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Photophysical and photochemical properties of tetrasulfonated silicon and germanium phthalocyanine in aqueous and non-aqueous media

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Abstract

The photophysical and photochemical properties of tetrasulfonated silicon and germanium phthalocyanine ($SiPcS_4$ and $GePcS_4$) in aqueous solution (phosphate-buffered saline (PBS) solution, pH 7.4) (in the presence and absence of cremophore EL (CEL)) and in dimethylsulphoxide (DMSO) were studied. The complexes have intense absorption in the visible/near-IR region though they highly aggregate in aqueous solution with a dimerization constant of $\sim 2 \times 10^4$ dm^3 mol^{-1}. The fluorescence excitation spectra however have only one band suggesting that only the monomer fluoresces. Both the quantum yields of the triplet state (Φ_T) and the triplet lifetimes (τ_T) were found to be higher in DMSO compared to in aqueous solution. Aggregation is hindered by addition of cremophore EL in aqueous solution and this induced disaggregation caused an increased Φ_T and τ_T probably due to the reduced interaction of the phthalocyanines with the aqueous medium in the presence of CEL.

Keywords: Silicon phthalocyanine; Germanium phthalocyanine; Aggregation; Cremophore; Triplet yields; Triplet lifetime

1. Introduction

Photodynamic therapy (PDT) of cancer is based on a non-invasive treatment of superficial tumours that is currently being used in a number of countries. PDT is based on the concept that a photosensitizer can be preferentially localized in malignant tissues, followed by activation of the photosensitizer with the light of the appropriate wavelength to generate cytotoxic radical and non-radical species of oxygen that lead to tumour destruction [1–5]. Metallophthalocyanines (MPcs) which are known as second generation photosensitizers have shown great prospects in PDT due to their intense absorption in the red region of visible light, non-toxicity, selective localization in tumours and efficient generation of singlet oxygen [6]. Incorporation of non-transition metals such as silicon and germanium in the center of the phthalocyanine (Pc) ring results in complexes with high triplet state quantum yields and long triplet lifetimes, which are required for efficient photosensitization [7]. Sulfonated MPcs are particularly interesting since anionic sulfonate group render them water soluble.

For PDT applications, it is important to study the behaviour of complexes in aqueous media, though most water soluble MPcs are aggregated in aqueous solution [8,9]. Aggregation can be hindered by addition of surfactants [10]. Transport of photosensitizers in the bloodstream using drug carriers has been shown to provide a more selective accumulation of the drugs in tissues [11]. Moreover for drug delivery *in vivo*, it is important to have a balance of the amphiphilic property and photodynamic activity of the photosensitizer [12], hence in this work we study the photophysical behaviour of $SiPcS_4$ and $GePcS_4$ in water (PBS pH 7.4) in the presence of cremophore EL (CEL). CEL is a nonionic surfactant which is an excipient to drugs and since surfactants are known to hinder aggregation [10], a study of Pcs in CEL is warranted.

The association of MPc complexes with bovine serum albumin (BSA) is also studied since hydrophilic dyes bind preferentially to serum proteins, such as BSA [13] and serum albumin is one of the key components in the body that influences drug delivery [14].

In addition, the study of the photostability of MPcs during photosensitized reactions as well as their ability to generate singlet oxygen is of immense importance. The photophysics and photochemistry of complexes containing a mixture of differently sulfonated derivatives ($GePcS_{mix}$ and $SiPcS_{mix}$) [10,15] have been reported as an average for the whole mixture with high

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triplet quantum yield and singlet oxygen. In this work, we investigate the photophysics and photochemistry of tetrasulfonated Si and Ge phthalocyanine ($SiPcS_4$ and $GePcS_4$) that contains only one species (tetrasulfonated) in aqueous medium (in the presence and absence of CEL) and in dimethylsulphoxide (DMSO).

2. Experimental and method

2.1. Materials

Silicon and germanium tetrasulfophthalocyanines ($SiPcS_4$ and $GePcS_4$) were synthesized, purified and characterized according to literature method [16]. Zinc tetrasulfophthalocyanine ($ZnPcS_4$), zinc phthalocyanine (ZnPc) and $ZnPcS_{mix}$ were also synthesized, purified and characterized according to literature methods [10,16,17]. Cremophore EL, 1,3-diphenylisobenzofuran (DPBF) and anthracene-9,10-bis-methylmalonate (ADMA) were obtained from Aldrich. Bovine serum albumin was obtained from Fluka. Phosphate-buffered saline (PBS) solution (0.01 M, pH 7.4) was prepared using appropriate amounts of Na_2HPO_4, KH_2PO_4 and chloride salts, dissolved in ultra pure water, from a Milli-Q Water System (Millipore Corp., Bedford, MA, USA).

2.2. Equipment

Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluoremeter. UV–visible spectra were recorded on a Varian 500 UV–vis/NIR spectrophotometer. Laser flash photolysis experiments were performed with light pulses produced by a Quanta-Ray Nd: YAG laser providing 400 mJ, 90 ns pulses of laser light at 10 Hz, pumping a Lambda-Physik FL3002 dye (Pyridin 1 dye in methanol). Single pulse energy ranged from 2 to 7 mJ. The analyzing beam source was from a Thermo Oriel xenon arc lamp, and a photomultiplier tube was used as a detector. Signals were recorded with a digital real-time oscilloscope (Tektronix TDS 360). The triplet life times were determined by exponential fitting of the kinetic curves using the program OriginPro 7.5. Photo-irradiations for photodegradation or singlet oxygen determination were performed using a general electric quartz line lamp (300 W). A 600 nm glass cut off filter (Schott) and water were used to filter off ultraviolet and infrared radiations, respectively. An interference filter (Intor, 670 nm with a band width of 20 nm) was additionally placed in the light path before the sample. Light intensity was measured with a POWER MAX5100 (Molelectron detector incorporated) power meter and was found to be 3.12×10^{16} photons s^{-1} for photobleaching and to be 1.25×10^{16} photons s^{-1} for singlet oxygen studies.

