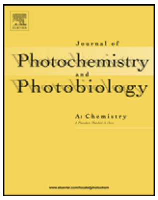
Journal of Photochemistry and Photobiology A: Chemistry 210 (2010) 82–88

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem



Reactive oxygen species produced by irradiation of some phthalocyanine derivatives

Jiří Černý[a,*], Marie Karásková[a], Jan Rakušan[a], Stanislav Nešpůrek[b]

[a] Research Institute of Organic Synthesis, Rybitví 296, 533 54 Rybitví, Czech Republic
[b] Institute of Macromolecular Chemistry AS CR, v.v.i., Heyrovský Sq. 2, 162 06 Prague, Czech Republic

ARTICLE INFO

Article history:
Received 24 September 2009
Received in revised form
11 November 2009
Accepted 23 November 2009
Available online 1 December 2009

Keywords:
Singlet oxygen
Photosensitizer
Phthalocyanine
Quantum yield

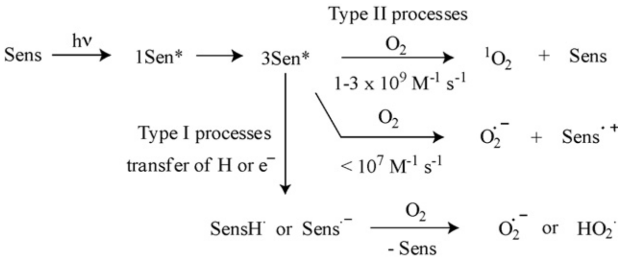
ABSTRACT

A comparative study of the reliability of methods used for the determination of singlet oxygen was carried out. The water iodide method, as well as the methods using 9,10-dimethylanthracene (DMA) and 1,3-diphenylisobenzofuran (DPIBF) in dimethylformamide (DMF) solutions, was used for the detection of singlet oxygen $^1O_2$ production in eight phthalocyanine photosensitizers. The iodide method is not only selective for the determination of $^1O_2$ but can also be used for the determination of other oxygen species. In addition, the results of the iodide method are affected by a possible formation of phthalocyanine dimers and by post-irradiation reactions. On the other hand, both the methods using indicators in DMF are highly selective for the determination of $^1O_2$. The quantum yield of the singlet oxygen production was determined for eight phthalocyanine derivatives; the following sequence was obtained: **5** ∼ **4** > **7** ∼ **2** ∼ **8** (standard) > **6** > **3** ∼ **1**, where **5** represents the ammonium salt of disulphonated zinc phthalocyanine, **4** sulphonated zinc phthalocyanine [(3-diethylammonium)-propylsulphonamide] citrate, **7** tetrakis(trimethylammonium)zinc phthalocyanine tetraiodide, **2** tetrakis(*N*-methylpyridinium-3-oxy)zinc phthalocyanine tetraiodide, **8** zinc phthalocyanine, **6** sodium salt of disulphonated aluminum phthalocyanine, **3** sulphonated aluminum phthalocyanine [(*N*-2-hydroxyethyl)aminoethylsulphonamide] and **1** tetra-methylenepyridinium chloride of hydroxyaluminum phthalocyanine.

1. Introduction

Phthalocyanine compounds are important photosensitizers used in photodynamic therapy (PDT) [1]. The photodynamic activity is utilized not only in cancer treatment [2] but also in inactivation of bacteria [3], viruses [4] and yeasts [5]. A combined application of photosensitizers and light of appropriate wavelength is a new trend in the inactivation of microorganisms.

The mechanism of photosensitization reactions was described in Refs. [6–8]. Absorption of light of appropriate wavelength results in the excitation of photosensitizers (1Sen*). After that a triplet state (3Sen*) of the photosensitizer (Sens) is activated by intersystem crossing and consecutive reactions occur according to the following scheme:



In the Type I process, 3Sen* interacts with another molecule (e.g. solvent) and transfer of electron ($Sens^{\bullet -}$) or hydrogen atom ($SensH^{\bullet}$) occurs. The process usually involves the production of radicals or ion-radicals which undergo further reactions with some present substances [9]. In the reaction with oxygen a superoxide radical [10–13] is formed which can disproportionate to hydrogen peroxide or react further.

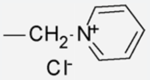
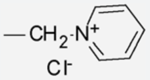
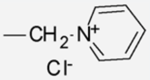
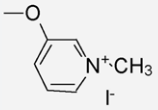
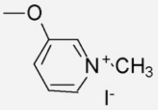
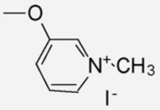
In the Type II process, the photosensitizer in the triplet state (3Sen*) interacts with diatomic oxygen, energy transfer from the photosensitizer to triplet oxygen occurs and singlet oxygen ($^1O_2$) formed [14]. The triplet state of photosensitizer is often quenched by a very fast reaction. A less frequent case of the Type II process (less than 1%) is an electron transfer leading to superoxide radi-

---

[*] Corresponding author. Tel.: +420 46 6825661; fax: +420 46 6823900.
E-mail addresses: jiri.cerny@vuos.com (J. Černý), marie.karaskova@vuos.com (M. Karásková), jan.rakusan@vuos.com (J. Rakušan), nespurek@imc.cas.cz (S. Nešpůrek).

1010-6030/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jphotochem.2009.11.016

Table 1
Chemical structures of phthalocyanines.

Compound	M	R^1	R^2	R^3, R^4
1	AlOH			
2	Zn			
3	AlOH	$SO_3^-Na^+$	$SO_2NHCH_2CH_2CH_2NHCH_2CH_2OH$	H
4	Zn	SO_3H	$SO_2NHCH_2CH_2NH^+(C_2H_5)_2$ citrate$^-$	H
5	Zn	$SO_3^-NH_4^+$	$SO_3^-NH_4^+$	H
6	AlOH	$SO_3^-Na^+$	$SO_3^-Na^+$	H
7	Zn	$N^+(CH_3)_3I^-$	$N^+(CH_3)_3I^-$	$N^+(CH_3)_3I^-$
8	Zn	H	H	H

cals ($O_2^{\bullet-}$) [10–13]. Nevertheless, superoxide radicals are formed predominantly by Type I process. Singlet oxygen as a very reactive compound is able to react with biomolecules. The quantum yield of singlet oxygen production strongly depends on the type of photosensitizer.

