

DOI: 10.1070/QEL16110

Photodynamic effect of radiation with the wavelength 405 nm on the cells of microorganisms sensitised by metalloporphyrin compounds

M.V. Korchenova, E.S. Tuchina, V.Yu. Shvayko, A.G. Gulkhandanyan, A.A. Zakoyan, R.K. Kazaryan, G.V. Gulkhandanyan, B.M. Dzhagarov, V.V. Tuchin

Abstract. **We have studied the photodynamic activity of photosensitisers based on metalloporphyrins. New metalloporphyrin compounds are synthesised and characterised, the quantum yields of the singlet oxygen formation are analysed. It is shown that when the photodynamic effect is implemented using the metalloporphyrins with Zn ions and butyl radical in the 3rd and 4th positions of the pyridine ring, the number of opportunistic bacteria, such as *Staphylococcus aureus* (antibiotic-sensitives and antibiotic-resistant strains), *Staphylococcus simulans* and *Escherichia coli* is efficiently reduced by 90%–99%.**

Keywords: *photodynamic therapy, radiation with the wavelength 405 nm, microorganisms, S. aureus, S. simulans, E. coli, metalloporphyrins.*

1. Introduction

For two decades the method of photodynamic therapy (PDT) finds wide application both in the treatment of numerous diseases of oncological and bacterial origin and for prophylactics and correction of physiological changes in the human organism. The photodynamic therapy is based on the photochemical reaction catalysed by oxygen, activated by the optical radiation with a definite wavelength and the photosensitiser [1–10].

The violet radiation (405–415 nm) activates the endogenic or exogenic photosensitisers and involve them into the photochemical reaction. Due to the shallow penetration depth of radiation into tissues (less than 1 mm), the violet radiation is mainly used in dermatology, stomatology and ophthalmology [5–7]. In PDT both laser and nonlaser light sources are used; in recent years due to the high efficiency and relatively low cost, diode lasers and light-emitting diodes have been widely used, as well as 1D and 2D arrays of these sources. In a number of cases, laser sources have essential advantages, caused by their high luminance, monochromaticity (provided that the photosensitiser absorption band maximum coincides with the laser wavelength), and the possibility of high-efficiency delivery of radiation to internal organs via optical fibres. In a number of problems, e.g., suppression of bacterial flora over large surfaces (skin, wound surfaces, operation filed, etc.), light-emitting diode sources appear to be optimal.

The efficiency of the violet light is significantly enhanced by using exogenic photosensitisers, including porphyrin compounds and protoporphyrin IX, induced in tissue in the case of topical application of aminolevulinic acid (ALA), which at the expense of strong absorption in the region 405–415 nm provides the effect of these wavelengths, greater by nearly 40 times than that of the commonly used red radiation at $\lambda =$ 630 m [8–10].

The first compounds, whose photosensitising nature has manifested itself under the action of UV and violet radiation, are porphyrin compounds. Porphyrins (PPs) are widely spread in living nature and are necessary for optimal behaviour of fundamental biological processes, such as photosynthesis, cell breathing, and metabolism. Hence, in spite of long years of study, the interest in their investigation still increases [11, 12].

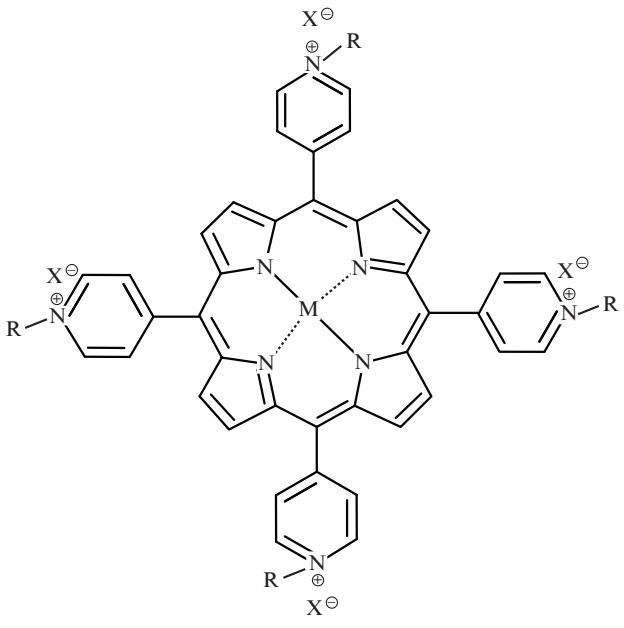
Thus, the aim of the present study was to assess the sensitivity of gram-positive and gram-negative microorganisms to the photodynamic impact of the violet (405 nm) radiation of a light-emitting diode under the conditions of sensitising them with cation porphyrins and metalloporphyrins.

