Photophysical Properties of Sodium zinc(II)-2,9,16,23-phthalocyanine Tetracarboxylate in Aqueous Solution

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Abstract

A photosensitizer as potential anticancer drug of sodium zinc(II)-2,9,16,23-phthalocyanine tetracarboxylate (ZnPc(COONa)₄) was investigated by means of steady-state spectroscopies in aqueous solution. The photosensitizer was found to be of far red-absorbing photosensitizer with a considerable singlet oxygen quantum yield (ΦΔ = 0.28). Upon illumination ZnPc(COONa)₄ could be considered a stable with photobleaching constant of 1.8×10⁻³cm²J⁻¹. Its photodynamic dose was found to be 1389 Jcm⁻². The mechanism for the photodegradation of ZnPc(COONa)₄ involves both singlet oxygen and radicals (mechanism of type II and type I).

Keywords: Fluorescence, Free radicals, Absorption, Phthalocyanine, Photobleaching.

Introduction

Metallophthalocyanines (MPcs), a family of aromatic macrocycles based on delocalized 18π electron system, have been intensively investigated due to their
unique catalic, optical, electronic, and structural properties [1]. The growing interest in the applications of phthalocyanines in a variety of new technological fields includes (but not limited) such as, electrochromic display devices [2], liquid crystals[3], semiconductor devices [4], Langmuir-Blodgett films [5], and as photosensitizers in photodynamic therapy (PDT) of cancer [6]. PDT is based on toxic reactions that follow light activation of the photosensitizer. The main process always includes the absorption of light by the sensitizer in the spectral range between 600–870 nm. After absorption of a photon, a transition of the photosensitizer occurs from the ground state (S0) to the first excited singlet state (S1), which can, besides radiative and thermal pathways, deactivates by intersystem crossing, produce the population of the long-lived first excited triplet state (T1) [7]. From T1 either electron transfer (type I reaction) or energy transfer to molecular oxygen (type II reaction) can occur. As a consequence, cytotoxic species, primarily activated oxygen such as, hydroxyl radical (OH•), superoxide anion (O2−), and singlet molecular oxygen (1O2) are generated. A type III reaction without participation of oxygen may occur under special conditions such as under an aerobic conditions or at large acceptor concentrations. The radical and non-radical species of oxygen lead to tumor destruction [6–10].

Incorporation of substituents of MPcs in their peripheral and non-peripheral positions can modify their chemical and physical properties. For instance, the incorporation can increase the π-electron density and makes solvation easier [11, 12]. Tetra-substituted phthalocyanines are normally more soluble than octa-substituted phthalocyanines because of the formation of constitutional isomers and the strong dipole moment that yields from the unsymmetrical non-transition metals such as zinc in the ring centre of the phthalocyanine (Pc) results in complexes with high intersystem crossing quantum yields and comparable long-triplet lifetimes, which are a demand for efficient photosensitization [12–14]. Since an ionic or cationic groups such as sulfonic, carboxylic, phosphoric or quaternized amino groups make MPcs water soluble, MPcs are particularly interesting [15–18].

Some cationic phthalocyanines have been reported to selectively attack sites in cells, demonstrating their significance in cancer necrosis and photoinactivation of bacteria [19–23]. Hydrophobic phthalocyanines have been found to have a higher tumor selectively, but they are accompanied with skin phototoxicity [24]. Water soluble phthalocyanines can be injected directly into the bloodstream, despite their aggregation tendency in hydrous medium; they are still considered the best targets of photosensitizers [25–27].

Upon illumination most photosensitizers applied in biomedical studies are photodegraded. In this process, a decrease in the absorption and fluorescence is observed. The photobleaching of certain compounds of porphyrins and phthalocyanines, have been reported in vitro and in vivo [28, 29]. It was shown in these studies that the photosensitizers photobleach at different rates in solutions, cells and tissues. In this context, it has been observed the photobleaching of Photofrin II in patients undergoing photosensitized tumor therapy [30].

