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Photodynamic applications of phthalocyanines

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Abstract

This paper reports the singlet oxygen has been detected under irradiation of red light while water-soluble sodium salt of sulfonated phthalocyanines ($MPc(SO_3Na)_4$) and organo-soluble tetrakis(2,9,16,23-*tert*-butyl) dysprosium bisphthalocyanines ($Dy(TBPc)_2$) are used as photosensitizers. Furthermore, the experimental results reveal that singlet oxygen can kill microorganism under photodynamic treatment.

Keywords: Phthalocyanine; Metallophthalocyanine; Microwave; Photosensitizer; Photodynamic

1. Introduction

Phthalocyanines have attracted much attention for many decades because they exhibit excellent photochemical properties [1,2]. They can be used in jet printing inks [3], catalysts [4], gas sensors [5] and photonic devices [6,7]. Recently, they are used as photosensitizers in the treatment of cancer [2,8,9] and intimal hyperplasia [10] for photodynamic therapy (PDT).

In photodynamic therapy (PDT), singlet oxygen is generated with appropriate kinds of photosensitizer and oxygen via several energy transfer steps [11]. It is found that phthalocyanines are better photosensitizers for PDT than others, such as porphyrins, naphthalocyanines, etc. They exhibit effective tissue penetration because of their chemical stability, photodynamic activity, and proper light absorption region [2]. Singlet oxygen has been known not only to be able to cause tumor necrosis [2] but also induce several biological reactions [12,13]. Nevertheless, the existence of singlet oxygen seems to be indefinite in the solution. The presence of singlet oxygen is essential in photodynamic therapy. In this paper, the existence of singlet oxygen has been investigated. Furthermore, we have also executed the qualitative and quantitative experiments with phthalocyanines to kill *Esherichia coli*, *Bacillus cereus*, and *Aurebacterium* sp. under photodynamic treatment.

2. Experimental

2.1. Preparation of sodium salt of sulfonated phthalocyanines ($MPc(SO_3Na)_4$, $M = H_2$, Zn, Cu, Co, AlCl)

Commercially available 50% aqueous 4-sulfophthalic acid solution (4 g, 8.12 mmol), urea (1.95 g, 32 mmol), and zinc chloride (0.55 g, 4.1 mmol) in the presence of ammonium chloride (1.56 g, 29 mmol) and ammonium molybdate (0.17 g, 0.14 mmol) as catalysts, were irradiated in a microwave oven at 560 W for 10 min. The mixture was then added into aqueous sodium hydroxide solution (100 ml, 10 wt.%), heated until boiling, and cooled to the room temperature. Finally, the solution was firstly poured into methanol (50 ml), and then *i*-propyl alcohol (100 ml) was added for precipitation. After drying, sodium salt of sulfonated zinc phthalocyanine (1.35 g) was obtained (yield: 68%). The same procedure was adopted in the preparation of the sodium salt of sulfonated copper, chloroaluminum, cobalt, and metal-free phthalocyanines, respectively, with appropriate catalysts. The experimental details and characteristic data of the prepared phthalocyanines are described in Table 1 (Scheme 1).

2.2. Preparation of tetrakis(2,9,16,23-tert-butyl) dysprosium bisphthalocyanines ($Dy(TBPc)_2$)

$Dy(TBPc)_2$ was synthesized from tetrakis(2,9,16,23-*tert*-butyl)phthalocyanines ($H_2(TBPc)$) by microwave irradiation

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Table 1
Sodium salt of sulfonated phthalocyanines, $MPc(SO_3Na)_4$, by microwave irradiation