The aim of this work is to compare the efficiency of the production of singlet oxygen in eight phthalocyanine photosensitizers which, in preliminary experiments, proved efficient antimicrobial effects. Antimicrobial PDT proceeds usually in aqueous media and hence a method of the measurement of $^1O_2$ production was determined in this medium.

The iodide method was developed by Mosinger [15] for the measurement of $^1O_2$ production generated by sulfo-derivatives of porphyrin. The sensitivity of the method was 10 times higher than the commonly used photobleaching of 4-nitroso-*N*,*N*-dimethylaniline (RNO) [16]. So far the iodide method has not been used for the measurement of the production of $^1O_2$ generated by the irradiation of phthalocyanines up to now. Another method, using 9,10-dimethylanthracene (DMA) method, utilizes nonaqueous medium, dimethylformamide (DMF) [17]. Both methods were also modified by adding some additives affecting the production of singlet oxygen. In the third method, 1,3-diphenylisobenzofuran (DPIBF) in DMF was used [18]. Zinc phthalocyanine was chosen as a standard, having the quantum yield of singlet oxygen production $\Phi = 0.56$ [19].

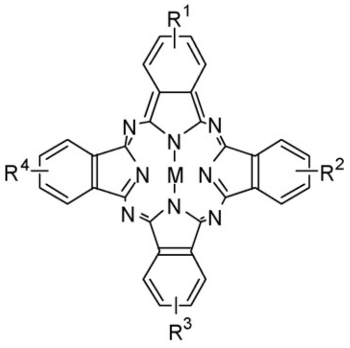
2. Materials and methods

2.1. Photosensitizers

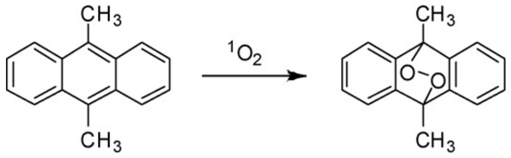
Phthalocyanine (Pc) photosensitizers used in this study are presented in Table 1. They were synthesized and purified by the methods published elsewhere [20–25]. Their structure and purity were confirmed by thin layer chromatography, elemental analysis and UV–vis spectroscopy (Scheme 1).

2.1.1. Other chemicals

Solvents, DMF, methanol and propan-1-ol (PrOH) were used as received from Penta (Czech republic). The indicators, DMA and DPIBF, potassium iodide, ammonium molybdate and sodium azide, were purchased from Aldrich.



Scheme 1. Phthalocyanine photosensitizers (M = central metal ion).



Scheme 2. The photobleaching reaction of DMA with $^1O_2$.

2.2. Chemical methods for singlet oxygen determination

2.2.1. The iodide method [15]

The method is based on the reaction of $^1O_2$ (generated by laser excitation of phthalocyanine) with iodide in the presence of ammonium molybdate. The reaction proceeds by the following multistep mechanism [26]:

$$^1O_2\,(^1\Delta_g) + I^- \longrightarrow IOO^- \xrightarrow{H_2O} IOOH$$

$$IOOH + I^- \longrightarrow HOOI_2^- \longrightarrow I_2 + HO_2^-$$

$$I_2 + HO_2^- \xrightarrow{I^-,\ H_2O} I_3^- + H_2O_2 + OH^-$$

Under weakly acidic conditions (buffer pH = 6.2), in the presence of ammonium molybdate as a catalyst, another reaction step takes place:

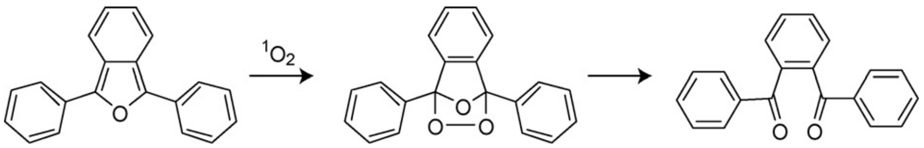
$$H_2O_2 + 2\,I^- + 2\,H^+ \longrightarrow I_2 + 2\,H_2O$$

$$I_2 + I^- \longrightarrow I_3^-$$

The amount of triiodide anion is directly proportional to the $^1O_2$ production. The reaction of iodine with iodide is a diffusion-controlled reaction ($k = 4 \times 10^{11}\,l\,mol^{-1}\,s^{-1}$) [27] which can be spectrometrically monitored at 351 nm (the triiodide anion band).

2.2.2. The DMA method

The process is based on the quenching of DMA by $^1O_2$ exclusively, in the chemical reaction [28,29] yielding 9,10-dimethyl-9,10-dihydro-9,10-epidioxyanthracene (Scheme 2).



Scheme 3. The photobleaching reaction of DPIBF with $^1O_2$.

The reaction is not affected by consecutive processes; the resulting endo-peroxide is stable under measurement conditions. The quenching reaction is fast; the rate constant in benzene solution is ∼3×10^{10} l mol^{-1} s^{-1} [28] — the value close to those existing in diffusion-controlled processes. The photobleaching rate of DMA is directly proportional to the amount of $^1O_2$ production and can be monitored spectrometrically at 381 nm.

2.2.3. The DPIBF method

The reaction is based on the photobleaching of DPIBF with singlet oxygen giving 1,2-dibenzoylbenzene [30,31] as a stable final product (Scheme 3).