When photobleaching the photosensitizer is probably attacked by the singlet oxygen 1O2 (1Σg−) it produces, besides free radicals which may also play a role [31]. This compilation is expected to establish for the significance of singlet oxygen
reactions in the photobleaching of dyes, polymers, pigments, etc, as well as in harmful and/or beneficial photo-oxidations in biological system [32].

A promising second-generation compound, sodium zinc(II)-2,9,16,23-phthalocyanine tetracarboxylate (ZnPc(COONa)₄) was found to have absorption peak at 670 nm [33]. ZnPc(COONa)₄ has several advantages: it is chemically pure compound; can be excited to the first excited-singlet state (S₁) by longer wavelength resulting in high tissue penetration which is a requirement for the photosensitizer to be applied in the photodynamic therapy [34–38].

The aim of the present work was to study the photophysical properties of ZnPc(COONa)₄, solved in water:NaOH solution to serve as anticancer drug. The illumination influence on the physical-chemical photobleaching of ZnPc(COONa)₄ was also investigated.

Materials and methods
Materials
9, 10 dimethylanthracene (DMA) and pyropheophorbide methyl ester (PPME) were purchased from Sigma-Aldrich. Sodium zinc (II)-2,9,16,23-phthalocyanine tetracarboxylate (ZnPc(COONa)₄) was prepared according to the protocol in reference [33]. As received, the compounds were used and kept in the dark at low temperature. PPME was taken as a reference compound. The structural formula of ZnPc (COONa)₄ is shown in Fig. 1.

Absorption spectroscopy
The electronic ground-state absorption spectra were recorded using spectrophotometer Shimadzu UV-1700 employing quartz cells (10×10 mm path length) at room temperature. The increment step of the measurement was 0.1 nm.

Steady-state fluorescence spectroscopy
The steady-state fluorescence spectra were collected on JASCO-FP 6500 spectroscopy using fluorescence quartz cell (1 cm×1 cm path length) at room
temperature. The parameters were constant for each sample enabling a maximum intensity of less than 21,000 counts.

**Photobleaching measurements**

Samples of 2.5 ml placed in 1×1 cm quartz cell were illuminated to produce photobleaching using He-Ne laser (emission wavelength: 632 nm; light irradiance: 23 mWcm⁻²). The steady-state fluorescence was detected perpendicularly to the direction of excitation in an L-shaped setup. The concentration was set to be 6 μM at the excitation wavelength of 632 nm for all samples of ZnPc(COONa)₄ and PPME. The steady state fluorescence spectra were recorded frequently for 125 minutes to observe the photobleaching. The absorption spectra were registered before and after exposure. The fluorescence bleaching constant $K_{Fl}$ of the investigated sensitizers can be estimated by the following formula [39]

$$\frac{\Delta F(t)}{F_0} = -K_{Fl}l(t)OD_{exc}$$  \hspace{1cm} (1)

where $F(t)$ is the integrated area under the fluorescence spectrum at an exposure time of $t$, $F_0$ is the integrated area under the fluorescence spectrum at an irradiated time of zero, $l(t)$ is the light intensity absorbed by the photosensitizer, and $OD_{exc}$ is the optical density at the excitation wavelength.

**Singlet oxygen measurements**

DMA which is specific singlet oxygen-scavenging agent, with a concentration of 0.6 mM and absorbance at wavelength of 402 nm was followed by absorption spectroscopy to calculate the singlet oxygen $^{1}O_2$ quantum yield ($\phi_A$) produced by the triplet excited state of ZnPc(COONa)₄ (type II). The absolute value of the singlet oxygen quantum yield of ZnPc(COONa)₄ ($\phi_A^{ZnPc(COONa)₄}$) in inhomogeneous medium of water: 0.1M NaOH was estimated using PPME as standard ($\phi_A^{PPME}$ is 0.19 in DMF) [39]. In the presence of DMA, $^{1}O_2$ generated by the triplet state of the photosensitizer undergoes several decay processes upon continuous stationary irradiation. The bleaching constant ($K$) is the rate at which DMA absorbance is consumed by $^{1}O_2$. Thus, we can write [1–3, 6]