Compound	Metal	Catalyst	Yield (%)	UV-Vis λ_{max} (nm)	MS: (*m*/*z*) (M^-)[a]	IR (KBr) (cm^{-1})
$ZnPc(SO_3Na)_4$	$ZnCl_2$	Ammonium chloride Ammonium molybdate	68	669, 636, 337[b] 676, 610, 344[c]	892.57	3439, 1664, 1646, 1612, 1572, 1553, 1535, 1505, 1469, 1450, 1413, 1399, 1332, 1192, 1107, 1037, 989, 912, 838, 805, 746, 698, 669, 647, 621, 470
$CuPc(SO_3Na)_4$	$CuCl_2$	Antimony Ammonium chloride, Ammonium molybdate	80 74	668, 631, 327[b] 672, 605, 341[c]	891.80	3439, 1664, 1649, 1598, 1553, 1535, 1505, 1469, 1450, 1432, 1399, 1336, 1192, 1152, 1115, 1070, 1034, 923, 901, 879, 835, 746, 698, 665, 635, 599, 565, 470
$ClAlPc(SO_3Na)_4$	$AlCl_3$	Antimony	66	676, 608, 342[b] 674, 609, 345[c]	889.74	3439, 1664, 1646, 1631, 1612, 1576, 1553, 1483, 1469, 1450, 1432, 1413, 1369, 1192, 1137, 1078, 1037, 838, 809, 724, 669, 624, 584, 545, 477, 418
$CoPc(SO_3Na)_4$	$CoCl_2$	Antimony Ammonium chloride, Ammonium molybdate	58 51	657, 626, 318[b] 661, 600, 326[c]	886.470	3439, 1664, 1631, 1590, 1553, 1480, 1465, 1443, 1432, 1410, 1384, 1196, 1155, 1129, 1074, 1037, 882, 831, 783, 753, 694, 635, 558, 481, 451, 407
$H_2Pc(SO_3Na)_4$	–	Ammonium chloride Ammonium molybdate, Antimony	72	668, 632, 335[b] 693, 658, 604, 340[c]	830.57	3439, 3322, 1664, 1646, 1631, 1612, 1572, 1553, 1535, 1517, 1505, 1450, 1369, 1336, 1153, 1030, 879, 746, 698, 617, 584, 473, 451

[a] The samples were soluble in the water. In the water, $MPc(SO_3Na)_4$ are ionized into $MPc(SO_3^-)_4$ and sodium ion (Na^+) so that $MPc(SO_3^-)_4$ can be detected by the mass spectrometry.
[b] In water.
[c] In aqueous ethanol solution (v/v = 1/1).

with the procedure published previously [14] and purified by column chromatography (silica gel, ethyl acetate/*n*-hexane = 1/5, w/w) (Scheme 2).

2.3. Generation of singlet oxygen under photodynamic treatment

Weldon and Ogilby [15] have reported that the singlet oxygen can be detected using absorption experiments with an infrared spectrometer at about 5200 cm^{-1}. The wavenumber, 5200 cm^{-1}, approximately equals to 1940 nm according to the question:

$$\lambda = \frac{1}{\bar{\nu}} \times 10^7 \quad (1)$$

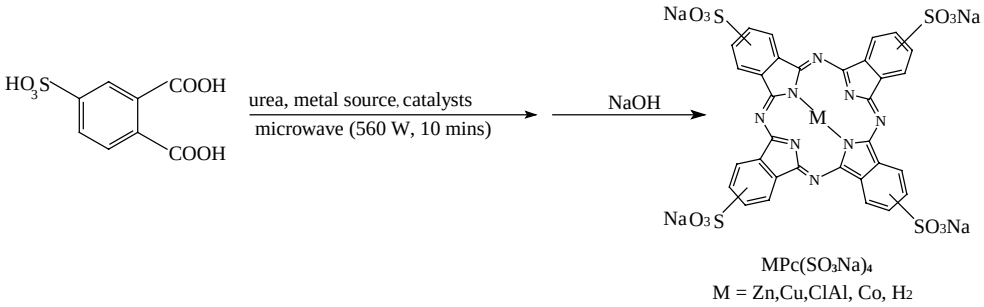
where λ represents the wavelength (nm) and $\bar{\nu}$ represents the wavenumber (cm^{-1}).

Therefore, we detected the singlet oxygen by another absorption instrument (UV-Vis/NIR spectrometer) at 1940 nm.

