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Tetra-2-[2-(dimethylamino)ethoxy]ethoxy substituted zinc phthalocyanines and their quaternized analoques: Synthesis, characterization, photophysical and photochemical properties

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ABSTRACT

The new peripherally and non-peripherally tetra-2-[2-(dimethylamino)ethoxy]ethoxy substituted zinc (II) phthalocyanine complexes (**2** and **4**) and their quaternized amphiphilic derivatives (**2a** and **4a**) have been synthesized and characterized for the first time. The quaternized complexes show excellent solubility in both organic and aqueous solutions, which makes them potential photosensitizer for use in photodynamic therapy (PDT) of cancer. Photophysical (fluorescence quantum yields and lifetimes) and photochemical (singlet oxygen generation and photodegradation under light irradiation) properties of these novel phthalocyanines are investigated in dimethylsulfoxide (DMSO) for non-quaternized complexes and in DMSO, phosphate buffered solution (PBS) or PBS + triton X-100 (TX) for quaternized complexes. In this study, the effects of the aggregation of the molecules, quaternization and position (peripherally or non-peripherally) of the substituents and nature of the solvents (DMSO, PBS or PBS + triton X-100) on the photophysical and photochemical parameters of the zinc (II) phthalocyanines are also reported. A spectroscopic investigation of the binding of the quaternized cationic zinc (II) phthalocyanine complexes to bovine serum albumin (BSA) is also presented in this work.

1. Introduction

One of the main targets in the chemistry of the phthalocyanines and their metallic complexes is the development of new materials that open new opportunities in advanced functional molecular materials such as catalysis, biomedicine, electronic and optoelectronic devices [1,2]. One of these new opportunities is using phthalocyanines in photodynamic therapy (PDT) of cancer as second generation photosensitizers [3].

Traditional cancer therapies such as surgery, chemotherapy and radiation therapy involve a delicate balance between removing or destroying diseased tissue and sparing surrounding healthy cells. These conventional treatments cause serious effects due to the death of normal cells by indiscriminate cytotoxic properties. PDT is an alternative method in the treatment of cancer therapies. Three fundamental requirements for PDT are oxygen, light and photosensitizer [4]. Each factor is harmless by itself, but their combination can produce cytotoxic agents such as singlet oxygen. The photodynamic activation often begins with an intravenous injection of a photosensitizer, such as phthalocyanines. In the design of a photosensitizer, one important goal is to maintain a balance of enough hydrophilicity for it to dissolve in aqueous solutions, and enough hydrophobicity for it to partition into malignant cells. Whereas, introduction of hydrophilic groups into substituted phthalocyanine derivatives was performed in order to be soluble in aqueous media, water solubility of phthalocyanine derivatives has a strong influence on the bioavailability and *in vivo* distribution [5,6]. In PDT administration, the drug is injected into the patient's blood stream, and since the blood itself is a hydrophilic system. The amphiphilic phthalocyanine derivatives are considered the best compounds for a new generation of photosensitizers for PDT [7,8]. Enhancement of the amphiphilicity of the phthalocyanine with the introduction of hydrophobic groups proved to increase the cell penetration [9,10]. Once the photosensitizer has been administered, and the sensitizer has reached the cells in question, the affected area is irradiated by light of wavelength appropriate for the sensitizer. Light absorbed by the sensitizer excites it, and the absorbed energy is transmitted to molecular oxygen (e.g., Type II mechanism) in its ordinary triplet ground state whereby it is excited into its singlet level. Within the cell, the hydrophobic lipid bilayer is considered to be the uptake location and locus of action of hydrophobic sensitizers [11].

An ideal photosensitizer has to localized in tumor tissue, be able to produce high singlet oxygen generation, possess a large absorption coefficient at wavelengths greater than 650 nm and

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have no dark toxicity. The first photosensitizers are hematoporphyrin derivatives and have already been described in detail in several articles [12,13]. Second generation photosensitizers such as phthalocyanines have also been introduced for PDT in research and clinical trials [14]. Photosens®, which is a mixture of sulfonated aluminium (III) phthalocyanines, is clinically used in Russia for the treatment of a range of cancers [15]. Pcs are known to be useful photosensitizers due to their high molar absorption coefficient in the red part of the spectrum, photostability and long lifetimes of the photoexcited triplet states [16–19]. Especially, zinc phthalocyanines (ZnPcs) have been extensively studied since the d^{10} configuration of the central Zn^{2+} ion. ZnPcs have intensive long wavelength absorption and high singlet and triplet yields, which make them valuable photosensitizer for PDT applications.

The photophysics and photochemistry of ZnPc complexes are well documented [20–25]. Such studies reveal that these complexes show great promise for photocatalytic and photosensitizing applications [26,27]. However, studies on amphiphilic ZnPc complexes are scarce in the literature [7,28–34]. The advantages that go with amphiphilic phthalocyanines are too striking to overlook, hence our interest in these complexes. The aim of our ongoing research is to synthesis amphiphilic ZnPc complexes to be used as potential PDT agents, since ZnPc complexes show good photophysical and photochemical properties which are very useful for PDT studies. Herein, we report the synthesis, characterization and spectroscopic characterization, aggregation behavior as well as photophysical (fluorescence quantum yields and lifetimes) and photochemical (singlet oxygen and photodegradation quantum yields) properties of ZnPc complexes tetra-substituted at the peripheral (**2**) and non-peripheral (**4**) positions with 2-[2-(dimethylamino)ethoxy]ethoxy group (Scheme 1) and their quaternized (**2a** for peripheral, and **4a** for non-peripheral position) complexes (Scheme 2).

Bovine serum albumin (BSA) and human serum albumin (HSA) are major plasma proteins, which contribute significantly to physiological functions and display effective drug delivery roles [35,36], hence the investigation of binding of drugs with albumin is of interest. A spectroscopic investigation of the binding of the amphiphilic ZnPc complexes (**2a** and **4a**) to BSA is also presented in this work.

2. Experimental

2.1. Materials

All reagents and solvents were of reagent grade quality and were obtained from commercial suppliers. Unsubstituted zinc phthalocyanine and 2-[2-(dimethylamino)ethoxy]ethanol were purchased from Aldrich. 9,10-Antracenediyl-bis(methylene)dimalonoic acid (ADMA) and 1,3-diphenylisobenzofuran (DPBF) were purchased from Fluka. All solvents were dried and purified as described by Perrin and Armarego [37]. 4-Nitrophthalonitrile [38] and 3-nitrophthalonitrile [39] were synthesized and purified according to well known literature.

2.2. Equipment

The IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Varian Mercury 200 MHz spectrometer in $CDCl_3$. Chemical shifts were reported (δ) relative to Me_4Si as internal standard. Mass spectra were measured on a Micromass Quatro LC/ULTIMA LC–MS/MS spectrometer. Melting points were measured on an electrothermal apparatus and are uncorrected. Absorption spectra in the UV–visible region were recorded with a Shimadzu 2001 UV spectrophotometer. Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1 cm pathlength cuvettes at room temperature.

Photo-irradiations were done using a General Electric quartz line lamp (300 W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations, respectively. An interference filter (Intor, 670 nm with a band width of 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX5100 (Molelectron detector incorporated) power meter.

2.3. Photophysical parameters

2.3.1. Fluorescence quantum yields and lifetimes

Fluorescence quantum yields (Φ_F) were determined by the comparative method using equation given in the references [40,41]. Unsubstituted ZnPc (in DMSO) ($\Phi_F = 0.20$) [42] was employed as the standard. The absorbance of the solutions at the excitation wavelength ranged between 0.04 and 0.05.