$$K = (1-10^{\text{OD}})I_{exc}\phi_A K_\Lambda$$

where $(1-10^{\text{OD}})I_{exc}$ stands for the total absorbed photons by ZnPc(COONa)₄ or PPME at the excitation wavelength, and $K_\Lambda$ is the total deactivation processes (physical and chemical) of $^{1}O_2$. $I_{exc}$ is identical for ZnPc(COONa)₄ and PPME in the same setup. $K_\Lambda$ should be the same for ZnPc(COONa)₄ and PPME because of the similar conditions. Hence, $\phi_A^{ZnPc(COONa)₄}$ can be obtained by rearrangement Eq. (2):

$$\phi_A^{ZnPc(COONa)₄} = \phi_A^{PPME} \frac{1-10^{\text{OD}_{ZnPc(COONa)₄}}}{1-10^{\text{OD}_{PPME}}} \frac{K_{ZnPc(COONa)₄}}{K^{PPME}}$$

where respectively, $OD_{ZnPc(COONa)₄}$ and $OD^{PPME}$ are the optical densities of ZnPc(COONa)₄ and PPME. Respectively, $K_{ZnPc(COONa)₄}$ and $K^{PPME}$ are the kinetic slopes of DMA disappearance photosensitized by ZnPc(COONa)₄ and PPME.
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Results
Absorption spectra
The absorption spectrum of substituted and unsubstituted phthalocyanines, as generally accepted, can be divided into different regions: the Q region located above 600 nm, the Soret or B region centered around 300 nm, and the C, L and N regions below 280 nm [40]. In this paper, we discuss the Q and B regions. The electronic absorption spectra of ZnPc(COONa)₄ is shown in Fig. 2. As clear, the shape of the absorption spectrum resembles those of other phthalocyanine derivatives with two strong absorption vibronic B-bands: B2 band centered at 279 nm (Table 1) with molar extinction coefficient of $\varepsilon_{279\text{nm}} = 2.41 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ and B1 at 342 nm with molar extinction coefficient of $\varepsilon_{342\text{nm}} = 1.58 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$. The other maxima of Q bands have less extinction coefficients with peaks centered at 641 nm (Q₂ band) and 691 nm (Q₁ band) with, respectively (Table 1), molar extinction coefficient of $\varepsilon_{641\text{nm}} = 1.81 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ and $\varepsilon_{691\text{nm}} = 1.31 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$. Solutions of ZnPc(COONa)₄ followed the Beer-Lambert law up to 28 μM pointing out that under such conditions the molecule is monomeric.

![Normalized absorption](image)

**Figure 2**: Electronic absorption spectrum of ZnPc(COONa)₄ in inhomogeneous solution of water: 0.1 M NaOH at room temperature.

**Table 1.** Ground state absorption of Q and B bands of ZnPc(COONa)₄ in a solution of water: 0.1 M NaOH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Q₁/nm</th>
<th>Q₂/nm</th>
<th>B₁/nm</th>
<th>B₂/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnPc(COONa)₄</td>
<td>691</td>
<td>641</td>
<td>341</td>
<td>278</td>
</tr>
</tbody>
</table>

Fluorescence
The fluorescence spectrum of ZnPc(COONa)₄ is shown in Fig. 3. The shape of the fluorescence spectrum of the molecule is similar to those of phthalocyanines. The maximum peak is at $\lambda_{\text{max}} = 702$ nm (see, Fig. 3). The estimations have shown that the fluorescence quantum yield ($\Phi_F$) is 0.07.
Figure 3: Fluorescence emission spectrum of ZnPc(COONa)$_4$ in inhomogeneous solution of water: 0.1 M NaOH at room temperature. Excitation wavelength ($\lambda_{exc}$) = 350 nm.

