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CHARACTERIZATION AND PHOTOPHYSICAL PROPERTIES OF PHTHALOCYANINE-CYTIDINE-SINGLE WALLED CARBON NANOTUBE ANTI-CANCER COMPLEX

Awolope R.O.[1], Ejidike I.P.[1], Nyokong T.[2]

[1]Department of Chemical Sciences, Faculty of Science and Science Education, Anchor University, Lagos, Nigeria
[2]Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa

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ABSTRACT

Background: The mortality rate of cancer, one of the leading causes of death globally is continuously increasing. Despite the progress in preparing and approving conventional chemotherapeutic drugs, new forms of the diseases are evolving. Hence, an urgent need for alternative therapy .
Objectives: To prepare combinatory drugs composed of nucleoside-metallophthalocyanine and a single-walled carbon complex as a potential anti-cancer agent.The complex is a major key player in photodynamic therapy. Cytidine, a nucleoside which is a major component of ribonucleic acid was chemically linked to zinc tetra-phenoxycarboxy phthalocyanine (1), a photoactive compound represented as ZnTCPC-cytidine (2). Complex 2 was adsorbed on single-walled carbon nanotube (SWCNT) represented as ZnTCPC-cytidine-SWCNT (3).
Methods: Complexes 1, 2 and 3 were characterized using UV-visible and Fourier transform infrared (FTIR) spectroscopy. The photophysical properties such as fluorescence quantum and triplet quantum yield were studied using fluorescence emission and laser flash spectroscopy respectively.
Results: The absorption spectra show that complex 1, 2 and 3 have the characteristics Q-band of metallophthalocyanine compounds, the FTIR spectra also confirmed that the cytidine was chemically linked to ZnTCPC, while ZnTCPC-cytidine was successfully adsorbed on SWCNT through π-π stacking. The fluorescence quantum yields were 0.10, 0.098 and 0.130, while the triplet quantum yield was 0.49, 0.76 and 0.78 for complex 1, 2 and 3 respectively.
Conclusions: The triplet quantum yield result showed that complexes 2 and 3 can generate high singlet oxygen, the cytotoxic agent responsible for the irreversible destruction of cancerous cells. Hence, these complexes would find application as potential anticancer drugs for photodynamic therapy.

Keywords: Zinc tetra-phenoxycarboxy phthalocyanine, Single-walled carbon nanotubes, cytidine, fluorescence and triplet quantum yields

INTRODUCTION

Photosensitizer bio-conjugate; a complex containing a receptor-targeting moiety and a photosensitzer (metallophthalocyanines (MPcs)) is one of the developing targeting strategies employed by researchers in photodynamic therapy (PDT) (Bonnett, 2000, Okura 2001). The low toxicity of MPc and ability to generate singlet oxygen, the chief cytotoxic specie responsible for cancer cell death makes MPc a good photosensitizer for photodynamic therapy(Ogbodu et al. 2015). MPcs are in different stages of clinical trials (Grove and Cheng 1996). However, there is a need for a more specific therapeutic system, which could be achieved by conjugating MPcs to cancer-specific agent.

Nucleoside is one of the major components of ribonucleic acid which is responsible for genetic expressions among others. Studies have shown that some nucleosides such as cytidine and uridine have anticancer properties (Grove and Cheng 1996, Guillemette et al. 2000, Nagar and Remmel 2006).

Haakensen et al. 2010 also shows that uridine 5′-diphospho-glucuronosyltransferase is over-expressed in breast cancer.The presence of cytidine in the macromolecule of MPc, will provide selectivity, as well as increase the potency to MPc in cancer ells. Some nucleoside-phthalocyanine conjugates have been reported, Shen et al. 2013 reported the spectra properties of zinc phthalocyanine conjugated to nucleoside, while the photophysical properties of silicon(iv) phthalocyanine-nucleoside conjugate was shown by Li et al. 2001. Herein, we report for the first time the photophysical properties of zinc tetraphenoxylcarboxy phthalocyanine (ZnTPCPc (**1**)) linked to cytidine represented as ZnTPCPc-cytidine (**2**).