2.3. Determination of percentage aggregation, equilibrium dimerization constants

Percentage aggregation (%Agg) was calculated using the following equation [18]:

$$\%\mathrm{Agg} = \frac{\mathrm{Abs_{SRF}} - \mathrm{Abs}}{\mathrm{Abs_{SRF}}} \times 100 \quad (1)$$

where $\mathrm{Abs_{SRF}}$ and Abs are the absorbances at the Q band maxima in the presence and absence of the surfactant (SRF), cremophore EL in this case.

The equilibrium constant for $2\mathrm{M} \leftrightarrow \mathrm{D}$ is defined by the following equation:

$$K_\mathrm{D} = \frac{[\mathrm{D}]}{[\mathrm{M}]^2} \quad (2)$$

where [M] and [D] are the concentrations of the monomer and the dimer, respectively, the total concentration of the MPcs being given by the following equation:

$$C = [\mathrm{M}] + 2[\mathrm{D}] \quad (3)$$

The absorbance of a solution containing both the monomer and the dimer is given by the following equation [19]:

$$\mathrm{Abs} = \frac{[1 - \sqrt{(1 + 8K_\mathrm{D}C)}][\varepsilon_\mathrm{D}/(2 - \varepsilon_\mathrm{M})] + 2\varepsilon_\mathrm{D}K_\mathrm{D}C]}{4lK_\mathrm{D}} \quad (4)$$

where C is the total concentration of the MPcs (monomer and dimer), ε_M and ε_D the extinction coefficients of the monomer and dimer, respectively and l is the path length.

An estimated value of ε_M obtained from the measurements in aqueous solution within the concentration range at which the Beer–Lambert's law holds for each MPc was substituted in Eq. (4) and the absorbance data were fitted to obtain ε_D and K_D by a non-linear least square procedure using MATLAB 6.1 software.

2.4. Photophysical and photochemical studies

Fluorescence quantum yields (Φ_F) were determined by comparative method [20] (Eq. (5))

$$\Phi_\mathrm{F} = \Phi_{\mathrm{F(Std)}} \frac{FA_\mathrm{Std}\eta^2}{F_\mathrm{Std}A\eta_\mathrm{Std}^2} \quad (5)$$

where F and F_Std are the areas under the fluorescence curves of the MPc derivatives and the reference, respectively. A and A_Std are the absorbances of the sample and reference at the excitation wavelength, and η and η_Std are the refractive indices of solvents used for the sample and standard, respectively. ZnPc in DMSO was used as a standard, $\Phi_\mathrm{F} = 0.2$ [21]. At least three independent experiments were performed for the quantum yield determinations. Both the sample and the standard were excited at the same relevant wavelength.

Triplet quantum yields were determined using a comparative method based on triplet decay, using the following equation:

$$\Phi_\mathrm{T}^\mathrm{Sample} = \Phi_\mathrm{T}^\mathrm{Std} \frac{\Delta A^\mathrm{Sample}\varepsilon^\mathrm{Std}}{\Delta A^\mathrm{Std}\varepsilon^\mathrm{Sample}} \quad (6)$$

where $A_\mathrm{T}^\mathrm{Sample}$ and $A_\mathrm{T}^\mathrm{Std}$ are the changes in the triplet state absorbance of the sample derivative and the standard, respectively. $\varepsilon_\mathrm{T}^\mathrm{Sample}$ and $\varepsilon_\mathrm{T}^\mathrm{Std}$ are the triplet state extinction coefficients for the sample and standard, respectively. $\Phi_\mathrm{T}^\mathrm{Std}$ is the triplet state quantum yield for the standard. $ZnPcS_4$ in aqueous solution, $\Phi_\mathrm{T}^\mathrm{Std} = 0.56$ [22] and ZnPc ($F_\mathrm{T}^\mathrm{Std} = 0.65$ in DMSO [23]) were used as standards. Quantum yields of internal conversion were obtained from Eq. (7) which assumes that only three processes

(fluorescence, intersystem crossing and internal conversion), jointly deactivate the excited singlet states of the complexes.

$$\Phi_{IC} = 1 - (\Phi_F + \Phi_T) \quad (7)$$

The values of photobleaching quantum yields (Φ_P) were determined using the following equation:

$$\Phi_{Pd} = \frac{(C_0 - C_t)VN_A}{I_{abs}St} \quad (8)$$

where C_t and C_0 are the MPc concentration in mol dm^{-3} after and prior to irradiation, respectively. V is the reaction volume, S the irradiation area of the cell, t the irradiation time, N_A the Avogadro's number and I_{abs} is the overlap integral of the radiation light source intensity and the absorption of the MPc in the region of the interference filter transmittance [24,25]. Eq. (9) was employed for calculating singlet oxygen quantum yields:

$$\Phi_\Delta = \Phi_\Delta^{Std} \frac{RI_{abs}^{Std}}{R^{Std}I_{abs}} \quad (9)$$

where F_Δ^{Std} is the singlet oxygen quantum yield for the standard ($ZnPcS_{mix}$, $\Phi_\Delta^{Std} = 0.45$ in aqueous solution) [26] and ZnPc ($\Phi_\Delta^{Std} = 0.67$ in DMSO [25]). R and R^{Std} are the ADMA or DPBF photobleaching rates in the presence of the respective MPcs under investigation and the standard, respectively. I_{abs} and I_{abs}^{Std} are the rates of light absorption by the MPcs and the standard, respectively. To avoid chain reactions, the concentration of ADMA was kept at $\sim 6 \times 10^{-5}$ mol l^{-1} while that of DPBF was kept at $\sim 3 \times 10^{-5}$ mol l^{-1}.

