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# Theranostics of skin neoplasms based on luminescence diagnostics in combination with photodynamic therapy in the absorption band of porphyrin

I.P. Shilov, A.S. Gorshkova, A.V. Ivanov, V.D. Rumyantseva, G.L. Danielyan, V.V. Kashin

*Abstract.* **We report the results of developing a technique for theranostics of skin neoplasms based on luminescence diagnostics in combination with photodynamic therapy (PDT) in the absorption band of porphyrin. It is shown that the therapeutic effect is achieved exclusively due to PDT, without the participation of the hyperthermia process, which occurs at temperatures above 42 °C. The Fluroscan gel [based on the dipotassium salt of the ytterbium complex of 2,4-di-(α-methoxyethyl)deuteroporphyrin IX (Yb-DMDP)] is used as a preparation for theranostics. The main photophysical properties and possible mechanisms of accumulation of nanosized low-toxic photosensitisers based on this compound are studied. It is shown that the Yb-DMDP compound in a DMSO solution (30 % aqueous DMSO) enhances photophysical characteristics (luminescence lifetime 5–10 μs, luminescence quantum yield up to 1 %, extinction coefficient $\sim 1.96 \times 10^5$ $M^{-1}$ $cm^{-1}$ at a wavelength of 398 nm). Experimental animals are used to test the proposed technique for theranostics of tumours using the Fluroscan gel and a fibre-optic laser fluorimeter.**

***Keywords:*** *theranostics, nanoscale photosensitisers, ytterbium complexes of porphyrins, photophysical properties, luminescence diagnostics, fibre-optic probe, pharmaceutical composition, skin neoplasms.*

## 1. Introduction

A modern trend in nanobiotechnology is the creation of multimodal nanostructures for theranostics of neoplasms, which combine diagnostic and therapeutic functions in one nanoparticle. Today, the main method of laser medical imaging and analysis of biological tissues for the presence of neoplasms is laser luminescence analysis [1]. There are two options for using luminescence diagnostics (LD) of neoplasms: using specially introduced exogenous tumouротropic fluorescent markers into the body and using fluorescence of endogenous chromophores, primarily porphyrins, accumulated in tumour and other rapidly proliferating biological tissues [1].

For early photodiagnostics of tumours, as well as luminescence diagnostics accompanying such therapeutic procedures as photodynamic therapy (PDT) of cancer, so-called therapeutic photosensitisers (PS's) are currently used: Photofrin, Photohem (first generation PS preparations), Foscan, Photosense, Radachlorin, Photoditazin, etc. (second generation preparations). Recently, oncological statistics have recorded a noticeable increase in neoplasms of superficial localisation, tumours of the skin and mucous membranes. Among the therapeutic PS's used to diagnose cancer of the skin and mucous membranes, a special place is occupied by the preparation Alasens, created on the basis of delta-aminolevulinic acid (5-ALA). 5-ALA does not fluoresce itself, but is a precursor of intracellular heme biosynthesis. One of the drawbacks in working with 5-ALA and its derivatives is the rather frequent false positive diagnoses. Thus, in Ref. [2], it was reported that when a normal mucous membrane was irradiated with a laser beam directed tangentially to the surface of the biological tissue, noticeable fluorescence appeared in the red region of the spectrum due to the excitation of many normal cells containing endogenous protoporphyrin IX. Such a rather strong fluorescence appeared to be comparable to the fluorescence of tumour tissue excited by a laser beam perpendicular to the biological tissue surface, which caused errors in diagnostics.

From the point of view of the primary diagnosis of cancer, the aforementioned therapeutic PSs, including Alasens with its derivatives, are not very promising, since their fluorescence in tissues is always accompanied by a much more probable process of generating singlet oxygen in both malignant and healthy tissues of the body, which causes a number of unwanted side reactions. Attempts to reduce to a safe level the extremely harmful role of singlet oxygen in LD by reducing the PS dose and the power of the radiation that excites them are futile, since this inevitably leads to a sharp decrease in the sensitivity of the LD. In the spectral range of fluorescence of these PSs (600–750 nm), for LD there is also background luminescence from biological tissues, including healthy ones, due to the presence of endogenous porphyrins in them, which reduces the signal-to-noise ratio and, in general, the sensitivity of the LD method.

Thus, it is obvious that the prospects of using therapeutic PS's for effective early diagnosis of cancer are rather doubtful, and the use of only the spectral range (600–750 nm) in the process of early diagnosis of neoplasms, in our opinion, is not optimal.

**I.P. Shilov, A.S. Gorshkova, V.D. Rumyantseva** Kotelnikov Institute of Radioengineering and Electronics, Fryazino Branch, Russian Academy of Sciences, pl. Akad. Vvedenskogo 1, 141190 Fryazino, Moscow region, Russia; e-mail: laserlab@ms.ire.rssi.ru;
**A.V. Ivanov** State Research Centre for Laser Medicine of the Federal Medical and Biological Agency of Russia, Studencheskaya ul. 40, stroenie 1, 121165 Moscow, Russia; Blokhin National Medical Research Centre of Oncology, Kashirskoe shosse 23, 115478 Moscow, Russia;
**G.L. Danielyan, V.V. Kashin** Prokhorov General Physics Institute of the Russian Academy of Sciences, ul. Vavilova 38, 119991 Moscow, Russia

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It has been established that the near infrared (NIR) range is the most promising at present for biomedical diagnostic research due to the greater depth of penetration of photons through biological tissue and minimal autofluorescence in this range of the spectrum [3]. Various NIR fluorochromes, such as cyanine dyes, are used for NIR fluorescence [4]. However, most of them (for example, indocyanine green) are not tumourotropic and are rapidly excreted from the body [5]. In addition, they all have a very small Stokes shift (the difference in wavelengths between the maxima of the emission and excitation bands), which complicates the technology of luminescence measurements.