The photooxidation of DPIBF proceeds through trans-annular peroxide [32] which rearranges to 1,2-dibenzoylbenzene. The rate constant for the quenching of DPIBF is about 7×10^8 l mol^{-1} s^{-1} [30]. The photobleaching of DPIBF is directly proportional to the amount of $^1O_2$ generated by the irradiation of photosensitizers. The photobleaching of the indicator is monitored spectrometrically at 417 nm.

2.3. Measurement conditions

2.3.1. The iodide method

Iodide reagent: A mixture of 6.81 g potassium dihydrogenphosphate, 2 mg ammonium molybdate, 19.92 g potassium iodide and 8.6 ml 1 M NaOH was diluted in 1 l of distilled water.

An appropriate quantity of the tested phthalocyanine derivative was dissolved in the iodide reagent to give the concentration of 4 mg l^{-1}. Then 2 ml of the resulting solution was put in a 1 cm quartz cuvette (3 ml) with a magnetic stirrer and irradiated with red laser light (40 mW, λ = 670 nm). The distance between the cuvette and laser light source was 0.5 cm. The laser light doses were in the range of 0.3–5 J cm^{-2}, depending on the phthalocyanine derivative. The spectrum of the solution was measured immediately after the irradiation and the absorbance of triiodide at 351 nm was recorded. For each measurement ca. 10 light doses were used to reach the absorbance of potassium triiodide about 1.9–2.0.

2.3.2. The DMA method

The procedure is the same as with the iodide reagent, except that the tested compounds were dissolved in DMF. The concentration of the compounds in DMF was 4 mg l^{-1} for nearly all of them except **5** and **8**. The concentrations of the compounds **5** and **8** were kept at the level of 1 mg l^{-1} due to their high molar absorption coefficients. A sufficient amount (∼0.05 ml) of the concentrated freshly prepared solution of DMA in DMF was added to the solution of 2 ml of photosensitizer in a cuvette, to get the initial absorbance of DMA solution at 381 nm equal to ca. 1.7–1.9. The laser light doses used were smaller ranging between 0.32 and 1 J cm^{-2}. For each measurement about 10 light doses were necessary to decrease the absorbance of DMA solution to ca. 0.2–0.3.

2.3.3. The DPIBF method

The procedure is the same as in the case of DMA method. A sufficient volume (∼0.05 ml) of the concentrated freshly prepared solution of DPIBF in DMF was added to the 2 ml solution of the photosensitizer in a cuvette, to get an initial DPIBF absorbance of about 0.9. The laser light doses used were smaller than in the DMA method and amounted to 0.01–0.1 J cm^{-2}. The power of the laser light was reduced to 10 mW. For every measurement about 10 light doses were used to decrease the absorbance of the indicator to ca. 0.1–0.2.

Table 2
Spectral characteristics of phthalocyanines **1–8** in DMF.

Compound	λ_{max} (nm)	ε (l mol^{-1} cm^{-1})
1	680	94 500
2	677	119 000
3	679	60 500
4	678	72 500
5	675	205 000
6	680	150 000
7	714	69 000
8	674	312 500

2.3.4. Determination of molar absorption coefficients

Fresh DMF solutions of the compounds under study were prepared and their VIS spectra were measured. The λ_{max} values were estimated and the corresponding absorbances were recorded. Extinction coefficients ε were calculated from the Lambert–Beer law. The obtained values are summarized in Table 2.

3. Results and discussion

3.1. Measurement of the quantum yield of singlet oxygen production by the iodide method

3.1.1. Standard iodide method

The production of singlet oxygen was measured by the standard iodide method for compounds **1–6**; compounds **7** and **8** were not measured due to their insufficient solubility in the reagent.

In Fig. 1 is shown typical VIS spectrum of phthalocyanine (compound **6**, 4 mg l^{-1}) in the iodide reagent without irradiation and the same after irradiation with laser light. From the graph it follows that

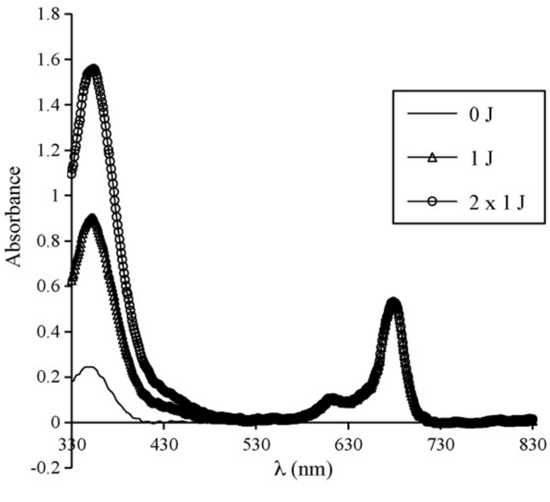


Fig. 1. The dependence of absorbance of compound **6** (4 mg l^{-1}) in the iodide reagent on the illumination doses (0 J, 1 J and 2 × 1 J, respectively).

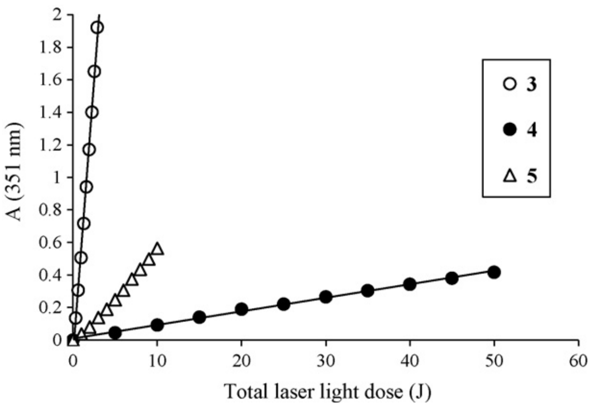


Fig. 2. The dependence of absorbance of potassium triiodide on the irradiation doses for compounds **3**–**5** at 4 mg l^{-1} in the standard iodide reagent (pH 6.2). The numbers in the figure refer to the numbers of compounds as listed in Table 1.