Solutions of the MPcs with an absorbance of 0.2 at the irradiation wavelength were prepared in the dark and irradiated at the Q band region, monitoring the ADMA and DPBF degradation at 380 nm and 417 nm, respectively. The error was ~10% from several values of Φ_Δ.

2.5. Binding of MPcs to BSA

The binding of the MPcs to BSA was studied by spectrofluorometry at room temperature. An aqueous solution of BSA (fixed concentration) was titrated with varying concentrations of the respective MPc solution. BSA was excited at 280 nm and fluorescence recorded between 290 and 500 nm. The steady decrease in BSA fluorescence with increase in MPc concentration was noted and used in the determination of the binding constants and the number of binding sites on BSA, according to the following equation [19,27,28]:

$$\log\left[\frac{(F_0 - F)}{(F - F_\infty)}\right] = \log k_b + n \log[MPc] \quad (10)$$

where F_0 and F are the fluorescence intensities of BSA in the absence and presence of MPc, respectively, F_∞ the fluorescence intensity of BSA saturated with MPc, k_b the binding constant, n the number of binding sites on a BSA molecule and [MPc] is the concentration of different MPc complexes. Plots of $\log[(F_0-F)/(F-F_\infty)]$ against log [MPc] would provide the values of n (from slope) and k_b (from the intercept). The changes in BSA fluorescence intensity were related to MPc concentrations by the Stern–Volmer relationship (Eq. (11)) [29]:

$$\frac{F_0^{BSA}}{F^{BSA}} = 1 + K_{SV}^{BSA}[MPc] \quad (11)$$

and K_{SV}^{BSA} is given by the following equation:

$$K_{SV}^{BSA} = k_q \tau_F^{BSA} \quad (12)$$

where F_0^{BSA} and F^{BSA} are the fluorescence intensities of BSA in the absence and presence of MPcs, respectively, K_{SV}^{BSA} the Stern–Volmer quenching constant, k_q the bimolecular quenching constant and τ_F^{BSA} is the fluorescence lifetime of BSA. τ_F^{BSA} is known to be 10 ns [30–32], thus from the values K_{SV}^{BSA} obtained from the plots of F_0^{BSA}/F^{BSA} versus [MPc], the value of k_q may be determined (Eqs. (11) and (12)).

3. Results and discussion

3.1. Spectrophotometric studies

Tetrasulfonated metallophthalocyanines ($MPcS_4$) complexes are well known and the $GePcS_4$ and $SiPcS_4$ gave satisfactory spectroscopic analyses similar to literature [33].

The ground state electronic spectra of the tetrasulfonated metallophthalocyanine complexes are shown in Fig. 1 with the inset representing the structure. The complexes are aggregated with the formation of two main bands at ~680 nm due to the monomeric species and ~640 nm due to the aggregated species, respectively. Aggregation in MPc complexes is due to a coplanar association of rings, resulting in splitting and broadening of spectra, with a blue shifted peak at ~630 nm being due to the aggregate. It is documented that with tetrasulfonated phthalocyanines, there is existence of dimers in aqueous solution, these dimers are not chemically bonded but exist as loosely associated species which can be dissociated by surfactants or in non-aqueous solvents. They are believed to be a result of association between the peripheral substituents of the phthalocyanine, which hold adjacent rings together in space. Also, water has been found to distinctively change the spectra of sulfonated

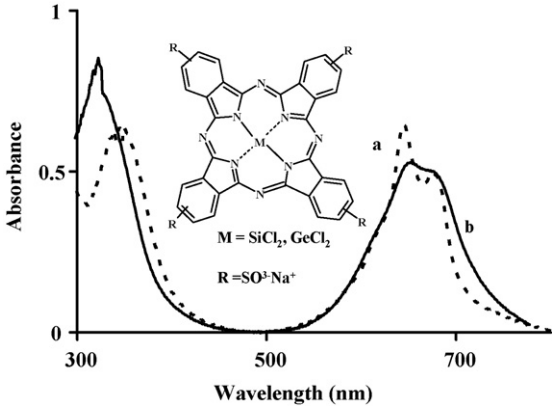

Fig. 1. Ground state electronic absorption spectra of the sulfonated MPc complexes in aqueous solution; (a) $GePcS_4$ and (b) $SiPcS_4$ (10^{-6} mol dm^{-3}). *Inset*: chemical structure of the tetrasulfonated substituted metallophthalocyanines.