Drug delivery agents are capable of increasing the PDT effects of MPcs. This can be done by adsorbing the MPc onto a drug delivery agent, such as single-walled carbon nanotubes (SWCNTs). SWCNTs are nanomaterials that possess hollow and cage-like interior through which they can transport drugs; its aspect ratio is high and surface area is large (Lin et al. 2004, Bianco et al. 2005). The effects of SWCNTs on the photophysical properties of MPc-nucleoside conjugate were examined. Complex **2** was adsorbed onto SWCNTs represented as ZnTPCPc-cytidine-SWCNT (**3**).

2. Experimental

2.1 Materials

Zinc phthalocyanine (ZnPc), dimethylaminopyridine (DMAP),cytidine,lipophilic sephadex LH-20 microbeades, and1-ethyl-3-(3-dimethylaminoproplyl) carbodiimide hydrochloride (EDC), were bought from Sigma Adrich. Single-walled carbon nanotubes (SWCNTs, 1-5 nm in diameter and 1-5 mm in length) were obtained from Nanolab. Dimethylsulphoxide (DMSO) was obtained from SAARCHEM. ZnTPCPcwere synthesized according to literature methods (Li et al. 2008).

2.2 Equipment

The Shimadzu UV-Vis 2550 spectrophotometer were used to record the absorption spectra and the fluorescence emission and excitation spectra were obtained from a Varian Eclipse spectrofluorimeter using a 360-1100 nm filter. A Perkin-Elmer Universal ATR Sampling accessory spectrum 100 FT-IR spectrometer were used to record infrared spectra.

The triplet decay kinetics were determined using laser flash photolysis details have been reported (Masilela and Nyokong 2011) In brief, a tunable laser system consisting of an Nd:YAG laser (355 nm, 135 mJ/4-6 ns) pumping an optical parametric oscillator (OPO, 30 mJ/3-5 ns) with a wavelength range of 420-2300 nm (NT-342B, Ekspla) was used to produce the excitation pulses. Sample solutions with Q-band maxima at 1.5 for triplet state studies under de-aeration with argon for 15 min before measurement. Triplet lifetimes were determined by exponential fitting of the kinetic curves using OriginPro 8 software.

2.3 Synthesis

2.3.1 Synthesis ZnTPCPc-cytidine (2) conjugate, Scheme 1A.

ZnTPCPc-cytidine (**2**) conjugate was synthesized using a modified literature method (Fashina et al. 2013). The carboxy group of the ZnTPCPc (**1**) (0.1g, 0.089 mmol) was activated by stirring it with 0.2g of EDC for 2 h in phosphate buffer saline (PBS). After this time, 0.87g of cytidine and 0.17 g of DMAP was added and the solution was further stirred for 48 h. Ethanol was used to precipitate the solid product, and the product was repeatedly washed with ethanol to remove unreacted EDC and DMAP which were soluble in ethanol. The solid product was washed with de-ionized water to remove unreacted cytidine. Size exclusion chromatography was used to further purify the synthesized conjugate.

ZnTPCPc-cytidine (**2**): UV–Vis λ_{max} nm 678, IR [(ATR) ν_{max}/cm^{-1}];3397-3048 (O-H str.), 1786 (C=O), 1600-1558 (C=N, amide), 1485 (C=C), 1391 (O-H bend),1231 (C-O-C str.).

2.3.2 Synthesis of ZnTPCPc-cytidine-SWCNT (3) conjugate, Scheme 1B

Complex **2** was adsorbed onto SWCNT -COOH following literature methods (Ogbodu and Nyokong 2015): 20 mg of SWCNT-COOH were ultrasonicated for 1 hr in 10 ml of phosphate buffer saline PBS to give a brown coloured suspension, after this time, it was centrifuged at 3500 rpm for 20 minutes to get rid of large bundles debris of SWCNT-COOH. The supernatant was used

for experiments. 0.02g of ZnTPCPc-cytidine (2) was mixed with the activated supernatant solution of SWCNT to give a blue suspension; it was then stirred for 7 days to give a dark blue solution indicating the adsorption of complex 2 onto SWCNT-COOH. Ethanol was added to extract PBS, and the solid products were obtained by centrifugation. The final product ZnTPCPc-cytidine-SWCNT (3) was purified using size exclusion chromatography.