2. Material and methods

Cation porphyrins and metalloporphyrins were chemically synthesised at the Yerevan State Medical University (Armenia). The technique of their synthesis, described earlier in Refs [13, 14], allows one to obtain compounds, in which active hydroxyethyl- and butyl-peripheral groups (–R) are bound in the 3rd or 4th position of the pyridine ring and have a different degree of hydrophobicity (Fig. 1, Table 1). All studied porphyrins possess high quantum yields of the singlet oxygen formation (~80%), the level of intrinsic fluorescence being as low as a few percent [15]. The quantum yield of the

M.V. Korchenova, E.S. Tuchina, V.Yu. Shvayko N.G. Chernyshevsky National Research Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia; e-mail: kliany@rambler.ru;
A.G. Gulkhandanyan, A.A. Zakoyan, G.V. Gulkhandanyan H.Kh. Buniatian Institute of Biochemistry, National Academy of Sciences of Armenia, 5/1 Paruir Sevak str., 0014 Yerevan, Republic of Armenia;
R.K. Kazaryan Yerevan State Medical University after Mkhitar Heratsi, 2 Koryun str., 0025 Yerevan, Republic of Armenia;
B.M. Dzhagarov B.I. Stepanov Institute of Physics, National Academy of Sciences of Belarus, prosp. Nezavisimosti 70, 220072 Minsk, Belarus;
V.V. Tuchin Research and Educational Institute of Optics and Biophotonics, N.G. Chernyshevsky National Research Saratov State University, ul. Astrakhanskaya 83, 410012 Saratov, Russia; National Research Tomsk State University, prosp. Lenina 36, 634050 Tomsk, Russia; Institute of Precision Mechanics and Control, Russian Academy of Sciences, ul. Rabochaya 24, 410028 Saratov, Russia; e-mail: tuchinvv@mail.ru

Received 21 April 2016
Kvantovaya Elektronika **46** (6) 521–527 (2016)
Translated by V.L. Derbov



R = CH_2–CH_2–CH_2–CH_3 TBut4PyP
R = CH_2–CH_2–OH TOEt4PyP

Figure 1. New cation porphyrins and metalloporphyrins with different peripheral groups (–R) in the 4th position of the pyridine ring: (M) metal; (X) halogen; (R) radical; (TBut4PyP) meso-tetra-[4-N-butylpyridyl]porphyrin; (TOEt4PyP) meso-tetra-[4-N-(2-oxyethyl)pyridyl]porphyrin.

singlet oxygen formation γ_Δ was measured in aqueous solutions at 20 °C (Table 1).

The absorption spectra were recorded using an MC122 spectrophotometer (Proscan, Belarus). All studied porphyrins had a maximum absorption in the violet–blue spectral region. Figure 2 presents the absorption spectrum of the porphyrin TOEt4PyP as the most typical one. Other porphyrin compounds under study possess the spectra of similar shape. The wavelengths of the absorption maxima λ_{max} in the region of the Soret band (400–440 nm) of the studied porphyrins and the values of absorption in relative units at these wavelengths and at $\lambda = 405$ nm are presented in Table 1. Note that less expressed absorption peaks are recorded also in the green–yellow (525–570 nm) and red (630–640 nm) regions of the spectrum (Fig. 2).

As the studied microorganisms we have chosen the methicillin-sensitive strain *S. aureus* 309P (MSSA), the methicillin-resistant *S. aureus* (MRSA), *E. coli* 113-13 and *S. similans* (Tarasevich State Institute of Standardization and Control of Biomedical Preparations, Moscow). The microorganisms

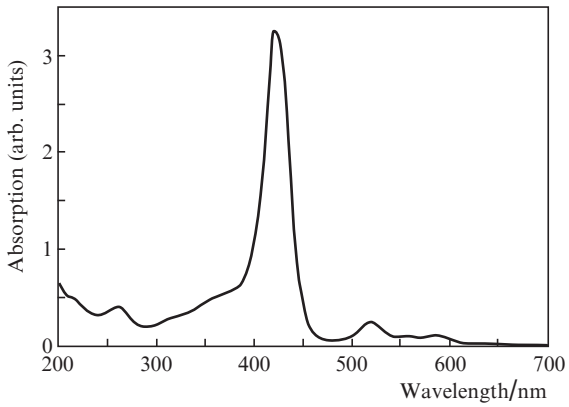


Figure 2. Optical absorption spectrum of the solution of the porphyrin TOEt4PyP in distilled water; [C] = 2×10^{-5} M.

were cultivated at the temperature 37 °C on the GRM agar (Obolensk, Russia).

The source of light was a light-emitting diode (LED) with a spectral emission maximum at $\lambda = 405 \pm 15$ nm and a power density of 47 mW cm^{-2}. In all experiments, we used the cw irradiation regime, the time of exposure varied from 5 to 30 min.

The porphyrin compounds were used in the concentrations 0.01 and 0.1 μg mL^{-1} in the saline solution. In the experiments, we followed the protocol (Fig. 3) elaborated earlier [10]. To create the aseptic conditions, the polystyrene 96-well plate was placed in a plastic case and the light source was located above the wells of the plate. In the experiments, we used 24-hour cultures of the studied strains. The bacterial suspension was prepared in sterile saline; the final concentration amounted to 5 thousand microbial cells (m.c.) per 1 mL. From the suspension with the concentration 10^4 m.c. mL^{-1} we took 0.1 mL and introduced it into 0.9 mL of porphyrin solution. Then the resulting mixture was incubated in the darkness during 15 min and from this dilution having the concentration 10^3 m.c. mL^{-1} the bacterial suspension was introduced into the wells of the plate in the volume of 0.1 mL and exposed during 5, 10, 15 and 30 min.

After the exposure, the bacterial suspension was transferred to the Petri dishes with a dense nutritive medium and incubated in a thermostat at the temperature 37 °C. The recording of the results was implemented by counting the number of colony-forming units (CFUs) in 24 hours. For control we used bacterial suspensions not treated with porphyrins and not exposed to radiation. Each experiment was repeated five times. For the statistical processing of the experimental data we used the Microsoft Excel 2010 programme.

