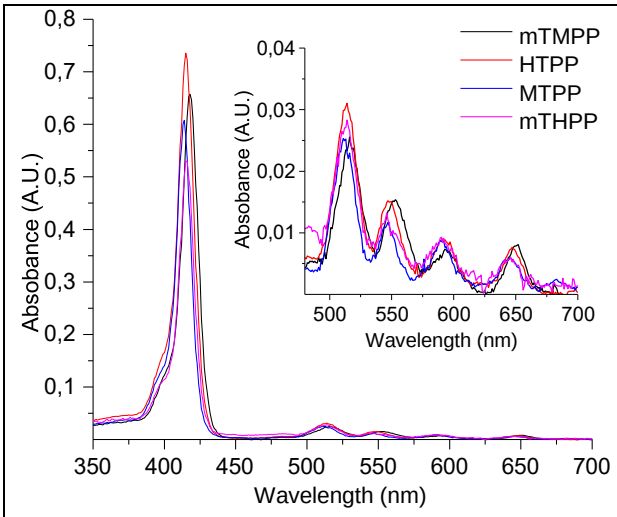
The results obtained from spectral studies of mTHPP, HTPP, MTPP, mTMPP in neat solvents and different biological solutions are shown in (Table 4.2).

The spectral characteristics of these photosensitizers are generally defined by their belonging to porphyrin-type compounds. Absorption spectra of PSs in organic solutions reveal the most prominent peaks in the region of **420 nm** and four monotonically decreasing Q bands. The absorption spectrum of chlorin-type PS mTHPC differs from other presented pigments spectra. In organic solvents, mTHPC has a typical absorption spectrum of chlorin-type compound with two main peaks at 416-422 nm (Soret band) and four Q bands, the longest wavelength band being the most intensive. (Fig. 4.2).

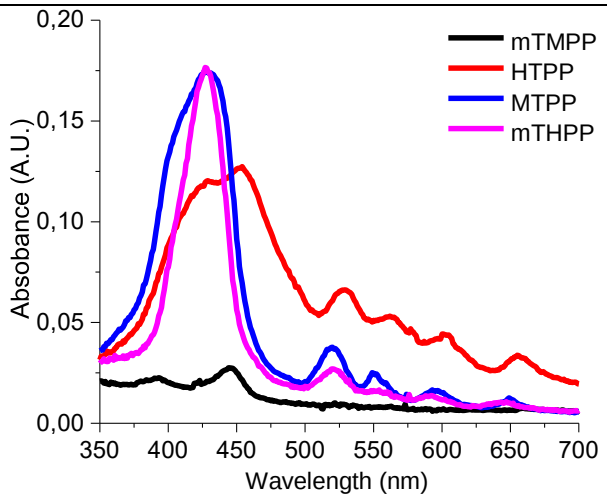
Table 4.2 - mTHPP, HTPP, MTPP, mTMPP in neat solvents and Different biological solutions

Solvent	D	λ(soret), nm	I, a.u.	λ(ex), nm	I_1, a.u.	λ_1(em), nm	I_2, a.u.	λ_2(em), nm
Ethanol	1,09	415	56,325	415	54,796	653	37,117	718
DPBS	0,31	423	5,241	421	5,084	656	3,460	720
Serum	0,55	421	30,634	421	30,567	654	20,413	719
D (20°C)	0.47	420	10,313	419	9,769	654	7,320	713
D (37°C)	0.38	423	40,891	418	40,166	653	27,423	716

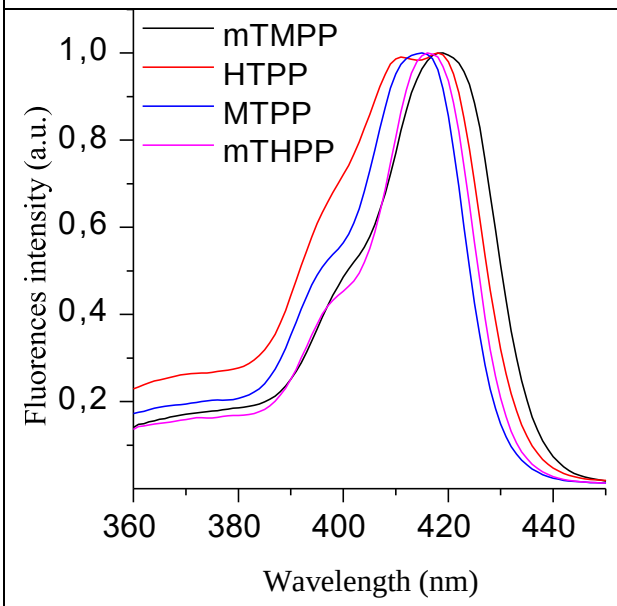
In organic solvents, all investigated compounds intensively fluoresce at 650-655 nm with a quantum yield around 0.04-0.09 and the fluorescence lifetime of 10 ns for chlorin and 5-6 ns for porphyrin-type pigments. In different organic solvents, we detected only minor spectral changes: shifts of 2-4 nm in the peaks and the relative fluorescence quantum yield varying within 10% compared to ethanol solution. The observed changes can be attributed to the effects of pigment solvation in media with different degrees of polarity.