ZnTPCPc-cytidine-SWCNT (3): UV–Vis λmax nm 78, IR [(ATR) vmax/cm-1]; 3466 -3000 (O-H str.), 1589(C=N, amide), 1490 (C=C), 1391 (O-H bend), 1236 (C-O-C str.).

2.4 Photophysical and photochemical parameters

2.4.1 Triplet quantum yields and lifetimes

Triplet quantum yields (Φ_T) were determined using a comparative method based on triplet decay (Tran-Thi 1989) using ZnPc as the standard, Eq. 1

$$\Phi_T = \Phi_{T\,(std)} \frac{\Delta A_T \cdot \varepsilon T^{std}}{\Delta A_T^{std} \cdot \varepsilon T} \quad (1)$$

where ΔA_T and ΔA_T^{std} are the changes in the triplet state absorbance of the complexes (**1**, **2** and **3**) and standard, respectively. ε_T and ε_T^{std} are the triplet state extinction coefficients for the complexes (**2**, **3**) and standard, respectively. $\Phi_{T\,(std)}$ is the triplet state quantum yield for ZnPc used as the standard in DMSO ($\Phi_{T\,(std)} = 0.65$ (Kubat, 1996)).

2.4.2 Fluorescence quantum yields

Fluorescence quantum yields (F_F) of the complexes (**2**, **3**) were determined using the comparative method (Frey-Forgues, 1999) Eq. (3):

$$\Phi_F = \Phi_{F\,(std)} \frac{F \cdot A_{std} \cdot n^2}{F_{std} \cdot A \cdot n^2_{std}} \quad (3)$$

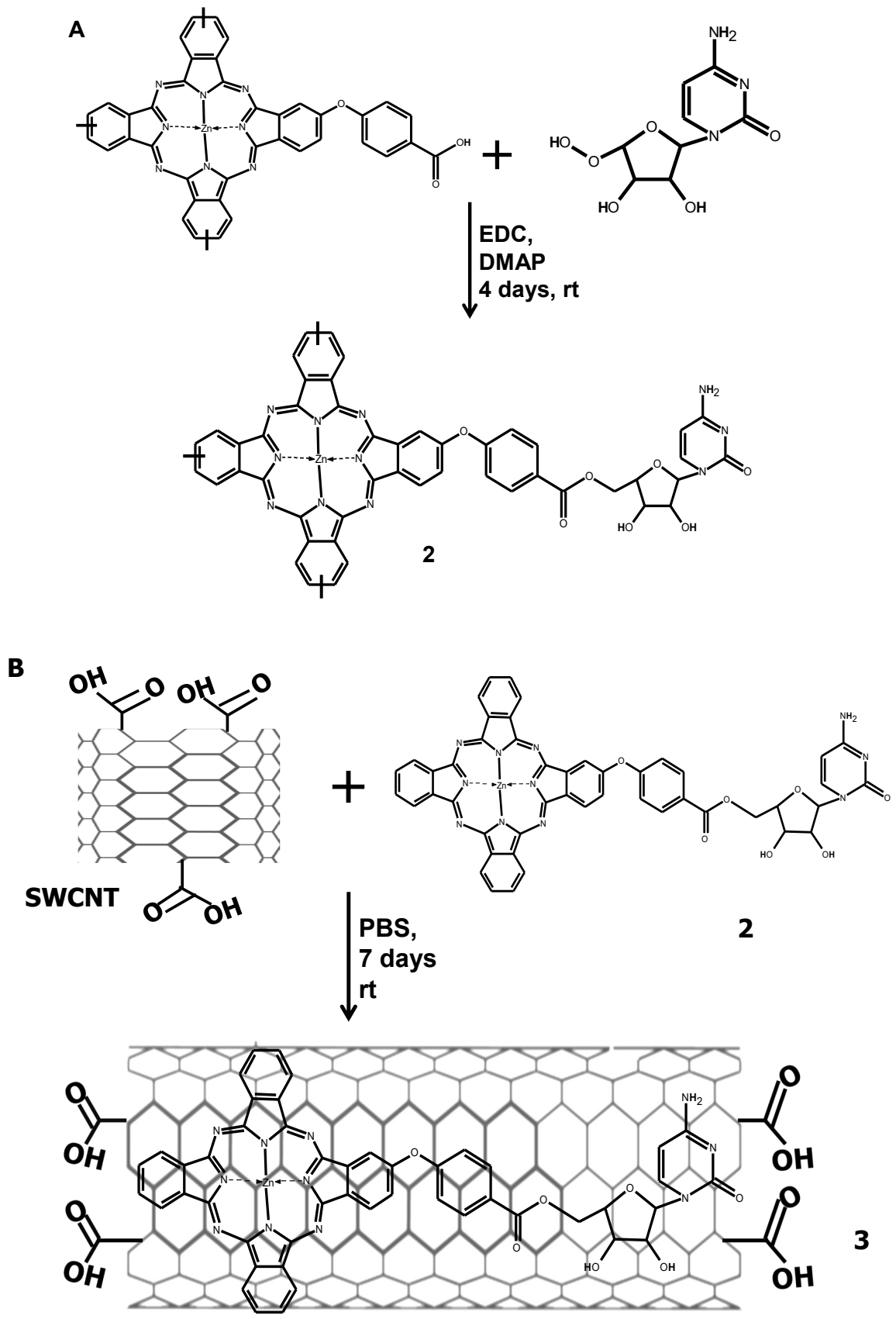
where F and F_{Std} are the areas under the fluorescence curves of the complexes (**2**, **3**) and the reference, respectively. A and A_{Std} are the absorbance of the sample and reference at the excitation wavelength, respectively, and n and n_{Std} are the refractive indices of solvents used for the sample and reference, respectively. ZnPc in DMSO was employed as a standard, Φ_F = 0.20 (Ogunsipe, 2003). Both the samples and reference were excited at the relevant wavelength (606 nm). The absorbance ranged between 0.04 and 0.05 at the excitation wavelength for all complexes.