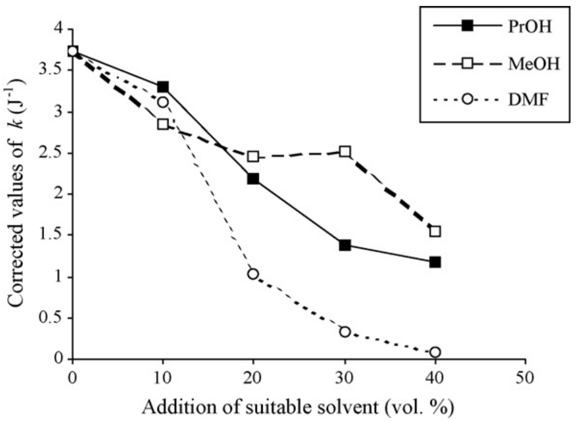


Fig. 3. The effect of the addition of methanol (MeOH), propan-1-ol (PrOH) and DMF to the iodide reagent on the corrected k (J^{-1}) values for the compound **3**.

phthalocyanine has low absorbance at the measured wavelength (351 nm) and in this way does not affect measurements.

The dependences of the absorbance of potassium triiodide solution on the irradiation doses for compounds **3**–**5** are shown in Fig. 2. The dependences are linear; the correlation coefficients (R^2) of the linear regression for all the compounds are greater than 0.993.

The slopes of the plots k_{obs} (J^{-1}) were calculated from the dependences of the absorbance of KI_3 on the irradiation doses. The slopes were corrected for the unit absorbance of phthalocyanines at 670 nm (λ_{max} of the used laser light) according to the relation $k = k_{obs}/A_{670}$, where k_{obs} is the observed slope in the dependence of potassium triiodide absorbance on the dose (Fig. 2) and A_{670} is the absorbance of the phthalocyanine in the iodide reagent at 670 nm. The k values are shown in Table 3.

3.1.2. Dimerization of the compounds

Two approaches to suppress a possible dimerization of phthalocyanine derivatives during the measurements were used. It is well known that the dimerization can be reduced by increasing pH value to the alkaline range [33]. Another possibility is the addition of a suitable solvent [34].

3.1.2.1. Effect of increasing pH. The iodide reagent was prepared in a similar way as in the standard iodide method; the pH value was increased to 8.0. The obtained results are summarized in Table 3.

For compounds **3** and **6** no distinct change in the k values were observed, for most other compounds (**1**, **2**, **4**) k decreased significantly. The reason for the decrease may lie in the fact that cationic phthalocyanines **1**, **2** and **4** are sensitive to higher pH and a partial decomposition may occur. On the other hand, compound **5** was found to be more effective photosensitizer at higher pH value. In this case, the increase of pH of iodide reagent suppressed the dimerization of this compound and higher amount of generated singlet oxygen was found.

Table 3
Slopes k calculated from the dependences of absorbance at 351 nm of potassium triiodide on laser light dose at pH 6.2 and pH 8.0, respectively, corrected for the unit absorbance of phthalocyanine compounds at 670 nm.

Compound	Iodide reagent pH 6.2	Iodide reagent pH 8.0
	Slope k (J^{-1})	Slope k (J^{-1})
1	1.562	1.137
2	0.471	0.138
3	2.879	2.752
4	0.123	0.075
5	0.363	0.526
6	1.746	1.676

Production of $^1O_2$ decreased in the order **3** > **6** > **1** > **2** > **5** > **4**.

A further increase in pH of the reagent to 11.0 adversely affected the formation of potassium triiodide.

3.1.2.2. Effect of addition of suitable solvents. Addition of some alcohols, such as methanol or propan-1-ol, as well as other protic solvent DMF, decreases the dimerization constant. Alcohol is less polar than water and has weaker hydrophobic interactions with phthalocyanines and thus, can provide some screening against the dimerization [35].

Certain amounts of suitable solvents (methanol, propan-1-ol or DMF) were added to the iodide reagent. Experiments were performed with compounds **1**, **3** and **6** (4 mg l^{-1}). Several iodide reagents with different amounts (10–40 vol.%) of methanol, propan-1-ol or DMF were prepared. The observed slopes k corrected for the unit absorbance at 670 nm for compound **3** are presented in Fig. 3 and for compound **1** in Fig. 4. The data for compound **6** are not shown as the dependences of k values are the same as for compound **3**.

From the results presented in Figs. 3 and 4 it follows that addition of various solvents significantly affects the k values. The effect varies for three compounds measured. For compound **1** the slopes k first increase with the increasing amount of the added methanol or DMF and then significantly decrease. The increase is more efficient after the addition of methanol, whereas the decrease is more

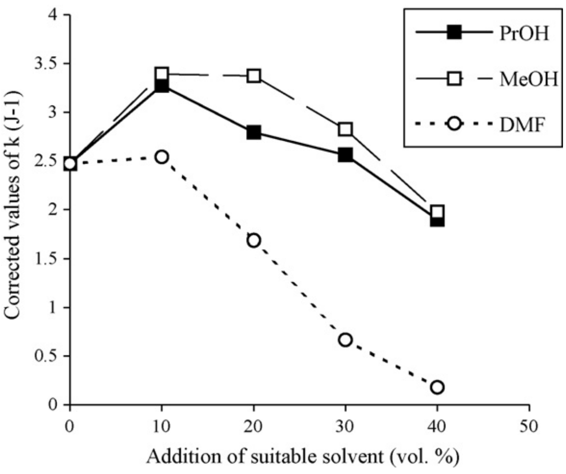


Fig. 4. The effect of the addition of methanol (MeOH), propan-1-ol (PrOH) and DMF to the iodide reagent on the corrected k (J^{-1}) values for the compound **1**.

Table 4
Dimerization extent D_r of compounds **1–6** in the iodide reagent with or without 30 vol.% propan-1-ol.