Photobleaching measurements
Upon irradiation of ZnPc(COONa)$_4$ molecules in water:NaOH solution a reduction in the absorption and fluorescence throughout the spectra occurs with no new band appearance or band shift over whole the registered spectral regions. The reduction is monotonic with increasing the illumination time. The bleaching constant was calculated to be $K_{Fl} = 1.8 \times 10^{-3} \text{ cm}^2 \text{J}^{-1}$ as presented in Fig. 4 and Table 2.

Figure 4: The kinetic fluorescence bleaching of ZnPc(COONa)$_4$ in water:NaOH at room temperature. The solid line is the fitted curve.

Table 2. Photobleaching parameters of ZnPc(COONa)$_4$ in a solution of water: 0.1M NaOH.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Phi$</th>
<th>$K_{Fl} \times 10^{-3}$</th>
<th>$K \times 10^3$</th>
<th>$PD$</th>
<th>$PD_{ZnPc(COONa)4}$</th>
<th>$T_{1/2}$</th>
<th>$T_{PPME}^{2/1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ZnPc(COONa)$_4$</td>
<td>0.28</td>
<td>1.8</td>
<td>2</td>
<td>1389</td>
<td>2.8</td>
<td>347</td>
<td>100</td>
</tr>
<tr>
<td>PPME</td>
<td>0.19</td>
<td>5</td>
<td>7</td>
<td>500</td>
<td>2.8</td>
<td>347</td>
<td>100</td>
</tr>
</tbody>
</table>

Normalized fluorescence

Wavelength (nm)

-0.2
-0.1
0.0
1.0

Illumination dose (J/cm$^2$)

-0.2
-0.1
0.0
Singlet oxygen generation
The singlet oxygen quantum yield of ZnPc(COONa)$_4$ (φ$_{Δ}$) was evaluated in water:NaOH solution. The used singlet oxygen-scavenger was DMA. The bleaching kinetics of the experimental data of DMA is linear. The singlet oxygen quantum yield of ZnPc(COONa)$_4$ (φ$_{Δ}$) was obtained by comparing the slope of DMA in the presence of ZnPc(COONa)$_4$ to the slope in the presence of PPME. Applying Eq. (3), φ$_{Δ}$ was calculated to be 0.28 (Table 2) with relative error of 2%.

Discussion
The carboxylate of ZnPc(COONa)$_4$ molecules significantly increases its solubility in polar solvents[33], therefore, ZnPc(COONa)$_4$ is highly water-soluble, which allows its intravenous administration without the need for alternative delivery systems. In contrast to the known solubility of the substituted MePs, ZnPc(COONa)$_4$ is insoluble in most organic solvents except in the inhomogeneous solution of water:NaOH. The incorporation of carboxylate to Pc prophetically, not only increases the solubility of these phthalocyanines in aqueous media, but also prevents their molecular aggregation, yielding superior photophysical properties [10]. Fig. 2 shows the absorption spectra of ZnPc(COONa)$_4$ in water:NaOH. Compared to metal-free phthalocyanines, the absorption of ZnPc(COONa)$_4$ exhibits a strong bathochromic shift of the Q-band transition which probably is due to the fourth butyloxy groups bound via the oxygen at the α-positions of the Pc. In this case, the π-system is enlarged by the electron-donating substituents. As a consequence, a strong red shift of the Q band occurs [1, 15]. This spectral shift forms an excellent therapeutic window as a demanded by photodynamic therapy. The presence of the metal gives higher efficiency of light absorption and a subsequent photosensation [33, 34] as noticed from the high value the extinction coefficients of Q and B bands (see Fig. 2). It also increases the symmetry of the compound and hence it is observed the existence of only two Q bands centered at $Q_1 = 691$nm and $Q_2 = 641$nm (Table 1), respectively. The wavelength of 691 is favorable in PDT due to the high penetration of the light in tissues at longer wavelengths. Moreover, Pcs are with optical properties similar to the porphyrins, but in contrast to them they have an allowed Q transition and, thus, an absorption transition of Q band in the red spectral region around 660 nm [11, 13, 41]. In addition, it was reported that the number of Q-bands in the absorption spectra of some Pcs was found to be solvent-dependent and it decreases with increasing its polarity [42].