Table 1. Porphyrin compounds used in the work: [C] = 2×10^{-5} M.

Porphyrin	Porphyrin name	Molecular mass/Da	λ_{max}/nm	Absorption (rel. units)		γ_Δ (%)
				$\lambda = \lambda_{max}$	$\lambda = 405$ nm	
PPI	Meso-tetra-[4-N-(2-oxyethyl)pyridyl]porphyrin (TOEt4PyP)	940	421	3.53	1.41	77
PPII	Zinc-meso-tetra[4-N-(2-oxyethyl)pyridyl]porphyrin (Zn-TOEt4PyP)	1003	~440	2.89	0.52	85
PPIII	Zinc-meso-tetra-[4-N-buthyl pyridyl]porphyrin (Zn-TBut4PyP)	1229	437	3.09	0.52	97
PPIV	Zinc-meso-tetra-[3-N-buthyl pyridyl]porphyrin (Zn-TBut3PyP)	1229	431	3.33	0.66	97

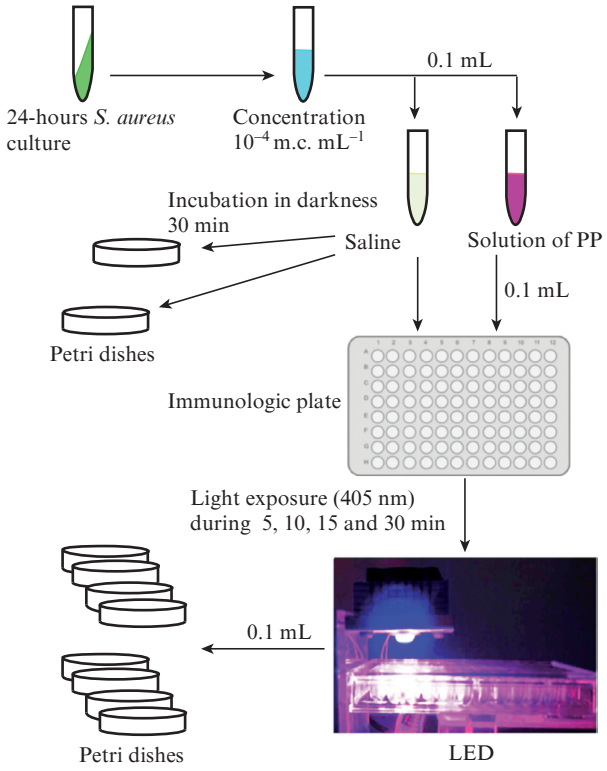


Figure 3. Schematic diagram of experiments on the photodynamic effect of LED radiation (λ = 405 nm) on microorganisms sensitised by porphyrin compounds.

3. Results and discussion

The test of porphyrin compounds for dark toxicity (Fig. 4) has shown that the treatment of cells of antibiotic-sensitive strain *S. aureus* MS with porphyrins having the concentration 1% reduced the number of CFUs on average by 35%. The treatment of cells with the porphyrins with lower concentrations did not lead to a significant reduction of the number of bacteria.

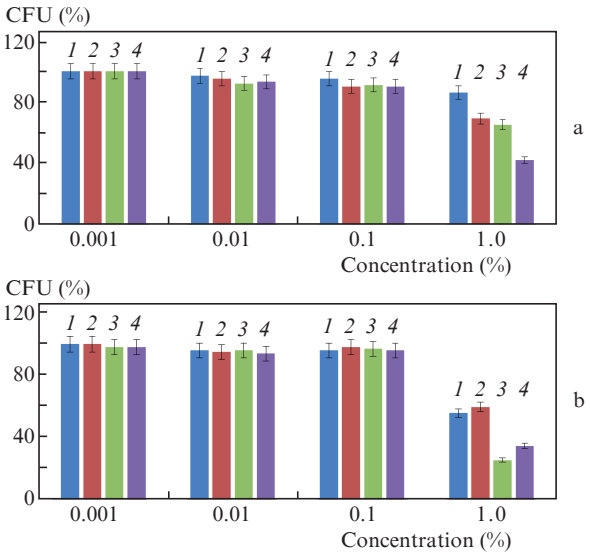


Figure 4. Effect of different concentrations of porphyrin compounds PPI (*1*), PPII (*2*), PPIII (*3*) and PPIV (*4*) on the number of microorganisms *S. aureus* MS (a) and *E. coli* (b).

Analogous results were obtained estimating the dark toxicity of porphyrins for the antibiotic-resistant strain *S. aureus* MS and the saprophyte *S. simulans*.

In the case of *E. coli* the treatment of cells with porphyrins having the concentration 1% caused a reduction of the CFU number on average by 65%. No statistically significant difference from the control values was revealed in the variation of the number of bacterial populations after the treatment with porphyrins having the concentrations 0.1%, 0.01% and 0.001%.

Based on the obtained data, for further studies on the photodynamic effect we have chosen the porphyrin concentrations 0.1% and 0.01%, which did not exert a toxic influence on the bacterial cells in the absence of light exposure.

At the next stage of the work, we have found that the LED radiation (405 nm) insignificantly suppressed the growth of the majority of the microorganisms under study (Fig. 5).