Compound	D_r, without PrOH	D_r, with 30 vol.% PrOH
1	2.49	15.70
2	0.95	1.68
3	2.47	6.24
4	0.86	2.63
5	0.90	4.12
6	6.33	8.07

efficient after the addition of DMF. For compounds **3** and **6** only a decrease was found. From Fig. 3 it follows that the decrease of k is larger for the additions of propan-1-ol and DMF than for methanol.

From these results it is evident that the addition of solvent to the iodide reagent has two effects. The positive effect is the suppression of the dimerization of phthalocyanines which leads to an increase of singlet oxygen production. The lifetime of singlet oxygen in methanol is about 7 μs [36]. This value is about 3 times higher than that in water. For propan-1-ol the exact value has not been found but we suppose that the value is similar (5–8 μs). The negative effect (expressed by the decrease of the k value) of the use of the solvents is probably not caused by quenching of singlet oxygen. It is most likely caused by steric effects of added solvents and it increases with the increasing size of solvent molecules.

The negative effect prevails for phthalocyanines bearing neutral or anionic moieties such as compound **3**. The positive effect asserts only for smaller additions of used solvents (till 10%) in the case of cationic phthalocyanine **1**.

3.1.2.3. *Dimerization of compounds.*

Further studies were oriented on dimerization of the studied phthalocyanines. The extent of dimerization can be described by D_r value [37]:

$$D_r = \frac{A_{\lambda_{\max}}}{A_{\lambda = 630\,\mathrm{nm}}},$$

where $A_{\lambda_{\max}}$ is the absorbance measured at $\lambda_{\max}$ of phthalocyanine and $A_{\lambda = 630\,\mathrm{nm}}$ is that measured at 630 nm (corresponding to the dimer). The ratio D_r is low when a high concentration of dimers is present. D_r increases with decreasing amount of dimers in solution.

To compare the extent of dimerization, the spectra of freshly prepared solutions of compounds **1–6** in the iodide reagent or the reagent containing 30 vol.% of propan-1-ol were recorded in the wavelength range 600–690 nm and the values D_r were calculated. The obtained results are shown in Table 4.

It follows from Table 4 that the dimerization occurred to some extent in all the compounds studied. The dimerization corresponds with the results mentioned in Table 3, where compounds **2**, **4** and **5** showed low production of $^1O_2$ in the iodide reagent due to the presence of dimers.

3.1.3. *Post-irradiation reaction*

In the measurements of the singlet oxygen production by the standard iodide method it was found that the formation of potassium triiodide continued for ca. 15 min after the end of irradiation. Therefore, this phenomenon was studied. The post-irradiation reaction was observed with nearly all the tested compounds. A typical dependence of an increase of absorbance after the end of irradiation is illustrated in Fig. 5 for the compound **6**. The reaction is of a first-order with the half-time being 146 s. For the same reaction without ammonium molybdate it was found that the half-time increased to 386 s. Ammonium molybdate catalyzed the post-irradiation reaction.

The post-irradiation reaction was also observed when the iodide reagent containing propan-1-ol was used. The reaction was much faster, the estimated half-time decreased to 17 s. The extent of the post-irradiation reaction was much smaller than that in the standard iodide reagent.

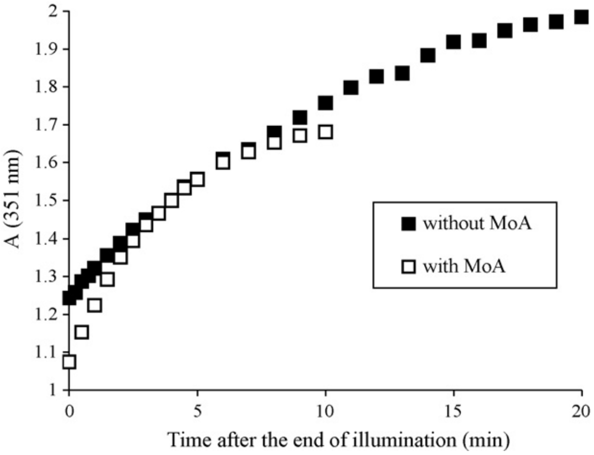


Fig. 5. The time dependence of absorbance of potassium triiodide solution after the end of illumination of compound **6** (4 mg l^{-1}, laser light dose 2 J cm^{-2}) in the presence or absence of ammonium molybdate.

From Fig. 5 and the short lifetime of singlet oxygen in solutions (microseconds) it can be deduced that singlet oxygen could not act as the oxidizing agent during the post-irradiation reaction.

In further experiments we added NaN_3 (which is known as a selective quenching agent for $^1O_2$ [38]) to iodide reagents. The production of potassium triiodide was measured for compound **1** partly in the standard iodide reagent containing 0.01 M NaN_3 and partly in the iodide reagent containing 30 vol.% of propan-1-ol and 0.01 M NaN_3. It was found that the values of k decreased in both cases to about 10% of their original values. The non-zero slopes indicate that also some other reactive oxygen species, which are not quenched by the action of NaN_3, are generated by irradiation of phthalocyanines. In addition, the post-irradiation reaction with similar kinetics occurred in the presence of NaN_3. This proved that NaN_3 had no effect on the post-irradiation reaction.

From these results we deduced that H_2O_2 could be the most probable species causing the post-irradiation reaction. Thus, several model experiments with addition of H_2O_2 to the iodide reagent were performed. The kinetics of the reaction with 6×10^{-5} M H_2O_2 in the iodide reagent, in the iodide reagent containing 30 vol.% of propan-1-ol and in the iodide reagent with 0.01 M NaN_3 were studied. The time dependences of absorbances are shown in Fig. 6.