Natural radiative lifetimes (τ_0) were determined using PhotochemCAD program which uses the Strickler–Berg equation [43]. The fluorescence lifetimes (τ_F) were evaluated using Eq. (1).

$$\Phi_F = \frac{\tau_F}{\tau_0} \quad (1)$$

2.4. Photochemical parameters

2.4.1. Singlet oxygen quantum yields

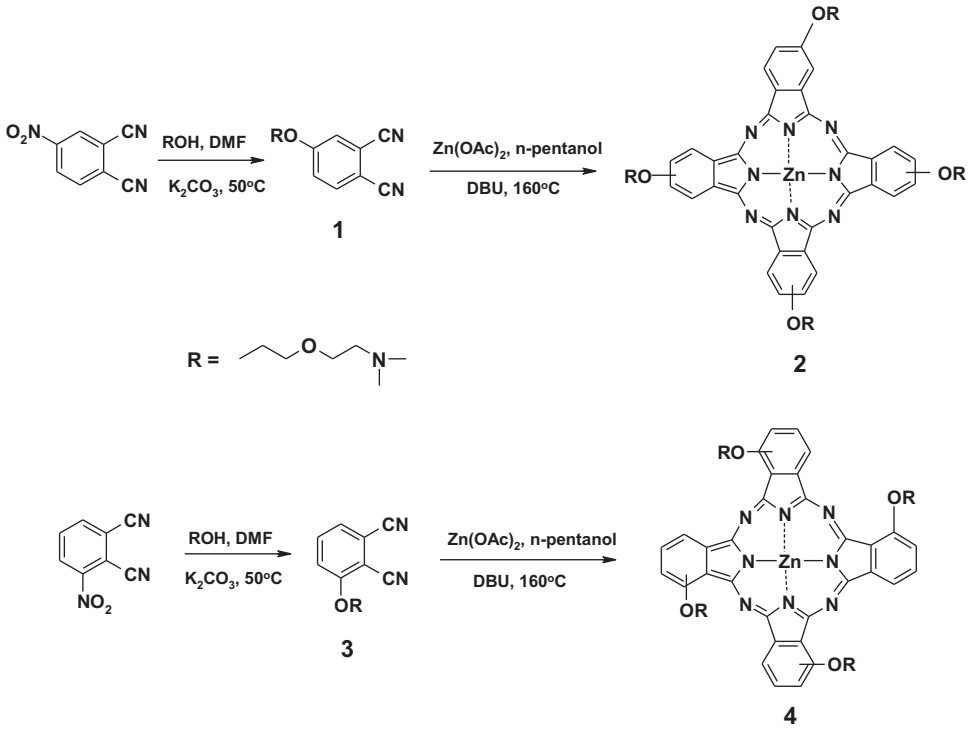
Singlet oxygen quantum yield (Φ_Δ) determinations were carried out using the experimental set-up described in literature [44,45]. Typically, a 3 mL portion of the respective unsubstituted, peripherally and non-peripherally tetra-substituted ZnPc solutions (concentration = 1×10^{-5} M) containing the singlet oxygen quencher was irradiated in the Q band region with the photoirradiation set-up described in Refs. [44,45]. Φ_Δ values were determined in air using the relative method with ZnPc (in DMSO) or $ZnPcS_{mix}$ (in aqueous media) as references. DPBF and ADMA were used as chemical quenchers for singlet oxygen in DMSO and aqueous media, respectively. The Φ_Δ values of the studied ZnPc complexes were calculated using equation given in the Ref. [45]. Unsubstituted ZnPc ($\Phi_\Delta^{Std} = 0.67$ in DMSO) [46] and sulfonated ZnPc ($ZnPcS_{mix}$, $\Phi_\Delta^{Std} = 0.45$ in aqueous media) [45] were used as standard. To avoid chain reactions induced by DPBF (or ADMA) in the presence of singlet oxygen [47], the concentration of quenchers (DPBF or ADMA) was lowered to $\sim 3 \times 10^{-5}$ M. Solutions of sensitizer (concentration = 1×10^{-5} M) containing DPBF (or ADMA) were prepared in the dark and irradiated in the Q band region using the setup described in literature [44,45]. DPBF degradation at 417 nm and ADMA degradation at 380 nm were monitored. The light intensity 6.57×10^{15} photons s^{-1} cm^{-2} was used for Φ_Δ determinations.

2.4.2. Photodegradation quantum yields

Photodegradation quantum yield (Φ_d) determinations were carried out using the experimental set-up described in literature [44,45]. Photodegradation quantum yields were determined using Eq. (2),

$$\Phi_d = \frac{(C_0 - C_t)\ V\ N_A}{I_{abs}\ S\ t} \quad (2)$$

where C_0 and C_t are the samples concentrations before and after irradiation, respectively, V is the reaction volume, N_A the Avogadro's constant, S the irradiated cell area and t the irradiation time, I_{abs} is the overlap integral of the radiation source light intensity and the absorption of the samples. A light intensity of 2.19×10^{16} photons s^{-1} cm^{-2} was employed for Φ_d determinations.



Scheme 1. Synthesis of tetra-2-[2-(dimethylamino)ethoxy]ethanol substituted zinc phthalocyanine complexes.

2.4.3. Binding of quaternized zinc phthalocyanine complexes to BSA

The binding of the quaternized ZnPc complexes (**2a** and **4a**) to BSA was studied by spectrofluorometry at room temperature. An aqueous solution of BSA (fixed concentration) was titrated with varying concentrations of the respective quaternized ZnPc solutions. BSA was excited at 280 nm and fluorescence recorded between 290 nm and 500 nm. The steady diminution in BSA fluorescence with increase in quaternized ZnPc concentrations was noted and used in the determination of the binding constants and the number of binding sites on BSA, according to Eq. (3) [48–50].

$$\log\left[\frac{(F_0 - F)}{(F - F_\infty)}\right] = \log \quad K_b + n \quad \log[\mathrm{Pc}] \tag{3}$$

where F_0 and F are the fluorescence intensities of BSA in the absence and presence of quaternized ZnPc complexes (**2a** and **4a**), respectively; F_∞, the fluorescence intensity of BSA saturated with quaternized ZnPc complexes; K_b, the binding constant; n, the number of binding sites on a BSA molecule; and [Pc] the concentration of quaternized ZnPc complexes. Plots of $\log[(F_0 - F)/(F - F_\infty)]$ against log[Pc] would provide the values of n (from the slope) and K_b (from the intercept). The changes in BSA fluorescence intensity were related to quaternized ZnPc concentrations by the Stern–Volmer relationship (Eq. (4)):

$$\frac{F_0^{BSA}}{F^{BSA}} = 1 + K_{SV}^{BSA}[\mathrm{Pc}] \tag{4}$$

and k_{SV}^{BSA} is given by Eq. (5):

$$K_{SV}^{BSA} = k_q \tau_{F(BSA)} \tag{5}$$

where F_0^{BSA} and F^{BSA} are the fluorescence intensities of BSA in the absence and presence of quaternized ZnPc complexes (**2a** and **4a**), respectively; K_{SV}^{BSA}, the Stern–Volmer quenching constant; k_q, the bimolecular quenching constant; and $\tau_{F(BSA)}$, the fluorescence lifetime of BSA. $\tau_{F(BSA)}$ is known to be 10 ns [51–53], thus from the values of K_{SV}^{BSA} obtained from the plots of F_0^{BSA}/F^{BSA} versus [Pc], the value of k_q may be determined using Eq. (5).