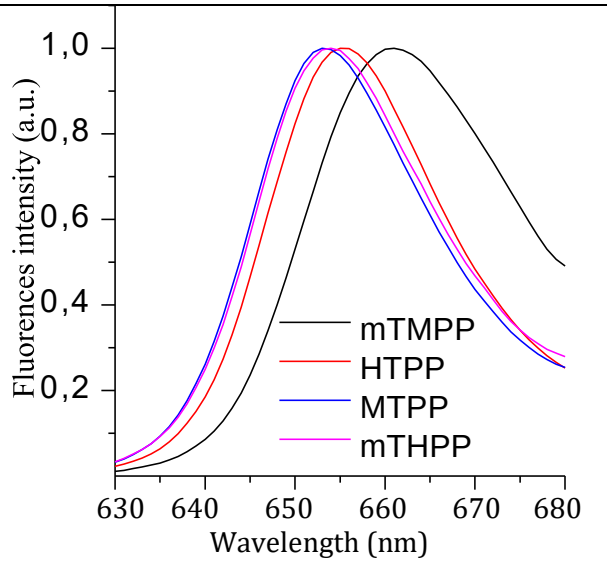
Absorption spectra of AP in ethanol, The concentration of AP 5 * 10^{-6} in methanol



Absorption spectra of AP in aqueous solution, The concentration of AP 5 * 10^{-6} in methanol



Normalized AP excitation spectra, The concentration of AP 5 * 10^{-6} in methanol



Normalized emission spectra of AP, The concentration of AP 5 * 10^{-6} in methanol

Figure 4.1 - In organic solvents mTHPC has a typical absorption spectrum of a chlorin-type compound with two main peaks at 416-422 nm (Soret band) and four Q bands, the longest wavelength band being the most intensive

Like many hydrophobic tetrapyrroles, mTHPP, HTPP, MTPP, mTMPP and mTHPC molecules in aqueous solutions tend to aggregate. Spectral changes, corresponding to aggregation, were observed after injection of their ethanol solutions in PBS (Fig 4.2). The aggregated species are characterized by a broadening of the Soret band and a shift of its peak to 430-450 nm with a concomitant decrease of extinction in all bands. The half-height bandwidth of PSs Soret band undergoes substantial increase upon introduction of sensitizer into the aqueous medium. According to our data mTHPP, HTPP, MTPP, mTMPP, and mTHPC show a drastic drop in fluorescence intensity after their removal from organic to water solution. Fluorescence yields are very sensitive to the aggregation state of porphyrin-like sensitizers with the monomeric. species solely responsible for fluorescence. addition of proteins, cells, and other biological structures to the aqueous medium is accompanied by the disaggregation of nonpolar porphyrins, and as a result, an increase in the intensity of fluorescence. Spectral modifications of investigated PSs in different environments demonstrate that all these changes are more pronounced in the case mTHPP and mTHPC. In model biological solutions, HTPP, MTPP, mTMPP fluorescence yields are significantly greater than in PBS solutions, but still is lower compared to the monomeric ethanol solution.