The fluorescence spectra of mononuclear ZnPc(COONa)$_4$ is shown in Fig. 3. It was found that the shape and spectral position of the fluorescence bands are independent of the excitation wavelength. It is worthy to notice that the fluorescence of ZnPc(COONa)$_4$ is red-shifted with relatively weak fluorescence signal having maximum at 708 nm. The low value of fluorescence quantum yield of the ZnPc(COONa)$_4$ (φ$_F$ = 0.07 ) should be well correlated with enhancing the nonradiative transition of internal conversion (IC) and intersystem crossing (ISC).
As well known, energy transfer between the triplet state of the photosensitizer and ground state of molecular oxygen (type II) leads to the generation of singlet oxygen $\left( \Phi_\Delta \right)$ [3, 4, 6]. Therefore, singlet oxygen quantum yield which is the efficiency of singlet oxygen generation should depend on the intersystem crossing quantum yield of the triplet state and its lifetime as well as on the efficiency of energy transfer [43]. In turn, it depends on the energy of the triplet state and the ability of the substituents to quench the triplet state among other factors [44].

The presence of zinc as the central metal ion gives some interesting characteristics such as short triplet lifetime, high triplet quantum yield, and high singlet oxygen quantum yield [1, 4]. These aspects of course results in increasing its photoactivity [45]. Moreover, the type of metal ion chelated within a phthalocyanine has been shown to have considerable effect on the tumor retention efficiency of the Pc [13]. These findings are enhanced by the considerable value of singlet oxygen of ZnPc(COONa)$_4$ which was estimated to be 0.28 in aqueous solution of NaOH. The high value of singlet oxygen quantum yield enhances the belief that the aggregation for the current investigated molecule is absent attributed to the fact that the aggregation of photosensitizers is often cited as a reason for the low singlet oxygen yields. This is important since aqueous medium tends to promote such aggregation, especially in the case of planar molecules. The situation may be ameliorated by the incorporation of carboxylate substituents as in ZnPc(COONa)$_4$. Similar findings were also reported [12]. Nevertheless, this value still, in general, smaller than those of MPcs which dissolved in solvents with lower viscosities. Probably, this is because as well known that the molecular oxygen has a lower diffusion coefficient in a solvent with a higher viscosity (like in the current study) [46], and hence a less accessibility of molecular oxygen to the triplet state of ZnPc(COONa)$_4$. In addition, the polar solvents are known to enhance the radiationless transitions of the internal conversion and the intersystem crossing transition, and hence they are in competition. The considerable value of photoinduced singlet generation in a polar solvent should indicate a high probability of ISC. This is consistent with the fact that, in general, closed shell diamagnetic ions, such as Zn$^{2+}$, Ga$^{3+}$ and Al$^{3+}$ give the phthalocyanine complexes high singlet oxygen quantum yields due to a high value of the product of triplet quantum yield (as a result of spin-orbital coupling or so-called heavy atom effect) and triplet lifetime [47]. This also could explain the considerable value of singlet oxygen that the ZnPc(COONa)$_4$ in the current study has (\(\Phi_\Delta = 0.28\)).

From the above discussion, it seems that ZnPc(COONa)$_4$ has convenient features that candidate it to be a therapeutic second generation photosensitizer from the point of view of its potential application in PDT of cancer. Furthermore, the structure of ZnPc(COONa)$_4$ is charged, hence, there may be consequences of such charge in terms of hydrophilicity regarding the activity and possibly pharmacology.

