

**PHOTOPHYSICOCHEMICAL AND
PHOTODYNAMIC STUDIES OF
PHTHALOCYANINES
CONJUGATED TO SELECTED DRUG
DELIVERY AGENTS**

A thesis submitted in fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Of

RHODES UNIVERSITY

By

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January 2012

DEDICATION

To my father **Wellington Mphathelwa Nombona**

Tata, you have taught me that the biggest deterrent to **anything** is excellence; I will always strive for excellence - this to me means following in your footsteps. Thank you *Zikode, mbhabala yakwa Mashiya*. I love you.

To my mother, **Nonzuzo Euticus Nomziwake Nombona**

Thank you for your love, encouragement and the laughter. You taught me what it means to persevere, to be strong, honest and humble. I love you, *ndiyabulela Nyawuza*.

Acknowledgments

I am reminded of my loving ancestors who passed away in the beginning and during the course of my studies. I remember you Ntshangase ('**Gogo**'), thank you. I remember you Mavumisa ('**Mha**'), thank you. I remember you Msuthu (*Tato'mkhulu Bread*), thank you. Thank you **Makazi** Nomandla, I am reminded of you.

Mr. and Mrs Nombona: *Ndiyabulela.*

I thank my supportive siblings, **Nwabisa, Patiswa, Lwazi** (twins for life!), **Thando, Zoleka, Zola**, and **Luleka**. I love you all.

I wish to thank the support and encouragement from my supervisor **Prof. Tebello Nyokong**. Thank you Prof., you have paved the way forward. *Ke ya le boha, thata.*

Dr. Edith Antunes, you are what I inspire to become. *Thank you ♥.*

I thank the chemistry department, in particular **Ms Gail Cobus**, a super woman!

I thank NRF, CSIR and Rhodes University for financial assistance.

A **BIG thank you** to my lab mates, **S22 iyashisa!** To my friends: **Vongani, Wadzi, Sammy, Lulama, Tanaka, Zandi, General, Christian, and Nozuko**. It was lovely 🎵.

Abstract

This work reports on the successful synthesis, characterisation and photophysical properties of new asymmetric metal free, magnesium and zinc phthalocyanines. The synthesis of symmetrical phthalocyanines is also reported. A selection of phthalocyanines have been conjugated, covalently linked, encapsulated or mixed with selected of drug delivery agents which include gold or silver nanoparticles, poly-L-lysine, liposomes and folic acid. The influence of delivery agent on the photophysical and photochemical properties of conjugated phthalocyanine is investigated. The studies showed that the Au nanoparticle significantly lowered the fluorescence quantum yield values of the phthalocyanines.

The photodynamic activity of Zn phthalocyanine- ϵ -polylysine conjugates in the presence of nanoparticles towards the inactivation of *Staphylococcus aureus* showed high photoinactivation in the presence of silver nanoparticles. The presence of silver nanoparticles from the minimal inhibition concentration (MIC₅₀) studies showed remarkable growth inhibition for the tested conjugates even at low concentrations. The conjugate also showed no dark toxicity when evaluated using the chick chorioallantoic membrane (CAM) assay.

The efficiency of selected zinc phthalocyanine as photodynamic therapy (PDT) agents was investigated. The production of reactive oxygen species (ROS) and phototoxicity of the photosensitizers were assessed. Healthy fibroblast cells and breast cancer (MCF-7) cells were treated with either free phthalocyanine or phthalocyanine bound to either gold nanoparticles or encapsulated in liposomes. Cell viability studies showed the optimum phototoxic effect on non-malignant cells to be 4.5 J.cm⁻². The PDT effect of the liposome bound phthalocyanine showed extensive damage of the breast cancer cells. Gold nanoparticles only showed a modest improvement in PDT activity.

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List of symbols

α	- Non-peripheral position
β	- Peripheral position
	- Full width at half maximum
ϵ	- Molar extinction coefficient
ϵ_S	- Singlet state extinction coefficient
ϵ_T	- Triplet state extinction coefficient
λ	- Wavelength
$\lambda_{\max}$	- Wavelength maximum
η	- Refractive Index
A	- Absorbance
C	- Concentration
F	- Fluorescence
I	- Intensity of light
I_{abs}	- Intensity of light absorbed
$^3\text{O}_2$	- Ground state molecular oxygen
$^1\text{O}_2$	- Excited singlet oxygen
P	- Phosphorescence

$^3\text{PS}^*$	- Triplet state of the photosensitiser
$\text{PS}^{\cdot+}$	- Radical specie of the photosensitiser
Q_{abs}	- Q-band absorption maximum
Q_{ems}	- Q-band emission maximum
Q_{exc}	- Q-band excitation maximum
S_0	- Ground singlet state
S_1	- Excited singlet state
S_{Δ}	- Efficiency of quenching of the triplet excited state by singlet oxygen
T_1	- First excited triplet state
ΔA	- Change in absorbance
τ_T	- Triplet lifetime
τ_F	- Fluorescence lifetime
Φ_{Δ}	- Singlet oxygen quantum yield
Φ_F	- Fluorescence quantum yield
Φ_{IC}	- Internal conversion quantum yield
Φ_T	- Triplet quantum yield

List of abbreviations

Ac₂O	- Acetic anhydride
ADMA	- tetrasodium α,α -(anthracene-9,10-diyl) dimethylmalonate
AFM	- Atomic force microscopy
AuNPs	- Gold nanoparticles
AgNPs	- Silver nanoparticles
CHCl₃	- Chloroform
DBU	- 1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	- Dicyclohexylcarbodiimide
DCM	- Dichloromethane
DMF	- Dimethylformamide
DMSO	- Dimethylsulfoxide
DPBF	- 1,3-Diphenylisobenzene-furan
EDC	- 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide
EtOH	- Ethanol
FT-IR	- Fourier transform-infrared
¹H-NMR	- Proton nuclear magnetic resonance
H₂Pc	- Metal-free phthalocyanine
HOMO	- Highest occupied molecular orbital
HPLC	- High performance liquid chromatography
IC	- Internal conversion

ISC	- Intersystem crossing
LUMO	- Lowest unoccupied molecular orbital
MeOH	- Methanol
MPc	- Metallophthalocyanine
NHS	- N-hydroxysuccinimide
PBS	- Phosphate buffer saline
Pc	- Phthalocyanine
PDT	- Photodynamic therapy
PL	- Poly-L-lysine
PS	- Photosensitizer
rt	- Room temperature
SPR	- Surface plasmon resonance
TCSPC	- Time correlated single photon counting
TEM	- Transmission electron microscopy
TFA	- Trifluoroacetic acid
THF	- Tetrahydrofuran
TLC	- Thin layer chromatography
TOABr	- Tetraoctylammonium bromide
UV/Vis	- Ultraviolet/visible
XRD	- X-Ray diffraction

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Chapter 1

Introduction

This thesis aims to develop phthalocyanines that can be effectively used in photodynamic therapy. The development of drug delivery systems for synthesized phthalocyanines is also explored; these include the use of liposomes, polymers, nanoparticles and folic acid. The phthalocyanines in combination with their delivery vehicles will be tested for their photodynamic therapy ability on healthy, tumour and bacteria cells.

1. Introduction

1.1 Photodynamic therapy

1.1.1 Background

Photodynamic therapy (PDT) has emerged as a promising treatment modality for various cancers. PDT relies on the simultaneous presence of three components: light, a photosensitiser (PS), and oxygen. The treatment consists of the systemic or topical administration of a photosensitiser that will localise in target tissue at non-toxic concentrations [1].

The effectiveness of PDT relies, to a great extent, on the retention of the PS in tumour cells followed by irradiation of the tumour with visible light. These steps of PDT are illustrated schematically in Figure 1.1. Step 1 involves the systematic administration of a PS. Step 2 entails a waiting period for the PS to localize on the tumour; the time period depends on the PS used and tumour type. This time will allow for maximum differentiation between normal and tumour tissue ensuring effective PS accumulation in tumour tissue. The tumour is then irradiated with visible light causing preferential tumour destruction. Upon light activation in step 2, the PS generates reactive oxygen species such as singlet oxygen, $^1\text{O}_2$, and free radicals that include $\text{HO}\bullet$, $\text{HO}_2\bullet$ and $\bullet\text{O}_2^-$. These species cause damage to cellular membranes, deoxyribonucleic acid (DNA) and other cellular structures, inevitably leading to cell death [2, 3], step 3.

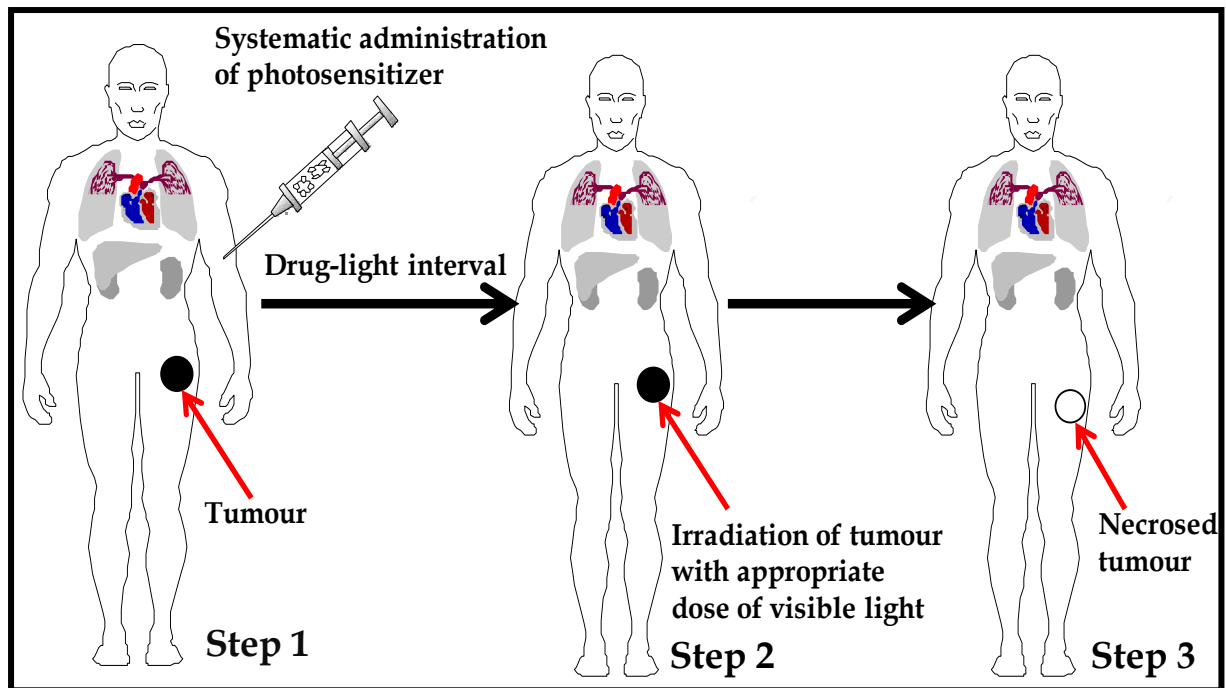


Figure 1.1: Schematic illustration of photodynamic therapy [3].

PDT has now reached the level of being an accepted treatment for a number of diseases such as various types of cancers and choroidal neovascularisation for age-related macular degeneration; countries such as Canada, Switzerland and Russia have approved the use of PDT for the treatment of various malignancies.

The term “photodynamic therapy” (*‘photodynamische Wirkung’*) was first introduced by H. von Tappeiner and A. Jodlbauer in 1904 [4]. This discovery came when Oscar Raab, a student of von Tappeiner, coincidentally observed odd effects of acridine on *in vitro paramecium* [5]. Performing two experiments, only one caused death of all *paramecia*, whereas the other showed no toxic effects of the acridine. The experiments were done under identical conditions, except Raab recorded the occurrence of a

thunderstorm during one of the experiments. Raab concluded that the transfer of energy from the light (lightning), to the chemical was important for inducing toxicity. In collaboration with a dermatologist, von Tappeiner utilized this photodynamic action to treat skin malignancies utilising eosin in the presence of white light [6]. A large amount of work on photosensitization was performed by the group and they later discovered that oxygen was required for the photodynamic effect [7].

One of the first PSs used for PDT was hematoporphyrin. This compound was first produced (in an impure form) by Scherer who removed iron from dried blood in 1841 by treatment with sulfuric acid [8]. In the 1908-1913 period a number of photobiological experiments were carried out with hematoporphyrin, demonstrating how it sensitized *paramecia*, erythrocytes, mice [9] and guinea pigs [10] in the presence of light. A German doctor, Friedrich Meyer-Betz was the first to demonstrate the human photosensitization effect of hematoporphyrin. After injecting himself with 200 mg of hematoporphyrin, Meyer-Betz experienced pain and swelling in skin areas exposed to ambient light, Figure 1.2, and remained photosensitive for more than two months [11].

The non-selective accumulation has been the major drawback in PDT. Figure 1.2 shows the generalised photosensitivity typical of a number of photosensitisers.

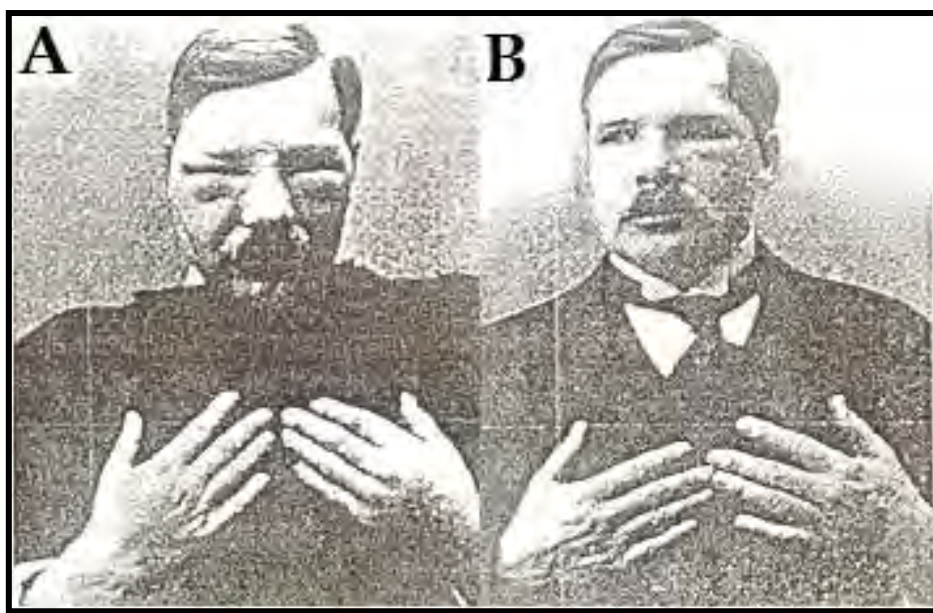


Figure 1.2: Friedrich Meyer-Betz (A) after injecting himself with 200 mg of hematoporphyrin and (B) 5 days later [11].

Pure hematoporphyrin showed poor localization in tumours; however treatment of hematoporphyrin with an acetic acid/sulfuric acid mixture gave components which had better properties with respect to tumor-localization. These components came to be known as ‘hematoporphyrin derivative’, HpD. HpD contains several porphyrin monomers, dimers and oligomers [12]. The widespread use of PDT is based on the ground-breaking work of Dougherty, who reported complete tumor response after administration of HpD in combination with red light [13].

In the 1980s, the active HpD fraction became commercially known as Photofrin (porfimer sodium) [14]. Photofrin was approved for PDT of recurrent superficial papillary bladder cancer by the Canadian Health Agency in 1993. This was the first

official approval for PDT in the world, marking it a milestone in PDT history. In Russia the first photosensitizer, Photoheme, was approved for clinical application in 1996. A number of clinical trials have shown PDT to be a safe and successful treatment option for cancer. In particular, PDT can be a useful alternative for drug-resistant tumours [15] and radiotherapy resistant cells [16]

1.1.2 Mechanisms of photodynamic therapy

The effectiveness of PDT is determined by the photo-activation of a PS and the subsequent formation of reactive oxygen species that induce cellular damage. The wavelength of the light has to be tuned to match the absorption band of the PS.

A Jablonski diagram is used to explain the photophysics of PDT (Figure 1.3). In the absence of photo-activation, PSs are in the singlet ground state, (S_0). When the PS absorbs light of appropriate wavelength, it is excited to a short-lived (~ 1 -100 ns) excited singlet state, S_1 . From S_1 , the PS can either relax back to the S_0 state (via fluorescence) or cross into a triplet state, T_1 , in a process called intersystem crossing (ISC).

The transition back to the S_0 state from the T_1 state, is spin-forbidden, and this results in a long lifetime (≥ 500 ns) of the PS in the triplet state, allowing the PS to interact with surrounding molecular oxygen. This long lifetime in the triplet state is essential for efficient photosensitized reactions [17-19].

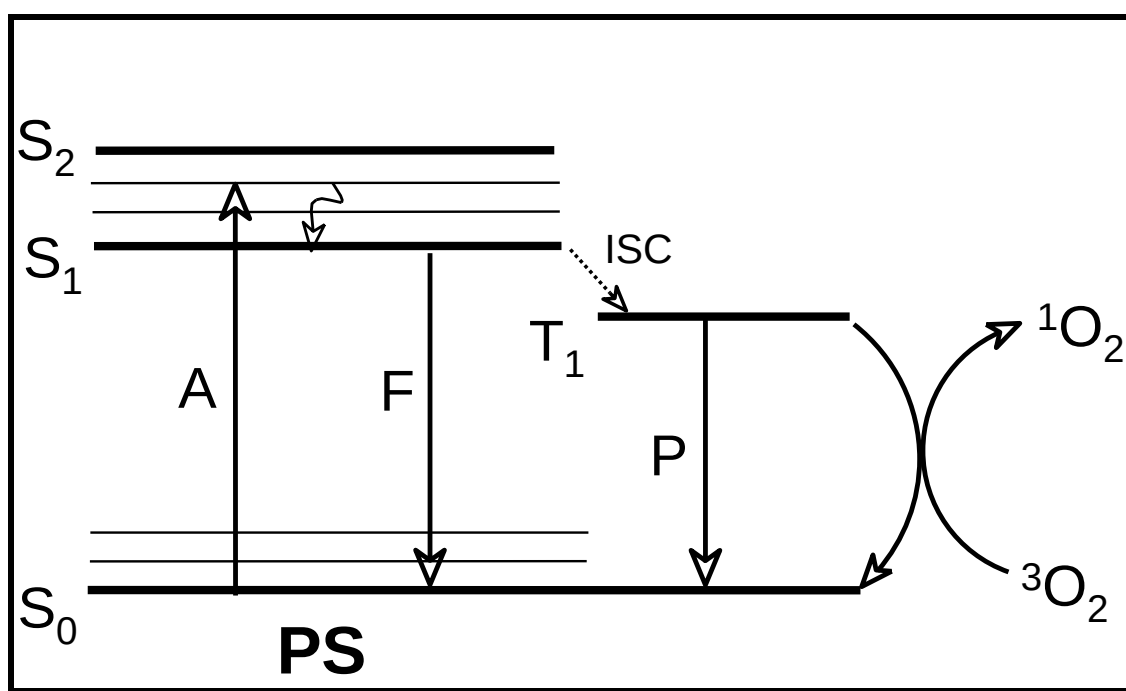
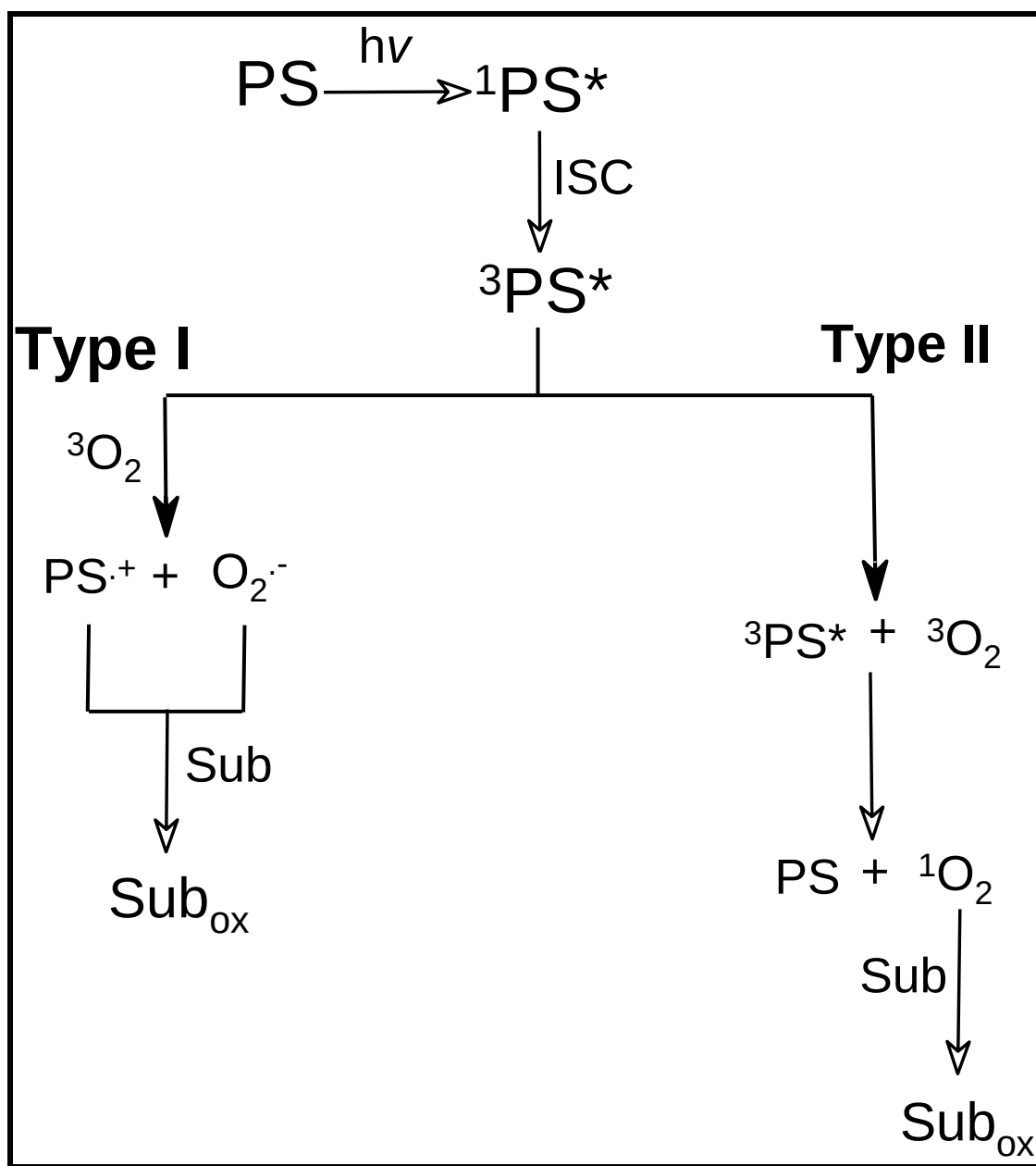


Figure 1.3: Jablonski diagram schematically illustrating transition processes

following absorption of light by a PS. A = absorbed energy, F = fluorescence, ISC = intersystem crossing, P = phosphorescence.

The photodynamic processes in PDT are referred to as type I and II reactions [18], Scheme 1.1. In the type I reaction, the PS in the T_1 state reacts with neighbouring substrate molecules via electron or hydrogen transfer processes. This leads to the formation of highly reactive radicals. These radicals often react with oxygen to form a number of reactive oxygen species (ROS) such as $HO^\bullet$ or $HO_2^\bullet$. Type I reactions are efficient at low oxygen and high substrate concentrations and are especially more efficient if there is an increase in the non-covalent interaction between the PS and the substrate molecule prior to light irradiation [18-20].



Scheme 1.1: Type I and Type II reaction mechanism of PSs.

The type II reaction involves energy transfer from a PS in the T_1 state to molecular oxygen (${}^3\text{O}_2$), yielding cytotoxic singlet oxygen (${}^1\text{O}_2$) with excitation energy of 94.4 kJ.mol⁻¹. In order to generate singlet oxygen, the energy of the PS in the T_1 state should not be less than 94.4 kJ.mol⁻¹, but should not exceed 215 kJ.mol⁻¹ to avoid

factors that inhibit $^1\text{O}_2$ generation. Triplet state energies higher than $215 \text{ kJ}\cdot\text{mol}^{-1}$ are known to lead to unfavourable Franck-Condon factors, which prevent efficient coupling of the photosensitizer to molecular oxygen, thereby inhibiting the generation of $^1\text{O}_2$ [18, 21, 22].

The ROS produced by the type I and II reactions can oxidize a wide variety of biomolecules and initiate biochemical and biophysical mechanisms that cause tumour necrosis [23, 24]. For example, the unsaturated bonds in lipids can be photo-oxidized through PDT which may lead to protein dysfunction, membrane damage and loss of enzymatic activity [25]. Such PDT induced structural and functional changes lead to necrosis. Since ROS are highly reactive with an estimated intracellular lifetime of 250 ns and diffusion distance of less than 50 nm, targeted cellular structures are largely determined by the site of $^1\text{O}_2$ production [26]. The site of PS cellular localisation is highly influenced by the chemical properties of the PS used. For instance, lipophilic PSs are known to accumulate well in membrane structures such as the plasma membrane or the mitochondria [27]. Both type I and II reactions can occur simultaneously and the ratio between the two depend on the PS, substrate, oxygen concentration and PS-substrate binding [28]. Singlet oxygen, however, is generally understood to be the major destructive species in PDT [2]. From this it is evident that PDT effects are highly dependent on oxygen.

1.1.3 Physical aspects of light-tissue interaction

When photons of light pass through tissue, processes of absorption, reflection, scattering, transmittance and fluorescence can occur. These processes can be used for medical purposes. In PDT, some of the treatment light will be lost by the tissue via diffuse scattering or absorption, and only part of the light transported into biological tissue will be transmitted [28, 29].

The term photosensitization covers all reactions in which a substance (PS), activated by light, causes a biological effect. The most commonly used PSs are relatively large and complex in their molecular structures. As a result, these PSs are usually administered via intravenous injection. However, recent research has shown intratumoral administration to be more efficient with improved selectivity at short light illumination times [30, 31]. The most important feature a PS should possess is light absorption at a wavelength range where the surrounding biological tissue is relatively transparent [32]. The porphyrin based PSs (first generation of PSs) show maximum light absorption within the UV region and less absorption in the visible region. Figure 1.4 shows the tissue optical window indicating the relative absorption of major chromophores present in tissue across the UV-vis spectral window. To reach a deeper tissue penetration, PSs that absorb in the red (visible) region are used. These are typically phthalocyanine (Pc) molecules (second generation of PSs). The research within this field aims at finding compounds which absorb far out in the red wavelength region, towards the borders of the infrared, where the penetration depth of the treatment light is at its maximum [33].

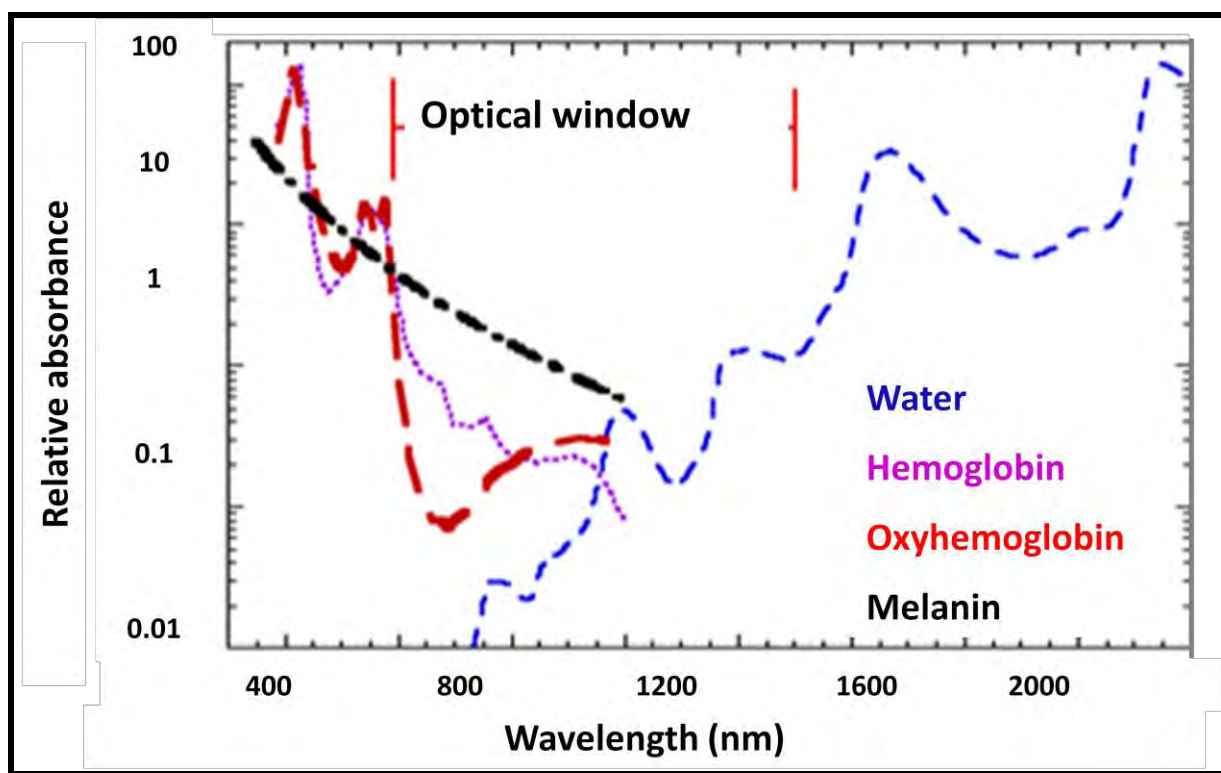


Figure 1.4: Tissue optical window [28].

1.1.4 Bio-distribution of PDT photosensitisers

Compounds of molecular weight greater than 500 Dalton show low permeability of the *stratum corneum*, a barrier of the skin, hence large or highly conjugated PSs are generally administered to patients through intravenous injection in clinical trials [34]. When a PS is injected into the bloodstream, depending on the delivery solvent or vehicle used for injection, the PS has to first equilibrate with the components of the circulating blood. This process often involves the disaggregation of the PS from itself or its delivery vehicle and consequent binding to various protein components of serum [35]. The PS then has to bind to the walls of blood vessels and penetrate

through these walls; PSs that bind weakly to the blood vessel wall will pass through quicker [35]. In general, the fast angiogenesis of tumour tissue results in vessels of a lower quality that have a tendency to 'leak', allowing macromolecules to pass into the interstitial compartment, which often is larger in malignancies. After 'leaking' out from the blood vessels, the PS will diffuse throughout the *parenchyma* of the organ or tumour to which it has been delivered, and lastly the PS will be eliminated from the tissue and excreted from the body [36].

1.1.5 Selectivity of PDT photosensitisers based on binding

Since PSs have different lipophilicity properties, their solubility in the aqueous part of biologic compartments will also differ [37]. Aggregated and hydrophilic PSs can be taken up by endocytosis and can accumulate in lysosomes [38, 39]. Most cationic PSs preferentially accumulate in the mitochondria due to the electrical potential gradient across the mitochondrial membrane [40, 41]. Lipophilic PSs are known to bind preferentially to lipoproteins and hydrophilic sensitizers to serum proteins such as albumin, post administration [42]. Increased selectivity in tumours has been observed for lipophilic PSs [43-45].

High mitotic activity in tumours results in an increased amount of low-density lipoprotein (LDL) receptors on the cell membrane. Lipophilic PSs, are known to solubilise within the lipid moiety of the lipoprotein particle without interfering with the receptor-mediated uptake process. From this, it appears useful to enhance the

accumulation of lipophilic PSs using liposomal vesicles as vehicles for drug selectivity [46]. This is particularly important for hydrophilic PSs, as they can be delivered to LDL *via* the PS-liposome complex.

It is important to note the difference between selective accumulation and selective retention. The selective accumulation of a PS in tumours is caused by fast pharmacokinetics of the PS. Selective retention on the other hand includes factors such as binding of the PS to LDL receptors or the leaking of PS from the hyper-permeable tumour neovasculature and the poorly developed lymphatic drainage system the tumours possess [47]. All these factors make elimination of PSs along the lymphatic route slow. To take advantage of this preferred retention of PSs in malignant tissue, one waits for some time after administering the PS, before light irradiation.

For HpD, the period most commonly applied is 2-3 days, however for the new generation of PSs, this period has been significantly shortened to a couple of hours [47]. The choice of delivery vehicle used highly influences the tumour selectivity of the PS. The encapsulation of PSs in liposomes has shown efficient tumour accumulation as opposed to free PSs in aqueous formulation [48-50].

It is important to acknowledge that tumours are not the only type of tissue which exhibit accumulation of PSs. Photosensitiser accumulation by intraocular choroidal neovascularisation (a characteristic of age-related macular degeneration), and the

plaques of psoriasis have been used to achieve encouraging therapeutic results [51, 52]. Microorganisms such as bacteria and fungi also exhibit selective accumulation of PSs relative to mammalian cells. This is caused by the differences in permeability of the microorganisms' cell walls or membranes [53, 54].

1.1.6 Drug delivery systems for PDT

The main difficulty PSs have is reduced selectivity in non-malignant or normal tissue, especially the skin, leading to prolonged photosensitivity lasting up to several weeks [55]. It is important therefore to develop suitable delivery systems such as liposomes or polymer-PS conjugates to enhance specific uptake by targeted tissue and ultimately improve PDT efficiency. The ideal drug delivery system should facilitate selective accumulation of the PS within the targeted tissue and the delivery of therapeutic concentrations of the PS while minimizing PS release to normal tissue. It is vitally important for the PS carrier to be able to incorporate the PS without loss or alteration of PS activity.

Liposomes, oil dispersions, biodegradable polymeric particles and hydrophilic polymer-PS conjugates are all considered as passive targeting systems. These delivery vehicles utilize the natural distribution patterns which are passive diffusion and phagocytosis [56].

1.1.6.1 Liposomes

Liposomes are microscopic spheres with an aqueous core surrounded by one or more outer shell(s) consisting of lipids arranged in a bilayer configuration. Liposomes have become the standard means of delivering drugs into cells. PSs can be incorporated in the interior of the liposomes, and such PSs have shown enhanced photocytotoxicity. A variety of PSs, including those that are hydrophilic, lipophilic, or amphipathic can be carried by liposomes. PS loading in liposomes can be achieved passively (PS encapsulated during liposome formation) or actively (PS encapsulated after liposome formation). Passive encapsulation of water-soluble PSs relies on the ability of liposomes to trap aqueous buffer containing a dissolved PS during vesicle formation. Trapping efficiencies of less than 30 % are often obtained due to restriction by the trapped volume contained in the liposomes and PS solubility. Alternatively, water-soluble drugs that have protonizable amine functions can be actively entrapped by employing pH gradients, which can result in trapping efficiencies approaching 100 % [57]. Since most liposomes are composed of naturally occurring phospholipids, they are non-toxic, biodegradable and non-immunogenic at pharmacological doses. Liposome bound PSs injected into the bloodstream reach the cells as a component of blood lipoproteins, the PS is redistributed upon contact of liposomes with serum proteins. Similar behaviour is observed with PSs administered into the organism as emulsions [58].

Hematoporphyrin (Hp) was the first PS whose pharmacokinetic behaviour was studied after incorporation into small unilamellar liposomes of dipalmitoylphosphatidylcholine (DPPC) (Table 1.1) [59]. In another study, the bio-distribution of 9-(glutamic acid glutaryl amide)-2,7,12,17-tetrakis(methoxyethyl)porphycene, (Glut(OH)₂GlamTMPn), intravenously administered, showed a fast clearance from the body even when bound in liposomes of dioleoylphosphatidylcholine (DOPC) [60], Table 1.1. The DOPC liposomes however induced a three times larger accumulation in the skin tumour and a slightly lower accumulation in the skin and other non-malignant tissue. These results show that the encapsulation of PSs into liposomes can significantly increase selectivity and simultaneously alter bio-distribution [60].

PSs often aggregate when entrapped in liposomes. This can be avoided with the use of an organic solvent dilution procedure to monomerise the PS. It was found that stable lyophilized zinc phthalocyanine entrapped in 1-palmityl-2-oleoylphosphatidylcholine (POPC) and 1,2-dioleoylphosphatidylserine (DOPS) liposomes showed uptake which proportionally increased with increasing monomeric function in Meth-A sarcoma-bearing Balb/c mice [61].

To improve the transport of liposomes by LDL, a germanium phthalocyanine (Ge(IV)Pc) with two axially ligated cholesterol moieties was entrapped in POPC/DOPS liposome formulation [62]. It was found that PS accumulation via lipoprotein pathway in MS-2 fibrosarcoma-bearing Balb/c mice was more effective

and doubling the Ge(IV)Pc dose increased accumulation in tumour two-folds with low dye accumulation in normal muscle and skin tissue [62].

Liposomes are currently used as PS vehicles in clinical trials to successfully treat diseases such as choroidal neovascularisation [63]. The liposomal benzoporphyrin derivative monoacid ring A (BPD-MA, Visudyne, QLT Inc., Canada) was recently approved in Switzerland and USA for the treatment of the wet form of age-related macular degeneration characterized by choroidal neovasculation.

As Table 1.1 shows [59-62, 64-78], there have been few phthalocyanines used for delivery using liposomes and no ring substituted phthalocyanines have been employed [59-62, 64-78]. In this thesis, substituted phthalocyanines are employed for increased selectivity for PDT.

In this work, egg yolk, highly purified L- α -Lecithin was used as a liposome source for the encapsulation of a phthalocyanine photosensitizer for improved photodynamic activity on a breast cancer cell line.

Table 1.1: Drug delivery systems and formulations used in PDT.

^a PS	^b Vehicle	Target	Host	Ref.
Hp	DPPC	MS-2	Mice	[59]
Glut(OH) ₂ GlamTMPn	DOPC	-	Mice	[60]
ZnPc	POPC/DOPS liposomes	Meth-A sarcoma	Balb/c mice	[61]
Ge(VI)Pc	POPC/DOPS liposomes	MS-2 fibrosarcoma	Balb/c mice	[62]
CMA	HPMA	Ovarian carcinoma	Cultured	[64]
m-THPC	PEG	Liver	Rat	[65]
Ce ₆	PL	EA.hy926, A431	Cultured	[66]
Pheophorbide a	Peptide linked PL	T-24	Cultured	[67]
Doxorubicin	PIHCA	M 5076	Mice	[68]
Pc-4	PEG-coated AuNP	Not revealed	Mice	[69]
Pc-4	PEG-coated AuNP	HeLa	Cultured	[70]
Paclitaxel	AuNP	HepG2	Cultured	[71]
C11Pc	AuNP	HeLa	Cultured	[72]
C11Pc	Anti-HER2-PEG coated AuNP	SK-BR-3 and MDA- MB-231	Cultured	[73]

Table 1.1 continued

^a PS	^b Vehicle	Target	Host	Ref.
MTX	Folic acid	CHO AA8	Cultured	[74]
Mitomycin C	Folic acid	M109	Balb/c mice	[75]
Ce ₆	Folic acid-graphene oxide	MGC803	Cultured	[76]
CdTe(S)-type QD	Folic acid	KB and HT-29	Cultured	[77]
AlClPcS ₄	Folic acid-Liposomes	KB	Cultured	[78]

^aHp = Hematoporphyrin, ZnPc = zinc phthalocyanine, Ge(IV)Pc = germanium phthalocyanine, CMA = mesochlorin mono-ethylenediamine, m-THPC = meso-tetra(hydroxyphenylchlorine), Ce₆ = chlorin_{e6}, Pc-4 = silicon phthalocyanine 4, C11Pc = (1,1',4,4',8,8',15,15',18,18',22,22'-tetradecakisdecyl-25,25'-(11,11'dithiodiundecyl)diphthalocyanine zinc), MTX = methotrexate, CdTe QDs = cadmium telluride quantum dots, AlClPcS₄ = chloroaluminum phthalocyanine tetrasulfonate, ^bDPPC = dipalmitoylphosphatidylcholine, Glut(OH)₂GlamTMPn = 9-(glutamic acid glutaryl amide)-2,7,12,17-tetrakis(methoxyethyl)porphycene, DOPC = dioleoylphosphatidylcholine, POPC = 1-palmityl-2-oleoylphosphatidylcholine, DOPS = 1,2-dioleoylphosphatidylserine, HPMA = N-(2-hydroxy-propyl)methacrylamide, PEG = polyethylene glycol, PL = cationic, anionic or neutral poly-L-lysine, PIHCA = poly(isohexylcyano-acrylate), AuNPs = gold nanoparticles,

1.1.6.2 Polymers

The use of polymers in medicine has evolved into a broad discipline referred to as polymer therapeutics that encompasses polymeric drugs. The stability of the drug-polymer bond is imperative in polymer-drug conjugates. On the basis of this stability, polymer conjugates are classified into two categories; the “permanent” conjugates, and prodrugs. In permanent polymer-drug conjugates, the drug is able to elicit the desired effect while conjugated on the polymer, whereas conjugates intended as prodrugs usually require the transformation of the prodrug to the active drug within the body, to elicit therapeutic action. Generally, a prodrug is a biologically inactive derivative of a parent drug molecule designed to circumvent problems associated with the delivery of the parent drug [79].

Polymer-PS conjugates have opened another possibility for delivering PSs to tumour sites. The specificity of PDT action can be greatly improved by conjugating PSs with a variety of polymers. Conjugation to polymers will not only extend the plasma half-life of PSs but also improve the hydrophilicity of the PS and enhance tumour uptake by exploiting the increased permeability of tumour microvasculature, leading to good PDT efficacy [79]. As mentioned earlier, the rapid angiogenesis of malignant tissue lead to tumour vessel walls that show an enhanced permeability and retention of macromolecules (known as the EPR effect). Low molecular weight substances are therefore not retained in solid tumours but returned to circulating blood by diffusion [80]. The EPR effect applies to most plasma proteins and biocompatible synthetic

polymers or their conjugates; this phenomenon has become essential for targeting macromolecular drugs to cancer tissue and in designing anti-cancer drugs. Polymers commonly used for conjugation to PSs include polyethylene glycol (PEG), poly(vinyl alcohol) (PVAL), cationic, anionic or neutral poly-L-lysine (PL) and N-(2-hydroxypropyl)methacrylamide (HPMA) (Table 1.1). The cellular uptake of drugs from polymer conjugates is either by binding to the plasma membrane or by endocytosis [81].

A study showed that when mesochlorin mono-ethylenediamine (CMA) was conjugated to HPMA, the conjugate showed increased accumulation in tumour compared to the free PS on ovarian carcinoma cells [64]. The photodynamic activity of meso-tetra(hydroxyphenylchlorine) (m-THPC) conjugated to PEG showed effective tumour necrosis in mouse tumour with minimal tissue damage [65].

The phototoxicity effect of charge on the interaction of cationic, anionic and neutral PL-chlorin_{e6} (Ce₆) conjugates on human epidermoid carcinoma (A431) and human endothelium (EA.hy926) cell lines showed that not only did conjugation facilitate better cellular uptake of Ce₆ compared to free Ce₆ but, the Ce₆ bound to the cationic polymer showed the highest intracellular concentration compared to the anionic and neutral polymer derivatives [66, 82]. The cationic PL-Ce₆ conjugate accumulates more in the cells due to binding of the positively charged conjugate to anionic regions of the plasma membrane. The plasma membrane of cancer cells has a more

negative net charge compared to normal cells due to the over expression of polysialic acid residues [66].

Cationic PL-PS conjugates, are widely used for the photo-inhibition of microorganisms. Cationic PSs or poly-cationic conjugates with PL have been reported to be more active than the anionic or neutral derivatives against Gram-positive and Gram-negative bacteria [83, 84]. The positive charges of the PL promote tight electrostatic interaction with the negative charges on the surface of the bacteria [84].

As Table 1.1 shows, the use of polymers for drug delivery has been limited to porphyrins. Thus this work presents the first use of polymer-bound phthalocyanines for PDT and for the photo-inactivation of bacteria. The toxicity of the polymer bound phthalocyanine was tested in chick chorioallantoic membrane (CAM).

1.1.6.3 Gold nanoparticles

Nanoparticle (NP)-based therapy is struggling to advance into clinical trials due to the early stages of development. However, great advances in the field of nanotechnology has made NP-based therapy a research hotspot. Nanoparticles are used as drug carriers in order to lower the drug dose while maintaining or improving efficacy and ultimately reducing side effects. Once in the bloodstream, conventional nanoparticles are more susceptible to the action of phagocytes and are particularly cleared by the fixed macrophages of the mononuclear phagocyte system (MPS) organs which include the liver, spleen, lungs and bone marrow [85]. This

biodistribution can thus be utilized for the treatment of MPS localized tumours. Since anticancer efficacies are limited to MPS organs for NPs, a new generation of nanoparticles 'invisible' to macrophages was developed; these are termed StealthTM nanoparticles. StealthTM nanoparticles are nanoparticles coated with neutral or hydrophilic polymers (such as PEG). These nanoparticles are characterized by a relatively long half-life in blood circulation, allowing them to selectively accumulate in tumours located outside the MPS regions [86].

The non-toxicity, biocompatibility and inert nature of gold nanoparticles (AuNPs) make them ideal as drug carriers. The size of AuNPs can easily be controlled to correspond with macromolecules such as proteins making their integration into tumours simple through the EPR effect. Due to the strong interaction between sulfur and gold (Au), the surface modification of AuNPs has been done through the self-assembly of thiolated PSs on the AuNP surface. The AuNP surface is multivalent and allows for the incorporation of therapeutic drugs via covalent and non-covalent conjugation on the surface [87]. Covalent conjugation makes NPs stable delivery systems. However, such systems often require intracellular processing of the prodrug. Non-covalent conjugation on the other hand allows for direct release of the active drug although premature drug release is a major obstacle. The high surface-area-to-volume which AuNPs possess provide a dense drug loading on the NP surface. An AuNP of 2 nm core diameter can covalently conjugate approximately 100 ligands [88].

In PDT, PEGylated AuNPs, Table 1.1, have been investigated as carriers for silicon phthalocyanine 4 (Pc-4), a hydrophobic PS currently under phase I clinical trials [89, 90]. The study showed that when Pc-4 is injected *in vivo*, it takes up to two days until sufficient Pc-4 reaches the tumor site [91], the PEG-AuNP-Pc-4 conjugate showed a significantly reduced accumulation time of less than two hours and renal clearance of the NPs over seven days [69].

In another study, the delivery and PDT effect of covalently (via thiol group) and non-covalently (*via* amide group) attached Pc-4 on AuNPs, Table 1.1, was investigated on human cancer (HeLa) cells [70]. The results showed that labile amino adsorption on the AuNP surface allowed for efficient drug release into the cancer cells resulting in efficient PDT. The covalent thiol bond led to the delivery of the drug into cell vesicles with no PDT effect observed, highlighting the importance of the bond that links the drug to the AuNP surface. AuNPs have also been shown to enhance the anti-proliferation and apoptosis of human hepatoma cells (HepG2) induced by Paclitaxel, a chemotherapeutic drug [71].

The phthalocyanine derivative (1,1',4,4',8,8',15,15',18,18',22,22'-tetradecakisdecyl-25,25'-(11,11'dithiodiundecyl) diphthalocyanine zinc), or C11Pc, Table 1.1, has been conjugated to AuNPs *via* thiol-Au association and has been found to produce sufficient singlet oxygen for use in PDT [72]. When incubated with HeLa cells, the C11Pc-AuNP conjugate was taken directly into the cell interior and the PDT efficiency of the nanoparticle conjugate was determined to be twice that obtained using the free phthalocyanine derivative [92].

Stuchinskaya and co-workers reported on the synthesis of a 4-component antibody-phthalocyanine-PEG-gold nanoparticle conjugate. The AuNPs were stabilised with C11Pc and a hetero-bifunctional (PEG) polymer, Table 1.1. Experiments done on breast carcinoma cell lines (SK-BR-3 and MDA-MB-231) demonstrated that the nanoparticle conjugate selectively targets cells that over expressed the HER2 epidermal growth factor cell surface receptor, and that the conjugate was an effective photodynamic therapy agent [73].

As Table 1.1 shows, phthalocyanines tested on cell lines in the presenct of gold nanoparticles are limited and no studies have been reported on the use of gold nanoparticles-phthalocyanine conjugates for bacteria inactivation.

In this work, the photophysicochemical properties of AuNPs conjugated to phthalocyanines will be investigated together with the PDT activity in breast cancer and bacteria cells.

1.1.6.4 Folate receptors

Irrespective of their diverse applications, the efficacy of drug delivery vehicles in PDT depends on the efficiency of specific targeting. Receptor targets have become ideal for accurate drug delivery. The receptor for vitamin folate has been identified as a marker for ovarian carcinomas and has been found to be over-expressed in a range of human cancers including brain, kidney and breast. Folate receptors (FRs), therefore present an attractive target for tumour-selective drug delivery. Folic acid

and its reduced counterparts are needed by eukaryotic cells for carbon transfer reactions required for the biosynthesis of nucleotide bases; cell survival and proliferation are consequently dependent on a cell's ability to acquire the vitamin [93-95]. FRs have the ability to transport both folic acid and folate-linked cargos such as folic acid linked to imaging agents, proteins, liposomes and nanoparticles. Once such folate conjugates are bound to a cell surface FR, they are transported into the cell through a process called receptor-mediated endocytosis [96]

Folate receptors are found at significant levels in normal epithelia involved in the retention and uptake of folate. However, these FRs are mostly inaccessible to blood-borne folate-drug conjugates, as they are restricted to the apical surfaces of polarized epithelia [93]. Folic acid has consequently become an attractive ligand for use in drug targeting and its low molecular weight (MW 441), water solubility, stability in diverse solvents, facile conjugation chemistry, lack of immunogenicity, together with the molecule's high affinity for its receptor, has made it a proficient drug delivery vehicle [97, 98].

In vitro studies showed that when folic acid was conjugated to an anticancer drug methotrexate (MTX) and to arabinogalactan (AG), a natural polysaccharide, this folate conjugate could differentially deliver the cytotoxic drug into Chinese hamster ovary (CHO) AA8 cells transfected with a pcDNA3.1 expression vector harbouring a human folate receptor. The conjugate presented a 6.3-fold increased cytotoxic activity to FR-overexpressing cells compared to their FR-lacking counterparts [74].

The phototoxicity of chloroaluminum phthalocyanine tetrasulfonate (AlClPcS_4), Table 1.1, delivered to the cytoplasm of folate-deficient KB cells *via* 1,2-di-*O*-(Z-1'-hexadecenyl)-*sn*-glycero-3-phosphocholine (DPPCs)-folate liposomes was found to be greater than the free sensitizer. This method offers the potential for boosting the efficacy of PDT *in vivo* by site-selectively delivering high concentrations of sensitizer to the cytoplasm of target tumor cells while maintaining the pharmacokinetic profile of the host liposome formulation [78].

Although there are few reports, these results suggest that folic acid-PS conjugates have great potential as effective drug delivery system in targeting PDT, only one phthalocyanine has been tested with folic acid bound in liposomes. This work will highlight the photophysical properties of phthalocyanines in the presence of folic acid.

1.1.7 Commonly used photosensitizers

Various phase II clinical studies have shown that there are several areas of oncology for which PDT can be a valuable feature [99]. Several PSs have undergone laboratory, pre-clinical, and clinical tests in the past decades. The main classes of photosensitizers are porphyrin derivatives, chlorines, phthalocyanines and porphycenes. The bulk of the clinical experience in PDT has however been with porphyrins [14, 25].

Porphyrin-based PSs are known as the first generation of photosensitizers, Photofrin (2) (Figure 1.5), is a well-known first generation PS approved for the treatment of lung, bladder, and early stages of gastric and cervical cancers. The absorption spectrum of Photofrin has five peaks with the weakest peak at 630 nm. The compound gives admirable singlet oxygen quantum yield (Φ_{Δ}) values (Table 1.2) depending on the solvent and drug vehicle used [101, 102].

Although the first generation sensitizers fulfill certain criterion for ideal sensitizers they have significant side effects. The major drawback of using the porphyrin family as PDT agents, is due to their limited absorption in the red region coupled with prolonged skin photosensitivity lasting up to a month post PDT treatment [100]. The unfavourable light absorption of Photofrin causes intense skin photosensitization and the PS takes 4-8 weeks clearance time post PDT treatment. In spite of these challenges, more than 5000 patients were treated in the USA with Photofrin between 1978–1993 [33].

Benzoporphyrin (Visudyne, (3)), Temoporfin, (4) and bacteriochlorins, (5) (Figure 1.5) are some of the newer porphyrin derivatives that have been recently used for PDT. These second-generation PSs have maximum absorption in the visible region coupled with *in vivo* phototoxicity [103].