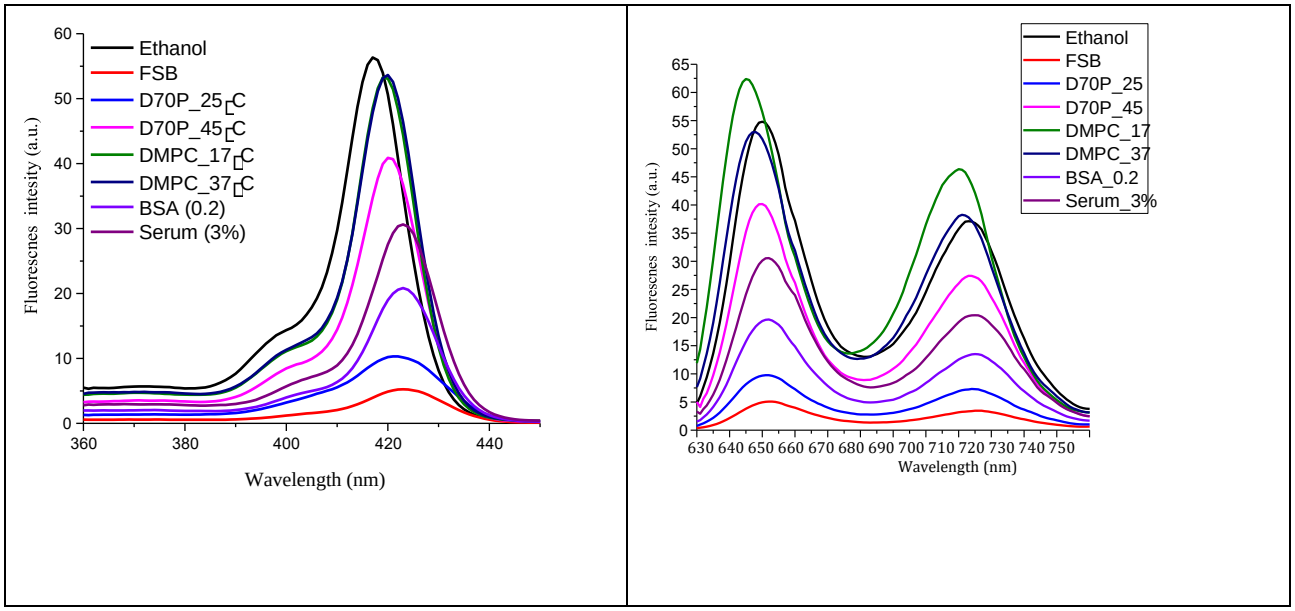


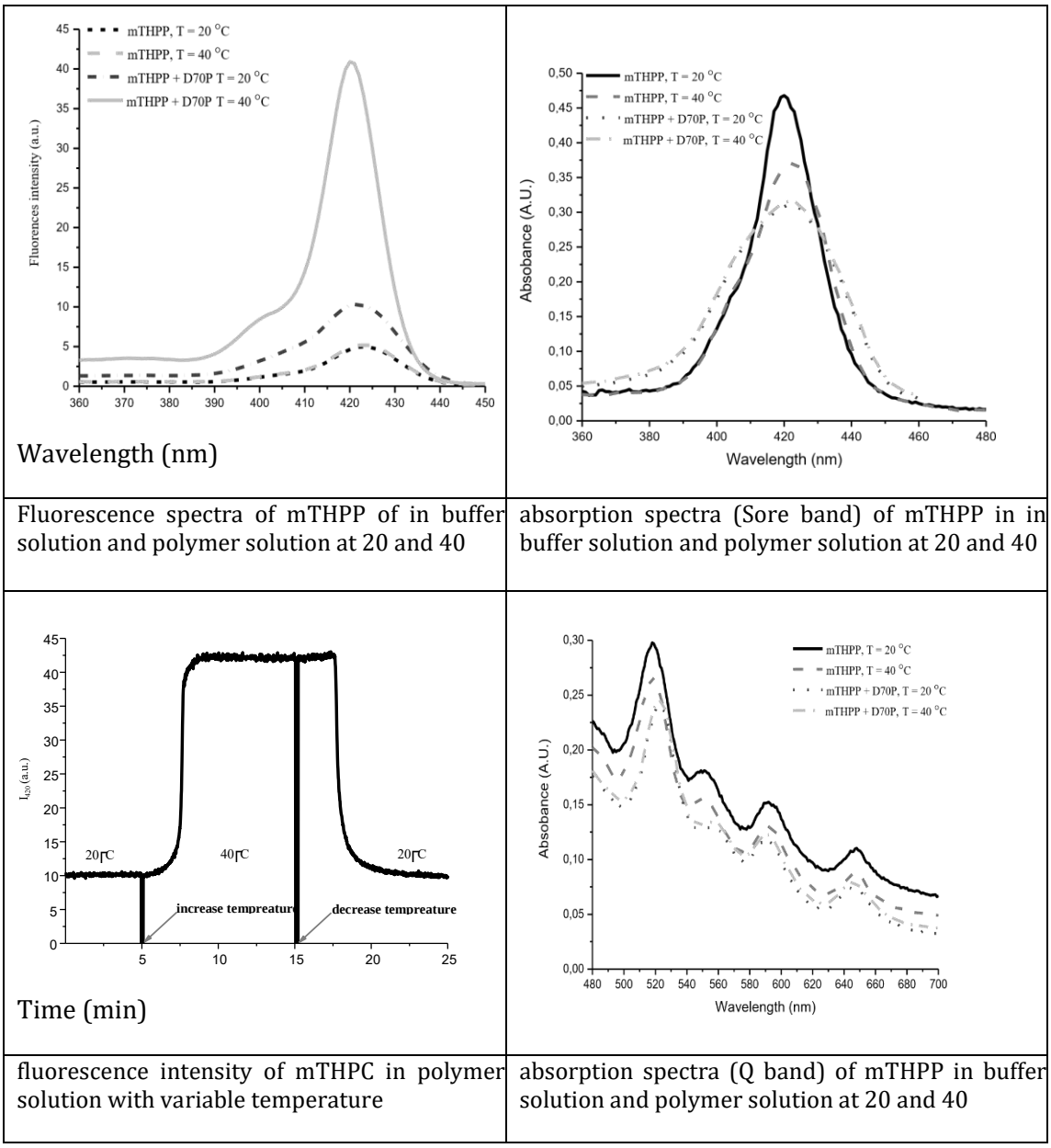
Figure 4.2 - injection of their ethanol solutions in PBS