During irradiation, the fluorescence quantum yield of the investigated molecules is reduced after 125 minutes. The absorbance or fluorescence decreases monotonically with increasing the illumination time. Respectively, the fluorescence quantum yields were 41% and 30% less than their initial values for ZnPc(COONa)$_4$ and PPME, respectively. It was not detected changes of the spectral position, shape of the absorption, and fluorescence bands. In addition, no recovery in the dark has been
detected when registering the absorption and fluorescence spectra after about three days. Based on these findings, the photobleaching of the investigated molecules generated as a result of illumination.

The kinetics of the photobleaching of ZnPc(COONa)₄ is presented in Fig. 4. The photobleaching efficiency of ZnPc(COONa)₄ is ²Fl/ZnPc(COONa)₄ = 1.8×10⁻³ cm²/J (Table 2). This value may be due to the presence of oxidative attack on the excited triplet state by singlet oxygen, since the triplet state is sufficiently long-lived to participate in photochemical reactions. As documented, the singlet oxygen has the ability to react with macrocyclic metal complexes [48]. On the contrary, the lack of the central Zn ion lowers the quenching constant [49, 50] which is probably attributed to less generation of singlet oxygen and other reactive species. These species attack the substituents and the macrocyclic of the sensitizer [6]. Hence, less generation of reactive species is less photobleaching efficiency and vice versa.

The significance of the photobleaching constants [6, 39] is that they can be used to calculate the dependency of the drug concentration on the illumination time applying the following equation

\[ [A] = [A]₀ \exp(-Kt) \quad (4) \]

where \([A]₀\) is the initial concentration of the sensitizer before illumination, and \(K\) (\(K_{\text{PPME}}\) or \(K_{\text{ZnPc(COONa)₄}}\)) is the decay constant of the photosensitizer deactivation. This constant can be formulated as [6]

\[ K = I(t) \times K_{F₁} \quad (5) \]

Using the value of \(K_{F₁}ZnPc(COONa)₄ = 1.8×10⁻³\) cm²/J for ZnPc(COONa)₄ and \(K_{F₁}PPME = 5×10⁻³\) cm²/J for PPME (Table 2) as well as taking into account that \(I(t)\) is 23 mWcm⁻², then Eq. (5) results in a value of \(K_{ZnPc(COONa)₄} = 0.002\) min⁻¹ for ZnPc(COONa)₄ and of \(K_{PPME} = 0.007\) min⁻¹ for PPME (Table 2). Substituting these values into Eq. (4), the drug concentration is monoexponentially decreases with illumination time and it is consumed faster in case of PPME compared with that of ZnPc(COONa)₄. This infers that ZnPc(COONa)₄ is more photostable. To quantify this behaviour, based on Eq. (4), we used the concept of the half life of the sensitizer (\(T_{1/2}\)), where the sensitizer concentration falls to its half-initial value after illumination:

\[ T_{1/2} = \frac{0.693}{K} \quad (6) \]

Thus, respectively, the half lives of ZnPc(COONa)₄ and PPME were calculated to be \(T_{1/2}ZnPc(COONa)₄ = 347\) min and \(T_{1/2}PPME = 100\) min (Table 2). The ratio between their half lives is about \(\frac{T_{1/2}ZnPc(COONa)₄}{T_{1/2}PPME} = 3.5\) (Table 2) indicating that under similar circumstances of irradiation, PPME is consumed faster than ZnPc(COONa)₄. For both compounds, the singlet oxygen quantum yield (\(Φ₄\)) has analogous trend: \(Φ₄ZnPc(COONa)₄ = 0.28 > Φ₄PPME = 0.19\). Since the photostability of the singlet oxygen-generated molecule generally depends on its singlet oxygen quantum yield, one should expect to have less photostability for ZnPc(COONa)₄ when compared to PPME. But, this is not the case
as we have seen from the photobleaching constants. Probably, the reason for this is that PPME was found to be attacked by singlet oxygen which generates as well as by other reactive species [39].