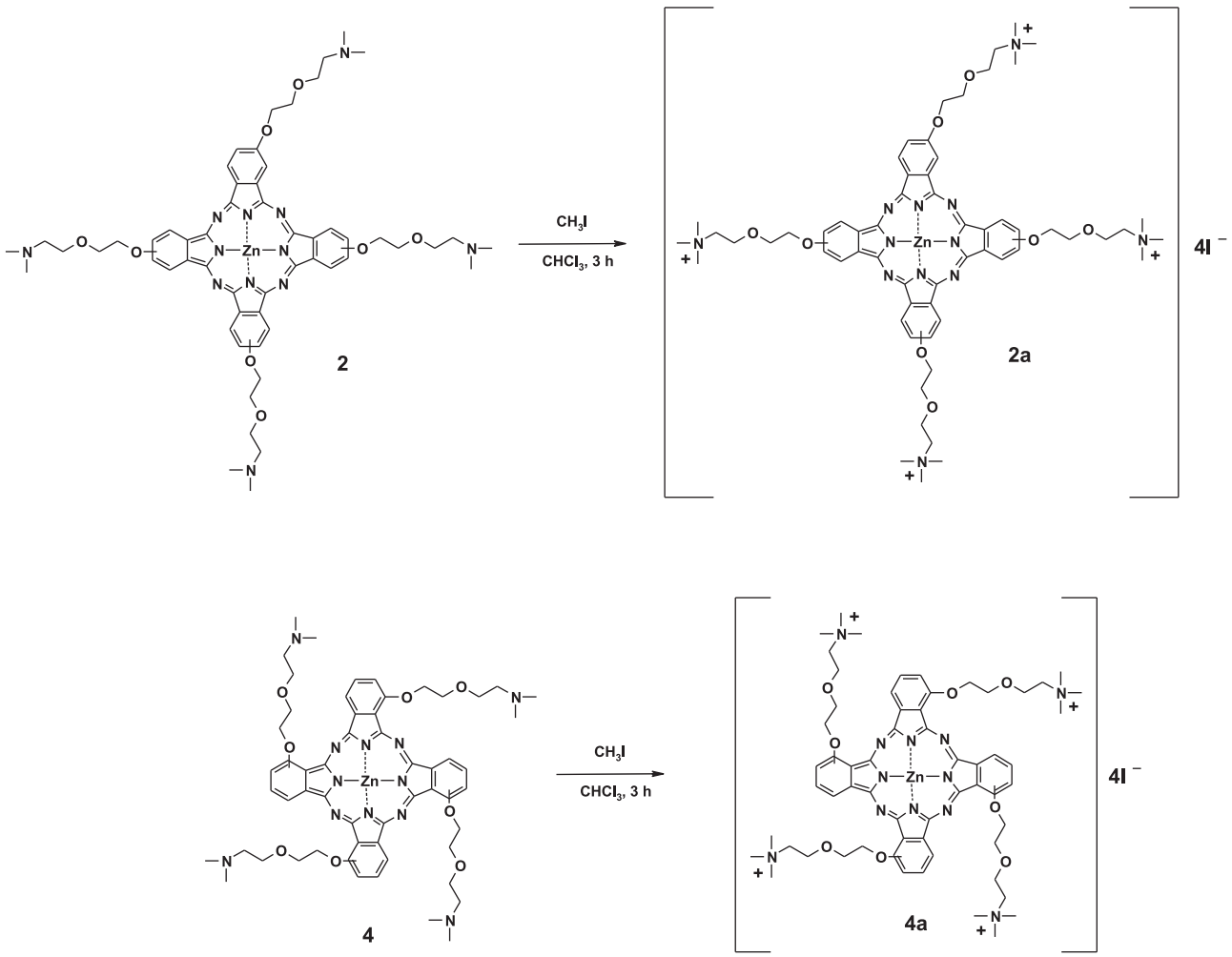
2.5. Synthesis

*2.5.1. 4-{2-[2-(dimethylamino)ethoxy]ethoxy}phthalonitrile (**1**)*

4-Nitrophthalonitrile (0.5 g, 2.89 mmol) was dissolved in anhydrous DMF (25 mL) under nitrogen and 2-[2-(dimethylamino)ethoxy]ethanol (1.15 g, 8.67 mmol) was added. After stirring for 10 min, finely ground anhydrous K_2CO_3 (3.98 g, 28.9 mmol) was added in portions over 2 h with stirring. The reaction mixture was stirred at 50 °C for 72 h under nitrogen. Then water (100 mL) was added and the aqueous phase extracted with chloroform (3 × 100 mL). The combined extracts were treated with water and dried over anhydrous magnesium sulfate and then filtered. Solvent was evaporated and the product was crystallized from ethanol. Yield: 0.3 g (40%), mp: 130–132 °C. IR (KBr pellet), ν_{max}/cm^{-1}: 3092 (Ar–H), 2953–2846 (Aliph. C–H), 2229 (C≡N), 1603, 1564, 1493, 1310, 1255, 1163, 1098, 1021, 882, 834, 693, 636, 524. ^{1}H NMR. ($CDCl_3$), (δ:ppm): 7.71 (d, 1H, Ar–H), 7.30 (s, 1H, Ar–H), 7.19 (d, 1H, Ar–H), 4.21 (t, 2H, –CH_2–O–Ar–), 3.83 (t, 2H, –CH_2–O–), 3.62 (t, 2H, –CH_2–O–), 2.51 (t, 2H, –CH_2–N), 2.25 (s, 6H, CH_3). ^{13}C NMR. ($CDCl_3$), (δ:ppm): 157.45, 130.69, 115.38, 115.01, 112.38, 111.29, 110.82, 102.29, 64.74, 64.26, 64.05, 54.01, 41.12. MS (ES^+), (m/z): 259 $[M]^+$.

*2.5.2. 3-{2-[2-(dimethylamino)ethoxy]ethoxy}phthalonitrile (**3**)*

Synthesis and purification were as outlined for compound **1** except 3-nitrophthalonitrile was employed instead of 4-nitrophthalonitrile. The amounts of the reagents employed were: 3-nitrophthalonitrile (0.5 g, 2.89 mmol), 2-[2-(dimethylamino)ethoxy]ethanol (1.15 g, 8.67 mmol), K_2CO_3 (3.98 g, 28.9 mmol) in anhydrous DMF (25 mL). Yield: 0.27 g (36%), mp: 114–116 °C. IR (KBr pellet), ν_{max}/cm^{-1}: 3087 (Ar–H), 2943–2868 (Aliph. C–H), 2229 (C≡N), 1583, 1472, 1355, 1295, 1181, 1130, 1063, 854, 796, 731, 689. ^{1}H NMR. ($CDCl_3$), (δ:ppm):



Scheme 2. Synthesis of quaternized tetra-2-[2-(dimethylamino)ethoxy]ethanol substituted zinc phthalocyanine complexes.

7.60 (d, 1H, Ar–H), 7.30 (s, 1H, Ar–H), 7.25 (d, 1H, Ar–H), 4.24 (t, 2H, $-CH_2-O-Ar-$), 3.84 (t, 2H, $-CH_2-O-$), 3.59 (t, 2H, $-CH_2-O-$), 2.43 (t, 2H, $-CH_2-N$), 2.18 (s, 6H, CH_3). ^{13}C NMR. ($CDCl_3$), (δ:ppm): 156.79, 130.36, 120.80, 118.76, 115.71, 112.96, 112.05, 100.06, 65.05, 64.826, 64.49, 54.05, 41.11. MS (ES^+), (m/z): 260 $[M+H]^+$.