Thus, both absorption and fluorescence measurements indicate that these PSs in all water solutions exist as a mixture of monomers and aggregates. The presence of aggregates was further evidenced by using resonance light scattering (RLS) spectroscopy. RLS effect is observed as increased scattering intensity at or very near the wavelength of the absorption maximum of aggregated molecular species. Taking into account the impossibility of HTPP, MTPP, mTMPP monomerization in model biological systems, we excluded the compounds from further studies. When studying the properties of complexes with stimulus-dependent polymers, only mTHPC and mTHPP were used.

6. Interaction of mTHPC and mTHPP with Polymer

At solution temperatures above 35 C, the addition of the polymer causes a significant increase in the fluorescence intensity of PSs, which suggests the destruction of molecular aggregates due to their interaction with the polymer. The shape of the absorption and fluorescence spectra is closed to

the same characteristics of these photosensitizers in organic solvents. The formation of complexes of mTHPP and mTHPC molecules with polymer nanoparticles is also evidenced by the results of measurements of chlorins fluorescence polarization degree. PS binding with copolymer nanoparticles restricts the rate of rotational relaxation and enhances the degree of fluorescence polarization. At temperatures below the critical point, adding a polymer does not affect the fluorescence of PSs in aqueous solutions. Low values of the relative quantum yield of fluorescence and the degree of fluorescence polarization, which practically coincide with the PSs characteristics in the absence of a polymer, allow us to conclude that in such conditions the processes of complex formation are ineffective. Cooling of an aqueous solution of complexes D70-g-PNIPAM15 with PSs to a temperature below LCST point is accompanied by changes in the fluorescence characteristics that are typical for aggregation of photosensitizers in an aqueous medium. Thus, the stimulus-dependent change in the phase state of the polymer is accompanied by the release of photosensitizer molecules from the complexes. It should be noted that if the solution contains structures that can bind chlorins, for example, serum proteins, aggregation processes are not observed. In this case, PSs molecules released from the complexes are bound by new acceptor structures, which is confirmed by the spectral analysis data (Figure 4.3).



Fluorescence spectra of mTHPP of in buffer solution and polymer solution at 20 and 40

absorption spectra (Sore band) of mTHPP in in buffer solution and polymer solution at 20 and 40

fluorescence intensity of mTHPC in polymer solution with variable temperature

absorption spectra (Q band) of mTHPP in buffer solution and polymer solution at 20 and 40

Figure 4.3 - PSs molecules released from the complexes are bound by new acceptor structures, which is confirmed by the spectral analysis data

Analysis of fluorescent characteristics allows us to make several conclusions on the behavior of polymer/Ps complexes The features of photosensitizer chemical structure influence interaction with copolymer D70-g-PNIPAM15 polymer. The structure of the copolymer molecule is a determinant of the ability of the photosensitizer molecule to form a complex. Temperature-dependent phase transition allows operating the process of photosensitizer release from its complex with a Copolymer molecule.

Our results show the high potential of the application of stimulus-dependent polymer as a nanocarrier for photosensitizer during photodynamic therapy.

7. Conclusions

The spectral characteristics of porphyrin fluorescence in polymer solutions are analyzed. It is shown that at low temperatures, the fluorescent characteristics of PSs in polymer solutions differ

slightly from those of aqueous solutions. When polymer solutions are heated, the fluorescence parameters change: the relative quantum yield of fluorescence increases, and the degree of fluorescence polarization increases. The temperature dependence of changes in the fluorescence characteristics correlates with temperature-dependent changes in the turbidity of solutions, which suggests that changes in the fluorescence parameters of porphyrin PS in polymer solutions are due to the influence of the phase state of polymers on the processes of their interaction with photosensitizers. It is established that a number of fluorescent parameters can be used the rate of release of photosensitizers from complexes with polymers. In the course of the research, new spectral methods were developed to control the processes of photosensitizer release from the complex with the polymer. These methods are used to analyze the processes of redistribution of photosensitizers from complexes in solutions

and in model biological systems. It is shown for the first time that temperature dependent changes in the polymer structure can act as a regulatory factor in the distribution of photosensitizers in biological systems. The results obtained are of interest for the development of the methodological base of research in the field of nanobiotechnology, for the production of new materials suitable as systems for the introduction of medicinal compounds.

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