In a number of papers [6, 7], for various biomedical applications, including diagnostics of neoplasms, it is proposed to use low-toxic photosensitisers based on some lanthanide porphyrin complexes (LaPCs), which luminesce in the NIR region of the spectrum. These include complexes of porphyrins based on Yb, Ho, Er, and Nd. To achieve an increased level of luminescence of lanthanide ions, it is necessary to ensure an efficient energy transition between the antenna (porphyrin ligand) and the emitter (lanthanide ions). The gap between the energy level of the antenna (source) and the energy level of the lanthanide ion (receiver) should ideally be 1000– 2000 $cm^{-1}$ [7]. LaPCs meet these requirements almost perfectly; moreover, they have an increased extinction coefficient in the visible and UV spectral ranges. In addition, the $Yb^{3+}$ ion has the highest internal quantum yield (up to 4%) among lanthanide ions, making it the most promising candidate for use in NIR luminescence diagnostics of neoplasms.

It should be also noted that the introduction of the $Yb^{3+}$ ion into the centre of the porphyrin matrix leads to a sharp decrease in the photochemical activity of the compound [8], while maintaining the tropism to malignant tumours characteristic of most porphyrins. It is obvious that ytterbium ions introduced into the corresponding derivatives of porphyrin complexes significantly reduce the quantum yield of singlet oxygen generation, since the luminescent level of the $Yb^{3+}$ ion lies somewhat below the triplet level of the organic part of the molecule, but higher than that of singlet oxygen (Fig. 1). As a result, the excitation of the porphyrin matrix under the influence of external light radiation is not transferred to oxygen, but is intercepted by the $Yb^{3+}$ ion, thereby sharply reducing the generation of singlet oxygen sensitised by porphyrin. Therefore, the phototoxicity of such ytterbium porphyrin complexes (YbPCs) significantly decreases, and upon excitation of the π-electron system of the organic part of the molecule, the Yb complexes exhibit luminescence due to the transitions of 4f electrons of the $Yb^{3+}$ ion: $^4F_{5/2} \rightarrow {}^2F_{7/2}$. [9].

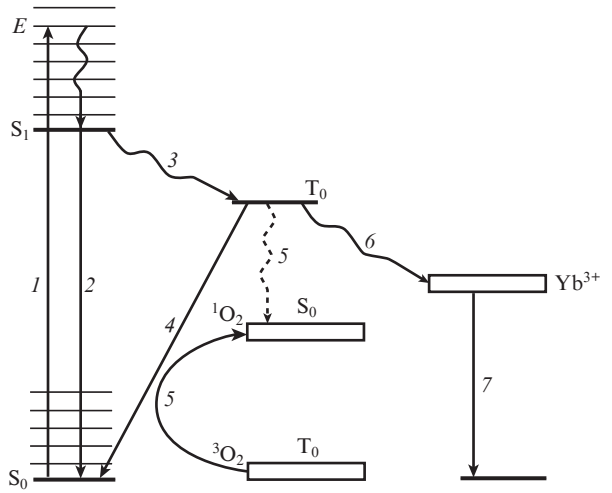


**Figure 1.** Diagram of electronic transitions of porphyrin sensitisers and the formation of singlet oxygen: (*1*) absorption; (*2*) fluorescence; (*3*) intercombination conversion; (*4*) phosphorescence; (*5*) transfer of excitation to oxygen and the formation of singlet oxygen $^1O_2$; (*6*) transfer of excitation to the Yb ion; (*7*) luminescence of the $Yb^{3+}$ ion.

It was established earlier [10] that one of the most promising compounds for IR luminescence diagnostics of neoplasms is the Yb-complex of 2,4-di(α-methoxyethyl) deuteroporphyrin IX (Yb-DMDP), which is a natural compound and, therefore, has low toxicity.

The combination of diagnostic and therapeutic functions in one nanostructure is the basic principle of theranostics, a new direction in biomedical photonics. Nanocomposites for cancer diagnostics and therapy, consisting of nanocarriers and nanoparticles encapsulated in them, have very great prospects in oncology.

The aim of this work is to create a technique for theranostics of superficial neoplasms (of skin and mucous membranes) based on luminescence diagnostics in combination with photodynamic therapy of tumours.

## 2. Materials and equipment

As a preparation for theranostics, we used the Fluroscan gel developed by us (Certificate No. POCCRU.0001.510608) based on Yb-DMDP [11]. The material presented in this paper was used to certify the Fluroscan gel pharmaceutical composition.

The absorption spectra of the synthesised complexes were measured using an LS-5B PerkinElmer spectrofluorimeter. The spectral kinetic characteristics were studied using the measuring stroboscopic stand with excitation in the visible range of the spectrum developed at the Kotelnikov Institute of Radioengineering and Electronics of the Russian Academy of Sciences (IRE RAS) [12]. The radiation source was an LS-2132 (Lotis-Tii) pulsed laser based on an yttrium aluminium garnet crystal with neodymium ions with a radiation wavelength of 532 nm. The size of nanoparticles in the suspension was determined by dynamic light scattering using a Kurs-3 laser correlation spectrometer [13], which allows measurements in the range 0.5– 104 nm.