3. Results and discussion

3.1 Characterization of complex 1-3

3.1.1 FTIR Spectra

Scheme 1A shows the synthetic route for the formation of complex **2**, **3** as an esterification reaction between ZnTPCPc (**1**) and cytidine nucleoside base compounds. Fig. 1A shows the FTIR spectrum of 1A(b) which exhibits a broad OH stretch between 3397 and 3048 cm^{-1}, the C=O peak was observed at 1786 cm^{-1}, the amide bond ν(C=N) was between 1600 and 1558 cm^{-1}, ν(C=C) was observed at 1485 cm^{-1}, OH bend was observed at 1391 cm^{-1}, and C-O-C stretch was observed at 1231 cm^{-1}. Similar shifts in peaks and the appearance of new peaks were observed for complex **3** as shown in Fig. 1B (b), **3** exhibited broad OH stretch between 3466 and 3000 cm^{-1}, amide bond ν(C=N) between 1601 and 1589cm^{-1}, ν(C=C) at 1490 cm^{-1}, OH bend at 1391 cm^{-1}, and C-O-C stretch at 1235 cm^{-1}. The shift in peaks or appearance of new peaks for complexes **2** and **3** were quite different for either ZnTCPc, or SWCNT-COOH alone indicating successful conjugation.



SCHEME 1: Synthetic route for the formation of complex **2** and **3**.

