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Photosensitized oxidation by dioxygen as the base for drinking water disinfection

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Abstract

Efficiencies of the series water-soluble anionic and cationic sensitizers have been studied in photodynamic natural water disinfection. It was found that only cationic sensitizers are efficient in photooxidative bacteria killing during photodynamic water treatment. The difference in photodynamic action towards different groups of microorganisms has been observed. The most vulnerable are enterococcus and enterococcus faecalis. Spores of sulfite-reducing clostridium are resistant to photodynamic action but, to provide drinking water, clostridium may be removed by sedimentation and filtration. The dependence of photodisinfection on treatment conditions was studied. It was found that sunlight along with artificial visible light sources may be used for photodynamic water treatment. The photodynamic step, arranged with artificial visible light source, was included in a process of conventional water purification instead of chlorine disinfection. Preliminary pilot testing have shown that photodynamic water disinfection in combination with coagulation, sedimentation, sand and carbon filtrations (latter—to remove sensitizer and products of its photolysis) provides water of high quality, free of bacteria and chemicals as well.
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Keywords: Bacteria; Drinking water; Photodynamic disinfection; Photosensitizer; Singlet oxygen

1. Introduction

Photodynamic technology uses a combination of a photosensitizer, light, and molecular oxygen to achieve destruction of a biological target. Energy from light excites the photosensitizer molecule to the excited singlet state. This excited state may then undergo intersystem crossing to the slightly lower energy, but longer lived, triplet state, which may then react further by one or both of two pathways known as the type I and type II photoprocesses, both of which require oxygen. The type I pathway involves electron-transfer reactions from the triplet state of photosensitizer with the participation of a substrate to produce radical ions that can then react with oxygen to produce cytotoxic species, such as superoxide, hydroxyl and hydroperoxide radicals. The type II pathway involves energy transfer from the photosensitizer triplet state to ground-state molecular oxygen (triplet) to produce excited-state singlet oxygen, which can oxidize many of biological molecules and lead to cytotoxicity. Photodynamic technology has now been extensively developed for therapeutic purposes to selectively destroy cancers. It has recently become clear that this field has a high potential, both for therapy and for non-therapeutic purposes such as sterilisation, including water sterilisation.

The aim of this work was to study the possibility of usage of photodynamic bacteria inactivation for water disinfection instead of chlorine disinfection in conventional technology of water purification.

2. Materials and methods

2.1. Chemicals

Commercial samples of Rose Bengal, eozine and methylene blue were used without additional purification. Sulfonated aluminium phthalocyanine $AlPcS_n$, with average number of sulfogroups per molecule n about 3, has been synthesized by direct sulfonation of corresponding Pc as described previously [1]. Octapyridiniomethyl substituted phthalocyanines of zinc ($ZnPcPym_8$) and aluminium ($AlPcPym_8$) were synthesized by chloromethylation of corresponding unsubstituted

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Table 1
The photosensitizing properties of dyes

Sensitizer	λ_{max} (nm)	Singlet oxygen quantum yield, φ_Δ	Photoinactivation of total coliform bacteria in natural water[a]		
			Concentration of sensitizer (mol dm^{-3})	Count in initial water, CFU per 0.1 dm^3	Count after photodisinfection, CFU per 0.1 dm^3
Rose Bengal	514	0.75 [4]	2×10^{-6}	1200	6
Eozine	550	0.52 [4]	4×10^{-6}	2200	460
$AlPcS_n$	679	0.38 [5]	4.5×10^{-6}	1200	18
$AlPcPym_8$	677	0.37	2×10^{-6}	1600	0
$ZnPcPym_8$	678	0.45	2×10^{-6}	1600	0
Methylene blue	665	0.52 [4]	5×10^{-6}	1100	0
Proflavine	441	<0.05	5×10^{-6}	2700	0

[a] Contact time of incubation: 1 h, irradiation time: 0.5 h.

metallophthalocyanines with bis (chloromethyl) ether followed by quaternization of intermediate octachloromethyl-derivatives with pyridine [2]. Proflavine was synthesized as described by Lushina et al [3].