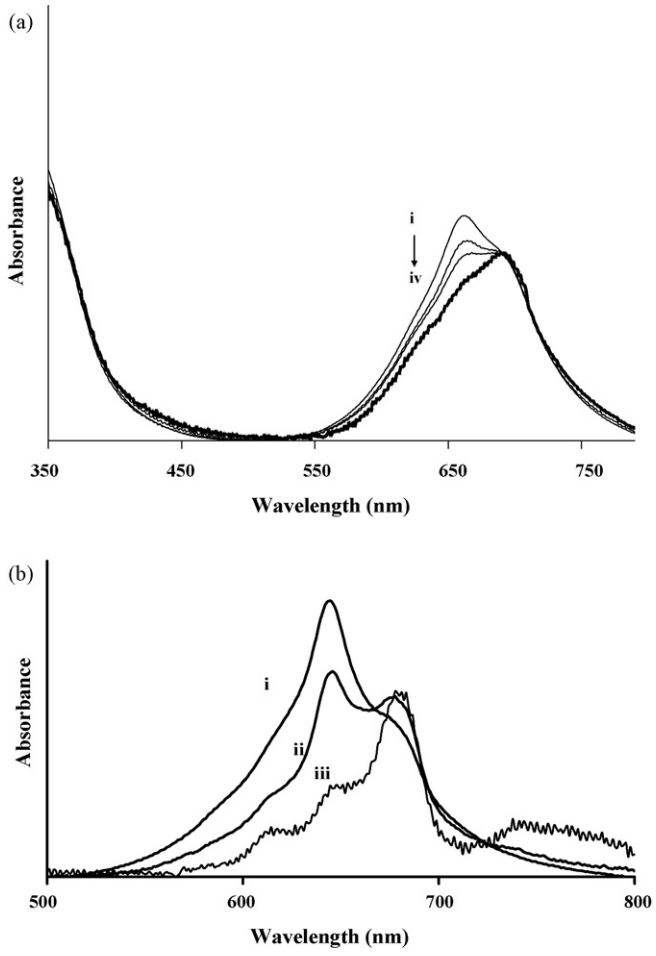

Fig. 2. (a) Electronic absorption spectra of $SiPcS_4$ in aqueous solution at four different concentrations; (i) 7.2×10^{-6} mol dm^{-3}, (ii) 3.1×10^{-6} mol dm^{-3}, (iii) 2.0×10^{-6} mol dm^{-3} and (iv) 5.1×10^{-7} mol dm^{-3}. (b) Electronic absorption spectra of $GePcS_4$ in aqueous solution at three different concentrations; (i) 3.6×10^{-6} mol dm^{-3}, (ii) 1.2×10^{-6} mol dm^{-3} and (iii) 1.8×10^{-7} mol dm^{-3}.

compounds [22,34–37]. In this present study, the unassociated species only began to appear at very low concentrations of about 10^{-7} mol dm^{-3} as shown in Fig. 2a and b. Beer's law was obtained at concentrations lower than this value for both complexes. It therefore means that at very low phthalocyanine concentration, the monomeric band is enhanced, due to decreased association between the species in solution. The addition of a surfactant drug carrier, CEL, resulted in a decrease in the intensity of the band due to the aggregates with an increase in the intensity of the band due to the monomeric species (Fig. 3a and b), which confirms that the complexes are aggregated in aqueous solution with Q band maxima given in Table 1. The disaggregation of $SiPcS_4$ occurred immediately after adding 4×10^{-3} mol dm^{-3} of CEL while for $GePcS_4$, disaggregation occurred with time, Fig. 3c, but the final spectrum still shows some aggregation suggesting that strong forces are responsible for the aggregation or that a high equilibrium between the monomer and the aggregated species occurs. It was observed, that disaggregation occurred in $GePcS_4$ when the complex was left in aqueous solution for 5 days without the addition of the surfactant which means it disaggregates by itself with time but since fresh solutions are needed

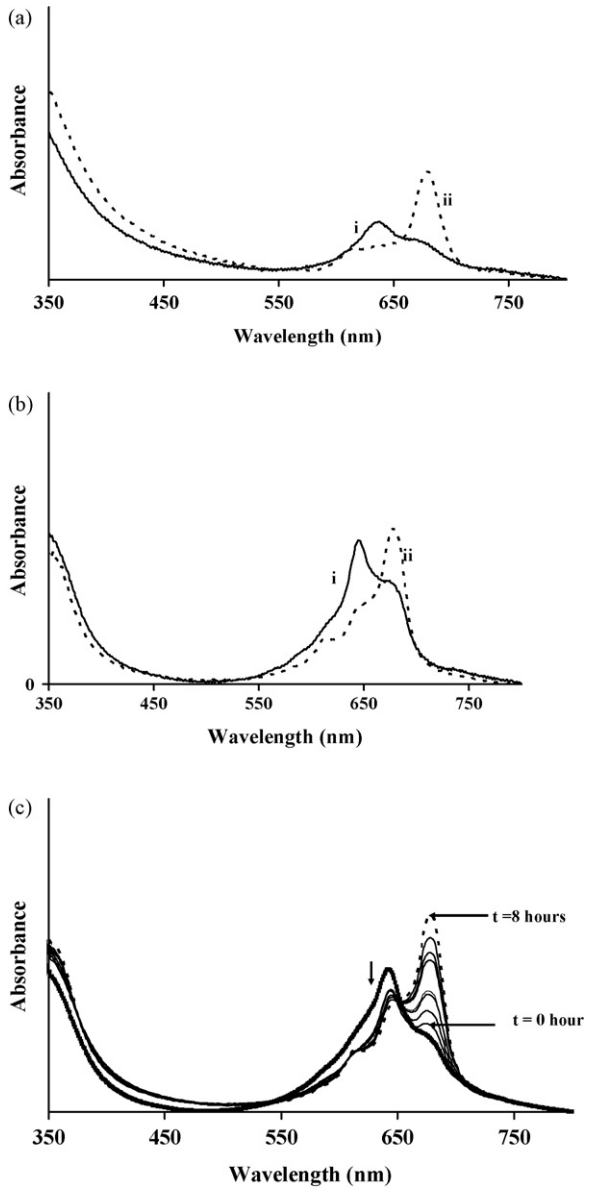

Fig. 3. (a) Absorption spectra of 1.8×10^{-6} mol dm^{-3} $SiPcS_4$ in the (i) absence and (ii) presence of 4×10^{-3} mol dm^{-3} CEL. (b) Absorption spectra of 1.7×10^{-6} mol dm^{-3} $GePcS_4$ in the (i) absence and (ii) presence of 4×10^{-3} mol dm^{-3} CEL. (c) $GePcS_4$ (1.7×10^{-6} mol dm^{-3}) absorption spectrum (thick line) showing disaggregation with time in the presence of 4×10^{-3} mol dm^{-3} CEL, $t = 0$–8 h.

for all experimental work, we base our results on its aggregated form.