2.5.3. 2(3),9(10),16(17),23(24)-Tetrakis{2-[2-dimethylamino)ethoxy]ethoxy}phthalocyaninato zinc (II) (**2**)

4-{2-[2-(Dimethylamino)ethoxy]ethoxy}phthalonitrile (**1**) (200 mg, 0.77 mmol), anhydrous $Zn(CH_3COO)_2$ (70 mg, 0.38 mmol) and 2 mL of n-pentanol were placed in a standard Schlenk tube in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.48 mL, 0.31 mmol) under a nitrogen atmosphere and held at reflux temperature for 16 h. After cooling to room temperature, the reaction mixture was precipitated by adding it drop-wise into diethylether. The crude product was collected by filtration and washed with water, ethanol and ether and then dried. Purification was achieved using column chromatography with basic alumina as column material and $CHCl_3$/MeOH (100:4) solvent system as eluent. Yield: 63 mg (30%). IR (KBr pellet) ν_{max}/cm^{-1}: 3065 (Ar–H), 2943–2868 (Aliph. C–H), 1607, 1488, 1448, 1393, 1359, 1315, 1242, 1123, 1091, 1057, 955, 841, 748, 635. ^1H NMR ($CDCl_3$), (δ:ppm): 7.49 (d, 4H, Ar–H), 6.98 (s, 4H, Ar–H), 6.86 (d, 4H, Ar–H), 3.97 (t, 8H, $-CH_2-O-Ar-$), 3.62 (t, 8H, $-CH_2-O-$), 3.37 (t, 8H, $-CH_2-O-$), 2.27 (t, 8H, $-CH_2-N$), 2.01 (s, 24H, CH_3). MS (ES^+), (m/z): 1103 $[M+H]^+$.

2.5.4. 2(3),9(10),16(17),23(24)-Tetrakis{2-[2-trimethylamino)ethoxy]ethoxy}phthalocyaninato zinc (II)iodide (***2a***)

Compound **2** (40 mg, 0.036 mmol) was dissolved in 3.5 mL of chloroform and methyl iodide (26 mg, 0.18 mmol) was added to this solution. The reaction mixture was stirred under reflux for 3 h. After cooling to room temperature, the green precipitate was filtered off, washed with acetone, diethyl ether and chloroform and then dried. Yield: 42 mg (70%). IR (KBr pellet) ν_{max}/cm^{-1}: 3010 (Ar–H), 2917–2857 (Aliph. C–H), 1618, 1544, 1481, 1445, 1382, 1314, 1278, 1237, 1215, 1119, 1097, 1059, 952, 872, 743. MS (ES^+), (m/z): 1655 $[M-CH_3]^+$.

2.5.5. 1(4),8(11),15(18),22(25){2-[2-dimethylamino)ethoxy]ethoxy}phthalocyaninato zinc (II) (***4***)

Synthesis and purification were as outlined for compound **2** except 3-{2-[2-(dimethylamino)ethoxy]ethoxy}phthalonitrile (**3**) was employed instead of 4-{2-[2-(dimethylamino)ethoxy]ethoxy}phthalonitrile (**1**). The amounts of the reagents employed were: 3-{2-[2-(dimethylamino)ethoxy]ethoxy}phthalonitrile (**3**) (200 mg, 0.77 mmol), anhydrous $Zn(CH_3COO)_2$ (70 mg, 0.38 mmol), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.48 mL, 0.31 mmol) in n-pentanol (2 mL). Yield: 59 mg (28%). IR (KBr pellet) ν_{max}/cm^{-1}: 3065 (Ar–H), 2930–2862 (Aliph. C–H), 1599, 1589, 1488, 1451, 1334, 1359, 1269, 1231, 1125, 1083, 885, 802, 745. ^1H NMR

Table 1
Absorption, excitation and emission spectral data for unsubstituted, non-peripheral and peripheral tetra-substituted zinc phthalocyanine complexes in DMSO and PBS.

Compound	Solvent	Q band λ_{max} (nm)	($\log \varepsilon$)	Excitation λ_{Ex} (nm)	Emission λ_{Em} (nm)	Stokes shift Δ_{Stokes} (nm)
2	DMSO	684	5.07	685	693	9
2a	DMSO	685	4.57	686	694	9
	PBS	635, 688	4.03, 3.88	–	–	–
	PBS + TX	686	4.29	687	696	10
4	DMSO	706	5.03	707	717	11
4a	DMSO	705	4.95	707	716	11
	PBS	654, 704	4.42, 4.45	–	–	–
	PBS + TX	705	4.90	706	716	11
ZnPc[a]	DMSO	672	5.14	672	682	10

[a] Data from Ref. [21].

($CDCl_3$), (δ:ppm): 7.43 (m, 4H, Ar–H), 6.97 (s, 4H, Ar–H), 6.84 (d, 4H, Ar–H), 4.09 (m, 8H, $-CH_2-O-Ar-$), 3.64 (m, 8H, $-CH_2-O-$), 3.40 (t, 8H, $-CH_2-O-$), 2.16 (m, 8H, $-CH_2-N$), 2.01 (s, 24H, CH_3). MS (ES^+), (m/z): 1125 $[M+Na]^+$.

2.5.6. 1(4),8(11),15(18),22(25)-Tetrakis{2-[2-trimethylamino)ethoxy]ethoxy}phthalocyaninato zinc (II)iodide (**4a**)

Synthesis and purification were as outlined for compound **2a** except compound **4** was employed instead of compound **2**. The amounts of the reagents employed were: compound **4** (40 mg, 0.036 mmol), methyl iodide (26 mg, 0.18 mmol) in chloroform (3.5 mL). Yield: 40 mg (67%). IR (KBr pellet) ν_{max}/cm^{-1}: 3005 (Ar–H), 2923–2862 (Aliph. C–H), 1613, 1589, 1486, 1382, 1322, 1267, 1231, 1168, 1113, 1065, 954, 883, 798, 744. MS (ES^+), (m/z): 1543 $[M-I]^+$.

3. Results and discussion

3.1. Synthesis and characterization

General synthetic route for the synthesis of new peripherally and non-peripherally substituted ZnPc complexes (**2** and **4**) and their quaternized derivatives (**2a** and **4a**) are given in Schemes 1 and 2, respectively. 2(3),9(10),16(17),23(24)-Tetrasubstituted (peripheral position) phthalocyanines can be synthesized from 4-substituted phthalonitriles while 1(4),8(11),15(18),22(25)-tetrasubstituted (non-peripheral position) phthalocyanines are obtained from 3-substituted analogues [54]. In both cases, a mixture of four possible structural isomers is obtained. The four probable isomers can be designed by their molecular symmetry as C_{4h}, C_{2v}, C_s and D_{2h}. In this study, synthesized peripherally and non-peripherally substituted ZnPc complexes are obtained as isomer mixtures as expected. No attempt was made to separate the isomers of complexes.

Phthalonitrile derivatives (**1** and **3**) were synthesized through base-catalyzed aromatic nitro displacement of 4-nitrophthalonitrile and 3-nitrophthalonitrile with 2-[2-(dimethylamino)ethoxy]ethanol using K_2CO_3 as a base in anhydrous DMF. The reactions were carried out at 50 °C under N_2 atmosphere for 72 h. The preparation of phthalocyanine derivatives from the aromatic nitriles occurs under different reaction conditions. For various substituted dinitriles, the reaction in the presence of strong non-nucleophilic bases such as DBU or 1,5-diazabicyclo[4.3.0]non-5-ene (DBN) either in n-pentanol or in bulk is most efficient in comparison to other methods. In addition, these reactions are easy to perform, work under relatively mild conditions and yield pure phthalocyanines [55].