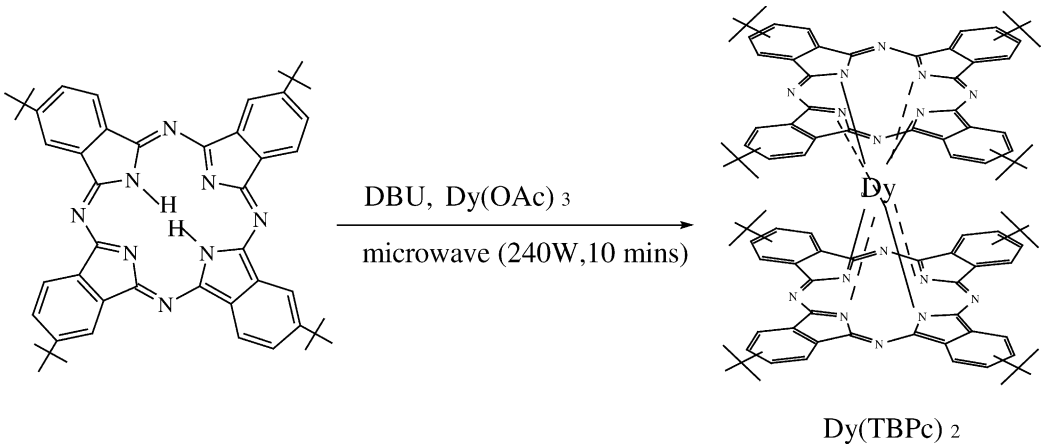
By the same procedure, however, we could not detect singlet oxygen in the case of aqueous $MPc(SO_3Na)_4$ solution due to the interference of water with UV-Vis/NIR absorption at the same position. Thus, we used lab-made $MPc(SO_3Na)_4$ as the photosensitizer in the oxidation of guanine into parabanic acid (Scheme 3) to demonstrate the existence of singlet oxygen.

2.3.1. Generation of singlet oxygen for $Dy(TBPc)_2$

Under irradiation by a 300 W halogen lamp (Saturn Co.) with a filter of red light (650 nm) for 5 min, we have measured the UV-Vis/NIR spectrum of $Dy(TBPc)_2$ in ethanol solution by a UV-Vis/NIR spectrometer (Perkin-Elmer



Scheme 1.



Scheme 2.

UV-Vis/NIR Spectrometer Lambda 19) to demonstrate the existence of singlet oxygen. The concentration of $Dy(TBPc)_2$ in ethanol solution was 5×10^{-6} M.

2.3.2. Generation of singlet oxygen for $MPc(SO_3Na)_4$

2.3.2.1. General procedure for oxidation of guanine into parabanic acid. Guanine (0.5 g, 3.1 mmol), $MPc(SO_3Na)_4$ (0.2 g), and water (50 ml) were aerated with oxygen for 20 min and stirred at room temperature. After irradiated by a 300 W halogen lamp (Saturn Co.) with a filter of red light (650 nm) for 5 min, the crude product was washed with water and purified by recrystallization with ethanol. After drying, parabanic acid (0.24 g) was obtained (yield: 63%). Its characterization data are identical with those of literature reported previously [16].

IR (KBr) (cm^{-1}): 3042, 2707, 2487, 1974, 1831, 1820, 1786, 1766, 1600, 1419, 1376, 1343, 1334, 1321, 1116, 994, 803, 762, 731, 703, 662, 596, 485, 428. MS: (*m*/*z*) (M^+) 115 (Scheme 3).

2.4. Quantum yield of singlet oxygen

The quantum yield of singlet oxygen for $Dy(TBPc)_2$ can be calculated from the following [17]:

$$\Phi(\text{sample}) = \Phi(\text{standard})\frac{S(\text{sample})}{S(\text{standard})} \quad (2)$$

where Φ represents the quantum yield and S represents the slope of the bleaching of the probe absorbance with irradiation time.

The standard compound used in the experiment was Rose bengal and Φ (Rose Bengal) was 0.76 [17].

2.5. The qualitative experiment for E. coli

Because *E. coli* can produce β-glucurinidase to decompose 4-methyl-umbelliferyl-β-D-glucuronide (MUG enzyme) [17], the cultural solution emits blue fluorescence (450–500 nm) when it is excited by the ultraviolet of 366 nm. Therefore, we fostered *E. coli* with MUG enzyme at 37 °C for 24 h. After adding the aqueous ethanol solution of $Dy(TBPc)_2$ (1000 ppm in ethanol/water = 95/5, w/w solution) into the cultural solution, the fluorescent spectrum was measured while it was excited by the ultraviolet of 366 nm.

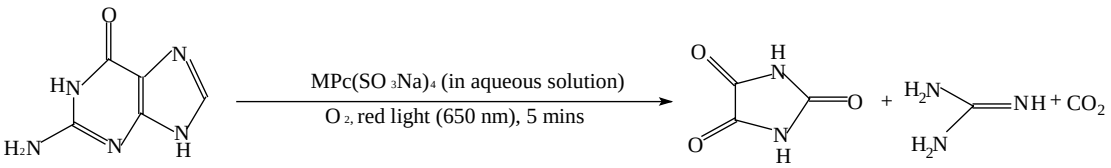
2.6. The quantitative experiment for B. cereus and Aurebacterium sp.