The LD procedures, as well as the subsequent PDT in the absorption band of the porphyrin, were carried out using the experimental fibre-optic laser fluorimeter (FOLF) developed at the Fryazino Branch of the Kotelnikov IRE RAS [14]. The main challenge in the development of the FOLF fibre-optic laser system was to provide the highest possible qualitative and quantitative assessment of the level of the luminescence response. Obviously, it is necessary to create a parametrically reliable laser system (LS) with the ability to adjust the laser output power directly at the sensitive end face of the fibre-optic probe (FOP), and the probe construction should provide the maximum signal-to-noise ratio [15]. The structural diagram of the improved LS is shown in Fig. 2. The system specific feature is that, for practical use, a procedure for tuning the excitation laser is introduced with an estimate of the power level directly at the output sensitive end face of the reflex-type FOP.

The PDT procedure of previously identified pathologically altered areas of biological tissue was carried out by treating them with a therapeutic laser at a wavelength of

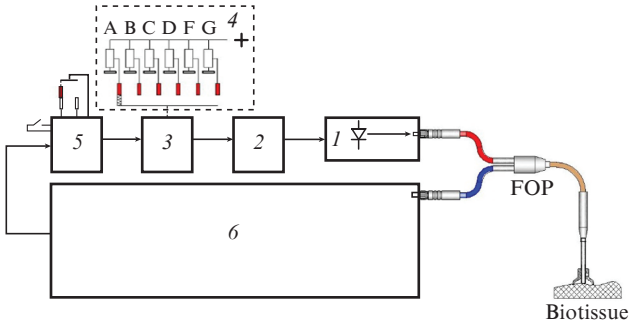


**Figure 2.** Scheme of the improved LS: (*1*) laser (405 nm, 100 mW) with a system of launching optical radiation into the FOP; (*2*) driver for stabilisation of the laser diode current, containing a control input for switching on pulses; (*3*) generator of pulse packets; (*4*) system for adjusting the levels of laser radiation power; (*5*) switch of the laser operation modes from manual control to control from the registration unit; (*6*) unit for recording the reflex reflection optical signal through 12 receiving fibres of the FOP.

635 nm (New Surgical Technologies LLC, Moscow). In this case, the exposure dose was ~300 J cm$^{-2}$.

For microwave thermography of the zone of malignant growth, a specially created multichannel hardware and software complex (MHSC) was used, which provides noninvasive detection of temperature anomalies of internal tissues of laboratory animals at a depth of several centimetres. MHSC determines the temperature of internal tissues by measuring the intrinsic electromagnetic radiation of laboratory animals in the microwave range, the operating frequency range of the receiving channel is 3150–3800 MHz. The power of the radiation received by the antenna is determined by the thermodynamic temperature, the parameters of the environment and the directional pattern of the antenna. The MHSC is equipped with receiving antennas of radio sensors, allowing its use indoors without special shielding.

## 3. Results and discussion

The development of a technique for theranostics of skin neoplasms was carried out by us based on luminescence diagnostics in combination with subsequent PDT in the absorption band of porphyrin.

The technique of tumour LD is based on two phenomena: the selective accumulation of the luminescent marker–photosensitiser in the neoplasm tissue and the possibility of its detection by characteristic luminescence in the area illuminated by laser radiation, as well as differences in the intensity and spectral composition of intrinsic fluorescence of healthy and pathologically altered tissues, when they are excited by laser radiation in UV spectral range in the Soret band of the PS. In this regard, the substance for LD must have enhanced photophysical characteristics (luminescence lifetime, luminescence quantum yield, and extinction coefficient); high tumourotropic properties; and low phototoxicity.

The possibility of using YbPCs for theranostic purposes was first reported in Ref. [16]. For PDT purposes, in the porphyrin absorption band online (immediately after the LD procedure), this substance should, with appropriate processing, also have high cytophototoxicity in one of the porphyrin absorption bands. Our studies have shown that the Yb-DMDP dipotassium salt, which under various conditions can exhibit both diagnostic and therapeutic properties, can satisfy the above requirements. The fact is that when the ytterbium ion is removed from the porphyrin matrix, the metal-free form of porphyrin already acquires increased cytophototoxic properties as a result of the formation of singlet oxygen $^1O_2$ after exposure to laser radiation at a certain wavelength. Figure 3 shows the absorption spectra of both Yb-DMDP and its metal-free form.

It is known that the absorption spectra of the initial metal-free porphyrins consist of an intense fundamental Soret band at 370–420 nm (B-band) and four so-called Q-bands in the region of 500–600 nm [Fig. 3, curve (*2*)]. One of the bands at 630 nm is dominant when using this form of porphyrin in PDT procedures. Based on this form, the Photogem photosensitiser was developed by the Lomonosov Moscow State University of Fine Chemical Technology, which is still used today. As to the absorption spectrum of Yb-DMDP, it is dominated by the Soret band near 400 nm and only two Q bands (at wavelengths of 530 and 570 nm) of significantly lower intensity [Fig. 3, curve (*1*)].