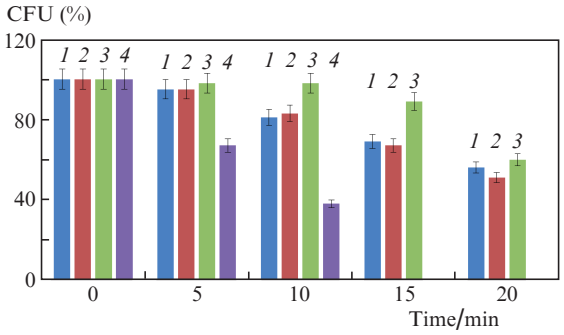


Figure 5. Effect of the exposure time (λ = 405 nm) on the viability of microorganisms *S. aureus* MS (*1*), *S. aureus* MR (*2*), *E. coli* (*3*) and *S. simulans* (*4*).

In the case of methicillin-sensitive *S. aureus* MS the significant reduction of the CFU number by 19% was observed after 10 min of irradiation, and by 49% after 30 min. The effect of blue light on the methicillin-resistant strain *S. aureus* MR was similar. The effect of the blue light on *E. coli* was yet weaker, in the first 10 min no significant changes in the number of microorganisms occurred. With an increase in the exposure time from 15 to 30 min, the number of bacterial populations of *E. coli* was reduced by 11% and 40%, respectively. It is interesting that in the case of irradiating the cells *S. simulans* the statistically significant reduction of the number of bacterial colonies was observed. Already after 5 min of irradiation, the number of CFUs was reduced by 33%, after 10 min of irradiation by 62%, and with an increase in the exposure time to 15 min the growth of microorganisms was almost suppressed. The sensitivity of the bacteria to the blue light is caused by the presence of endogenic chromophores, mainly porphyrins [3, 12, 16–23]. It is known that due to the specificity of the habitat and the evolution of stability factors the cells of staphylococci contain more these compounds than the enterobacteriaceae [3, 12].

The treatment of microorganisms with the porphyrin compounds significantly increased the suppressing effect of the radiation at the wavelength 405 nm. The study of the effect of 0.01% metalloporphyrins concentration on the *S. aureus* yielded the following results. The irradiation of antibi-

otic-sensitive staphylococcus MSSA during 5 min after the treatment with four types of porphyrins (CTOEt4PyP, Zn-TOEt4PyP, Zn-TBut4PyP, Zn-TBut3PyP) led to insignificant reduction of the bacterial population number, smaller than $0.8\log_{10}$ (by 11%, 25%, 81% and 87%). An increase in the exposure time from 10 to 30 min reduced the number of CFUs by $2.9\log_{10}$ (by 60%–99%) (Fig. 6a).

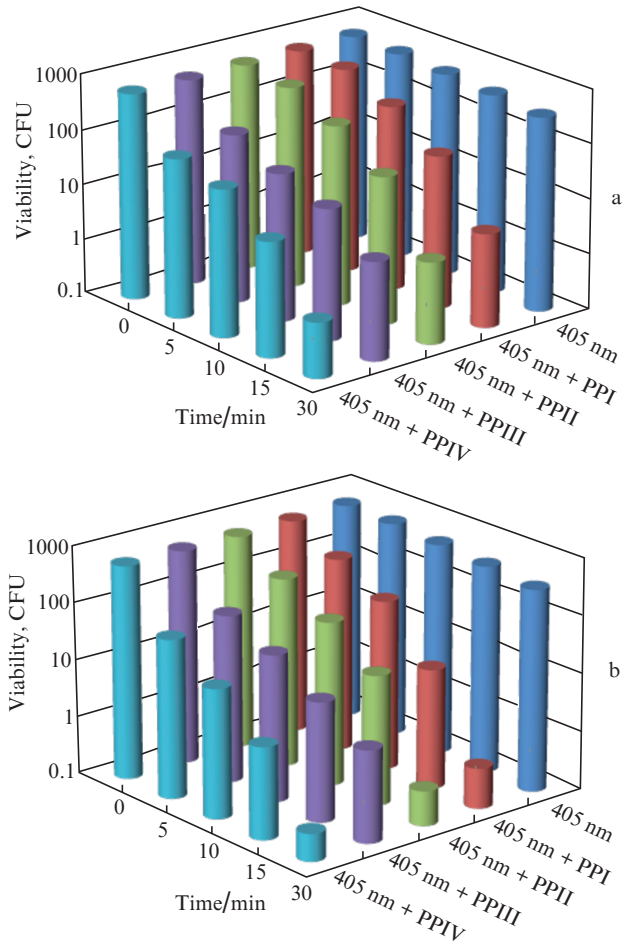


Figure 6. Viability variation of the MSSA staphylococcus cells under the action of the radiation with $\lambda = 405$ nm and porphyrin compounds with the concentration 0.01% (a) and 0.1% (b).

In the course of treating the methicillin-sensitive staphylococcus with porphyrin compounds in the concentration of 0.1% we observed a more expressed reduction of the CFU number: the exposure during the first 5 min led to the reduction of the bacterial population by $1\log_{10}$ (by 60%, 67%, 86%, and 90%). An increase in the exposure time from 10 to 30 min reduced the number of CFUs by $2.9\log_{10}$ (by 91%–99%) (Fig. 6b).