Since the generation of the superoxide anion ($O_2^-$, type I) should be expected in a polar solvent [27–30], there is a possibility that $O_2^-$ contributes besides singlet oxygen to the bleaching of ZnPc(COONa)$_4$. To further configure the photobleaching process of ZnPc(COONa)$_4$ by $O_2^-$, superoxide dismutase (SOD, bovine erythrocytes) was used as a quencher of superoxide anion radical ($O_2^-$) to investigate its effect on the photobleaching process of ZnPc(COONa)$_4$. With the introduction of SOD in the solution of ZnPc(COONa)$_4$, the photobleaching process was slightly prohibited. This infers that the superoxide anion radical mechanism may be also involved in the self sensitization of ZnPc(COONa)$_4$ (Fig. 5).

![Figure 5: The proposed routes for the photobleaching of ZnPc(COONa)$_4$ in a solution of water:NaOH.](image)

It seems reasonable to propose that upon illumination ZnPc(COONa)$_4$ in water:NaOH with visible light, the self-sensitized photooxidation of ZnPc(COONa)$_4$ by singlet oxygen yields a product of ZnPc(COONa)$_4$O$_2$ which seems to be unstable at room temperature with the regeneration of ZnPc(COONa)$_4$ itself (shown in Fig. 5). This result is based on the fact that when ZnPc(COONa)$_4$ underwent self-sensitized photooxidation to form the product of ZnPc(COONa)$_4$O$_2$, the absorption spectrum of ZnPc(COONa)$_4$ decreased. Once the light was turned off, the product of ZnPc(COONa)$_4$O$_2$ underwent principally a loss of molecular oxygen with regeneration of ZnPc(COONa)$_4$ itself gradually, leading to increase the intensity of absorption spectrum and recovering that of ZnPc(COONa)$_4$. This infers that the singlet oxygen was released from the photoformed product of ZnPc(COONa)$_4$O$_2$. Moreover, the absorption intensity of ZnPc(COONa)$_4$ in water:NaOH did not recover completely to that seen before illumination. This could be understood if we assume that the superoxide anion is existent in the aqueous solution of NaOH and it reacts with ZnPc(COONa)$_4$ leading to formation the product ZnPc(COONa)$_4$O$_2$ (Fig. 5). Thus, this product contributes to the photobleaching of ZnPc(COONa)$_4$ and it seems that it does not regenerate ZnPc(COONa)$_4$. This conclusion is based on the finding...
that after turning off the light for long time the absorption spectrum did not retrieve its initial optical density before illumination.

To calculate the photodynamic dose ($PD$) [50] we define relative critical photobleaching as the dose needed to obtain the half maximum value of fluorescence bleaching ($\frac{\Delta F}{F_0} = 0.50$) (see, Eq. (2)). Based on this definition, the photodynamic dose of $PD = 1389 \text{ Jcm}^{-2}$ (Table 1) is needed for ZnPc(COONa)$_4$ to bleach a fifty percent of its initial fluorescence intensity. Whereas, for PPME it is required much less photodynamic dose of $PD = 500 \text{ Jcm}^{-2}$ (Table 2) to bleach the same ratio of the fluorescence intensity. This infers that, under similar conditions of illumination, the photobleaching life time of ZnPc(COONa)$_4$ is about 2.8 times longer than that of PPME. The longer photobleaching lifetime of ZnPc(COONa)$_4$ is advantage because the cancerous tissue needs a less amount of photosensitizers (drugs); hence a less damage for the healthy tissue.