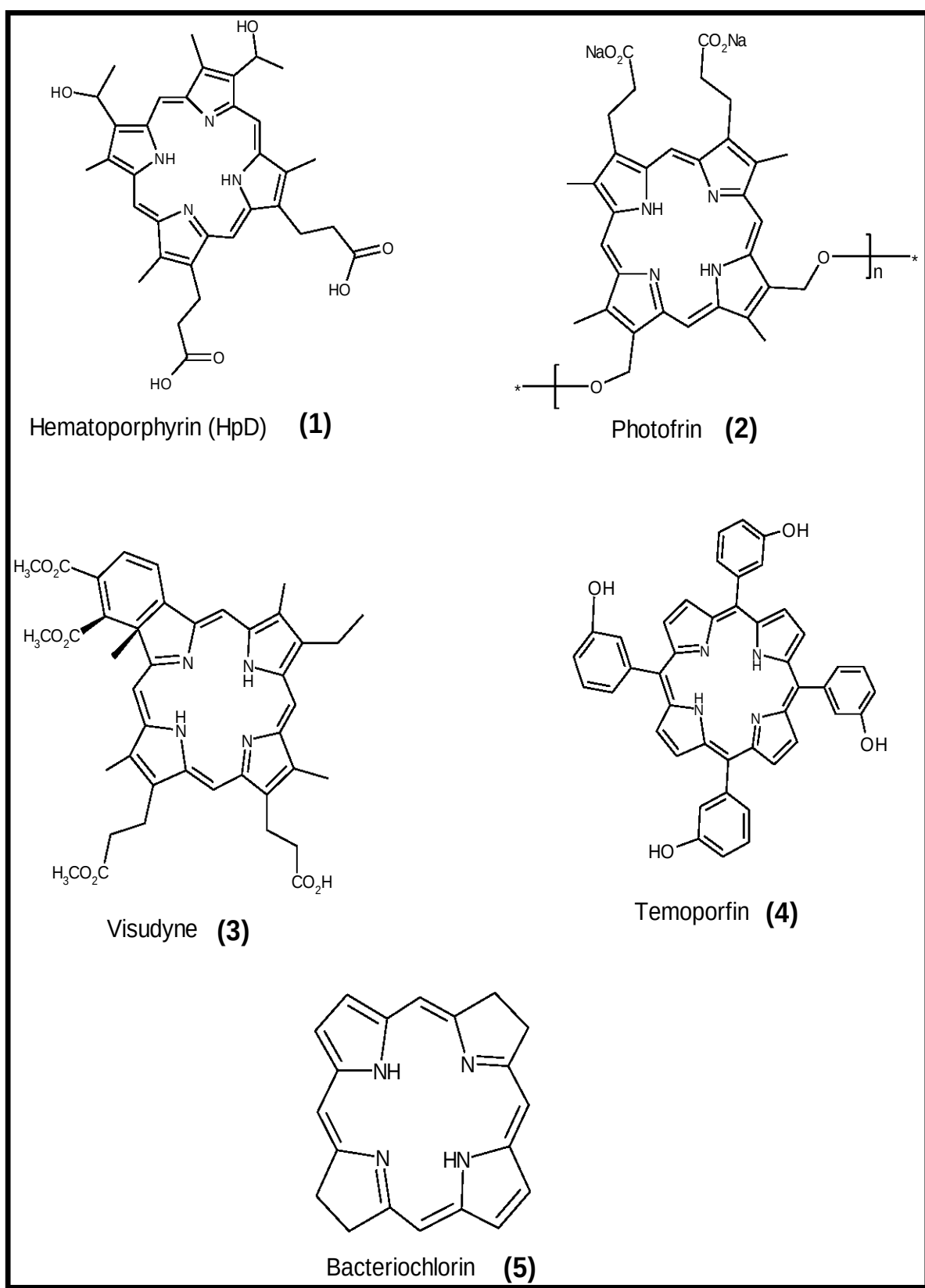


Figure 1.5: Structure of porphyrin based photosensitizers in pre- and clinical trials.

Table 1.2: Photophysical properties of a few selected photosensitisers (PS).

^a PS	^b Φ_F	^c Φ_T	^d τ_T (μ s)	^e Φ_Δ	^f Solvent	Ref
Photofrin	<0.1	0.60 (monomer) 0.20 (aggregate)	-	0.60 (monomer) 0.20 (aggregate)	2% DMSO in serum	[108]
ZnPc	0.2	0.50	330	0.67	Toluene/py	[109]
ZnPcS ₄	0.07	0.88	470	0.46	DMSO	[110]
ZnPcS ₂	-	0.46	270	0.52	Methanol	[111, 112]
Photosens	0.34	0.45	2.90	0.49	PBS	[113]
AlPcS ₂	-	0.24	775	0.27	Methanol	[113,114]
GaPcS ₂	-	0.36	390	0.38	Methanol	[111,112]
GaPcS ₁ ^t Bu ₃	-	-	440	0.36	Methanol	[111]
ZnttblPc	<0.1	0.82	180	0.59	DMSO	[115]

^aZnPc = zinc phthalocyanine, ZnPcS₄ = tetrasulfonate zinc phthalocyanine, ZnPcS₂ = disulfonate zinc phthalocyanine, AlPcS₂ = disulfonate aluminium phthalocyanine, GaPcS₂ = disulfonate galium phthalocyanine, ZnttblPc = Tris{9 (10), 16 (17), 23 (24)-(tert-butyl)imidophthalcyaninato}zinc(II), ^b Φ_F = fluorescence quantum yield, ^c Φ_T = triplet quantum yield, ^d τ_T = triplet lifetime, ^e Φ_Δ = singlet oxygen quantum yield. ^fpy = pyridine.

Among the more promising PSs are phthalocyanines (Pcs), Figure 1.6. Pcs have a very strong absorption peak in the far region of the visible spectra, where tissue penetration by visible light is great in addition to an improved light absorption capacity that is two-fold over that of the highest absorbing HpD (Pc molar extinction coefficient, $\epsilon \sim 10^5 \text{ M}^{-1} \text{ cm}^{-1}$; HpD $\epsilon \sim 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) [104]. Pcs differ from porphyrins through extended conjugation by benzene rings fused to the β -positions on each pyrrolic sub-unit. This extended conjugation strengthens the absorption of the Pc at longer wavelengths. It is important to establish the fluorescence, triplet-state and singlet-oxygen generating properties of these compounds [17, 105].

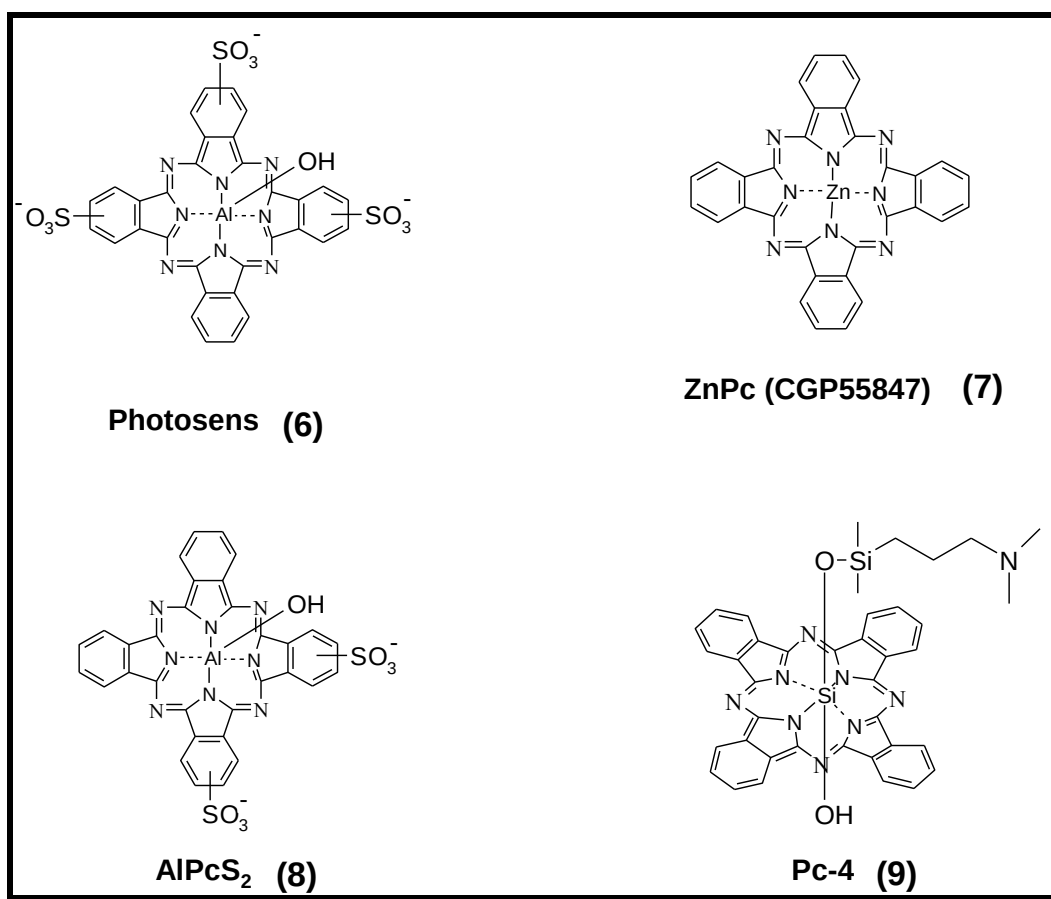


Figure 1.6: Structure of phthalocyanine based photosensitizers in clinical trials.

Pcs that contain diamagnetic metal ions such as Zn^{2+} , Al^{3+} and Ga^{3+} , are known to produce high triplet quantum yields ($\Phi_{\text{T}} > 0.4$), high triplet lifetimes ($\tau_{\text{T}} > 200 \mu\text{s}$) coupled with good singlet oxygen quantum yields ($\Phi_{\Delta} > 0.3$) hence they have been studied more for PDT [106, 107]. Table 1.2 shows a selection of Pcs together with their photophysical parameters [108-115].

Aluminum sulfophthalocyanine (Photosens, (6)) was developed in Russia; this drug passed through clinical trials and has been approved by the Russian Ministry of Public Health to treat skin, breast, liver, gastrointestinal and lung malignancies [77]. Photosens is a mixture of AlPcs bearing di- and tri-sulfonic acid substituents. Sulphonation significantly increases Pc solubility in polar solvents eliminating the need for delivery vehicles. This PS can be formulated for intravenous use, direct lesion injection and aerosol formulation. Selective tumour uptake has been found to be greatly affected by the degree of AlPc sulphonation. AlPcS₂ (8) has also been developed for various cancers.

Since PDT action is increased in highly monomeric Pcs, lipophilic Pcs therefore require biocompatible delivery vehicles. A liposomal formulation of a zinc phthalocyanine (CGP55847, Figure 1.6, (7)) was developed in Canada, sponsored by Ciba-Geigy (Switzerland). This PS has been in early Phase I/II clinical studies in Switzerland for treatment of upper gastrointestinal carcinomas [116].

A great deal of attention has been given to a silicon Pc derivative, Pc-4, (**9**), bearing a long-chain amino axial ligand ($\text{HOSiPcOSi}(\text{CH}_3)_2(\text{CH}_2)_3\text{N}(\text{CH}_3)_2$) (Figure 1.6). *In vitro* and *in vivo* studies have shown Pc-4 to be a promising PDT agent and is currently under clinical trials for the PDT treatment of neoplasms and has been examined for the sterilization of blood components. The axial ligation of Pcs has proven to be an ideal form of substitution as not only will it prevent aggregation or increase solubility but it eliminates the formation of isomers resulting in a pure complex.

In this work, unsymmetrically substituted phthalocyanines which are aimed at being more selective are developed for PDT. Synthesis of such phthalocyanines is more difficult than axial ligation. The latter can only be achieved for a few central metals.

1.1.8 Antibacterial photodynamic inactivation

Bacteria are divided in two classes, depending on the cell's reaction to a staining method called Gram stain. Gram-negative bacteria do not retain the violet dye during Gram staining and Gram-negative bacteria will retain the dye. The distinction between gram-positive and gram negative bacteria is due to differences in cell wall structure and chemical composition. Gram-positive bacteria have thick cell walls made out of peptidoglycan and gram-negative bacteria cell walls have another layer, called an outer membrane. These structural differences result in differences in the biochemical composition of the cell walls/membranes [117, 118].

Since the discovery of penicillin by Alexander Fleming in 1928 [119], there have been different families of antibiotics approved for the treatment of various microbial

infections. Antibiotics have since become a remedy of all infections. In the past decades, there have been an increased number of clinical drug-resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA). Some fungal strains such as *Candida albicans* have already developed resistance to antifungal agents such as amphotericin B. The photo-inactivation of microorganisms has been studied as a means to eliminate the problem of drug resistance [120].

For several decades, the discovery of antibiotics together with the resistance of Gram-negative bacteria to photosensitization by photosensitisers such as porphyrins and phthalocyanines hampered research on the use of PDT against microbial infections [121]. There is little historical evidence for the use of phthalocyanines in the treatment of microorganisms and the extensive current interest on this class of photosensitizers is directed at the development of PDT for cancer treatment. Application of phthalocyanines and local irradiation in mice showed a significant reduction of bacteria infected wounds and burns, better healing of wounds and no sepsis development [122].

The structure-activity relationship for phthalocyanines, showed little correlation between the antiviral strength and the central atom of the phthalocyanine. However, the degree of phthalocyanine sulphonation and butylation was found to affect both the antiviral activity and the extent of haemolysis [123].

When 16 strains of MRSA suspensions were irradiated with light from a laser diode in the presence of AlPcS₂. The strains were found to be susceptible to killing. The

bactericidal effect was determined to be dependent on AlPcS₂ concentration and the light dose [123].

Antibacterial testing of phthalocyanines has been carried out *in vitro*. Photo-inactivation of *Streptococcus sanguis* in biofilms [124] and of MRSA by aluminium phthalocyanine [125], Table 1.3, has been reported. Metal-free tetra(tert-butyl)phthalocyanine has been incorporated into polymer films as a photo-bactericidal material which was found to be effective against *Staphylococcus aureus* (*S. aureus*) [126], Table 1.3. The fact that the bacteria were killed only upon irradiation with light suggests that the site of action must be the cell wall. It is proposed that the Pc was immobilized on the cell wall and could not enter the bacterial cell. Thus singlet oxygen generated at the polymer–cell interface would react immediately on contact with the cell wall. When the antibacterial efficiency of anionic, cationic and neutral zinc phthalocyanines was tested against Gram-positive and Gram-negative bacteria, the results showed that only the positively charged species, a cationic water soluble pyridinium zinc phthalocyanine (PPC), was active [127]. This signifies the presence of a specific site of action for the active species since the neutral phthalocyanine showed similar uptake without activity. It has also been shown that the cationic silicon phthalocyanine (Pc-4) can incite the photo-inactivation of bloodborne pathogens such as *Plasmodium falciparum* and *Trypanosoma cruzi* [127-129].

Table 1.3: Summary of substances and microorganisms tested for antimicrobial activity.

^a Photosensitiser	^b Microorganism	Ref
PL-Ce ₆	<i>Actinomyces viscosus</i> ,	[84]
AlPcS ₂	MRSA, <i>streptococcus auers</i>	[124]
H ₂ Pc ^t Bu	<i>S.auers</i>	[126]
PPC	<i>E.coli</i>	[127]
Pc-4	<i>Plasmodium falciparum</i>	[127]
ZnPc-PL	<i>Porphyromonas gingivalis</i>	[133]
Porphyrin	<i>S.auers</i> , <i>E.coli</i>	[134]
AgNPs	<i>E.coli</i>	[135]

^aPL-Ce₆ = poly-L-lysine-chorin conjugate, AlPcS₂ = disulfonate aluminium phthalocaynine, H₂Pc^tBu = tertiary butyl phthalocyanine, PPC = pyridinium zinc phthalocyanine, Pc-4 = silicon phthalocyanine 4, ZnPc-PL = zinc phthalocyanine-poly-L-lysine conjugate, AgNPs = silver nanoparticles, ^bMRSA = methicillin-resistant *Staphylococcus aureus*, *S. auers* = *Staphylococcus aureus*, *E.coli* = *Escherichia coli*.

Cationic PSs, such as positively charged phthalocyanine [130] or poly-cationic conjugate bearing poly-L-lysine, Table 1.3, [131-133] have been reported to be more active than the corresponding anionic or non-ionic compounds against both Gram-positive and Gram-negative bacteria. The photosensitizing efficiency of porphyrin

derivatives conjugated to poly-L-lysine tested against Gram-positive (*S. aureus*) and Gram-negative (*Escherichia coli*, *E. coli*) bacteria, showed that poly-L-lysine significantly increased the activity of the photosensitizers. The photo-inactivation of *E. coli* occurred only in the presence of the cationic derivatives implying that positive charges are required for the photo-inactivation of Gram-negative bacteria [134], Table 1.3. Poly-L-lysine-chlorin_{e6} (*ce6*) conjugates have also been studied for targeted photo-destruction towards gram-positive (*Actinomyces viscosus*) and gram-negative (*Porphyromonas gingivalis*) oral species while sparing an oral epithelial cell line (HCPC-1) [84].

It has been demonstrated that highly reactive metal oxide nanoparticles display admirable antibacterial action against Gram-positive and Gram-negative bacteria [129]. For this reason, the preparation, characterization, surface modification, and functionalization of nanosized inorganic particles opens the possibility of formulation of a new generation of antibacterial drugs. Silver ions and silver-based compounds are known to be highly toxic to microorganisms [135, 136], Table 1.3 and show strong biocidal effects on as many as 16 species of bacteria [137]. Tiller and co-workers showed that silver nanoparticles hybrids with amphiphilic hyper-branched macromolecules exhibit effective antimicrobial surface coatings [138]. AuNPs have also shown antimicrobial activity [139-142].

As Table 1.3 shows, the use of phthalocyanine-nanoparticle conjugates has not been employed for the inactivation of bacteria. The combination of NPs with

phthalocyanines will result in synergistic effects inevitably leading to improved photo-inactivation.

The photodynamic antimicrobial therapy could become an effective alternative treatment for bacterial infections caused by multi-resistant strains compared to other bacterial inactivation processes [143-146].

1.2 Phthalocyanines

1.2.1. History and general applications

The interest in phthalocyanines (Pcs) and their structurally related derivatives has grown tremendously. Phthalocyanines (Pcs) were accidentally discovered at the Grangemouth plant of Scottish Dyes Ltd in 1928 during the industrial preparation of phthalimide from phthalic anhydride. During the synthesis of phthalimide, the glass-lined reaction vessel cracked exposing the outer steel casing to the reaction; this resulted in the formation of a blue-green material. The study of this compound was taken up by Professor J. T. Thorpe who gave it to Reginald Patrick Linstead (1902-1966) for investigation. Using a combination of elemental analysis, molecular mass determination and oxidative degradation, Linstead was able to come up with the correct structure for this material. He called this compound phthalocyanine due to its phthalic anhydride origin (phthalo) and deep blue color (cyanine) [147]. A series of papers describing the synthesis and chemical properties of phthalocyanines later followed.

Phthalocyanines (**10**) are macrocyclic compounds that are closely related to the porphyrin (**11**) ring system (Figure 1.7), the structural difference being the four isoindole subunits and nitrogen atoms at the four *meso* positions, thus phthalocyanines are often referred to as *tetra-benzotetraazaporphyrins* [148].

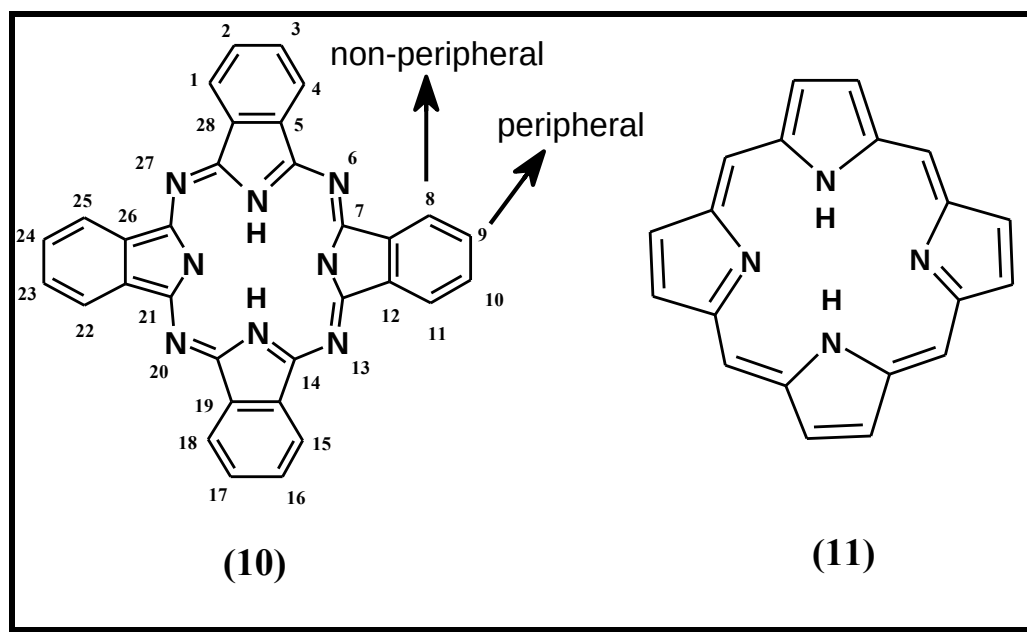


Figure 1.7: Phthalocyanine structure (**10**) with possible substitution sites at benzo-positions numbered using the accepted notation and (**11**) is the structure of a simple porphyrin (porphine).

Pcs are aromatic compounds owing to their planar conjugated display of 18 π -electrons. Pcs show increased stability, architectural flexibility, diverse coordination properties and improved spectroscopic characteristics. Pcs are used in a number of applications such as chemical sensors [149-151], liquid crystals [152, 153],

semiconductors [154], non-linear optics [155, 156], and photodynamic therapy (PDT) [3, 157].

Phthalocyanines are light absorbing compounds, particularly red light. The positions of the absorption bands in phthalocyanines, (particularly the Q-band) are affected to a varying degree, by the central metal, axial ligation, solvents, peripheral and non-peripheral substitution, aggregation and by extension of the conjugation [158].

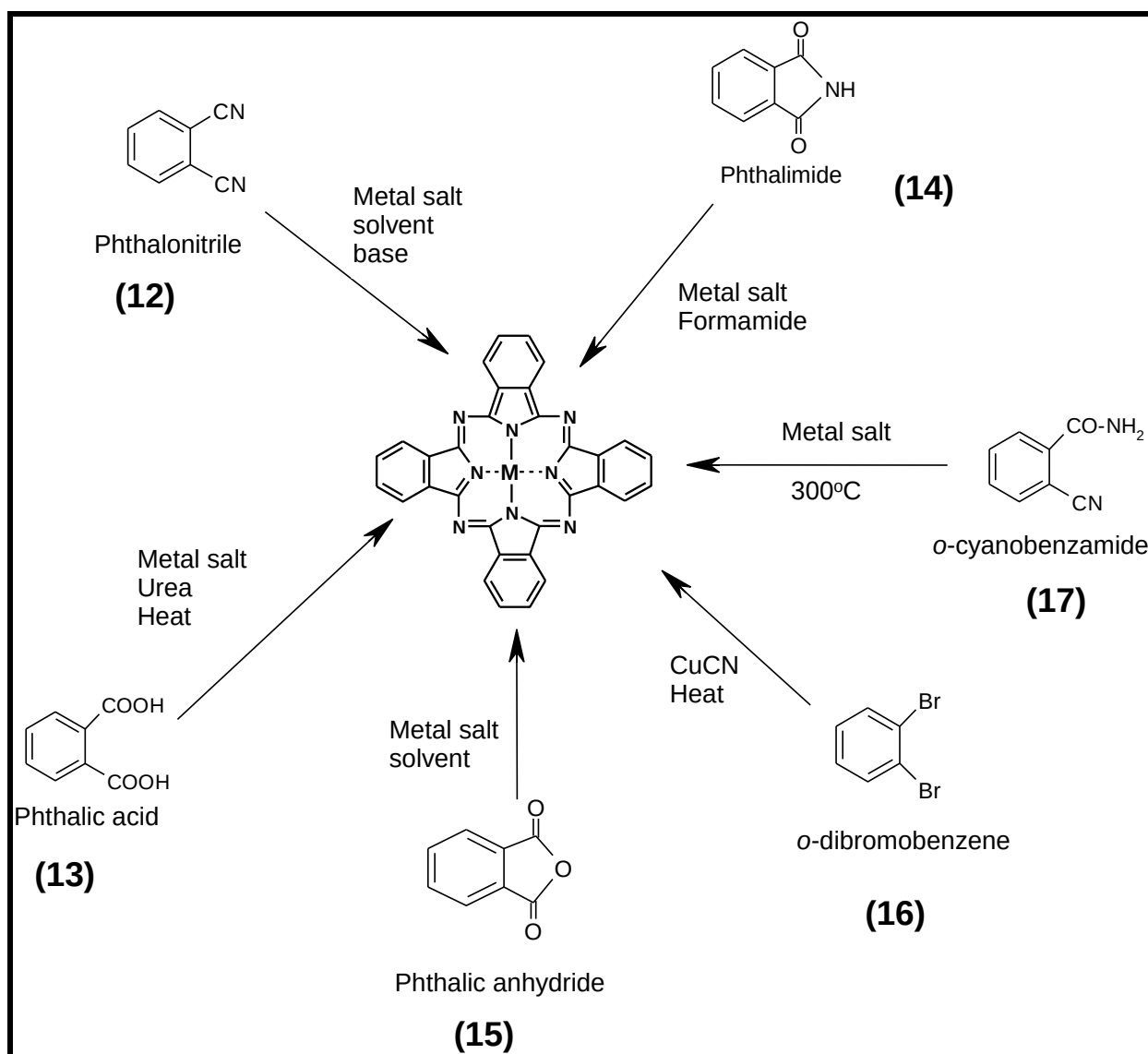
1.2.2 Synthesis of phthalocyanines

Unsubstituted phthalocyanine derivatives are insoluble in a number of solvents. This stems from the hydrophobicity of the aromatic core and planarity of the Pc causing Pcs to stack on each other forming highly stable structures. Insolubility of Pcs is very important for their use as dyes as it ensures durability. However, most Pc applications require solubility in common organic solvents.

Solubility is induced through the functionalization of the Pc framework. The flexible structure of a Pc ring allows for the incorporation of different substituents on the peripheral {(2,3), (9,10), (16,17) or (23,24)} or non-peripheral {(1, 4), (8,11), (15,18) or (22,25)}, (Figure 1.7), positions. Such structural manipulations greatly improve the solubility of phthalocyanines in organic and aqueous media and help diminish aggregation. Phthalocyanine aggregation is usually depicted as the coplanar association of rings progressing from monomer to dimer to higher order complexes, occurring through the π - π interaction of Pc rings [159].

A number of ortho-disubstituted benzene derivatives can act as Pc precursors, these include phthalonitrile (**12**), phthalic acid (**13**), phthalimide (**14**), phthalic acid anhydride (**15**), *o*-cyanobenzamide (**16**), *o*-dibromobenzene (**17**), and other derivatives (Scheme 1.2). Phthalonitriles are the more popular choice for laboratory synthesis given their high yields for the Pc product [160].

Unsubstituted metal-free Pcs can be conveniently prepared by the Linstead method [161] using a phthalonitrile as the starting material in a refluxing alcohol solution containing lithium, sodium or magnesium alkoxides. This results in the corresponding metallophthalocyanine (MPc) that is easily demetallated by the addition of a dilute acid (i.e. glacial acetic acid) affording the metal-free Pc (H₂Pc). Other methods include the use of a base (ammonia, or 1,8-diazabicyclo[5.4.0]undec-7-ene, DBU) or organic reducing reagents that create the ideal environment for the cyclotetramerisation of the phthalonitrile [162]. Lastly the use of microwave irradiation offers a quick and solvent free alternative [163].



Scheme 1.2: Synthetic routes of metallophthalocyanines (MPcs) from starting materials.

MPcs are formed through the substitution of the hydrogens in the cavity of metal-free Pcs by metal atoms. Over seventy different metallic and non-metallic cations have been inserted in the cavity of the Pc moiety. This enables the control of the oxidation potential and electrical properties of Pc complexes. The synthetic

procedures of MPcs are shown in Scheme 1.2. The majority of MPcs are generally synthesized by temperature cyclotetramerization of phthalonitrile pre-cursors in the presence of the corresponding metal or metal salt.

Symmetrically and unsymmetrically substituted phthalocyanines are reported in this work, hence are discussed

1.2.2.1 Symmetrically substituted phthalocyanines

The development of new derivatives that have improved chemical properties has lead to the synthesis of an assortment of substituted Pcs. Substituted Pcs can be accomplished through direct substitution onto a pre-existing Pc. Sulphonated Pcs for instance are synthesized by heating a Pc in oleum. This results in complex isomeric mixtures of varying degrees of sulphonation and is usually not ideal as the mixture lacks a distinct structure. The other method of making substituted Pcs is done using phthalonitrile precursors that contain the desired substituent.

Tetra-substituted Pcs are formed from 3- and 4-substituted precursors substituted either at the α or β -position respectively. The synthesis of tetra-substituted phthalocyanines results in the formation of four possible constitutional isomers with different symmetry. These isomers, shown in Figure 1.8 can be isolated using high performance liquid chromatography (HPLC) [164].

Phthalocyanines tetra-substituted at the peripheral positions have been extensively synthesized and studied more than the non-peripheral derivatives despite the fact

that substitution at the non-peripheral positions reduces phthalocyanine aggregation tendencies more than substitution at the less sterically crowded peripheral position, see Figure 1.7 for definitions.

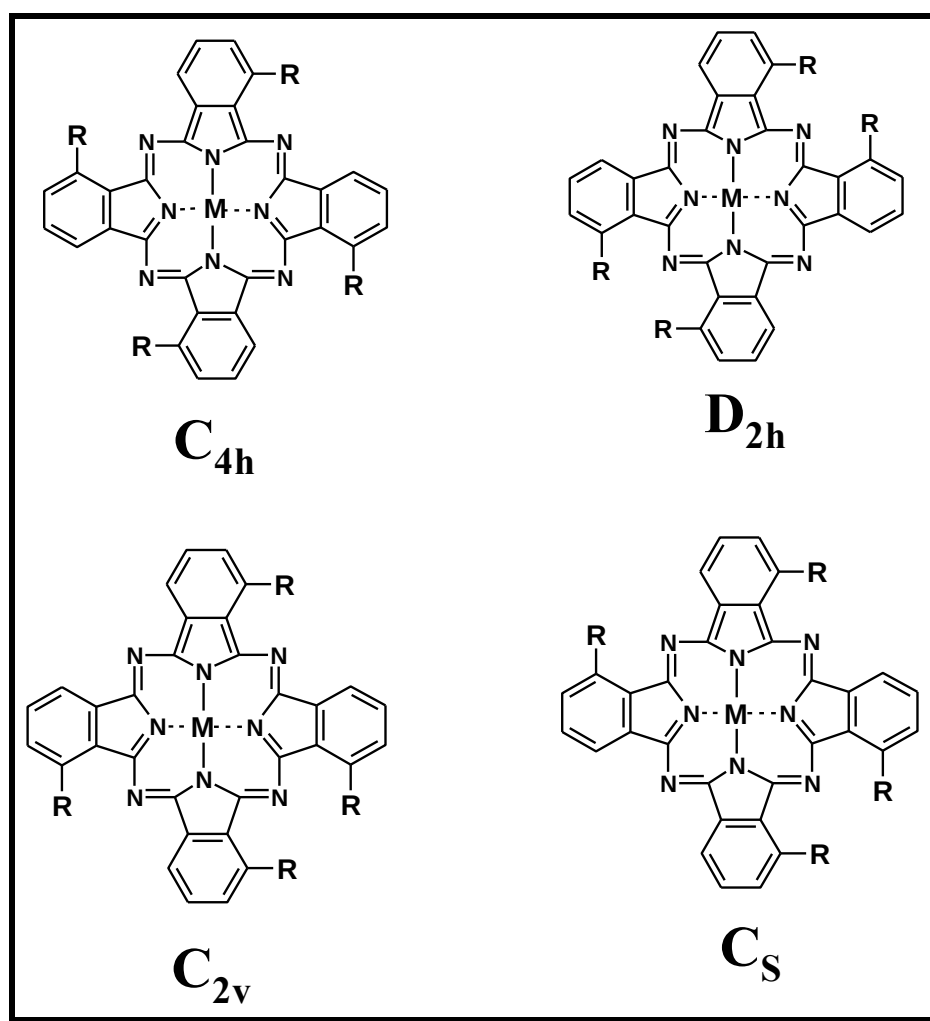


Figure 1.8: Constitutional isomers of non-peripherally substituted MPcs. R represent a substituent.

Octa-substituted Pcs are synthesized from 3,6- or 4,5-disubstituted phthalonitriles to form non-peripherally or peripherally octasubstituted Pcs, see Figure 1.7 for numbering. Their structures are shown in Figure 1.9. The non-peripherally octasubstituted Pcs are more soluble in common organic solvents due to an out-of-plane arrangement of the substituents on these positions [165]. Nevertheless, non-peripheral octasubstituted Pcs are easier to synthesize and have better yields.

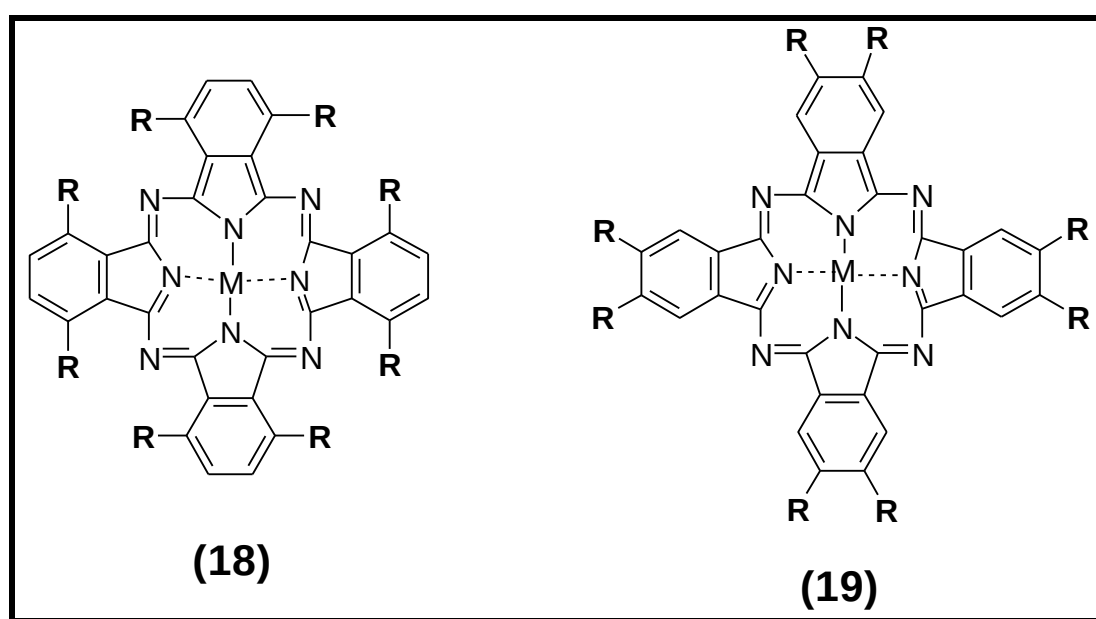


Figure 1.9: Structure of 1,4,8,11,15,18,22,25- (18) and 2,3,9,10,16,17,23,24- (19) octasubstituted MPcs.

The preparation of thiol-derivatized metallophthalocyanine complexes is relatively more difficult than other MPc derivatives, which explains why MPcs containing sulfur donors have been less explored compared to other metallophthalocyanine derivatives in literature. The presence of thiol groups at the end of substituents of

the Pc could improve dye amphiphilicity and in this form the Pcs can be easily linked to carriers and their attachment on nanoparticles is made simple [166-169]. There are limited reports on the photophysicochemical properties, in particular of phthalocyanines tetrasubstituted with thiol groups at the end of peripheral or non-peripheral Pc substituents. The great interest in the development of the above-mentioned compounds has led us to investigate the synthesis, spectroscopic characterization and photophysicochemical properties of novel thiol-derivatized zinc (II) phthalocyanine complexes. To our knowledge, only the synthesis and characterization of [2,9,17,23-tetrakis[(3-propoxythiol)phthalocyaninato]zinc (II) (**23**), (Figure 1.10) has been reported so far [170]. This Pc is tetrasubstituted with thiol groups at the end of the alkyl peripheral substituents. This work reports on another tetrasubstituted thiol complex. The rest of the complexes in Figure 1.10 contain one thiol group.

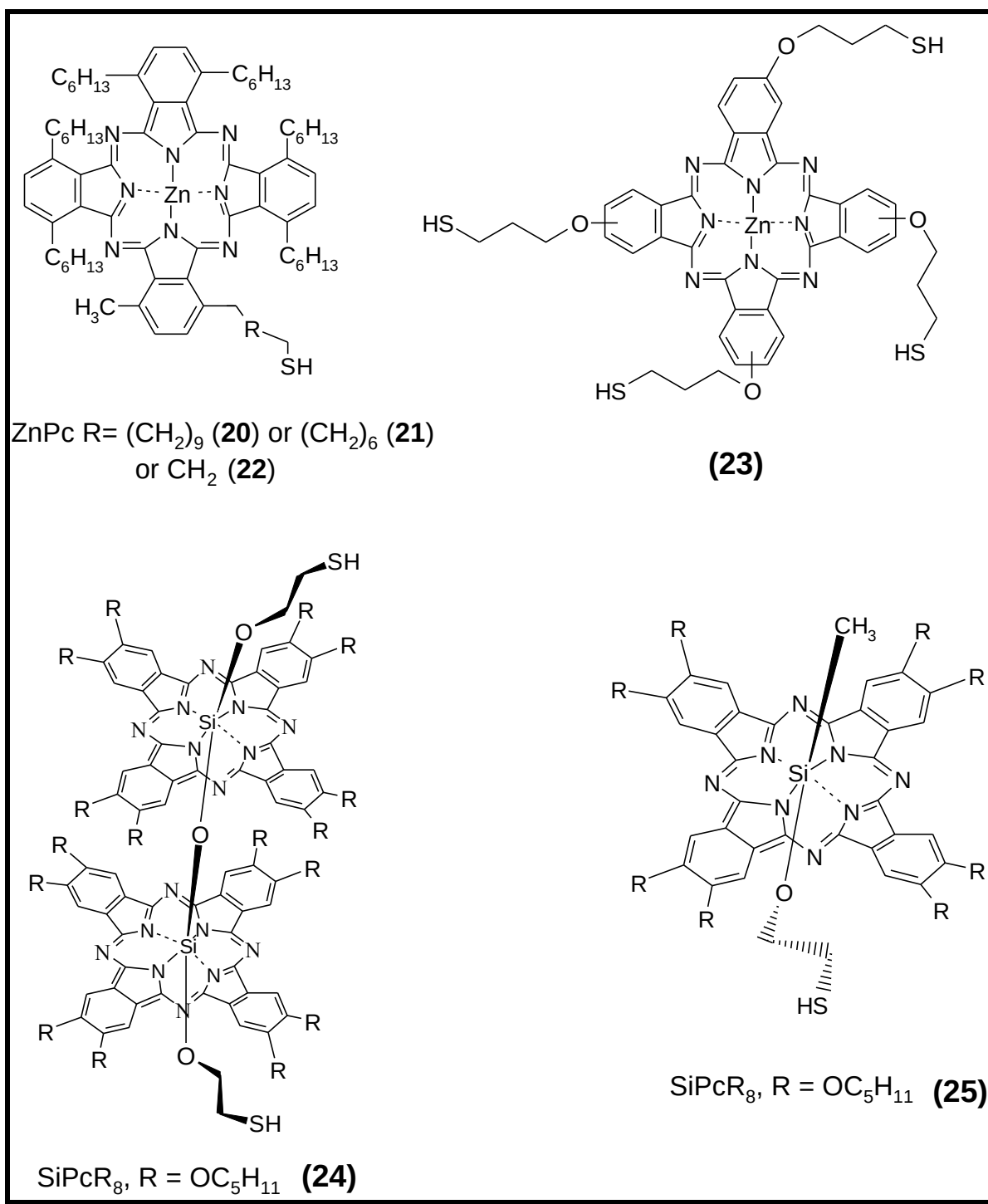


Figure 1.10: Synthesized thiol-derivatized zinc phthalocyanines.

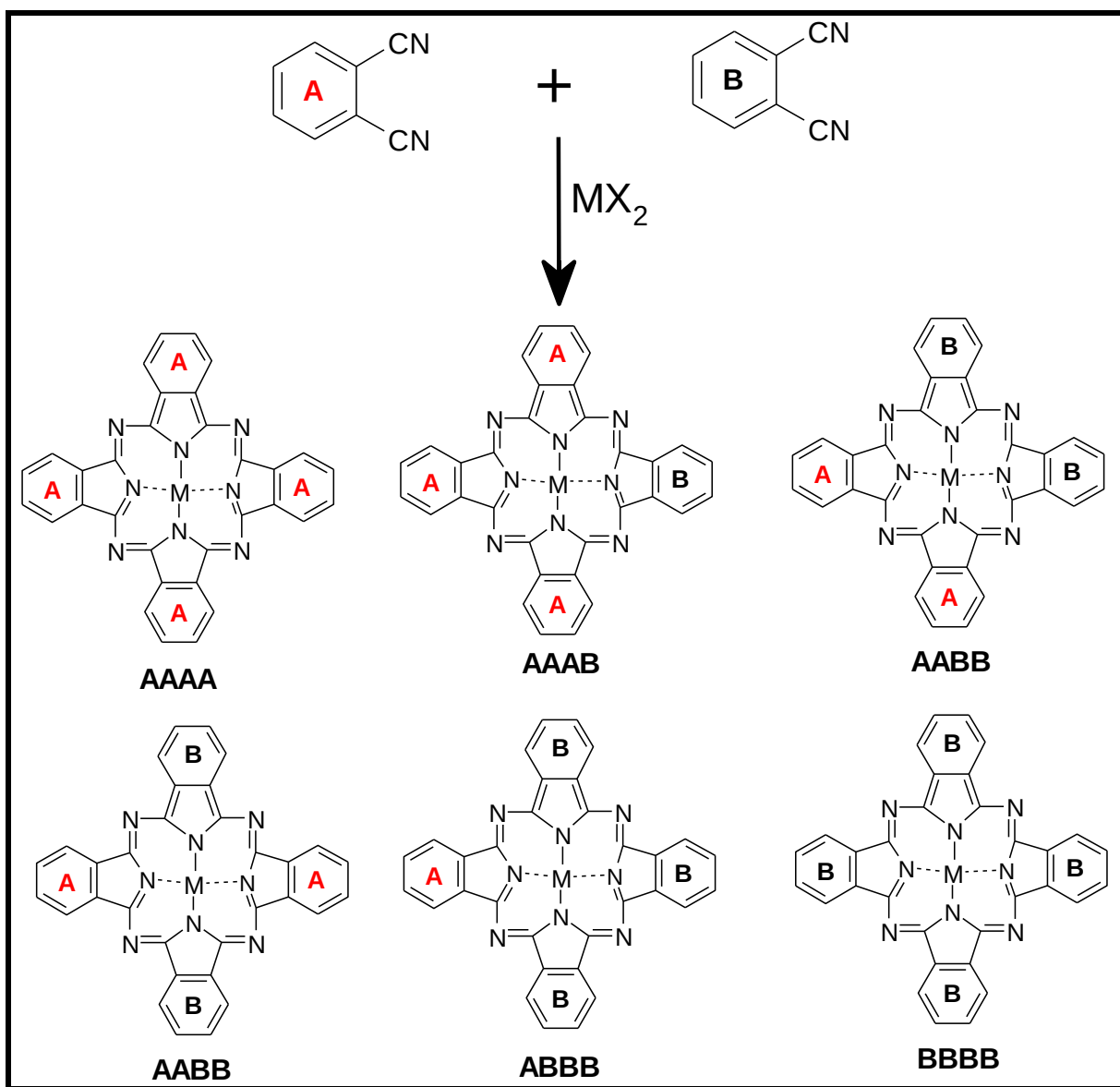
1.2.2.2 Unsymmetrically substituted phthalocyanines

The synthesis of unsymmetrically substituted Pcs has attracted enormous interest in recent years. The presence of different functional groups on the same Pc molecule provides unique co-existing features for various applications that include non-linear optics or specific conjugation with biological molecules [171, 172]. Pcs comprising of three identical (A) and one different (B) isoindole subunits (A_3B type) have been the target of many studies for such applications.

Statistical condensation is the most widely used strategy for the synthesis of A_3B type Pcs. This method utilizes one different isoindole and three identical subunits [173, 174]; this reaction however produces a mixture of six compounds which requires lengthy and strenuous chromatographic techniques for their subsequent isolation [175] (Scheme 1.3).

For this kind of synthesis it is often customary to have at least one of the two different phthalonitriles to bear bulky groups to prevent aggregation or one that will provide a different solubility feature which will allow the separation of the different unsymmetrical Pcs by virtue of the dissimilar solubilities of the Pc compounds present in the statistical mixture [176].

The maximal conversion of phthalonitriles to the A_3B type Pc is largely determined by stoichiometry manipulation.



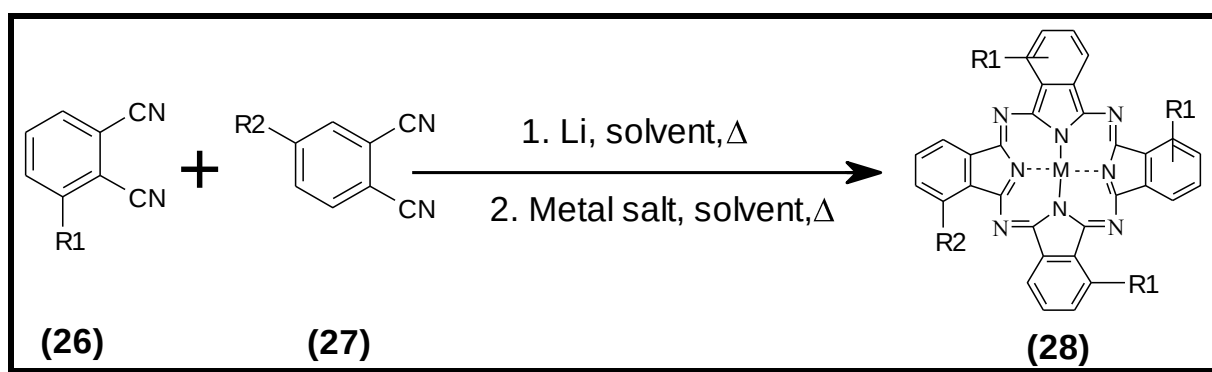
Scheme 1.3: Statistical condensation of two different phthalonitriles (A and B) resulting in six possible phthalocyanine products [175].

The statistical condensation approach predicts that if two precursors of similar reactivity react in a 3:1 molar ratio, the resultant mixture will contain the following percentages; A_4 (33 %), A_3B (44 %) and the rest of the products will have a total of 23

% combined. A 3:1 molar ratio is thus commonly used to favour A₃B Pc formation [177-179]. Since the electronic properties or position of substituent(s) can affect the ratio of products formed, the stoichiometry of phthalonitrile precursors may be modified to 9:1 or to even higher A:B ratios if B is more reactive than A. The ratios can also be inverted when B is less reactive than A [180-182].

In this work, focus is given to the A₃B type phthalocyanines with the synthesis of novel mono-functionalized carboxy metal free, zinc and magnesium phthalocyanine. The carboxylic acid functional group on the phthalocyanine allows site specific covalent attachment of biological markers such as monoclonal antibodies or lipoproteins, to enhance the target specificity of phthalocyanine as photosensitizer in photodynamic therapy.

The A₃B Pcs were prepared using the statistical condensation approach by reacting two differently substituted phthalonitriles (**26**) and (**27**) precursors (Scheme 1.4).

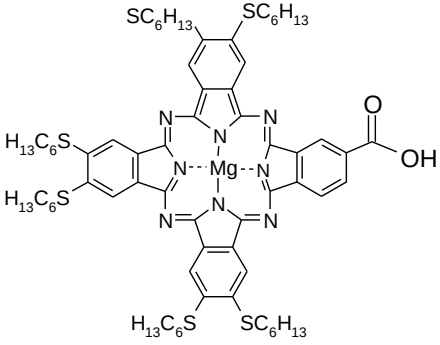
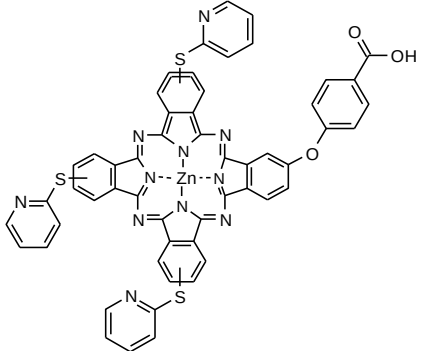
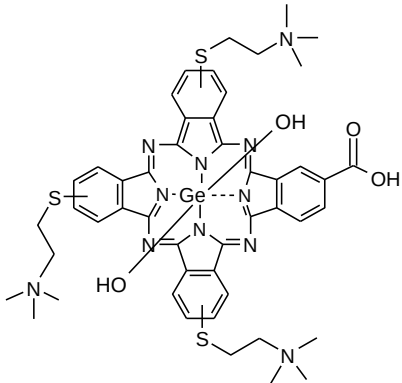


Scheme 1.4: Synthesis of A₃B type MPcs.

Mono-functionalized carboxy substituted zinc and magnesium phthalocyanines show fascinating spectroscopic chemistry although their photophysicochemistry studies are limited [183, 184]. There is a lack of photophysical data pertaining to mono-functionalized carboxy substituted Pcs, for this reason this work aims to determine the properties of novel unsymmetrically substituted MgPc together with the metal-free and zinc derivatives. Table 1.4 shows a selection of mono-functionalized carboxy metallophthalocyanines (**29-31**) together with their photophysical and photochemical properties [185-190].

The Table shows that these complexes are still limited, so are their photophysical properties.

Table 1.4: Photophysical and photochemical properties of mono-functionalized MPcs.

MPc	Solvent	Φ_F	τ_F	Φ_T	τ_T	Φ_Δ	Ref
 (29)	DMSO	0.06	-	0.64	310	-	[185]
 (30)	DMF	0.25	-	0.68	8	0.63	[186]
 (31)	DMF	0.09	1.61	0.70	70	-	[187]

1.2.3 Spectral properties of phthalocyanines

Phthalocyanines are characterized by a distinct absorption in the visible region of the spectrum, namely the Q (Q_{00}) band and a weaker absorption called the B or Soret band [191], Figure 1.11. The assignment of the Q and B bands is based on the Gouterman's four-orbital model (Figure 1.12), [191-195]. According to this model, the Q band arises from $\pi\text{-}\pi^*$ (x/y polarized) transitions best explained in terms of the transitions from a_{1u} , the highest occupied molecular orbitals (HOMO) of the Pc ring to the lowest unoccupied molecular orbitals (LUMO), e_g^* .

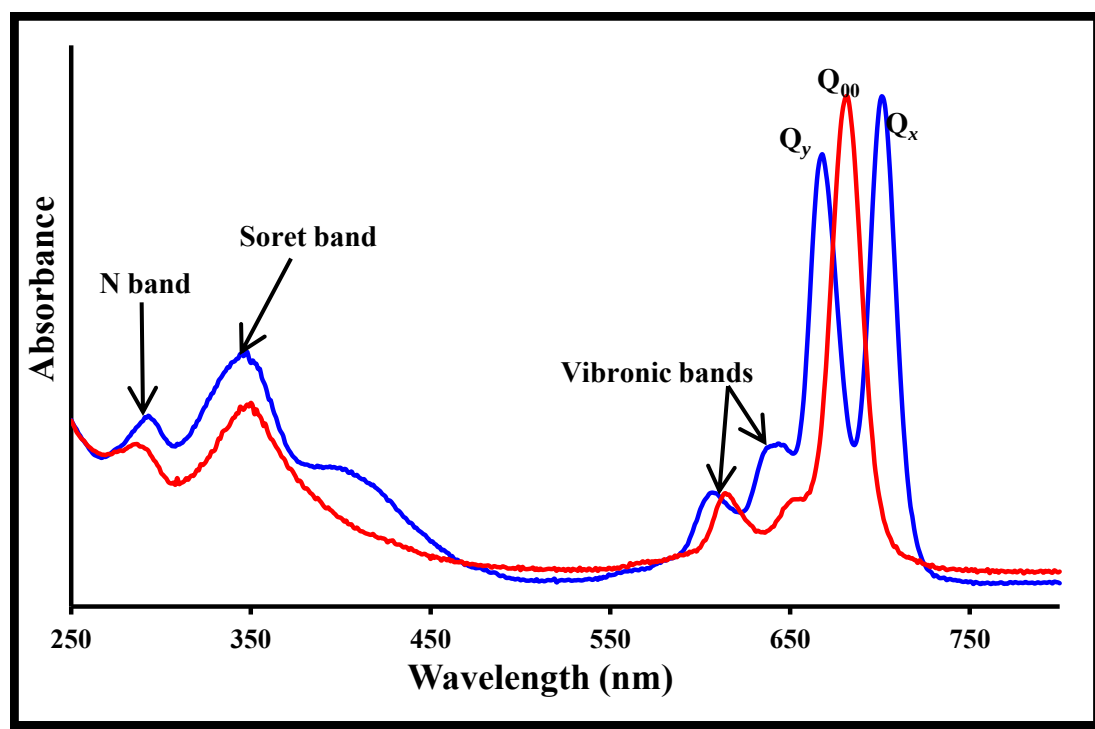


Figure 1.11: Absorption spectra of metallated (red) and metal-free (blue) phthalocyanines showing typical phthalocyanine absorption bands.

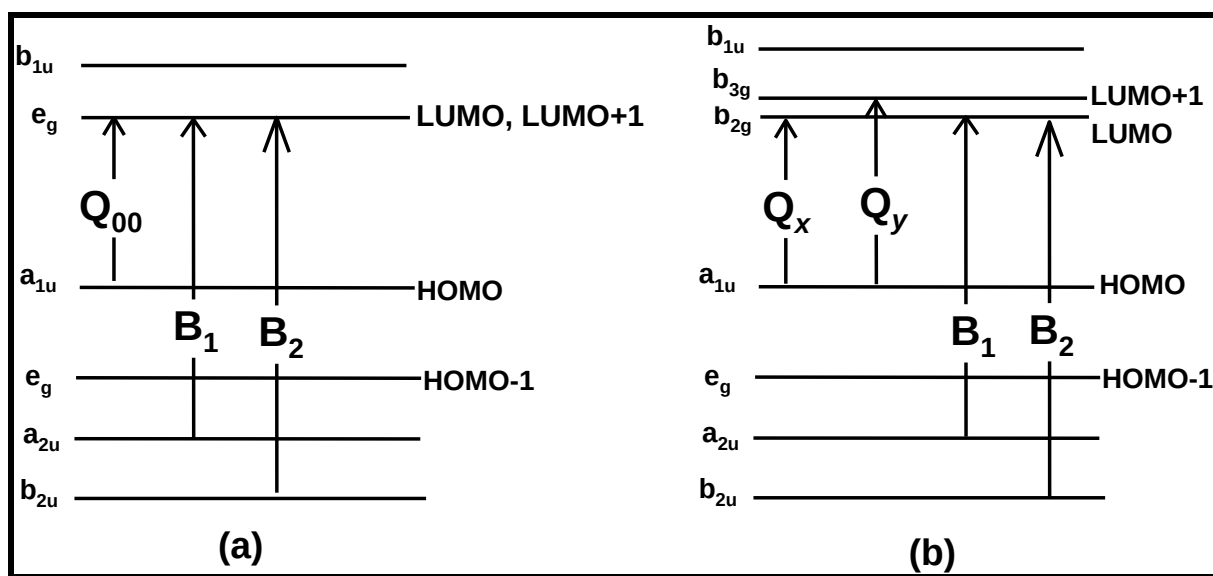


Figure 1.12: Electronic transitions (a) in symmetrical MPcs showing the origin of the Q_{00} band and B_1 and B_2 absorption bands [195] and (b) in symmetry lowered MPcs showing the origin of the Q_x and Q_y bands.