2.6.1. The blank experiment

B. cereus and *Aurebacterium* sp. were cultured in plate count agar (PCA, Difco Co.) at 30 °C. The cultural periods of *B. cereus* and *Aurebacterium* sp. were 24 and 48 h, respectively.

2.6.2. The photodynamic experiment

The same procedure for the blank experiment was used in the photodynamic experiment except for addition of the aqueous ethanol solution of $Dy(TBPc)_2$ (0.1 wt.% in ethanol/water = 95/5, w/w solution) or the aqueous solution of $MPc(SO_3Na)_4$ (0.1 wt.%) under irradiation of red light for 5 min.



Scheme 3.

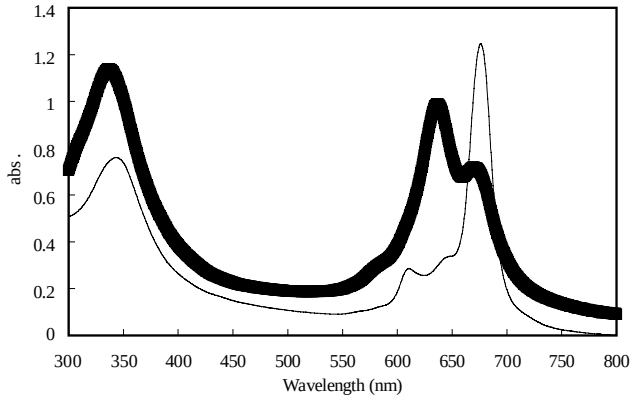


Fig. 1. The UV-Vis spectrum of $ZnPc(SO_3Na)_4$. Thick line: in water; thin line: in aqueous ethanol solution (v/v = 1/1).

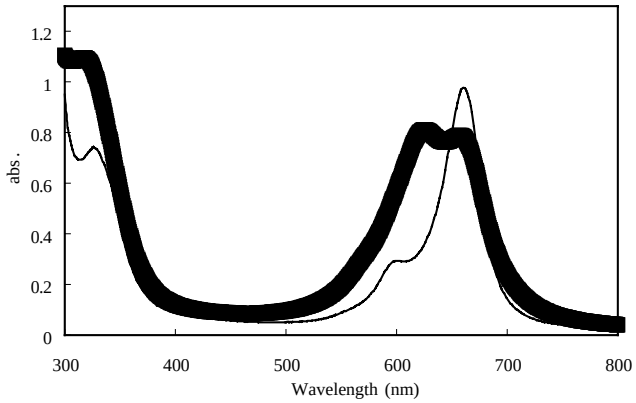


Fig. 3. The UV-Vis spectrum of $CoPc(SO_3Na)_4$. Thick line: in water; thin line: in aqueous ethanol solution (v/v = 1/1).

The death rate was calculated form the following:

$$\text{death rate (\%)} = \left[1 - \frac{\text{the tribal numbers of germ for the photodynamic experiment}}{\text{the tribal numbers of germ for the blank experiment}}\right] \times 100 \quad (3)$$

3. Results and discussion

3.1. Water-soluble $MPc(SO_3Na)_4$

In order to improve the solubility of phthalocyanines in water, this study introduces four hydrophilic sulfonated groups at the periphery of the ring structure. This makes phthalocyanines soluble in water and feasible for spin-coating technology in various industrial applications.

Quick characterization can be established with UV-Vis spectra, which show the characteristic absorption band of the phthalocyanines. As shown in Figs. 1–5, all of the figures have Q-band (around 600–800 nm) and B-band (around 300–400 nm). However, the absorption band in water and that in aqueous ethanol solution differs because aggregation takes place in water [1,18], which makes the Q-band blue-shift due to the exciton coupling effectively raising the energy level of the excited state [19]. With addition of ethanol to the aqueous solution, disaggregation takes place as shown in Figs. 1–4. Sodium salt of sulfonated chloroaluminum phthalocyanine is an exception, as shown in Fig. 5, since it has non-planar structure, which refrains from aggregating layer by layer.