2.2. Photosensitized inactivation of bacteria

Contaminated water of the Moscow river was used as sewage. Some experiments were performed with museum coliform bacteria, pseudomonas, enterococcus, enterococcus faecalis, and sulfite-reducing clostridium strains. The tested sensitizer was added to the bacteria-contaminated water typically at concentration of 1.5–5 $\times$ 10^{-6} mol dm^{-3}. After 0.5–1.0 h of incubation at ambient temperature, the samples under air bubbling conditions (flow set at about 1 dm^3 min^{-1}) were exposed to irradiation with light of 350–700 nm spectral range in cylindrical glass vessel, containing 0.3 dm^3. The vessel was provided with a jacket for the cooling water. The radiation source was 500 W halogen lamp placed at 15 cm distance from the sample, which provided the fluent rate of about 240 W m^{-2} on the surface of the sample. After 0.5 h exposure to light, the water samples were inoculated on differential media directly or with membrane filtration and the number of colonies formed after 18–24 h incubation at a temperature of 37 °C were counted. The number of germ in colony-forming units (CFU) was determined in initial water samples before applying sensitizer and when photodynamic treatment was accomplished.

3. Results

In Table 1 absorption maxima in visible spectral range along with singlet oxygen quantum yields (φ_Δ) and data on photoinactivation of natural strains of coliform bacteria for series of common photosensitizing dyes (Fig. 1) are presented. With

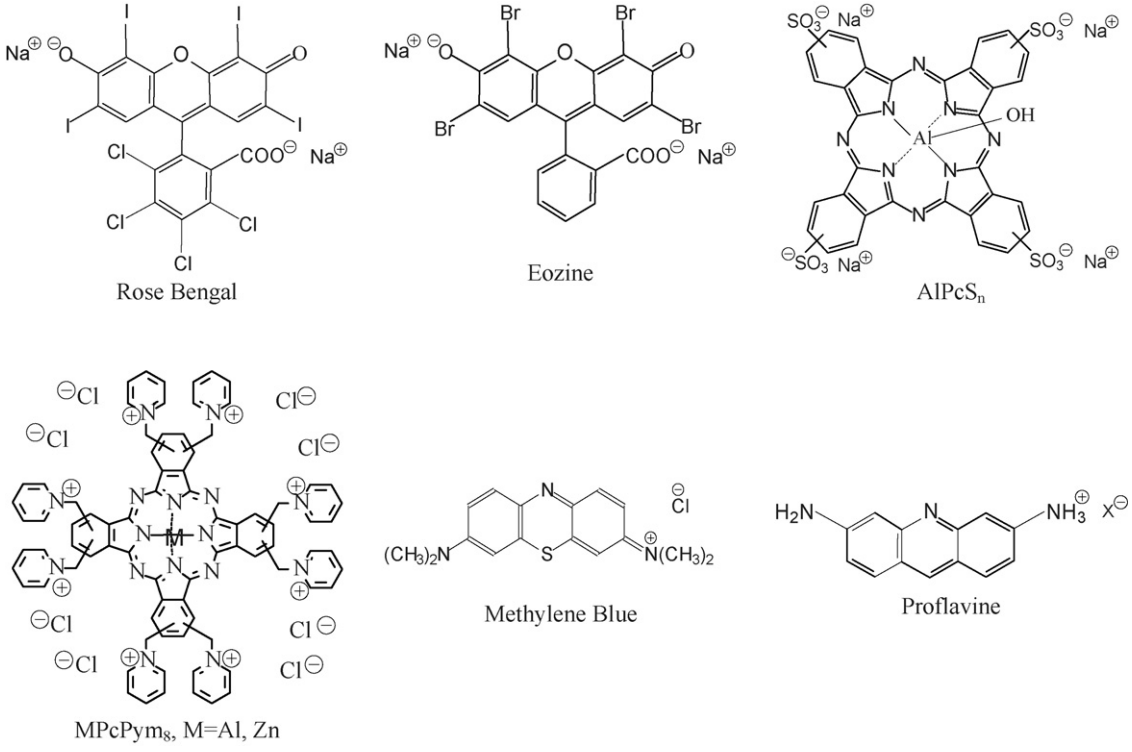

Fig. 1. Chemical structures of sensitizers.