Phthalocyanine symmetry becomes important in understanding their spectral properties, for instance a metal-free Pc has D_{2h} symmetry whereas a metallated Pc has D_{4h} symmetry. The symmetry of the MPc is attributed to the degenerate e_g orbital, however the H_2Pc has a non-degenerate e_g orbital (e_{gx} and e_{gy}) resulting in a loss of symmetry. The consequence of this is seen in the UV/Vis spectra where the H_2Pc has two Q-bands and the MPc has one as shown in Figure 1.11. There also exist charge transfer bands which can be from metal to the Pc ring (Metal to Ligand charge transfer) or vice versa (Ligand to Metal charge transfer). These bands are usually observed in the 550 nm - 400 nm range [194]. The next lowest energy bands

are called the N, L and C bands which commonly appear around 290 nm, 240 nm and 190 nm respectively.

1.2.4 Photophysical and photochemistry of phthalocyanines

The photophysical properties of phthalocyanines are exceptionally central in PDT. The Jablonski diagram (Figure 1.3) is used to represent and explain the radiative and non-radiative routes a Pc can take going back to the ground state following photo-excitation to higher energies. [17-19].

1.2.4.1 Fluorescence quantum yield and lifetime

The fluorescence quantum yield (Φ_F) is defined as the number of photons emitted relative to the number of photons absorbed. Thus Φ_F may be defined as the ratio of molecules fluorescing to the number of photons absorbed. The Φ_F is used to determine and quantify the efficiency of emission from the singlet state. The Φ_F value is determined comparatively [196] using compounds of known fluorescence quantum yields, for example ZnPc in DMSO (Φ_F) = 0.20 [197], using equation 1.1

$$\Phi_F = \Phi_{F(\text{Std})} \frac{F \cdot A_{\text{Std}} \cdot n^2}{F_{\text{Std}} \cdot A \cdot n_{\text{Std}}^2} \quad (1.1)$$

where F and F_{std} are the areas under the fluorescence curves of the sample and the standard respectively. A and A_{std} are the respective absorbances of the sample and

the standard at the excitation wavelength and n and n_{std} are the refractive indices of the solvents used for the sample and standard respectively.

The fluorescence lifetime, τ_F , of an excited species is the time needed for a concentration of the species to decrease to $1/e$, of its original value [198]. The τ_F is related to the radiative lifetime, τ_0 , defined by equation 1.2.

$$\Phi_F = \tau_F / \tau_0 \quad (1.2)$$

Time domain measurements are used to determine the fluorescence lifetime using time-correlated single-photon counting (TCSPC) techniques [199, 200]. Figure 1.13 shows a typical fluorescence decay curve for Pcs, obtained using a TCSPC set-up. The fluorescence lifetimes of MPcs are usually of the order of a few nanoseconds.

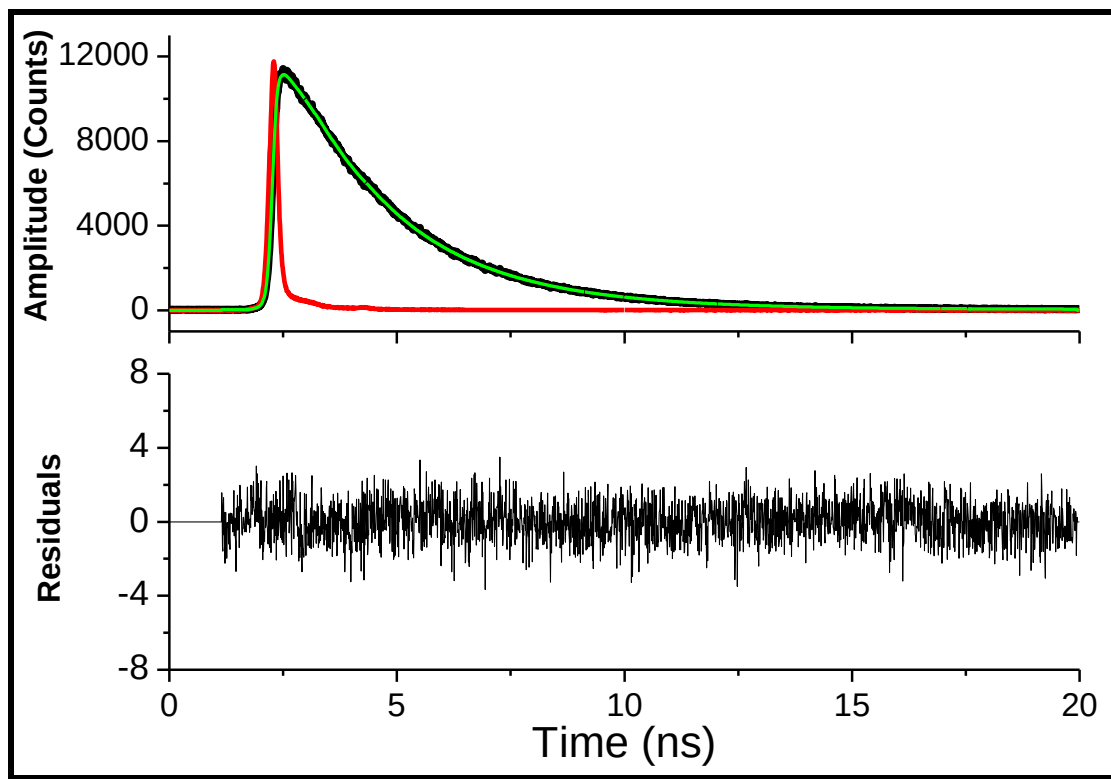


Figure 1.13: A typical fluorescence decay curve of a phthalocyanine [187].

1.2.4.2 Triplet quantum yield and lifetimes

The triplet quantum yield is the number of molecules that undergo ISC from the first excited state, S_1 , to the triplet state T_1 . Phthalocyanine triplet quantum yields (Φ_T) and triplet lifetimes (τ_T) are generally determined by laser flash photolysis.

This technique uses intense laser light together with white light to measure and monitor the absorption of the molecule from the excited T_1 state to higher energy states, T_n . A triplet decay curve of change in absorbance (ΔA) versus time in seconds is obtained from the experiment and from this the triplet lifetime (τ_T) can be determined. Most Pcs exhibit triplet-triplet absorption around the 500 nm region, far from the ground singlet state absorption making it possible to conduct these measurements. [201, 202]. A typical decay curve is shown in Figure 1.14.

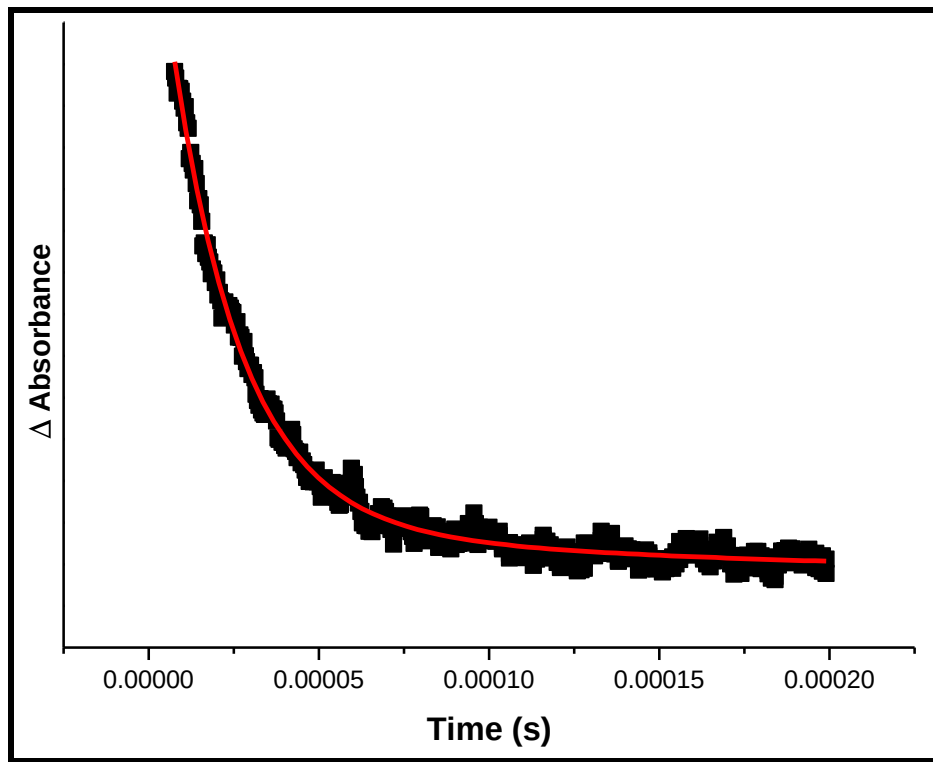


Figure 1.14: Typical triplet decay curve of Pcs [186].

A comparative method using zinc phthalocyanine as a standard is employed for triplet quantum yields (Φ_T) calculations, equation 1.3

$$\Phi_T = \Phi_T^{\text{Std}} \cdot \frac{\Delta A_T \cdot \epsilon_T^{\text{Std}}}{\Delta A_T^{\text{Std}} \cdot \epsilon_T} \quad (1.3)$$

where ΔA_T and ΔA_T^{Std} are the changes in the triplet state absorbances of the sample and standard, respectively. ϵ_T and ϵ_T^{Std} are the triplet state molar extinction coefficients for the sample and standard, respectively [203]. Fitting of the triplet decay curves (as shown in Figure 1.14), using OriginPro 7.5 software has been used in this work for determination of the lifetimes of the Pc transients.

1.2.4.3 Singlet oxygen quantum yield

Singlet oxygen ($^1\text{O}_2$) is a highly reactive species responsible for the light-induced oxidative destruction of malignant or bacteria/virus infected tissue. $^1\text{O}_2$ is generated when oxygen in its triplet ground state ($^3\text{O}_2$) interacts with a sensitizer in a process called photosensitization, (Scheme 1.1, Type II mechanism).

Singlet oxygen may be determined by two main methods, using chemical quenchers or using luminescence at 1270 nm [204]. In this work singlet oxygen scavengers, 1,3 diphenylisobenzofuran (DPBF) or anthracene-9,10-bis-methylmalonate (ADMA), known quenchers in organic and aqueous solvents respectively, were employed. The quencher quickly reacts with the singlet oxygen in a 1:1 ratio without side reactions or decomposition products that may interfere with the detection of singlet oxygen

[204]. This chemical method is undoubtedly the most common method used for quantifying singlet oxygen in the laboratory. As soon as singlet oxygen is generated, it can be trapped using a singlet oxygen quencher. The disappearance of the quencher is followed spectroscopically.

The quantum yield of singlet oxygen (Φ_{Δ}) is used to measure the efficiency with which various photosensitizers generate singlet oxygen. The values of Φ_{Δ} are determined using equation 1.4.

$$\Phi_{\Delta} = \Phi_{\Delta}^{Std} \cdot \frac{R I_{abs}^{Std}}{R^{Std} I_{abs}} \quad (1.4)$$

where Φ_{Δ}^{Std} is the singlet oxygen quantum yield for the ZnPc. R and R^{Std} are the photodegradation rates of ADMA or DPBF in the presence of a sensitizer under investigation and the standard respectively. I_{abs} and I_{abs}^{Std} are the rates of light absorption by the sensitizer and standard, respectively.

1.3 Nanoparticles

Nanotechnology is a powerful technology that holds promise for the design and development of various types of novel products with potential medical applications. Metal nanoparticles represent a new class of biocompatible vectors capable of fulfilling this promise through selective cell targeting which will provide new means for the site-specific diagnosis and treatment of medical conditions. Nanoparticles are defined as atomic arrangements with nanometric dimensions and usually with a small number of constituent atoms [205]. The novelty of nanoscale materials comes from the fact that with decreasing size, the properties of nano-materials change.

Researchers around the world have made numerous attempts to discover and expand the synthetic procedures and characterization techniques for metal nanoparticles. Controlling the shape and size of nanoparticles has become increasingly more attractive due to the shape/size-dependent functional properties metallic nanoparticles have [206]. This work outlines the methodology for conjugation of gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) with phthalocyanines. Cytotoxic and antibacterial effects of the conjugates will be examined.

1.3.1 Chemical and physical properties of gold and silver nanoparticles

Gold and silver are generally inert and, are not attacked by reactive O_2 to a significant degree [207], making AuNPs and AgNPs suitable for PDT applications. Both Au and Ag have a high affinity for sulfur, this implies that the surface of such nanoparticles can be functionalized with organic thiols. The sulfur becomes the electron donor whereas the metal atom becomes the acceptor. Also, metal electrons are partially delocalised in molecular orbitals formed between the filled d -orbitals of the metal and the empty d -orbitals of sulfur [207]. Therefore, AuNPs and AgNPs may be easily tagged with various proteins and bio-molecules rich in amino acids leading to important biomedical applications including targeted drug delivery [208, 209], cellular imaging [210], and biosensing [211].

Solutions of AgNP have been used as bactericidal agents because the Ag^+ ions interfere with bacteria metabolism and act as bactericides [207].

The surface plasmon resonance, SPR, is a major physical property of metal nanoparticles. The SPR is due to the coherent oscillation of $6s^1$ (Au) or $5s^1$ (Ag) conduction band electrons in the presence of electromagnetic waves [212]. For spherical AgNP and AuNP having a diameter between 2 and 100 nm, the position of the surface plasmon absorption is at ~ 400 nm for AgNPs and ~ 520 nm for AuNPs. This displacement of conduction electrons from their equilibrium positions around positive ionic core, produces strong electric fields on the AuNP surface, with intensity depending on their composition and assembly [213-215]. Thus the SPR scattering may be tuned by changing the size and shape of metal nanoparticle.

The most obvious example of the SPR for AuNPs is the color change from yellow to scarlet red when bulk gold is converted into AuNPs. This SPR band is used as an indicator of product formation during the synthesis of AuNPs from their precursor salts [216, 217].

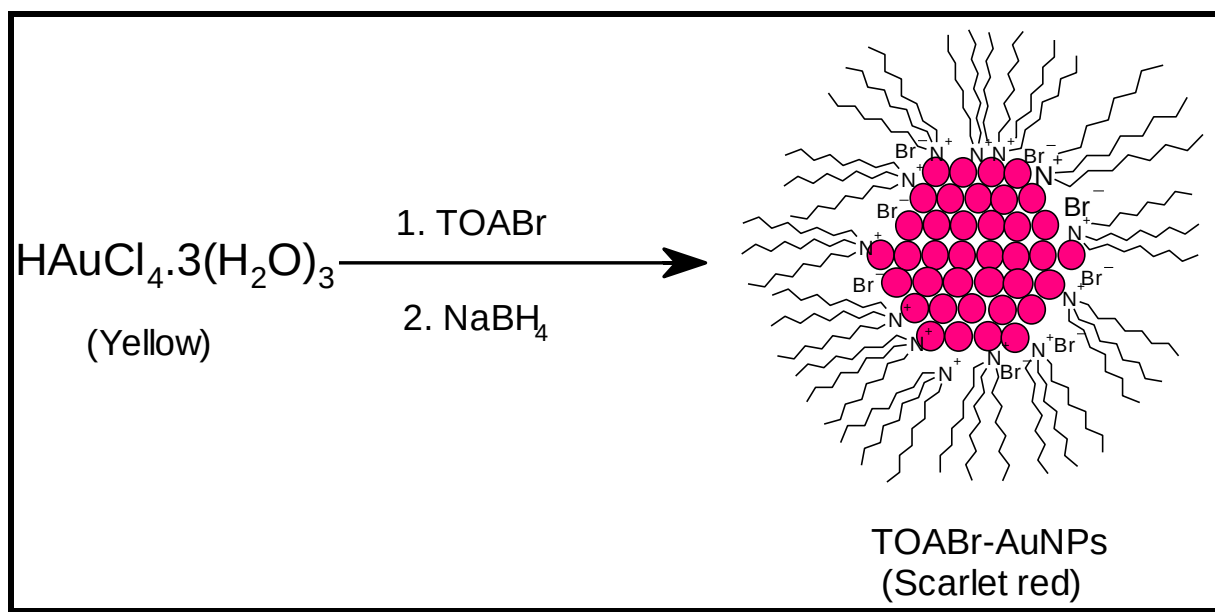
1.3.2 Synthesis and characterization of gold and silver nanoparticles

The synthesis of metal nanoparticles is divided into physical and chemical methods. Physical methods, that include ion implantation and sputtering techniques, are used to obtain metal nanoparticles that are supported on substrates or embedded in solid matrices. The chemical method to yield gold nanoparticles is the most commonly used synthetic procedure [218]. The chemical approach involves the reduction of Ag^+ or Au^+ ions followed by chemisorption or physisorption of ligands on the surface of metal nanoparticles to avoid aggregation and subsequent precipitation. The Turkevitch method is by far the most popular for obtaining aqueous solutions of gold nanoparticles that are easily functionalizable [219]. This method involves the reduction of HAuCl_4 in a boiling aqueous solution of sodium citrate. The citrate molecules act both as reducing and stabilizing agents. The average particle diameter can be tuned in the range of 10 – 100 nm. The same procedure can be used to reduce any Ag salt; however, in this instance particle size control is rather limited [218, 219].

Nanoparticle surface functionalization occurs *via* ligand exchange whereby the citrate is displaced by the desired water soluble molecules. Brust *et al.* developed a

two phase method, which is now the most commonly used procedure for thiol stabilized metal nanoparticles in organic solvents [220]. Essentially, HAuCl_4 or AgNO_3 is dissolved in water and subsequently transported in toluene by tetraoctylammonium bromide (TOABr), which acts as a phase transfer agent. The toluene solution is then mixed and thoroughly stirred together with an aqueous solution of NaBH_4 in the presence of thioalkanes or aminoalkanes. In this case particle size can be tuned in the 1 – 30 nm range [220].

Imaging systems to visualize nanoparticles are required for characterization at the nanoscale. For such purposes, several systems exist: scanning tunneling-(STM), atomic force-(AFM), scanning electron-(SEM), and transmission electron (TEM)-microscopies. High resolution TEM (HRTEM) analysis allows accurate measurement of particle average size and size distribution and is useful for the determination of NP crystalline or defective structure. TEM analysis becomes almost ineffective for particles smaller than about 1 nm in size, depending on the TEM performances. X ray diffraction (XRD) analysis is also essential for accurate size determination of nanostructures. In this work, Ag and Au NPs were synthesized according to the Turkevitch or Brust methods. The AgNPs were capped with citrate and the AuNPs were either capped with TOABr (Scheme 1.5) or citrate



Scheme 1.5: General synthetic procedure of gold nanoparticles.

1.3.3 Conjugation of nanoparticles to phthalocyanines

Gold and silver nanoparticles show photoactivity under UV-visible irradiation as is evident from the surface plasmon resonance band. Binding a photoactive molecule such as a phthalocyanine on the surface of nanoparticles would enhance the photochemical activity of the former [221, 222].

Attempts have been made to stabilize gold and silver nanoparticles by capping them with various molecules such as thiols, carboxylic acid and amines [220, 223, 224]. Macrocycles such as phthalocyanines, porphyrins, dendrimers and polymers have also been used, but to a very limited extent [169, 225–227]. The ability of gold or silver nanoparticles to bind with specific functional groups makes them potentially useful in the development of biological probes [228], photonic materials [142, 229],

chemical sensors [230] and Pcs can be functionalized for binding to AuNPs and AgNPs.

There are only limited reports available in the literature for the use of phthalocyanines as capping agents for metal nanoparticles. Generally, thiol functionalized phthalocyanines have been used as capping agents to prepare uniformly distributed gold nanoparticles and further used as photosensitizers on tumours (Table 1.1) [72, 73, 92, 169]. In one of the studies, the surface bound phthalocyanine showed a 50 % enhancement of the singlet oxygen quantum yield as compared with the free phthalocyanine [92]. Stabilization of silver nanoparticles using phthalocyanines is even more limited in literature [231] with no reports on the use of Pc-AgNPs for the photo-inactivation of microorganisms.

As yet there have been no reports on the use of Pc-NPs conjugates for the photo-inactivation of bacteria hence this work reports on the use of Pc-Au or -AgNPs conjugates for the photo-inactivation of *Staphylococcus aureus*.

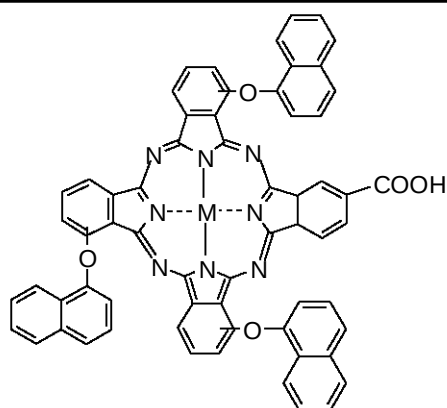
1.4 Summary of Aims

1. Synthesis and characterization of symmetrically and unsymmetrically (mono-carboxy functionalized) substituted phthalocyanines. These macromolecules will be characterized by elemental analysis, mass spectroscopy (MS), nuclear magnetic resonance (NMR) spectroscopy, UV-vis spectroscopy and infrared (IR) spectroscopy.
2. Photophysical properties (fluorescence quantum yield, fluorescence lifetimes, triplet quantum yield, triplet lifetimes and singlet oxygen quantum yields) of the newly synthesised macromolecules will be investigated.
3. Synthesis and characterization of gold and silver nanoparticles.
4. Conjugation of gold and silver nanoparticles to thiol derivatized phthalocyanines.
5. Spectroscopic, photophysical and photochemical properties of nanoparticle-phthalocyanine conjugates and phthalocyanines mixed with folic acid.
6. Investigation of the photosensitizing abilities of phthalocyanines liposome bound or conjugated to gold nanoparticles against breast cancer and non-malignant fibroblast cells.
7. Investigation of the photo-inactivating abilities of nanoparticle-phthalocyanine and poly-L-lysine-phthalocyanine conjugates against *Staphylococcus aureus* and chick chorioallantoic membrane.

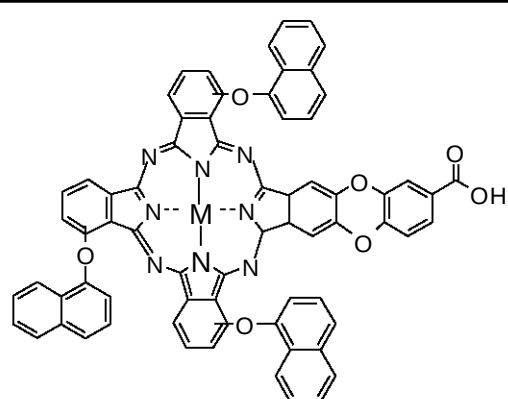
Phthalocyanines under investigation in this thesis are (Figure 1.15):

[8,15,22-Tris-(naphtho)-2-(carboxy)] phthalocyaninato (**32a**), [8,15,22-tris-(naphtho)-2-(carboxy)phthalocyaninato] magnesium(II) (**32b**), [8,15,22-tris-(naphtho)-2-

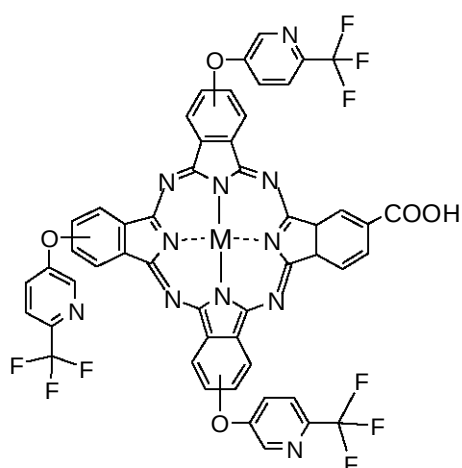
(carboxy) phthalocyaninato]zinc(II)(**33c**), [8,15,22-tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenyl)]phthalocyaninato (**33a**), [8,15,22-tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenoxy) phthalocyaninato]magnesium(II) (**33b**), [8,15,22-tris-(naphtho)-4,5-(3-carboxy-1,2dioxyphenoxy) phthalocyaninato]zinc(II) (**33c**), [9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)]phthalocyaninato (**34a**), [9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato]magnesium(II) (**34b**), [9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato]zinc(II) (**34c**), [9, 16, 23-Tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)]phthalocyaninato (**35a**), [9,16,23-tris-(5-trifluoromethyl-2-mercaptopyridine)-2(carboxy)phthalocyaninato]magnesium (II) (**35b**), [9,16,23-tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato]zinc(II) (**35c**), [8,15,22-tris-(naphtho)-2(amidoethanethiol)phthalocyaninato]zinc(II)(**36**), [2, 9, 17, 23-tetrakis-(1, 6-hexanedithiol)phthalocyaninato]zinc(II) (**37**), and zinc (II) tetrasulfonated phthalocyaninato (**38**).



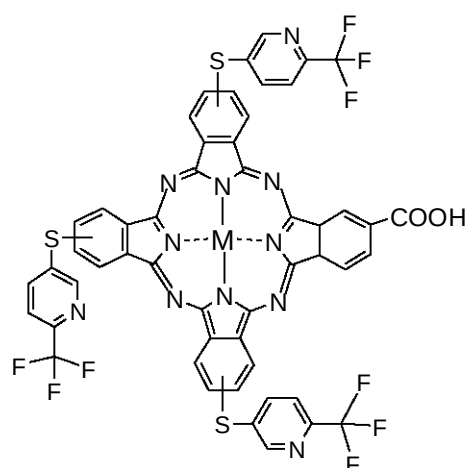
M = H₂ (32a)
= Mg (32b)
= Zn (32c)



M = H₂ (33a)
= Mg (33b)
= Zn (33c)



M = H₂ (34a)
= Mg (34b)
= Zn (34c)



M = H₂ (35a)
= Mg (35b)
= Zn (35c)

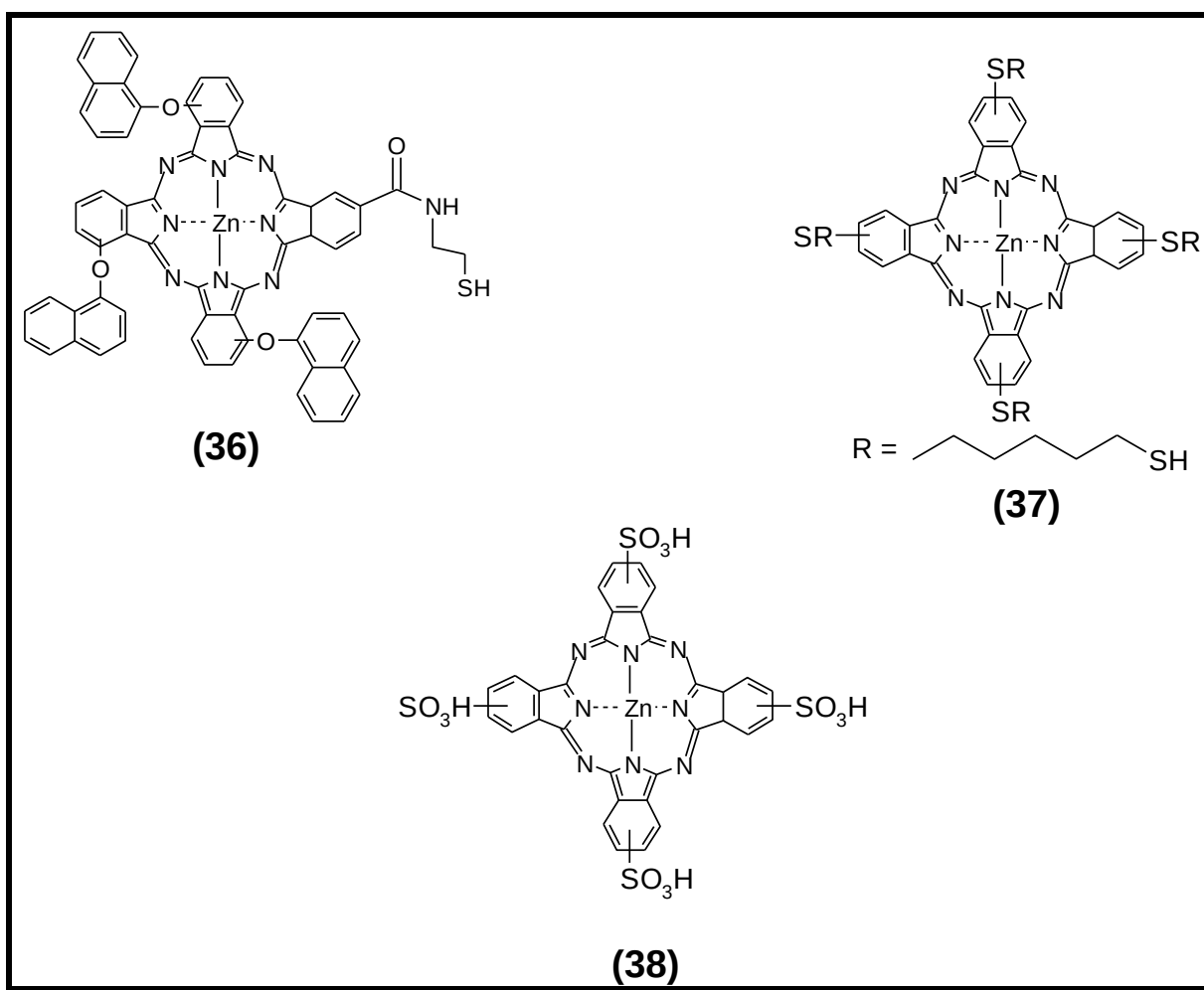


Figure 1.15: Phthalocyanines synthesized in this work.

Chapter 2

Experimental

**This chapter combines all experimental procedures and methods of
characterization for molecules employed in this work.**

2. Experimental

2.1 Solvents

Acetone, chloroform (CHCl_3), dimethylformamide (DMF), dimethylsulfoxide (DMSO), tetrahydrofuran (THF), dichloromethane (DCM), methanol, ethanol, toluene, glacial acetic acid, pentanol, sodium hydroxide, pyridine, acetonitrile, deuterated chloroform (CDCl_3), deuterated dimethylsulfoxide ($\text{DMSO-}d_6$), deuterated tetrahydrofuran ($\text{THF-}d_8$), acetic anhydride (Ac_2O), thionyl chloride, 25 % aq ammonium hydroxide solution, n-hexane, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), formamide, hydrochloric acid, trifluoroacetic acid (TFA), thiourea, Triton-X 100, O-(7-azabenzotriazol-1-yl)-N,N,N,N-tetramethyluroniumhexafluorophosphate (HATU), 4-sulfophthalic acid were purchased from SAARChem or Sigma-Aldrich. All solvent used for high performance liquid chromatography (HPLC) were HPLC grade. Ultra pure water was obtained from a Milli-Q Water System (Millipore Corp, Bedford, MA, USA). The rest of the solvents were obtained from commercial suppliers and used as received.

2.2 Reagents

Folic acid, sodium borohydride, zinc acetate, magnesium chloride, lithium metal, trimellitic anhydride, 1,3-diphenylisobenzofuran (DPBF), egg yolk (highly purified L- α -Lecithin), N,N-diisopropylethylamine (DIPEA), 3-nitrophthalic acid, 2-naphthol, potassium carbonate, sodium carbonate, urea, tetraoctylammonium bromide (TOABr), gold(III) chloride trihydrate, dicyclohexylcarbodiimide (DCC), cysteamine

hydrochloride, magnesium sulphate, dihydroxy carboxylic acid, methanesulfonylchloride, ϵ -poly)lysine HBr (ϵ -PL HBr, 25 KDa), ammonium chloride, urea, ammonium molybdate, thionyl N,N' -dicyclohexylcarbodiimide (DCC), sodium dodecyl sulphate (SDS), silver nitrate, N-succinimidyl (1-methyl-3-pyridinio)formate iodide (NICO-NHS), tri-sodium citrate, Agar bacteriological BBL Mueller Hinton broth and nutrient agar, disodium hydrogen phosphate, potassium dihydrogen phosphate, potassium chloride, were purchased from SAARChem or Merck or Sigma-Aldrich.

Column chromatography was performed on silica gel 60 (0.04 – 0.063 mm) or on Bio-beads S-X1 200-400 mesh purchased from BioRad. Dulbecco's modified eagles medium (DMEM), eagles minimal essential medium (EMEM), 10 % Foetal Bovine Serum (FBS), 1 % Penicillin/Streptomycin, were purchased from Lonza. Trypsin/ethylene diamine was purchased from Invitrogen and Cell Titer Blue (CTB) reagent purchased from Promega. Breast cancer cells (MCF-7; Human malignant breast cancer) and primary skin dermal fibroblast cells were received from the University of Pretoria and University of Cape Town respectively.

Staphylococcus aureus (*S. aureus*) 6538 TM was purchased from Microbiologics

2.3 Equipment

1. Ultraviolet-visible (UV-vis) spectra were recorded on Shimadzu 2001 UV Pc spectrophotometer.
2. FR-IR spectra were recorded on a Perkin-Elmer Universal ATR Sampling accessory spectrum 100 FT-IR spectrometer.
3. ^1H NMR spectra were obtained using a Bruker AMX 400 MHz or a Bruker Advance II+ 600 MHz NMR spectrometer.
4. Elemental analysis was performed using a Vario-Elementar Microcube ELIII.
5. Mass spectra data were collected with a Bruker AutoFLEX III Smart-beam TOF/TOF mass spectrometer. The instrument was operated in positive ion mode using a m/z range of 400–3000 amu. The voltage of the ion sources were set at 19 and 16.7 kV for ion sources 1 and 2, respectively, while the lens was set at 8.50 kV. The reflector 1 and 2 voltages were set at 21 and 9.7 kV, respectively. The spectra were acquired using dithranol as the MALDI matrix, using a 354 nm nitrogen laser.
6. Fluorescence emission and excitation spectra were obtained on a Varian Eclipse spectrofluorimeter.
7. Fluorescence lifetimes were measured using a time correlated single photon counting setup (TCSPC) (FluoTime 200, Picoquant GmbH), Figure 2.1. The fluorescence lifetime of phthalocyanine derivatives and conjugates were determined using a diode laser (LDH-P-670 with PDL 800-B, Picoquant GmbH, 670 nm, 20 MHz repetition rate, 44 ps pulse width). Fluorescence was detected under the magic angle with a Peltier cooled photomultiplier tube (PMT) (PMA-C 192-N-M, Picoquant) and

integrated electronics (PicoHarp 300E, Picoquant GmbH). A monochromator with a spectral width of about 4 nm was used to select the required emission wavelength band. The response function of the system, which was measured with a scattering Ludox solution (DuPont), had a full width at half-maximum (FWHM) of 300 ps. All luminescence decay curves were measured at the maximum of the emission peak and lifetimes were obtained by deconvolution of the decay curves using the FLUOFIT software program (PicoQuant GmbH, Germany). The support plane approach was used to estimate the errors of the decay times.

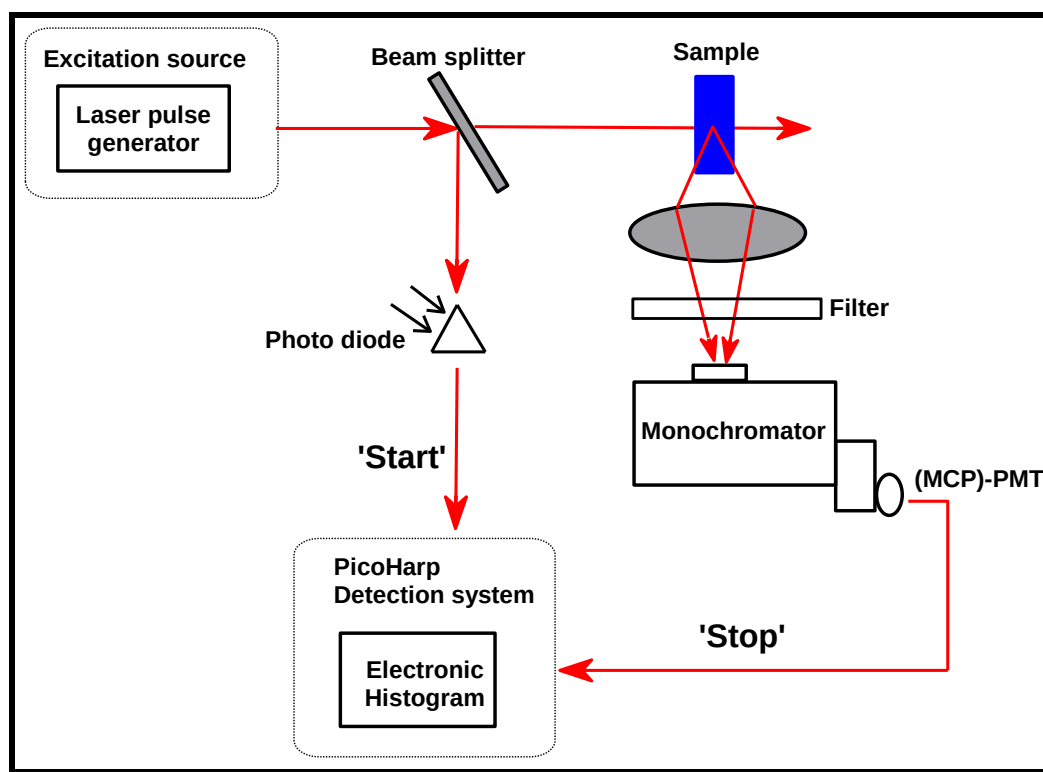


Figure 2.1: Schematic diagram of the TCSPC.

8. A laser flash photolysis system (Figure 2.2) was used for the determination of triplet decay kinetics. The excitation pulses were produced by a Quanta-Ray

Nd:YAG laser (1.5 J/9 ns), pumping a Lambda Physik FL 3002 dye laser (Pyridin 1 in methanol). The analyzing beam source was from a Thermo Oriel 66902 xenon arc lamp, and a Kratos Lis Projekte MLIS-X3 photomultiplier tube was used as the detector. Signals were recorded with a two-channel, 300 MHz digital real time oscilloscope (Tektronix TDS 3032C); the kinetic curves were averaged over 256 laser pulses. Triplet lifetimes were determined by exponential fitting of the kinetic curves using ORIGINPRO 8 software.

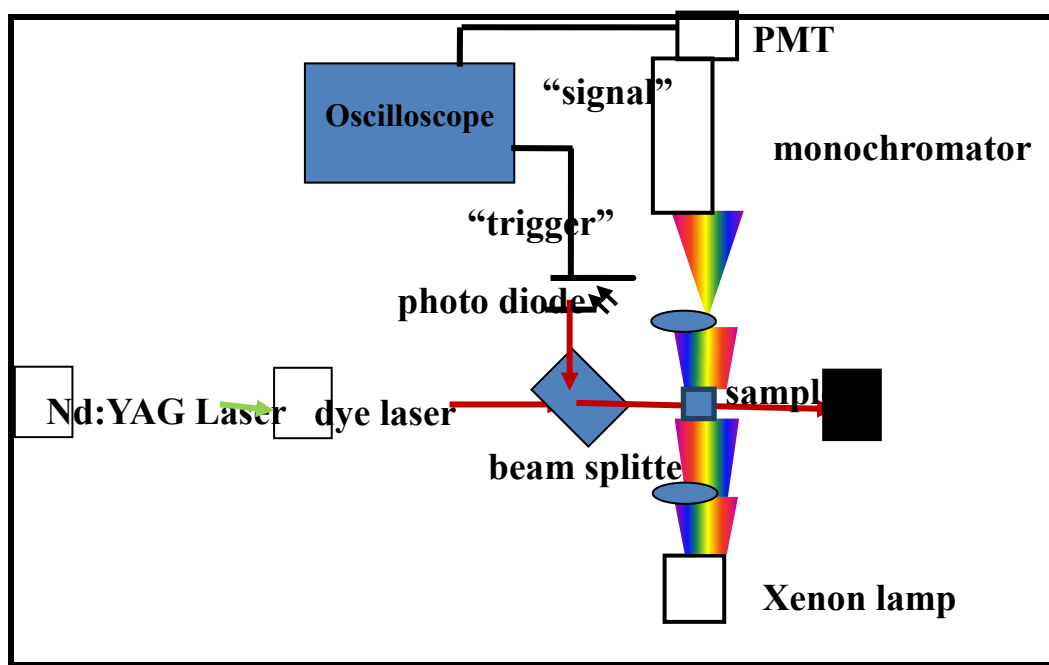


Figure 2.2: Schematic diagram of a laser flash photolysis set-up.

9. 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, Figure 2.3.

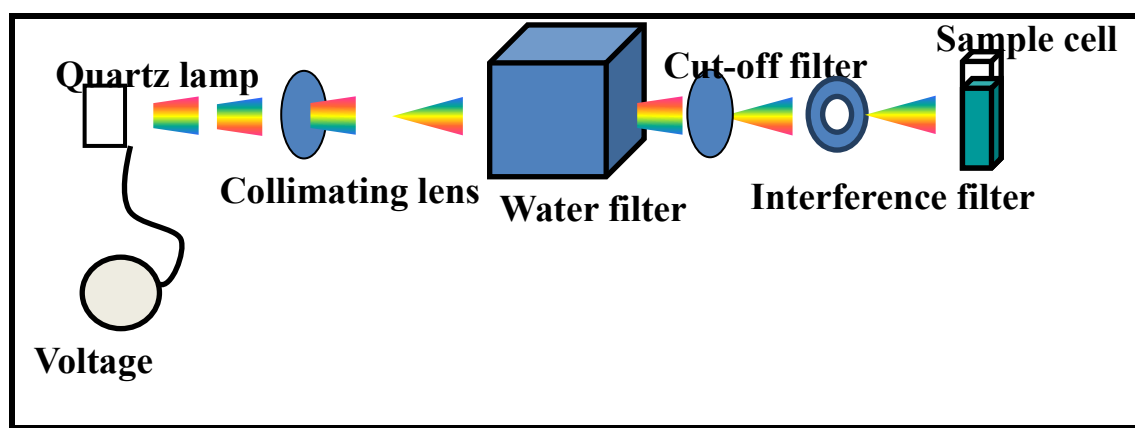


Figure 2.3: Schematic diagram of a photochemical set-up.

10. Light intensities were measured with a POWER MAX5100 (Mol-electron detector incorporated) power meter.

11. High performance liquid chromatography (HPLC) analysis were performed using an Agilent 1200 Series HPLC-pump and a 5- μm Eclipse XDB C₁₈ column all from Agilent Technology. The eluting solvent used was 60 % water and 40 % acetonitrile and the flow rate was kept at 1 ml/min for 10 minutes. The absorption was measured at 550 nm by an Agilent DAD UV-vis detector.

12. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was performed using a BioRAD mini PROTEAN Tetracell with an unstained protein molecular weight marker 120KDa Plus DNA ladder from Thermo Scientific.

13. Plate readings for bacteria work were obtained using the LEDETECT 96 computer controlled microplate reader for *in vitro* diagnostic from LABXIM products and Fluorimetric measurements for cell studies were obtained using Fluostar Optima Labtech.

14. X-ray powder diffraction (XRD) patterns were recorded on a Bruker D8,

Discover equipped with a proportional counter, using Cu-K α radiation ($\lambda = 1.5405 \text{ \AA}$, nickel filter). Data were collected in the range from $2\theta = 5^\circ$ to 60° , scanning at 1° min^{-1} with a filter time-constant of 2.5 s per step and a slit width of 6.0 mm. Samples were placed on a silicon wafer slide. The X-ray diffraction data were treated using the freely-available Eva (evaluation curve fitting) software. Baseline correction was performed on each diffraction pattern by subtracting a spline fitted to the curved background and the full-width at half-maximum values used in this study were obtained from the fitted curves.

15. Atomic force microscopy (AFM) images were recorded in the non-contact mode in air with a CP-11 Scanning Probe Microscope from Veeco Instruments (Carl Zeiss, South Africa) at a scan rate of 1 Hz. Samples for AFM were prepared by spin coating onto glass slides.

16. Transmission electron microscope (TEM) images were obtained using a JEOL JEM 1210 transmission electron microscope at 100 kV accelerating voltage. Samples ($\sim 10 \mu\text{l}$) were dried on a carbon grid for a period of 1h prior viewing under the microscope.

17. CAM fluorescence images were recorded using a CAM auto-fluorescence microscope (BH2-RFC; Olympus, Tokyo, Japan) using a slow-scan 16 bit CCD camera (EEV P86231; Wright, Endfield, UK) equipped with an objective (S.Plano Apo; Olympus; 4x/0.16; working distance, 9.8 mm). Fluorescence excitation was controlled by an excitation shutter (Uniblitz Driver D122; Vincent, Rochester, NY). The egg was placed under the microscope. Before injection, a 5-mm sterile Teflon ring was placed onto the CAM surface at a distance of approximately 10 mm from

the injection site, to demarcate the irradiation site. The embryos were injected with 20 μ L of freshly prepared Pc solution.

2.4 Photophysical and photochemical parameters

2.4.1 Fluorescence quantum yields and lifetimes

The fluorescence spectra of the synthesised Pcs together with the zinc phthalocyanine (ZnPc) standard, were prepared such that the absorbance of each at their respective excitation wavelength, was ~ 0.05 . Due to differences in solubility, the emission spectra of the Pcs and ZnPc standard were measured in different solvents. However, the differences in refractive indices were corrected and the area under the emission curves measured to calculate the fluorescence quantum yields using equation. 1.1. The ZnPc standard was in DMSO ($\Phi_F = 0.20$) [197].

2.4.2 Triplet quantum yields and lifetimes

Triplet quantum yields (Φ_T) and lifetimes (τ_T) were determined by laser flash photolysis set-up (Figure 2.2) described in Section 2.3. Argon saturated solutions of each of the complexes under investigation together with the ZnPc standard was adjusted to an absorbance of ~ 1.5 and introduced into a 1 cm path length spectrophotometric cell. The samples were then irradiated at the wavelength where the Q-bands of the sample and standard intersect.

The triplet quantum yields of the samples were determined using equation 1.3 with ZnPc in DMSO ($\Phi_T = 0.65$ [232] or in DMF $\Phi_T = 0.58$, [233]). In aqueous solution (PBS), tetrasulfonated zinc phthalocyanine (ZnTSPc) was used as a standard ($\Phi_T =$

0.56 [234]). The triplet lifetimes were determined by exponential fitting of the kinetic curves using OriginPro 7.5 or 8.0 software to fit the kinetic curves.

2.4.3 Singlet oxygen quantum yields

The singlet oxygen quantum yield (Φ_{Δ}) determinations for phthalocyanines were recorded with the set-up shown in Figure 2.3. The studies were carried out in air using a 2 mL solution of a phthalocyanine sample mixed with a singlet oxygen chemical quencher (DPBF in organic solvents, or ADMA in aqueous solution). The photochemical reaction was done in a 1 cm path length spectrophotometric quartz cell fitted with a tight-fitting stopper and then photolysed at the Q-band using a 300W General electric quartz lamp, as described in section 2.3. The DPBF absorbance was corrected for the absorbance of sensitizer at the respective detection wavelength. The light intensity reaching the reaction vessel was calculated to be $\sim 4.12 \times 10^{16}$ photons $\text{cm}^{-2} \text{s}^{-1}$. Φ_{Δ} values were determined (using equation 1.4) by monitoring the absorbance decay of ADMA at 380 nm in aqueous media or DPBF at 414 or 415 nm in DMF with time. To avoid chain reactions the concentrations of ADMA or DPBF were kept at $\sim 6 \times 10^{-5} \text{ mol dm}^{-3}$. ZnTSPc was used as the standard in aqueous solutions ($\Phi_{\Delta} = 0.30$ in PBS [237]) and ZnPc was employed as a standard in DMF ($\Phi_{\Delta} = 0.56$ [235]) or DMSO ($\Phi_{\Delta} = 0.67$ [236]).

2.5 Cell Culture

Breast cancer cells (MCF-7) were grown in DMEM supplemented with 10 % Foetal Bovine Serum and 1 % Penicillin/Streptomycin. Primary skin dermal fibroblast cells

were also grown in supplemented DMEM. Fibroblast and MCF-7 cells were grown in T-25 flasks until 80 % confluency in a moisturised atmosphere maintained at 37 °C with 5 % CO₂. For cytotoxic assays the confluent cells were detached by trypsinization using 1× trypsin/ethylene diamine. Each cell line was seeded onto 24-well plates and allowed to settle and attach for 24 h. The cell plating volume was 1 mL per well with a seeding density of 2×10^4 .

2.5.1 Photodynamic treatment of cells

A dark toxicity study was conducted to determine if phthalocyanine complexes in their inactive state (without any laser irradiation) have cytotoxic effects on the fibroblast and breast cancer cells. The MCF-7 and fibroblast cells of 2×10^4 cell density were cultured for 24 hours before being exposed to the photosensitizer. The culture medium was removed from each well, the cells were washed with PBS and treated with Pc solutions (alone or liposome bound or linked to AuNPs). For each set of experiments, cells containing no Pc were used as a control and each concentration was tested in triplicate. The plates were wrapped in aluminium foil and incubated at a humidified temperature of 37 °C in a 5 % CO₂ incubator for the required times. Preliminary experiments were conducted with incubation times of 1 h, 2 h, 4 h, 18 h and 24 h. This was done in order to determine the incubation time required for laser treatment. The 4 h, 18 h and 24 h incubation periods were potentially cytotoxicity to the cells without laser activation, hence incubation times of 1 h and 2 h were employed. Morphological changes in the cell cultures were evaluated.

For phototoxicity studies, the cells were first cultivated for 24 h and then photosensitized with the drugs for 1 or 2 h. The cells were irradiated with a diode laser (676 nm, doses of 4.5, 10, 20, and 25 J.cm⁻²) with an output power of 50 mW. The cell plates were then incubated for a further 24 h.

2.5.2 Cell viability assay

The viability of cells was determined by means of Cell Titer Blue (CTB) assay for fluorescence microscopy. The quantitative changes of cell viability in relation to phthalocyanine concentrations and irradiation doses were proved by fluorimetric measurements. The cells were seeded into 24-well microculture plates at 2×10^4 cells/well and allowed to attach overnight. Following this, the medium was removed and replaced with the respective Pc solutions in PBS. For phototoxicity, the cells were incubated for 1 or 2 h and then irradiated for PDT. After PDT, the cells were incubated for 24 h before cell viability was measured for treated and control cells using Cell Titer Blue viability assay. Control cells included cells treated with 2 % DMSO, cell with no drugs and cells with drugs without laser irradiation. Then after the cells were washed twice in PBS and 40 μ L of Cell Titer Blue in 50 μ L EMEM was added to each well. The cell plates were incubated for 3 h before emission reading. The emission was determined using a fluorometer spectrophotometer at 590 nm. Mitochondrial function was expressed as a percentage of viable treated cells relative to untreated.

2.6 Bactericidal assays

S. aureus was grown aerobically at 37°C in nutrient broth prepared according to manufacturer's specifications. The optical density at 600 nm (OD 600) was adjusted to ~0.8 in nutrient broth in order to give inoculums of $\sim 10^4$ to 10^5 CFU (colony forming unit)/mL. In 96-well micro plates, 5 μ l aliquots of the bacteria broth was added to 100 μ l of liquid broth contained in each well. In the first well, 100 μ l of the Pc drug was added. Then 100 μ l of this stock solution was added to the second well resulting in a two-fold dilution of the Pc concentration. This serial dilution was repeated for the rest of the wells.

Irradiated and non-irradiated samples were prepared by adding suitable volumes of the sensitizer solutions to the cell suspensions. Samples were incubated in the dark for 60 min, then irradiated with white light (39.6 mW/cm²) for 10 min. Irradiated and non-irradiated cells were incubated on a rotatory shaker (~200 rpm) in the dark at 37°C overnight. The microorganisms survival values were corrected with controls which included the OD of bacteria alone (growth control), broth alone (sterility control), broth and Pc, and Pc alone.

2.7 Chick choriallantoic membrane (CAM) assay

Fertilized eggs were placed in an incubator at 37 °C and 65 % relative humidity. On day 4 a hole was drilled through the egg shell at the narrow apex and albumen withdrawn from all eggs. The hole was covered with adhesive tape and the eggs were left in the incubator for a further 12 days without rocking. On day 12 the hole

was enlarged to expose the CAM vasculature. The schematic representation of the process is shown in Figure 2.4. The embryos were placed under the objective of the fluorescence microscope. Before injection a teflon ring was placed on the CAM surface a distance away from the injection site. Twenty μL of the photosensitizer was injected and comparisons of the vascular effects (on the area with the teflon ring) were made before and after injection.

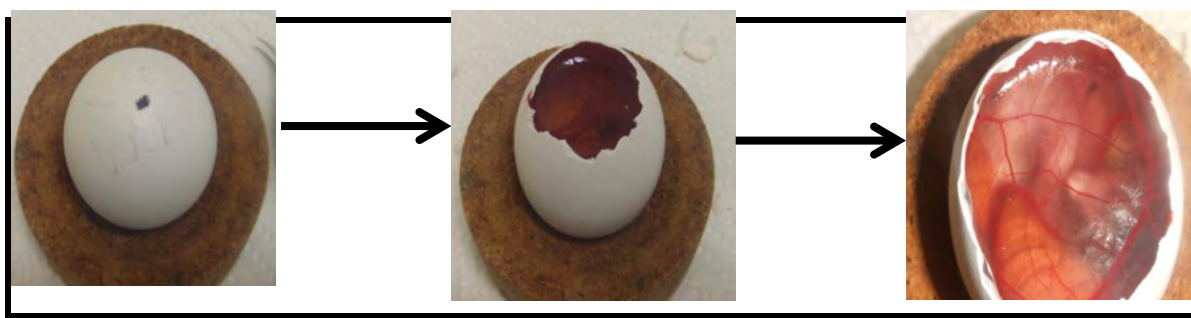


Figure 2.4: Schematic representation showing the process to chick embryo CAM vasculature exposure.