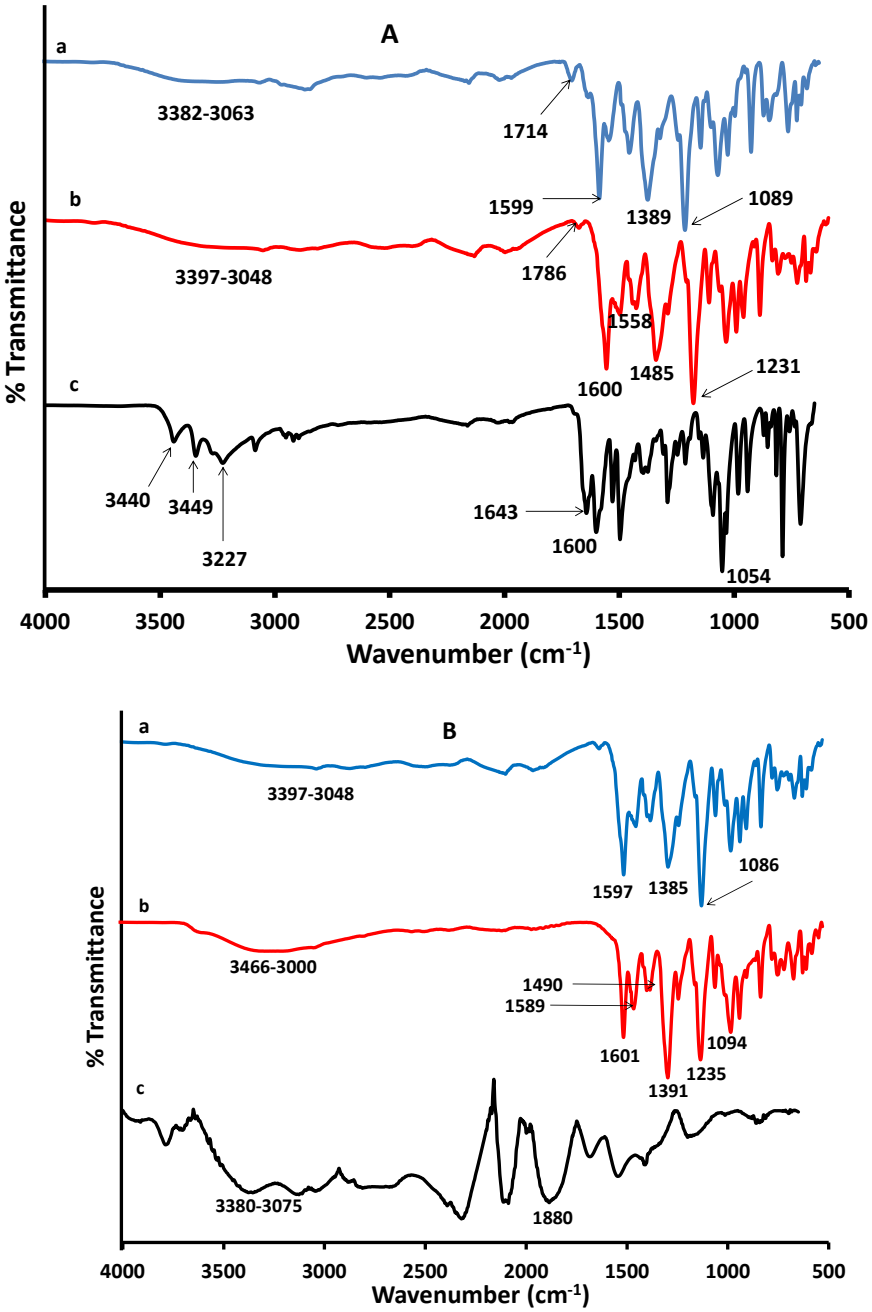


Fig. 1: FTIR Spectra of (**A**): ZnTPCPc (a) ZnTPCPc-cytidine, **2** (b), cytidine (c); (**B**) ZnTPCPc-cytidine, **2** (a), ZnTPCPc-cytidine-SWCNT **3** (b), SWCNT (c).

3.1.2 UV-Vis Spectra

Fig. 2 shows the absorption spectra of complex ZnTPCPc, **2** and **3**,there was no significant change in the Q-band maxima (678 nm) of ZnTPCPc on conjugation to cytidine or on adsorption of ZnTPCPc-cytidine into SWCNT-COOH to form ZnTPCPc-cytidine-SWCNT with a base peak at about 610 nm, the B-band maxima were observed at about 350 nmas seen in Fig. 2. The presence of the nucleoside, cytidine or the drug delivery agent SWCNT-COOH did not change the spectra properties of ZnTCPc. These peaks were similar to what has been reported in the literature. Metallophthalocyanines have two characteristic peaks, known as the Q-band (the most intense band) and the B-band (less intense). The Q-band is usually from 650 to 1000 nm depending on the substituents on the phthalocyanine molecule, while the

B-band is between 300 - 400 nm (Edwards 1970, Henrikson and Soundbom 1972). Using Gouterman's four orbital model (Gouterman 1978) the Q-band is due to the transition between the ground state a_1u highest occupied molecular orbital (HOMO) to eg lowest unoccupied molecular orbital (LUMO), while the B-bands correspond to the a_2u to eg and b_2u to eg transitions. Symmetrically substituted MPc are characterized by a single Q-band as shown in complex **2** and **3** while unmetallated Pcs and unsymmetrically substituted MPcs might display split Q-band depending on the solvent.