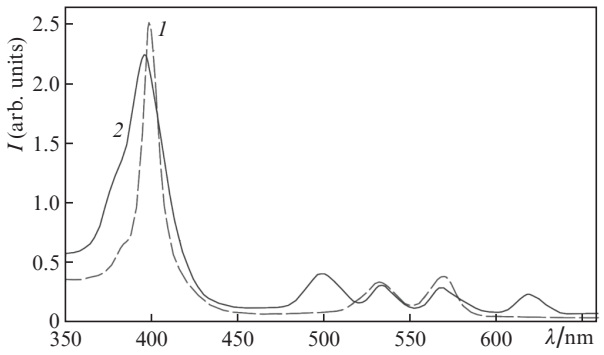


**Figure 3.** Absorption spectra of (*1*) Yb-DMDP (concentration $10^{-4}$ M, 20% aqueous solution of DMSO) and (*2*) its metal free form.

Studies have shown that it is possible to remove the $Yb^{3+}$ ion from the porphyrin matrix by treating the substance with weak acids (ascorbic, citric). Figure 4 shows the results of treatment of the PS solution with various acids. The data obtained confirm the release of the Yb ion from the porphyrin matrix, since treatment with acids leads to the appearance of four Q-bands in the region of 500–600 nm. Particularly effective, as can be seen from Fig. 4, is the treatment with ascorbic acid at a concentration of 1% (pH = 4.3). For com-

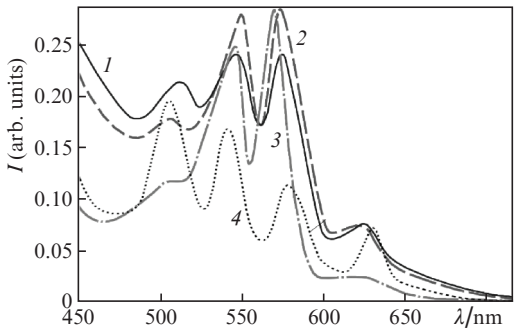


**Figure 4.** Absorption spectra of Yb-DMDP: (*1*) Yb-DMDP + ascorbic acid; (*2*) Yb-DMDP + citric acid; (*3*) Yb-DMDP; (*4*) metal free DMDP.

parison, the absorption spectrum of metal-free DMDP is shown [curve (*4*)].

The luminescence spectrum of Yb-DMDP (Fig. 5) demonstrates that this substance exhibits a narrow and rather bright luminescence band characteristic of rare-earth ions, which for $Yb^{3+}$ is in the IR range (975–985 nm), where the intrinsic luminescence of biological tissues is practically absent. Figure 6 shows the results of studies of the spectral and kinetic characteristics of Yb-DMDP. The luminescence lifetime $\tau$ of this YbPC in a 100% DMSO solution was about 20 μs. Of practical interest for use in medicine are YbPC substances in 20%–30% DMSO solution, the use of which is permitted in medicine. For such DMSO concentrations, the lifetime is $\tau \sim 1-5$ μs. The total luminescence quantum yield $\Phi_{tot}$ for Yb-DMDP was calculated according to the method described in Ref. [17] using a solution of Zn-tetraphenylporphyrin in ethanol as a standard, the quantum yield of which is 0.03 [17]. The calculated value of $\Phi_{tot}$ obtained was ~0.9%. We also emphasise that the $Yb^{3+}$ ion in the entire energy range, up to energies corresponding to the UV spectral region, has only two energy levels: ground ($^2F_{7/2}$) and excited ($^4F_{5/2}$) [18]. In this case, the electronic levels $^4F_{5/2}$ and $^2F_{7/2}$split into 3 and 4 degenerate levels, respectively. For this reason, the 4f luminescence of the $Yb^{3+}$ ion in YbPC is observed in a rather wide spectral NIR range (920–1080 nm) upon excitation in the 300–630 nm range. This property was used to create the fibre-optic laser fluorimeter.

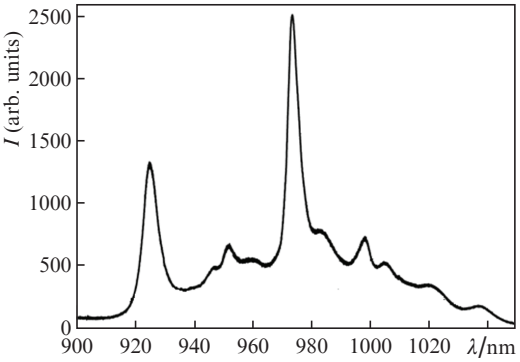


**Figure 5.** Luminescence spectrum of Yb-DMDP (20% DMSO solution, concentration $10^{-4}$ M).

Thus, it was confirmed that Yb-DMDP possesses enhanced photophysical characteristics (the luminescence lifetime is $\tau \sim 1-10$ μs, the luminescence quantum yield $\Phi_{tot}$ is about 1%, the extinction coefficient is $\sim 1.96 \times 10^5$ $M^{-1}$ $cm^{-1}$ at a wavelength of 398 nm).