2.8 Synthesis

2.8.1 Synthesis of nanoparticles

2.8.1.1 Tetraoctylammonium bromide stabilised nanoparticles (TOABr-NPs)

The gold nanoparticles were synthesized according to a procedure previously reported in literature [220]. Briefly, a gold solution was prepared by stirring $\text{AuCl}(\text{H}_2\text{O})_3$ (0.019 g, 25 mM) in toluene (2 mL). A solution of TOABr (0.139 g, 85 mM) in 3 mL toluene was added to the gold solution and the mixture was vigorously stirred until the colour of the mixture changed from yellow to a

brownish-yellow colour. Thereafter NaBH_4 (0.002 g, 36 mM) in 2 mL of water was added drop-wise to the gold solution until the solution changed colour from brownish-yellow to milky then scarlet and finally to a purple colour. The mixture was left to stir for a further 30 minutes. The gold nanoparticles were washed repeatedly with water to remove the reducing agent and were stored in a minimum amount of toluene. The absorption coefficient of the TOABr-AuNPs was estimated to be $9.5 \times 10^6 \text{ M}^{-1}\text{cm}^{-1}$ at 527 nm, the concentration was estimated using equations 2.1 and 2.2 [238]:

$$\text{---} \quad (2.1)$$

where C is the concentration of AuNPs, N_{Total} is the amount of Au salt used, N is the number of Au atoms per nanosphere, V is the volume of the reaction solution and N_A is avogadro's number. This equation however, assumes 100 % Au reduction hence C is an estimation. N is calculated using equation

$$\text{---} \quad (2.2)$$

where D is the average core diameter, M is the molar mass of the Au salt and ρ is the density for fcc gold (19.3 g/cm^3) [239, 240].

2.8.1.2 Citrate stabilized silver nanoparticles (citrate-AgNPs)

The synthetic route developed for citrate stabilized silver nanoparticles (~7.5 nm) was a modified version of the procedure described by Solomon *et al* [241]. The synthesis involved the cooling of an aqueous solution of sodium borohydride (2 mM in 30 mL) to ~3 °C, which was then added dropwise to an excess aqueous solution of AgNO₃ (1 mM, 10 mL) and the reaction was left to stir for a further 30 minutes. To the stirring mixture, sodium citrate (85 mM, 2 mL) was added and the stirring was continued for a minimum of 30 min.

2.8.1.3 Citrate stabilized gold nanoparticles (citrate-AuNPs)

Gold nanoparticles (~13 nm) were synthesised according to a well documented method [242]. Briefly, an aqueous solution of gold(III) chloride trihydrate (1 mM, 100 mL) was refluxed for 10 min. An aqueous solution of sodium citrate (39 mM, 10 mL) at 55 °C was added to the refluxing solution and the mixture was left to reflux until the solution reached a scarlet colour. The AuNPs were filtered using a 0.45 µm Millipore syringe filter and the filtrate was stored in the dark at room temperature.

2.8.2 Synthesis of substituted phthalonitriles

The syntheses of **38** and phthalonitriles **41**, **43**, **46**, **47**, **48** and **49** have been reported [243-246].

3-(1-naphthoxy) phthalonitrile (39) (Scheme 3.1)

1-Naphthol (**40**, 2.11g, 14.6 mmol) was dissolved in dry DMSO (13 mL) and 3-nitrophthalonitrile (**41**, 2.5g, 14.4 mmol) was added under inert atmosphere. To this reaction mixture finely ground anhydrous potassium carbonate (4.15g, 30 mmol) was added. After 4 h of stirring at room temperature further potassium carbonate (1.06g, 7.66 mmol) was added and this same amount was added again after 24 h of stirring. After 48 h of stirring, the reaction mixture was poured into water (50 mL) resulting in the formation of brown precipitates. The brown product was centrifuged and recrystallised from methanol.

Yield: 3.7g (67 %). IR [(KBr) ν_{max} /cm⁻¹]: 2228 (CN), 1273 (C-O-C). ¹H NMR (DMSO-*d*₆): δ , ppm 8.21 (1H, d, Ar'-H), 7.95 (1H, t, Ar'-H), 7.85 (1H, d, Ar'-H), 7.75 (1H, t, Ar-H), 7.7-7.55 (4H, m, Ar'-H), 7.35 (1H, d, Ar-H), 7.15 (1H, d, Ar-H).

4,5-(3-carboxy-1,2-dioxyphenyl) phthalonitrile (42) (Scheme 3.1)

A mixture of 4,5-dichlorophthalonitrile (**43**, 6g, 30.5 mmol), 3,4 dihydroxy carboxylic acid (**44**, 4.72g, 30.5 mmol) and dry DMSO (23 mL) was stirred at 90°C, whilst finely ground anhydrous potassium carbonate (2.12g, 15.3 mmol) was added every five minutes until eight portions were added. The reaction was stirred for 60 min and cooled to room temperature. Water (50 mL) was added to the reaction mixture and the pH of this solution was dropped to one, with stirring, using a 10 % HCl solution.

The product was extracted with ethyl acetate and the organic extract was washed with water and dried under MgSO_4 . The product was obtained as a brown solid.

Yield: 4.3g (71 %). IR [(KBr) $\nu_{\text{max}}/\text{cm}^{-1}$]: 3050 (OH), 2235 (CN), 1718 and 1337 (C-O). ^1H NMR ($\text{DMSO}-d_6$): δ , ppm 7.80-7.84 (2H, d, Ar-H), 7.64-7.58 (1H, d, Ar'-H), 7.32 (1H, s, Ar'-H), 7.10-7.14 (1H, d, Ar'-H).

4-(6-Mercaptohexan-1-ol) phthalonitrile (**45**) (Scheme 3.1)

6-Mercaptohexan-1-ol (1.48 mL, 11.22 mmol) and 4-nitrophthalonitrile (**46**) (1.68 g, 9.35 mmol) were dissolved in DMSO (80 mL) under a stream of nitrogen and the mixture stirred at room temperature for 15 min. Thereafter, finely ground K_2CO_3 (2.11 g, 15.31 mmol) was added portion wise over a period of 4 h and the reaction mixture left to stir for a further 24 h at room temperature. The mixture was then added to water (150 mL) and stirred for 30 min. The resulting precipitate was filtered off, thoroughly washed with diethyl ether and acetone, dried and recrystallized from ethanol.

Yield: 90 %. IR [(KBr) $\nu_{\text{max}}/\text{cm}^{-1}$]: 3007 (C-H), 2230 ($\text{C}\equiv\text{N}$), 1668, 1583 ($\text{C}=\text{C}$), 1437, 1408, 1387 (C-H) 884, 666 (C-S-C). ^1H NMR (600 MHz, $\text{DMSO}-d_6$): δ , ppm: 7.65 (d, 1H, Ar-H), 7.57 (d, 1H, Ar-H), 7.50 (dd, 1H, Ar-H), 3.65 (t, 2H, O- CH_2), 3.03 (t, 2H, S- CH_2), 1.75 (m, 2H, CH_2), 1.61-1.43 (m, 6H, CH_2).

2.8.3 [2, 9, 17, 23-Tetrakis-(1, 6-hexanedithiol)phthalocyaninato] zinc (II) (37) (Scheme 3.2)

Complex **37** was prepared according to a procedure previously reported [247]. A mixture of anhydrous zinc (II) acetate (0.51 g, 2.8 mmol), 4-(6-mercaptohexan-1-ol)phthalonitrile (**45**) (0.5 g, 1.6 mmol), DBU (0.55 mL, 4 mmol) and pentanol (15 mL) was refluxed at 160 °C for 5 h. Formed complex **50** (111 mg) was dissolved in dry DCM (20 mL) in the presence of dry TEA (10 eq. 0.92 mmol). Methanesulfonyl chloride (10 eq. 0.92 mmol) was added to the solution with cooling to 10°C. The solution was stirred and allowed to warm to room temperature (10 min). The product in DCM was washed with water, dried using MgSO₄, filtered and DCM removed under reduced pressure. This mesylate salt (**51**) was dissolved in a THF (10 mL) / EtOH (3 mL) mixture and degassed with sonication for 30 min. The solution was brought to reflux under N₂ in the dark. Thiourea (30 mg, excess) was added and the reflux continued for a further 8h. N₂ gas was flushed through the reaction mixture, degassed aqueous NaOH (20 %, 6 mL, 30 min sonication) was added and the reflux continued for a further 2 h. This mixture was subsequently poured into a dilute HCl/ice mixture, extracted with DCM (2 x 40 mL) and dried over MgSO₄, filtered and the solvent evaporated. The final phthalocyanine (**37**) was purified twice with BioBeads using DCM as the eluent.

Yield: 12 %. UV/vis (DMSO): λ_{max} nm (log ε); 706 (4.64), 638 (4.08), 364 (4.33). IR (KBr): ν_{max}/cm⁻¹; 2997 (C-H), 1436, 1406 (C=C), 1307 (C-H), 667 (C-S-C). ¹H NMR (600 MHz, DMSO-d₆) δ ppm: 7.70-7.69 (dd, 4H, Ar-H), 7.53-7.51 (dd, 4H, Ar-H), 6.97 (s, 4H, Ar-H), 4.22 (m, 8H, CH₂-S-Ar), 1.67 (m, 8H, CH₂-S), 1.43-1.24 (m, 32H, CH₂).

SH protons not observed. Calc. for $C_{56}H_{64}N_8S_8Zn$: C; 57.38, H; 5.59, N; 9.56, Found: C; 57.35, H; 5.44, N; 9.65. MALDI-TOF-MS m/z : Calc: 1171.08; Found $[M]^+$: 1170.56.

2.8.4 Synthesis of unsymmetrical phthalocyanines (Schemes 3.3, 3.4 and 3.5)

2.8.4.1 Synthesis of unmetallated phthalocyanines

8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyanine (32a) (Scheme 3.3)

In refluxing dry pentanol (25 mL), one equivalent of 4-carboxyphthalonitrile (**47**) and three equivalents of 3-(1-naphthoxy) phthalonitrile (**39**) were stirred for 10 min. To the reaction mixture, 100 mg of lithium was added and refluxing continued for 2 h. The solution was left to cool to room temperature, and then glacial acetic acid (40 mL) was added to the mixture. The resulting precipitates were centrifuged and washed with water. The product was purified by repeated re-precipitation from THF into methanol followed by column chromatography on silica gel as substrate using THF:NH₄OH:H₂O in a 1:1:1 ratio as the eluting solvent mixture.

Yield: <5 %, UV-Vis [(THF/ λ_{max} /nm, (log ϵ))] 714 (4.96), 684 (4.93), 653 (4.46), 621 (4.33), 351 (4.59). IR [(KBr) ν_{max} /cm⁻¹]: 2958-2869 (carboxylic acid OH), 1157 (C-O), 1096 (N-H). ¹H NMR (THF-*d*₈): δ , ppm 8.80 (2H, s, Pc-H), 7.22 (3H, broad s, Pc-H), (1H, s, OH) not observed, 6.32-5.10 (28 H, m, Ar-H, Pc-H), -1.02 (2H, s, H₂Pc). Calc. for: $C_{70}H_{66}N_8O_5$: C; 76.48, H; 6.05, N; 10.19 %; Found C; 75.14, H; 5.91, N; 9.23 %. MALDI-TOF-MS m/z Calc: 984.28; Found $[M]^-$: 985.0.

8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenyl)phthalocyanine (33a)**(Scheme 3.3)**

Synthesis and purification was as described for **32a** using 4,5-(3-carboxy-1,2-dioxyphenyl) phthalonitrile (**42**) instead of **47**. The amounts used for the synthesis of **33a** were the same as those used for **32a**.

Yield: <5 %, UV-Vis [(THF/ $\lambda_{\max}$ /nm, (log ϵ))] 710 (4.51), 680 (4.47), 646 (4.05), 617 (3.93), 354 (4.29). IR [(KBr) $\nu_{\max}$ /cm⁻¹]: 3053-2853 (carboxylic acid OH), 1246 (C-O), 1082 (N-H). ¹H NMR (THF-*d*₈): δ , ppm 9.00 (2H, s, Pc-H), (1H, s, OH) not observed, 7.15 (3H, m, Ar-H), 6.40 (4H, m, Pc-H), 5.93 (5H, d, Pc-H), 5.80-5.25 (21H, m, Ar-H), -2.00 (2H, s, H₂Pc). C₇₆H₆₈N₈O₅: C; 75.72, H; 5.69, N; 9.30 %; Found C; 75.27, H; 5.62, N; 10.08 %. MALDI-TOF-MS *m/z* Calc: 1090.29; Found [M⁻]: 1090.9.

9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato (34a) (Scheme 3.5)

In 10 mL of dry pentanol, one equivalent of **47** (0.2 g, 1.35 mmol) and three equivalents of **48** (1.07 g, 4.05 mmol) were stirred under reflux in an argon atmosphere at 140 °C for 10 minutes. To the reaction mixture, 100 mg of lithium was added and the reaction mixture was refluxed for a further 2 hours. Thereafter the reaction mixture was left to cool to room temperature. Glacial acetic acid (16 mL) was added to the mixture generating the desired product. The resulting precipitates were centrifuged and washed with water. The product was purified using column chromatography on silica gel and DCM:MeOH (5:0.8) as the eluent. The product was further purified on silica gel using THF as the eluting solvent.

Yield: 13 %, UV-Vis [(DMSO/: $\lambda_{\max}$ /nm, (log ϵ))] 676 (4.30), 612 (3.87), 366 (4.02). IR [(KBr) $\nu_{\max}$ /cm⁻¹]: 2890 (Ar-CH), 1542 (C=C), 1331, 1294 (C-F), 1061 (C-O-C). ¹H NMR (CDCl₃): δ , ppm -0.12 (2H, s, Pc-H), 8.25-7.50 (21H, m, Pc-H, Ar-H, 1H, s, OH). C₅₁H₂₄N₁₁O₅F₉: Calc. C; 58.80, H; 2.31, N; 14.79, Found C; 59.06, H; 3.24, N; 13.92 %. MALDI-TOF-MS m/z : Calc. 1041.80, Found [M]⁺ 1042.89.

9, 16, 23-Tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyanine (35a) (Scheme 3.5)

Using a mortar and pestle, 0.109g (3 equivalent) of 4-[5-(trifluoromethyl)-2-mercaptopyridine]-phthalonitrile (**49**) and 0.0204g (1 equivalent) of **47** were mixed and ground. The powder was refluxed in dry pentanol (5mL) under argon for 2h with dissolved lithium (0.8 mmol). Thereafter the reaction mixture was left to cool to room temperature and glacial acetic acid (20 mL) was added and precipitates of crude complex **35a** were centrifuged and washed with water. The product was washed repetitively in hot methanol. Column chromatography was carried out using a CHCl₃:DMF (9:1) mixture as the eluent.

Yield: ~13 %. UV-vis (CHCl₃): $\lambda_{\max}$ nm (log ϵ): 699 (4.34), 664 (4.31), 635 (4.03), 603 (3.86), 340 (4.27). IR $\nu_{\max}$ /cm⁻¹: 1595 (C-O coupled to O-H), 1323 (Aryl NH), 742 (C-S). ¹H NMR (CDCl₃): δ , ppm 8.21-6.35 (21H, m, Ar-H), (1H, s, OH) not observed. Calc. for: C₅₁H₂₄N₁₁S₃O₂F₉: C; 60.80, H; 4.51, N; 9.45, S; 8.12 %; Found C; 60.40, H; 3.96, N; 9.42, S; 8.60 %. MALDI-TOF-MS m/z Calc: 1089.98; Found [M]⁻: 1090.20

2.8.4.2 Synthesis of magnesium phthalocyanines (MgPc)

[8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyaninato]magnesium(II) (32b)

(Scheme 3.3)

In 5 mL of dry THF:DMF mixture (1:9), **32a** (0.3 mg, 0.29 mmol) was refluxed for 1 hour in the presence of dry magnesium chloride in excess. The mixture was cooled to room temperature and the product was purified on silica gel using a DMF:diethyl ether (1:9) eluting mixture. Yield: <10 %, UV-Vis [(THF: λ_{max} /nm, (log ϵ))] 694 (4.95), 636 (3.54), 360 (3.55). IR [(KBr) ν_{max} /cm⁻¹]: 1547 (C=C), 1333, 1297 (C-F), 1060 (C-O-C). ¹H NMR (CDCl₃): δ , ppm 8.00-7.30 (33H, m, Pc-H, Ar-H), The OH proton was not observed. Calc. for: C₆₃H₃₄N₈O₅: C; 75.12, H; 3.40, N; 11.12, Found C; 74.14, H; 2.91, N; 10.82 %. MALDI-TOF-MS m/z : Calc. 1007.3, Found [M⁺] 1009.1.

[8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenoxy)phthalocyaninato]magnesium (II) (33b) (Scheme 3.3)

Synthesis and purification was as described for **32b** using **33a** (0.3 mg, 0.28 mmol) instead of **32a**. Yield: <10 %, UV-Vis [(THF: λ_{max} /nm, (log ϵ))] 740 (5.12), 711 (4.83), 666 (3.87), 650 (3.66). IR [(KBr) ν_{max} /cm⁻¹]: 1541 (C=C), 1335, 1294 (C-F), 1061 (C-O-C). ¹H NMR (CDCl₃): δ , ppm 7.60-7.20 (35H, m, Pc-H, Ar-H). The OH proton was not observed. Calc. for: C₆₈H₃₆N₈O₅: C; 76.38, H; 3.39, N; 10.48, Found C; 76.14, H; 2.91, N; 9.82 %. MALDI-TOF-MS m/z : Calc. 1069.3, Found [M⁺] 1071.2.

[9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato]magnesium(II)**(34b) (Scheme 3.5)**

In a round bottom flask, **34a** was refluxed pentanol in the presence of excess dry magnesium (II) chloride and six drops of DBU in for 2 hr. The product was dissolved in a acetone:hexane (1:0.9) mixture and column chromatography was performed using this solvent mixture as the eluent. The product was washed with water followed by acetone. Yield: 6 %, UV-Vis [(DMSO/: λ_{max} /nm, (log ϵ))] 677 (4.66), 612 (3.92), 366 (4.26). IR [(KBr) ν_{max} /cm⁻¹]: 2929 (Ar-CH), 1545 (C=C), 1330, 1292 (C-F), 1061 (C-O-C). ¹H NMR (CDCl₃): δ , ppm 8.25-6.55 (21H, m, Ar-H, 1H, s, OH). Calc. for: C₅₁H₂₂N₁₁O₅F₉.2H₂O: C; 55.58, H; 2.56, N; 13.98; Found C; 55.88, H; 2.64, N; 14.07 %. MALDI-TOF-MS m/z : Calc. 1064.09 Found [M^+] 1066.10.

[9,16,23-tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato]magnesium (II) (35b) (Scheme 3.5)

Complex **35b** was synthesized by refluxing compound **35a** (0.2 mg, 0.2 μ mol) in dry DMF (5 mL) in the presence of excess magnesium chloride. After an hour, the reaction was cooled to room temperature and the green product was precipitated out of solution and thoroughly washed with hot methanol followed by acetone. The product was further purified on silica gel using DMF:THF (1:9) as the eluent.

Yield <10 %, UV-Vis [(DMSO: λ_{max} /nm, (log ϵ))] 684 (5.01), 616 (3.49), 358 (3.87). IR [(KBr) ν_{max} /cm⁻¹]: 1547 (C=C), 1333, 1297 (C-F), 695 (C-S-C). ¹H NMR (DMSO-*d*₆): δ , ppm 7.90-7.20 (21H, m, Pc-H, Ar-H), (1H, s, OH) not observed. Calc. for:

$C_{51}H_{24}N_{11}O_3F_9Mg \cdot H_2O$, Calc. C; 54.19, H; 2.14, N; 13.63, Found C; 55.79, H; 1.91, N; 12.82 %. MALDI-TOF-MS m/z : Calc. 1112.3, Found $[M^+]$ 1105.3.

2.8.4.3 Synthesis of zinc phthalocyanines (ZnPc)

[8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyanato]zinc(II) (32c) (Scheme 3.3)

The unmetallated phthalocyanine (**32a**) was refluxed for 1h, in the presence of excess zinc (II) acetate in pentanol (20 mL). Methanol (30 mL) was added to the product to precipitate out the dark green crude product. For purification, the procedure for **32a** was followed.

Yield: <5 %, UV-Vis [(THF/ λ_{max} /nm, (log ϵ))] 680 (4.15), 651 (3.40), 614 (3.44), 357 (3.95). IR [(KBr) ν_{max} /cm⁻¹]: 2955-2854 (carboxylic acid OH), 1244 (C-O). ¹H NMR (THF- d_8): δ , ppm 10.82 (2H, s, Pc-H), 9.10 (3H, broad s, Pc-H), 8.22-6.91 (28 H, m, Ar-H, Pc-H), (1H, s, OH) not observed. Calc. for: $C_{70}H_{64}N_8O_5Zn$: C; 72.19, H; 5.71, N; 9.62 %; found C; 71.44, H; 5.87, N; 8.07 %. MALDI-TOF-MS m/z Calc: 1046.19; Found (M^+): 1045.7.

[8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenoxy)phthalocyanato]zinc(II)**(33c) (Scheme 3.3)**

Synthesis and purification of compound **33c** was carried out in the same manner as compound **32c** except **33a** was used instead of **32a**. The amounts used for the synthesis of **33c** were the same as those used for **32c**.

Yield: <5 %, UV-Vis [(THF/ λ_{max} / nm, (log ϵ))] 685 (3.98), 654 (3.21), 355 (3.60). IR [(KBr) ν_{max} /cm⁻¹]: 3052-2849 (carboxylic acid OH), 1237 (C-O). ¹H NMR (THF-d₈): δ , 9.00 (1H, br s, OH), 8.25 (3H, br d, Pc-H), 7.78 (6H, m, Pc-H), 7.60 – 7.19 (21H, m, Ar-H), 7.15 (2H, d, Pc-H), (1H, s, OH) not observed, 6.80 (3H, d, Ar-H). Calc. for: C₇₆H₆₆N₈O₅Zn: C; 70.83, H; 5.39, N; 10.82; Found C; 69.94, H; 6.12, N; 10.14 %. MALDI-TOF-MS m/z Calc: 1152.20; Found [M]: 1153.2.

[9,16,23-tris-(5-trifluoromethyl-2-pyridyloxy)-2-(carboxy)phthalocyaninato] zinc (II) (34c) (Scheme 3.5)

The synthesis of compound **34c** was carried out by refluxing compound **34a** in the presence of excess zinc (II) acetate and six drops of DBU in pentanol for 2 hours. The resulting product was dissolved in acetone:hexane (1:0.9) mixture and column chromatography was performed twice using this solvent mixture as the eluent. The product was repeatedly washed with water followed by acetone. The desired product was isolated and further purified on silica gel using THF as the eluent. The complex was then dried under vacuum.

Yield: 8 %. UV-Vis [(DMSO/: $\lambda_{\max}$ /nm, (log ϵ))] 678 (6.60), 613 (6.30), 368 (4.89). IR [(KBr) $\nu_{\max}$ /cm⁻¹]: 2920 (Ar-CH), 1545 (C=C), 1330, 1293 (C-F), 1061 (C-O-C). ¹H NMR (CDCl₃): δ , ppm 8.25-7.50 (21H, m, Ar-H, 1H, s, OH). Calc. for: C₅₁H₂₂N₁₁O₅F₉: Calc. C; 55.42; H; 2.18, N; 13.94, Found C; 56.38, H; 1.98, N; 13.87 %. MALDI-TOF-MS m/z : Calc. 1105.17 Found [M + 2H]²⁺ 1103.13.

[9,16,23-tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato] zinc (II) (35c) (Scheme 3.5)

Synthesis and purification of compound **35c** was carried out in the same manner as compound **35b** except MgCl₂ was used instead of zinc acetate. The amounts used for the synthesis of **35c** were the same as those used for **35b**.

Yield <10 %, UV-Vis [(DMSO: $\lambda_{\max}$ /nm, (log ϵ))] 682 (4.81), 616 (3.14), 355 (3.50). IR [(KBr) $\nu_{\max}$ /cm⁻¹]: 1540 (C=C), 1332, 1298 (C-F), 720 (C-S-C). ¹H NMR (DMSO-*d*₆): δ , ppm 7.80-7.30 (21H, m, Pc-H, Ar-H), (1H, s, OH) not observed. Calc. for: C₅₁H₂₄N₁₁O₃F₉Zn.H₂O, Calc. C; 52.29, H; 2.07, N; 13.15, Found C; 53.84, H; 2.31, N; 12.21 %.MALDI-TOF-MS m/z : Calc. 1153.4, Found [M⁺] 1151.1.

[8,15,22-Tris-(naphtho)-2-(amidoethanethiol)phthalocyanato] zinc(II)(36) (Scheme 3.4)

Under an Ar atmosphere, complex **32c** (2 mg, 2 μ mol) and DCC (0.4 mg, 2 μ mol) were dissolved in a mixture of dry DMSO (2 mL) and pyridine (0.8 mL). After 10 min of stirring at 0°C, cysteamine was added to the mixture and the reaction was left

to stir at room temperature for 48 h. The product was purified using column chromatography on BioBeads. THF was used as an eluent. Yield: ~10 %. UV-vis (THF): $\lambda_{\max}$ nm (log ϵ): 733 (4.57), 687 (4.31), 655 (4.03), 615 (3.86), 353 (4.27). IR $\nu_{\max}/\text{cm}^{-1}$: 3000 (amide N-H), 2562 (S-H), 1693 (C=O coupled to N-H), 1323 (Aryl NH). ^1H NMR (600 MHz, CDCl_3) δ ppm: 8.20-6.72 (m, 34H, Ar-H, N-H), 1.50-0.82 (m, 4H, methylene). Calc. for: $\text{C}_{65}\text{H}_{39}\text{N}_9\text{SO}_4\text{Zn}$: C; 70.49, H; 3.55, N; 11.36, S; 2.89, Found C; 69.97, H; 3.81, N; 11.77, S; 2.66 %. MALDI-TOF-MS m/z Calc: 1107.51; found (M-H): 1108.24.

2.8.5 Synthesis of phthalocyanine conjugates

2.8.5.1 Self assembly of phthalocyanines onto nanoparticles

In 1 mL THF or chloroform, phthalocyanine complexes (1 μM) and TOABr-AuNP (0.5 μL in toluene) were stirred at room temperature for 48 h under Ar atmosphere and protection from light. Purification of the Pc-AuNP conjugates was performed using column chromatography on Bio-Beads with THF or chloroform as the eluting solvent. For cell studies 2 mL of the Pc-AuNP conjugate was added to 98 mL of PBS and the mixture was vigorously stirred. The final concentration of the complex was 100 $\mu\text{g}/\text{mL}$.

2.8.5.2 Preparation of tetrasulfonylchloride ZnPc conjugated to ϵ -PL (52- ϵ -PL)

The synthesis of zinc tetrasulfonylchloride, $\text{ZnPc}(\text{SO}_2\text{Cl})_4$ has been reported [248, 249]. The preparation of the conjugate (52- ϵ -PL) was carried out according to

literature methods [250, 251] by dissolving 100 mg (0.15 mmol) of $\text{ZnPc}(\text{SO}_2\text{Cl})_4$ and 50 mg of ϵ -PL HBr in DMF (1 mL). The mixture was stirred overnight and thin layer chromatography (TLC) was used to confirm the formation of the new product. The reaction occurred without the use of coupling agents and at room temperature. HPLC and SDS-PAGE, were employed to characterize the new product.

Yield: < 48 %, UV-vis (H_2O): 658, 607 nm. MW (SDS-PAGE): ~25.79 KDa

2.8.5.3 Conjugation of 4-tetrakis-(5-trifluoromethyl-2-pyridyloxy) phthalocyaninato zinc(II) to ϵ -PL (**34c- ϵ -PL**) [252, 253].

The activation of 4-tetrakis-(5-trifluoromethyl-2-pyridyloxy) phthalocyaninato zinc(II) (**34c**) was achieved by mixing **34c** (0.3 mg) in a DMF/pyridine solvent mixture (0.6:0.4 mL) in the presence of 0.169 mg DCC (used as a coupling agent). TLC was used to confirm the formation of a new product by the appearance of a new band. To this mixture, ϵ -PL HBr (25 KDa) (1.2 mg, 8.21×10^{-6} mol of ϵ - NH_2 functions) together with DIPEA (3 equiv per ϵ - NH_2 , 3.2 mg) were added under argon while stirring. DIPEA is used as a base. This mixture was left to stir overnight under argon to give **34c-PL**. In order to quench the remaining free amino groups on the polymer chain as well as increase the water solubility of the product, NICO-NHS (1.1 equiv based on free ϵ - NH_2 functions, 3.3 mg) was added to the reaction and the mixture was left to stir for one hour. The mixture was thereafter quenched by adding water/acetonitrile (70/30) mixture (1 mL) and TFA to pH 3. The product was purified on a C-18 column and dried under vacuum to yield a light green solid.

UV-vis (H₂O): 604, 658 nm. MW (SDS-PAGE): ~ 25.79 KDa

2.8.5.4 Conjugation of Pc- ϵ -PL with AuNP or AgNPs

The conjugation of **34c- ϵ -PL** and **52- ϵ -PL** to nanoparticles was achieved by stirring a 3 mL aqueous solution of the polymer conjugates with an aqueous solution of citrate stabilized gold (AuNP) or silver nanoparticles (AgNP) (1 mL). The reactions were left to stir for 15 minutes, following which the formed conjugates were purified using BioBeads.

2.8.6 Preparation of liposome bound phthalocyanine

The liposomes were prepared according to a previously reported procedure [254]. Briefly, egg-yolk lecithin (1.75 mg) was dissolved in ethanol (275 μ L) and 10 μ L of this solution was added to a DMSO/PBS solution of the relevant Pc while stirring. The final concentration of the Pc was 100 μ g/mL.

2.8.7 Preparation of phthalocyanines for cell studies

A 100 μ g/mL stock solution was prepared by dissolving the Pc in dimethylsulfoxide (DMSO) where the final concentration of DMSO was less than 2 % (v/v) in phosphate buffer saline (PBS). 2 % DMSO was used to aid in phthalocyanine solubility while PBS, which is often used to wash cultured cells as it provides the right environment for the cells to grow, was used to make up the phthalocyanine solution and thus reduce the amount and toxic effect of DMSO. The stock solution was subsequently diluted in increments to obtain desired concentrations of the Pc (100 μ g/mL–10 μ g/mL).

Results and Discussion

This section includes the following chapters

Chapter 3: Synthesis and characterization

Chapter 4: Photophysical and photochemical properties

Chapter 5: Phototherapeutic properties

Publications

The results discussed in the following chapters are based on work contained in the following publications which have been published in or submitted to peer-reviewed journals. The articles are not referenced in the chapters.

1. **Synthesis and fluorescence behaviour of phthalocyanines unsymmetrically substituted with naphthol and carboxy groups.**

Nolwazi Nombona, Edith Antunes, Tebello Nyokong, Dyes and Pigments 86 (2010) 68.

2. **Photophysical behaviour of asymmetrically substituted metal free, Mg and Zn phthalocyanines in the presence of folic acid.**

Nolwazi Nombona, Wadzanai Chidawanyika, Tebello Nyokong, Polyhedron 30 (2011) 654.

3. **Synthesis and photophysical studies of phthalocyanine-gold nanoparticle conjugates.**

Nolwazi Nombona, Edith Antunes, Christian Litwinski, Tebello Nyokong, Dalton Trans. 40 (2011) 11876.

4. **Synthesis of phthalocyanine conjugates with gold nanoparticles and liposomes for photodynamic therapy.**

Nolwazi Nombona, Kaminee Maduray, Edith Antunes, Aletta Karsten, Tebello Nyokong, J. Photochem. Photobiol. B: Bio. 107 (2012) 35.

5. **Synthesis, photophysics and photochemistry of phthalocyanine- ϵ -polylysine conjugates in the presence of metal nanoparticles against *Staphylococcus aureus*.**

Nolwazi Nombona, Edith Antunes, Wadzanai Chidawanyika, Phumelele Kleyi, Zenixole Tshentu, Tebello Nyokong, J. Photochem. Photobiol. A: Chem., *In Press*.

6. **Spectroscopic and physicochemical behaviour of magnesium phthalocyanine derivatives mono-substituted with a carboxylic acid group.**

Nolwazi Nombona, Wadzanai Chidawanyika, Tebello Nyokong, J. Mol. Struct. *In Press*.

7. **Photophysical and photochemical behaviour of sulfur containing phthalocyanine derivatives in the presence of folic acid.**

Nolwazi Nombona, Tebello Nyokong, Inorganica Chimica Acta, Submitted.

Chapter 3

Synthesis and characterization

The synthesis, characterization and photophysicochemical properties of MPs employed in this work are presented. The preparation and photophysicochemical studies of phthalocyanine functionalized nanoparticles are discussed. The interaction of synthesized phthalocyanines with liposomes, poly-L-lysine, folic acid or nanoparticles is conveyed.

3. Synthesis and characterization

3.1 Phthalocyanines

3.1.1 Phthalocyanine precursors

Table 3.1 shows a summary of symmetrically and unsymmetrically tetra substituted phthalocyanines synthesized in this work.

Table 3.1: Phthalocyanines synthesized in this work.

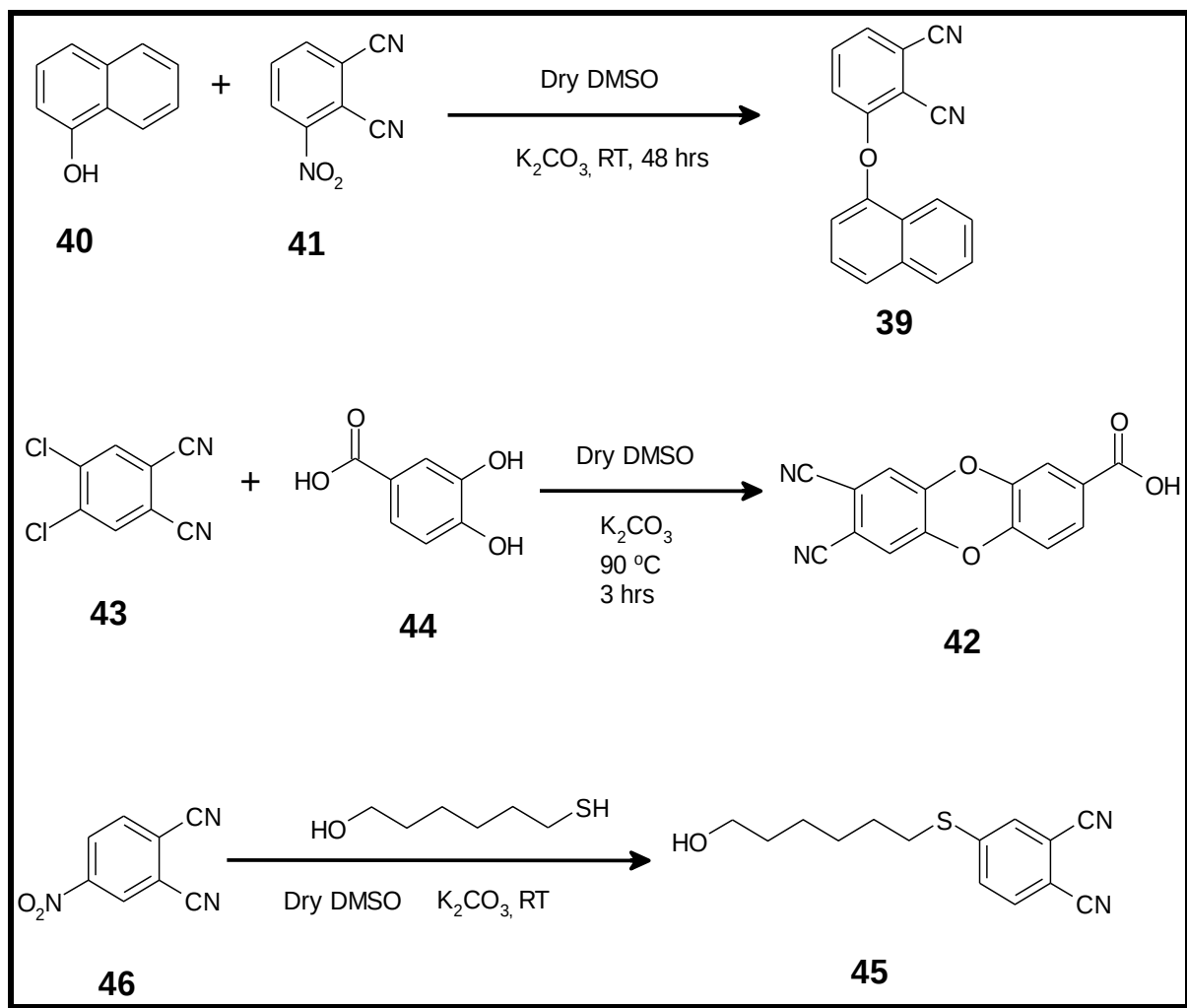
Phthalocyanine	No.
8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyanine	32a
[8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyaninato]magnesium(II)	32b
[8,15,22-Tris-(naphtho)-2-(carboxy)phthalocyaninato]zinc(II)	32c
8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenyl)phthalocyanine	33a
[8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenoxy)phthalocyaninato]magnesium (II)	33b
[8,15,22-Tris-(naphtho)-4,5-(3-carboxy-1,2-dioxyphenoxy)phthalocyaninato]zinc(II)	33c
9,16,23-Tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyanine	34a

Table 3.1 continued

Phthalocyanine	No.
[9,16,23-Tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato]magnesium(II)	34b
[9,16,23-Tris-(5-trifluoromethyl-2-pyridyloxy)phthalocyaninato]zinc(II)	34c
9,16,23-Tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyanine	35a
[9,16,23-Tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato]magnesium (II)	35b
[9,16,23-Tris-(5-trifluoromethyl-2-mercaptopyridine)-2-(carboxy)phthalocyaninato] zinc (II)	35c
[8,15,22-Tris-(naphtho)-2-(amidoethanethiol)phthalocyanato] zinc(II)	36
[2, 9, 17, 23-Tetrakis-(1,6-hexanedithiol)phthalocyaninato] zinc (II)	37
Tetrasulfonated zinc phthalocyanine	38

Scheme 3.1 shows the synthetic procedures used to obtain 3-(1-naphthoxy) phthalonitrile (**39**), 4,5-(3-carboxy-1,2-dioxyphenyl) phthalonitrile (**42**) and 4-(6-mercaptohexan-1-ol) phthalonitrile (**45**). The electron-withdrawing capability of the dinitrile functionalities makes **41**, **43** and **46** susceptible to nucleophilic attack. The base catalyzed nucleophilic substitution of the nitro groups (**41**, **46**) was performed in dry DMSO at room temperature under an inert nitrogen atmosphere. The substitution reaction for compound **42** was performed at 90°C for 3 h under argon atmosphere. Good yields were obtained for all reactions. As an example, the ^1H NMR spectrum of compound **39** is shown in Figure 3.1 together with assignments of peaks. The ^1H -NMR spectra confirmed the purity of phthalonitriles by observation of the proton peaks in their respective regions, as described Chapter 2.

The phthalonitriles were also characterized by FT-IR and ^1H NMR. The distinct $\text{C}\equiv\text{N}$ stretch between 2228 cm^{-1} and 2235 cm^{-1} , in the IR spectra of all phthalonitriles was observed together with the characteristic C-S-C vibration at $\sim 666\text{ cm}^{-1}$ for **45**.



Scheme 3.1: Synthesis of mono-substituted phthalonitriles **39**, **42** and **45**.

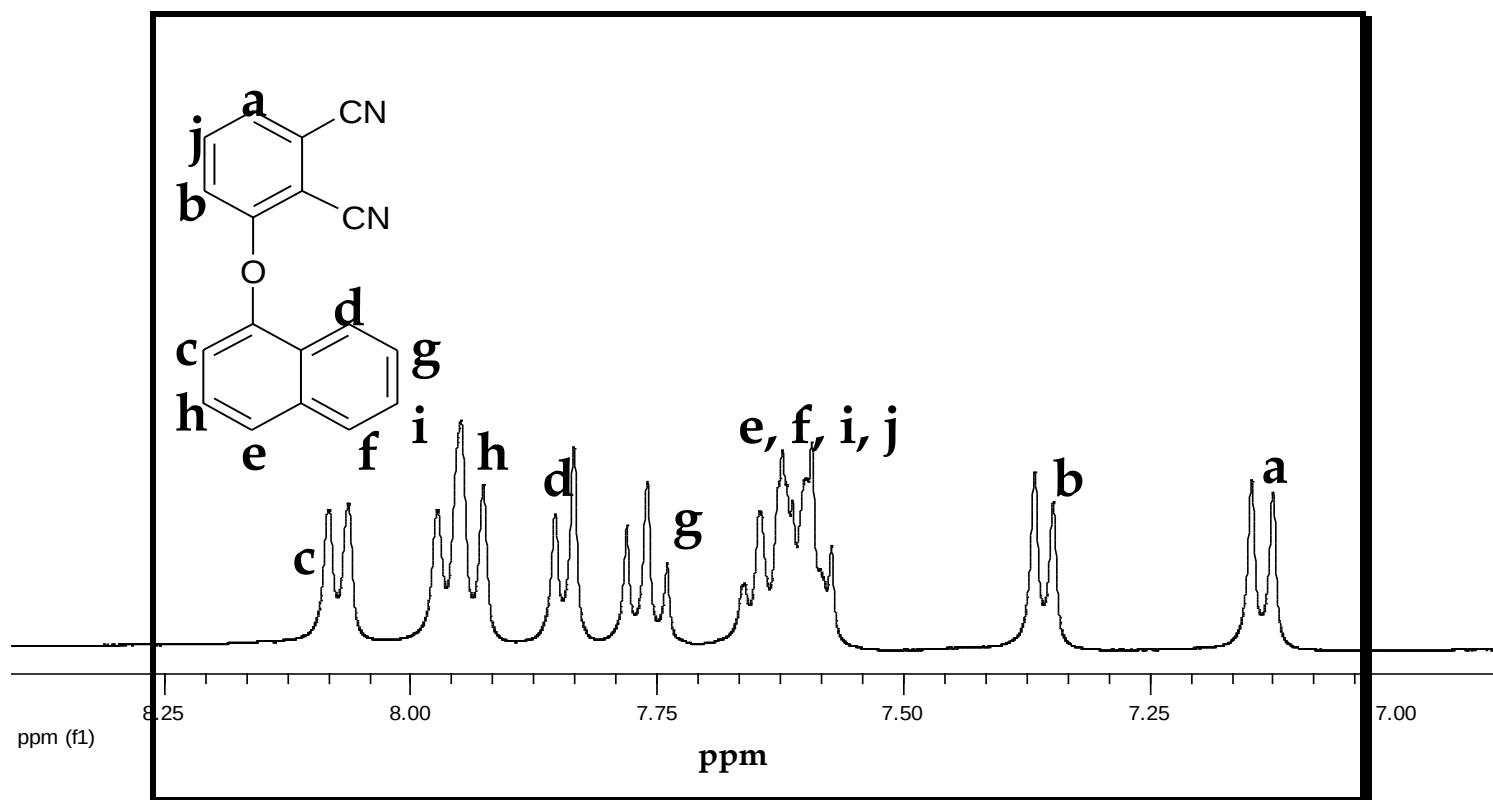
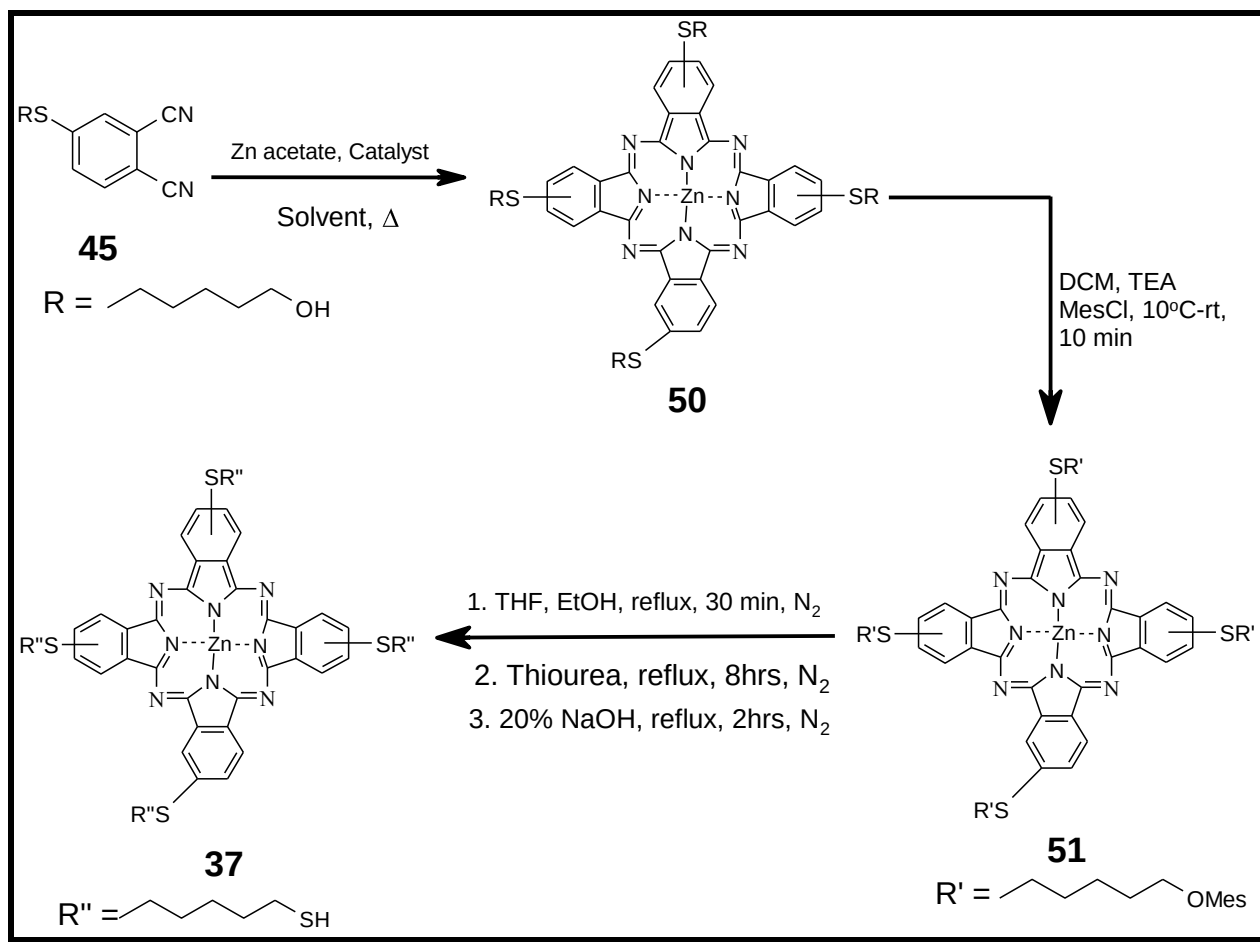


Figure 3.1: ^1H NMR spectrum of compound **39** in $\text{DMSO}-d_6$.

3.1.2 Symmetrically substituted phthalocyanine (**37**)

Scheme 3.2 shows the chemical structure and synthetic route of compound **37**. The conversion of **45** into the corresponding hydroxyl substituted zinc phthalocyanine (**50**) was accomplished in pentanol in the presence of a catalyst (DBU) and zinc acetate. The terminal hydroxyl groups on complex **50** were converted into their mesylate counterparts using triethanolamine (TEA) followed by the addition of methanesulfonyl chloride at low temperature under standard reaction conditions to give the substituted phthalocyanine **51**.



Scheme 3.2: Synthetic routes for tetra substituted zinc phthalocyanine (**37**).

Finally, the terminal thiol functional group was obtained by refluxing **51** in thiourea, under an inert atmosphere in the absence of light, in a previously degassed THF/ethanol solvent mixture. The resultant product was then hydrolysed using 20 % NaOH (also previously degassed). The inert atmosphere inhibits the formation of disulfides *via* aerobic oxidations and ensures the formation of complex **37** at a moderate yield.

Characterization of complex **37** was achieved using FT-IR, UV-vis, MALDI-TOF and ^1H NMR spectroscopies as well as elemental analyses. The absence of the sharp $\text{C}\equiv\text{N}$ vibration ($\sim 2230\text{ cm}^{-1}$) for compound **45** in the IR spectra of **37** confirmed phthalonitrile cyclisation. Complex **37** was found to be pure by ^1H NMR. Aromatic protons were observed between 7.70 and 6.97 ppm integrating for 12 and CH_2 protons were between 4.22 and 1.24 ppm, integrating for 48 protons, in total, as expected. The mass spectra of the complex showed a molecular ion peak at 1170.56 amu which is consistent with predicted structure.

Figure 3.2 shows the UV-vis spectra of complex **37** in chloroform, DMF and DMSO. The figure illustrates typical monomeric behaviour with Q-band maxima at 704 nm (Table 3.2) in chloroform. However the Pc showed slight aggregation in DMSO and DMF.

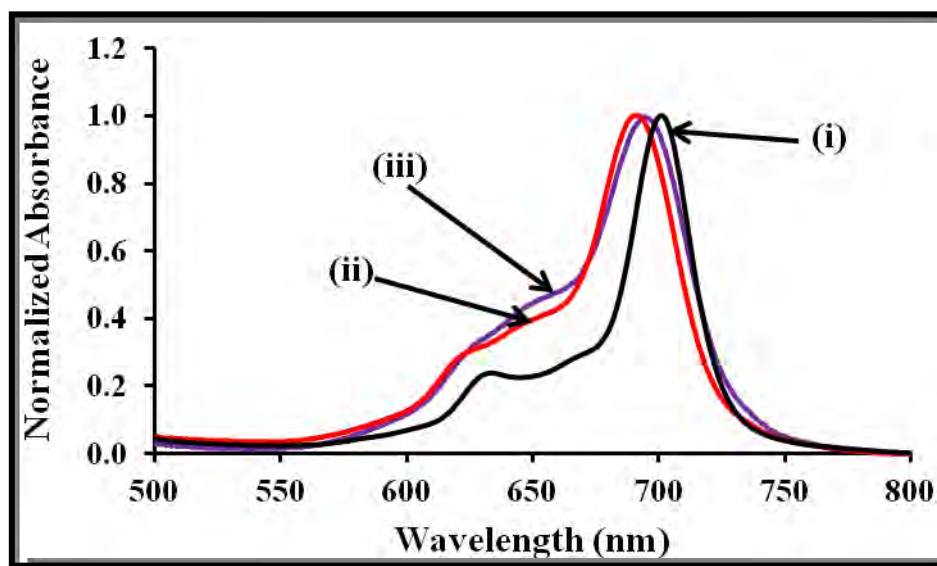


Figure 3.2: Ground state electronic absorption spectrum of **37** in (i) chloroform, (ii) DMF and (iii) DMSO. Concentration = $\sim 1 \times 10^{-5}$ M.

3.1.3 Unsymmetrically substituted phthalocyanine derivatives

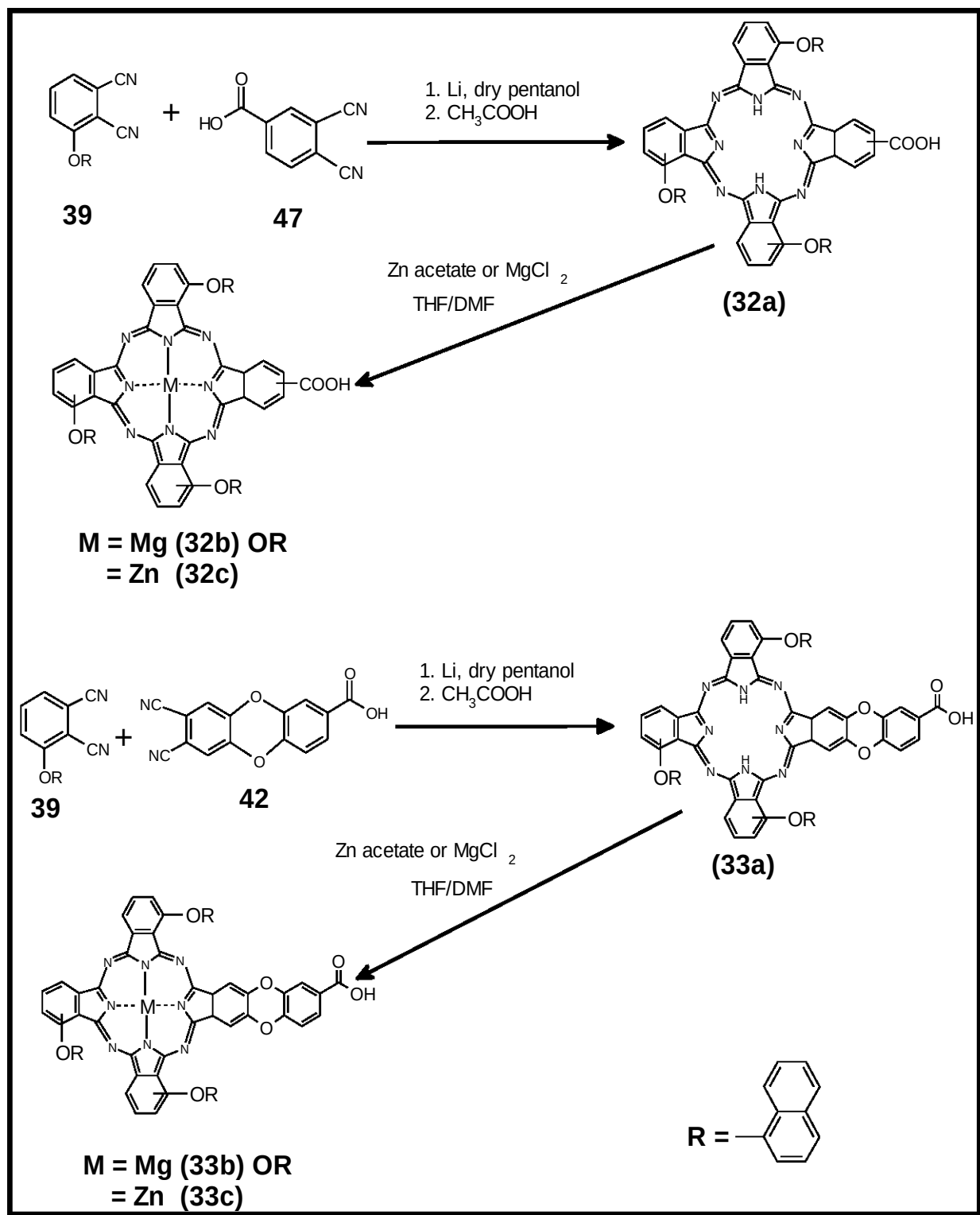
3.1.3.1 Naphthoxy substituted Pcs (32, 33 and 36)

The synthetic procedure outlined in Scheme 3.3 shows the statistical condensation approach used for the synthesis of the A₃B type H₂Pcs. This method is based on the reaction of two differently substituted phthalonitriles in a ratio of 3:1 or higher. Thin layer and column chromatographic techniques were used for the isolation of the desired product and the A₃B type Pcs were isolated with very low (<10 %) percentage yields. The asymmetric unmetallated phthalocyanines (**32a** and **33a**) were soluble in THF and only partially soluble in other organic solvents including ethanol, chloroform, DMF, DCM and DMSO. The characterization of the complexes involved a combination of methods including elemental analysis, mass spectrometry, ¹H NMR, IR and UV-Vis spectroscopy. The disappearance of the C≡N stretch (~ 2230 cm⁻¹) in the IR spectra of **32a** and **33a** confirmed the conversion into the corresponding phthalocyanine analogues. The ring cavity protons for **32a** and **33a** were observed as weak peaks at -1.02 and -2.00 ppm as anticipated in the ¹H NMR spectra.