Therefore, ZnPc complexes (**2** and **4**) were obtained by using the anhydrous metal salt [$Zn(CH_3COO)_2$] in n-pentanol in the presence of a strong organic base such as DBU at reflux temperature. Water soluble tetra-cationic ZnPc complexes (**2a** and **4a**) were obtained from the reaction of corresponding ZnPc complexes (**2** and **4**) with methyl iodide as quaternization agent in chloroform. After reaction with methyl iodide, the quaternized ZnPc complexes (**2a** and **4a**) are very soluble in water. The structures of the target compounds were confirmed using IR, ^1H NMR, ^{13}C NMR and mass spectral data. All the results were consistent with the predicted structures as shown in the Section 2. The NMR spectra of the quaternarized phthalocyanine complexes (**2a** and **4a**) showed more unresolved patterns compared to non-quaternarized derivatives due to the aggregation of these complexes in deuterated DMSO.

3.2. Ground state electronic absorption spectra

The electronic absorption spectra of the studied ZnPc complexes (**2**, **2a**, **4** and **4a**) showed characteristic absorptions in the Q band region at around 684–706 nm in DMSO, Table 1. The B bands were observed at around 350 nm (Fig. 1a). The spectra showed monomeric behavior evidenced by a single (narrow) Q band, typical of metallated phthalocyanine complexes in DMSO [56]. The red-shifts were observed for ZnPc complexes follow-

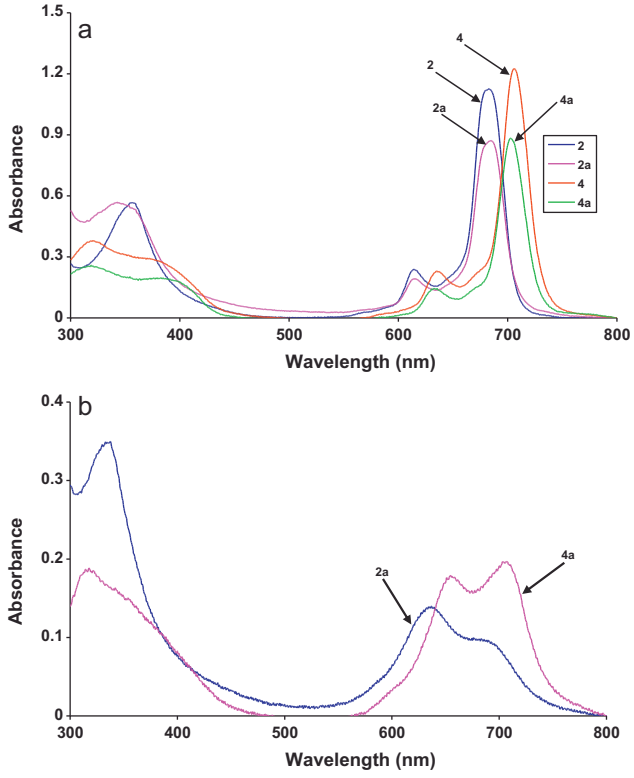


Fig. 1. Absorption spectra of: (a) tetra-substituted zinc phthalocyanine complexes (**2**, **2a**, **4** and **4a**) in DMSO, (b) cationic tetra-substituted zinc phthalocyanine complexes (**2a**, and **4a**) in PBS. Concentration = 1×10^{-5} M.

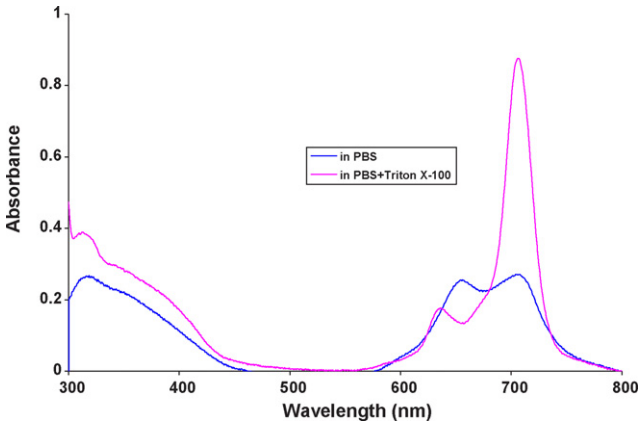


Fig. 2. Absorption spectral changes for complex **4a** observed on addition of triton X-100 (0.1 mL) in water. [**4a**] = 1×10^{-5} M.

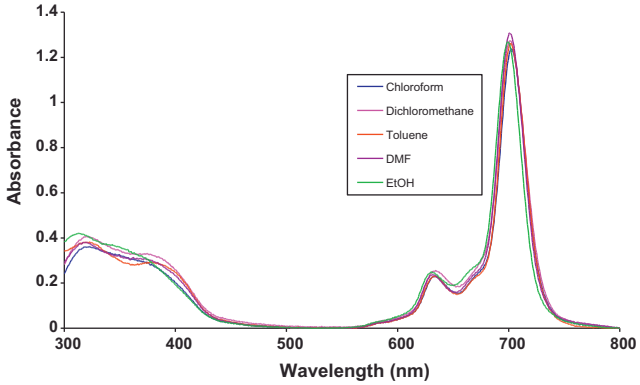


Fig. 3. Absorption spectra of complex **4** in different solvents. Concentration = 1.0×10^{-5} M.

ing substitution. (For interpretation of the references to color in this text, the reader is referred to the web version of the article.) The Q bands of the non-peripherally substituted complexes are red-shifted when compared to the corresponding peripherally substituted complexes in DMSO (Fig. 1a). The red-shifts are 22 nm between **2** and **4**, 20 nm between **2a** and **4a**. The observed red spectral shifts are typical of Pcs with substituents at the non-peripheral positions and have been explained in the literature [57,58]. The B-bands are broad due to the superimposition of the B_1 and B_2 bands in the 350 nm region.

3.3. Aggregation studies

Aggregation is usually depicted as a coplanar association of rings progressing from monomer to dimer and higher order complexes. It is dependent on the concentration, nature of the solvent, nature of the substituents, complexed metal ions and temperature [59,60]. In PBS, the absorption spectra of quaternized complexes (**2a** and **4a)** showed cofacial aggregation (H-aggregation), as evidenced by the presence of two non-vibrational peaks in the Q band region, Fig. 1b. The lower energy (red-shifted) bands at 688 for **2a** and 704 for **4a** are due to the monomeric species, while the higher energy (blue-shifted) bands at 635 for **2a** and 654 nm for **4a** are due to the aggregated species. The peripherally substituted cationic complex (**2a**) more aggregated than non-peripherally substituted complex (**4a**) in PBS, suggesting that more sterically hinderence of the substitution on the non-peripheral position.

Addition of triton X-100 (0.1 mL) to a PBS solution of quaternized ZnPc complexes (**2a** and **4a**) (concentration = 1.0×10^{-5}) brought about considerable increase in intensity of the low energy side of the Q band (Fig. 2 as an example for complex **4a**), suggesting that the molecules are aggregated and that addition of triton X-100 breaks up the aggregates between the Pc molecules.

In this study, the aggregation behavior of the ZnPc complexes (**2**, **2a**, **4** and **4a**) were investigated in different solvents (Fig. 3 as an example for complex **4**). The non-ionic complexes (**2** and **4**) did not aggregate in $CHCl_3$, CH_2Cl_2, DMSO, DMF, toluene and ethanol. The ionic complexes (**2a** and **4a**) did not also aggregate in DMSO, DMF and ethanol. The aggregation behavior of the studied ZnPc complexes (**2**, **2a**, **4** and **4a**) were also studied at different concentration in DMSO. In DMSO, as the concentration was increased, the intensity of absorption of the Q band also increased and there were no new bands (normally blue shifted) due to the aggregated species for the ZnPc complexes (**2**, **2a**, **4** and **4a**) (Fig. 4 as an example for complex **4**). (For interpretation of the references to color in this text, the reader is referred to the web version of the article.) Beer-Lambert law was obeyed for ZnPc complexes (**2**, **2a**, **4** and **4a**) in the concentrations ranging from 1.2×10^{-5} to 2×10^{-6} M.