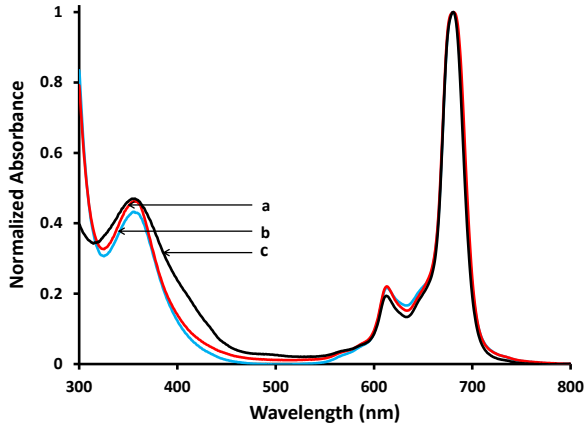


Fig. 2: Absorption spectra of ZnTPCPc (a), ZnTPCPc-cytidine-SWCNT, **3** (b), ZnTPCPc-cytidine, **2** (c).

3.2 Photophysical and photochemical parameters

3.2.1 Triplet quantum yield (ϕ_T)

The MPcs studied in this work contained zinc as the central metal to encourage intersystem crossing to a triplet state (long-lived), where the molecule can interact with molecular oxygen ($^3O_2$) to generate singlet oxygen ($^1O_2$) which is of particular importance in PDT. The triplet quantum yield (ϕ_T) values for complexZnTCPc (**1**),ZnTCPc-cytidine (**2**) and ZnTCPc-cytidine-SWCNT (**3**) are 0.49,0.76 and 0.78 respectively, A typical triplet decay curve for complex **3** which obeyed first-order kinetics is shown in Fig 3. The triplet quantum yield of complex **2** and **3** was about 27 % higher than **1**. The high triplet quantum yield exhibited by complex **2** may be a result of the presence of the phenoxyl carboxy group that serves as a linker between ZnTCPc and cytidine moiety. The presence of phenyl link has been shown to support spin-orbit charge transfer intersystem crossing (SOCT-ISC) mechanism, which causes a rapid intersystem crossing rate from excited singlet state to triplet state (Colvin et al., 2012).

On adsorbing ZnTCPc-cytidine (**2**) onto SWCNTs to give ZnTCPc-cytidine-SWCNT (**3**), there was no decrease in the triplet quantum yield (ϕ_T) which is surprising. SWCNTs are electron accepting group that is known to accept electrons from electron-donating phthalocyanine ring, this process of electron transfer usually cause deactivation of the photoexcited state of the phthalocyanine (Bottari et al. 2010, Bottari et al. 2011). The increase rather than decrease in triplet quantum yield observed in complex **3** may be due to the formation of radical pair by the two complexes which have been shown to support radical-pair intersystem crossing (RP-ISC), RP-ISC depends on the length of the linker between the phthalocyanine ring and carbon nanotube structure ((Bottari et al. 2010, Bottari et al. 2011, Suzuki and Obi 1995). A short linker such as the cytidine nucleoside base used in this work produces a highly short-lived radical ion pair, such that the charge recombination process is extremely fast (Bottari et al. 2011) and quenching of the excited state by CNTs is not observed. A similar observation of an unquenched triplet state by carbon nanotube complex observed in Pc-fullerene dyad has been reported by Sastre.et al. (1999).