Table 2
Efficiency of $ZnPcPym_8$ photodynamic action towards museum microorganisms

Microorganisms	Count in initial water	Count after photodisinfection	Photodisinfection efficiency (%)
Colony count per 10^{-3} dm^3 at 37 °C	3900	0	100
Total coliform bacteria per 10^{-1} dm^3	35000	0	100
Termotolerant coliform bacteria per 10^{-1} dm^3	23000	0	100
Glucose-positive coliform bacteria per 10^{-1} dm^3	45000	0	100
Pseudomonas per 10^{-1} dm^3	75000	0	100
Enterococcus per 10^{-1} dm^3	10000	0	100
Enterococcus faecalis per 10^{-1} dm^3	1100	0	100
Spores of sulfite-reducing Clostridium per 2×10^{-2} dm^3	80	80	0

Sensitizer concentration: 1.5×10^{-6} mol dm^{-3}, contact time of incubation: 1 h, irradiation time: 0.5 h.

exception of proflavine, all tested dyes have high quantum yields for generation of cytotoxic singlet oxygen. However, data of Table 1 evidence for the absence of relationship between φ_Δ values and photodynamic efficiency of dyes against Gram-negative coliform bacteria in water samples. Thus, Rose Bengal and eozine, which have the highest values of quantum yields for singlet oxygen photogeneration among the tested dyes, possess low efficiency in coliform bacteria photoinactivation. Data of Table 1 show that substantial amount of coliform bacteria survive after photodynamic disinfection with these dyes. Considering the chemical structures of sensitizers (Fig. 1) we can infer that high photodisinfection activity possess sensitizers, positively charged in aqueous media. Positive charge endows sensitizer molecule high affinity to negatively charged outer membrane of cell, which is essential for photodynamic bacteria killing. This finding is in agreement with the dependence of photodynamic antibacterial activity on sensitizer charge, reported in literature [6]. Taking this into account, in our further work we have focused on the cationic photosensitizers. Among the cationic dyes, studied in this work, $AlPcPym_8$, $ZnPcPym_8$ and methylene blue are type II sensitizers, whereas proflavine has low efficiency of singlet oxygen formation and operates according to the type I radical mechanism.

The wide range of microorganisms was studied under photodynamic treatment with cationic photosensitizing dyes. For example, data on $ZnPcPym_8$ photodynamic activity are presented in the Table 2. It may be seen that photodisinfecting influence of $ZnPcPym_8$ was effective (100%) for all indices (colony count at 37 °C, total coliform bacteria, termotolerant coliform bacteria, glucose-positive coliform bacteria, pseudomonas, enterococcus, enterococcus faecalis), except spores of sulfite-reducing clostridium. Detailed studies have revealed the difference in photodynamic action towards different groups

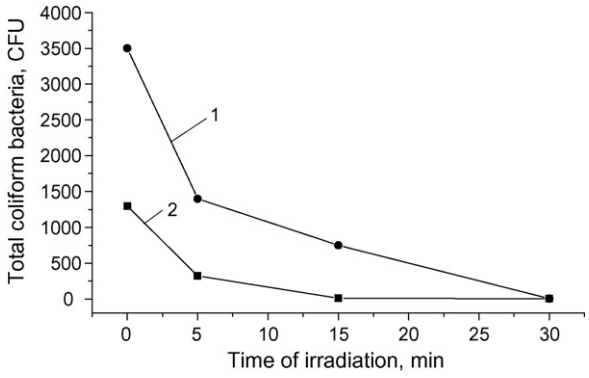

Fig. 3. Dependences of total coliform bacteria survival (in CFU per 10^{-1} dm^3) on irradiation time during photodynamic inactivation with $AlPcPym_8$ (1) and methylene blue (2). Concentrations of $AlPcPym_8$ and methylene blue are 3×10^{-6} and 5×10^{-6} mol dm^{-3}, correspondingly. Contact time of incubation: 1 h.