3.4. Fluorescence spectra

Fig. 5 shows fluorescence emission, absorption and excitation spectra of complex **2** in DMSO as an example of the studied ZnPc complexes. Fluorescence emission peaks were listed in Table 1. The observed Stokes shifts were within the region observed for ZnPc complexes. All studied ZnPc complexes (**2**, **2a**, **4** and **4a**) showed similar fluorescence behavior in DMSO (Fig. 5 for complex **2** as an example). The excitation spectra were similar to absorption spectra and both were mirror images of the fluorescent spectra for all ZnPc complexes in DMSO. The proximity of the wavelength of each component of the Q-band absorption to the Q band maxima of the excitation spectra for all ZnPc complexes suggests that the nuclear configurations of the ground and excited states are similar and not affected by excitation. The water soluble quaternized ZnPc complexes (**2a** and **4a**) are non fluorescent in PBS due to aggregation. Aggregated phthalocyanines are not known [20] to fluorescent since aggregation lowers the photoactivity of molecules through dissipation of energy by aggregates. The addition of triton X-100 increased the fluorescence emission of the studied fluorescent in PBS by lowering the aggregation, thus the quenching by the aggregated species.

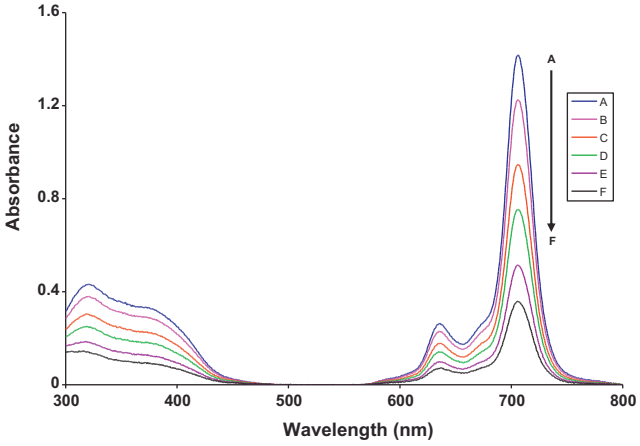


Fig. 4. Absorption spectral changes for **4** in DMSO at different concentrations: 12×10^{-6} (A), 10×10^{-6} (B), 8×10^{-6} (C), 6×10^{-6} (D), 4×10^{-6} (E), 2×10^{-6} (F) M.

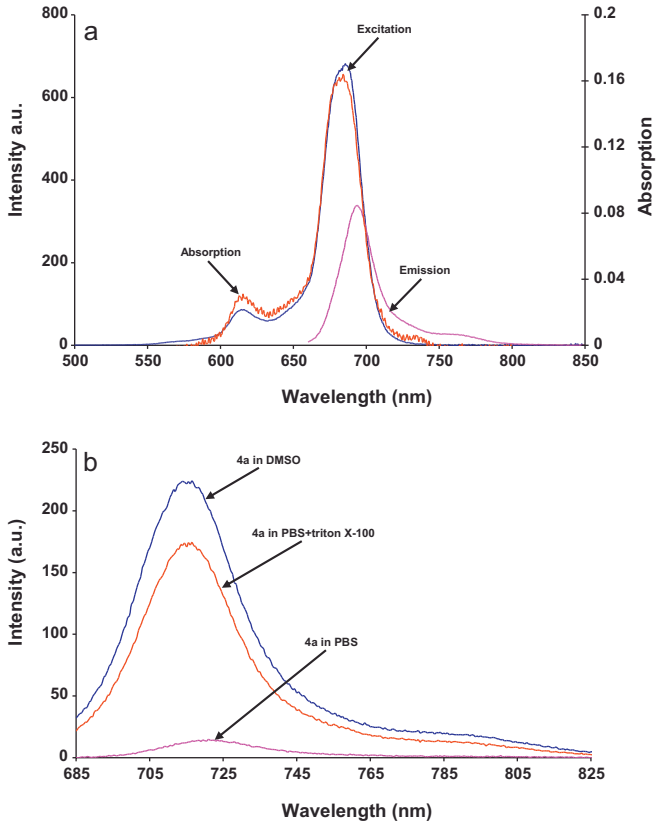


Fig. 5. (a) Absorption, excitation and emission spectra of **2** in DMSO (excitation wavelengths: 650 nm), (b) emission spectra of **4a** in DMSO, PBS and PBS + triton X-100 (excitation wavelengths: 675 nm).

3.5. Fluorescence quantum yields and lifetimes

The fluorescence quantum yields (Φ_F) for non-ionic ZnPc complexes (**2** and **4**) in DMSO and for quaternized ionic ZnPc complexes (**2a** and **4a**) in DMSO, PBS and PBS + triton X-100 are given in Table 2. The Φ_F values of all studied ZnPc complexes are typical of zinc phthalocyanine complexes [20] in DMSO. The Φ_F values of the substituted ZnPc complexes (**2**, **2a**, **4** and **4a**) are lower compared to unsubstituted ZnPc complex in DMSO, which implies that the presence of the 2-[2-(dimethylamino)ethoxy]ethoxy substituents certainly results in fluorescence quenching. The peripherally substituted complexes (**2** and **2a**) show higher Φ_F values in DMSO, compared to non-peripheral substituted complexes (**4** and **4a**), suggesting not as much of quenching of the excited singlet state by peripheral substitution compared to the non-peripheral substitution. In PBS + triton X-100 solution, the Φ_F value of the non-peripherally substituted ZnPc complex (**4a**) is higher than peripherally substituted complex (**2a**), suggesting that the non-peripherally substituted complex shows less aggregation than peripherally substituted complex in this solution. The non-ionic ZnPc complexes (**2** and **4**) show higher Φ_F values compared to the corresponding quaternarized ionic complexes (**2a** and **4a**) in DMSO.

Fluorescence lifetime (τ_F) refers to the average time a molecule stays in its excited state before fluorescing, and its value is directly related to that of Φ_F; i.e., the longer the lifetime, the higher the quantum yield of fluorescence. Any factor that shortens the fluorescence lifetime of a compound indirectly reduces the value of Φ_F. Such factors include internal conversion and intersystem crossing. As a result, the nature and the environment of a compound determine its fluorescence lifetime. τ_F values were calculated using the Strickler–Berg equation and these values are given in Table 2. The τ_F values of the substituted ZnPc complexes (**2**, **2a**, **4** and **4a**) are higher compared to unsubstituted ZnPc complex in DMSO. τ_F values are lower for non-peripheral complexes (**4** and **4a**) when compared to peripheral complexes (**2** and **2a**), Table 2, suggesting more quenching by peripheral substitution compared to non-peripheral substitution. However the τ_F values are typical for ZnPc complexes [20] in DMSO. The natural radiative lifetime (τ_0) and the rate constants for fluorescence (k_F) values are also given in Table 2. In DMSO, τ_0 values of the substituted ZnPc complexes are higher, but the k_F values are lower than unsubstituted ZnPc complex. While the quaternization of the ZnPc complexes caused to increasing of the τ_0 values, decreasing of the k_F values in DMSO.