Aggregated MPcs are expected to disaggregate and exhibit monomeric behaviour in organic solvents [10,38], and this was the case with both complexes in dimethylsulphoxide, $SiPcS_4$ showed mainly monomeric behaviour, while $GePcS_4$ remains slightly broadened in DMSO (Fig. 4), suggesting that complete dissaggregation was not achieved in this solvent for $GePcS_4$ which may be due to the fact that some of the forces responsible for the aggregation may be too strong to be broken by the organic solvent, DMSO.

Percentage aggregation (%Agg) was calculated for the two complexes with $SiPcS_4$ having a higher value of 65.8% com-

Table 1
Spectral and photophysicochemical parameters for TSMPcs complexes in aqueous and non-aqueous media

Sample	Solvent	λ_Q (nm)	log ε	λ_F (nm)	Φ_F	Φ_T	Φ_{IC}	τ_T (μs)	Φ_Δ	Φ_{Pd} ($\times 10^5$)
$SiPcS_4$	PBS	679,636	4.96	692	0.01	0.49	0.50	200	0.50	4.48
$SiPcS_4$	PBS + CEL	679	–	691	0.03	0.57	0.40	270	0.55	5.82
$SiPcS_4$	DMSO	679	4.44	692	0.05	0.67	0.28	310	0.68	8.62
$GePcS_4$	PBS	680,646	4.83	688	0.05	0.61	0.34	180	0.64	3.66
$GePcS_4$	PBS + CEL	681	–	689	0.09	0.70	0.21	240	0.68	5.54
$GePcS_4$	DMSO	683	4.32	691	0.12	0.84	0.04	640	0.31	8.57

CEL: cremophore EL.

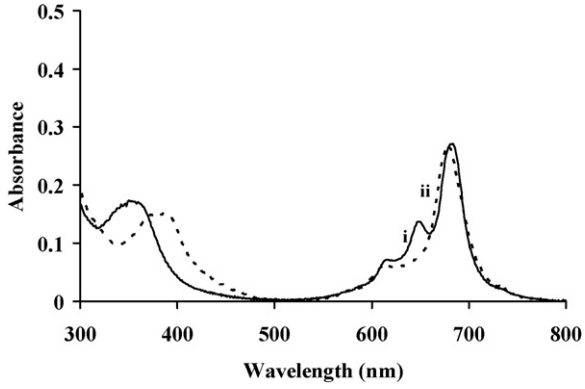

Fig. 4. Ground state electronic absorption spectra of the sulfonated MPc complexes in DMSO; (i) $GePcS_4$ (solid line) and (ii) $SiPcS_4$ (dotted line) (10^{-5} mol dm^{-3}).

pared to $GePcS_4$ (35.2%) as shown in Table 2. This higher %Agg for the former may lead to a low photoactivity of the complex in aqueous solution since aggregation diminishes the photosensitizing ability of MPc complexes. The low value of %Agg for $GePcS_4$ could be due to incomplete disaggregation which could not be achieved in CEL or organic solvent. The values of equilibrium constant (K_D) for dimerization are the same for both complexes Table 2 indicating a strong association between the aggregates. The values of ε_D (Table 2) and ε_M (Table 1) are within the range reported for some MPc complexes [18,37].

Absorption and fluorescence excitation spectra of MPcs are meant to be similar and mirror images of their emission spectrum, but for these complexes in aqueous medium, there is a lack of agreement between these spectra as shown in Fig. 5a and b. The band around 640 nm, associated with the dimer is not seen in the fluorescence excitation spectra suggesting that only the monomer fluoresces. It has been documented before that dimers are non-photoactive [22]. The Stokes' shifts were in the neighbourhood of 10 nm, typical of MPc complexes. In DMSO, the absorption and fluorescence excitation spectra of $SiPcS_4$ are similar and are mirror images of their emission spectrum, however, for $GePcS_4$, only a single band was observed in the fluorescence

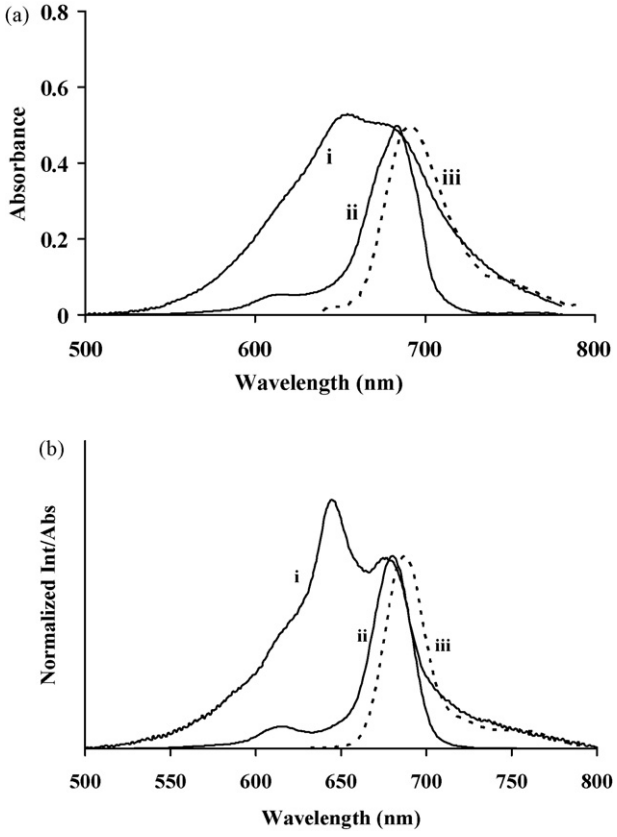

Fig. 5. (a) Normalized absorption (i), fluorescence excitation (ii) and emission (iii) spectra of $SiPcS_4$ in aqueous medium. (b) Normalized absorption (i), fluorescence excitation (ii) and emission (iii) spectra of $GePcS_4$ in aqueous medium.

excitation spectrum in contrast with the slight broadness in its absorption band. Fig. 6 shows the fluorescence excitation and emission spectra of the complexes in aqueous medium in the presence of CEL, they are both mirror images of each other. The excitation spectrum is similar to the absorption spectrum in the presence of CEL for $SiPcS_4$ which further emphasizes the disaggregation induced by CEL. For $GePcS_4$, the absorption spectrum still had some aggregation even in CEL.