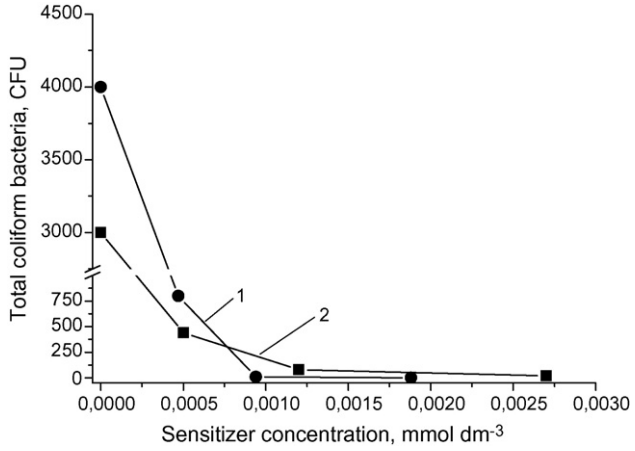

Fig. 2. Effect of sensitizer concentration on total coliform bacteria survival (in CFU per 10^{-1} dm^3) for photodynamic treatment with $ZnPcPym_8$ (1) and methylene blue (2). Contact time of incubation: 1 h, irradiation time: 0.5 h.

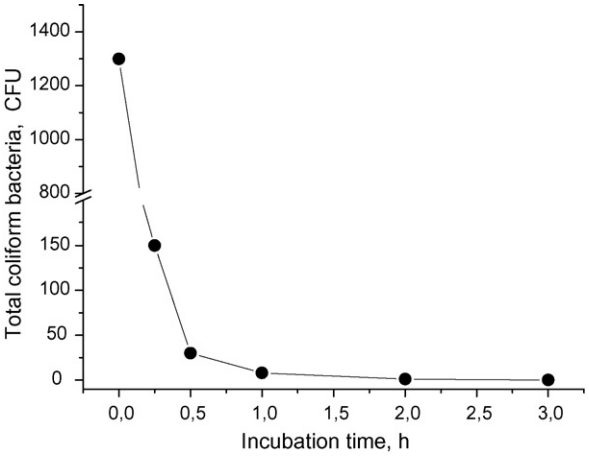

Fig. 4. Total coliform bacteria survival (in CFU per 10^{-1} dm^3) dependence on incubation time for photodynamic treatment with methylene blue. Sensitizer concentration: 5×10^{-6} mol dm^{-3}, time of irradiation: 0.5 h.

Table 3
Bacteriological and chemical indices for treated water along technological scheme of pilot set-up for drinking water provision

Indices	Initial water	After sedimentation	After photodynamic step	After carbon filter	After chloroammonization
Colony count per 10^{-3} dm^3	90	5	3	12	1
Total coliform bacteria per 10^{-1} dm^3	450	18	0	0	0
Termotolerant coliform bacteria per 10^{-1} dm^3	450	18	0	0	0
Clostridium per 2×10^{-2} dm^3	19	0	0	0	0
Coliphags per 10^{-1} dm^3	40	1.4	0	0	0
Turbidity, mg dm^{-3}	5.4	0.289	0.548	0.077	0.256
Color of water, deg.	66	30	57	0	0
pH	8.02	7.49	8.10	7.80	7.91
Permanganate water oxidizability, mg dm^{-3}	6.6	3.92	4.24	0	0
Al, mg dm^{-3}	–	0.061	–	0.042	–

of microorganisms. The most vulnerable were enterococcus and enterococcus faecalis.

The dependence of photodisinfection efficiency on concentration, time of irradiation, time of incubation was studied for proflavine, methylene blue, AlPcPym$_8$ and ZnPcPym$_8$. Examples of obtained relationships are presented on Figs. 2–4. The main results of these studies are considered below.

During incubation period, the sensitizer interacts with cell outer membrane, penetrates it and localizes in appropriate cell compartment. It is worthy to note that localization of sensitizer inside bacteria is important for it photodynamic killing. It was found that extent of sensitizer interaction with bacteria depends both on sensitizer structure and bacteria morphology. Thus, for killing of coliform bacteria the 5–10 min incubation period is quite sufficient for proflavine and ZnPcPym$_8$, whereas efficient photodynamic disinfection by means of methylene blue and AlPcPym$_8$ requires incubation time of about 0.5–1.0 h (Fig. 4). The most crucial for efficient photodisinfection are doses of sensitizer and light (Figs. 2 and 3).