It follows from Fig. 6 that the reaction of H_2O_2 with iodide has the same half-time as the post-irradiation reaction. The addition of NaN_3 to the iodide reagent had no effect on the kinetics of this

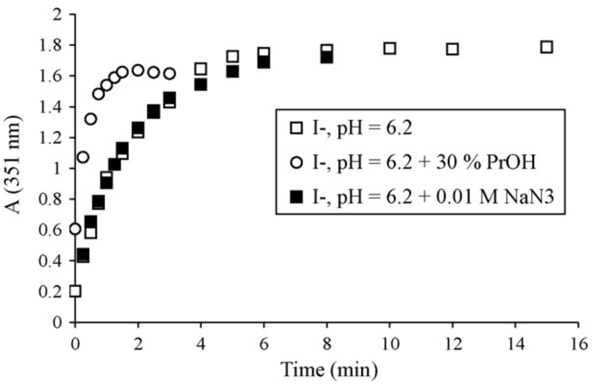


Fig. 6. The time dependence of absorbance of potassium triiodide for the reaction of H_2O_2 with the iodide reagent.

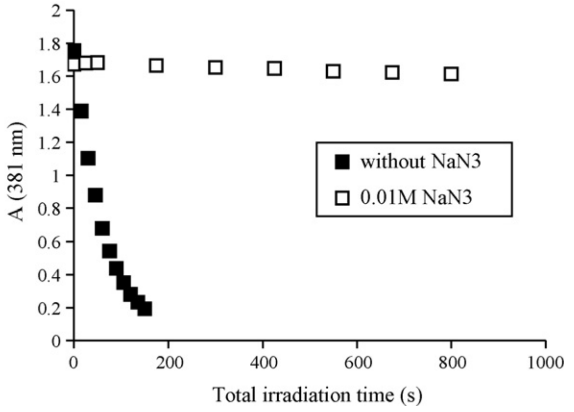


Fig. 7. The effect of NaN_3 on the DMA photobleaching by the action of singlet oxygen generated by irradiation of phthalocyanine **6** in DMF.

reaction. With the addition of propan-1-ol the reaction was much faster. These indirect proofs confirmed the presence of H_2O_2 in the reaction mixture during irradiation of phthalocyanines with laser light. Direct determination of H_2O_2 was not successful due to its very low concentration.

3.2. Measurement of the quantum yields of singlet oxygen using the DMA and DPIBF methods in DMF

The singlet oxygen production was measured in DMF in the presence of the indicators DMA or DPIBF. A typical kinetics of photobleaching of DMA in DMF is shown in Fig. 7.

The photobleaching is a first-order process. Half-times ($\tau_{1/2}$) of the decays of indicator were determined. The obtained half-times were corrected for the unit absorbance of phthalocyanines at 670 nm (A_{670}) according to the relation $\tau_{1/2}^{\mathrm{corr}} = \tau_{1/2} \times A_{670}$. The quantum yields were related to the standard – compound **8** ($\Phi = 0.56$) [19] – by the relation: $\Phi_{\mathrm{PS}} = \Phi_{\mathrm{S}}(\tau_{1/2}^{\mathrm{corr(S)}}/\tau_{1/2}^{\mathrm{corr(PS)}})$, where Φ_{PS} is the quantum yield of phthalocyanine compounds **1–7**, Φ_{S} is the quantum yield of the standard (compound **8**), $\tau_{1/2}^{\mathrm{corr(S)}}$ is the corrected half-time for the standard and $\tau_{1/2}^{\mathrm{corr(PS)}}$ is the corrected half-time for the phthalocyanine compounds **1–7**.

The corrected half-times for compounds **1–8** determined using DMA and DPIBF are summarized in Table 5. It was found that the decomposition of both indicators was negligible ($\tau_{1/2} \sim 2$ h) compared with that in the presence of $^1O_2$ generated by phthalocyanines **1–8**. Note that the decomposition of the photosensitizers by the action of laser light was very low.

Table 5
Corrected half-times $\tau_{1/2}^{\mathrm{corr}}$ (s) of photobleaching of indicators (DMA or DPIBF) in DMF and obtained quantum yields Φ_{PS} in the presence of compounds **1–8** (at 1 or 4 mg l^{-1}).

Compound	DMA method		DPIBF method	
	$\tau_{1/2}^{\mathrm{corr}}$ (s)	Φ_{PS}	$\tau_{1/2}^{\mathrm{corr}}$ (s)	Φ_{PS}
1	14.31	0.19	5.411	0.19
2	4.542	0.61	1.797	0.57
3	13.04	0.21	5.577	0.18
4	3.610	0.76	1.276	0.80
5	3.321	0.83	1.293	0.79
6	9.251	0.30	3.590	0.28
7	6.105	0.45	1.750	0.58
8	4.930	0.56	1.825	0.56

Contrary to the iodide method, no post-irradiation reaction was observed.

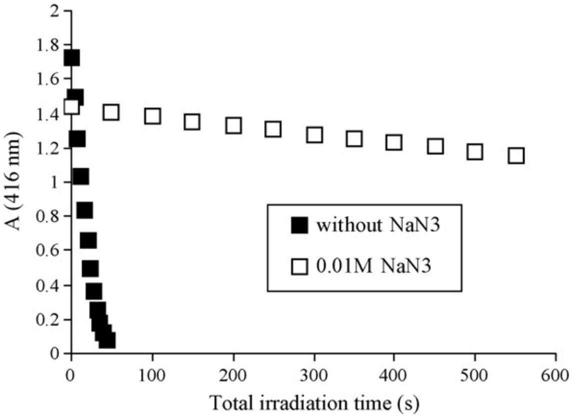


Fig. 8. The effect of NaN_3 on the DPIBF photobleaching by the action of singlet oxygen generated by irradiation of phthalocyanine **6** in DMF.

Next, the influence of NaN_3 on the kinetics of photobleaching of the indicators was studied. A typical effect on the kinetics for both indicators is shown in Figs. 7 and 8.