Table 2
Aggregation parameters and BSA binding data for the MPcs in aqueous medium

Complex	%Agg	log ε_D	K_D ($\times 10^{-4}$ dm^3 mol^{-1})	K_{SV}^{BSA} ($\times 10^{-5}$ M^{-1})	k_q ($\times 10^{-13}$ M^{-1} s^{-1})	k_b ($\times 10^{-7}$ dm^3 mol^{-1})	n
$SiPcS_4$	65.8	4.69	1.98	2.51	2.51	3.02	1.4
Ge PcS_4	35.2	4.53	1.99	2.21	2.21	2.38	1.4

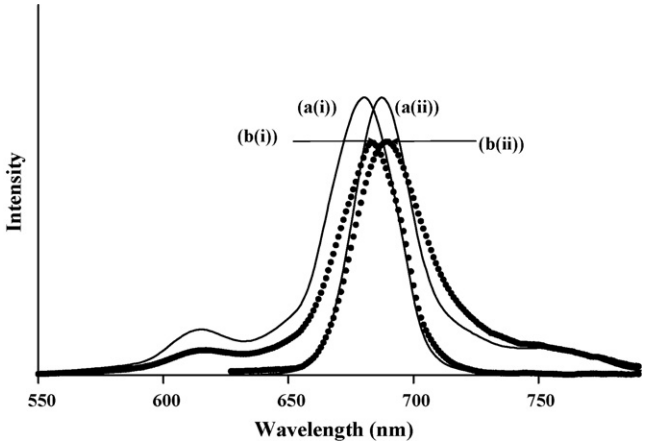

Fig. 6. Normalized fluorescence excitation (i) and emission (ii) spectra of $GePcS_4$ (a) (solid line) and $SiPcS_4$ (b) (dotted line) in the presence of cremophore EL in aqueous medium.

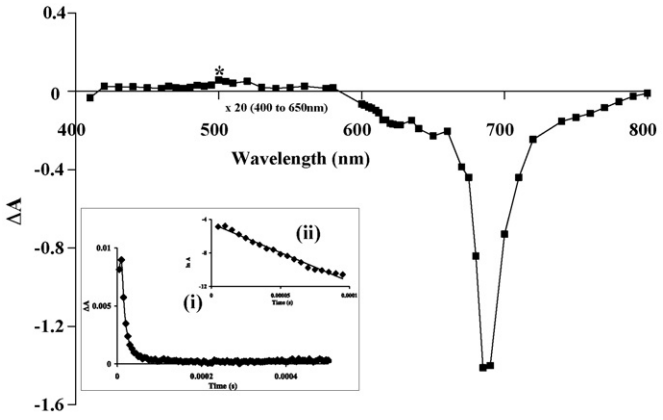

Fig. 7. Transient absorption spectrum for $GePcS_4$ in PBS pH 7.4 in the presence of CEL. (*) = triplet state absorption at $\lambda = 500$ nm. *Inset*: (i) triplet decay curve of $GePcS_4$ in the presence of CEL in PBS 7.4 and (ii) plot of ln *A* vs. time for $GePcS_4$ in the presence of CEL. $[GePcS_4] = \sim 2.3 \times 10^{-5}$ mol dm^{-3}, $\lambda_{exc} = 685$ nm.

3.2. Photophysical and photochemical parameters

The fluorescence quantum yields Φ_F of the complexes are listed in Table 1. The two complexes have relatively low yields of fluorescence, even in CEL where $SiPcS_4$ is totally disaggregated. Aggregation is known to dissipate the electronic energy of the excited singlet state, thereby lowering fluorescence. Monomerization of aggregates leads to enhanced fluorescence and this is noticed in the Φ_F value of the complexes in the presence of CEL in aqueous medium and in DMSO compared to that in aqueous medium alone. The Φ_F values followed the trend PBS 7.4 < PBS 7.4 + CEL < DMSO for both complexes. The Φ_F values are however low compared to MPcs in general [7].

Φ_T values depended on heavy atom effect. Φ_T values were higher in all the media for $GePcS_4$ compared to $SiPcS_4$. Φ_T values were highest in each case in DMSO, which is probably due to the fact that intersystem crossing is favoured in DMSO than in aqueous solution. As expected, the presence of CEL gave increased Φ_T values since the $MPcS_4$ complexes are disaggregated in this case, monomers have greater tendencies to undergo intersystem crossing because less energy is lost through internal conversion [10]. Fig. 7 shows the transient absorption spectrum of $GePcS_4$ in the presence of CEL in PBS showing the monomer peak with slight broadening. The absorption of the triplet state is centered at 500 nm as shown with the asterisk in Fig. 7. The shape of the Q band is similar to the ground state absorption spectrum indicating that there is no change occurring in the MPc in the presence of CEL at the triplet state. The triplet absorption curve in the presence of CEL is shown in Fig. 7 (inset i), confirming a monoexponential decay. Plots of ln *A* versus time (inset ii) are linear, showing a first order dependence of the triplet state.