Complexes **32b** and **33b** were prepared by refluxing **32a** or **33a**, respectively with magnesium (II) chloride in a THF/DMF mixture (1:9) for one hour. The unmetallated phthalocyanines (**32a** and **33a**) were only partially soluble in DMF hence a small amount of THF was added to aid in solubility for the metallation. Complexes **32b** and **33b** showed good solubility in solvents including chloroform, DMF, DCM and DMSO compared to their H₂Pc and ZnPc derivatives. The

metallation of Pcs **32a** and **33a** in dry pentanol using zinc (II) acetate gave Pcs **32c** and **33c** with relatively low percentage yields after purification.

IR data also confirmed the conversion of **32a** and **33a** to the respective Mg and Zn phthalocyanines (**32b**, **33b**, **32c** and **33c**) by the disappearance of the N-H stretches at approximately 1000 cm^{-1} . The disappearance of the inner protons in the NMR spectra of **32a** or **33a** also confirmed metallation. The non-peripheral ring protons were observed as two signals at 8.80 and 7.22 ppm for **32a**, between 8.00-7.30 ppm for **32b** and at 7.15 and 6.40 ppm for **32c**.



Scheme 3.3: Synthesis of naphthoxy substituted phthalocyanines.

Table 3.2: Spectral data for phthalocyanines. DMF contains <1 % THF for complexes **32** and **33** to aid solubility.

Pc	Central metal	Substituents	Solvent	Q-band (nm)
32a	H ₂	-(O-C ₁₀ H ₇) ₃ and CO ₂ H	THF	710, 680
			DMF	709, 679
32b	Mg	-(O-C ₁₀ H ₇) ₃ and CO ₂ H	THF	697
			DMF	689
32c	Zn	-(O-C ₁₀ H ₇) ₃ and CO ₂ H	THF	680
			CHCl ₃	691
			DMF	684
33a	H ₂	-(O-C ₁₀ H ₇) ₃ and C ₇ H ₃ O ₄	THF	715, 685
			DMF	713, 680
33b	Mg	-(O-C ₁₀ H ₇) ₃ and C ₇ H ₃ O ₄	THF	740, 710
			DMF	743, 716
33c	Zn	-(O-C ₁₀ H ₇) ₃ and C ₇ H ₃ O ₄	THF	685
			DMF	686
34a	H ₂	-(O-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	676
34b	Mg	-(O-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	676
			MeOH	670
34c	Zn	-(O-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	678
			MeOH	667
35a	H ₂	-(S-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	682
35b	Mg	-(S-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	682

Table 3.2 continued

Pc	Central metal	Substituents	Solvent	Q-band (nm)
35c	Zn	-(S-C ₆ H ₄ NF ₃) ₃ and CO ₂ H	DMSO	684
36	Zn	-(O-C ₁₀ H ₇) ₃ and C ₃ H ₇ NOS	DMSO	694
			CHCl ₃	692
37	Zn	-(C ₆ H ₁₃ S) ₄	DMSO	691
			DMF	694
			CHCl ₃	704
			DMSO(2 %)/PBS	642
			DMSO(2 %)/PBS/Triton X-100	681, 642
38	Zn	-(SO ₃ H) ₄	DMSO	679

The ¹H NMR spectra of all complexes were complicated and gave broadened peaks due to possible intermolecular aggregation of the Pc as well as the presence of structural isomers. The ¹H NMR spectra of **32a**, **33a**, **32c** and **33c** were taken in THF-

d_8 while ^1H NMR spectra of **32b** and **33b** was taken in CDCl_3 . For complexes **32a**, **32b** and **32c**, **35**, **33** and **33** protons were obtained respectively. In a similar manner complex **33a**, **33b** and **33c** gave **37**, **35** and **36** aromatic protons respectively. The ^1H NMR spectral data for **32b**, **33b**, **32c** and **33c**, were similar to that of respective complexes **32a** and **33a** except that the proton signals for the ZnPc derivatives were deshielded. This may be caused by the electron withdrawing effect caused by the insertion of the Zn metal. The carboxylic acid proton was only observed for complex **33c** as a broad peak at 9.00 ppm. The acid proton is often difficult to locate due to its varying chemical shift and is hardly observed. The integration of the peaks corresponded suitably to the expected number of protons for all complexes, further confirming the relative purity of the complexes. The purified monofunctional phthalocyanines were further characterized by elemental analysis and mass spectrometry.

Complexes **32a,c** and **33a,c** were not adequately soluble in DMF or DMSO, hence a small amount (<1 %) of THF was added to aid solubility, Table 3.2.

The ground state electronic absorption spectra of the Pcs in THF, shown in Figure 3.3 (Table 3.2) compare the unmetallated (**32a**), MgPc (**32b**) and the ZnPc derivatives (**32c**). Figure 3.4 compares the spectrum of complex **33a** with that of the magnesium (**33b**) and zinc (**33c**) derivatives in THF. The absorption spectra show characteristic sharp Q-bands. There is a blue shift in the wavelength position of the Q-band for compounds **32** relative to corresponding **33**, Table 3.2. This implies that the electron density of compound **33** is higher than that of compound **32** due to the dioxyphenyl

substituent. This results in the lowering of the HOMO-LUMO gap of complexes **33a**, **33b** and **33c**.

The aggregation behavior of the Pcs were investigated in THF (Figure 3.5 using complex **32a** as an example). Upon dilution, no new bands were observed for all complexes, signifying no aggregation behavior at these concentrations, probably due to the bulky nature of the ring substituents. Beer's law was observed for all complexes for concentrations less than 5×10^{-5} M.

The Q-band of **32b** at 697 nm (Figure 3.3) shows monomeric behaviour; however there is an extra band observed for this complex at 736 nm. This band is more pronounced for complex **33b**, Figure 3.4. The split in the Q-band can suggest demetallation, but since the wavelengths of the two components of the Q-band for both **32b** and **33b** are different from the corresponding **32a** and **33a**, demetallation is ruled out.

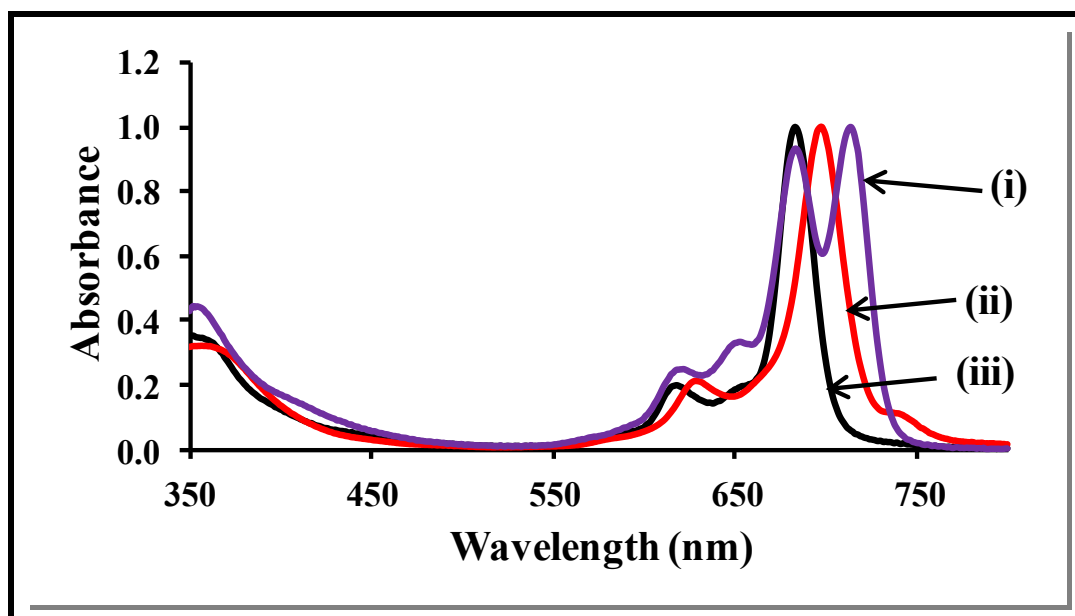


Figure 3.3: Ground state electronic absorption of (i) 32a, (ii) 32b and (iii) 32c in THF.

Concentration = $\sim 1 \times 10^{-5}$ M.

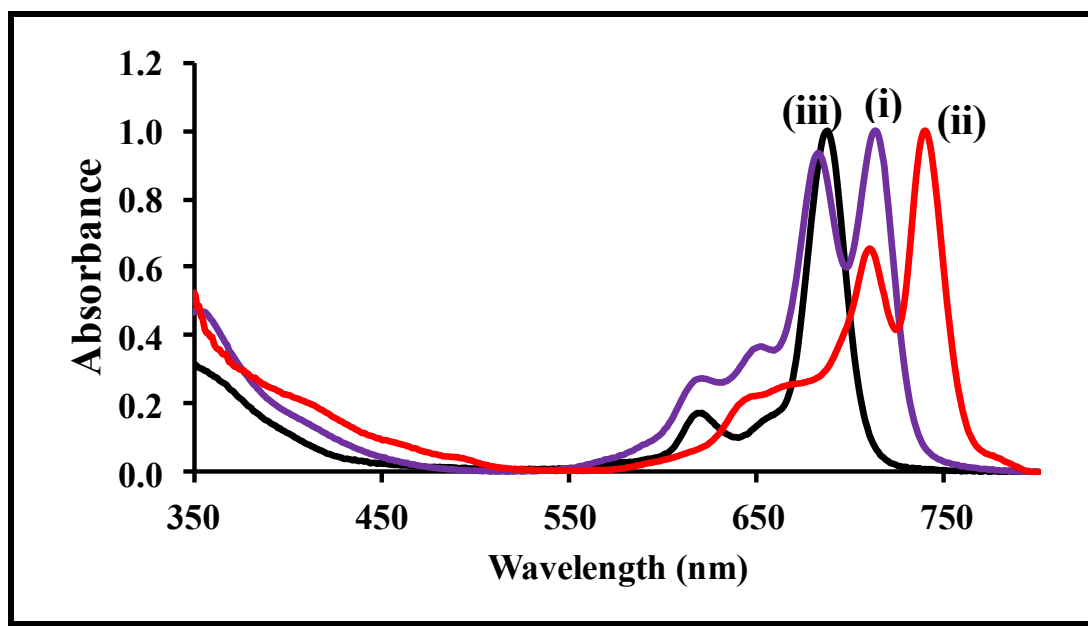


Figure 3.4: Ground state electronic absorption of (i) 33a, (ii) 33b and (iii) 33c in THF.

Concentration = $\sim 1 \times 10^{-5}$ M.

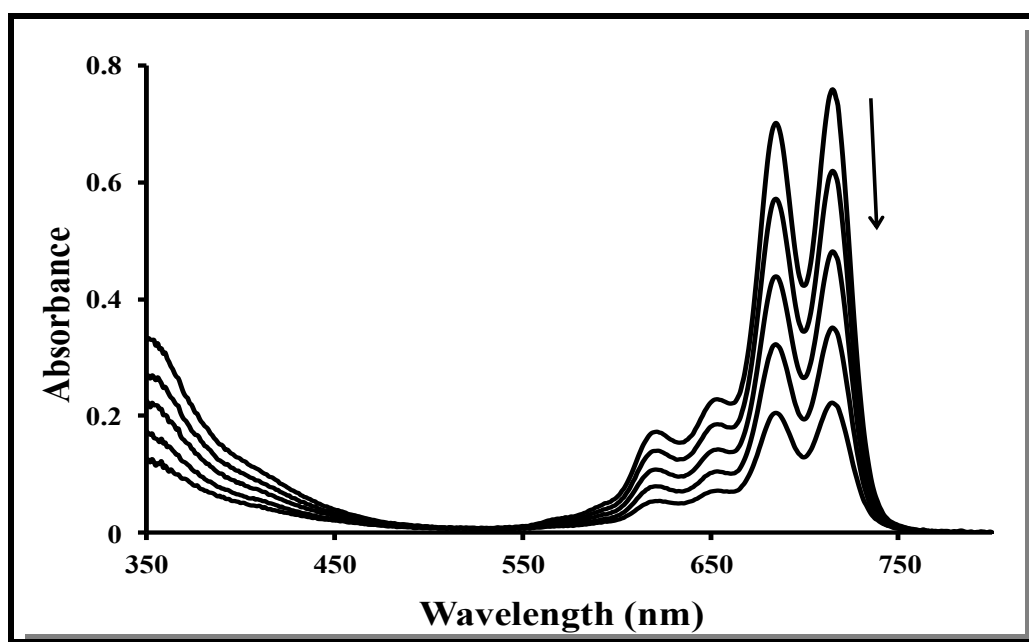


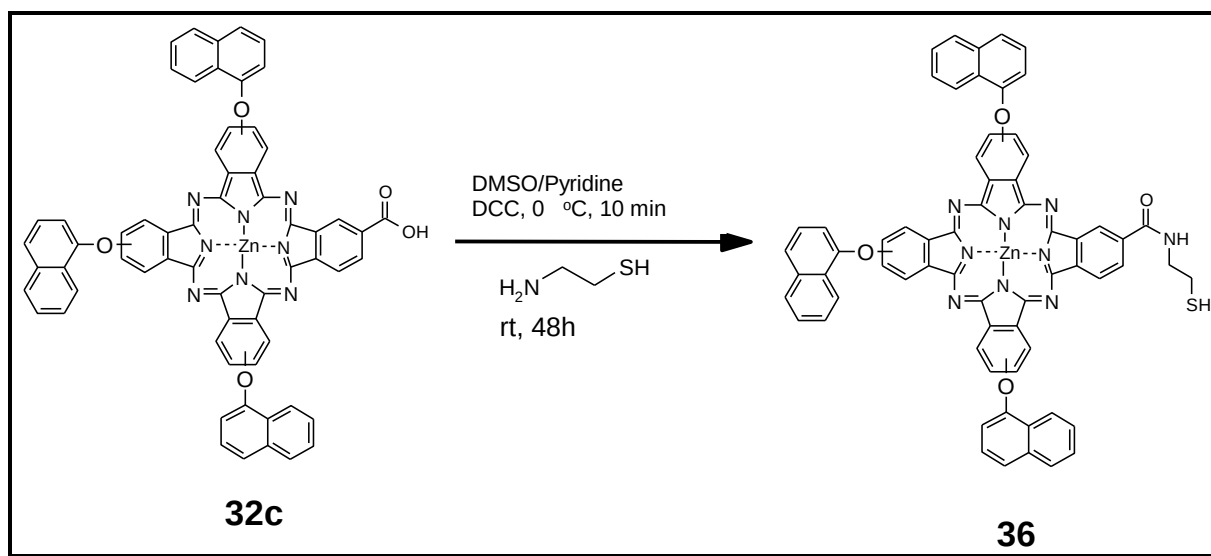
Figure 3.5: Absorption spectra of compound **32a** in THF at different concentrations. Concentration range $\sim 2 \times 10^{-6}$ – 9×10^{-6} M.

Another possibility would be loss of symmetry due to protonation. The presence of minor amounts of acid, in solvents such as DCM, CHCl_3 and THF, is also said to cause protonation of the inner Pc ring nitrogen atoms, causing molecular loss in symmetry and the appearance of a red shifted band [255]. This band was also observed in chloroform, DMSO and THF/DMF, ruling out the possibility of inner ring protonation due to the acidic nature of THF. The split in the Q-band has been observed when phenoxy groups are attached to the phthalocyanine ring. It has been suggested that this band is caused by the flexible attachment of the phenoxy (C–O–C) groups at the Pc ring [256–258]. This flexibility allows the groups to ‘twist’ about the bond and induce a slight loss in symmetry, hence splitting the Q-band [259]. Since the Mg ion is more out of plane of the ring than the Zn ion [260, 261], the latter

are expected to show less pronounced loss of symmetry [260]. The extra band has been observed mainly in the solid state for MgPc derivatives, with a few reports in solution depending on the substituent [261]. The presence of the naphthol group is expected to show twisting in the same manner as phenoxy groups stated above. In addition, the out of plane nature of the Mg ion has resulted in the enhanced splitting of the Q-band in solution as opposed to the generally observed single Q-band.

When comparing complex **32b** to its zinc derivative (**32c**), the magnesium metal is seen to induce a red shift in the Q-band position of the Pc. This is evident by the 17 nm red shift of the Q-band for complex **32b** in THF. This implies that the insertion of Mg metal significantly lowers the HOMO and LUMO gap compared to the Zn metal (**32c**). The absorbance of the Mg complex (**33b**) also shows a red shifting of 25 nm (high energy band) in the Q-band relative to the Zn derivative (**33c**). The deformation of the Pc ligand is known to cause red shifting in the Q-band [262], hence the observed red shift for MgPc derivatives.

Complex **32c** was covalently linked to cysteamine in the presence of an amide coupling agent dicyclohexylcarbodiimide (DCC) in a DMSO/pyridine mixture to form complex **36** (Scheme 3.4). DCC reacts with the carboxylic acid to form the O-acylisourea anhydride intermediate. This intermediate is more reactive than the resonance stabilised carboxylic centre and can directly react with the amine to yield complex **36**. The formation of possible by-products was diminished by reacting **32c** with DCC in the presence of a base (pyridine) at 0°C prior to the addition of cysteamine. Characterization of complex **36** was achieved using infra-red (IR), ultraviolet-visible (UV-Vis), mass and ^1H NMR spectroscopies as well as elemental analyses. The IR spectroscopy data for complex **36** confirmed amide bond formation with a peak observed at 1693 cm^{-1} .



Scheme 3.4: Synthesis of mono-thiol functionalized phthalocyanine derivative.

The ^1H NMR for complex **36**, showed NH and aromatic protons between 6.72 and 8.20 ppm integrating for 34 protons and CH_2 protons being observed between 0.82 and 1.50 integrating for 4 protons as expected. MALDI-TOF MS further confirmed the formation of complex **36** with a mass peak observed at 1108.24 amu.

Figure 3.6 shows that complex **32c** and **36** display similar spectra in terms of Q-band maxima, showing that the presence of one SH band does not significantly affect the spectra although MPc complexes containing sulfur groups are often considerably red-shifted. Complex **36** shows a split in the Q-band which could be due to the loss of symmetry as a result of unsymmetric substitution (which should also affect **32c**) or due to protonation of the aza nitrogens caused by the acidic chloroform [255]. The protonation would be substituent specific since it depends on the basicity of the ring; hence it is suggested that the split in the Q-band is due to loss of symmetry as a result of unsymmetrical protonation of the inner nitrogen atom.

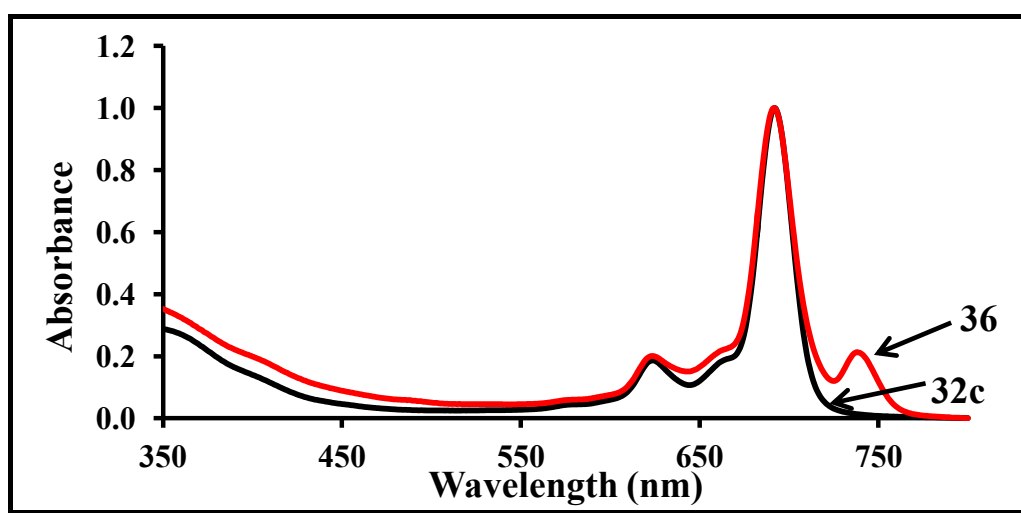


Figure 3.6: Absorption spectra of **32c** and **36** in chloroform. Concentration = $\sim 1 \times 10^{-5}$

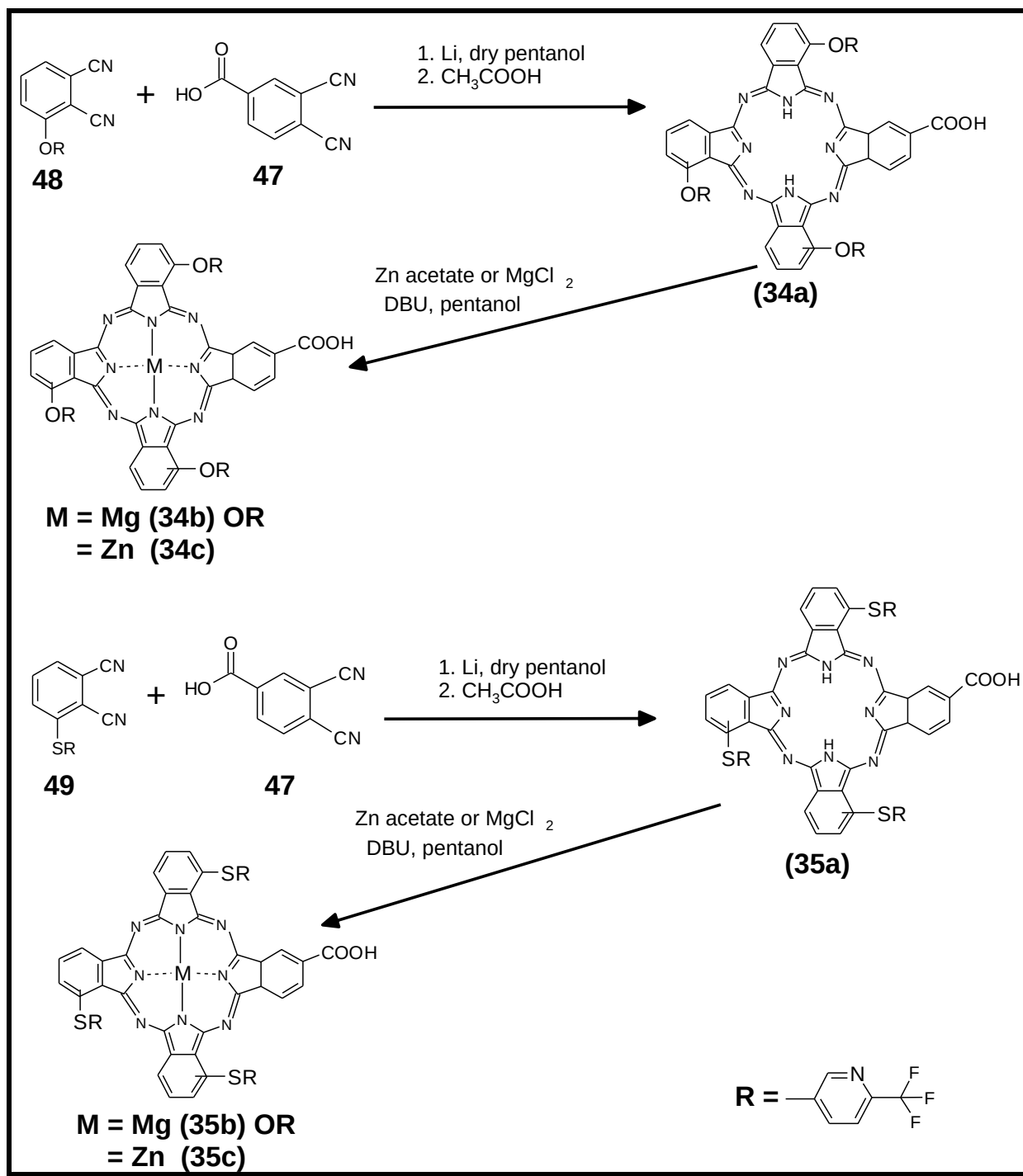
M.

3.1.3.2 Fluorine substituted mono-carboxy Pcs

The conversion of **48** and **47** into the metal-free phthalocyanine (**34a**) was accomplished using the statistical condensation method (Scheme 3.5). Lithium was used as a catalyst and source of metal and later removed using glacial acetic acid. A portion of **34a** was used to synthesize complex **34b** and **34c**. Metallation of **34a** was achieved by refluxing in a high boiling point solvent such as pentanol in the presence of DBU and corresponding metal salt.

The phthalocyanines (**34a-34c**) were soluble in most organic solvents including CHCl_3 , DCM, THF, acetone, MeOH (except for **34a**), DMSO and DMF. The solubility of these phthalocyanines in polar solvents is explained by the extreme electronegative nature of the fluorine atoms [263, 264]. After purification low yields (6-13 %) of the Pcs were obtained.

The conversion of **48** and **47** to complex **34a** was confirmed by the absence of the $\text{C}\equiv\text{N}$ stretch ($\sim 2227\text{ cm}^{-1}$, observed for compounds **48** and **47**) in the IR spectra. The inner core NH stretch was observed at $\sim 3356\text{ cm}^{-1}$ and was not observed for the metallated Pc derivatives.



Scheme 3.5: Synthesis of mono-carboxy phthalocyanines derivatives.

The ^1H NMR spectrum for complex **34a** showed broad and split peaks between 8.25-7.50 ppm due to aryl protons. The shielded inner core N-H protons were observed as a single peak at -0.12 ppm. These protons were not observed for **34b** and **34c** confirming metallation. Complex **34a**, **34b** and **34c** showed similar ^1H NMR spectra, with broad peaks observed between 8.20 and 6.55 ppm. These were attributed to aryl protons. MALDI-TOF and elemental analysis results were consistent with the predicted structures shown in the experimental section.

To increase the product yield of the statistical condensation approach, compounds **49** and **47** were ground together (Scheme 3.5). The powder was added to a refluxing solution of dry pentanol containing dissolved lithium. A relatively good yield for the unsymmetrical phthalocyanine (**35a**) was obtained (13 %) after continuous washing in hot methanol and purification by column chromatography. The different lipophilicities of **49** and **47** assisted in the purification of **35a** from by-products due to different silica-binding properties in the solvent mixture employed. The phthalocyanine (**35a**) showed partial solubility in most organic solvents including DMSO, DCM, methanol, acetone and toluene. However it showed good solubility in CHCl_3 , DMF and THF. Both **35b** and **35c** were obtained by refluxing **35a** in the presence of magnesium chloride or zinc acetate respectively, in DMF for an hour. Purification of the products was carried out by taking advantage of the products' insolubility in highly hydrophilic organic solvents. The metallated complexes (**35b** and **35c**) showed excellent solubility in DMF, DMSO, THF and CHCl_3 . Characterization of the compounds was carried out by UV-Vis, IR, ^1H NMR, MALDI-TOF and elemental analysis.

Infrared spectra observed for complexes **35a-35c** showed a band at $\sim 720\text{ cm}^{-1}$ (C-S vibration) which is characteristic of a sulfide functionality. Aggregation and short relaxation times often results in broad signals in the ^1H NMR spectra of phthalocyanines [265]. Both **35a** and **35b**, as expected, showed broad ^1H NMR signals that did not give information about the partition of Pc protons. The integration of proton peaks observed at 7.90-7.20 ppm for **35b** gave 21 H and protons observed at 7.80-7.30 ppm for complex **35c** corresponded to the expected 21 H. The OH proton for both complexes was not observed. The elemental analysis and mass spectra results for **35b** and **35c** were in agreement with the calculated values for the proposed structures.

Figure 3.7 shows the UV-Vis absorption spectra of complexes **34a** to **34c** in DMSO. Table 3.2 gives a summary of the Q-band maxima of the complexes. Unmetallated phthalocyanine complexes show a split Q-band. However in basic solvents such as pyridine and DMSO, the Q-band is not split [266]. Figure 3.7b shows the split nature of the Q-band for **34a** in chloroform. The Soret band of the complexes was observed as a very broad peak at $\sim 360\text{ nm}$. This broadness is a result of the presence of two overlapping bands (B_1 and B_2) [266]. The aggregation behaviour of the Pcs was investigated in DMSO. No new bands were observed for all complexes on increasing the concentration up to $2 \times 10^{-5}\text{ M}$, signifying lack of aggregation at these concentrations. Below $2 \times 10^{-5}\text{ M}$ Beer's law was observed.

The ground state electronic absorption spectra of compounds **35a-c** in DMSO is shown in Figure 3.8. The spectra show Q-bands characteristic of metallated

phthalocyanines between 682-684 nm (Table 3.2). The unmetallated compound (**35a**) does not show the typical split Q-band caused by transitions to the non-degenerate e_g orbital of H_2Pcs . A closer look at the spectrum for **35a** shows a slight split of the Q-band due to the unmetallated nature of the complex. Compound **35a** shows a pronounced broadening between 600 and 650 nm which cannot be due to unresolved splitting since it is at a much lower energy than expected for the components of the Q-band. The broadening is due to aggregation, as determined by dilution and also observed to a lesser extent for **35b** and **35c**.

In summary, Table 3.2 shows that the most red shifted complexes are **37**, **36**, and **35c** (containing Zn). This is due to the presence of sulfur as terminal or linkage in DMSO. Complexes **32** and **33** (except **32b** and **33b**) were not soluble in DMSO, Table 3.2.

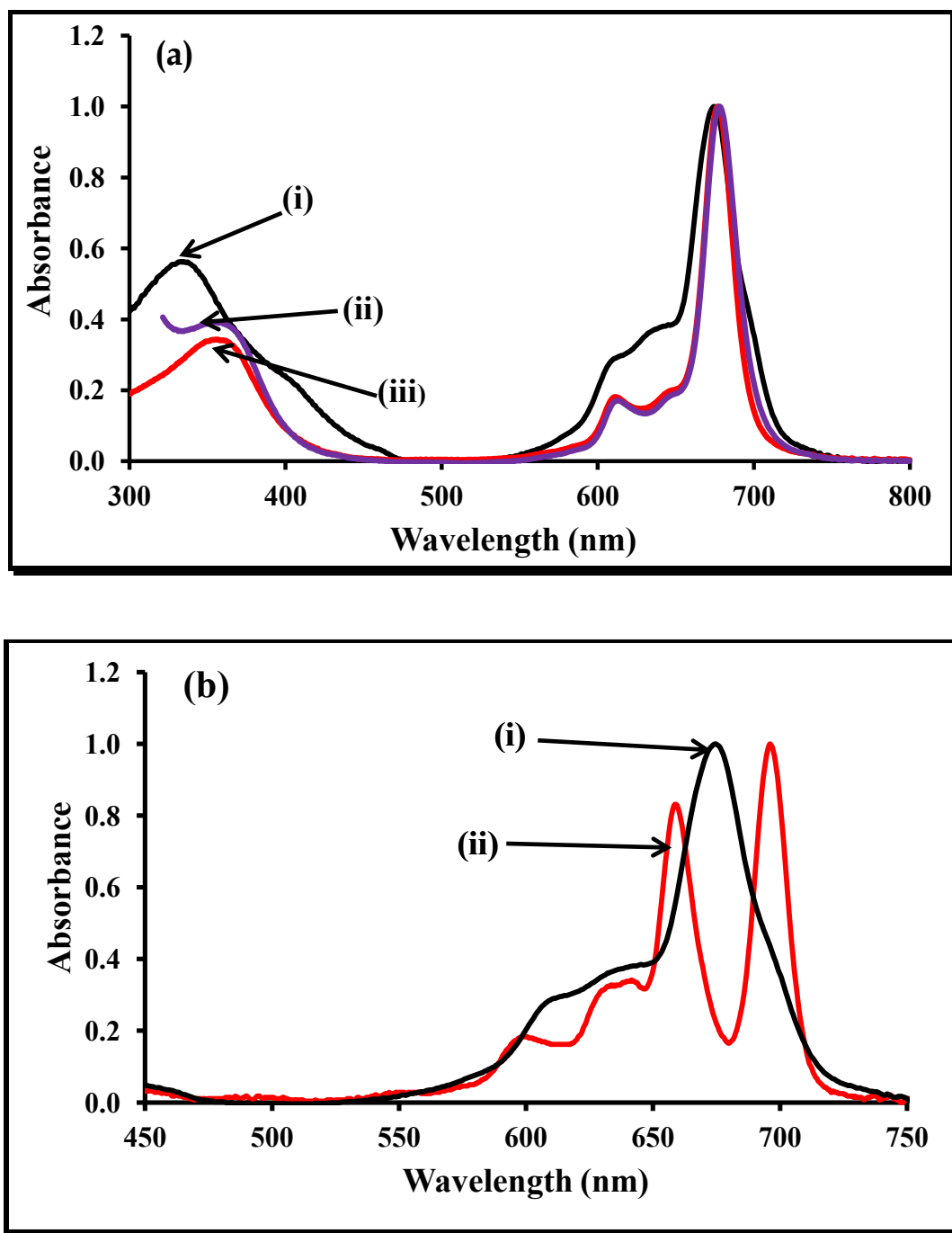


Figure 3.7: (a) Absorbance spectra of compounds **34a**, (i), **34b** (ii) and **34c** (iii) in DMSO, (b) spectrum of **34a** in (i) DMSO and (ii) chloroform. Concentration $\sim 1 \times 10^{-5}$ M.

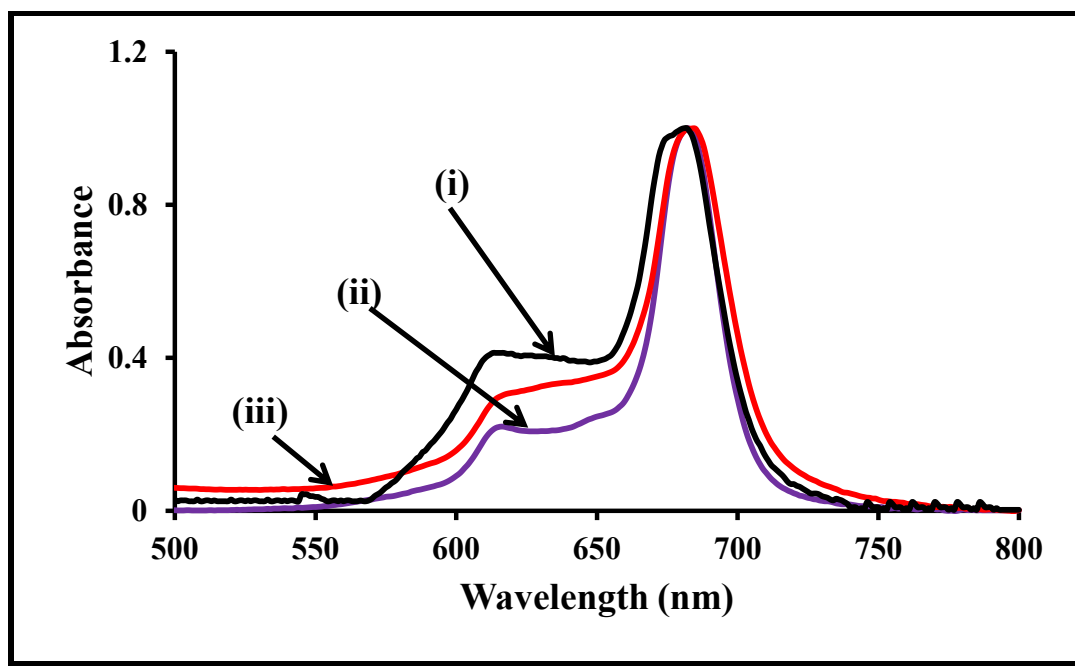


Figure 3.8: Absorbance spectra of compounds (i) **35a**, (ii) **35b** and (iii) **35c** in DMSO. Concentration = $\sim 1 \times 10^{-5}$ M.

3.2 Synthesis of nanoparticles (NPs)

Gold and silver nanoparticles (AuNPs and AgNPs) used in this work were synthesized as previously reported [219, 220] with consistent spectroscopic features as characterized in literature.

The surface plasmon resonance (SPR) absorption band for TOABr-AuNP or citrate stabilized-AuNP and AgNP was determined from UV-vis spectrum to be at 530 nm for TOABr stabilized AuNPs and at 527 and 390 nm for citrate stabilized AuNPs and AgNPs, respectively (Figure 3.9).

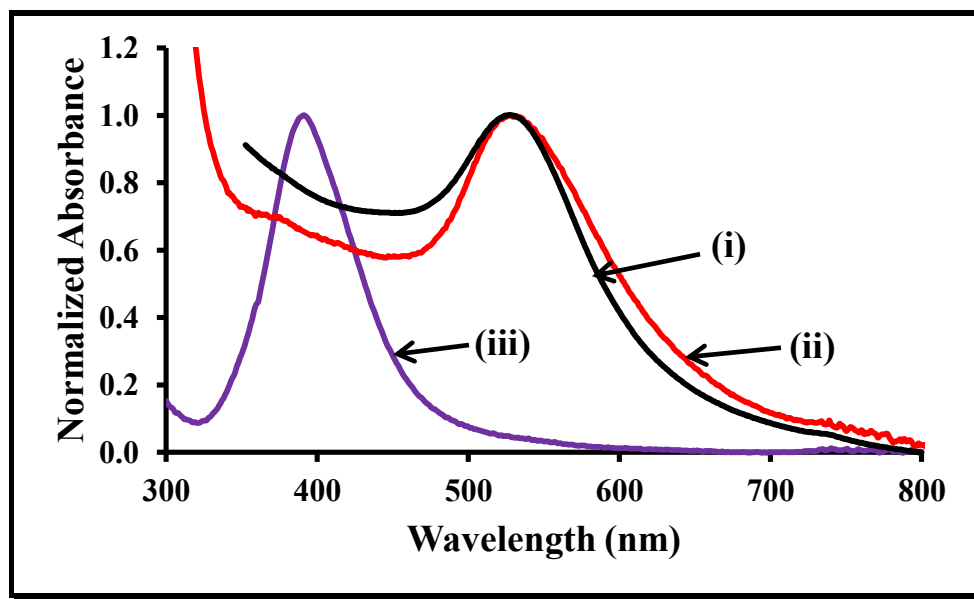


Figure 3.9: Absorption spectra of TOABr-AuNPs in chloroform (i), citrate-AuNPs (ii) and citrate-AgNPs (iii) in water.

The nanoparticles were characterised using UV-vis, XRD and TEM. To determine the size of the nanoparticles the XRD technique was employed. The Debye-Scherrer equation (equation 3.1) was used to calculate the size of the gold nanoparticles and Pc conjugates [267].

$$d(\text{\AA}) = \frac{k\lambda}{\beta \cos \theta} \quad (3.1)$$

where k is an empirical constant equal to 0.9, λ is the wavelength of the X-ray source, (1.5405 Å), β is the full width at half maximum of the diffraction peak, and θ is the angular position of the peak.

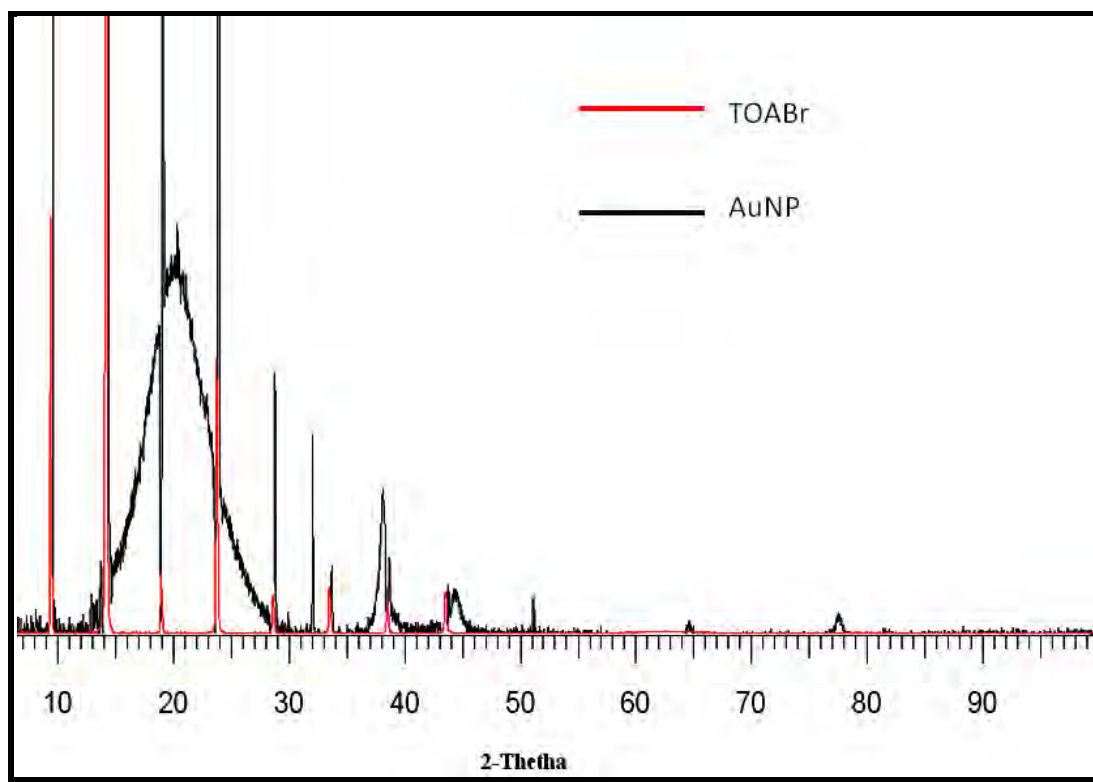


Figure 3.10: XRD of TOABr-AuNPs (black) and TOABr (red).

Figure 3.10 shows the XRD pattern for the TOABr-AuNPs and the corresponding TOABr pattern. The sharp TOABr peaks suggest bulk and broad peaks confirm the small size of the AuNPs. The average size for the AuNPs was calculated to be 5.37 nm. This size was further confirmed by atomic force microscopy where the average size of the nanoparticles ranged between 3.5 and 4.5 nm.

Figure 3.11a shows the atomic force microscope image of TOABr-AuNPs on a cross section of a glass surface coated from toluene solution. Figure 3.11b shows the corresponding size distribution (1- 6 nm) of the gold nanoparticles as determined by AFM. The sizes of the citrate stabilized nanoparticles were also confirmed using AFM where the size of the AuNPs was determined to be 13.5 nm and the AgNPs

were found to have a smaller diameter of 7.5 nm. The size distribution of the silver nanoparticles as determined by AFM is shown in Figure 3.11c to be 3.5 to 12 nm.

Figure 3.12a shows the TEM image of the TOABr-AuNP and Figures 3.12b and 3.12c show the TEM images of citrate-AuNPs and citrate-AgNPs, respectively. These TEM images show the NPs as spheres with different sizes dispersed in a disorderly manner, these are seen as dark spots on the images. The size of the nanoparticles determined from the TEM image was found to range between 5.97- 7.87 nm for TOABr-AuNPs. The size of AuNPs (~ 5 nm) determined using XRD is expected to be more reliable, unlike TEM which is an estimation due to partial aggregation. The size determined using AFM is closer to that determined using XRD.

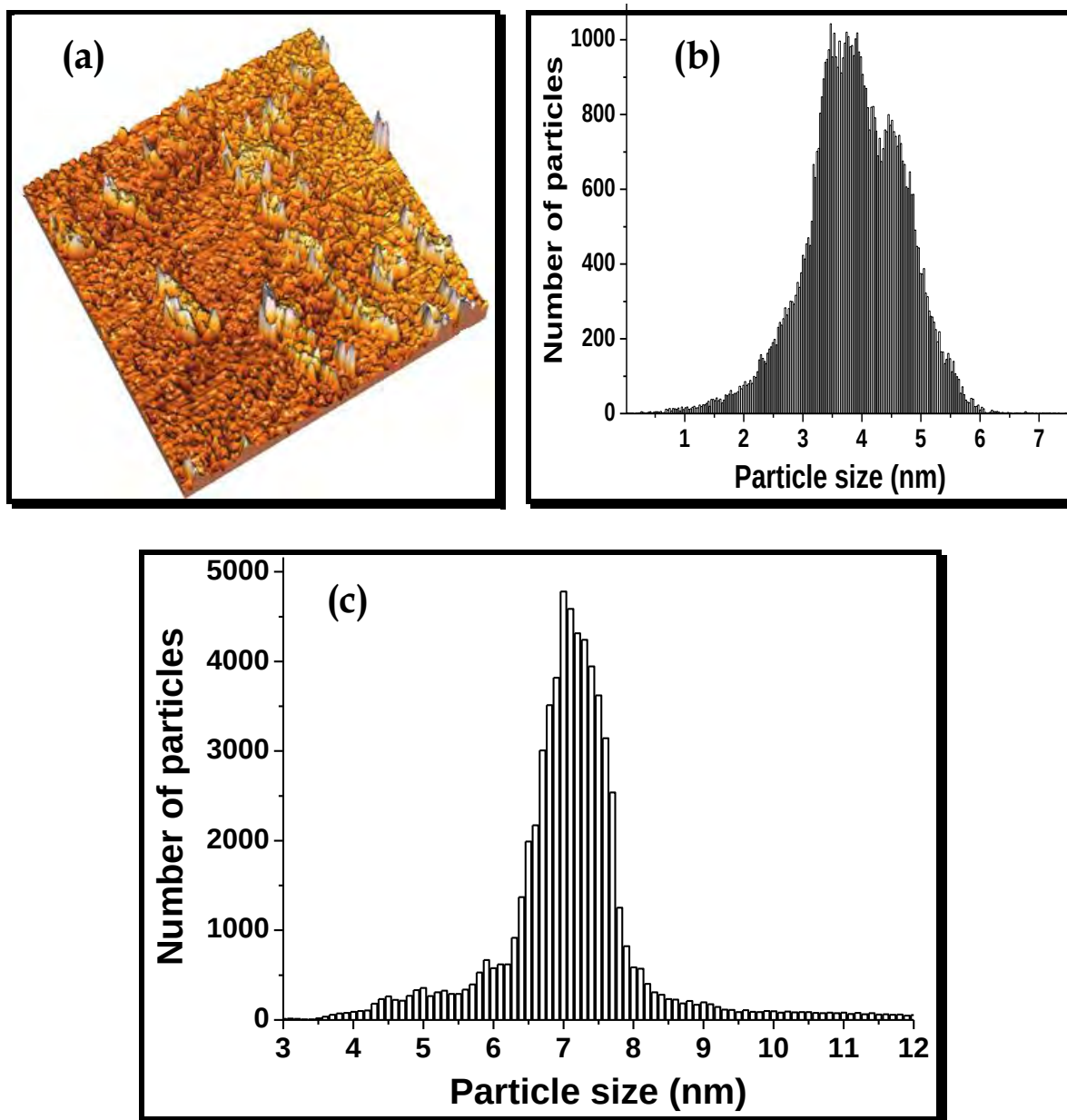


Figure 3.11: (a) AFM image of TOABr-AuNPs deposited on a glass surface from a toluene solution with (b) the corresponding size distribution of TOABr-AuNPs, (c) shows the AFM size distribution histogram of citrate-AgNPs.

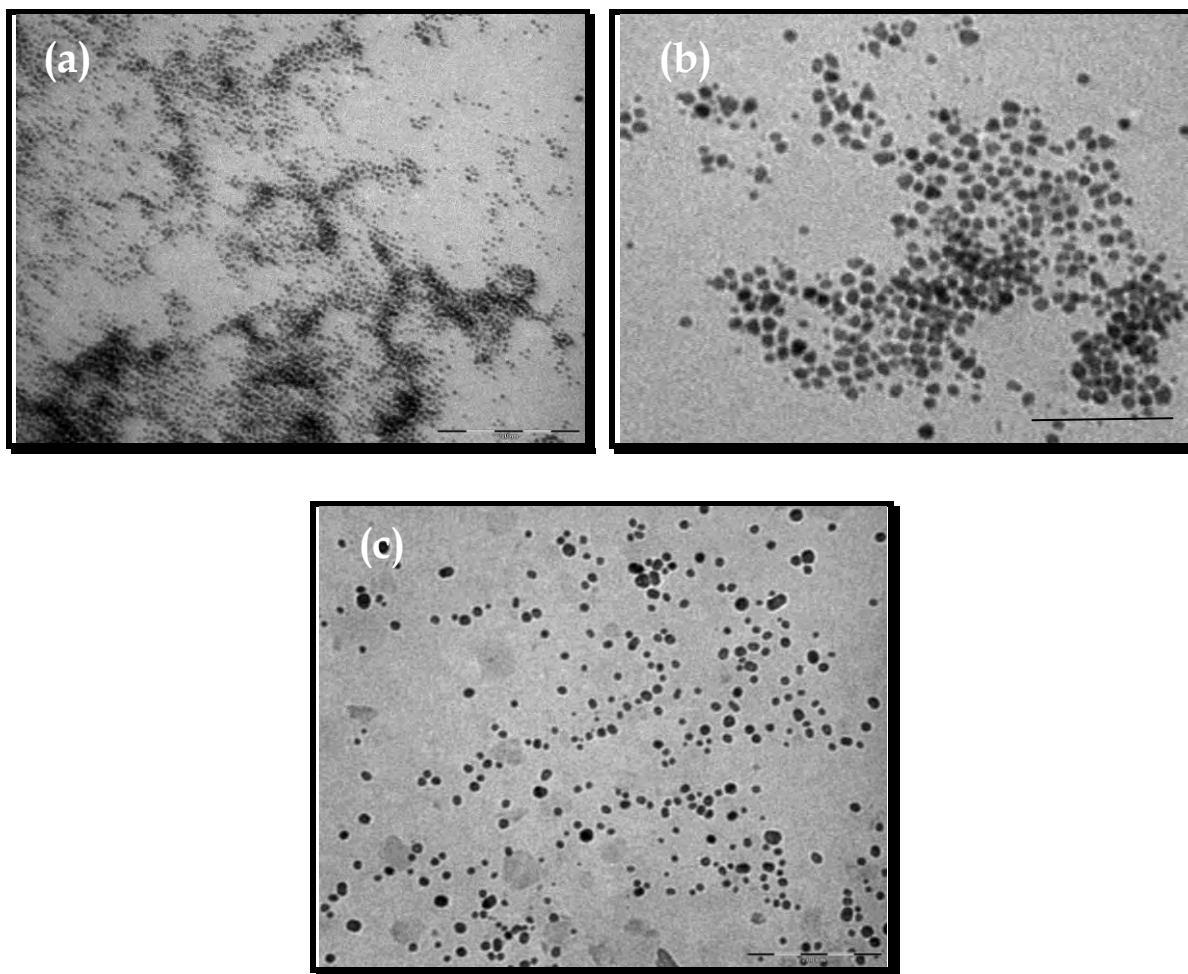


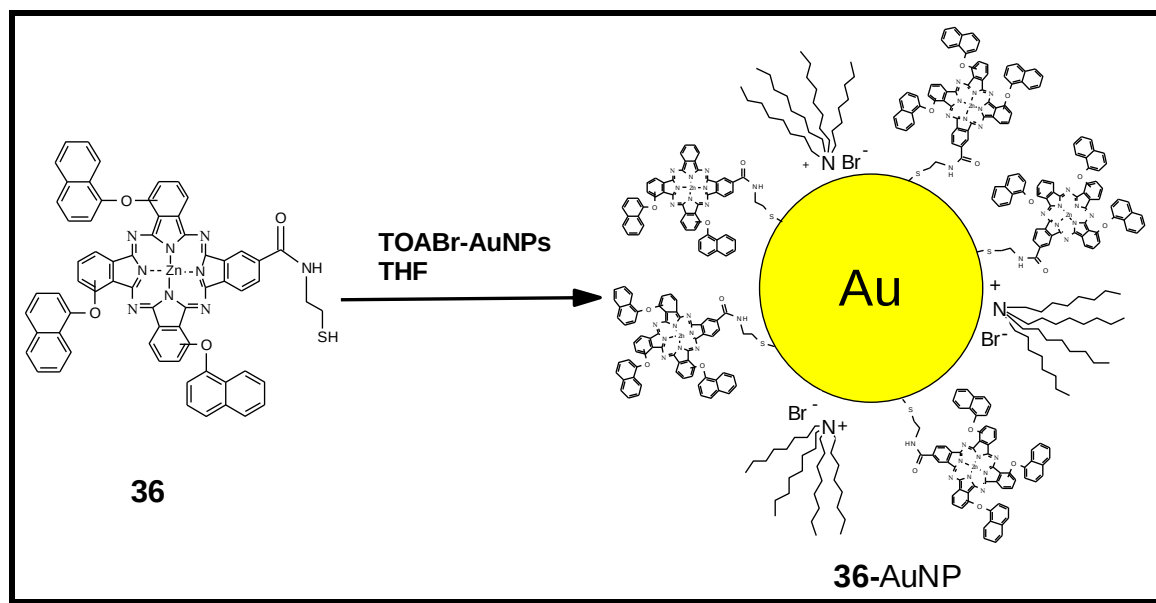
Figure 3.12: TEM images of (a) TOABr-AuNPs, (b) Citrate-AuNPs and (c) Citrate-AgNPs. Scale 200 nm.