A necessary requirement for the substances used in this theranostic technique is also the presence of increased tumourotropic properties, i.e., increased selectivity of the substance accumulation in tumour tissues. The ability of porphyrins and their metal complexes to accumulate in malignant tumours underlies the PDT and LD methods of oncological diseases. In human blood, porphyrins bind to serum proteins, including lipoproteins, globulins, and albumins [19]. The longest (48 h) binding of porphyrins is observed with lipoproteins and albumins. And since proliferating tumour cells create a large number of lipoprotein receptors, they capture the conjugates of porphyrin complexes with serum proteins. In this case, the selectivity of the accumulation of porphyrins in

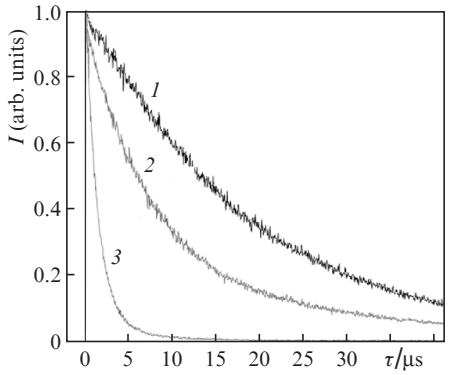


**Figure 6.** Dependences of the Yb-DMDP luminescence lifetime on the DMSO concentration: (*1*) YbPC in 100% DMSO; (*2*) YbPC in 30% DMSO; (*3*) YbPC in 100% aqueous solution.

tumour tissues increases. The selectivity of the accumulation of YbPCs and their conjugates with endogenous transporters (serum albumins) can also be associated with their sizes, which *a priori* lie in the nanometre range. Among the methods for measuring the size of nanoparticles in liquid media, the optimal method is dynamic light scattering, which is implemented in laser correlation spectrometers.

Measurements carried out on a KURS-3 laser dynamic light scattering spectrometer showed that more than 95% of the total amount of elements of the Yb-DMDP substance at a concentration of $10^{-4}$ M have a size of about 5 nm, which is about half the average size of a serum albumin molecule (9 nm) [20] (Fig. 7). The smaller the nanoparticles, the less likely they are captured by the reticuloendothelial system of the body and the longer the time of their circulation in the bloodstream in the body and the greater the possibility of the formation of conjugates with albumin molecules. The dynamics of the formation of Yb-DMDP conjugates with bovine serum albumin (BSA) is shown in Figs 7c and 7d. The conjugates appearing in small amounts 55 min after mixing the solutions in the form of a shoulder on the histogram of the particle size distribution (Fig. 7c), in a day stand out into an independent fraction with a significant concentration and an average size of 20–25 nm (Fig. 7d).

Thus, it is not surprising that the maximum accumulation of the Yb-DMDP substance in the tumour, established in the study of pharmacokinetics, is 45–48 h after the administration [8], since the transport function of albumin begins to work effectively only a day after its administration. As shown in Ref. [21], the total number of special centres in the albumin molecule capable of binding many types of drugs is more than two. As a result, the average size of the Yb-DMDP/BSA conjugate, measured by the laser correlation spectroscopy, was 20–25 nm considering the dispersion. The presented conjugate has a high selectivity of accumulation in the tumour, which is confirmed by studies of the pharmacokinetics and biodistribution of YbPC in biological tissues [8]. In our opinion, this result is associated with the dimension of the conjugate and Yb-DMDP (long circulation time in the bloodstream), with the natural tumourotropic property of porphyrins, as well as with the amphiphilic property of Yb-DMDP. The third argument in favour of using Yb-DMDP in this theranostic technique is due to its low cytophototoxicity [22].

For the purpose of theranostics of neoplasms of the skin and mucous membranes, we have developed a pharmaceutical composition (PC) Fluroscan, consisting of Yb-DMDP