Φ_{IC} values were generally high due to aggregation, which is due to the dissipation of electronic energy by the aggregates. Φ_{IC} values decreases in the order, PBS 7.4 > PBS 7.4 + CEL > DMSO which suggests that molecules are more photoactive in DMSO than in aqueous solution. The decrease in Φ_{IC} values in the presence of CEL is due to its monomerizing effect on the $MPcS_4$ complexes. Φ_{IC} values are lower in $GePcS_4$ than in $SiPcS_4$ due to the higher photosensitizing ability of the former.

There is a striking increase in triplet lifetimes (τ_T) in the order, PBS 7.4 < PBS 7.4 + CEL < DMSO. The lowest τ_T values exhibited in PBS 7.4, are likely due to the fact that water quenches the triplet states of MPcs as has been reported before [21]. Values of τ_T in DMSO are high; this may be attributed to the fact that the absorption of DMSO at the region of the energy of the triplet states of MPcs (~1108 nm) is not as intense as that of water [21]. This stresses the effect of solvent on triplet lifetimes. The increase in τ_T values in the presence of CEL compared to PBS alone may be due to the reduction in the exposure of the phthalocyanine to the aqueous medium because of the presence of the CEL.

Energy transfer between the triplet state of photosensitizers and ground state molecular oxygen leads to the production of singlet oxygen, Φ_Δ. Therefore, singlet oxygen quantum yield which is the efficiency of singlet oxygen generation, should depend on the triplet state quantum yield and lifetime, the efficiency of energy transfer, which depends on the energy of the triplet state and the ability of the substituents to quench the triplet state amongst other factors. An efficient production of Φ_Δ in all media was observed, though the Φ_Δ value is unexpectedly low for $GePcS_4$ in DMSO. This may be connected to the fact that this complex is not fully disaggregated even in DMSO.

Oxidative attack on the excited triplet state of MPcs by singlet oxygen brings about photodegradation, since the triplet state is sufficiently long lived to participate in photochemical reactions. It is believed that singlet oxygen has the ability to react with macrocyclic metal complexes [38]. Photodegradation quantum yields in the MPc complexes were higher in DMSO than in PBS pH 7.4 and also higher in the presence of CEL. In both DMSO and CEL, Φ_Δ values are higher than for pH 7.4 for $SiPcS_4$. Thus, photodegradation is dependent on singlet oxygen for $SiPcS_4$. For $GePcS_4$, the Φ_Δ value in DMSO is low, yet the photodegradation quantum yield is higher than in both pH 7.4 alone and in CEL, this implies that for this complex in DMSO, the degradation occurs because the triplet state is sufficiently long lived.

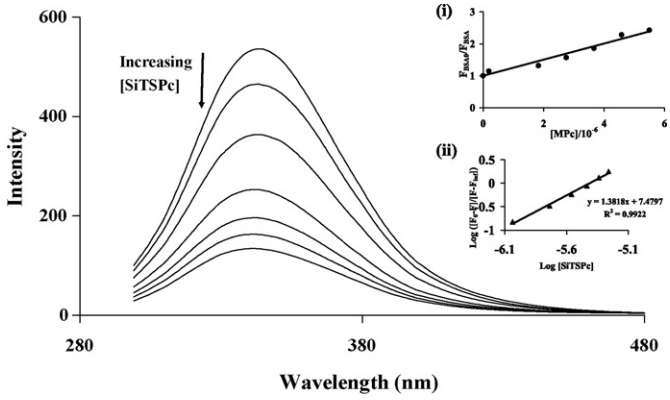

Fig. 8. Spectral changes of BSA (3×10^{-6} mol dm^{-3}) on addition of $SiPcS_4$ (0–5.5×10^{-6} mol dm^{-3}) in aqueous medium. *Inset*: (i) is the Stern–Volmer's plot and (ii) determination of binding constants.

3.3. Interaction of MPcs with BSA

3.3.1. Binding constants and fluorescence quenching

Fig. 8 shows quenching of BSA fluorescence by $SiPcS_4$. This quenching in fluorescence was used to estimate the binding constants (k_b) and the binding stoichiometry (n) using Eq. (10) (Fig. 8, inset ii). The values of k_b and n are shown in Table 2. The k_b values for both complexes are in the same range though slightly higher in $SiPcS_4$ compared to $GePcS_4$ indicating that $SiPcS_4$ binds more strongly to BSA than $GePcS_4$ which may be due to the fact that $SiPcS_4$ disaggregates easily and binds to BSA. The binding stoichiometry in these experiments is ~1 suggesting a single binding site for the MPcs which is characteristic for MPc–BSA interactions in aqueous medium [19,39].

The inset (i) in Fig. 8 shows the Stern–Volmer plot which indicates that within the investigated range of concentrations, Stern–Volmer equation (Eq. (11)) is obeyed. The K_{SV}^{BSA} values were determined from the slopes of plots of Fig. 8 (inset i). Table 2 shows the K_{SV} values with $SiPcS_4$ having a higher value compared to $GePcS_4$ suggesting that the BSA fluorescence quenching is more effective in the former. This also shows that there is some agreement between K_b and K_{SV}. Using Eq. (12) and the approximate values of fluorescence lifetime of BSA (10 ns) [30–32], the bimolecular quenching constants (k_q) were determined for the individual MPcs. As shown in Table 2, the k_q values are higher (being in the order of 10^{13} dm^3 mol^{-1} s^{-1}) than the proposed value for dynamic quenching (10^{10} dm^3 mol^{-1} s^{-1}) [40] indicating that quenching mechanism in this case is static.

4. Conclusion

The photophysical and photochemical properties of silicon and germanium tetrasulfonated phthalocyanine were studied and were found to be dependent on the central metal and solvents used. The complexes were highly aggregated in aqueous solution, but the addition of cremophore EL hindered aggregation resulting in increased triplet quantum yields (Φ_T), lifetimes (τ_T) and singlet oxygen (Φ_Δ) compared with aqueous medium, suggesting an improved photosensitizing ability of these complexes in the presence of CEL.