3.6. Singlet oxygen quantum yields

The amount of the singlet oxygen quantum yield (Φ_Δ) is an indication of the potential of the compounds as photosensitizers in photocatalytic applications such as PDT. The Φ_Δ values were determined using a chemical method (DPBF in DMSO and ADMA in both PBS and PBS + triton X-100 solutions). The disappearance of DPBF or ADMA was monitored using UV-vis spectrophotometer (Fig. 6a using DPBF for complex **2a** in DMSO and Fig. 6b using ADMA for complex **4a** in PBS + triton X-100 solution). Many factors are responsible for the magnitude of the determined quantum yield of singlet oxygen including; triplet excited state energy, ability of substituents and solvents to quench the singlet oxygen, the triplet excited state lifetime and the efficiency of the energy transfer between the triplet excited state and the ground state of oxygen. There was no decrease in the Q band of formation of new bands during Φ_Δ determinations (Fig. 6). Φ_Δ values of the ZnPc complexes are given in Table 2 and it shows that the Φ_Δ values are higher for substituted ZnPc complexes (**2**, **2a**, **4** and **4a**) when compared to respective unsubstituted ZnPc complex in DMSO. The Φ_Δ values of non-peripherally substituted complexes (**4** and **4a**) are higher when compared to the peripherally substituted complexes (**2** and **2a**) in all studied solutions, suggesting that the non-peripherally

Table 2
Photophysical and photochemical parameters of unsubstituted, non-peripheral and peripheral tetra-substituted zinc phthalocyanine complexes in DMSO and PBS.

Compound	Solvent	Φ_F	τ_F (ns)	τ_0 (ns)	[a] $k_F/10^8$ (s^{-1})	$\Phi_d/10^{-4}$	Φ_Δ
2	DMSO	0.19	1.94	10.23	0.98	1.21	0.77
2a	DMSO	0.15	4.36	28.86	0.34	0.20	0.73
	PBS	–	–	–	–	0.73	0.05
	PBS + TX	0.04	2.47	61.71	0.16	23.43	0.34
4	DMSO	0.11	1.42	12.97	0.77	0.82	0.88
4a	DMSO	0.10	1.44	14.47	0.69	1.92	0.79
	PBS	–	–	–	–	0.25	0.13
	PBS + TX	0.06	0.72	11.94	0.83	36.64	0.44
ZnPc[b]	DMSO	0.20[c]	1.22	6.80	1.47	0.26	0.67

[a] k_F is the rate constant for fluorescence. Values calculated using $k_F = \Phi_F/\tau_F$.
[b] Data from Ref. [21].
[c] Data from Ref. [42].

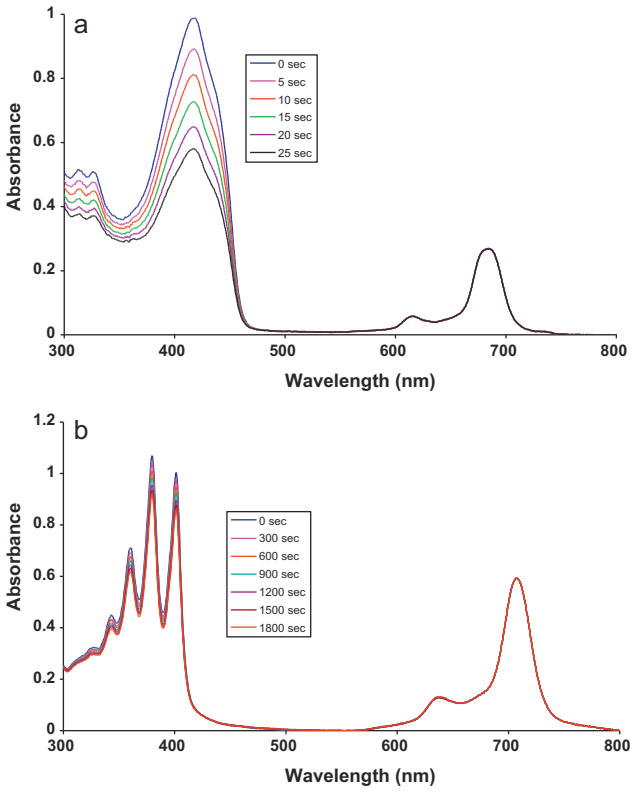


Fig. 6. A typical spectrum for the determination of singlet oxygen quantum yield of: (a) **2a** in DMSO using DPBF as a singlet oxygen quencher and (b) **4a** in PBS + triton X-100 using ADMA as a singlet oxygen quencher. Concentration = 1×10^{-5} M.

substituted complexes absorb light at long wavelength. Table 2 shows that lower Φ_Δ values are observed in aqueous solution compared to in DMSO suggesting that aggregation of the ionic ZnPc complexes this solution. The addition of the triton X-100 which is a surfactan to aqueous solution breaks up the formed aggregates between ionic ZnPc molecules in aqueous solution. However, the ZnPc complexes (**2a** and **4a**) have good Φ_Δ values in PBS + triton X-100 solution and these values make them as potential sensitizers for PDT applications. Quaternarized ionic ZnPc complexes (**2a** and **4a**) showed lower Φ_Δ values when compared to corresponding non-ionic complexes (**2** and **4**) in DMSO.

3.7. Photodegradation studies

Degradation of the molecules under light irradiation can be used to study their stability and this is especially important for those molecules intended for use in photocatalytic application such as PDT. The collapse of the absorption spectra without any distortion of the shape confirms clean photodegradation not associated with phototransformation into different forms of MPc absorbing in the visible region. Generally, photodegradation depends on the structure of the molecule, concentration, solvent and light intensity. It is believed that photodegradation is a singlet oxygen mediated process, since singlet oxygen is highly reactive and it can react with phthalocyanines. For DPBF which is used as chemical quencher for singlet oxygen a [4 + 2] cycloaddition with singlet oxygen under formation of *o*-dibenzoylbenzene is known to occur [61]. A similar reaction is supposed to occur between phthalocyanine derivatives and singlet oxygen. In the case of phthalocyanines, phthalimide residue was found to be the photooxidation product following degradation [62].

The spectral changes observed for all ZnPc complexes (**2**, **2a**, **4** and **4a**) in all studied solvents during irradiation are as shown

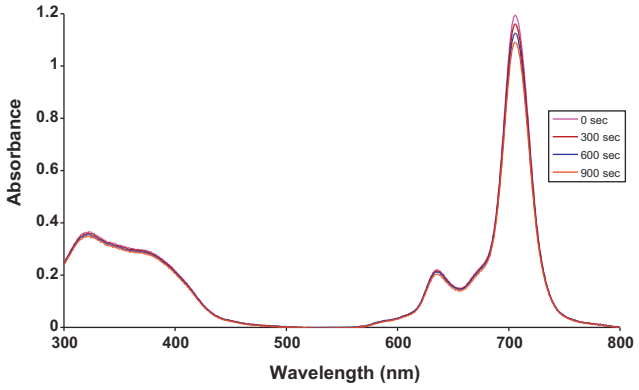


Fig. 7. The photodegradation of **4** in DMSO showing the disappearance of the Q-band at five minutes intervals.

in Fig. 7 (using complex **4** in DMSO as an example). The collapse of the absorption spectra without any distortion of the shape confirms clean photodegradation not associated with phototransformation for complexes.

The photodegradation quantum yield (Φ_d) values of the ZnPc complexes in DMSO, PBS and PBS + triton X-100 solutions are given in Table 2. Stable phthalocyanine molecules show Φ_d values as low as 10^{-6} and for unstable molecules, values of the order of 10^{-3} have been reported [20]. While all substituted ZnPc complexes (**2**, **2a**, **4** and **4a**) have an intermediate photostability in both DMSO and PBS, they are less stable in PBS + triton X-100 solution. The quaternization of the studied ZnPc complexes reduced to Φ_d values and caused to increasing of the stability of these complexes.