A similar effect was caused by the porphyrin compounds in the methicillin-resistant strain of the staphylococcus MRSA at the concentration 0.01%: during the first 5 min one could observe the reduction of the number of bacterial colonies by $0.9\log_{10}$ (by 22% 30%, 88% and 83%). A further increase in the exposure time from 10 to 30 min reduced the number of CFUs by $(2–2.9)\log_{10}$ (by 61%–99%) (Fig. 7a). At a higher concentration of dyes (0.1%) during the first 5 min of exposure, the number of bacteria was reduced by

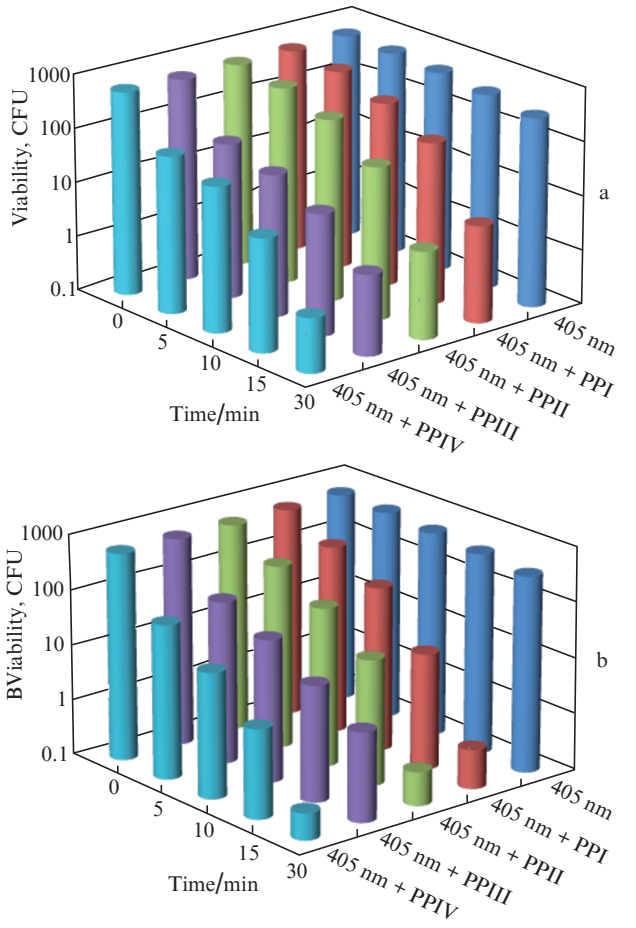


Figure 7. Viability variation of the MRSA staphylococcus cells under the action of the radiation with $\lambda = 405$ nm and porphyrin compounds with the concentration 0.01% (a) and 0.1% (b).

$1\log_{10}$ (by 60%, 67%, 90%, 87%). A further increase in the exposure time from 10 to 30 min reduced the number of CFUs to $2.8\log_{10}$ (90%–99%) (Fig. 7b).

The study of the combined effect of the radiation from the light-emitting diode and the porphyrin compounds on *E.coli* yielded the following results (Fig. 8). The treatment of the microorganisms with porphyrins having the concentration 0.01% led to the change in the CFU number within $2\log_{10}$ (by 25%–48%). With an increase in the concentration to 0.1%, the population number was reduced by $2.8\log_{10}$ (by 99%).

It was also shown that the combined effect of the radiation at $\lambda = 405$ nm and metalloporphyrins having the concentrations 0.01% and 0.1% on *S. simulans* already in the course of five-minute exposure caused a significant (within $2\log_{10}$) reduction of the number of microorganisms, and at the 15th minute of irradiation the population number decreased by $3\log_{10}$ (by 99.99%) (Fig. 9).

Analysing the obtained results one can conclude that the studied porphyrin compounds in combination with the LED source of radiation ($\lambda = 405 \pm 15$ nm) provide efficient destruction of different microorganisms. The reduction of the number of bacteria in all cases was dose-dependent. The most strongly expressed suppression of the number of microorganisms was observed at the concentration of porphyrins 0.1% and 30-min exposure with the given density of the radiation power 47 mW cm^{-2}; for MSSA 99.6%, for MRSA 99.7%, for *E. coli* 98.9%, and for *S. simulans* 100% of bacteria died.

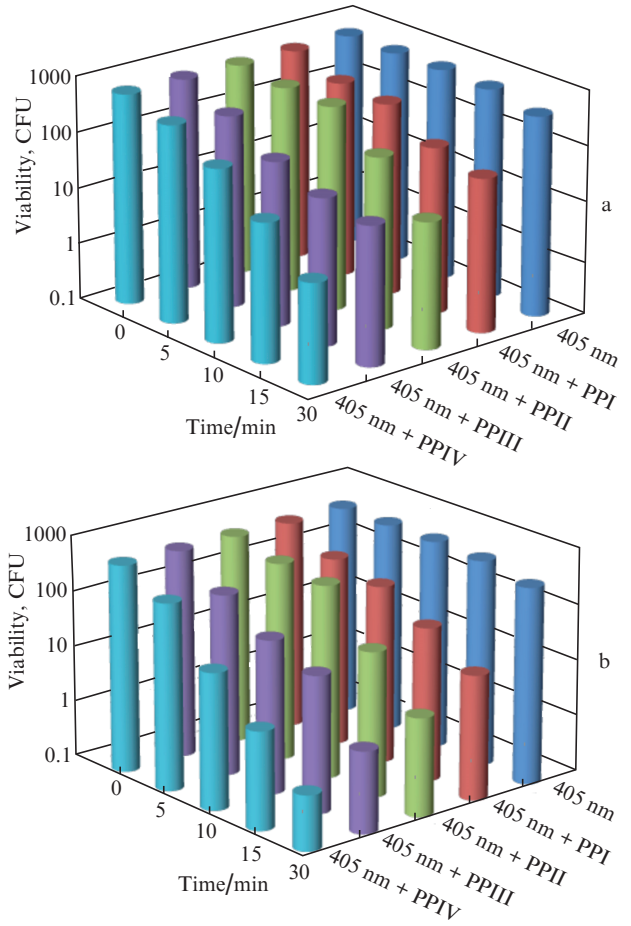


Figure 8. Viability variation of the *E. coli* cells under the action of the radiation with $\lambda = 405$ nm and porphyrin compounds with the concentration 0.01% (a) and 0.1% (b).