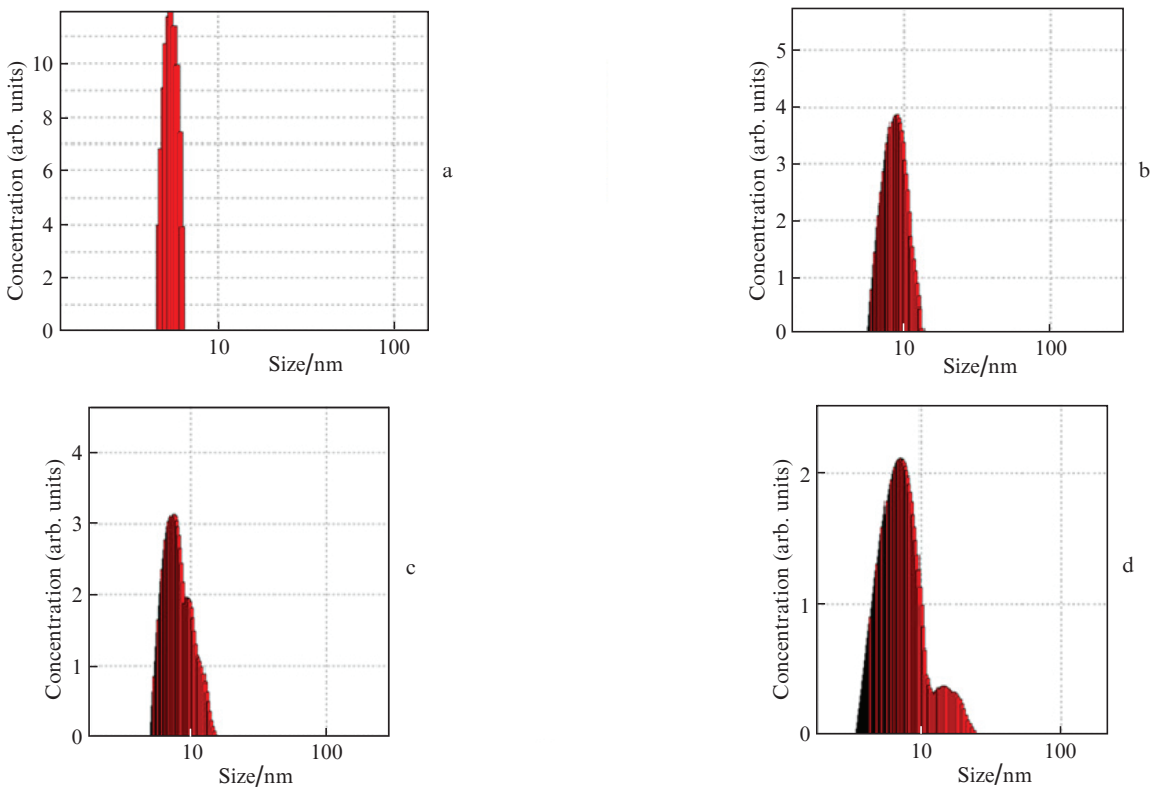


**Figure 7.** (Colour online) Histograms of size and concentration distributions obtained with a KURS-3 laser dynamic light scattering spectrometer: (a) Yb-DMDP ($10^{-4}$ M, 20% DMSO solution); (b) bovine serum albumin (BSA), concentration 5 mg $mL^{-1}$; (c) Yb-DMDP (0.5 mL) with BSA (2 mL, 5 mg $mL^{-1}$) 55 min after mixing the solutions; (d) Yb-DMDP with BSA 24 h after mixing.

dipotassium salt, luminescent in the NIR region of the spectrum (900–1100 nm), and various gels (Cremophor, Tizol) using DMSO, glucosamine and glycerol, which provide good penetration into the skin and mucous membranes (so-called penetrators) [11]. The high selectivity of YbPC accumulation in the skin was confirmed by the results [10], obtained during a diagnostic examination of volunteer patients using this gel.

The presence of porphyrin in the PC provides the predominant accumulation of the metal complex in pathologically altered tissues and mucous membranes, and the ytterbium ion, when irradiated with a laser with a wavelength of 405 nm, luminesces in the near-IR region of the spectrum (900–1100 nm), where the background fluorescence of the body's tissues is almost completely absent.

Since Yb-DMDP has practically no phototoxicity, the application of these gels to the skin and mucous membranes does not cause negative effects on the body in the form of adverse dermatological reactions. It should be noted that we chose the application method of using the preparation because of its simplicity, accessibility, and lack of trauma and general systemic action, as well as in accordance with the initial task: theranostics of superficial tumours, tumours of the skin and mucous membranes. In addition, such tumours, as a rule, are not highly invasive. Intravenous administration of preparations based on Yb-DMDP requires at least 24-hour exposure for selective accumulation in neoplasms (see [8]).

Our proposed new method for theranostics of malignant neoplasms of the skin and mucous membranes based on PC Fluroscan was tested on laboratory animals (C57BL mice with transplanted LLC lung adenocarcinoma, Lewis tumour).

The technique of theranostics of malignant neoplasms of the skin and mucous membranes using this PC is implemented as follows. First, the LD procedure is performed, in which suspicious areas of the skin and mucous membranes are treated with PC gel. After 30–40 min after the treatment of skin neoplasms and closely spaced healthy tissues with this preparation using FOLF (optical power up to 5 mW), the diagnostic contrast index (the neoplasm/norm ratio of the tissue luminescence intensities) is measured, which can range from 3 to 15, depending on the type of skin lesion and the concentration of YbPC in the PC. The technique of NIR luminescence diagnostics makes it possible to reveal objective differences between morphologically normal and pathologically altered tissues in the diagnosis of skin diseases.

Further, before the PDT stage, the affected biological tissue is treated with ascorbic acid. The surface of the skin is first treated with a solution of 96% ethyl alcohol, and then a saturated (1 g in 3 mL of 0.9% NaCl) solution of ascorbic acid is applied. This procedure takes 10–15 min, after which the integral intensity of luminescence in the NIR range is measured in various parts of biological tissues. In this case, the integral luminescence intensity decreases almost threefold (Fig. 8, region C), which confirms the intense release of the ytterbium ion from the porphyrin macrocycle. Thus, the PC accumulated in biological tissue contains mainly the metal-free form of the porphyrin complex.

At the next stage of theranostics, the PDT of previously identified affected areas of biological tissue is performed by treating them with a therapeutic laser at a wavelength of 635 nm (in the PS absorption band of 630 nm). The exposure dose was ~300 J $cm^{-2}$; the irradiation time was 10 min. With such a gentle irradiation regime, there are practically no side thermal effects. After laser irradiation of the tumour surface, the luminescence signal slightly decreases, which is due to the

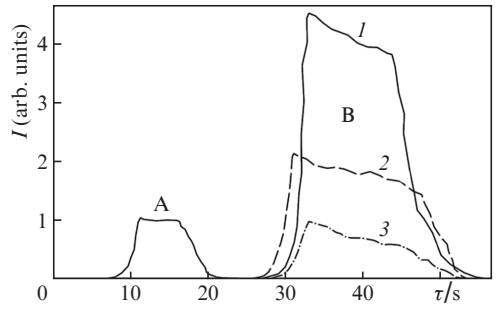


**Figure 8.** Integral luminescence intensity depending on the time of laser irradiation during various skin treatment procedures: (*1*) before treatment with ascorbic acid; (*2*) after treatment with ascorbic acid; (*3*) after the PDT procedure; (A) gel-treated skin area near the tumour area; (B) the area of the skin affected by the tumour, treated with gel and acid.