3.3 Phthalocyanine conjugates

3.3.1 Assembly of phthalocyanine-nanoparticle structures

Phthalocyanine functionalised gold nanoparticles were synthesized using a ligand exchange process, where the loosely bound TOABr or citrate ligands were partially exchanged by Pcs that bind to the nanoparticle surface. The phthalocyanine complexes are most likely attached to the AuNPs surface via Au-S bonding by the sulfur groups on the periphery of the phthalocyanine ring, for complexes **35a**, **36** and **37**. A schematic representation of the conjugation of **36** with the TOABr-AuNP is shown in Scheme 3.6. For complex **36** containing one thiol group, the arrangement of the phthalocyanines will be by linkage with this one group, resulting in a perpendicular arrangement shown in Scheme 3.6. For complexes **35a** and **37**, it is possible that one or more of the SR or SH groups are attached.

On conjugation with Pc complexes TEM images (using **36**-AuNP and **37**-AuNP as examples), show a highly ordered arrangement, Figures 3.13a and 3.13b.



Scheme 3.6: Representation of the formation of **36-AuNP**.

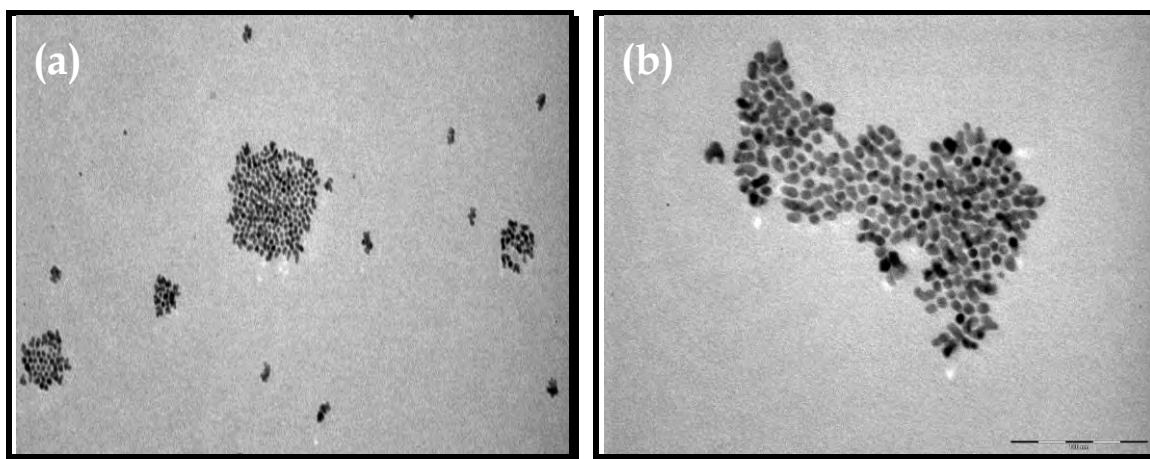


Figure 3.13: TEM images of (a) **36-AuNP** and (b) **37-AuNP**.

The UV-vis spectra of the Pc-TOABr-AuNP conjugates are shown in Figure 3.14a-c for **35a-AuNP**, **36-AuNP** and **37-AuNP** and together with their unconjugated phthalocyanine derivatives. It is known that the absorption spectrum of the Pc-AuNP shows a broadening of the phthalocyanine Q-band absorption. This was

attributed to a tight packing of the phthalocyanines on the gold [268].

In Figure 3.14a for **35a**-AuNP this broadening is observed, in addition there is a small blue shift since the SR groups are now engaged with the AuNPs reducing their electron donating abilities. Similarly, a slight blue shift is observed for **36**-AuNP in Figure 3.14b and for **37**-AuNP in Figure 3.14c. For **36**-AuNP, there is an increase in a split in the Q band, which suggests that the splitting due to loss of symmetry is enhanced by attachment of AuNPs.

A closer look at Figure 3.14c for **37**-AuNP, shows the SPR peak in the conjugate to be larger than for **35a**-AuNP or **36**-AuNP, suggesting more coordinated AuNPs, in the latter two conjugates. The diminished and broad surface plasmon band has been found to be indicative of surface complexation; hence the SPR band serves as a probe to monitor the interaction between AuNPs with surface bound molecules [269].

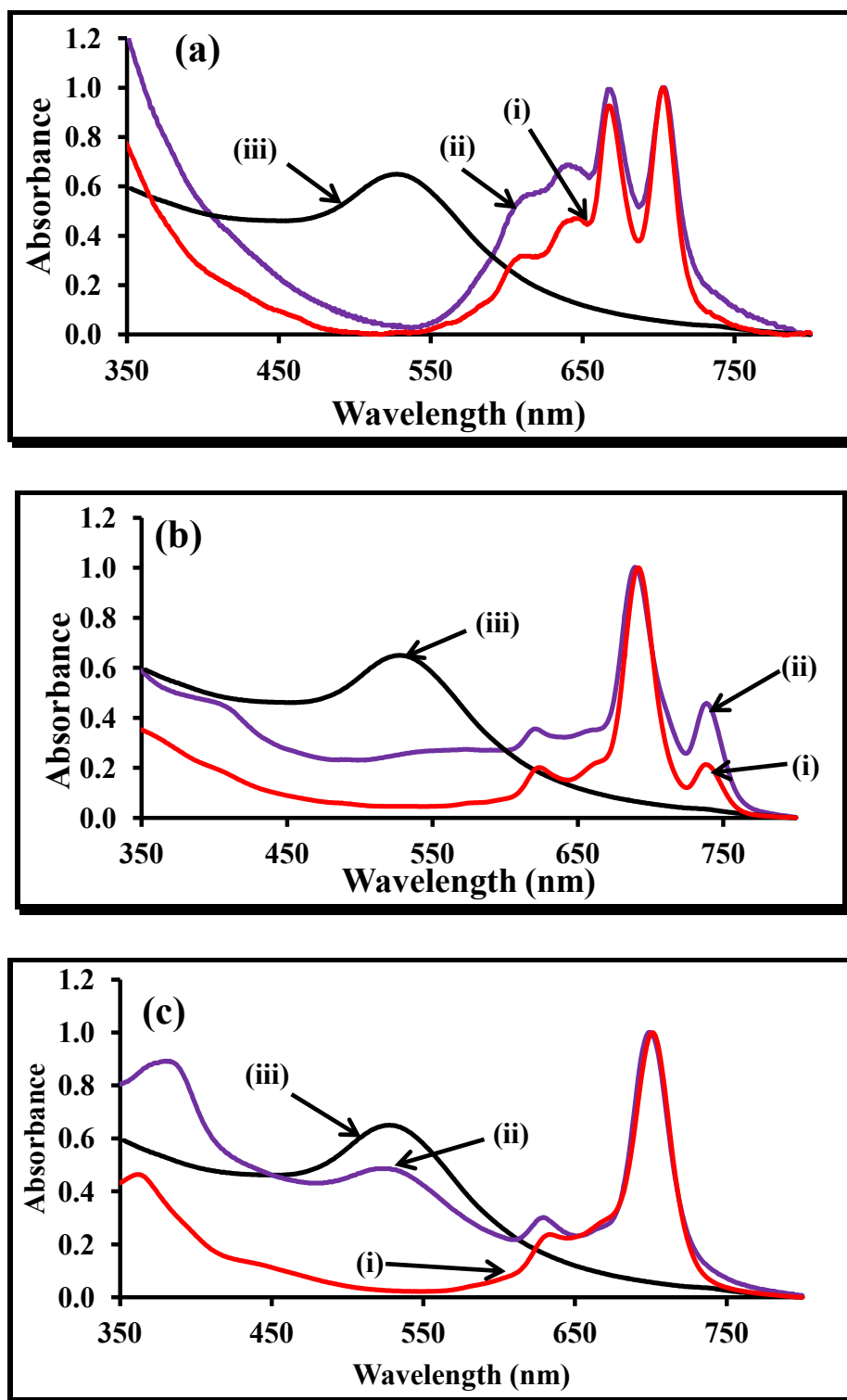


Figure 3.14: Absorption spectra of (a) compound 35a (i), 35a-AuNP (ii), TOABr-AuNPs (iii), (b) compound 36 (i), 36-AuNP (ii), TOABr-AuNPs (iii), (c) compound 37 (i), 37-AuNP (ii), TOABr-AuNPs (iii). Concentration = $\sim 1 \times 10^{-5}$ M.

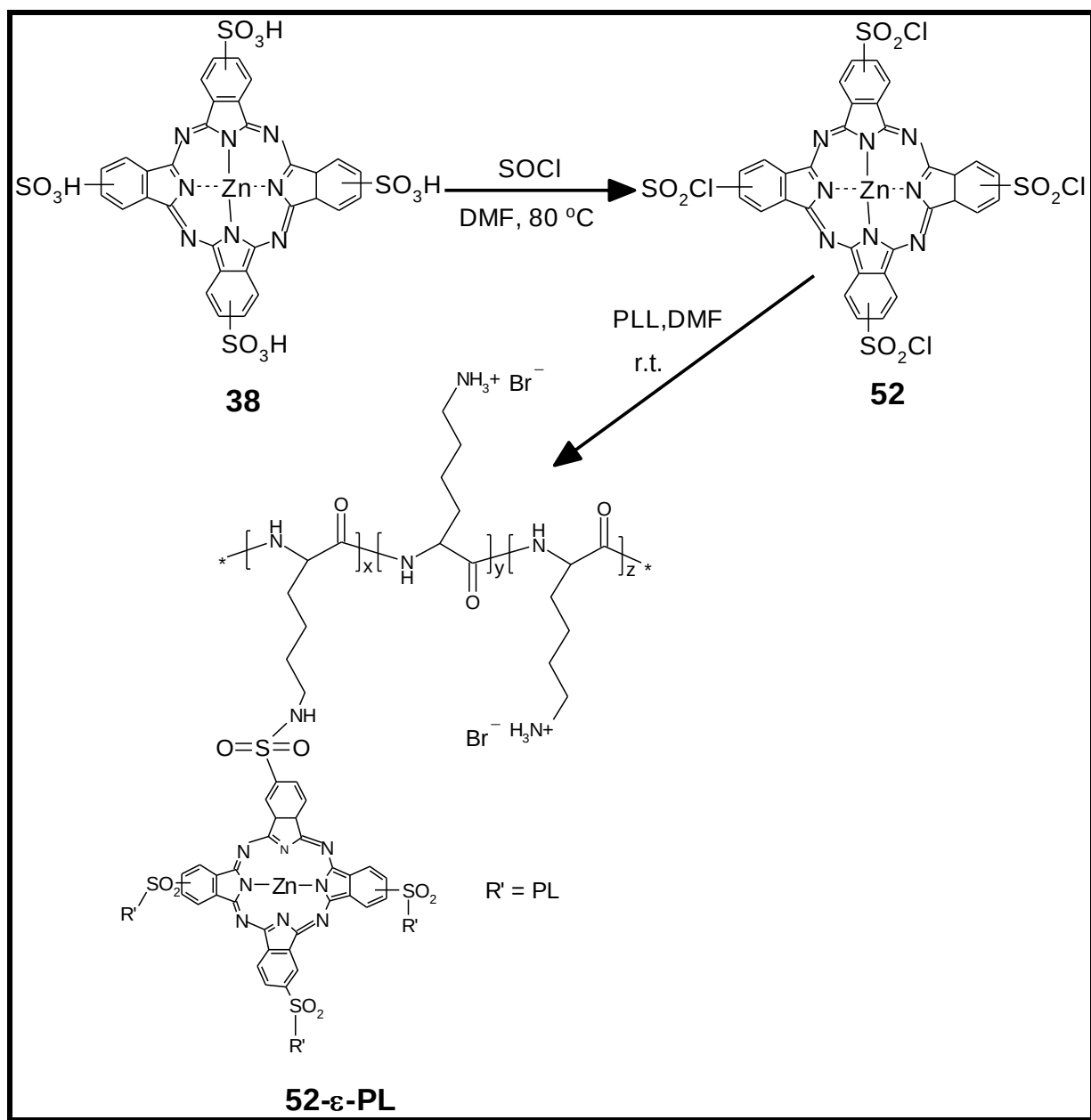
3.3.2 Assembly of phthalocyanine-poly-L-lysine structures

3.3.2.1 Synthesis and characterization of $\text{ZnPc}(\text{SO}_2\text{-}\epsilon\text{-PL})_4$ conjugate (**52- ϵ -PL**)

Tetrasulfonylchloride $\text{ZnPc}(\text{SO}_2\text{Cl})_4$ (**52**) was obtained from $\text{ZnPc}(\text{SO}_3)_4$ (Scheme 3.7, **38**) using well known procedures [270]. The complex is very unstable and is used as an intermediate without isolation [271]. Conjugation of complex **52** to ϵ -polylysine to form **52- ϵ -PL** was carried out in DMF by stirring the complex with the polymer overnight under argon atmosphere. TLC was used to confirm the formation of the conjugate, and the product was precipitated out of solution using methanol. **52- ϵ -PL** is soluble in water, even though **52** is not, confirming successful coordination of ϵ -PL to **52**.

Figure 3.15 shows the absorption spectrum of the conjugate in water. The **52- ϵ -PL** conjugate appears aggregated and the addition of Triton X 100 does not break the aggregation. The absorption spectrum of **52- ϵ -PL** is similar to that reported for the coordination of PL to $\text{CoPc}(\text{COOH})_4$ [272]. Additionally, SDS-PAGE and HPLC were used for characterization, discussed below.

HPLC analysis revealed that conjugation had occurred through the appearance of product peaks at retention times of 2.8, 3.0, 3.4, and 4.2 minutes (Figure 3.16b) compared to the single peak observed at 4.0 minutes (Figure 3.16a) for complex **38** in water. Using a diode array detector in the HPLC analyses also enables the confirmation of the presence of a Pc in the conjugate as judged by the presence of the Q-band.



Scheme 3.7: Synthesis of $\text{ZnPc(SO}_2\text{-poly-L-lysine)}_n = \text{52-}\epsilon\text{-PL}$ conjugate. n could be 1 to 4.

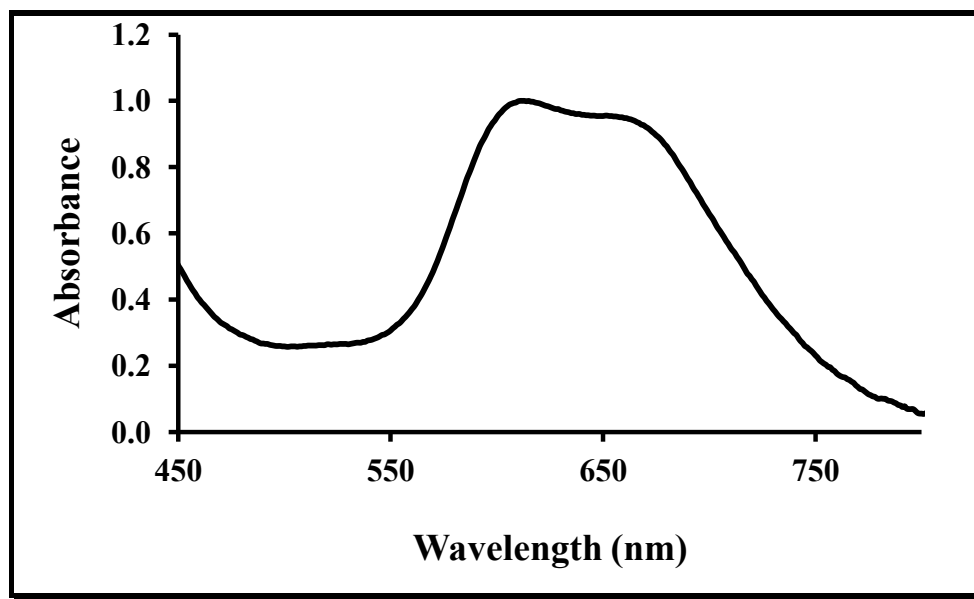


Figure 3.15: Absorption spectra of 52- ϵ -PL in water (pH 7.4).

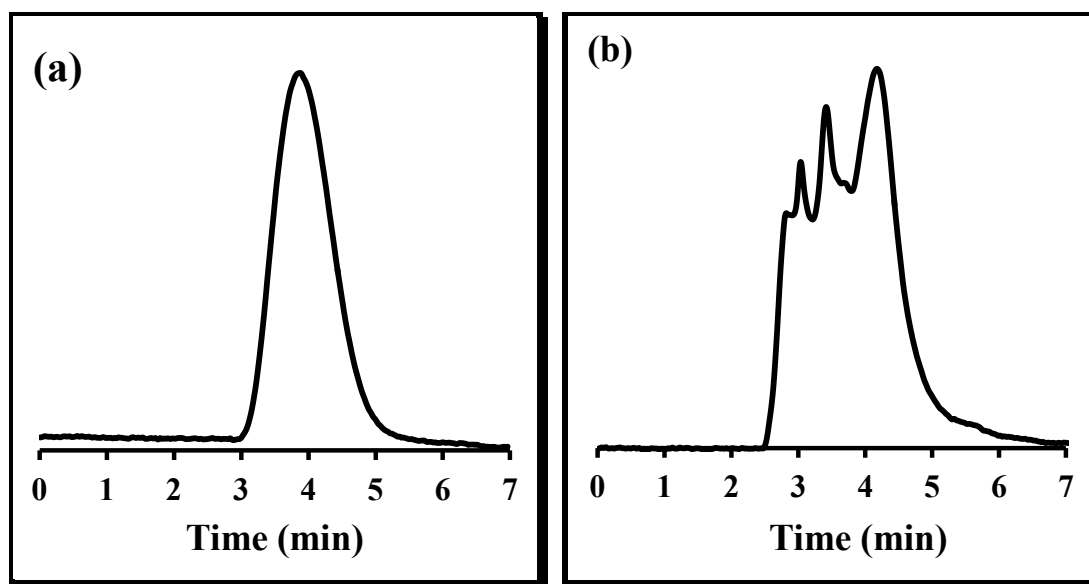
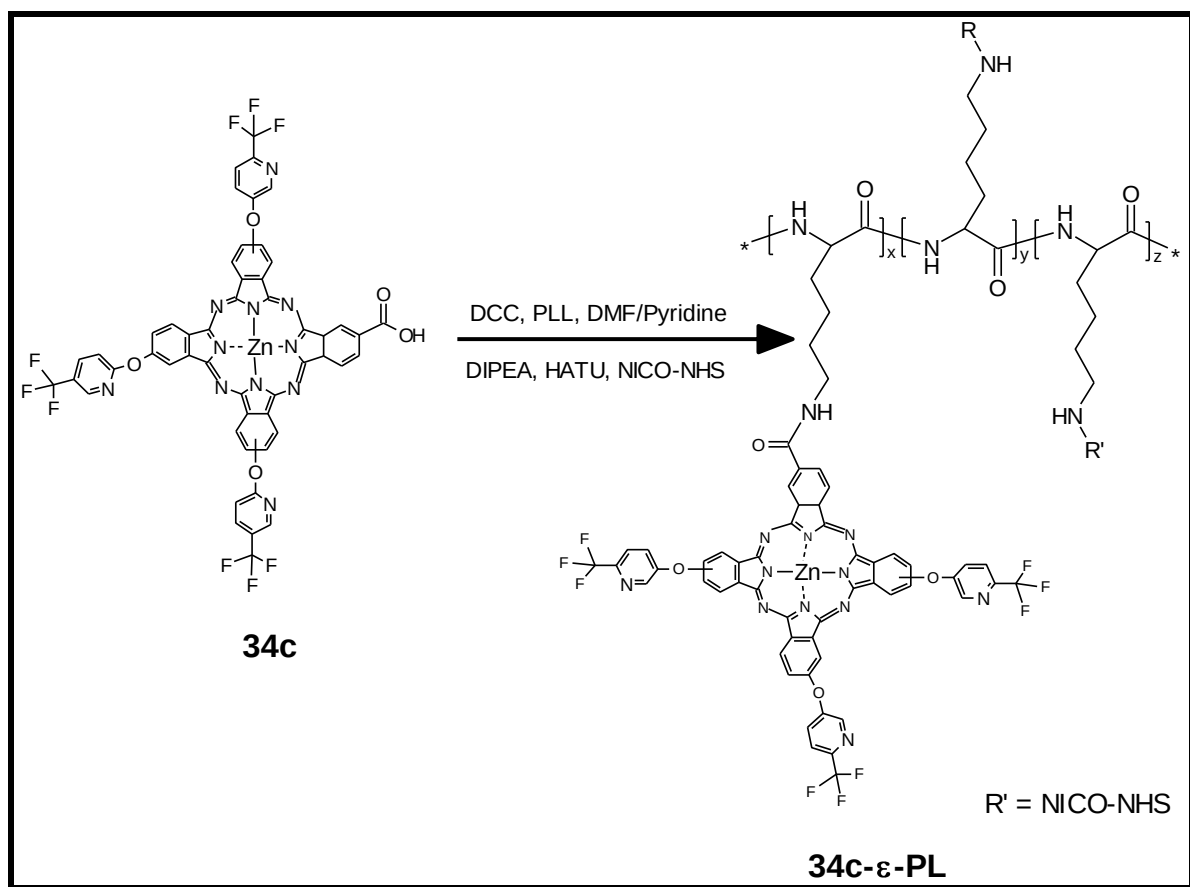


Figure 3.16: Analytical HPLC traces of (a) compound 38 and (b) 52- ϵ -PL.

3.3.2.2 Synthesis and characterization of 4-tetrakis-(5-trifluoromethyl-2-pyridyloxy) phthalocyaninato zinc(II)- ϵ -PL conjugate (**34c- ϵ -PL**).

Activation of the carboxylic group on complex **34c** was performed using DCC in the presence of a base in DMF as shown in Scheme 3.8. The lysine amino groups on the polymer chain were activated using DIPEA which ensured the essential ionization for reactivity. The activated complex **34c** was covalently coupled via the C-terminus of the polymer linked to the ϵ -NH₂ functional groups with an estimated 20 Pc units per polymer chain (using mole ratios). The remaining ϵ -NH₂ functions were capped with NICO-NHS. The coordination resulted in the water solubility even though **34c** is not water soluble. Figure 3.17 shows the absorbance spectrum of complex **34c** (in DMSO) and **34c- ϵ -PL** (in water). Complex **34c** has a Q-band absorption at 676 nm in DMSO and when conjugated to the polymer, the complex shows a resolved peak at 600 nm and a very broad peak at ~690 nm. A similar spectrum was obtained for CoPc(COOH)₄ in the presence of PL and diethylaminoethyl cellulose (DEAE) dextran, a positively charged resin [272]. HPLC analysis (Figure not shown) was used to show the formation of the product as was done for **52- ϵ -PL**.

Both conjugates (**52- ϵ -PL** and **34c- ϵ -PL**) migrated as broad, single bands on the SDS-PAGE, with the bands detected by Coomassie blue staining (Figure 3.18a). A standard curve (Figure 3.18b) was constructed from the distances migrated by each marker protein and the distance migrated by the conjugates was used to calculate the molecular weight by interpolation. The conjugates were found to have an average molecular weight of 25.79 kDa. This mass suggests the presence of both Pcs and ϵ -PL in the conjugate.



Scheme 3.8: Synthesis **34c-ε-PL** conjugate. R could be another molecule of complex **34c**.

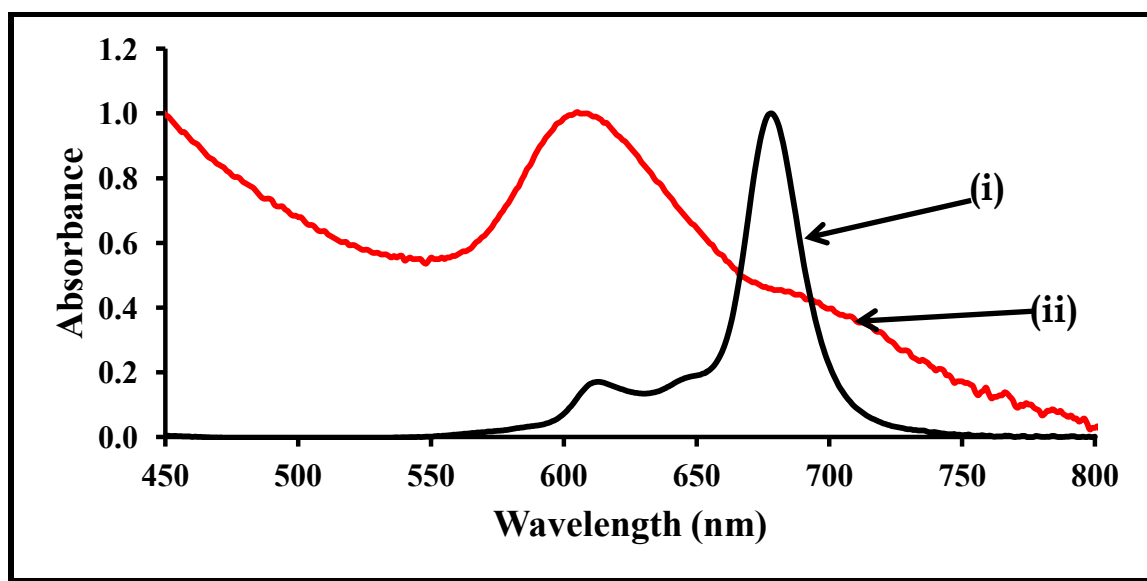


Figure 3.17: Absorption spectra of (i) **34c** in DMSO and (ii) **34c-ε-PL** in water.

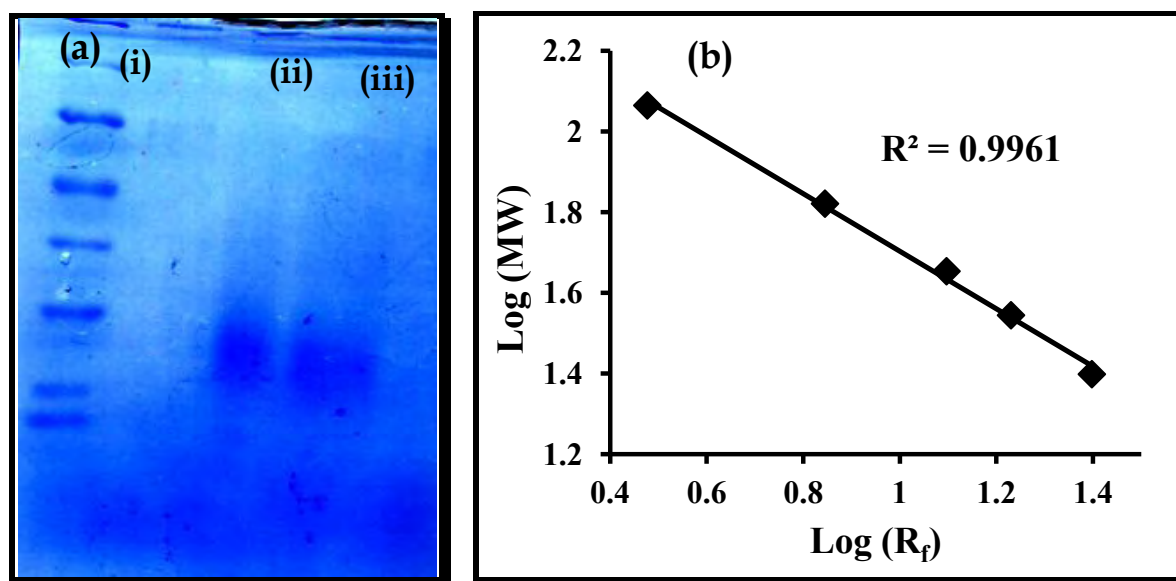
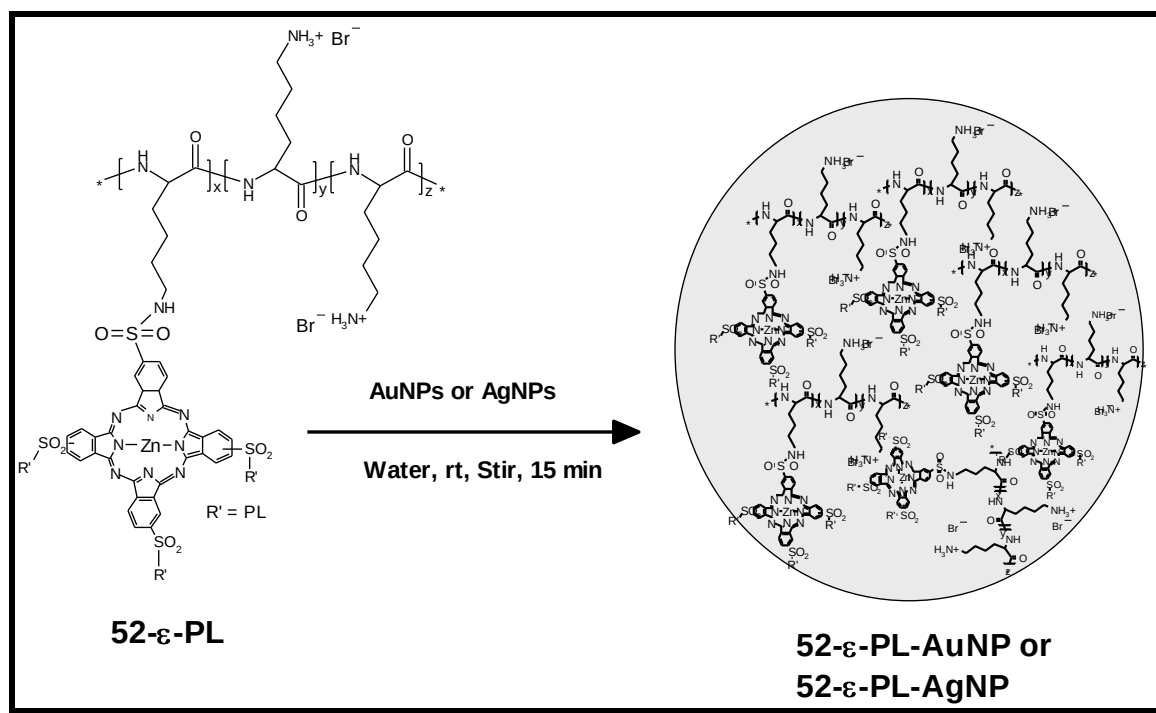


Figure 3.18: SDS-PAGE gel (a) molecular weight analysis from a protein marker (i), 52-ε-PL (ii) and 34c-ε-PL (iii) and the corresponding protein marker M_r (logarithm scale) vs relative mobility (logarithm scale) on SDS-PAGE (b).

3.3.2.3 Conjugation of phthalocyanine-polymer derivatives with nanoparticles

Conjugation was achieved by stirring a solution of either Au or Ag nanoparticles stabilized by citrate in the presence of complex 52-ε-PL or 34c-ε-PL. The mixture was left to stir for 15 minutes, as increased times lead to the formation of black precipitates. The conjugation of complexes 52-ε-PL and 34c-ε-PL to either gold or silver nanoparticles is shown in Scheme 3.9 and it relies upon the known [273] adsorption of amino groups onto gold and silver. Alkylamines have been observed to adsorb strongly onto gold nanoparticles [274, 275] via self-assembly [276]. Characterization of such amine functionalised nanoparticles indicated that the amine-gold interaction can be described by a weak covalent bond [277]. The same

behaviour is expected for the silver nanoparticles [278]. In this work the NH/NH₂ groups on **52-ε-PL** or **34c-ε-PL** are expected to adsorb onto the metallic nanoparticles.



Scheme 3.9: Synthetic route for complex **52-ε-PL** (or **34c-ε-PL**) conjugation of AuNP or AgNP.

Figure 3.19 (a and b) shows the TEM images of the nanoparticles. Both gold and silver nanoparticles appear to be well dispersed, see also Figure 3.12. However, in the presence of **52-ε-PL** or **34c-ε-PL**, the nanoparticles (Figure 3.19c and d) show an ordered form of aggregation suggesting the formation of **52-ε-PL-AuNP** or **52-ε-PL-AgNP**.

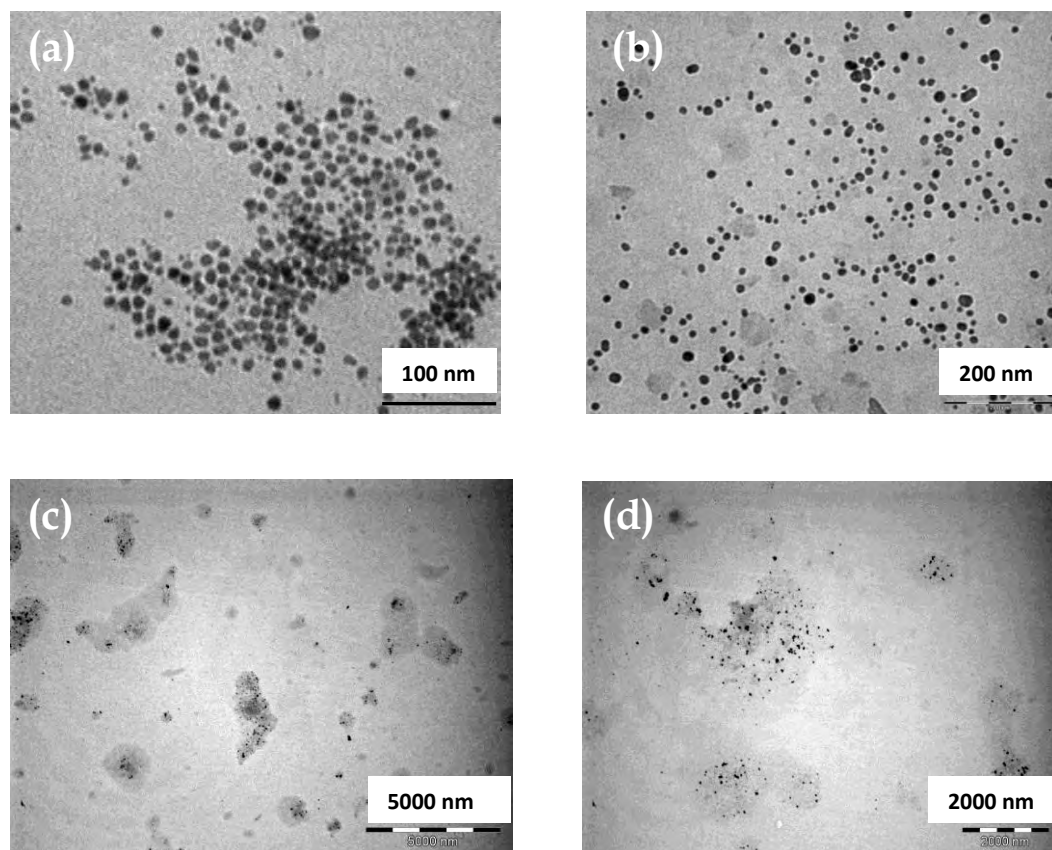


Figure 3. 19: TEM images of (a) citrate-AuNPs, (b) citrate-AgNPs (Scale: 200 nm) and 52- ϵ -PL in the presence of (c) AuNPs or (d) AgNPs.

3.3.3 Interaction of phthalocyanine with folic acid

There have been no studies on the influence of folic acid on the behaviour of phthalocyanines. Photodynamic activity of porphyrins in the presence of folic acid has however been reported [279]. In this work, we compare the effect of sulfur (complexes **35**, **36**, **37**, and **38**) versus oxygen (**34**) bridges on the photophysical behaviour of phthalocyanines in the presence or absence of folic acid. In the presence of folic acid (0.11 mmol) the absorption spectra of **34a** is split in DMSO due to the acid environment, which offsets the basic nature of DMSO (Figure not shown). In the presence of folic acid (Figure 3.20), the Q-band of compound **35a** showed enhanced splitting but aggregation was still prevalent. There were no spectral changes observed for complexes **34b**, **34c**, **35b**, **35c**, **36**, **37** and **38** in the presence of folic acid.

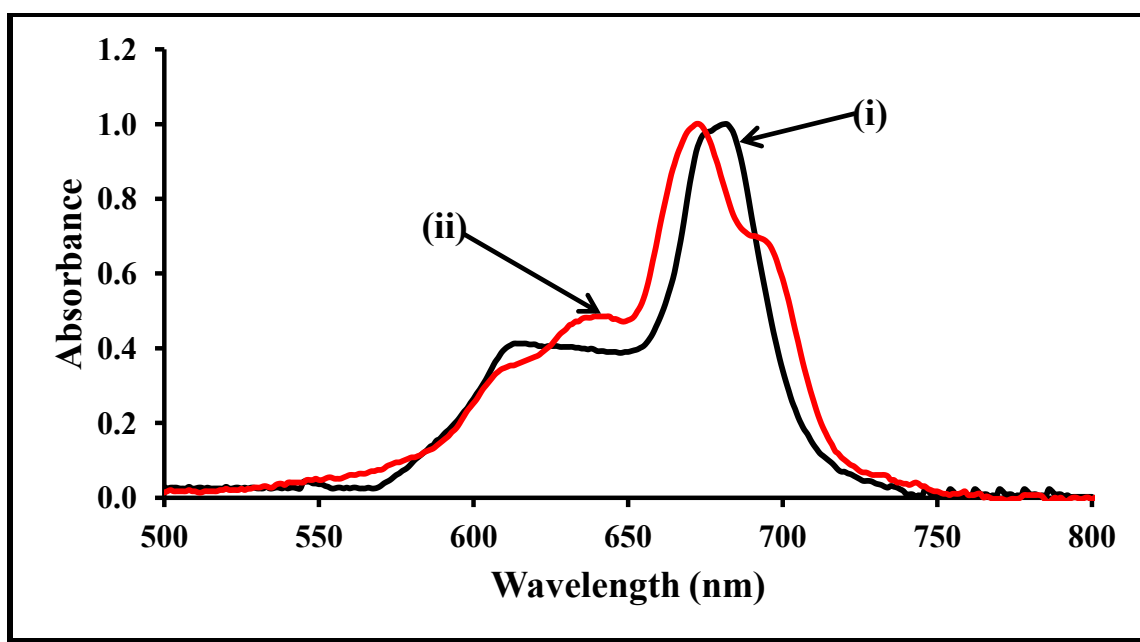


Figure 3.20: Absorbance spectra of **35a** in the absence (i) and presence (ii) of folic acid, in DMSO. Concentration = $\sim 1 \times 10^{-5}$ M.

3.3.4 Preparation of phthalocyanines for cell studies

An aqueous solvent mixture was employed to allow phthalocyanine complexes to dissolve while keeping the media still favorable for cells. The normalized ground state electronic absorption spectra using complex **37** as an example in DMSO (2 %)/PBS and DMSO(2 %)/PBS/Triton X 100 are shown in Figure 3.21. In the DMSO (2 %)/PBS mixture complex **37** was highly aggregated and the addition of a surfactant (Triton X 100) could not break the aggregation. The aggregation is due to the coplanar association of the rings, resulting in splitting and broadening of spectra, with a blue shifted peak due to the aggregate. Aggregation is not a desired feature for PDT since the aggregates are non-photoactive.

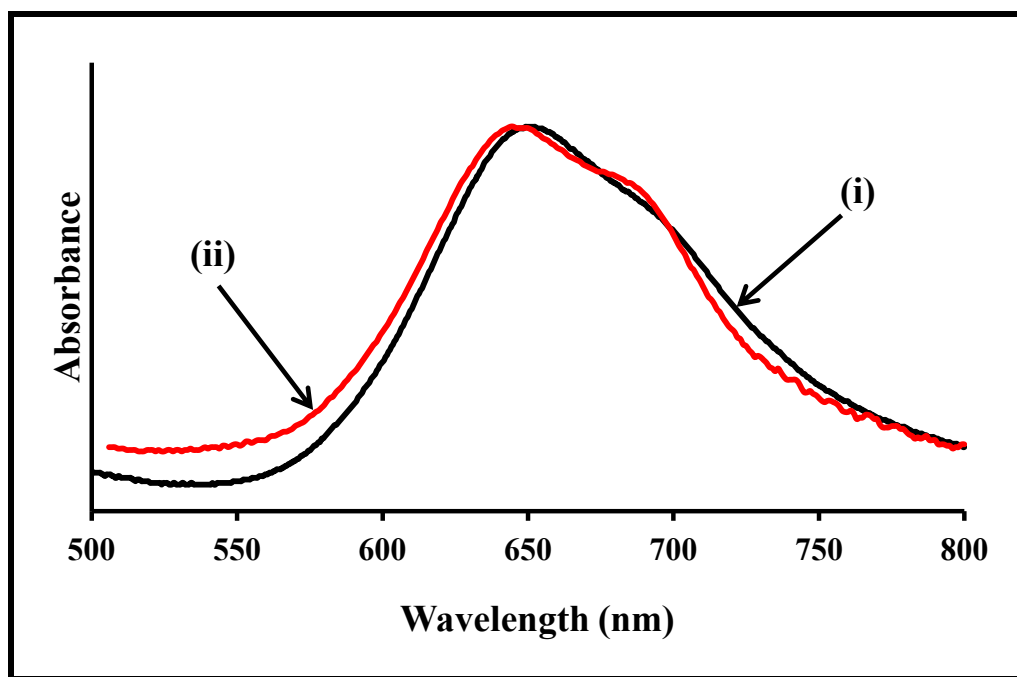


Figure 3.21: Absorption spectra **37** in (i) DMSO(2 %)/PBS, and in (ii) DMSO(2 %)/PBS/Triton X.

3.4 Conclusions

Novel low-symmetry metal-free, magnesium, zinc phthalocyanine and symmetrical zinc phthalocyanine derivatives have been successfully synthesized and characterized by spectroscopic methods. The complexes showed good solubility in THF with limited solubility in DMSO, DMF and DCM. The synthesis and spectroscopic characterization of phthalocyanines conjugated to drug delivery agents has also been reported. All complexes synthesized gave spectroscopic data which indicated high purity and in accordance with the predicted structures.

Chapter 4

Photophysical and photochemical properties

This chapter focuses on the photophysicochemical properties of synthesized phthalocyanine derivatives together with their corresponding conjugates.

4. Photophysical and photochemical properties

4.1. Fluorescence spectra, quantum yields and lifetimes

4.1.1 Phthalocyanine derivatives

4.1.1.1 Symmetrically substituted phthalocyanine derivative: **37**

The fluorescence behaviour of **37** in DMF or chloroform was similar in that the fluorescence spectra were mirror images of their excitation spectra. However, the excitation spectra were narrower than absorption spectra due to some aggregation in DMSO, Figure 4.1a. The complex showed a fluorescence emission peak at 704 nm in DMF, 706 nm in DMSO and 716 nm in chloroform, Table 4.1. The closeness of the Q-band absorption wavelength to the Q-band maxima of the excitation spectra in DMF suggests that the molecules in the ground and excited states are the same before and after excitation. The slight red shift in DMSO could reflect change in symmetry on excitation in this solvent.

Fluorescence was also recorded in DMSO/PBS which is the solvent mixture used for cell studies. The complex showed extreme aggregation in this solvent mixture which could not be broken by Triton X 100, Figure 4.1b(i). Aggregation is judged by the high energy band near 640 nm.

In the DMSO/PBS mixture, no fluorescence was observed for **37** due to the non-photoactive nature of aggregates. However a very weak emission was observed when Triton X 100 (Figure 4.1b) was added in this solvent mixture. A lack of agreement was observed between the absorption and fluorescence excitation spectra; the high energy band associated with the dimer was not observed in the fluorescence excitation spectra since only the monomer fluoresces [280].

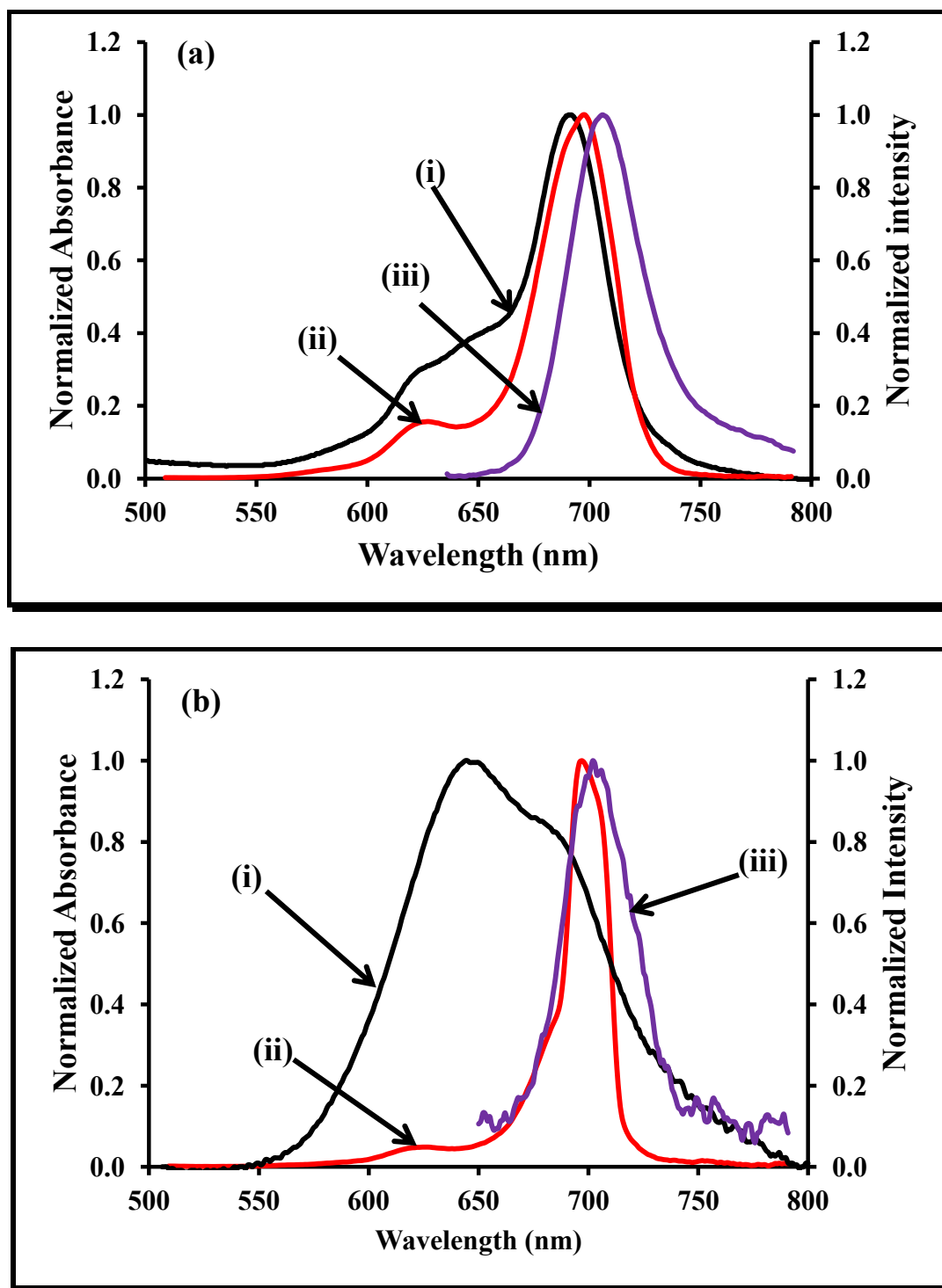


Figure 4.1: Absorption (i), excitation (ii), and emission spectra (iii), of complex 37 in (a) DMSO and (b) DMSO/PBS/Triton X.

Table 4.1 shows the fluorescence quantum yields (Φ_F) obtained for compound **37**. The highest fluorescence quantum yield was observed in chloroform with a value of 0.15, followed by 0.14 in DMSO and 0.11 in DMF. The fluorescence quantum yield was determined to be lower than 0.01 for complex **37** in the DMSO/PBS/Triton X 100 solvent mixture. For aggregated species, selection rules entail a large reduction in the fluorescence quantum yield, due to the increased rate of internal conversion from the first excited singlet state.

Figure 4.2 shows the time-resolved fluorescence decay curve of **37** in DMSO, indicating a mono-exponential decay. χ^2 of near unity was obtained for the fit in Figure 4.2. Similar behavior was observed for the complex in chloroform and DMF. As expected, in the DMSO/PBS/Triton-X 100 mixture, complex **37** gave a low fluorescence lifetime value of 0.07 ns due to the Pcs highly aggregated nature, Table 4.2. In chloroform, DMF and DMSO however, typical phthalocyanine τ_F values of 2.51 ns, 2.32 ns and 2.35 ns respectively were obtained (Table 4.2) [281]. Two lifetimes (0.07 and <0.01) were observed for the complex in the DMSO/PBS/Triton-X 100 mixture. The first lifetime one is due to free monomeric species and the other due to monomers interacting or mixed with aggregates which account for the reduced lifetime and abundance.

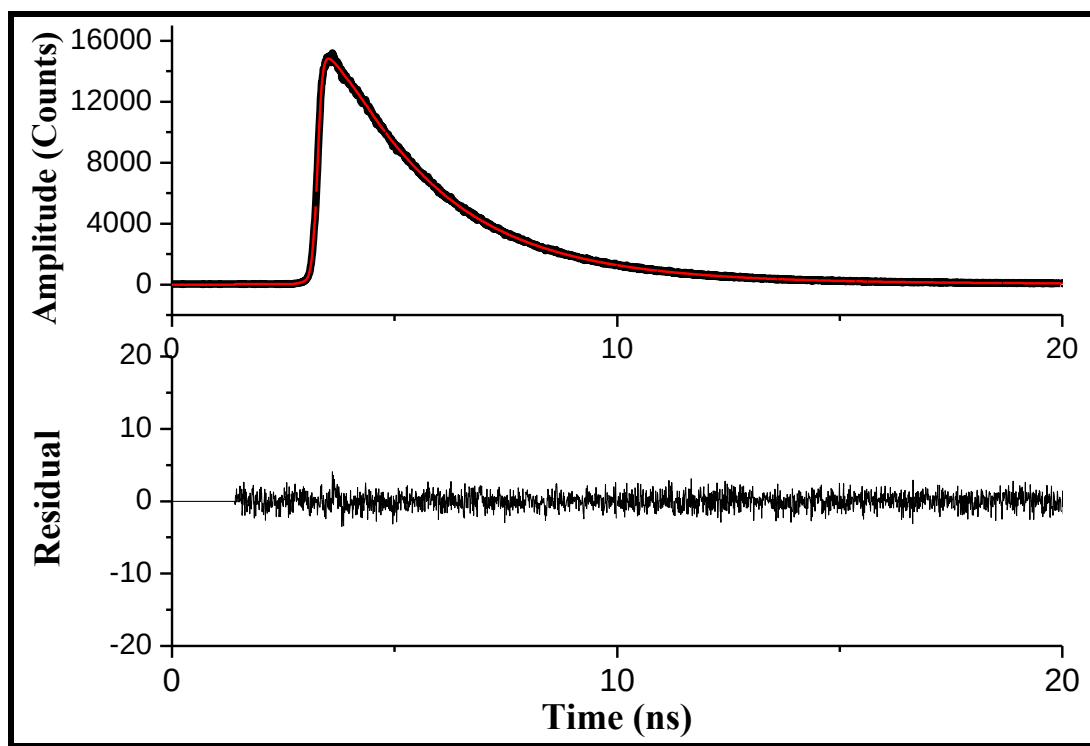


Figure 4.2: TCSPC trace of **37** in DMSO.

Table 4.1: Fluorescence parameters of synthesized phthalocyanines. DMF contains <1 % THF for complexes **32** and **33** to aid solubility.

Pc	Solvent	Q_{abs} (nm)	Q_{exc} (nm)	Q_{ems} (nm)	Φ_{F} (± 0.02)
32a	THF	710, 680	713, 678	718	0.16
	DMF	709, 679	711, 683	716	0.14
32b	THF	697	695	703	0.23
	DMF	689	693	702	0.25
32c	THF	680	680	685	0.05
	DMF	684	686	690	0.09
	CHCl ₃	691	697	708	0.10
33a	THF	715, 685	716, 687	722	0.10
	DMF	713, 680	715, 682	721	0.07
33b	THF	740, 710	743, 713	749	0.21
	DMF	743, 716	745, 718	751	0.22
33c	THF	685	687	698	0.12

Table 4.1 continued

Pc	Solvent	Q_{abs} (nm)	Q_{exc} (nm)	Q_{ems} (nm)	Φ_{F} (± 0.02)
33c	DMF	686	685	693	0.09
35a	CHCl ₃	703, 667	701, 669	708	0.04
	DMSO	682	685	693	0.09
35b	DMSO	682	686	693	0.35
35c	DMSO	684	687	693	0.13
36	CHCl ₃	692	696	704	0.05
	DMSO	694	695	706	0.06
37	CHCl ₃	704	705	716	0.15
	DMF	694	694	704	0.11
	DMSO	691	698	706	0.14
	DMSO(2 %)/PBS	642	-	-	-
	DMSO(2 %)/PBS/Triton X-100	690 ^a , 642	695	699	<0.01
38	DMSO	679	676	683	0.07

a = an approximation

Table 4.2: Time correlated single photon counting (TCSPC) parameters of synthesized phthalocyanine derivatives.

Pc	Solvent	τ_{F1} (ns) (± 0.01)	Relative Amplitude (A_1) (%)	τ_{F2} (ns) (± 0.04)	Relative Amplitude (A_2) (%)
32a	THF	2.41	100	-	-
32b	THF	5.13	100	-	-
32c	THF	2.31	100	-	-
	CHCl ₃	4.50	100	-	-
33a	THF	2.52	100	-	-
33b	THF	5.23	100	-	-
33c	THF	2.51	100	-	-
34a	DMSO	2.80	92	1.21	8
34b	MeOH	5.31	100	-	-
	DMSO	5.13	100	-	-
34c	MeOH	2.92	100	-	-
	DMSO	2.72	100	-	-

Table 4.2 continued

Pc	Solvent	τ_{F1} (ns) (± 0.01)	Relative Amplitude (A ₁) (%)	τ_{F2} (ns) (± 0.04)	Relative Amplitude (A ₂) (%)
35a	CHCl ₃	2.51	100	-	-
	DMSO	2.42	93	0.82	7
35b	DMSO	4.93	100	-	-
35c	DMSO	2.51	100	-	-
36	CHCl ₃	2.83	100	-	-
	DMSO	2.72	100	-	-
37	CHCl ₃	2.51	100	-	-
	DMF	2.32	100	-	-
	DMSO	2.35	100	-	-
	DMSO(2 %)/PBS/Triton	0.07	80	<0.01	20
38	DMSO	2.74	98	0.5	2

4.1.1.2 Unsymmetrically substituted phthalocyanine derivatives

Naphthoxy phthalocyanine derivatives (**32**, **33** and **36**)

The data was in DMF containing <1 % THF due to low solubility in DMF. The solubility was even worse in DMSO even with <0.1 % of THF; hence in this chapter complexes **32** and **33** are in a THF/DMF solution. Complexes **32b** and **33b** were soluble in DMSO but for comparison, THF/DMF was used.