3.2.3 Fluorescence quantum yield (Φ_F) and lifetimes (τ_f)

The fluorescence quantum yields (Φ_F) are dependent on several factors,which include: the nature of the central metal atom, aggregation, solvent properties, concentration, excitation wavelength, substituent type and photo-induced energy transfer (Bonnett, 2000). The complexes were excited at approximately the same wavelength (610 nm) and absorbance of 0.05 for the measurements to eliminate most of these factors. All studies were done in dimethylsuphoxide (DMSO). Typical absorption (b), emission (a) and excitation (c) spectra of ZnTCPc-cytidine **2** (as an example) are shown in Fig. 4. The absorption and excitation spectra were found to be similar and they are mirror images of the emission spectrum, showing that the molecule emitting is the same as the one absorbing. The Φ_F of ZnTCPc (**1**), ZnTCPc-cytidine (**2**) and ZnTCPc-cytidine-SWCNT (**3**) are given as 0.10, 0.12, 0.099 respectively. There was no significant difference in the fluorescence quantum yield of the complexes.

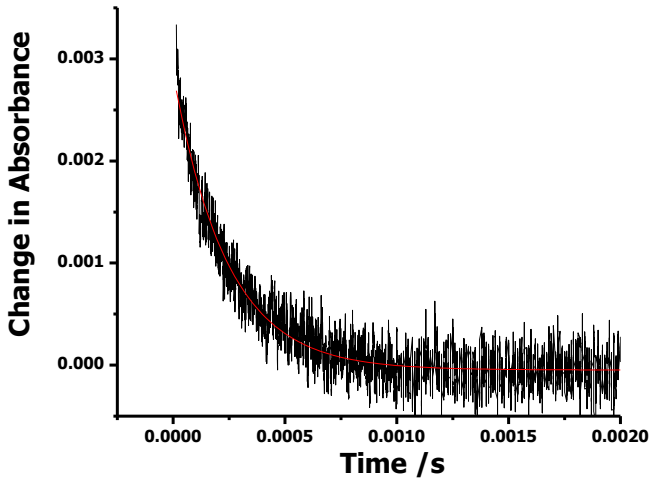


Fig. 3: Mono-exponential triplet decay curve of ZnTPCPc-cytidine, **2** in DMSO.

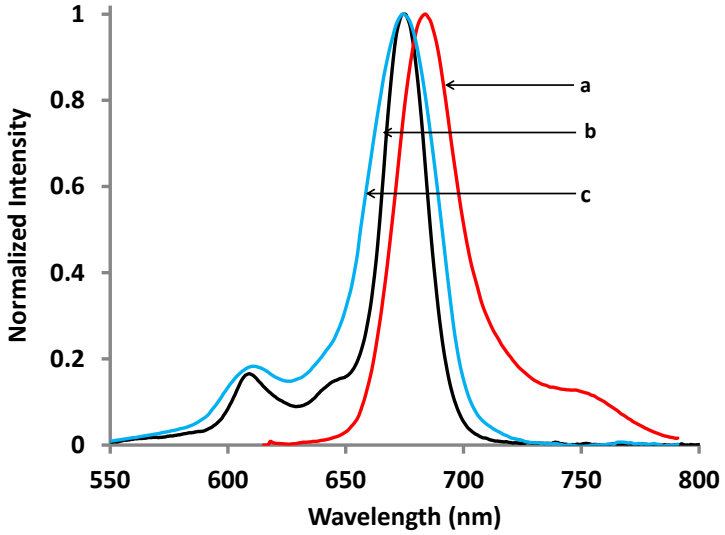


Fig. 4:Absorption (a) Excitation (b) and Emission (c) spectra of ZnTPCPc-cytidine, **2** in DMSO. Excitation wavelength = 610 nm. The concentration of ZnTPCPc-cytidine 6.5 x10^{-6} M.

4.0 Conclusion

ZnTPCPc-cytidine, **2** and ZnTPCPc-cytidine-SWCNT, **3** were synthesized using simple chemical methods. The characteristics Q-band of metallophthalocyanine were exhibited by complex **2** and **3**, which have similar absorption maxima with ZnTPCPc indicating that the presence of cytidine or SWCNT did not affect ZnTPCPc. The triplet quantum yield result showed that complexes **2** and **3** can generate high singlet oxygen, the cytotoxic agent responsible for the irreversible destruction of cancerous cells. Hence, these complexes would find application as potential anti-cancer drugs for photodynamic therapy.

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