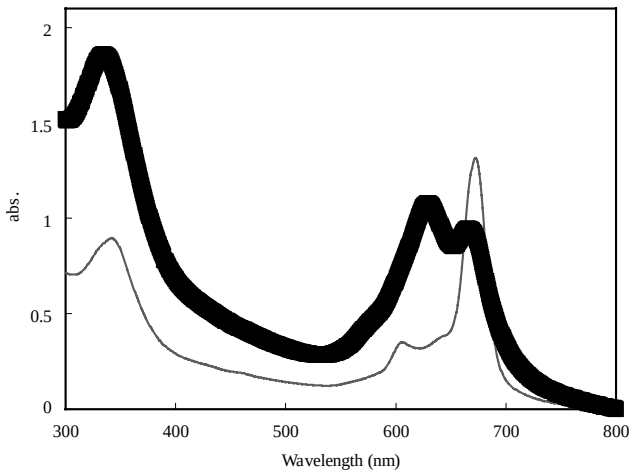


Fig. 2. The UV-Vis spectrum of $CuPc(SO_3Na)_4$. Thick line: in water; thin line: in aqueous ethanol solution (v/v = 1/1).

3.2. Generation of singlet oxygen and its quantum yield

The presence of singlet oxygen is essential in photodynamic applications. As shown in Fig. 6, we have successfully detected single oxygen in the aqueous ethanol solution of $Dy(TBPc)_2$ under illumination of red light by its UV-Vis/NIR absorption at 1940 nm. Without illumination of red light, no significant absorption can be detected.

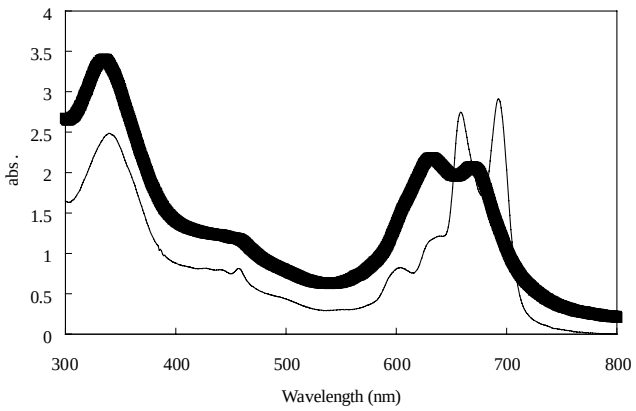


Fig. 4. The UV-Vis spectrum of $H_2Pc(SO_3Na)_4$. Thick line: in water; thin line: in aqueous ethanol solution (v/v = 1/1).

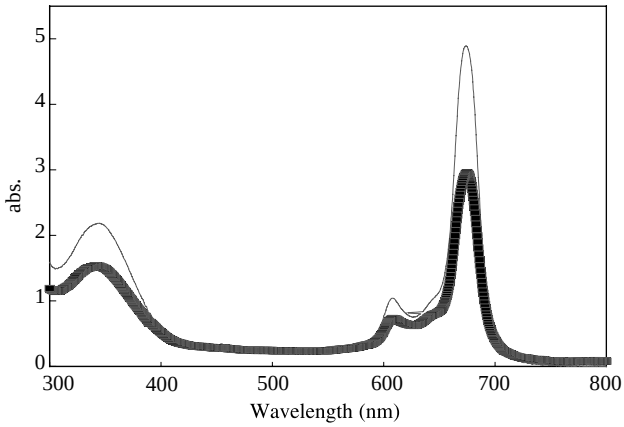


Fig. 5. The UV-Vis spectrum of $AlClPc(SO_3Na)_4$. Thick line: in water; thin line: in aqueous ethanol solution (v/v = 1/1).

Furthermore, we have also calculated the quantum yield of singlet oxygen produced by $Dy(TBPc)_2$ from Eq. (2). The quantum yield is 0.60.

$MPc(SO_3Na)_4$ have also been successfully used as photosensitizers for the oxidation of guanine to parabanic acid under photodynamic treatment. In the blank test (in the absence of $MPc(SO_3Na)_4$), the oxidized reaction does not occur. This demonstrates that the singlet oxygen is generated with $MPc(SO_3Na)_4$. The starting material, guanine, is an important component in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) [20]. Since guanine is converted into parabanic acid in the presence of $MPc(SO_3Na)_4$ under photodynamic treatment, the basic structures of DNA and RNA are altered, and the succeeding procedure (e.g. transcription, translation, and so forth [20]) will also be deterred. We speculate that this is the explanation why $MPc(SO_3Na)_4$ can be used as a photosensitizer for the photodynamic therapy (PDT).