further release of the ytterbium ion from the porphyrin macrocycle [Fig. 8, curve (*3*)]. Three groups of mice (six individuals in each) participated in the theranostic procedure: group 1 – control (without PDT), group 2 – for PDT with a dose of 300 $cm^{-2}$ and group 3 – for PDT with a dose of 150 J $cm^{-2}$. A suspension of tumour cells was transplanted intradermally into the surface of the thigh of a mouse according to standard technique. After the development of the primary tumour node, the skin flap over the zone of its growth was epilated and the mice were immobilised on a wooden support. The size of the tumour surface in all mice was approximately 1 $cm^2$. All groups of mice underwent the LD procedure under the same conditions, and then for the second and third groups, a PDT session with a given radiation dose was carried out.

Before, during and after exposure to laser radiation, a thermogram of the tumour node was recorded. The averaged thermogram typical for the second group of mice is shown in Fig. 9. It can be seen that temperature fluctuations in the growth zone of the primary tumour node after exposure to laser radiation were about 5 °C, while the temperature did not reach 40 °C. After the termination of laser irradiation, the background temperature fluctuations in the tumour tissue become low-frequency and low-amplitude (less than 0.5 °C). This indicates a violation of the bioenergetics and microcirculation of the blood flow of the tumour tissue due to its destructive changes caused by the performed PDT. Apparently, we can talk about partial necrosis of tumour cells. As can be seen from Fig. 9, the therapeutic effect was achieved entirely due

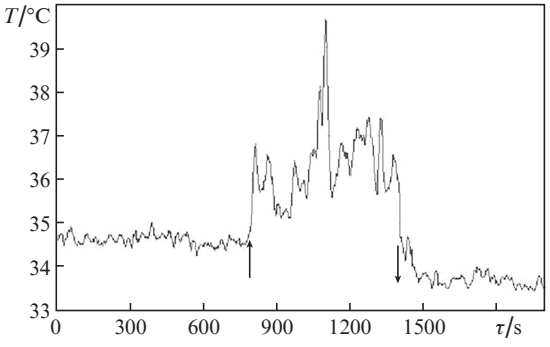


**Figure 9.** Averaged thermogram typical for the second group of laboratory mice (with Lewis lung adenocarcinoma).

to PDT, and not to hyperthermia, which occurs at temperatures above 42 °C.

The average survival rate of the second group of mice (with the highest radiation dose) turned out to be the maximum among all groups and amounted to two months. For comparison: the survival rate of the first group was two weeks, and the third, about one month, which preliminary confirms the effectiveness of the proposed scheme as a whole. For a complete cure, apparently, not a single procedure is needed, but a course of three or four procedures. Work in this direction will continue.

It is known from the literature that the microenvironment of tumour tissues is characterised by low pH values [23, 24]. On this basis, it could be assumed that the transformation of ytterbium porphyrin complexes into the metal free form of porphyrin will occur in tumours to some extent selectively. However, we did not record the fact of transformation of ytterbium porphyrin complexes into the metal free form of porphyrin in tumours under normal conditions. While the probability of such a process exists, the real values of the pH difference (between a healthy tissue and a tumour) are apparently not enough to break the coordination bond between Yb and the porphyrin ring. Artificially lowering the acidity of the environment allows overcoming this barrier. We hypothesise that this is due to the structure of the tissue water matrix (microenvironment). Note also that diagnostic procedures are usually limited in time. For this reason, in our opinion, acid treatment makes it possible to solve more effectively the problem of converting the entire ytterbium complex into a metal-free form for theranostics purposes.

It should be also noted that in the case of measuring the size of elements by the method of dynamic light scattering, their hydrodynamic radius is meant, which coincides with the geometric size mainly for hard spherical particles. For porphyrin molecules, the hydrodynamic radius will slightly differ from the actual size of the molecule. Nevertheless, it can be assumed that in an aqueous solution, the elements of the Yb-DMDP substance are mainly present in the form of dimers with a total size of about 5 nm (see Fig. 7).

The preliminary results of the use of ascorbic acid for treating the tumour node confirm the fact that, when exposed to 1% acid, not all ytterbium, apparently, leaves the porphyrin matrix [see Fig. 4, curves (*1*) and (*4*)]. However, from a practical point of view, it does not make much sense to increase the acid concentration significantly. Work in this direction will continue with the participation of volunteer patients.

## 4. Conclusions

The results obtained indicate the promising nature of the developed theranostics technique that uses the NIR luminescence diagnostics of skin neoplasms and mucous membranes based on a substance of the Yb-DMDP type and PDT in the absorption band of porphyrin. It is shown that the therapeutic effect is achieved entirely due to PDT, without the participation of the hyperthermia process, which occurs at temperatures above 42 °C. Studies of the basic photophysical properties have shown that Yb-DMDP has enhanced photophysical characteristics (luminescence lifetime 1–10 μs, luminescence quantum yield up to 1%, extinction coefficient $\sim 1.96 \times 10^5$ $M^{-1}$ $cm^{-1}$ at a wavelength of 398 nm).