The addition of NaN_3 to the indicators had a pronounced effect on the method of $^1O_2$ determination: the half-times of all the compounds decreased at least 50 times. This confirmed our previous results obtained by the iodide method where also a decrease in the slopes was observed.

The addition of 0.01% H_2O_2 to the DMA solution had only a negligible effect on the decomposition of indicator by the action of laser light. Hence, the method using DMA is very selective for the determination of $^1O_2$ production. A similar selectivity was found also for DPIBF indicator.

3.3. Comparison of all methods

The order of the efficiency of the singlet oxygen production using phthalocyanines determined by the standard iodide method is **3** > **6** > **1** > **2** > **5** > **4**. However, the results are affected by the formation of dimers and by post-irradiation reaction. It was found that the dimerization can be suppressed by the addition of a certain amount of other solvent to the iodide reagent. The optimum amount of used solvent depended on the type of the studied compound. For this reason the iodide method is not reliable for the determination of singlet oxygen production.

The quantum yields obtained by two methods in DMF are nearly the same. These methods are very selective for the determination of singlet oxygen. However, the sensitivity of the DMA method is about 10-fold lower than that of the DPIBF method.

3.4. Antimicrobial activity

The phthalocyanine compounds **1–7** were tested against bacteria *Staphylococcus aureus* 5887, *Escherichia coli* 5276 and yeast *Candida albicans*. The most effective phthalocyanine against all tested microorganisms was compound **1** where low singlet oxygen production was found. From this result it follows that correlation between singlet oxygen production and antimicrobial activity is not simple and another factors (binding to the bacteria membranes) take place.

Further details about antimicrobial activity for phthalocyanines **1–7** will be included in a subsequent article.

4. Conclusions

Photodynamic activity based on the singlet oxygen production was compared for eight phthalocyanine photosensitizers. Three chemical methods – the iodide, DMA and DPIBF methods – were used for the singlet oxygen determination. The results obtained by the iodide method are considerably affected by dimerization of phthalocyanines and also by the post-irradiation reaction (due to the presence of other reactive oxygen species after the irradiation like hydrogen peroxide, which was proved indirectly by model reactions). Therefore, one can conclude that the iodide method is not reliable for the determination of singlet oxygen quantum yield for phthalocyanine derivatives.

The addition of solvents (methanol, propan-1-ol or DMF) to the iodide reagent to suppress the dimerization and increase singlet oxygen production, showed also a negative effect, which led to an decrease in singlet oxygen production. The negative effect was likely caused by the steric effects of molecules of the added solvents. The effect correlates with the size of the solvent molecules, the most prominent effect was found for DMF. The effect of the addition of used solvents was not the same for all phthalocyanine compounds under study, owing to the different levels of their solvatation.

Both methods using DMF as solvent are highly selective for the singlet oxygen determination (no post-irradiation reaction occurs) and the values of quantum yields determined by both DMA and DPIBF methods are nearly the same. The order of the photodynamic activity of the studied phthalocyanines determined from the singlet oxygen production is **5** ∼ **4** > **7** ∼ **2** ∼ **8** (standard) > **6** > **3** ∼ **1**, where **5** represents the ammonium salt of disulphonated zinc phthalocyanine, **4** sulphonated zinc phthalocyanine [(3-diethylammonium)-propylsulphon-amide] citrate, **7** tetrakis(trimethylammonium)zinc phthalocyanine tetraiodide, **2** tetrakis(*N*-methylpyridinium-3-oxy)zinc phthalocyanine tetraiodide, **8** zinc phthalocyanine, **6** sodium salt of disulphonated aluminum phthalocyanine, **3** sulphonated aluminum phthalocyanine [(*N*-2-hydroxyethyl)aminoethylsulphonamide] and **1** tetramethylenepyridinium chloride of hydroxyaluminum phthalocyanine.

Acknowledgements

This research work was supported by the Ministry of Education, Youth and Sports (National Programme of Research II – Project No. 2B06104) and by the Grant Agency of the Academy of Sciences of the Czech Republic (grant KAN400720701).

References

[1] E. Ben-Hur, I. Rosenthal, The phthalocyanines: a new class of mammalian cells photosensitizers with a potential for cancer phototherapy, Int. J. Radiat. Biol. 47 (1985) 145–147.

[2] E.A. Lukyanets, Phthalocyanines as photosensitizers in the photodynamic therapy of cancer, J. Porphyrins Phthalocyanines 3 (1999) 424–432.

[3] A. Minnock, D.I. Vernon, J. Schofield, J. Griffiths, J.H. Parish, S.B. Brown, Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both gram-negative and gram-positive bacteria, J. Photochem. Photobiol. B: Biol. 32 (1996) 159–164.

[4] A.N. Vzorov, L.G. Marzilli, R.W. Compans, D.W. Dixon, Prevention of HIV-1 infection by phthalocyanines, Antiviral Res. 59 (2003) 99–109.

[5] P.F.C. Menezes, C. Bernal, H. Imasato, V.S. Bagnato, J.R. Perussi, Laser Phys. 17 (2007) 468–471.

[6] G.O. Schenck, Photosensitization, Ind. Eng. Chem. 55 (1963) 40–43.

[7] G.O. Schenck, E. Koch, Zwischenreaktionen bei photosensibilisierten Prozessen in Lösungen, Z. Elektrochem. 64 (1960) 170–177.

[8] C.S. Foote, in: W.A. Pryor (Ed.), Free Radicals in Biology, vol. II, Academic Press, New York, 1976, pp. 85–133.

[9] C.S. Foote, Mechanisms of photosensitized oxidation, Science 162 (1968) 963–970.

[10] A. Kepka, L.I. Grossweiner, Photodynamic oxidation of iodide ion and aromatic amino acids by eosin, Photochem. Photobiol. 14 (1972) 621–639.

[11] V. Kasche, L. Lindqvist, Transient species in the photochemistry of eosin, Photochem. Photobiol. 4 (1965) 923–933.