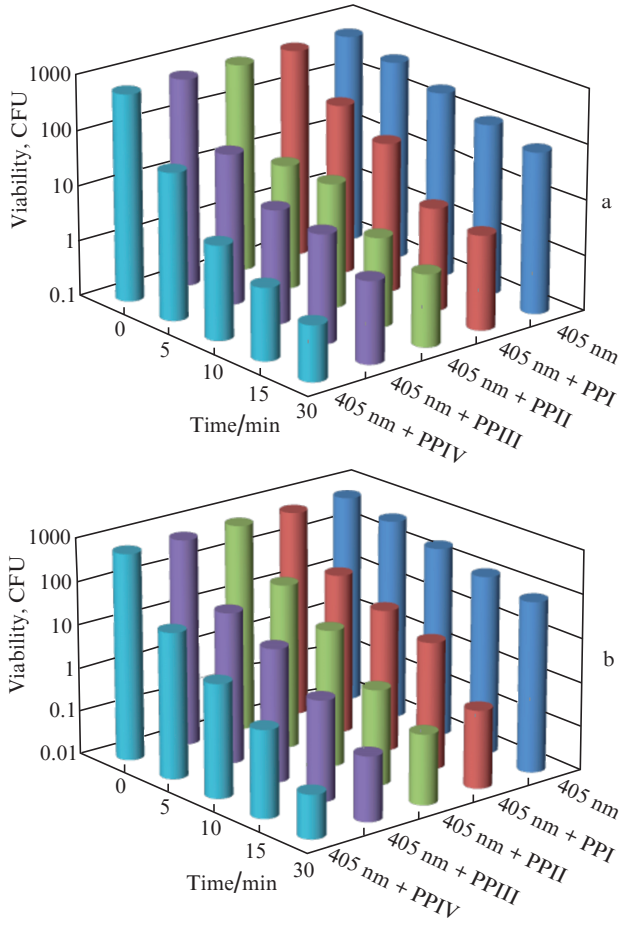


Figure 9. Viability variation of the *S. simulans* cells under the action of the radiation with $\lambda = 405$ nm and porphyrin compounds with the concentration 0.01% (a) and 0.1% (b).

In the experiments we used new cation porphyrins and metalloporphyrins [13–15], possessing a number of essential differences from other porphyrins. The most important difference is that the active hydroxyethyl and butyl peripheral groups (–R) of the compounds can bind in the 3rd and 4th position of the pyridine ring and also that these groups can have a different degree of hydrophobicity.

Metalloporphyrins possess the ability to bind additional axial ligands directly to the metal ion, which is one of the most important functional properties. Due to this interaction, the binding of chlorophyll and haem with the protein is provided, the reversible oxygenation of haemoglobin and myoglobin is implemented, and many biochemical reactions are catalysed and controlled.

Recently, a special attention has been paid to the synthesis of modified cation pyridyl porphyrins and metalloporphyrins with additional amphiphilic terminal groups. Their potential in PDT and photodynamic inactivation of microorganisms is also studied [17–20].

One of the most important parameters of the porphyrin efficiency is the quantum yield of the singlet oxygen formation γ_Δ, which is a ratio of the number of formed molecules of singlet oxygen to the number of photosensitiser porphyrin molecules absorbing the light quanta in the ground state. The measurements of γ_Δ were carried out using the method thoroughly described in Ref. [15], which implies the direct registration of the intrinsic glow of singlet oxygen, formed in the course of quenching of the triplet state of the etalon photosensitiser and the studied one with the molecular oxygen. As the etalon compound we used TMe4PyP ($\gamma_\Delta = 77\%$). For the new synthesised porphyrins the quantum yield of the singlet oxygen production is by 8%–20% greater than for the known photosensitiser 5,10,15,20-tetrakis(4-N-methylpyridyl)porphyrin (H2TMe4PyP) [2]. Note that the values of γ_Δ for Zn-derivatives of metalloporphyrins (PPII, PPIII, PPIV) are significantly higher than those of the free porphyrin bases (PPI) due to an increase in the efficiency of intercombination (intersystem) conversion, when the metal atom is involved in the porphyrin macrocycle [15].

These data correlate with the effect of cation porphyrins on microorganisms, since in the course of the study a significantly higher efficiency of Zn-derivatives of metalloporphyrins was demonstrated.

The replacement of [3-pyridyl]porphyrins with [4-pyridyl] derivatives of porphyrins leads to a small increase in the rate constant of the quenching of the porphyrin molecule in the excited triplet states from $\sim 1.4 \times 10^9$ to $\sim 1.7 \times 10^9$ M^{-1} s^{-1} [15]. This increase in the quenching rate constant can explain a somewhat smaller efficiency of the metalloporphyrin Zn-CTBut4PyP as compared to ZnTBut3PyP for both strains of *S. aureus*.