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References

[1] R. Bonnett, In Chemical Aspects of Photodynamic Therapy, Gordon and Breach Science Publishers, Amsterdam, 2000.
[2] R.K. Panday, J. Porphyrins Phthalocyanines 4 (2000) 368.
[3] I. Rosenthal, Photochem. Photobiol. 53 (1991) 859.
[4] S.G. Brown, C.J. Tralau, P.D. Colendge-Smith, D. Akdemir, T. Wieman, J. Cancer 54 (1987) 43.
[5] J.D. Spikes, J. Photochem. Photobiol. B: Biol. 6 (1990) 259.
[6] W.S. Cahn, N. Brasseur, C. La Madeleine, R. Quellet, J.E. van Lier, Eur. J. Cancer 33 (2001) 1855.
[7] T. Nyokong, Coord. Chem. Rev. 251 (2007) 1707.
[8] N.A. Kuznetsova, N.S. Gretsova, V.M. Derkacheva, O.L. Kaliya, E.A. Lukyanets, J. Porphyrins Phthalocyanines 7 (2003) 147.
[9] R. Edrei, V. Gottfried, J.E. van Lier, S. Kimel, J. Porphyrins Phthalocyanines 2 (1998) 191.
[10] A. Ogunsipe, T. Nyokong, J. Photochem. Photobiol. A: Chem. 173 (2005) 211.
[11] G. Storm, D.J.A. Crommelin, Pharm. Sci. Technol. Today 1 (1998) 19.
[12] S. Xu, S. Chen, M. Zhang, T. Shen, Z. Xiaoxing, W. Zhengping, Photochem. Photobiol. 78 (2003) 411.
[13] G. Jori, in: B.W. Henderson, T.J. Dougherty (Eds.), Photodynamic therapy basic principles and clinical applications, Marcel Dekker, New York, NY, 1992.
[14] A.A. Bhattacharya, S. Curry, N.P. Franks, J. Biol. Chem. 275 (2000) 38731.
[15] A.O. Ogunsipe, T. Nyokong, J. Porphyrins Phthalocyanines 9 (2005) 121.
[16] J.H. Weber, D.H. Busch, Inorg. Chem. 4 (1965) 469.
[17] H. Tomoda, S. Saito, S. Shiraishi, Chem. Lett. 12 (1983) 313.
[18] S. Khene, A. Ogunsipe, E. Antunes, T. Nyokong, J. Porphyrins Phthalocyanines 11 (2007) 109.
[19] S.M.T. Nunes, F.S. Squilla, A.C. Tedesco, Braz. J. Med. Biol. Res. 37 (2004) 273.
[20] S. Fery-Forgues, D.J. Lavabre, Chem. Ed. 76 (1999) 1260.
[21] A. Ogunsipe, J.Y. Chen, T. Nyokong, New J. Chem. 7 (2004) 822.
[22] A. Harriman, M.C. Richoux, J. Chem. Soc. Faraday Trans. 2 76 (1980) 1618.
[23] T.H. Tran-Thi, C. Desforge, C. Thies, J. Phys. Chem. 93 (1989) 1226.
[24] I. Seotsanyana-Mokhosi, N. Kuznetsova, T. Nyokong, J. Photochem. Photobiol. A: Chem. 140 (2001) 215.
[25] N. Kuznetsova, N. Gretsova, E. Kalmykova, E. Makarova, S. Dashkevich, V. Negrimovskii, O. Kaliya, E. Lukyanets, Russ. J. Gen. Chem. 70 (2000) 133.
[26] F. Wilkinson, W.P. Helman, A.B. Ross, J. Phys. Chem. Ref. Data 22 (1993) 113.
[27] D.M. Chipman, V. Grisaro, N. Sharon, J. Biol. Chem. 242 (1967) 4388.
[28] S. Lehrer, G.D. Fashman, Biochem. Biophys. Res. Commun. 23 (1966) 133.
[29] J. Rose, Advanced Physico-chemical Experiments, 1st edn., Sir Isaac Pitman and Sons Ltd., London, 1964, p. 257.
[30] M. Guo, J.W. Zou, P.G. Yi, Z.C. Shang, G.X. Hu, Q.S. Yu, Anal. Sci. 20 (2004) 465.
[31] C.Q. Jiang, M.X. Gao, J.X. He, Anal. Chim. Acta 452 (2002) 185.
[32] J.R. Lakowicz, G. Weber, Biochemistry 12 (1973) 4161.
[33] A.G. Gurek, G. Appel, R.P. Mikalo, D. Schmeiber, J. Porphyrins Phthalocyanines 5 (2001) 751.
[34] L.D. Rollman, R.T. Iwamoto, J. Am. Chem. Soc. 90 (1980) 1455.
[35] K. Bernauer, S. Falab, Helv. Chim. Acta 44 (1961) 1287.
[36] H. Kobayashi, Y. Torii, N. Fukada, Nippon Kagaku Zasshi 81 (1960) 694.

[37] M.J. Stillman, T. Nyokong, in: C.C. Lexnoff, A.B.P. Lever (Eds.), Pthalocyanines: Properties and Applications, vol. 1, VCH, New York, 1989, p. 139.
[38] G. Schnurpfeil, A.K. Sobbi, W. Spiller, H. Kliesch, D. Worhle, J. Porphyrins Phthalocyanines 1 (1997) 159.
[39] A. Ogunsipe, T. Nyokong, Photochem. Photobiol. Sci. 4 (2005) 510.
[40] S.L. Murov, I. Carmichael, G.L. Hug, Handbook of Photochemistry, 2nd edn., Marcel Decker, New York, 1993, p. 207.