[12] M. Nemoto, Y. Usui, M. Koizumi, The occurrence of a D–D mechanism in the ethanol solution of eosine, Bull. Chem. Soc. Jpn. 40 (1967) 1035–1040.

[13] C. Balny, P. Douzu, Production of superoxide ions by photosensitization of dyes, Biochem. Biophys. Res. Commun. 56 (1974) 386–391.

[14] C.S. Foote, Photosensitized oxygenations and the role of singlet oxygen, Acc. Chem. Res. 1 (1968) 104–110.

[15] J. Mosinger, Z. Mička, Quantum yields of singlet oxygen of metal complexes of meso-tetrakis(sulphonatophenyl)porphine, J. Photochem. Photobiol. A: Chem. 107 (1997) 77–82.

[16] I. Kraljic, S. El Mohsni, A new method for the detection of singlet oxygen in aqueous solutions, Photochem. Photobiol. 28 (1978) 577–581.

[17] I. Scalise, E.N. Durantini, Synthesis and photodynamic activity of tetracationic and non-charged zinc phthalocyanine derivatives in homogeneous and biological media, 7th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-7), http://www.mdpi.net/ecsoc-7, 1–30 November 2003.

[18] J. O'Neill, M. Wilson, M. Wainwright, Comparative antistreptococcal activity of photobactericidal agents, J. Chemother. 15 (2003) 329–334.

[19] P.P.S. Lee, P.-Ch. Lo, E.Y.M. Chan, W.-P. Fong, W.-H. Ko, D.K.P. Ng, Synthesis and in vitro photodynamic activity of novel galactose-containing phthalocyanines, Tetrahedron Lett. 46 (2005) 1551–1554.

[20] J. Griffiths, J. Schofield, M. Wainwright, S.B. Brown, Some observations on the synthesis of polysubstituted zinc phthalocyanine sensitisers for photodynamic therapy, Dyes Pigments 33 (1997) 65–78.

[21] S. Gaspard, T.-H. Tran-Thi, A new method for the synthesis of zinc tetrakis(3-methylpyridyloxy)phthalocyanine and the physical properties of dimers formed by complexation of the cationic phthalocyanine with anionic porphyrins, J. Chem. Soc., Perkin. Trans. 2 (1989) 383–389.

[22] S.B. Brown, A.L. Bell, J. Griffiths, J. Schofield, Photosensitisers, World Patent WO 02/24226 A2 (2002).

[23] M. Karásková, J. Rakušan, S. Nešpůrek, K. Palewska, J. Sworakowski, Light-induced effects in sulfonated aluminum phthalocyanines photosensitizers in the photodynamic therapy, in: Moscow, Russia, 6–11 July, Fifth International Conference on Porphyrins and Phthalocyanines, 2008, p. 410 (book of abstracts).

[24] B.N. Achar, K.S. Lokesh, Studies on tetra-amine phthalocyanines, J. Organomet. Chem. 689 (2004) 3357–3361.

[25] H. Uchida, H. Tanaka, H. Yoshiyama, P.Y. Reddy, S. Nakamura, T. Toru, Novel synthesis of phthalocyanines from phthalonitriles under mild conditions, Synlett 10 (2002) 1649–1652.

[26] J. Mosinger, B. Mosinger, Photodynamic sensitizers assay: rapid and sensitive iodometric measurement, Experientia 51 (1995) 106–109.

[27] E.A. Moelwyn-Hughes, The Kinetics of Reactions in Solution, Clarendon Press, Oxford, 1947, p. 153.

[28] B. Stevens, B.E. Algar, The photoperoxidation of unsaturated organic molecules. I. Relaxation and oxygen-quenching parameters of the sensitizer singlet state, J. Phys. Chem. 72 (1968) 2582–2587.

[29] B. Stevens, B.E. Algar, The photoperoxidation of unsaturated organic molecules. II. The autoperoxidation of aromatic hydrocarbons, J. Phys. Chem. 72 (1968) 3468–3474.

[30] E.A. Mayeda, A.J. Bard, The production of singlet oxygen in electrogenerated radical ion electron transfer reactions, J. Am. Chem. Soc. 95 (1973) 6223–6226.

[31] J. Olmsted, T. Akashah, Photooxidation of isobenzofurans. A dual mechanism process, J. Am. Chem. Soc. 95 (1973) 6211–6215.

[32] A. Le Berre, R. Ratsimbazafy, Sur l'autoxydation du diphénylisobenzofuranne, Bull. Soc. Chim. France (1963) 229–230.

[33] R.B. Ostler, A.D. Scully, A.G. Taylor, I.R. Gould, T.A. Smith, A. Waite, D. Phillips, The effect of pH on the photophysics and photochemistry of di-sulphonated aluminum phthalocyanine, Photochem. Photobiol. 71 (2000) 397–404.

[34] Y.-C. Yang, J.R. Ward, R.P. Seiders, Dimerization of cobalt (II) tetrasulfonated phthalocyanine in water and aqueous alcoholic solutions, Inorg. Chem. 24 (1985) 1765–1769.

[35] A.W. Snow, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), Phthalocyanine Aggregation, The Porphyrin Handbook, vol.17, Academic Press, San Diego, 2003, p. 168.

[36] D.R. Adams, F. Wilkinson, Lifetime of singlet oxygen in liquid solution, J. Chem. Soc., Faraday Trans. 2 (1972) 586–594.

[37] J. Zhou, W. Jian, Ch. Naisheng, H. Jinling, Substituted number and solvent effects on the aggregation of b-sulfonate-substitutes ZnPc, in: Fifth International Conference on Porphyrins and Phthalocyanines, Moscow, Russia, 6–11 July, 2008, p. 405 (book of abstracts).

[38] A.K. Gupka, K.K. Rohatgi-Mukherjee, Solvent effect on the photosensitized oxidation of iodine ion by anthracene sulphonates, Photochem. Photobiol. 27 (1978) 539–543.