Earlier it was shown [16, 23–25] that the reaction of individual strains of the same species of bacteria to the combined action of radiation and photosensitisers can be different,

which manifests itself in the degree of the growth suppression; however, at the same time the dynamics of the population variation has a similar character. A different trend can be observed in the comparison of different physiological groups of microorganisms, e.g., possessing gram-positive and gram-negative type of the cell wall.

Thus, the gram-negative bacteria demonstrate a greater resistivity to the PDT effect. The cell wall of gram-negative microorganisms has a multicomponent structure with an expressed outer liposaccharide layer [3]. The lifetime of free radicals does not exceed 10^{-6} s, and this is not enough for the migration through the outer membrane of gram-negative bacteria and for stimulation of photooxidation processes.

It is known that the hydrophilicity and hydrophobicity of cell walls of microorganisms strongly vary depending on the species and the strain, the conditions of the cells extraction and cultivation [26]. Thus, the protein of *S. aureus*, causing its pathogenic properties, enhances the hydrophobic properties of peptidoglycan, and the presence of the capsule and other polysaccharide structures increases the hydrophilicity. It is generally assumed that the cell wall of the gram-positive type possesses hydrophilicity, and the cell wall of gram-negative type is hydrophobic due to a variety of additional components [26, 27]. Therefore, providing the new porphyrin sensitisers with certain properties one can selectively control the efficiency of the photodynamic effect. A higher efficiency of butyl derivatives of porphyrins is probably caused by deeper incorporation of the porphyrin molecules with the butyl hydrophobic group into the bacterial membrane as compared to the oxyethyl group (incorporating the OH hydrophilic terminal), which can lead to the cell destruction not only over the surface, but also throughout the depth of the cell wall.

Possibly, this is the explanation of the greater sensitivity of gram-positive staphylococci to the combined effect of the violet radiation (405 nm) and the porphyrin compounds, as compared to the gram-negative colon bacillus.

According to the data of Refs [5, 21] and the data presented in Fig. 2, the coefficient of porphyrin excitation in the violet–blue region is by 20–40 times greater than in the red region of the spectrum (630 nm); therefore, the violet light exerts a stronger photodynamic effect than the red light.

The reduction of the number of bacteria under the combined effect of the violet light and metalloporphyrins for the MSSA and MRSA bacteria had a similar character. Thus, the developed method can be used for efficient elimination of microorganisms with a different degree of resistance to antibiotics.

The *S. simulans* bacterium is a component of normal microflora of human skin. The significant reduction of the number of these staphylococci already at the first minutes of photodynamic treatment may be a negative effect, since it can lead to the population of the microflora by pathogenic microorganisms. Therefore, in the clinical practice the technique should be used with care at those stages of treating the wounds, ulcers and burns, when the risk of uncontrollable reproduction of antibiotic-resistant pathogens exists.

The maximal efficiency was demonstrated by the metalloporphyrins PPII and PPIV, containing Zn and the butyl radical in the 3rd position of the pyridine ring; their use reduced the CFU number of microorganisms by $2\log_{10}$ already after 5 min of irradiation (by 70%–95%), and in 30 min it achieved 98% and even 100%.

Note that the proposed technique can be implemented using relatively inexpensive diode lasers, operating at the wavelengths 405, 410 and 445 nm, which can essentially increase the efficiency of antimicrobial PDT. Besides that, the standard laser medical instrumentation, designed for intravenous laser irradiation of blood at the wavelengths 405 and 445 nm (VLOK-405 and VLOK-445) [21], can be an efficient tool for suppressing the pathogens, sensitised by porphyrins, in the treatment of wounds and ulcers at the skin surface or mucosa, as well as in the blood irradiation.

It seems promising also to analyse *in vivo* the level of singlet oxygen and photosensitiser accumulation in cells of microorganisms in the course of the photodynamic treatment. For example, for the microorganisms populating the biofilms, formed at the surface of soft or hard tissues (skin, wound surfaces, tooth and bone tissues) one can use the method of diffuse backscattering, described in Ref. [28]. These data will allow the determination of the optimal time of the PDT procedure.

The use of new porphyrin compounds as component of the nanocomposites, aimed at the selective binding with microorganisms, are expected to provide an essential increase in the antimicrobial therapy efficiency [22–25].

4. Conclusions

The analysis of the efficiency of new metalloporphyrin compounds in combination with the action of the radiation at the wavelength 405 nm demonstrated high sensitivity of the studied microorganisms to the PDT. The most strongly expressed reduction of the population of both gram-positive and gram-negative bacteria was detected after 15–20 min treatment with the use of the solution of the porphyrin Zn-TBut3PyP (PPIV) having the concentration 0.1%, as well as the other Zn derivatives of metalloporphyrins (PPII and PPII), in which the quantum yield of the singlet oxygen is essentially higher than in free porphyrin bases (PPI). Besides that, the efficiency of binding the dye molecules with the cell structures can be also of certain importance.

Apparently, the use of the laser radiation with wavelengths 420–440 nm and a higher power density (100–200 mW cm^{-2}) will allow an essential increase in the antimicrobial efficiency of the technique, reducing the exposure time to a few minutes or even seconds, which will be certainly demanded by the biomedical practice.

Acknowledgements. The work was partially supported by the RF President's Grants Council (Grant No. NSh-7898) and the Government of the Russian Federation (Grant No. Z50.31.0004), as well as by the D.I. Mendeleev Foundation of the Tomsk State University.