The excitation spectra of **32a** and **33a** were similar to the corresponding absorption spectra in that there was no change in Q-band positions, but the relative intensities of the split pair were different. This suggests a slight change in symmetry upon excitation, Figures 4.3a and 4.3b. Low symmetry Pc complexes, such as that of unmetallated Pcs, fluoresce with only one main peak which is assigned as the 0-0 transition of the fluorescence [280], hence the observation of a single main emission peak in Figures 4.3a and 4.3b.

The steady-state fluorescence emission spectra of MgPcs (**32b** and **33b**) were performed in THF (Figure 4.4a and 4.4b). The figures show a typical Pc absorbance and emission spectra with the excitation spectra still showing a split Q-band. However, Pcs tend to change symmetry upon excitation as observed for complex **33b** where the high energy band of the excitation spectrum (710 nm) appears to be less defined with a lower intensity upon excitation. The emission spectra show one peak, as expected for low symmetry Pc complexes. The corresponding zinc phthalocyanines (**32c** and **33c**) show excitation spectra which are similar to

absorption spectra with both being mirror images of emission, Figure 4.5a and 4.5b. The small Stokes shifts observed indicates that the excited state energy is nearly identical to the ground state energy of the singlet state.

The values of fluorescence quantum yields (Φ_F) obtained for all complexes in THF and DMF are summarized in Table 4.1. As expected the MgPc complexes showed the highest quantum yield values, this is due to the small nature of the Mg central atom that encourages fluorescence as opposed to intersystem crossing (ISC) to populate the triplet state, observed for heavy metals such as Zn. For complexes **32** and **33**, complex **32b** had the largest value ($\Phi_F = 0.23$) followed by complex **33b** ($\Phi_F = 0.21$) in THF. The ZnPc derivatives exhibited low Φ_F values compared to the corresponding MgPc derivatives, thus further emphasizing the principle that the presence of a heavy metal encourages intersystem crossing to the triplet state. The H₂Pcs, **32a** and **33a** show mediocre fluorescence quantum yield values

Time-resolved fluorescence measurements showed a mono-exponential decay (Table 4.2) for complexes **32** and **33**, with fluorescence lifetimes (τ_F) between 2.31 and 2.52 ns in THF for the unmetallated and Zn derivatives. The MgPcs showed much higher lifetimes of 5.13 and 5.23 ns for **32b** and **33b** respectively, which is not surprising given the high Φ_F values observed for both Pcs. The fluorescence lifetime values fall within the range typical for most monomeric phthalocyanines [281].

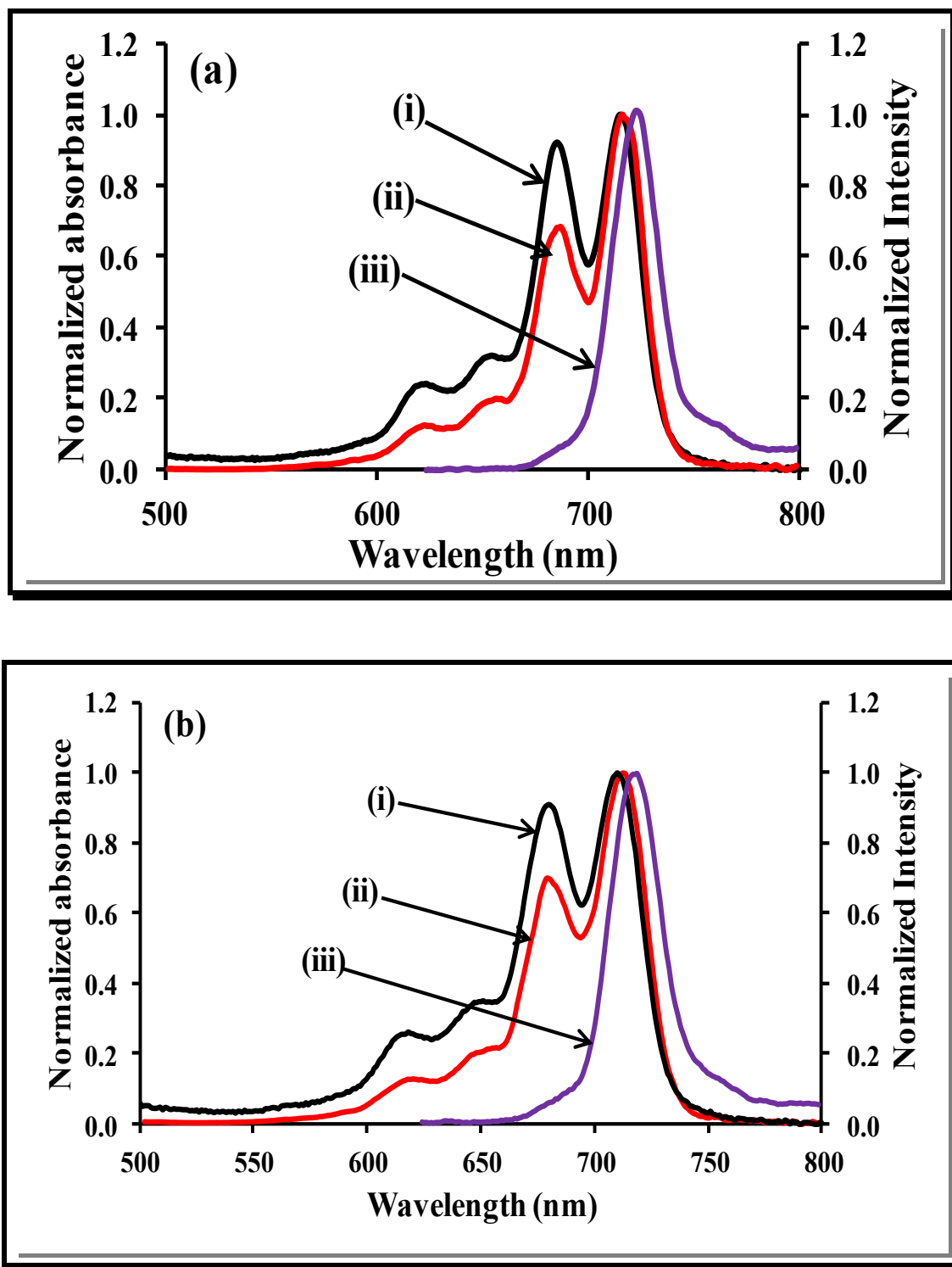


Figure 4.3: Absorption (i), excitation (ii), and emission (iii), spectra of (a) 32a and (b) 33a in THF.

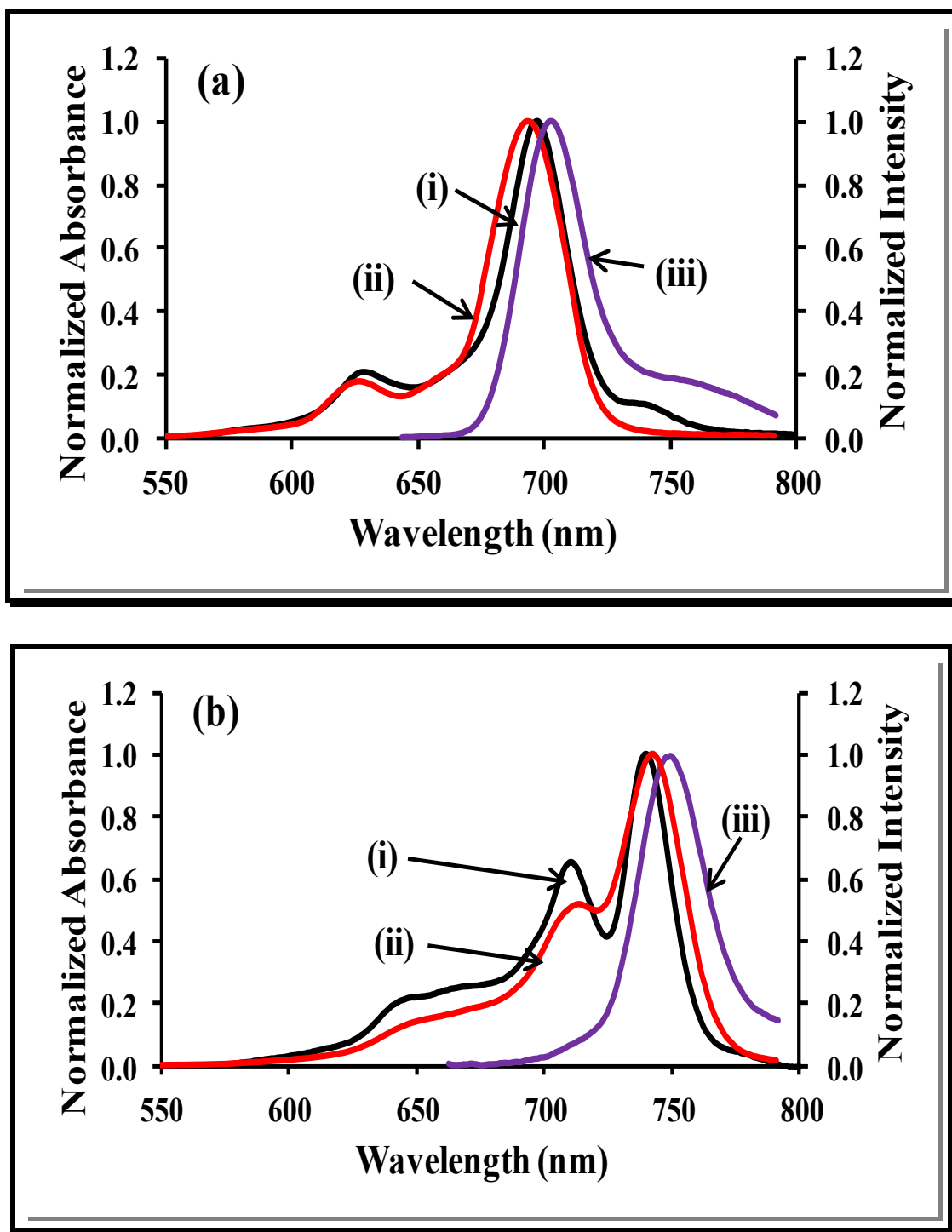


Figure 4.4: Absorption (i), excitation (ii), and emission spectra (iii), of (a) **32b** and (b) **33b** in THF.

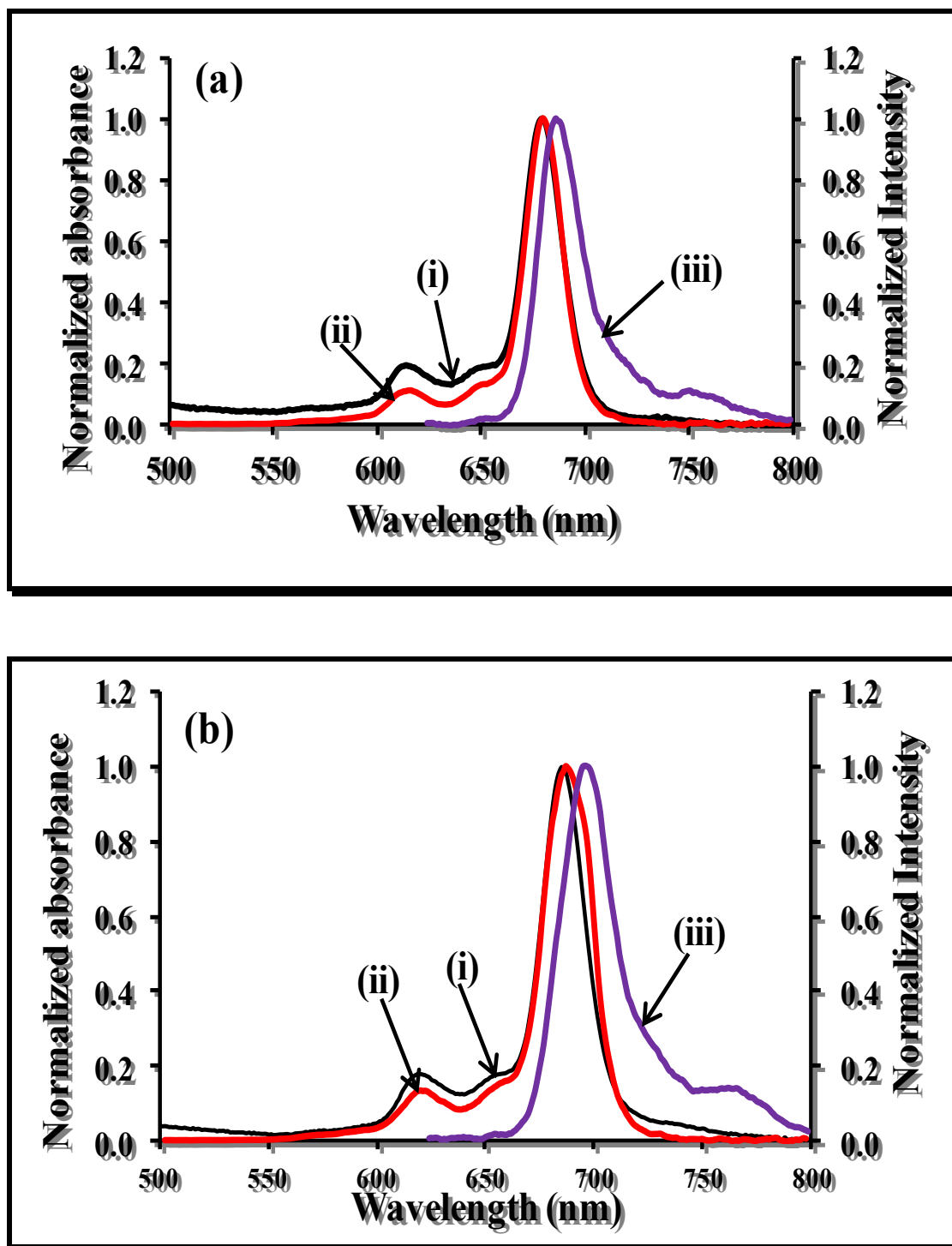


Figure 4.5: Absorption (i), excitation (ii), and emission spectra (iii), of (a) 32c and (b) 33c in THF.

The fluorescence excitation spectrum of **36** (in chloroform and DMSO) was a mirror image of the emission spectrum and was similar to the absorption spectra as shown in Figure 4.5a for **32c**. Again, the closeness of the Q-band maxima for absorption and excitation spectra shows that there are no changes in the configuration of the complexes following excitation. Comparing **32c** and **36** shows that the presence of the thiol group in complex **36** lowered the fluorescence quantum yield value from 0.10 (**32c**) to 0.05 for **36**, in chloroform, Table 4.1. Time-resolved fluorescence measurements for complex **36** indicate a mono-exponential decay (Table 4.2), with fluorescence lifetimes (τ_{F1}) of 2.83 (CHCl₃) and 2.72 (DMSO) ns indicating only one species in the solution.

Fluorinated phthalocyanine derivatives (**34** and **35**)

Figures 4.6a-c show the absorption, emission and excitation spectra of complexes **34a** in DMSO and **34b** in methanol. As discussed in chapter 3, the Q-band is not split in DMSO in Figure 4.6a for **34a**, due to the basic nature of the solvent. An incomplete splitting of the Q-band is observed on excitation, Figure 4.6a(ii). The emission spectrum for **34a** is however not split, as explained above. The metallated complexes **34b** and **34c**, showed absorption and excitation spectra which were similar and mirror images of emission spectra (Figures not shown) in DMSO. Fluorescence behaviour (for **34b** and **34c**) were also evaluated in methanol since the toxicity of this solvent is very low in biological systems [282], making it compatible with biological media (complex **34a** is not soluble in methanol). Figure 4.6b shows the absorption,

emission, and excitation spectra of complex **34b** in methanol. Some broadening is observed in Figure 4.6b for the complex due to aggregation. However, the excitation and emission spectra are narrower since aggregates do not fluoresce.

The fluorescence quantum yield (Φ_F) for **34a** was low at $\Phi_F = 0.09$ (Table 4.1). Unmetallated phthalocyanines have been reported to give low Φ_F values depending on the substituents, with the values actually improving on insertion of the Zn central metal [283]. This is contrary to what would be expected based on the heavy atom effect where Zn would be expected to show low Φ_F values since it encourages intersystem crossing to the triplet state. Thus unmetallated **34a** gave $\Phi_F = 0.09$ compared to **34c** with $\Phi_F = 0.20$, Table 4.1. Complex **34b** containing Mg in the central cavity, showed a larger Φ_F value (0.52) in DMSO compared to complex **34c** containing Zn central metal ($\Phi_F = 0.20$). MgPcs have been shown to have high Φ_F values [284, 285]. The fluorescence quantum yield value of **34c** in methanol was considerably lower than the value obtained in DMSO whereas for **34b** the value was slightly higher than that obtained in DMSO. The intense aggregation of complex **34c** (figure not shown) in methanol significantly decreased its quantum yield value.

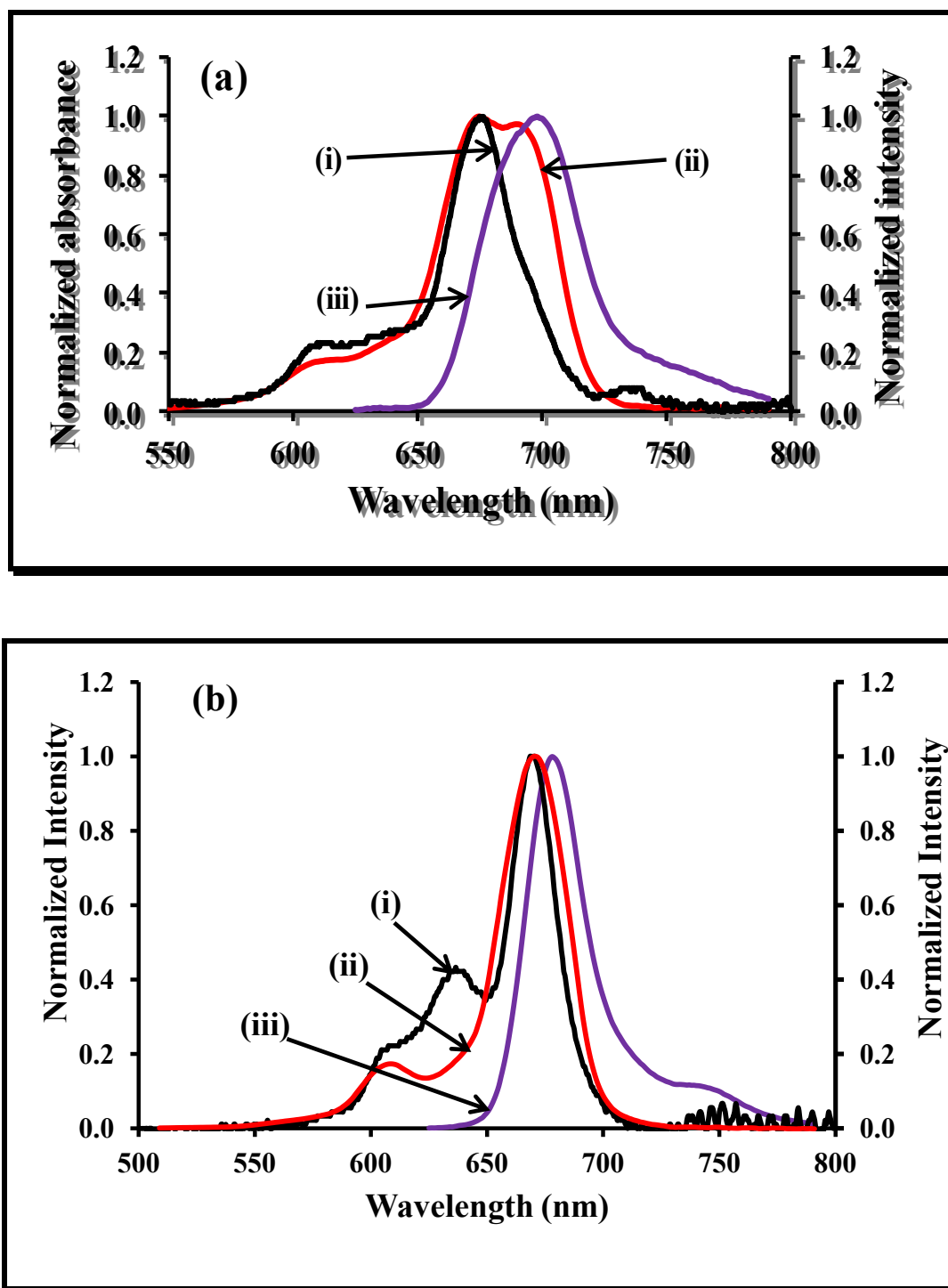


Figure 4.6: Absorption (i), excitation (ii), and emission (ii), spectra of (a) **34a** in DMSO and (b) **34b** in MeOH.

Time-resolved fluorescence measurements indicate a bi-exponential decay (Table 4.2) for complex **34a**, with fluorescence lifetimes (τ_F) of 2.80 and 1.21 ns. The first component is due to the deprotonated phthalocyanine derivative in DMSO and the second component may be due to protonated derivatives. Complexes **34b** and **34c** showed mono-exponential decay with lifetime values of 5.13 and 2.72 ns, respectively in DMSO. For complexes **34b** and **34c** in methanol, the lifetimes were 5.31 and 2.92 ns, respectively. Again, these values fall within the range typical for most monomeric metallated phthalocyanines [281]. As expected, complex **34b** had a higher lifetime compared to **34c** (in both DMSO and methanol) and **34a** (in DMSO), Table 4.2. The complex had high fluorescence quantum yield values and thus should be accompanied by corresponding high fluorescence lifetimes as reflected by the data shown in Table 4.2.

Figure 4.7a shows the absorbance, emission and excitation spectra of **35a**. The differences in the excitation and absorption spectra in the 630 nm region, Figure 4.7a, are due to aggregation in the latter and aggregates do not fluoresce. The fluorescence quantum yield value was determined to be 0.09 in DMSO, Table 4.1. Figure 4.7b shows the absorption, emission and excitation spectra of **35a** in chloroform. The excitation spectra showed the typical split Q-band at the same wavelength positions as the absorption spectra. Fluorescence quantum yield was determined to be 0.04, Table 4.1.

The absorption and fluorescence excitation spectra of **35b** and **35c** (Figures not shown) in DMSO are almost identical implying that the absorbing species do not differ much from the fluorescing species. The emission spectra were mirror images

of the absorption spectra except for the slight broadening in the latter due to aggregation as discussed above.

The time-resolved fluorescence lifetime measurements of **35a** in DMSO showed a bi-exponential decay suggesting two species in solution (Table 4.2) with fluorescence lifetimes (τ_F) of 2.42 and 0.82 ns for the same reasons given for compound **34a**. The metallated phthalocyanines (**35b** and **35c**) showed mono-exponential decay with lifetime values of 4.93 and 2.51 ns, respectively. These values also fall within the range typical for most monomeric metallated phthalocyanines [281]. Complex **35b** had the longer lifetime compared to **35a** and **35c**, Table 4.2.

In summary, the proximity of the wavelength of each component of the Q-band absorption to the Q-band maxima of the excitation spectra for symmetrical and unsymmetrical metallated phthalocyanine complexes (Table 4.1) suggest that the nuclear configurations of the ground and excited states are similar and not affected by excitation, with a few exceptions. The Stokes' shifts range from 1 to 15 nm, which is usual for ZnPc derivatives [286]. For most complexes in Table 4.2, one lifetime is observed in DMSO or THF, with the exception of unmetalated **34a** and **35a**. One lifetime shows that there is one species in solution confirming the purity of the complexes. For unmetalated Pcs in DMSO, the deprotonation leads to two lifetimes. One lifetime represents the protonated Pc and the other lifetime represent Pcs that are not deprotonated.

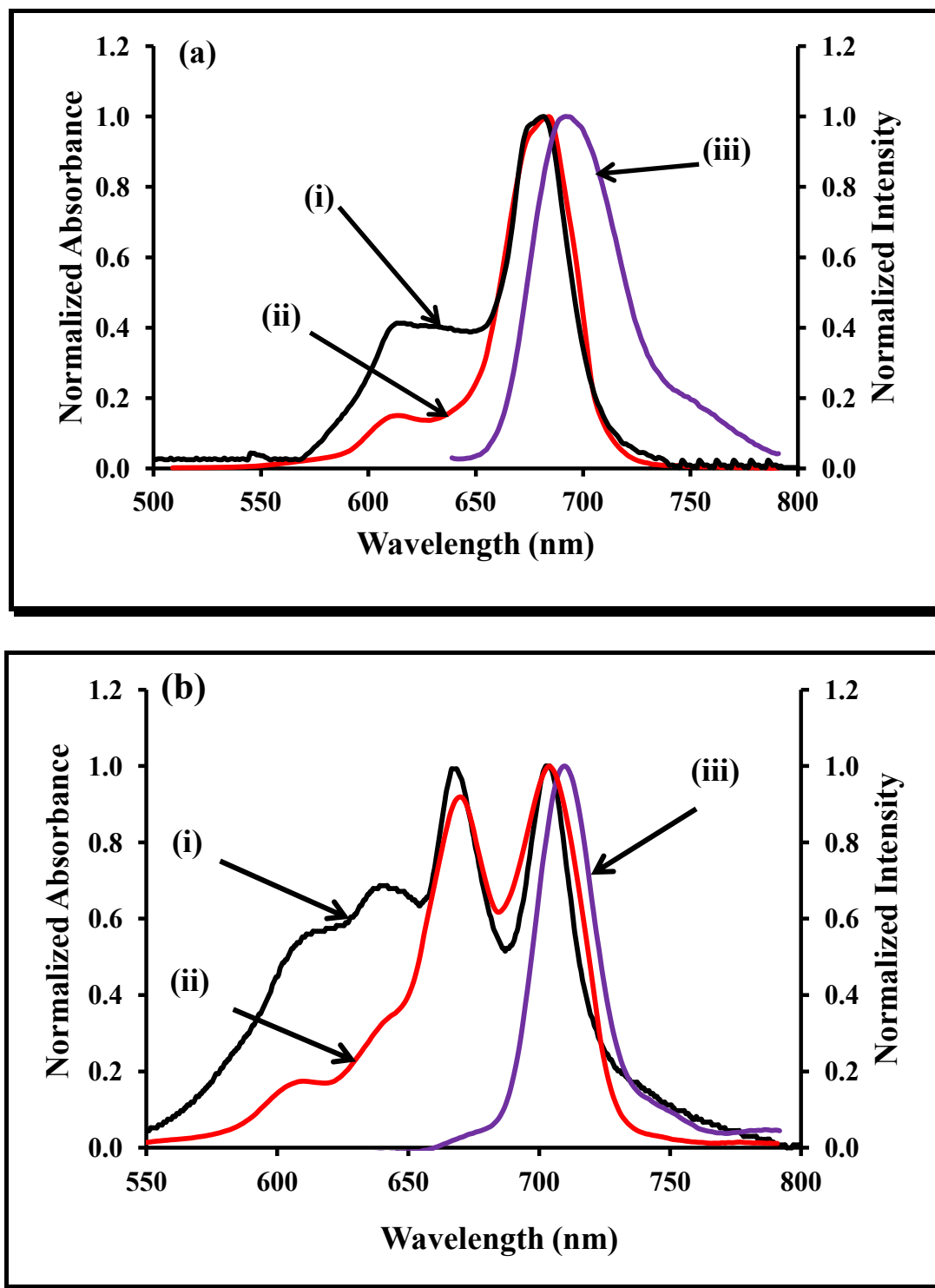


Figure 4.7: Absorption (i), excitation (ii), and emission (iii), spectra of (a) 35a in DMSO and (b) 35a in chloroform.

4.1.2 Phthalocyanine conjugates

4.1.2.1 Phthalocyanine-nanoparticle conjugates

The absorption, emission and excitation spectra of **35a**-AuNP in chloroform showed similar behaviour to complex **35a** alone. The nearness of the absorption Q-band wavelength (especially the low energy component) with the excitation Q-band wavelength (for **35a** or **35a**-AuNP) implies that the nuclear configurations of the ground and excited states are similar and not affected by excitation, Table 4.3. The fluorescence quantum yield of **35a** was slightly higher than for **35a**-AuNP (Table 4.3) confirming that the presence of AuNPs lowers the Φ_F , and that AuNPs quench fluorescence as has been reported before [268], contrary to when cobalt tetraamino phthalocyanine interacts with AuNPs [287]. The quantum yield value for **36**-AuNP was however similar to that of the free phthalocyanine. The conjugation of AuNPs did not have a significant effect on the Φ_F value for **36**-AuNP.

A decrease in the fluorescence quantum yield of **37**-AuNP compared to **37** was rather significant confirming that the presence of the AuNPs strongly quenches fluorescence as was also the case for **35a**-AuNP. **37**-AuNP did not show any fluorescence behaviour in DMSO(2 %)/PBS or DMSO(2 %)/PBS/Triton X 100.

Typically, Pcs in close proximity with AuNPs experience emission quenching which can originate either from enhanced intersystem crossing due to the heavy atom effect or through direct energy transfer from the Pc to the AuNPs. This energy transfer is called surface energy transfer (SET) as the photo-excited Pc transfers its energy to the AuNP, upon relaxation back to the ground state. This phenomenon generally occurs for gold nanoparticles with small diameters [288] and that are larger than for Foster

resonance energy transfer (FRET) to occur [289]. It is possible that the slight aggregation observed for phthalocyanines when conjugated to AuNPs can result in self-quenching decreasing the fluorescence quantum yields further.

Conjugate **35a**-AuNP showed a bi-exponential decay in chloroform where the first component (with τ_{F1} of 2.61) may be due to the presence of free phthalocyanines and the second component (with τ_{F2} of 1.12 ns, Table 4.4) may be due to derivatives conjugated to the nanoparticles. Conjugate **36**-AuNP also showed bi-exponential decay in chloroform with $\tau_{F1} = 2.52$ ns, and $\tau_{F2} = 0.82$ ns, Table 4.4. The second components for **35a**-AuNP and **36**-AuNP were short-lived with low abundance. Conjugate **37**-AuNP, however, showed a mono-exponential decay suggesting that the phthalocyanines attached on the gold surface are highly quenched to the point where they are not fluorescent and only the fluorescence lifetime of the free Pc is observed.

Table 4.3: Absorption and fluorescence parameters of phthalocyanine conjugates.

Conjugate	Solvent	^a Q _{abs} (nm)	Q _{exc} (nm)	Q _{ems} (nm)	^a Φ _F (±0.01)
35a-AuNP	CHCl ₃	704, 667 (703, 667)	703, 670	709	0.02 (0.04)
36-AuNP	CHCl ₃	689 (692)	694	701	0.04 (0.05)
37-AuNP	CHCl ₃	698 (704)	704	716	0.07 (0.15)
34a + folic acid	DMSO	665, 692 (676)	703	666, 697	0.09 (0.09)
34b + folic acid	DMSO	676 (676)	689	678	0.49 (0.52)
34c + folic acid	DMSO	678 (678)	690	681	0.20 (0.20)

^a = values in brackets are for Pc complex alone.

Table 4.3 continued

Conjugate	Solvent	^a Q _{abs} (nm)	Q _{exc} (nm)	Q _{ems} (nm)	^a Φ _F (±0.01)
35a + folic acid	DMSO	698, 670 (682)	703, 675	710	0.07 (0.09)
35b + folic acid	DMSO	683 (682)	685	693	0.33 (0.35)
35c + folic acid	DMSO	682 (684)	683	693	0.15 (0.13)
36 + folic acid	DMSO	695 (694)	706	697	0.03 (0.06)
37 + folic acid	DMSO	691 (691)	694	701	0.09 (0.14)
38 + folic acid	DMSO	680	676	684	0.04 (0.07)
34c-ε-PL	Water	683, 589	-	-	-
52-ε-PL	Water	655, 606	-	-	-

^a = values in brackets are for Pc complex alone.

Table 4.4: TCSPC parameters of phthalocyanine conjugates.

Conjugate	Solvent	$^a\tau_F$ (ns) (± 0.02)	Relative Amplitude (A ₁) (%)	$^a\tau_{F2}$ (ns) (± 0.04)	Relative Amplitude (A ₂) (%)
35a -AuNP	CHCl ₃	2.61 (2.51)	70	1.12	30
36 -AuNP	CHCl ₃	2.52 (2.83)	80	0.82	20
37 -AuNP	CHCl ₃	2.53 (2.51)	100	-	-
34a + folic acid	DMSO	2.90 (2.80)	0.98	0.83 (1.21)	0.02
34b + folic acid	DMSO	5.23 (5.13)	0.93	1.11	0.07
34c + folic acid	DMSO	2.74 (2.72)	0.90	0.74	0.10
35a + folic acid	DMSO	2.21 (2.42)	0.88	1.44 (0.82)	0.12

a = values in brackets are for Pc complex alone.

Table 4.4 continued

Conjugate	Solvent	$^a\tau_F$ (ns) (± 0.02)	Relative Amplitude (A ₁) (%)	$^a\tau_{F2}$ (ns) (± 0.04)	Relative Amplitude (A ₂) (%)
35b + folic acid	DMSO	4.81 (4.93)	0.91	0.92	0.09
35c + folic acid	DMSO	2.53 (2.51)	0.95	1.13	0.05
36 + folic acid	DMSO	2.10 (2.72)	0.98	0.71	0.02
37 + folic acid	DMSO	2.22 (2.35)	0.92	1.00	0.08
38 + folic acid	DMSO	2.51 (2.74)	0.90	1.22	0.1
34c- ϵ -PL	Water	<0.01	-	-	-
52- ϵ -PL	Water	<0.01	-	-	-

a = values in brackets are for Pc complex alone.

4.1.2.2 Phthalocyanine-folic acid interactions

Figure 4.8 shows absorption, excitation and emission spectra of complexes **34a**, **34c** and **35a** in the presence of folic acid. The Q-band of complex **34a** is split in DMSO in the presence of folic acid, Figure 4.8a, but unsplit in the same solvent without folic acid. The ratio of the components of the split changes on excitation, but emission occurs with one band as explained earlier. Figure 4.8b (i) shows an increase in absorption in the B-band region on complex **34c**, due to absorption by folic acid. Figure 4.8c shows the absorbance, emission and excitation spectra of **35a**, in the presence of folic acid. The excitation spectrum shows a change in the relative intensities of the split Q-band compared to the absorption spectrum, suggesting a significant change in symmetry upon excitation. For **34b**, **35b**, **35c**, **36**, **37** and **38**, the presence of folic acid did not show changes in the fluorescence profile confirming that the same species are responsible for the fluorescence emission.

More than one lifetime was observed for all complexes in the presence of folic acid. The first, long lifetime may be associated with monomeric non-interacting MPc molecules, while the second may be attributed to quenching as a result of the interaction between the MPc and folic acid. Such a quenched system is short-lived with a low abundance, Table 4.4. Further proof for such a quenching interaction is obtained from time resolved emission spectroscopy (TRES) detected between 670 nm and 800 nm as shown in Figure 4.9a, which shows a strong emission spectrum of **34c** with a Q-band emission peak maxima at 686 nm and a weak emission at 683 nm (insert) for the Pc interacting with folic acid.

The TRES for **35a** detected between 670 and 800 nm (Figure 4.9b), shows a strong emission for **35a** at 696 nm and a weak emission when **35a** is interacting with folic acid at 703 nm. The slight shift in emission peak wavelength position is also indicative of two different species in solution. Thus for the rest of the complexes, two species occur in solution, judged by the TCSPC data, and they are expected to give similar TRES traces.

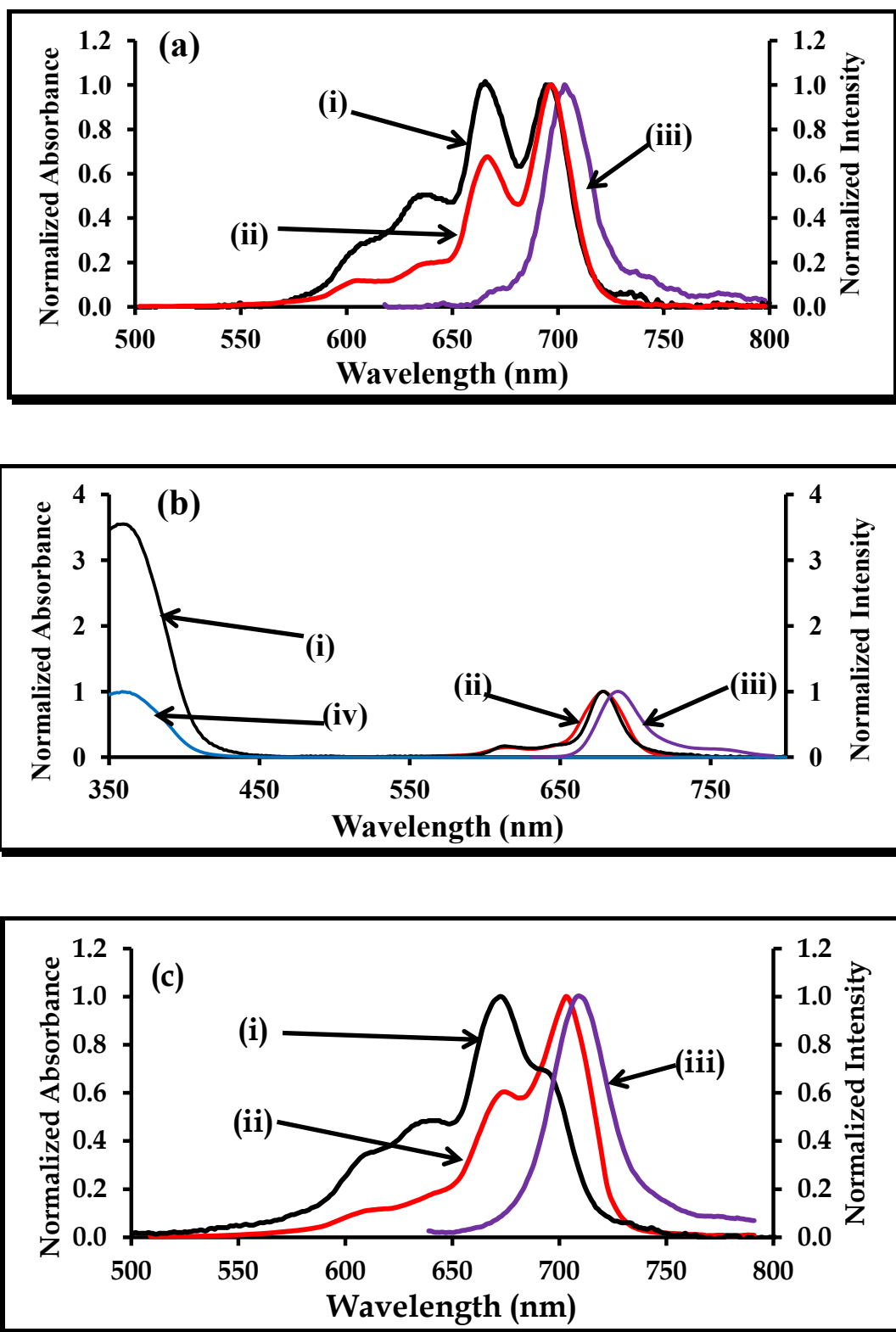


Figure 4.8: Absorption (i), excitation (ii) and emission (iii) spectra of (a) **34a**, (b) **34c** and (c) **35a** in DMSO in the presence of folic acid ((iv) in (b) represents the absorption spectrum of folic acid).

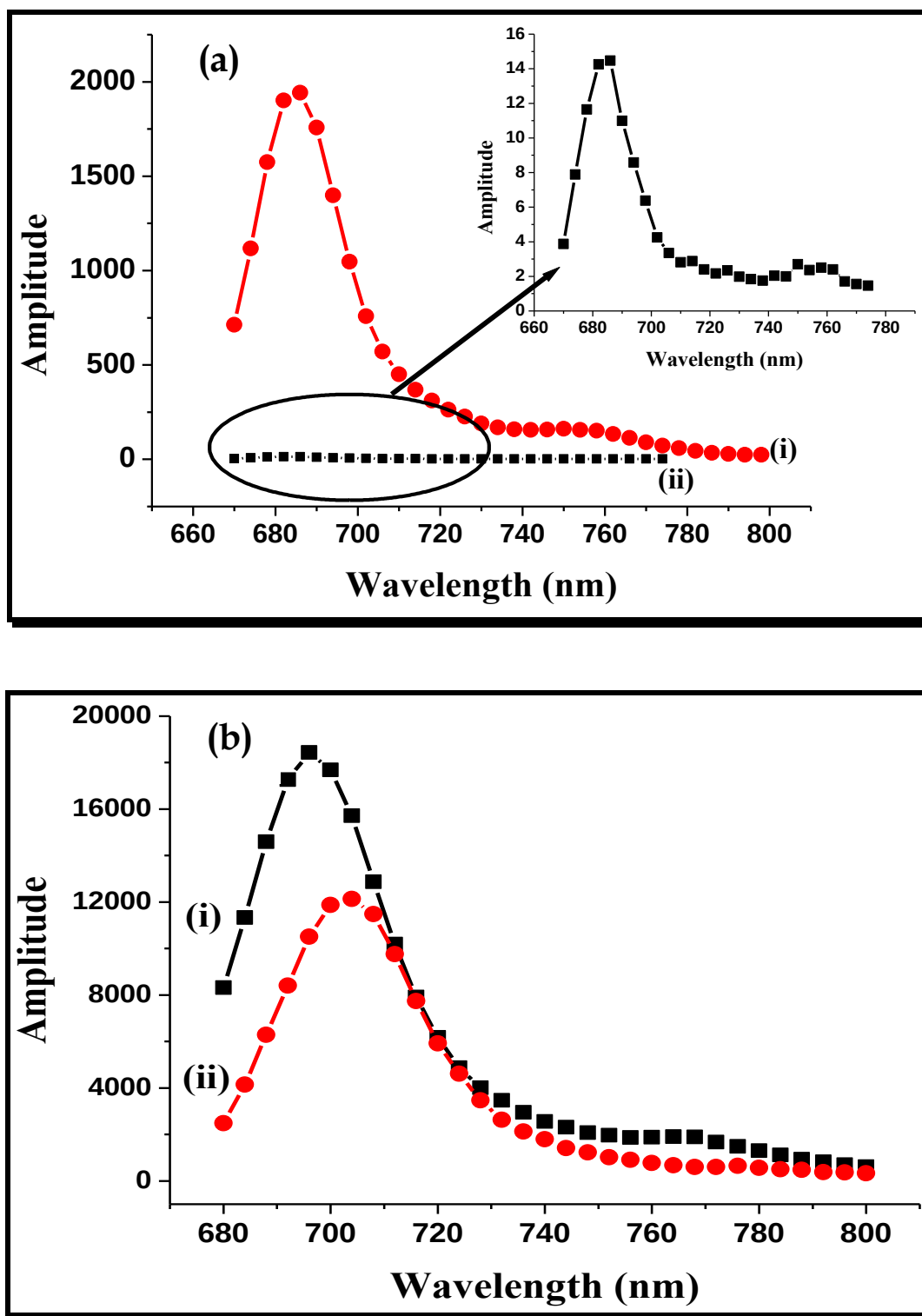


Figure 4.9: Time resolved emission spectra (TRES) of (a) **34c** and (b) **35a** in the presence of folic acid, (i) long lifetime, (ii) short lifetime, in DMSO Insert in (a): expansion of (ii).

Folic acid has amino groups and these are known to quench fluorescence [290]. However this was not the case for **34a** and **34c** where the Φ_F values did not change and for **35c**, where there was an increase. For complexes **36**, **37** and **38**, nevertheless there is a significant decrease in Φ_F values in the presence of folic acid showing the effects of quenching. The Φ_F values will be affected by aggregation in addition to the heavy atom effect. Comparing sulfur bridged complexes (**35**) with the oxygen bridged ones **34**, in the presence and absence of folic acid, shows that the latter have larger Φ_F values, this could be related to the heavy atom effect of sulfur which encourages intersystem crossing to the triplet state. This was also observed in the absence of folic acid except for **34a** and **35a**.

4.1.2.3 Phthalocyanine-poly-L-lysine interactions

34c- ϵ -PL and **52- ϵ -PL** did not show steady state fluorescence and the fluorescence quantum yield could not be determined. However **52- ϵ -PL** and **34c- ϵ -PL** showed a TSCPC signal (Figure 4.10a), and fluorescence lifetimes were estimated to be low (< 0.01 ns) showing extensive quenching. The close packing of the conjugate causes the phthalocyanines to be non-fluorescent. As previously mentioned, amino groups are also known to quench fluorescence [290]. It is also possible that the fluorescence lifetimes of the conjugates are too fast to be detected. Figure 4.10a (inset) shows the TRES trace of the conjugate in water where the Q-band was observed at 720 nm. Figure 4.10b compares the steady state fluorescence spectrum of complex **34c** in DMSO together with the TRES of **34c- ϵ -PL** in water.

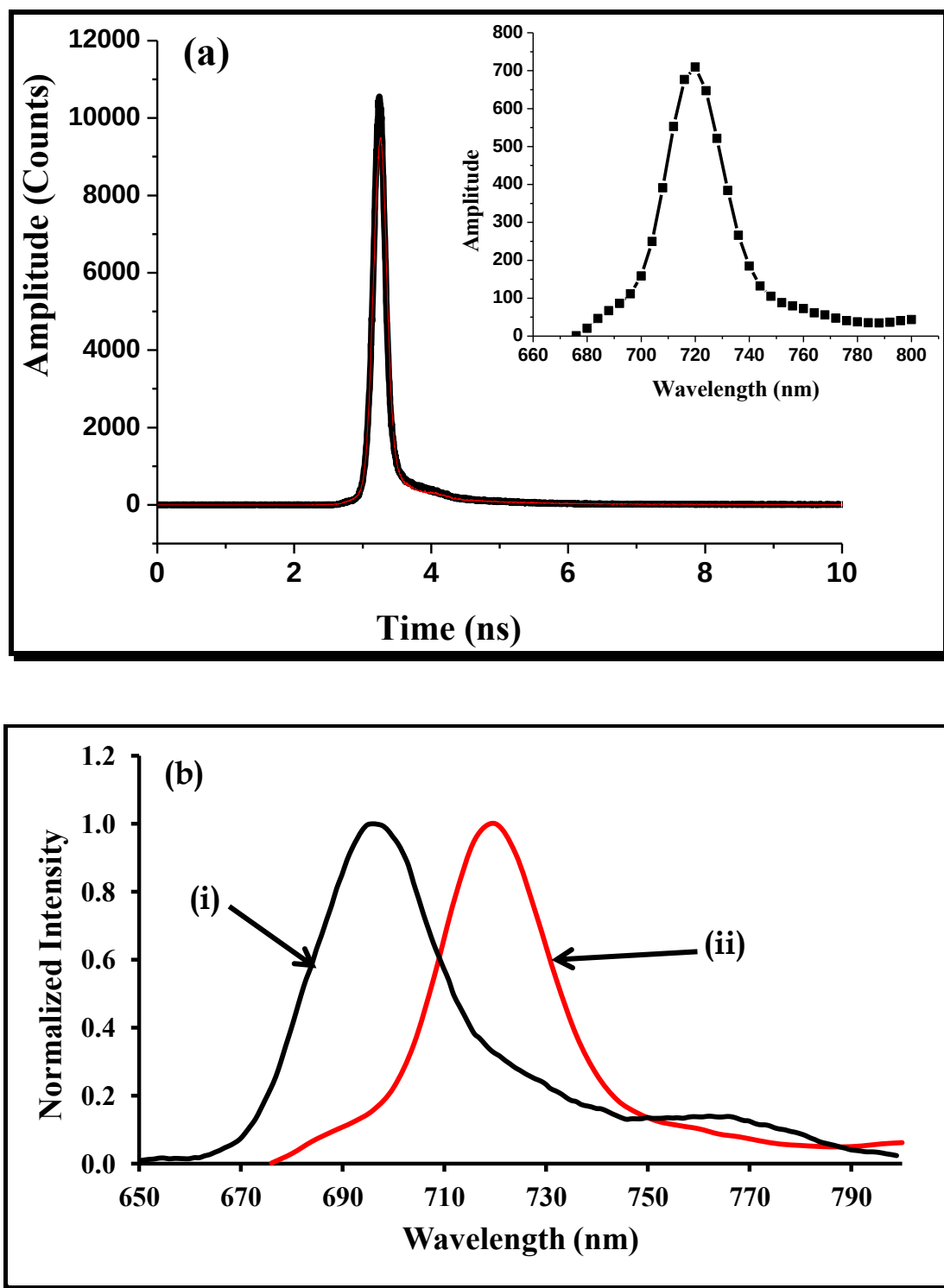


Figure 4.10: (a) Fluorescence decay curve of complex **34c-ε-PL** in water. Insert: Time resolved emission spectra (TRES) of **34c-ε-PL**. (b) fluorescence emission curve for (i) complex **34c** in DMSO and (ii) TRES **34c-ε-PL** in water.

4.2 Triplet state quantum yields and lifetimes

4.2.1 Phthalocyanine derivatives

4.2.1.1 Symmetrically substituted phthalocyanine derivative: **37**

The triplet quantum yield (Φ_T) is a measure of molecules that undergo intersystem crossing to occupy the triplet state. Therefore, factors which induce spin-orbit coupling will indeed populate the triplet excited state. The triplet quantum yields and lifetimes (τ_T) were determined by flash photolysis experiments in argon saturated solutions of Pc in DMSO or DMF. The data for complexes **32** and **33** was obtained in DMF containing small amount of THF (<1 %). However, the DMF standard does not contain any THF. The small volume of THF will not affect the Φ_T or τ_T values. Table 4.5 gives the triplet state parameters, Φ_T and τ_T . Figure 4.11 shows the triplet decay curve for complex **37**, which is similar to those of the other complexes. All triplet decay curves showed second order kinetics, typical for MPc complexes at high concentrations ($>1 \times 10^{-5}$ M) [291] caused by triplet-triplet annihilation (TTA). The TTA process corresponds to the short-range energy transfer process in which two triplet molecules yield a singlet excited state and another ground state molecule. In addition, other processes such as delayed fluorescence and internal conversion as well as intersystem crossing may be produced. At such high concentrations of triplet molecules (as is the case in this work), the contribution of TTA to the relaxation kinetics dominates. The rate constant for TTA (k_{TTA}) may be described by the equation 4.1 [292, 293].

$$\text{---} \quad (4.1)$$

where $[T]$ is the concentration of the triplet state calculated using triplet state extinction coefficients. The values in Table 4.5 are of the same order of magnitude or higher than reported for phthalocyanines [292], confirming a strong contribution of TTA.

Table 4.5 shows a high Φ_T value for **37** (0.79) in DMSO suggesting that a significant number of the molecules undergo intersystem crossing to the triplet state. A considerable decrease of the Φ_T value in the DMSO(2 %)/PBS mixture (0.11) and in DMSO(2 %)/PBS/Triton X-100 (0.34) was observed due to aggregation for **37**. The triplet lifetime values of complex **37** in DMF or DMSO were determined to be 304 and 340 μs respectively. The triplet lifetime (τ_T) value of complex **37** in the DMSO(2 %)/PBS mixture (20 μs) was much lower than τ_T values for the complex in DMF or DMSO (304 and 340 μs respectively). The presence of Triton X 100 did not show an increase in the τ_T in aqueous media.

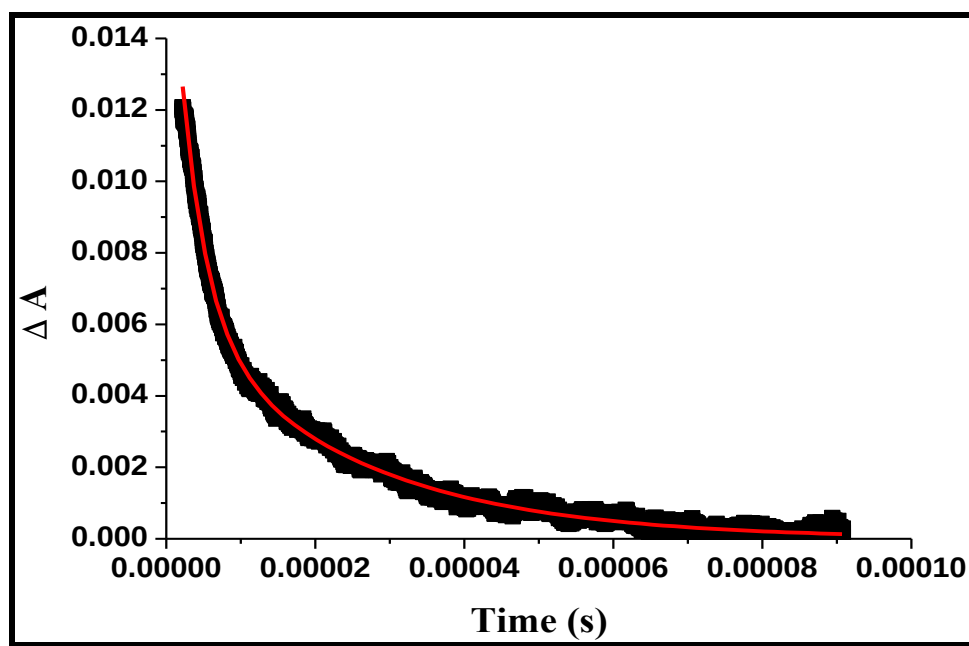


Figure 4.11: Triplet decay curve for complex **37** in DMSO.

Table 4.5: Photophysical and photochemical properties of synthesized phthalocyanines. DMF contains <1 % THF for complexes **32** and **33** to aid solubility.

Pc	Solvent	Φ_T (± 0.05)	τ_T (μs)	Φ_Δ	S_Δ	$K_{TTA}/10^7$ $M^{-1} s^{-1}$
32a	DMF	0.63	320	0.55	0.87	6.17
32b	DMF	0.56	260	0.45	0.80	4.33
32c	DMF	0.71	360	0.60	0.84	8.17
33a	DMF	0.66	320	0.56	0.85	7.53
33b	DMF	0.65	360	0.58	0.89	2.44
33c	DMF	0.75	450	0.62	0.83	9.55
34a	DMSO	0.62	150	0.53	0.85	5.73
34b	DMSO	0.49	275	0.30	0.61	4.21
34c	DMSO	0.74	240	0.64	0.86	8.71
35a	DMF	0.68	110	0.55	0.81	6.78
	DMSO	0.65	270	0.58	0.89	6.56
35b	DMSO	0.52	210	0.27	0.54	5.29
35c	DMSO	0.71	350	0.61	0.86	9.01
36	DMF	0.71	140	0.51	0.81	8.73

Table 4.5 continued

Pc	Solvent	$^a\Phi_T$ (± 0.05)	$^a\tau_T$ (μs)	$^a\Phi_\Delta$	S_Δ	$K_{TT}/10^7$ $\text{M}^{-1} \text{s}^{-1}$
36	DMSO	0.63	140	0.53	0.84	8.21
37	DMF	0.75	304	0.61	0.81	8.73
	DMSO	0.79	340	0.66	0.83	9.11
	DMSO(2 %)/PBS	0.11	20	0.04	0.36	1.24
	DMSO(2 %)/PBS/Triton	0.34	20	0.18	0.52	1.11
38	DMSO	0.88 [291]	470 [291]	0.46 [291]	0.52	-

a= References in square brackets.