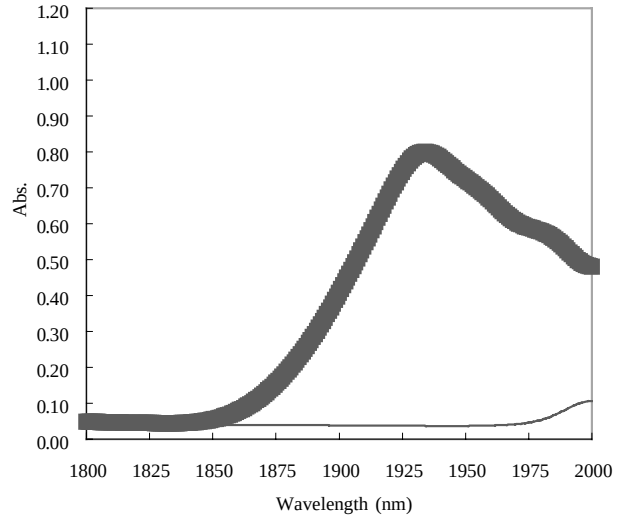


Fig. 6. The UV-Vis/NIR absorption of $Dy(TBPc)_2$. Thick line: under illumination of red light; thin line: without illumination of red light.

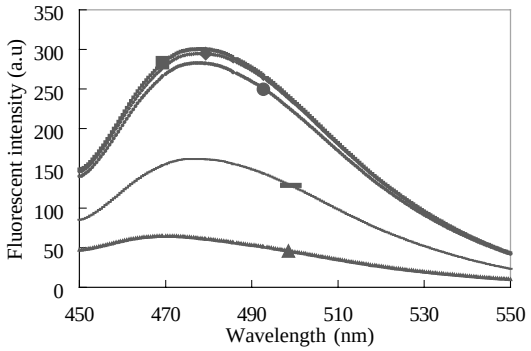


Fig. 7. The fluorescent spectrum of *E. coli* with MUG enzyme and $Dy(TBPc)_2$ (1000 ppm in ethanol/water = 95/5; w/w solution) (●): without $Dy(TBPc)_2$ and without illumination of red light; (◆): without $Dy(TBPc)_2$ and with illumination of red light for 1 min(■): with $Dy(TBPc)_2$ and without illumination of red light (—): with $Dy(TBPc)_2$ and with illumination of red light for 1 min (▲): with $Dy(TBPc)_2$ and with illumination of red light for 5 min.

Table 2
The death rates of *B. cereus* and *Aurebacterium* sp.

Photosensitizer	The death rate of *B. cereus* (%)	The death rate of *Aurebacterium* sp. (%)
$Dy(TBPc)_2$	92.29	99.98
$AlPc(SO_3Na)_4$	91.20	99.29
$CuPc(SO_3Na)_4$	79.30	87.80

3.3. The qualitative experiment for *E. coli*

As shown in Fig. 7, the fluorescent intensity of *E. coli* was decreased dramatically after addition of aqueous ethanol solution of $Dy(TBPc)_2$ and illumination of red light for 5 min. This reveals that most of *E. coli* has been killed.

3.4. The quantitative experiment for *B. cereus* and *Aurebacterium sp.*

After the photodynamic treatment described in Section 2.6, the death rates of *B. cereus* and *Aurebacterium* sp. with $Dy(TBPc)_2$, $ClAlPc(SO_3Na)_4$, and $CuPc(SO_3Na)_4$ as photosensitizers were shown in Table 2. The killing effect of $Dy(TBPc)_2$ was higher than that of $MPc(SO_3Na)_4$. Moreover, $ClAlPc(SO_3Na)_4$ exhibits better killing effect than $CuPc(SO_3Na)_4$ because of non-aggregation in the aqueous solution, resulting in higher absorption of red light.

Furthermore, we have also executed the quantitative experiment for *B. cereus* and *Aurebacterium* sp. with the powder of $Dy(TBPc)_2$, $ClAlPc(SO_3Na)_4$ as well as $CuPc(SO_3Na)_4$ by the same procedure and their death rates were approximately zero. This means $Dy(TBPc)_2$ and $MPc(SO_3Na)_4$ powders exhibit no phototoxicity.

4. Conclusion

The existence of singlet oxygen and its quantum yield have also been investigated. Moreover, $Dy(TBPc)_2$ and

$MPc(SO_3Na)_4$ have also been utilized as a photosensitizer under photodynamic treatment to kill microorganism, including *E. coli*, *B. cereus* and *Aurebacterium* sp. The experimental results reveal that phthalocyanines are potential photosensitizers for the photodynamic therapy.

Acknowledgements

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