References

1. Bonnett R. *Chem. Soc. Rev*., **24**, 19 (1995).
2. Grinholc M., Szramka B., Kurlenda J., Graczyk A., Bielawski K. *J. Photochem. Photobiol. B*, **90**, 57 (2008).
3. Wainwright M.J. *Antimicrob. Chemotherapy*, **42**, 13 (1998).
4. Barolet D. *Semin. Cutan. Med. Surg*., **27** (4), 227 (2008).
5. Babilas P., Schreml S., Landthaler M., Szeimies R.M. *Photodermatol. Photoimmunol. Photomed*., **26** (3), 118 (2010).
6. Gursoy H., Ozcakir-Tomruk C., Tanalp J., Yilmaz S. *Clin. Oral Investig*., **17**, 1113 (2013).
7. Sidorenko E.I., Filatov V.V., Filatova N.V., Fedorov A.A., Muravyov M.V. *Ross. Detskaya Oftalmol*., **1**, 67 (2012).

8. Aponiene K., Luksiene Z. *J. Photochem. Photobiol. B*, **142**, 257 (2015).
9. Elsaie M.L.T. *Photodynamic Therapy – New Research* (New York: Nova Sci. Publ. Inc., 2013).
10. Shelest N.A., Volkova E.., Kozina K.V., Korchenova M.V., Tuchina E.S., Zakharevich A.M., Kochubey V.I., Tuchin V.V. *Izv. Saratov. Univer.*, **4**, 62 (2014).
11. Geynits A.V., Sorokatyi A.E., Yagudaev D.M., Trukhnanov R.S. *Lazernaya Med.*, **11**, 62 (2007).
12. Stranadko E.F. *Lazernaya Med.*, **11**, 42 (2007).
13. Madakyan V.N., Kazaryan R.K., Khachatryan M.A., Stepanyan A.S., Kurtikyan T.S., Ordyan M.B. *Khim. Geterotsiklich. Soed.*, **2**, 212 (1986) [*Chem. Heterocycl. Compoun.*, **2**, 167 (1986)].
14. Tovmasyan A., Ghazaryan R., Sahakyan L., Gasparyan G., Babayan N., Gyulkhandanyan G. *Techn. Abstr. Summaries. Europ. Conf. Biomed. Opt*. (Munich, Germany, 2007) p. 71.
15. Stasheuski A.S., Galievsky V.A., Knyukshto V.N., Ghazaryan R.K., Gyulkhandanyan A.G., Gyulkhandanyan G.V., Dzhagarov B.M. *J. Appl. Spectrosc.*, **80** (6), 823 (2013).
16. Tuchina E.S., Tuchin V.V., Altshuler G.B., Yaroslavsky I.B. *Yestestv. Tekh. Nauki*, **34** (2), 90 (2008).
17. Gyulkhandanyan G.V., Ghambaryan S.S., Amelyan G.V., Ghazaryan R.K., Arsenyan F.H., Gyulkhandanyan A.G. *Proc. SPIE Int. Soc. Opt. Eng*., **6139**, 613911 (2006).
18. Gyulkhandanyan G.V., Paronyan M.H., Hovsepyan A.S., Ghazaryan R.K., Tovmasyan A.G., Gyulkhandanyan A.G., Gyulkhandanyan A.G., Amelyan G.V. *Proc. SPIE Int. Soc. Opt. Eng*., **7380**, 73803I (2009).
19. Kovaleva O.A., Tsvetkov V.B., Shchyolkina A.K., Borisova O.F., Ol'shevskaya V.A., Makarenkov A.V., Semeikin A.S., Shtil A.A., Kaluzhny D.N. *Europ. Biophys. J*., **41**, 723 (2012).
20. Moan J., Iani V., Ma L.W.*Proc. SPIE Int. Soc. Opt. Eng*., **2625**, 544 (1996).
21. Moskvin S.V. *Effektivnost lazernoy terapii* (Efficiency of Laser Therapy) (Moscow-Tver: Triada, 2014).
22. Khlebtsov B.N., Tuchina E.S., Khanadeev V.A., Panfilova E.V., Petrov P.O., Tuchin V.V., Khlebtsov N.G. *J. Biophotonics*, **6** (4), 338 (2013).
23. Tuchina E.S., Petrov P.O., Kozina K.V., Ratto F, Centi S., Pini P., Tuchin V.V. *Kvantovaya Elektron.*, **44** (7), 683 (2014) [*Quantum Electron.*, **44** (7), 683 (2014)].
24. Khlebtsov B., Tuchina E., Tuchin V., Khlebtsov N. *RSC Advances*, **5**, 61639 (2015).
25. Tuchina E.S., Tuchin V.V., Khlebtsov B.N., Khlebtsov N.G. *Kvantovaya Elektron.*, **41** (4), 354 (2011) [*Quantum Electron.*, **41** (4), 354 (2011)].
26. Reifsteck F., Wee S., Wilkinson B.J. *J. Med. Microbiol.*, **24** (1), 65 (1987).
27. Rozgonyi F., Ljungh A., Mamo W., Hjerten S., Wadström T. *Bacterial Cell-Surface Hydrophobicity in Pathogenesis of Wound and Biomaterial-Associated Infections* (Berlin: Springer, 1990).
28. Stratonnikov A.A., Meerovich G.A., Ryabova A.V., Savel'yeva T.A., Loshchenov V.B. *Kvantovaya Elektron.*, **36** (12), 1103 (2006) [*Quantum Electron.*, **36** (12), 1103 (2006)].