Our studies have shown that sunlight along with artificial visible light sources may be used for photodynamic water disinfection.

The photodynamic step with artificial visible light source was designed for proflavine as sensitizer. Excitation of sensitizer was carried out by luminescent lamps like Blue OSRAM 18 W/67 lamps, emission spectrum of which perfectly overlaps absorption of proflavine. Moreover, lamps Blue OSRAM in comparison with other light sources have high coefficient of electric energy conversion to energy of light [7]. In pilot set-up the photodynamic treatment was carried out during water flow through four cameras of 100 dm^3 volume with submersible lighting fitting. The photodynamic step was included in a process of conventional water purification instead of chlorine disinfection. It should be noted that photodynamic disinfection is less efficient than chlorination. Thus, spores (Clostridium) are resistant to photodynamic treatment but may be removed by means of sedimentation and filtration. The photodynamic technology employs dissolved sensitizer and has the drawback of undesirable contamination by the sensitizer itself and by products of its photolysis. Hence after photodynamic step the sorption on carbon was used. Bacteriological and chemical indices for treated water along technological scheme of pilot set-up are presented in Table 3. Tests (Table 3) have shown that photodynamic water disinfection in combination with coagulation, sedimentation, sand and carbon filtrations provides water of high quality, pure from bacteria and some chemicals as well.

4. Conclusions

The combined approach of the photophysical and photobiological investigations is useful for the understanding of the mechanism by which sensitizers induce antibacterial activity. The discussed experimental data show that singlet oxygen production is not the only factor determining dye activity in photodynamic water disinfection. Positive charge of sensitizer is necessary for efficient interaction with bacterium outer membrane, which precedes photoinactivation.

Studies of regularities of common cationic dyes photodynamic action against coliform bacteria permitted to found affective doses of sensitizer and light. The difference in photodynamic action towards different groups of microorganisms has been revealed. In general the bacteria are susceptible photodynamic inactivation except for the family of spores, which nevertheless should be removed from drinking water also. Therefore, photodynamic technology cannot be used alone to provide drinking water. However, spores may be removed by sedimentation and filtration—the customary steps of water purification, which are obligatory for all water-treatment technologies. Taking this into account, the photodynamic step was included in a process of conventional water purification instead of chlorine disinfection with sorption on carbon to remove sensitizer and products of its photolysis. Preliminary pilot tests have shown that photodynamic water disinfection in combination with coagulation, sedimentation, sand and carbon filtrations provides water of high quality, pure from bacteria and chemicals as well.

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References

[1] N. Kuznetsova, N. Gretsova, V. Derkacheva, O. Kaliya, E. Lukyanets, Sulfonated phthalocyanines: aggregation and singlet oxygen quantum yield in aqueous solutions, J. Porphyrins Phthalocyanines 7 (2003) 147–154.

[2] D.A. Makarov, O.A. Yuzhakova, V.M. Negrimovsky, L.K. Slivka, N.A. Kuznetsova, O.L. Kaliya, E.A. Lukyanets, Cationic Zn and Al Phthalocyanines: synthesis, spectroscopy and photosensitizing properties, J. Porphyrins Phthalocyanines, in press.

[3] O.T. Lushina, A.A. Pryanishnikov, M.I. German, Reagents and High-Purity Substances, IREA, Moscow, 1977 (in Russian).

[4] M.C. DeRosa, R.J. Crutchley, Photosensitized singlet oxygen and its applications, Coord. Chem. Rev. 233–234 (2002) 351–371.

[5] J.M. Fernandez, M.D. Bilgin, L.I. Grossweiner, Singlet oxygen generation by photodynamic agents, J. Photochem. Photobiol., B 37 (1997) 131–140.

[6] G. Jori, S.B. Brown, Photosensitized inactivation of microorganisms, Photochem. Photobiol. Sci. 3 (2004) 403–405.

[7] OSRAM GmBH Catalogue.