This LD technique also allows identifying objective differences between morphologically normal and pathologically

altered tissues in the diagnosis of skin diseases. The technique is distinguished by high sensitivity – the intensity of luminescence from tumour tissues increases 3–15 times compared to the norm. The study of the luminescence level is a promising direction for the development of a new method for diagnosing pathological conditions in dermatology. In the future, this technique can be used for differential diagnosis of skin cancer, detection of hidden foci of tumour growth and control of the effectiveness of therapy.

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## References

1. Tuchin V.V. (Ed.) *Optical Biomedical Diagnostics* (Bellingham: SPIE Press, 2002) Vol. II.
2. Zaak D., Karl A., Knüchel R., Stepp H., Hartmann A., Reich O., Bachmann A., Siebels M., Popken G., Stief Ch. *BJU International*, **96**, 217 (2005).
3. Kumar R., Ohulchanskyy T.Y., Roy I., Gupta S.K., Borek C., Thompson M.E., Prasad P.N. *ACS Appl. Mater. Interfaces*, **1**, 1474 (2009).
4. Achilefu S. *Nature Photonics*, **1**, 496 (2007).
5. Perumal V., Sivakumar P.M., Zarrabi A., Muthupandian S., Vijayaraghavalu S., Sahoo K., Das A., Das S., Payyappilly S.S., Das S. *Photochem. Photobiol. B: Biology*, **199**, 111630 (2019).
6. Comby S., Bunzli J., in *Handbook on the Physics and Chemistry of Rare Earths* (Amsterdam: Elsevier Science, 2007) p. 217.
7. Bulach V., Sguerra F., Hosseini M.W. *Coord. Chem. Rev*., **256**, 1468 (2012).
8. Ivanov A.V., Rumyantseva V.D., Shchamkhalov K.S., Shilov I.P. *Laser Phys*., **20**, 2056 (2010).
9. Gainov V.V., Shaidullin R.I., Ryabushkin O.A. *Quantum Electron*., **41**, 637 (2011) [*Kvantovaya Elektron*., **41**, 637 (2011)].
10. Alekseev Yu.V., Rumyantseva V.D., Shilov I.P., Ivanov A.V., Shumilova N.M., Mislavsky O.V. *Laser Medicine*, **20** (2), 20 (2016) [*Lazernaya Meditsina*, **20** (2), 20 (2016)].
11. Shchelkunova A.E., Boltukhina E.V., Rumyantseva V.D., Shilov I.P., Karakotov S.D. *Macroheterocycles*, **12**, 382 (2019) [*Makrogeterotsikly*, **12**, 366 (2019)].
12. Markushev V.M., Rumyantseva V.D., Shilov I.P., Gorshkova A.S. *Zh. Radioelektron*., **8** (2018).
13. Ivanov A.V., Pevgov V.G. *Integral*, **59**, 6 (2011).
14. Shilov I.P., Kochmarev L.Yu., Novichikhin E.P. *Biomed. Eng*., **54**, 371 (2020) [*Med. Tekh.*, **6**, 1 (2020)].
15. Danielyan G., Shilov I., Zamyatin A.A., Kochmarev L.Y., Makovetskii A., Savosin S., in *Clinical and Preclinical Optical Diagnostics II* (Proc. SPIE, 2019) pp 11075_38, Vol. EB101.
16. Khlebtsov B., Panfilova E., Khanadeev V., Bibikova O., Terentyuk G., Ivanov A., Rumyantseva V., Shilov I., Ryabova A., Loshchenov V., Khlebtsov N. *ACS Nano*, **5**, 7077 (2011).
17. Korovin Yu.V., Rusakova N.V., Popkov Yu.A., Dotsenko V.P. *J. Appl. Spectrosc*., **69**, 841 (2002) [*Zh. Prikl. Spektrosk*., **69**, 732 (2002)].
18. Boldyrev K.N., Popova M.N., Bezmaternykh L.N., Bettinelli M. *Quantum Electron*., **41**, 120 (2011) [*Kvantovaya Elektron*., **41**, 120 (2011)].
19. Mishra P.P., Patel S., Datta A. *J. Phys. Chem. B*., **110**, 21238 (2006).
20. Alekhin A.P., Boleiko G.M., Gudkova S.A., Markeev A.M., Sigarev A.A., Toknova F., Kirilenko A.G., Lapshin R.V., Kozlov E.N., Tetyukhin D.V. *Nanotechnologies in Russia*, **5**, 696 (2010) [*Ross. Ranotekhnol*., **5**, 128 (2010)].
21. Dobretsov G.E., Syreishchikova T.I., Gryzunov Yu.A., Smolina N.V., Komar A.A. *Biophys*., **55**, 182 (2010) [*Biofiz*., **55**, 213 (2010)].
22. Shilov I.P., Ivanov A.V. *Zh. Radioelektron*., **9** (2020).
23. Persi E., Duran-Frigola M., Damaghi M., Roush W.R., Aloy P., Cleveland J.L., Gillies R.J., Ruppin E. *Nature Commun*., **9**, 2997 (2018).
24. Huber V., Camisaschi C., Berzi A., Ferro S., Lugini L., Triulzi T., Tuccitto A., Tagliabue E., Castelli C., Rivoltini L. *Seminars Cancer Biol.*, **43**, 74 (2017).