3.8. Interaction of quaternized cationic ZnPc complexes with BSA

The fluorescence emission spectra of BSA in the presence of different concentrations of **4a** in PBS show as an example in Fig. 8. The quaternized ZnPc complexes are mixtures of aggregated and unaggregated species. The total concentrations of the complexes are mixture of the monomer and aggregated species. We calculated the percentage aggregation (% Agg) of these complexes using the equation described in the literature [63,64]. The aggregation percentages are 70% for **2a** and 62% for **4a**. The non-peripherally substituted zinc phthalocyanine complex (**4a**) having lower % Agg values which may be due to reduced aggregation tendencies when the substitution is at the non-peripheral position of the Pc skeleton [65].

The BSA fluorescence at 348 nm is mainly attributable to tryptophan residues in the macromolecule. BSA and the respective

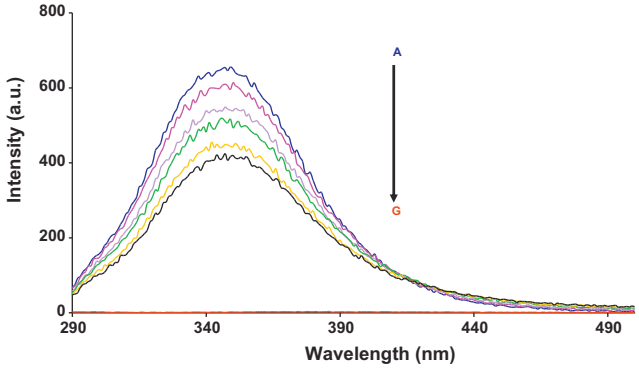


Fig. 8. Fluorescence emission spectral changes of BSA ($C = 3.00 \times 10^{-5}$ M) on addition of varying concentrations of **4a** in PBS. [**4a**]: A = 0, B = 1.66×10^{-6}, C = 3.33×10^{-6}, D = 5.00×10^{-6}, E = 6.66×10^{-6}, F = 8.33×10^{-6} M, G = saturated with 4a.

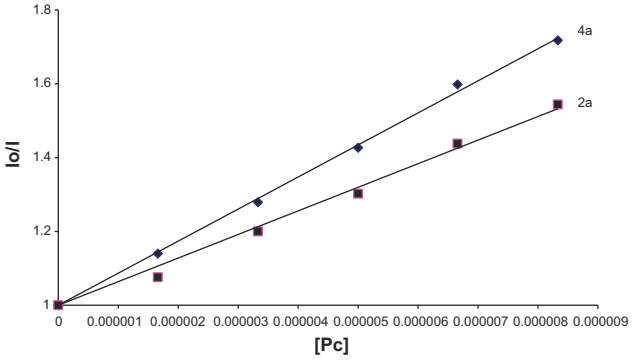


Fig. 9. Stern–Volmer plots of tetra substituted zinc phthalocyanines quenching of BSA in PBS. [BSA] = 3.00×10^{-5} M and [Pc] = 0, 1.66×10^{-6}, 3.33×10^{-6}, 5.00×10^{-6}, 6.66×10^{-6}, 8.33×10^{-6} M in PBS.

Table 3
Binding and fluorescence quenching data for interaction of BSA with quaternized zinc phthalocyanine complexes in PBS.

Compound	$K_{SV}^{BSA}/10^5$ (M^{-1})	$k_q/10^{13}$ (M^{-1} s^{-1})	$K_b/10^{-6}$ (M^{-1})	n
2a	0.64	0.86	4.26	1.22
4a	0.86	0.86	5.28	1.04

quaternized cationic ZnPc complexes exhibit reciprocated fluorescence quenching on one another; hence it was possible to determine Stern–Volmer quenching constants (K_{SV}). The slope of the plots shown at Fig. 9 gave K_{SV} values and listed in Table 3, suggest that BSA fluorescence quenching is more effective for quaternized non-peripherally substituted ZnPc complex (**4a**) than quaternized peripherally substituted ZnPc complex (**2a**) in PBS. Using the approximate fluorescence lifetime of BSA (10 ns) [52,53], the bimolecular quenching constant (k_q) was determined by Eq. (5). These values are of the order of 10^{13} M^{-1} s^{-1}, which exceed the proposed value of 10^{10} M^{-1} s^{-1} for diffusion-controlled (dynamic) quenching (according to the Einstein–Smoluchowski approximation) at room temperature [66]. This also, is an indication that the mechanism of BSA quenching by quaternized ZnPc complexes (**2a** and **4a**) is not diffusion-controlled (i.e., not dynamic quenching, but static quenching). The k_q values are larger for quaternized non-peripherally substituted ZnPc complex (**4a**) than quaternized peripherally substituted ZnPc complex (**2a**) in PBS. The binding constants (K_b) and number of binding sites (n) on BSA were obtained using Eq. (5) and the results are shown in Table 3. The slope of the plots shown at Fig. 10 gave n values and the intercepts of these plots gave K_b values. The values of K_b and n are typical of MPc–BSA

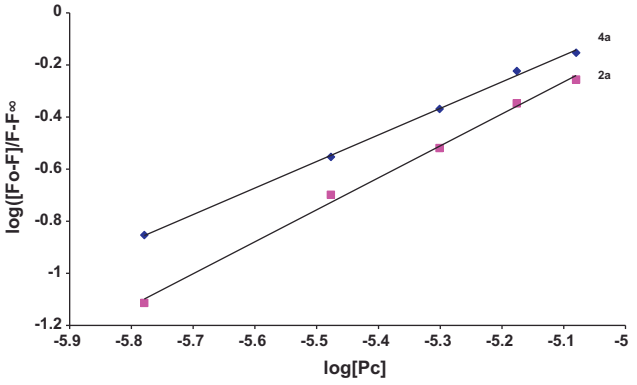


Fig. 10. Determination of quaternized zinc phthalocyanine–BSA binding constant and number of binding sites on BSA. [BSA] = 3.00×10^{-5} M and [Pc] = 0, 1.66×10^{-6}, 3.33×10^{-6}, 5.00×10^{-6}, 6.66×10^{-6}, 8.33×10^{-6} M in PBS.

interactions in aqueous solutions [49,67]. The higher K_b value for quaternized non-peripherally substituted ZnPc complex (**4a**) implies that a non-peripherally substituted ZnPc complex binds more strongly to BSA than the quaternized peripherally substituted ZnPc complex (**2a**), probably due to less aggregation tendency of the complex at the non-peripheral position. n values of near unity suggest that both non-peripherally and peripherally substituted ZnPc complexes form 1:1 adducts with BSA. The decrease in the intrinsic fluorescence intensity of tryptophan with quaternized ZnPc concentration indicates that these complexes readily bind to BSA, which implies that the phthalocyanine molecules reach subdomains where tryptophan residues are located in BSA. This also suggests that the primary binding sites of these molecules are very close to tryptophan residues, since the occurrence of quenching requires molecular contact.

4. Conclusion

In conclusion, this work has described the synthesis, characterization, aggregation behavior, photophysical and photochemical properties of new non-peripherally and peripherally tetra-2-[2-(dimethylamino)ethoxy]ethoxy substituted amphiphilic zinc phthalocyanine photosensitizers to candidate for PDT. The effect of quaternization on these properties is also presented. Solvent effect (DMSO, PBS or PBS + triton X-100 solution) on the aggregation, photophysical and photochemical properties of the quaternized derivatives is investigated. Although, the photophysical and photochemical properties relevant for photosensitization gave more attractive values in DMSO, the values in water are still enough for PDT applications. Especially, the addition of triton X-100 to PBS solution improved these properties due to lowering of aggregation. This work will certainly enrich the hitherto scanty literature on the potentials of amphiphilic zinc phthalocyanines as photosensitizers in PDT. This study reveals that the water-soluble complexes bind strongly to serum albumin; hence they can easily be transported in the blood.

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