4.2.1.2 Unsymmetrically substituted phthalocyanine derivatives

Naphthoxy phthalocyanine derivatives: **32**, **33** and **36**

The zinc complexes (**32c** and **33c**) had the highest triplet quantum yields ($\Phi_T = 0.71$ and 0.75 respectively, in THF/DMF) compared to the rest of complexes **32a,b**, **33a,b** and **36**, Table 4.5. These high values suggest efficient intersystem crossing from the singlet excited state to the triplet excited state. Spin orbit coupling (due to heavy atom effect of Zn) is known to enhance intersystem crossing of the molecules to populate the excited triplet state, and can thus be used to account for the high Φ_T values for **32c** and **33c**. The relatively high fluorescence quantum yields obtained for the MgPcs resulted in the lowest triplet quantum yields consistent with expectations, considering the small size of Mg compared to Zn. The unmetallated derivatives (**32a** and **33a**) gave larger Φ_T values than MgPcs, corresponding to lower Φ_F in the former. The data in Table 4.5 indicates larger Φ_T values for Pcs substituted with carboxy-dioxyphenyl groups (**33b** and **33c**) relative to the corresponding carboxy derivatives (**32b** and **32c**). The lifetimes obtained showed that complexes with high triplet quantum yields had the longest lifetimes with complex **33c** having the longest τ_T value of $450\ \mu\text{s}$. The expected trend is the decrease in triplet lifetime with increase in triplet quantum yield. However, long triplet lifetimes are important in ensuring efficient energy transfer from the triplet excited state to ground state molecular oxygen.

The covalent linking of **32c** with cysteamine to form **36** resulted in the lowering of τ_T values in DMF from $360\ \mu\text{s}$ to $140\ \mu\text{s}$ respectively, Table 4.5.

Fluorinated phthalocyanine derivatives: **34** and **35**

The magnesium phthalocyanines (**34b** and **35b**), as expected, had the lowest Φ_T values of 0.49 and 0.52 (Table 4.5) respectively, compared to **34a**, **34c**, **35a** and **35c**, due to the small size of the Mg ion that does not promote intersystem crossing. The oxygen bridged complexes generally had lower triplet quantum yields corresponding to higher fluorescence quantum yields, Table 4.5 with the exception of **34c**. The Table also shows that the zinc phthalocyanines (**34c** and **35c**) had the highest triplet quantum yield values of 0.74 and 0.71 in DMSO respectively. This is due to the heavy atom effect of Zn as explained earlier. The τ_T values for the complexes were relatively long (110-350 μ s) in DMF and DMSO, with compound **35a** having the lowest lifetime in DMF (Table 4.5) but good enough for energy transfer to molecular oxygen.

4.2.2 Phthalocyanine-nanoparticle conjugates

4.2.2.1 Nanoparticles

These studies were done in DMF since the conjugates dissolved effectively in this solvent compared to DMSO, also the triplet quantum yield (Φ_T) values could not be determined in CHCl_3 due to the lack of standard in this solvent. Table 4.6 shows high triplet quantum yield (Φ_T) value for the conjugate of **35a** in DMF compared to the free phthalocyanine where the Φ_T value was 0.68, whereas **36**-AuNP had the same Φ_T value as **36** and the Φ_T value of **37**-AuNP was lower than the Pc alone. The presence of the AuNPs for **35a** encourage intersystem crossing due to the heavy

atom effect. For complex **37** containing four thiol groups, there is a surprising decrease in Φ_T value in the presence of AuNPs. This could be due to the slight aggregation of **37** in DMF discussed in Chapter 3. The conjugates showed a decrease in triplet lifetimes compared to free phthalocyanines with **36**-AuNP having the shortest lifetime of 70 μ s compared to 140 μ s for the free Pc. The speedy deactivation of the triplet state for the conjugates may be explained by the heavy atom effect.

4.2.2.2 Phthalocyanine-folic acid photophysical interactions

On addition of folic acid, there was no significant change in the triplet quantum yield value for **34a** but a drastic decrease in the triplet lifetime was observed (from 150 μ s to 70 μ s), Table 4.6. Triplet quantum yield values for **34b** and **34c** decreased from 0.49 and 0.74 to 0.37 and 0.60 respectively, on conjugation. This decrease was accompanied by an increase in lifetimes in the presence of folic acid, Table 4.6.

The presence of folic acid did not significantly reduce Φ_T for complexes sulfur bridged complexes **35a-c**, however, the triplet lifetimes were significantly lowered. For complexes **36** and **37** in the presence of folic acid, there was no change in the Φ_T within experimental error, however for **38**, there was a large decrease in Φ_T in the presence of folic acid.

The triplet lifetimes (τ_T) were shorter in the presence of folic acid for complexes **36**, **37** and **38**. The τ_T values are relatively long overall and good enough for energy transfer to molecular oxygen, Table 4.6.

In conclusion, for all complexes in the presence of folic acid, there is a general decrease in the triplet lifetime compared to the Pc alone, suggesting quenching.

Table 4.6: Photophysical parameters of phthalocyanine conjugates. a = values in barckest are for Pcs alone

Pc	Solvent	$^a\Phi_T$ (± 0.05)	$^a\tau_T$ (μs)	Φ_Δ
35a-AuNP	DMF	0.71 (0.68)	92 (110)	
36-AuNP	DMF	0.71 (0.71)	70 (140)	-
37-AuNP	DMF	0.69 (0.75)	87 (304)	-
34a + folic acid	DMSO	0.63 (0.62)	70 (150)	-
34b + folic acid	DMSO	0.37 (0.49)	290 (275)	-
34c + folic acid	DMSO	0.60 (0.74)	290 (240)	-
35a + folic acid	DMSO	0.66 (0.65)	250 (270)	-
35b + folic acid	DMSO	0.51 (0.52)	150 (210)	-
35c + folic acid	DMSO	0.68 (0.71)	170 (350)	-
36 + folic acid	DMSO	0.59 (0.63)	110 (140)	-
37 + folic acid	DMSO	0.72 (0.79)	270 (340)	-
38 + folic acid	DMSO	0.75 (0.88)	320 (470)	-
34c- ϵ -PL	Water	-	-	0.11
52- ϵ -PL	Water	-	-	0.22

The phthalocyanine-poly-L-lysine conjugates were too weak for triplet lifetime determinations.

4.3 Singlet oxygen quantum yield

4.3.1 Phthalocyanine derivatives

Singlet oxygen is formed via energy transfer between the triplet state of a Pc and ground state molecular (triplet) oxygen. The efficiency of singlet oxygen generation depends upon, amongst others, the triplet state quantum yield together with the triplet state lifetime. High efficiency of energy transfer between the excited triplet state of the Pc and the ground state of oxygen to generate large amounts of singlet oxygen is essential. The amount of singlet oxygen produced was determined by monitoring the decrease in absorbance intensity of DPBF or ADMA spectroscopically over a period of time.

Figure 4.12 shows a typical decay of DPBF on photolysis, using complex **37** as an example. There was no change in the Q-band intensity for all Pcs during the singlet oxygen quantum yield (Φ_{Δ}) determinations, confirming that the complex is not degraded during the studies. Similar decay was observed for the rest of the phthalocyanine derivatives. The Φ_{Δ} values are dependent on the triplet quantum yield of the photosensitizer and how efficiently energy is transferred from this state to ground state molecular oxygen; hence the complexes with high Φ_T are expected to give high Φ_{Δ} values. Quantum yields of singlet oxygen generation (Φ_{Δ}) of all complexes in DMSO or DMF are presented in Table 4.5.

It is clear from Table 4.5 that the ZnPc derivatives (**32c**, **33c**, **34c**, and **35c**, in their respective solvents) show higher Φ_{Δ} values than their H₂Pc and MgPc counterparts in response to the high triplet yields. This could be attributed to the higher triplet state population in ZnPc derivatives.

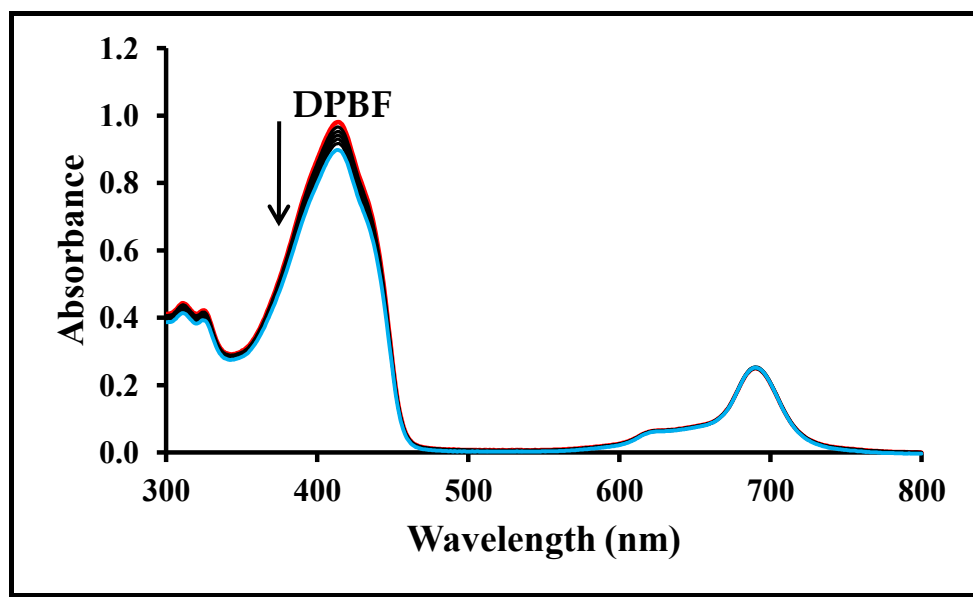


Figure 4.12: Time-dependent photobleaching of DPBF absorption in the presence of **37** in DMSO.

Considering data obtained in DMF and DMSO, the lowest Φ_{Δ} value observed was for the magnesium phthalocyanine, **35b** at 0.27, even though this complex did not have the lowest Φ_T value, suggesting inefficient energy transfer from the triplet state to molecular oxygen.

The magnitude of the S_{Δ} (equation 4.2) represents the efficiency of quenching of the triplet excited state by singlet oxygen.

(4.2)

All complexes showed S_{Δ} of near unity except for complex **34b**, **35b** and **38** in DMSO, and **37** in PBS/DMSO mixture (Table 4.5), suggesting efficient quenching of the triplet state by singlet oxygen. The most efficiency however was found to be with complex **33b** and **35a** at $S_{\Delta} = 0.89$.

4.3.2 Singlet oxygen quantum yield of phthalocyanine conjugates

The singlet oxygen quantum yields for phthalocyanine-nanoparticle conjugates could not be determined due to interference of the nanoparticles with the absorption of ADMA or DPBF. The same was true for the folic acid-phthalocyanine interaction study.

Phthalocyanine-poly-L-lysine photochemical interactions

Singlet oxygen quantum yields were determined in water using ADMA as a quencher for Pc- ϵ -PL conjugates. The photodegradation of ADMA over time was monitored by UV-vis spectroscopy. Singlet oxygen quantum yield values are highly affected by a number of factors which include the triplet excited state lifetime and the efficiency of energy transfer between the triplet excited state and the ground state of oxygen. The Φ_{Δ} value of complex **52- ϵ -PL** (0.22) was higher when compared to complex **34c- ϵ -PL** (0.11). This suggests that the presence of more phthalocyanine moieties on the polymer chain increases Φ_{Δ} values (Table 4.6). It is well known [110] that water absorption can quench singlet oxygen and this may be a significant

contributor to the low Φ_{Δ} values. The highly aggregated nature of the phthalocyanine conjugates also has a negative effect on the Φ_{Δ} values. The measured Φ_{Δ} values show that the conjugates are promising photosensitizers that may be applied for the photo-inactivation of microorganisms.

4.4 Conclusions

The photophysical and photochemical properties of synthesized phthalocyanines were determined. The effect that mono-substituted phthalocyanines have on the splitting of the Q-band is highly influenced by the electronic properties of the mono-substituent and the nature of the central metal. The use of Mg as a central metal has a large influence on the fluorescent properties associated with the phthalocyanine moiety and thus indicates similar MgPc derivatives may be of particular use for fluorescence imaging applications. The accompanying photophysical properties are also significant, with relatively good triplet quantum yields and long lifetimes which will be particularly effective if used as photosensitizers in PDT.

The general trend observed for metallated Pc molecules is that high triplet quantum yields often results in low triplet lifetimes. This is almost usually the case for heavy metals, the heavy metal effect encourages intersystem crossing inevitably leading to a fast relaxation back to the ground state. Factors such as change in symmetry of the Pc molecules that leads to the distortion of the conjugation of the Pc may also influence the time Pc molecules stay in the excited triplet state. However, long triplet lifetimes are important in ensuring efficient energy transfer from the triplet excited

state to ground state molecular oxygen. It was found that conjugation of phthalocyanine molecules to gold nanoparticles results in a slight decrease in the triplet quantum yields with a large decrease in the triplet lifetime. In the presence of folic acid, the triplet quantum yield values were still relatively high. To conclude photophysical and photochemical parameters of the phthalocyanines studied together with their corresponding conjugates shows these molecules as potential PDT agents.

Chapter 5

Phototherapeutic properties

This chapter focuses on the phototherapeutic properties of selected phthalocyanines of against normal human cells, breast cancer cells and *S. aureus*. The PDT effect of the corresponding phthalocyanine-nanoparticle conjugation is also explored.

5. Phototherapeutic properties

5.1 Cytotoxicity studies

Phthalocyanines are commonly reported as lethal photosensitizers for both cancer and bacterial cells. This work thus aims to explore the photodynamic properties of three Zn phthalocyanine derivatives, i.e. **34c**, **37** and **52**. The Pcs were also covalently linked to poly-L-lysine (Scheme 3.9), conjugated to nanoparticles or encapsulated by liposomes.

5.1.1 *In vitro* cytotoxicity evaluation of healthy and cancer cells by Cell Titer Blue assays

For these studies **34c** and **37** using AuNPs or liposomes for delivery were employed. Complex **37** was chosen due to its ability to bind to AuNPs and **34c** represents all the ZnPc derivatives.

Regardless of the growth conditions, *in vitro* cultured cells exist as a heterogeneous population. This suggests that cells exposed to drugs will not all respond in the same manner. Cells exposed to drugs may respond over the course of several hours or days (delayed toxicity). The bioavailability of a drug is influenced by a number of factors which include the route of drug administration, tissue distribution, extent of drug metabolism, and drug clearance. Such factors may directly affect the response of a cell and the difference in drug concentration at the tumor site is expected to create discrepancies in antitumor effects. As a result of culture heterogeneity, the data from plate-based assay formats represent an average of the signal from the

population. There are a number of screening strategies used for the identification of compounds with activity against a wide range of solid tumors. The Cell Titer-Blue cell viability assay is a fluorometric method for estimating the number of viable cells present in multi-well plates. This method is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Nonviable cells rapidly lose metabolic capacity, cannot reduce the indicator dye, and thus do not generate a fluorescent signal.

The *in vitro* cytotoxicity of complexes **34c** (alone or bound to liposomes) and **37** (alone or linked to gold nanoparticles or bound to liposomes) was evaluated against human breast cancer (MCF-7) and healthy human fibroblast cells by Cell Titer Blue assays following exposure for 24 h and irradiation with laser light. The intrinsic cytotoxicity of the complexes was assessed by dark toxicity treatment. Figure 5.1 shows that both complexes (alone) become toxic with increase in concentration and incubation time for MCF-7 cells with complex **34c** showing more toxicity compared to complex **37**, Table 5.1. An incubation time of 1 h was chosen for subsequent experiments as this time showed the least dark toxicity for both complexes.

The fibroblast cells however indicated a lower dark toxicity effect from both complexes compared to the breast cancer cells (Figure 5.2), with complex **34c** still showing an increased dark toxicity compared to **37**, Figure 5.2(b), Table 5.1.

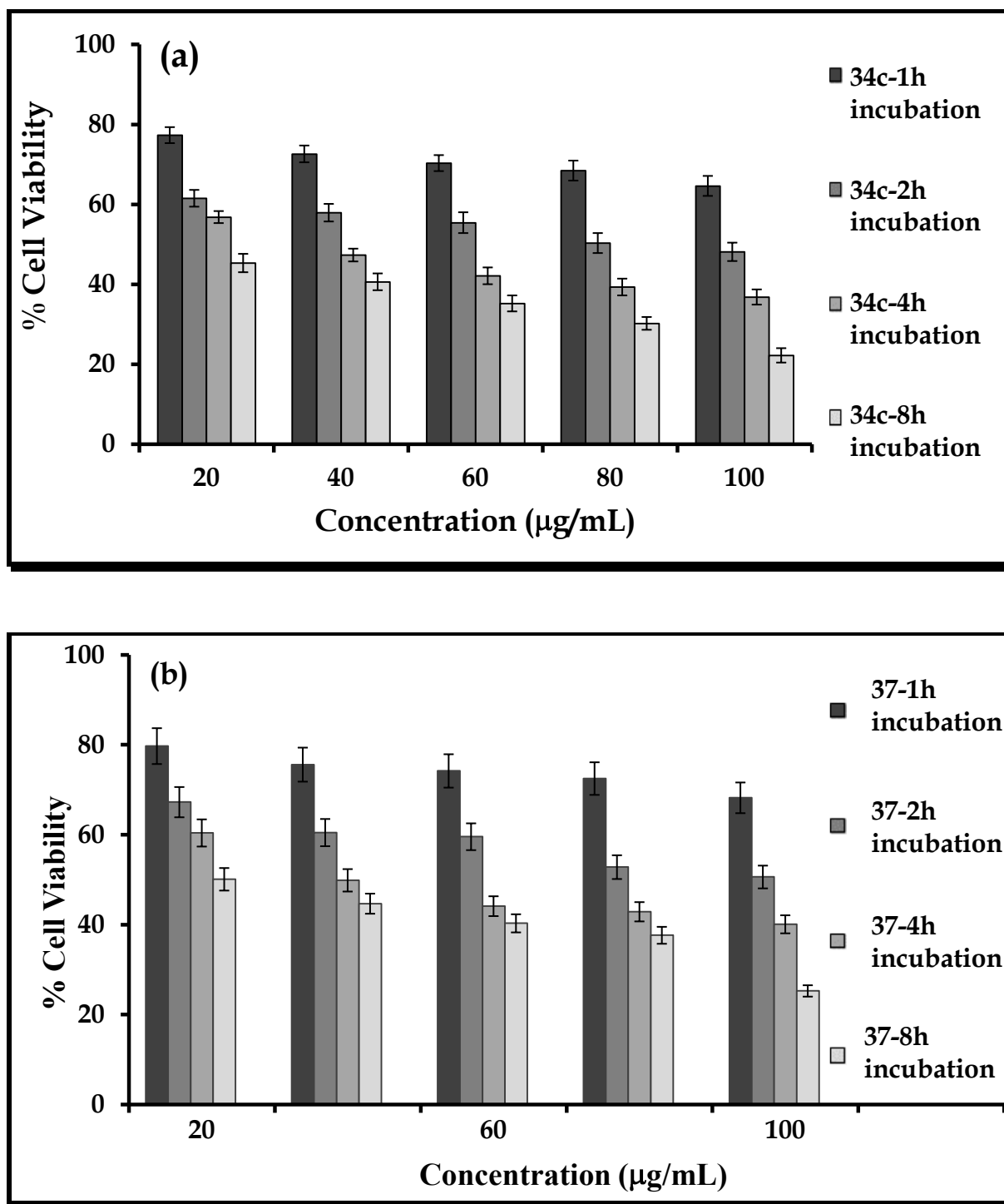


Figure 5.1: Concentration and incubation time effect of (a) **34c** and (b) **37** on MCF-7 cells in the absence of light.

Table 5.1: Photodynamic properties of 20 µg/mL of **34c** and **37** with corresponding conjugates after 1 h incubation in 2 %DMSO/PBS solution mixture.

Pc	Delivery agent	Cell line	Cell viability (%)	Light dose (J.cm ⁻² , 676 nm)
Control	-	Fibroblast	99.2	4.5
Control	-	MCF-7	98.7	4.5
34c	-	Fibroblast	89.8	0
	-	MCF-7	77.3	0
	-	Fibroblast	87.3	4.5
	-	MCF-7	56.5	4.5
	Liposomes	MCF-7	42.7	4.5
37	-	Fibroblast	93.5	0
	-	MCF-7	79.72	0
	-	Fibroblast	91.1	4.5
	-	MCF-7	63.8	4.5
	AuNPs	MCF-7	60.1	4.5
	Liposomes	MCF-7	51.9	4.5

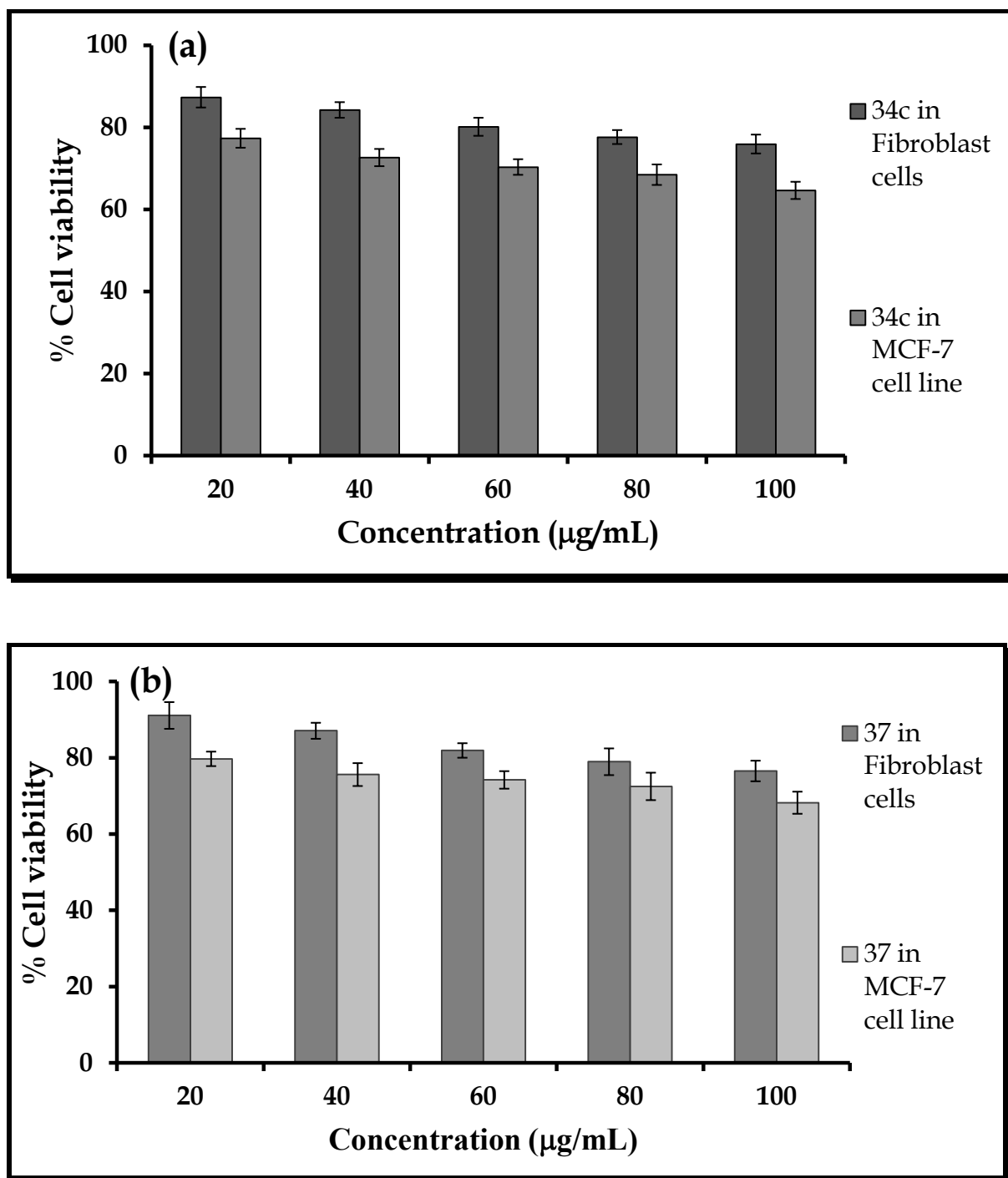


Figure 5.2: Concentration and incubation time effect of (a) **34c** and (b) **37** on MCF-7 and fibroblast cells after 1 h incubation in the absence of light.

The photodynamic activity of the complexes following laser light (676 nm) irradiation was determined at different light doses (4.5, 10, 20 and 25 J.cm⁻²) following 1h incubation, Figure 5.3. Control cells are in the absence of dye. Post irradiation, the cells showed a significant cell viability decrease with increase in light doses. The majority of the cells (96.1 % and 94.7 % for **34c** and **37**, respectively, Figure 5.3) were killed when comparing 0 to 25 J.cm⁻², through photodynamic action. This intense laser light (25 J.cm⁻²) proved to be very harmful to healthy fibroblast cells whereas a light dose of 4.5 J.cm⁻² gave good percentage cell viability (91.1 % for fibroblast cells and 63.8 % for breast cancer cells for **37** and 87.3 % for fibroblast cells and 56.5 % for breast cancer cells for **34c**, Table 5.1). The light doses of 4.5 J.cm⁻² was consequently chosen as the light dose for the rest of the experiments. In general the MCF-7 cells were more sensitive to PDT treatment compared to healthy fibroblast cells, Figure 5.3. The control cells incubated with 2 % DMSO showed a minimal toxicity effect with a cell viability of 99.2 % for fibroblast cells and 98.7 % for breast cancer cells, Table 5.1.

Figure 5.4a shows the photo-cytotoxic effect of complex **34c** bound to liposomes and Figure 5.4b shows for complex **37** when linked to AuNPs or bound to liposomes. Following PDT, a 56.5 % cell viability for MCF-7 was observed for complex **34c** alone whereas 42.7 % cell viability was observed for the liposome bound Pc. This shows that the use of liposomes as a delivery agent for complex **34c** appreciably improved its PDT efficacy.

A 60.1 % cell viability was observed for the **37**-AuNP conjugate against MCF-7 whereas 51.9 % cell viability was observed for the liposome bound Pc. This is a

moderate decrease compared to complex **37** alone at 63.8 %. There was a significant change in the morphology of the cancer cells after PDT action as shown in Figure 5.5 for complex **37**. The treated cells appear scattered, unattached and distorted whereas the untreated cells were attached to each other and intact. This is indicative of cell death on the treated cancer cells.

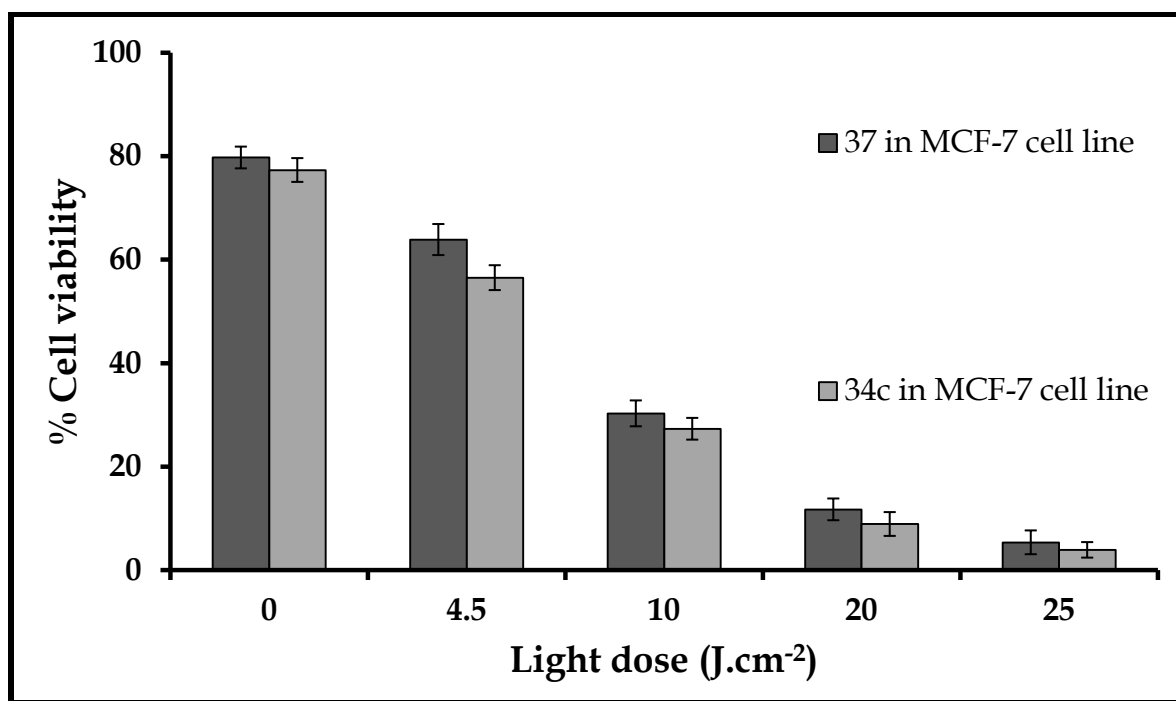


Figure 5.3: Effect of light dose on MCF-7 cells after 1 h incubation with 20 µg/mL of complexes **34c** and **37** at 676 nm.

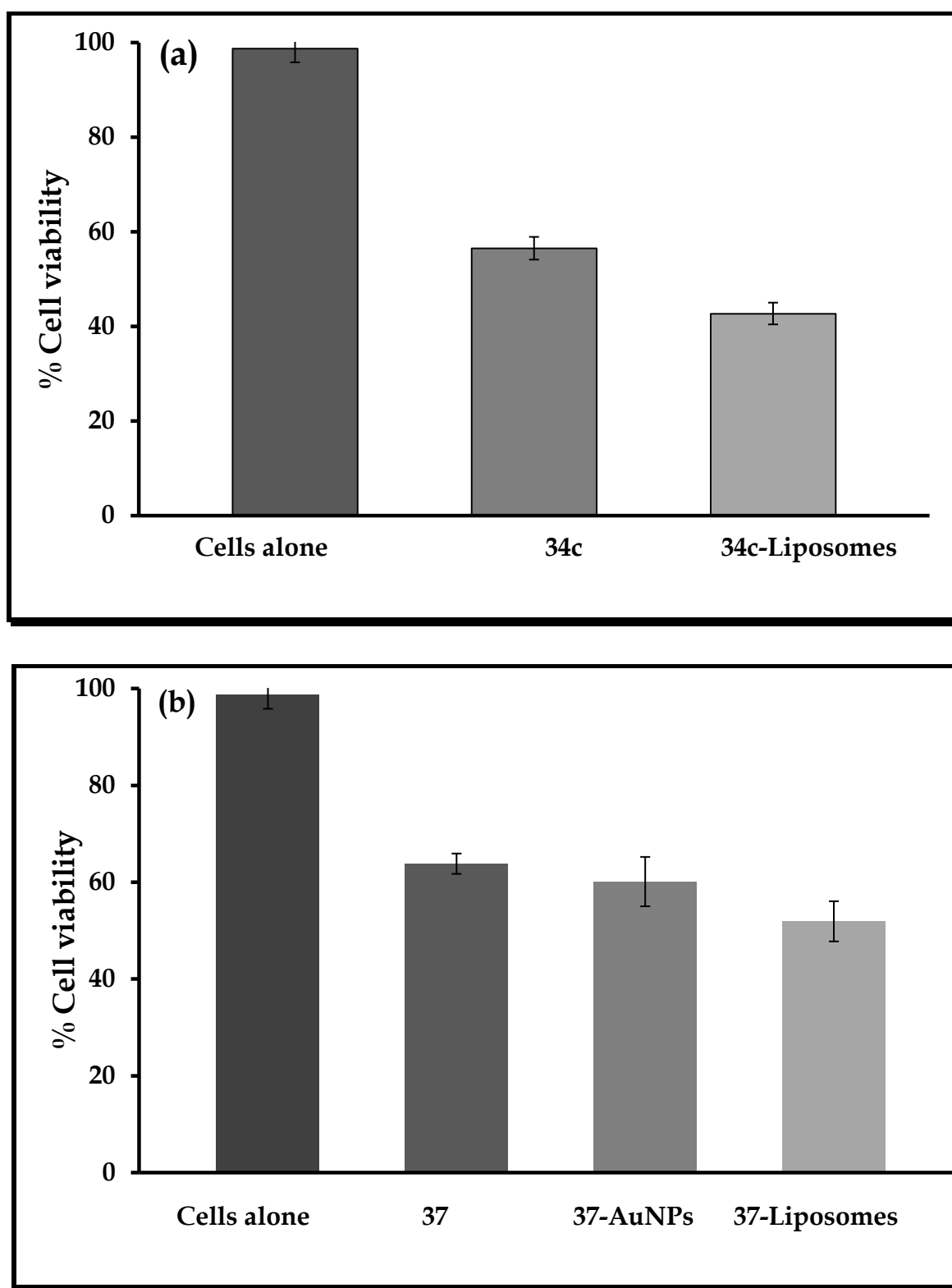


Figure 5.4: PDT effect of 20 $\mu\text{g/mL}$ of (a) **34c** and (b) **37** on MCF-7 cells in the presence of delivery vehicles at 4.5 $\text{J}\cdot\text{cm}^{-2}$ after 1 h incubation.

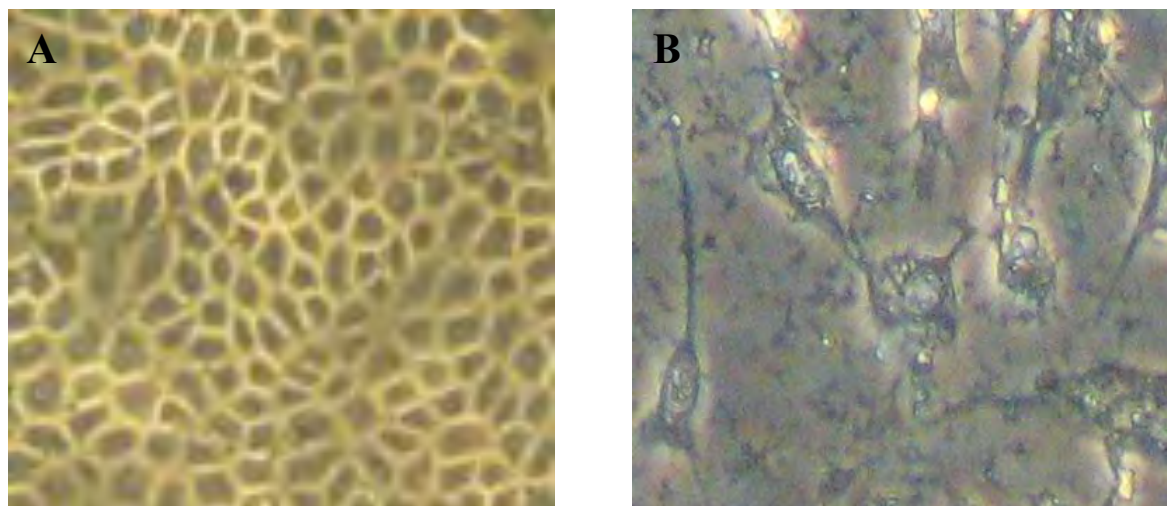


Figure 5.5: Morphology changes observed on MCF-7 cells before (A) and after PDT treatment (B) with complex **37** at 25 J.cm⁻².

5.1.2 *In vitro* photodynamic inactivation of *S. aureus* by Pc-PL conjugates

For these studies, conjugates **34c-ε-PL** and **52-ε-PL** were employed.

Phthalocyanines are known to exhibit photodynamic microbial damage, with the singlet oxygen pathway (Type II) being responsible for photooxidative cellular destruction. It has been established that singlet oxygen will react with molecules responsible for the maintenance and structure of the cell wall/membranes and these include phospholipids, peptides and sterols [53].

The synthesized poly-L-lysine-phthalocyanine conjugates were tested for the photo-inhibitory activity against *S. aureus* as a model to access the microorganism inactivation (Table 5.2). The relative concentration of the PS contained in the bacterial suspension was determined by UV-vis spectroscopy to be in the 10⁻⁹-10⁻⁸ M range for the synthesized conjugates. Photodynamic antimicrobial chemotherapy

(PACT) studies using the **34c-ε-PL** and **52-ε-PL** conjugates resulted in considerable antimicrobial activity against *S. aureus* following irradiation at a light dosage of 39.6 mW/cm² for 10 min (Figure 5.6). The photo-inactivation was observed to increase with an increase in photosensitizer dose for both conjugates.

The minimum inhibitory concentration required to inhibit 50 % growth (MIC₅₀) method was used to determine the susceptibility of *S. aureus* to these potential antimicrobials agents. The MIC₅₀ concentrations obtained for complex **52-ε-PL** for the dark toxicity experiments were 0.75 μM and 0.187 μM for the irradiated samples. In the case of complex **34c-ε-PL**, the same is true in terms of decreased growth inhibition in the presence of light, however the concentrations were lower for both dark (0.375 μM) and light (0.046 μM) experiments, making **34c-ε-PL** a better photosensitizer contradicting the lower singlet oxygen values obtained for **34c-ε-PL** (0.11, Table 4.6) compared to **52-ε-PL** (0.22, Table 4.6). The highly amphiphilic nature of complex **34c-ε-PL** relative to complex **52-ε-PL** may enhance easy attachment on the bacteria cell wall causing greater cell damage. It can be suggested that **34c-ε-PL** may have proper adhesion to the cell membrane that is sufficient for light-activated destruction of the bacterium.

While the two conjugates can be considered as active agents against the tested microorganism, when **34c-ε-PL** and **52c-ε-PL**, are conjugated with AgNPs (to form **34c-ε-PL-AgNP** or **52-ε-PL-AgNP**, respectively), they are found to be effective light activated antimicrobials with a growth reduction of 94.0 % for **34c-ε-PL-AgNP** as compared to **34c-ε-PL-AuNP** showing a 80.0 % reduction. A 87.6 % growth

reduction was observed for **52- ϵ -PL-AgNP** compared to the 77.6 % growth reduction observed for **52- ϵ -PL-AuNP**. A higher percentage of photo-inactivation was observed for all conjugates, at the highest concentration of 3 μ M in the assay employed (Figure 5.7).

MIC₅₀ values of the drugs were also investigated in the presence of gold and silver nanoparticles. The results showed that the presence of AgNPs greatly inhibited percentage growth of the microorganism for both tested complexes, Table 5.2. The enhancement in bacterial killing observed with the nanoparticle conjugates demonstrates that the antimicrobial efficacy of PSs can be enhanced by the presence of gold and more especially silver nanoparticles.

Table 5.2: *S. aureus* photo-inhibition at fluence of 39.6 mW/cm² for 10 min irradiation time at MIC₅₀ concentrations for **34c-ε-PL** and **54-ε-PL** in pH 7.4

Conjugate	MIC ₅₀ (μM) Dark	MIC ₅₀ (μM) Light	MIC ₅₀ (μM) with AuNPs	MIC ₅₀ (μM) with AgNPs	% growth reduction with AuNPs	% growth reduction with AgNPs
34c-ε-PL	0.375	0.046	<0.046	<0.0058	80.0	94.0
52-ε-PL	0.75	0.187	0.046	0.0058	77.6	87.6

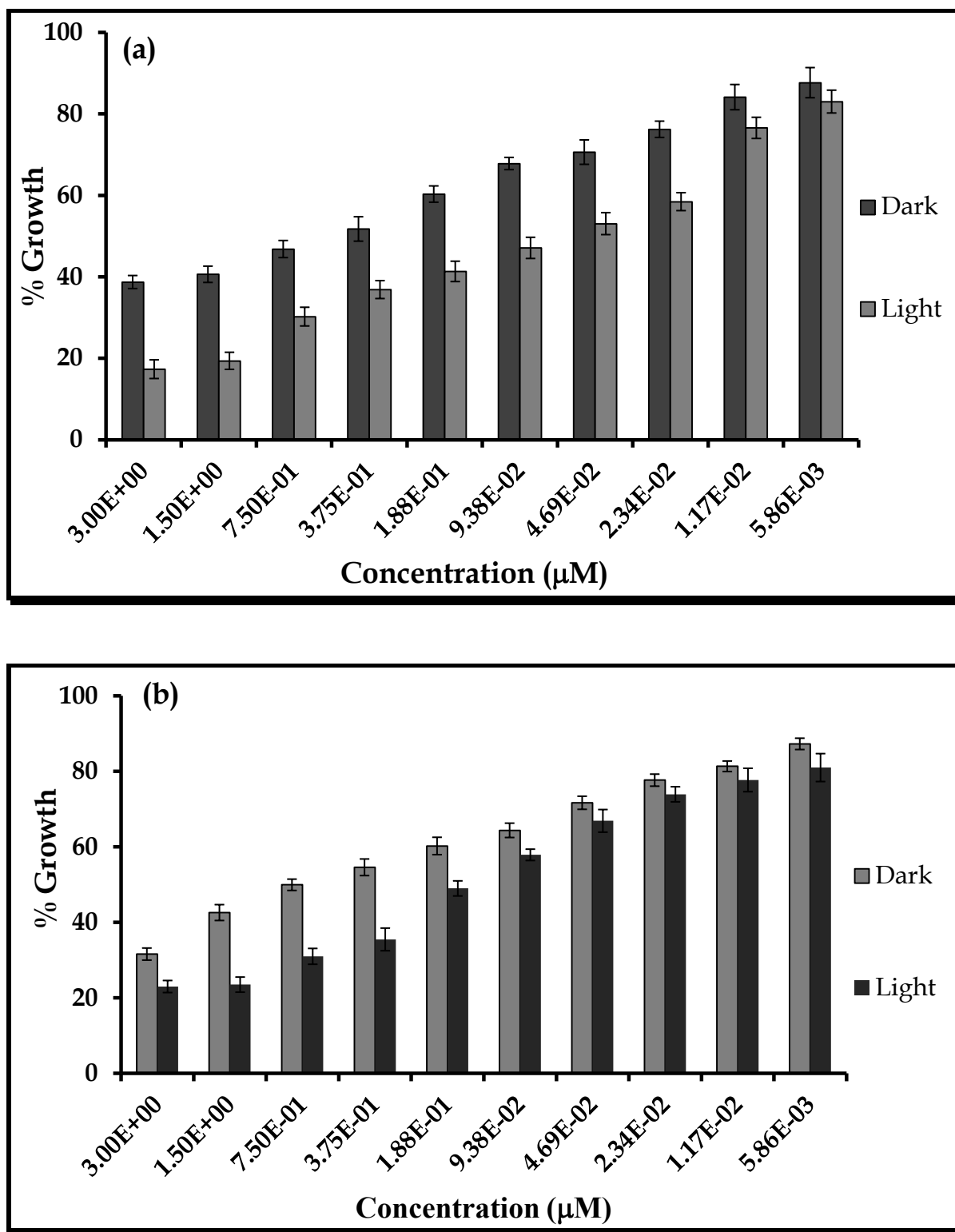


Figure 5.6: Antibacterial activity of (a) **34c- ϵ -PL** and (b) **52- ϵ -PL** against *S. aureus* in the presence and absent of light.

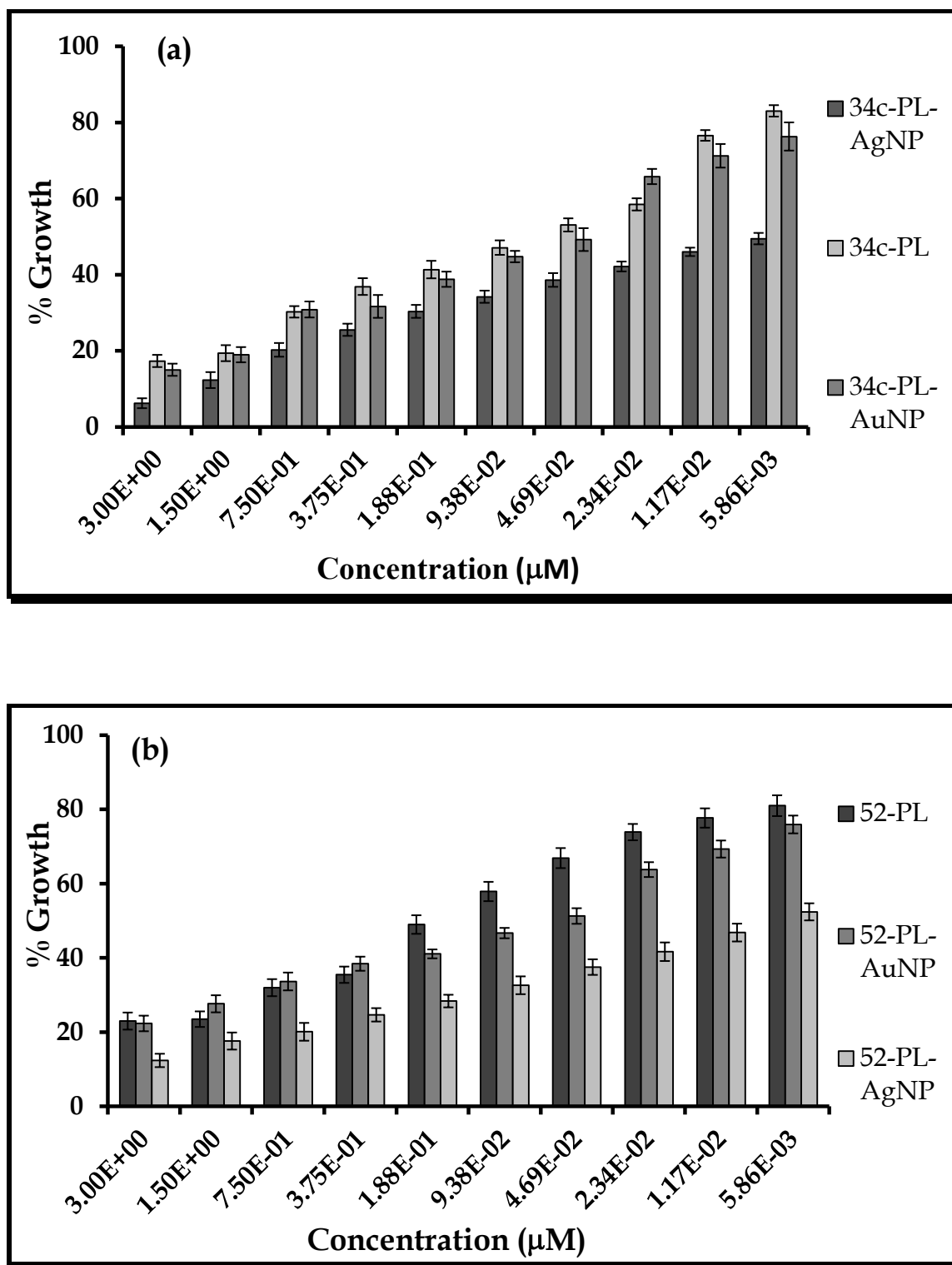


Figure 5.7: Growth inhibition process of (a) 34c-ε-PL and (b) 52-ε-PL in the presence of Au or Ag nanoparticles post light irradiation.

5.1.3 Chick chorioallantoic membrane (CAM) evaluation of a phthalocyanine-poly-L-lysine conjugate

Tumor growth is highly dependent on angiogenesis, as a result, antiangiogenic drugs are useful in cancer therapy as they have the ability to cause occlusion of the blood vessels that feed the tumor. The chick embryo is a practical method to screen the effects of anti-cancer agents on vascular acclusion of CAM. Its transparency provides quantitative assessment and real time documentation of PDT damage on the micrometer scale, both to vasculature and to implanted tumours.

In this work, the CAM model was used to evaluate the extent of vascular occlusion caused by **34c- ϵ -PL** in the absence of light. The results from the images show that **34c- ϵ -PL** did not exert any toxic effects as the blood vessel morphology of the CAM did not change as depicted in Figure 5.8. This result suggest that the conjugate is suitable for PDT studies as it is non-toxic in the absence of light

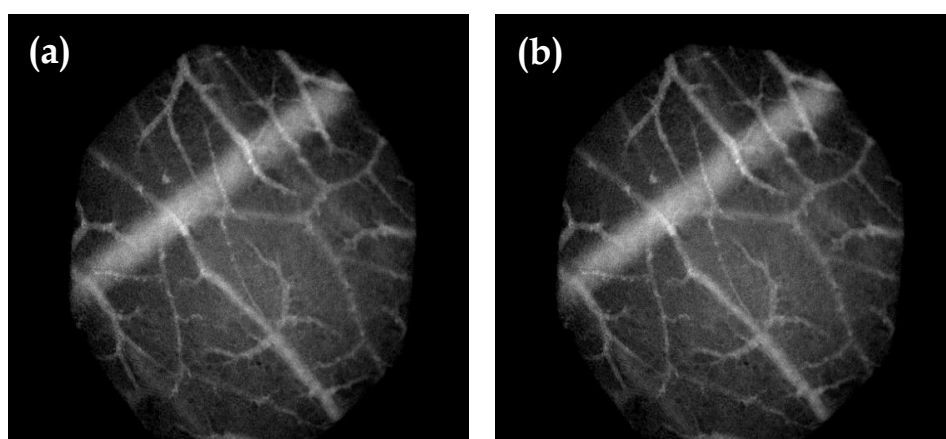


Figure 5.8: Fluorescence angiography of the CAM (a) before and (b) after injection with **34c- ϵ -PL** after 24 hrs.

Laser studies for the CAM assay could not be conducted as a result of a damaged laser system, since this study was carried out at the University of Geneva Switzerland, PDT studies could not be performed.

5.2 Conclusions

Photodynamic studies confirmed that the conjugation of phthalocyanines to carrier molecules improves PDT efficiency. The low dark toxicity of the phthalocyanine in cells is very promising and further studies on the synthesis of a water soluble derivative is essential in order to improve the photophysical and photochemical properties of the Pc in cells with regards to better singlet oxygen generation. AuNP had a moderate effect on the PDT activity of complex **37**, while liposomes showed a much more enhanced activity compared to **34c** or **37** alone indicating that liposomes are better drug delivery agents compared to gold nanoparticles in this case.

The antimicrobial activity of **Pc- ϵ -PL** conjugates in the absence and presence of Ag or Au nanoparticles was tested on *S. aureus* as a model microorganism. This type of bacterium is rather problematic due to the permeable outer membrane that allows for the diffusion of agents. Hence, as a defence mechanism such bacteria tend to develop resistance to anti-microbial agents such as the MRSA strains. The results of the minimum inhibitory concentration of the conjugates against *S. aureus* indicate that the presence of silver nanoparticles greatly decreases the growth of the microorganism for both **34c- ϵ -PL** and **52- ϵ -PL** conjugates. The studies showed that the **34c- ϵ -PL-AgNP** conjugate is a more effective photosensitizer compared to **52- ϵ -**

PL-AgNP in the photodynamic microbial inactivation at 39.6 mW/cm². The present research suggests that the delivery of photosensitising agents to bacterial cells can be improved by their association with polylysine moieties and nanoparticles.

Chapter 6

General conclusions and future prospects

This chapter summarizes the results reported in this thesis together with future prospects.

6. Conclusions and future prospects

6.1 General Conclusions

Novel unsymmetrical metal-free, magnesium and zinc tetra-substituted phthalocyanine complexes have been successfully synthesized in this work using the statistical condensation approach. The synthesis of a novel symmetrical thiol zinc phthalocyanine (**37**) is furthermore presented. The complexes were fully characterized by spectroscopic methods and elemental analysis confirmed the purity of the complexes. Gold and silver nanoparticles were synthesized and fully characterized. Selected sulfur containing phthalocyanines were conjugated to nanoparticles and the resulting conjugates were characterized. The fluorinated low-symmetric zinc phthalocyanine (**34c**) and the tetrasulfonylchloride zinc phthalocyanine (**38**) were successfully linked to poly-L-lysine and characterized; this rendered the phthalocyanines water soluble and highly valuable for PDT.

A study of the photophysical properties of the phthalocyanines, corresponding conjugates and their interaction with folic acid was conducted. The magnesium phthalocyanine derivatives gave the highest fluorescence quantum yield (Φ_F) values together with high lifetimes (τ_F) compared to the metal-free and zinc derivatives. Conjugation of the complexes with delivery agents caused quenching of the Φ_F or τ_F in some cases suggesting photoinduced energy transfer from the phthalocyanine to the delivery agent. Sufficient singlet oxygen was generated by the complexes. Conjugation of complexes with drug delivery agents resulted in a

significant decrease in the triplet lifetimes (τ_T), however slight variations of the Φ_T were observed for most conjugates.

Complexes **34c** and **37** were tested for their PDT activity against MCF-7, a breast cancer cell line and against non-malignant fibroblast cells. The complexes were encapsulated in liposomes for effective drug delivery or conjugated to gold nanoparticles. Complex **34c** gave the lowest cell viability when bound to liposomes compared to complex **37** conjugates.

Phthalocyanine-poly-L-lysine conjugates were tested for their antimicrobial activity against *S. aureus*, the results showed that the presence of light reduced the growth of the organisms as a result of singlet oxygen production. However, the conjugates were more lethal in the presence of silver nanoparticles with minimum inhibitory concentrations (MIC_{50}) of less than 0.0058 mM for **34c- ϵ -PL** conjugate in the presence of light.

Dark toxicity evaluation of **34c- ϵ -PL** using the CAM model showed that this conjugate poses no toxic effects on the vasculature without photo-activation, making it a prospective PDT agent.

6.2 Future prospects

The covalent linking of phthalocyanines to folic acid needs to be explored together with the photophysicochemical effects and PDT abilities. Laser treatment using the CAM model is also necessary to establish the PDT effect of the conjugates *in vivo*.

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