For a photosensitizer with a bleaching constant of $K_{Fl}$ ($K_{Fl}^{\text{ZnPc(COONa)}} = 1.8\times10^{-3} \text{ cm}^2/\text{J}$ or $K_{Fl}^{\text{PPME}} = 5\times10^{-3} \text{ cm}^2/\text{J}$) a simple definition of the photodynamic dose ($PD$) was applied [51]

$$PD = \frac{[A]_0}{K_{Fl}}\left[1 - \exp\left(-I K_{Fl}\right)\right] \quad (7)$$

where, $[A]_0$ is the initial concentration of the photosensitizer ($\mu$gkg$^{-1}$) at zero illumination time, and $I$ is the incident light dose in unit of Jcm$^{-2}$. Fig. 6 shows for ZnPc(COONa)$_4$, the dependence of the photodynamic dose on the incident intensity according to Eq. (7). As seen, it describes an exponential behaviour and the curvature trends to be a linear due to the small bleaching constant. Furthermore, the curve is fitted linearly giving a bleaching constant of $2\times10^{-3} \text{ cm}^2/\text{J}$ which is very close to the found one ($K_{Fl}^{\text{ZnPc(COONa)}} = 1.8\times10^{-3} \text{ cm}^2/\text{J}$). This result is in accordance with other works that for a small photobleaching constant less than $0.01 \text{ cm}^2/\text{J}$ the relationship between the photodynamic dose and light dose is a linear [17, 30]. Furthermore, Eq. (7) can be applied safely regardless of the value of the photobleaching constant whether it is small or large. In this frame, it was reported that under hypoxic conditions with a bleaching constant of $23\times10^{-3} \text{ J}^{-1}\text{cm}^2$, the photobleaching of protoporphyrin IX was fitted according to monoexponential decay, referring to a first order photobleaching mechanism [6]. Moreover, based on Eq. (7), it is required an illuminated light dose of $385 \text{ J/cm}^2$ for the photodynamic dose of ZnPc(COONa)$_4$ to decay to its half initial value. Due to fact that the molecular oxygen is more soluble in the lipid membrane than in the aqueous environment, this photobleaching should be more rapid in vivo [52], which means that more singlet oxygen is generated. After generation, the singlet oxygen rapidly begins the production of lipid peroxides in membrane, leading to autocatalytic propagation of peroxidative reactions in the presence of molecular oxygen [52]. Consequently, in vivo, the peroxidative reactions could have a role in the oxidative destruction of ZnPc(COONa)$_4$. It is worthy to mention that the bleaching should proceed essentially through reactions involving singlet oxygen when the initial concentration of sensitizer is high. As the concentration of the photosensitizer is
reduced, photosensitizer triplet reactions would also play an increasingly important role.

Like some photosensitizers [53], it seems that the photobleaching of ZnPc(COONa)$_4$ could be used as a ruling basis for a singlet oxygen dose. This is because the photobleaching kinetics are explicitly related to singlet oxygen production as they were previously demonstrated that they can be used to calculate singlet oxygen dose in vitro [53]. Thus, ZnPc(COONa)$_4$ could be useful as possible metric singlet oxygen dose.

\[\text{Figure 6: For ZnPc(COONa)$_4$, the dependence of the photodynamic dose (PD) on the incident light dose (I) in a solution of water:NaOH at room temperature. The solid line is the fitted curve.}\]

**Conclusions**

We studied photophysical properties of peripherally sodium zinc(II)-2,9,16,23-phthalocyanine tetracarboxylate (ZnPc(COONa)$_4$) in aqueous medium of NaOH. The photosensitizer was found to be far red-absorbing photosensitizer with considerable singlet oxygen quantum yield. Our results showed that the photobleaching of ZnPc(COONa)$_4$ in water:NaOH was mainly through self-sensitized photooxidation by singlet oxygen besides the superoxide anion radical. In vitro, a relationship was quantified between the photobleaching constant and the photodynamic dose for the investigated molecule. The photodegradation of ZnPc(COONa)$_4$ is less efficient than that of the reference compound of PPME, but the photodynamic dose of ZnPc(COONa)$_4$ is greater than that of PPME. The photobleaching life time of ZnPc(COONa)$_4$ is about 2.8 times longer than that of PPME under similar conditions of illumination.

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References

Photophysical Properties of Sodium zinc(II)-2,9,16,23-phthalocyanine


